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Enhancement strategies for sustainability in the agrifood field and characterization methodologies of products from biorefinery

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Strategie di recupero valorizzativo per la sostenibilità in ambito agrifood e metodologie di caratterizzazione dei prodotti da bioraffineria

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ABSTRACT

Questo lavoro di ricerca si pone obiettivi che, in modalità interdisciplinare, coniugano i principi dell'economia circolare, la bioeconomia e la sostenibilità in ambito agrifood, per individuare percorsi di recupero valorizzativo di scarti agroindustriali mediante un approccio tipico delle applicazioni di bioraffineria.
Il focus principale è dedicato alle grandi produzioni massive di *Cucurbitaceae*, con particolare riferimento alle cultivar di *Citrullus Lanatus* (cocomero) e *Cucumis Melo* (melone), poiché il comparto agroalimentare rappresentato da queste colture primarie genera immense quantità di materiali di scarto lungo tutta la filiera '*from farm to fork*'.
L'idea cardine - pivotale del progetto di dottorato, consiste nella possibilità di estendere la 'catena del valore', che attualmente sembra interrompersi con l'abbandono in pieno campo dei prodotti di scarto per varie ragioni. Alcune di esse sono: (i) smaltimento di prodotti genericamente devalorizzati per non conformità morfologica; (ii) per sovraproduzione primaria; (iii) per sovramaturazione; (iv) per qualunque altra ragione che ne impedisca la distribuzione commerciale ed il regolare consumo.
Ripristinare la 'catena del valore', in questi casi significa darle continuità, evitando lo spreco in tutte le direzioni possibili, soprattutto dove le produzioni massive e le dimensioni dello smaltimento possono rappresentare un problema ambientale in aggravio alle situazioni di contesto tipiche dei nostri territori.
Le modalità più coerenti con questa enunciazione prevedono l'implementazione di nuove piattaforme tecnologiche per applicare efficacemente le strategie operative della bioraffineria che, mediante trasformazioni degli scarti recuperati, genera la metamorfosi 'da problema a soluzione con valore aggiunto'.
Le sfide dell'innovazione tecnologica di prodotto e di processo applicate in campo agroalimentare consentirebbero di utilizzare i materiali di scarto per conseguire obiettivi di valorizzazione che sfociano in ogni direzione (ingredienti funzionali per *food&feed*, monomeri per bioplastiche, biofuel, chimica fine e secondaria, ecc.), finalità comunque tutte orientate secondo i target di programma dell'Agenda 2030.
Pur riconoscendo la valenza strategica dell'intero pool di driver del cambiamento identificati dal programma ONU, abbiamo scelto di cogliere le maggiori sfide per convertire gli scarti agronomici da *Cucurbitaceae* in opportunità di contesto *food&feed*, trascurando al momento gli aspetti direttamente connessi col recupero energetico da biomasse residuali e la valorizzazione mediante processi di compostaggio.
Pertanto, mediante efficace frazionamento a partire dalle matrici selezionate, è stato possibile ricavare
● fibre alimentari di origine cellulosica, sia naturali "tal quali", sia sbiancate per trattamenti con H_2O_2 (da *Melone Inodorous*);
● fibre rosse ad alto contenuto di licopene (da *Citrullus Lanatus*) ;
● pigmenti e coloranti naturali da altre frazioni e sottoprodotti da esocarpo;
● prodotti della canditura a partire dalla frazione mesocarpo (albedo) del cocomero.
Per la caratterizzazione estesa delle diverse frazioni, abbiamo applicato una varietà di tecniche strumentali, metodologie analitiche e chimico-fisiche, compresa la preparazione adeguata dei campioni, che si possono così riassumere:
➢ spettroscopia UV-Vis in stato solido e in soluzione, valutazione del punto colore con metodo CIELab;
➢ tecniche separative cromatografiche GC-MS, SPME-GC-MS, HPLC-MS;
➢ analisi ICP-OES per la valutazione del contenuto di metalli;
➢ metodi termici di analisi (TG/DTG/DTA);
➢ analisi elementare (CHNS);
➢ analisi morfologica con tecniche di superficie (SEM);
➢ proprietà tecnologiche e funzionali: Water Holding Capacity (WHC), Water Swelling Capacity (WSC), Oil Holding Capacity (OHC).

La relazione finale per il conseguimento del titolo PhD è strutturata per argomenti capitolari che trattano le varie tematiche in modalità e formato autoconsistente.

Il capitolo 1 apre la discussione, ponendo le basi per contestualizzare l'argomento e le scelte motivate per l'attività di ricerca. Inoltre, fornisce una panoramica di letteratura che propone obiettivi e stato dell'arte relativo a questo progetto di lavoro, incentrato su alcuni aspetti relativi alla sostenibilità in campo agroalimentare.

Nel capitolo 2 si motivano le scelte effettuate per quanto concerne le matrici da processare con tecniche di bioraffineria (*Cucurbitaceae*), anche tenendo conto di alcune specificità produttive dei nostri territori.

Nel 3° capitolo abbiamo focalizzato l'attenzione su alcuni aspetti correlati alle frazioni maggiormente rappresentative dei sottoprodotti a valore aggiunto che si possono estrarre e recuperare, soprattutto da materiali in condizioni pre-waste.

Nel capitolo 4 sono raccolte tutte le informazioni relative ai materiali, alle procedure separative, alle metodologie analitiche applicate per estrarre le informazioni quali- e quantitative necessarie per costruire il quadro di riferimento per l'elaborato finale.

Il 5° capitolo è dedicato alle attività focalizzate sulla matrice *Melone Inodorous*, Cucurbitacea a pasta bianca, da cui si può recuperare la fibra alimentare neutra dal punto di vista organolettico, un potenziale ingrediente di formulazione per alimentazione umana, ecc.

Il capitolo 6 descrive in dettaglio i risultati ottenuti applicando la tecnica SPME-GC-MS alla matrice ed ai sottoprodotti di frazionamento del *Melone Inodorous*.

Nel capitolo 7 sono illustrati e discussi i risultati relativi alle indagini effettuate sulla matrice 'cocomero', avendo selezionato i prodotti rappresentativi di 3 cultivars particolarmente interessanti per lo stesso settore merceologico.

Il capitolo 8 riporta i risultati raccolti nel corso delle osservazioni sperimentali distribuite nell'arco temporale di 2 anni. In questo periodo d'indagine, abbiamo investigato le caratteristiche aromatiche della 'fibra rossa' da *Cucurbitaceae*, al fine di valutarne lo stato di conservazione, e gli effetti di ageing sulle caratteristiche della fibra medesima.

Nel capitolo 9 abbiamo ripreso le problematiche relative agli effetti dell'invecchiamento delle fibre. Qui sono discussi in dettaglio i risultati ottenuti in merito alla valutazione del punto colore, con un focus specifico dedicato alla cultivar Crimson Sweet.

Il capitolo 10 propone i risultati ottenuti valutando un set di proprietà tecnologiche e funzionali delle diverse fibre alimentari ottenute nel corso di questo lavoro di ricerca. Tali proprietà descrivono il comportamento meccanico per assorbimento di acqua ed olio, e sono particolarmente utili per la qualificazione tecnologica delle fibre di interesse applicativo. In realtà, nel capitolo mettiamo a disposizione una panoramica estesa anche ad altre matrici di nostro interesse, seppure di origine diversa dalle *Cucurbitaceae*.

Nel capitolo 11 è illustrata una possibile applicazione per la valorizzazione delle bucce di scarto da cocomero. Un vero e proprio caso di studio di trasferimento tecnologico che, oltre agli elementi di novità scientifica, mette in evidenza gli aspetti della sostenibilità a tutto campo, proponendo un piccolo e modestissimo contributo anche per risolvere il problema della fame nel mondo.

La relazione si chiude con il capitolo 12, dove riportiamo in forma conclusiva gli aspetti più significativi di tutto il percorso di attività di dottorato, oltre a sottolineare alcuni passaggi che rappresentano i focus points per gli eventuali sviluppi futuri.

A questo punto, come postilla delle attività di dottorato, possiamo rivolgere uno sguardo critico ai target conseguiti. Estendere la catena del valore ai materiali di scarto può consentire di acquisire competitività per il sistema agrifood, ma solo a fronte di sforzi adeguati per investimenti in ricerca ed innovazione.

L'analisi riportata nel testo della tesi descrive alcune caratteristiche strutturali di un comparto produttivo che, in ambito regionale e nazionale, è correlato alle performance del sistema agroalimentare sovradimensionato, poiché tende a sprecare risorse pre-waste ancora recuperabili. Proiettare questi risultati, che esprimono potenzialità competitive, in un orizzonte produttivo grintoso e di consumo ragionato, potrà garantire anche solo un piccolo contributo fattivo alla sostenibilità alimentare globale.

Enhancement strategies for sustainability in the agrifood field and characterization methodologies of products from biorefinery

Laura Maletti

ABSTRACT

This research work sets goals that, in an interdisciplinary context, combine the circular economy, bioeconomy and sustainability principles in the agri-food sector, to identify possible paths for the valorization recovery of agro-industrial wastes, through an approach typical of biorefinery applications. The main focus is dedicated to the large and massive productions of *Cucurbitaceae*, with particular attention to *Citrullus Lanatus* (watermelon) cultivars and *Cucumis Melo* (melon), since the agri-food sector represented by these primary crops generates huge quantities of waste materials along the entire "from farm to fork" supply chain.

The basic and key idea of the PhD project consists in the possibility of extending the "value chain", which currently seems to be interrupted for many reasons. Some of these can be: (i) abandonment of waste products in the open field; (ii) disposal of products generically written down due to morphological non-compliance; (iii) for primary overproduction; (iv) for over-ripening, and so on. In any case, it can be any reason that prevents commercial distribution and regular consumption.

Restoring the "value chain" in these cases means giving it continuity, avoiding waste in all possible directions, especially where massive production and the disposal size can represent an environmental problem in aggravation to context situations typical of our territories.

The methods most consistent with this statement involve the implementation of new technological platforms to effectively apply the operational strategies of the biorefinery which, by transforming the recovered waste, generates the transition "from problem to solution, with added value".

Therefore, the challenges of product and process technological innovation applied in the agri-food sector would allow the use of waste material to achieve valorization goals that cover every direction (functional ingredients for *food&feed*, monomers for bioplastics, biofuels, fine and secondary chemicals, etc.). All these purposes are oriented according to the program targets of the 2030 Agenda.

Although recognizing the strategic value of the entire pool of change drivers identified by the UN program, we have chosen to seize the greatest challenges to convert agronomic waste from *Cucurbitaceae* into opportunities for a *food&feed* context, neglecting for now the aspects directly related to energy recovery from residual biomass and valorization through composting processes. Therefore, by effective fractionation starting from the selected matrices, it was possible to obtain:

- dietary fibers of cellulosic origin from *Cucumis Melo* var. *Inodorus* (winter melon), both natural "as it is" and bleached by H_2O_2 treatments;
- red fibers from *Citrullus Lanatus* (watermelon) with a high content of lycopene;
- natural pigments and dyes from other fractions and exocarp by-products;
- candied products starting from the watermelon mesocarp fraction (albedo).

For the extended characterization of the different fractions, we have applied a variety of instrumental techniques, analytical and chemical-physical methodologies, including the adequate preparation of the samples, which can be summarized as follows:

- UV-Vis spectroscopy in solid-state and solution, color point evaluation with CIELab method;
- GC-MS, HS-SPME-GC-MS, HPLC-MS-ESI-IT chromatographic separative techniques;
- ICP-OES analysis for the metal content evaluation;
- thermal analytical methods (TG / DTG / DTA);
- elemental analysis (CHNS);
- morphological analysis with surface techniques (SEM);
- technological and functional properties: Water Holding Capacity (WHC), Water Swelling Capacity (WSC), Oil Holding Capacity (OHC).

The final report for the PhD degree achievement is structured by chapter topics that deal with the various themes in a self-consistent manner and format.

Chapter 1 opens the discussion, laying the groundwork for contextualizing the topic and the motivated choices for the research activity. In addition, it provides a literature survey that proposes objectives and state of art relating to this work project, focusing on some aspects related to sustainability in the agri-food sector.

Chapter 2 explains the decision taken regarding the matrices to be processed with biorefinery techniques (*Cucurbitaceae*), also considering some production specificities of our territories.

In the 3rd chapter, we focused on some aspects related to the most representative fractions of value-added by-products, extractable and recoverable especially from materials in pre-waste conditions.

Chapter 4 collects all the information related to the materials, the separative procedures, the analytical methodologies applied to extract the qualitative and quantitative information necessary to build the reference framework for the final report.

The 5th chapter is dedicated to the activities centered on the melon *Inodorous* matrix, a white-fleshed Cucurbit, from which the organoleptically neutral dietary fiber can be recovered, as a potential formulation ingredient for human nutrition, etc.

Chapter 6 describes in detail the results obtained by applying the HS-SPME-GC-MS technique to the matrix and fractionation by-products of the *Inodorus* melon.

In chapter 7, the results of the surveys carried out on the 'watermelon' matrix are illustrated and discussed. The representative products of 3 cultivars were selected, particularly interesting for the same agronomic product sector.

Chapter 8 reports the results collected during the experimental observations distributed over a period of 2 years. We studied the aromatic characteristics of the 'red fiber' from *Cucurbitaceae*, to evaluate the aging effects of the fiber itself.

In chapter 9 we have resumed the problems related to the chromatic variation of the fibers. Here the results produced regarding the evaluation of the color point are discussed in detail, with a specific focus dedicated to the Crimson Sweet cultivar.

Chapter 10 proposes the results obtained by evaluating a set of technological and functional properties of the different dietary fibers obtained during this research work. These properties describe the mechanical behavior by water and oil absorption, particularly useful for the technological qualification of the fibers of application interest. The chapter provides an overview extended to some other matrices of our interest, although having different origins from *Cucurbitaceae*.

Chapter 11 illustrates a possible application for the watermelon waste peels (rinds) valorization. A real case study of technology transfer which, in addition to the elements of scientific novelty, highlights the aspects of sustainability all-round, proposing a small and very modest contribution also to solve the problem of hunger in the world.

The thesis closes with chapter 12, where we report in a conclusive form the most significant aspects of the entire course of PhD activity, as well as underlining some passages that represent the focus points for any future developments.

At this point, once the PhD activities are over, we can take a critical look at the targets achieved. Extending the value chain to waste materials allows to acquire competitiveness for the agri-food system, but only through adequate efforts for investments in research and innovation. The analysis reported in the thesis text describes some structural characteristics of a production sector which, at a regional and national level, is related to the oversized agri-food system performance, since it tends to waste ‘pre-waste’ resources that are still easily recoverable. Projecting these results, which express competitive potential, in a gritty production horizon and reasoned consumption, can guarantee even a small effective contribution to global food sustainability.

Abbreviations

DF	Dietary Fiber
M	Melon
M_AI	Melon pomace “as it is”
M_AI_V	Melon pomace “as it is”, Vacuum-freeze dried
M_AI_O	Melon pomace “as it is”, Oven dried
M_B	Melon pomace Bleached with H_2O_2 at Room Temperature
M_B_{50}	Melon pomace Bleached with H_2O_2 at T=50 °C
M_B_V / M_B_O	Melon pomace Bleached with H_2O_2 at Room Temperature, Vacuum-freeze dried or Oven dried
M_B_{50}_V / M_B_{50}_O	Melon pomace Bleached with H_2O_2 at T=50 °C, Vacuum-freeze dried or Oven dried
W	Watermelon
W_AI	Watermelon pomace “as it is”
W_*w*	Watermelon pomace *washed* with distilled water
W_AI_V	Watermelon pomace “as it is”, Vacuum-freeze dried
W_AI_O	Watermelon pomace “as it is”, Oven dried
W_*w*_V / W_*w*_O	Watermelon pomace *washed* with distilled water, Vacuum-freeze dried or Oven dried
W-seed-flour	Defatted watermelon seed flour
TDF	Total Dietary Fiber = “as it is”
SDF	Soluble Dietary Fiber
IDF	Insoluble Dietary Fiber = “washed” or “bleached”
WHC	Water Holding Capacity
SRC	Solvent Retention Capacity
OHC	Oil Holding Capacity
WSC	Water Swelling Capacity
GLU	Glucose
SUC	Sucrose
CA	Citric Acid
AHP	Aesculus Hyppocastanum Pure
AHH	Aesculus Hyppocastanum Hybrid
AXC	Aesculus X Carnea

Ex nihilo nihil fit
Lucrezio

CHAPTER 1

1 - INTRODUCTION

If we look at the Earth in the images that come from a great distance (400 MKm), collected by the Rovers sent to Mars with different space missions, we are fascinated by that blue dot which, after all, contains us.
But if we reduce the distance of the filming (400 Km), and we are pleased with satellite images, or collected by the International Space Station (ISS), we observe that embryonic dot beginning to take shape, showing excellent details, but sometimes not, and in some cases take away the previous charm.
Because the images become merciless for ourselves, they express a condition of discomfort that preludes the condemnation for the human race, for our irresponsible behavior.
Oceanic plastic islands, deforestation, enormous forest fires, intensive agriculture, increasingly frequent floods, climate change, and other devastating situations appear.
The first formulation of the planetary 'theory of limits to growth' dates back to 1972 [1], formulated by D. Meadows during the annual meeting of The Club of Rome, an informal and international organization, properly described as the 'invisible college' of Prof. Aurelio Peccei.
Half a century after the theoretical hypothesis was enunciated, now we can only collect the proofs: humankind is now very close to the growth limits established from the physical constraints of the planet we live on.
In this context, as humanity continues to race headlong towards total and irreversible disaster,
in full awareness of the consequences of our actions, we must express an option between only two choices: i) accept our collective irresponsibility, or ii) change the way we behave.
If we want to pursue the second and right goal, we must replace the current development model "business-as-usual" introduced with the industrial revolution of three centuries ago, with the "planet-at-first" conservative one.
The effects of the contrast between these two models are macroscopic and evident: the predation of 'economically vital' resources in favor of a few, against sustainable development in favor of all.
The consequences are becoming perceptible and dramatically visible in the current food shortage, reduction of the availability of fossil raw materials, impoverishment of mineral sites, increase in conflicts for the hoarding of resources of any kind, among many other critical issues.
If we then add the periodic, or systemic, crises of global financial systems, the lack of trust we place in our political systems, beyond the thousand uncertainties that also undermine the health conditions of living beings, we can identify some reasons that rationalize in part the pervasive frenzy of our erroneous behaviors [2,3].
The United Nations recognized the first symptoms and possible negative fallout of this global crisis in the early 1980s.
Following those indications provided almost with a spirit of preaching, reiterating the concepts relating to the limited resources of the planet, and proposing the idea that 'a large system is not an infinite system anyway', a growing consensus has emerged on the need to take care and respect for the natural resources necessary for human survival.
So, after many decades of unbridled, uncontrolled and irresponsible exploitation, aimed at an anthropo-centric and eco-denier development, a more moderate and conservative approach has begun to gain some theoretical acceptance.
A further trust in this direction also came from the advance of popular movements inspired by new principles of the green-blue economy and world preservation. By gradually gaining political representation and decision-making consensus, they have largely contributed to spreading new ideas in support of an increasingly eco-centric development in an attempt to marginalize the anthropocentric one.
Thus, partially renouncing to the devastating instinct of the rule 'business-first-of-all', in the "Brundtland Report" of 1987, the model of *sustainable development* appeared for the first time, defined as "development

that satisfies the needs of the present without compromise the ability of future generations to satisfy theirs needs" [4].
This definition represents an equity pact between present and future generations, provided that every production chain (food, materials, transports, and so on) follows the wave of responsible transformation.
This will lead to the creation of new jobs, cleaner infrastructure and a resilient future for each of us.
All these actions will ensure better health and full respect for human rights, enabling people to live with dignity on a healthy planet.
In early 2021, the United Nations Environment Program (UNEP), the leading global environmental authority promoting the consistent implementation of Sustainable Development within the United Nations, published a report entitled "Making Peace with nature: a scientific project to tackle climate emergencies, biodiversity and pollution" [5].
Among the various problems that emerged in the UNEP report, we also find the increase in the irrational use of natural resources and the exponential production of waste.
In fact, to meet the growing demands from human activities and industrial production, the exploitation of soil, energy and water resources has become progressively more aggressive over time.
Consequently, they are depleting and we assisted to an excessive production and accumulation of waste materials.
The UN 2030 Agenda for the Sustainable Development Goals can also be defined as an "action plan" to achieve the fundamental goals for humanity and the planet.
The need to put an end to poverty and hunger, the protection of the planet by promoting sustainable consumption and production, ensuring that all human beings can enjoy good living conditions, are now essential objectives.
To reach some of these goals and targets, the actions suggested to educational and scientific organizations include the transformation of food, water and energy systems to meet the growing human needs, in a fair and resilient way and respectful for nature.
Within this global intervention plan for the European Community, all projects that place the topic of sustainability at the center of research activities are strongly encouraged, allocating substantial economic resources to all the proposals related to the issues "ecological transition".

This PhD study fits perfectly into this context, having the aim of responding, albeit with a small and modest contribution, to the development of knowledge relating to the increasingly widespread problem of food loss and waste and overproduction in the agri-food sector.
FAO has estimated that about 14 % of the food globally produced for consumption is lost each year between harvest and the wholesale market, while approximately 17 % of total global food production is wasted (11% in households, 5 % in the food service and 1 % in retail) [6].
In particular, FAO defines **food loss** as the discarded and disposed food along the supply chain, starting from post-harvest but excluding retailers, food service providers and consumers. Unfortunately, this material is not re-used in any other productive sector, for example feed. This is particularly true for fruits and vegetables, where fresh products that deviates from what is considered optimal for consumers, in terms of shape, size, color or because of defects or damages, are removed from the supply chain during sorting operations.
However, the same behavior is also adopted downstream of the supply chain, for example by consumers.
In this case, according to FAO, it falls into the concept of food waste.
In fact, **food waste** refers to the decrease in the quantity or quality of food as a consequence of decisions and actions by retailers, food service providers and consumers.
An example is represented by foods that are close to, at or beyond the "best-before" date which are often discarded by retailers and consumers.
Concerning fruit and vegetables, it must be also added the large quantities of material that is discarded during the field production, and by the agroindustry during the processing operations of these foods.
Bearing all this in mind, it is evident that reducing food loss and waste is fundamental not only to reduce the number of people affected by hunger but also to make our food system more sustainable.

When food is lost or wasted also all the resources used to produce it are wasted (water, soil, energy, among others), in addition to the problem of the disposal of agri-food wastes in landfills, which leads to greenhouse gas emissions, contributing to climate change.
The UN has declared 2021 the International Year of Fruits and Vegetables, with the aim to promote the consumption of these foods thanks to their nutritional and health benefits, but also to address new policies for the reduction of loss and waste of these highly perishable products at each step of the supply-chain, starting from the farm [7].

Figure 1 shows the Sustainable Development Goals related to fruit and vegetables.

Figure 1 - Sustainable Development Goals related to fruit and vegetables according to FAO [7]

Therefore, one objective to be pursued is the transformation and conversion of poor materials that for someone is "waste", available in large quantities and apparently without any economic value, into a resource and a value-added product, which can be then reintroduced in some production chain. This can be achieved by applying the biorefinery concept. In biorefinery, almost all the types of biomass feedstocks can be separated in several fractions and sub-fractions, such as oils, fibers, bioactive compounds, pigments, etc… which, then, can be converted again in other products, chemicals and biofuels through jointly applied conversion technologies. Moreover, through the integration of green chemistry into biorefineries and the use of low environmental impact technologies, the future sustainable production of high-added value products from vegetable biomasses, such as agro-food wastes, can be achieved.
So, the implementation of the fundamental principles of sustainable development allows to minimize the use and handling of raw materials, and to face any new challenge considering the relationship between environmental, economic and social aspects.

All these concepts are fully included in the context of **circular economy**, a model of production and consumption which implies the reduction of waste to a minimum by reusing, repairing, refurbishing and recycling existing materials and products as long as possible, extending their life cycle.

Starting from these assumptions, my PhD project aims to identify effective splitting up processes and extractive methodologies to obtain several fractions, products and by-products from the selected biomasses, to study and characterize them as accurately as possible, with the purpose of enhance some agro-food waste materials.
The cornerstone of the project is the need to reduce waste in the agronomic field, to satisfy new food needs, to defend quality agriculture, to guarantee the sustainability of primary productions, to attribute new life and new value to products and materials recovered on the dis-value chain.

Among the several biomasses available and potentially selectable, thinking about the types of primary agricultural productions diffused in our territories, we have decided to study fruits belonging to the *Cucurbitaceae* family. In particular, we selected different watermelon (*Citrullus Lanatus*) cultivars and one type of melon (*Cucumis Melo*), which will be better described in the following sections.
According to FAOSTAT [6], the production of watermelon in Italy passed from ~ 580 Ktons in 2018 to ~ 650 Ktons in 2020, with a cultivated area of about 13400 ha. Emilia-Romagna region contributes to the national production for about 7 - 7.5 % (ISTAT data) [8]. ISTAT also estimated that during the cultivation cycle ≈ 8.5 % of the total production is not harvested, corresponding to about 55 Ktons/year [8].

As previously mentioned, the sorting operations carried out during the cultivation cycle generate the "food loss", because of fruits defects caused by hail, skin spots, fruit malformations, over-ripening, product left on the growing field at the end of the cultivation season. To these quantities it is then necessary to add the amount of fruits that are further discarded or unsold by retailers and the by-products derived from industrial processing. These operations, instead, generate "food waste".
The previously reported data become impressive if considering the worldwide production of watermelon. In 2017-2018, about 42 million tons of watermelon by-products have been generated during transformation, preparation and consumption of the fruit [9]. To this, it must be added the amount of fresh product that annually remains in the field, which can reach ≈ 20 % of the total production [10].

In addition to the waste of materials and resources, also the disposal of these large biomasses of *Cucurbitaceae* is a problem. In fact, fruits with a high-water content and rich in fermentable sugars, if immediately spread on the land, can originate large amount of alcohols, i.e. methanol and ethanol. These species give serious consequences for the microbial flora of the soil, to the point of even procuring sterility [11].
Bearing this in mind, the PhD project is aimed to transform melon and watermelon fractions and sub-fractions into new commodities for increasing *food&feed* availability, and to reintroduce them in some production chains.
We specify that during this study we refer to materials that are in a pre-waste condition, i.e. rejected or discarded for the above-mentioned reasons, but that have not been attacked by molds, spores or fermentative processes of sugars. Therefore, they don't present any health risk. In fact, biomass which is potentially dangerous for health (for example, due to microbial contamination) is not the topic of this study and, to the best of our knowledge, is typically intended for composting or for the production of biofuels.

*Cucurbitacea*e family is very large, includes numerous varieties of watermelons, melons, pumpkins and cucumbers. Among the various watermelon (*Citrullus Lanatus*) cultivars, we decided to study Crimson Sweet and Asahi Miyako, typically cultivated in the territories of the Po plain, and a variety produced in Sardinia which has been called Gavina®.
Each of them has different intrinsic characteristics, depending on genotype factors, the geographical origin, so the climate in which it develops and matures, the chemical composition of the soil in which is cultivated, among others. These peculiarities made the characterization phase particularly interesting and intriguing.
As for melons (*Cucumis Melo*) concerns, we selected the Yellow Canary type, also called winter melon, which belongs to the *Inodorus* family.

These biomasses provide interesting raw materials from which to obtain products with high added value such as dietary fiber, seed oil, dyes and pigments, protein, starch, thirst-quenching juice, among others, and we proceeded with the isolation and the accurate characterization of some of them.
Even though each fraction obtainable from these fruits constitutes an interesting subject of study, during this thesis we have dealt, in particular, with the fiber extracted from the endocarp of the aforementioned fruits.

For watermelon, using different methods of investigation, we have studied:
● the chromatic stability over time of the dietary fiber, stored in different conditions;
● extraction tests were conducted for lycopene (the natural dye prevalent in this matrix) which gives the natural intense carmine red chromatic characteristic, in order to obtain some semi-quantitative information on its concentration in the fiber;
● the aromatic profile of the VOCs present in the fiber was assessed over time, with the aim to establish a useful correlation between some analytes that can play the role of marker and the degree of aging of the fiber itself;
● some chemical-physical properties were monitored such as water holding capacity (WHC), solvent retention capacity (SRC), water swelling capacity (WSC), oil holding capacity (OHC), the fundamental parameters for technological applications.

Similarly, also for melon some different analytical techniques have been applied to:
● estimate the effectiveness of the bleaching processes applied to the dietary fiber obtained from the fruit pulp, by monitoring the color stability over time;
● evaluate the behavior of the pulp fiber subjected to thermal stress (thermal analysis);
● identify the VOCs responsible for the aroma of the fiber in order to monitor the compositional variations induced by the bleaching process;
● monitor the physico-chemical properties previously cited (WHC, SRC, WSC, OHC) addressed to technological applications;
● characterize extracts from melon peel, to obtain natural dyes and pigments.

The study of the materials obtained from *Cucurbitaceae* took up most of the time of my PhD, allowing me to deepen aspects previously analyzed by our research group [12].

However, since it is a part of a broader and more focused project about the valorization recovery of biomass in general, we have also considered other materials of interest, such as those coming from *Hippocastanaceae* [13].
In fact, the biorefinery and Circular Economy concepts and thus the operative methodologies exploited, can also be applied to other matrices, by introducing the appropriate adjustments that can allow the processability of any other species.

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CHAPTER 2
2 – THE SELECTED *CUCURBITACEAE* MATRICES

Melon (*Cucumis melo* L.) and watermelon (*Citrullus Lanatus* L.) are two of the most important horticultural crops worldwide. They belong to the *Cucurbitaceae* family, which includes approximately 825 species among which also cucumber and pumpkin. It seems that both originated in Africa (melon had also a secondary center of origin which comprises a broad area between Iran, India and east China) and then widespread across wide areas of the world, becoming one of the most popular fruit cultivated in countries with temperate climate like Asia, America, Europe and Africa [1], [2].

2.1. MELON (*Cucumis Melo* L.)

The term "melon" can be referred to either the plant or the fruit, which is a kind of berry (a pseudo-berry) and more specifically a "pepo", which presents a great variation in size, shape, color of the skin and of the pulp, but also flavor, taste and texture depending on the cultivars.
In fact, the peel may be smooth, netted or ribbed with a color that can vary from different shades of green to ivory, up to bright yellow, while the flesh can assume a slight yellowish-green color (in some cases it seems almost white) or, on the other hand, it has an intense orange color. Finally, seeds are typically beige-colored and over 10 mm long in ripe fruits.
According to FAOSTAT, in 2018 the global production of melon fruit exceeded 40 million tons (FAOSTAT 2018, https://www.fao.org) and, in particular, in the period 2008-2016, the first producer has been Asia with almost 74 % of the world production (32 million tons), followed by America (11.9 %) and Europe (7.2 %) [1].
Among all the varieties, the three more common and diffused in Italy are:

- C. melo var. Cantalupensis: its fruits are round or slightly oval, with a gray-green skin, lightly ribbed and a sweet and very aromatic orange pulp;
- C. melo var. Reticulatus: it is a round melon with a firm green-yellowish pulp. Owes its name to the characteristic morphology of the peel, a net-like surface, gray-green or light yellow in color;
- C. melo var. Inodorus: it has oval fruits characterized by a very pale flesh, greenish-yellow or almost white and a skin which can be rough or smooth. Its cultivars are later in maturity than cantalupensis and have very good storage characteristics.

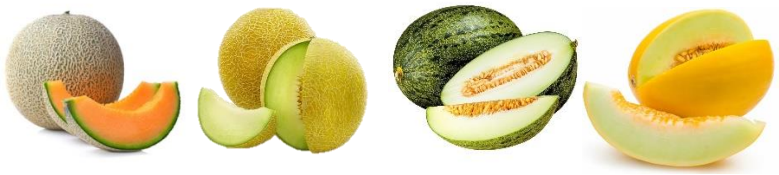

Figure 1 - Examples of some pepo from different melon cultivars: Cantalupensis, Reticulatus and Inodorus, respectively.

The weight of melon fruit typically ranges from 0.8 to 2.5 Kg and the edible pulp is the most precious fraction, representing around 65% of the whole fruit, while the peel and seeds make up 25 – 27 % and 5 – 7 %, respectively [3, 4].
It is particularly appreciated for being a very tasty fruit, low in calories, moderately satiating and quite thirst-quenching thanks to the high-water content. Melon is also an excellent source of bioactive compounds and mineral salts, beneficial for human health. In particular, the potential health benefits are mainly attributable to the phenolic fraction and their high antioxidant activity as well as to the organic acids and amino acids components [5].
Typically, the intake of the above-mentioned bioactive principles occurs through the consumption of the edible fraction of the melon, the endocarp, but it is increasingly recognized that even the fractions that are normally discarded, i.e. seeds and peel, can actually be an important source of nutrients and compounds with biological activity. Of course, this is true not only for melon but also for watermelon and, in general, for all fruit and vegetables belonging to the *Cucurbitaceae* family.

Not surprisingly, in recent years the interest in products and by-products deriving from *Cucurbitaceae* has increased a lot, with the aim of using them as a starting material for the recovery and extraction of bioactive compounds and other sub-fractions having possible technological applications [3, 6].

The industrial manufacturing processes involving these materials, as well as producing consumers commodities such as juices, jams, snacks and so on, generate huge quantity of by-products still rich in vitamins, oils, mineral salts, fibers, polyphenols and carotenoids. These by-products, instead of being lost, should be recovered and re-used for some potential applications focused on human nutrition, for example developing new food supplies or novel functional foods. In this way only the truly waste material, for example the one attacked by molds, bacteria, spores or involved in fermentation processes would be destined for composting and for obtaining energy or as biofuel precursors.
This would certainly be a starting point to at least try to reduce (if not even solve) the problems related to food loss, waste, landfills and pollution. For these reasons, their management and re-utilization in the context of valorization, namely cost reduction and less use of toxic methodologies, are the main challenge in the field of biotechnology to solve pollution problems and to meet the growing demand for food and feed supplies [3]. As far as the melon concern, among the different varieties mentioned above, we have focused our attention on the Yellow Canary melon type, which belongs to the *Inodorus* group. Actually, someone call it "yellow melon" for the color of the peel while it becomes "white melon" if you look at the color of the pulp or, again, it is called "winter melon" because it can be consumed until winter months, thanks to its long shelf-life.
In fact, it is characterized by a smooth and yellow skin, while the pulp is almost white and fleshy (to be precise it presents pale greenish or yellowish shades in relation to the degree of ripeness), fresh and juicy, very pleasant even if more delicate and less sweet than the orange pulp melons. To someone, its watery and sugary flavor is reminiscent of that of watermelon and pear.
Moreover, what differentiates it from the typical summer melon with the orange pulp is the crop cycle and the storage period: while a netted melon ripens in about two months from the fruit setting, the yellow one takes almost double the time. Finally, a curious aspect is related to the fact that it's not possible to smell its aroma externally when the fruit is intact but only after cutting it into slices, making the term "Inodorus" perfect.

Figure 2 - Cucumis Melo var. Inodorus, Yellow Canary type.

The characteristics just mentioned make this fruit particularly interesting as its pulp can represent a good starting material from which to obtain a fiber as neutral as possible which could meet the ever-increasing industrial demand for gelling and thickening agents for the food field, among others. This formulation ingredient should give to the finished product the desired technological properties without, however, making any contribution in terms of color, aroma and flavor to the mixture to which it is added.
To complete the picture of information relating to this matrix, the peel was also manipulated and moderately characterized in order to identify some potential applications as a starting material for any future studies and insights.

Fractionation scheme and mass balance for Cucumis Melo var. *Inodorus*

The first essential information to estimate the potential application of these pre-waste materials was obtained from the fractionation of winter melon fruits produced in Sicily (Italy) and purchased at a local

supermarket in Modena city. Five different fruits were taken and considered in order to give a representative analytical sample. Melons were readily used in the full ripening conditions at the purchasing time.
This allowed us to define the mass balance showed in Figure 3.

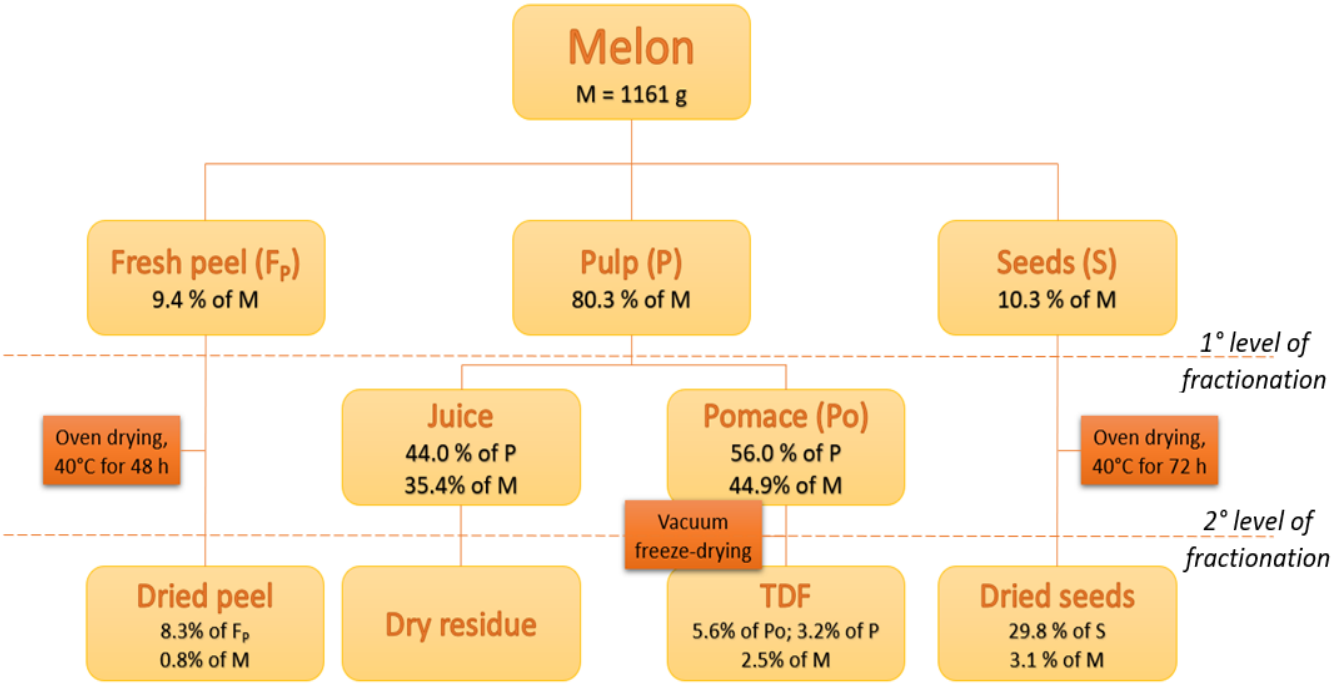

Figure 3 - Fractionation scheme and related mass balance for winter melon.

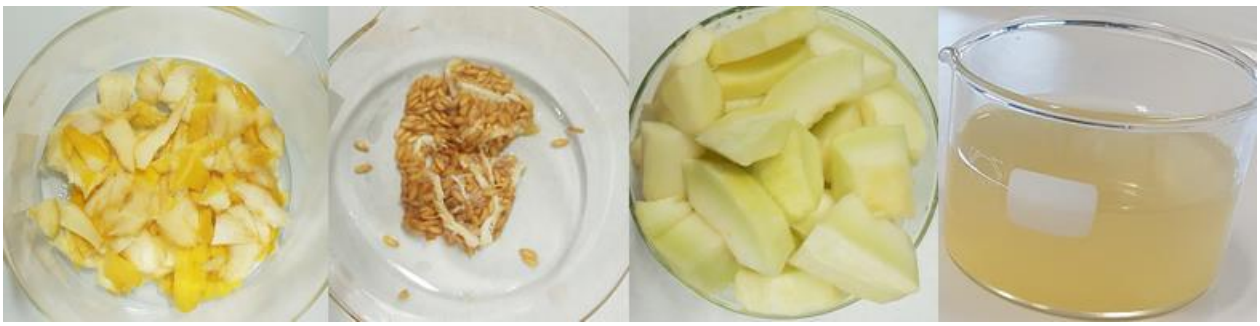

Figure 4 - Main fractions obtained from winter melon.

Fundamental unitary operations: the fruits were carefully washed with distilled water, weighed and cut, separating the peel from the pulp and carefully removing the seeds. It is worth pointing out that, for our purposes, the peel fraction was obtained by separating as effectively as possible the bright yellow exocarp from the inner pulp (endocarp).

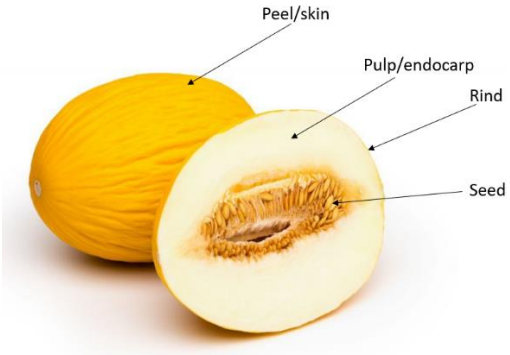

Figure 5 – Cross section of a melon fruit and identification of the constituent fractions.

In the following chapters we will identify the contribution of endocarp and the mesocarp as "pulp or total endocarp". Doing this, the peel was cut into strips of ~ 1 - 2 mm thick and then repeatedly washed again with distilled water and finally oven dried at 40 °C for 48 h. The material thus obtained does not show marked variations in terms of color if compared to the "fresh" one, keeping its golden shade, even if a partial loss of the initial brilliance is observed.
Such a mild heating should preserve as much as possible the properties and characteristics of the starting material, therefore, this drying temperature and contact time were used for all the oven dried fibers we prepared during this study (unless otherwise specified).
Before being used for any other operation, the dried peel was ground in a grinding mill equipped with Ti blades, to a fine powder having an average grain size of 0.5 mm. To preserve the thermolabile and volatile molecules, and the bioactive compounds, the rotor that houses the blades has been preliminarily refrigerated with some few drops of liquid N_2. The obtained powder was preserved in closed vessels at - 20 °C and under N_2 conditioned atmosphere until analyses were carried out.
All the fibrous samples we have obtained from various fractions of our matrices have been ground to the same particle size (0.5 mm) to be sure that this variable does not affect the results obtainable from the laboratory tests. All these materials have also been stored in the same conditions previously mentioned, to preserve their initial characteristics as much as possible.

Figure 6 - Winter melon peel oven dried at 40 °C for 48 h, before (left) and after grinding (right).

As for the melon seeds, after being separated from the pulp and isolated from the filamentous fraction they are in contact with, they were accurately washed with distilled water and then dried in a vented oven at 40 °C for 72 hours. Even if they are not the topic of our investigation, they can surely represent an interesting starting material for future studies, as already demonstrated in some scientific papers [3].
For what concern the endocarp fraction, it has been shredded with a common kitchen blender to obtain a homogeneous slurry-meal, light yellow in color and having the characteristic aroma of the ripe fruit. It was then centrifuged for obtaining the rapid sedimentation of the suspended micelles and finally filtered with filter paper and using a vacuum filtration system to separate the juice from the fibrous residue, which is

called pomace.
Table 1 summarizes our values for the above-mentioned fractionation procedure, and the data have been compared with some literature ones.

Table 1 - Results of the fractionation procedure of Yellow Canary melon, and comparison with some literature data.

Sample		**Values %**					
		Figure 3	[5]	[7]	[3]	[4]	[8]
Edible fraction (endocarp)		80.3	52 - 65	47	65	66	53-56
	Pomace	44.9				19	
	Juice	35.4				47	
Waste fraction		19.7		53	32	32	
	Rind	9.4 (fw)*	25 - 44	43.7	25	27	39-41
	Seeds	10.3 (fw)* 3.1 (db)#	3.4 - 7	3.4	7	5	4-6

*fw = fresh weight
#db = dry basis (residual moisture after 72 hours at 40 °C)

The comparison of the values in Table 1 shows a great variability of results. This observation is entirely compatible with the fact that different researchers used fractionation strategies which, probably, are not always perfectly overlapping. On the other hand, it is also known that even in fruits belonging to the same cultivar and having the same initial mass, the compositional ratios between the different fractions can significantly change depending on the overall morphology, the degree of ripeness, the thickness of the skin which is removed, etc. Therefore, it is quite obvious that this comparison has a purely symbolic meaning, since the ranges of variability of the qualitative and quantitative indices are definitely wide.

Peel – Our value (9.4% fw and 0.8% db) is very far from the literature range (25 ÷ 45 %), reasonably because we removed a thickness which never exceeded 1 ÷ 2 mm. In fact, for our studies, the yellow outer skin may have a rather important intrinsic value, since the recovery of the natural golden yellow dye, which is present in this vegetative district, could be one of the objectives to be pursued by applying separation strategies via biorefinery techniques [3].
The compositional characteristics of exocarp and endocarp are quite different and, given the high potential of the skin, which contains very interesting bioactive ingredients and analytes, it is certainly worth optimizing as much as possible the fractionation of the fruit to then make the best use of each part that is obtained.
In fact, it should be remembered that at industrial level, especially in the food field, among the most sought-after natural dyes there are precisely the yellow ones (in addition to those having a dark-black color). So, the possibility of achieving this goal, associated with the fact that the analytes responsible for this coloring could be bio-pharmacologically active compounds with beneficial effects on human health, make this fraction even more interesting and valuable.

Seeds – This fraction (3.1 %) shows a value which is aligned with the literature data (3.4 – 7 %) reported in the previous table. The upper value may be attributed to the characteristics of a genotype, among others, of the selected cultivar.
Despite the small percentage of this fraction if compared to the whole fruit, the global annual production of melon seeds reaches almost 1 megaton [FAOSTAT 2018], representing an environmental and management problem which could be tackled by applying the concept of bio-refinery to this fraction as well, in order to solve pollution problems and to meet the growing demand for food&feed supplies.

Endocarp – Literature values show that the edible fraction of the fruit represents on average 55 % (47 ÷ 65 %) of the total mass, while our current data even reaches the threshold of 80 %, with an excess of almost +30

% points on average. As already explained above, this difference can reasonably be attributed to a different approach applied during the fractionation step, that allowed us to obtain the maximum possible amount of the inner part of melon. Furthermore, we must probably add the effect of a different degree of ripeness between our fruits and the literature ones, since the thickness of the mesocarp under the skin is gradually and progressively converted during ripening. This process converts the mesocarp which becomes less and less thick and inedible, assuming instead a consistency comparable to that of the soft edible pulp. For our purposes it was necessary to apply this strategy because both the endocarp and mesocarp are particularly interesting starting material from which to derive an almost white dietary fiber (another highly sought-after product by the food industry). In detail, the quantities of juice and pomace obtained by Miller et al. [4] show a clear predominance of the former with respect to the residual solid, quite far from our values which, in turn, are fairly equally divided between the two sub-fractions, with 10 % more pomace than juice.
Again, a different degree of ripeness, melon cultivar and / or method of squeezing and filtration can reasonably explain these differences.
For the purposes of this research activity, we paid particular attention to the endocarp fraction in order to obtain dietary fibers having peculiar characteristics, such as whiteness and neutral taste and aroma, which could be used as formulation ingredient in the food industry. To increase the knowledge about this fiber, we proceeded with a wide and extensive characterization applying different techniques on properly handled samples. The results of these activities are reported in the following chapters.

2.2. WATERMELON (*Citrullus Lanatus* L.)

Watermelon (*Citrullus Lanatus* L.) is another annual crop, typical of summer season, appreciated by many people across the world as a fresh sweet fruit, thirst-quenching and low in calories, usually consumed as fresh slices, diced in mixed fruit salads or as juice.
The nutritional potential of watermelon has been evaluated by several studies in literature according to which its intake is associated with a reduction in the risk of the onset of cardiovascular and neurodegenerative diseases, the prevention of metabolic disorders, such as insulin-resistance and diabetes, and certain types of cancers [9–11].
The beneficial properties of this fruit are attributed to the presence of important phytochemical molecules, including lycopene, β-carotene and polyphenols, all bioactive species able to exert a powerful antioxidant and anti-inflammatory effect [10]. Furthermore, it is a precious source of mineral salts, in particular K, Mg, Ca and Fe [11] and of some vitamins: one cup of diced watermelon covers about 21 % of the daily requirement of vitamin C and 17 % of vitamin A [9].
In addition, watermelon is an important source of the non-essential amino acid L-citrulline, a precursor of L-arginine: the latter is very important in the transmission of nerve signals in various neurological and immune bio-processes [10]. Furthermore, it plays a fundamental role in the regulation of the cardiovascular system [12]. It should be noted, however, that the amount of antioxidants, vitamins, mineral salts and other bioactive components depends strongly on the cultivar, genotypic differences, and external factors such as environmental conditions, degree of ripeness of the fruit at the time of harvest, post-harvest handling.
Watermelon fruit is a pseudo-berry called “pepo” as for melon, with a smooth, thick and hard rind (exocarp), mid- to dark green and usually mottled or striped, even if there is no lack of fruits characterized by a fairly thin skin with a light green color. The fleshy and sugary pulp (endocarp), made up of over 90 % water, is typically bright red in color or, less frequently, orange, yellow or white depending on which carotenoid are mostly present.

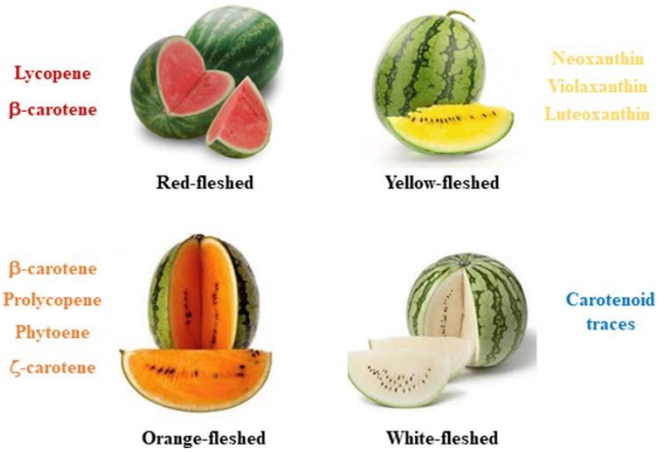

Figure 7 - Main groups of carotenoids and xanthophylls responsible for the different colors of watermelon pulp [13].

Some cultivars also present an intermediate layer of vegetable tissue (mesocarp) between them, white in color and which can have different thicknesses depending on the variety and the degree of ripeness of the fruit. Making a comparison, it is the equivalent of the "albedo" in citrus fruits.
In addition to fruits which contain seeds spread throughout the flesh, there are numerous seedless hybrid varieties that were initially developed since the mid-1900s and that became increasingly popular and widespread in the 21st century, occupying a more and more important part of the global market.

The watermelon cultivation extends over an area that covers about 7 % of the total land surface dedicated to the global production of fruit and vegetables [14] and its commercial availability, in the territories with a temperate climate, goes indicatively from May to September.
Also in this case, among the many cultivars that currently exist, we had to choose just few varieties to investigate and the choice fell on "Crimson Sweet", "Asahi Miyako" and "Gavina®", some of the most commonly diffused in Italy.
The first two are more representative of the typical productions of our territories (Po valley), while Gavina® is a more recent variety selected and registered as IGP product by the Consorzio Ortofrutticolo Campidanese (Sardinia).
Crimson Sweet is one of the most popular and enjoyed varieties of watermelon, native to America, it has a rounded or slightly elongated shape with an average weight of 8 ÷ 12 Kg, a thick skin with light and dark green stripes and a bright red crunchy pulp. It is sugary, firm in consistency and easy to digest. The seeds are dark brown in color and about 10 mm long when fully ripe, while they are much smaller, soft and whitish when unripe. Crimson Sweet is quite resistant to over ripening and transport, thanks to the thickness of the rind, and has an average shelf life of 15-20 days.
Asahi Miyako pepo are round in shape, with a thin and quite easily breakable skin which does not allow long-distance transportation or long storage periods after harvesting, even if some new selections belonging to this cultivar have a thicker bark and a longer shelf life. It has a medium weight of 4 - 5 Kg, a firm pulp bright red, very sugary and a light green rind streaked with dark green. It contains seeds that look very similar to those of Crimson, only slightly smaller. It is an early-ripening hybrid of Japanese origin, which is well suited to forced cultivation in tunnels and whose harvesting period goes indicatively from early June to August.
The **Gavina®** variety, instead, belongs to the category of mini watermelons, having a weight that ranges between 1.5 ÷ 2.5 Kg. It's a seedless hybrid, however if present, the seeds are very few and small, white and soft. It is obtained from the hybridization of a tetraploid watermelon (created in the laboratory) and a diploid (typical watermelon) [15].
This cultivar has been specifically obtained to give fruits with excellent organoleptic characteristics and small dimensions. The latter aspect is increasingly required by consumers, thanks to the advantage of being practical, space-saving and quick to consume.
The thin skin, light green in color with slight dark streaks, accounts for about 15 % of the total weight compared to an average of 25 - 30 % of other watermelon cultivars [5, 16, 17]. This means that the Gavina®

edible fraction is strongly increased in term of mass percentage. The red pulp is crunchy and very sweet thanks to a sugar content that can exceed 13 °Brix. It is typically commercialized from June to mid-September.

In over 15 years this cultivar has been constantly improved to refine its own important organoleptic characteristics and increase the crunchiness and shelf life so that, today Gavina, is a unique product of its kind, also very appreciated in Europe where it has recently roused great commercial interest.

Figure 8 - Different Citrullus Lanatus L. varieties: Crimson Sweet, Asahi Miyako and Gavina.

As previously done for melon, we proceed in the same way for watermelon. As an example, we show the fractionation scheme and the mass balance related to the Crimson Sweet cultivar (Figure 9).
The information reported below (Figure 9 and Table 2) refer to watermelons purchased from local producers near Modena city, while Gavina watermelons have been purchased in a local supermarket. In particular, three different fruits for each cultivar were taken and considered in order to give a representative analytical sample. All fruits were readily used in the full ripening conditions at the purchasing time.

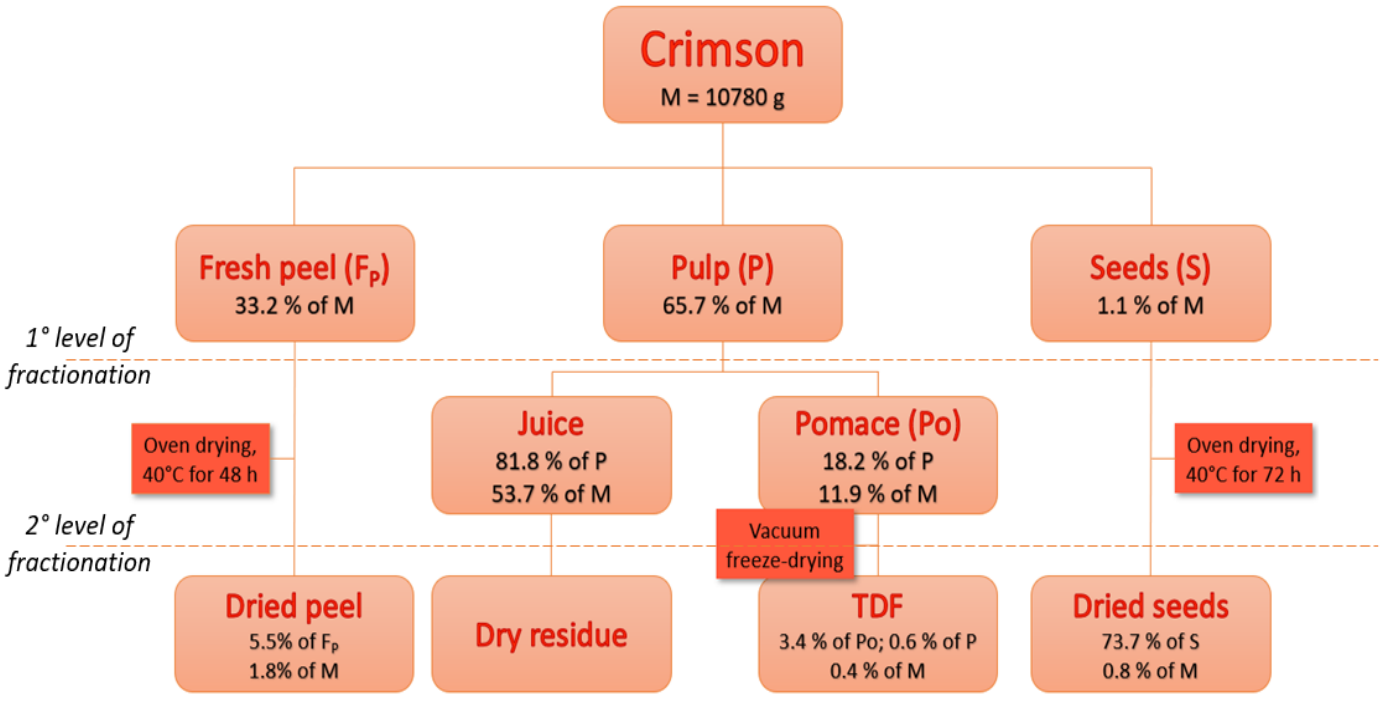

Figure 9 - Fractionation scheme for Crimson Sweet watermelon and related mass balance.

Also in these cases all the fruits were immediately processed, washing them with distilled water and then going further with the main fractionation steps, that is the separation of the green skin (epicarp), the rind (mesocarp) from the edible inner pulp (endocarp) and, finally, the seeds (when present).

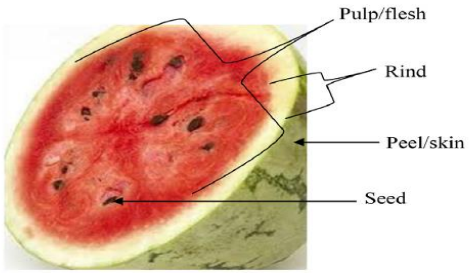

Figure 10 - Cross section of a watermelon fruit and identification of the constituent fractions.

The seeds have been carefully washed with distilled water to remove the residual sugar, oven at 40 °C for 72 hours and stored in closed vessels at - 20 °C and under N_2 conditioned atmosphere until analyses were carried out. This fraction represents another good starting material deriving from the processing of fruit and vegetables and which deserves to be enhanced.
In fact, the results of studies conducted on watermelon seeds have shown that they contain many nutrients such as proteins, fibers and minerals as well as being an alternative source of vegetable oil, making them an interesting material that can be used in the food sector [18].

To better understand the information collected so far, below we summarize the data relating to the yields of the first and second fractionation steps of each watermelon cultivar and, for comparison, some literature ones.

Table 2 - Results of the fractionation procedure for Crimson Sweet, Asahi Miyako, Gavina watermelons, and comparison with some literature data.

Sample		**Values %**					
		Crimson Sweet[@]	Asahi Miyako[@]	Gavina[@]	[16]	[17][**]	[5]
Edible fraction (endocarp)		65.7	71.0	83.6	68		57 - 68
	Pomace	11.9	9.2	7.9		10.4 (including seeds) 8.9 – 23.6[**]	
	Juice	53.7	70.5	75.7		55.3 41.5 – 60[**]	
Waste fraction							
	Rind and skin	33.2	28.3	15.5	30	31.5 31 – 49.6[**]	30 - 41
	Seeds	1.1 (fw)[*] 0.8 (db)[#]	0.9 0.7	trace	2		2

[@]values referred on the total mass weight of the fruit
[*]fw = fresh weight
[#]db = dry basis (residual moisture after 72 hours at 40 °C)
[**]and references therein quoted

The data reported in Table 2 show that there are substantial differences between the Gavina variety and the other two, which can be attributed precisely to genotype factors. In fact, on the other hand, the fractionation procedure of the Crimson and Miyako cultivars, carried out always applying the same methodology and the same criterion, leads to comparable results in terms of endocarp (65.7 % for Crimson and 71.0 % for Miyako), rind (33.2 % and 28.3 % for Crimson and Miyako, respectively) and seeds (1.1 % and 0.9 %).

The amount of the fractions isolated from Crimson Sweet and Asahi Miyako watermelons are perfectly comparable with some literature data, which report an average value of around 68% for the edible fraction and around 30% for the green peel including mesocarp, regardless of the origin of the fruit and the cultivar [5, 16, 17].
As for the seed fraction concern, however, there is a substantial difference between the Crimson sweet (1.1 %) and Miyako (0.9 %) values resulting from our fractionation procedure if compared to 2 % average of literature [19]. About that, it must be considered that this data may vary even by a factor of about 200 % depending on the cultivar, if compared with our values (fw) reported in Table 2.
Moving on to analyze the values relating to the Gavina variety, we have the confirmation of what was previously reported, namely that this "mesocarp-free" fruit has an amount of rind which is about half that of the other cultivars analyzed and a ≈ 22 % more edible fraction.

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CHAPTER 3
3 – DIETARY FIBER, A NOT UNIVOCAL DEFINITION

In the last years, the application of biomaterials in food and non-food industries is greatly increased with the intention of obtaining products which contain value-added factors like dietary fibers and bioactive molecules. At the same time, consumers are becoming increasingly interested in maintaining a healthy diet and lifestyle and in using more natural products (for example in the cosmetic field).
However, on the other hand, it is good to remember that with current trends in demographic changes in populations around the world, people with obesity, diabetes and old age become the main "social problem" in many countries. This is the reason why, in the last years, specific needs for targeted food products rapidly increased, like low-calories foods for obesity, high-fiber and low-glycemic ones for diabetics and high-fiber healthy foods for the elderly [1].
Parallel to this, it cannot be ignored that nowadays up to a third of fruit and vegetables, is discarded during industrial processing [2]. To this it must be added the large quantities of material rejected because of damages, due to visual defects, over-ripening and unsold products at the end of the typical cultivation period, creating huge amount of "waste".
Fortunately, more and more studies show that these by-products may be used as potential added ingredients [3-5], having a high nutritional value and being capable to improve some technological properties of the doughs and formulations in which they are used. Among these properties there are, for example, gelling, thickening and texturizing capability, in addition to increase water holding capacity and fat binding ones.
This is the perfect demonstration of how health claims will not only benefit the consumers but also the functional food and non-food industry, always looking for new cheaper healthy ingredients such as dietary fibers.
Over the years, it was quite difficult to give a univocal definition of dietary fiber, as it has been changed several times, up to one of the latest versions approved by the Codex Alimentarius Commission during the 32nd Session at FAO Headquarters, Rome, 2009 (ALINORM 09/32/A) [6]:

Dietary fiber means carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories

- edible carbohydrate polymers naturally occurring in the food as consumed;
- carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities;
- synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

Basically, dietary fiber is primarily made of non-digestible carbohydrate polymers (non-starch polysaccharides) which compose plant cell walls, such as cellulose, hemicelluloses [7-9] and pectins [10 - 14], as well as other polysaccharides of plant origin like resistant starch, fructo-oligosaccharides, modified celluloses and synthesized carbohydrate polymers. Also lignin and minor compounds (waxes, polyphenols, phytosterols) are included as they are extracted with the polysaccaridic matrix in several fiber analytical methodologies. However, when these compounds are isolated, they are not considered dietary fiber anymore, except for lignin [15].
Despite the unambiguous definition of "dietary fiber" produced by FAO (ALINORM 09/32/A), from a practical point of view, it is not always the one to refer to. For technological applications of various kinds, in fact, the dietary fiber used as a formulation additive is very often a fraction obtained readily from the processing of an edible raw material, of any origin. This means that, in order to extract the fiber according to Codex Alimentarius, it is necessary to carry out at least some refining operations beyond the minimum ones necessary to recover a generic fraction that contains the "crude", "raw, "whole" fiber. For these empirical reasons, very often, operators of production chains rely on the alternative description introduced by Larrauri [15], who described the "perfect fiber" as having the following characteristics:

• It must not contain any components that are nutritionally offensive
• To maximize its use, it must express a high potential even if added in small quantities
• It should have no taste and no negative odour (off-flavor)
• It should be colourless, without texture effects (unless specifically requested)
• It should contain a balance between soluble and insoluble fiber with an acceptable presence of bioactive compounds
• Its addition must not affect the food to which is added, and it must also have a long shelf-life
• It should work harmoniously with food processing
• It should have a positive consumer image
• It should contain the expected physiological effects
• It should be in an adequate price [15, 16].

In addition to the above recommendations, some further general criteria should be followed when preparing dietary fibers, these being no nutritionally objectionable components. In particular, dietary fiber (DF) must be compatible with the methods of food processing employed, while still having positive physiological effects [17].
Larrauri's guidelines seemed particularly consistent with the objectives proposed in this research work. Therefore, we have chosen to recover the dietary fiber from the selected matrices, minimizing the complexity of the biorefinery operations, following the block diagram of Figure 1.

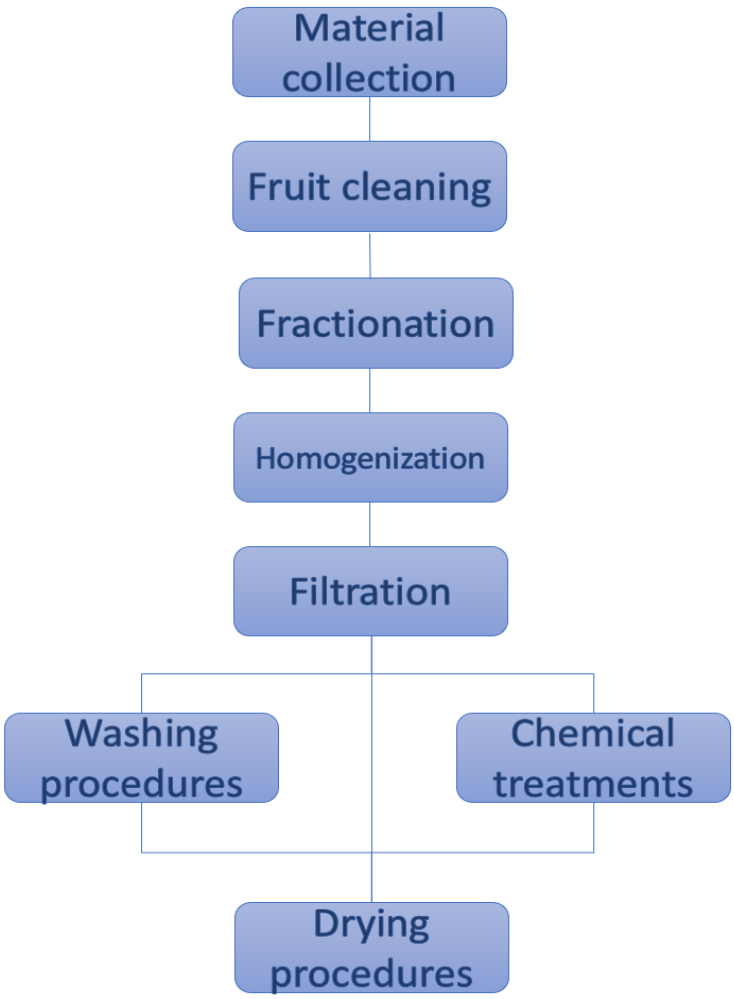

Figure 1 – Flow chart of the extractive process of dietary fibers from Cucurbitaceae matrices

Therefore, dietary fibers obtained from the matrices of our interest, and reported in this PhD thesis, will be represented according to these formalisms:

● TDF = Total Dietary Fiber, the whole fraction containing DF, the raw fiber "as-it-is". It has been recovered only by mechanical operations, without being subjected to any other chemical or chemical-physical treatment including washing procedures with water, and finally dried. This fraction contains all the soluble compounds (sugars, simple starches, mineral salts, organic acids, vitamins, etc.) which remain bounded to the polymeric fibers (i.e. polysaccharides and proteins) or mechanically trapped in the dried material.
● IDF = Insoluble Dietary Fiber, the residual fiber fraction after repeated washing procedures with distilled water at room temperature, to eliminate all soluble compounds in these conditions. IDF has also been obtained after chemical treatments with H_2O_2 conducted on the raw fiber. The IDF, according to our definition, contains only polymers, namely cellulosic polysaccharides, lignins, higher starches, proteins.
● SDF = Soluble Dietary Fiber, the fraction of soluble material which is eliminated by washing the whole and raw fiber.

In the case of our initial matrices, the SDF is a subfraction of the whole and raw fiber, which contains a large amount of mineral salts, in addition to all the other functional compounds that are lost with the washing operations of the TDF. Therefore, the IDF is notably depleted of bioactive molecules compared to raw fiber. For these reasons, the whole dietary fiber from *Cucurbitaceae*, especially the red TDF rich in lycopene from *Citrullus Lanatus* and containing the wide range of naturally occurring bioactive compounds, could be much more nutritionally effective. However, if the *Cucurbitaceae* fiber was intended as a formulation ingredient for certain productions, the whole and raw fraction (TDF) or the IDF subfraction could both be particularly suitable or alternatives, due to the functionalities to be achieved in the finished product. Certainly, when the material undergoes very few extraction-preparative operations, it maintains and retains at least in part the natural color, taste and aromatic fragrances of the initial matrix. Therefore, these residual characteristics in DF are not always to be considered "negative" since some industrial applications could derive incremental advantages and benefits from them.

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CHAPTER 4

4 – MATERIALS and ANALYTICAL METHODOLOGIES

This chapter describes the operating methodologies and analytical procedures applied in hierarchical form to the materials selected and used in order to achieve the aims of the PhD thesis.
The preparation of the fractions deriving from the fruits and the manipulations necessary to obtain the various samples, has already been reported in Chapters 2, 5 and 7.
Table 1 summarizes the specimens obtained from the Melon *Inodorus* cultivar and the drying methods adopted, while Table 2 shows the representative samples deriving from *Citrullus Lanatus* matrices.

Table 1 - Identification of Melon var. Inodorus – dietary fiber samples and specific drying conditions

Sample	Drying conditions
Melon pomace “as it is”, Oven dried (M_AI_O)	48 h at 40°C
Melon “as it is”, Vacuum-freeze dried (M_AI_V)	12 h ; 8 h at P = 1 mbar and 4 h at P = 0.001 mbar
Melon pomace Bleached with H_2O_2 at Room Temperature, Oven dried (M_B_O)	48 h at 40°C
Melon pomace Bleached with H_2O_2 at T=50 °C, Oven dried (M_B_{50}_O)	48 h at 40°C
Melon pomace Bleached with H_2O_2 at Room Temperature, Vacuum-freeze dried (M_B_V)	12 h ; 8 h at P = 1 mbar and 4 h at P = 0.001 mbar
Melon pomace Bleached with H_2O_2 at T=50 °C, Vacuum-freeze dried (M_B_{50}_V)	12 h ; 8 h at P = 1 mbar and 4 h at P = 0.001 mbar

Table 2 - Identification of Citrullus Lanatus – dietary fiber samples and specific drying conditions

Sample	**Drying conditions**
Watermelon pomace "as it is", Oven dried (W_AI_O)	48 h at 40°C
Watermelon pomace "as it is", Vacuum-freeze dried (W_AI_V)	12 h ; 8 h at P = 1 mbar and 4 h at P = 0.001 mbar
Watermelon pomace *washed* with distilled water, Oven dried (W_*w*_O)	48 h at 40°C
Watermelon pomace *washed* with distilled water, Vacuum-freeze dried (W_*w*_V)	12 h ; 8 h at P = 1 mbar and 4 h at P = 0.001 mbar

In order to obtain an effective evaluation of the differences that may occur on the DF samples to be processed, we have always applied two different preparation methods of the specimens, working with alternative drying techniques: i) 'hot method', oven-dried at 40 °C; ii) 'cold method', vacuum freeze-dried.
In the specific case of oven-drying, the temperature was maintained at 40 °C for 48 h, in order to avoid the onset of fiber browning phenomena, mainly due to the establishment of Maillard reactions. In addition, it is necessary to avoid the oxidation in air of residual polyphenolic compounds which, in the case of white fibers, would nullify the efforts dedicated to achieving the maximum compatible whitening point. Arranging the raw fiber on a thin layer, at the end of the thermal cycle the material is perfectly dry to the touch, rigid and compact, in fragile flakes, since they are extremely thin, of a color similar to that of the initial matrix, devoid of significant alterations.
The vacuum-freeze dryer is a CHRIEST, Mod. Alpha 1-2 LDplus (Direct Industry, Germany).

4.1. DETERMINATION OF WATER CONTENT, ASHES, PROTEINS AND pH

Total water content, residual moisture content at 101 °C and ashes were determined according to the AOAC methods [1].
Total water content was quantified by oven-drying the samples at 105 °C up to the final constant weight. The residual moisture was determined by dehydration in an electric oven at 101 °C [2].
Ashes were obtained by incineration of the dry residue in a muffle furnace at 550 °C up to the final constant weight.
Protein content in our samples was determined by Kjeldahl method [1].
Crude protein was calculated by multiplying the value of N % obtained from titration by the correction factor 5.6, unless otherwise specified [3].
The pH of the freshly homogenized melon and watermelon pulp was measured by using a pH-meter (Crison Basic 20, Crison Instruments S.A., Alella, Barcelona, Spain).
For each sample, all the analyses listed above were conducted in triplicate and the results expressed as the average value with the associated standard deviation (s_3).

4.2. DETERMINATION OF SUGARS

Glucose, fructose and sucrose in fresh melon and watermelon juices have been separated and quantified through HPLC analysis, according to the method of Villanueva et al. [4].
Fresh fruit juices were centrifuged (5000 rpm for 15 min), then the supernatant was recovered and diluted 50 times in MilliQ water, it was filtered with a 0.45 µm CA filter and finally 20 µL of the obtained sample were injected in the HPLC system.
The system is composed of a Waters 2690 Separation Module (Waters, Milford, MA), equipped with an in-line vacuum degasser, a double reciprocating pump, an autosampler injector, and a column heater.
The chromatographic conditions set were: isocratic elution with degassed MilliQ water as mobile phase, at constant flow rate of 0.8 ml/min and 85 °C column temperature (Aminex Carbohydrate HPX-87C, 300 mm x 7.8 mm, particle size 9 µm, Bio-Rad, Richmond, CA). The detector was a differential refractometer (Waters 2410), set at 30 °C.

For the identification and quantification of each sugar, an external standard was used to prepare calibration curves over the range of 175-1000 mg/kg (ppm), yielding resolutions with linear correlation coefficient values greater than 0.99.
The total sugar content, i.e. the sum of the three individual sugars, has also been calculated.
For each sample, the measurements were conducted at least in triplicate and the results expressed as the average value with the associated standard deviation (s_3).

4.3. DETERMINATION OF TITRATABLE ACIDITY

Melon and watermelon fresh juice samples were centrifuged (5000 rpm for 15 min) and then filtered to ensure the complete elimination of solid particles. Acidity was then determined by titrating the clear juice with NaOH 0.01 M, using a couple of drops of phenolphthalein indicator until obtaining a persistent pink coloration. Furthermore, we have monitored the titration progress by potentiometric method [5].
The acidity was expressed as mg/ml of citric acid and also as mg/ml of citric acid monohydrate in order to better compare our results with the literature data [6].

4.4. CHNS ELEMENTAL ANALYSIS

About 500 µg of dried samples obtained from some fractions of melon and watermelon fruits were weighted into Sn microcrucibles on a microbalance. The determination of carbon, hydrogen, nitrogen and sulfur was performed using a Thermo-Scientific CHNS Analyzer mod. Flash2000 (Thermo-Scientific, Waltham, Massachusetts, USA) after calibration with thiourea as standard. The data processing was simply performed by the software implemented in the instrument and the results are expressed in percentage of each element in the sample.
The analysis was performed in triplicate and the results are indicated as the average values with the associated standard deviation (s_3).

4.5. VOLATILE ORGANIC COMPOUNDS ANALYSIS THROUGH HS-SPME-GC-MS

Solid Phase Micro-Extraction (SPME) was used for sampling the Volatile Organic Compounds (VOCs) present in the vial Head Space (HS), which characterize the aroma of some our specimens (i.e. melon and watermelon dried fibers, in addition to Crimson Sweet mesocarp treated in Chapter 11).
A SPME holder containing a fused-silica fiber coated with Carbowax/Divinylbenzene/Polydimethylsiloxane (CW/DVB/PDMSO) from Supelco Inc. (Bellefonte, PA, USA) was used to manually perform the headspace analysis. This type of coating is highly performing as it promotes the retention capacity and improves the sensitivity of the sampling if compared to other types of similar devices.
Furthermore, given the characteristics of different polarity of the three constituents of the coating, this fiber seems suitable for the capture of analytes with significantly different molecular features, resulting adequate for the characterization of complex matrices as in these cases [7, 8].
The number of samples analyzed as well as the methodologies adopted for their preparation depends on the type of material considered, therefore these details are reported in the specific chapter of each matrix, together with the list of reagents used and accompanied by the discussion of the results obtained. The same applies to the oven temperature settings adopted during the various chromatographic runs.
Anyway, regardless of the type of sample and the handling procedure it has previously undergone, all the samples have always been introduced in glass vials tightly sealed with Teflon / silicone septa and sonicated for 30 min in a thermostated bath at 40.0 ± 0.1 °C to favor the transfer of volatile compounds from the matrix to the headspace. The SPME fiber was finally exposed in the HS for 15 min at 40.0 ± 0.1 °C.
Some blank tests, corresponding to the analysis of an external standard solution containing 1-decanol (conc. 150 µg/g ethanolic solution) were performed periodically after a certain number of chromatographic runs (5) relating to real samples.
Generally, all measurements were performed in at least 3 replicates for each sample/vial.
Analyses were conducted using an Agilent 6890N Network gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 5973 Network mass spectrometer. Volatiles were separated on a capillary column DB-5MS UI (60 m x 0.25 mm i.d., 1.00 µm film thickness; J&W Scientific, Folsom, CA, USA). The carrier gas used was He with a constant flow rate of 1 ml/min and a column head pressure of 15 psi.

Injections were performed in splitless mode, setting the injection port at 250 °C and introducing the fiber into the injector for 15 min. The transfer line was heated to 270 °C and the detector started operating immediately after injection.
Mass spectra were obtained by electron ionization (EI) at 70 eV with a scan range from *m/z* 25 to 350, in full scan acquisition mode. All chromatograms and mass spectra were evaluated using the ChemStation software (G1791CA, Version C.00.00, Agilent Technol.). Volatile compounds were identified in some different ways:
(A) by comparison of their mass spectra with those of the libraries provided with the data analysis software of the GC-MS (NIST14; NIST05; WILEY275; NBS75K), National Institute for Standards and Technology database (https://webbook.nist.gov) and Mass Bank of North America database (https://mona.fiehnlab.ucdavis.edu)
(B) by comparison with mass spectra found in the literature;
(C) by injection of some standard analytes (when available, that will be specified in the "Materials and Methods" section of each matrix analyzed);
(D) comparing the Linear Retention Index values (LRI) of our analytes with those reported in the literature or in NIST database (https://webbook.nist.gov), considering only values referring to analyses carried out under the same operative conditions (instrumental specifications and heating ramp).
Linear Retention Index of compounds have been calculated from a series of n-alkanes (C6, C9, C12, C14, C16) (Carlo Erba Reagents, Milano, Italy) subjected to the same analysis procedure adopted for the samples. This choice is valid whichever the samples we have analyzed in this research activity.
The volatile compounds such as silane and siloxane derivatives, or volatile organic compounds related to the sorbent fiber were discarded and are not reported in the GC-MS output tables.
The estimation of the amount of each volatile identified in the SPME-GC-MS analysis was expressed as the Total Ion Current (TIC) peak area.

4.6. HPLC-MS-ESI-IT

The analysis was performed for the qualitative identification of polyphenols, among other analytes, in some aqueous and hydro-organic extracts obtained from the selected matrices.
HPLC analyses were carried out using an Agilent Series 1200 Ion Trap LC-MS (n) system (Agilent Technologies, CA, USA), equipped with a vacuum degasser, binary pump and autosampler. The column used for the chromatographic separation was a Cortecs C_{18} (2.7 µm, 2.1 mm x 100 mm) (Waters Co., Milford, MA, USA). The analysis was performed at a constant flow rate of 0.3 ml/min, using a gradient elution with acidified water (0.1 % formic acid) and acetonitrile 80:20 (mobile phase A), and acetonitrile 100% (mobile phase B). The gradient was programmed as follows: 0 min, 20 % B; 25 min, 98 % B held for 5 min to then return to the initial conditions. Finally, a conditioning cycle of 10 min with the initial conditions was adopted.
The effluent from the HPLC column was split before being introduced into the mass spectrometer (split ratio 1:10) to provide a stable spray and reproducible results. When necessary, samples have been properly diluted from 1:10 to 1:50. The injection volume was always 20 µl.
HPLC system was coupled to an Ion Trap (IT) mass spectrometer equipped with Electrospray Ionization (ESI) operating in negative mode. The detection was made considering a mass range of 50-1200 u.m.a.

4.7. METALS CONTENT VIA ICP-OES SPECTROSCOPY

The analysis was conducted by applying the Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES), Perkin Elmer - Optima 4200 DV, on samples mineralized in a microwave digestion system (wet method) or on solutions obtained from the solubilization of the ashes (dry method) of the starting materials. The spectrophotometer is equipped with an ultrasonic nebulizer (Cetac Technologies Inc.; Omaha, NE, USA) and Charge-Coupled Device (CCD) area detector complete the instrumental setup.

Mineralization of samples

All mineral acids and oxidants (HNO_3 and H_2O_2) used, were of the highest quality (Suprapure, Merck). All the plastic vessels and glassware were cleaned by soaking, with overnight contact, in a 10% HNO_3 solution and then rinsing with deionized water (from a Milli-Q Plus system, Millipore). Samples of flours of about 0.2-0.3 g (3 replicate runs, in addition to the fortified and spiked one to evaluate recovery factors for each sample)

were digested with 6 ml of HNO_3 (65%) and 4 ml of H_2O_2 (30%) in a microwave digestion system (CEM MARSX), then diluted to 50 ml as final volume with deionized water. The final solutions are perfectly clear and apparently colorless, stable with time and without turbidity or precipitate formation (wet method). A blank and fortified blank spiked digests were carried out in the same way for each carousel of six Teflon liners – model XP 1500+ (digestion conditions for microwave were: 2 min at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400 W, 8 min at 550 W, and vent for 8 min). This procedure was adopted because it was accurate with respect to both time and recovery values for such a type of natural matrix.
To dissolve ashes, the residues have been treated following the same procedure as mentioned above, working with the same aliquots of reagents into Pt crucibles, without the mineralization step (dry method).
Merck ICP standards and multistandards (10-1000 mg/L) were used to prepare the reference solutions.

4.8. FT-IR SPECTROSCOPY

The FT-IR pattern of some samples was measured by using a Jasco 4200 FT-IR (JASCO International Co. Ltd., Tokyo, Japan). The solid samples were blended with KBr powder. The spectra were acquired in the range 4000-400 cm^{-1}. Alternatively, the samples have been processed with ATR device.

4.9. UV-VIS SPECTROSCOPY

This instrumental analysis technique has been used both for the characterization of solid samples, i.e. fibers from melon and watermelon pomace, and on sample solutions. In the first case we collected the UV-Vis spectrum of solid samples to identify the absorption bands present and to monitor the evolution of these spectra over time, during a natural ageing study. We also obtained information about the color point of the fibers themselves. Instead, for sample solutions, we exploited the UV-Vis technique to carry out a semi-quantitative determination of lycopene extracted with n-hexane from the pomace fibers of the three selected watermelon varieties. We also collected some spectra of extracted solutions obtained from dried melon powdered skin, using different extraction solvents (organic and aqueous ones).
All spectroscopic measurements were performed with a UV-Vis spectrophotometer JASCO V-570 (JASCO International Co. Ltd., Tokyo, Japan). The instrumental parameters set for each type of analyzes are reported in the following sections.

Solid state sample analysis and color point evaluation (L*a*b*)

About 200 mg of powdered fiber (grinded at 0.5 mm average granulometry) has been subjected to a pressure of about 20 atm for 1 minute (Hydraulic Press, PerkinElmer, Waltham, Massachusetts, United States) to obtain ϕ = 13 mm round tablets and ≈ 1 mm thick. No other component was added to the pads.
All spectroscopic measurements were performed with a UV-Vis spectrophotometer equipped with the integrating sphere device, calibrated with a white standard tile ($BaSO_4$) in the spectral range 200 ÷ 800 nm, placing the pad on a suitable support. Furthermore, we proceeded with an objective evaluation of the color point of some our fiber samples collecting the values of the parameters L*, a*, b* provided directly by the instrument, defined within the CIELab color space (Commission Internationale de l'Éclairage, 1976). In particular, L* refers to the lightness and can assume values between 0 (black) to 100 (white), a* is the coordinate that ranges between red (positive values) and green (negative values), while +b* indicates yellow and -b* blue color.

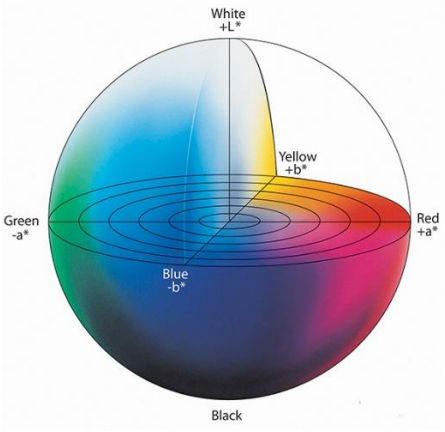

Figure 1 - Representation of the CIELab color space

The color coordinates L*a*b* were obtained from the same equipment previously described, set to use the standard illuminant D65 and the 10° standard observer. Illuminant D65 is the most widely light source used in measurement of food color (Commission Internationale de l'Eclairage) and corresponds to the spectral distribution of midday daylight in Western Europe [9].
The value of 10° standard observer, represents the best average spectroscopic response of human observers [10].
For our purposes we also calculated the total color change (ΔE), that is the distance between two colors in the chromatic space. It is defined by the following equation:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

where L*, a* and b* are the coordinates of our sample at t time of ageing, while L_0*, a_0* and b_0* are the color parameters of the material used as a reference with respect to which we calculate the color variation (fiber at t_0 time).
This measurement has been carried out to evaluate the color point:

- of melon pulp fiber "as it is" and after the bleaching treatments with H_2O_2. We also monitor the color change over time of the above-mentioned fibers
- of some commercial fibers
- of watermelon (Crimson Sweet) dried pomace fibers, studied over ageing time and stored in different conditions.

As far as ΔE concern, the higher the value, the greater the color variation the fiber has undergone after the whitening process or during aging, respectively.
The results relating to the values of L*, a* and b* are always expressed as the average of three replicates for each reference condition, accompanied by the standard deviation (s_3).
Information on the set of specimens, storage conditions, etc., will be detailed in the chapters dealing specifically with the related experimental results.

Qualitative analysis of extraction solutions from Yellow Canary melon skin

Generally, unless otherwise specified, solutions have been filtered through a 0.2 µm syringe filter to ensure the complete removal of solid particles. Depending on the maximum value of absorbance of the pure extracts, the extraction solutions were properly diluted to bring absorbance values below 1. If necessary, the diluted solutions have been filtered again. The UV-Vis spectra of clear extracts have been immediately recorded, in the wavelength range 260 – 600 nm, at room temperature and using quartz cells having an optical path of 2 mm. The fresh solvent corresponding to each extract has been used as a blank.

Semi-quantitative estimation of lycopene content

n-hexane was used as organic solvent to extract lycopene from the "as it is" watermelon dried pulp fibers through a Conventional Solvent Extraction (CSE) procedure as indicated by Rahimi and Mikani [11], with some slight modifications. Hexane has been chosen because it is a non-polar solvent and thus able to solubilize the analyte of interest. It is also one of the greenest solvents, quite sustainable, biocompatible and currently already used in different applications.

A mass of 0.5 g ground fiber has been extracted with 5 ml of n-hexane for 1 h at room temperature, under constant stirring and subdued light to prevent lycopene degradation and isomerization. The system has been centrifuged (10.000 rpm for 15 min) and the supernatant removed. The same procedure was repeated with fresh solvent on the previous solid residue until the fiber no longer yields dye to the hexane. For each sample the various aliquots of extract were collected and diluted to the final volume of 50 ml.

The UV-Vis spectra of the extracts, previously filtered with a 0.2 µm filter have been recorded with a UV-Vis spectrophotometer JASCO V-570 (JASCO International Co. Ltd., Tokyo, Japan), in the wavelength range 200 – 800 nm, at room temperature and using quartz cells having an optical path of 0.2 and 1 cm. Fresh n-hexane was used as a blank.

The semi-quantitative estimation of lycopene content in each solution was determined exploiting Lambert-Beer's law at 503 nm, i.e. at the longest λ and, therefore, the less interfered by the presence of other carotenes and/or carotenoids [12].

$$A_{(\lambda)} = \varepsilon_{(\lambda)} * b * C$$

where $A_{(\lambda)}$ is the absorbance recorded at the selected λ, $\varepsilon_{(\lambda)}$ is the molar extinction coefficient of lycopene at chosen λ, b is the optical path and C is the concentration of the analyte in solution.

The estimated amount of lycopene expressed as mg/Kg of fresh pulp is obtained by applying the following equation:

$$\text{estimated lycopene content (mg/kg fresh fiber)} = \frac{A(\lambda) * D * V * MW}{\varepsilon(\lambda) * \mathrm{b} * \mathrm{M}} * 1000$$

with $\mathbf{A_{(\lambda)}}$ and $\boldsymbol{\varepsilon_{(\lambda)}}$ that are the absorbance value and the molar extinction coefficient of lycopene at λ wavelength, respectively; **D** is the dilution factor; **V** is the total volume of hexane used for the extraction procedure (50 ml unless otherwise specified), **MW** is the molecular weight of lycopene, **b** is the optical path and **M** is the weighed mass of dried fiber, referred to the fresh pulp and expressed in Kg.

4.10. THERMAL METHODS OF ANALYSIS

These methods of analysis were used to evaluate the behavior of some dried fiber samples from melon and watermelon pomace, under thermal stress using a SETARAM Labsys instrument.

For the TG/DTG/DTA measurements, about 10 - 15 mg of the grinded fibers were weighed in a Pt crucible and the analyses were performed in presence of Helium, setting a heating ramp of 10 °C/min from 0 °C to 630 °C.

4.11. SCANNING ELECTRON MICROSCOPY (SEM)

Morphological and elemental analyses were performed using a Scanning Electron Microscope (ESEM Quanta-200, FEI Company, Hillsboro, Oregon, USA) on solid samples fixed on a circular metal stub with double-sided sticky tape. Samples have been exposed to an electronic beam at accelerating voltage ranging from 20 to 30 kV, recording the signal in secondary electron imaging mode.

Images were collected at different magnifications depending on the type of sample analyzed and, therefore, on the level of detail to be achieved.

4.12. PHYSICOCHEMICAL AND TECHNOLOGICAL PROPERTIES

Before discussing in detail the methodological aspects connected with the technological properties, it is necessary to specify that, by definition, the acronyms reported in this section will be maintained as reported in the literature (WHC, SRC, WSC, OHC). In this way we attribute the same meaning to each property, even if the fibrous matrices reported in this PhD work are not perfectly consistent with the definitions of the literature (TDF, SDF, IDF). In fact, the technological properties should refer to the single IDF and SDF fractions, as previously highlighted, identified according to the Codex Alimentarius [13] and Larrauri [14].
The IDF and SDF subfractions are obtainable by extraction from the initial matrices only after long and subsequent manipulations, adequate (enzymatic treatments) to effectively separate the polymeric compounds representative of the various constituent classes: cellulosic polysaccharides, starch polysaccharides and proteins.
For reasons of expediency, typically related to the technological transfer procedures of the research results, we have found it much more convenient to work with the raw fiber "as it is" (TDF). Alternatively, we have chosen at most to wash and bleach the pomace with H_2O_2, reducing the biorefinery operations to the minimum degree of complexity. This allowed to accelerate the recovery process and identify some potential applications of the fibers obtained from complex matrices.

Water Holding Capacity (WHC)

The ability of a fiber to retain water under the application of an external stress was determined according to the method described by Zhang et al. [15], with some slight modifications.
In particular, the analysis was performed on two different types of "simplified systems": i) **binary systems,** consisting only of a known amount of fiber and distilled water {fiber (1)-H_2O (2)} ; and ii) **ternary systems,** where other solutes commonly used in the food industry have also been added, one by one, to the previous materials for obtaining a mixture consisting of suspended {fiber (1)-solute (2)-H_2O (3)}. By doing this, we assume that the "fiber" represents a single component, regardless of its actual composition.
The chosen solutes we used in aqueous solutions:

- Glucose (GLU) 5-10-15-20-25 %
- Sucrose (SUC) 5-10-15-20-25 %
- Citric Acid (CA) 1-2-3-4-5 %
- NaCl 1-2-3-4-5 %

It should be emphasized that the nature of the solutes and the range of concentration selected for each one is reasonably compatible with the quantities used in food mixtures, depending on whether they are salty or sweet products.
In a pre-weighed centrifuge tube, 0.1 g of fibrous (or starch-proteinic flours) sample was dispersed in 10 ml of distilled H_2O for binary systems, or previous cited solutions for ternary systems, making sure that the latter was in excess of the solid.
Each of the systems obtained have been kept under constant agitation on a vibrating plate at room temperature (ca. 25 °C) for 24 hours, and then centrifuged for 15 minutes at 5000 rpm to effectively separate the solid fraction from the liquid in excess. Finally, the supernatant was removed and the tubes were weighed again.

For **binary systems** WHC (g/g) value was calculated as:
$WHC = [(m_2 - m_0) - m_1] / m_1$
where m_0 is the tare of centrifuge tube, m_1 is the weight of the fiber before hydration and m_2 is the weight of the centrifuge tube with hydrated fiber after the removal of the supernatant.

For **ternary systems** the relevant calculation of WHC (g/g) are derived from the expression:
$WHC = [(m'_2 - m_0) - m_1] / m_1$
where m_0 is the tare of centrifuge tube;
m_1 is the weight of the fiber before hydration;

m'_2 is the weight of the centrifuge tube with hydrated fiber in presence of solute, after the removal of the supernatant.
$m'_2 - m_0$ is the mass of the hydrated fiber in presence of the added solute.
$m'_2 - m_0 = (m_1 + m_w^{WHC} + m_s)$
where m_w^{WHC} is the mass of water as obtained from the WHC procedure;
m_s is the mass of solvated solute retained by the hydrated fiber

Results are expressed as mean ± standard deviation of three replicates.

Based on the initial choice to use the TDF instead of the IDF, by definition the results obtained are a little more similar to the SRC property (Solvent Retention Capacity) than to the WHC. The treatment of our whole fibers with distilled water or with a generic solution involves at least partial dissolution of the soluble fiber fraction. Certainly, if TDF were used as a formulation ingredient, all the analytes removed from the fiber would still remain dispersed within the finished product mass, without any loss of active ingredients or water-soluble functional compounds. This results in an immediate and considerable application advantage, since the TDF possesses qualitative and quantitative compositional requirements higher than the IDF and SDF subfractions, possibly obtainable by operating as required by the general definition [14].

Water Swelling Capacity (WSC)

In association with the WHC, the WSC parameter (Water Swelling Capacity) is also generally reported. This property expresses the swelling capacity of dietary fibers following the absorption of water up to the point of saturation.
The presence of electrolytic compounds, especially mineral salts, favor the volume contraction of the “wet” material due to the specific positive interactions between the components of the heterogeneous system. Despite the hydrophilicity of the cellulosic-polysaccharide constituents, very often these analytes tend to swell to a greater extent than the volume of water absorbed. This effect can be the result of the sum of two countertrend contributions detectable within the same polysaccharide micelle. These two effects can be described as follows: (i) the positive “contraction” interactions between the hydroxyl groups and the water molecules, (ii) the negative repulsive ones between the apolar hydrocarbon groups and the solvent molecules.
From the nutritional point of view, WSC is an extremely positive factor, directly proportional to the ability to satiate the body, without however altering the amount of calories introduced with food. This property is also very useful from the technological point of view, since it allows to evaluate, even if in predictive form, the behavior and volume effects of complex formulations.
For the measurement, ~ 500 mg of DF (or starchy-proteic flour) was weighed in a calibrated cylinder and hydrated in 30 mL of deionized water. The suspension was slowly stirred for 15 min to prevent sample agglomeration. Then, the cylinder was covered and left for 6 h to incubate at room temperature (20–25 °C). The final bed volume was recorded and expressed as volume mL/g, then converted to g/g of the DF dry weight.

Oil Holding Capacity (OHC)

The adsorption capacities of oil were determined according to the method described by Benavent-Gil and Rosell [16] with slight modifications. The samples, both DFs or starchy-proteic flours, were mixed with excessive soybean oil (10 g) at room temperature for 1 hour until oil adsorption equilibrium has been established, and then centrifuged at 5000 rpm for 15 min.
When the sediment was placed on the filter paper and the surface soybean oil was absorbed, the weight of the pellet was measured.
The adsorption oil capacity was calculated in analogous way as for WHC, as shown in the previous paragraph.

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CHAPTER 5

5 - CHARACTERIZATION PROCEDURES APPLIED TO *CUCUMIS MELO*

Referring to some official documents of several international organizations such as FAO, World Health Organization (WHO), World Food Program (WFP), International Fund for Agricultural Development (IFAD), ONU, UNICEF, ecc… the world population is estimated to increase to 9 billion people by 2050. Therefore, global food production (net of biomass used to produce biofuels) should increase by about 70 % to ensure conditions of fair and sustainable development also extended to populations currently living in critical situations. However, if agri-food production were to increase so significantly, it is not possible to predict a parallel increase for the consumption of N, P, K and water for an intensive agriculture capable of satisfying the needs expressed so far. Therefore, if it will not be possible to achieve this objective in accordance with the global policy choices, it is necessary to identify alternatives to meet these needs. The first objective is represented by the reduction of food loss and waste, by applying sustainability models and strengthening the enhancement and promotion of the sustainable use of resources in compliance with the natural functionalities that ecosystems offer.

Taking onto account the previous opinions and considerations, we paid particular attention to the recovery of added-value fractions, materials and by-products from the agro-industrial processing of the melon Cucumis Melo var. *Inodorus*, Yellow Canary type, among the *Cucurbitaceae* family.

During the development of our research activities, we have dealt with:

- endocarp fraction, manipulated by applying different methodologies and procedures to obtain **Dietary Fiber (DF)** and juice;
- juice fraction, as thirst-quenching beverage;
- yellow skin, intended for obtaining and recovering natural dyes and pigments.

The choice of the selected variety is linked to the fact that our aim is to obtain an almost white dietary fiber, (as in the case of winter melon pulp), possibly neutral also for what concern the aromatic properties, which can be used as a formulation ingredient in several food products, without altering the final characteristics induced by the other ingredients eventually present.

5.1. ENDOCARP FRACTION

Initially, the endocarp fraction from **melon** (**M**) was shredded with a common kitchen blender to obtain a homogeneous slurry-meal, light yellow in color and having the characteristic aroma of the ripe fruit.
The pulp was then centrifuged and filtered with paper through a vacuum filtration system to separate the juice from the pomace, which was then handled in different ways in terms of drying procedure and bleaching treatment.
We will talk about a fiber "**as it is**" (M_**AI**) when we refer to the material containing all components naturally present in the fruit, which can be dried in a vented **Oven** (M_**AI_O**) or **Vacuum-freeze dried** (M_**AI_V**). Alternatively, the pomace has been bleached with H_2O_2 to obtain an almost white-odorless-tasteless fiber, as much as possible. In this way, it was then possible to characterize, evaluate and compare the composition and some physicochemical properties of the obtained DF which, despite having the same origin, have

different peculiarities due to different obtaining processes.

Starting from some preliminary research [1 - 5], the pomace has been **bleached with H_2O_2** (conc. 10 %), using H_2O_2 : pomace ratio of 1:3 (by mass), maintaining the system under continuous stirring for two hours and working at **room temperature** (M_**B**) or a **50 °C** (M_**B_{50}**).
During these procedures no pH modification were performed by adding reagents, but the system has always self-balanced without any external input.
The pomace has been washed repeatedly with distilled water both before treatment, to free it as much as possible from water-soluble analytes, and at the end of the same to remove the residual H_2O_2.
In both cases washing operations were carried out using a volume of distilled water equal to about three times the initial mass of pomace.
Hydrogen peroxide was chosen as it is used as an eco-friendly whitener, avoiding the problems caused by the use of active chlorine-based whiteners or other reagents. Finally, a not negligible advantage is represented by the fact that it is a relatively low expensive reagent.
Finally, both the bleached fibers have been rapidly filtered again with the same previous procedure and the residues have been both oven dried and vacuum-freeze dried as described in Chapter 4.

The samples obtained with the two drying procedures are perfectly dry to the touch but, at first sight, they look quite different. The vacuum-freeze dried fibers (M_AI_V, M_B_V and M_B_{50}_V) are spongy, crumbly, not very compact and with low bulk density, while the oven-dried ones (M_AI_O, M_B_O and M_B_{50}_O) are much more rigid and compact and show a certain resistance to breaking even if the material is fragile due to the very thin final thickness. The crude fibers (M_AI) have maintained a very pleasant aroma as well as the original pale greenish-yellow color, although slightly less bright. They are a little sticky to the touch due to the presence of residual sugars.
The bleached fibers (M_B and M_B_{50}) appear almost white in color and almost devoid of volatile aromas.

At any earlier convenience, we list the identifying acronyms of the melon-based fibers samples in the following scheme.

M_AI_V	Melon pomace “as it is”, Vacuum-freeze dried
M_AI_O	Melon pomace “as it is”, Oven dried
M_B_V	Melon pomace Bleached with H_2O_2 at Room Temperature, Vacuum-freeze dried
M_B_O	Melon pomace Bleached with H_2O_2 at Room Temperature, Oven dried
M_B_{50}_V	Melon pomace Bleached with H_2O_2 at T=50 °C, Vacuum-freeze dried
M_B_{50}_O	Melon pomace Bleached with H_2O_2 at T=50 °C, Oven dried

Scheme 1 – List of the identifying acronyms of melon-based fibers samples

Also in this case, immediately after the drying procedures, all fibers have been grinded and then stored as previously described along the text of this dissertation thesis.

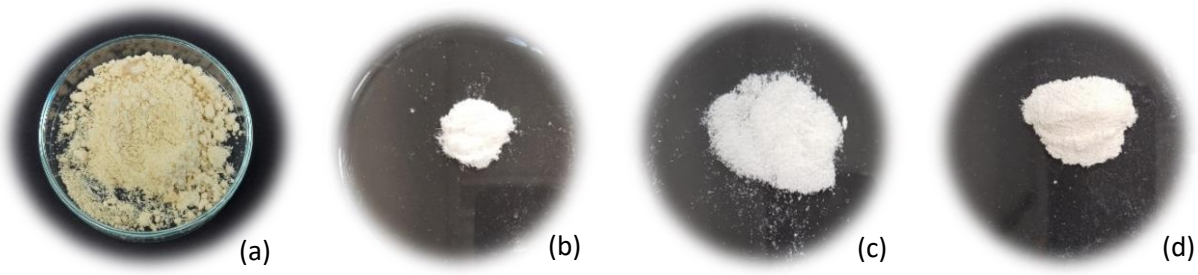


Figure 1 – Images of some fibers obtained from winter melon pomace: (a) M_AI_V; (b) M_B_V; (c) M_B_{50}_V and (d) M_B_O

Figure 2 shows a partial mass balance, related to the pulp fraction and the sub-fractions resulting from the different handling processes to which it has been subjected to obtain the different types of DF.
As an example, we report the data associated to the vacuum-freeze dried fiber "as it is" and the one obtained by bleaching with H_2O_2 at room temperature and dried with the same technique.

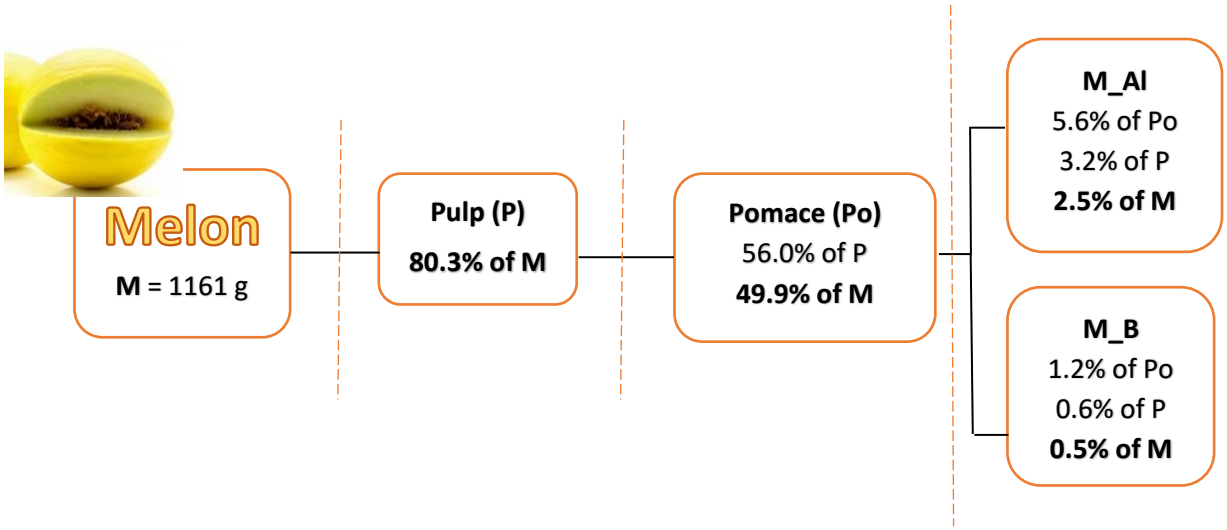


Figure 2 – Mass balance for the Cucumis Melo pulp fraction, with the resulting DFs

This scheme (Figure 2) confirms that depending on how melon pomace is processed, different amounts of dry fiber are obtained. This behavior can be explained by the fact that by bleaching and washing the fiber, soluble analytes (mineral salts, organic acids, simple sugars, among others) are removed, with a consequent decrease in the residual fiber mass.

On average, it has been verified that the amount of M_AI fiber obtained regardless of the drying technique adopted (setting the parameters we selected) is around 2.5%, with values ranging in the 2.2 – 2.9% interval for some replicates, calculated with respect to the total mass of the fruits. For the fiber treated with H_2O_2, instead, it is estimated to obtain an average quantity of DF between 0.4 - 0.6%, starting from fruits having the same characteristics of those used in this study.

In the following sections we report the results obtained from the various analyses carried out on the different types of melon pomace fibers, starting from the proximate analysis and continuing with some instrumental analyses.

Proximate analysis

Table 1 collects an accurate comparison among the samples of winter melon fresh pulp, the fibers obtained from this fraction through different procedures and some literature data. Among the several types of bleached fibers, in this table we report as an example the M_B_V one.

Table 1 – Proximate analysis representative of some fractions obtained from Cucumis Melo var. Inodorus, Yellow Canary type.*

	FRESH PULP	**M_AI_V**	**M_B_V**	**[6]$**	**[7]$**
Total water content %	92.20 ± 0.26			83.6 – 84.7	
Moisture % at 40 °C	17.30 ± 0.46				
Moisture % at 101 °C	6.33 ± 0.31	5.33 ± 0.25	4.91 ± 0.32		
Residue % at 105 °C (db)	7.82 ± 0.26	5.51 ± 0.70	5.40 ± 0.46		8.49 – 15.54
ashes % (db)	7.26 ± 0.12	7.23 ±0.33	3.46 ± 0.09	6.8 – 7.5	4.407 – 6.685
ashes % (fw)	0.55 ± 0.03	0.54 ± 0.03	0.26 ± 0.02	0.68 – 0.75	
pH	5.83 ± 0.02			6.05 - 6.55	6.36 – 6.94
Sugars (%)#					
Reducing	3.17 ± 0.14			2.35 – 3.00	
Glucose	1.24 ± 0.10			1.30 - 1.56	
Fructose	1.93 ± 0.10			1.05 – 1.44	
Sucrose	1.34 ± 0.11			0.95 – 0.97	
Total	4.51			3.30 – 3.97	
Titratable acidity#					
mg/mL citric acid	0.91 ± 0.07			0.7 - 0.6	0.475 – 0.809
mg/mL citric acid monohydrated	0.99 ± 0.08				
Proteins (g/kg fw)		8.67 ± 0.55	2.83 ± 0.42	10.1 – 11.4	6.625 – 10.23
C %		37.35 ± 0.21	40.21 ± 0.22		
H %		6.34 ± 0.14	6.21 ± 0.10		
N %		2.10 ± 0.10	0.65 ± 0.07		
S %		-	-		

*mean of three replicates (± standard deviation, $s_{(3)}$).
#measurements conducted on fresh juice
db = on dry basis ; fw = on fresh weight
$ the data used for comparison refer to melon cultivars different than ours

As can be seen from Table 1, the most significant differences were detected between the bleached fiber and the other two samples of integral material. In fact, the procedures adopted during the treatment with H_2O_2 have led to an important variation in the compositional characteristics of the obtained fiber. As a consequence, a reduced ash and protein fraction content was detected compared to the starting material, both due to the strongly oxidizing effect of H_2O_2 and to the leaching effect.
In fact, considering the values on fw, there is a reduction of about 50 % as regards the ash in M_B_V if compared to fresh pulp and M_AI_V fiber, while ≈ - 68 % for protein content.
Crude fiber, on the other hand, shows amounts of ash and proteins that are comparable with literature data, even if considering that the comparison is made between different cultivars.
However, some of the differences showed in Table 1 can be traced back to the different origin of the fruits used in the various studies cited, to differences in cultivation practices, degree of ripeness and genotypic factors. In fact, plant mineral absorption can differ according to soil mineral composition, plant variety, and plant growth stage [8]. In particular, melon shows a sensitivity towards the salinity of the soil in which it is

cultivated and, also for this reason, its quality is strictly related to its chemical composition and to the irrigation system used [7].
Similar considerations can also be developed for pH values.
It is well known that harvesting time, in association with genotype factors and environmental parameters are crucial in defining the chemical composition and fruit quality traits, including sugar content. For melon, accumulation of sugars during the fruit development is of great importance due to the high relation that exist between sugar content and acceptability of the product by consumers.
Fructose and glucose levels increase during melon fruit development for being subsequently converted in sucrose, during the ripening stage [6].
Their relative ratios together with the contribution given by organic acids define the taste and the perception of sweetness of the melon pulp.
Our results show a high sugar content in melon juice (4.51% for total sugars and 3.17% for the reducing ones), and titratable acidity in accordance with the literature data.
Even the elemental analysis confirms the sample that has undergone oxidative treatment with H_2O_2 mainly consists of C and H, evidence of a material having a mainly polysaccharide-fibrous-cellulosic structure.

UV-Vis spectroscopy analysis

The UV-Vis analysis technique was applied to melon pomace fiber samples to evaluate the spectrum profile and absorption bands which characterize the solid material and, also, the associated color point parameters. The evaluation of this latter aspect is particularly important for this study, as one of the main objectives is to obtain a material that is as colorless as possible. For doing this we collected the spectra of the selected samples and we also recorded the color point parameters L*, a* and b* defined within the CIELab color space.
The parameters which define the "absolute" white, that we would like to achieve with the bleaching treatment, are L* = 100, a* = 0, and b* = 0.
We then proceeded to measure the UV-Vis spectra of the fibers bleached with H_2O_2, obtained by applying the different experimental conditions previously described, and of the “as it is” melon pomace fiber (M_AI_V). The measurements were carried out with total reflectance technique, operating in the range of 200 ÷ 800 nm and using the instrumentation and methods listed in Chapter 4.
Below we report the list of the analyzed samples:

1. Vacuum-freeze dried melon pomace, “as it is” (M_AI_V).
2. Melon pomace Bleached with H_2O_2 at room temperature, Vacuum-freeze dried (M_B_V)
3. Melon pomace Bleached with H_2O_2 at room temperature, Oven dried at 40 °C for 48 h (M_B_O).
4. Melon pomace Bleached with H_2O_2 at 50 °C, Vacuum-freeze dried ($M\_B_{50}\_V$).
5. Melon pomace Bleached with H_2O_2 at 50 °C, Oven dried at 40 °C for 48 h ($M\_B_{50}\_O$).

We have prepared at least three pads for each fibrous sample listed in the previous diagram. All samples were stored in closed glass vials, in contact with the residual atmosphere, in the dark and at room temperature. The samples were periodically analyzed and processed with the UV-Vis analysis technique for the entire duration of the study (24 months).
All measurements conducted at each ageing step were carried out by processing at least three tablets, randomly exposing them to the incident radiation beam.

Figure 3 shows the UV-Vis spectra of the fibers obtained from the pomace of the winter melon, manipulated following the various procedures mentioned above.

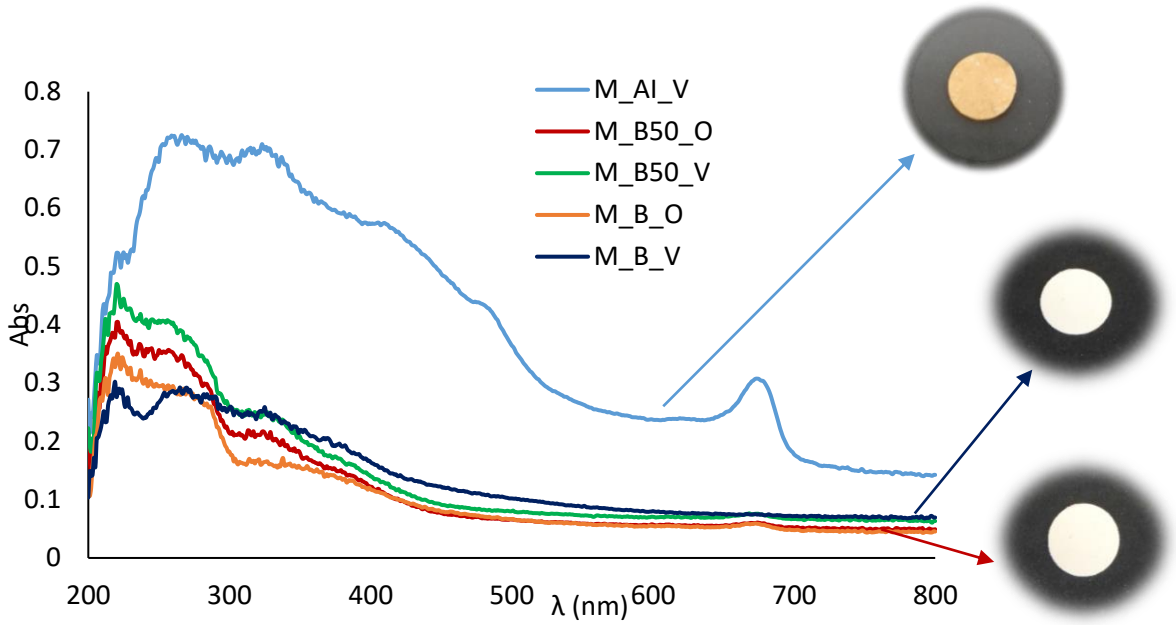


Figure 3 – Comparison of UV-Vis spectra of winter melon fiber samples in tablet form.

As can be seen, the trend of the spectra of the fibers bleached with H_2O_2 are comparable one each other, while the spectrum relative to the vacuum-freeze dried fiber "as it is" differ considerably from the previous ones. In particular, the M_AI_V spectrum shows an intense absorption band between 280 – 320 nm, followed by a broad and decreasing absorption band around 410 nm with expansion up to ≈ 500 nm. In addition, an isolated absorption band centered around 675 nm is detected. The absorption of electromagnetic radiation at visible wavelengths is responsible for the greenish-yellow coloration of the vacuum-freeze dried winter melon pulp.

It is reasonable to assume the analytes that contribute to this coloring may be flavonoids, flavones, flavonols, among other molecules, with a pale yellow color (identified in methanolic extracts from the peel of the same melon), as well as xanthophylls and chlorophylls. In fact, it is known that carotenes and xanthophylls absorb in the range from about 400 nm to about 500 nm, while the typical chlorophyll absorbance is in the blue and red wavelength regions [9].

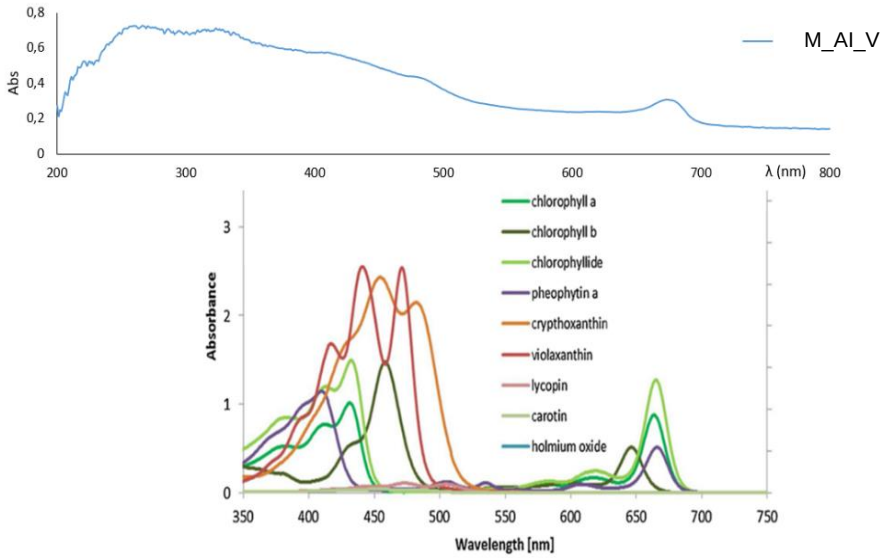


Figure 4 – Comparison between UV-Vis spectra of chlorophylls and carotenoids (in solution) taken from literature [9], and a sample of melon fiber M_AI_V (recorded in solid state)

We specify that the literature spectra used for comparison with those of our samples refer to solutions containing the individual molecules indicated in the caption, while in our case we have UV-Vis spectra of real and complex matrices, recorded in solid state.
Their direct comparison was therefore useful to us to develop hypotheses on the presence of chlorophylls in the pulp of the winter melon, based on the presence of the isolated and characteristic absorption band at about 670 nm, but it cannot be extended to the rest of the spectral window since the nature of the analyzed samples is too different.
On the other hand, the UV-Vis spectra of the bleached samples do not show these absorptions in the visible region but are mainly characterized by an intense absorption band at about 220 nm, followed by a broad one around 250 nm with expansion up to ≈ 320 nm.
These absorptions in the UV region underlie the most energetic transitions, compatible with the structure of the polysaccharide matrix and with the presence of nitrogen compounds (for example amino acids, proteins, nucleic acids) [10, 11].
This shows that the action of hydrogen peroxide leads to the degradation of the natural pigments originally present in the pulp of the "Canary type" melon, allowing to obtain an almost white fiber, in all the experimental conditions tested.
Actually, the spectra profiles of the oven dried samples (M_B_O and M_B_{50}_O), show a very weak hint of the absorption band centered at ≈ 675 nm. Despite having a very low intensity, this small contribution is not entirely negligible. The overall trend of the spectra confirms what has been observed by the visual inspection of the two fiber samples: the one dried by mild heating has a slightly ivory color, while the vacuum-freeze dried one appears of a "cleaner" white.

Table 2 shows the values of the L*, a*, b* coordinates and ΔE in the CIELAb color space, associated with each of the spectra shown in Figure 3.

Table 2 - Color point parameters of fiber samples obtained from Yellow Canary melon pulp, pressed in the form of tablets[#].

	L*	a*	b*	ΔE
M_AI_V	77.2 ± 0.1	-4.51 ± 0.04	22.6 ± 0.2	/
M_B_V	94.7 ± 0.1	-0.44 ± 0.02	2.71 ± 0.02	26.8
M_B_{50}_V	93.6 ± 0.2	-0.47 ± 0.02	2.84 ± 0.02	26.0
M_B_O	89.2 ± 0.2	-0.53 ± 0.02	5.65 ± 0.04	21.1
M_B_{50}_O	91.2 ± 0.1	-0.45 ± 0.01	5.48 ± 0.03	22.5
ABSOLUTE WHITE COLOR	100	0	0	

[#] values represent the mean of 3 replicated measurements, ± standard deviation $s_{(3)}$.

Looking at the data in Table 2 it is even more evident that the bleaching operations allowed to obtain L*, a* and b* values close to our reference, i.e. the white color. In fact, the action of H_2O_2 led to a significant reduction of the weight of the b* parameter associated with the greenish-yellow color of the initial matrix. This indicates that the analytes containing the chromophore groups responsible for this color have been degraded. Conversely, we observe an important increase in the values of L* parameter (relative to brightness) in all bleached samples, which increases by about 22 % if compared to the value of the M_AI_V fiber.
Furthermore, we can also notice that both M_B_O and M_B_{50}_O are characterized by b* values that are about twice those of the respective vacuum-freeze dried samples, in contrast to the L* parameter which is greater for M_V samples than for M_O specimens. The reason why b* values of the bleached and oven dried samples are higher than the analogous vacuum-freeze dried, may be due to interaction phenomena between sugars and amino acids / proteins, catalyzed by the presence of organic acids and favored by the oven drying temperature of 40 °C (Maillard reaction). This reaction typically occurs when heating food at high temperature and leads to the formation of brown color compounds (intensity of coloration is variable), but it can begin at much lower temperature, generating products having only a slight ivory color, as in our case.

However, the decrease of b* values (yellow-blue coordinate) as a result of the bleaching process is significant, with approximately - 87 % for vacuum-freeze dried fibers and about - 75 % for oven dried ones. Finally, the a* parameter almost does not contribute to the residual coloring of our fibers. In this case, in fact, all values are around zero and therefore very close to the coordinate of the white color.

Going further with the analysis of our data, two-way ANOVA test was applied to better understand which factors have a significant incidence on the total color variation (ΔE) of the bleached melon pomace fibers. ΔE was calculated by applying the equation:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

where L*, a* and b* are the coordinates of the bleached fibers, while L_0*, a_0* and b_0* are the color parameters of the material used as a reference with respect to which we calculate the color variation (M_AI_V fiber).

The factors we considered are:
- the temperature set during the bleaching treatment (room temperature or 50 °C)
- the drying technique adopted (Vacuum-freeze drying or Oven drying).

From this analysis it appears that both factors investigated, "Vacuum-freeze drying/Oven drying" and "Temperature", and their interaction, are statistically significant. In particular, for the "Vacuum-freeze drying/Oven drying " factor a significance level of 99.9% was obtained ($p < 0.001$), for the "Temperature" one, the significance level is 95% ($p < 0.05$) and, finally, for the interaction we obtained a significance level of 99.9% ($p < 0.001$).

This confirms that especially the vacuum-freeze drying technique allowed to obtain fibers with a better whiteness than the oven dried ones. In fact, if comparing the ΔE values we can notice that the experimental conditions we tested lead to an overall variation in the color of the fibers that is around 26.5 for the vacuum-freeze dried ones and ≈ 22 for the oven dried samples.
For our purposes, achieving the highest possible whiteness means moving as far away as possible from the initial color of the M_AI_V fiber (L*= 77.2; a*= -4.51; b*= 22.6), to obtain L* values which tend to 100, while a* and b* should approach 0 value.
So, balancing the values of L*, a* and b* for each sample with the above-mentioned formula and considering the coordinates of the M_AI_V fiber as the starting point (L_0*, a_0*, b_0*), the higher the value of ΔE, the greater the color variation of the fiber and the better the whiteness reached.
From an application point of view, the obtaining of the whitest dietary fiber (DF) is essential if we think about its usage in products such as ice creams, yoghurts, etc ... which necessarily must maintain a neutral-white color, while for bakery and pastry products a DF with a slightly ivory color can be already employed.
On the basis of these considerations, we can say that all the conditions we have adopted allowed us to obtain extremely satisfactory results in terms of whiteness achieved. In fact, also the oven drying process allows to obtain an acceptable product with the considerable advantage of being much cheaper than the vacuum-freeze drying one, both in terms of energy consumption and costs associated with the production plant.
These assessments are very important in order to contain production costs and energy expenditure as much as possible and trying to limit the resources associated with the production process necessary to obtain the bleached fiber. This must be even more underlined since our aim is to enhance a waste raw material and, therefore, devoid of any commercial value to obtain a material hopefully finalized to a wide audience.

As previously mentioned, in an attempt to pursue goals of effective technological transfer starting from waste materials to recover high-added value products (as DF), we consider it necessary to make some comparisons with some commercial fibers and others obtained during this PhD study. The results are shown in Table 3 and Figure 5.

Table 3 - Color point parameters of fiber samples obtained from Yellow Canary melon pulp, of some commercial and other types of fibers, pressed in the form of tablets[#].

	L*	a*	b*
M_AI_V	77.2 ± 0.1	-4.51 ± 0.04	22.6 ± 0.2
M_B_V	94.7 ± 0.1	-0.44 ± 0.02	2.71 ± 0.02
$M\_B_{50}\_V$	93.6 ± 0.2	-0.47 ± 0.02	2.84 ± 0.02
M_B_O	89.2 ± 0.2	-0.53 ± 0.02	5.65 ± 0.04
$M\_B_{50}\_O$	91.2 ± 0.1	-0.45 ± 0.01	5.48 ± 0.03
Wheat starch	95.1 ± 0.2	-0.15 ± 0.01	0.28 ± 0.01
Corn starch	95.1 ± 0.2	-1.03 ± 0.01	5.68 ± 0.03
Potato starch	92.2 ± 0.1	-0.02 ± 0.01	1.54 ± 0.02
Baobab powder	82.8 ± 0.1	3.54 ± 0.03	13.8 ± 0.1
Horse-chestnuts seed flour (AHP)	85.2 ± 0.2	-0.73 ± 0.01	14.8 ± 0.1
Defatted watermelon seed flour (W-seed-flour)	89.1 ± 0.3	0.18 ± 0.04	10.0 ± 0.1
ABSOLUTE WHITE COLOR	100	0	0

[#]values represent the mean of 3 replicated measurements, ± standard deviation $s_{(3)}$.

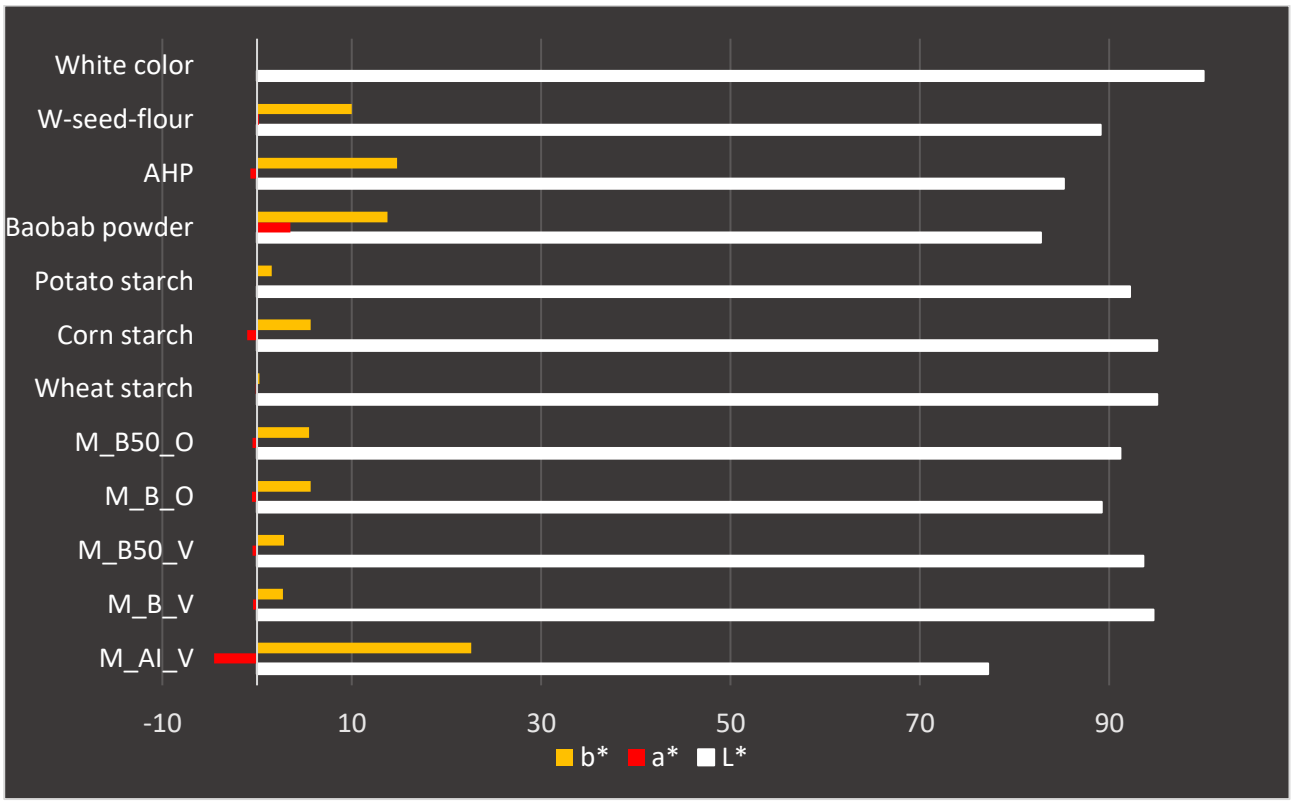


Figure 5 – Histogram representation of the data shown in Table 3.

We specify that the commercial products analyzed have been used as they are commercialized, within the expiration date indicated on the package and stored in a cool and dry place, in the dark.

For example, baobab fruit is consumed as basic food in many regions of Central and West Africa as an ingredient of traditional recipes. The pulp and the seeds of baobab fruit are highly nutritious, being an important source of energy, proteins, vitamin C, antioxidants, and mineral salts. The benefits associated with the consumption of baobab fruit powder have allowed its widespread diffusion all over the world. Currently it is used as a functional ingredient in many foods and beverages, such as baking products, yogurt, ice-creams, in energy and nutrition bars, among others.

Also horse-chestnuts seed flour has been extensively analyzed by our research group in some previous studies [12, 13], even during my PhD [14, 15], finding that, thanks to its compositional characteristics, it is

used in pharmaceutical formulations, cosmetic preparations, herbal remedies and commodities such as shampoos, shower gel, creams, lotions, sun products, dermo protectives, and tooth-pastes.
Defatted watermelon seed flour was obtained from Crimson Sweet seeds, shelled to separate the cuticle from the kernel. Then, the inner part (germplasm fraction) has been subjected to extraction processes with organic solvents to remove the oil fraction, as already done during a previous study [16].
Among the several tests carried out, we point out that the best quantitative results in term of oil extraction were obtained using n-hexane and extracting in a thermostated ultrasonic bath at T = 60 °C for 60 min. For these reasons, in this PhD project we used the same extraction conditions to obtain the defatted watermelon seed flour.
Watermelon seeds represent an important source of vegetable proteins, oil, vitamins, minerals and bioactive compounds. They are typically used to prepare snacks or added to salads, but they are also milled to obtain flour which is used for sauces or inserted in bakery products. Also the oil that can be extracted from watermelon seeds is used in cooking and for cosmetic formulations [17].
Observing the values of L*, a* and b* coordinates of the samples examined for comparative purposes (Table 3), baobab fiber is the one that differs most from the white color, being characterized by a light brown coloring due to the presence of chromophore molecules, including tannins [18].
Instead, we can affirm that the color of our bleached fibers is comparable with some of the most common starches used in food preparations. This applies especially to the L* and a* parameters of vacuum-freeze dried fibers (M_B_V and M_B_{50}_V), while b* is characterized by greater variability even among commercial fiber samples. In fact, b* value for corn starch is 5.68, quite far from the reference white color but perfectly comparable with the values of our bleached and oven dried melon fibers (M_B_O and M_B_{50}_O). In addition, these samples show a* values which are about half that of corn starch.
As already reported, although differences are recorded between the values of the individual coordinates, we must remember that the color of a material is given by the combination of these three parameters.
Therefore, based on these considerations, we can affirm that the overall color of our bleached fibers does not differ excessively from that of wheat, corn and potato starches, demonstrating that our whitening process has been effective. In addition, if our fibers would be used in some doughs or industrial formulations, they would allow to obtain a product with a coloration close to neutral one.
Among the other fibers obtained during this PhD study, also defatted watermelon and horse-chestnuts seed flours have a very pale ivory color, given in particular by the b* parameter (+ 10.0 and + 14.8, respectively), associated to the coordinate defined within the CIELab color space ranging from yellow (+b*) to blue (-b*).
Although these fibers are not perfectly white, using them in a small percentage (for example 0.1 % – 1 %, in mixtures) may not affect the neutral color required for the final product.

Finally, we have monitored the evolution of L*, a*, b* values over time for each of the selected melon fibers, to detect any changes in their color during aging. Each sample was analyzed at t_0 time and after 6, 12 and 24 months of storage in the dark at room temperature, in closed glass vials but in contact with the residual atmosphere. The results obtained are shown in Table 4, remembering that each value is the average of at least three replicates.

Table 4[#] - L, a*, b* values of melon pomace fiber samples, over time.*

	M_Al_V			**M_B_V**			**M_B_{50}_V**			**M_B_O**			**M_B_{50}_O**		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
t_0	77.2[a]	-4.51[a]	22.6[a]	94.7[a]	-0.44[a]	2.71[a]	93.6[a]	-0.47[a]	2.84[a]	89.2[a]	-0.53[a]	5.65[a]	91.2[a]	-0.45[a]	5.48[a]
t_{6m}	75.3[b]	-3.55[b]	17.1[b]	92.3[b]	0.17[b]	4.43[b]	91.2[b]	0.19[b]	5.44[b]	90.1[b]	-0.31[b]	6.14[b]	91.1[a]	-0.43[a]	6.95[b]
t_{12m}	74.8[b]	-3.40[c]	16.4[b]	92.4[b]	0.17[b]	4.73[bc]	91.2[b]	0.18[b]	5.60[b]	90.2[b]	-0.34[b]	5.85[c]	91.5[a]	-0.45[a]	6.90[b]
t_{24m}	74.6[b]	-3.33[c]	15.4[c]	92.8[b]	0.16[b]	5.12[c]	91.4[b]	0.18[b]	5.82[c]	90.3[b]	-0.35[b]	5.49[a]	91.6[a]	-0.43[a]	6.85[b]

[#] in a column, values with a different superscript letter indicate a statistically significant difference between each other ($p < 0.001$)

One-way Analysis of Variance (ANOVA) was performed in order to assess if there is a statistically significant difference between the values of each color point parameter at different ageing steps.

The comparison of the data collected in Table 4 shows that all the samples analyzed, except for the M_B_{50}_O one, show a significant variation ($p < 0.001$) of all three parameters associated with their color point, passing from t_0 time to six months of storage (6m). However, continuing with ageing, the values of L*, a* and b* coordinates of most bleached fibers do not change markedly.

This suggests that the overall color of these samples, stored in the conditions previously indicated, remains stable over time, allowing their conservation and possible use in the medium-long term. In particular, the parameters of M_B_{50}_O sample vary the least over time; it suggests that the combination of the effects given by the bleaching and drying temperatures allowed to obtain a fiber that has already reached its maximum whiteness at t_0 time.

Statistical analysis in Table 4 has shown some significant variations between the values of some of the measured parameter. However, the treatments with H_2O_2 allowed to obtain satisfactory results in terms of “whiteness point", already reached at t_0 time, which remains almost unchanged after two years.

Thermal Analyses

The knowledge of the thermal stability of easily degradable polymers, such as DF, is an indispensable feature which contributes to a great extent to define their behavior when subjected to heating, since it allows to optimize their uses and applications. The scarce ability of polymers to withstand thermal stress implies the possibility of being degraded, making them unsuitable materials for particular food applications. This is especially true in cases where the material is thermally treated through different operations in industrial processes, such as sterilization and cooking.

Therefore, TGA/DTG/DTA runs were performed to evaluate the thermal behavior of different melon fiber samples, subjected to various preliminary handling operations. These tests are essential to obtain information about the thermal stability of the materials we examined.

In the following discussion, we will study the thermal runs performed in the temperature range between 0 °C and 630°C, working in an inert atmosphere for He.

Figure 6 shows the thermal analysis profiles of a fiber sample obtained from the pomace of winter melon, bleached with H_2O_2 at room temperature, washed with distilled water to eliminate all the soluble components, including Soluble Dietary Fiber (SDF), and finally vacuum-freeze dried. Therefore, M_B_V sample, represents the amount of Insoluble Dietary Fiber (IDF), net of the oxidative bleaching process.

The descriptive elements of the thermal profiles of Figure 6 are collected in Table 5.

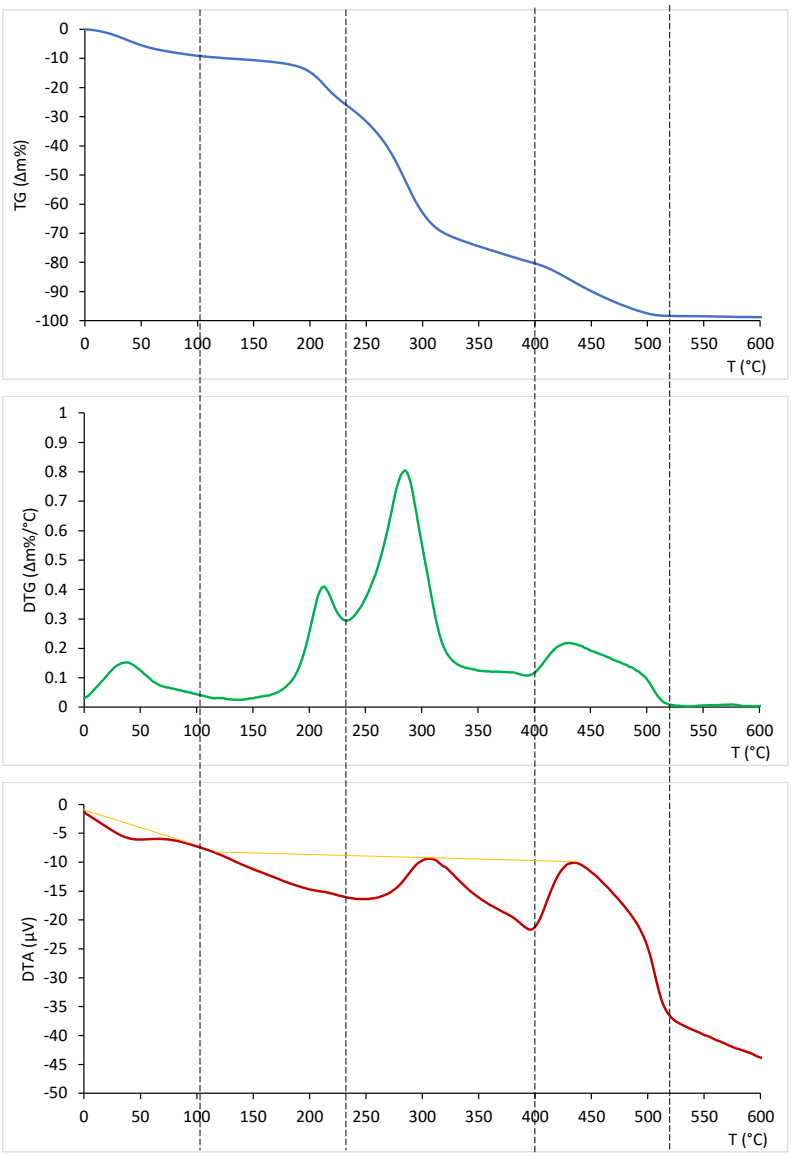


Figure 6 – TG, DTG and DTA thermal analysis profiles for a sample of M_B_V fiber, measured in He atmosphere.

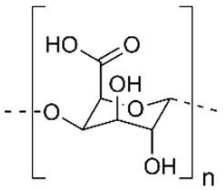
Table 5 – Representative values of the TG/DTG/DTA profiles of Figure 6, obtained in inert atmosphere (He)

Peak	Δm (%)	T_o (°C)	T_p (°C)	T_c (°C)	Processes
1	- 9.9	20	44.4	107.3	Elimination of VOCs and moisture
2	- 18.3	107.3	214.6	239.2	Elimination of bound water, NH_3, low-boiling VOCs, CO and CO_2, caramelization of polysaccharides.
3	- 48.7	239.2	287.1	384.9	Elimination of constitution water, VOCs and SVOCs, decarboxylation with CO_2 loss, degradation of polysaccharides, sample greification
4	- 21.2	384.9	429.1	517.5	Elimination of hydrocarbons and volatilization of carbon residues (C6 – C20)
---	- 0.4	517.5	---	630	Volatilization of carbon residues (C20 - C40)
Residue at 630 °C = 1.5 %					Inorganic material and carbon residues

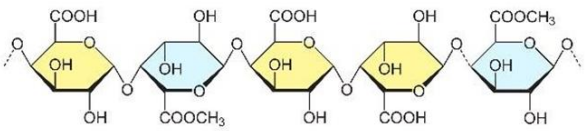
T_o = Onset Temperature (peak start)
T_p = Peak temperature (central T value of the peak)
T_c = Closure Temperature (end of the peak)

For an effective description of the experimental results, we divided the total thermal range into 5 wide intervals (Figure 6), which subtend the peaks relating to processes and structural deformations involving the sample analyzed.

- 1st interval: 0 < t/°C < ~ 105 - The elimination of the SDF fraction simplifies the *faces* of the residual sample subjected to pyrolysis (IDF), and consequently, reduces the complexity of the thermal analysis profiles. Peak 1 underlies the elimination of residual moisture, together with the more volatile VOC fraction present in the sample.
- 2nd interval: ~ 105 <t/°C < ~ 240 - This thermal range is characterized by the presence of peak 2, clearly identifiable even if partially superimposed on peak 3. The estimated mass loss of about -18 % underlies some of the processes described in Table 5. The effects of pyrolysis initially involve thermal elimination processes of bound and constitution H_2O, auto-oxidation and decomposition of polysaccharide polymers, with random breaking of some glycosidic bonds [19]. This is reasonably associated with the elimination of CO and CO_2 by the more thermolabile compounds. Probably, this behavior is mainly attributed to structural deformations of pectins, proteins and some other molecules and polymers [20].

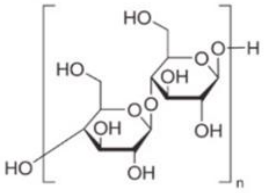


Pectic acid or polygalacturonic acid

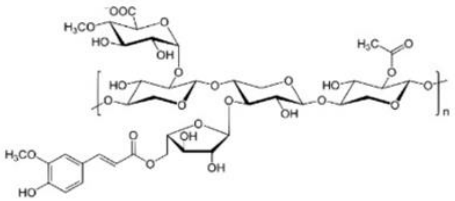


Pectins

- 3rd interval: ~ 240 < t/°C < ~ 400 - Peak 3, present in this temperature range, is centered at ~ 287°C and expresses the greatest mass loss of the entire thermogram, with Δm% = -48.7%. The peak represents the progressive deformations of hemicelluloses, a large family of predominantly xylanoid compounds (together with xyloglucans, β-glucans, mannans and glucomannans), each of them can show a completely original "*faces*" [21]. An example is produced in the following representation.



Cellulose



Structure of xylans, belonging to hemicellulose family

Analyzing the structure of hemicellulose and comparing it with the cellulosic chains, i.e. the typical linear homopolymer of glucose, the greatest differences are immediately detected. As far as xylans concerns, being a complex mixture consisting of very different compounds (hexose-pentose heteropolymers, branched chains, with the presence of free or partially esterified carboxylic acid groups, ether groups and polyphenolic substituents, etc.), it is evident that different analytes undergo thermal stress and structural degradation over a wide range of temperatures [22]. In the tail part of the peak, where there is a plateau, it cannot be excluded that at least a fraction of the residual mass sample may plasticize, while another fraction could reach the greification limit with the consequent appearance of a pseudo-glassy phase.

- 4^{th} interval: ~ 400 < t/°C < ~ 520 - The behavior of the sample at the exothermic peak 4 can be represented as a solidification of the residual mass, which transfers the latent heat of solidification due to the transition from the pasty-pseudo-glassy phase to a solid one. Then the solid, by decomposing, generates partially hydrogenated nanoparticles carbon residues, with typical C6-C20 dimensions.
- 5^{th} interval: ~ 520 < t/°C < ~ 630 - The behavior of the sample at the last thermal window probably simulates that of a solid carbonaceous mass brought to red heat which, by decomposing, sublimates partially, generating hydrogenated nanoparticle carbon residues with typical C20-C40 dimensions.

At the end of the thermal degradation processes of the IDF sample, the residual fraction consisting of mineral salts, metal oxides and carbonaceous ashes, expresses 1.5 % of the initial mass.

Figure 7 shows the thermal profiles relating to a fibrous sample extracted from the pomace of Cucumis Melo, var. *Inodorus*, vacuum-freeze dried “as it is” (M_AI_V). In this case we can therefore talk about Total Dietary Fiber (TDF). Obviously, this sample contains both the soluble components (monosaccharides, disaccharides, mineral salts, etc.), the soluble fiber fraction (oligosaccharides with MW up to ≈ 2000 Da) and the insoluble one (polysaccharides in general, of MW > 2000 Da, such as pectins, glycoproteins, starches, hemicelluloses, etc.).

As can be noticed from Figure 7, the thermal stress causes significant changes which can be mainly described within the same 5 temperature ranges previously identified for Figure 6, while Table 6 shows the interpretative elements associated with the thermal profiles for M_AI_V sample.

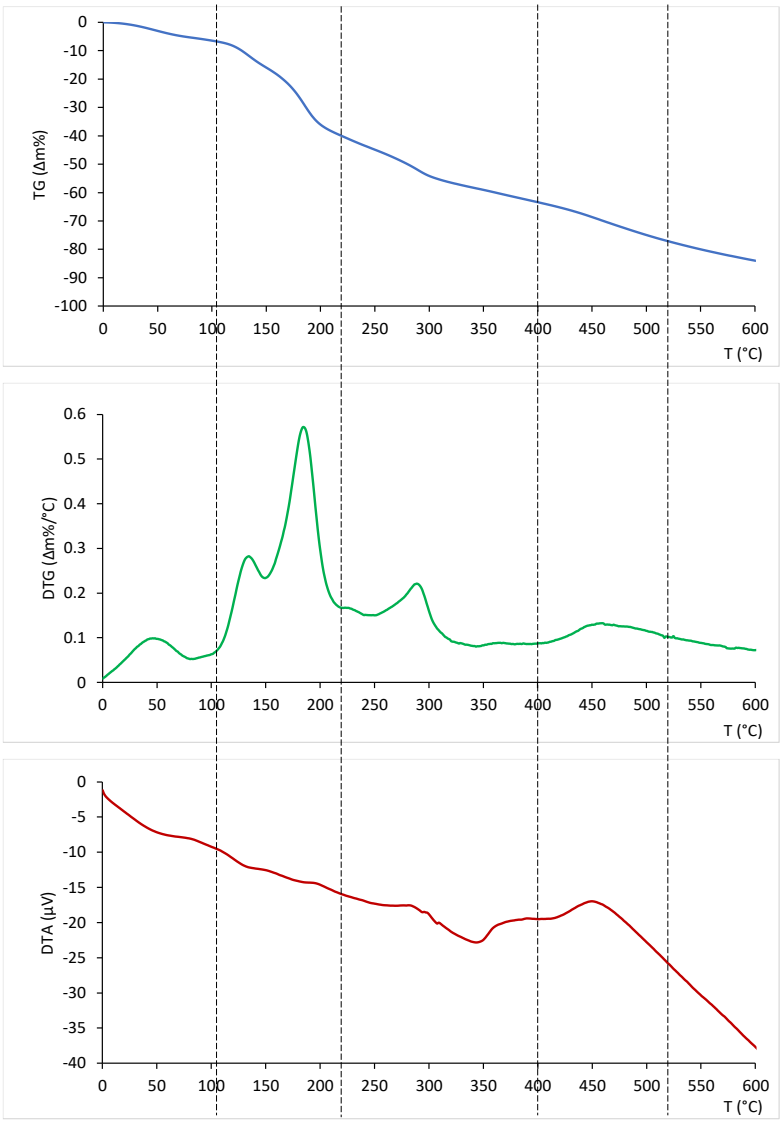


Figure 7 - TGA, DTG and DTA thermal analysis profiles for a sample of M_AI_V fiber, measured in He atmosphere.

Table 6 – Representative values of the TG/DTG/DTA profiles of Figure 7, obtained in inert atmosphere (He) for a M_AI_V fiber sample.

Peak	Δm (%)	T_o (°C)	T_p (°C)	T_c (°C)	Processes
1	- 6.3	20	44.4	105	Elimination of VOCs and moisture
2	- 12.1	105	139.5	158.5	Elimination of bound water, NH_3, low-boiling VOCs, initial of decarboxylation processes with CO_2 loss
3	- 21.7	158.5	172.9	207.5	Elimination of bound water, VOCs, CO and CO_2 loss, elimination of constitution water, caramelization of sugars and polysaccharides
4	- 7.2	207.5	223.1	239.2	Elimination of SVOCs, carbonylic and carboxylic groups, constitution water and degradation of polysaccharides
5	- 10.4	239.2	279.6	311.3	Elimination of constitution water, sample greification
6	- 8.1	311.3	381	399.8	Elimination of low MW hydrocarbons and carbon residues (C6-C20)
7	- 10.9	399.8	443.9	520	Elimination of higher MW hydrocarbons and carbon residues
---	- 9.1	520	---	630	Volatilization of carbon residues (C20-C40)
Residue at 630 °C = 14.2 %					Inorganic material and carbon residues

T_o = Onset Temperature (peak start)
T_p = Peak temperature (central T value of the peak)
T_c = Closure Temperature (end of the peak)
Mass loss is a common feature of the entire TGA profile.

- 1st interval: 0 <t/°C < ~ 105 - In the first temperature range, residual moisture up to 105 °C, and particularly volatile and low MW VOCs are eliminated.
- 2nd interval: ~ 105 <t/°C < ~ 220 - The most consistent mass loss is recorded in the 2nd temperature window, where two well-profiled peaks are present, associated to the processes described in Table 6. However, the residual solid mass can undergo some other transformations involving compounds resilient to heating. Among these secondary processes, at the peak centered around 139 °C, the phase transition of pectins could occur, passing from a crystalline structure, characterized by absorbed and strongly bound water, to an amorphous form [23]. The second endothermic transition in this interval, associated to the major peak centered around 173 °C, demonstrates the great instability of the material in this range of temperature, since a great variety of thermal degradation processes involving the hemicelluloses are activated.
- 3rd interval: ~ 220 <t/°C < ~ 400 - In this interval there are 3 peaks. The first one detected (peak 4 of Table 6), centered at ~ 223 °C, poorly resolved on the TGA/DTG traces and quite small, can probably be attributed to pectins pyrolysis peak [20, 24]. Despite the low resolution, caused by the shoulder peak leaning against the previous one centered at ~ 173°C, the DTA curve shows a wide endothermic band (190 - 280°C), compatible with structural deformations related to some components present in the sample.

  The second peak within the interval (peak 5), centered at ~ 280 ° C, underlies a rather important Δm%, despite a relatively modest endothermic flow, probably attributable to the completion of greification processes of some residual components in the material, with the acquisition of a pseudo-glassy consistency.

  Peak 6, centered at 381 °C, strongly endothermic but modest in size, probably subtends a plasticization process of the sample, due to total or only partial melting of some components.
- 4th interval: ~310 <t/°C < ~ 520 - In the 4th thermal range, there is only a peak. Peak 7, centered at ~ 444 ° C, is exothermic due to the probable release of the latent heat of solidification of the plasticized components in the 410 - 480 °C range. Actually, it is the result of a variety of degradative processes with a continuous and very consistent mass loss, due to the volatilization of heavy hydrocarbons and carbon compounds, only partially hydrogenated.
- 5th interval: ~ 520 <t/°C < ~ 630 - There are no peaks in this thermal window, although continuous mass loss is observed. The quantitative analysis is resolved in the ash residue (14.2 %) up to the final temperature of 630°C.

To conclude the comments on the results obtained from the thermal analyses on melon fiber samples, in Figure 8 we show a comparison between the DTG thermal profiles of IDF (M_B_V) and TDF (M_AI_V) samples (in He).

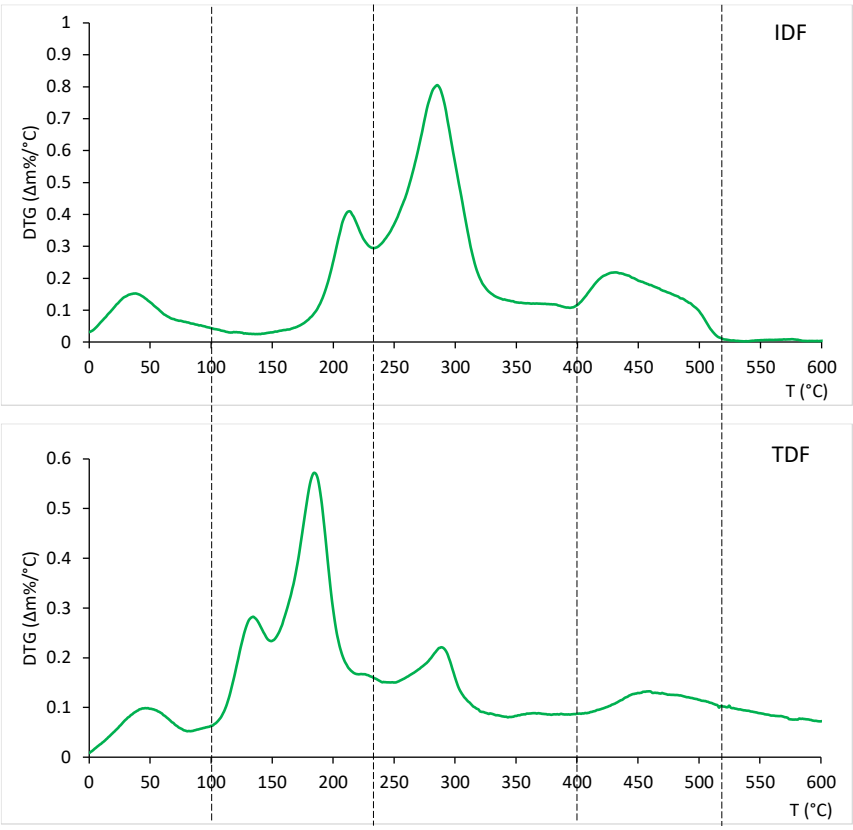


Figure 8 – DTG thermal analysis profiles for IDF (M_B_V) and TDF (M_AI_V) melon samples (in He).

The parallel comparison of the DTG curves related to the samples indicated in Figure 8 highlights some particularly evident and significant differences.

- The presence of the soluble components in the TDF sample shifts both thermal stress strain peaks of pectins (T_p_TDF = 139.5°C) and hemicellulose (T_p_TDF = 172.9°C) within the 2nd temperature range (≈ 105 - 230°C). In IDF sample, the peak produced by pectins is positioned on the upper limit of the 2nd thermal window (T_p_IDF = 214.6°C), while the peak related to hemicelluloses (T_p_IDF = 287°C) is present in the 3rd interval (≈ 220 - 400°C). We can evaluate the thermal shift, which stands at ΔT = 75 °C for the pectin peak, and ΔT = 114 °C for the xylanoid compounds. Intuitively, the analytes underlying these two peaks thermally interact since the profile does not appear to be affected by the presence or the absence of the SDF fraction. Conversely, a stretching of the curve is observed, equivalent to a tail expansion towards higher temperatures for the IDF sample.
  Therefore, it can be stated that the mutual position and centering of these two peaks can be considered the preliminary evidence of specific thermal interactions between pectins and xylans during the pyrolysis processes.

- The IDF sample loses peaks 4 and 5, present in the TDF sample. Evidently, these peaks underlie structural deformations compatible with the presence of the SDF fraction (monosaccharides, oligosaccharides, mineral salts, organic acids, amino acids, etc.), consisting of analytes that provide strong thermal interactions during pyrolysis processes. On the other hand, it is known that the presence of different compounds during heating of mixtures favors the plasticization of the mass, also lowering the melting temperature due to the appearance of eutectic phases otherwise not possible for pure components. Evidently, the presence of simple sugars and metal salts, which are excellent catalysts of chemical processes, favors the decomposition of pectins and xylans at lower temperatures in the TDF fraction.

In conclusion, the IDF fraction is much more stable than the corresponding TDF fraction and can be particularly recommended as a formulation ingredient since it can withstand more the thermal stress without undergoing significant structural deformations and thermally activated decompositions.

Finally, we can still point out some not negligible peculiarities, which emerged from this study.

(i) Xylans are certainly the most important components of hemicelluloses found in these fiber samples, although other constituent species of the same fraction could be present, such as glucomannans, albeit in much smaller quantities [25].

(ii) The possible network of specific interactions established between the various constituents of the fiber sample, whose effective action emerges from the thermograms and from the pyrolytic behavior of pectins and hemicelluloses, cannot represent a sum of independent contributions and cannot be described as the sum of the behavior of the single components [26]. Evidently, the intrinsic structural differences among these complex species, present in a heterogeneous mixture of physical simulation of an analogous system, induce consistent deviations from the behavior of a real biomass of similar composition.

(iii) The removal of an individual component from a fiber sample or from a real biomass, without altering the physical structure and the specific interactions networks present in the residual sub-system, is highly unlikely. This means that it is very difficult to separate the SDF fraction, or any other fraction or component, without altering the real structure, the characteristics and the properties of a generic sample of dietary fiber [27].

5.2. SKIN FRACTION

It is well known that melon skin, despite being considered a waste material, deserves to be enhanced thanks to the considerable abundance of bioactive compounds present, such as carotenes/carotenoids, fatty acids and polyphenols, among others [28].
Polyphenols are natural antioxidants, synthesized by photosynthetic organisms, capable of counteracting the negative effects caused by oxidative stress, i.e. oxidation exerted by peroxide radicals (R-O-O*, H-O-O*) and by some other particularly reactive and harmful species for cells, such as nitrogen oxides (N_xO_y). Furthermore, polyphenols exert a regulatory action in genetic transcription processes. Interest towards these compounds has progressively increased over the years, thanks to the beneficial effects they exert on human health. In addition to their strong antioxidant capacities, in fact, they show a neuroprotective action and a probable anticancer function [29].
For these reasons, the yellow skin of winter melon can represent an interesting material from which bioactive compounds and natural pigments/dyes can be obtained. In fact, it could be used (i) in the form of dried and finely ground powder, to increase the DF content and give a yellow color to the mixtures to which it is added, but also (ii) be subjected to extraction processes to obtain solutions containing the bioactive compounds responsible for the intense yellow color of the skin itself.
The first option (i) may seem the quickest and most practical to apply, however, it should be remembered that the quantity of analytes responsible for the chromatic aspect represents only a modest percentage of the total skin mass. Therefore, these analytes undergo a sort of dilution effect caused by all other components of the fiber. This means that, in order to impart an intense yellow color to some generic doughs, blends or formulations, a significant amount of peel should probably be used. This, however, could be counterproductive if an excess of DF from melon skin would not allow to confer to the final product the physicochemical and technological properties necessary for consumers acceptability. Therefore, extraction of the molecules responsible for the intense yellow color of Yellow Canary melon skin could be a much more effective and performing method for obtaining a natural dye, widely requested by the food industry, starting from the waste biomass itself. Furthermore, from an application point of view, the use of a coloring solution is much more versatile and can extend the range of products and formulations in which this could be used.
Based on what has been reported so far, in our study, we decided to carry out some extraction tests on dried melon skin powder, using both organic (MeOH), hydro-organic (MeOH : H_2O) solvents and aqueous solutions containing electrolytes. The choice to use electrolytic solutions as extraction media, among others, was made on the basis of some information found in the literature [30, 31, 32].

It is well known that polyphenolic compounds exist both in free and bound forms in plant cells. Free phenolic compounds can be extracted quite easily using different solvents (e.g. MeOH, MeOH:H_2O mixtures, acetone, etc…), while bound phenolic compounds, which are covalently bound to the plant matrix, cannot be extracted into water or aqueous/organic solvents mixtures without first proceeding with pre-treatment operations. In fact, bound phenolic compounds are covalently conjugated to cellulose, pectins and polysaccharides through ester bonds, and can be very difficult to extract. Alkaline, acidic or enzymatic hydrolysis methods can be used to release this fraction of analytes [32]. To the best of our knowledge, in most of the studies conducted with this aim, bound phenolic compounds were released by alkaline hydrolysis.
For these reasons we decided to use both alcoholic and hydro-alcoholic solutions and alkaline ones, in order to verify which condition is most effective for the extraction of the analytes responsible for the yellow color of winter melon skin. Therefore, alcoholic and hydro-alcoholic solution should convey what is defined "free phenolic fraction", instead, by treating the skin with highly alkaline pH solutions (pH range 11 – 13), we attempt to extract the bound polyphenolic content as well.
Furthermore, the choice of using aqueous solutions fits with the increasing attention towards environmental protection and the progressive decrease in the use of organic solvents, selecting some more eco-friendly ones. Certainly, among the various possible solvents, water has the lowest environmental impact, as shown in Figure 9.

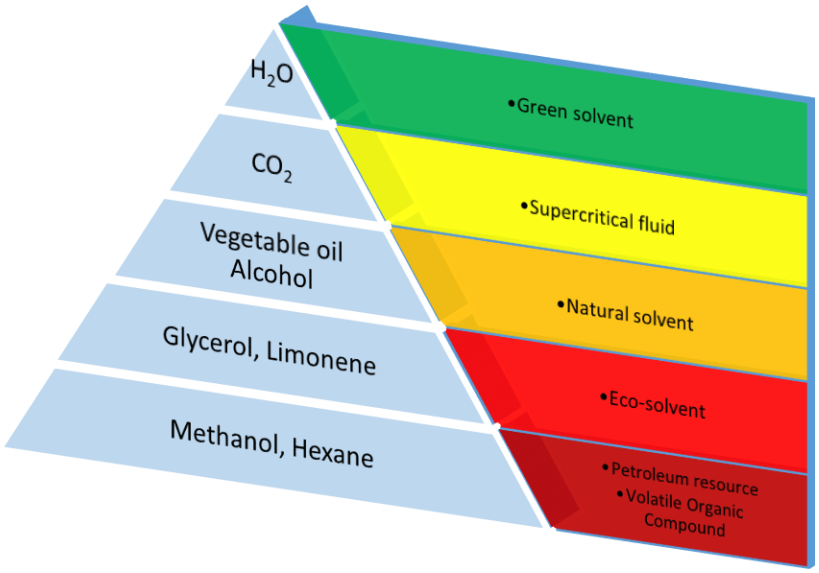


Figure 9 – Pyramid of biocompatibility and environmental impact of various extraction solvents

Not surprisingly, the number of studies about the recovery of bioactive compounds from waste biomasses, employing extraction techniques based on water replacement of organic solvents and the use of mechanical processes to prevent harmful and costly chemical substances, have increased. Furthermore, obtaining extracts in aqueous solution would make them more biocompatible (Figure 9), facilitating their possible use, for example in the food sector. In our case, these are only preliminary tests which, unfortunately, we were unable to deepen during this PhD project, but which could represent an interesting starting point for future studies.

Materials and methods

Chemicals

Methanol and formic acid are analytical grade Sigma-Aldrich products for HPLC-MS chromatography, distributed by Merck KGaA, Darmstadt, Germany. NaOH and Na_2CO_3 are commonly used laboratory reagents, purchased from Carlo Erba Reagents, Milano (Italy). Ultrapure water was produced from Millipore system (Millipore Sigma, Darmstadt, Germany).

Preparation of melon peel extracts

All extraction tests were carried out on samples of washed and dried skin, ground at an average particle size of 0.5 mm and stored at -20 °C until analysis. In particular, the solvents used are 100% methanol [30], 80-20% methanol-water [30, 31], NaOH 3M (pH = 13) [31] and Na_2CO_3 aqueous solution (concentration of 110

g/L, equal to half saturation; pH = 11). About 0.5 g dried melon powder skin were extracted with 5 mL of each solvent previously listed, through Ultrasound Assisted Extraction (UAE) technique, working in a thermostated bath at T = 60°C for 60 min. The UAE, thanks to cavitation phenomena, leads to the rupture of cell walls, increasing the mass transfer and the penetration of solvents, allowing to accelerate the extraction process. Furthermore, this technique allows to preserve as much as possible the initial properties and characteristics of the matrix and extracts.

Extracts in basic aqueous solutions were then acidified to pH ≈ 2 by continuous addition of HCl 6M. The obtained solution has been washed five times with 5 ml ethyl acetate (25 ml final volume). Ethyl acetate extracts were combined and evaporated to dryness using a rotary vacuum evaporator at 30 °C. Polyphenolic dried extracts were reconstituted in MeOH:H_2O (80:20), filtered through a 0.2 µm syringe filter and immediately analyzed.

Instead, organic and hydro-organic extracts were directly centrifuged at 5000 rpm for 15 minutes, the supernatant was filtered through a 0.2 µm syringe filter and immediately analyzed. All extracts have been characterized through UV-Vis spectroscopy and via HPLC-MS-ESI-IT. Details of the instrumentation and the conditions set during the characterization steps are reported in Chapter 4. All the spectroscopic and spectrometric determinations have been conducted for qualitative investigations.

Results and Discussion

Since this is preliminary study, unfortunately, we were not able to adequately investigate both the characterization of the dried peel and of the extracts obtained by applying the Ultrasound Assisted Extraction (UAE) technique. However, in this section, we report the most significant results obtained from the analyses through UV-Vis spectroscopy and HPLC-MS-ESI-IT technique of some extracts from winter melon skin.

Figure 10 shows the extracted solutions obtained by applying UAE technique to Yellow Canary melon skin aliquots, using different extraction solvents.

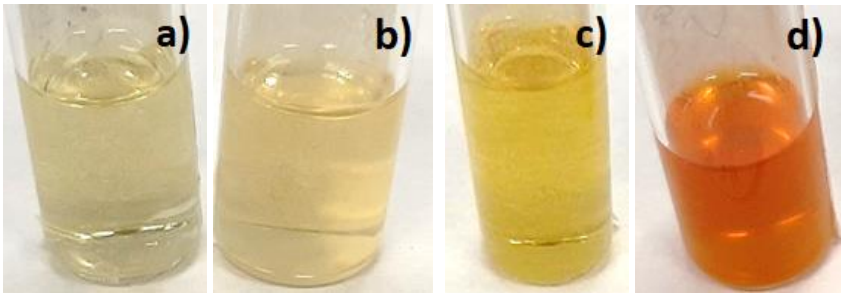


Figure 10 - Extracts in: (a) MeOH 100% , (b) MeOH:H2O (80:20), (c) Na_2CO_3 semi saturated, (d) NaOH 3M.

Looking at Figure 10, it appears evident that, under the same extraction conditions, the ability of the selected solvents to extract the analytes responsible for the color of winter melon peel is significantly different. In fact, extracts in 100% MeOH and MeOH : H_2O mixture (80:20) show only a very pale yellow color, while extracts in aqueous alkaline solution are intensely yellow colored (extracted in Na_2CO_3) or even yellow-orange (NaOH extracts). The alkaline salification of polyphenolic systems involves a red-shift of the absorption bands and the resulting appearance of intense colors, typical of anionic forms. For the non-dissociated compounds, whose hydroxyl and carboxylic groups are characterized by low values of Ka, the chromophore groups are instead particularly active in the near UV spectral window.

UV-Vis spectroscopy

The characterization of colored solutions cannot disregard the analysis through UV-Vis spectrophotometer. Spectra were obtained by diluting the extracts: dilutions between 10 (methanol extracts) and 50 times (aqueous extracts), because the starting samples present maximum absorbance values greater than 1. Hence, all the spectra shown below refer to diluted solutions. This investigation is particularly interesting in this first characterization step, trying to at least understand if the extracts contain, on average, the same molecules or families of molecules (presence of absorptions in the same λ intervals). In Figure 11 we report an effective comparison between the UV-Vis spectra of all the extracts obtained by UAE technique from winter melon dry skin.

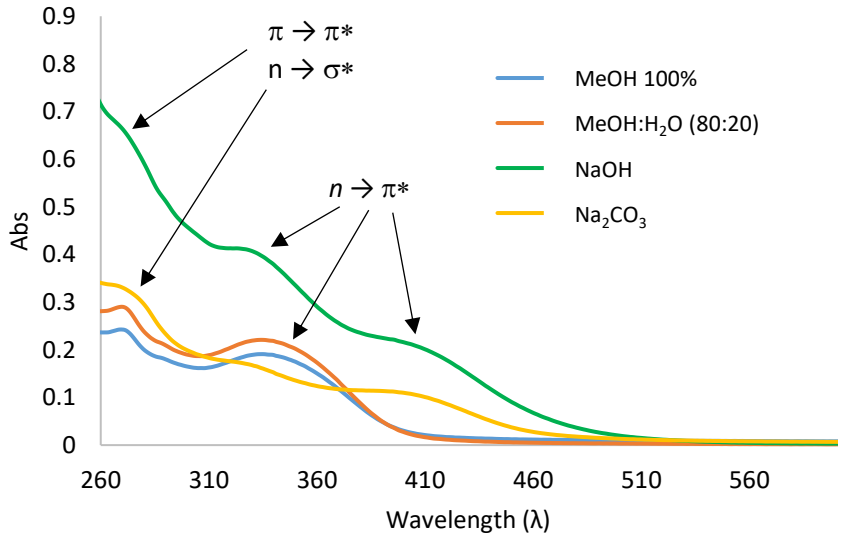


Figure 11 – Comparison of UV-Vis spectra of all extraction solutions obtained by UAE technique

From the comparison of the spectra collected in Figure 11, rather significant differences can be noticed between the number and position of absorption bands which characterize the alcoholic/hydroalcoholic extracts and the aqueous ones. UV-Vis spectra of the extracts in MeOH 100% and MeOH : H_2O show two main absorption bands, one centered at about 270 nm and the other at ≈ 345 nm, while the spectra of aqueous solutions are characterized by the presence of three absorptions, centered at ≈ 270 nm, 330 nm and 410 nm, respectively. Therefore, it can be assumed that at alkaline pH, in addition to obtaining the salification of weak acids, there may be a partial modification of the aqueous extracts with respect to alcoholic ones. Methanolic extraction is certainly milder and does not involve chemical reactions with the compounds present in the skin. In fact, spectral profiles of the two alcoholic/hydroalcoholic extracted solutions and those of the two aqueous extracts show a perfectly comparable trend, in pairs, except for the absorbance values, with absorption bands on average centered at the same wavelengths. Moreover, the spectra related to methanol-water solvent show slightly higher absorbances than those in methanol. The same occurs for the extract in NaOH solution, compared to that in Na_2CO_3. The increase in absorbance values could be explained with a higher concentration of analytes responsible for the absorptions identified in the spectra. Unfortunately, dealing with a very complex mixture of molecules, with an unknown composition, it is not possible to define a ε value which allows to obtain quantitative information relating to some particular species present in our extracts. Reasoning only in qualitative terms, we can therefore deduce that extraction in NaOH solution is more effective than that in Na_2CO_3, given the higher absorbance values recorded at the three absorption bands.

HPLC-MS-ESI-IT analysis

To obtain some other information, it was appropriate to further characterize some of the obtained extracts in order to better define their compositional characteristics, identifying the structure of some molecules. As an example, in Figure 12, we report the results obtained from the analysis of the MeOH : H_2O (80:20) extract sample, analyzed using the HPLC-MS-ESI-IT technique.

The chromatogram shows a series of peaks related to the complex composition of the examined sample. This observation is coherent with the fact that, for complex matrices, the probability of recording coelutions due to the high number of molecules extracted, the presence of different isomers or molecules with similar chemical structure increase.

The description of the molecules identified during this study, with the proposed fragmentation pattern, is reported below. Moreover, Table 7 collects the information obtained from the MS/MS fragmentation pattern, with the abundances of the fragments. On the basis of these data, which have been compared with the literature ones, we have tentatively identified some molecules present in our skin extracts.

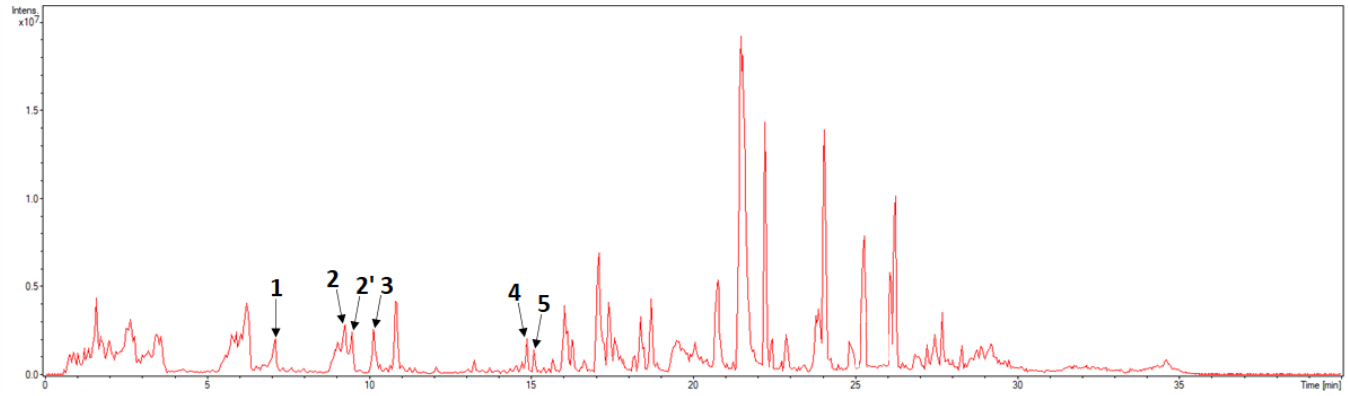


Figure 12 – HPLC-MS-IT (ESI$^-$) chromatogram of a MeOH:H_2O (80:20) extract sample obtained from winter melon dried powdered skin

Table 7 - HPLC-MS-ESI-IT data of some compounds identified (chromatogram in figure 12), for a MeOH:H_2O extract sample

Peak number	Rt (min)	-MS^1 (m/z) [M-H]$^-$	-MS^2 (m/z) MS/MS fragments
1	7.1	447.2	429.0 (46%) - 357.0 (100%) - 327.0 (72%)
2	9.3 (**2**)	271.0	176.9 (38%) - 150.9 (100%)
	9.5 (**2'**)	271.0	176.9 (24%) - 150.9 (100%)
3	10.1	329.3	311.0 (28%) – 229.0 (100%) – 211.9 (31%) – 210.9 (24%) – 170.9 (37%)
4	14.9	293.2	131.1 (100%) – 113.1 (11%)
5	15.1	341.3	179.1 (100%) – 135.2 (23%)

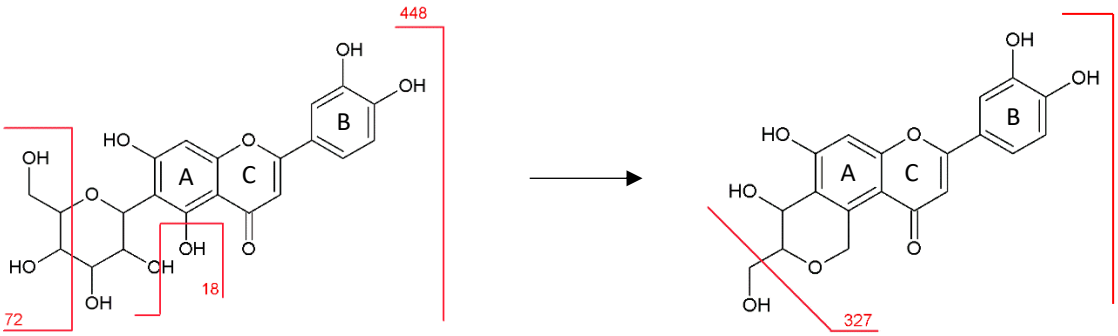


Figure 13 – Proposed fragmentation scheme for luteolin-6-glycoside molecule (Peak 1)

The molecule identified at Rt = 7.1 min through the analysis of the MS^1 and MS/MS spectra, is luteolin-6-glycoside. It is a flavonoid, already identified in melon peel [31], whose proposed fragmentation pattern is reported in Figure 13.

Peak 1 has been identified as luteolin-6-glycoside since the 2nd order MS spectrum shows fragments at m/z 429, 357 and 327, already partially described in the literature [31]. This fragmentation pattern corresponds to the initial loss of a water molecule, released as a result of the condensation between the C5-hydroxyl group and the one belonging to the monosaccharide bound in C6 position at the aromatic ring. This leads to the formation of a six-membered cycle, followed by the loss of a fragment having m/z 72 from the breakdown of the cyclic form of the starting glycoside. Finally, the fragment corresponding to m/z 327 is obtained as a result of further fragmentation of the original monosaccharide, with loss of the CH_2OH group.

Figure 14 shows the structure of naringenin with the proposed fragmentation pattern.

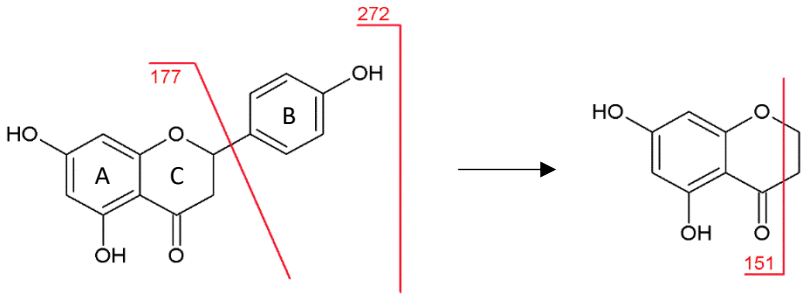


Figure 14 - Proposed fragmentation scheme for naringenin molecule (Peaks 2 and 2')

Similarly to what was previously done, the fragmentation pattern related to another flavonoid identified in the chromatogram of Figure 12 is reported in Figure 14. In this case, the molecule identified thanks to the fragmentation information collected in Table 7 and those from literature [31], is naringenin.
The first fragment at m/z 177 is generated by the loss of the aromatic ring "B", while the fragment at m/z 151 derives from the breaking of the "C" cycle. The reason why the same analyte generates two consecutive peaks, not perfectly resolved but still distinct, could be due to the formation of an H-bridge between the carbonyl of the "C" cycle and the hydroxyl group in position 5, which could lead to slight differences in the ability of interaction and distribution between the mobile and stationary phases, resulting in elution at two slightly different retention times.
Flavonoids, a group of natural substances with various phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, wine, etc… These natural products and bioactive molecules are well known for their beneficial effects on health, therefore, the interest towards them is constantly increasing as well as the efforts for their isolation and extraction from matrices in which are contained. Their accurate characterization is of primary importance in order to understand their biological functions and their possible application in the health sector. Indeed, flavonoids are considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their antioxidant, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties, coupled with their ability to modulate certain enzymatic functions at cellular level, even if information on the functioning mechanisms of flavonoids has not yet been fully understood.
Going into more detail, referring to the two identified flavonoids, luteolin belongs to the subclass of flavones as shown in Figure 15.

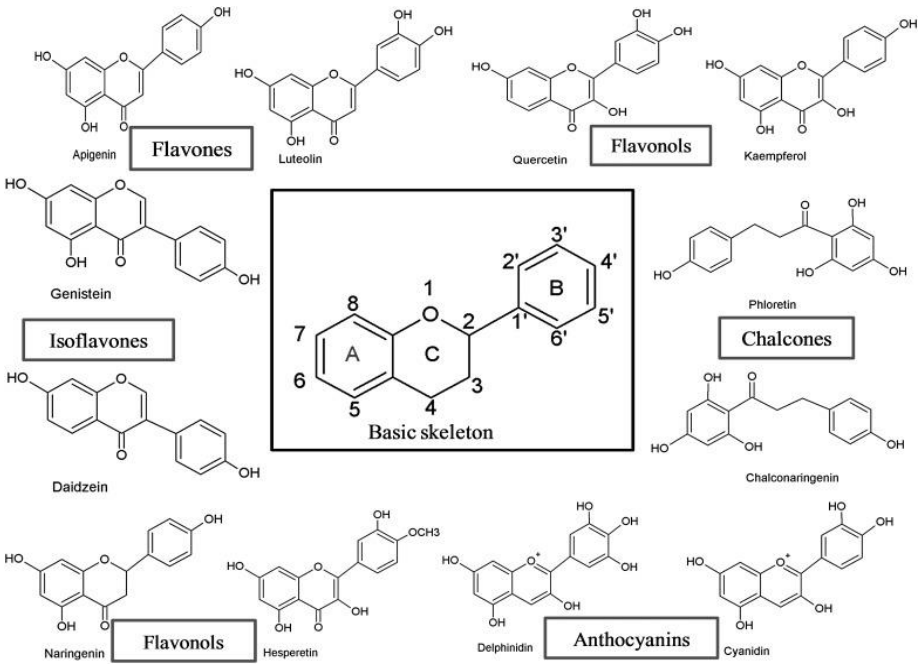


Figure 15 - Examples of molecular structures characteristics of six subclasses of flavonoids

Flavone family is a subclass of flavonoids, molecules widely present in fruit and vegetables, whose basic structure consists of three rings, A, C and B, respectively. The three functional groups characteristic of this family of compounds are the hydroxyl, the carbonyl ones and the presence of the double bond on the heterocyclic C ring. They are soluble in water and methanol, while in crystalline form they have a color which generally ranges from white to pale yellow (as shown in Figure 16, left); however, when they are in alkaline solutions the yellow color becomes more intense. Naringenin, on the other hand, is an odorless and colorless flavanone (Figure 16, right).

Figure 16 - Luteolin-6-glycoside (left) and naringenin (right) in crystallized form

Actually, naringenin can be categorized as flavanone. Flavanones are another important class of phenolic compounds, present in various plant species but especially in the peel and juice of citrus fruits, to which they give the characteristic bitter taste. These analytes exert interesting pharmacological effects by acting as antioxidants, anti-inflammatory, cardioprotectors and contributing to lowering lipids and cholesterol levels [33].
Flavanones, also called dihydroflavones, are characterized by the absence of the double bond between positions 2 and 3 within the "C" ring, as the only structural difference compared to flavones.

In addition to polyphenolic compounds, we have identified a molecule belonging to the family of gibberellins, Gibberellin A7 (Figure 17).

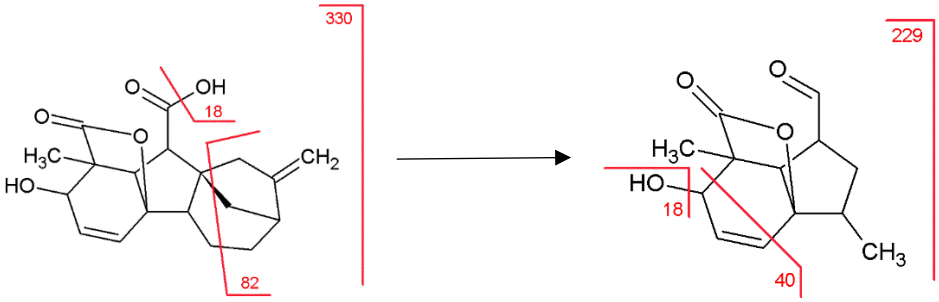


Figure 17 – Proposed fragmentation pattern for Gibberellin A7 (Peak 3)

Gibberellin A7 has been identified through the analysis of the MS^1 and MS/MS spectra and comparing our data with the literature ones [29].
Gibberellins represent a large family of hormones of plant origin and diterpenic nature. In nature they are produced by fungi and higher plants, including melons, through hormonal activity. This large family includes more than 125 identified molecules. Gibberellins have a structure similar to diterpenes with 19-20 carbon atoms, grouped in 4 or 5 rings. Their synthesis is regulated by enzymes present in plant cells, which lead to the obtaining of the several molecules belonging to this group of analytes. Gibberellins perform important biological functions in plant systems: for example, they play a fundamental role in cell division and growth, regulate flowering, stimulate the germination of the seeds of many dicotyledons, as well as inducing the elongation of internodes in plants [34].

Finally, we also tentatively identified hydroxybutanoic acid ethyl ester-hexoside (Rt = 14.9 min) and caffeic acid-O-hexoside (Rt = 15.1 min), already detected in other *Cucumis melo* peel samples [29, 35]. In Figure 18 is reported the proposed fragmentation pattern for caffeic acid-O-hexoside.

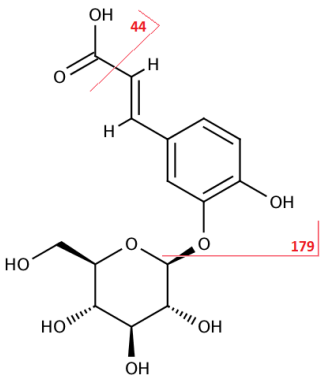


Figure 18 – Proposed fragmentation pattern for caffeic acid-O-hexoside.

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CHAPTER 6

6 - VOLATILE AROMAS OF *CUCUMIS MELO* FIBERS

6.1. BACKGROUND

The global market of dietary fibers represents a rapidly growing and quickly expanding commercial sector, always looking for new possibilities and technological applications. This is quite evident thinking about their use as formulation ingredients in baked goods, ice cream, pre-packaged foods, to obtain a framework where dietary fibers (DF) also play a functional role as stabilizers, gelling agents, adjuvants, etc.

In fact, in several well-documented reports, including FAO ones, the increase of the consumption of dietary fibers in human diet is strongly recommended [1–3].

These suggestions are aimed, above all, at populations who have adopted a Western-world lifestyle, and consequently a diet typically characterized by a high consumption of proteins, lipids and carbohydrates, but not particularly healthy due to the low daily intake of fibers.

In addition to these considerations, we can also report that the forcing of the production rhythms of foodstuffs is becoming less and less globally sustainable, as the amount of products and waste materials that are not used for food purposes is growing exponentially.

Nevertheless, the problem of hunger in the world is a condition that afflicts about 700 million people, while a series of indicators for the multidimensional measure of the problem suggest that about 2 billion people face moderate or severe levels of food insecurity [4 and reference therein cited].

To maximize the spillover effects due to agronomic overproductions, and at the same time mitigate the anti-health effects due to both excessive consumption of food or food deprivation, it is necessary to sensitize producers, consumers, industrial processors, stakeholders and industry sector players. In fact, the problem of waste and loss of food can become a source of unexpected resources if compared to the most common current behavioral models. From this framework arises the need to recover the largest possible amount of nutritional components starting from the agro-industrial by-products and from the residual primary agronomic productions. With this term we refer to the massive quantity of fruit and vegetables products that remain on the crop-field in a pre-waste condition, i.e. abandoned and not harvested due to overproduction, or over-ripening, or for aesthetic reasons that do not comply with quality standards (deformed products, non-commercial sizes, etc.). Among the agri-food products particularly rich in fibers, and widespread on most of the globe, *Cucurbitaceae* have always occupied a prominent position. To deepen our knowledge about the enhancement of these crops, we have chosen to pay particular attention to the dietary fiber obtainable from melon (Cucumis Melo var. *Inodorus*, Yellow Canary type). In some recent studies a broad overview of the compositional characteristics of several melon cultivars, to some extent representative of the world production of these fruits, has been provided [5, 6].

Till now, the aromatic profile of non-climacteric *Inodorus* melons has received little attention in the literature, even if these melons have great interest as fresh-cut or processed fruits thanks to their longer shelf-life than climacteric ones [7].

Despite the term *Inodorus*, it has been demonstrated that the aromatic profile of these fruits is however pretty consistent and varied from a compositional point of view. This aspect could represent the best empirical basis to favor and optimize the cultivation and the consumption of this type of melons. In fact, in this chapter of the PhD thesis, we have tried to deepen the knowledge related to the VOCs fraction of the pomace fiber extracted from Yellow Canary melon type. During this study, this fruit will be also called “winter melon”.

In particular, we have analyzed both the Melon pomace "As it Is", Vacuum freeze-dried (M_AI_V) and the Oven dried Bleached fiber (M_B_O) obtained through the process described in Chapter 5. This choice allowed us to characterize the aroma of the starting material and, also, to monitor the VOCs variations as a consequence of the bleaching treatment. Thinking about a possible use of these fibers as a functional ingredient within food products belonging to the above-mentioned categories, we also analyzed the M_B_O stored for a long period of time (24 months).
The aging of all food products can be described as the natural reduction of the initial characteristics due to the increase in age, which causes compositional changes and alterations due to unwanted but unavoidable processes such as oxidation, photolysis and thermal decomposition. In addition to the action of chemical and physical agents that increase the instability of the fibers, other negative contributions can be added, such as the eventual presence of bacterial strains which can metabolize some analytes or classes of compounds initially present at t_0 time, i.e. at the time of preparation of the fibers themselves.
Bearing this in mind, in this study, we have characterized the whole dried melon pomace fiber and we followed the evolution of the volatile fraction of the bleached fibers over two years, to identify some markers associated to the conservation status and the level of ageing of the investigated material.

6.2. MATERIALS AND METHODS

Fiber samples preparation

The dried melon pomace fiber samples analyzed in this study were prepared as reported in Chapter 5.
The resulting fibers were homogenized in a grinding mill equipped with a rotor made in Ti and a sieve with 500 μm mesh, maintaining the equipment at low temperature by refrigerating it with some few drops of liquid N_2. This strategy has been adopted in order to preserve as much as possible the aromatic characteristics of the fibers. Then a consistent set of samples were prepared as follows: about 0.5 g of material were transferred in 10 mL glass vials which were sealed tightly with Teflon/silicone septa.
The vacuum-freeze dried specimens (M_AI_V) were immediately analyzed after they have been prepared (at t_0 time) in order to characterize the VOCs aromatic fraction of the starting material (M_AI_V_t_0). On the contrary, the bleached fiber was analyzed at t_0 time (M_B_O_t_0), and after a storage period of 24 months (M_B_O_t_{24}), preserving the samples in the dark, at room temperature and in the same closed vials, without ever opening them during the aging period.
Repeatability and reproducibility of experimental procedures have been established working with at least three replicated samples of the same matrix and making at least three different measures for each vial.

Chemicals

The chemicals used during the study are listed below:
(i) 2-methyl-1-propanol; 1-pentanol; 2-ethyl-1-hexanol; phenylethyl alcohol; 2-phenoxyethanol; 3-methyl-butanal; 2-ethyl-2-butenal; ethyl butanoate; ethyl-3-hydroxybutanoate; 3-hydroxy-2-butanone (acetoin); 2-methylbutanoic acid and benzothiazole were obtained from Sigma – Aldrich product, distributed by Merck KGaA, Darmstadt, Germany.
(ii) decanal; methyl acetate; 1-decanol; n-hexane; nonane; dodecane, tetradecane and hexadecane were obtained from Carlo Erba Reagents, Milano (Italy).

HS-SPME-GC-MS analysis

The extraction of volatile compounds was performed by manually exposing a 2 cm long SPME fiber to the headspace of each prepared vial, according to the procedure explained in Chapter 4. The volatile compounds desorbed from the SPME fiber have been analyzed through a GC – MS, Agilent Technologies 6890N Network gas chromatograph.
The oven temperature was programmed from 40 °C (held for 5 min) to 160 °C with a heating rate of 10 °C/min and then from 160 °C to 270 °C with a heating rate of 8 °C/min, while the transfer line was heated to 270 °C. The final temperature of 270 °C was held for 5 minutes.
All the other instrumental setup are described in detail in Chapter 4.
The estimation of the amount of each volatile identified in the SPME-GC-MS analysis was expressed as the Total Ion Current (TIC) peak area.

6.3. RESULTS AND DISCUSSION

The first aim of this research was to develop a straightforward methodology providing an effective characterization of the VOCs aromatic profile of dietary fiber recovered by pre-waste melon fruits.
Furthermore, it would be desirable to obtain from this study a suitable and reproducible screening method which, starting from the sampling of the VOCs generated by melon pomace and fibers at different stages of handling and aging, can produce a robust correlation between the characteristics of the cultivar and the chemical shelf-life of the dietary fiber itself.
Figure 1 shows the vacuum-freeze dried melon pomace "as it is" at t_0 time (M_AI_V_t_0), the bleached fiber immediately after the whitening treatment (M_B_O_t_0) and after a storage period of 24 months in the closed vial, in the dark and at room temperature (M_B_O_t_{24}).

Figure 1 - Images of the M_AI_V_t_0 (left), M_B_O_t_0 and M_B_O_t_{24} (right) samples from winter melon pomace fiber.

Figure 2 shows the HS-SPME chromatogram obtained from the vacuum-freeze dried melon pomace "as it is", immediately processed with the GC-MS instrumentation (t_0 time), and Table 1 summarizes the identified compounds.

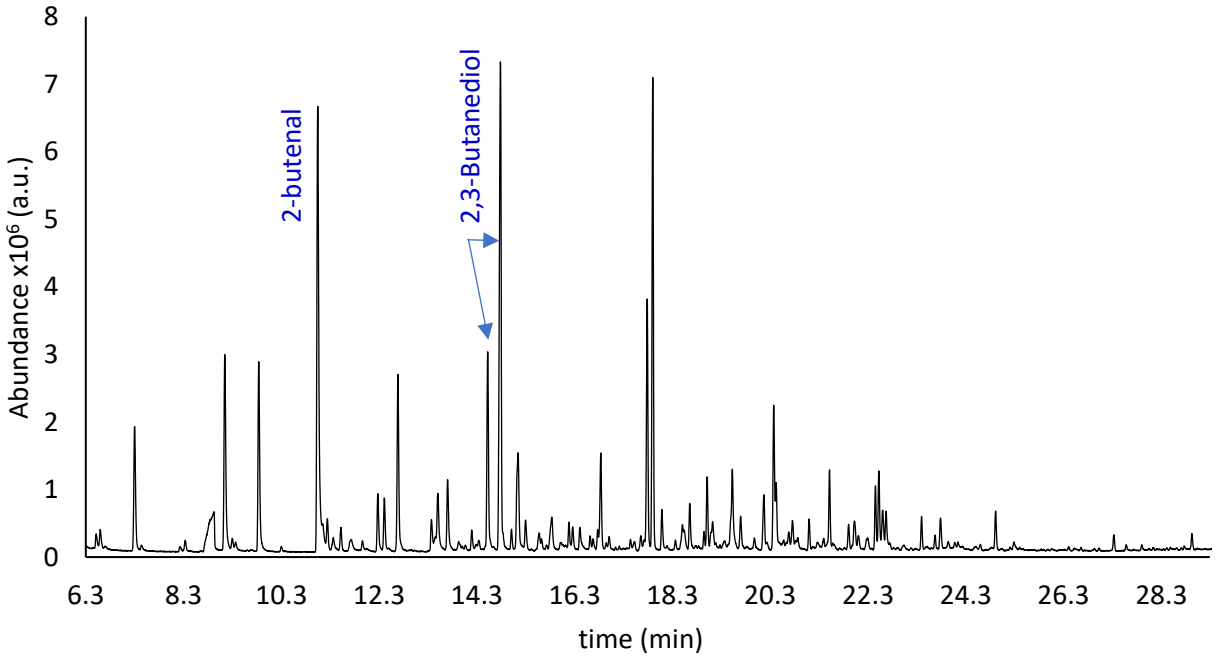

Figure 2 - Total ion current chromatogram of the volatile organic compounds from M_AI_V_t_0 fibrous sample, obtained by HS-SPME-GC-MS (a.u. – arbitrary units).

Table 1 - VOCs composition of vacuum-freeze dried melon pomace, at t_0 time (M_AI_V_t_0) identified through HS-SPME-GC-MS analysis, grouped by chemical classes.

RT/min	LRI	Analyte	Identification[#]	Aroma	TIC Area x 10^{-7}	REF
Alcohols						
8.23	538	1-Propanol	A, B	Alcoholic, fermented	0.220	-
10.30	639	2-methyl-1-Propanol	A, B, C	Ethereal	0.241	[8]
11.97	720	1-Penten-3-ol	A, B	Ethereal, green, vegetable	0.745	[9–11]
13.38	789	Propylene glycol	A, B	-	1.19	[12]
14.20	829	1-Pentanol	A, B, C	Pungent	0.778	[9, 10, 13–15]
14.53	845	(R,R)-2,3-Butanediol	A, B, D	Creamy, fruity, buttery	7.37	[15, 16]
14.79	858	(R,S)-2,3-Butanediol	A, B, D	Creamy, fruity, buttery	20.0	[15, 16]
15.15	875	3-Pentanol	A, B	Sweet, herbal, oily, nutty	5.19	[9, 10, 13-15]
16.41	937	3-Hexen-1-ol	A, B	Green, leafy	1.24	[5, 17, 18]
16.62	948	1-Hexanol	A, B	Green, flowery	0.526	[5, 9, 13–15, [17–19]
16.78	955	1-acetoxy-2-Propanol	A, B	-	0.670	-
17.01	967	2-acetoxy-1-Propanol	A, B	-	0.495	-
19.02	1065	1-Octen-3-ol	A, B	Mushroom, earthy, green	2.38	[5, 13, 18]
19.98	1112	2-ethyl-1-Hexanol	A, B, C	Citrus, fresh, floral, sweet	0.628	[5, 9]
20.38	1131	Benzyl alcohol	A, B	Sweet, floral, fruity	4.86	[5, 11, 13, 18, 20]
22.03	1212	Phenylethyl alcohol	A, B, C	Floral, sweet	1.45	[5, 20]
22.11	1216	2,6-dimethyl-Cyclohexanol	A, B	-	0.716	[8]
22.46	1233	3-Nonen-1-ol	A, B	Green, melon	1.93	[5, 9, 10, 18, 20]
22.53	1236	3,6-Nonadienol	A, B	Fresh, green, melon, cucumber	2.69	[5, 13, 17]
22.68	1243	1-Nonanol	A, B	Fresh, fatty, floral	1.78	[5, 10, 13, 18, 20]
23.95	1306	2-phenoxyethanol	A, B, C	Floral, balsamic	0.461	[20]
Aldehydes						
6.60	458	Propanal	A, B	Ethereal, pungent, earthy, alcoholic	0.638	[13]
8.34	543	2-methyl-Propanal	A, B	Malty	0.491	[8, 20]
11.05	675	2-Butenal	A, B	Flower	19.8	[9]
11.25	685	3-methyl-Butanal	A, B, C	Fruity, green, cocoa	1.28	[9, 18–20]
11.52	698	2-methyl-Butanal	A, B	Malty, cocoa, almond	0.900	[8, 9, 19, 20]
12.41	742	Pentanal (valeraldehyde)	A, B	Fermented, bready, almond, malt	2.11	[13, 15, 18, 19, 21]

13.71	805	2-methyl-2-Butenal	A, B	Pungent, green, ethereal	2.87	-
15.63	899	2-ethyl-2-Butenal	A, B, C	-	0.352	-
16.02	918	Furfural	A, B	Woody, almond, sweet, fruity, floral	0.634	[8]
16.26	930	2-ethenyl-2-Butenal	A, B	-	1.01	-
17.45	988	Heptanal	A, B	Fresh, fatty, green, herbal	0.550	[5, 9, 10, 13, 15, 18, 19]
17.53	992	2-ethyl-3-methyl-Butanal	A, B	-	0.450	
17.73	1002	2,4-Hexadienal	A, B	Fatty, sweet, green	0.391	[18]
18.66	1047	2-Heptenal	A, B	Green, fatty	1.63	[5, 9, 10, 13, 18]
19.10	1069	Benzaldehyde	A, B	Bitter almond-like, fruity	0.731	[5, 9, 11, 13, 17–19]
20.69	1146	Benzeneacetaldehyde	A, B	Green, floral, honey	0.976	[5, 13, 20]
21.52	1187	Nonanal	A, B	Waxy, fresh, green, citrus, cucumber-like	3.16	[5, 9, 13, 18, 19]
23.40	1279	Decanal	A, B, C	Sweet, waxy, citrus, green melon	0.986	[5, 9, 13, 18]
24.92	1353	Benzeneacetaldehyde, α-ethylidene	A, B	-	1.55	-
Esters						
7.44	499	Methyl acetate	A, B, C	Ethereal, fruity	0.324	[5, 13, 14, 21]
9.84	616	Ethyl acetate	A, B	Ethereal, fruity, sweet	7.16	[5, 13, 14, 18, 21]
15.01	869	Ethyl butanoate	A, B, C	Fruity	0.762	[5, 11, 13, 20]
16.19	926	Ethyl 2-methyl-Butanoate	A, B	Fruity	0.975	[20]
18.09	1019	Ethyl 3-hydroxy-Butanoate	A, B, C	-	1.23	-
18.95	1061	1-Butanol, 3-methyl-, propanoate	A, B	-	0.619	-
19.70	1098	Ethyl 2,3-epoxybutyrate	A, B	-	1.39	[12]
20.18	1121	2,3-Butanediol diacetate	A, B	Earthy, soily-like odor	2.19	[13, 17]
20.43	1134	2,3-Butanediol diacetate	A, B	Earthy, soily-like odor	2.17	[13, 17]
Ketones						
6.52	454	Acetone	A, B	Solvent, ethereal	0.462	[19]
9.15	582	2,3-Butanedione	A, B	Buttery, sweet, creamy	7.88	[20, 22]
9.30	590	2-Pentanone	A, B	Sweet, fruity, ethereal	0.583	-
11.37	691	1-hydroxy-2-Propanone	A, B	Sweet, caramellic	0.844	-
12.28	735	2,3-Pentanedione	A, B	Buttery, sweet, caramellic	2.07	[8, 20, 22]

12.69	755	3-hydroxy-2-Butanone (Acetoin)	A, B, C	Acid, yogurt, creamy, fruity	6.45	[11, 18]
13.50	795	3-Penten-2-one	A, B	Fruity	3.24	-
16.95	964	4-hydroxy-5-methyl-2-Hexanone	A, B	-	0.297	-
19.13	1070	6-methyl-5-Hepten-2-one	A, B	Citrus, green, fruity	1.04	[5, 9, 18]
20.87	1155	3,5-Octadien-2-one	A, B	Fruity, fatty, mushroom	0.621	[13, 18, 20]
27.59	1484	6,10-dimethylundeca-5,9-dien-2-one (geranylacetone)	A, B	Floral, fresh, green	0.268	[5, 20]
Acids						
8.92	571	Acetic acid	A, B	Sour, pungent	5.34	[11, 18, 20]
11.74	709	Propanoic acid	A, B	Pungent, acidic	1.08	[22]
13.46	793	2-methyl-Propanoic acid	A, B	Acidic, sour	0.499	[8]
15.58	897	3-methyl-Butanoic acid (Isovaleric acid)	A, B	Sour, cheesy	1.34	-
15.84	909	2-methyl-Butanoic acid	A, B, C	Cheesy	2.05	[11, 20]
16.84	958	2-Hydroxy-2-methylbutyric acid	A, B	-	3.51	-
18.51	1040	Hexanoic acid	A, B	Sour, fatty, sweet, cheesy	1.80	[11]
24.08	1312	Nonanoic acid	A, B	Waxy, cheesy, dairy	0.343	[5]
Sulfur-derived compounds						
7.30	492	Dimethyl sulfide	A, B	Sulfurous, onion, corn, vegetable, cabbage, tomato	4.68	[19, 20, 23, 24]
13.93	816	Dimethyl disulfide	A, B	Sulfurous, onion, cabbage	0.731	[14, 15, 19, 20, 24]
16.12	923	Dimethyl Sulfoxide	A, B	Fatty, oily, cheesy	0.260	[23]
17.66	998	3-(methylthio)-Propanal (Methional)	A, B	Cooked potato, earthy, tomato, vegetable, musty	0.575	[5, 11, 18, 20]
19.19	1073	Ethyl (methylthio)acetate	A, B	Fruity, sulfurous, green	0.240	[5], [13]
23.79	1298	2,3-dihydro-Thiophene	A, B	-	1.17	-
Alkanes						
23.20	1269	Tridecane	A, B	-	0.161	[20]
26.67	1438	Tetradecane	A, B, C	Waxy	0.114	[13, 17]
Others						
16.68	950	methoxy-phenyl-Oxime	A, B	-	0.595	-

21.10	1167	3-ethyl-2,5-dimethyl-Pyrazine	A, B	Nutty, potato, cocoa, rosted	0.978	-
22.61	1240	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	A, B	-	1.65	[15, 25–27]
24.61	1338	Benzothiazole	A, B, C	vegetable, cooked, coffe-like	0.237	-
27.91	1499	1-Tridecene	A, B		0.204	[17]

[#]The identification is indicated by: (A) mass spectral data of the libraries supplied with the operating system of the GC-MS and from mass spectra databases; (B) mass spectra found in the literature; (C) mass spectra and retention time of an injected standard; (D) LRI values, typically used for the identification of isomers

According to Table 1, in M_AI_V_t_0 sample, 81 compounds have been detected, including 19 aldehydes (ALD), 21 alcohols (ALC), 9 esters, among which, 5 non-acetate esters (NAE) and 4 acetate ones (ACE), 11 ketones (KET), 8 acids (ACD), 2 alkanes (AHA), 6 sulfur-derived compounds (SDC) and 5 other compounds (OTH).

Figure 3 shows the HS-SPME chromatogram obtained from the oven dried bleached fiber from winter melon pomace (M_B_O_t_0), immediately processed with the GC-MS instrumentation described in Chapter 4.

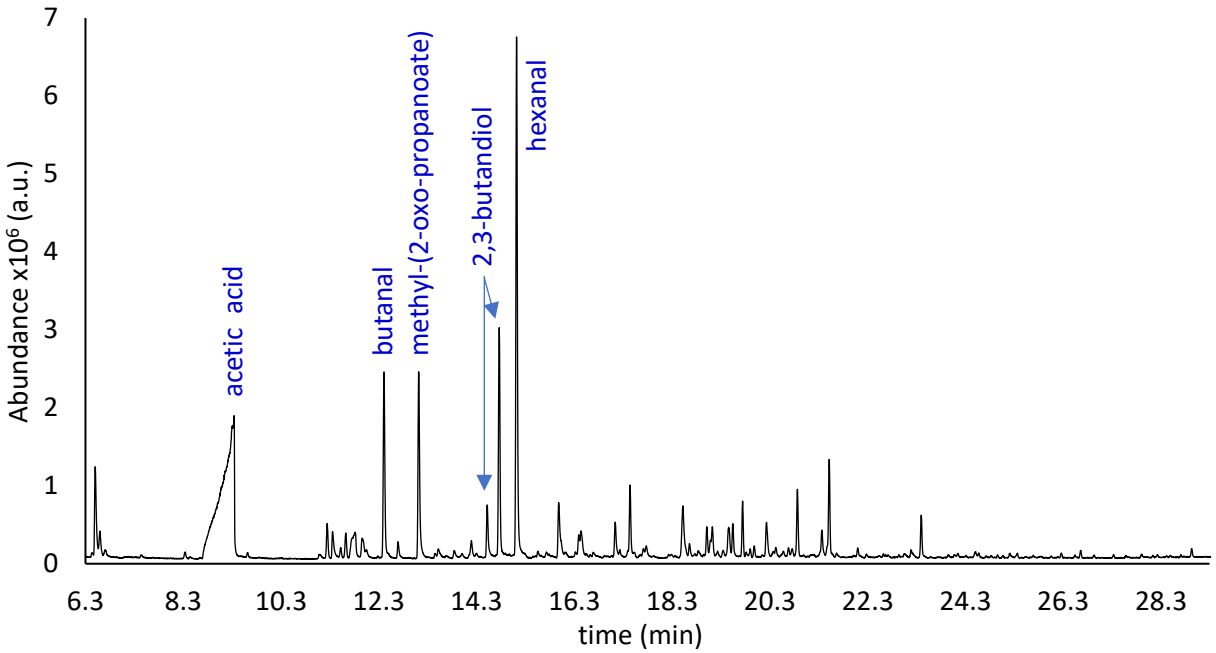

Figure 3 - Total ion chromatogram of the volatile organic compounds from M_B_O_t_0 fibrous sample, obtained through HS-SPME-GC-MS. (a.u. – arbitrary units).

At first sight the comparison between the chromatograms in Figures 2 and 3 shows a lower number of peaks in the case of the sample bleached and oven dried. Evidently the bleaching process and washing steps remove significant quantities of some analytes. Table 2 collects the results of the HS-SPME-GC-MS analysis of the sample of winter melon pomace, bleached with H_2O_2, oven dried and analyzed immediately after its preparation.

Table 2 - VOCs composition of bleached melon pomace at t_0 time (M_B_O_t_0) identified through HS-SPME-GC-MS analysis, grouped by chemical classes.

RT/min	RI	Analyte	Identification[#]	Aroma	TIC Area x 10^{-6}	REF
Alcohols						
11.96	720	1-Penten-3-ol	A, B	Ethereal, green, vegetable	14.9	[9–11]
14.20	829	1-Pentanol	A, B, C	Pungent,	7.55	[9, 10, 13–15]
14.52	845	(R,R)-2,3-Butanediol	A, B, D	Creamy, fruity, buttery	19.4	[15, 16]
14.77	857	(R,S)-2,3-Butanediol	A, B, D	Creamy, fruity, buttery	71.4	[15, 16]
16.33	933	2-Furanmethanol	A, B	Alcoholic, bready, caramellic	1.69	-
19.02	1065	1-Octen-3-ol	A, B	Mushroom, earthy, green	10.4	[5, 13, 18]
19.99	1112	2-ethyl-Hexanol	A, B, C	Citrus, fresh, floral, sweet	4.19	[5, 9]
22.10	1215	2,6-dimethyl-Cyclohexanol	A, B	-	3.69	[8]
Aldehydes						
6.60	458	Propanal	A, B	Ethereal, pungent, earthy, alcoholic	9.60	[13]
8.34	543	2-methyl-Propanal	A, B	Malty	2.11	[8, 20]
11.08	677	2-Butenal	A, B	Flower	2.03	[9]
11.25	685	3-methyl-Butanal	A, B, C	Fruity, green, cocoa	10.2	[9, 18–20]
11.52	698	2-methyl-Butanal	A, B	Malty, cocoa, almond	3.66	[8, 9, 19, 20]
12.41	742	Pentanal (valeraldehyde)	A, B	Fermented, bready, almond, malt	58.6	[13, 15, 18, 19, 21]
15.12	874	Hexanal	A, B	Grass, green, fat	155	[5, 13, 15, 19–21]
15.98	916	Furfural	A, B	Woody, almond, sweet, fruity, floral	26.6	[8]
17.44	988	Heptanal	A, B	Fresh, fatty, green, herbal	24.5	[5, 9, 10, 13, 15, 18, 19]
18.66	1047	2-Heptenal	A, B	Green, fatty	5.13	[5, 9, 10, 13, 18, 20]
18.84	1056	5-methyl-2-Furancarboxaldehyde	A, B	Sweet, caramellic, spicy	3.91	-
19.55	1091	Octanal	A, B	Waxy, fatty, citrus, green	11.1	[5, 13, 15, 20]
19.82	1104	2,4-Heptadienal	A, B	Green, pungent, fruity, spicy	1.89	-
20.69	1146	Benzeneacetaldehyde	A, B	Green, floral, honey	3.44	[5, 13, 20]
21.52	1187	Nonanal	A, B	Waxy, fresh, green, citrus, cucumber-like	30.5	[5, 9, 13, 18, 19]
23.40	1279	Decanal	A, B, C	Sweet, waxy, citrus, green, melon	12.4	[5, 9, 13, 18]
Esters						
13.12	776	2-oxo-Propanoic acid, methyl ester	A, B	-	59.9	-

20.43	1134	Isopropyl 3-methylbutanoate	A, B	-	6.47	[13, 17]
Ketones						
6.5	453	Acetone	A, B	Solvent, ethereal	30.3	[19]
11.36	690	1-hydroxy-2-Propanone	A, B	Sweet, caramellic	10.6	-
12.70	756	3-hydroxy-2-Butanone (Acetoin)	A, B, C	Acid, yogurt, creamy, fruity	5.70	[11, 18]
13.52	796	3-Penten-2-one	A, B	Fruity	4.24	-
16.44	939	1-(acetyloxy)-2-Propanone	A, B	Fruity, buttery	8.67	[17]
17.14	973	2-Heptanone	A, B	Fruity, spicy, sweet, herbal	12.8	[18]
19.13	1070	6-methyl-5-Hepten-2-one	A, B	Citrus, green, fruity	14.8	[5, 9, 18]
19.24	1075	2-Octanone	A, B	Earthy, woody, herbal	3.20	-
20.24	1124	3-Octen-2-one	A, B	Earthy, herbal, spicy	13.6	-
20.87	1155	(E,E)-3,5-Octadien-2-one	A, B, D	Fruity, fatty, mushroom	20.2	[13, 18, 20]
21.37	1180	(E,Z)-3,5-Octadien-2-one	A, B, D	Fruity, fatty, mushroom	11.2	[13, 18, 20]
Acids						
9.34	591	Acetic acid	A, B	Sour, pungent	326	[11, 18, 20, 22]
11.82	713	Propanoic acid	A, B	Pungent, acidic	22.3	-
13.45	793	2-methyl-Propanoic acid	A, B	Acidic, sour	1.69	-
15.56	896	3-methyl-Butanoic acid (Isovaleric acid)	A, B	Sour, cheesy	4.80	-
18.53	1041	Hexanoic acid	A, B	Sour, fatty, sweet, cheesy	12.3	-
Alkanes						
23.19	1269	Tridecane	A, B	-	3.68	[8]
24.97	1355	3-methyl-Dodecane	A, B	-	0.726	-
26.67	1438	Tetradecane	A, B, C	Waxy	1.96	[13, 17]
Others						
13.85	812	Pyrrole	A, B	Sweet, nutty	3.55	-
16.69	951	methoxy-phenyl-Oxime	A, B	-	4.61	-
17.72	1001	2(5H)-Furanone	A, B	Buttery	3.03	[20]
19.35	1081	2-pentyl-Furan	A, B	Fruity, green, earthy	3.00	[9, 13, 15, 18, 19]
27.91	1499	1-Tridecene	A, B	-	1.12	[17]

#The identification is indicated by: (A) mass spectral data of the libraries supplied with the operating system of the GC-Ms and from mass spectra databases; (B) mass spectra found in the literature; (C) mass spectra and retention time of an injected standard; (D) LRI values, typically used for the identification of isomers

For the bleached fiber, at t_0 time (M_B_O_t_0) 50 VOCs were identified, among which 16 aldehydes (ALD), 8 alcohols (ALC), 2 non-acetate esters (NAE), 11 ketones (KET), 5 acids (ACD), 3 alkanes (AHA) and 5 other

compounds (OTH). Finally, in M_B_O_t_{24} only 30 volatiles have been determined and classified in 9 ACD, 8 ALD, 7 KET and 6 ALC, as reported in Table 3.

Figure 4 shows the HS-SPME-GC-MS chromatogram of a sample of bleached melon pomace fiber, oven dried and stored for 24 months in closed vessel, in the dark, at room temperature. Table 3 reports its descriptive elements.

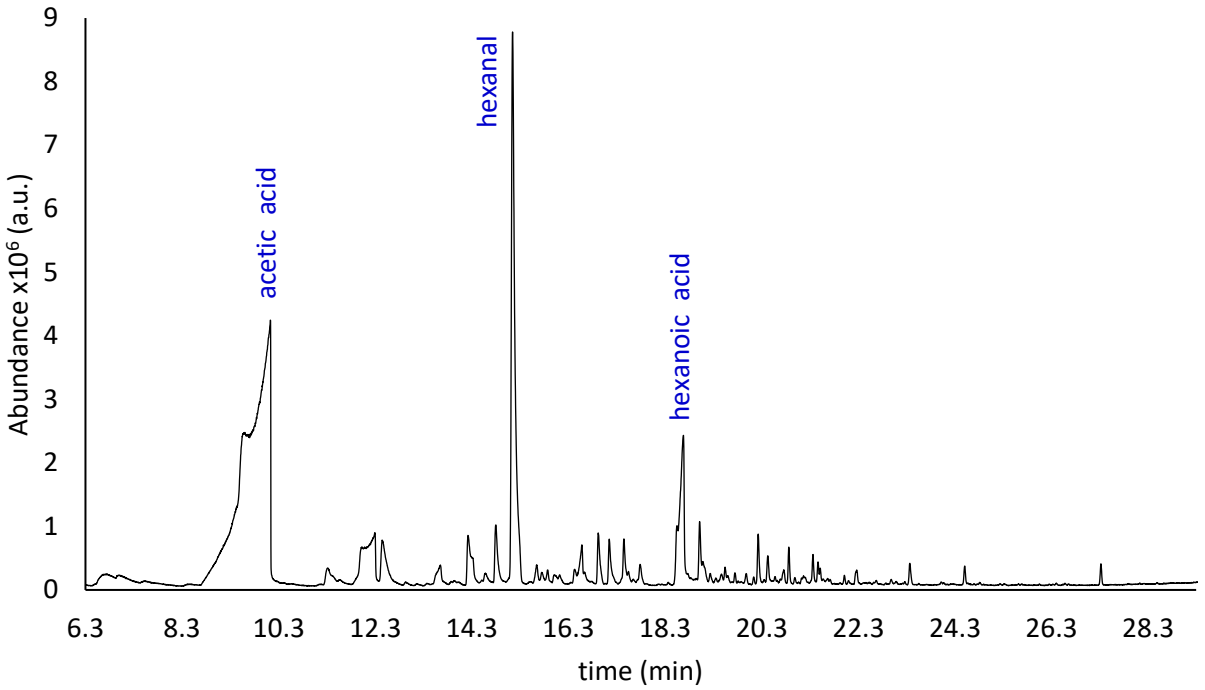

Figure 4 - Total ion chromatogram of the volatile organic compounds from M_B_O_t_{24} fibrous sample, obtained by HS-SPME-GC-MS analysis (a.u. – arbitrary units).

Table 3 - VOCs of the bleached melon pomace fiber after 24 months of storage (M_B_O_t_{24}) identified through HS-SPME-GC-MS analysis, grouped by chemical classes.

RT/min	RI	Analyte	Identification [#]	Aroma	TIC Area x 10^{-6}	REF
Alcohols						
14.23	831	1-Pentanol	A, B, C	Pungent	34.1	[9, 10, 13–15]
14.58	848	(R,R)-2,3-Butanediol	A, B, D	Creamy, fruity, buttery, green, leafy	16.6	[15, 16]
14.80	859	(R,S)-2,3-Butanediol	A, B, D	Creamy, fruity, buttery, green, leafy	45.8	[15, 16]
19.02	1065	1-Octen-3-ol	A, B	Mushroom, earthy, green	49.5	[5, 13, 18]
19.98	1112	2-ethyl-1-Hexanol	A, B, C	Citrus, fresh, floral, sweet	5.34	[5, 9]
22.10	1215	2,6-dimethyl-Cyclohexanol	A, B		1.62	[8]
Aldehydes						
11.32	688	3-methyl-Butanal	A, B, C	Fruity, cocoa, green	28.5	[9, 18–20]
11.57	701	2-methyl-Butanal	A, B	Malty, cocoa, almond	9.37	[8, 9, 19], [20]
12.45	744	Pentanal	A, B	Fermented, bready, almond, malt	64.0	[13, 15, 18, 19, 21]

15.15	876	Hexanal	A, B	Grass, green, fat	448	[5, 13, 15, 19–21]
16.01	918	Furfural	A, B	Woody, almond, sweet, fruity, floral	9.24	[8]
16.43	938	2-Hexenal	A, B	Green, fruity, pungent, vegetable-like	13.1	-
17.45	988	Heptanal	A, B	Fresh, fatty, green, herbal	35.4	[5, 9, 10, 13, 15, 18, 19]
19.55	1091	Octanal	A, B	Waxy, fatty, citrus, green	13.1	[5, 13, 15, 20]
Ketones						
6.74	464	Acetone	A, B	Solvent, ethereal	32.9	[19]
17.15	973	2-Heptanone	A, B	Fruity, spicy, sweet, herbal	30.2	[18]
19.24	1075	2-Octanone	A, B	Earthy, woody, herbal	6.75	-
20.23	1124	3-Octen-2-one	A, B	Earthy, herbal, spicy	21.2	-
20.86	1155	(E,E)-3,5-Octadien-2-one	A, B, D	Fruity, fatty, mushroom	13.3	[13, 18, 20]
21.17	1170	Acetophenone	A, B	Sweet, pungent, almond	8.39	
21.36	1179	(E,Z)-3,5-Octadien-2-one	A, B, D	Fruity, fatty, mushroom	12.2	[13, 18, 20]
Acids						
6.99	477	Formic acid	A, B	Pungent, vinegar	21.3	
10.11	630	Acetic acid	A, B	Sour, pungent	823	[11, 18, 20]
12.28	736	Propanoic acid	A, B	Pungent, acidic	143	[22]
13.64	802	2-methyl-Propanoic acid	A, B	Acidic, sour	12.2	-
15.64	900	3-methyl-Butanoic acid	A, B	Sour, cheesy	13.9	-
15.87	911	2-methyl-Butanoic acid	A, B, C	Cheesy	10.4	[11, 20]
16.58	945	Pentanoic acid	A, B	Acidic, cheesy	29.5	-
18.67	1048	Hexanoic acid	A, B	Sour, fatty, sweet, cheesy	137	[11]
20.43	1134	Heptanoic acid	A, B	Sour, cheesy	16.2	-

[#]The identification is indicated by: (A) mass spectral data of the libraries supplied with the operating system of the GC-Ms and from mass spectra databases; (B) mass spectra found in the literature; (C) mass spectra and retention time of an injected standard; (D) LRI values, typically used for the identification of isomers

Although winter melon belongs to the *Inodorus* group, it is still possible to note, from Figure 2 and Table 1, a considerable richness and complexity of the aromatic fraction composition of the pomace fiber (M_AI_V_t_0). This volatile fraction is mainly characterized by very pleasant green, fresh, sweet, fruity and floral notes, which undergoes a significant modification due to the bleaching process (Figure 3, Table 2) to which the fiber has been subjected (M_B_O_t_0). This treatment leads to a significant increase in the amount of some analytes, such as acetic and propanoic acid, as well as the appearance or disappearance of some others, like C9 alcohols and sulfur-derived compounds. Obviously, the natural aging process of the fiber (Figure 4, Table 3) also leads to a change in the composition of the aromatic fraction, which, after 24 months, is considerably depleted of many original molecules.

As mentioned along the text, we are looking for dietary fibers that are stable over time and as neutral as possible in terms of color, aroma and flavor. Even if we are aware that a lower number of VOCs does not directly translate into the absence of odor and aroma, we can however notice that our findings represent a very interesting and encouraging result for the purposes of our studies.

Below, we report the most significant and representative experimental results of the VOCs detected in the analyzed samples, treating them separately according to macro-families of compounds.

Aldehydes

Aldehydes are known to be some key compounds which define the flavor and aroma profile of fruits and vegetables.

These analytes are usually present in higher concentrations in non-ripe melon cultivars, then will be gradually replaced by esters and alcohols as the degree of ripeness progresses [17]. Therefore, most of them (especially Cn with n < 8) are not detected, or recovered only in very small quantities, in ripe fruits [21].

Nevertheless, Güler et al. [21], and Shi et al. [5] agreed with us, reporting that aldehydes were the most abundant compounds in some *Inodorus* melon varieties. These literature informations are in agreement with our experimental results, where aldehydes represent a very important family of the volatile organic fraction of the M_AI_V_t_0 fiber (≈ 25 %), together with alcohols (≈ 34 %).

This is the reason why to *Inodorus* melons is attributed the lowest odor perception but the highest freshness score, being generally characterized by a "green-like" and herbaceous aroma [21].

Previous studies suggest that saturated and unsaturated C9 aliphatic aldehydes, together with C9 alcohols, play a key role in melon and watermelon aroma [5, 18, 28]. In the specific case of melons belonging to the *Inodorus* group, the main analytes having this function are (Z)-6-nonenal, nonanal, (E,Z)-2,6-nonadienal, (E)-2-nonenal, 6-nonen-1-ol and 1-nonanol [5 and reference therein cited].

All these molecules contribute to some extent to impart waxy, fresh and green notes to the smell of winter melon. Among these aldehydes, we only detected nonanal, both in M_AI_V_t_0 and in M_B_O_t_0 samples, with an amount of $3.16x10^7$ and $30.5x10^6$ respectively (in terms of peak TIC area), confirming that this analyte particularly characterizes the fibrous matrix and persists even after the strong invasive treatment with H_2O_2. The reason why the other aldehydes were not detected could be attributed to many factors including their very low quantity in our samples (below the detection limit), the selected cultivar, origin and cultivation practices, fruit ripening, handling and post-harvest processing. All these variables, not controllable by us in this study, can influence the concentration of volatile compounds in melons [19], altering their aromatic profile.

Among aldehydes, we observed that also 2-butenal and 2-methyl-2-butenal are present in quite high level in the HS of M_AI_V_t_0 ($19.8x10^7$ and $2.87x10^7$, respectively) but, however, only the first persist after the H_2O_2 bleaching process in smaller quantities than the starting material by about two orders of magnitude.

Pentanal and heptanal, on the other hand, show an exactly opposite behavior, increasing their concentration of about 3 - 4 times, respectively, immediately after the treatment of the fiber with H_2O_2 (M_B_O_t_0). During the ageing of the white melon fiber, these two aldehydes are partially converted to their most oxidized form, namely pentanoic and heptanoic acid, respectively. This may be due to radical autoxidation processes reasonably initialized by the action of the oxygen present in the residual atmosphere inside the vials in which the sample is stored.

The branched-chain molecules 2-methylbutanal and 3-methylbutanal, described as 'malty-like' aroma, have been detected in all three samples we have analyzed. These analytes are suggested to be synthesized from isoleucine and leucine, respectively, even though the pathways are not well recognized yet [19]. However, it cannot be established which fraction of the total quantity of 2-methylbutanal and 3-methylbutanal is not directly linked to the aforementioned amino acids.

It is assumed that also 2-methylpropanal is an intermediate product in the catabolism of two amino acids (leucine and valine) and, together with 2-methylbutanal, is considered a key compound in several foods, including melon. Following the suggestions of the literature, it should then be converted into parental compounds, the oxidized one, 2-methylpropanoic acid, or the reduced one, 2-methylpropanol [8] (Figure 5).

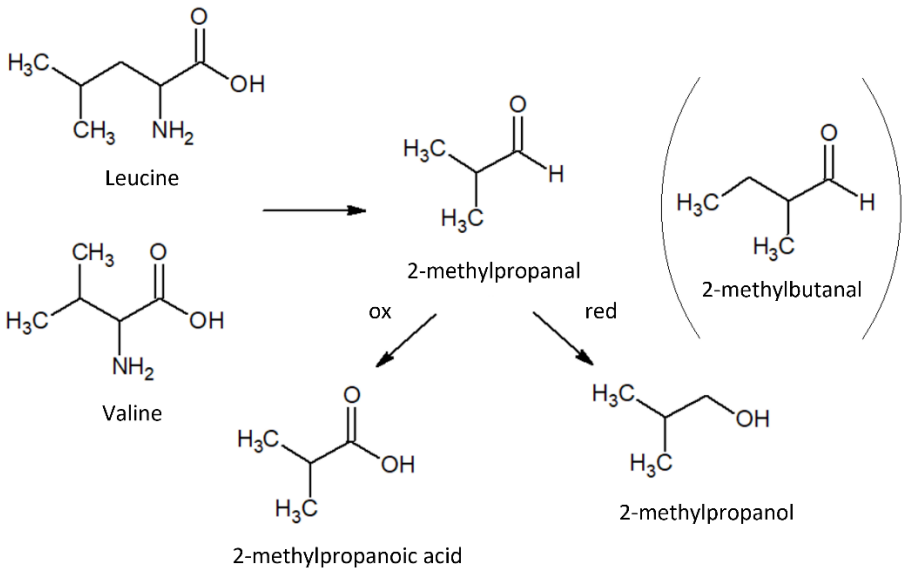

Figure 5 – Scheme of 2-methylpropanal biosynthetic pathway and red-ox molecular evolution

Six-carbon aldehydes such as hexanal and (E)-2-hexenal, as well as their corresponding alcohols and straight-chain C9 compounds, such as (E,E)-2,6-nonadienal and (E,Z)-2,6-nonadienal, are typically produced by lipoxygenase activity in damaged plant tissues [15, 19]. For this reason, these compounds increase in number and concentrations after disruption of cells and tissues.
These observations perfectly match our results, as these analytes are absent in the M_AI_V_t_0 fibrous sample, while hexanal was detected in both bleached samples, becoming the aldehyde present in the largest absolute quantity compared to all the others. In fact, in M_B_O_t_0 sample it has been detected with an amount, in terms of TIC area, of $155x10^6$ which correspond to a relative percentage area of 43 % with respect to the class of aldehydes. In M_B_O_t_{24} sample it reaches a quantity of $448x10^6$, that is almost 72 % of all aldehydes. It confers grassy, green-like and fatty notes to the aroma of our bleached fibers.
It is reasonable to think that this behavior is caused by the synergistic effect of damage to plant tissues and the oxidative action of H_2O_2 towards the molecules originally present in the matrix.
In fact, hexanal is used as an index of the degree of oxidation of fatty acids in food chemistry [21].

Among the aromatic aldehydes identified, benzaldehyde, benzeneacetaldehyde and α-ethylidene-benzeneacetaldehyde, were detected even if in small quantities. They cover only about 2 % of the entire volatile fraction of the vacuum freeze-dried melon pulp (Table 1).
In particular, benezeneacetaldehyde was the only one initially identified both in the M_AI_V_t_0 fiber and after the bleaching treatment (M_B_O_t_0), but none of the three molecules was preserved during the aging period.
Unlike what reported by Güler at al. [21], in our case the presence of acetaldehyde was not detected, reasonably for the same reasons previously suggested for the C9 aldehydes. Furthermore, we should not forget that most of the studies found in the literature refer to "fresh" fruit pulp or juice and not to dehydrated material. This aspect, too, certainly contributes to the differences we find compared to previous studies.
On the other hand, to the best of our limited knowledge, this is the first time that α-ethylidene-benzeneacetaldehyde (2-phenyl-2-butenal), is mentioned among the melon VOCs and, for this reason, we don't know what its contribution may be in terms of perceived aroma.
Finally, in all three samples, we have also identified furfural. It is a natural dehydration product of five-carbon sugars, such as xylose and arabinose, which are typically found in the hemicellulose fraction of fruit and vegetables [29]. In particular, its quantity (in terms of peak TIC area) increases about four times passing from the M_AI_V_t_0 sample ($0.634x10^7$, Table 1) to the M_B_O_t_0 ($26.6x10^6$, Table 2). After the long ageing period the fiber present a furfural amount of $9.24x10^6$ (Table 3).

Alcohols

Alcohols are the most abundant analytes in the HS of the M_AI_V_t_0 sample, both in terms of number of analytes (21) and quantity, since they cover about the 34 % of the total volatile organic fraction.
In particular, among them, 2,3-butanediol is the most abundant (≈ 49 %, calculated considering the sum of the two stereoisomers), together with benzyl alcohol (≈ 8.7 %), 3-pentanol (≈ 9.4 %) and 1-octen-3-ol (≈ 4.3 %) in the above-mentioned sample.
They confer to the aromatic profile a variety of creamy, buttery, fruity, sweet and earthy notes.

Benzyl alcohol is a very important volatile compound for the melon aroma, with its sweet and fruity contribution, having already been identified in many different cultivars even if it was not present in high quantities [5]. The 2,3-butanediol species was identified as the major volatile compound in banana fruit [30] and was detected in two of its isomers in our study, (R,R)-2,3-butanediol and (R,S)-2,3-butanediol. It was found in all three samples analyzed, but its quantity was drastically reduced after the treatment of the fiber with H_2O_2, passing from $2.74x10^8$ in M_AI_V_t_0 fiber, to $9.08x10^7$ in the M_B_O_t_0 sample (≈ -67 %) and finally to $6.24x10^7$ after 24 months of storage (≈ -77 %).
Its presence is related to that of butanedione and 3-hydroxy-2-butanone (acetoin) as will be discussed in the subsection dedicated to the category of ketones.

As already mentioned for aldehydes, also aliphatic C9 alcohols have been previously reported as the main characteristic odorants in melon and watermelon fruits, where 3,6-nonadienol, 3-nonen-1-ol, 6-nonen-1-ol and 1-nonanol are the prevalent compounds of this class [5, 9, 31].
Among these molecules, 3,6-nonadienol, 3-nonen-1-ol and 1-nonanol have been detected in the M_AI_V_t_0 fiber with a total amount of $6.40x10^7$ (peak TIC area), which correspond to ≈ 11.5 % in terms of relative area percentage calculated with respect to the alcohol class. They impart a fresh, green-like aroma and 3-nonen-1-ol is even called “melon aroma” [9].
It is believed that some of the C9 active aromatic compounds of the *Cucurbitaceae* family may not be present in all melon varieties due to genetic or cultivar differences, post-harvest fruit handling, degree of ripeness at harvest, climate and cultivation method [21]. Furthermore, it is reasonable to assume that some differences found in the flavor profile may be attributed to the extraction, sampling technique and chromatographic conditions applied.
Anyway, we observe a decrease in the overall quantity of alcohols passing from the crude fiber (M_AI_V_t_0: $5.55x10^8$) to bleached one (M_B_O_t_0: $1.33x10^8$) and, again, to aged fiber (M_B_O_t_{24}: $1.53x10^8$). This could be related to the development of analytes characterized by more oxidized functional groups, such as ketones and organic acids.

Ketones

As can be seen from Table 1, the M_AI_V_t_0 fiber is characterized by the presence of 3-hydroxy-2-butanone (acetoin) and butanedione detected in similar amounts in terms of TIC area ($6.45x10^7$ and $7.88x10^7$, respectively). Among the analytes belonging to this class of molecules, these two are the ones detected in the highest quantities in the previously mentioned sample.

Acetoin has a pleasant creamy-yogurt smell, generally used in the food industry to enhance the flavor of some products. It has been identified in several dietary products such as yogurt and cheese, vinegar, fruits, vegetables and some type of flours [32].
In relation to the category of melons, it has previously been detected in some cultivars in Italy [33].
It also has good biocompatibility and solubility, which extends its usage in soaps, lotions and cosmetics [34].
In general, acetoin has three natural origins: (i) microbial fermentation, (ii) vegetable synthesis by fruits or plants, and (iii) animal synthesis. Its biosynthesis in plant cells was confirmed in the literature; it is also reported that, along with other odorous compounds, it confers to plants rich fragrances with the purpose to attract insects and higher animals to help their pollination and propagation [32]. Being a very active molecule, acetoin generates many derivatives which can be detected through HS-SPME-GC-MS analysis.
For example, 2,3-butanediol is a product of the acetoin pathway in many bacterial species.

This alcohol is produced from pyruvate during fermentation processes via several intermediate metabolites where different enzymes are involved.
At present, it is not well known what the exactly metabolic function of 2,3-butanediol is, but it may have a role in avoiding an excessive intracellular acidification through the conversion of an acid compound (pyruvic acid) in a neutral one [34, 35], as represented in Figure 6.

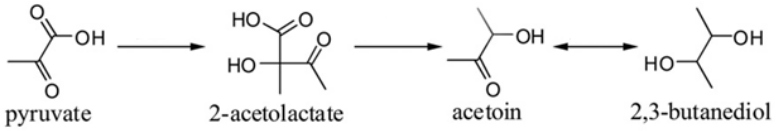

Figure 6 - Scheme of acetoin and 2,3-butanediol biosynthetic pathway [34].

Also butanedione is produced at the beginning of fermentation processes by yeasts, but it is generally rapidly reduced to acetoin and 2,3-butanediol. Together with acetoin, the diol imparts a buttery aroma to the food in which it is contained [36].
In our case it is quite unlikely that fermentation processes have taken place markedly, given the speed of handling the melon pulp from the fresh fruit to the dried fiber, so the most of acetoin could instead have a natural origin. However, since all metabolites and intermediates previously cited have been detected, it cannot be excluded at priori that some microorganism has initiated the metabolic processes just described.

Among other ketones, 6,10-dimethylundeca-5,9-dien-2-one (geranylacetone) which is present only in M_AI_V_t_0 sample, is known to derive from the natural degradation of long-chain terpenes, typically β-carotene and lycopene, giving a floral aroma to the ripe fruit [5]. The same occurs for 6-methyl-5-hepten-2-one. In this case, given the pale yellowish-greenish color of the winter melon flesh, we suppose that it may originates from some other carotenoid or xanthophyll naturally present in this fruit and not from lycopene or β-carotene. These two molecules may also result from the degradation of ζ-carotene, a linear carotenoid having a weak yellow color [37, 38].

Finally, among ketones, C8 compounds are the main ones in the HS of both the bleached fibers, with 2-octanone, 3-octen-2-one and 3,5-octadien-2-one covering an amount of about 35 % and ≈ 43 %, respectively in the M_B_O_t_0 and M_B_O_t_{24} samples (calculations of the relative area percentages refer only to the ketones class).
In particular, 3,5-Octadien-2-one is present in greatest quantities among the three species above mentioned, existing in the form of two conformational isomers.

Esters
Fruit esters are generated by the action of enzymes and / or fermentative metabolism processes which can be enhanced by various factors, such as maturation (intrinsic factors), microbial growth (biotic factors), as well as climate and seasonal temperature (extrinsic factors) [12].
The aroma quality assessment of 39 melon cultivars conducted by Shi et al. [5] shows that ethyl acetate was present in the highest amount among esters, followed by 2,3-butanediol diacetate.
This perfectly agree with our experimental results, where 2,3-butanediol diacetate was even detected as two stereoisomers.
Also Güler et al. identified ethyl acetate as the main ester in several varieties of melon, both climacteric and non-climacteric ones [21].
This volatile compound contributes to the complexity of the aroma and gives positive notes but, if present at too high level, it becomes an off-flavor, conferring the typical "solvent" or "ethereal" odor to the over-ripened fruit.

The biosynthetic pathway of a huge number of plant VOC compounds can be traced back to primary metabolism. For example, some aliphatic esters can be produced by the oxidation of free fatty acids, such as

linoleic and linolenic acids, generating short-chain compounds, or starting from some amino acids such as valine and aminobutyric acid [17, 21].
It is known that biochemical reactions are regulated by various factors such as physical conditions, kinetic factors and enzymatic activity. As regards the esters formation, it is generally believed that they can also be enzymatically formed by the combination of an alcohol with an acyl group. In particular, ethyl esters and acetate esters can be formed following two different biosynthetic pathways (Figure 7), but they have pyruvic acid as the same precursor [14].

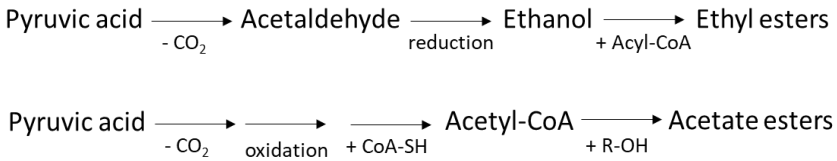

Figure 7 - Scheme of the biosynthetic pathway which leads to the formation of ethyl and acetate esters starting from pyruvic acid [14].

Pyruvic acid is the key product of glycolysis, a biological process which occurs in the cytoplasm of the cell and that is present in all living organisms [39, 40]. This process consists of a chain of reactions where several enzymes are involved and where glucose is converted in two molecules of pyruvic acid. In plants, glucose is constantly available in large quantities, being the final product of photosynthesis, and as it represents the most suitable form of carbohydrate storage.

Acids
Organic acids mainly contribute to the chemical composition of the HS of the bleached fibrous sample, particularly the one aged two years. As can be seen from Table 3, in M_B_O_t_{24} we have detected 9 acids where acetic, propanoic and hexanoic acids are those present in higher quantity (peak TIC area) within this class of compounds.
For what concern acetic acid, there is a progressive and significant increase in its amount, passing from the untreated fiber (M_AI_V_t_0: $5.34x10^7$) to the bleached ones, both for the sample at t_0 time (M_B_O_t_0: $3.3x10^8$) and for the one aged 24 months (M_B_O_t_{24}: $8.2x10^8$). This trend is always associated with the complete loss of 2,3- butanedione.
Indeed, it has been shown that 2,3- butanedione reacts with H_2O_2 to give acetic anhydride, which is rapidly and completely hydrolyzed to acetic acid [22]. Since this is a reaction involving diketones and having detected the complete loss of 2,3-pentanedione as well, it is reasonable to hypothesize that this compound has also been completely oxidized to give still acetic and propanoic acids. Not by chance, we have observed a gradual and progressive increase in the quantity of the latter as well.
Finally, hexanoic acid probably derive from the oxidation of some precursors, such as C6 alcohols and aldehydes.

Sulfur-derived compounds
Compounds containing sulfur can have a major impact on the overall flavor perception of fruits and vegetables. However, many of them are recognized as off-flavors of many foods due to their characteristic unpleasant odors associated with a low sensory detection threshold [24]. Off-notes typically increase their quantity as a consequence of heat treatment, such as sterilization techniques, used to ensure safety and extend the shelf-life of perishable foods [20, 24].
The concept of off-flavor can be considered partly subjective, depending on various factors, such as the type of analyzed matrix, the quantity of analytes considered as such, and the impact that their characteristic aroma has on the entire aromatic component of the food. In addition, we must remember that only odor-active compounds contribute significantly to the unpleasant taste notes.
Therefore, we will limit ourselves to commenting on the results obtained for this family of analytes, comparing them with the literature data, without speculating how marked their impact is on the entire aromatic profile of our samples.

In particular, we have identified some sulfur compounds in the M_AI_V_t_0 fiber: dimethyl sulfide (DMS), dimethyl disulfide, dimethyl sulfoxide (DMSO), 3-methylthio-propanal, ethyl-methylthioacetate, 2,3-dihydro-thiophene and benzothiazole.
Most of them have previously been identified in thermally treated melon juices [15, 19, 20, 24].
It has also been reported that some of these compounds, in particular dimethyl sulfide and dimethyl disulfide, originate from the degradation of sulfur amino acids, such as methionine, cysteine and their derivatives, when involved in some reactions induced by heat (for example Maillard reactions).
However, there is a big difference between our samples and those reported in the papers just mentioned. In our case, the fiber has not undergone any heat treatment, but it has been vacuum-freeze dried in order to preserve the original characteristics of the pulp as much as possible.

It was interesting to find that dimethyl sulfide (DMS), dimethyl sulfoxide (DMSO) and, to a lesser extent, dimethyl sulfone, are widely distributed in nature in various fruits, vegetables (including cucumber), forage and also in some beverages [23].
In particular, DMS, is responsible for the aroma of many foods and is supposed to play an important role in the natural transfer of sulfur of biological origin. Moreover, it has been shown in the literature that its photolysis in the presence of air generates dimethyl sulfoxide [23].
However, it cannot be overlooked that DMSO is widely used as an organic solvent also for the production of pesticides, and therefore its presence could be partly due to some form of external contamination.
In addition, we also detected 2,3-dihydrothiophene, which is known to contribute to the aroma of white truffle [41], and benzothiazole. These sulfur compounds have been found in various foods such as persimmons, potatoes and tea [41], but may also partially result from Maillard reactions [20]. Ethyl-methylthio-acetate has also been previously found in various fruits, including melon [17, 41]. Finally, we report that none of the sulfur compounds were detected in the H_2O_2 bleached fibrous samples.

Other compounds
In this heterogeneous group, we collected the residual analytes, which apparently do not seem to be related to the compounds identified and previously classified on the basis of molecular structure and functional groups.
Although some molecules such as methoxy-phenyl-oxime, some nitrogenous compounds such as pyrrole and pyranone, as well as epoxybutyrate have been detected in our samples and in other fruits, there is a lack of information on the chemical and biochemical cycles that rationalize their presence in nature.
Finally, it is worth noting that for propylene glycol, identified in the M_AI_V_t_0 sample, we did not find mention in the specific literature about *Cucurbitaceae* fruits.

Table 4, and Figure 8 for greatest visual impact, summarize the results obtained from the HS-SPME-GC-MS analysis for the different fibrous samples from winter melon pomace (M_AI_V_t_0, M_B_O_t_0 and M_B_O_t_{24}), collected in comparative form based on the classes of compounds identified.
One-way analysis of variance (ANOVA) was performed to assess whether there is a statistically significant difference between the quantities of each compound class among the M_AI_V_t_0, M_B_O_t_0, and M_B_O_t_{24} samples.

Table 4[#] - Compound classes identified in the HS-SPME-GC-MS analysis[*] of the samples obtained from winter melon pomace: M_AI_V_t0, M_B_O_t0 and M_B_O_t24.

Compound class	**M_AI_V_t0**	**M_B_O_t0**	**M_B_O_t24**	**p-value**
	$\bar{x} \pm s_{(3)}$	$\bar{x} \pm s_{(3)}$	$\bar{x} \pm s_{(3)}$	
ALC (alcohols)	33.8 ± 3.2	12.3 ± 2.3	7.3 ± 0.9	p<0.05
ALD (aldehydes)	24.7 ± 2.4	33.2 ± 3.9	29.5 ± 2.7	p<0.05
KET (ketones)	14.5 ± 2.6	12.5 ± 2.2	5.9 ± 0.8	p<0.05
ACD (acids)	9.7 ± 1.7	33.8 ± 3.7	57.3 ± 4.4	p<0.05
ACE (acetate esters)	7.2 ± 1.0	---	---	
SDC (sulfur-derived compounds)	4.7 ± 0.8	---	---	
NAE (non-acetate esters)	3.0 ± 0.6	6.1 ± 0.7	---	p<0.05
AHA (alkanes)	0.2 ± 0.2	0.6 ± 0.4	---	p>0.05
OTH (other compounds)	2.2 ± 0.5	1.4 ± 0.9	---	p>0.05

[#]Data are expressed as mean % of each class to the total normalized peak areas on the basis of the sum of the TIC area.
[*]mean of three replicates of the chromatograms, ± standard deviation, $s_{(3)}$

The p-value, for almost all the investigated compounds (excluded AHA and OTH compounds,) is smaller than the significance level (0.05), so we can conclude that globally the investigated fibers are statistically different.

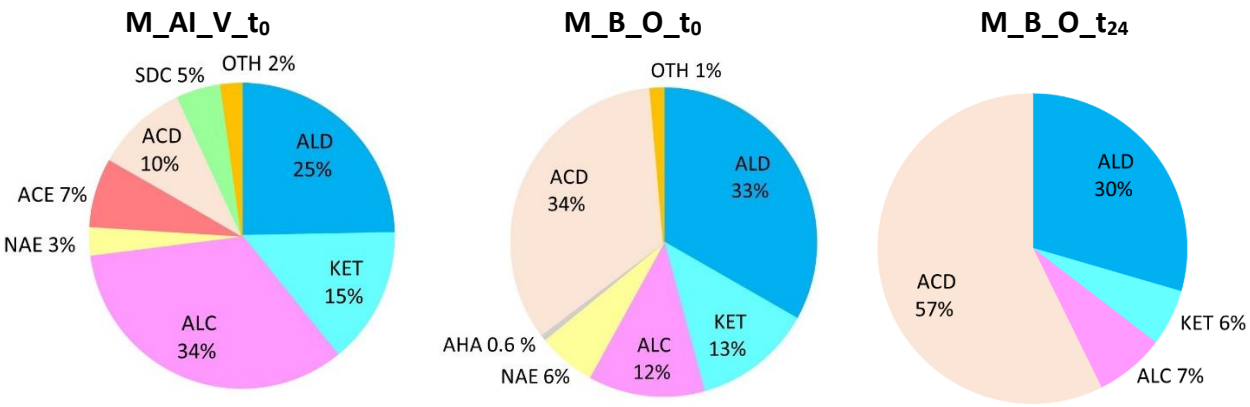

Figure 8 - Graphic representation of the compound classes identified through HS-SPME-GC-MS analysis of the samples obtained from winter melon pomace: M_AI_V_t0, M_B_O_t0 and M_B_O_t24.

The fibers obtained after the bleaching process with H_2O_2 at t_0 time (M_ B_O_t_0) and the sample stored for 24 months (M_B_O_t_{24}) presents a HS chemical composition significantly different from that of the "as it is" pomace fiber vacuum-freeze dried (M_AI_V_t_0). In particular, some specific considerations can be developed for some classes of molecules listed in Table 4.

i) volatile compounds typical of the ripe fruit (**alcohols, esters**). Alcohols cover about one third of the VOCs of the M_AI_V_t_0 fiber, while immediately after the bleaching treatment they represent ≈ 12 % of the volatiles (M_B_O_t_0) and only ≈ 7 % in the aged sample (M_B_O_t_{24}). Acetate esters are present only in the M_AI_V_t_0 sample in a relative percentage of about 7 % and completely disappears in the treated ones. On the other hand, non-acetate esters persist even after the chemical whitening process of the melon pomace fiber, reaching ≈ 6 % in M_B_O_t_0 sample, but not after the long storage period.

ii) volatiles completely lost during the bleaching process (**sulfur-derived compounds**). 6 SDCs have been detected only in the crude fiber (M_AI_V_t_0), around 4.5 %, while none of them have been identified in the whitened ones. The complete loss of this class of analytes may be caused by their intrinsic volatility in combination with hydrolysis processes in which they can be involved, being in presence of organic acids and residual moisture. Finally, even the repeated washing of the fibers may have contributed to the removal of some analytes present in the "as it is" pomace.

iii) molecules developed from the melon pomace fiber treatment with H_2O_2 (**organic acids**). The fraction of acids is the largest in the M_B_O_t_{24} sample (≈ 57 %), while it covers only about 10 % of the total volatile organic fraction of the M_AI_V_t_0 and reaches ≈ 34 % in the M_B_O_t_0.
Certainly, the action of hydrogen peroxide has strongly contributed to the development of these analytes, oxidizing some of the molecules originally present in the starting matrix, to give products with acid functional groups. Furthermore, even the oxygen present in the residual atmosphere of the vials in which the fiber was stored has led to a further increase in the amount of organic acids over time, acting as a starter for radical autoxidation processes.
In general, it is quite evident that the bleaching of the fiber from winter melon pomace and, even more the aging, leads to a simplification of its aroma as there is a progressive reduction of the classes of molecules detected, passing from the initial 9 to only 4 after 24 months.

6.4. SOME CONCLUDING REMARKS

The results presented in this chapter clearly indicated the validity of a VOCs sampling methodology and the strategic value for establishing the typical aromatic profile of the fibrous matrix obtained from the *Inodorous* melon, as it can be obtained for any other material.
The HS-SPME technique is extremely performing and has produced highly reproducible results even for analytes present in limited quantities in the processed samples.
This method is suitable for the effective screening of a wide variety of volatile and semi-volatile compounds related to specific aromatic profile of the analyzed material, such as alcohols, aldehydes, ketones, esters, sulfur compounds, among many others.
The results clearly demonstrate that a large number of VOCs have been adsorbed/absorbed by the multiphase SPME sampling system, and subsequently detected.
A diversified range of analytes allowed to discriminate between fiber samples, both as a function of possible preliminary treatment and for the ageing of the fiber itself.
It can be noticed that the bleaching treatment with H_2O_2 reduced the number of VOC classes present at t_0 time, conversely increasing the quantitative ratios of the oxidized molecules such as acids, at the detriment of alcohols and ketones. These compositional ratios change progressively and propagate even during the ageing of the fiber over time.
We recall that one of the aims of this research work was the possibility to obtain a dietary fiber having neutral characteristics as much as possible, which can be used as a functional ingredient in food products, without altering the color, taste and aroma. Therefore, the ideal fiber should be colorless, have inert properties from a “flavoring” point of view, in addition to having a shelf-life as long as possible.
Nevertheless, it seems particularly useful being able to identify some target compounds that can assume the role of qualitative and quantitative marker in order to correlate the compositional characteristics and the ageing level of a generic sample of *Inodorus* melon fiber.
From what has been observed so far, it seems that a fair number of particularly significant and representative VOCs of the *Inodorus* melon pomace can assume the role of quality indicators. In fact, some of them were detected in this study, and have already shown to be key components within the molecular bouquet that expresses the fragrance of the melon cultivar we examined (C9 and C6 aldehydes and alcohols, including nonanal, 3,6-nonadienol, 3-nonen-1-ol and 1-nonanol, among others).
The data collected and presented in this study, related to the fiber from winter melon endocarp, with the purpose of a potential enhancement of the pre-waste material, widely justify further investigations on the fruit itself.
For example, it could be useful to study the variations of the aromatic profile during the development and ripening of the melon fruit, characteristics which will then be partly transmitted to the extractable endocarp fiber and that may be applied also for identifying the origin of the cultivar.

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CHAPTER 7

7 - CHARACTERIZATION PROCEDURES APPLIED TO WATERMELON

ENDOCARP FRACTION

Proximate analysis

UV-Vis spectroscopy

Analysis of fiber samples in solid-state

Semi-quantitative estimation of lycopene content

Thermal analysis

Bearing in mind the objectives of enhancing as much as possible the agro-industrial waste materials deriving from *Cucurbitaceae*, we start to examine in detail the activities carried out on *Citrullus Lanatus* L. matrix, presenting and discussing the results obtained.
The main purpose is to obtain dietary fibers (DF) which should maintain the bioactive and functional components present in the initial matrix as much as possible, opening the way to different and possible applications as functional ingredients having an important added value in sectors such as food&feed or cosmetic, among others.

Starting from this chapter and in the following ones, we will show the results obtained from the characterization of:

- Endocarp fraction (the edible pulp), manipulated by applying different methodologies and procedures to obtain Dietary Fiber (DF) and juice;
- Mesocarp fraction (Chapter 11);
- Seed fraction, from which it is possible to extract oil and to obtain defatted proteic-starch.

ENDOCARP FRACTION

The red fiber obtainable from the inner edible part of watermelon fruit represents a very interesting material, since it combines the beneficial properties of DF (already described in this PhD thesis), and those related to the numerous bioactive compounds present in the pulp of the fresh fruit itself. In fact, some of these important molecules remains in the fibrous fraction despite the handling procedures necessary to obtain it.
For each **watermelon** (**W**) selected cultivar, the endocarp fraction (i.e. the red pulp) was shredded with a common kitchen blender to obtain a homogeneous slurry-meal, bright red in color and having the characteristic aroma of the ripe fruit. The pulp was then centrifuged and filtered with paper through a vacuum filtration system to separate the juice from the pomace, which was then handled in different ways.
We will talk about the "**as it is**" pomace fiber (W_**AI**) when we refer to the material containing all components naturally present in the fruit, which has been dried in a vented **Oven** (W_**AI_O**) or **Vacuum-freeze dried** (W_**AI_V**), in order to obtain the TDF fraction.
Alternatively, the pomace has been washed with distilled water (W_***w***), in a quantity equal to about three times that of pomace (mass/mass), to remove soluble components as much as possible, pursuing the aim of obtaining a fibrous material as neutral as possible from the organoleptic point of view. As already fully mentioned, this is a particularly sought-after feature in food industry, cosmetic sector, etc... because a fiber with taste and aroma not particularly intense is much more versatile for being used in different types of mixtures and formulations.
Finally, also the **washed** watermelon pomace was rapidly filtered again with the same previous procedure and the residue in part has been oven dried (W_*w*_O) and in part vacuum-freeze dried (W_*w*_V) as described in Chapter 4.
Doing this, it was then possible to characterize, evaluate and compare the composition and some physicochemical and technological properties of the obtained DF which, despite having the same origin (watermelon pomace), have different peculiarities due to different obtaining processes. These procedures were applied to all three selected watermelon cultivars. This allowed us to make some comparisons between a few compositional characteristics of the fibers obtained from the endocarp of Crimson Sweet, Asahi Miyako

and Gavina.
The samples obtained with the two drying procedures adopted are perfectly dry to the touch but, at first sight, they look quite different. For all watermelon varieties, all the vacuum-freeze dried fibers "as it is" (W_AI_V) are spongy, crumbly, not very compact, with low bulk density and have maintained a very pleasant aroma. They still appear red in color, even if less bright. The oven-dried crude fibers (W_AI_O), instead, are much more rigid, compact and show a certain resistance to breaking even if the material is fragile due to the very thin final thickness. In this case, the color is deep red and the perceived aroma is still pleasant even if a little weaker than that of the vacuum-freeze dried samples.
All dried, washed fibers (W_*w*_V and W_*w*_O) have maintained the red color although it is a little less intense than that of TDF ones, still present a little note of pleasant aroma, but they are not sticky to the touch at all. Reasonably, washing process has been effective in the removal of most of the sugars and water-soluble analytes naturally present in the raw material.

At earlier convenience, in the following scheme, we report the identifying acronyms of the obtained watermelon-based fibers samples.

W_AI_V	Watermelon pomace "as it is", Vacuum-freeze dried
W_AI_O	Watermelon pomace "as it is", Oven-dried
W_*w*_V	Watermelon pomace *washed* with distilled water, Vacuum-freeze dried
W_*w*_O	Watermelon pomace *washed* with distilled water, Oven dried

Scheme 1 - List of the identifying acronyms of watermelon-based fibers samples.

Immediately after the end of drying procedures, all fibers have been ground (average particle size 0.5 mm) and stored as described along the text of this dissertation thesis.

Figure 2 – Images of some fibers obtained from watermelon pomace: (a) W_AI_V; (b) W_w_V

Figure 2 shows a partial mass balance, related to the pulp fraction and to the sub-fractions obtained from different handling processes to which it has been subjected for obtaining the different types of DF.
As an example, we report the data related to Crimson Sweet watermelon cultivar, indicating the average data associated with the vacuum-freeze dried fiber "as it is", and the one obtained by washing it with distilled water and dried with the same technique.

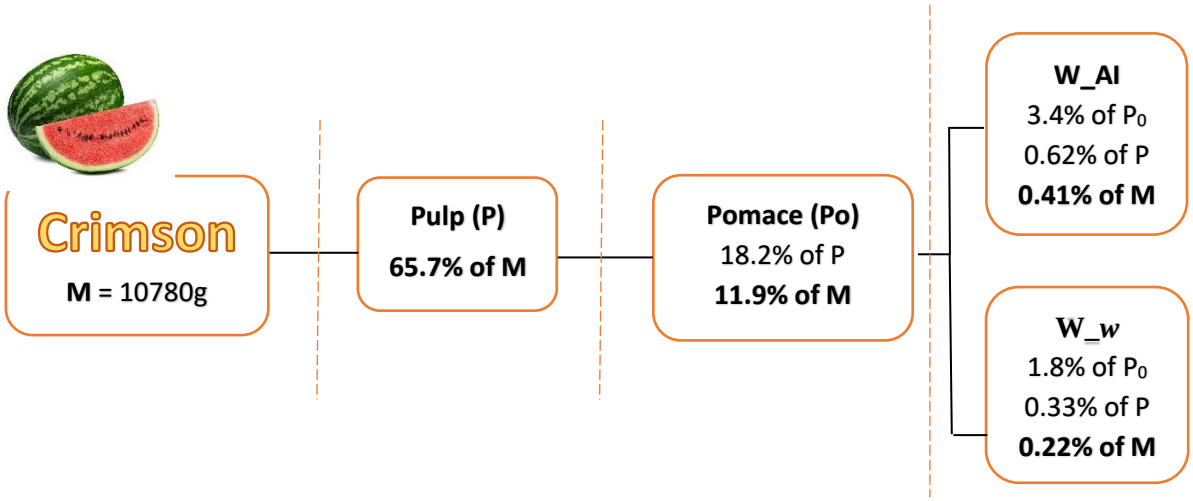

Figure 2 - Mass balance for the Crimson Sweet pulp fraction, with the resulting DFs

Washing operations are aimed to obtain a “neutral” polymer material, at least in terms of taste and aroma, mainly reducing the amount of sugars and other water-soluble molecules. Moreover, a DF consisting essentially of the insoluble stable components, could be stored for a longer period of time without undergoing alteration phenomena and microbial spoilage. This is a not negligible aspect considering that we are working with seasonal matrices, not available throughout the year, whose fractionation products could be obtained and used by downstream users at very different times.
However, we must consider that depending on how watermelon pomace is processed, different amounts of dry fiber are obtained (Figure 2). In fact, as previously mentioned, by washing the fibers, soluble analytes (mineral salts, organic acids, simple sugars, soluble oligosaccharides, among others) are removed, with a consequent decrease in the residual fiber mass.
On average, for Crimson Sweet, it has been verified that the amount of W_AI fiber obtained regardless of the drying technique adopted (setting the parameters we selected) is around 0.41 %, with values ranging in the 0.38 – 0.45 % interval for some replicates and calculated with respect to the total mass of the fruits.
For fibers washed with distilled water, instead, we estimated to obtain an average quantity of IDF between 0.20 - 0.25% starting from fruits having the same characteristics as those used in this study.

In the following sections, we report the results obtained from the various analyses carried out on the different types of watermelon pomace fibers, specifying from time to time the selected cultivar, starting from the proximate analysis and continuing with some instrumental determinations.
Currently, it has emerged that particularly in-depth studies have not yet been conducted regarding the Gavina variety, therefore it was considered appropriate to proceed with a characterization, albeit partial, of the fiber obtained from the pulp of this fruit.

Proximate analysis

Table 1 collects the results of the proximate analysis conducted on the samples of fresh pulp from Crimson Sweet, Asahi Miyako and Gavina watermelons. Moreover, we report some literature data as a term of comparison.

Table 1 - Proximate analysis representative of the pulp fraction obtained from three selected cultivars of Citrullus Lanatus.*

	CRIMSON PULP	MIYAKO PULP	GAVINA PULP	REF[$]
Total water content %	92.70 ± 0.34	91.3 ± 0.42	91.9 ± 0.38	
Moisture % at 40 °C	17.7 ± 0.31	16.40 ± 0.25	15.7 ± 0.22	
moisture% at 101 °C	3.60 ± 0.22	3.40 ± 0.16	3.60 ± 0.25	
residue% at 105 °C (db)	7.30 ± 0.17	8.73 ± 0.22	8.10 ± 0.21	
ashes % (fw)	0.09 ± 0.02	0.12 ±0.03	0.18 ± 0.03	
ashes % (db)	3.6 ± 0.03	2.9 ± 0.03	4.6 ± 0.02	
pH	5.80 ± 0.02	5.58 ± 0.03	5.70 ± 0.03	5.4 – 6.2[1] 5.20 – 5.80 [2] 4.50 – 5.38 [3]
Sugars (%)[#]				
Reducing	4.52 ± 0.16	4.90 ± 0.14	4.44 ± 0.16	7.88 – 8.12 [3] ; 3.3 – 7.4 [4]
Glucose	1.11 ± 0.11	1.26 ± 0.11	1.27 ± 0.13	2.75 – 2.77 [3] ; 0.9 - 2.3[4]
Fructose	3.41 ± 0.13	3.64 ± 0.14	3.17 ± 0.14	5.13 – 5.35 [3] ; 2.4 - 5.1[4]
Sucrose	2.37 ± 0.12	3.95 ± 0.15	3.56 ± 0.16	2.28 – 2.67 [3] ; 1.0 – 4.2[4]
Total	6.89	8.85	8.00	10.16 – 10.79 [3] ;4.3 – 11.6 [4]
Titratable acidity[#]				
mg/mL citric acid	0.49 ± 0.09	0.68 ± 0.12	0.56 ± 0.10	
mg/mL citric acid monohydrated	0.54 ± 0.10	0.74 ± 0.13	0.61 ± 0.11	
Proteins (% fw)	0.61 ± 0.16	0.71 ± 0.18	0.60 ± 0.14	
C%[£]	39.64 ± 0.22	42.35 ± 0.23	38.93 ± 0.24	
H%[£]	6.92 ± 0.11	6.33 ± 0.13	6.49 ± 0.16	
N%[£]	1.73 ± 0.11	2.41 ± 0.13	1.35 ± 0.10	
S%[£]	n.d.	n.d.	n.d.	

[*]mean of three replicates (± standard deviation, $s_{(3)}$).
[#]measurements conducted on fresh juice
db = on dry basis ; fw = on fresh weight
[$]the data used for comparison refer to watermelon cultivars different than ours
[£]for each cultivar, elemental analysis was conducted on W_AI_V fibers (TDF)

It is well known that watermelon is one of the fruits with the highest total water content, which makes it particularly thirst-quenching but, on the other hand, very susceptible to rapid deterioration if not consumed quickly.

The results of the proximate analysis conducted on Crimson Sweet, Miyako and Gavina fresh pulp show overall accordance and comparability, among them and with some literature data, while highlighting some interesting peculiarities associated with the individual cultivars. For example, while total water content and

residual moisture values at the various selected temperatures seem independent of the variety, the same cannot be affirmed for ashes content. Indeed, the pulp of Gavina watermelon shows an ash content (db) which is significantly higher than that in the other two samples (about +25% and +55% compared to Crimson Sweet and Miyako, respectively). It is reasonable to assume that these differences mainly depend on the different place of cultivation of these fruits and that the soil of Sardinia, very rich in mineral salts and trace elements, provides a greater contribution of these species to Gavina watermelon. This results in a greater amount of residual inorganic fraction in pulp ashes. Even the pH of fully ripe watermelon fruits may vary depending on the variety and production area, among other factors. It typically ranges from 5.4 – 6.2, while pH values from 6.5 upwards indicate overripening [1].

In association with pH values, also the low titratable acidity which was determined demonstrates that the fruits we used during our study were perfectly ripe.
According to literature, malic and citric acids are the main organic ones in ripe watermelon fruits. They play an important role, because their presence in different quantity may modulate the perception of sweetness [4, 5].
Soluble sugars and organic acids can be considered the key components which define fruit flavor, also having a great influence on the overall organoleptic fruit quality and metabolism. The most important quality trait of watermelon is sweetness, which also contributes to determine its commercial value. Variation in sweetness among cultivars and during fruit growth and ripening is influenced by the different proportions of the three main sugars found in watermelon juice, i.e. glucose, fructose and sucrose [6, 7, 8].
Reducing sugars, i.e. glucose and fructose, start to accumulate in the early development stages of watermelon to then decrease in concentration during ripening, while sucrose follows an opposite trend.
Usually, fructose is the main monosaccharide found in ripe fruits, followed by sucrose and glucose. This trend is perfectly identifiable in all the watermelon juice samples analyzed in our study, where fructose is always ≈ 2.5 – 3 times higher than glucose while, in Miyako and Gavina juice, sucrose is slightly higher than fructose. Although genetic factors may slightly affect the sugar profile, it can be noticed that both the quantity of reducing and total sugars found in the juice of watermelons we analyzed, are perfectly comparable with literature data reported in Table 1.

It is well known that watermelon is a natural source of L- citrulline and L-arginine (Figure 3), among other amino acids. These molecules, although not present in most foods, are found in quite high amounts in watermelon fruits, and, in association with other precious bioactive compounds, can bring significant healthy beneficials [9-11]. They surely contribute to the protein content of watermelon pulp, even if in presence of other species.

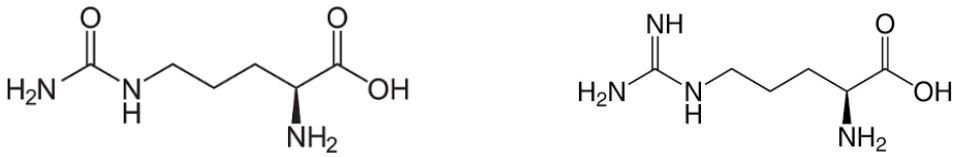

Figure 3 – Chemical structure of L-citrulline (left) and L-arginine (right).

The protein content of Asahi Miyako pulp is higher than that of Gavina and Crimson Sweet ones of about +18%, maybe due to the presence of traces of small and unripe (white) seeds which remained inside the pulp during the fractionation operations. They may have increased the nitrogen content of the fruit flesh, having themselves an important percentage of proteins [12].
Moreover, they probably have also augmented the free fatty acids content of Asahi Miyako pulp.
Looking at the results of the elemental analysis, we can still observe these differences in the N% content, in association with some other variations in the values related to C% and H%. Probably, these differences are not particularly significant, since the common backbone of all these samples are cellulose, polysaccharides and carbohydrates. As already mentioned along the text of this thesis, the possibility of recovering particularly valuable “food-additive” fractions, starting from agri-food waste by-products, can be motivated by the beneficial effects that may derive from the aware consumption of DF and functional foods.

Not surprisingly, there are now several studies in the literature that provide for the addition of small amounts of fibers obtained from some watermelon fractions, including pulp, as functional ingredients to doughs and foods [13, 14].

UV-Vis spectroscopy analysis

- Analysis of fiber samples in solid-state

The UV-Vis analysis technique was applied to dried watermelon pomace fiber samples to evaluate the spectrum profile and absorption bands which characterize the solid material. We also recorded the associated color point parameters. The evaluation of this latter aspect is described in Chapter 9.
We proceeded to measure the UV-Vis spectra of the fibers of each watermelon cultivar (Crimson Sweet, Asahi Miyako and Gavina), both washed with distilled water (IDF) and "as it is" (TDF), according to the experimental conditions previously described. The aim was to evaluate whether the different manipulations and drying procedures, applied to obtain IDF and TDF samples, have introduced some significant compositional variations which can be evaluated with this analytical technique.
The measurements were carried out with total reflectance technique, operating in the range of 200 ÷ 800 nm and using the instrumentation and methods listed in Chapter 4.
We have prepared at least three pads for each fibrous sample, processing each of them through a random exposure to the incident radiation beam. Fibers were analyzed immediately after they have been prepared (t_0 time).

From the analysis of the obtained results, we point out that the UV-Vis spectra of "as it is" (AI) fibers deriving from the three different varieties are perfectly comparable to each other, in the whole λ range considered. The same occurs within the class of fibers washed with distilled water (*w*). Even the two different drying techniques we applied did not produce appreciable differences. Conversely, for each cultivar, the most significant variations have been detected by comparing the spectra of the TDF and IDF fibers. As an example, in Figure 4, we report the comparison between the UV-Vis spectra of the four samples W_AI_V, W_AI_O, W_*w*_V and W_*w*_O samples, obtained from Asahi Miyako cultivar.

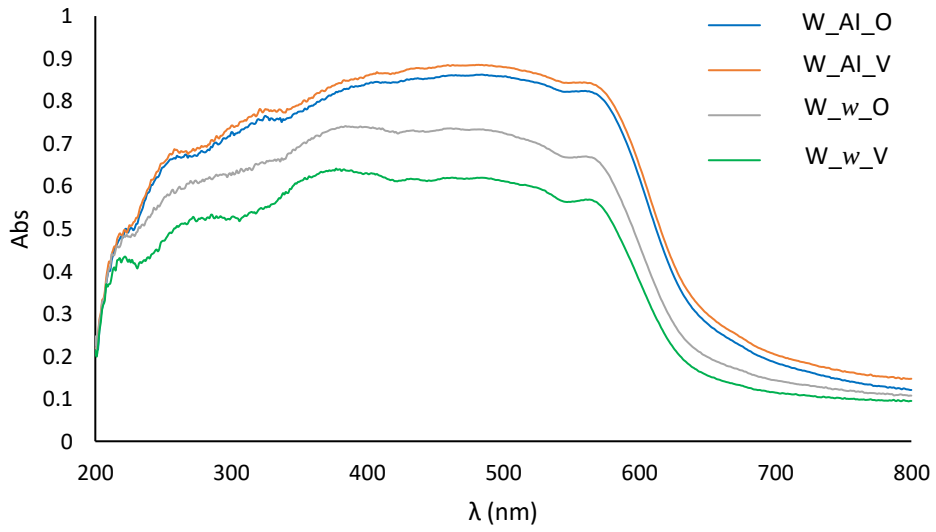

Figure 4 – Comparison of UV-Vis spectra collected at t_0 time for the four fiber samples in tablet form, obtained from Asahi Miyako cultivar.

Observing Figure 4 we can affirm that all the spectral profiles show a comparable trend in the entire spectral window considered. This suggests that the analytes which contribute to the absorption bands are generally the same in all fiber samples, regardless of the handling steps required for their preparation. However, it is reasonable to think that among different samples there may be differences in their relative quantities. In

fact, it can be noticed that passing from the row material (TDF) to the washed one there is a general decrease in absorbance values. In particular, the two TDF samples (W_AI_O and W_AI_V) from Miyako pomace show Abs values comparable to each other and greater than those of the two IDFs ones washed with distilled water (W_*w*_O and W_*w*_V). It is reasonable to think that the amount of spectroscopically active compounds remaining in W_*w*_O and W_*w*_V samples is lower than that in W_AI_O and W_AI_V ones. This could appear unusual considering that lycopene and other carotenoids in general exhibit characteristics of fat-solubility rather than hydro-compatibility. However, the presence of other compounds such as sugars, organic acids, fatty acids, amino acids and protein aggregates, could promote the formation of cluster-complexes with important implications on the hydrophilicity of fat-soluble chromophores, thus favoring their partial elimination with the washing water. We recall that washing operations certainly allow to obtain "neutral" fibers from an organoleptic point of view (flavor, aroma), but unfortunately also lead to their depletion as a consequence of the partial elimination of desirable compounds, such as lycopene, among others.

Literature studies reports that lycopene is lodged within cellular corpuscles (Figure 5) whose membrane could literally explode due to an osmotic pressure gradient produced by the interaction with the distilled H_2O used for washing. This leads the consequent expulsion of the cellular content, which can be released in solution and subsequently eliminated with water during filtration [15].

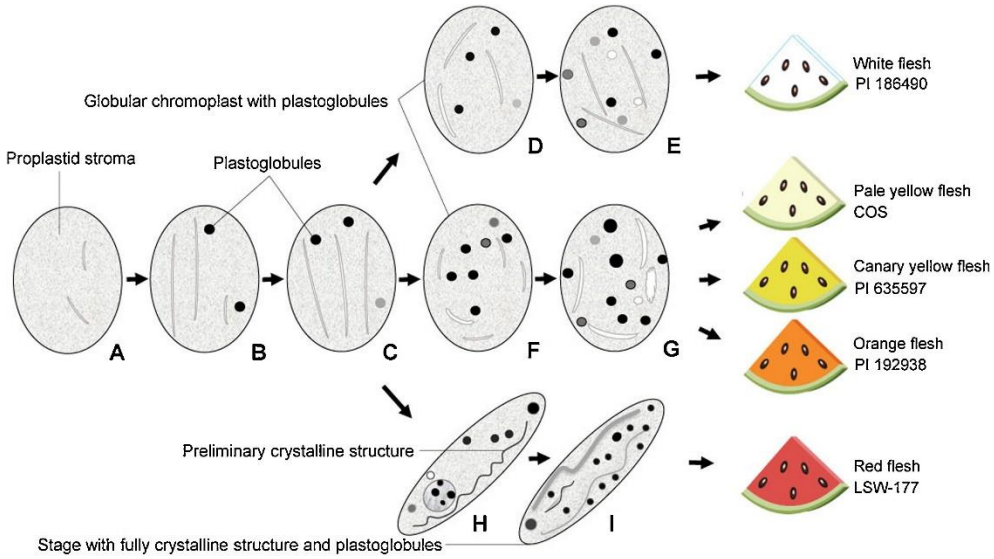

Figure 5 - Representation of chromoplasts and plastoglobules containing lycopene and carotenoids, undergoing differentiation during the growth process until ripening, in different watermelon cultivars [15].

From Figure 4, it can be noticed that all the recorded spectra are characterized by a wide absorption band covering the whole range 230-650 nm, from which a quite narrow band centered around 560-570 nm clearly emerges. It is one of the characteristic absorptions of lycopene, the main carotene naturally present in watermelon pulp, to which it confers the characteristic bright red color. Actually, the typical absorption bands of a lycopene solution in n-hexane, in the visible region, fall around 444 nm, 470 nm and 502 nm (Figure 6). However, the absorption spectra of analytes in solution can be significantly different from those recorded on a solid-state sample. This is even more true if the analyzed analyte is lodged within real and complex matrices like ours.

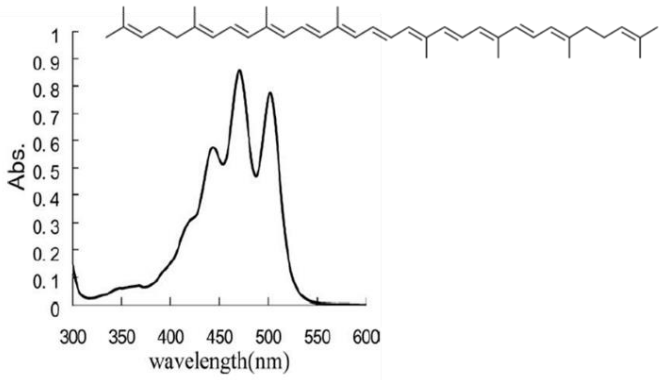

Figure 6 - UV-Vis absorption spectrum of lycopene in n-hexane [16]

It is evident that the chemical environment which surrounds and interacts with an analyte of interest (in this case lycopene), can significantly influence the absorption capacity of the latter. What has been said so far is confirmed by some literature papers where it is evaluated the absorption capacity of supramolecular aggregates of lycopene in the presence of biomolecules, obtained at a laboratory scale [17, 18].
In both cases, in fact, the ability of numerous carotenoids, including lycopene, to form supramolecular assemblies in hydrated organic solvents and / or in aqueous solutions due to their high hydrophobic character is highlighted.

It is now quite well known that lycopene is present as microcrystals (otherwise called crystalloids) inside the chromoplasts of some fruits and vegetables like watermelon, gac fruit and tomatoes [17, 18]. As said before, the exposure to a hydrophilic environment leads to the instauration of intermolecular interactions between lycopene molecules to form aggregates which have a particular packing arrangement. This process depends on two main factors: the steric properties related to the structure of the molecules and the attractive forces between neighboring hydrophobic species (Van der Waals forces, dipole interactions, H-bridges, among others).
This induces variations in the electronic state of lycopene molecules, leading to the shift of its UV-Vis absorption bands towards longer or shorter wavelengths (if compared to those characteristics of the monomer in solution), depending on the type of aggregate which is formed.

In particular, two types of carotenoids assemblies have been reported in literature: H- and J-aggregates, which correspond to strongly and weakly coupled ones, respectively [17-19].
Briefly, in an ideal crystal structure, the H-type is a strongly coupled aggregate formed by molecules whose chains are disposed quite parallel to each other, generating a structure which can be described as “card-packed”. On the other hand, J-aggregates have a loosely packed structure, where the hydrocarbon chains are supposed to interact in a “had-to-tail” manner which is less effective than the H-type [18, 19].
This different association caused a significant hypsochromic shift (or blue-shift) of the H-aggregates absorption band in the UV-Vis region, compared to the main band of the related monomer dissolved in organic solvents. On the contrary, the UV-Vis absorption spectrum of J-aggregates shows a bathochromic effect (red-shift), leading also to a significant color deepening [18, 19].
This is the reason why UV-Vis spectroscopy is one of the analytical techniques which can be used to identify the presence of this type of aggregates, both through the absorption bands shift and by the spectrum shape.

Going more into detail, Hempel et al. [19] accurately studied this phenomenon for several carotenoids, including lycopene, both in different solvent mixtures and in solid-state (thin films), through UV-Vis and CD spectroscopy, light microscopy and also performing computational modeling to estimate intermolecular distances in aggregate dimers.

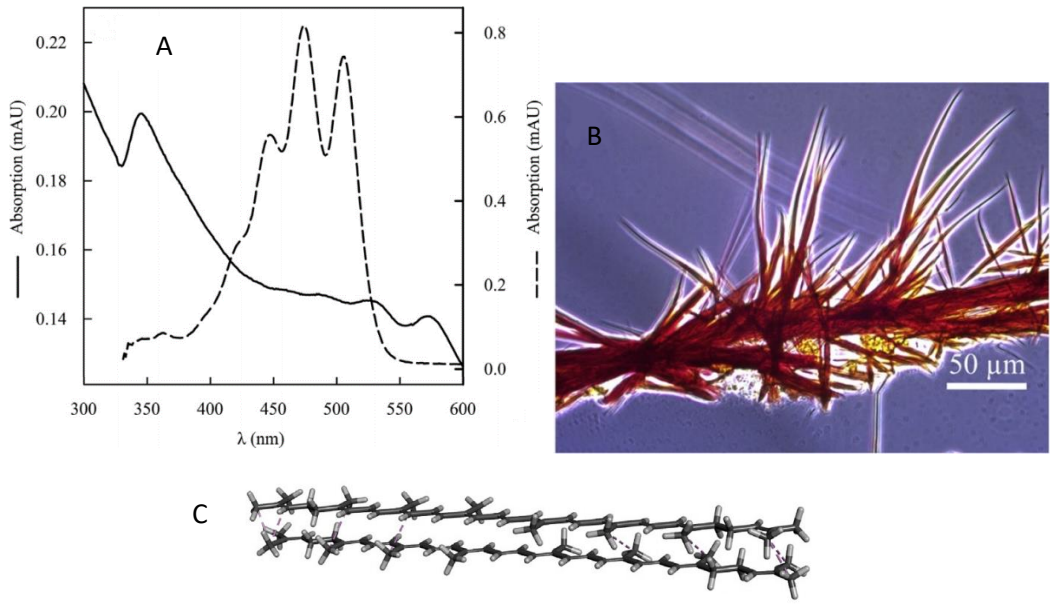

Figure 7 - UV-Vis spectra of lycopene dissolved in acetone (dashed line) and aggregated as thin film (solid line) on quartz slide (A), light micrographs of lycopene aggregates in phase contrast mode (B) and molecular model of (all-E)-lycopene dimer (C) [19].

Looking at Figure 7, it emerges that lycopene in different state of aggregation generates two spectral profiles extremely different from each other. When it is solubilized in a solvent such as acetone, lycopene is present as a monomer and its acyclic chromophore made of 11 conjugated double bonds gives a spectrum with a characteristic profile and three absorption maxima at about 445, 475 and 505 nm.
Conversely, the solid line related to lycopene in solid-state shows a strong maximum of absorbance at ≈ 350 nm (blue-shift) and two other weak peaks at 537 and 582 nm, corresponding to a red-shift.
At this point, it is necessary to deepen some aspect related to the structure of these aggregates, reporting some more information from the literature.

Figure 7 (C) shows the modeled structure for an (all-E)-lycopene dimer [19]. The model assumes a parallel arrangement of the two lycopene molecules with the establishment of intermolecular interactions between some of the double bonds present. This fits with a generally accepted assumption according to which carotenoid molecules, in an aqueous environment and/or in solid-state, can aggregate in many parallel two-dimensional layers. Molecules belonging to each layer are aligned in the same direction, while the interaction, bending and tilt angle of neighboring layers, determines the type of supramolecular aggregate [20].
Lycopene has a rod-like structure consisting of a hydrocarbon open chain without any chiral center and any cyclic end group. Each lycopene molecule can interact with neighboring ones to give dimers and higher aggregates, which are effectively stabilized by π-π stacking interactions among the double bonds of the polyene chains. Obviously, the possibility to establish this type of interactions requires a certain degree of planarity of the molecules, at least in correspondence with some double bonds.
Going on, dimers can assume a card-pack like arrangement where the above mentioned π-π stacking interactions are maximized, giving H-type aggregate and the associated blue-shift of the absorption bands with respect to the monomer. However, dimers can also interact in a head to tail manner (J-aggregate), where the molecular centers of lycopene dimers are shifted from each other because of some strong dispersion effect due to the presence of other species (e.g. polar and/or nonpolar molecules other than lycopene) [20].

Figure 8 shows the proposed arrangement of lycopene dimers within H aggregates and J ones.

Figure 8 – Configurational disposition of lycopene H-aggregates and J-ones through polar and/or non-polar molecules ( )

Obviously, Figure 8 schematically represents a situation that was obtained by working at laboratory level, in the presence of complex mixtures of hydro-organic solvents. The non-polar solvents used are able to interact with the dimers that are formed by creating a sort of layer on their surface but, at the same time, can remain trapped between the dimers themselves, increasing the degree of structural disorder due to phase shifts between vicinal planes.

It can be hypothesized that within watermelon pomace fibers, regardless of the selected cultivar, there may be several molecules which can interact with the lycopene dimers and which could interpose themselves to give rise to the aggregates as indicated in Figure 8. An example can be represented by long-chain fatty acids, triglycerides, long-chain hydrocarbons, among others, which despite being present in very modest quantities, can effectively act as a "dispersing agent", interposing themselves between the various dimers and between the different planes, leading to situations similar to the ones reported above.
Despite new discoveries in this field, to the best of our knowledge, neither the precise aggregation state of lycopene in these agglomerates, nor the mechanism(s) of this red-shift are precisely known and described yet [20, 21].
As far as this last observation concern, it can be assumed that, in molecular aggregates, lycopene can be considered dissolved in the neighboring carotene molecules. As a consequence, the red-shift of absorption energy can be associated to an induced elongation of the conjugation length of lycopene if compared to that of the corresponding monomer.

From the comparison of the spectrum of Figure 7 (A, solid line) with those relating to our fibers (Figure 4) there seems to be only a partial correspondence. However, in our case, lycopene is not found deposited in a thin layer but is part of an extremely complex biological system, within which it probably interacts with other molecules, thus finding itself in a different condition from that described by Hempel et al [19]. Furthermore, the different spectral profile we obtained from all watermelon fiber samples, could be due to the coexistence of the two types of aggregates to which lycopene can give rise [17]. In addition to this, the different size of the aggregates also plays an important role in defining in which direction the shift of the absorption band occurs. In fact, if lycopene is present in the form of microcrystals, i.e. large supramolecular structures, the proportion of J-aggregate increase, causing an important red shift and an increasing intensity of the principal absorption band. An opposite behavior occurs with smaller aggregates, where the H-type typically prevails [17]. Finally, within the UV-Vis spectra of watermelon pomace fibers, all the analytes present in the matrix other than lycopene, also contribute to the absorption values at the various wavelengths.
Molecular self-assembly of carotenoids does not only take place in "artificial" systems but it is also typical of biological ones, where it plays a crucial role in ensuring that their functioning is correct, as happens for example, for the photosynthetic apparatus. At a biological level, the different aggregates forms significantly influence the photochemical properties of the biological systems in which are contained and, perhaps, also their own bioavailability and stability [21]. For this reason, the study of carotenoid aggregation, especially at biological level, is attracting more and more interest.

It is common opinion that lycopene is typically related to tomatoes, which are the most studied matrices due to the high content of this analyte. In fact, lycopene is typically recovered from the huge amount of industrial wastes deriving from the processing of tomatoes.
Our results perfectly match with what has been reported in some interesting studies, conducted precisely on tomatoes, which dealt with this carotene by exploiting the potential of spectroscopic analysis

techniques (UV-Vis, Raman, CD) to better understand the aggregation of lycopene both in vitro and in vivo in tomato samples. [18, 21].

In the following figures (Figures 9, 10 and 11) some experimental spectroscopic evidence related to these matrices of interest are reported (*Citrullus Lanatus* and tomato).

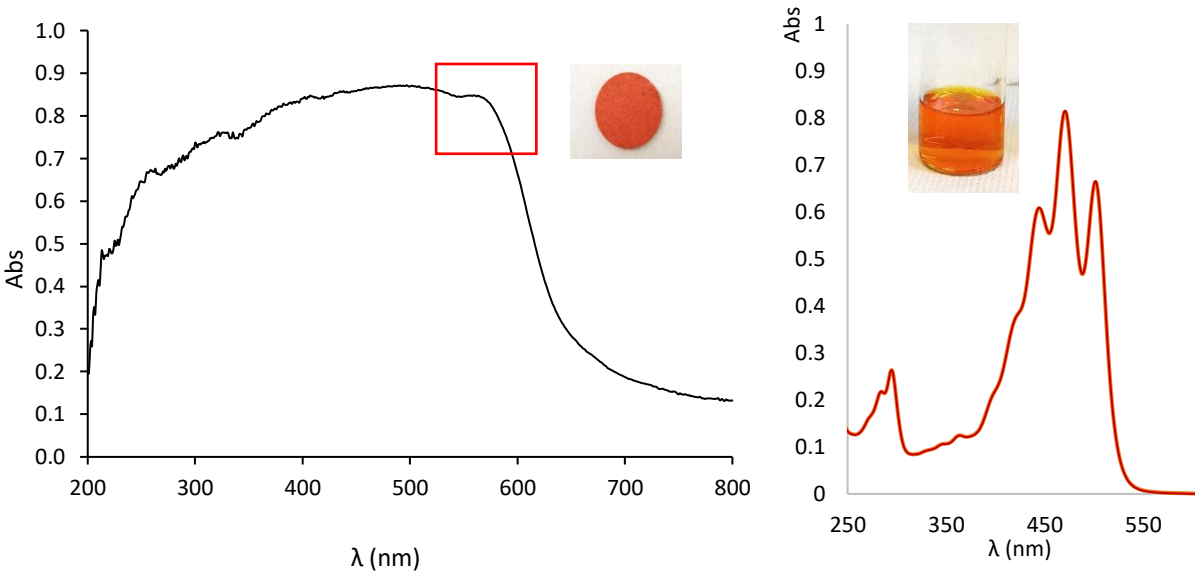

Figure 9 - UV-Vis spectrum of a sample of Crimson Sweet TDF, vacuum-freeze dried, pressed in tablet form (this study).

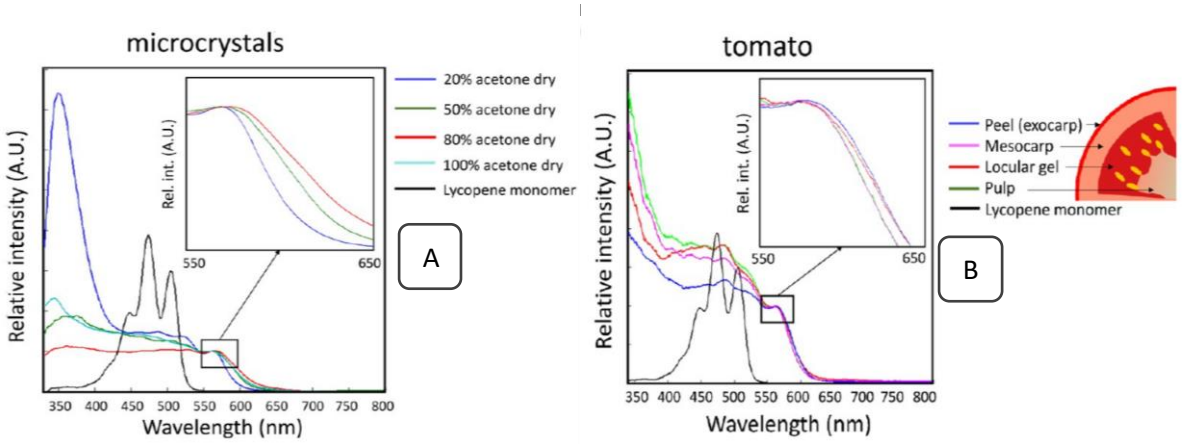

Figure 10 - UV–vis spectra of lycopene monomer and aggregates in: (A) microcrystals and (B) tomato fruits [18].

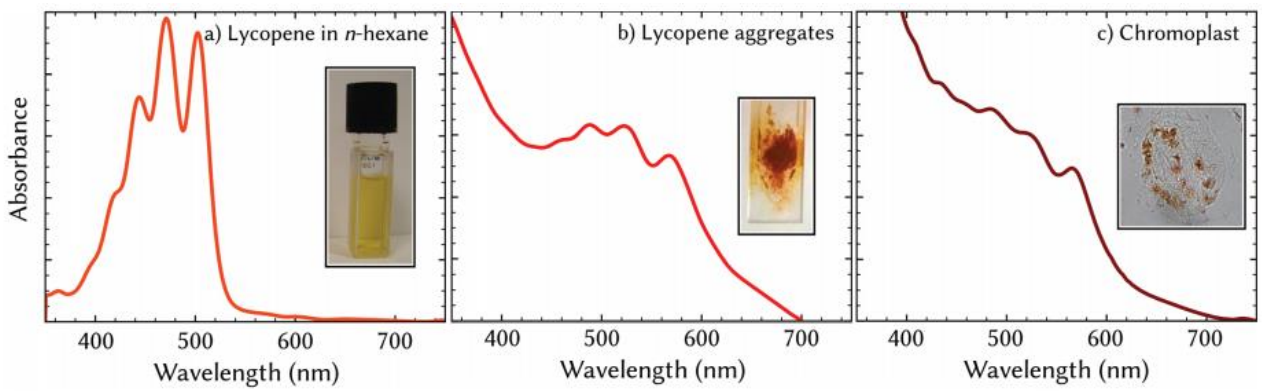

Figure 11 - Absorption spectra of lycopene in n-hexane (a), lycopene aggregates (b), and tomato chromoplasts (c) [21].

The spectral profiles of our watermelon pulp fiber samples (Figures 4 and 9) are comparable with those relating to lycopene in aggregate form in solid state, with those obtained from the analysis of tomato chromoplasts and with the spectra directly recorded on the tomato itself (Figures 10 and 11). The same can be said for the spectra of lycopene extracted in n-hexane solution (Figures 9, 10, 11). In each of the spectra recorded on solid state samples it is possible to clearly notice the presence of the absorption band centered around 560-570 nm, which emerges from the spectrum and that could probably be attributed to the J-aggregate of lycopene.
The similarities between the spectra of Figures 9 and 10 also continue by evaluating the rest of the wavelength range. In fact, the spectra are characterized by a wide absorption band which extends over the entire range considered, with absorbance values that are also quite significant, to which other analytes present in the analysed plant matrices also contribute. Moreover, it cannot be excluded that the H-aggregate of lycopene also contributes to these absorptions, since the coexistence of both types of supramolecular aggregates has been reported in the various literature studies mentioned above.

- **Semi-quantitative estimation of lycopene content**

UV-Vis spectroscopy was applied not only for the analysis of fiber samples from watermelon pomace, but also to conduct a semi-quantitative estimation of lycopene content in the TDFs of the three selected watermelon cultivars.

Lycopene ($C_{40}H_{56}$) is one of the most extensively studied natural carotenoids. It is a fat-soluble molecule with 11 conjugated double bonds. It is one of the most powerful natural antioxidants and it has been proved to be effective against cardiovascular disease and some types of cancer [16]. Thanks to its pharmacological, anti-carcinogenic and antioxidant activity, the interest toward lycopene is continuously growing as is the study of alternative materials to tomatoes, from which it can be extracted.

In order to effectively extract lycopene, it was necessary to use an organic, non-polar solvent, such as n-hexane, since our analyte of interest shows lipophilic affinity. Furthermore, among the various possible solvents, n-hexane was selected because it is one of the greenest, eco-sustainable and used in several applications.
The aim was to make a comparison between the lycopene content in TDFs from Crimson Sweet, Asahi Miyako and Gavina watermelon pomace. Considering the extreme sensitivity of lycopene towards the drying temperature, we decided to perform the extraction on W_AI_V fiber samples obtained from each selected cultivar. Lycopene extraction operations were carried out by Conventional Solvent Extraction (CSE) as indicated in Chapter 4, on aliquots of W_AI_V fibre samples obtained from each variety, immediately after they have been prepared (t_0 time). The measurements were carried out recording the entire UV-Vis spectra, operating in the range 250 ÷ 600 nm and using the instrumentation and methods listed in Chapter 4.
UV-Vis spectroscopic measurements have been performed at least in duplicate on fresh extracted solutions.

Figure 12 shows the average spectra recorded on the extracted solutions for different runs, normalized on the basis of the weight of ground fiber and on the final extraction volumes, as well as on the respective dilutions necessary to bring the absorbances in the linearity range of the instrumental response.

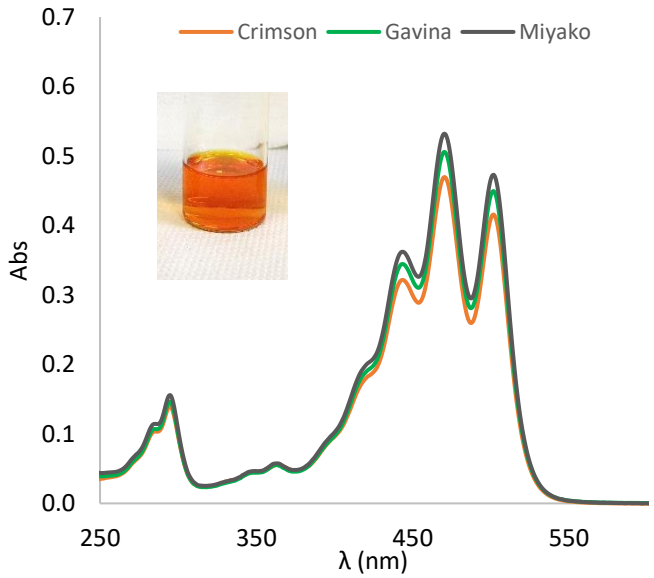

Figure 12 – UV-Vis spectra recorded on extracts obtained from Asahi Miyako, Crimson Sweet and Gavina watermelon pomace fibers (W_AI_V)

At the end of the extraction processes, extracts shows an intense orange color (Figure 12), typical of lycopene in monomeric form in solution [21].
Before moving on to the estimation of lycopene amount in the samples analysed, let's quickly survey the UV-Vis spectra shown in Figure 12. The main absorptions are present within the visible window, with three distinct maxima centred at 444, 471 and 503 nm respectively. Furthermore, in the UV region there are two non-resolved peaks of lower intensity around 290 and 300 nm, as well as a pair of other two peaks at 350 and 360 nm, having the lowest intensity. The comparison with literature data [22, 23] shows that all the absorption bands detected are characteristic of lycopene in n-hexane (Figure 13).

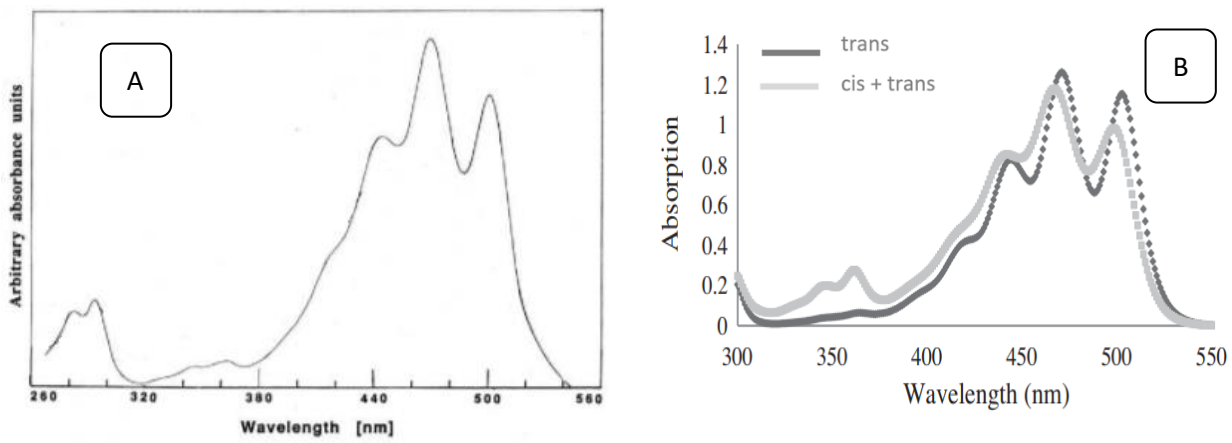

Figure 13 – Literature UV-Vis spectra of all-trans-lycopene (A) and of a cis-trans lycopene mixture, extracted in n-hexane (B) [22, 23]

The hypothesis of having extracted mainly lycopene is therefore confirmed by the correspondence between the spectra of our watermelon TDFs extracts and those reported above. From Figure 13 it is interesting to note that, passing from a solution where only the all-trans-lycopene isomer is present to a mixture of cis-trans isomers, there is a slight blue-shift for the three bands present in the visible window. In addition, a significant increase in the absorption values of the peaks around 350 - 360 nm is detected. For this reason, the absorption bands recorded around 350 nm are called “cis-peaks” [22, 23].
Depending on the different cis-forms present in the mixture and the reciprocal amount of cis- and trans-lycopene isomers, the intensity of the “cis-peaks” may be greater or lesser. The spectra we recorded (Figure 12) could be the result of a mixture of different isomers in an unknown percentage. However, given the low intensity of the so-called “cis-peaks”, it can be hypothesized that trans isomer seems to be the predominant

one in our extracts.
Literature data concerning the compositional analysis of the pulp of different watermelon, report that lycopene is the carotene present in greater quantity, followed by a significantly lower amount of β-carotene [24, 25].
This means that we cannot exclude the presence of other carotenes and carotenoids within our watermelon fibre samples although, based on our UV-Vis spectra, they do not appear to produce an observable contribution. It is reasonable to assume that if these analytes are present, their amounts are significantly lower than that of lycopene and, therefore, not easily detectable by this spectroscopic technique. We can than state that the analyte which mainly contributes to the red color of watermelon flesh is lycopene. Based on these considerations, even if we do not exclude the possibility that other carotenoids (or pigments in general) are present, albeit in very modest quantities, we will refer only to lycopene.
From the UV-Vis spectra of the hexane extracts obtained from the TDFs from each watermelon cultivars, it was possible to carry out a semi-quantitative estimation of lycopene within each sample and then make a comparison between the three selected varieties. This technique has already been used for this purpose, as it is a non-destructive, fast and performing analysis [26, 27, 28].
However, several approximations are necessary to achieve the results, including that:

- only lycopene has been extracted or, at least, this analyte is not subjected to interference from other molecules which absorb at the same wavelength used for the semi-quantitative estimate;
- all the extracted lycopene is present in all-trans form, since the molar extinction coefficients reported in the literature and used for calculations refer to this isomer.

Finally, we specify that we didn't use a standard lycopene reagent since it is a very thermodynamically unstable molecule, highly sensitive to light exposure, heat, oxidizing species and ageing, which cause its isomerization and degradation. The acquisition of a standard would have been of doubtful utility as it would not have been certain the concentration and degree of isomerization at the time of performance of the tests. Therefore, the semi-quantitative analysis was carried out using Lambert-Beer's law, selecting the 503 nm wavelength, i.e. the longest λ peak in the visible region. This wavelength has been previously used in the literature for these purposes [24, 28].

The molar extinction coefficient of lycopene at 471 nm would allow for greater sensitivity, however, the measurements could be partially interfered by the presence of other carotenes / carotenoids in watermelon pomace. Some of them, in fact, at 471 nm show non-negligible absorbance and molar extinction coefficient values (Figure 14). For this reason, the absorption band centered at the highest λ (503 - 506 nm) is generally taken as a reference absorption to discriminate lycopene from other carotenoids. Despite carotenoids show very similar spectral profiles, normally absorb at wavelengths slightly lower than the values previously cited (Figure 14). The 503 nm absorption band, being the least interfered in the case of a mixture of carotenoids, is used to carry out quantitative measurements of lycopene by spectrophotometry. [25, 28, 29].

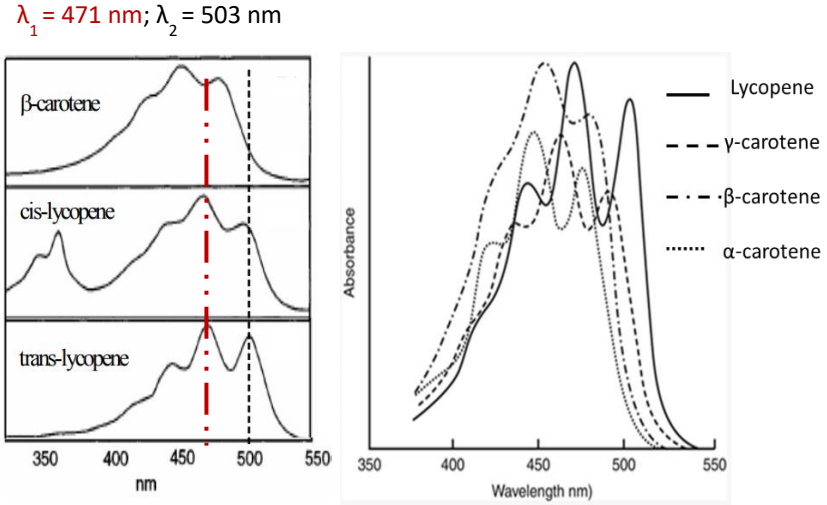

Figure 14 – Comparison among UV-Vis absorption spectra of different carotenes extracted in organic solvents [27, 29].

Not being able to exclude the presence of other carotenes / carotenoids, for our purposes, we have conducted the semi-quantitative estimation of lycopene content selecting λ = 503 nm and applying the Lambert-Beer's law (equation reported in Chapter 4). For the calculations, the value of $\varepsilon = 1.72 \times 10^5\ M^{-1}cm^{-1}$ has been selected [27, 30, 31]. Following literature suggestions, we choose to refer it to all-trans lycopene form.
The semi-quantitative results obtained are collected in Table 2, relating to extracts in n-hexane from TDFs, vacuum-freeze dried (W_AI_V), at t_0 time, for each of the three selected watermelon cultivars.

Table 2 - Semi-quantitative values# of lycopene content in the pulp fraction of the three selected watermelon varieties, calculated according to ε values reported in the literature [27, 30, 31].

	Lycopene content (mg/Kg fw)*
Crimson Sweet	98 (95 – 104)
Asahi Miyako	139 (131 – 143)
Gavina	114 (105 – 124)

*Data are expressed as mg lycopene/Kg fresh watermelon pulp
#data indicates statistical dispersion intervals

Values collected in Table 2 are perfectly comparable with some literature data related to many different watermelon cultivars, including hybrids and seedless ones: 40-120 mg(lyc)/kg (fw)[32], 2.65-151.75 mg/kg (fw)[33] e 144.27 mg/Kg (fw)[34]. Small differences between the three selected varieties also emerge from our results, but it is well known that genotype factors, methods and place of cultivation, degree of ripeness and meteoclimatic factors, among others, contribute to define the compositional characteristics of watermelon fruits (including lycopene content).

Thermal Analysis

Since the 80s, several studies have demonstrated how the consumption of foods rich in dietary fiber (DF) significantly reduces the risk of developing chronic diseases, mainly thanks to the reduction of cholesterol [35] and glucose levels in the blood [36], as well as providing beneficial effects that can potentially reduce risk factors for other related diseases [37].
For these reasons, in recent years, the recommended daily intake of DF has been increased to 25-30g for a 2000 kcal diet. This need has become a priority especially in Western countries with a high rate of development. Here the daily diet can lead to imbalances between ingested kcal and DF content, with potentially serious consequences on the quality of life (e.g. obesity and related pathological dysfunctions). Consequently, many industries along the supply chains producing packaged foods, tend to properly increase the DF content to satisfy the recommended and indispensable health requirements. Parallel to these new needs, the international research community has been questioning for a long time about how to express some indications relating to the composition of the DF fraction, since various definitions and alternative determination methodologies have been proposed [38 - 40].

For the purposes of this work, we preferred to consider the TDF fraction obtained by drying the whole pulp, after having squeezed and removed the clear juice. The fiber thus obtained maintains a content of different components (SDF, IDF, mineral salts, lycopene and other nutraceutical components present in traces) which constitute a complex matrix, still representative of the initial product.
The thermal methods of analysis applied to pomace fibers obtained from the endocarp of Gavina watermelon have produced the results we are going to examine in detail below.
Figure 15 reports the thermograms related to a sample of Gavina TDF, vacuum-freeze dried (W_AI_V), heated in an inert atmosphere (He).
Table 3 collects the representative elements of the thermal profiles showed in Figure 15 as well as some descriptions of the thermal processes associated to the structural deformations which occur within 5 large intervals identified.

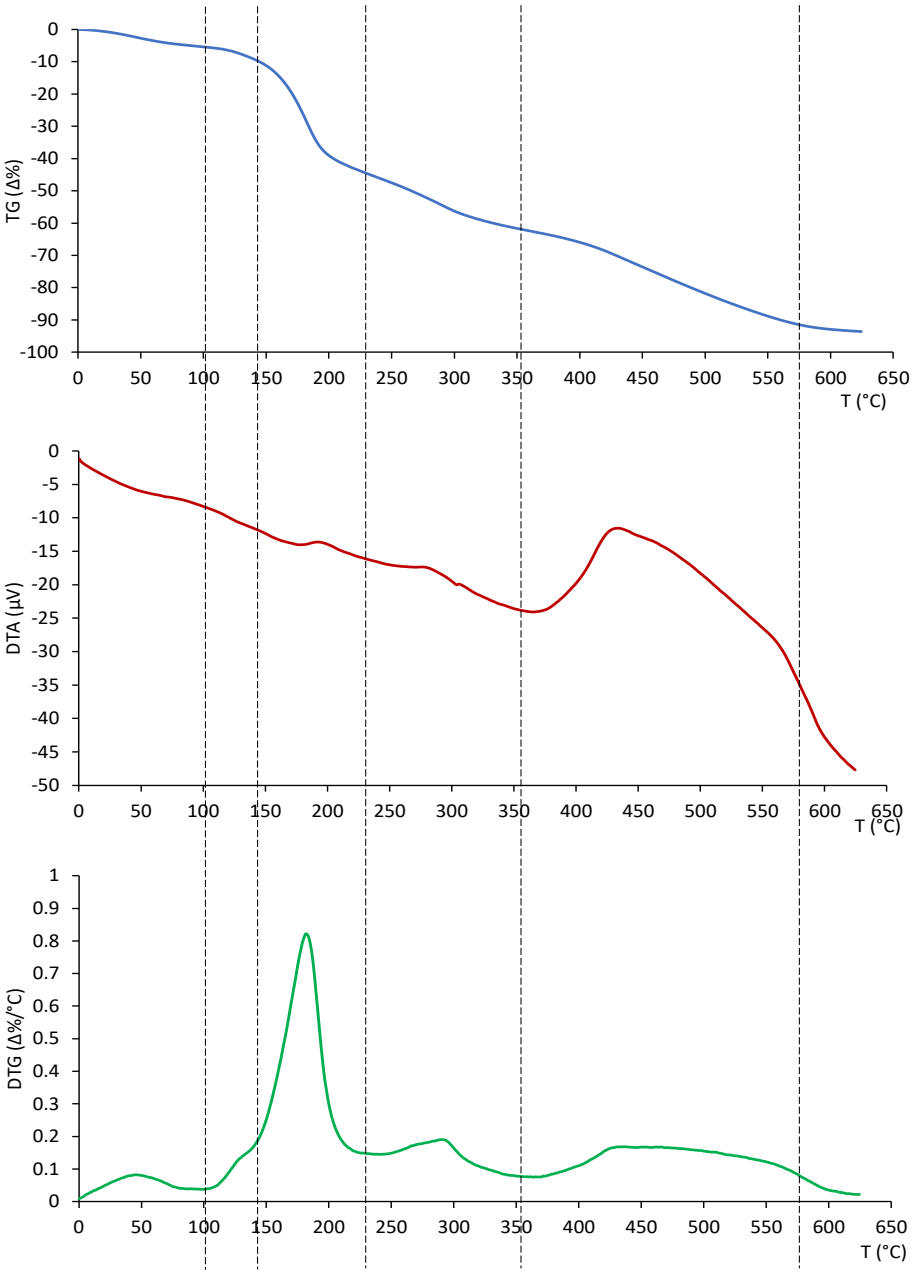

Figure 15 - TG, DTG and DTA thermal analysis profiles for a sample of TDF (W_AI_V) from Gavina watermelon pomace, measured in He.

Table 3 - Representative values of TG/DTG/DTA profiles of Figure 15, obtained in an inert atmosphere (He).

Peak	T_o /°C	T_p /°C	T_c /°C	Δm%	Processes
1	20	47.7	105	- 6.1	Elimination of VOCs and moisture
2	105	128.2	140.1	- 3.1	Elimination of bound water, NH_3 from glycoproteins, low-boiling VOCs, CO and CO_2, caramelization of polysaccharides
3	140.1	181.9	238.5	- 34.2	
4	238.5	289.2	356.3	- 19.3	Elimination of constitution water, nitrogen compounds, VOCs and SVOCs, loss of CO and CO_2, degradation of polysaccharides, plasticization of the mass and vitrification of the sample
5	356.3	426.4	570	- 26.8	Elimination of hydrocarbons and volatilization of carbon residues C6-C20, crystallization of residual material
---	570	---	630	-3.4	Elimination of hydrocarbons and volatilization of carbon residues C20-C40
residue at 630 °C = 7.1 %					Inorganic compounds and carbon residues

T_o = Onset Temperature (peak start)
T_p = Peak temperature (central T value of the peak)
T_c = Closure Temperature (end of peak)

- → 1st interval (0 <T/°C < ~105) - Heating to 105 °C leads to the elimination of residual moisture and of some VOC species with high vapor pressure.
- → 2nd interval (~105 <T/°C < ~240) - In this window a modest signal is present, centered at ~128°C with Δm% = -3.1%, which probably underlies the elimination of water strongly bound to inorganic compounds. This signal appears as a shoulder of peak 3. Peak 3 is the most representative of the entire thermogram, centered at ~182°C and associated to Δm% = -34.2%. In this region, structural deformations attributable to pectins and hemicelluloses prevail, in presence of a network of specific thermal interactions which also involve other polysaccharides such as sugars and starches [41].
- → 3rd interval (~240 < T/°C < ~360) - Peak 4 is probably attributable to structural deformations of the soluble compounds, such as monosaccharides and simple sugars, oligosaccharides and other analytes which can exert some thermal stress actions capable of compressing the deformations of pectins and hemicelluloses at lower temperatures (inside the 2nd interval). A similar behavior had already been observed for the fibers obtained from melon and described in Chapter 5. The mass loss (Δm% = -19.3%) is probably associated with partial or total plasticization and vitrification of some components of the sample, or of the whole one [42, 43].
- → 4th interval (~360 < T/°C < ~570) - This large interval underlies a gradual and progressive deformation of the thermal profiles of Figure 15, showing a very broad band, without any defined peak. The DTA curve seems to confirm that the overall process is exothermic, although the variety of analytes present may suggest some differentiation of behavior.
- → 5th interval: (~570 < T/°C < ~630) - The last stage of the programmed thermal run allows the probable elimination and volatilization of carbon residues (C20-C40), leaving final ashes constituted of inorganic compounds and some carbon deposits, equivalent to 7.1%.

Below we report the results obtained from the thermal analyses conducted on Gavina pomace fibers subjected to effective washing operations with distilled water to obtain the IDF (Insoluble Dietary Fiber).
Figure 16 shows the TGA/DTG/DTA thermograms of a sample of Gavina IDF, vacuum-freeze dried (W_*w*_V), heated in an inert atmosphere (He).

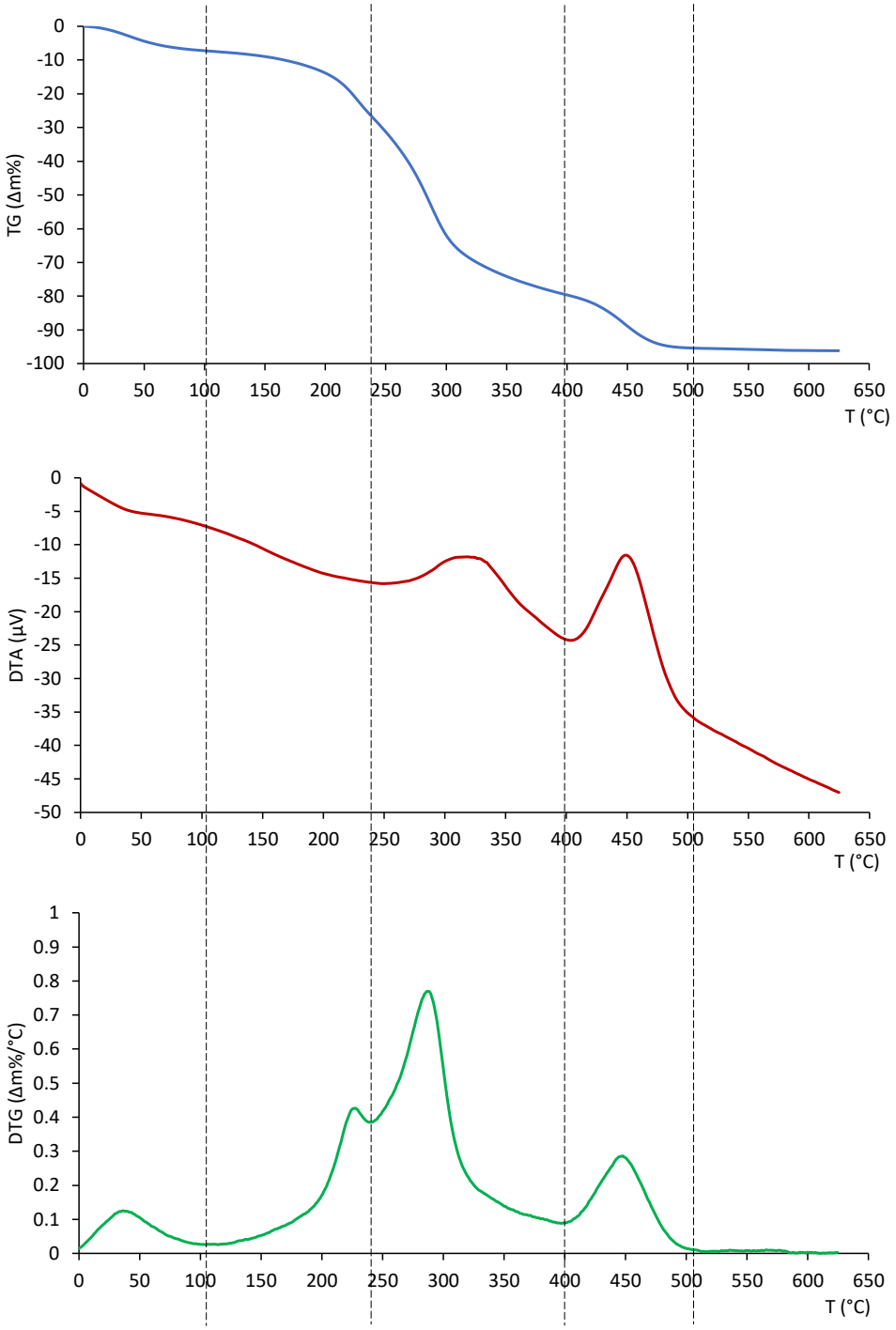

Figure 16 - TGA, DTG and DTA thermal analysis profiles for a sample of IDF (W_w_V) from Gavina watermelon pomace, measured in He.

The interpretative reading of the thermal profiles shown in Figure 16 can be conducted following the same methods already applied for melon fibers (Chapter 5). The entire thermal window has been divided into 4 temperature ranges where the most significant processes occur. In the last interval only very modest deformations are observed in the sample, which reaches the condition of ash mineral residue.

Table 4 - Representative values of TG/DTG/DTA profiles of Figure 16, obtained in an inert atmosphere (He).

Peak	To /°C	Tp /°C	Tc /°C	Δm%	Processes
1	20	32.3	104.4	- 8.0	Elimination of VOCs and moisture
2	104.4	226.6	241.5	-17.1	Elimination of bound water, NH_3 from glycoproteins, low boiling VOCs, loss of CO and CO_2, caramelization of sugars
3	241.5	286.2	399.5	-54.9	Elimination of constitution water, NH_3, VOCs and SVOCs, decarboxylation of acids with CO_2 loss, degradation of polysaccharides, plasticization and vitrification of the sample
4	399.5	445.7	506.9	-15.3	Elimination of hydrocarbons and volatilization of carbon residues (C6-C20)
---	506.9	---	630	- 0.2	Volatilization of carbon residues (C20-C40)
Residue at 630 °C = 4.3%					Inorganic material and carbon residues

T_o = Onset Temperature (peak start)
T_p = Peak temperature (central T value of the peak)
T_c = Closure Temperature (end of peak)

- → 1st interval: (0 < T/°C < ~105). In this window the elimination of residual moisture (Δm% = -8%) is observed, as well as that of some VOCs having high vapor pressure. Comparing this peak with the analog one of Figure 15 (Δm% = -6.1%), it can be observed that the mass loss for the "washed" sample (IDF) is about 2% higher. The data is perfectly compatible with the fact that the "as it is" fiber (TDF) retains a greater amount of mineral salts, analytes with a marked tendency to retain crystallization water which will be eliminated at higher temperatures.
- → 2nd interval: (~105 < T/°C < ~240) - The washed fiber of Gavina watermelon, losing its soluble components, presents constitutive characteristics which make its behavior very similar to the IDF fraction already observed and described for melon. Peak 2, located in a borderline position at the edge of the interval and not well resolved being the shoulder signal of the next peak, is centered at ~226°C and can be mainly attributed to the pectin fraction [44]. Sample degradation processes can involve elimination of VOCs, as well as low MW molecules such as strongly bound water (especially to inorganic compounds), water of crystallization, constitutional water, NH_3 from glycoproteins, CO and CO_2 from caramelization of sugars and decarboxylation of acids, etc.
- → 3rd interval: (~240 <T/°C <~400) - Peak 3, mainly attributable to structural deformations of hemicelluloses, extends over the entire thermal range and underlies the most consistent mass loss (Δm% = -54.9). Given the complexity of the matrix, the set of thermal processes which take place in the interval shows cumulative endothermic effects, even if the individual processes could generate both exothermic and endothermic contributions. The tail of the peak probably underlies deformations attributable to partial or total plasticization, caramelization and vitrification processes of the sample mass [45].
- → 4th interval: (~400 < T/°C < ~510) - Peak 4 is present in this thermal window, it is exothermic for the probable crystallization of previously plasticized compounds, with mass loss Δm% = -15.3%. In this thermal range the elimination processes of highly degraded compounds are completed, by volatilization of heavy hydrocarbons and carbon residues (C20-C40), typical polycyclic aromatic compounds. The peak closes on the TGA curve with a very slow descent which opens onto the next window.
- → 5th interval: (~510 < T/°C < 630) – In this thermal window, a very modest mass lost occurs, ~0.2%, with a progression that extends on the entire range in which the TGA and DTG thermal profiles flow in a plateau, until the closure of the analysis at 630°C. The final mass consists of mineral compounds and carbon residues.

Finally, as previously done for Cucumis Melo pomace fibers, we report the comparison between some thermal profiles related to Gavina's pomace fibers.
The parallel comparison of DTG curves obtained for the TDF and IDF samples from Gavina watermelon (Figure 17), underlines some significant differences already partially reported in the discussion of the experimental results in the previous paragraphs.

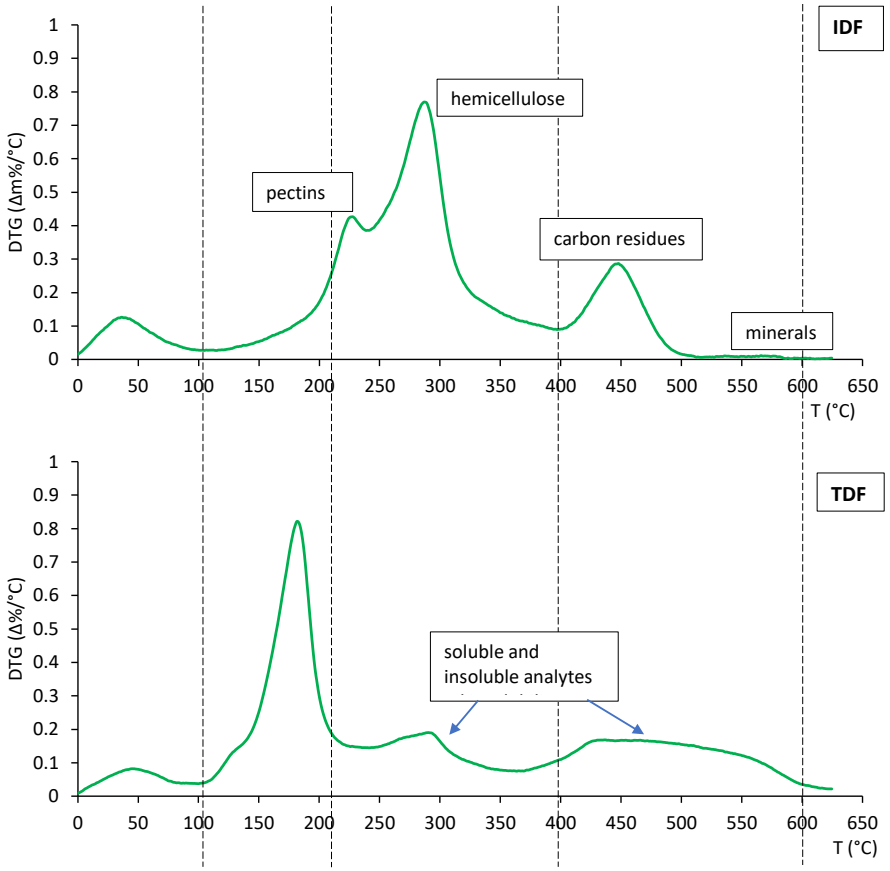

Figure 17 - DTG thermal analysis profiles for IDF (W_w_V) and TDF (W_AI_V) samples from Gavina (in He).

The presence of soluble components in TDF sample seems to favor the appearance of a network of specific thermal interactions which compresses both heat stress deformation peaks related to pectins and hemicelluloses within the 2nd temperature range (~105 – 240°C). For IDF sample, the peak generated by pectins rests on the upper limit of the 2nd thermal window (T_p_IDF = ~226°C), while the peak related to hemicelluloses (T_p_IDF = ~286°C) is present in the 3rd interval (~240-400°C). By evaluating the thermal shift of Peak 3 related to hemicelluloses (Figure 17), a ΔT~104°C is detected, with a subsequent strong stabilization of xylanoid compounds in the absence of the SDF fraction during the pyrolytic processes of the sample.

Peak 4 detected in the TDF sample is not present in the thermal profile of the IDF specimen. Moreover, the wide band present between 400 – 600 °C in TDF sample, is converted in a more defined peak in IDF one. Evidently, Peaks 4 and 5 in TDF specimen underlie structural deformations compatible with the presence of soluble fraction (monosaccharides, oligosaccharides, mineral salts, among others), consisting of analytes that provide strong thermal interactions during pyrolytic processes.
Mixtures of different compounds favor the plasticization of the mass during the heating step, significantly reducing the melting temperature due to the formation of eutectic phases. Polysaccharides and other soluble analytes tend to caramelize with heating. They transform themselves into plastic and pitchy matrices which certainly also involve other insoluble species such as pectins and hemicelluloses, favor the decomposition of insoluble constituents at lower temperatures in the TDF fraction.

In conclusion, the IDF fraction from Gavina watermelon pomace is much more stable than the corresponding TDF one towards medium high temperature heating. In perfect accordance with what has been previously reported for melon's fibers, it follows that IDFs are particularly recommended as a formulation ingredient, since they can better withstand thermal stresses without undergoing significant structural deformations and thermally activated decompositions [46].

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CHAPTER 8
8 - VOLATILE AROMAS OF WATERMELON FIBERS

8.1. BACKGROUND

The analysis of the volatile fraction of food is essential for understanding, planning or improve the sensory characteristics of new possible products [1]. The aromas of fruit and vegetables strictly depend on their chemical composition, as well as on any possible constitutive variation of the molecular bouquet [2,3]. Furthermore, their odor is determined by unique combinations of volatile species with different physico-chemical properties and different perceptual thresholds [4]. These impart specific quality characteristics and represent a valuable sensory parameter of extreme interest, especially in the food industry. In fact, the information deriving from the measurement and the aroma identification of a food product, is directly related to its acceptability by consumers, in addition to its nutritional and qualitative characteristics, also in terms of healthiness and safety of the product itself [3]. Furthermore, in the specific case of fruits, they allow to identify and highlight any difference between various cultivars of the same species, due to geographical origin, cultivation site, production methods, harvesting technologies, degree of ripeness and aging [2,3,5]. The identification of the volatile analytes responsible for the aroma of a food product is very important, since it allows to detect the possible presence of compounds capable of altering the matrix from an organoleptic point of view. By monitoring the composition of the VOC fraction over time, it is possible to achieve various objectives. For example, it is possible to hypothesize, or possibly define the origin of the food, as well as to establish its authenticity and product traceability. These food control activities can become more effective the more it is possible to identify the molecules that can take on the role of markers of quality, or uniqueness. Furthermore, some particular analytes may develop over time, reflecting the age of the product. In addition, they could result from the preparative manipulations to which the material is subjected (i.e. more or less prolonged thermal stress, sterilization, cooking, drying, etc.). This kind of study is very important because, among the countless VOC analytes, some of these are not particularly desired, since they impart unpleasant aromas (the "off-flavors"), compromising the acceptability of the product by the final consumers [6-9]. From numerous literature papers concerning the volatile fraction of watermelon (*Citrullus Lanatus*), it emerges that its aroma originates from a combination of different classes of molecules: esters, alcohols, organic acids, aldehydes, ketones, lactones, some phenols, heterocyclic compounds, terpenoids and volatile apocarotenoids [3–5,10]. Furthermore, the great variety of molecules that define the aromas in vegetables is the result of different metabolic pathways, biosynthetic or degradative, which involve lipids, amino acids, carotenoids, among others, giving rise to a rich and complex VOCs population. Bearing this in mind, the following study aims to analyze the VOCs of a set of Gavina® watermelon fiber samples, by applying the SPME-GC-MS technique. We started immediately after obtaining the fiber itself (t_0) and we investigated its evolution over time, considering two aging steps: 30 days (t_1) and 24 months (t_2) of storage. The compositional stability and resistance to aging processes of the watermelon fibers was evaluated both in macroscopic form following the time-evolution of the color, but also in microscopic-molecular form through the variation of the VOC fraction. For example, when β-carotene and lycopene degrade, they release numerous volatile analytes, the identification of which indicates the loss of these valuable bioactive components. It should be noted that, for this investigation, we have processed only specimens prepared with vacuum-freeze dried fibers. The choice is consistent because, even if carried out at a low temperature (40°C), it is unavoidable that oven drying involves a massive loss of the volatile fraction. In addition, the thermal stress of the sample favors and accelerates the degradation processes of functional molecules, thus reducing the representativeness of the initial matrix. Limited to the initial stage of investigation at t_0 time, the same

procedure was applied in parallel on the vacuum-freeze dried fiber samples of the three different cultivars examined: Gavina®, Crimson Sweet and Miyako. The objective of this comparative survey consists in the characterization of the VOCs of the starting materials, to identify possible common markers or any analytes that differentiate the cultivars, respectively.

8.2. MATERIALS AND METHODS

Fiber samples preparation

Since in this chapter we are dealing with samples originating only from watermelons but representative of three different cultivars, it was decided to adopt an alternative formalism to identify the processed samples. In the following scheme we report the identifying acronyms of the obtained watermelon-based fibers samples.

G_AI_V	Gavina pomace "as it is", Vacuum-freeze dried
C_AI_V	Crimson pomace "as it is", Vacuum-freeze dried
Mi_AI_V	Asahi Miyako pomace "as it is", Vacuum-freeze dried

Scheme 1 – List of the identifying acronyms of watermelon-based fibers samples.

For each cultivar, dried watermelon pomace fiber samples were prepared as reported in Chapters 4 and 7. The resulting fibers were homogenized in a grinding mill equipped with a rotor made in Ti and a sieve with 500 µm mesh, maintaining the equipment at low temperature by refrigerating it with some few drops of liquid N_2. This strategy has been adopted in order to preserve as much as possible the aromatic characteristics of the fibers. Then a consistent set of samples were prepared as follows: about 0.5 g of material were transferred in 10 mL glass vials which were sealed tightly with Teflon/silicone septa.
As previously mentioned, the fiber of each variety was analyzed at t_0 time (G_AI_V, C_AI_V, Mi_AI_V) while for Gavina the study continued over time, with the analysis of the VOCs after 30 days (G_AI_V_t_1) and after 24 months (G_AI_V_t_2) of storage, preserving the samples in the dark, at room temperature and in the same closed vials, without ever opening them during the ageing period.
For each cultivar, repeatability and reproducibility of experimental procedures have been established working with at least three replicated samples of the same matrix and making at least three different measures for each vial.

Chemicals

The chemicals used during the study are the same described in Chapter 6.

HS-SPME-GC-MS analysis

The extraction of volatile compounds was performed by manually exposing a 2 cm long SPME fiber to the headspace of each prepared vial, according to the procedure explained in Chapter 4. The volatile compounds desorbed from the SPME fiber have been analyzed through a GC – MS, Agilent Technologies 6890N Network gas chromatograph.
The oven temperature was programmed from 40 °C (held for 5 min) to 160 °C with a heating rate of 10 °C/min and then from 160 °C to 270 °C with a heating rate of 8 °C/min, while the transfer line was heated to 270 °C. The final temperature of 270 °C was held for 5 minutes.
All the other instrumental setup are described in detail in Chapter 4.
The estimation of the amount of each volatile identified in the SPME-GC-MS analysis was expressed as the Total Ion Current (TIC) peak area.

8.3. RESULTS AND DISCUSSION

- **Aroma characterization of Gavina® pomace fibers over time**

Figures 1a, 1b, 1c show the chromatograms obtained by HS-SPME-GC-MS from a sample of Gavina watermelon red fiber at t_0 time, after 30 days (t_1) and after 24 months (t_2), respectively.

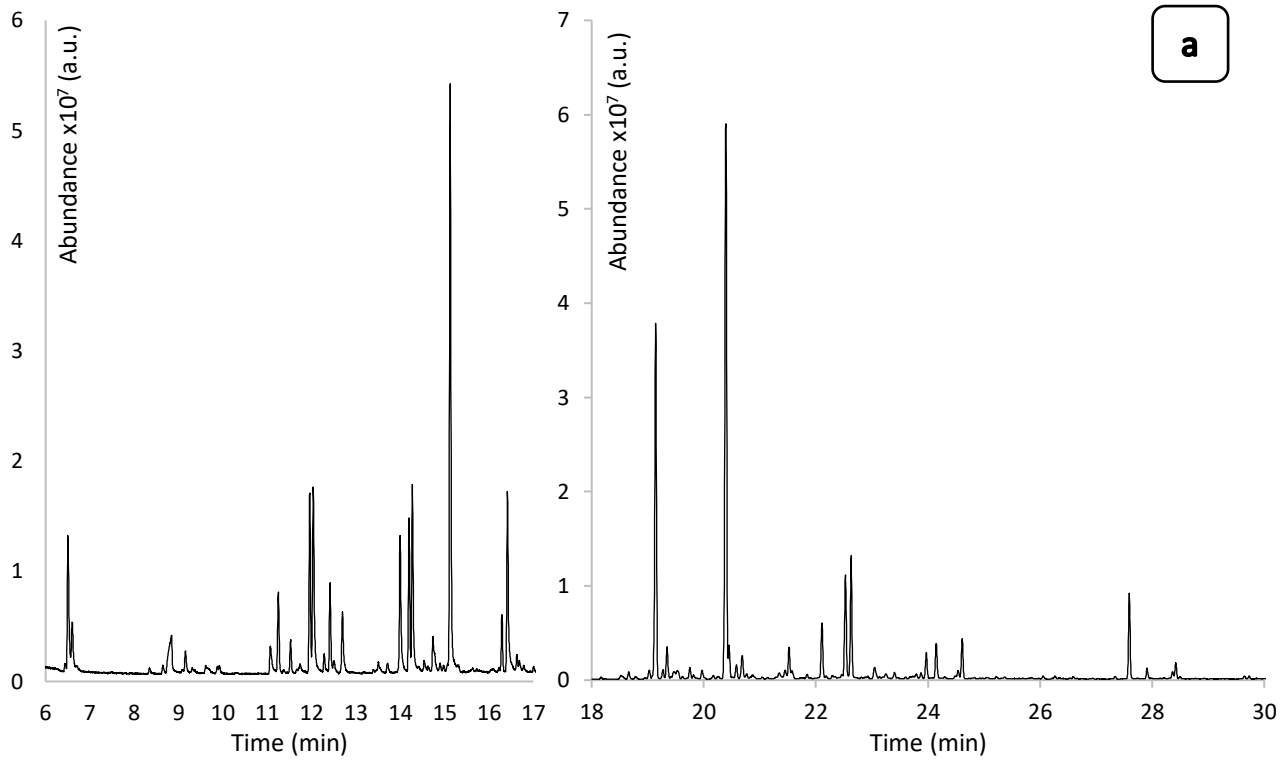

Figure 1a - Chromatogram obtained by HS-SPME-GC-MS from a sample of Gavina watermelon red fiber at t_0 time (G_AI_V_t_0).

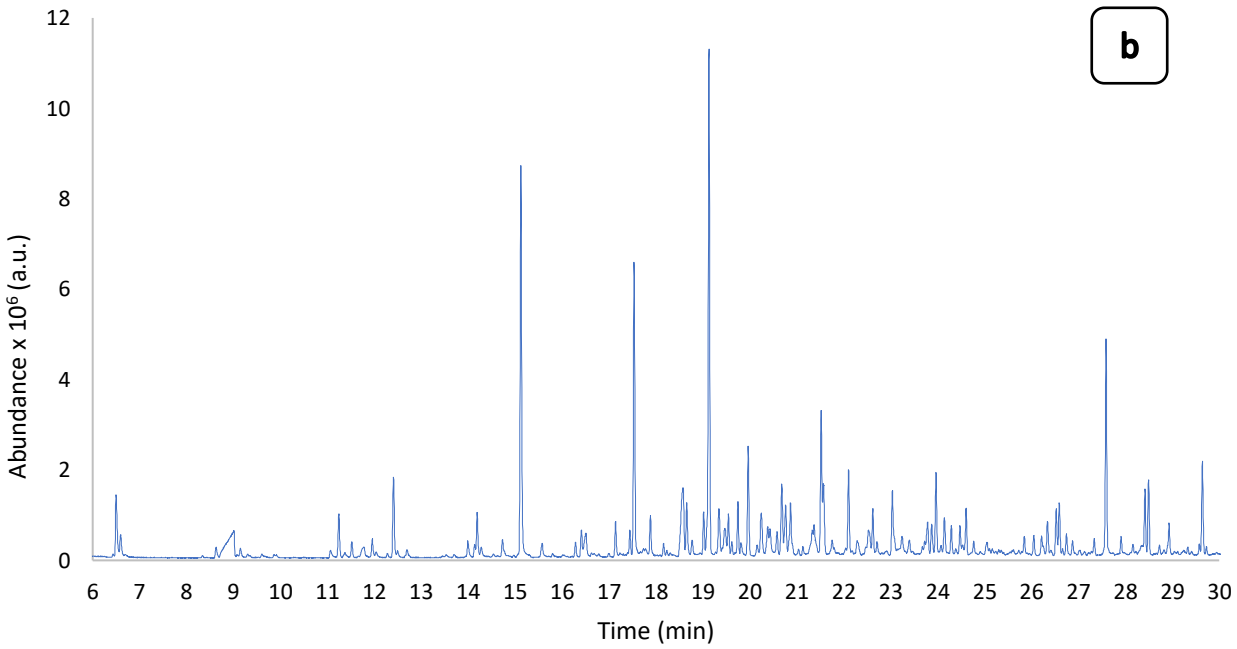

Figure 1b - Chromatogram obtained by HS-SPME-GC-MS from a sample of Gavina watermelon red fiber after 30 days (t_1) of ageing.

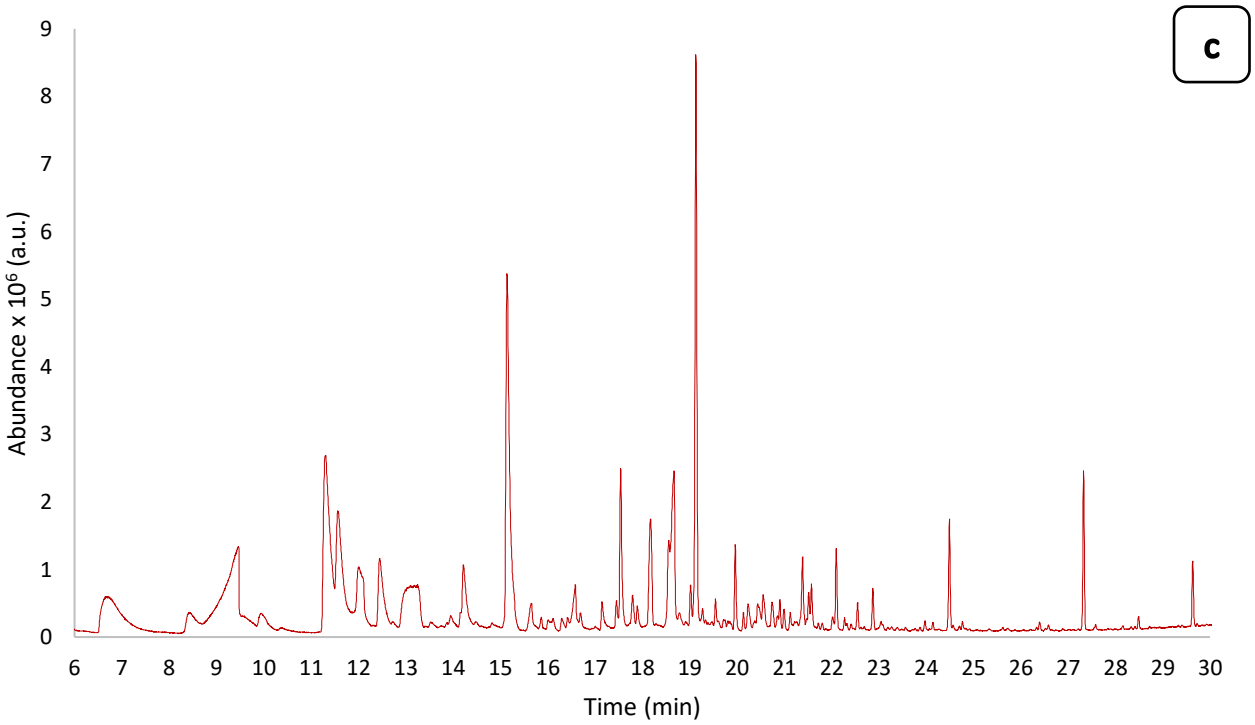

Figure 1c - Chromatogram obtained by HS-SPME-GC-MS from a sample of Gavina watermelon red fiber after 24 months (t_2) of storage (G_AI_V_t_2).

For a better representation of the experimental results of the chromatographic runs relative to the TDF sample of Figure 1a, we have partitioned the profile into two sections, with expansion of the scales to visualize also the peaks of lesser intensity. By comparing the scales on the ordinate for the three runs, a general decrease in the analytical signals is observed with the increase of ageing time of the specimens themselves.

In Figure 1a (G_AI_V_t_0), 102 compounds were detected, including 34 aldehydes, 15 alcohols, 1 ester, 8 ketones, 2 acids, 7 hydrocarbons, 25 terpenes and derivatives, 10 other compounds.
After 30 days of ageing (G_AI_V_t_1), 88 compounds were identified, among which 27 aldehydes, 14 alcohols, 2 esters, 10 ketones, 7 acids, 3 hydrocarbons, 16 terpenes and derivatives, 9 other compounds.
Finally, in the sample stored for 24 months (G_AI_V_t_2), just 44 analytes were determined, including 9 aldehydes, 7 alcohols, 2 esters, 2 ketones, 7 acids, 1 hydrocarbon, 10 terpenes and derivatives, 6 other compounds. These informations are schematically reported below.

Compound class	Gavina® t_0	Gavina® t_1	Gavina® t_2
ALC	15	14	7
EST	1	2	2
KET	8	10	2
OTH	10	9	6
AHA	7	3	1
ALD	34	27	9
ACD	2	7	7
TER	25	16	10

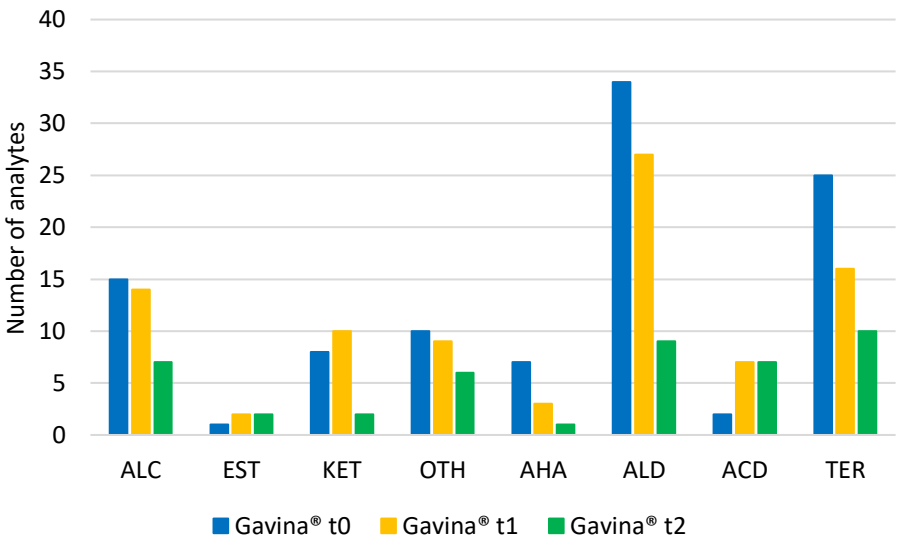

Figure 2 – Histogram representation of the number of analytes belonging to each class, for the G_AI_V sample at different aging times.

The identified analytes are listed in the tables below, divided into their chemical classes, in order to allow a better interpretation of the obtained results. The tables show, for each peak of each analyte, the value of the absolute area (TIC) and the percentage area calculated with respect to the total sum of the area of each identified chromatographic peak (counts relating to the Total Ionic Current, TIC). The value of the absolute peak area allows to make a comparison between different chromatographic runs performed under the same conditions, while that of the relative percentage area allows to understand which analytes are predominant in the HS of the same sample.

Aldehydes

Aldehydes are the prevalent species at t_0 time, for a total of 34 molecules out of 102 detected, with an overall relative percentage area of 23.3%. From a preliminary analysis it can be said that, during storage, a decrease in the number of identified molecules takes place (27 at t_1, 9 at t_2), but the relative percentage area undergoes a progressive increase, reaching 28.6% after 30 days and 38.2% after 24 months. Therefore, some aldehydes during storage become the species that most characterize the aroma of the samples G_AI_V, probably due to the combined effect of the increase in their abundance following the degradation of complex molecules from which they originated. As a consequence, we note the decrease in the abundance of other unstable VOCs, thus reducing over time the contribution to the overall aromatic bouquet of the analyzed matrix.

Table 1 - Composition of the volatile fraction of Gavina watermelon pomace fiber over time: compounds identified by HS-SPME-GC-MS analysis – Aldehydes class

		t_0		t_1 (30 days)		t_2 (24 months)		
Analyte	**Aroma**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Ref**
Acetaldehyde	Pungent, fresh, penetrating, fruity	54.6 1.15%	435	3.96 0.19%	435	-	-	[2, 10–14]
Propanal	Ethereal, pungent, fruity	13.5 0.28%	498	12.5 0.59%	498	-	-	[11, 14]
2-Methyl-propanal	Fresh, aldehydic, floral, green	1.57 0.03%	559	1.17 0.06	559	48.1 1.95%	562	-
(Z)-2-Butenal	-	2.31 0.05%	570	6.55 0.31%	569	-	-	-
Butanal	Pungent, cocoa, green, malty	2.06 0.04%	593	2.25 0.11%	593	-	-	-
(E)-2-Butenal	Green, fruity	8.18 0.17%	655	6.49 0.31%	655	-	-	[5, 10, 11, 13]
3-Methyl-butanal	Ethereal,chocolate peach, fatty	17.2 0.36%	661	22.1 1.04%	661	257 10.43%	663	[5, 15, 16]
2-Methyl-butanal	Chocolate, nutty, furfural	7.19 0.15%	671	8.55 0.40%	671	195 7.93%	673	[5, 15]
Pentanal	Fermented,fruity, berry nuances	19.0 0.40%	703	40.3 1.90%	702	93.5 3.80%	704	[5,10,11, 15,17,18]
2-Methyl-2-butenal	Pungent, green, penetrating, anisic	2.79 0.06%	748	2.19 0.1%	748	-	-	-
(E)-2-Pentenal	Pungent, green, fruity, apple	33.6 0.71%	758	9.52 0.45%	758	-	-	[5, 10–15, 17]
3-Methyl-2-butenal	Sweet, fruity, pungent, nutty	9.29 0.20%	784	12.6 0.59%	784	-	-	-
Hexanal	Green, fatty,fruity, woody nuance	119 2.51%	798	196 9.2%	798	298 12.13%	799	[2,5,10–13,17,18]
2-Ethyl-3-methylbutanal	-	-	-	-	-	8.17 0.33%	833	-
(Z)-2-Hexenal	Green	1.06 0.02%	837	-	-	-	-	[5]
(E)-2-Hexenal	Green leaf, almond, fruity	42.2 0.89%	843	14.1 0.66%	843	-	-	[5,10,11,13, 14,17–19]
4-Heptenal	-	3.25 0.07%	878	2.43 0.11%	878	-	-	[10 - 13]
Heptanal	Fresh, fatty, green, grass	11.9 0.25%	880	12.6 0.59%	880	13.9 0.56%	880	[5,10–13, 15,16]
(E,E)-2,4-Hexadienal	Green, fruity, citrus, waxy	3.36 0.07%	890	5.41 0.25%	890	-	-	[10, 15]
(E)-2-Heptenal	Green, fatty, oily, fruity	18.7 0.39%	923	29 1.36%	923	-	-	[5,10–13, 17, 18]
Octanal	Orange, waxy, green peel	7.35 0.16%	954	22.0 1.03%	954	13.3 0.54%	954	[5,10,11,13, 15,17,18]
(E,E)-2,4-Heptadienal	Fatty, sweet, melon, spicy	10.9 0.23%	963	8.04 0.38%	963	-	-	[5,10,11,13, 15,17]
Benezenacetaldehyde	Honey, sweet, rose floral, talc	64.7 1.36%	994	41.1 1.93%	994	-	-	[11, 13]
(E)-4-Nonenal	Fruity	18.1 0.38%	1017	-	-	-	-	[5, 10, 13, 15]
Nonanal	Waxy, citrus, lemon, cucumber	74.2 1.56%	1023	70.9 3.33%	1023	12.9 0.53%	1023	[2,5,10, 15–18,20]
(Z,Z)-3,6-Nonadienal	Fresh-cut watermelon	7.88 0.17%	1046	-	-	-	-	[11–13, 16,17, 19]
(Z)-2-Nonenal	Cucumber, fatty, waxy	6.91 0.15%	1052	-	-	-	-	[5,12-16,19]
(E,E)-2,6-Nonadienal	Melon, cucumber, fatty nuances	4.47 0.09%	1054	-	-	-	-	[5,12,15]
(E,Z)-2,6-Nonadienal	Cucumber, melon, green, fatty	237 4.98%	1059	21.7 1.02%	-	-	-	[2,10,12,13, 15–17,20]
(E)-2-Nonenal	Fatty,green,melo-ne, cucumber, waxy	266 5.59%	1062	23.4 1.10%	1062	-	-	[2, 5, 10–14,20]
Decanal	Zest, waxy, orange, floral	19.7 0.41%	1090	11.7 0.55	1090	-	-	[5, 11,15]
(E,E)-2,4-Nonadienal	Cucumber, melon, waxy, citrus	6.56 0.14%	1101	6.64 0.31%	1101	-	-	[5, 10, 11,13,16]
(E)-2-Decenal	Waxy, fatty, earthy, coriander	8.67 0.18%	1128	15 0.70%	1128	-	-	[5,13,15, 20]

(E,Z)-2,4-Decadienal	Fatty, orange, citrus, fresh	4.66 0.10%	1165	-	-	-	-	[5, 11, 13,17]
Dodecanal	Soapy, waxy, citrus, floral	2.36 0.05%	1214	-	-	-	-	[5]

The C9 aldehydes, identified in different species belonging to the *Cucurbitaceae* family, are considered responsible for their characteristic flavor and aroma [2,3,11,13,19–21]. In particular, in our samples, we have identified 4-nonenal, nonanal, (Z,Z)-3,6-nonadienal, (Z)-2-nonenal, (E,E)-2,6-nonadienal, (E)-2-nonenal and 2,4-nonadienal, analytes already reported in previous studies about analogous matrices [13,16,22]. It's reasonable to state that they are among the species that mainly contribute and define the characteristic and pleasant aroma of watermelon, since they are present in rather significant quantities in the sample analyzed at t_0 time. This observation perfectly agrees with what is reported in the previously cited literature. The other aldehydes, on the other hand, are considered to contribute to a lesser but not negligible extent [11,16]. In particular, we identified the presence of acetaldehyde, 3-methyl- and 2-methyl-butanal, pentanal, hexanal, heptanal, octanal, benzenacetaldehyde, all molecules with very pleasant fragrances, that enrich and complete the aroma of the watermelon pulp's fiber.

At a biological level, the aliphatic aldehydes C9 are produced following the enzymatic oxidation of polyunsaturated fatty acids (PUFA), in particular linolenic and linoleic acids, by lipoxygenase [5,19]. This radical process involves the formation of C9 hydroperoxides, highly unstable and subject to degradation, typical precursors of aldehydes mainly unsaturated.
In order to confirm this phenomenon, several studies report the correlation between the concentration increase of 2-nonenal and 2,6-nonadienal and the concentration decrease of linoleic and linolenic acid. Other enzymes, such as alcohol dehydrogenase and cis/trans isomerase, then convert these compounds to the corresponding alcohols and cis/trans isomers, respectively [16].
(Z,Z)-3,6-nonadienal is often cited in the literature, but not easily identified, since it's a particularly labile molecule, subject to both isomerization and oxidation. It represents the most characteristic aroma of *Citrullus Lanatus*, thanks to its low threshold and to its strong odor of "freshly-cut watermelon"; for this reason, some authors has defined it "watermelon aldehyde" [16]. However, given its instability, it's scarcely used in the context of the food industry as added natural flavoring, since it's difficult to preserve.
The most common and recurrent hypothesis is that it isomerizes rapidly to the 2,6-nonadienal and 2-nonenal isomers [18,19,23], especially in matrices that have undergone stressful mixing and homogenization processes [19].

Particularly unstable are also 2,6-nonadienal and 2-nonenal, extensively studied especially in cucumber, since they represent the two species that characterize its "green" and "waxy" aroma.
In fact, in our samples a progressive decrease in their quantity over time takes place, until complete disappearance after 24 months. Various mechanisms have been proposed in the literature to justify this decrease during storage [14]: hydration followed by condensation [23], oxidation to the corresponding acids, or enzymatic reduction [19]. For example, 2,6-nonadienal degrades to acetaldehyde and 2-heptenal [23]. In addition to C9 aldehydes, also hexanal, 2-hexenal, 4-heptenal, 2,4-hexadienal, 2-heptenal, 2,4-heptadienal, 2,4-decadienal can be generated by the oxidation of polyunsaturated fatty acids and, to a lesser extent, by their autoxidation [1,3,5,24]. These molecules, already detected in some watermelon cultivars, are characteristics of foods rich in fatty acids, such as, for example, avocado, fish oil and olive oil [24].

The C6 aldehydes hexanal and 2-hexenal play a very important role in the food and perfume industry; they impart a pleasant, extremely characteristic "green" and fresh fragrance, notes particularly appreciated and sought after [1]. In addition, some authors [25] suggest the possible formation of hexanal (although in small quantities) as a result of lycopene degradation, a carotene abundantly present in the watermelon fruit, to which a specific paragraph is dedicated below. Actually, the abundance of this aldehyde increases significantly during the ageing of the Gavina pomace fibers, reaching an amount 2.5 times higher after 24 months (t_2) than that at t_0 time. This difference is represented by the values 2.98×10^6 and 1.19×10^6 on the TIC scale, respectively. Moreover, at t_2 time the relative percentage area is about 12%, compared to the initial

2.5%; it can therefore reasonably be said that hexanal progressively contributes during its storage to the aroma of the samples.

Pentanal and decanal, which impart fruity and floral notes, are assumed to originate from the enzymatic degradation of oleic acid [15], while benzeneacetaldehyde ("floral, sweet, honey") could be produced by the degradation of the amino acid phenylalanine. The values of the peak areas in terms of TIC of many aldehydes decreases during the fibers aging. They probably undergo degradation due to oxidative, biological and photochemical processes, thus giving rise to other species and leading to a change in the chemical composition of the VOCs of the samples.
The main example is represented by C9 aldehydes, which are completely absent in the 24-month aged sample, with the exception of nonanal. Actually, the most unstable and rapidly degrading species (4-nonenal, (Z)-2-nonadienal, 3,6-nonadienal and (E,E)-2,6-nonadienal) were not detected after only 30 days (t_1). We can therefore argue that during aging the dried fiber of watermelon pomace progressively loses the aroma of the fresh product, since these molecules are extremely characteristic. Already after 30 days, in fact, the "green" and "herbaceous" notes prevail with some "fruity" nuances, which however disappear completely at t_2 time, where the pleasant fragrance perceived is given only by the first two aromas (nonanal and C6 aldehyde hexanal).

On the other hand, the behavior of some other aldehydes, such as 2-methyl-propanal, pentanal, 2-methyl-butanal and 3-methyl-butanal, is in clear contrast. They increase their quantities over time, passing from $1.57x10^6$ (t_0) to $48.1x10^6$ TIC values (t_2) for the first, from $19.0x10^6$ (t_0) to $93.5x10^6$ (t_2) for the second, from $7.19x10^6$ (t_0) to $195x10^6$ (t_2) for 2-methyl-butanal and, finally, from $17.2x10^6$ (t_0) to $257x10^6$ (t_2) for 3-methyl-butanal. The origin of these last two compounds has been associated with some amino acids [26], in particular with the catabolism of leucine, in a complex degradation process that involves some sugars, certainly present in the analyzed samples. It's interesting to underline that the oxidation of 2-methyl-butanal and 3-methyl-butanal give rise to volatile compounds present only in aged samples, such as 2-methyl butanoic and 3-methyl butanoic acids, species absent at the t_0 time and present, in increasing quantities, respectively, at t_1 and t_2 time (as indicated in the acids-Table 4). In addition to the two aldehydes mentioned above, 2-methyl-propanal is also assumed to have the same origin [26] and this could actually explain the significant increase in its concentration over time.

Ketones

Ketones occupy only a restricted portion of the samples' headspace, both for the number of the identified analytes and for their relative abundance. At t_0 time their percentage area represents the 2.71% of the total, reaches 8.88% after 30 days and 7.38% after 24 months.

Table 2 - HS-SPME-GC-MS analysis of the VOCs of Gavina® watermelon pomace fiber over time: ketones class

		t_0		t_1 (30 days)		t_2 (24 months)		
Analyte	**Aroma**	**Area(·10^{-6}) Area %**	**LRI**	**Area(·10^{6}) Area %**	**LRI**	**Area(·10^{6}) Area %**	**LRI**	**Ref**
Acetone	Solvent, ethereal, apple, pear	31.1 0.65%	495	32.5 1.53%	494	166 6.79%	501	-
3-Methyl-2-butanone	Camphor	6.14 0.13%	588	6.43 0.30%	588	-	-	-
1-Penten-3-one	Pungent, peppery, garlic, mustard, onion	45.0 0.95%	689	52.7 2.48%	689	-	-	[10, 11, 13, 27]
2,3-Pentanedione	Buttery, nutty, toasted, caramelized	4.20 0.09%	698	2.83 0.13%	698	-	-	[10, 11]
Acetoin (3-Hydroxy-2-butanone)	Sweet,butterycreamy,milk	15.0 0.32%	713	4.9 0.23%	713	-	-	[15]
3-Penten-2-one	Bitter, fruity	-	-	2.58 0.12%	742	-	-	-
1-(1-Cyclohexen-1-yl)-ethanone	-	6.57 0.14%	869	17.3 0.81%	869	14.5 0.59%	869	-
3,5-Octadien-2-one (E,Z)	Fatty fruity, green hay	14.1 0.30%	1000	37.9 1.78%	1000	-	-	[11, 24]
3,5-Octadien-2-one (E,E)	Fruity, green, grassy	6.21 0.13%	1018	23.4 1.10%	1018	-	-	[11]
2-Cyclopenten-1-one	-	-	-	8.49 0.40%	1065	-	-	-

3,5-Octadien-2-one, 1-penten-3-one and 2,3-pentanedione derive from lipid autoxidation reactions and are related to the degradation of polyunsaturated fatty acids (PUFA). Some authors, in fact, have proposed their use as indicator of the oxidative state of vegetable oils [24]. The quantities of 3,5-octadien-2-one and 1-penten-3-one increase after 30 days (t_1 time), passing from 20.3x10^6 to 61.3x10^6 TIC values and from 45.0x10^6 to 52.7x10^6, respectively, and then reset to zero after 24 months (t_2 time).

Acetone, on the other hand, was detected in high quantities at t_2 time, compared to the previous steps where its abundance was comparable. Probably it originates from the degradation of complex molecules of different nature, present in the initial matrix, following paths of oxidative reactions that lead to this stable product.

Acetoin has a pleasant smell of yogurt, with buttery and creamy notes, it's used as a food additive in order to enhance the aroma of some preparations [28,29]. It's widely distributed in nature, since it is synthesized and produced by some microorganisms and insects through various enzymes and metabolic pathways. It's a precursor species of various molecules, often detected together with 3-hydroxy-2-butanone by HS-SPME-GC-MS analysis, including 2,3-butanediol, identified in the G_AI_V sample. Since they are molecules with a similar biological and biosynthetic pathway, they show an equally similar behavior over time: albeit in different quantities, both were detected only at t_0 time (15.0x10^6 TIC for acetoin, and 4.21x10^6 for 3-hydroxy-2-butanone), and at t_2 time (4.9x10^6 and 2.8x10^6 on a TIC basis, respectively).

Alcohols

In the HS of the fiber samples analyzed at t_0 time, alcohols are 15 out of 102 total analytes and they cover an overall percentage area of 7.79%. During storage, a reduction in their number is observed (14 in the sample at t_1 time and 7 at t_2 time), which cover 8.66% and 7.08% of the relative percentage area of the samples, respectively. Through this preliminary analysis we can say that the alcohol class do not define the aroma of the G_AI_V samples in a predominant way, and this is true for all the three aging steps examined.

Table 3 - HS-SPME-GC-MS analysis of the volatile organic compounds of Gavina® watermelon pomace fiber over time: alcohols class

		t_0		t_1 (30 days)		t_2 (24 months)		
Analyte	**Aroma**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Ref**
Ethanol	Alcoholic	22.5 0.47%	466	2.16 0.10%	467	-	-	[11, 12, 17, 18]
2-Methyl-3-buten-2-ol	Grass, earthy, oily	1.81 0.04%	613	1.67 0.08%	613	39.4 1.61%	616	-
1-Penten-3-ol	Pungent,horseradish,green,fruity	35.8 0.76%	686	10.4 0.49%	686	-	-	[5, 10, 12, 17,18]
2-Methyl-1-butanol	Ethereal,alcoholic,fatty,wine	3.59 0.08%	741	-	-	-	-	[5, 11, 17, 18]
1-Pentanol	Pungent, bread, fermented, yeast	29.8 0.63%	765	22.1 1.04%	765	62.8 2.56%	766	[5,11–13, 15,17,25]
2-Penten-1-ol (Z)	Green, phenolic, ethereal	41.8 0.88%	768	7.84 0.37%	768	-	-	[10,12,13, 17,18]
2,3-Butandiol	Fruity, creamy, buttery	4.21 0.09%	777	2.8 0.13%	777	-	-	-
2,5-Dimethyl-1,5-hexadien-3-ol	-	11.2 0.24%	839	7.4 0.35%	839	6.89 0.28%	840	-
1-Hexanol	Pungent, oleic, fruity, alcoholic	3.67 0.08%	851	2.17 0.10%	851	-	-	[2,5,10,11,13,15,17,18]
1-Heptanol	Must, pungent, green leaf, fruity	-	-	10.1 0.48%	926	-	-	[2, 10, 11]
1-Octen-3-ol	Mushroom,earthy,green,oily	23.6 0.50%	936	21.5 1.01%	935	19.2 0.79%	935	[10,11,13, 15,17,18]
2-Methyl-6-hepten-1-ol	-	-	-	-	-	11.1 0.45%	944	-
Benzyl alcohol	Sweet,floral, fruity	-	-	20.2 0.95%	983	5.97 0.24%	983	[10, 11, 14, 17]
1-Octanol	Waxy,green, citrus,floreal	15.2 0.32%	997	32.3 1.52%	997	-	-	[5, 10–13, 17,18]
2,6-Dimethylcyclohexanol	-	132 2.78%	1044	37.3 1.75%	1044	28.2 1.15%	1044	-
2,6-Nonadien-1-ol	Cucumber, green	10.3 0.22%	1051	-	-	-	-	[13, 17, 18, 20]
6-Nonen-1-ol	Green,melon, cucumber	11.2 0.24%	1056	-	-	-	-	[2,5,10,12, 13,17,18, 20]
3,4-Dimethylcyclohexanol	-	22.0 0.46%	1129	6.11 0.29%	1129	-	-	-

In contrast to what has been suggested by several authors in the literature [2,5,10,13,17], C9 aliphatic alcohols are not the prevalent species, since only 2,6-nonadien-1-ol and 6-nonen-1-ol have been identified at t_0 time.

Both are present in decidedly comparable quantities (respectively $10.3x10^6$ and $11.2x10^6$ on TIC basis), which corresponds to about 0.2% in terms of relative percentage area. These compounds, together with 3,6-nonadien-1-ol, 2-nonen-1-ol and 3-nonen-1-ol, have been detected in several fresh samples of *Cucurbitaceae* fruits. They are considered particularly characteristic and dominant of their aroma [2,5], as they give "green, fresh, waxy and cucumber or melon notes". Not surprisingly, these molecules were detected by the HS-SPME-GC-MS analysis conducted on our melon (var. *Inodorus*) fiber samples, the results of which are reported in Chapter 6.

In particular, the VOC 3,6-nonadien-1-ol, is considered one of the volatile alcohols that most contribute to defining the watermelon's aroma [2,10,17,18]. Probably, the manipulation of the fresh pulp and the subsequent vacuum-freeze drying led to a strong decrease of the concentration of this characteristic analyte, not detected in any of the G_AI_V samples. On the contrary, the amount of 1-pentanol significantly increases from the t_1 to t_2 time sample ($22.1x10^6$ and $62.8x10^6$ on a TIC basis, respectively). This compound originates mainly from the radical oxidation of linoleic acid and, to a lesser extent, from the degradation of lycopene [25]. The only aromatic alcohol is benzyl alcohol, detected after 30 days (t_1) and, to a lesser extent, after 24 months (t_2) of storage. It imparts sweet, floral and fruity notes and it's associated with the degradation of phenylalanine.

Acids

Organic acids are present in extremely small quantities in the fiber samples analyzed immediately after their preparation (t_0), where they cover a relative percentage area of less than 1%. They increase both in number and abundance in the aged samples.

Table 4 - HS-SPME-GC-MS analysis of the VOCs of Gavina® watermelon pomace fiber over time: acids class

		t_0		t_1 (30 days)		t_2 (24 months)		
Analyte	**Aroma**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Ref**
Acetic acid	Vinegar, acid, pungent, strong	21.8 0.46%	576	210 3.17%	582	283 11.6%	598	[15]
Propanoic acid	Vinegar,cheese, acid,pungent	-	-	13.8 0.65%	680	-	-	-
Propanoic anhydride	-	-	-	-	-	113 4.6%	688	-
3-Methyl-butanoic acid	-	-	-	9.2 0.43%	814	21.8 0.89%	817	-
2-Methyl-butanoic acid	-	-	-	2.4 0.11%	822	7.22 0.29%	824	-
Butanoic acid	Bitter,milk,cheese,buttery,fruity	-	-	21.1 0.99%	847	38.2 1.56%	849	-
Hexanoic acid	-	11.7 0.25%	918	69.1 3.25%	919	176 7.17%	923	-
Octanoic acid	-	-	-	9.94 0.47%	1050	4.9 0.20%	1050	-

For example, the amount of acetic acid progressively increases over time ($21.8x10^6$, $219x10^6$ and $283x10^6$, at t_0, t_1 and t_2 times, respectively), probably due to the combined effects of degradation and oxidation of molecules of different kinds originally present in the initial matrix (i.e. sugars, but not only). The 3-methyl-butanoic and 2-methyl butanoic acids, as already described above, could reasonably originate from the oxidation of the respective aldehydes, 3-methyl-butanal and 2-methyl-butanal [26]. In general, it can be assumed that all the acids formed over time derive from chemical and biochemical processes similar to those indicated for acetic acid.

Esters

Observing Table 5, it is evident that esters are not particularly abundant in the G_AI_V samples. However, our results are in line with what has been reported by several studies in the literature concerning some cultivars of seedless watermelons [4,5,10].

Table 5 - HS-SPME-GC-MS analysis of the VOCs of Gavina® watermelon pomace fiber over time: esters class

		t_0		t_1 (30 days)		t_2 (24 months)		
Analyte	**Aroma**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Ref**
Isopropil formiate	Ethereal,solvent cocoa, tropical	-	-	0.97 0.05%	739	-	-	-
3-Methylcyclopentyl acetate	-	50.6 1.07%	883	141 6.64%	883	77.5 3.17%	883	-
Methyl hexanoate	fruit, pineapple, apricot,banana	-	-	-	-	20.8 0.85%	892	[2, 11]

In contrast to other cultivars, in fact, esters are not characteristic of the aroma of these fruits, unlike aldehydes, alcohols and terpenes. Actually, in seedless watermelons the synthesis of ethylene, a molecule required to give rise to all those enzymatic activities related to the production of volatile esters, is minimal [10], while it is synthesized in higher quantities in seeded cultivars and in some melon varieties. This information is very interesting, suggesting that, among the various factors affecting the aroma of the plant species considered, the genetic ones play a particularly important and significant role.

Hydrocarbons

A fairly small number of molecules identified belong to this class of compounds, which progressively decreases passing from t_0 to t_2 time. Their abundances are also quite low in the three analyzed samples; this is reasonably due to their low vapor pressure, since they have a high molecular weight. As a consequence, only a very small fraction of them passes into the headspace during the sampling phase.

Table 6 - HS-SPME-GC-MS analysis of the VOCs of Gavina® watermelon pulp fiber over time: hydrocarbons class

		t_0		t_1 (30 days)		t_2 (24 months)		
Analyte	**Aroma**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Ref**
3-Ethyl-2-methyl-1,3-hexadiene	-	69.7 1.47%	986	16.0 0.75%	985	20.2 0.82%	985	[11]
4,5-Dimethyl nonane	-	7.77 0.16%	1159					
Octadecane	-	3.44 0.07%	1170	-	-	-	-	[11, 17, 30]
Tetradecane	Waxy, soft	2.20 0.05%	1204	40.8 0.15%	1204	-	-	[11, 30]
1-Tridecene	-	24.5 0.52%	1248	9.63 0.45%	1248	-	-	-
Heicosane	Waxy	1.82 0.04%	1256	-	-	-	-	[30]
Heneicosane	Waxy	1.77 0.04%	1279	-	-	-	-	[17, 30]

Terpenes and derivatives

Terpenes, also called isoprenoids, are a large class of natural organic compounds produced by the combination of a progressive number of isoprenic units [$(C_5H_8)_x$ with $x > 2$] through the mevalonic acid metabolic pathway [34]. Terpenes are hydrocarbons classified according to the number of isoprene units, starting from the lightest monoterpenes up to compounds formed by four or more isoprene structures. On the other hand, the term terpenoids typically indicates compounds derived from hydrocarbon skeletons of the terpene type, functionalized with groups containing heteroatoms, generally oxygen atoms, introduced by oxidation. In particular, monoterpenoids play a fundamental role in defining the aroma and fragrance of fruits, flowers and spices [1,4]. Together, terpenes and terpenoids probably constitute one of the largest groups of compounds known in nature, with approximately 30000 molecules characterized to date, isolated from plants, microorganisms and animals.

Table 7 - HS-SPME-GC-MS analysis of the VOCs of Gavina® watermelon pomace fiber over time: terpenes class (and derivatives)

		t_0		t_1 (30 days)		t_2 (24 months)		
Analyte	**Aroma**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area%**	**LRI**	**Ref**
6-Methyl-5-hepten-2-one	Fruity,apple, moldy,cream	810 17.1%	940	240 11.3%	940	236 9.63%	940	[2,4,5,11-13,18,25]
β-Terpinene (4-Methylene-1-(1-methylethyl)-Cyclohexene	-	22.8 0.48%	944	-	-	-	-	[30]
Isoterpinelene (3-Methyl-6-(1-methylethylidene)cyclohexene	-	-	-	-	-	3.99 0.16%	963	[30]
γ-Terpinene (1-Isopropyl-4-methyl-1,4-cyclohexadiene)-	Terpenic,sweet,citrus, tropical	2.52 0.05%	970	-	-	-	-	[30]
Cymene (4-Isopropyltoluene)	Bitter,woody nuance,terpenic,citrus	10.5 0.22%	979	-	-	-	-	[31]
Limonene (1-Methyl-4-(1-methylethenyl)-cyclohexene)	Citrus,orange, sweet,fresh	1330 28.0%	984	-	-	-	-	[5, 11, 15, 30]
2,2,6-Trimethyl-cyclohexanone	Pungent, honey	-	-	-	-	21 0.65%	989	[4]

Citronellol (3,7-Di methyloct-6-en-1-ol)	Floral, rose, sweet, citrus	37.8 0.80%	990	13.3 0.63%	990	-	-	-
Linalool oxide	Woody,nuance,floral,green	5.18 0.11%	1010	6.52 0.31%	1010	6.78 0.28%	1010	[30]
Linalool	Citrus,orange, floral,waxy	26.7 0.56%	1021	-	-	-	-	[30]
6-Methyl-3,5-heptadien-2-one	Sweet,green, spicy,fresh	22.8 0.48%	1025	41.1 1.93%	1025	18.1 0.74%	1025	-
Isocitral ((Z)-3,7-Di methyl-3,6-octadienal)	-	8.14 0.17%	1073	-	-	-	-	-
Borneol (1,7,7-Tri methyl-bicyclo[2.2.1] heptan-2-ol)	Balsamic,woody,nuance,fresh camphor	18.0 0.38%	1084	14,8 0,7%	1084	-	-	-
α-Terpineol (2-(4-Methyl-1-cyclosen-3-enyl)propan-2-ol)	Pine, woody, nuance,resinous,citrus	5.82 0.12%	1097	-	-	-	-	[30]
5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde	-	16.9 0.35%	1106	18.2 0.86%	1106	-	-	-
2,3-Epoxygeranial (3-Methyl-3-(4-Methyl-3-pentenyl)-oxirancarboxaldehyde	-	61.0 1.29%	1110	40.9 1.92%	1110	-	-	[4, 25]
β-Cyclocitral (2,6,6-Trimethyl-1-cyclohexen-1-carboxaldehyde)	Saffron,tropical,grass,rose, fruity	-	-	-	-	3.32 0.14%	1116	[3,4,11–13, 30,32, 33]
β-Citral ((Z)-3,7-Dimethyl-2,6-octadienal)	Fresh, lemon, sweet, green	85.4 1.80%	1116	21.6 1.02%	1116	-	-	[4,5,10,11, 13,18, 32]
1,4-Dimethyl-3-cyclohexene-1-carboxaldehyde	-	7.59 0.16%	1121	15.5 0.73%	1121	-	-	-
α-Citral ((E)-3,7-Dimethyl-2,6-octadienal)	Tea,refreshing,mint,fruity	89.8 1.89%	1132	24.1 1.13%	1132	-	-	[4,5,11,17, 18, 25, 32]
β-Homocyclocitral (2, 6,6-Trimethyl-1-cyclohexen-1-acetaldehyde)	Camphor,refreshing,woody nuance, fruity	4.72 0.1%	1140	-	-	-	-	[30]
Isopulegol (5-Methyl-2-(1- methylethenyl)-cyclohexanol	Mint,refreshing,woody nuance, grass	8.34 0.18%	1154	-	-	-	-	-
Isocitral ((E)-3,7-Di-methyl-2,6-octadienal	-	2.23 0.05%	1180	-	-	-	-	-
Cubebene	Grass, waxy	1.38 0.03%	1217	-	-	-	-	-
Geranylacetone (6,10-Dimethyl-5,9-undecadien-2-one)	Rose,fresh,leaf,floral,green	181 3.82%	1237	104 4.88%	1237	2.36 0.1%	1237	[3–5,10–13, 18]
β-Ionone (4-(2,6,6-Trimethyl-1-cyclohexen-1-il) 3-buten-2-one)	Sweet,wood, violet,floral,fruity	35.8 0.75%	1266	31.2 1.47%	1266	0.93 0.04%	1266	[3,12,17,18, 20,28,32,33]
5,6-β-Ionone epoxide (4-(2,2,6-Trimethyl-7-oxabicyclo [4.1.0]hept-1-yl) 3-buten-2-one)	Fruity,sweet, woody, violet, iris	5.78 0.12%	1269	34.4 1.62%	1269	4.19 0.17%	1269	[3,17,18,20, 30]
Carvone oxide (1-Methyl-4-(prop-1-en-2-yl)-7-oxabicyclo [4.1.0]heptan-2-one)	Mint, green	-	-	4.26 0.2%	1298	-	-	-
Pseudoionone(3,5,9-Undeca-trien-2-one,6,10-dimethyl-)	Sweet, waxy, citrus, floral	-	-	4.79 0.23%	1307	-	-	[3], [32]
Dihydroactinidiolide (5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	Apricot, red fruit, wood	7.65 0.16%	1309	44.7 2.1%	1309	22.0 0.89%	1309	[4,30,32]

The lighter species are important constituents of essential oils and plant resins, while the heavier ones include carotenes and carotenoids. These classes of compounds take part in several biological processes in plants, including the biosynthesis of various hormones and vitamins. Their oxidation leads to the formation of a family of analytes and metabolites called apocarotenoids [33,35].

Apart from some species related to the degradation of lycopene and β-carotene, the main monoterpenes and monoterpenoids identified by the analysis of the G_AI_V fibers are mainly present at t_0 time.

Subsequently, already after 30 days (t_1) these volatile analytes are not detected, because they have probably undergone degradation.
The HS-SPME-GC-MS analysis highlighted the presence of limonene in an unexpected and positive way. In fact, this analyte is one of the characteristic constituents of citrus family, but it has been found in essential oils and in various other fruits, including watermelon, as reported in some literature studies [5,11,15]. It is a particularly sought-after molecule, appreciated in the food, cosmetic and soap industry, since it gives a very pleasant citrus and fresh fragrance.
In the analyzed samples it is the most abundant species at t_0 time, but it was not detected in any of the aged specimens; natural limonene is highly unstable, since it undergoes isomerization and autoxidation processes [36].
Another monoterpene is linalool, with the structure shown in Figure 3, typically present in the essential oils of lavender, citrus, jasmine and in the essence of rosewood, to which it gives citrus and floral notes. This species undergoes epoxidation followed by rearrangement, which leads to the formation of trans-linalool oxide. This explains the absence of linalool already at t_1 time and the increase in the aged samples of its oxidized product.

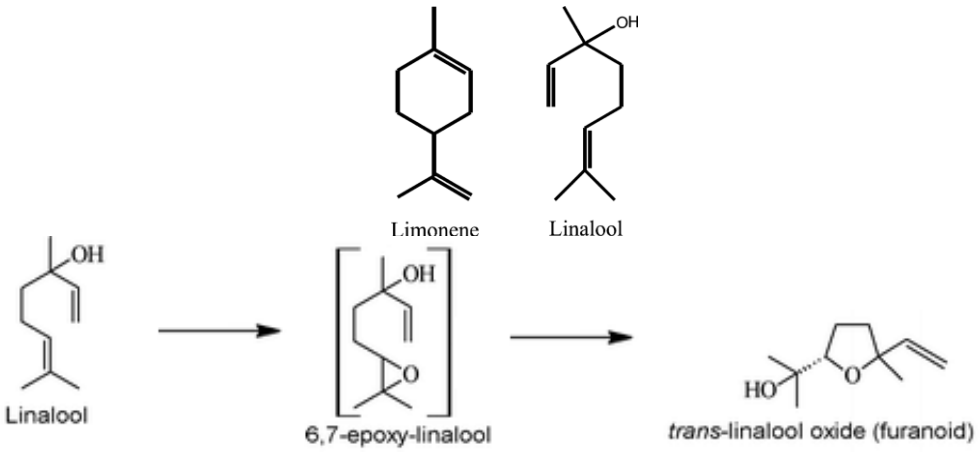

Figure 3 - Epoxidation of linalool to linalool oxide [37]

β-Terpinene, γ-Terpinene and α-Terpineol (Figure 4) are cyclic monoterpenes only present in the freshly prepared sample (t_0), while isoterpinolene was detected only after 24 months of storage. We don't know if this analyte is formed over time or if it was already present at t_0 time and had not been identified due to the high complexity of the chromatogram of the initial sample. Although present in rather modest quantities, they help to enrich and complete the aroma of the Gavina® watermelon pomace fiber with floral, citrus and woody notes (Table 7).

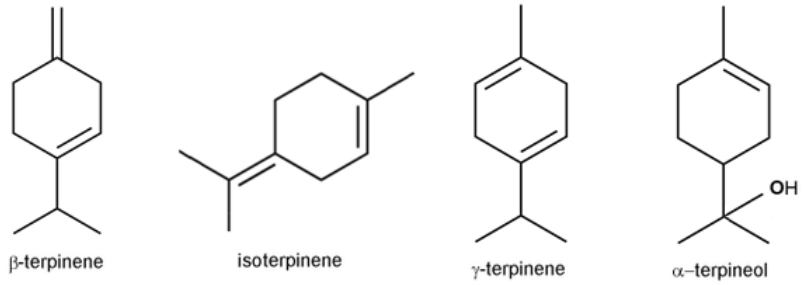

Figure 4 - Structure of some cyclic monoterpenes identified in the Gavina® watermelon fiber samples.

Cymene was identified only in the fiber analyzed at t_0 time and in none of the two subsequent aging steps, demonstrating that the analytes belonging to the terpene family have a relatively short lifetime. It is an aromatic volatile compound very common in thyme and oregano essential oils, but has been found in more than 100 plants and 200 fruits [1,31]. It performs various biological functions such as, for example, the increasing in the activity of antioxidant enzymes. It also possesses anxiolytic, anticancer and antimicrobial properties, as well as antiviral and antifungal activities [1].

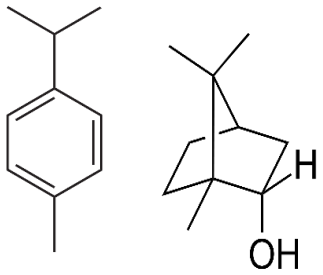

Figure 5 - Chemical structure of cymene (left) and borneol (right)

On the other hand, borneol (Figure 5), it's a bicyclic molecule with a fresh and woody fragrance, detected in the G_AI_V sample at t_0 and t_1 times, in comparable quantities ($18.0x10^6$ and $14.8x10^6$ on TIC basis, respectively).

Carotenes, carotenoids and derivatives

Carotenoid and the products of their catabolism, the apocarotenoids, form a group of more than 700 fat-soluble pigments. Carotenoids belong to the group of terpenes, which includes innumerable classes of metabolites, both primary and secondary. The primary ones include carotenoid, gibberellins and phytosterols which are necessary for various cellular functions, such as growth and maintenance, while the secondaries play roles mostly attributable to the interactions of the plant with the surrounding environment [38].
Likely to monoterpenes, carotenoids are also biosynthesized starting from geranyl diphosphate (Figure 6), which through further couplings of isoprene units, each catalyzed by specific enzymes, progressively forms derivatives with a lipophilic chain gradually longer, up to the tetraterpenes (C_{40}).

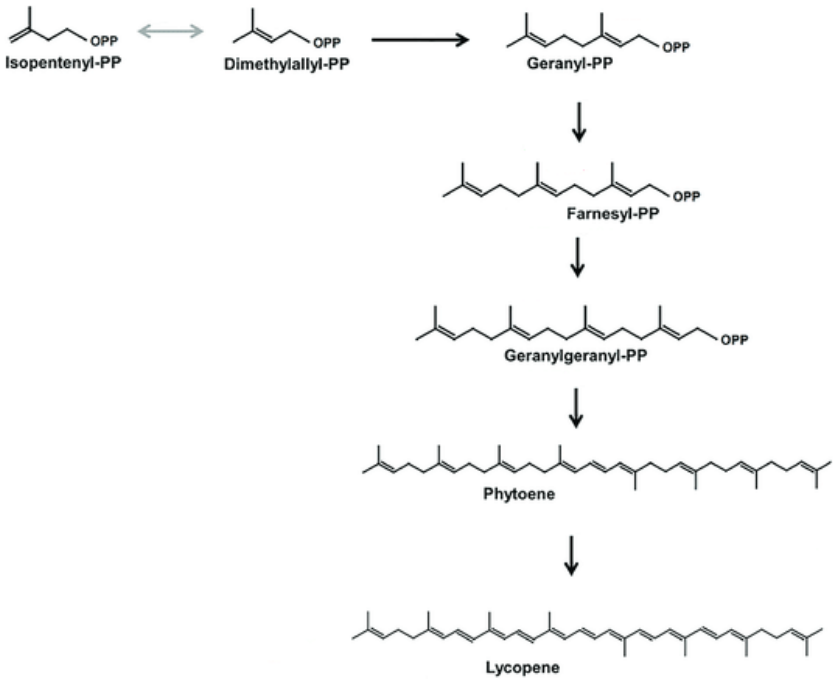

Figure 6 - Lycopene, a carotenoid biosynthesized starting from geranyl pyrophosphate [39].

Carotenoids typically have a 40-carbon atom chain backbone, they are lipophilic molecules and among the most important pigments in fruits and flowers [4,33]. They show different colors, from the yellow of lutein, to the orange of β-carotene, to the red of lycopene, thanks to their system of conjugated double bonds, on which their particular chemical, physical and biological properties depend. The high delocalization of π electrons give these molecules efficient antioxidant properties, since it allows them to stabilize reactive intermediates, such as carbocations or radical, by resonance. It is therefore known that carotenoids protect cells from oxidative stress induced by particularly reactive species, such as free radicals, giving the foods that contain them a great contribution of added value. Furthermore, from a nutritional point of view, their intake

is strongly recommended, since it is associated with the prevention of heart disease and the stabilization of blood pressure [4,33]. Due to the high number of double bonds, however, these molecules are particularly sensitive to degradation phenomena induced by heat, by biological processes of oxidation or by atmospheric oxygen and by exposure to electromagnetic radiation, leading to the formation of characteristic volatile compounds. For this reason, many carotenes, carotenoids and apocarotenoids have a considerable commercial value in the food and cosmetic industry, not only as pigments but also as substances capable of imparting pleasant aromas to products [35]. Given the continuous increase in demand for natural products and the problems related with the synthesis of these analytes, the flavor and fragrance industry is increasingly moving towards the search for new methods to obtain natural substances to replace synthetic counterparts. Among the emerging production methods, the use and transformation of carotenoids and the matrices containing them is increasingly invasive, since they represent a natural alternative capable of supplying precious substances [32, 40]. The detection and monitoring of carotenoid degradation compounds in commercial products can provide information on storage time, product quality and acceptability, nutritional value, vitamin and aroma content [32], color and therefore, on the visual impact. It has also been seen that from the identification of the VOCs in the HS of some fruits, it is possible to draw conclusions on the presence or the absence of a given carotenoid. By making this estimate, albeit approximate, one can discuss and speculate about the abundance of one analyte over another, of one cultivar over another, and so on [4].

Lycopene

Lycopene is accumulated in plant tissues and typically acts as a simple precursor for the biosynthesis of other carotenoids [4], but in some fruits, such as tomato and watermelon, it is instead present in high quantities, giving the distinctive red color.

As can be seen from Figure 7, the degradation of lycopene gives rise to various molecules with lower molar masses, following various degradation pathways.

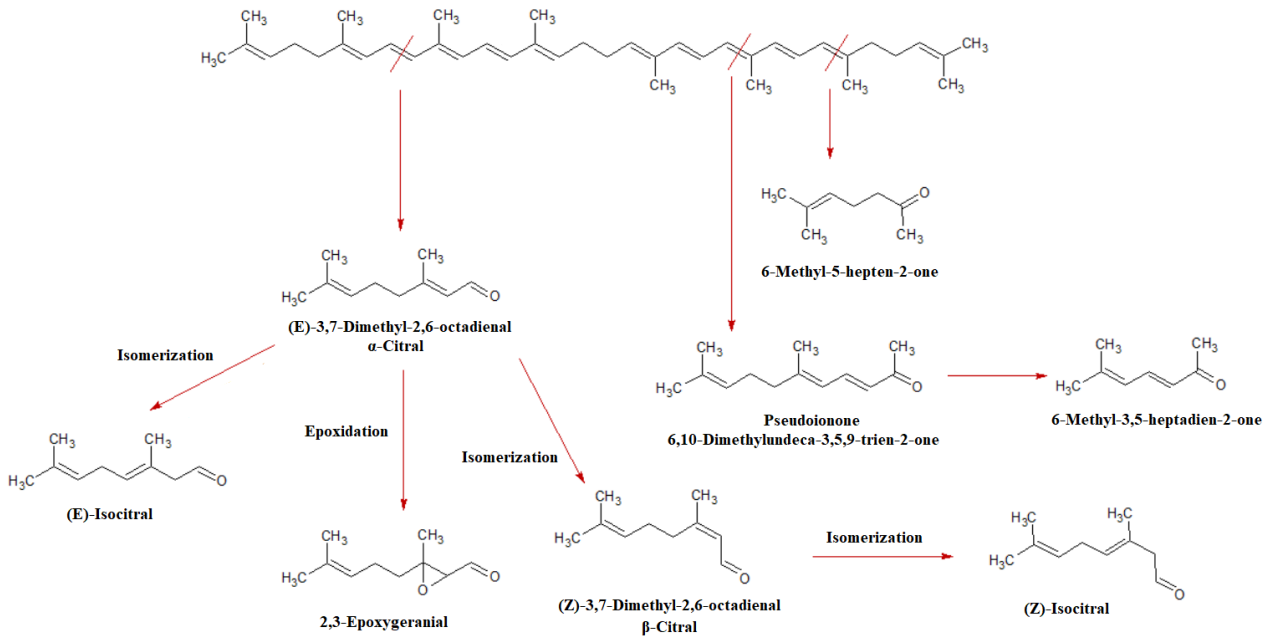

Figure 7 – Fragmentation scheme of the lycopene molecule and analytes of successive orders

With the term “citral”, or 3,7-Dimethyl-2,6-octadienal, we mean the cis- and trans- mixture of the neral and geranial acyclic monoterpenes, unsaturated aldehydes that impart a “citrus-like, fresh and fruity” aroma. In nature they are found mainly in citrus fruits and are particularly exploited in the food and cosmetic industry for their characteristic and pleasant fragrance. However, these are quite unstable molecules and sensitive to oxidation [25].

In our Gavina watermelon fibers samples, the highest concentration of (Z)-3,7-dimethyl-2,6-octadienal and (E)-3,7-dimethyl-2,6-octadienal was detected at t_0 time (85.4 x10^6 and 89.8x10^6 on a TIC basis, or 1.80% and 1.89% as relative % area, respectively).
These quantities are reduced to about a quarter at time t_1 (21.6x10^6 for the (Z) isomer and 24.1x10^6 for (E)) and reduced to zero after 24 months of storage, since neither molecule was detected in older samples.
Both isomers have been identified in different types of plants. In addition to watermelon, they are also present in some cultivars of apples, tomatoes, paprika [2,13] and, more generally, in species containing high levels of lycopene or its precursors [4]. The literature [2,13,25] suggests that they are degradation by-products of lycopene, carotene present in high quantities in *Citrullus Lanatus*, due to its interaction with atmospheric oxygen [4,25]. Therefore, the detection of α-citral and β-citral is considered a marker of the oxidation of lycopene. In addition to citral, we also have identified its epoxidation product, 2,3-epoxy-geranial, a compound already detected by other authors in similar matrices [25], and which is formed following a mechanism that is easily established in contact with the atmospheric oxygen. Furthermore, only in the sample analyzed at t_0 time, the citral structural isomer is present, isocitral, in a cis- trans- mixture. Some authors [15] actually classify these compounds as "off-flavor", as they alter the original aroma of the starting matrix.

Finally, the most abundant species associated with the degradation of lycopene is certainly 6-methyl-5-hepten-2-one, which covers about 17% of the VOCs fraction of the G_AI_V sample at t_0 time. It imparts a fruity, waxy, green fragrance with citrus notes. Its abundance tends to decrease strongly (about -70%) already after 30 days, but it remains almost constant passing to t_2 time. Several authors correlate its formation to the oxidation or degradation of lycopene, α-farnesene, citral or conjugated trienols [2,10,13].

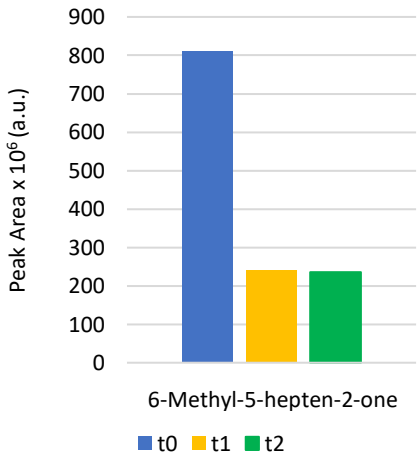

Figure 8 – Change in the abundance of 6-methyl-5-hepten-2-one over time in a sample of G_AI_V fiber.

The fact that 6-methyl-5-hepten-2-one is present in a non-negligible quantity already at t_0 time, makes us understand how much lycopene is an unstable molecule, which rapidly undergoes to the degradation processes indicated schematically in Figure 8.
Although vacuum-freeze drying has been selected as a technique which stresses the matrix to a lesser extent than oven-drying, the degradation processes still take place, probably due to the preliminary isolation procedures of the fibrous fraction.

The histogram in Figure 9 shows the variation over time in the content of terpenes released following the degradation of lycopene in a sample of fiber obtained from the Gavina® cultivar, vacuum-freeze dried as it is.

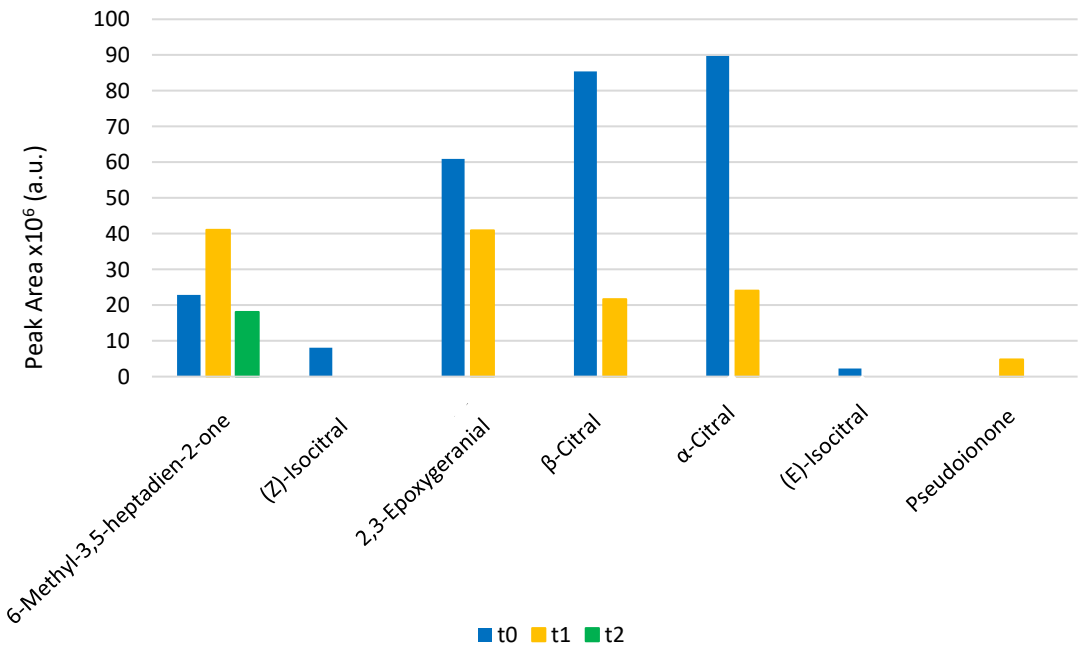

Figure 9 – Representation of the VOCs abundance from lycopene degradation, and their timing evolution for a sample of Gavina red fiber (G_AI_V).

β-Carotene

β-Carotene is found in high concentration in various vegetables, such as carrots, sweet potatoes, spinach, pumpkin and its intake, from a nutritional point of view, is particularly important as a precursor of vitamin A [33].

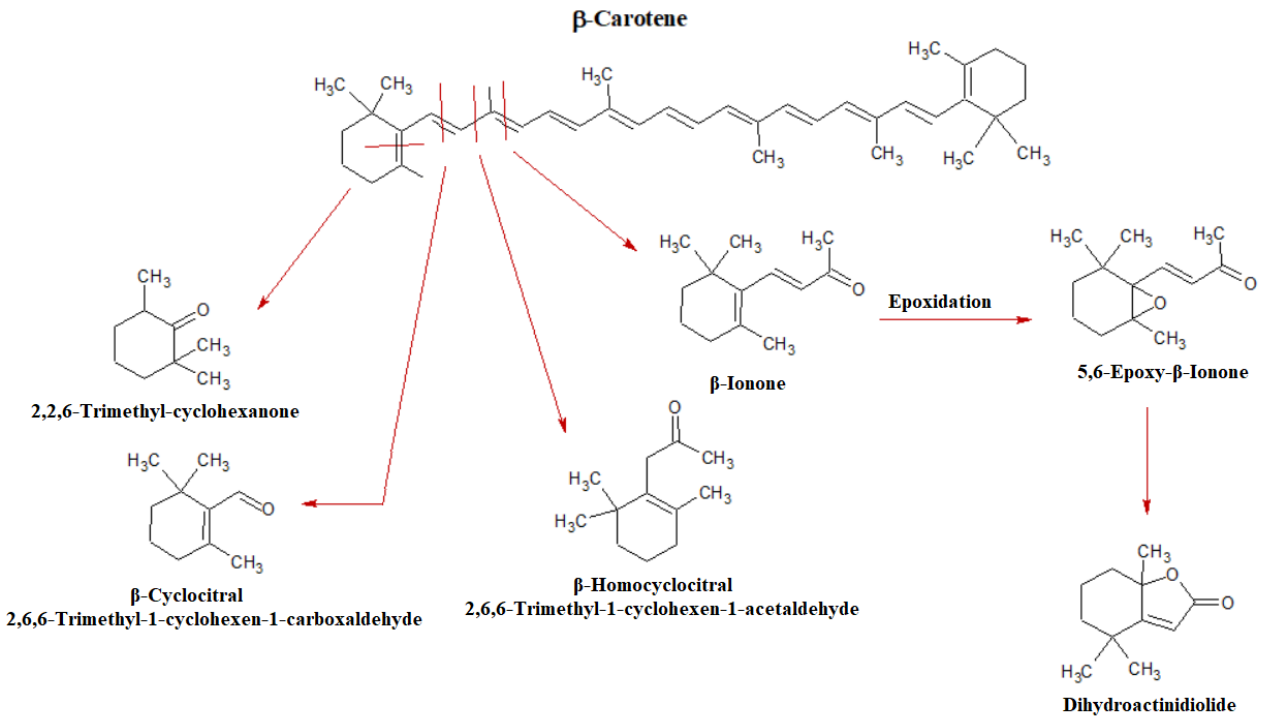

Figure 10 – Fragmentation scheme of β-carotene molecule and analytes of successive orders

The species shown in Figure 10 are commonly related to the degradation of β-carotene [13,32] by breaking some bonds in the different structural positions of the molecule. Among the degradation products, the most representative ones are:

● β-Ionone, produced following the breaking of the C9-C10 bond, it is present in comparable quantities at t_0 and t_1 time ($35.8x10^6$ and $31.2x10^6$, respectively), while it is much less abundant after 24 months of storage ($0.93x10^6$). It is a molecule with a floral, slightly fruity and pleasant aroma [33], widely used in all sectors of perfumery, as well as being used industrially for the synthesis of vitamin A [1]. In our samples, β-Ionone was also identified in its epoxidized form (5,6-Ionone epoxide), which reaches the higher abundance at t_1 time ($34.4x10^6$). At this aging step, the quantities of β-Ionone and 5,6-Ionone epoxide are comparable. As already reported for 2,3-epoxy-geranial, the epoxidation process of these terpenes occurs quite easily in the presence of atmospheric oxygen, therefore, its detection is not unexpected. Since these two species are the most abundant among those related to the degradation of β-Carotene, it is reasonable to hypothesize that the C9-C10 bond is the most susceptible to breaking.

● Dihydroactinidiolide, whose formation mechanism is not well understood, it is hypothesized to form starting from 5,6-Ionone epoxide according to a radical oxidation mechanism [33] or following the oxidation of the C8-C9 bond [32]. Like 5,6-Ionone epoxide, also dihydroactinidiolide reaches its maximum quantity after 30 days of storage and then halved after two years ($44.7x10^6$ at t_1 and $22.0x10^6$ at t_2) and gives the aged fiber a fruity aroma with woody notes.

Although β-Carotene is not the most present carotenoid in watermelon pulp, it contributes quite significantly to its aroma and fragrance, since its degradation products mentioned above have a particularly low odor threshold. Therefore, even if present in small quantities, they are easily perceived by the human sense of smell [4,32].

In addition to the three species mentioned above, after 24 months of storage, the analysis of the headspace of these samples reveals the presence of some species considered secondary in the degradation process of β-carotene, such as 2,2,6-trimethylcyclohexanone and β-Cyclocytral, while the previous ones decrease significantly in terms of abundance (as already specified). The histogram of Figure 11 shows the main degradation products of β-carotene found in the samples of vacuum-freeze dried fiber obtained from Gavina® watermelon, and their variation during the three aging steps examined.

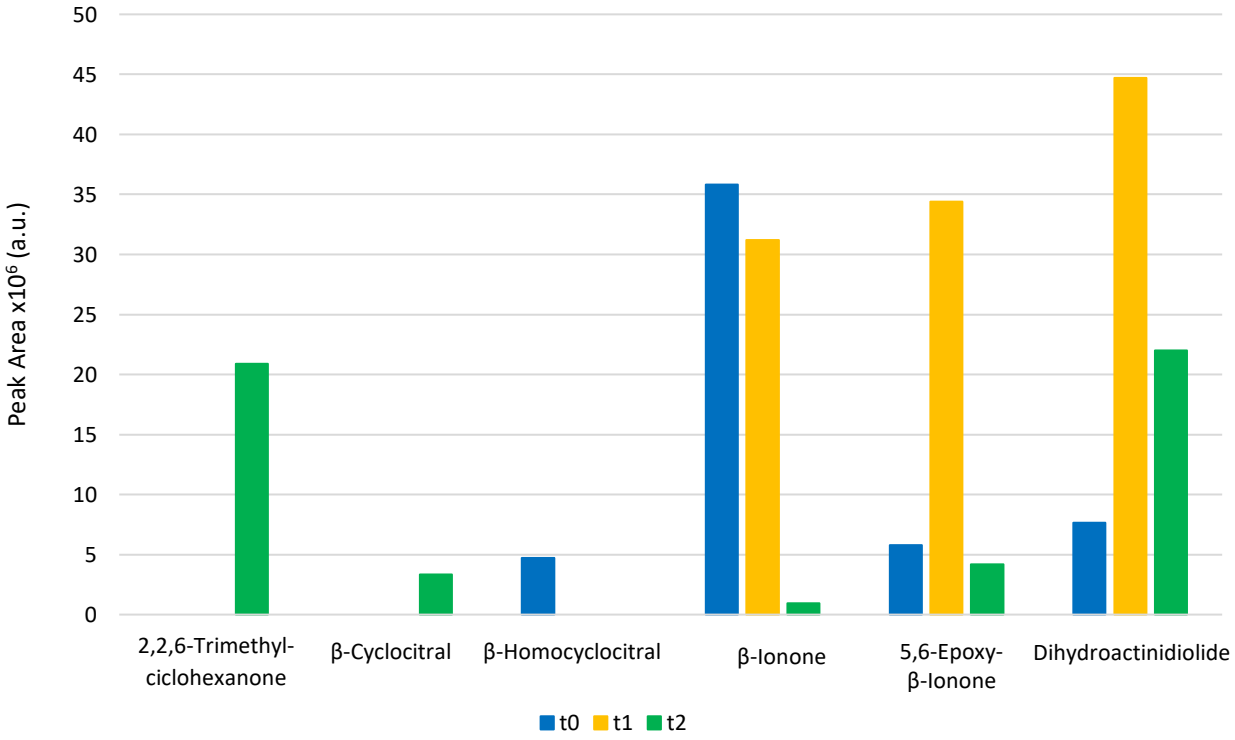

Figure 11 - Histogram representation of the abundance of volatile degradation compounds from β-Carotene and their timing evolution for a sample of Gavina red fiber.

From the SPME-GC-MS analysis we can deduce that β-carotene should be present in our samples, even if it was not detected by the UV-Vis spectra of the fiber extracts in hexane. Probably, routine UV-Vis spectroscopy

applied in our experimental conditions is a less sensitive technique than GC-MS, making difficult to identify the β-carotene bands. Furthermore, the high concentration of lycopene could prevent the identification of β-carotene, hiding its characteristic low intensity peaks.

ζ-Carotene

ζ-Carotene is an acyclic carotenoid precursor of lycopene and β-carotene, structurally very similar to lycopene, but with a lower number of unsaturations (Figure 12). Its presence has been hypothesized in several fruits containing carotenoids, including watermelon and tomato, and its characteristic color is a faint yellow [4].

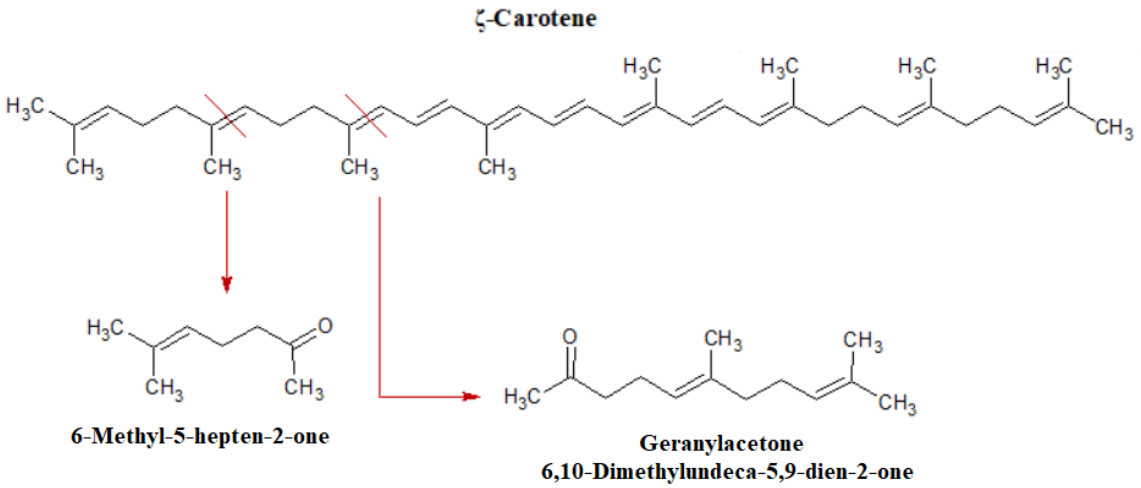

Figure 12 – Fragmentation scheme of the ζ-Carotene molecule and higher order analytes.

We can assume that this analyte is also present in our samples because its main degradation product, namely geranylacetone, was detected at each of the aging steps, albeit in progressively lower quantities over time ($181x10^6$ at t_0, $104x10^6$ at t_1 and $2.36x10^6$ at t_2, on TIC basis). Geranylacetone is a volatile molecule with a floral and fruity aroma, with a rather high odor threshold [27], lacking a distinctive effect on the overall fragrance of our samples. Furthermore, 6-methyl-5-hepten-2-one derives from ζ-carotene, a ketone already described above in relation to lycopene, with a high odor threshold too [14]. Since 6-methyl-5-hepten-2-one can result from the simultaneous degradation of two precursor analytes, the significant amount detected in the G_AI_V fiber samples could thus be justified.

Others

Table 8 shows some analytes of different nature, among which only a small number of them have already been identified and described in the literature as volatile aromas related to samples of *Citrullus Lanatus*. Among the identified molecules, there are some furans, a phenolic derivative (2,4-dimethyl phenol) and some more complex analytes with different functional groups, i.e. the 3-hydroxy-2,2,4-trimethilpentyl ester of 2-methyl propanoic acid.

Table 8 - HS-SPME-GC-MS analysis of the VOCs of Gavina watermelon pomace fiber over time: other compounds

		t_0		t_1 (30 days)		t_2 (24 months)		
Analyte	**Aroma**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Ref**
2-Methylfuran	Ethereal,vinegar,chocolate	1.83 0.04%	604	2.26 0.11%	604	-	-	-
3-Methylfuran	-	2.05 0.04%	615	1.44 0.07%	615	-	-	-
2-Ethylfuran	Sweet,malty, earthy	3.49 0.07%	706	4.18 0.20%	706	-	-	[10]
Dimethyl disulfide	Cabbage,vegetable,onion	-	-	-	-	12.8 0.52%	757	-

Furfural	Sweet, brown, woody,bread	-	-	-	-	8.98 0.37%	829	[13]
Methional	Tomato,potatoes,yeast	7.81 0.16%	887	-	-	-	-	-
2,4-Dimethylphenol	Dark, roast, smoked	4.89 0.10%	905	6.94 0.33%	905	-	-	-
2-Pentylfuran	Green, waxy, caramel	76.1 1.6%	947	29.5 1.39%	947	4.96 0.20%	946	[1,5,11,14, 15,17]
2-(2-Pentenyl)furan	-	23.3 0.49%	953	-	-	-	-	[13]
3,4-Dimethyl-2,5-furandione	-	-	-	29.7 1.4%	978	16.9 0.69%	978	-
5-Ethyldihydro-2(3H)-furanone	Creamy,fatty, fruity,coconut	-	-	-	-	15.9 0.65%	996	-
5-Hydroxy-2-Methyl-3-esenoic acid	-	4.38 0.09%	1193	17.7 0.83%	1193	-	-	-
5-Heptyldihydro-2(3H)-furanone	-	2.16 0.05%	1196	3.43 0.16%	1195	-	-	-
2-Methyl propanoic acid, 3-Hydroxy-2,2,4-trimethylpentyl ester	-	5.87 0.12%	1202	25.4 1.2%	1202	2.14 0.09%	1202	-

Some authors [1, 17] associate 2-pentylfuran to the radical peroxidation of fatty acids, a mechanism already mentioned above and from which various analytes identified originate in the headspace of the examined Gavina samples.

3,4-Dimethyl-2,5-furandione has been described as a bioactive molecule naturally produced by various plants, with an efficient insecticidal and biofumigant activity [41]. This analyte was identified in the sample aged 30 days in a modest quantity (relative area % = 1.4 %) and decreases by about 40 % after 24 months of storage.

Table 9 - Compound classes identified in the HS-SPME-GC-MS analysis of the Gavina watermelon pomace fiber at t_0, t_1 (30 days) and t_2 (24 months) storage time.*

Compound class	Gavina®t_0	Gavina®t_1	Gavina®t_2
ALC %	7.8 ± 0.8	8.7 ± 0.7	7.0 ± 0.5
EST %	1.1 ± 0.2	6.7 ± 0.5	4.0 ± 0.4
KET %	2.7 ± 0.5	8.9 ± 0.7	7.4 ± 0.6
OTH %	2.8 ± 0.4	5.7 ± 0.6	2.5 ± 0.2
AHA %	2.4 ± 0.5	1.3 ± 0.3	0.8 ± 0.1
ALD %	23.3 ± 1.8	28.6 ± 2.2	38.2 ± 2.4
ACD %	0.7 ± 0.1	9.1 ± 0.9	26.3 ± 3.1
TER %	59.2 ± 3.1	31.0 ± 2.6	12.8 ± 1.3

*mean of three replicates of the chromatograms, ± standard deviation, $s_{(3)}$

Compound classes: ACD, acids; EST, esters; AHA, alkanes; ALC, alcohols; ALD, aldehydes; KET, ketones; OTH, others; TER, terpenes.

The data in Table 9 and Figure 13, are expressed as mean % of each class with respect to the total normalized peak areas, on the basis of the sum of the total peak ion current (TIC).

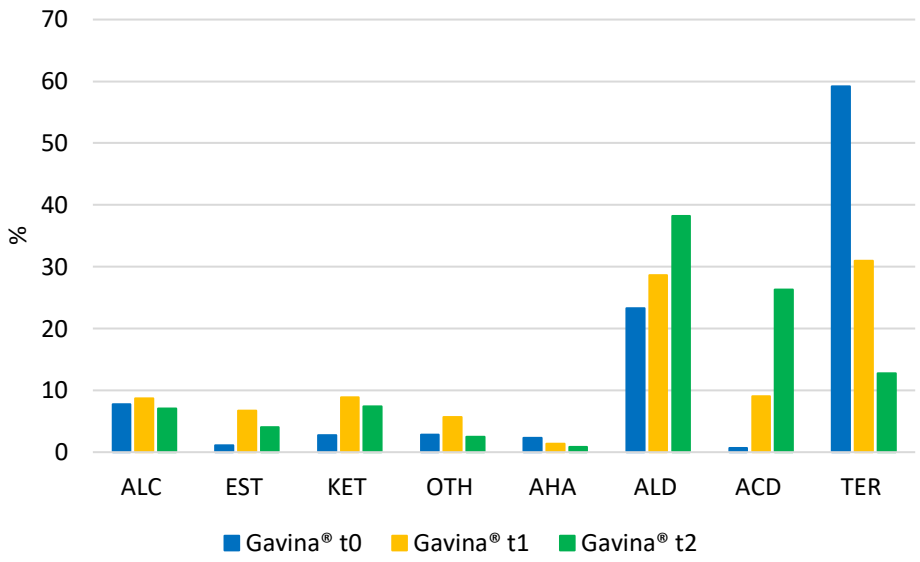

Figure 13 – Graphical representation of the timing evolution of VOCs classes (area % on TIC basis) for Gavina watermelon pomace fibers.

Analyzing the summary data of table 9, we observe that during storage the molecules of the terpenes class undergo the greatest decrease. These complex species derived from isoprene decompose into simpler analytes belonging to the other chemical classes. They give the samples a characteristic and pleasant aroma and their decrease contributes to the loss of the typical fragrance of the initial fresh matrix.
During storage there is a progressive increase mainly of esters, ketones, aldehydes and acids. This increase, parallel to the aging time, is attributed to the degradation of more complex molecules. The increase recorded for the class of aldehydes is particularly significant, attributed to the degradation of some fatty acids, leading above all to the formation of pentanal and hexanal. These molecules are responsible for the typical green grass fragrance of the dietary fiber sample.
In addition, the concentration upgrading of 3-methyl-butanal and 2-methyl-butanal, both structural isomers produced by the decomposition of the amino acid leucine, is observed. A similar situation exists for acetic acid, as this analyte originates from many molecular degradation pathways, being a common product of many natural processes. Furthermore, the loss of C9 aldehydes progressively takes place, both in number of identified analytes and in their abundance. As mentioned above, these compounds are extremely specific and impart the typical fragrance of the *Cucurbitaceae* fruits.
Overall, we can say that our fibers lose the aromatic characteristics typical of the fresh matrix during storage. This is consistent with the significant decrease in the terpene population, the loss of watermelon fragrances and the simultaneous increase of a new generation analytes responsible for a different aroma than the original one.

- **Comparison of the aroma profile among the three selected cultivars**

The results shown below aim to highlight and define the differences in terms of chemical composition of the headspace of the watermelon fiber samples deriving from the three different cultivars, Crimson Sweet, Asahi Miyako and Gavina, examined during this study.
The aromatic and fragrant characteristics of a fruit can also vary significantly when passing from one cultivar to another, since the VOCs are produced through metabolic pathways that develop during the ripening, harvesting and storage processes, all strongly affected and variety-dependent factors. The variables relating to the soil where the fruit is grown must also be considered, i.e. the geographical position and the micro-climate of the place [2,4]. This means that the identification of the main VOCs is essential, since it allows to define the sensorial identity and the characteristic aroma of the fruit [3]. Figure 14a, 14b, 14c shows the chromatograms obtained by the HS-SPME-GC-MS technique applied on the samples G_AI_V, Mi_AI_V and C_AI_V. Also in this case, to preserve the aromatic properties of the watermelon pomace, we decided to

work on vacuum freeze-dried fibers and to compare the results of the samples analyzed immediately after their preparation.

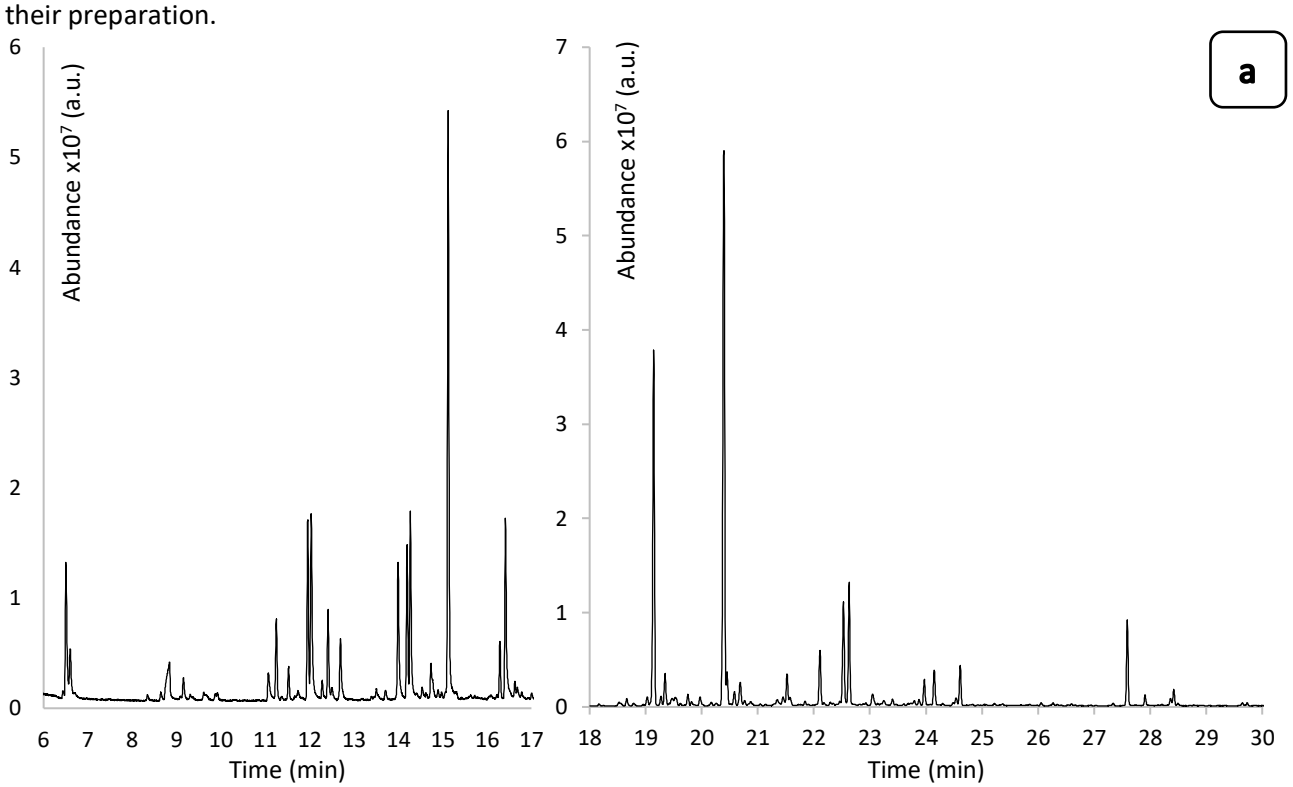

Figure 14a – Chromatogram obtained by HS-SPME-GC-MS from a sample of Gavina watermelon red fiber (G_AI_V) at t_0 time.

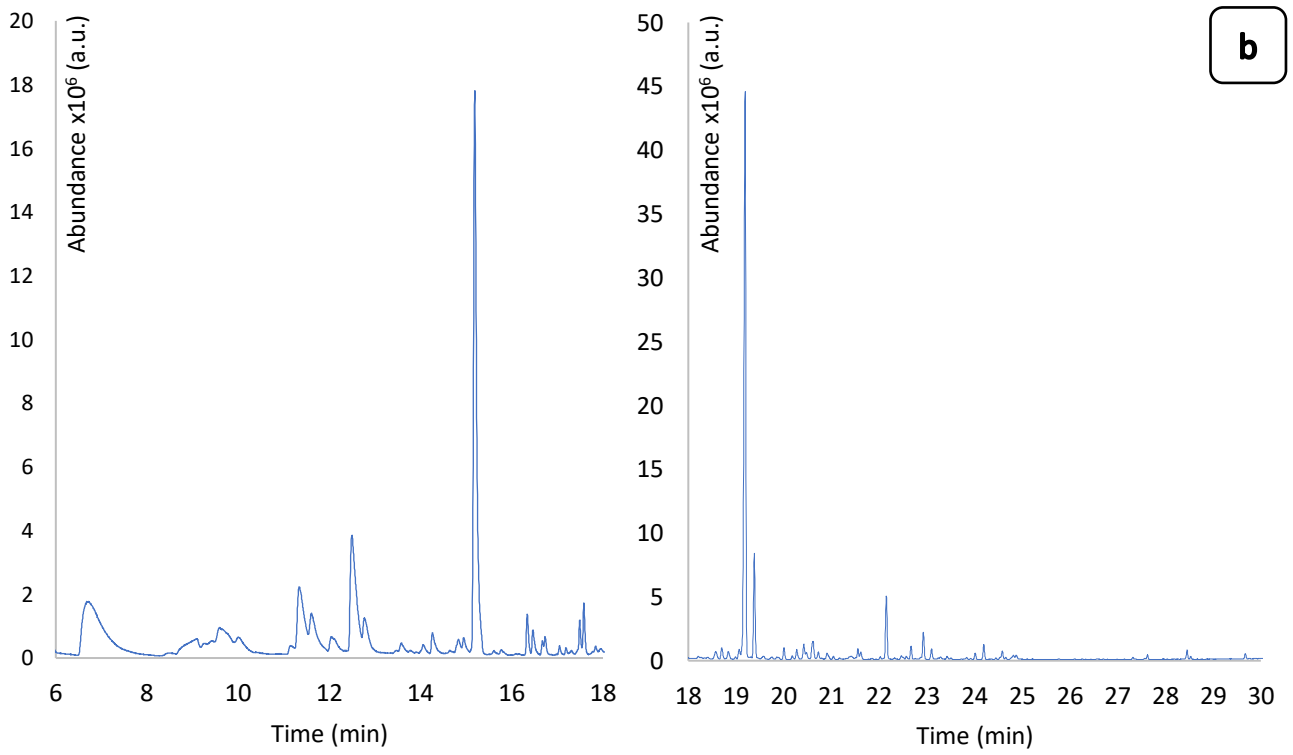

Figure 14b –Chromatogram obtained by HS-SPME-GC-MS from a sample of Asahi Miyako watermelon red fiber (Mi_AI_V) at t_0 time.

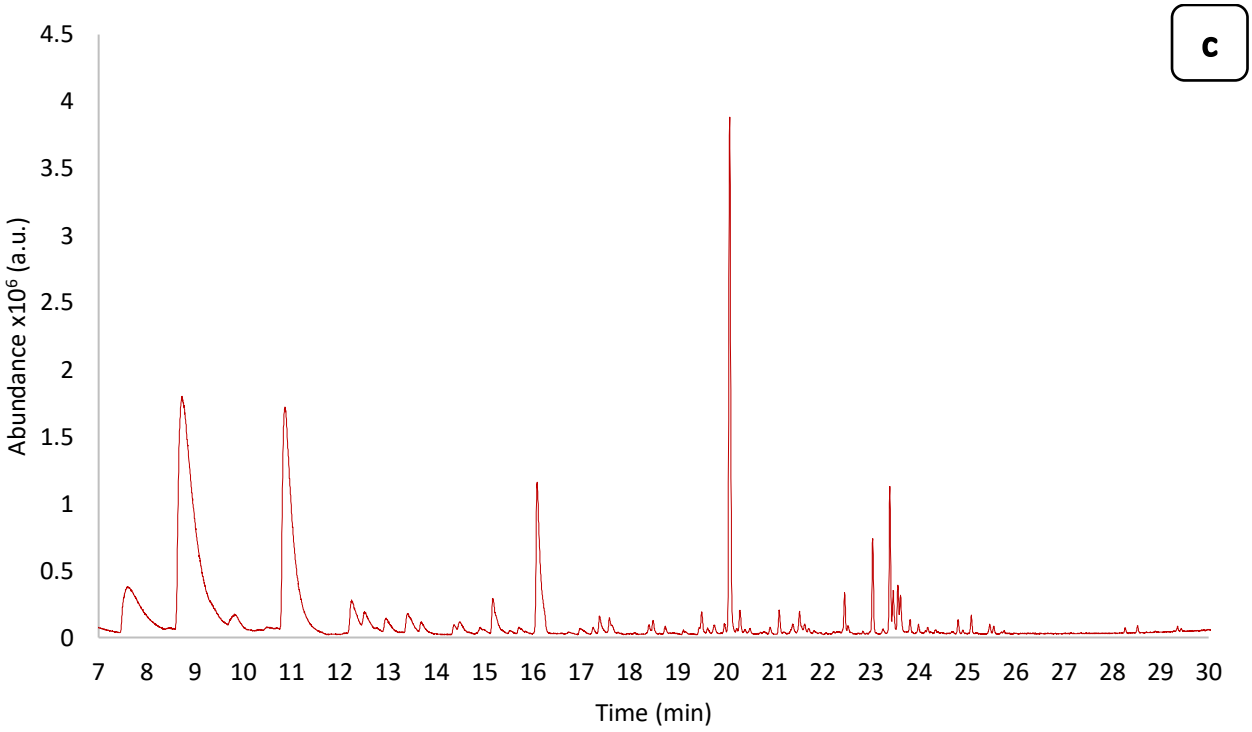

Figure 14c – Chromatogram obtained by HS-SPME-GC-MS from a sample of Crimson Sweet watermelon red fiber (C_AI_V) at t_0 time.

From a quick preliminary examination, it is observed that the chromatogram relating to the Crimson Sweet sample is the simplest, both for the number of peaks and for their intensity. On the other hand, those relating to the Miyako and Gavina cultivars are more complex, with a greater number of peaks and analytes. The compounds identified are listed in the tables below, with their absolute quantities (peak area on TIC basis) and the respective aromas, also divided by chemical class.

Aldehydes

At first sight, it can be seen that the G_AI_V specimen have a headspace populated by a greater number of molecules belonging to the class of aldehydes, with a total of 34 species detected, compared to 19 and 13 of the Mi_AI_V and C_AI_V samples, respectively.

Table 10 - Comparison of the composition of volatile organic fraction among cultivars Gavina, Miyako and Crimson via HS-SPME-GC-MS analysis – Aldehydes class

		G_AI_V		Mi_AI_V		C_AI_V		
Analyte	**Aroma**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Area(·10⁶) Area %**	**LRI**	**Ref**
Acetaldehyde	Pungent,fresh,penetrating	54.6 1.15%	435	-	-	-	-	[2, 10–14]
Propanal	Ethereal,pungent,fruity	13.5 0.28%	498	-	-	-	-	[11,14]
2-Methyl-propanal	Fresh,aldehydic,floral	1.57 0.03%	559	11.8 0.22%	564	-	-	-
(Z)-2-Butenal	-	2.31 0.05%	570	26.4 0.50%	572	-	-	-
Butanal	Pungent, green, malty	2.06 0.04%	593	-	-	-	-	-
(E)-2-Butenal	Green, fruity	8.18 0.17%	655	24.6 0.46%	658	0.58 0.08%	656	[5, 10, 11, 13]
3-Methyl-butanal	Ethereal,aldehydic,peach	17.2 0.36%	661	230 4.33%	665	24.6 3.33%	661	[5, 15, 16]
2-Methyl-butanal	Chocolate,nutty, furfural	7.19 0.15%	671	165 3.12%	674	15.4 2.08%	670	[5, 15]

Pentanal	Fermented,fruity, berry	19.0 0.4%	703	351 6.63%	705	15.2 2.06%	702	[5,10,11,15, 17,18]
2-Methyl-2-butanal	Pungent,green,penetrating	2.79 0.06%	748	-	-	-	-	-
(E)-2-Pentenal	Pungent,green,fruity,apple	33.6 0.71%	758	26.2 0.49%	760	-	-	[5, 10–15, 17]
3-Methyl-2-butanal	Sweet,fruity, pungent,nut	9.29 0.2%	784	19.8 0.37%	788	2.87 0.39%	783	-
Hexanal	Green, fatty, fruity, woody	119 2.51%	798	850 16.0%	800	59.8 8.09%	796	[2,5,10,13, 17,18]
(Z)-2-Hexenal	Green	1.06 0.02%	837	-	-	-	-	[5]
(E)-2-Hexenal	Green,almond,fruity	42.2 0.89%	843	33.7 0.64%	845	6.35 0.86%	842	[5,10,11,13, 14,17]
4-Heptenal	-	3.25 0.07%	878	5.39 0.10%	879	-	-	[10–13]
Heptanal	Fresh, fatty, green, grass	11.9 0.25%	880	30.3 0.57%	881	2.01 0.28%	878	[5,10-13,15,16]
(E,E)-2,4-Hexadienal	Green, fruity, citrus, waxy	3.36 0.07%	890	3.84 0.07%	891	-	-	[10, 15]
(E)-2-Heptenal	Green, fatty, oily, fruity	18.7 0.40%	923	26.5 0.50%	924	1.17 0.16%	921	[5, 10–13, 17]
Octanal	Orange,waxy peel green	7.35 0.16%	954	4.46 0.08%	955	1.32 0.18%	952	[5,10,11,13, 15,17,18]
(E,E)-2,4-Heptadienal	Fatty, sweet, melon, spicy	10.9 0.23%	963	5.31 0.10%	965	-	-	[5,10,11,13, 15,17]
Benzeneacetaldehyde	Honey,sweet, rose floral	64.7 1.37%	994	-	-	2.12 0.29%	992	[11, 13]
(E)-2-Octenal	Green, nut, fat	-	-	16.4 0.31%	995	-	-	[5,10,11,13, 15,17,20]
(E)-4-Nonenal	Fruity	18.1 0.38%	1017	-	-			[5, 10, 13, 15]
Nonanal	Citrus,lemon, cucumber	74.2 1.57%	1023	-	-	6.99 0.94%	1021	[2,5,10,15-18,20]
(Z,Z)-3,6-Nonadienal	Fresh-cut watermelon	7.88 0.17%	1046	-	-	-	-	[11–13,16, 17,19]
(Z)-2-Nonenal	Cucumber, fatty, waxy	6.91 0.15%	1052	-	-	-	-	[5,12–16, 19]
(E,E)-2,6-Nonadienal	Melon,cucumber,fatty	4.47 0.09%	1054	-	-	-	-	[5, 12, 15]
(E,Z)-2,6-Nonadienal	Cucumber, melon,green	237 5.00%	1059	7.36 0.14%	1060	-	-	[2,10,12,13, 15–17, 20]
(E)-2-Nonenal	Fatty,green, melon,waxy	266 5.61%	1062	22.7 0.43%	1064	7.93 1.07%	1060	[2,5,10–14,20]
Decanal	Zest, waxy, orange, floral	19.7 0.42%	1090	-	-	-	-	[5, 11, 15]
(E,E)-2,4-Nonadienal	Cucumber, melon, waxy	6.56 0.14%	1101	1.55 0.03%	1102	-	-	[5,10, 11,13,16]
(E)-2-Decenal	Waxy,earthy, coriander	8.67 0.18%	1127	-	-	-	-	[5, 13, 15, 20]
(E,Z)-2,4-Decadienal	Fatty,orange, citrus, fresh	4.66 0.10%	1165	-	-	-	-	[5, 11, 13, 17]
Dodecanal	Soapy, waxy, citrus, floral	2.36 0.05%	1214	-	-	-	-	[5]

However, with regard to the abundance on TIC-based analytes, the sample with the greatest amount is Mi_AI_V (≈1862x10^6), followed by G_AI_V (≈1100x10^6) and finally C_AI_V (≈146x10^6). Furthermore, the Gavina sample presents a more complex HS chromatogram for the number of aldehydes, while the bouquet of the Miyako sample is characterized by a lower number of these analytes, but with higher concentrations.

As far as the C9 aldehydes concerns, only (E)-2-nonenal (7.93x10^6) was detected in the sample of fiber from Crimson Sweet. In the sample from Miyako cultivar, the species (E)-2-nonenal, 2,4-nonadienal and (E,Z)-2,6-nonadienal were detected in a total amount of 3.16x10^7. Finally, in the fiber sample from Gavina, 8 aldehydes (7 unsaturated and 1 saturated compounds) were identified, in a total amount of 6.21x10^8, which corresponds to approximately 13% of the VOCs fraction.

In the Mi_AI_V sample, hexanal and pentanal are particularly abundant and cover, respectively, 16% and 6.62% of the VOC fraction. These species appear among the main natural degradation products of linoleic acid [1,5]. It can be hypothesized that this fatty acid is present in the Miyako cultivar in greater quantities

than in the others examined, where pentanal and hexanal do not cover such a significant % area. However, given the limited knowledge in the literature, it is very difficult to establish what the origin of these two aldehydes (pentanal and hexanal) may be.

Among the aldehydes found in each fibrous sample of the three cultivars, there are 3-methyl-butanal and 2-methyl-butanal, present in greater quantities for Miyako, followed by Crimson and finally by Gavina ($395x10^6$, $40x10^6$ and $24.4x10^6$, respectively). These compounds could also derive, in part, from the degradation of the amino acid leucine [26]. It is reasonable to think that this amino acid is indiscriminately present in the three cultivars, although probably in rather small quantities, since leucine is not among the most characteristic amino acids of watermelon.

Ketones

Here too, as already reported for the aldehydes, the C_AI_V fiber sample is the one with the lowest number of ketones, both in number and quantity, if compared with the other two cultivars. In terms of abundance (expressed as TIC), the Miyako specimen is the one with the greatest amount of ketones (≈ $821x10^6$), followed by Gavina (≈ $128x10^6$) and finally Crimson Sweet (≈ $91x10^6$).

Table 11 - Comparison of the composition of volatile organic fraction among cultivars Gavina, Miyako and Crimson via HS-SPME-GC-MS analysis – Ketones class

		G_AI_V		Mi_AI_V		C_AI_V		
Analyte	**Aroma**	**Area(·10^{-6}) Area %**	**LRI**	**Area(·10^{-6}) Area %**	**LRI**	**Area(·10^{-6}) Area %**	**LRI**	**Ref**
Acetone	Solvent,ethereal, apple,pear	31.1 0.66%	495	550 10.4%	502	84.6 11.4%	498	-
3-Methyl-2-butanone	Camphor	6.14 0.13%	588	31.1 0.59%	592	-	-	-
2-Butanone	Ethereal, fruity, camphor	-	-	44.1 0.83%	597	-	-	-
1-Penten-3-one	Pungent,pepper,garlic,onion	45.0 0.95%	689	-	-	-	-	[10, 11, 13, 27]
2,3-Pentanedione	Buttery,nutty, caramelized	4.20 0.09%	698	-	-	-	-	[10, 11]
Acetoin	Sweet,buttery, creamy,milk	15.0 0.32%	713	113 2.14%	715	6.10 0.82%	712	[15]
1-(1-Cyclohexen-1-yl)-ethanone	-	6.57 0.14%	869	-	-	-	-	-
2-Heptanone	Fruity,spicy, sweet,grass	-	-	7.77 0.15%	871	-	-	[17]
2-Methyl-1-hepten-6-one	-	-	-	21.2 0.40%	929	-	-	-
3-Octen-2-one	Earthy, oily, sweet	-	-	22.0 0.41%	979	-	-	[10]
(E,Z)-3,5-Octadien-2-one	Fatty fruity, hay green	14.1 0.30%	1000	16.9 0.32%	1002	0.56 0.08%	998	[11, 24]
Acetophenone	-	-	-	1.75 0.03%	1013	-	-	-
(E,E)-3,5-Octadien-2-one	Fruity, green, grassy	6.21 0.13%	1018	13.1 0.25%	1019	-	-	[11]

The most evident difference found within this class of molecules, between the samples of the three different cultivars, concerns acetone. Its abundance is significantly greater in the C_AI_V and Mi_AI_V samples compared to the G_AI_V sample (about 6.5 times for the first and ≈ 18 times for the second).
Some previous studies link the presence of acetone to the radical peroxidation mechanism of lipids, suggesting the measurement of this analyte as an indicator of the oxidative state of fatty substances [42]. It can therefore be hypothesized that the Mi_AI_V sample is richer in some fatty acids, the degradation of which can give rise to this volatile molecule. The impossibility of perfectly separating the pulp from the small immature white seeds present in the fruits of the Miyako watermelon, probably makes the fibrous samples richer in some lipid components. Although present in modest quantities, they could significantly contribute to the aromatic bouquet of the matrices examined.

Alcohols

Alcohols are a class of compounds that significantly contribute to define the aroma of the fruits.
From a preliminary analysis of the results, it can be noticed that the number of alcohols present in the samples is on average comparable: 15 in G_AI_V, 11 in Mi_AI_V and 14 in C_AI_V, respectively. The Miyako cultivar is the one with the greatest abundance (799x10^6), followed by Gavina (369x10^6, -53.8% compared to Mi_AI_V) and, finally, Crimson Sweet (114x10^6, which corresponds to -85.7% compared to Miyako).

Table 12 - Comparison of the composition of the VOCs among cultivars Gavina, Miyako and Crimson via HS-SPME-GC-MS analysis – Alcohols class

		G_AI_V		Mi_AI_V		C_AI_V		
Analyte	**Aroma**	**Area(·10^6) Area %**	**LRI**	**Area(·10^6) Area %**	**LRI**	**Area(·10^6) Area %**	**LRI**	**Ref**
Ethanol	Alcoholic	22.5 0.47%	466	-	-	13.6 1.84%	468	[11, 12, 17, 18]
2-Methyl-3-buten-2-ol	Grass, earthy, oily	1.81 0.04%	613	-	-	-	-	
1-Penten-3-ol	Pungent, green, fruity	35.8 0.76%	686	74.9 1.41%	690	10.7 1.44%	686	[5, 10, 12,17,18]
3-Methyl-1-butanol	Alcoholic	-	-	-	-	3.24 0.44%	736	-
2-Methyl-1-butanol	Ethereal,fatty, wine, cocoa	3.59 0.08%	741	31.3 0.59%	744	6.24 0.84%	740	[5, 11, 17, 18]
1-Pentanol	Pungent, ferm ented, yeast	29.8 0.63%	765	434 8.18%	768	16.6 2.24%	764	[5, 11–13, 15, 17, 25]
(Z)-2-Penten-1-ol	Green, pheno lic, ethereal	41.8 0.88%	768	-	-	-	-	[10, 12, 13, 17, 18]
2,3-Butandiol	Fruity, crea my, buttery	4.21 0.09%	777	11.4 0.22%	785	1.72 0.23%	777	-
2,5-Dimethyl-1,5-hexadien-3-ol	-	11.2 0.24%	839	43.3 0.81%	841	1.84 0.25%	837	-
1-Hexanol	Pungent, fru ity, alcoholic	3.67 0.08%	851	12.7 0.24%	853	4.50 0.61%	849	[2,5,10,11,13,15,17,18]
1-Octen-3-ol	Mushroom, earthy, green,	23.6 0.50%	936	22.2 0,42%	937	2.02 0.27%	933	[10,11,13, 15,17,18]
Benzyl alcohol	Sweet, floral, fruity	-	-	33.4 0,63%	985	1.00 0.14%	981	[10, 11, 14, 17]
1-Octanol	Waxy, green, citrus, florale	15.2 0.32%	997	-	-	-	-	[5,10-13,17,18]
2,6-Dimethylcyclohexanol	-	132 2.78%	1044	115 2.16%	1046	15.5 2.1%	1041	-
2,6-Nonadien-1-ol	Cucumber, green	10.3 0.22%	1051	-	-	-	-	[13, 17, 18, 20]
3,6-Nonadien-1-ol (Z,Z)	Watermelon fresh,melon	-	-	-	-	7.18 0.97%	1056	[2,5,11,12, 18–20,22]
(Z)-6-Nonen-1-ol (Z)	Green, melon, cucumber	11.2 0.24%	1056	-	-	-	-	[5,10-13, 17,18,20]
(Z)-3-Nonen-1-ol (Z)	Fresh, waxy, green, melon	-	-	5.05 0.10%	1058	22.6 3.06%	1054	[2,5,12,13, 18–20,22]
1-Nonanol	Fresh, clean, floral, grass	-	-	-	-	6.83 0.92%	1061	[2,5,11,12,17,18,20,22]
3,4-Dimethylcyclohexanol	-	22.0 0.46%	1129	15.5 0.29%	1131	-	-	-

1-Pentanol is the absolute most abundant alcohol found in the HS of the Mi_AI_V sample (434x10^6).
It is one of the main degrading products of linoleic acid. Its high abundance, together with that of pentanal and hexanal, coming from the same fatty acid, would strengthen the hypothesis that linoleic acid is present to a greater extent in the Miyako cultivar than the other two examined.
As already extensively described in the previous section related to the HS-SPME-GC-MS analysis of Gavina fiber over time, it is well-known that C9 alcohols are characteristic compounds of the aroma of numerous *Cucurbitaceae* fruits and vegetables [2,5,10–13]. However, neither in the Mi_AI_V nor in the G_AI_V sample, numerous C9 alcohols were identified. In both cases, their relative percentage area is actually less than 1%. In contrast, in the headspace of the C_AI_V sample, these analytes cover a higher percentage abundance, approximately 5%. Among these, 3,6-nonadien-1-ol is one of the species most characterizing the aroma of *Citrullus Lanatus*, since it has a strong scent of fresh watermelon and a low perception threshold [16]. By

considering the previous results relating to aldehydes, it seems that the Mi_AI_V sample has an aromatic profile scarcely influenced by both C9 aldehydes and alcohols. The most significant contribution is given by molecules produced mainly by the degradation of linolenic acid, especially hexanal, acetone and 1-pentanol. The Gavina and Crimson cultivars, on the other hand, show a composition of the headspace and an aromatic profile more like with what is reported in the literature, albeit with some differences. The first, in fact, is more characterized by C9 aldehydes while the second by C9 alcohols.

Esters

Only two esters were identified in the headspace of the analyzed samples. G_AI_V and Mi_AI_V present the same species, 3-methylcyclopentyl acetate, with comparable abundance, while in the C_AI_V sample only ethyl acetate was identified in a rather significant quantity (32.7%).

Table 13 - Comparison of the chemical composition of the VOCs fraction among cultivars Gavina, Miyako and Crimson via HS-SPME-GC-MS analysis – Esters class

		G_AI_V		Mi_AI_V		C_AI_V		
Analyte	**Aroma**	**Area(·10^6) Area %**	**LRI**	**Area(·10^6) Area %**	**LRI**	**Area(·10^6) Area %**	**LRI**	**Ref**
3-Methylcyclopentyl acetate	-	50.6 1.07%	883	48.9 0.92%	885	-	-	-
Ethyl acetate	Fruit,pineapple,apricot	-	-	-	-	242 32.7%	612	[2, 11]

These experimental data are in line with what has been reported by several authors in the literature, who confirm the almost complete absence of esters in various seedless *Citrullus Lanatus* cultivars [5,10]. The reason is attributed to the fact that watermelon is a non-climacteric fruit, that is, it doesn't continue to ripen after being harvested, unlike apples, bananas, peaches, apricots, pears, etc. which are defined as climacteric fruits.

For the latter, ethylene, which acts as a plant hormone and is produced during ripening, has the task of activating a series of biochemical and enzymatic mechanisms linked to the production of volatile esters that characterize the aroma of ripe climacteric fruits. This observation is very interesting and shows that genetic factors play a fundamental role in the definition of some distinctive traits of the fruit considered, especially as regards aroma, flavor, organoleptic characteristics, etc. Not surprisingly, although watermelon is a non-climacteric fruit, Beaulieu et al. [10] does not rule out the possibility that there may be differences between seeded and seedless cultivars regarding the content of volatile esters. Our results seem to confirm this hypothesis, albeit limited to the Crimson Sweet cultivar.

Terpenes and derivatives

It is well known that terpenes and terpenoids play a fundamental role in defining the aroma of plants, flowers and fruits. The G_AI_V sample shows numerous monoterpenes and monoterpenoids that are absent in Mi_AI_V and C_AI_V, such as limonene, terpinene and its isomers, citronellol, borneol, terpineol, cubebene, isopulegol.

Table 14 - Comparison of the aromatic fraction composition of the Gavina, Miyako and Crimson cultivars through HS-SPME-GC-MS analysis – Terpenes and derivatives

		G_AI_V		Mi_AI_V		C_AI_V		
Analyte	**Aroma**	**Area(·10^{-6}) Area%**	**LRI**	**Area(·10^{-6}) Area %**	**LRI**	**Area(·10^{-6}) Area %**	**LRI**	**Ref**
6-Methyl-5-hepten-2-one	Fruity, apple, must, creamy	810 17.1%	940	1200 22.6%	941	102 13.8%	937	[2,4,5,11–13,18,25]
6-Methyl-5-hepten-2-ol	Sweet,coriander, green, oily	-	-	5.27 0.1%	946	0.854 0.12%	942	[5,10,12]
β-Terpinene	-	22.8 0.48%	944	-	-	-	-	[30]
γ-Terpinene	Terpenic,sweet,citrus	2.52 0.05%	970	-	-	-	-	[30]
Cymene	Bitter,woody nuance,citrus	10.5 0.22%	979	-	-	-	-	[31]
Limonene	Citrus,orange, sweet, fresh	1330 28.0%	984	-	-	-	-	[5, 11, 15, 30]
Citronellol	Floral, rose, sweet, citrus	37.8 0.80%	990	-	-	-	-	-
Linalool oxide	Woody,floral, re,freshing	5.18 0.11%	1010	3.00 0.06%	1011	-	-	[30]
Linalool	Citrus,orange, floral,waxy	26.7 0.56%	1021	-	-	-	-	[30]
6-Methyl-3,5-heptadien-2-one	Sweet,green, spicy,fresh	22.7 0.48%	1025	16.2 0.3%	1027	1.6 0.21%	1023	-
4-Terpineol (4-Methyl-1-(1-methylethyl)-3-cyclohexenol	Woody,citrus, spicy	-	-	2.67 0.05	1035	-	-	-
Cymen-3-ol	Spicy,phenolic, camphor	-	-	5.16 0.1%	1041	-	-	-
α-Cyclocitral (2,6,6-Trimethyl-2-Cyclohexen-1-carboxyaldehyde)	Saffron,tropical,grass,rose, fruity	-	-	3.5 0.07%	1051	-	-	-
Isocitral	-	8.14 0.17%	1073	-	-	-	-	-
Borneol	Balsamic,woody,camphor	18.0 0.38%	1084	-	-	-	-	-
α-Terpineol	Pine,woody,resinous,citrus	5.82 0.12%	1097	-	-	-	-	[30]
5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde	-	16.9 0.35%	1106	2.49 0.05%	1108	2.55 0.3%	1103	-
2,3-Epoxygeranial	-	61.0 1.29%	1110	11.0 0.21%	1111	-	-	[4, 25]
β-Cyclocitral	Saffron,tropical,grass,fruity	-	-	26.2 0.49%	1117	2.90 0.39%	1113	[3–5, 11,12, 30, 32, 33]
β-Citral	Fresh,lemon, sweet,green	85.4 1.80%	1116	-	-	-	-	[3-5,10,11, 17,18,32]
1,4-Dimethyl-3-cyclohexen-1-carboxaldehyde	-	7.59 0.16%	1121	1.45 0.03%	1123	-	-	-
α-Citral	Tea,refreshing,mint,fruity	89.8 1.89%	1132	3.82 0.07%	1133	1.25 0.17%	1129	[4,5,10,17, 18,20,25,32]
β-Homocyclocitral	Camphor,refreshing,woody	4.72 0.1%	1140	9.18 0.17%	1141	0.674 0.09%	1137	
Isopulegol	Mint,refreshing,grass	8.34 0.18%	1154	-	-	-	-	-
Isocitral	-	2.23 0.05%	1180	-	-	-	-	-
Cubebene	Grass, waxy	1.38 0.03%	1217	-	-	-	-	-
α-Ionone (4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one)	Sweet,wood, violet,floral, fruity	-	-	2.22 0.04%	1236	-	-	[5]
Geranylacetone	Rose, fresh, leaf, floral, green,aldehydic	181 3.82%	1237	7.35 0.14%	1238	1.12 0.15%	1234	[3–5, 10–12,18]
β-Ionone	Sweet,wood, violet,floral	35.8 0.75%	1266	16.06 0.3%	1267	0.842 0.11%	1264	[3,5,11,12,20,28,32,33]

5,6-β-Ionone epoxide	Fruity,sweet, wood, violet	5.78 0.12%	1269	4.94 0.09%	1270	-	-	[3,17,18, 20,30]
Dihydroactinidiolide	Apricot, red fruit, wood	7.65 0.16%	1309	8.45 0.16%	1310	-	-	[4,30,32]

These results indicate that Gavina cultivar possesses an aroma characterized by the presence of numerous terpene species. These molecules, as already pointed out, although following fairly common biosynthetic pathways, possess specific and peculiar fragrant characters, which are then transmitted to the fiber samples prepared with Gavina® pomace. Compounds related to lycopene degradation (6-methyl-5-hepten-2-one, α-citral, isocytral, β-citral, 2,3-epoxigeranial) are more abundant in the G_AI_V sample than in the other two cultivars, with the exception of 6-methyl-5-hepten-2-one. The latter component is detected in very high quantities in the pomace fiber of Miyako watermelon ($1200x10^6$ vs. $810x10^6$ and $114x10^6$ for the Gavina and Crimson Sweet samples, respectively). Another peculiarity of the Miyako cultivar samples is linked to the presence in their HS of α-ionone, together with β-ionone, although in a small quantity. α-ionone originates primarily from α-Carotene, isomer of β-Carotene, with similar nutritional properties, including the activity as a precursor of vitamin A.
α-ionone has very similar characteristics to its β-isomer, it has a low threshold [4,32], a pleasant floral and fruity aroma and, for this reason, it is widely used in the production of food flavorings and fragrances in the perfume industry. The C_AI_V fiber samples, on the other hand, have a particularly poor headspace in terpene species. Here, only some compounds deriving from the degradation of lycopene and β-carotene were detected, but in small quantities if compared with the samples obtained from the other two cultivars.
We can conclude that terpenes and terpenoids do not significantly characterize the aroma of the fibers obtained from Crimson Sweet watermelon, since they are poorly detected both in terms of analyte number and relative abundance.

Figure 15 shows the abundance of compounds related to the degradation of lycopene in the samples obtained from the three cultivars.

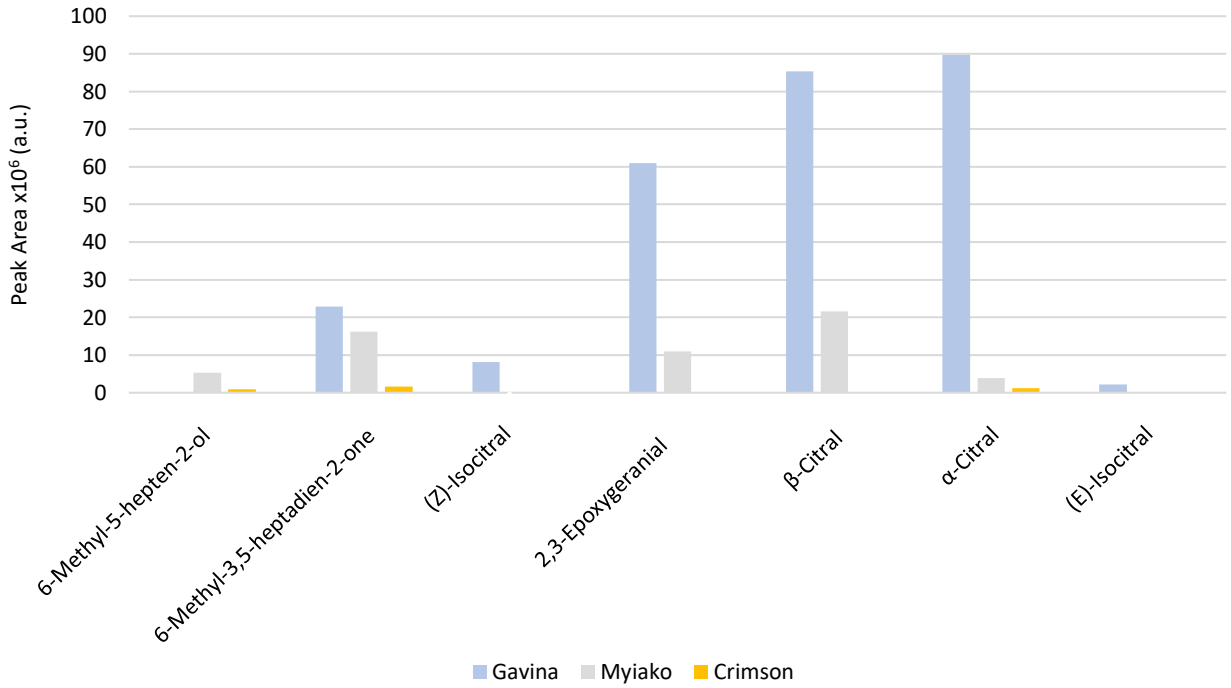

Figure 15 – Graphical representation of the abundance of VOCs from lycopene degradation in the samples of the three selected cultivars.

As far as the degradation of β-carotene concerns, we can see that the main species originating from this carotenoid, namely β-ionone, 5,6-ionone epoxide and dihydroactinidiolide, are mainly present in the samples

G_AI_V (49.2x10^6) and Mi_AI_V (29.5x10^6), while only β-ionone was detected in C_AI_V in very small quantities (0.842x10^6).
The histogram of Figure 16 shows the abundance of compounds related to the degradation of β-carotene in the samples obtained from the three cultivars.

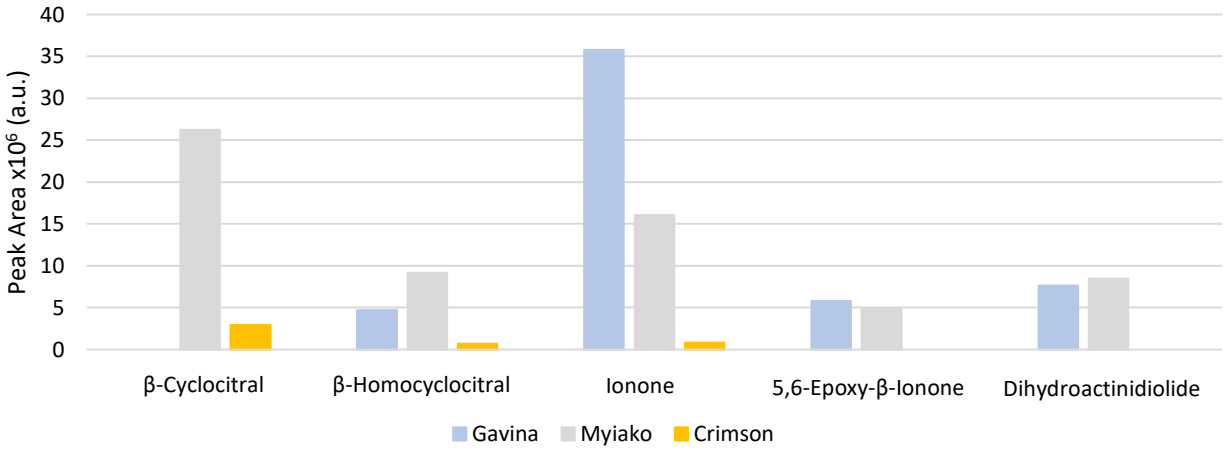

Figure 16 – Relative abundance of some compounds originated by β-carotene degradation.

Others

Table 15 shows analytes belonging to various chemical classes of different nature, detected in our fibrous samples. The main differences found between the samples of the three cultivars concern the 2-methylfuran and 2-pentylfuran compounds, present with significantly greater abundance for the Miyako cultivar (90.3x10^6 and 189x10^6 respectively) compared to that measured in the G_AI_V and C_AI_V samples.

Table 15 - Comparison of the aromatic fraction composition of the Gavina®, Miyako and Crimson cultivars through HS-SPME-GC-MS analysis – Other compounds

		G_AI_V		Mi_AI_V		C_AI_V		
Analyte	**Aroma**	**Area(·10^{-6}) Area %**	**LRI**	**Area(·10^{-6}) Area %**	**LRI**	**Area(·10^{-6}) Area %**	**LRI**	**Ref**
2-Methylfuran	Ethereal,vinegar, chocolate	1.83 0.04%	604	90.3 1.70%	618	-	-	-
3-Methylfuran	-	2.05 0.04%	615	-	-	-	-	-
2-Ethylfuran	Sweet, malty, earthy	3.49 0.07%	706	-	-	-	-	[10]
Dimethyl disulfide	Cabbage, vegeta-ble,sulphurous	-	-	-	-	2.15 0.29%	755	-
Furfural	Sweet, bread, caramel	-	-	-	-	3.20 0.43%	828	[13]
2-n-Butyl furan	Faint, fruit, sweet	-	-	7.96 0.15%	875	-	-	
Methional	Tomato, potato, yeast,	7.81 0.16%	887	-	-	-	-	-
2,4-dimethylphenol	Dark, roast, smoked	4.89 0.10%	905	8.88 0.17%	905	0.974 0.13%	903	-
2-Pentylfuran	Green, waxy, caramel	76.1 1.6%	947	189 3.57%	948	4.52 0.61%	944	[1,5,11, 14,15,17]
2-(2-Pentenyl)furan	-	23.3 0.49%	953	6.91 0.13%	948	-	-	[13]
5-Hydroxy-2-meth yl-3-hexenoic acid	-	4.38 0.09%	1193	-	-	-	-	-
5-Heptildihydro-2(3H)-furanone	-	2.16 0.05%	1196	-	-	-	-	-
2-Methyl propano ic acid, 3-Hydroxy-2,2,4-trimethyl pentyl ester	-	5.87 0.12%	1202	-	-	-	-	-

Some authors [1,17] associate 2-pentylfuran with the radical peroxidation of fatty acids, a mechanism already described and from which originate various analytes identified in the headspace of all the fibrous samples examined. The high abundance of 2-pentylfuran (3.57% of the total) in the Mi_AI_V samples is consistent with the previous observations, i.e. the aromatic profile of the fibrous samples obtained from the Myiako cultivar is particularly influenced by lipid peroxidation processes.

8.4. SOME CONCLUDING REMARKS

As a final remark, we propose, in comparative form in Table 16 the results obtained from the experimental investigations carried out on the pomace fibers of the 3 selected watermelon cultivars. Table 16 shows the quantitative data collected for the 8 classes of compounds identified and described along the text of the chapter.

Table 16- Compound classes identified in the HS-SPME-GC-MS analysis of the Gavina, Miyako and Crimson watermelon pomace fiber. Data are expressed as mean % of each class with respect to the total normalized peak areas on the basis of the sum of the total peak ion current.*

Compound class	Gavina®	Miyako	Crimson
ALC %	7.8 ± 0.8	15.0 ± 1.6	15.3 ± 1.7
EST %	1.1 ± 0.2	0.9 ± 0.1	32.7 ± 2.9
KET %	2.7 ± 0.5	15.5 ± 1.0	12.3 ± 0.8
OTH %	2.8 ± 0.4	5.7 ± 0.5	1.5 ± 0.3
AHA %	2.4 ± 0.5	0.9 ± 0.2	0.2 ± 0.1
ALD %	23.3 ± 1.8	35.1 ± 2.7	19.8 ± 1.6
ACD %	0.7 ± 0.1	1.8 ± 0.2	2.4 ± 0.3
TER %	59.2 ± 3.1	25.0 ± 1.9	15.3 ± 1.7

*mean of three replicates of the chromatograms, ± standard deviation, $s_{(3)}$

Compound classes: ACD, acids; EST, esters; AHA, alkanes; ALC, alcohols; ALD, aldehydes; KET, ketones; OTH, others; TER, terpenes

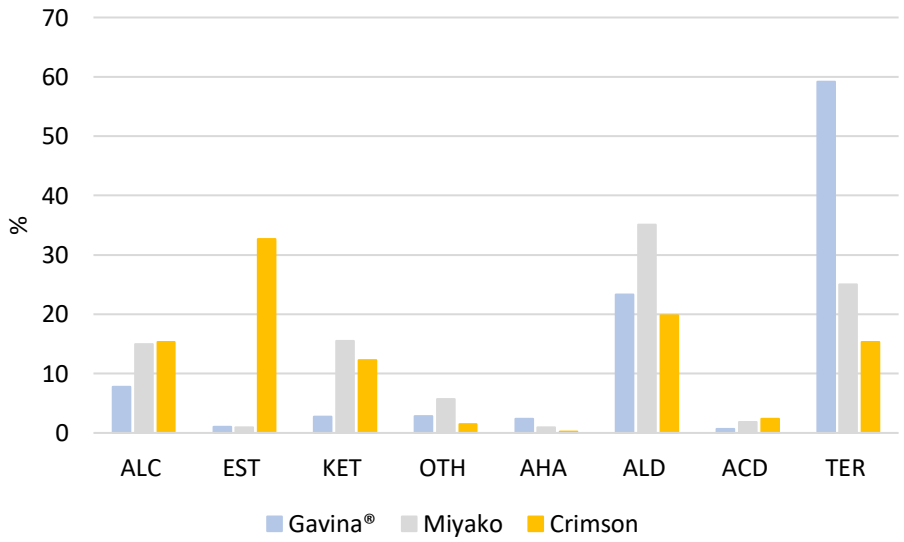

Figure 17 – Graphical representation of compound classes expressed as % detected in Gavina, Miyako and Crimson Sweet fibers

Analyzing the results grouped by analyte classes, it can be observed that the examined samples have HS chromatographic profiles that characterize the 3 cultivars, both for the different chemical composition and for the quantitative ratios within the individual classes of analytes. This observation is particularly significant, as it may underlie the possibility of identifying some suitable markers of product quality and product traceability:

▪ the fibers from Gavina show an aromatic bouquet defined mainly by the class of terpenes (TER);

▪ for the samples of the Miyako cultivar, the class of aldehydes (ALD) is the most representative one;
▪ the samples of the Crimson Sweet cultivar have the highest concentration of esters (EST).

At this research step, and limited to our investigations, the most complex and characteristic fragrance is certainly that of the Gavina® sample, since the terpenes are extremely aromatic, conferring pleasantness with strong effects on the perception and appreciation of the consumers [5]. Furthermore, the Gavina samples also show a high level (number and concentration) of C9 aldehydes, the compounds that most affect the overall aromatic bouquet of watermelon. Although Miyako samples have the highest percentage of aldehydes (≈ 35%), the open question remains whether they are endogenous compounds, produced by some metabolic pathways, or whether they probably develop due to the degradation of some fatty acids. Esters are abundantly present in the Crimson Sweet samples and scarcely present in those of the Gavina and Miyako cultivars. This observation agrees with several studies in the literature [5,10], as esters are reported to have a very low concentration in the HS of seedless watermelon.

Finally, a few underlining notes about Gavina cultivar, where the highest concentration of terpenes and terpenoid compounds was found. In particular, the compounds that possibly can be attributed to the degradation processes of lycopene have been detected in significant amounts. Unfortunately, at the moment we are not able to establish whether their origin is strictly related to the presence in significant quantities of the precursor compound lycopene, or what are the biosynthetic pathways from which they can be generated. Obviously, if they came from the main carotenoid present in this fruit, they could give a further contribution of added value to the red fiber obtained from the recovery of waste material. The Gavina cultivar has a rather recent history, if compared with those of the other varieties studied within this PhD thesis. In fact, very few studies and little information are reported in the literature. However, this product is already known as it has acquired the quality mark and the right recognition through the attribution of the international brand Gavina®. Nonetheless, our modest contribution to the development of knowledge about these topics could activate other paths to deepen some further scientific aspects, increase interest in the feasibility of technology transfer, and stimulate entrepreneurship dedicated to sustainability through the total valorization recovery of crop waste.

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CHAPTER 9

9 – AGEING AND CHROMATIC EVOLUTION OF WATERMELON FIBERS

9.1. BACKGROUND

Food quality is not a single and well-defined attribute, but it includes many properties and characteristics. One of the most important sensory quality attributes of fresh or processed foods is the appearance, an inclusive term involving size, shape, texture, mass, gloss, color, among others. The color of fruit and vegetables is the first quality parameter evaluated by consumers and it is particularly critical for the product acceptance and willingness to purchase: colors which indicate loss of freshness, suggest a lack of ripeness or over-ripening can discourage consumers from buying a certain product [1].

Actually, color is a physical property of fundamental importance, since it is strongly correlated with other physical, chemical and sensory indicators of commodities quality [2]. In fact, the visual aspect of vegetable foods usually provides an indication of freshness and, as a consequence, is often also associated to flavor quality, as well as providing information about the presence of some important chromophore molecules such as carotenoids, anthocyanins, etc… It is well known that color originates from natural dyes synthesized in fruits and vegetables, whose quantity depends on the kind of food, cultivar, harvesting and degree of ripeness, storage conditions. For these reasons, measurements of color point parameters can be used as quality indices in food routine analyses, to ensure conformity to specifications, to monitor color changes as a result of food processing and/or storage, among others.

In the specific case of watermelon ripe fruit, the characteristic and typical red color reflects the high lycopene content in its pulp [3]. This color is partially maintained in dried pomace, even if less bright than in the starting material. Lycopene is a powerful antioxidant, extremely sensitive to heat, light and oxygen exposure [4], so it can be very useful to identify the best storage conditions which can allow preserving this precious analyte over time as much as possible. A reduction in the amount of lycopene in dried watermelon pomace is associated with a loss of color of the fibers themselves, which pass from the initial red color to a progressively less intense one, until its disappearance at long ageing times. The evaluation of the chromatic variation of watermelon dried pomace fibers during storage could therefore provide useful information to monitor the variation of their qualitative characteristics, also associated to compositional ones, linked to the presence of analytes having beneficial properties. In general, the monitoring and control of color of processed foods, can be fundamental since its variation is usually associated to the loss of high-added value bioactive molecules having nutraceutical, antioxidant and beneficial effects on human health.

Thus, the aim of the present study was to evaluate if and how different drying methodologies and storage conditions can affect the stability of the color of Crimson Sweet watermelon dried pomace. This can be useful for identifying the best handling procedures and storage conditions for pomace fibers, in order to preserve lycopene as much as possible over time. To this aim, we have evaluated and monitored both the evolution of the UV-Vis spectra of the selected samples, over time, as well as the variation of the color point parameters L*, a* and b* defined within the CIELab color space.

9.2. MATERIALS AND METHODS

The study lasts 18 months, where the measurements started immediately after the drying of watermelon pomace (t_0 time) and have been completed at 540 days of storage. During this period we have identified several ageing steps (seven), at different time ranges, at which we recorded the spectra. Measurements were carried out with a UV-Vis spectrophotometer, by total reflectance technique, operating in the spectral range 200 ÷ 800 nm and using the instrumentation and methods described in Chapter 4.

Such a wide time window has been chosen in agreement with the average-standard ageing that a dietary fiber undergo when used as a functional ingredient, for example, in foodstuffs.

To obtain as much information as possible about the behavior of polysaccharide material during ageing, samples were stored in transparent glass vials holding a screw caps, to allow an effective closure, in different conditions of light exposure, temperature, and composition of the residual atmosphere present inside the containers themselves.

The complete list of preparation and testing storage conditions of the samples is reported in Table 1 with the following notation:

- W_AI_O (**W**atermelon Crimson Sweet pomace **A**s it **I**s and then **O**ven dried);
- W_*w*_O (**W**atermelon Crimson Sweet pomace ***w**ashed* with distilled water and then **O**ven dried);
- W_AI_V (**W**atermelon Crimson Sweet pomace **A**s it **I**s and then **V**acuum freeze-dried)
- W_*w*_V (**W**atermelon Crimson Sweet pomace ***w**ashed* with distilled water and then **V**acuum freeze-dried).

Table 1 - List of watermelon pomace fiber samples subjected to ageing study and color point evaluation, stored in different conditions.

	Atmosphere	Temp	Crimson W_AI_V	Crimson W_*w*_V	Crimson W_AI_O	Crimson W_*w*_O
daylight	air	Room Temperature (RT)	✓	✓	✓	✓
	N_2	RT	✓	✓	✓	✓
dark	air	RT	✓	✓	✓	✓
		4 °C	✓	✓	✓	✓
	N_2	RT	✓	✓	✓	✓

For each sample, at each ageing step, both the UV-Vis spectra and the color point parameters L*, a* and b* were measured on pads obtained as previously described in Chapter 4.

We have prepared at least three tablets for each fiber sample (4 fiber types) and related to each storage condition (5 different conditions), listed in the previous table. The faces of the tablets have also been marked to be able to always identify them during the handling operations to which they undergo during the aging period. For the entire duration of the study, the measurements were always carried out on the same marked face, i.e. the upper faces of the pads stored in the vials, therefore exposed to the action of the residual atmosphere and any luminous radiation. This strategy allowed us to keep the specimens always in the same condition between a working session and the following one, maintaining the same configuration throughout the aging study period. In addition, for the samples stored in an inert N_2 atmosphere, after each measurement carried out on the specimens extracted from the storage vial, the nitrogen atmosphere was restored, and the tablets were stored again. On the basis of the macroscopic behavior it was possible to hypothesize that a correlative trend between the color evolution of the tableted material and the spectroscopic response factors may exist. If this is verified, it will be possible to trace a rather significant and reproducible “ageing profile” for the studied materials and future developments.

The UV-vis spectra and L*, a*, b* parameters obtained on the three replicated tablets corresponding to the same condition, listed in Table 1, have been averaged. Thus, the results will always refer to the averaged data.

The total color change ΔE has been calculated by applying the following equation:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

where L*, a* and b* are the coordinates of our sample at *t* time of ageing, while L_0*, a_0* and b_0* are the color parameters of the material used as a reference with respect to which we calculate the color variation (fiber at t_0 time). The higher the ΔE value, the greater the color variation the fiber has undergone during the natural ageing.

Data analysis

Data analysis was performed on the data matrix consisting of the averaged UV-Vis spectra. The 300 ÷ 700 nm spectral range has been considered in the statistical analysis since it is the λ region that contains the most significant absorptions for our purposes, and where the major variations of the spectroscopic signal occur.

Principal Component Analysis (PCA) was used as exploratory data analysis tool in order to evaluate the effect of DF preparation and storage conditions on the UV-Vis spectrum profile over time. In fact, a variation of the UV-Vis spectrum profile is associated with a modification of the compositional characteristics of the analyzed matrix. In this specific case we are mainly interested in monitoring the degradation of lycopene over time, which can be depicted by the consequent loss of the initial red color of watermelon pomace fiber.

The Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) method [5] was used aiming at recovering the spectral profiles of single components, i.e. the "pure" chemical constituents such as lycopene, etc., and how their content varies during time (ageing).

MCR-ALS has become a popular chemometric method used for the resolution of multiple component responses in unresolved and/or unknown mixtures [5].

This methodology of data processing is based on the recovery of the pure response profiles (spectra, pH profiles, time profiles, elution profiles, ….) of the chemical constituents or species of an unresolved mixture obtained in chemical processes.

MCR is a decomposition method that can extract pure contributions from overlapped signals. This approach is very useful for the analysis of spectroscopic data generated by complex matrices, as in our case, where the spectra collected consists of the absorbance contributions of multiple analytes present in each selected sample. In fact, MCR-ALS, differently from PCA, assumes the data follow a Lambert-Beer model, it treats the input data as a mixture of pure signals mixed in different ratios, the concentrations. Resolving such a mixture implies obtaining the pure contributions (the resolved spectra) and their "mixing ratios", the concentrations, according to the equation:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{\mathrm{T}} + \mathbf{E}$$

where **D** is the data set holding the UV-vis spectra, **S** holds the pure resolved spectral profile of each chemical component, **C** is the concentration matrix and **E** holds the part of data which is not modelled.

Given that pure signals are obtained, MCR also works as a method for filtering the data: undesired sources of variability such as noise or background effects can be efficiently removed, and end up in the residual matrix **E**.

Software

PCA was performed by PLS Toolbox ver. 8.9 (Eigenvector Research Inc., Wenatchee, WA, USA) running in the Matlab® 2019b environment (The Mathworks Inc., Natick, MA, USA).

MCR-ALS analysis was performed by using the MCR-GUI v2 toolbox [6] as well implemented in MatLab.

9.3. RESULTS AND DISCUSSION

As an example, below we report some images showing the comparison between the UV-Vis spectra collected at the various ageing steps, for some Crimson Sweet watermelon pomace samples.
Details related to the type of fiber and storage conditions are indicated in the captions of each figure.

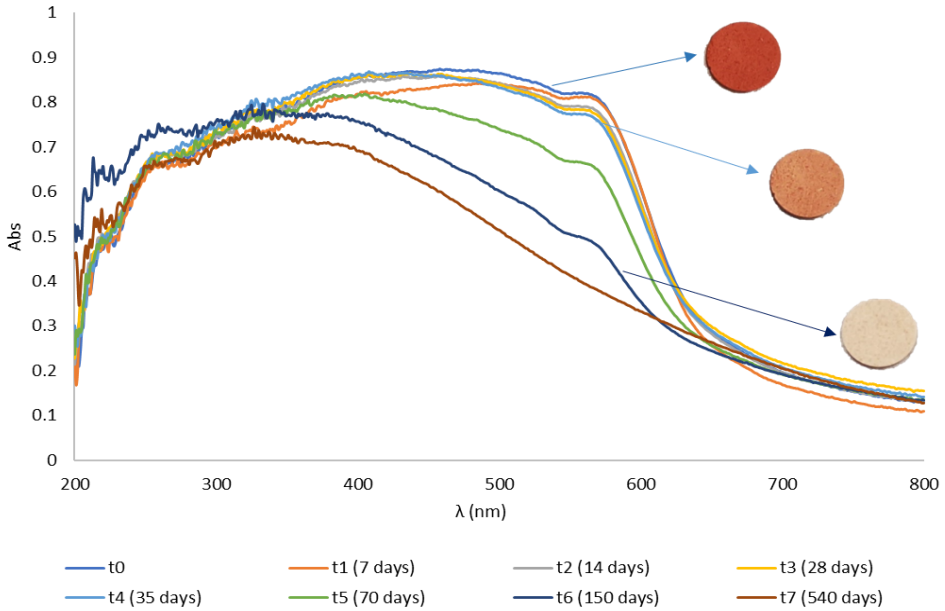

Figure 1 - Comparison of the UV-Vis spectra collected at different ageing steps, for a sample of Crimson Sweet watermelon pomace (W_AI_O), stored in a closed container, in contact with air, at room temperature, in the dark.

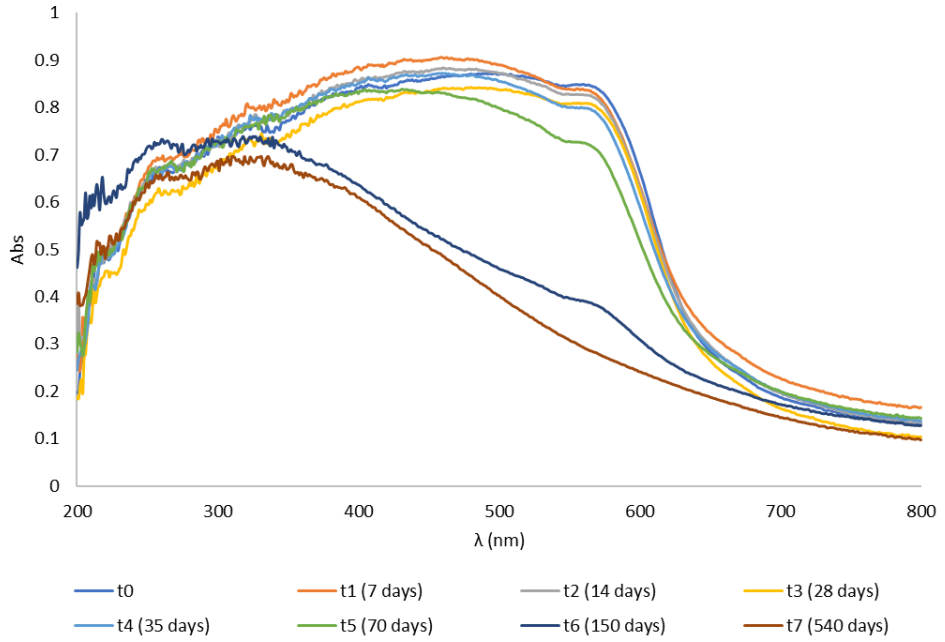

Figure 2 - Comparison of the UV-Vis spectra collected at different ageing steps of a Crimson Sweet watermelon pomace (W_AI_V), stored in a closed vial with an inert atmosphere (N_2), at room temperature and exposed to natural daylight.

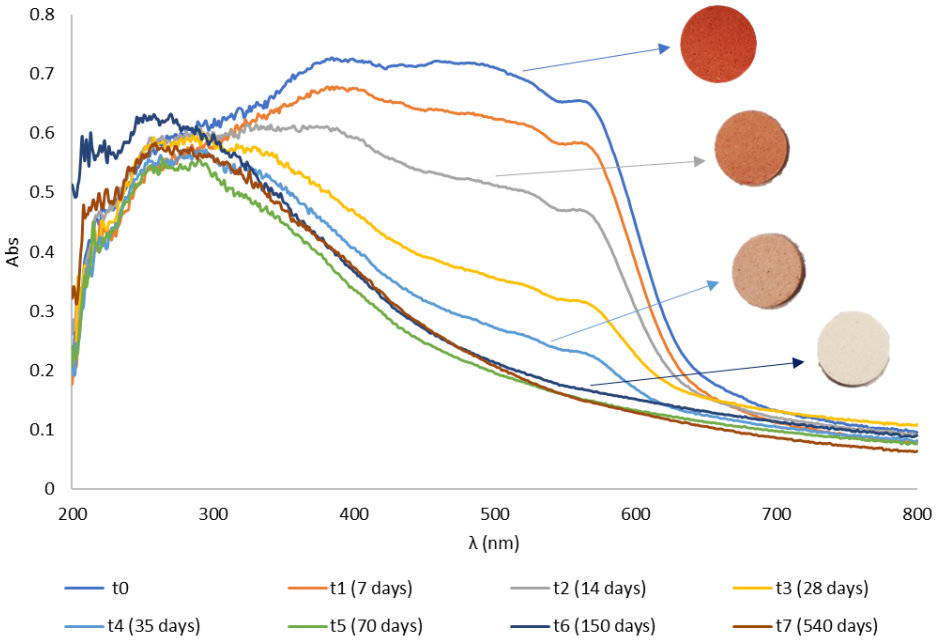

Figure 3 - Comparison of the UV-Vis spectra registered at different ageing steps, of a Crimson Sweet watermelon pomace sample (W_w_O) stored in a closed container in contact with air, at room temperature and exposed to natural daylight.

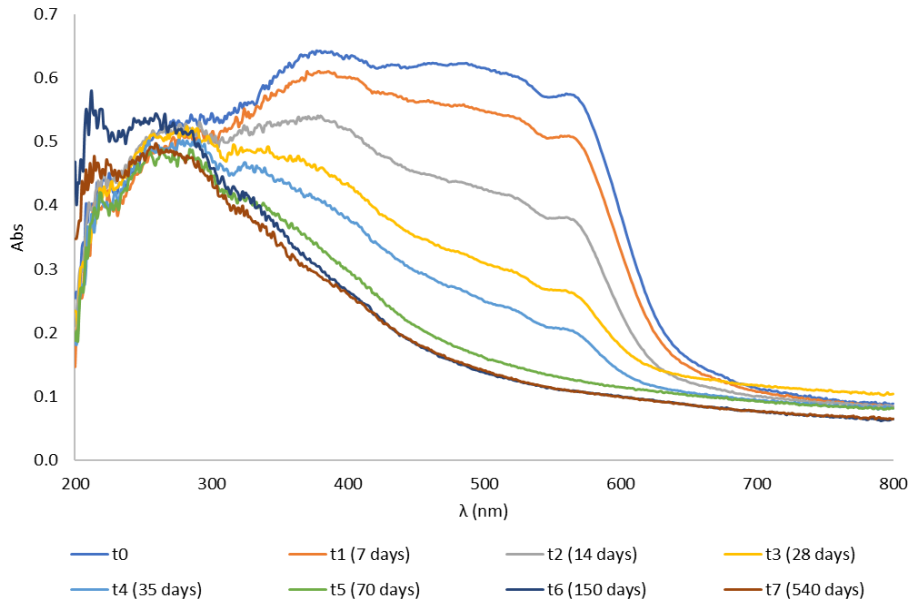

Figure 4 - Comparison of the UV-Vis spectra collected at different ageing steps, for a Crimson Sweet watermelon pomace (W_w_V) stored in a closed container with N_2 atmosphere, at room temperature, in the dark.

As already discussed in Chapter 7, all the spectra at t_0 time (Figures 1-4), shows the presence of a wide absorption band that extends throughout the range 230 ÷ 700 nm and, above all, the shoulder centred at about 560 - 570 nm which is attributable to the presence of lycopene in aggregate form. The intensities of these absorption bands decrease over time, more or less markedly and rapidly, depending on how the fibers have been prepared and / or stored. This trend can be observed in the previous figures (Figure 1, 2, 3, 4). At glance, it can be noticed a first important distinction between the "as it is" (W_AI) fibers and those washed with distilled water (W_*w*).

In fact, regardless of the storage conditions and the drying method used, W_AI samples are characterized by overlapped spectra, at least up to t_4 (35 days), while the spectra of the W_*w* fibers show a gradual and progressive decrease of the absorbance values already starting from the first ageing step (t_1). Furthermore, considering the spectra recorded at the longest ageing step (i.e. t_7), TDF have a wide continuous band in the visible region (Figures 1 and 2, curve colored in deep red-brown), which always has absorbance values on average two times higher than IDF washed with distilled water.
Probably, the drying processes of TDF material under the conditions we adopted (40 °C, 48 h and vacuum-freeze drying) do not significantly alter the matrix, allowing the molecules originally present (including lycopene) to maintain their thermodynamic stability for a longer time. The same cannot be affirmed for washed fibers (W_*w*_V and W_*w*_O). In this case, in fact, washes with distilled water impoverish the fiber of soluble analytes (e.g. sugars, metal cations, anions, amino acids, etc.), and this could alter not only the chemical composition but, probably, also the equilibria and structures of some of the analytes originally present in watermelon pomace. This hypothesis has already been advanced in Chapter 7, comparing the UV-Vis spectra of the “as it is” and washed fibers, at t_0 time, that showed different absorbance values. This is also confirmed by a faster variation of the spectral profiles of the washed material and faster loss of its initial red color over time.

In general, Figures 1, 2, 3 and 4 show that both the shape of the UV-Vis spectra and the absorbance values change as watermelon fiber samples age, i.e. for all the analysed samples the absorptions in the visible range (in particular, between 400 - 600 nm) gradually decrease ("flattening" of the band) while maintaining a quite marked absorption capacity only in the UV region. On the basis of our results so far, it seems reasonable to interpret the disappearance of the band centred at 560-570 nm as mainly related to the degradation of lycopene [7, 8]. Instead, the absorptions recorded in the UV region, which persist over time, are mainly due to the polysaccharides that constitutes the backbone of watermelon fibers, the amino acid / protein fraction and by the nitrogenous bases present in the matrix [9, 10].
The natural discoloring phenomenon the dried watermelon fibers underwent during this aging study was monitored and objectively defined also through the recording of the L*, a* and b* values related to the color point of the samples themselves.
Even the data and comments associated to these aspects will be reported and analyzed in this chapter.

PCA Results

Prior to PCA, spectral data were preprocessed by applying baseline correction (Automatic Weighted Least Squares) and mean centered.

A two-components model was chosen, explaining more than 90 % of the total variance (68.73 % for PC1 and 26.05 % for PC2). The scores plot of the first two principal components is shown in Figure 5.

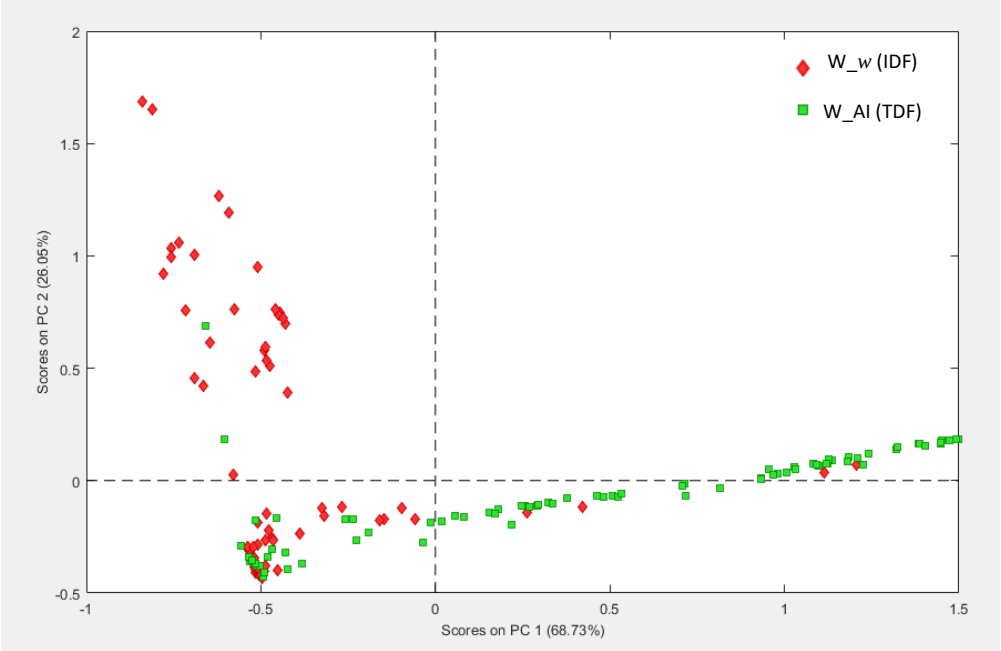

Figure 5 - PC1 vs PC2 scores plot (PCA of the watermelon UV-Vis spectra data set). Green squares identify the "as it is" (TDF) fibers, red diamonds the washed ones (IDF).

In Figure 5, almost all the "as it is" (W_AI) samples are uniformly distributed on PC1, showing negative values of PC 2; while the washed ones (W_*w*) lie mainly along PC2, with negative values of PC1. From these trends, it seems that PC 1 follows the evolution over time of the UV-Vis spectra of the "as it is" samples and PC2 describes the behaviour of the washed ones.

However, the two trends appear to be quite different; in fact, while the W_AI samples follow a more linear trend, some W_*w* samples are quite grouped at negative PC1 and PC2 values and others are spread along positive PC2 values. These trends are confirmed in Figure 6, showing the same scores plot, now colored according to the ageing time of the samples (see legend), thus highlighting the evolution of the UV-Vis spectra collected over time for the different types of conditions.

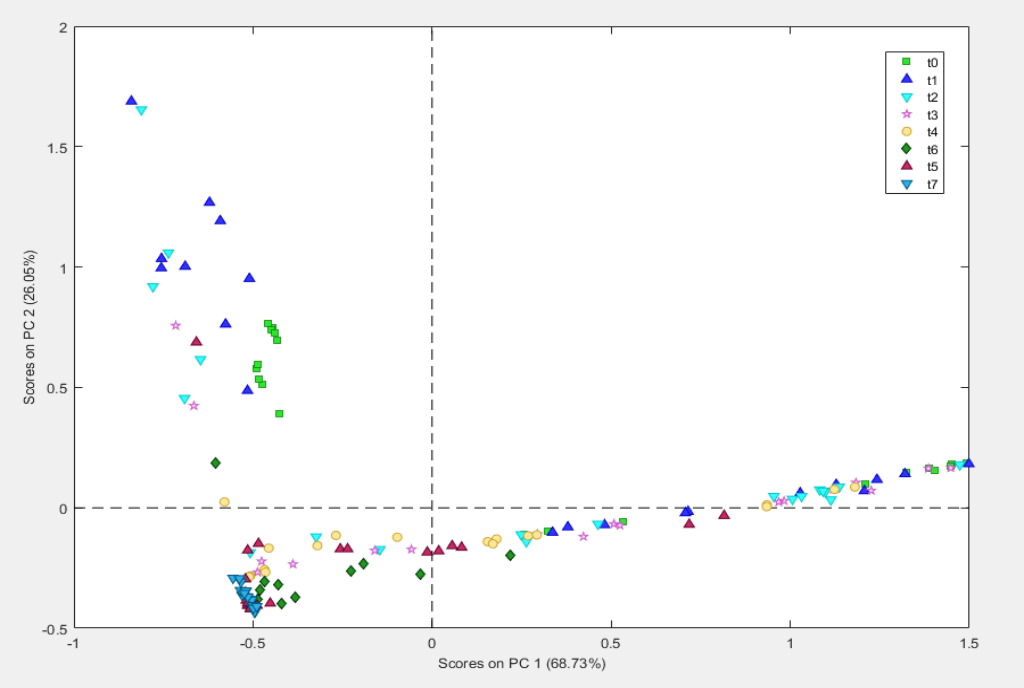

Figure 6 - PC1 vs PC2 scores plot (PCA of the watermelon UV-Vis spectra data set). Samples colored according to ageing as shown in the legend.

From Figure 6 it is evident that the spectra of washed watermelon IDF fibers, subjected to washing with distilled water, change much more rapidly than the "as it is" (TDF) ones. In particular, the UV-Vis spectra of washed samples (W_*w*_V, W_*w*_O) seem to maintain a profile comparable to the one recorded at t_0 time only for the first two ageing steps, then drastically change from 35 days (t_3).

In fact, starting from t_3 step, the spectra related to this family of samples become indistinguishable from those recorded at longer ageing times. For TDF fibers, however, the variation of the UV-Vis spectra occurs, on average, more slowly and more gradually. These trends also confirm that the washing process modify the characteristics of the physiological environment in which lycopene is lodged. As a consequence, its stability decreases over time probably because of its greater susceptibility to oxidizing agents or photodegradation.

Furthermore, observing the position in the scores space of samples corresponding to t_0 time (green square ■), it appears that the washing makes the starting conditions more homogeneous (see the green square cluster at positive PC2 values which relates to W_*w* (IDF), colored red in figure 5) with respect to the crude TDF samples.

Probably, removing soluble analytes also reduces the small differences both on the spectral profiles and on the intensity of the absorption bands of the washed samples. These differences, instead, remains among the row fibers and are highlighted by the PCA analysis.

Finally, all spectra recorded at the longest ageing time (t_7 = 540 days) are indistinguishable for both TDF and IDF samples. At this ageing step, in fact, the absorption bands present in the 300 - 700 nm region are mainly generated by analytes that constitute the polysaccharide components common to all samples regardless the manipulation operations to which they were initially subjected.

What has been reported so far, emerges even more clearly by the comparison of scores with their respective PC1 and PC2 loading values, shown in Figures 7 and 8, respectively.

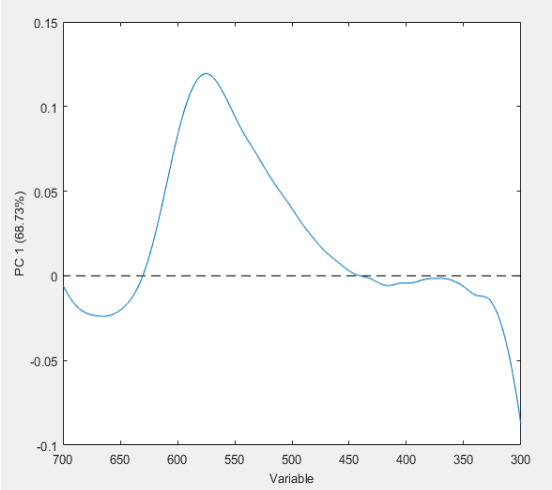

Figure 7 – PC 1 loadings plot vs UV-Vis wavelength.

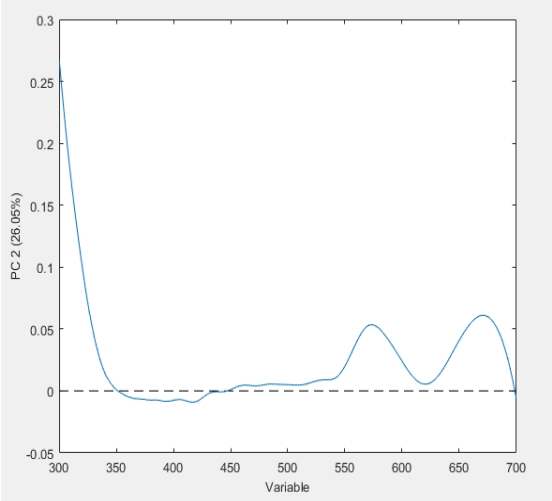

Figure 8 - PC 2 loadings plot vs UV-Vis wavelength.

From Figure 7, it is highlighted that there are two main UV-Vis-bands which mainly contributes to the differences between the "as it is" TDF samples from the washed ones. In particular, the wide absorption band covering the spectral window 450 ≤ λ/nm ≤ 630 nm, centred around 570 nm, is mainly contributing to the distinction between TDF and IDF samples. In general, samples with negative scores are characterized by a less intense absorption band centred at about 570 nm (having positive loadings), and by a more intense band present at ≈ 300 nm (having negative loadings). For IDF samples spectra, the 570 nm band always shows lower values than those for the TDF ones, since the PC1 scores associated to IDFs always have negative values, except for some in the first measurement steps. Conversely, PC2 is more sensitive to variations of the IDF spectra.

The trend reported in Figure 8 shows that the most important contribution in defining the spectra of watermelon IDFs over time, is found in the 300 - 350 nm window, mainly attributable to the components that constitute the backbone of the fibers itself.

From the comparison of PC1 and PC2 loadings plot with the corresponding scores shown in Figure 6, it is possible to better follow the evolution trends of the UV-Vis spectra of the several samples, over time.

Observing the evolution over time of the scores on PC 1 (associated with the TDF samples) it can be noticed a decreasing trend from positive to negative values on PC 1. This means that, within the UV-Vis spectra, the bands having the greatest impact in defining the evolution of the spectral profile over time progressively decrease. Evaluating the loadings profile in Figure 7, this decrease, for all watermelon TDF samples, is observed in the region ≈ 400 - 650 nm, in particular attributable to lycopene in solid state (characteristic absorption band at ≈ 570 nm). Finally, it can be noticed that most specimens become indistinguishable from

step t_6 onwards, all having completely comparable UV-Vis spectra at t_7 time, regardless of the fiber treatment and the conditions in which they have been stored over time (Figure 6).

Furthermore, from PC 2 scores and loadings (Figures 6 and 8), it can be noticed that the signals defining the profile of the UV-Vis spectra of washed fibers (IDF) gradually decrease over time, except for the passage from step t_0 to t_1 where, instead, there is an increase.
This behaviour is difficult to rationalize but, given the complexity of the matrix analyzed, it could be attributed to different mutual "weights" that some absorption bands may acquire, if compared to others, within the entire λ range considered. However, already after 7 days of ageing, all absorbance values gradually decrease. Finally, IDF samples are indistinguishable already starting from t_4 time (35 days), regardless of the conditions in which they have been stored. For TDF ones, instead, this occurs between 70 and 150 days. This means that after 28 days, washed specimens have lost their initial color due to the degradation of lycopene (and other molecules containing chromophore groups), resulting in a consequent reduction of absorptions in the visible region.
Conversely, the UV absorption bands remain, being generated by analytes which constitute the backbone of the fibers, such as polysaccharides, proteins and other nitrogenous compounds, etc ... [9, 10].
According to PC 1 e PC 2, neither the two different drying methods (vacuum-freeze drying vs oven), nor the different storage conditions (air /N_2; room temperature / 4 °C) lead to significant differences among the various samples, except for daylight /dark one (this has been observed coloring scores plots for the respective conditions, figure not shown for the sake of brevity).
Some additional considerations can be formulated regarding the impact of storage conditions:

- W_AI_O samples stored exposed to daylight degrade faster than those stored in the dark (the first ones reach negative scores already at time t_5), with the same residual atmosphere in contact with the material.
- For W_AI_V samples, on the other hand, different lighting conditions do not seem to have a significant influence, since all samples stored at room temperature degrade approximately with the same rate (from t_4 the scores values are comparable).

Temperature does not seem to particularly affect the color preservation of watermelon fibers. In fact, the trends described by scores on PC 1 related to W_AI_O and W_AI_V specimens stored at 4 °C, in the dark and in contact with air inside the vial, are comparable with those of the same fibers type, stored under similar conditions but at room temperature. Similar considerations can also be extended to the two different types of atmosphere tested (conventional air atmosphere and inert N_2 one), which, in general, do not seem to have a significant influence on the storage of our samples.

MCR-ALS Analysis

In the following section we will present the results obtained by using Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) analysis on the same UV-Vis data set analysed by PCA, exploiting treatments, conditions and ageing described previously and reported in Table1. In particular, we exploited MCR-ALS to verify which "pure" components can be recovered and whether the loss of fibers color and the variation of UV-Vis spectra profiles, over time, is actually attributable to lycopene degradation.
Nonnegativity constraints were imposed both on concentration and spectral profiles, meaning that both spectral profiles and concentrations should have only positive values. In addition, unimodality constraint was imposed on concentration. This impose that concentration profile of pure component should not show multiple maxima or minima, as it is expected for a kinetics. The Alternating Least Squares optimization algorithm was initialized by using initial estimates of spectral profiles provided by a pure variable selection method based on SIMPLISMA [11]. Once the initial estimates are obtained, the concentration profiles matrix (C) and the spectral profiles one (S^T) are alternatingly optimized in each iteration. As stopping convergence criterion the relative difference in percentage of lack of fit (LOF) between consecutive iterations was used [12].
Two MCR components were found relevant, so the algorithm selected, as initial guess, two pure spectra among the purest column variables, which correspond to the signal collected (1) at the beginning of storage period and (2) at the end of the monitoring.

The two components MCR model explained 99.86% of the data variance.

Figure 9 reports the two resolved pure spectral profiles.

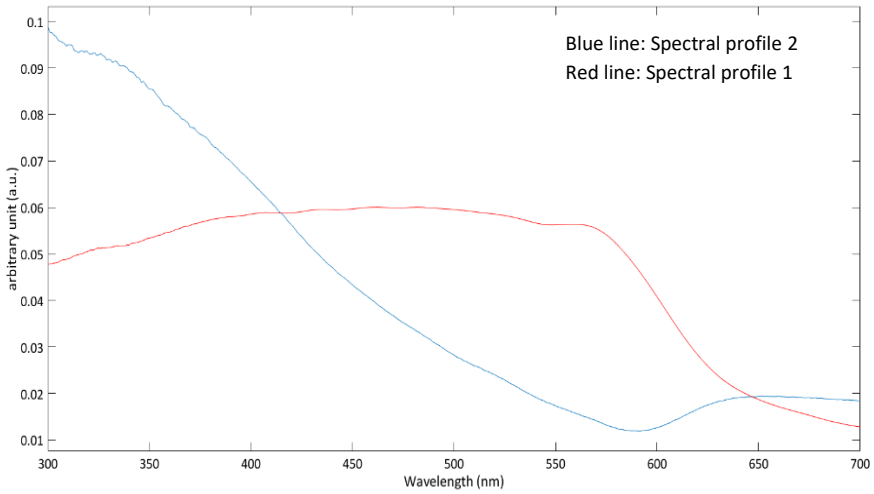

Figure 9 - Pure spectral profiles resolved by MCR-ALS analysis.

They can be interpreted as follow:
i) the first component spectral profile (red) showing a broad absorption band ranging from about 300 - 550 nm and a second band centred around 570 nm (characteristic of lycopene in solid state) can be matched to lycopene. Unfortunately, it has not been possible for us to experimentally record a UV-Vis spectrum of standard lycopene in solid state, to then make a direct comparison with those obtained from our fibrous samples, due to the high thermodynamic instability of the analyte itself. However, the confirmation of the presence of lycopene and information related to its UV-Vis spectra are reported in various literature studies [7, 8];
ii) the second component (blue) show absorptions mainly in the initial part of the spectrum towards the ultraviolet λ region, and it could be given by the analytes which constitute the structure of the fiber.

This interpretation is consistent with the concentration profiles which show for the first component (Figure 10a and 11a) a sharp decrease with ageing and an opposite behaviour for the second component (Figure 10b and 11b): as the ageing of watermelon pomace fibers proceeds, the intensity of lycopene absorption bands decreases until completely disappears when the samples have completely lost their initial color. In this condition, fibers acquire a pale ivory color and the most significant absorptions are due to fiber polysaccharides constituents.

Figures 10a and 10b report the MCR concentration profiles for TDF samples aged up to 540 days under different storage conditions.

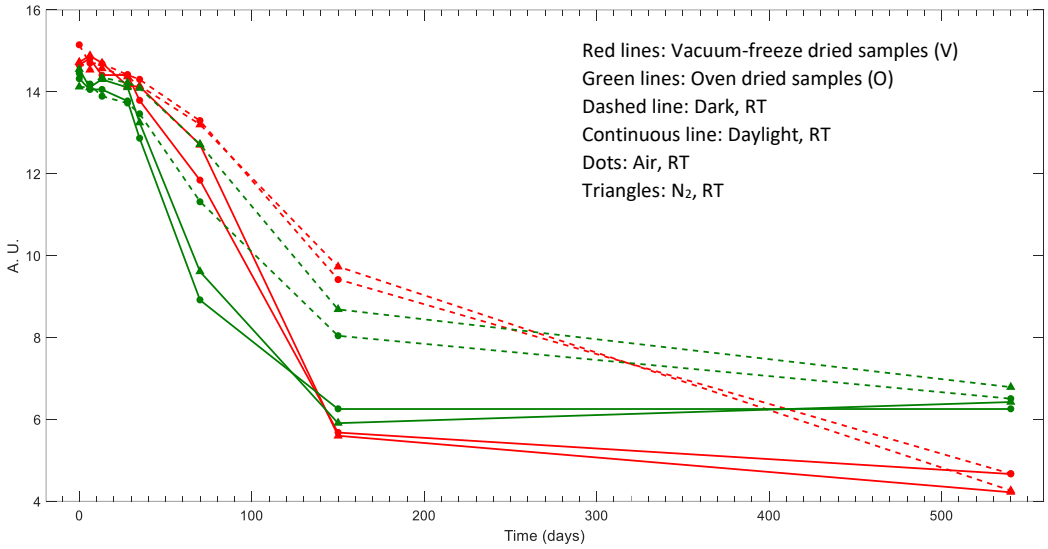

*Figure 10a - MCR concentration profiles for the first component corresponding to the red spectral profile of Figure 9 (**lycopene**). Only **TDF** samples are shown, each line corresponds to fiber stored in different conditions as reported in the legend.*

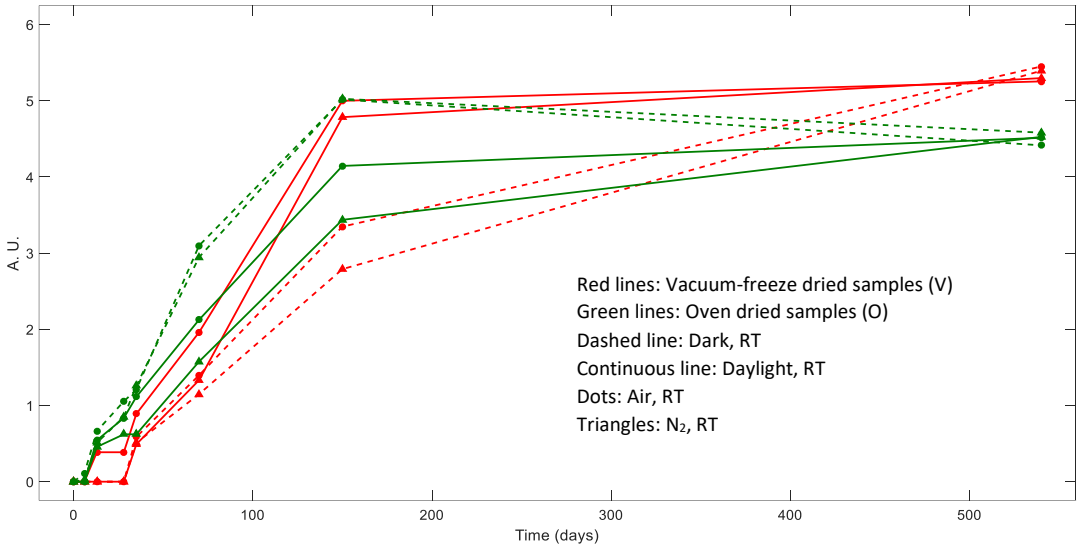

*Figure 10b - MCR concentration profiles for the second component corresponding to the blue spectral profile of Figure 9 (**backbone of fibers**). Only **TDF** samples are shown, each line corresponds to fiber stored in different conditions as reported in the legend.*

Figures 11a and 11b report MCR concentration profiles for IDF samples aged up to 540 days under different storage conditions.

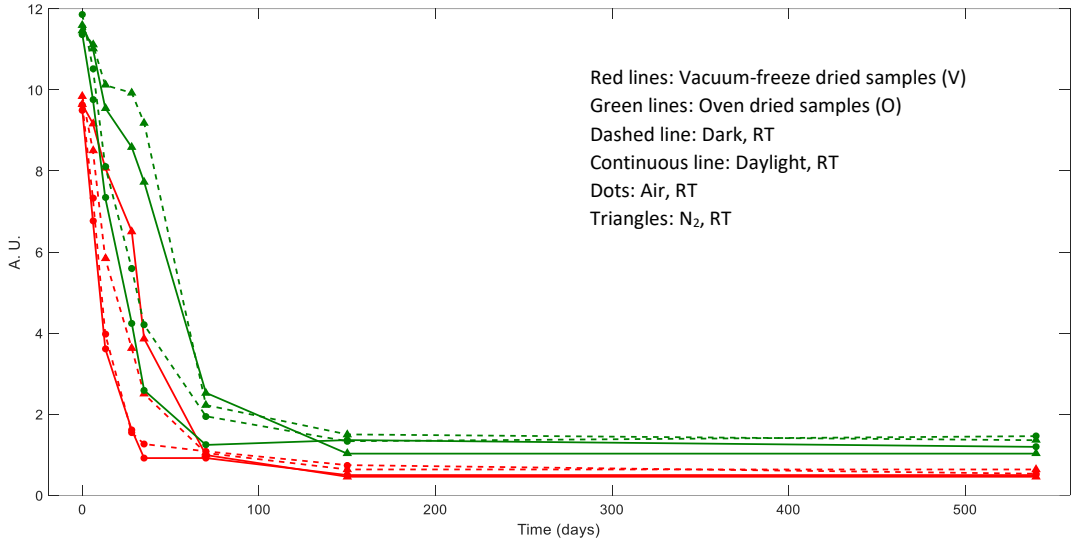

*Figure 91a - MCR concentration profiles for the first component corresponding to the red spectral profile of Figure 9 (**lycopene**). Only **IDF** samples are shown, each line corresponds to fiber stored in different conditions as reported in the legend.*

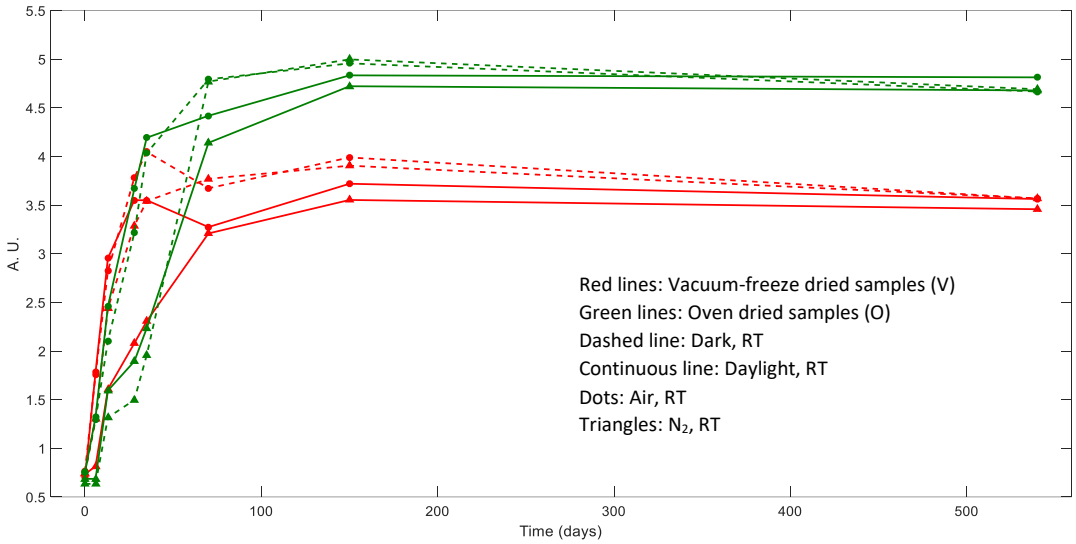

*Figure 11b - MCR concentration profiles for the second component corresponding to the blue spectral profile of Figure 9 (**backbone of fibers**). Only I**DF** samples are shown, each line corresponds to fiber stored in different conditions as reported in the legend.*

Observing Figures 10a and 11a, it can be noticed that for each analyzed sample, there is a progressive decrease over time of the concentration profiles associated with lycopene, which start from the maximum value at t_0 time, and then reach the minimum between steps t_6 and t_7 for TDF fibers. For most of the IDF samples, profiles are the same but minimum values are already reached at time t_5 (70 days). Conversely, concentration profiles of Figures 10b and 11b, associated with the analytes which constitute the core structure of the fibrous materials, describe an exactly opposite trend. This occurs for each of the storage conditions tested.

The processing of UV-Vis spectroscopic data through MCR-ALS analysis furnishes a much clear view of the ageing process with respect to the preliminary PCA. In particular, from Figure 10a it can be noticed that in

TDF samples stored to daylight exposure and at room temperature, the signal associated to lycopene is always lower than that in fibers aged in the dark in the same temperature condition, regardless of the composition of the residual atmosphere in contact with the samples. Moreover, at least for "as it is" fibers, both vacuum-freeze dried and oven dried, stored in the dark, the inert atmosphere (N_2) seems to slightly slow down lycopene degradation throughout all the ageing period (540 days). For the same specimens exposed to daylight, this is verified only until 70 days, after which the effect of different atmosphere composition is no longer significant. This behavior perfectly agrees with the literature data which report that lycopene degradation is favored by the presence and action of oxidizing agents, such as atmospheric oxygen, and by exposure to light sources. Finally, we point out that the different selected storage temperatures (4°C and room temperature) did not produce significant different effects in allowing the preservation of lycopene over time.
These results demonstrate the potential and effectiveness of using tools such as MCR-ALS analysis for the resolution of complex mixtures. In fact, it allowed to identify the species which mainly undergoes changes during ageing and contribute to the UV-Vis spectra of dried watermelon pomace fibers (i.e. lycopene and the polysaccharide backbone of the fibers), and to monitor them during the evolution of the sample over time. In addition, it highlighted some small details which were not easy to notice in the PCA plots.

9.4. COLOR POINT EVALUATION

In the following section we report the most significant results obtained from the monitoring of L*, a*, b* color point coordinates, collected at different ageing times (eleven step), and the calculation of the color variation ΔE. These parameters characterize the Crimson Sweet dried pomace fibers stored in different conditions, over time, as indicated in Table 1. In each figure we compare a single parameter, related to a homogeneous set of samples as far as the preliminary handling process adopted to obtain the fibers concern. In addition, each homogeneous sample set is referred to each different storage condition.

Evolution of L* coordinate over time

The following figures show the trends defined by L* parameter during the ageing of the different Crimson Sweet dried pomace fibers, stored in the selected conditions. Within the CIELab color space, L* represents the brightness of a color and ranges from 0 (black) to 100 (white). Details related to the type of fiber considered and storage conditions are specified in the caption and legend of each figure.

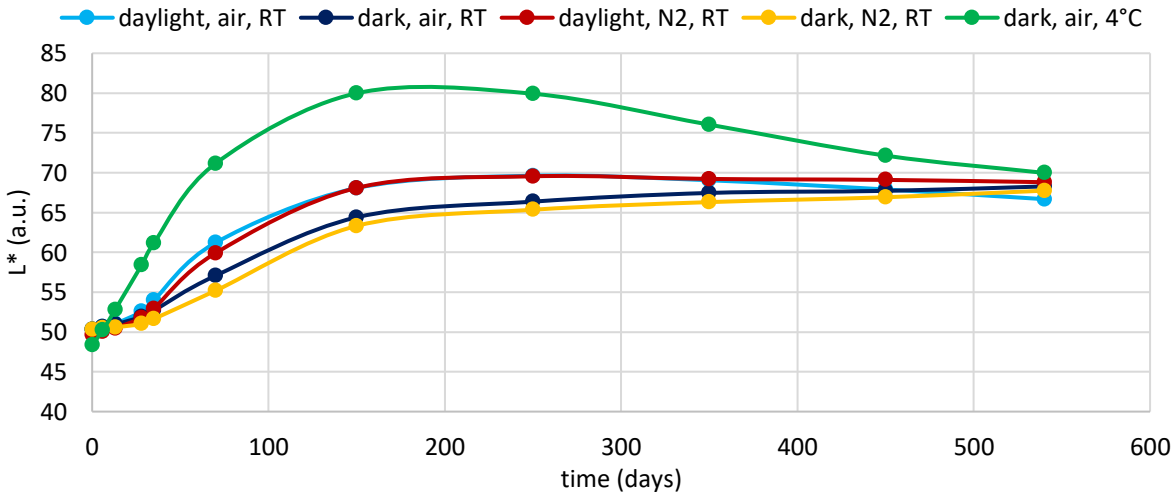

Figure 12 - Trends defined by L parameter vs. ageing time for W_AI_O samples from Crimson Sweet cultivar, stored in different conditions.*

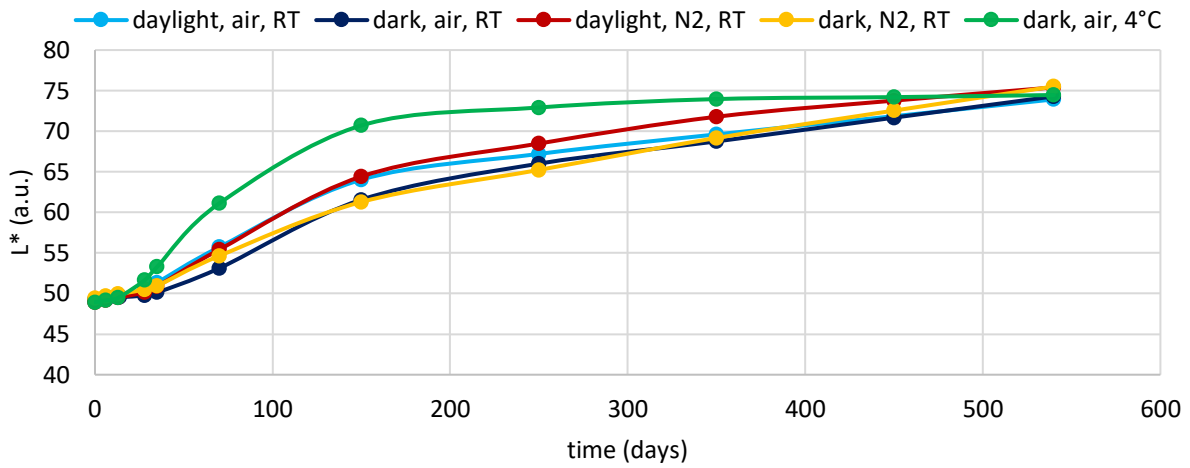

Figure 13 - Trends defined by L parameter vs. ageing time for W_AI_V samples from Crimson Sweet variety, aged in different storage conditions.*

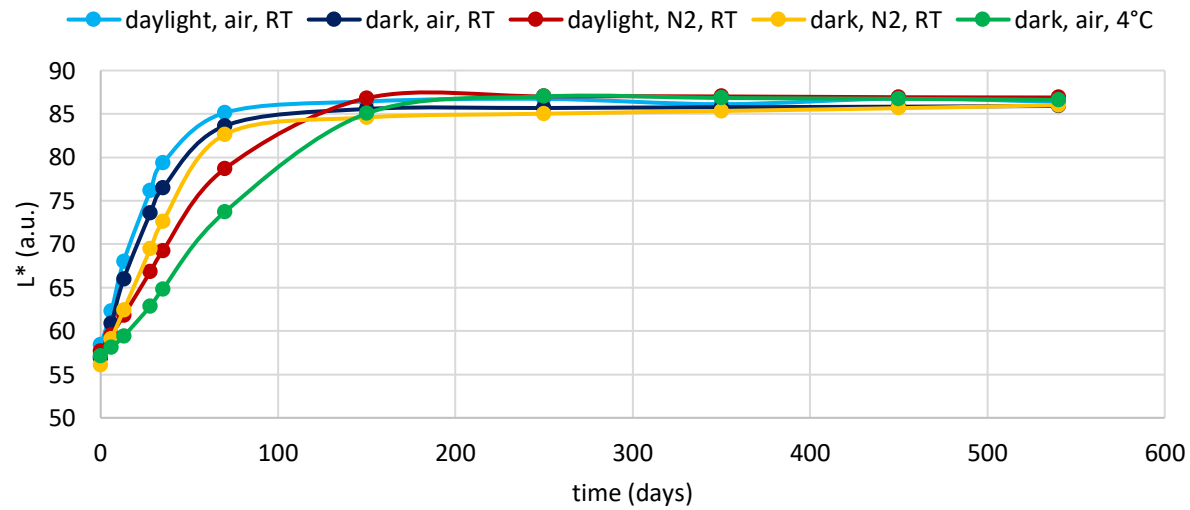

Figure 14 - Trends defined by L parameter vs. ageing time for W_w_O samples from Crimson Sweet cultivar, aged in different storage conditions.*

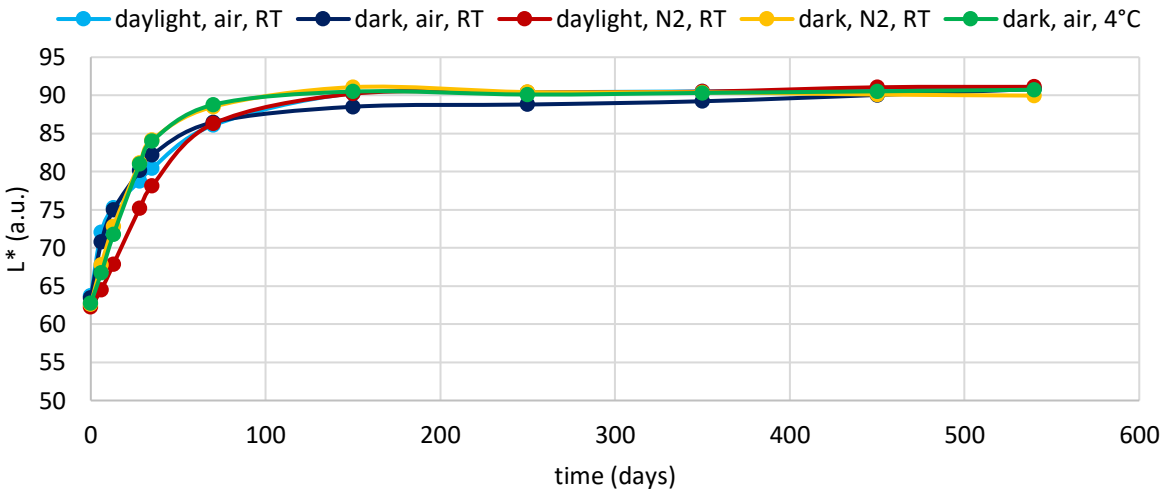

Figure 15 - Trends defined by L parameter vs. ageing time for W_w_V samples from Crimson Sweet watermelon, stored in different conditions.*

From Figures 12, 13, 14 and 15 it can be noticed that the L* values at t_0 time are comparable among all four types of fibers, while the values reached at t_{540} are significantly different between TDF and IDF samples. In fact, despite L* value progressively increases over time for all specimens, indicating an increase in fibers brightness, IDFs always reach higher values than TDFs at t_{540}, regardless of the drying methods and for each storage conditions tested. In particular, at the longest ageing step, W_*w*_V and W_*w*_O assume L* values around 85 (a.u.), while W_AI_V and W_AI_O ranges from ≈ 86 – 74 (a.u.), respectively. Furthermore, IDF samples undergo a much more noticeable variation of L * parameter than TDFs already in the first ageing steps, reaching a plateau around 70 days, on average. Conversely, L* parameters of "as it is" fibers change much more gradually throughout the investigated period, defining much less accentuated trends over time.

This is very evident by comparing, for example, Figure 13 and Figure 15. These profiles confirm that washing processes have modified the characteristics of the physiological environment in which lycopene is lodged, leading to a decreasing of its stability over time due to a greater susceptibility towards oxidizing agents or photodegradation. With the same type of fiber selected, these considerations are valid almost for all the investigated storage conditions. The only exception is observed on the W_AI_O and W_AI_V specimens, stored in the dark, in contact with the residual atmosphere (air), at 4 °C. They present an average higher increase in brightness than the same fibers stored at room temperature, to finally reach a comparable L* value at t_{540}.

Evolution of a* parameter over time

Within CIELab color space, a* is the coordinate which ranges from red (positive values) to green (negative values). For our matrix, i.e. an initially red fiber, the a* values recorded at t_0 time have always been positive regardless the handling procedures adopted to obtain the samples (TDF or IDF). The results obtained from the monitoring of a* for the selected fibers, over time, stored in different conditions are described below.

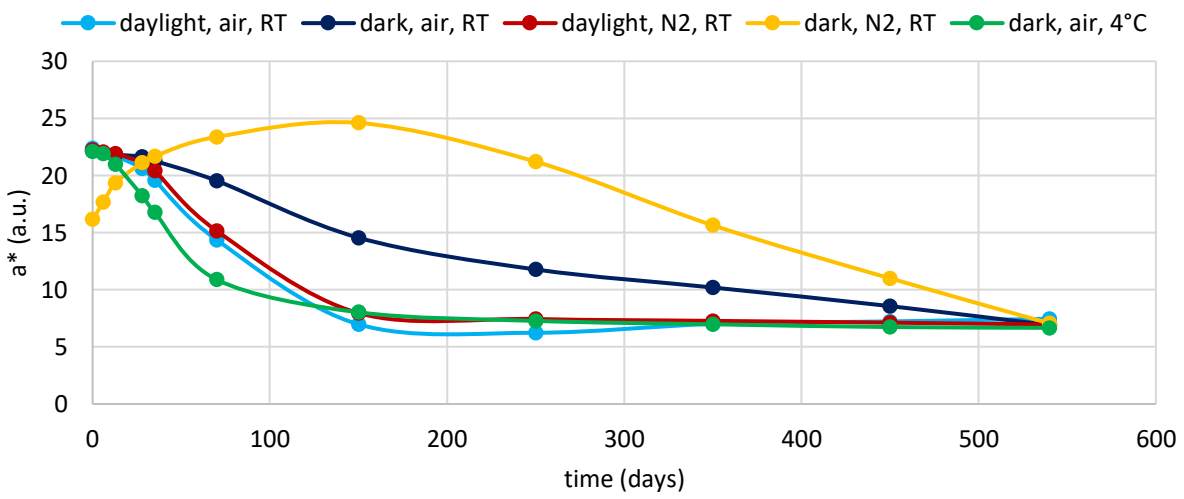

Figure 16 - Trends defined by a parameter vs. ageing time for W_AI_O samples from Crimson Sweet variety, stored in different conditions.*

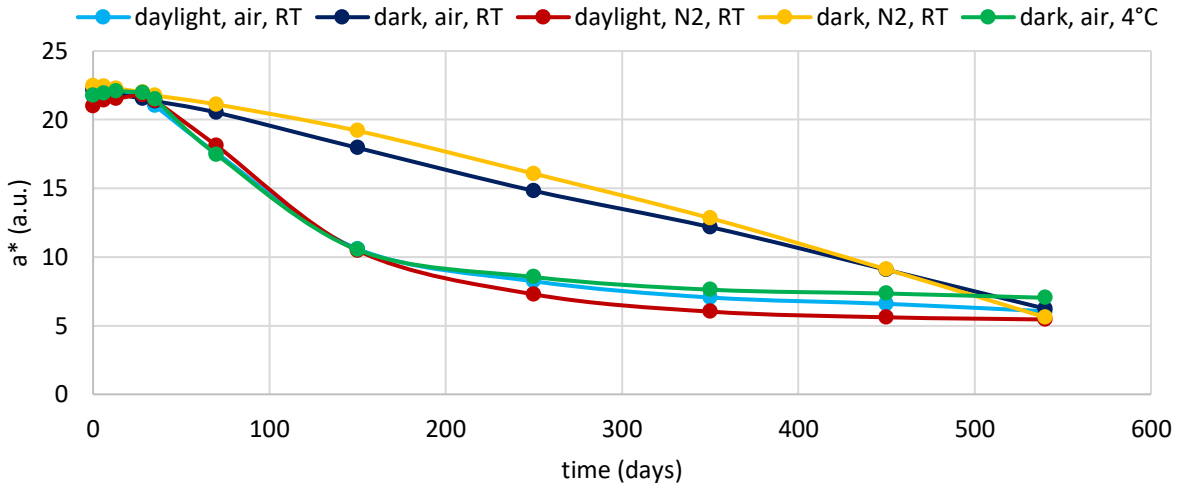

Figure 17 - Trends defined by a parameter vs. ageing time for W_AI_V samples from Crimson Sweet cultivar, aged in different storage conditions.*

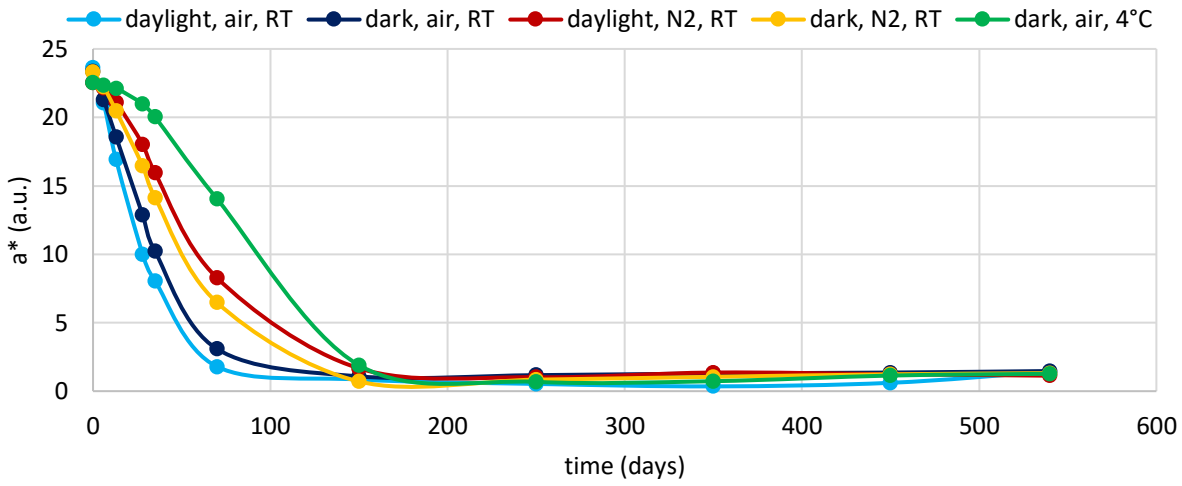

Figure 18 - Trends defined by a parameter vs. ageing time for W_w_O samples from Crimson Sweet watermelon, aged in different storage conditions.*

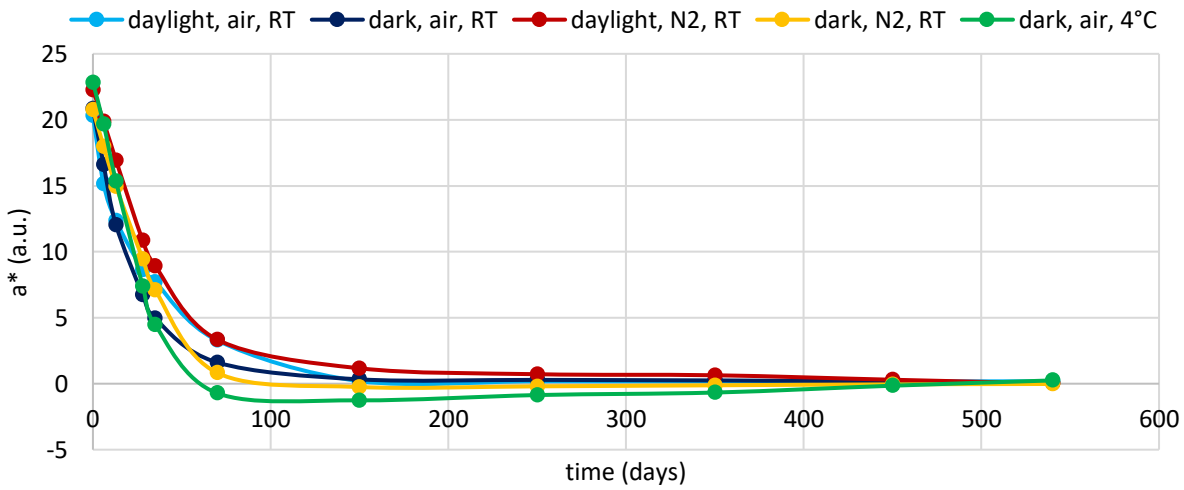

Figure 19 - Trends defined by a parameter vs. ageing time for W_w_V samples from Crimson Sweet cultivar, stored in different conditions.*

Looking at Figures 16, 17, 18 and 19, a progressively lowering of a* values is observed in all TDF and IDF samples passing from the initial analysis step (t_0) to the final one (t_{540}). Also in this case, this reduction is more pronounced for IDF fibers than TDF ones. In fact, for all W_*w*_O and W_*w*_V samples, a* parameter quickly decreases, assuming the lowest values (around 0 a.u.) at t_{150}, which remain almost constant until the end of the study (540 days). Conversely, the lowering of a* values associated to W_AI_O and W_AI_V fibers, describes more slow and gradual trends. This progressive reduction is observed during all 18 months, especially for "as it is" fibers stored in the dark and at room temperature, both in contact with air or N_2 (Figures 16 and 17). The decrease in the positive values of a* objectively indicates the loss of the initial characteristic red color of dried watermelon pomace, to finally assume a pale ivory color (Figure 2). This is due to lycopene degradation over time, which occurs for all samples in all the storage conditions tested.

Going into more detail, it can be noticed that while the starting values (t_0 time) are generally comparable among all types of fibers, the final ones (t_{540}) are quite different. W_*w*_O and W_*w*_V are characterized by a* values which ranges from ≈ 0.2 a.u. to ≈ 1.2 a.u., while for W_AI_O and W_AI_V fibers they vary between 5 – 7 a.u. Combining the information relating to the L * parameter, this results in a slightly more marked

residual color of the TDF specimens (a little more intense ivory color) than the IDF ones ("cleaner" final white color).
Considering the impact that different storage conditions produce on the values assumed by a* over time, it can be affirmed that all fibers washed with distilled water age and discolor in a comparable way, regardless of how they were stored. Conversely, some differences can be noticed as far as TDF samples concern. Both for W_AI_O and W_AI_V, storage in dark rather than to daylight exposure, regardless of the residual atmosphere present in the containers, allowed to preserve the red color of dried pomace fibers for a longer time, slowing down lycopene degradation processes. This perfectly fits with literature data which report the high sensitivity of lycopene to light exposure, which accelerates its photodegradation [4]. So, it can be affirmed that storage of this type of samples in presence or absence of light, represents a particularly impacting factor for a* parameter. However, TDFs stored in the dark, in air, at 4 °C represent an exception, since they show trends comparable with those stored in presence of daylight. Curiously, in this case, low temperature seems not able to preserve lycopene, negatively affecting a* parameter and leading to a faster decrease, if compared to samples always stored at room temperature (Figures 16 and 17).

In Figure 16 there is another profile very difficult to rationalize, i.e. the one associated to W_AI_O aged in the dark, at room temperature in presence of inert atmosphere (N_2). First thing, the initial value of a* is significantly lower than that of analogous fibers stored in the other conditions examined. Since at t_0 time the samples have not been subjected to the different storage conditions yet, all these values should have been comparable. Moreover, the whole trend associated to this W_AI_O specimen is very different from all the others, showing a progressive increase of a* up to 150 days, to then progressively decrease till reach a final value comparable with the other ones.
Looking at Figures 18 and 19 related to IDF samples, it can be affirmed that there aren't important differences between the described profiles. All specimens have almost completely lost the initial red color at 150 days: from this ageing step the variations of a* values are very small and a plateau is defined until the end of the studied period.

Evolution of b* parameter over time

Within CIELab color space, b* is the coordinate which ranges from yellow (positive values) to blue (negative values). In all our samples, b* values recorded at t_0 time have always been positive, both for TDF and IDF samples, and have also maintained positive values throughout all the ageing study (18 months). The results obtained from the monitoring of b* parameter over time for the selected fibers, stored in different conditions, are described below.

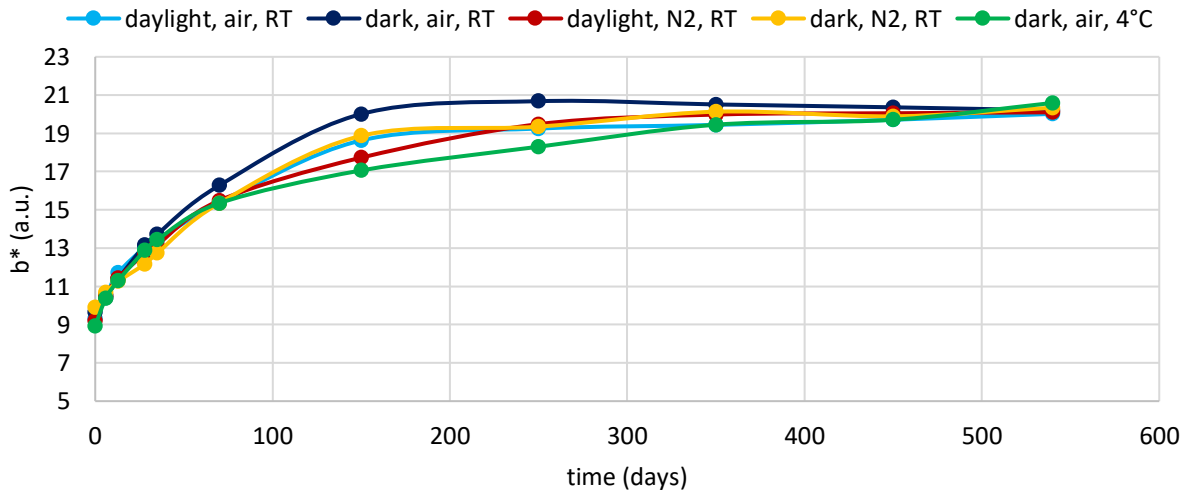

Figure 20 - Trends defined by b parameter vs. ageing time for W_AI_O samples from Crimson Sweet watermelon, stored in different conditions.*

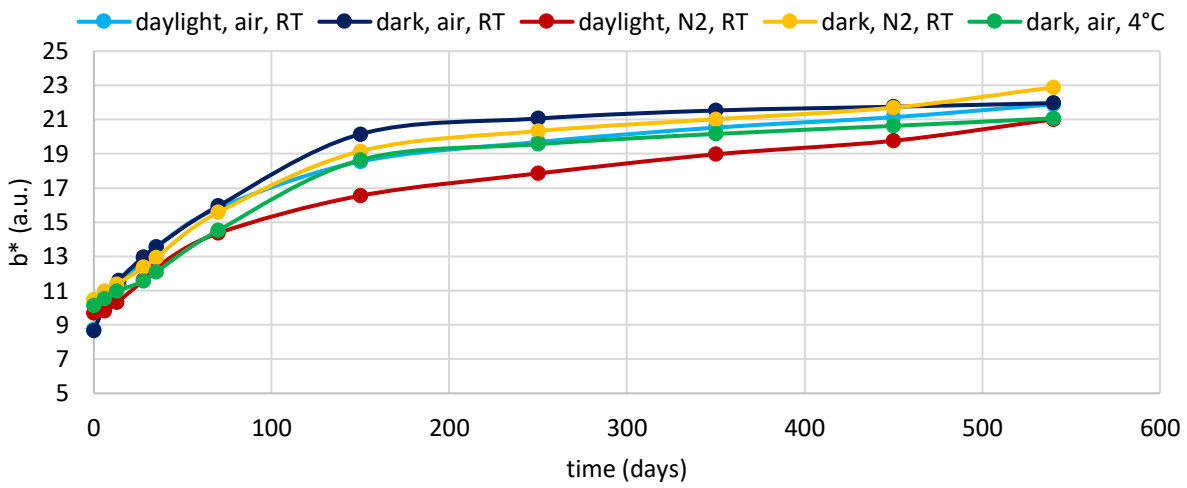

Figure 2110 - Trends defined by b parameter vs. ageing time for W_AI_V samples from Crimson Sweet cultivar, aged in different storage conditions.*

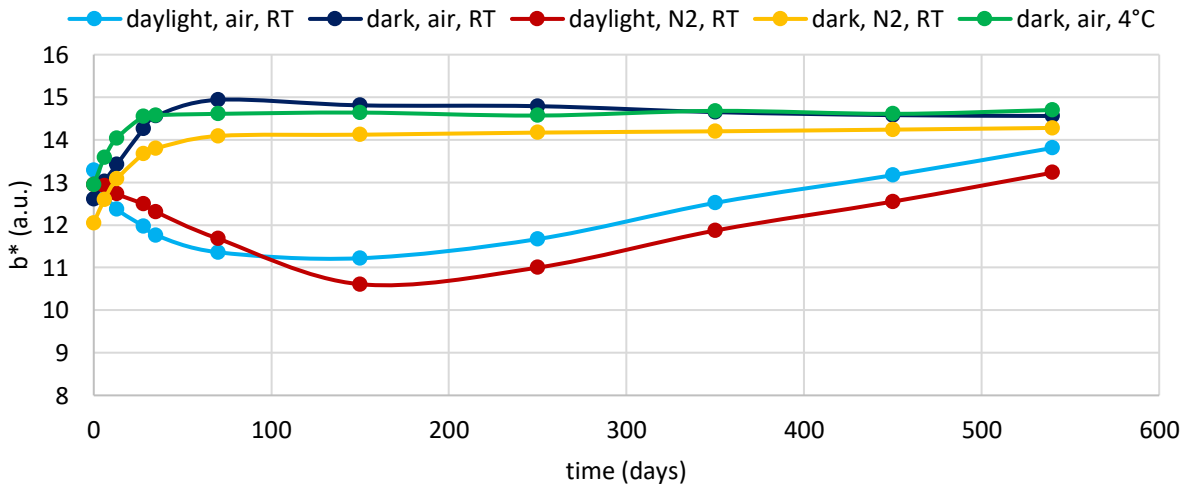

Figure 22 - Trends defined by a parameter vs. ageing time for W_w_O samples from Crimson Sweet watermelon, aged in different storage conditions.*

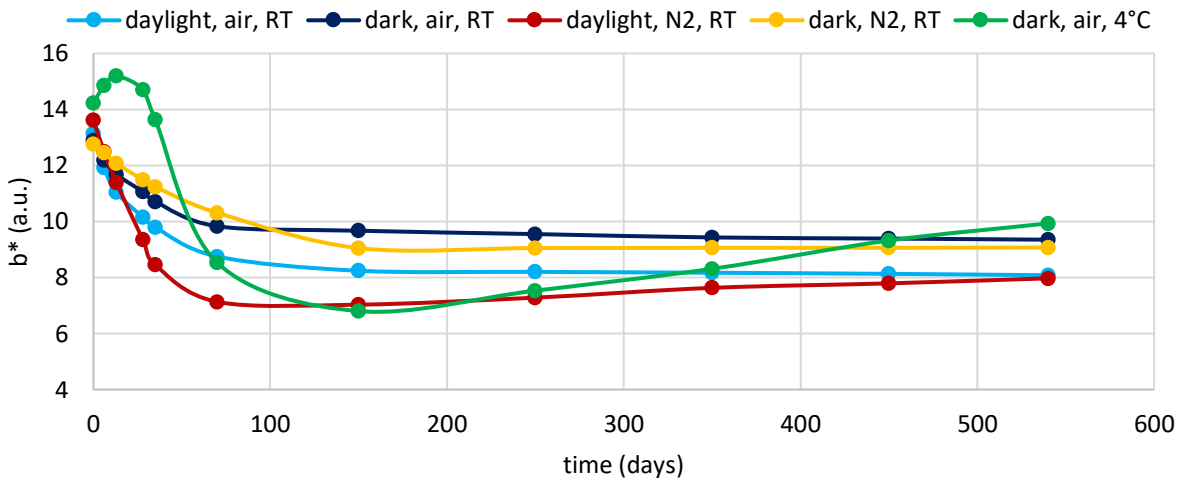

Figure 11 - Trends defined by b parameter vs. ageing time for W_w_V samples from Crimson Sweet cultivar, stored in different conditions.*

Figures 20, 21, 22 and 23 show that the evolution of b* over time describes profiles quite different from those defined by the previously analyzed parameters. In particular, all W_AI_O and W_AI_V samples exhibit a gradual increase of b* values, reaching increases of approximately +50 % (passing from t_0 data of ≈ 10 a.u. to t_{540} ≈ 20 a.u.). This is verified for all storage conditions tested. On the other hand, profiles associated to washed IDF specimens, are quite variable and depends on the storage method adopted. For W_*w*_O fibers, b* values slightly increase (≈ +16%) to then stabilize, for all samples stored in the dark, while those aged exposed to daylight show an opposite trend (Figure 22). Probably, in these types of fibers, the analytes responsible for the yellowish note are sensitive to photodegradation phenomena. Finally, W_*w*_V specimens are always characterized by a progressive decrease of b*, except for the storage at low temperature (4 °C), where this behavior is observed only starting from 28 days (Figure 23).
The always positive values of the b* coordinate, indicate that watermelon pomace fibers are also characterized by a yellow shade, not perceived so easily by visual impact due to the intense initial red color of the fibers themselves. However, as lycopene degrades, the slightly yellowish note emerges. Based on what has been reported so far, this is more evident for TDF “as it is” fibers. In fact, already at a first visual impact, they always show a slightly more intense ivory color than the washed IDF samples. Fibers washed with distilled water, having overall lower a* and b* values (in addition to higher L* ones) than the TDF samples, generally have a lighter and “cleaner” ivory color.

Comments on the overall ΔE color variation

ΔE value provides an indication of the overall color change the samples have undergone, at each ageing step, with respect to the starting conditions (t_0). As far as dried watermelon pomace fibers concern, the higher the ΔE values, the greater the natural discoloration of the samples over time. The obtained results are shown in the following graphs.

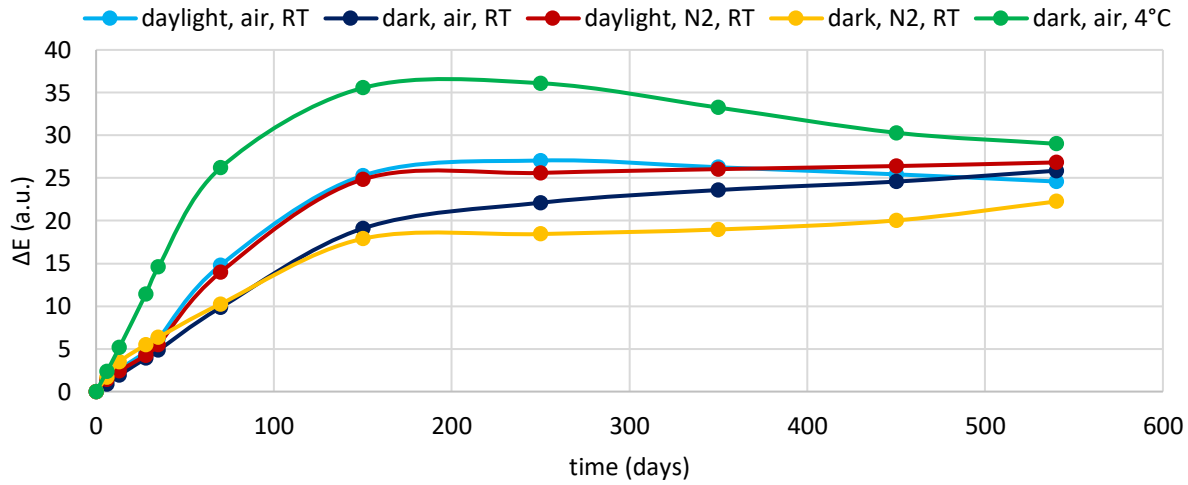

Figure 24 - Trends defined by ΔE values vs. ageing time for W_AI_O samples from Crimson Sweet watermelon, stored in different conditions.

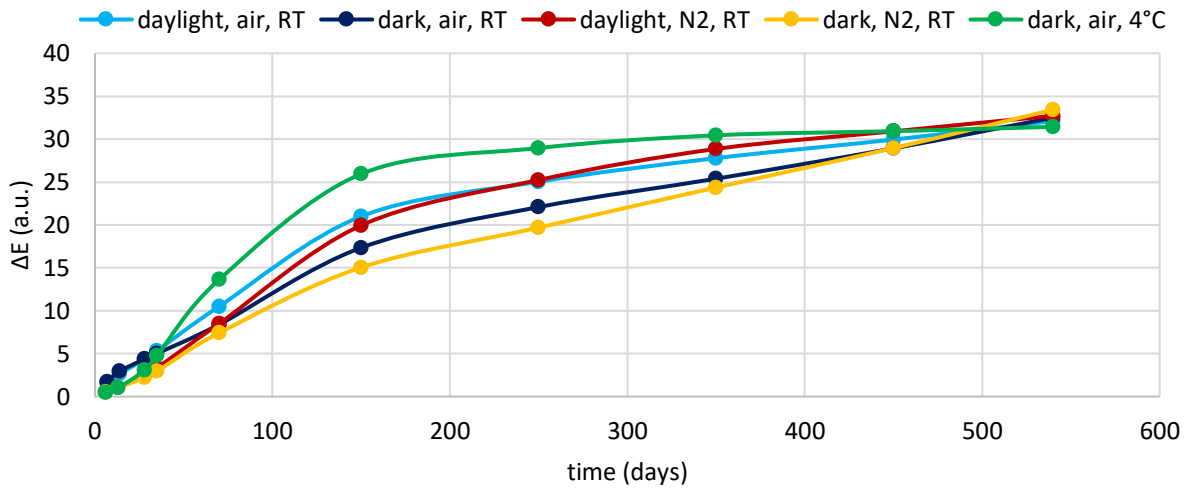

Figure 25 - Trends defined by ΔE vs. ageing time for W_AI_V samples from Crimson Sweet cultivar, aged in different storage conditions.

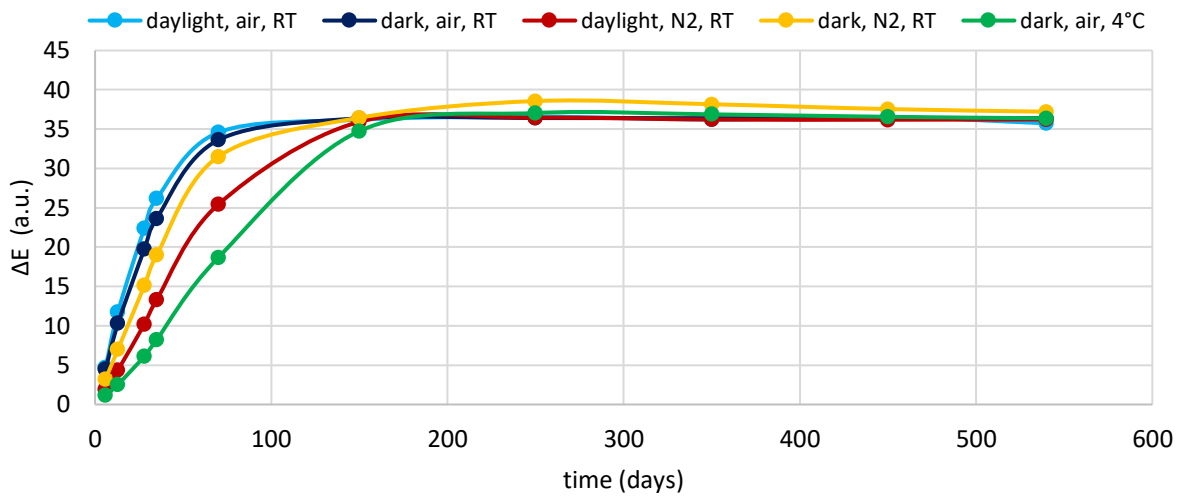

Figure 26- Trends defined by ΔE vs. ageing time for W_w_O samples from Crimson Sweet watermelon, aged in different storage conditions.

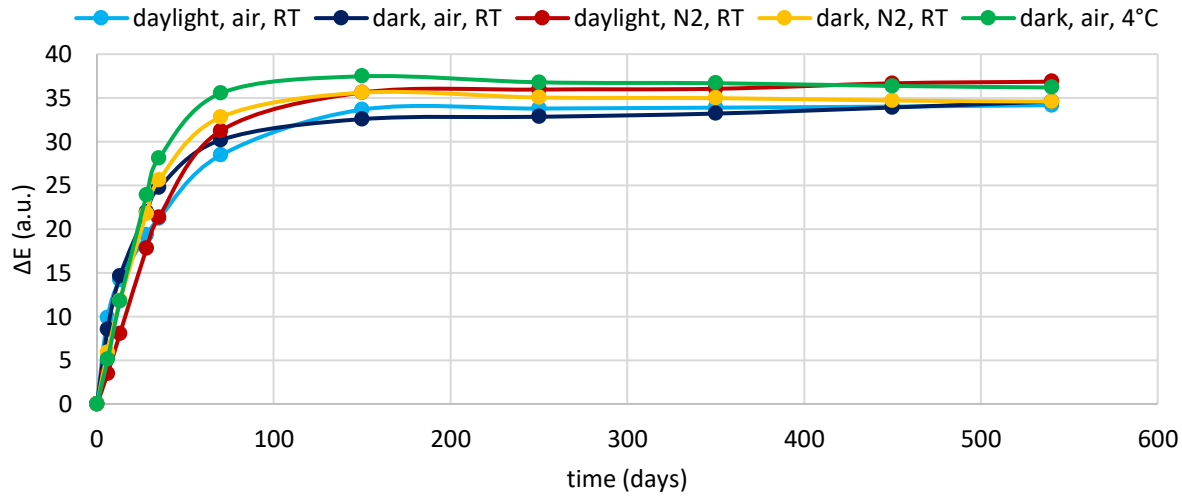

Figure 27 - Trends defined by ΔE values vs. ageing time for W_w_V samples from Crimson Sweet cultivar, stored in different conditions.

As can be seen from Figures 24, 25, 26, 27, regardless of the preliminary handling processes adopted to obtain the fibers (TDF or IDF), the drying methods and the storage conditions, all samples undergo a chromatic variation over time. This is associated to a progressively loss of the starting red color caused by lycopene degradation, to finally obtain an almost neutral ivory fiber. After 540 days (18 months) of ageing, none of them have maintained none red shade. In general, within the same homogeneous set of samples, the trends are all comparable to each other, on average. The washed fibers show the most homogeneous profiles, regardless of the storage method adopted, and lose their original color typically in the first 70 days of ageing. Instead, as far as the "as it is" specimens regard, chromatic variation occurs more gradually over time. In particular, the fiber which appears to discolor more gradually among the four types of fibers analyzed is W_AI_V, as can be seen from Figure 25.

9.5. SOME CONCLUDING REMARKS

As far as the storage conditions tested concern, overall, none of them allowed to preserve the original red color of watermelon dried pomace fibers for the entire ageing period considered (18 months). However, for "as it is" TDF fibers samples, appears that the combination between the presence of N_2 atmosphere and the absence of light allows to slow down the lycopene degradation process mostly. This emerged from the

evaluation of the parameters L*, a*, b*, by the calculation of ΔE and analyzing the UV-Vis spectroscopic data through MCR-ALS toolbox. This is followed by storage in the dark, at RT, in presence of the residual air, to then consider the conditions of exposure to daylight. In these situations, the two different types of residual atmospheres in contact with the samples produce comparable results.
Finally, it can be noticed that although in some cases the trends of the individual L*, a* and b* parameters were not perfectly in accordance with each other, the general behavior of the various samples is perfectly described when all three are combined together, through ΔE value. To conclude, we can affirm that IDFs washed with distilled water undergo a faster variation of the color point parameters L*, a* and b* if compared to the TDFs samples, as confirmed by the previously reported graphs. This is generally valid for all storage conditions investigated. Washing operations which lead to the removal of soluble components, have modified the characteristics of the physiological cells environment in which lycopene is lodged. This significantly affects the behavior of IDF fibers over time, with a faster lycopene degradation due to the effect of oxygen, temperature and light exposure, with the resulting faster loss of color. The preliminary handling operations of the pomace to obtain the two types of fibers ("as it is" and washed with distilled water) have been more incisive than the drying methods adopted (vacuum-freeze drying and oven drying), in the definition of samples behavior over time.

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CHAPTER 10

10 – SOME TECHNOLOGICAL PROPERTIES OF DIETARY FIBERS

10.1. BACKGROUND

Nowadays it is well known that the consumption of dietary fiber brings many health benefits like cholesterol reduction, cardiovascular diseases prevention, regulation in glucose and insulin responses. It also can improve intestinal function and feeling of satiety, leading to weight management, beyond being able to improve textural and rheological properties of food products to which is added [1–4].

Typically, TDF has both soluble (SDF) and insoluble (IDF) fractions and the relative ratio between these two components defines its technological characteristics, in association with other physicochemical parameters like particle size, mass weight and porosity [1, 5].

Among the various technological properties mentioned above, in this chapter we have selected some of the most representative ones, focusing our attention on Water Holding Capacity (WHC), SRC (Solvent Retention Capacity), WSC (Water Swelling Capacity) and OHC (Oil Holding Capacity).

These properties partially complete the characterization of the fibers obtained as previously reported, with the description of some potential applications.

Often the terms "Water hydration capacity", "Water retention capacity" or "Water binding capacity" are used equivalently referring to the ability of a fiber to interact and retain water after being subjected to a stress, such as the application of force, pressure, centrifugation, or heating [6, 7].

Let us now take a closer look at these technological properties.

● The **Water Holding Capacity** (WHC) represents an important protein–polysaccharides-water interaction that occurs in various food systems where water is a fundamental component. WHC expresses the ability of a cited polymeric-foodstuff-material to absorb and retain bound, hydrodynamic, capillary, and physically entrapped water against gravity [8]. Looking in the literature, we find some distinctive WHC definitions, to the point that it seems possible to distinguish the context in which the experimental measures themselves are placed.

Many authors attribute the distinctive character of the WHC parameter to the single fractions of soluble dietary fiber (SDF) and to the insoluble one (IDF).

As in this research work we have chosen to study the behavior of total fiber samples (TDF), not resolved in their subfractions, probably the WHC measure can be more correctly indicated as SRC (Solvent Retention Capacity).

In fact, this last definition seems much more consistent with what is reported in the literature, especially due to the fact that the TDF contains a large amount of soluble compounds, such as mineral salts, acids, simple sugars, among others, which heavily modulate the SRC values of real systems.

However, to avoid misleading, if the solvent in contact with the fibers is water, we have chosen to use the acronym SRC to indicate the behavior of integral fibers in presence of some other added solute.

Polysaccharides are hydrophilic and the great number of hydroxyl groups guarantees the formation of hydrogen bonds network with water molecules.

Therefore, either soluble and insoluble polysaccharides exhibit WHC. However soluble fibers, i.e. pectins, have a higher WHC than cellulose fibers.
WHC is also related to the porous structure of the polysaccharide chains, which through hydrogen bonding continue to trap and retain water.
This way, mechanical factors, i.e. grinding, or any other such as thermal stress, or pH conditions, can cause alteration of fibers structure, affecting WHC and swelling capacity.
However, in a generic food matrix of vegetable origin, both macromolecules (especially polysaccharides) and small molecules are present. Therefore, the interactions that exist and which are established between these components are a rather intriguing topic [9].
To this respect, technological properties constitute a family of techniques and exploratory methodologies which allow to monitor some peculiarities valid both for the single analytes in the presence of the solvent, as well as for groups of them considered in their chemical environment constituted by the same complex matrix.
Hydrophobic and hydrophilic interactions become the driving forces that, if well mediated and balanced through the contribution of each analyte, can adequately structure the system, keeping the components together in a distributed and apparent pseudo-homogeneous form.
It should be noted that the ability of each component to interact with the solvent and with the other constituents can generate a synergistic network of cross-linking interactions capable of stabilizing the complex system in a condition of interphase equilibrium (Figure 1).

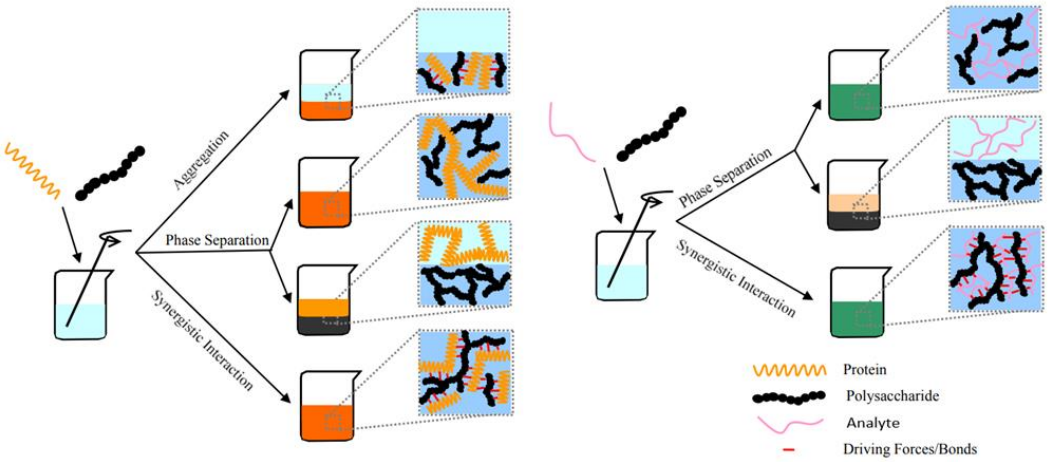


Figure 1 - Representation of specific interactions between natural foodstuff polymers (proteins, polysaccharides) and other analytes in solution, leading to different structures which have different impact on food properties and influence applications of crude DF.

If this condition breaks down and is not respected, the system can destabilize up to inconvenient phase separation. Turning around, technological properties can provide useful information about these equilibrium conditions for very complex systems.

● The **Water Swelling Capacity** (WSC) is the parameter that descends directly from the WHC for each 'wettable' material that comes into contact with water.
This technological property expresses the swelling level of the fiber, also related to the characteristics of each individual component and to the matrix complexity.
Consequently, it is related to the tunable water content and to the mechanical performance of the system.
Some general considerations will be useful to better understand the ways in which specific interactions occur in hydrophilic / hydrophobic systems. The sketch of Figure 2 represents the range of dipolar interactions that can be detected between the components of dietary fiber and water. The consequent properties of bound water are briefly modeled and examined here.

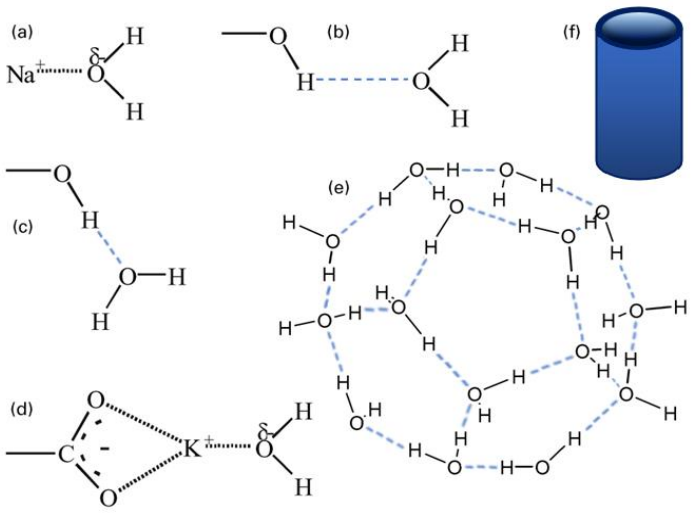


Figure 2 - Fiber can directly bind water in a number of ways: (a) polar effects involving both anions and cations orient and attract water; (b) weak hydrogen bonding involves bent or extended hydrogen bonds; (c) strong hydrogen bonding involves straight, directed and short hydrogen bonds; (d) ionic interactions involve strongly held and oriented water, here shown through interactions between a carboxylate group and potassium ion; (e) hydrophobic (surface) effects involve water clathrate formation at surfaces where water molecules form a flattish network of pentagons and hexagons; (f) enclosure of water involving capillary action (not to scale).

Obviously, the characteristics of the analytes directly affect the structural properties of liquid water (Figure 3).

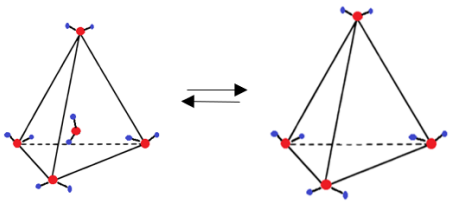


Figure 3 - Representation of the structure of liquid water in tetrahedral clusters, with body-centered pentamers (high density and short H-bonds) and tetramers (low density, long H-bonds).

The hydrophilic components produce water breaking structure in the immediate vicinity of the accessible surface. The clusters tend to break apart to provide adequate solvation, and then recombine immediately beyond the wettable surface. This mechanism is responsible for a compression of hydrophilic species, with a result of small swelling effect.
The hydrophobic components, on the other hand, repeal the solvent molecules from the accessible surface, due to repulsive effects. Therefore, the water molecules are forced to arrange themselves in expanded geometric domains that support the high surface tension cavities where the unwetted analytes are lodged.
Besides the hydrophobic or hydrophilic character of the fiber components, it must be remembered that the results of these interactions vary with the structural flexibility and deformation ability of the surface exposed to the solvent too.

● **Solvent retention capacity** (SRC) is the weight of solvent retained by a wet, swollen system pellet after centrifugation, and is expressed as a percentage of the original powder weight (adjusted to the moisture basis). For binary systems (DF + Solvent), the description of the real behavior of the SRC parameter is relatively simple. Conversely, the interpretative description of SRC values for ternary systems (DF + W + S), or superior, is more difficult as many more variables come into play.
In this research, four different aqueous solutions containing citric acid (CA), NaCl, glucose (GLU) and sucrose (SUC) at various concentrations, are used to correlate and predict the performance of the selected floured functional fibers.
The solutes selected for this study represent some of the most common formulation ingredients for food preparation. In addition to modulating the organoleptic characteristics of the finished products, in many

cases they also exert a preservative action, as they regulate the pH, increase salinity, reduce the amount of free water and its activity.
As they alter the osmotic pressure of the complex system, they contribute to limit or prevent the growth and development of bacterial colonies, thus also reducing the risk factors for the human health.

➢ Water-SRC (W-SRC) is associated with all constituents of DF, thus providing an overview of water retention ability in a generic product system. This property has already been referred to as WHC.

➢ Citric Acid-SRC (CA-SRC) could be related to the protein content of the fibrous sample, although it has been widely demonstrated that it is a very valuable ingredient when formulated in association with starchy and polysaccharidic components [10].
It is interesting to note that the heat treatment of dietary fiber with CA can convert the soluble form into an insoluble citrate-derived form, with great advantage from the nutraceutical point of view, as it reduces digestibility and the amount of absorbed calories.

➢ Sodium Chloride-SRC (NaCl-SRC) should be primarily associated with hydrophilic - specific interactions of polysaccharides, and indirectly, with fiber hardness [11]. However, carboxylate groups ($-COO^-$) are always active towards Na^+ and metal cations whatever their origin.

➢ Glucose-SRC (GLU-SRC) should be primarily associated with hexosides content of dietary fiber**.**

➢ Sucrose-SRC (SUC-SRC) should be associated with the pentosan content, as specific interactions between the pentatomic rings present in the fiber chains, will be favoured with the same fructosidic groups of sucrose.

Therefore, characterization of dietary fiber, based on SRC analysis, should highlights some behavioral picture about chemical, rheological, and baking aspects.

The **Oil Holding Capacity** (OHC) of DF is mainly related to the total charge density present on the surface of the sample micelles, the hydrophobicity of the analytes, the surface properties of the matrix constituents and to the conditions of preliminary treatment, among others.

10.2. MATERIALS AND METHODS

The selected powdered fibrous samples prepared as described along the text of the thesis are indicated in the following scheme.

M_AI_V	Melon pomace “as it is” (TDF), Vacuum-freeze dried
M_B_V	Melon pomace Bleached with H_2O_2 at Room Temperature, Vacuum-freeze dried (IDF)
M_skin	Melon skin, Oven dried
C_AI_V	Crimson Sweet pomace “as it is” (TDF), Vacuum-freeze dried
C_*w*_V	Crimson Sweet pomace *washed* with distilled water, Vacuum-freeze dried (IDF)
C_rind	Crimson Sweet rind, Oven dried
Mi_AI_V	Asahi Miyako pomace “as it is” (TDF), Vacuum-freeze dried
Mi_*w*_V	Asahi Miyako pomace *washed* with distilled water, Vacuum-freeze dried (IDF)
Mi_rind	Asahi Miyako rind, Oven dried
G_AI_V	Gavina pomace “as it is” (TDF), Vacuum-freeze dried
G_*w*_V	Gavina pomace *washed* with distilled water, Vacuum-freeze dried (IDF)
G_rind	Gavina rind, Oven dried
W-seed-flour	Defatted watermelon seed flour
Baobab	Baobab powder
AHP	Aesculus Hyppocastanum Pure
AHH	Aesculus Hyppocastanum Hybrid
AXC	Aesculus X Carnea

Scheme 1 – List of the samples and their specific acronyms, processed in this PhD thesis

We specify that baobab powdered fiber is a commercial product, already widespread in the food industry [12, 13].

As previously mentioned, in addition to fiber and water, other solutes commonly used in the food field have also been added (10 mL) to obtain a ternary system consisting of fiber-monosolute-H_2O. In particular, we used:

- Glucose (GLU) 5-10-15-20-25 %
- Sucrose (SUC) 5-10-15-20-25 %
- Citric Acid (CA) 1-2-3-4-5 %
- NaCl 1-2-3-4-5 %

where the concentrations are expressed by mass.
The detailed description of the procedures adopted for the determination of WHC, WSC and OHC are reported in Chapter 4.
Results are expressed as mean ± standard deviation of three replicates (Table 1).

10.3. RESULTS AND DISCUSSION

Table 1 shows the results obtained from the evaluation of WHC, WSC and OHC for some selected fiber samples analyzed during this study.

Table 1 - Experimental data of some technological properties, collected for some dietary fiber specimens.

		WHC (g / g)	WSC (g / g)	OHC (g / g)
MELON	M_AI_V	9.37 ± 0.30	8.6 ± 0.3	3.21 ± 0.29
	M_B_V	20.87 ± 0.33	19.6 ± 0.4	6.29 ± 0.35
	M_skin	10.16 ± 0.29	11.4 ± 0.4	3.77 ± 0.30
CRIMSON	C_AI_V	7.45 ± 0.32	7.3 ± 0.3	2.86 ± 0.28
	C_*w*_V	10.36 ± 0.39	10.6 ± 0.4	4.64 ± 0.21
	C_rind	8.85 ± 0.36	8.5 ± 0.4	2.51 ± 0.31
ASAHI MIYAKO	Mi_AI_V	6.27 ± 0.23	6.1 ± 0.3	3.12 ± 0.26
	Mi_*w*_V	8.53 ± 0.33	8.8 ± 0.3	4.41 ± 0.23
	Mi_rind	7.41 ± 0.38	7.8 ± 0.4	2.89 ± 0.31
GAVINA	G_AI_V	13.59 ± 0.38	14.4 ± 0.4	3.07 ± 0.27
	G_*w*_V	13.52 ± 0.42	13.9 ± 0.4	3.70 ± 0.19
	G_rind	13.64 ± 0.42	11.3 ± 0.4	1.94 ± 0.33
OTHER FIBERS	W-seed-flour	1.90 ± 0.11	2.6 ± 0.2	2.74 ± 0.31
	Baobab	3.67 ± 0.16	4.5 ± 0.2	3.52 ± 0.30
	AHP	2.86 ± 0.10	3.9 ± 0.1	3.07 ± 0.23
	AHH	2.42 ± 0.09	3.8 ± 0.1	3.41 ± 0.20
	AXC	2.95 ± 0.10	3.7 ± 0.1	3.35 ± 0.21

Water Holding Capacity (WHC)

Binary systems {DF + Water}

The evaluation of the intrinsic characteristics of the fibrous matrices of our interest cannot disregard the knowledge that can be acquired by studying their behavior in real systems. However, as the real multi-component systems are very complex, it is generally necessary to start from less chaotic conditions, thus preparing binary systems, where the second component is water.
In Table 1 are collected the results of the WHC tests on our fibrous samples.

As we can see, not all prevailing-polysaccharidic fibers behave in the same way towards H_2O since the degree of interaction of the active sites present on the fibers with water molecules results in a different ability to retain them. Polysaccharides are hydrophilic materials and their abundant free hydroxyl groups form hydrogen bonds with water, as well as the proteic fraction, which is always present in plant matrices although in different quantities depending on their origin. As said before, WHC is related to both soluble and insoluble polysaccharides (for example pectins have a higher WHC than cellulose) but it also depends on the intrinsic chemical composition, structure and porosity of the fibers. Each of these items plays a role in the ability to trap and retain water. In fact, many studies report how the functional properties of polysaccharidic materials change by the effect of grinding and micronization, or after some extraction and fractionation steps [5, 14, 15].
To remove the variableness related to grinding, we remember that all our samples were ground with the same mill and the same sieve, which gave the flours an average grain size of 0.5 mm. This way, all the differences detected are exclusively attributable to the different nature of the matrices and to the handling processes to which they have been subjected.

Analyzing the results, it can be seen that the TDFs obtained from both melon and Crimson, show lower WHC values than those related to specimens treated with H_2O_2 and washed with distilled water, respectively.
Whole fiber (TDF) contains a variety of soluble compounds such as sugars and salts, all hydrophilic components which are conversely lost with washing and bleaching.
At the time of rehydration, the “as it is” material should have shown a greater capacity to hold water thanks to these hydrophilic analytes, but this is not the rational explanation, as it contrasts with the experimental evidence.
Probably, the dehydration process of the raw sample leads to blocking a large number of active polar sites which, potentially, should have been restored when in contact with water again. On the contrary, it seems that a stable perturbation of the material occurs, which modifies the structure of its polymeric fibers due to specific interactions that are established between molecules of simple sugars, organic acids, metal cations (soluble salts), among others. Robertson and Eastwood agree with us, and state that the more severe the method of drying the lower is the WHC of vegetable fibers [16]. Furthermore, they report that differences in WHC are probably more a function of fiber structure than chemical composition, since chemical analysis does not appear to change within each fiber source whereas structure and WHC did change.

In fact, it is known that the coordination compounds formed by cations of alkali metals with corona ethers or polyhydroxylated compounds such as polysaccharides, are particularly stable [17].
For example, pectins are a group of substances found in fruit and many other plant cell walls which exhibit such a structure with Ca^{+2} ions encased in an “egg box” of polygalacturonate chains, thus conferring an ability for strong cohesion.
So, if stable compounds are formed because of the dehydration process, the rehydration water would have no chance of displacing the sugar molecules and metal cations from the positions they occupy for stable coordination, leading only to a partial rehydration of the fibers [18].

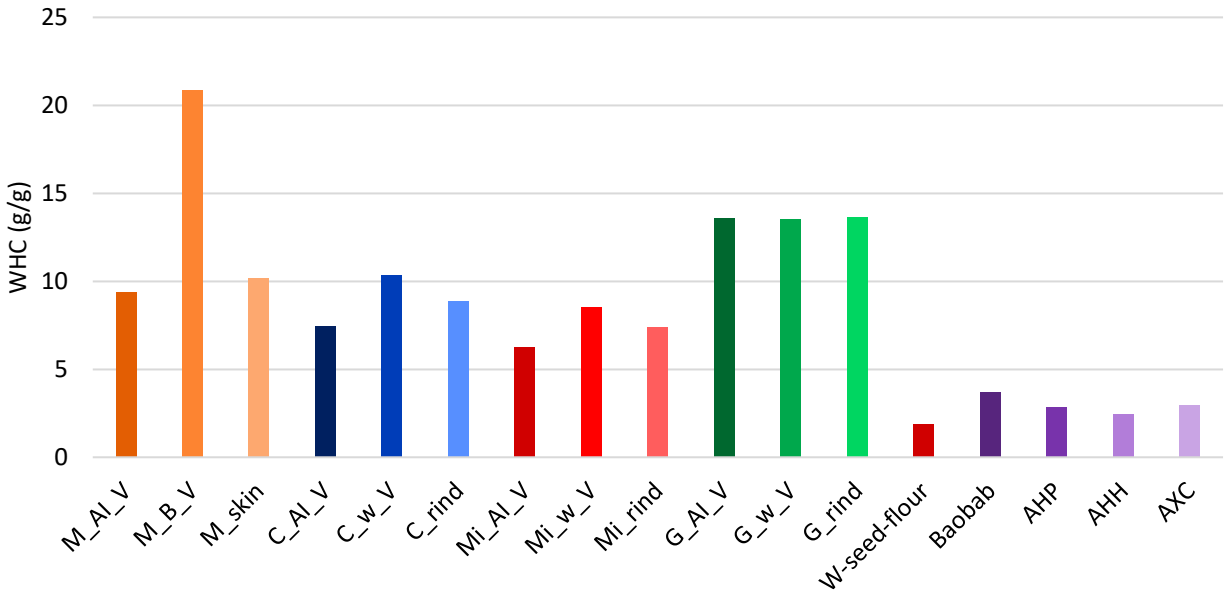


Figure 4 - Comparison of the WHC values of the fibrous-polysaccharide materials tested

The trend of the values in Figure 4 offers an excellent overview of the results obtained from our tests, with a very significant impact for the M_B_V sample.
Evidently, the oxidizing treatment eliminates a quantity of "ballast" analytes, releasing the fiber which can now interact much more effectively with water, up to + 255% compared to the M_Al_V fiber.
Not surprisingly, some research has shown that real chemical treatments could improve or worsen the physicochemical and technological properties of dietary fibers, due to the change in their composition and microstructure. For example, acidic or alkaline conditions could break the glycosidic bonds in DF, affecting the ratio of IDF to SDF, as well as H_2O_2 can [19]. Yue et al [20], observed a significant increase in WSC (+276%) and WHC (+197%) of citrus fibers obtained through alkaline H_2O_2 treatment, going from 3.64 ml/g to 10.04 ml/g and from 6.24 g/g to 12.28 g/g, respectively.
The increase of +255% for our bleached melon fiber is perfectly aligned with these data, with the advantage that it has been obtained without altering the initial pH of the matrix. So, it is reasonable to think that also for our sample, the action of H_2O_2 in our treatment conditions may have broken some H-bonding, leading to the loss of the original structure of the fiber and with the exposure of more hydrophilic hydroxyls group to water [5].

Also for watermelon fibers by Crimson cultivar, there is a greater WHC for C_*w*_V (10.36 g/g) rather than for C_Al_V sample (7.45 g/g). On the other hand, Gavina fibers show the same WHC value (13.52 - 13.59 g/g) regardless of the handling processes applied to pomace for obtaining TDF fibers or washed ones.
Such a mild treatment should not be able to alter the ratio between SDF and IDF.
If this were the case, a decrease in the WHC of the washed fibers would have been expected, as the post-treatment residue should have a higher % IDF.
These fibers, having a more rigid structure, should be less subject to deformation due to interaction with water, and therefore retain less of it [19].
As observed, it is reasonable to hypothesize that the WHC of dehydrated TDFs may also depend on the chemical composition of the matrix, which will differ not only for the cultivar but also for the degree of ripeness of the fruit.
Thinking about the use of these materials in some industrial formulations, in order to have a fiber as neutral as possible (in terms of color, aroma and flavor), it may be more appropriate to work with washed or bleached material rather than with the raw one.

As far as the skins concerns, they seem to have higher values of WHC than the pomace of the same fruit, with the greatest increase for the Crimson cultivar (+ 10.2% with respect to C_AI_V), while it is almost invariant for the Gavina variety (13.64 g/g).
The dark green fiber of the watermelon skin and the bright yellow peel of the winter melon could therefore be suitable for food formulations, i.e. in doughs that after cooking may have a brown color, as already reported in some recent papers [21, 22].

Finally, the "proteic-starch" samples from Crimson seeds (W-seed flour) and the "baobab fiber" are placed in the lowest WHC value range, in relation to the whole set of tested matrices (1.90 and 3.67 g/g, respectively). These results are consistent with literature data, as it is known that starch-protein fibers from cereals are much less effective in absorbing water than vegetable fibers with a cellulose character [16].
In our case, the analogy of behavior of protein-starch from Crimson seeds and baobab fiber, compared to those from cereals, legumes and oilseeds, suggests that there is some compositional and structural affinity between the functional biopolymers constituting this DF set [23].
To give an idea, let us recall that the WHC value for a common commercial Type 00 wheat flour is about 1.02 g/g [24].
These results are particularly encouraging, considering a possible technological transfer that includes the recovery and use of watermelon seeds as a functional ingredient, having strong application potential.

Solvent Retention Capacity (SRC)

Ternary systems {DF + Water + Solute}

As already highlighted, for formulation purposes in the food sector (but not only), many ingredients are used in addition to DFs as functional additives, to develop specific characteristics of the final product.
In an attempt to simulate their behavior in real systems by somewhat increasing the degree of complexity, we conducted an exploratory study by preparing some *ternary* systems, placing the fibers (DF) in aqueous suspension (W) obtained by adding an aqueous solution of ingredient (S).
Here, the indication 'ternary' roughly means that we consider DF as if it was a single chemical species.

Figure 5 shows some samples of the ternary system {DF + W + NaCl}, already sedimented after the preparatory treatment cycle.

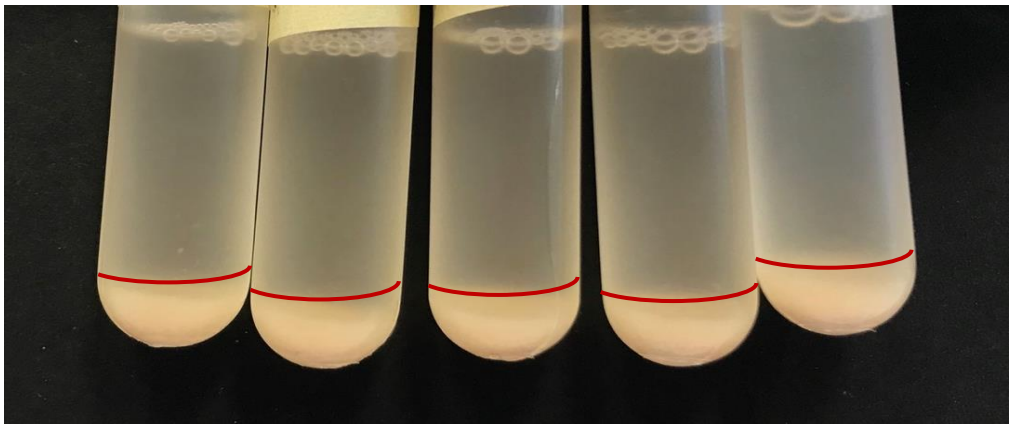


Figure 5 - Ternary system obtained using baobab fiber and adding NaCl solutions at different concentrations.

In the following sections we will examine in detail the behavior of the ternary systems thus prepared, graphically describing the experimental observations collected.

- **Melon pomace “as it is” (TDF), Vacuum-freeze dried: M_AI_V**

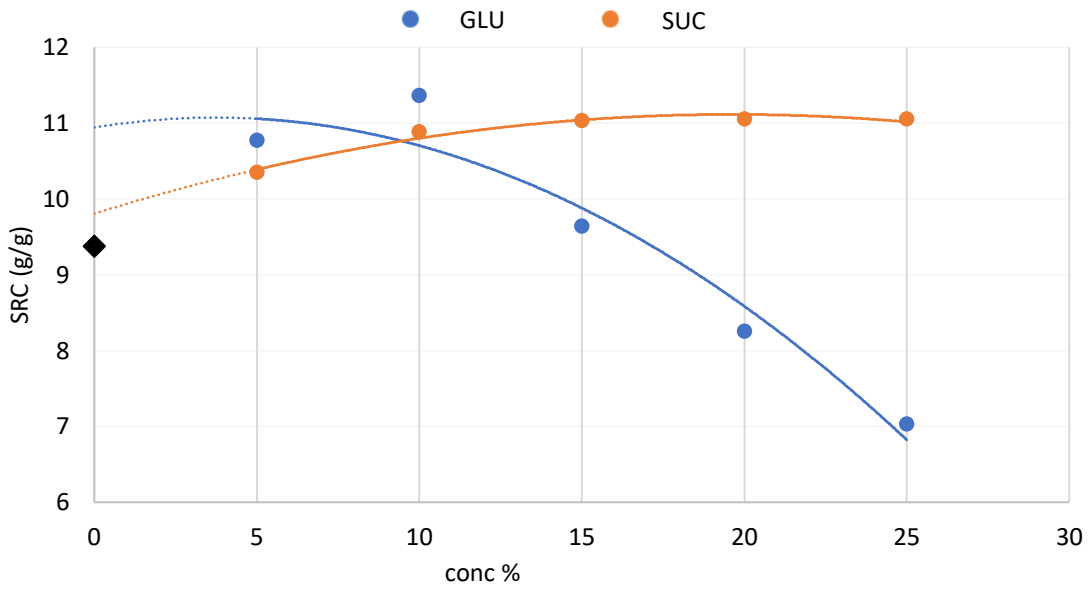


*Figure 6 - **SRC** curves for **M_AI_V** sample, obtained with **GLUcose** and **SUCrose** solution at various concentrations.*

M_AI_V fiber increases its solvent retention capacity (SRC) when in contact with GLU solution, up to a critical value of SRC ≈ 11 g / g, with an increase from the zero point (WHC = 9.37 g/g) of ~ 15%. After saturation of the fiber active sites through specific interactions with GLU molecules, the function decreases and the system shows reduced solvent retention ability in the range 15 - 25% concentration. Several factors could intervene to modulate this behavior. For example, the increase in analyte concentration can increase the osmotic pressure of the solution, with the effect of recalling the solvent previously absorbed by the fiber. Furthermore, the GLU-solution could carry in the liquid phase other soluble compounds present in the fiber, such as sugars, mineral salts, etc., which in turn could contribute to increase the osmotic gradient effects from the solid phase to the solution.

The SUC species, on the other hand, shows an alternative trend to that described now for the GLU. In fact, an increase is observed starting from the zero point (WHC = 9.37 g/g) reaching a plateau zone around 15-20%, and then keeping the SRC profile approximately constant at 11.1 g / g (+ 18%) up to the limit of the studied range. Figure 6 shows that the SUC component produces an incremental effect of the SRC property on this fiber, unlike to what observed in the case of the GLU solute. Furthermore, we anticipate that M_AI_V fiber exhibits an SRC profile similar to C_*w*_V sample (Figure 15).

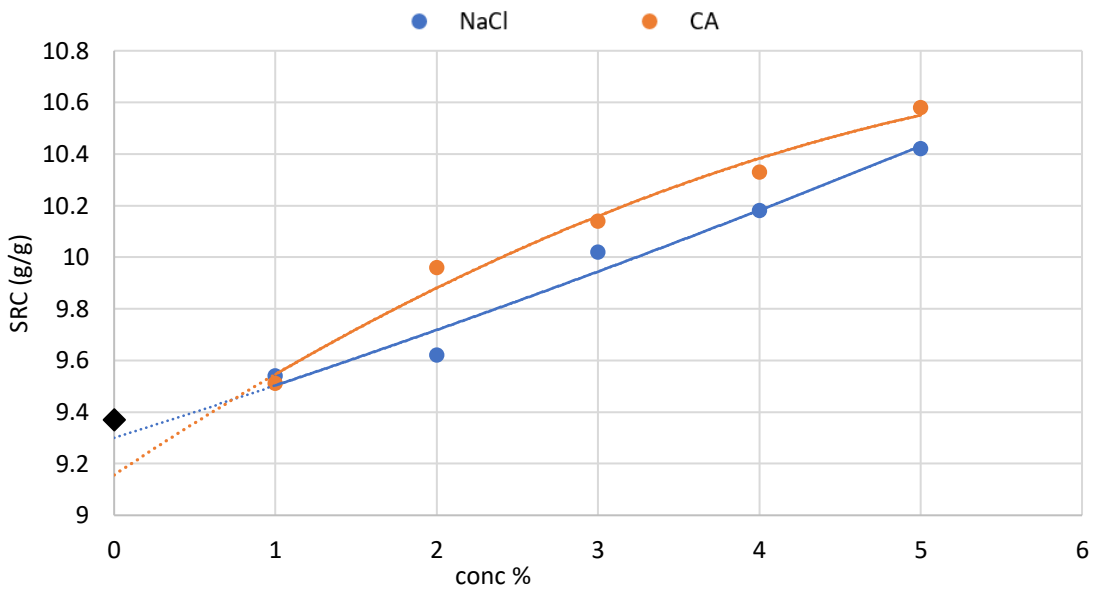


*Figure 7 - **SRC** curves for **M_AI_V** fiber, obtained with **NaCl** and **Citric Acid** solutions at various concentrations*

The effects of NaCl on SRC values for melon TDF (M_AI_V) are shown in Figure 7. As can be seen, the function increases throughout the composition range of the solution, although it can be assumed that there are no significant specific interactions between the polysaccharide fibers and the electrolytic analyte. The increase in the SRC parameter could therefore be attributed to the mechanical absorption of the hydrated solute rather than the adsorption by direct electroactive interactions between components.
The presence of CA in solution produces effects similar to those induced by NaCl, since there is a gradual increase in the SRC parameter throughout the range of concentrations investigated. Probably the trend can be attributed to mechanical absorption of the solvated analyte rather than to specific interactions of electrostatic nature.

- **Melon pomace Bleached with H_2O_2 at Room Temperature, Vacuum-freeze dried: M_B_V**

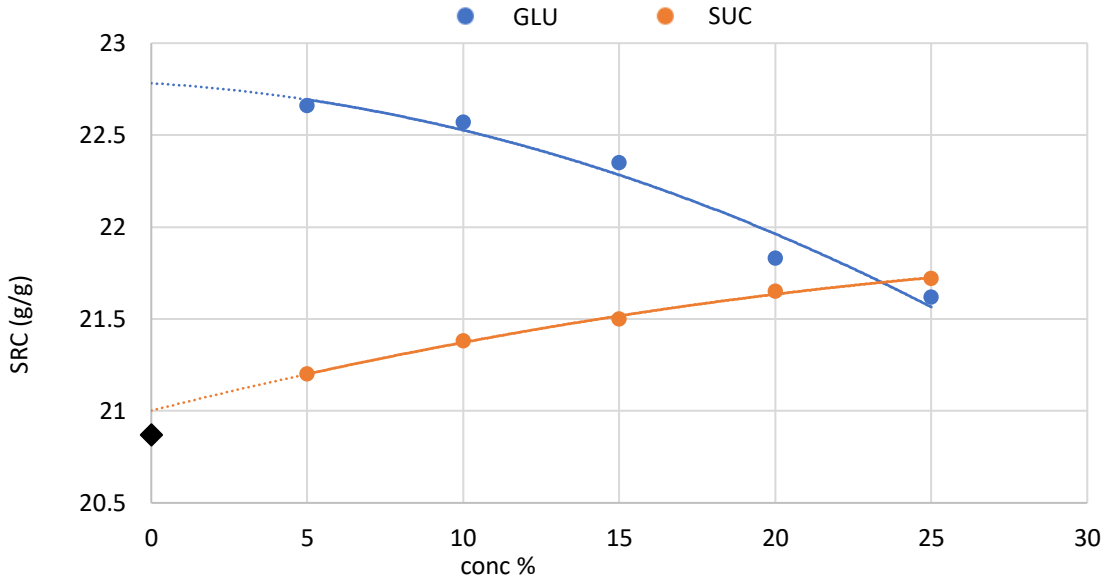


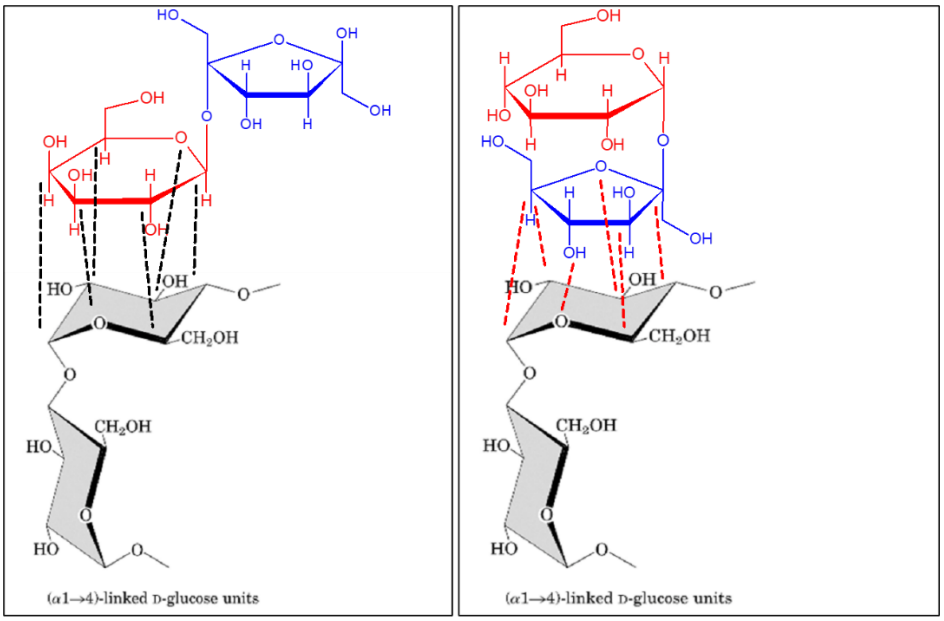
*Figure 8 - **SRC** curves for **M_B_V**, obtained with **GLUcose** and **SUCrose** solutions at various concentrations.*

The SRC of M_B_V fibers (Figure 8), differs significantly in relation to the solutes used for this purpose. Initially, the great interest is represented by the value at the zero point, since the W-SRC for the bleached fiber increases by approximately +120% compared to the TDF one (M_AI_V).

At least two countertrend effects could intervene to modulate this data at the zero point, but with important consequences within the entire window of explored concentrations.
In fact, the trend of the SRC function vs concentration % for the selected analytes is strongly affected by the interactive ability of the fiber with the solvent in presence of exogenous species.
The first positive effect which increases the SRC could derive from the possible oxidation, due to H_2O_2, of some substituent groups on the polysaccharide chains, with a parallel increase in the polar sites and greater interaction ability with the solute - solvent molecules.
Conversely, the second detractive effect of SRC could be attributed to the washing away of soluble and polar components such as sugars, organic acids, mineral salts, soluble starches and proteins, etc. Nonetheless, experimental evidence suggests that the positive and strongly incremental effects of SRC function prevail.

Observing the trend in Figure 8, the highly perturbative effect of the GLU on the behavior of M_B_V fiber is evident. In fact, even just considering the presence of the analyte in traces, an upper-shift of the SRC is observed which is worth ~ 1.6 points (+ 8% with respect to the zero point). The best-fit curve has the virtual intercept at 22.8, against the experimental value 20.9.
For these reasons, the best-fit curve is interpolated in polynomial form excluding the zero point, since the function is not defined in the first section.
The negative slope of the trend indicates that the saturation of the active sites in solid state is reached immediately in the first section, where the function is not defined.
However, it can be hypothesized that the specific interactions of the GLU are mainly produced by dipolar exchange of the hydroxylated sites by partial parallelization with the glucosidic units of the fibro-cellulosic polymer chains.

For the sucrose, the trend of SRC function is different than in the previous case, since the specific fiber-solute interactions do not seem as effective as observed for the GLU. The continuous increase in SRC values seems to be partly attributable:
● to the interactions *via* adsorption of the glucoside ring through parallelization of H bonds that are established with the monomer units of the polysaccharide fibers (Figure 9);
● to the mechanical absorption, or capillarity, of the solvation water associated with the added SUC molecule.



Glucose-glucose: favourable stacking interactions

Glucose-fructose: unfavourable stacking interactions

Figure 9 - Stacking interactions between a polysaccharide chain and a sucrose molecule: favourable (SX) and unfavourable (DX) specific interactions

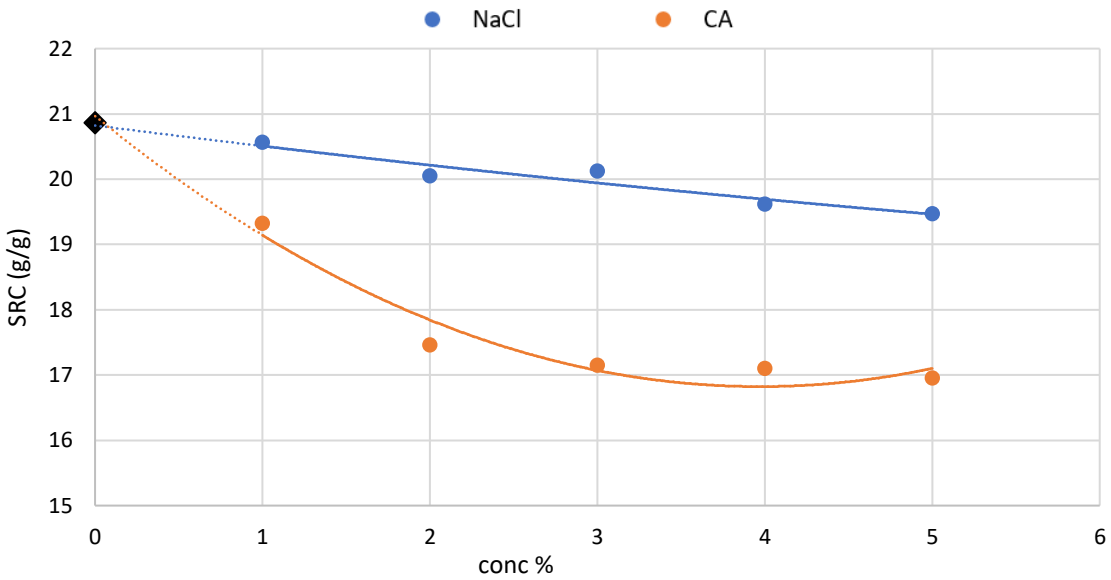


*Figure 10 - **SRC** curves for **M_B_V** fiber, obtained with **NaCl** and **CA** solutions at various concentrations.*

The behavior of the NaCl solute is quite negative towards the SRC property for M_B_V fiber sample (Figure 10), since due to osmotic effects it progressively reduces the water content retained by the polysaccharides. It should be noted that for the M_AI_V specimen (Figure 7), the SRC trend is always positive.
Like NaCl, CA also produces contrasting effects on the fiber: positive for the TDF, negative on the SRC values for the bleached one. Evidently, CA poorly interacts with the polysaccharides, especially in absence of the mineral cations. However, the SRC for M_B_V seems to be stabilized around the plateau value ~ 17 g/g, still very far from the value recorded for M_AI_V sample (SRC ~ 11 g / g , for concentration CA = 5%), Figure 7.

- **Melon skin, Oven dried: M_skin**

The skin of the melon, dried and ground, maintains its natural lemon-like yellow color, and therefore can act as a pigment in *food&feed* formulations of various kinds. As a formulation component, it can come in contact with other analytes which can modify its behavior. It is interesting to note that the SRC of the pulverized peel, with respect to the GLU solution, presents a trend similar to that of melon fibers, both TDF (M_AI_V) and bleached with H_2O_2 (M_B_V).

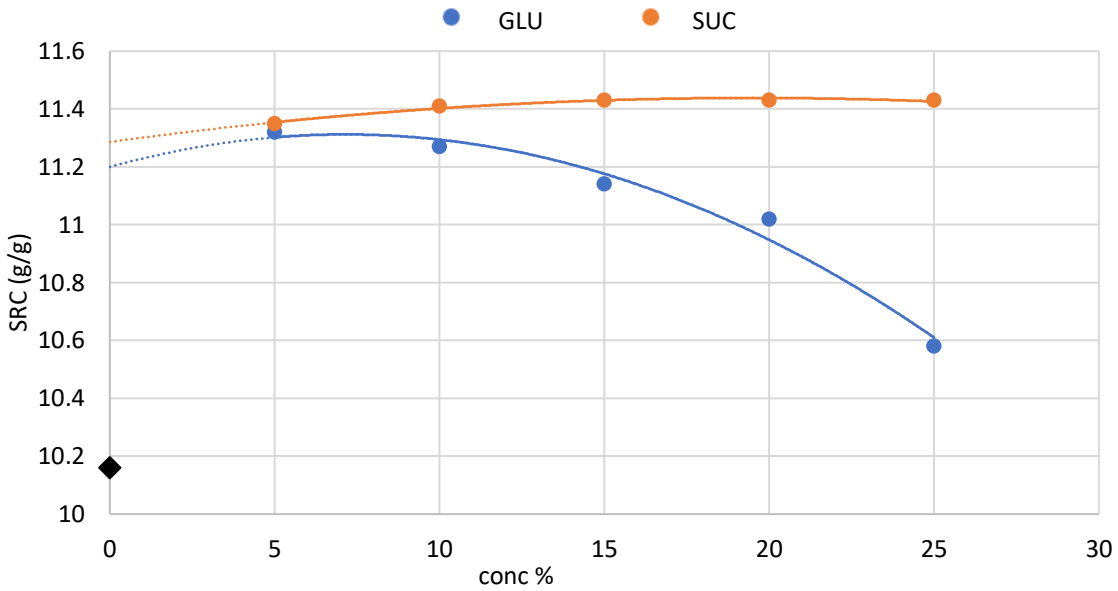


*Figure 11 - **SRC** curves for powdered **M_skin**, obtained with **GLUcose** and **SUCrose** solutions at various concentrations.*

The fiber of the skin is much harder, shows much more 'woody' characteristics than the fibers from the edible fraction. However, the GLU-SRC of this material (Figure 11), exceeds the property of the M_AI_V sample (Figure 6) by ~ 10 %, while it separates by ~ 50 % from the value of the bleached fiber (M_B_V). In all 3 cases of examined fibers, a strong perturbative effect is observed due to the presence of the GLU solute which, interacting effectively with the polysaccharide structures, significantly increases the SRC values by entrapment of solvation water molecules.
SUC also produces incremental effects of the SRC in a similar way to TDF, reaching a plateau value of around 11.4 g/g. On the other hand, this last finding seems quite common both to pomace TDF and to the two tested saccharides (GLU, SUC). Our observations seem to confirm that the number of active sites, the glucosidic monomers of the fibers, committed to saturation through dipolar interactions and H-bonding, remains unchanged despite the different nature of the fibers themselves.

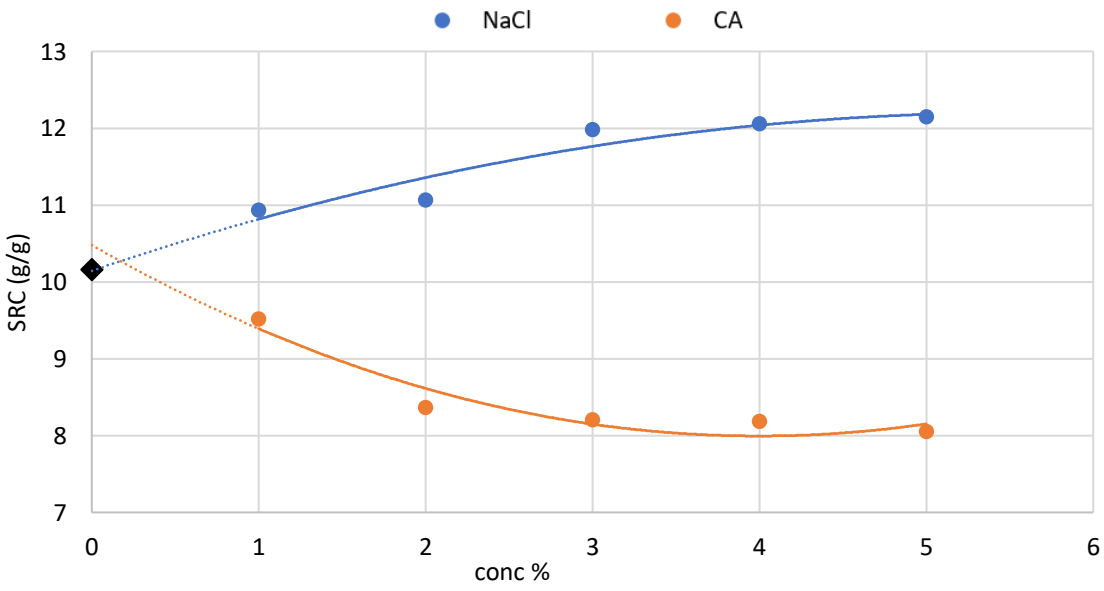


*Figure 12 - **SRC** curves for powdered **M_skin**, obtained with **NaCl** and **CA** solutions at various concentrations.*

The solute NaCl increases the SRC, but as previously observed, it does not appear to interact with the fibrous-cellulosic skeleton (Figure 12).

The SRC increase can only be attributed to factors of mechanical absorption, capillarity and entrapment of water solvation molecules of the electrolyte.
The presence of CA in solution reduces the SRC by about 2 g/g, up to a plateau value of ~ 8 g/g. This behavior could derive from the ability of the solute to extract soluble analytes from the fibrous support, thus lowering the solvation capacity of the residual species in the solid state.

- **Crimson Sweet pomace "as it is" (TDF), Vacuum-freeze dried: C_AI_V**

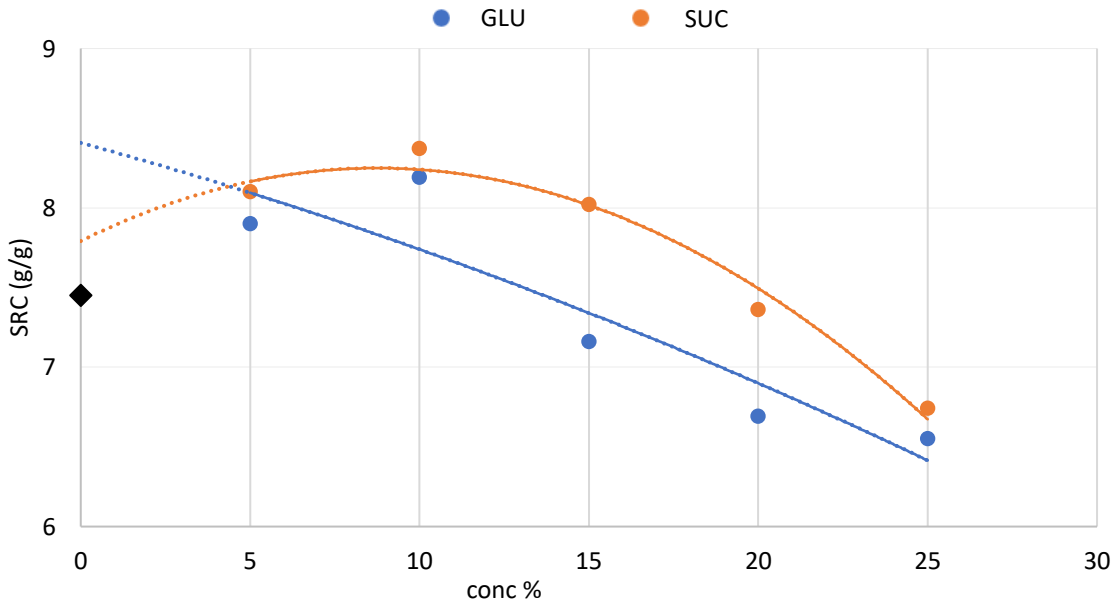


*Figure 13 - **SRC** curves for **C_AI_V** fiber, obtained with **GLUcose** and **SUCrose** solutions at different concentrations.*

From the trend of GLU-SRC values in Figure 13, it can be inferred that the 3-component system based on Crimson pomace fiber (C_AI_V - GLU - W) is strongly perturbed by the presence of the added analyte.
In fact, the best fit equation that models the experimental values identifies the intercept at the point of SRC = 8.4 g/g, against the experimental data for the binary system W-fiber set at 7.45 g/g.
As if to say: a few traces of Glucose are enough to rapidly increase the water retention values of the fibrous substrate by about 14%. Experimental evidence suggests that TDF effectively retains glucose on the surface through rather important specific interactions (H-bonds) until the active sites are saturated, thus increasing the availability of hydroxyl groups capable of producing interactions with water molecules.
Beyond the saturation of the solid phase with glucose, the trend follows a decrease resulting in a progressive reduction of water retention.
Probably, in such conditions the solid phase balances itself with the solution, losing water previously absorbed due to the incremental effect of the osmotic pressure in the solution.
Similarly, we can model the behavior of C_AI_V fiber towards sucrose.
In this case there is a modest shift of the intercept from the experimental value SRC = 7.45 g/g to the value 7.79 g/g of the best fit interpolating function.
Sucrose, a bulky and sterically hindered molecule, increases the retention of the solvent up to a concentration close to 10%, corresponding to the saturation of the active sites on the solid phase.

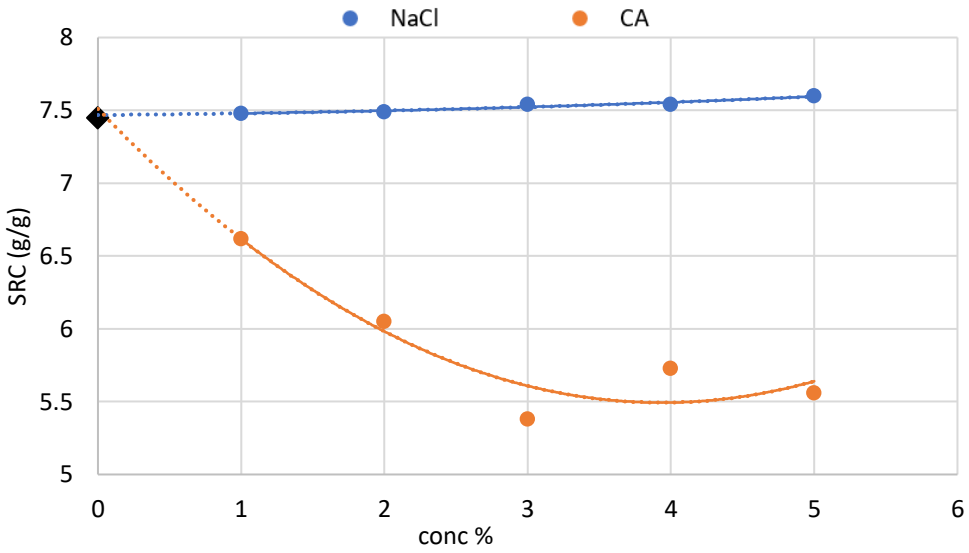


*Figure 14 - **SRC** curves for **C_AI_V** sample, obtained with **CA** and **NaCl** solutions at different concentrations.*

The presence of NaCl in solution (Figure 14), leads to a constant increase in the absorption / retention capacity of water by the TDF fiber (C_AI_V). It should be emphasized that the intercept of the best-fit function (7.47 g/g) is very close to the experimental value (W-SRC = 7.45 g/g). To rationalize this behavior it can be suggested that the fibrous sample does not interact effectively with the solute, and therefore the best-fit function also contains the starting point (zero concentration of solute NaCl).
Conversely, a greater quantity of NaCl that remains trapped in the TDF leads to a progressive increase in water retained due to the solvation of the soluble salt.
For citric acid (CA), an always decreasing trend is observed starting from the intercept generated by the best fit equation (CA-SRC = 7.51 g/g), close to the experimental value related to the pure solvent (7.45 g/g).
Subsequently, we reach the saturation threshold (CA ≈ 3%), beyond which there is a condition of near-invariance with the appearance of a plateau that could continue even for solute concentrations after the maximum investigated value. Also in this case, based on the trend, we can hypothesize a poor interaction capacity of the added solute with respect to the fibrous matrix TDF.

- **Crimson Sweet pomace *washed* with distilled water, Vacuum-freeze dried: C_*w*_V**

Before examining the behavior of Crimson Sweet fibers, it is interesting to note that the TDF (C_AI_V) shows a value of W-SRC = 7.45 g / g, while the C_*w*_V fiber (IDF), washed with distilled water, reaches a value of 10.36 g / g. These data are difficult to rationalize, especially if we consider the compositional variation induced by the washing process. The elimination of soluble solutes increases the retention capacity of the solvent in the solid phase (IDF) by about 30%.
At the moment, a plausible explanation can be given: the presence of the soluble fraction in the TDF could exert a compressive action of the insoluble components (cellulose, starches, proteins, etc., present in the washed fiber), thus limiting their swelling capacity for the effective solvent absorption.
These observations could have very important consequences in application field, if the fibers were intended for use as formulation ingredients for the food sector. Just think of the variations induced on the overall characteristics of the final formulation, with fluctuations in the thickening and gelling properties, etc.
These properties can be associated with the different water content which would appear, with the same added mass, partly in free form (for the TDF) or completely bound (probably for the washed IDF).
The presence of any solutes can significantly contribute to modulate the overall characteristics of multicomponent systems.

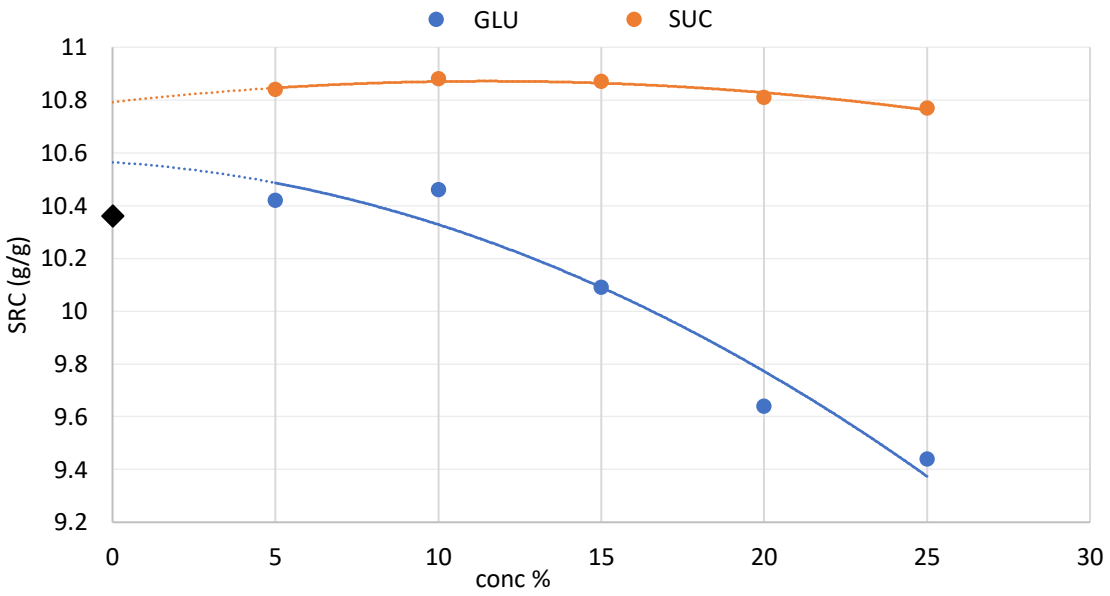


*Figure 15 - **SRC** curves for **C_w_V** fibers, obtained with **GLUcose** and **SUCrose** solutions at different concentrations.*

The SRC parameter for Crimson Sweet IDF (C_*w*_V), treated with Glucose solutions at different concentrations % (Figure 15), shows an initial trend with a plateau, which slowly increases to around 10.4 g/g, and then slowly decreases, similarly to that observed for the TDF (C_AI_V) sample (Figure 13).
The replacement of Glucose with Sucrose moves the SUC-SRC curve upwards by about 0.5 points, with a plateau effect around 10.9 g/g for IDF sample, with saturation of the active sites in the solid phase at a concentration of about 15%. Subsequently, a modest drop to SRC values ≈ 10.8 g/g is detected in the investigated concentration range. The trend of the best-fit function is similar to that observed for the TDF sample (Figure 13).

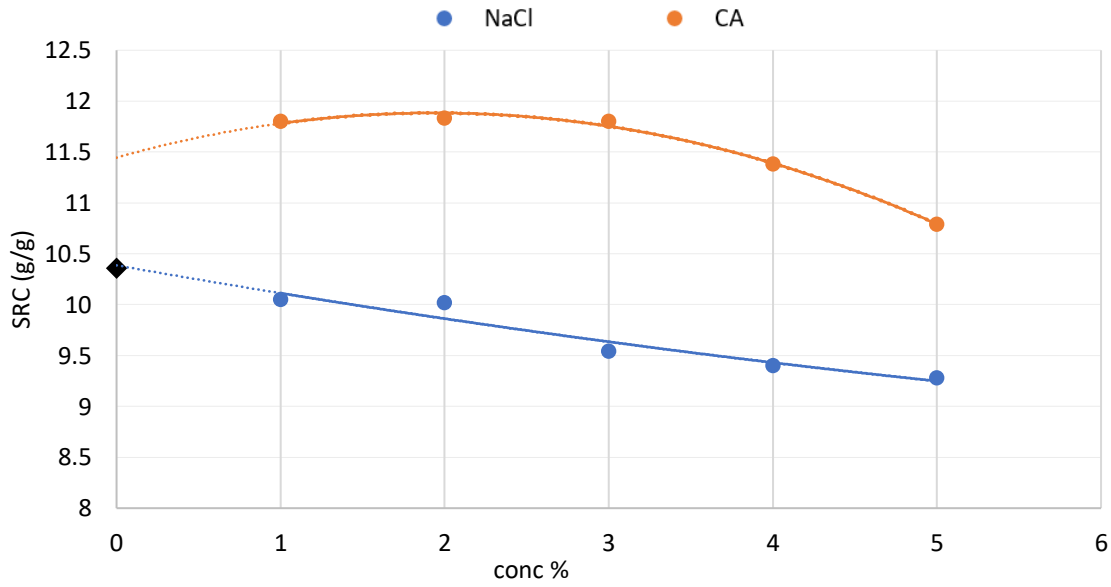


*Figure 16 - **SRC** curves for **C_w_V** (IDF), obtained with **CA** and **NaCl** solutions at different concentrations.*

For the washed fiber (IDF) in contact with NaCl and CA (Figure 16), there is a trend in contrast with that of the TDF ones (Figure 14). Evidently the presence of the soluble fraction (sugars, acids, mineral salts, amino acids, simple proteins, etc.) in the TDF seems to favor the dipolar interaction with the ions of the NaCl solute. For IDF specimen, the contact with the NaCl-based electrolyte solution leads to a gradual and progressive decrease in SRC vs. concentration%, an indication that the specific interactions of the salt with the fiber are not significant.

Indeed, the increase in the concentration of NaCl reduces the amount of water absorbed, producing an extractive effect from the mass of solid material impregnated with solvent.
This behavior could be mainly related to the absence of electrolytic solutes in the washed fiber (C_*w*_V), in particular the organic acids that are eliminated during washing. In fact, organic acids can interact effectively with metal cations, especially with those with a high oxidation state.
The best-fit equation identifies the intercept at the SRC value = 10.36 g/g, the condition of perfect overlap with the zero point for the experimental data (W-SRC = 10.36 g/g) obtained in the binary system {IDF + W}.
Contrary to what is reported for the TDF (always decreasing function), in the case of citric acid a trend similar to that of carbohydrates is observed.
Initially, there is a rapid increase in CA-SRC values up to the threshold of about 11.8 g/g, on a plateau zone where the active sites in solid state are saturated, at the CA concentration ≈ 2 - 3%.
Subsequently we observe a decrease up to the limit of the concentration range investigated.
Based on the trend, we can hypothesize a fair capacity for interaction of the added solute with the washed fiber (IDF), compared to what is observed for the TDF matrix.

Water Swelling Capacity (WSC)

In general, the swelling power of DFs in contact with water or other solvents is mainly associated with their starch and protein levels. This functional property is characteristic of all natural polymeric materials, especially polysaccharide and protein components, typically found in starch-protein flours obtained from seeds and grains. However, it is essential to bear in mind that the protein fraction, especially if low hydrophilic and present in large quantities in the fiber, is very often responsible for the effects of confinement, i.e. the encapsulation of the starch fraction, thus controlling the access of water to the starch microgranules and liming its swelling capacity [25].

In the TDFs obtained from *Cucurbitaceae* the major constituent fraction is represented by the cellulosic polysaccharide components, while the starchy and protein fractions are strongly minority. Therefore, the swelling capacity in contact with water indicates the presence of a network of hydrogen-bonding specific interactions. Swelling mechanisms have been extensively described in the literature [26, 27]. These studies show that more structured polysaccharide materials, with a high level of self-aggregation and synergistic interactions (H-bond, Coulomb forces, dipolar interactions, etc.) tend to pack tightly, thus resulting much more rigid. These materials show greater swelling resistance, due to the extensive and strongly bonded pseudo-micellar structure, which affects the ability to interact with water molecules.

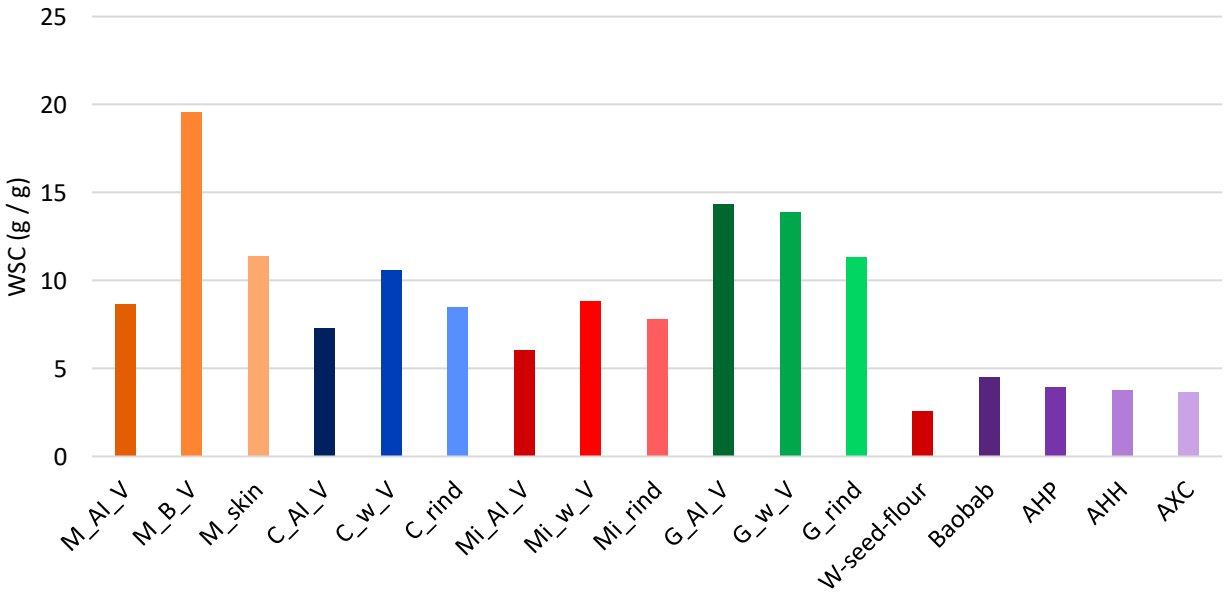


Figure 17 - Comparison of the WSC values of the fibrous-polysaccharide materials tested

As can be observed from the values of Table 1 and in Figure 17, the swelling power is maximum for M_B_V fiber (19.5 g/g). This experimental observation seems to confirm the reasons for the large swelling expressed above. The bleaching treatment with H_2O_2 is very invasive and is probably able to destroy, at least partially, the protein fraction which stands between the polysaccharide polymer and the water. The elimination of the barrier effect induced by protein, unblocks the hydroxylated sites of the cellulosic polymer, allowing free access to water molecules, which can consequently swell the solid-state insoluble material. Subsequently, in the WSC value scale for melon, we find M_skin with 11.4 g/g and, lastly, the M_AI_V sample with 8.6 g/g. It should be noted that the oxidative bleaching treatment with H_2O_2 produces a significant ~125% increase compared to the TDF (M_AI_V) fiber.

As far as the dietary fibers from watermelon concern, it is noted that, with the same treatment, for different samples of the three cultivars, the sequence WSC_Gavina> WSC_Crimson> WSC_Miyako is observed. For example, WSC of G_AI_V pomace fiber (14.4 g/g) is 97% higher than the analogous fiber of Crimson (C_AI_V: 7.3 g/g) and 140% than that obtained from Miyako cultivar (Mi_AI_V: 6.0 g/g). This trend is also common for the other two types of fiber analyzed (washed fibers from pomace and fibers from the rinds).

Finally, some considerations about the starch-protein flour samples from seeds of different origins. Baobab seed flour, a commercial product widely used as a formulation ingredient for baking and ice cream products, exhibits higher WSC (4.5 g/g) than other samples of the homogeneous set. The WSC of W-seed-flour is modest (2.6 g/g), even if it is still very rich in proteins (45 – 50%) [28, 29]. Proteins could generate a strong barrier effect for the water molecules, preventing them from approaching the microgranules of starch, perfectly hydrocompatible but scarcely swellable in these conditions.
The evaluation of the functional properties for horse-chestnut seed flours partly completes the characterization of these matrices, which have also aroused our curiosity, given the interest and relative importance that these waste materials have in the nutraceutical-cosmetic-pharmacological field [30].
The TDF flours obtained from the AHP, AHH and AXC seeds have fairly low WSC values, even if it is possible to trace a trend for WSC (g/g):

WSC (g/g)	AHP (3.9) > AHH (3.8) > AXC (3.7)
Proteins %	AHH (1.82) < AHP (2.64) < AXC (3.16)
Lipids %	AHH (5.10) > AXC (4.34) > AHP (4.13)
Cold water solubility %	AHH (48.6) < AHP (53.9) < AXC (55.1)

Observing the data reported in parallel for other compositional aspects too, it can be deduced that the greater swelling capacity for the AHP cultivar is related to the low values of proteins and lipids, while the soluble fraction seems to go against the trend, since the higher solubility corresponds to the lowest swelling capacity recorded for the AXC sample [31]. It should be noted that, in addition to the barrier effect of the protein components against hydrophilic species (cellulosic and starchy polysaccharides), the contribution of the lipid fraction, which tends to disperse in the matrices heterogeneous-hetero-aggregated mass, is added even more. However, the presence of a large amount of compounds with a strong surfactant action (aescins, polyphenols, hydrocolloids, among others) should favor the transport of the soluble fraction in solution, simultaneously favoring the dispersion of the solvent molecules within the insoluble solid mass.

Oil Holding Capacity (OHC)

Before developing considerations and making comparisons with literature data, it is necessary to specify that our samples are based on Dietary Fibers (DF), obtained through low-degree manipulation processes, in order to evaluate its possible applicability through technological transfer in any context where they are useful and required as formulation ingredients.
The samples of our interest shown in Table 1 and Figure 18, can be mainly divided into 2 groups:
- fibers obtained from different fractions of *Cucurbitaceae*, which have undergone different processes, with OHC values included in the range 1.94 g/g (G_rind) ÷ 6.29 g/g (M_B_V);

- fibers with starch-protein characteristics obtained from seeds of different origins, with OHC values in the range 2.74 g/g (W-seed-flour) ÷ 3.52 g/g (baobab).

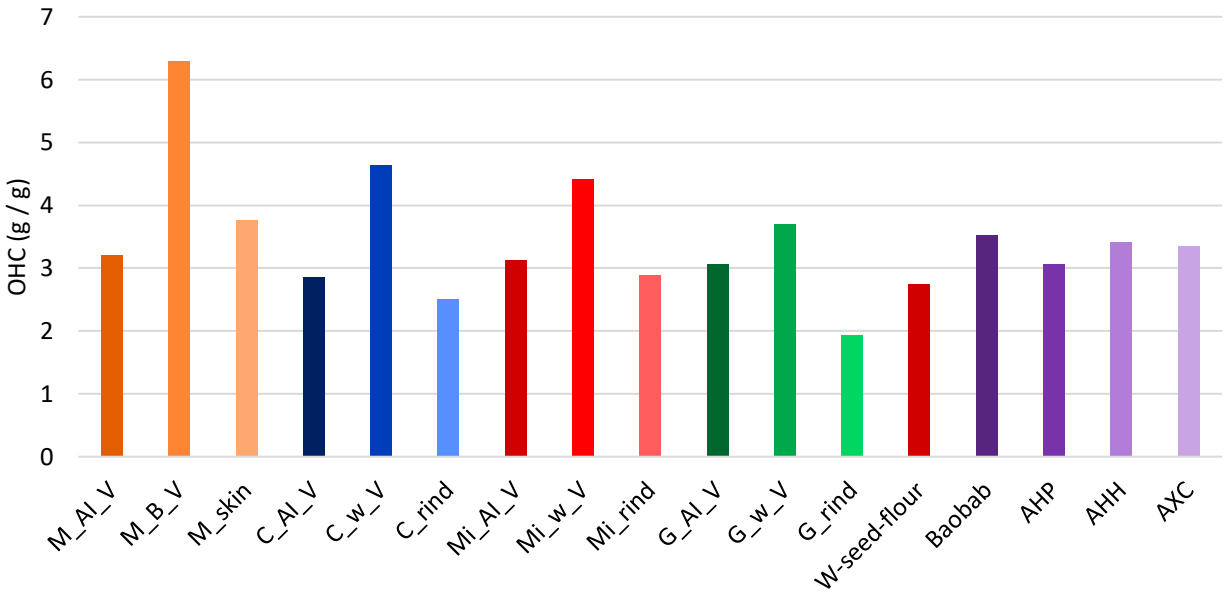


Figure 18 - Comparison of the OHC values of the fibrous-polysaccharide materials tested

These observations appear to be in line with literature data related to different matrices, despite the preliminary processes of preparing the samples themselves.
Despite the macroscopic differences existing between the raw integral fiber (TDF) and the SDF and IDF sub-fractions, our OHC values seem to reflect a general trend which results from the classification of DF according to its origin. The OHC of DF from vegetables ($1.01 \leq$ OHC (g/g) ≤ 25.8) is on average higher than that of DF from fruits ($0.65 \leq$ OHC (g/g) ≤ 9.91) and cereals ($0.96 \leq$ OHC (g/g) ≤ 29.00), although with some exceptions [32].

Given the overall characteristics of the physicochemical and technological properties measured for *Cucurbitaceae* fibers, we can assume that they probably could represent excellent formulation ingredients with functional properties for composite foods (bakery products, pastries, ice cream, prepared and precooked foods, etc.). In fact, they combine good water retention capacities associated with fat absorption and, therefore, are good promoters of emulsifiability. Other fibers obtained from Crimson watermelon seeds (dehulled - ground – defatted) and commercial baobab fibers, because of their starchy-protein nature, could be much more similar to fibers from legumes [33] and cereals, rather than from other sources.
The consumption of food products based on watermelon seeds is relatively common in Asian countries, while the fibers obtained from the baobab fruits are distributed and spread all over the world as they are effective ingredients for a wide variety of food applications [34 - 36].
Moreover, given the potential application reserved to the seeds of *Aesculus Hippocastanum* (AH), in some recent studies we have dealt with the characterization of these materials with particular attention to the 3 cultivars that are more widespread in the Mediterranean regions:
i) AHP, the genetically Pure species with white flowers; ii) AHH, the Hybrid species with pink flowers; iii) *Aesculus X Carnea* (AXC) with carmine red flowers [30, 37 - 39].
The main applications of products from *Hippocastanaceae* seeds are found in pharmacological - nutraceutical - cosmetic fields.
In order to increase the knowledge acquired so far, in addition to the chemical-physical characteristics, in this work we have also investigated the technological properties of wholemeal flours obtained from naturally dried, husked and ground seeds.
Although the values are distributed in narrow ranges, the WHC is maximum for the AXC species (+22% compared to AHH), while AHH shows the highest OHC values with small differences compared to AXC (+1.8%)

but more significant than AHP (+11%). Given the more or less significant differences recorded for the WHC, the swelling capacity of these whole fibers, expressed by the WSC values, averages around 3.8, with minimal differences (± 0.15) among the 3 cultivars examined. Conversely, the OHC is maximum for the AHH species (3.41 g/g), which is 2% higher than the AXC one and 11% higher than AHP (3.07 g/g). The presence of a large amount of bioactive compounds such as aescins, saponins, several polyphenols and other components having antithetical properties, as they are partly hydrophilic and partly lipophilic [40], probably promotes the absorption of fats in quite important quantities for all the samples examined. Reasonably, this may help to increase the emulsifying power and stabilizing the polyphasic micellar system.

10.4. INVESTIGATIONS WITH SEM TECHNIQUE

At this point, it is necessary to take a look at the structure of some fibers by exploiting the potential of SEM technique.

The figures in sequence below (Figures 19, 20 and 21) reproduce some SEM images collected from defatted starchy-protein fiber samples obtained from Crimson Sweet watermelon seeds.

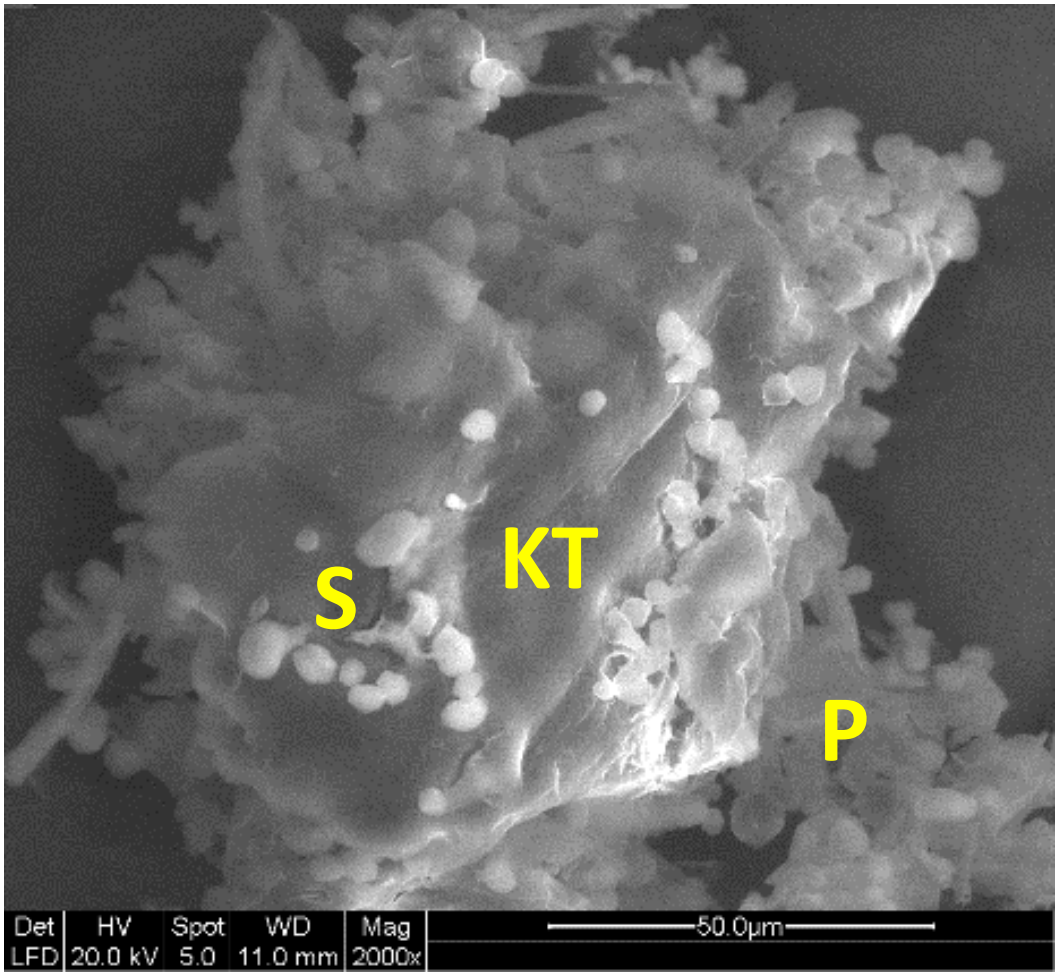


Figure 19 - SEM image of a defatted starchy-protein fiber aliquot from Crimson seeds: S = starch microgranules; P = germplasm proteins; KT = kernel tissue.

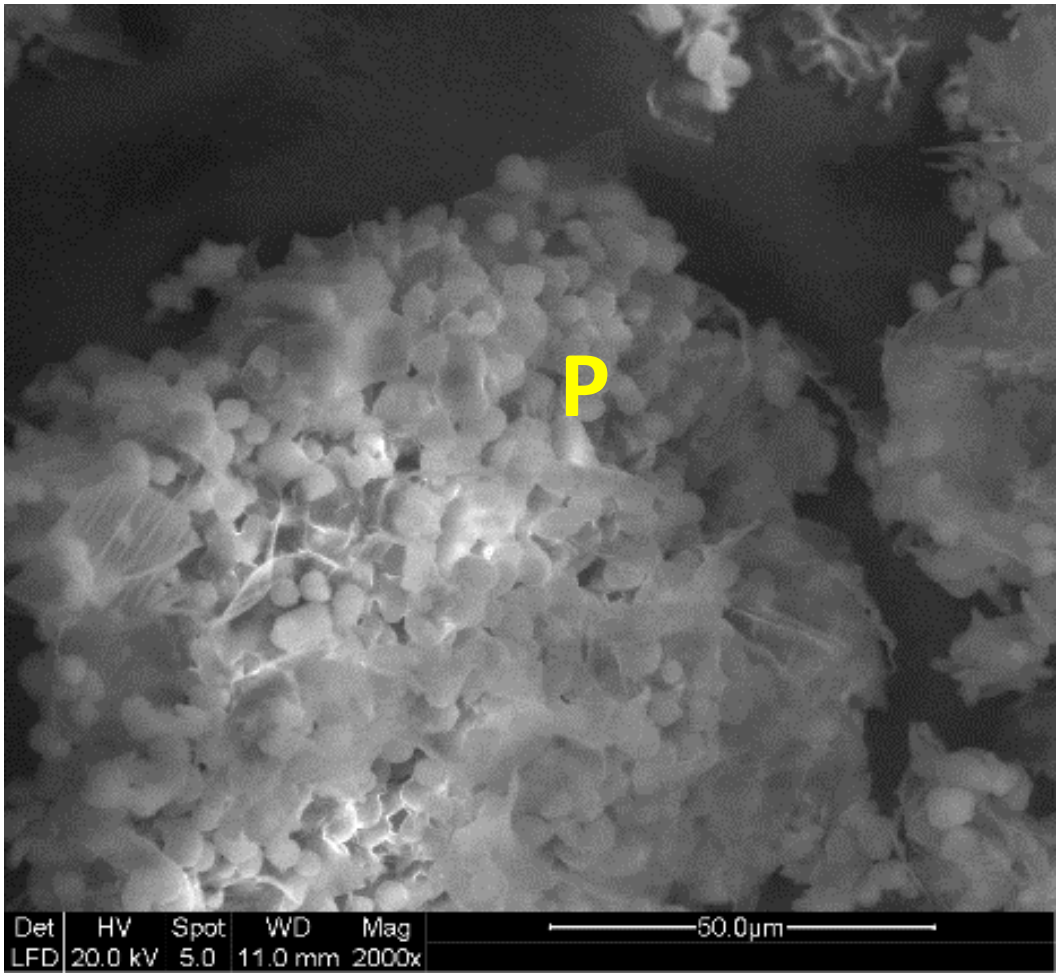


Figure 20 - SEM image of a defatted starchy-protein fiber aliquot from Crimson seeds: P = germplasm proteins

The defatted flour of watermelon seeds is mainly composed of at least two different globular structures, each of them preferably self-aggregated, tightly bonded and close to the alveolar tissue structures mainly consisting of cellulosic material.

The starch microgranules (S) in Figure 19, seem to show a quite homogeneous shape, being pseudo-spheroidal with dimension ~ 1 - 5 µm.

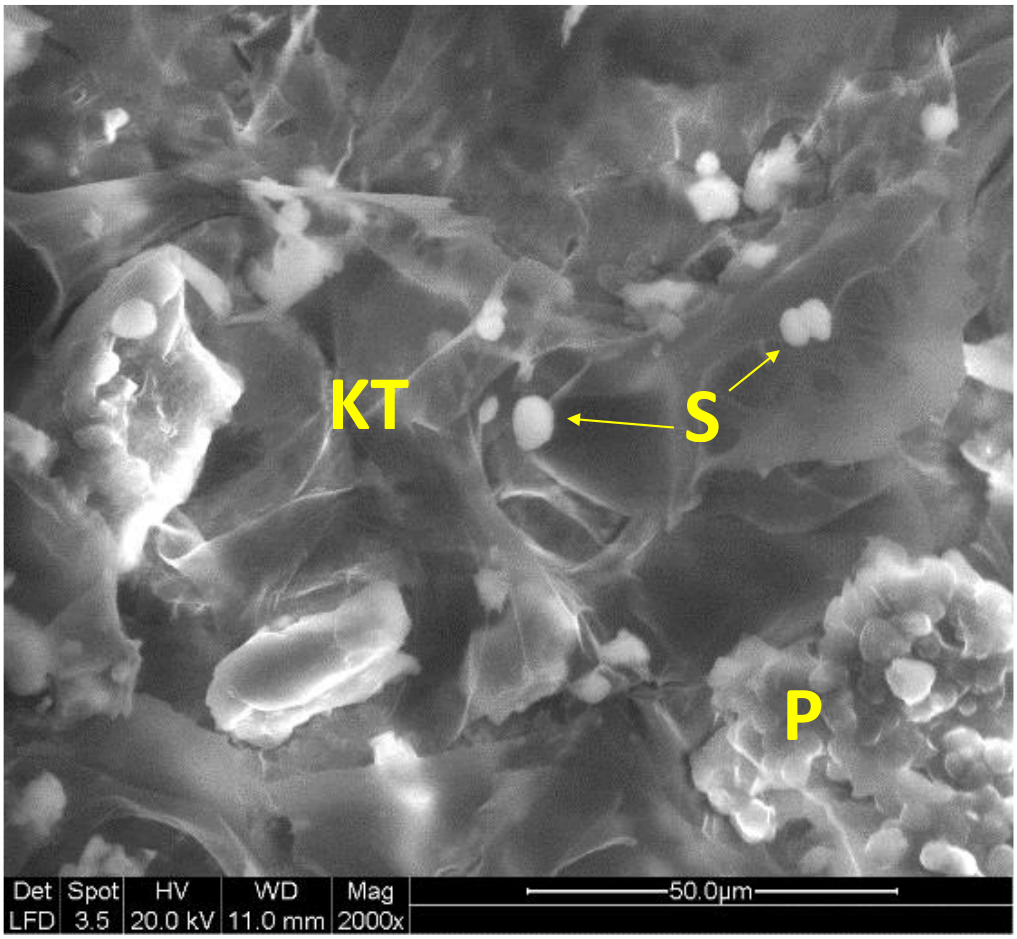


Figure 21 - SEM image showing the structural morphology of the inner parietal surface of a watermelon Crimson seed sectioned along the vertical axis

Figure 21 perfectly shows the natural starch granules of watermelon seeds, with a spheroidal shape and significantly bigger than the proteic components. Starch microgranules are much more spread and are housed in the alveolar cavities of cellulose-based inner structural tissues.
The details allow to distinguish the agglomerates of protein particles (P), whose corpuscles are strongly embedded, almost in the form of a protein paste and are held together by gelatinous binders, hydrocolloids and fatty matter [41]. Here, it is confirmed that the occurrence of starch granules located together with pseudo-spherical protein bodies could be found in starchy endosperm of some cereals as well as in cotyledon of some legumes and nuts and that, in general, the size of starch granules is larger than that of protein bodies [42]. On the basis of these information, comparing the literature structures with those identified in our floured samples and taking into account the results of the elemental analysis, we can consider all these analogies may explain the comparable values of WHC of the above-mentioned samples.
On the other hand, analyzing the structure of the fibers having the highest WHC values, we immediately notice that they are completely different from the previous ones, as shown in Figure 22 (M_B_V) and Figure 23 (C_*w*_V).

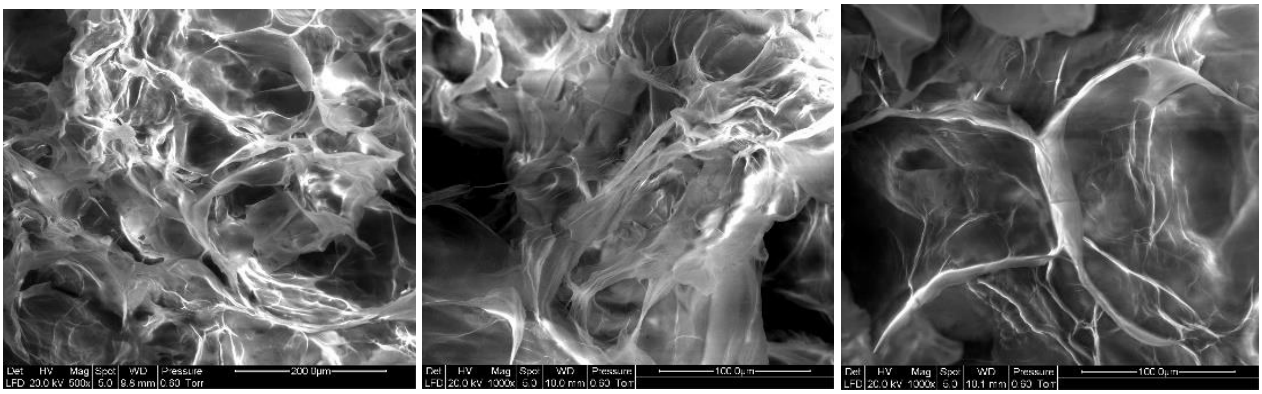


Figure 22 - SEM images of a M_B_V fiber sample

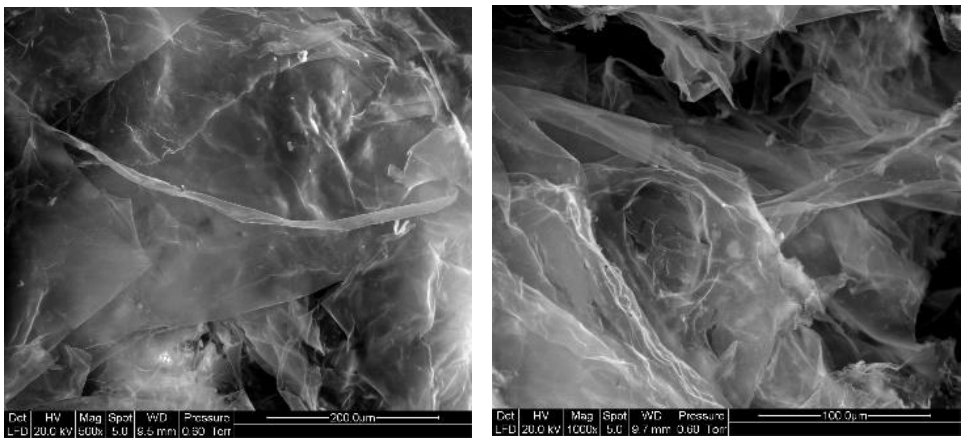


Figure 23 - SEM images of a C_w_V fiber sample

Both samples are characterized by the absence of starch granules and protein bodies, having instead a filamentous-type structure, with fibers interconnected in a fairly random way, without regular units and not particularly compact in appearance. This is quite similar to that of potato fiber (Figure 24), where it can be distinctly identified the cell wall material which comprises the fiber, even if it has undergone different handling processes [16].

It is known that this material consists of a complex mixture of polysaccharides and other polymers that are secreted by the cell, linked together by both covalent and noncovalent bonds and assembled to form a network. Plant cell walls also contain structural proteins, enzymes, phenolic polymers, and other materials that modify the wall's physical and chemical characteristics.

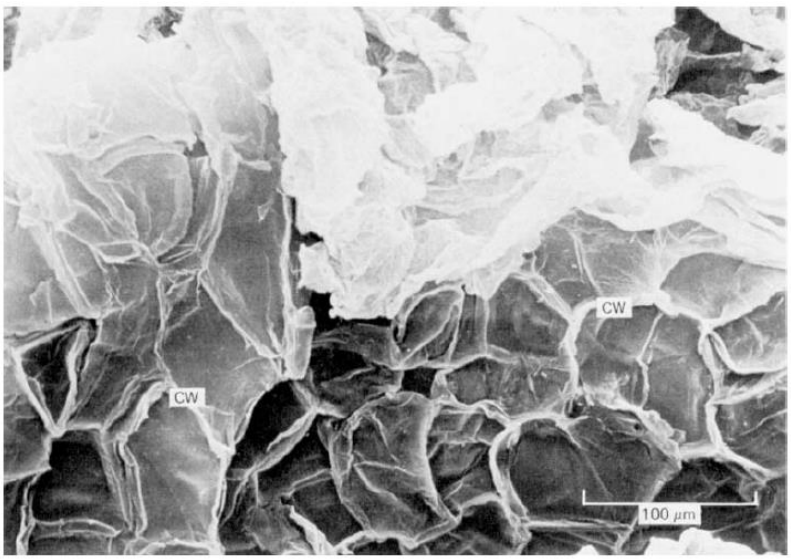


Figure 24 - Potato fiber after neutral detergent fiber preparation, from [16].

All this is further confirmed if we consider the WHC values of hydroxypropyl methylcellulose (1784 % ± 96.55 %), microcrystalline cellulose (1404 % ± 219.0 %) and agar (1274 % ± 149.7 %) samples and their morphology [43]. In this case, we are certain to deal purely with polysaccharide matrices whose structure and water absorption capacity find strong similarities with some of our samples (i.e. IDFs from melon and watermelon). The absence of starch and protein granules suggests the high WHC of vegetable fibers may be due to their greater ability to trap water within the fiber structure and cell matrix than cereal or seeds ones [16].
The reasons for this behaviour can probably be found in a marked structural rigidity of the matrices, attributable to a greater degree of crystallinity of the components. In particular, the starchy - amylopectin fraction has geometric domains in which the crystalline characteristics prevail over the amorphous components, typically attributable to the amyloid fraction content.
Similar reasoning, even if they are different analytes, can be extended to the cellulosic component of baobab fibers. Some particularly relevant issues for these fibers are the meteoclimatic factors to which they are exposed, since it is known that both water and thermal stress tend to stiffen polysaccharide structures, increasing their crystallinity levels.

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CHAPTER 11

11 - Candying process for enhancing pre-waste watermelon rinds to increase food sustainability

Abstract

This work draws attention to meet a current challenge that the world of agronomic productions will have to face in a short transient period, with definitive choices to ensure more and more food sustainability. To combat hunger and food paucity, it will be increasingly necessary to reduce food wastage and recover, as much as possible, leftover materials in pre-waste conditions, to transform them into as many safe food products. We have made an attempt to obtain 'candied fruit' from the fresh rind of Crimson Sweet watermelon, the mesocarp of the fruit, a typical waste material considered inedible, of low or zero nutritional value, based on hard cellulosic tissues, suitable for protect the endocarp, the edible fraction. Our experimental candying process, by slow osmotization cycle lasting 24 weeks at room temperature, was activated with granular sucrose according to two alternative laboratory methods, WET and DRY. The fresh rind of the watermelon, decidedly unappetizing, is transformed into candied fruit with an excellent taste, with volatile - gustatory impact aromas and of great quality, with high pleasantness levels. The aromas that can be perceived are associated to sweet perfumes, cooked sugars and fruity. The aromatic profile of the fresh rinds and the candied ones was characterized with the HS-SPME-GC-MS technique. The results highlighted some significant differences in the composition of the VOCs, attributable both to the cultivar but, above all, to the two candying methods, as verified also by a panel test.

1 Introduction

The concept of sustainability, in general, is a set of extremely articulated and interdisciplinary ideas, and attention is rarely paid to the fact that several factors contribute to modeling its contents and representation in the real world.

Thus, in order to ensure sustainable development conditions at global level, we cannot ignore some social variables (public security, globalized inclusion, availability of food resources for all people, etc.), environmental ones (reduction of predatory activities of fossil resources, elimination of pollution sources for a greener and cleaner world, etc.), and also economic ones (availability of resources equally distributed to satisfy the primary needs of populations, etc.). Bearing all this in mind, any general problem relating to the aspects of sustainability can be addressed [1].

It turns out that, in order to identify the most convenient solution, it is necessary to use the principles of the circular economy.

Apparently, it is surprising to see how the same principles, namely a set of simple and elementary rules, can be effectively implemented for interventions aimed at reducing emissions responsible for climate change, or to mitigate the negative effects of hunger in the world [2].

Circular economy and increasing the availability of food starting from pre-waste material, are the main topics that inspired this applied research work.

It is generally observed that the fruit and vegetable production of the geographic areas with a temperate climate are characterized from a large edible fraction and moderate amounts of waste materials such as husks, peels, seeds and kernels.

However, in contrast for some primary crops such as *Cucurbitaceae*, significantly higher discarded products and by-product ratios arise from the harvest and fruit processing [3].

To give an idea about these quantities, we report that our region (Emilia Romagna) produces about 40 - 60 kilotons/year of watermelon waste, while the national production (Italy) is estimated at ~ 150 - 200 kilotons/year.

The data become impressive if considering the worldwide production of watermelon in 2017–18, because approximately 42 million tons of watermelon by-products (rind and seeds) was generated by numerous fruit juice processing industries, restaurants or others public exercises during transformation, preparation and consumption of the fruit [4]. The mass of watermelon waste increases further if one adds the product that remains directly discarded by the farmers on the cultivation land.
The American Agricultural Marketing Resource Center agency also reports similar evaluations, underlining that the quantity of fresh product that annually remains in the field, rejected for surface blemishes or because fruit are misshapen, is around 20 % of the total production, for a total mass of 350 - 400 kiloton / year [5].
These are quite significant amounts, and if it were possible to improve the recovery of even a fraction of them through processing into other foods, this could help achieve at least 3 goals, such as (i) increasing availability to cover basic needs [6], (ii) minimizing waste and environmental impact, and (iii) maximizing agricultural profit [7].
It should be remembered that the implementation of innovative ways to extend the availability of new forms of food will also require new technologies and new business models for the production and marketing of new goods and services, fully confirming the general indications provided by the new schools of modern thought [8].
Because of significant increase in intensive agricultural productions, disposal can represent a growing problem since plant material with a high water content is usually subject to rapid spoilage due to microbial fermentations, thus limiting further exploitation.
On the other hand, the high costs of drying, storage, formulation and distribution of by-products can become economically limiting factors making prohibitive conditions for any further application of agronomic waste along the value chain.
For these reasons, both waste products abandoned in the field and having characteristics that do not comply with sales quality standards, or of agro-industrial origin if generated by transformation processes (the production of juices is the main one; for more information the readers should refer to www.imarcgroup.com), are often destined for animal feed, landfilling or composting before dispersed as agricultural amendments.
However, it must be remembered that the livestock sector's demand for the supply of feed must follow precise rules in terms of masses and volumes which also require storage procedures.
Conversely, the availability of by-products and waste materials from the agronomic supply chains could be variable and dependent on agricultural yields, as well as seasonal in nature.
Therefore, these aspects seem to go against the trend since there is no correlation between the variables that can be identified for the functional control of production management in all its specificities.
On the other hand, the disposal of waste and by-products whatever their origin, can be a significant problem as it is generally regulated in different way following the policies of different countries, and therefore it could be further heightened from legal restrictions.
To this unfavorable aspect, it can be added that the immediate spreading on the land of agri-food chains waste residues can be somewhere inappropriate, as in the case of mature *Cucurbitaceae*.
These fruits, being too rich in fermentable sugars, can develop large quantities of alcohols with dramatic consequences for the microbial flora of the soils, up to procuring sterility.
For these reasons, many waste productions potentially harmful towards the microbacterial stability of the soils must undergo some composting processes, both of the anaerobic -reductive and / or aerobic - oxidative type, in order to eliminate the negative potential related to the possible appearance of dangerous substances for the biotic stability of the land. Among these species, methanol and ethanol should be mentioned as very toxic for the soil biocenosis [9].
Therefore, the correct and efficient use of these materials, economic and respectful of the environment, is becoming increasingly important especially from the point of view of the extension of LCA cycles and strictly related to the profitability.
This observation is valid both in the case of any management savings due to the reduction of costs and minimization of disposal problems, but mostly for the enhancement induced by some commercial application of the by-products [10].

Bearing this in mind, we can address some attention to a case study represented by watermelon cultivation and processing pre-waste. To our knowledge, the rind of the watermelon constitutes a rather substantial fraction of the fruit, varying from about 15 to 30% of the total mass for the various commercial cultivars.
However, Tarazona-Díaz et al. [11] reported other data, indicating that watermelon rind approximately accounts for more than one-third of the total fruit mass (31 - 40 %) and is usually discarded, despite being edible, but most times it is avoided due to its too hard consistency and unappealing flavor.
The peels therefore remain the form of massive waste by-product most important among the agri-food waste generated by many players operating along the value-chain, but above all, by producers in the farmers and by food industries that produce vegetable juices [12].
This view is strongly shared in literature, and many other authors agree with us [13, 14 and references therein].
Accordingly, the transformation for the development of new value-added products starting from these peels could be of great interest for the food chain industries.
In previous literature studies, it has been shown that watermelon rinds have some potential for the possible recovery of derived materials.
For example, after processing- drying and grinding the mesocarp, the white and thick intermediate layer, a neutral dietary fiber can be obtained for use in the pharmaceutical industry [15], for the preparation of nutraceutical-enriched flours for the bakery production, pasta, pastry and ice cream products, stabilizing agent of water content [12] and as a natural food additive [16, 17], a useful product for new forms of packaging materials [18], among others.
In addition to all the opportunities described above, in this paper, we report the results from a series of candying tests that we carried out on some samples of mesocarp obtained from thick peels of Crimson sweet watermelon, in an attempt to integrate the panorama of possible applications and enhancement the recovery of these agro-waste materials.
Watermelon rinds have a very high moisture content, about 95% on a wet basis.
These materials are highly unstable in the fresh state, being susceptible to rapid deterioration and easily perishable, exposed to the aggression of molds and the rapid growth of bacterial colonies responsible for undesirable fermentation processes [19].
This means, for example, that the logistics of the production system requires the extension of the cold line for the proper storage of the rinds discharged from the production line of watermelon juice, waiting for the parallel processing for candying, since the latter operation requires longer time than the pressing of the juice.
The candying process involves the reduction of the moisture content to safe levels, slowing down and inhibiting the microbial growth and enzymatic activity associated with the fermentation processes, thus extending the shelf life of the obtainable products [20].
Obviously, these last are very sweet products with great added value, mainly intended for the confectionery sector, ice cream production, yoghurt and dairy products, as well as candied fruits in general, among others.
In particular, we have monitored the composition of the volatile aromas associated to different candying processes, in relation to the characteristics of the fresh product.
In fact, the fresh product is absolutely unappetizing, it is almost devoid of aromas that could make it interesting and recognizable for its particular scent, as in the case of other fruits. Conversely, the candying process associated with some partial fermentation processes leads to the development of volatile analytes that express a very pleasant aromatic profile, making the finished product unique and perfectly recognizable.
Unfortunately, extensive literature and market research shows that in the panorama of candied products, both fruits and vegetables, there is no other species that can resemble our finished products. This fact makes very difficult any possible comparison with any matrix, alternative or substitute, of commodity interest.
Currently, the global market seems to follow periodic fluctuations in time, starting in 2006, and in space: strong growth in Far Eastern countries, especially China and Thailand, while contracting in Western European countries and the USA. However, the overall balance is positive, with important and well localized increases in the market growth rate.
Due to the growing demand for candied fruits worldwide, the market is expected to show an upward trend in consumption over the next decade. The market trend is expected to accelerate, expanding at a compound annual growth rate of 4-6 % for the period from 2021 to 2030 (www.indexbox.io).

2 Materials and Methods

2.1 Samples preparation

The fresh rinds taken from 3 Crimson sweet watermelons, about 2 cm thick, have been completely cleaned, carefully eliminating both the internal part (the endocarp, the edible fraction) until the red fiber is completely removed, and the streaked green external cuticular fiber (the exocarp, the rigid layer with a nearly woody-like consistency, having a thickness of about 3-4 mm, the peel). Subsequently, the peeled rinds were cut into pieces approximately 5 x 5 x 1.5 cm in size for the candying process.
For the candying of the white mesocarp, we have identified 2 alternative very slow osmotic procedures (lasting 24 weeks), which we indicate WET and DRY, and which are very different from industrial-type processes.
The pieces of rinds (about 1 kg) were immersed and layered in a bed of solid sucrose in a rectangular glass container [dimensions 32 (length) x 16 (width) x 8 (depth) cm] until completely filled (mass ratio rinds : sucrose = 1 : 1).
The containers were covered with a steel plate onto which weights equivalent to 2 kg were loaded to exert a constant pressure.
The system was then covered with a plexiglass cloche to avoid equilibrium exchange with external atmospheric oxygen.
The dataset provided in this study is related to fruits collected in the Modena territory and processed in the year 2020.

WET process: the glass container was placed in a horizontal position and maintained in this initial condition for the entire duration of the candying process. In this way, the rinds always remained in contact with the concentrated sucrose solution that was slowly formed as the osmosis process contributes to remove the water from the peels themselves.
DRY process: the container was inclined to a suitable extent to allow the major part of the saccharide solution to constantly drain into another external collection pan. In this case, the rinds have undergone a particularly stressful and effective dehydration process, in the absence of continuous contact with the exceeding preserving liquid, the physiological solution generated autonomously. In this way, the rinds remain in contact only with a very small fraction of the concentrated and saturated solution. Therefore, they remain strongly impregnated with thick and viscous saccharide solution, with the result that the final product is particularly dehydrated and scarcely dripping.
Once the candying cycle was completed, the rinds were collected and placed in jars with their residual contact solution, highly sugary, dense, with a honey-like consistency, for the tests necessary to evaluate the qualitative and aromatic characteristics of the final product.

2.2 Materials

The dichloromethane used is an analytical grade Sigma-Aldrich product for HPLC and GC chromatography, distributed by Merck KGaA, Darmstadt, Germany.
Others chemicals such as: 2-methyl-1-propanol; 3-methyl-1-butanol; 2-ethyl-1-hexanol; phenylethyl alcohol; 3-methy-l-butanal; ethyl butanoate; ethyl hexanoate; 3-hydroxy-2-butanone (acetoin); b-pinene; b-Caryophyllene and limonene were obtained from Sigma – Aldrich product, distributed by Merck KGaA, Darmstadt, Germany.
Decanal; methyl acetate; 2-methylfuran and 2-ethylfuran were obtained from Carlo Erba Reagents, Milano (Italy).
n-hexane, nonane, dodecane, tetradecane and hexadecane, obtained from Carlo Erba Reagents, Milano (Italy), have been injected both for the for the identification of analytes through the mass spectra and for the calculation of the Linear Retention Index (LRI).
A Solid Phase Micro-Extraction (SPME) holder (Supelco Inc., Bellefonte, PA, USA) was used to manually perform the SPME headspace (HS) analysis.

The SPME device consists of a fused silica fiber coated with Carbowax/Divinylbenzene/ Polydimethylsiloxane (CW/DVB/PDMSO).
The triple fiber covering material CW/DVB/PDMSO is a mixed coating that contains a liquid polymer, a semi-solid polymer and solid particles. This type of coating is highly performing as it combines both the absorption and adsorption properties of the different coating particles with a synergistic effect.
This multiple effect facilitates the balance of analyte distribution between the stationary phase and the gas phase in the headspace, promoting the retention capacity and improving the sensitivity of the multiple-coated fiber compared to other types of similar devices.
Furthermore, given the characteristics of different polarity of the 3 constituents of the coating, the fiber seems suitable for the capture of analytes with significantly different molecular properties and dimensions, resulting adequate for the characterization of complex matrices as in this case [21,22].

2.3 HS-SPME Analysis

Initially, a set of specimens was prepared consisting of a freshly shredded mesocarp sample, representative of the material subjected to candying process.
The rinds were quickly ground with a mixer, obtaining a rather coarse and relatively dry pomace, therefore without significant losses of endogenous liquids.
Each sample consists of 10 ± 1 g of pomace, introduced in 20 mL vials which corresponds to a solid phase to headspace volume ratio of ~ 0.5, and sealed tightly with teflon / silicone septa.
The vials thus prepared were processed in different ways, in order to verify the compositional characteristics of the matrix with reference to the volatile aromatic fraction. Some vials have not undergone any other manipulation.
Some vials have been loaded with 7 mL of CH_2Cl_2 to facilitate the extraction of any soluble and easily volatilizable compounds from this phase.
Other vials were added with 7 mL of supersaturated NaCl solution to favor the effect of displacement from the pomace and relative salting out of the volatile compounds. Reproducibility of experimental procedures have been established working with at least three replicates samples of the same matrix and making many different measures for each vial.
All the samples, regardless of the manipulations undergone, were sonicated for 30 min in a thermostated bath at 40.0 ± 0.1 °C to favor the transfer of volatile compounds from the matrix to the headspace.
After this step, the SPME fiber was manually inserted into the headspace of the sample vial thermostated at 40.0 ± 0.1 °C, with exposure to volatile compounds for 15 min.
Some blank tests, corresponding to the analysis of an external standard solution containing 1-decanol (conc. 150 µg/g ethanolic solution) were performed periodically after a certain number of chromatographic runs (5) relating to real samples.
All measurements were performed in at least 3 replicates for each sample/vial.
The candied rinds obtained according to the 2 methods previously described (WET and DRY) were drained of their syrup until the dripping disappeared.
The samples then obtained were prepared and analyzed following the same procedures described above for the fresh peels, regardless of both the type of osmotization process of the initial matrix.
However, the much soft consistency of the candied rinds allowed to obtain a much more homogeneous pomace from the grinding, if compared to the analogue obtained from fresh and relatively hard peels.

2.4 GC-MS Analysis

The liquid dichloromethane extracts and the volatile compounds desorbed from the SPME fiber were analyzed in GC – MS, Agilent Technologies 6890N Network gas chromatograph.
The GC was equipped with a 60 m x 0.25 mm i.d., 1.00 µm film thickness, DB-5MS UI capillary column (J&W Scientific, Folsom, CA, USA) connected to an Agilent 5973 mass spectrometer.

The SPME injections were performed in splitless mode and the temperature of the injector was setted at 250 °C.
Some specific chromatographic conditions were used:
(i) for CH_2Cl_2 liquid extracts (0.5 μl) were injected in the injection port lined with a splitless glass liner. The detector started to operate after 8 min of injection (solvent delay);
(ii) for SPME samples, the injection port was lined with a splitless glass liner.
The fiber was introduced into the injector for 15 min.
The detector started operate immediately after injection.
The oven temperature was programmed from 40 (held for 5 min) to 250 °C with a scan rate of 10 °C / min up to 160°C, and 8 °C/min up to 250 °C, while the transfer line was heated to 270 °C. The final temperature of 250 °C was held for 5 min.
The carrier gas (He) was fluxed at 1 mL/min, with a column head pressure of 15 psi.
The mass spectrometer was used in electron impact mode at 70 eV, scanning the range *m/z* 25–300 and in full scan acquisition mode. The identification of volatile compounds was obtained from comparison of the retention times of the GC, by comparing the mass spectra with literature, with those of the libraries supplied with the operating system of the GC-MS (NIST14/NIST05/WILEY275/NBS75K), National Institute for Standards and Technology (NIST database https://webbook.nist.gov), Mass Bank of North America (https://mona.fiehnlab.ucdavis.edu), and spectra of pure standard compounds (when available).
The estimation of concentrations for all compounds identified in the SPME analysis was carried out by comparing the GC peak area, expressed as total peak ion current.
All the data shown in the tables relate to values obtained from analysis performed at least in triplicate.
The reproducibility of the results was expressed as standard deviation in the tables.

2.5 Metals content

The ashes obtained by incineration of the samples, about 100 – 150 mg, were directly solubilized in a volumetric flask with 5 mL of HNO_3 65% (Suprapure, Merck; Darmstadt, Germany) and 5 mL of deionized water (from a Milli-Q Plus, Millipore system; Burligton, MA, USA); a clear solution was immediately obtained. Afterwards, final volume was adjusted up to 50 mL with deionized water.
The final solutions are perfectly clear and apparently colorless, stable over time, and we have never observed the formation of turbidity or precipitates.
Along with a batch of 9 samples (3 original ones, each of which replicated 3 times), a blank was also prepared in the same way. Quantification of selected metals was done using the ICP-OES spectrophotometer (Perkin Elmer - Optima 4200 DV) [23].
The spectrophotometer is equipped with an ultrasonic nebulizer (Cetac Technologies Inc.; Omaha, NE, USA) and Charge-Coupled Device (CCD) area detector complete the instrumental setup.
Working Merck ICP standards for tested metals were prepared from stock solutions (1000 mg/L). Optimum analytical conditions maintained on ICP spectrophotometer have been reported in our recent paper [24].

2.6 Other determinations

The UV-vis spectrophotometric determination (Jasco V-570 UV/VIS/NIR spectrophotometer, JASCO Corporation, Tokyo, Japan) of phosphate is based on the molybdenum-blue chemistry, recording the absorbance at 880 nm. Ascorbic acid is the common reductant in the manual analysis of phosphate [25].
Estimation of total sugar content in our samples was carried out using Fehling's solution reagents as described in the literature [26]. For easiness of interpretation and comparison between different samples, we expressed all the results as sucrose equivalents.
Water activity hygrometer LabSwift-aw (Novasina, Switzerland) was used to determine the a_w of the fresh and candied watermelon rinds at room temperature.

2.7 Statistical Analysis

Experimental data were compared by applying analysis of variance (one-way ANOVA), by running in the Matlab® 2019b environment (The Mathworks Inc., Natick, MA, USA). The level of significance was determined at $p<0.05$ to see whether there are statistical differences between the mean values.

2.8 Sensory evaluation

Sensory evaluation of the different samples was performed using 7 levels hedonistic test to compare the degree of acceptability of FRESH watermelon rinds and the candied ones obtained by applying the two alternative WET and DRY methodologies. The 7 levels were denoted as: 1 = extremely dislike; 2 = slightly dislike; 3 = moderately dislike; 4 = neither like or dislike; 5 = moderately like; 6 = slightly like; 7 = extremely like. A total of 30 panelists consisting of inexperted or semi-trained people, such as students, technicians and teachers of our Department, participated in sensory evaluation.

The people engaged in this activity for a taste test, are equally distributed between males and females, are aged between 21 and 65 years, constitute a group that represents an average population of various cultural backgrounds, able to perceive different aromatic and gustatory sensations, and able to express a critical judgment according to the suggested indexes and scale of values.

Each panelist, completely unaware about the nature of the samples to be tasted, received three anonymized specimens: i) FRESH rind, easily identifiable because very different from the candied ones, but unrecognizable the origin and the cultivar; ii) two samples, WET and DRY candied rinds, coded by a letter and two-digit random number to avoid bias. In addition, bread and water to rinse the palate before or in the middle of the evaluation procedure have been provided.

Among other, panelists were asked to evaluate the attributes including colour, aroma, sweetness, sourness, and overall acceptability of the given samples.

All variables, factors and parameters needed to perform these assessments were considered unweighted and all equally balanced (seven-level hedonistic representation, as defined above).

3 Results and Discussion

The industrial candying processes carried out by osmotic dehydration, require that the water content of the fruits is reduced while the sugar content is gradually brought to about 70-75 °Brix. In these cases, the fresh fruits cut into pieces are firstly soaked in sugar syrup, working in batch systems, at temperatures in the range of 30-80 °C. At the end of the candying cycle, the product is then semi-dried, up to the residual water content comprised, on average, in the range of 15-20 % (w / w). In addition, industrial processes are much faster than our conditioning time (24 weeks) set to obtain the final product, since they reach the objective of candying in the short storage period of 1 - 2 weeks. Vice versa, the process used for this study is of historical interest, the most traditional one, consisting in a long and delicate operation that, thanks to the respect of the natural rhythms lasting some months, allows the fruits to express all their best and peculiar characteristics [27].

Since the chopped material was immersed in granular solid sugar, the physiological candying solution was slowly formed as the dehydration process proceeds. We also have chosen to work at room temperature for the entire duration of the process, avoiding thermal stress on the rinds.

Figure 1 shows the initial state of the fresh mesocarp and the final result of the candying process carried out according to the previously illustrated WET and DRY methods.

Figure 1 - Images of fresh rinds, WET and DRIED candied ones. The colored scales are represented at 1 cm.

Table 1 shows the values of the proximate analysis of the fresh material and of the finished products obtained by the two different candying procedures adopted.

Table 1 - Proximate analysis of some samples obtained from the starting material (FRESH rinds of Crimson sweet watermelon) and candied products from different procedures (WET and DRY).*

	FRESH rinds	**WET candied**	**DRY candied**
water content %	94.5 ± 0.4	53.1 ± 0.5	28.2 ± 0.4
water activity - a_W	0.985 ± 0.003	0.875 ± 0.002	0.641 ± 0.002
moisture % at 101 °C	0.42 ± 0.04	1.28 ± 0.03	1.27 ± 0.04
residue % at 105 °C (db)	5.4 ± 0.5	46.7 ± 0.4	71.5 ± 0.5
ashes % (db)	1.20 ± 0.03	1.01 ± 0.03	0.97 ± 0.03
total soluble glucides % (db)	0.2 ± 0.1	32.6 ± 1.2	49.8 ± 1.4
rind residual % (db)	5.2 ± 0.5	14.1 ± 0.7	21.7 ± 0.8
Metal content, expressed as mg/100 g sample db (dry basis)			
K	2766 ± 75 *2998 – 3065*	2675 ± 59	2721 ± 62
Ca	264 ± 29 *353 – 372*	216 ± 15	236 ± 34
PO_4^{3-}	815 ± 32 *972 - 1006*	802 ± 20	807 ± 13
Mg	195 ± 18 *181.6 – 234.9*	185 ± 16	192 ± 25
Na	17 ± 5 *21.3 – 26.2*	12 ± 6	10 ± 7
Cu	0.25 ± 0.08 *0.43 – 0.28*	0.27 ± 0.09	0.20 ± 0.07
Fe	2.07 ± 0.04 *2.66 – 3.07*	2.04 ± 0.05	2.40 ± 0.05
Mn	1.33 ± 0.05 *1.02 – 1.42*	1.38 ± 0.06	1.35 ± 0.08
Zn	2.26 ± 0.06 *1.97 – 1.34*	2.15 ± 0.06	2.69 ± 0.08

*mean of three replicates and uncertainties expressed as standard deviation, $s_{(3)}$
Values in italic characters were taken from [28].

The water content [29] measured on fresh rinds is perfectly aligned with the general trend of literature [30, 31].
Conversely, our candied products have a water content (~ 53 % WET, and ~ 28 % DRY) that is far from the average values of industrially produced candied fruit, which are estimated on average around 15-20 % [32]. Furthermore, the WET candied contains ~ 90 % more water than the DRY type.
To this respect, industrial candied seem really dry to the touch, apparently stiff and rigid, while those obtained from this work are very moist, relatively soft and elastic. The consistency of our samples could be on the borderline between that of industrially dry candied products and cooked fruit in syrup drowned in its own liquid. However, let's remember that even with a water content of about 53% for wet candied, the stability of our product is ensured by a high value of osmotic pressure which prevents bacterial and mold growth.
The residual moisture at 101 °C seems stabilized at around 1.27-1.28 % for candied products. This type of residual moisture represents the amount of strictly bounded water, mainly coordinated to the cationic metals and inorganic compounds present in the matrix. In fact, if the data are associated with the ash content (1.01 % and 0.97 % for WET and DRY, respectively), it is evident that there is a very close correlation between them, confirming the previous hypothesis.
As regards the values of a_W, we observe that the DRY sample (a_W = 0.641) is perfectly aligned with the literature data that establish adequate levels of food safety ($a_W \leq 0.78$) and product stability against the onset of bacterial growth. The WET sample (a_W = 0.875) is placed at the limit of the variability domain of a_W to have shelf life stability of products intended for long storage periods ($a_W \leq 0.88$). However, also in this case the saturation with crystalline sucrose leads to osmotic pressure values incompatible with the proliferation of undesired species, guaranteeing stability and food safety even if in presence of enzymatic activity.
The dry residue at 105 °C consists of all the non-volatile components present in the sample. The DRY candied product, with a lower water content, has a dry residue value that exceeds the equivalent WET candied fruit by 53 %.
As regards the content of total soluble glucides, we pass from a not very significant content in the FRESH material (~ 0.2 % wet basis), to increasing values for the candied products WET (~ 32 %) and DRY (~ 50 %). Noteworthy is the fact that industrial candied fruits have a total sugars content between 70 and 90 %, while the reference value of about 81 % is reported by the Agricultural Research Service [32]. The glucidic content of candied fruit is a stabilizing factor, which allows it to be stored for a long time, but which makes it unsuitable for diabetics and obese people.
We are fully aware that the strategy of using sweets as a form of enjoyment is not the best one, as sugar consumption is increasingly discouraged, especially in Western countries where obesity is a fairly widespread and impactful pathology. However, for the products we obtained with this home-made experiment, the reduced sugar content certainly helps to limit risk factors and health damage.

Table 1 also shows data relating to some metal content of macro- and micro-constituents, in addition to the quantity of phosphorus determined as PO_4^{3-}, i.e. the most significant components of the inorganic fraction, the ashes.

Potassium is the most abundant analyte for all the samples. For FRESH rinds, our results (2766 mg/100 g db) differ to some extent from those reported by [28]. These researchers have obtained very interesting results comparing two techniques of sample drying, vacuum freeze-drying and oven heating. For the sake of completeness, their results are reported, in italic characters, as an interval also in Table 1, taking into account that the first value relates to cryo-technique while the second to hot drying.
Obviously, the cold technique is respectful of the composition of the rinds as it maintains the bioactive ingredients such as vitamins and other compounds which, in turn, being thermolabile are lost by applying the hot technique.
In addition to the above considerations, we observe that the K content does not vary significantly for both candied samples. The same trend is valid for the other main components Ca, PO_4^{3-}, Mg and Na. Similarly, a good agreement is also observed for the microelements Cu, Fe, Mn and Zn. All the data related to the FRESH

rinds that we have listed in Table 1 can be attributed, among others, to the diversity of the soils, the crop varieties, the genotypes, the environmental and pedoclimatic conditions of plant development, the management practices of the sites and the ripeness degree at the harvesting time.
Nonetheless, it is reasonable to think that some of these differentiating elements of the fresh matrix can also be transferred to some extent in derived products, as in the case we are examining.
It is common opinion that watermelon rinds, as well as many other *Cucurbitaceae* fruits, are a rather 'hard and poor' natural matrix, since at first glance they are considered inedible, scarce in bioactive principles and of little or no nutritional value. In fact, the compact appearance and natural hardness of this material seems more suitable to protect the edible internal fraction than to suggest alternative use.
Also the organoleptic aspects do not make it particularly palatable, as it is weakly perfumed and therefore very poor in volatile and gustatory aromas. The combination of all these not particularly pleasant characteristics contribute to make it a real waste material. However, even if poor in volatile compounds, for completeness of information, the fresh mesocarp was also analyzed in this work. Most of the studies reported in the literature are related to the aroma of the edible fraction, which expresses the best qualities of the fruit. Contrary to the case of watermelon cultivars in which exocarp (rinds) and mesocarp do not communicate any olfactory information to the consumer, the other large family of *Cucurbitaceae* represented by melons, excluding the Inodorous group, releases aromas and fragrances that are extremely important for the attractiveness of the product [33,34].

Before describing the results obtained with the HS-SPME analysis, it is necessary to remember that the samples extracted with CH_2Cl_2 as a solvent, introduced into the GC-MS system did not produce any signal other than the solvent itself. This fact denotes the total lack of analytes soluble in CH_2Cl_2 for all the samples, although several attempts have been made with ultrasound and microwave techniques, to displace in solution at least some particularly resistant compound.
On the other hand, this evidence is not quite unexpected, being CH_2Cl_2 a moderately dipolar solvent with little affinity for polar and hydroxylated compounds, especially due to its low solvating power and modest ability to form hydrogen bonding networks.

Figure 2 shows the HS-SPME chromatogram obtained from a mesocarp sample freshly grinded, manipulated as illustrated in section 2.3, and immediately processed with the GC-MS instrumentation.

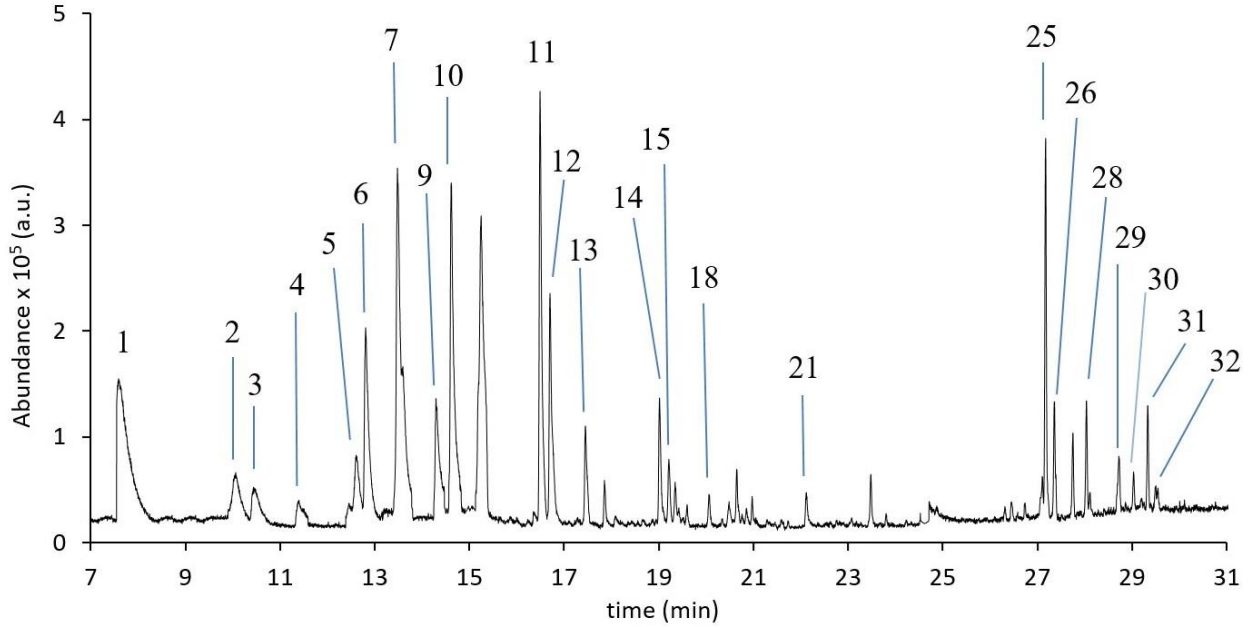


Figure 2 - Total ion chromatogram of the volatile compounds from FRESH mesocarp sample of Crimson sweet watermelon, obtained by HS-SPME-GC-MS. (a.u. – arbitrary units).

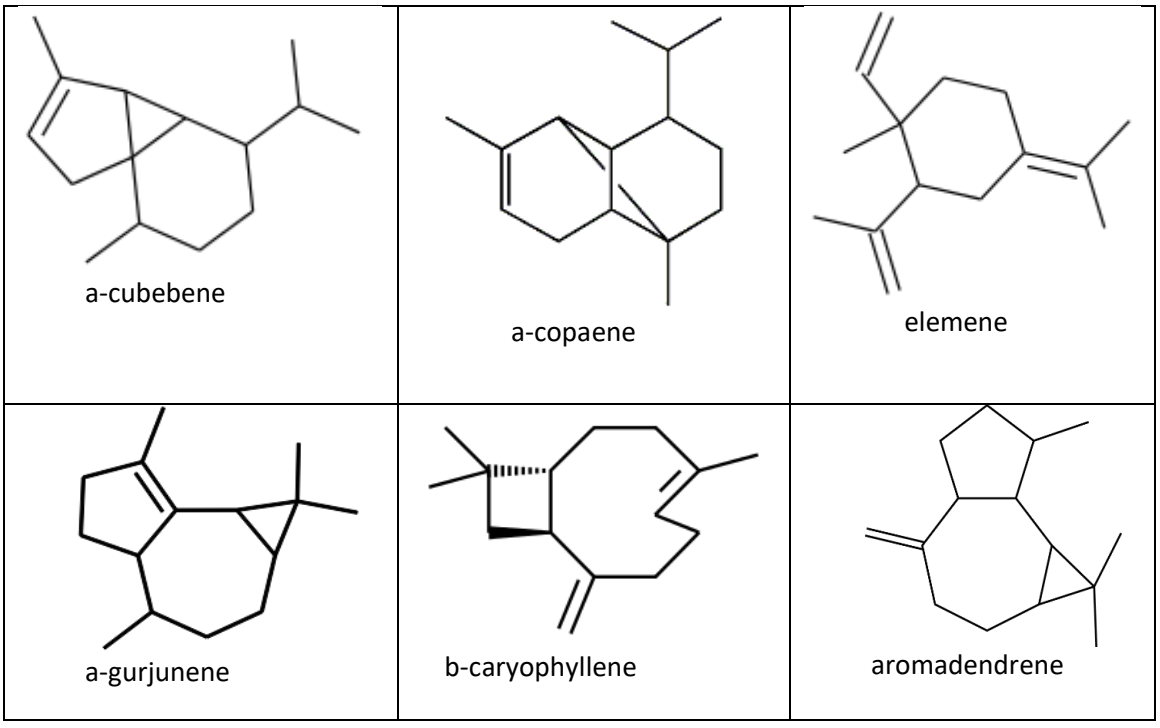
Table 2 - Composition of the volatile fraction of FRESH watermelon rinds, identified by HS-SPME-GC-MS analysis; substances grouped by chemical classes.

Peak n°	LRI	Analyte	Identification[#]	Aroma	Area x 10^{-6}	REF
		Alcanes				
13	988	nonane	A, B, C	waxy	3.19±0.58	
		Alcohols				
3	646	2-methyl-1-propanol	A, B, C	ethereal	3.57±0.34	
7	794	3-methyl-1-butanol	A, B, C	winey, fruity	15.2±1.3	
8	799	2-methyl-1-butanol	A, B	winey, fruity	7.12±0.62	[35]
9	834	1-pentanol	A, B	fruity, buttery	3.72±0.31	[35]
10	850	2,3-butanediol	A, B	green, leafy	13.1±1.1	
11	941	3-hexen-1-ol	A, B	green, flowery	15.5±1.3	[35]
12	952	1-hexanol	A, B	fruity, alcoholic	9.92±0.83	[35]
14	1065	phenol	A, B	pungent	4.19±0.35	
18	1116	2-ethyl-hexanol	A, B, C	citrus, fresh	0.896±0.070	[35]
19	1137	benzyl alcohol	A, B	floral, fruity	0.678±0.060	
21	1216	phenylethyl alcohol	A, B, C	floral, sweet	1.09±0.09	
		Aldehydes				
4	692	3-methyl-butanal	A, B, C	fruity, cocoa	1.21±0.30	[34,35]
20	1160	3,7-Dimethyl-2,6-octadienal	A, B	fresh, lemon	0.432±0.110	
22	1283	decanal	A, B, C	citrus, melon	1.25±0.31	[35]
		Esters				
1	506	methyl acetate	A, B, C	ethereal, winey	18.6±3.0	
		Ketones				
6	761	3-hydroxy-2-butanone (acetoin)	A, B, C	acid, yogurt	11.7±2.9	
15	10.74	6-methyl-5-hepten-2-one	A, B	citrus, fruity	1.97±0.51	[34,35]
		Terpenoids				
16	1081	β-myrcene	A, B	spicy, peppery	1.03±0.18	[35,36]
17	1093	β-pinene	A, B, C	fresh, pine,	0.396±0.070	[36]
23	1428	α-cubebene	A, B	herbal,	0.458±0.080	[36]
24	1460	α-copaene	A, B	spicy, honey	0.792±0.138	[33,34,36]
25	1463	α-elemene	A, B	herbal, fresh	8.02±1.44	[33,36]
26	1472	α-Gurjunene	A, B	woody, balsamic	2.26±0.41	[33]
27	1492	β-cadinene	A, B	green, woody	1.77±0.32	
28	1506	β-Caryophyllene	A, B, C	sweet, spicy	2.56±0.46	[33,34,36]
29	1539	Aromadendrene	A, B	woody	1.63±0.29	[33,36]
30	1554	α-muurolene	A, B	woody	0.871±0.160	
31	1569	δ-cadinene	A, B	herbal, woody	2.19±0.39	[33]
32	1577	calamenene	A, B	herbal, spicy	0.529±0.090	
		Others				
2	627	2-methyl-furan	A, B, C	ethereal	4.49±2.12	
5	752	2-ethyl-furan	A, B, C	sweet, malty	4.35±2.07	[35]

[#]The identification is indicated by: (A) mass spectral data of the libraries supplied with the operating system of the GC-Ms and from mass spectra databases; (B) mass spectra found in the literature; (C) mass spectra and retention time of an injected standard.

Table 2 collects the descriptive elements of the chromatogram of Figure 2. As can be seen, 32 analytes have been identified, which are based on as many peaks, among which the class of alcohols predominates (11 ALC) with a relative amount of 51.8 % of the total area evaluated as the total ionic current of the selected peaks in the chromatogram. This class is followed by the group of terpene compounds (12 TER) with the 15.6 %, acetic acid-methyl ester as the only non-acetate ester (1 NAE; 12.9 %), ketones (2 KET) with a total amount of 9.4 %, alkanes (1 AHA; 2.2 %) and, finally, aldehydes (3 ALD) and other compounds (2 OTH) that are present in a quantity equivalent to 2.0 % and 6.1 %, respectively.
Tables 2, 3 and 4 show the peak area expressed as Total Ion Current (TIC) instead of the relative area%. This choice is justified considering that the HS compositions of these samples are quite diverse owing to the different matrices compared.
The specific literature seems rather poor in information regarding the volatile aromatic fraction (VOCs) of this fresh material. However, in a very recent study, Ramirez J.L. agreed with us about the presence of a relevant number of volatile components as reported in Table 2 [35].
Among the analytes listed in the table, the class of terpene compounds arouses great interest. These molecules, some of which are displayed in Scheme 1, are generally identified in most plant species as they play different roles in nature and, in particular, they seem to be mainly engaged in developing communication signaling and defense capabilities against herbivores, insects, aphids and other parasites [37].

SCHEME 1 – Molecular structures of some volatile terpenoid compounds identified in fresh rinds and/or candied ones from Crimson sweet watermelons.



Apart from the aroma expressed on the outside of the fruit for cultivars having this characteristic, the physiological role and phylogenetic function of the sesquiterpenes present in the peel/rind of *Cucurbitaceae* have not yet been completely understood. Nevertheless, the results obtained by other researchers [33] are very interesting. Working with some cultivars of melons, they have shown that the group of sesquiterpenes is present only in the outer layers of the fruit. In fact, the enzymatic activity of the sesquiterpene-synthases is detectable only in cell extracts derived from the peel and not from the endocarp. In addition, this enzymatic activity in melons is completely lacking in young and unripe fruits, while it develops starting from the ripening stage and in fruits ready for harvest.

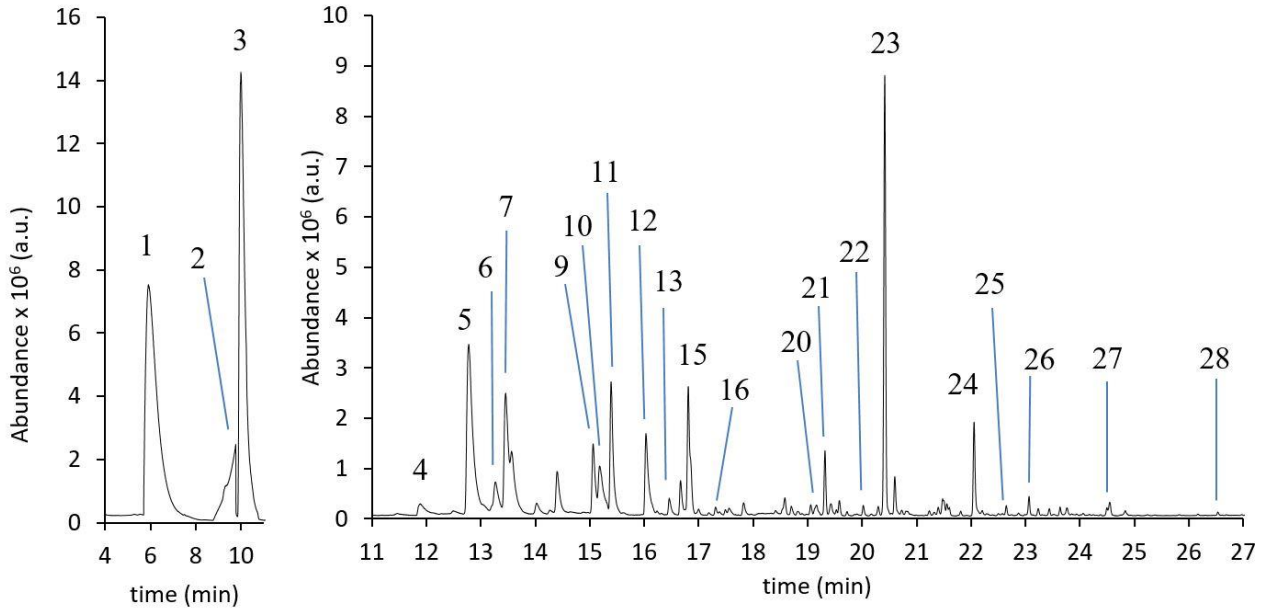


Figure 3 - Total ion chromatogram of the volatile compounds from WET candied rind sample of Crimson sweet watermelon, obtained by HS-SPME-GC-MS.

Figure 3 shows the HS-SPME-GC-MS chromatogram of a sample of candied mesocarp (WET procedure) and Table 3 reports the descriptive elements.

Table 3 - Composition of volatile fraction of WET candied watermelon rinds; substances identified by HS-SPME-GC-MS analysis, grouped by chemical classes.

Peak n°	LRI	Analyte	Identification#	Aroma	Area x 10^{-7}	REF
		Acids				
2	611	acetic acid	A, B	sour, pungent	59.1± 6.4	
		Alcohols				
1	425	ethanol	A, B	alcoholic	261± 23	[35]
6	793	3-methyl-1-butanol	A, B, C	winey, fruity	12.3± 1.1	
7	798	2-methyl-1-butanol	A, B	winey, fruity	8.07± 0.69	[35]
13	940	3-hexen-1-ol	A, B	green, leafy	1.16± 0.11	[35]
14	950	1-hexanol	A, B	green, flowery	2.12± 0.18	[35]
19	1067	1-octen-3-ol	A, B	mushroom	0.515±0.045	
23	1142	5-nonanol	A, B		1.62± 0.14	
24	1213	phenyl-ethyl alcohol	A, B, C	floral, sweet	4.41± 0.38	
		Aldehydes				
10	878	hexanal	A, B	grass, green	3.08± 0.72	[34,35]
12	919	furan-2-carbaldehyde (furfural)	A, B	woody, almond	7.42± 1.74	
17	990	heptanal	A, B	fresh, herbal	0.292± 0.069	[34,35]
18	1050	2-heptenal	A, B	green, fatty	0.586± 0.131	[34,35]
21	1092	octanal	A, B	waxy, citrus,	0.678± 0.157	[34,35]
		Esters				
3	624	ethyl acetate	A, B	fruity, sweet	257± 22	[34,35]

8	840	2-methylpropyl acetate	A, B	sweet, fruity	3.84± 0.33	
9	872	ethyl butanoate	A, B, C	fruity	5.32± 0.92	
11	888	ethyl-2-hydroxy-propanoate	A, B	Fruit, acidic	10.2± 1.8	
15	957	3-methyl-1-butanol acetate	A, B	sweet, fruity	9.46± 0.81	
16	982	ethyl pentanoate	A, B	apple, green	0.425± 0.073	
20	1080	ethyl hexanoate	A, B, C	fruity green	2.93± 0.50	[34]
25	1242	diethyl succinate	A, B	fruity, sweet	0.393± 0.068	
26	1263	ethyl octanoate	A, B	fruity, waxy	0.788± 0.136	[34]
27	1349	ethyl nonanoate	A, B	waxy, winey	0.210± 0.036	
28	1432	ethyl decanoate	A, B	fruity, apple	0.118± 0.020	
Ketones						
4	760	3-hydroxy-2-butanone (acetoin)	A, B, C	acid, yogurt,	27.1± 7.2	
Terpenoids						
22	1133	limonene	A, B, C	citrus, fresh	20.9± 4.2	
Others						
5	784	2,4,5-trimethyl-1,3-dioxolane	A, B, C	green, earthy	4.45± 2.96	

[#]The identification is indicated by: (A) mass spectral data of the libraries supplied with the operating system of the GC-Ms and from mass spectra databases; (B) mass spectra found in the literature; (C) mass spectra and retention time of an injected standard.

In particular, 28 analytes have been identified, which are based on as many peaks, among which the class of alcohols predominates (8 ALC) with a relative amount of 41.3 % of the total area computed as the total ionic current of the selected peaks in the chromatogram. The ALC group is followed by the class of acetate esters (3 ACE) that are present in a quantity equal to 38.3 %, organic acids (1 ACD), the only one of which is acetic acid with 8.3 %, 3-hydroxy-2-butanone as the only ketone (1 KET, 3.8 %), limonene (1 TER; 3.0 %), non-acetate esters (8 NAE; 2.9 %) and, finally, aldehydes (5 ALD) and other compounds (1 OTH) present at 1.7 % and 0.6 %, respectively.

The most significant compound of this chromatogram is represented by ethanol (37.0 %) and ethyl acetate (36.4 %) in quantities greater than any other analyte. The sum of these two components is approximately 73 % of the volatile fraction. Evidently, the extraction of the vegetation water from the rind favors the alcoholic fermentation of the physiological solution which is gradually formed, while the osmotic equilibrium between the saccharide solution penetrating the skin tissues and the liquid phase itself is stabilized. Acetic acid is the third component of the VOCs fraction with 8.4 % which probably derives from the subsequent oxidation stage of ethanol. Conversely, terpene compounds are almost no longer detected in candied fruit. Probably their thermodynamic stability could be compromised by the candying procedure since the presence of atmospheric oxygen under the plexiglass cloche still guarantees oxidizing conditions for the entire duration of the process.

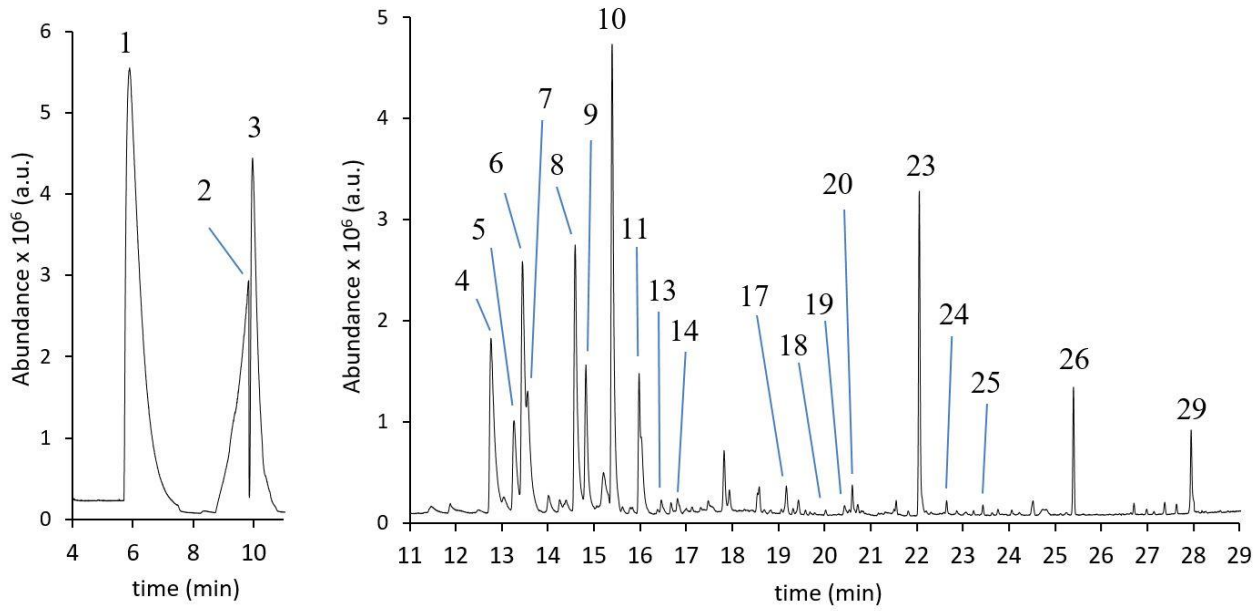


Figure 4 - Total ion chromatogram of the volatile compounds from DRY candied rind sample of Crimson sweet watermelon, obtained by HS-SPME-GC-MS.

Figure 4 shows the HS-SPME-GC-MS chromatogram of a sample of candied mesocarp from Crimson Sweet (DRY procedure) and in Table 4 the descriptive elements of the same profile are collected.

Table 4 - Composition of volatile fraction of DRY candied watermelon rinds; substances are identified by HS-SPME-GC-MS analysis, grouped by chemical classes.

Peak n°	LRI	Analyte	Identification#	Aroma	Area x 10^{-7}	REF
		Alcanes				
27	1441	tetradecane	A, B, C	waxy	0.183±0.179	
		Acids				
2	615	acetic acid	A, B	sour, pungent	75.7± 5.6	
		Alcohols				
1	423	ethanol	A, B	alcoholic	184± 16	[35]
6	793	3-methyl- 1-butanol	A, B, C	winey, fruity	12.8± 1.1	
7	798	2-methyl-1-butanol	A, B	winey, fruity,	7.70± 0.68	[35]
8	849	2,3-butanediol	A, B	fruity, buttery	11.8± 1.0	
9	860	2,3-butanediol		fruity, buttery	6.26± 0.55	
11	917	3-ethoxy-1-propanol	A, B	fruity	3.74± 0.33	
13	940	3-hexen-1-ol	A, B	green, leafy	0.584±0.052	[35]
14	950	1-hexanol	A, B	green, flowery	0.344±0.032	[35]
18	1114	2-ethyl-1-hexanol	A, B, C	citrus, sweet	0.108±0.010	[35]
19	1135	benzyl alcohol	A, B	floral, fruity	0.367±0.033	
20	1143	1-octen-4-ol	A, B	fresh, yeasty	0.485±0.043	
23	1213	phenylethyl alcohol	A, B, C	floral, sweet	7.88±0.70	
29	1502	1-dodecanol	A, B	earthy, waxy	1.90±0.17	
		Aldehydes				
12	918	furan-2-carbaldehyde (furfural)	A, B	woody, almond	3.05±0.68	

17	1093	octanal	A, B	waxy, citrus	0.123±0.027	[34,35]
21	1148	benzeneacetaldehyde	A, B	floral, honey	0.175±0.039	
22	1189	nonanal	A, B	waxy, green	0.308±0.068	[35]
25	1281	decanal	A, B, C	citrus, melon	0.230±0.051	[35]
		Esters				
3	622	ethyl acetate	A, B	fruity, sweet	71.2±7.7	[34,35]
10	888	ethyl 2-hydroxy-propanoate	A, B	sweet, fruity	18.6±2.5	
15	957	3-methyl-1-butanol acetate	A, B	sweet, fruity	0.586±0.063	
24	1242	diethyl succinate	A, B	fruity	0.349±0.048	
		Ketones				
4	759	3-hydroxy-2-butanone (acetoin)	A, B, C	acid, yogurt	12.4±4.3	
16	1072	6-methyl-5-hepten-2-one	A, B	citrus, fruity	0.914±0.324	[34,35]
28	1486	6,10-dimethyl-5,9-undecadien-2-one	A, B	floral, fresh	0.193±0.068	
		Others				
5	784	2,4,5-trimethyl-1,3-dioxolane	A, B	herbal, woody	5.34± 3.65	
26	1377	2-ethylhexyl glycidyl ether	A, B	floral, sweet	2.64± 1.80	

[#]The identification is indicated by: (A) mass spectral data of the libraries supplied with the operating system of the GC-Ms and from mass spectra databases; (B) mass spectra found in the literature; (C) mass spectra and retention time of an injected standard.

In this case, 29 analytes have been individuated, based on as many peaks, among which the class of alcohols predominates (13 ALC) with a relative amount of 55.3 % of the total area evaluated as the total ionic current of the selected peaks in the chromatogram. Acetic acid is the only one belonging to the class of organic acids (ACD; 17.6 %), followed by acetate esters (2 ACE) with a total amount of 16.7 %, non-acetate esters (2 NAE) present at 4.4 %, ketones (3 KET) at 3.1 % and, finally, aldehydes (5 ALD), alkanes (1 AHA) and other compounds (2 OTH) in a quantity equivalent to 0.9 %, <0.1 % and 1.9 %, respectively.
The DRY candying method leads to the formation of ethanol at about 42.8 % (+ 16 % compared to the WET condition, ≈ 37 %), while ethyl acetate reaches 16.6 % (about – 54 % compared to the WET procedure, equal to 36.4 %). These strongly aromatic compounds describe about 59 % of the VOCs fraction, while acetic acid represents ≈ 17.6 % (+ 110 % compared to WET sample, ≈ 8.4 %). From the comparison with the similar results obtained by the WET procedure, it can be concluded that ethanolic fermentation is guaranteed in both experimental candying conditions. However, the constant elimination of the physiological solution during DRY process reduces the reactivity of acetic acid and ethanol, which are converted more slowly to ethyl acetate. Again, the presence of terpenoid compounds is considerably reduced compared to the case of fresh material, and therefore the same considerations previously developed for the WET procedure are still valid for the DRY one.

Table 5 summarizes the results obtained from the HS-SPME-GC-MS analysis for the different samples of Crimson sweet watermelon rinds (fresh, WET and DRY candied), represented in comparative form for the classes of compounds identified.

Table 5# - Compound classes identified in the HS-SPME-GC-MS analysis of the Crimson sweet watermelon rind: FRESH, WET and DRY candied. Data are expressed as mean % of each class with respect to the total normalized peak areas on the basis of the sum of the total peak ion current.*

Compound class	**FRESH**	**WET**	**DRY**
	$\bar{x} \pm s_{(3)}$	$\bar{x} \pm s_{(3)}$	$\bar{x} \pm s_{(3)}$
ALC	$51.8^{ab} \pm 4.3$	$41.3^{a} \pm 3.6$	$55.3^{b} \pm 4.9$
TER	$15.6^{a} \pm 2.8$	$3.0^{b} \pm 0.6$	---
NAE	$12.9^{a} \pm 2.1$	$2.9^{b} \pm 0.5$	$4.4^{b} \pm 0.6$
KET	$9.4^{a} \pm 2.4$	$3.8^{b} \pm 1.0$	$3.1^{b} \pm 1.1$
OTH	$6.1^{a} \pm 2.9$	$0.6^{b} \pm 0.4$	$1.9^{ab} \pm 1.3$
AHA	$2.2^{a} \pm 0.4$	---	$0.1^{b} \pm 0.1$
ALD	$2.0^{a} \pm 0.5$	$1.7^{ab} \pm 0.4$	$0.9^{b} \pm 0.2$
ACE	---	$38.3^{a} \pm 3.3$	$16.7^{b} \pm 1.8$
ACD	---	$8.3^{a} \pm 0.9$	$17.6^{b} \pm 1.3$

*mean of three replicates of the chromatograms (± standard deviation, $s_{(3)}$)

Compound classes: ACD, acids; ACE, acetate esters; AHA, alkanes; ALC, alcohols; ALD, aldehydes; KET, ketones; NAE, non-acetate esters; OTH, others; TER, terpenes

#In a row, results with a different superscript letter indicate a statistically significant difference between each other ($p < 0.05$).

The final product obtained with two alternative methodologies, WET and DRY, contains volatile compounds that derive from very different origins, basically represented by three large groups of substances that we comment as follows.

i) VOCs typical of the fruit and probably of the selected cultivar (acids, terpenoids and esters). For example, free organic acids are totally absent in the fresh mesocarp of the Crimson Sweet watermelon, while they increase to about 8 % in the WET product and to ≈ 17 % in the DRY one. Acetate esters are also absent in the fresh rinds, while the contrary is true for the candied material. In fact, they reach 38 % in the WET product, while their concentration is halved to about 17 % in the case of the DRY one. Furthermore, terpenoid compounds are present in high concentration in the selected initial matrix (≈ 15 %), are reduced to 3 % in the WET product while they tend to zero in the DRY one.

ii) volatiles lost during the long osmotic treatment (furans and terpenoid compounds). Furans are preferably formed when the production processes of candied fruit are thermally activated, as industrially it happens. To carry out this study, we did not use heat sources. Therefore, the absence of furan compounds in both WET and DRY final products could be due to the choice of always working at room temperature throughout the candying process. Regarding terpenoid molecules, the loss of analytes due to completely natural causes is observed. Among these causes, we can hypothesize their volatilization or their metabolic degradation induced by particular chemical reactions.

iii) the third group is represented by flavoring compounds deriving from fermentation processes (ethanol and other alcohols, esters and acetic acid). The fraction of alcohols is definitely the largest group (52 % in fresh, 41 % and 55 % in WET and DRY, respectively). Given this classification, it should be noted that some compounds may have different origins.

Evidently their different origin is due to enzymatic and fermentation processes that work differently for the 2 candied samples. To this we can add that we did not start from sterile material, as the rinds were only carefully washed and dried on a cloth and exposed to the air.

On the other hand, the effect of osmotic pressure alone cannot explain these differences.

Since the ingredients used are only rinds and solid sugar, the activity of free water extracted from the substrate, probably plays a significant role in the compositional evolution of the 2 samples during the candying process.

Therefore, the hypothesis that different fermentation pathways accompanied the candying process of the 2 WET and DRY samples, seems quite plausible.

Table 6 - Some most representative analytes obtained from HS-SPME-GC-MS for the three samples of Crimson Sweet rinds: FRESH, WET and DRY.*

	FRESH	WET	DRY
	$\bar{x} \pm s_{(3)}$	$\bar{x} \pm s_{(3)}$	$\bar{x} \pm s_{(3)}$
3-hydroxy-2-butanone	$1.17^{a} \pm 0.29$	$27.1^{b} \pm 7.2$	$12.4^{c} \pm 4.4$
3-methyl-1-butanol	$1.52^{a} \pm 0.13$	$12.3^{b} \pm 1.1$	$12.8^{b} \pm 1.2$
2-methyl-1-butanol	$0.712^{a} \pm 0.062$	$8.07^{b} \pm 0.70$	$7.70^{b} \pm 0.69$
3-hexen-1-ol	$1.55^{a} \pm 0.13$	$1.16^{b} \pm 0.11$	$0.584^{c} \pm 0.052$
1-hexanol	$0.992^{a} \pm 0.083$	$2.12^{b} \pm 0.18$	$0.345^{c} \pm 0.032$
phenylethyl alcohol	$0.109^{a} \pm 0.009$	$4.41^{b} \pm 0.38$	$7.88^{c} \pm 0.70$

*Data are expressed as TIC Area x10^{7} ± standard deviation, $s_{(3)}$. In a row, results with a different superscript letter indicate a statistically significant difference between each other ($p < 0.05$).

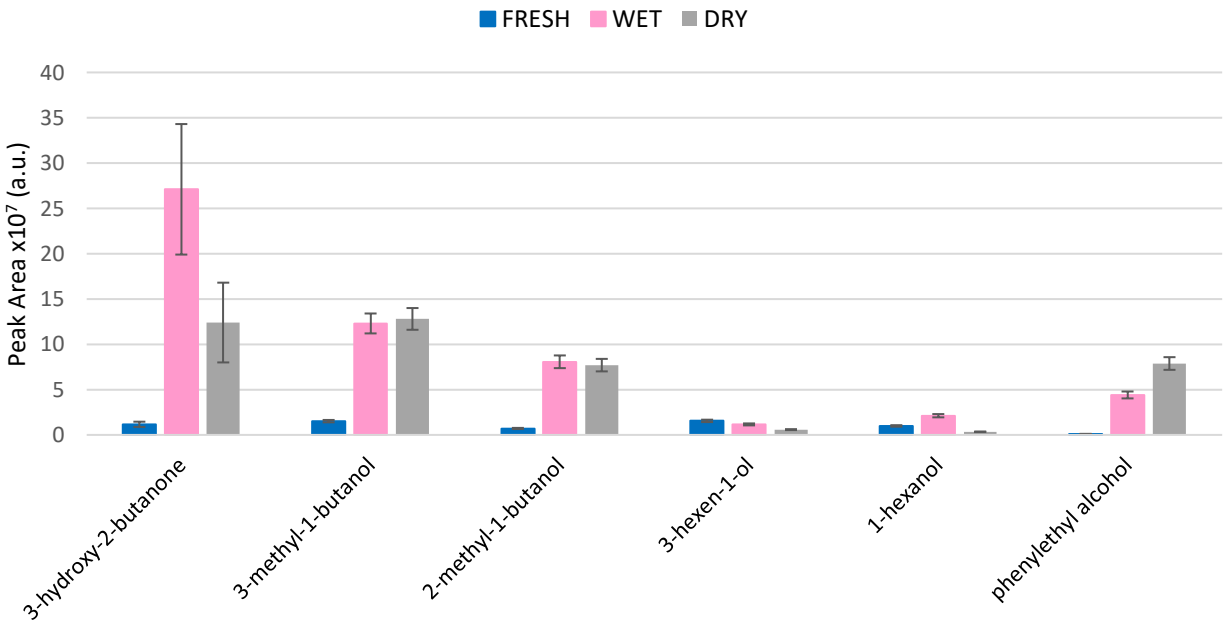


Figure 5 - Some most representative analytes obtained from HS-SPME-GC-MS for the samples of Crimson sweet rinds: fresh and candied with different procedures (WET and DRY).

In Table 6 and Figure 5 we have collected some experimental evidence obtained from this study, which we believe to be particularly significant.

For example, the two methyl-derivative isomers of 1-butanol, both strongly flavoring compounds of pleasant perception [35], are present in low concentration in the initial matrix (expressed as total ion current of peak area), while they seem to develop in equivalent quantities in the candied samples through the two different procedures. However, the 3-methyl-1-butanol isomer seems to be favored over the 2-methyl-1-butanol one, as its concentration increases of about 50 % in candied products.

Instead, the case of 3-hydroxy-2-butanone (acetoin) is different: it is scarcely present in the fresh mesocarp (1.17x10^{+7}), it rises to 12.4x10^{+7} in the DRY candied sample, to 27.1x10^{+7} in the WET one, with an increase of about 120% with respect to the DRY form.

This molecule derives from metabolic fermentation processes of various kinds and provides a mix of pleasant aromas ranging from mildly acidic, recalling the creamy notes of yogurt, to the pleasantness of the fruity odor.

The presence of acetoin seems closely related to that of 2,3-butanediol. Both of these compounds are currently undergoing an in-depth study, as their performance as bio-based platform chemicals is being evaluated [38]. They are considered platform compounds because they are applied in a wide variety of

contexts, from the heavy chemical industry - fine and secondary, cosmetics, food, agriculture, and pharmaceutical productions.
The specimen 3-hexen-1-ol undergoes increasing depletion starting from the fresh peel sample ($1.55x10^{+7}$), passing to the candied WET form ($1.16x10^{+7}$), and being reduced to a minimum in the DRY one ($0.584x10^{+7}$). This compound has a typical freshly cut green grass aroma [35].
An analyte having a singular behavior is certainly 1-hexanol. In the volatile aromatic fraction of the fresh peel it is present with a peak area equivalent to 0.990 $x10^{+7}$, it doubles the concentration in the WET product ($2.12x10^{+7}$), while it halves in the DRY candied one ($0.345x10^{+7}$). This compound contributes to the aroma with a freshly cut grass scent, as well as a pleasant floral fragrance [35].
In this examination of the experimental results, also the phenyl-ethyl alcohol concentrations trend is represented in Figure 5.
As can be seen, its presence in the volatile fraction of fresh skins is minimal and limited to $0.109x10^{+7}$, increases up to $4.41x10^{+7}$ in the WET sample and reaches the maximum value ($7.88x10^{+7}$) for the DRY one, gaining ≈ +180 % with respect to WET. It should be noted that it is a particularly valuable compound in the context in which it is found. In fact, it plays a role as flavoring and natural antimicrobial, antiseptic, disinfectant and preservative agent in the food industry, pharmaceutical, cosmeceutical and perfumery [39]. Furthermore, phenyl-ethyl alcohol is a typical plant metabolite that plays a growth retarding action, it is present in the metabolic cycles of Aspergillus as well as in the fermentative processes induced by Saccharomyces cerevisiae and other biocenosis [40]. Its presence ennobles the candied final product and confers a sweet aroma, recalling that of peach.

3.1 Sensory analysis

For the 3 samples submitted to the panel, the elements of perceived quality were evaluated according to the attributes: appearance (including color, chromatic brilliance, compact or spongy consistency); gustatory sensation (softness on the palate, crunchiness, pleasant taste); persistence after tasting (aftertaste, duration of the perceived sensation); aroma (recognizability of the product, distinguishability from substitutes because of fragrance uniqueness, or evocative perfumes of other origin); spiciness (prevalence or hint of pungent aromas); sweetness (sugarness level to taste); sourness (acidity, flavor); and overall sensory score (pleasantness, and overall acceptability of the product).
One-way analysis of variance (ANOVA) was performed to assess whether there is a statistically significant difference between the sensory scores of the FRESH, WET, and DRY products.
The numerical results of the sensory evaluation of the panel test are shown in Table 7.

Table 7 - Sensory profile attributes for the 3 samples tested.*

sample / attribute	FRESH rinds	WET candied	DRY candied
appearance	1.83 ± 0.95 ^{a}	3.97 ± 0.67 ^{b}	5.83 ± 0.65 ^{c}
mouth feel	2.80 ± 0.92 ^{a}	5.30 ± 0.65 ^{b}	5.87 ± 0.82 ^{c}
after taste	3.33 ± 0.71 ^{a}	5.57 ± 0.58 ^{b}	6.13 ± 0.57 ^{c}
aroma	3.63 ± 0.49 ^{a}	6.10 ± 0.55 ^{b}	6 .13 ± 0.57 ^{b}
pungency	3.93 ± 0.25 ^{a}	4.57 ± 0.57 ^{b}	5.50 ± 0.63 ^{c}
sweetness	3.83 ± 0.38 ^{a}	5.87 ± 0.35 ^{b}	6.50 ± 0.51 ^{c}
sourness	1.43 ± 0.57 ^{a}	3.60 ± 0.56 ^{b}	4.57 ± 0.68 ^{c}
overall sensory score	3.37 ± 0.76 ^{a}	6.07 ± 0.37 ^{b}	6.23 ± 0.63 ^{b}

* The values represent the mean of the scores obtained ± standard deviation $s_{(30)}$.
In a row, results with a different superscript letter indicate a statistically significant difference between each other ($p < 0.05$).

ANOVA results show how the sensory scores of the three different products present, in almost all cases, a statistically significant difference ($p < 0.05$) between them. The only exceptions are represented by aroma and overall sensory score of WET and DRY products, which achieved comparable responses.
The aroma is generally developed from the type of vegetable raw material used in the candying process. Obviously, fruits and vegetables bring their own typical and distinctive contributions, but the same osmotization method, if carried out at room temperature, may favor the appearance of specific analytes due to particular fermentation processes, or to singular chemical reactions between reactive species. A typical case is the spontaneous condensation between alcohols and acids to generate esters, since both the reactive species and the ACE and NAE products in the candied rinds are widely detected.

Figure 6 offers a much more effective impact of the panel test results, as it simultaneously reports the three different profiles of the samples checked, and the judgments collected well detailed through the specific descriptors.

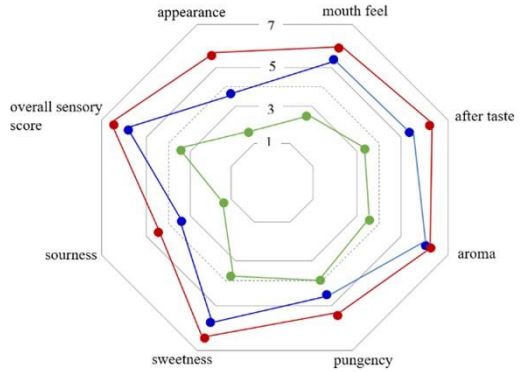


Figure 6 - Spider plot of acceptability specific attributes for each sample of watermelon rinds subjected to the panel test: FRESH ( ), WET ( ) and DRY ( ) candied ones.

As can be seen, the FRESH sample systematically received judgments below the dashed line of level 4, which corresponds to the total indifference of the panelists towards the product. In other words, the level 4 curve of our radar plot can be considered representative of the minimum threshold of edible acceptability of the material. This result is perfectly compatible with what has been indicated so far, i.e. the mesocarp is not considered palatable, even if there are no contraindications for human consumption.
On the contrary, both candied products far exceed the level of acceptability, however showing a fair competitive advantage for the DRY sample over the WET one.
As mentioned above, discussing the results of the HS-SPME-GC-MS analysis, it is noted from the panelists opinions that the aromatic fraction of these candies, although moderately different somewhere in the composition by classes of analytes, do not differ significantly to palate and smell ($p < 0.05$).
The greater distance of the sensory analysis for the WET and DRY samples is found for the contribution of 'appearance'. The fine brownness is very similar for both products, but the chromatic brilliance and compactness of DRY candied rind, Figure 1, make it much more similar to other different candied fruits of industrial origin. Probably, this observation increases the edonistic compliance of the panelists towards the DRY product, so their consensus become more manifest rather than for WET sample.

4 Interdisciplinary context

As suggested in the introductory premises, when we want to address issues related to food sustainability, we cannot ignore the interdisciplinary aspects connected to the application of the principles of Circular Economy. In particular, we want to focus on three relevant topics:
i) the energy content of the products that can be obtained by enhancing the recovery of pre-waste watermelon rinds;

ii) the feasibility of the plants, through the implementation of enabling technologies to achieve the target of candying of the rinds;
iii) the economic equilibrium point in terms of costs and benefits, including economic viability.

i) As regards the energy content of the raw materials and products obtained from this study (Table 8), it should be noted that fresh watermelon rinds give a very modest contribution (~ 0.8 Kcal / 100 g), almost negligible (~ 0.04 %) compared to what is necessary for the daily intake of an average person (~ 2000 - 3000 Kcal). Despite being rich in bioactive substances and nutrients, the energy supply would be insignificant even for a malnourished child. A very different situation arises when it comes to the candied product. The WET form provides 135 Kcal / 100 g, which rise to 200 for the DRY type (about 10% of the daily energy requirement). We remind that the characterization was carried out on the drained and non-dripping candied rinds. Consequently, these values rise further to 160 and 255 Kcal for WET and DRY, respectively, if we refer to 100 g of syrup completely free of polysaccharide fibers, cellulose and other non-energetic substances.
Therefore, the energy balance is at the values included in the ranges 135-160 Kcal for 100 g of standard WET product, and 200-255 Kcal for the DRY candied portion.

Table 8 - Some reference interdisciplinary indices for a food sustainability project aimed at enhancing the recovery of watermelon rinds.

sample / index	FRESH rind	WET candied	DRY candied
energetic content (Kcal/100 g smpl)	~ 0.8	~ 135 (drained)	~ 200 (drained)
		~ 160 (100 g syrup)	~ 255 (100 g syrup)
cost (artisan product)	0 value	15 - 20 $/Kg	20 - 25 $/Kg
cost (industrial product)	0.1 $/Kg	5 - 8 $/Kg	7 - 10 $/Kg

ii) The recovery methodology of watermelon rinds can be realized and represented on a 2-level scale-up:
1) home-made, or artisanal level for small quantities, with zero repercussions to help solve the problem of malnutrition and hunger in underdeveloped countries;
2) at an industrial level, for the transformation of large masses of raw materials into value-added products, with some significant repercussions on the aforementioned world problems. The large-scale candying process has been introduced in France for two centuries now, even if the Arabs and the ancient Romans knew the method of food preservation through the use of sugars and honey. Modern industrial production technologies are so specialized that the transformation times from the raw material to the finished product do not exceed an average of 1 - 2 weeks. They are common technologies (tanks, conveyor belts, solution recirculation pumps, crystallization systems, packaging machines, etc.) that are neither particularly expensive nor highly energy-intensive. However, the greater incisiveness of costs seems to relate to the cold-chain for the correct conservation and safe storage of the raw material pending processing. It should be noted that the waste of these processing companies can be destined for animal feed, which is a form of further economic recovery that reduces economic losses and integrates the company's profit. As industrial platforms already exist, it is sufficient to implement them in the appropriate places to reduce food waste and increase the productivity of food for human consumption.

iii) This is not the place to produce or analyze a business plan, but some elements about the economic aspects have also been estimated in Table 7. The commercial data per unit of candied product are the result of a survey conducted among the major producers in Italy. For the artisanal process, the cost of the raw material is zero, but the remunerative price of the finished product can reach quite important levels, even if not prohibitive. The price of the finished product is made up of a series of contributions which in turn, for modest

quantities of processing, have a heavy impact on the final value. Processing times are very invasive for the raw material when artisanal productions are carried out. They are much longer than those on an industrial scale, therefore the 'quality / price' ratio is definitely advantageous for the artisan product which generally does not contain chemical additives. For industrial candied fruits, in fact, the opposite is true, being generally elaborated by chemical treatments with anti-fermentation compounds, such as SO_2, or other stabilizing agents. To estimate the values relating to industrial costs, a symbolic price is attributed to the raw material, probably representative of the transport costs to the transformation platform. The unit price is reduced to 20-30% of the cost of a similar artisanal product, also considering the share of margins for the remuneration of the entrepreneurial management. It is also highly competitive and highly encouraging to take decisions by policy makers and sector players, which could make the difference between 'YES, it can be done', or 'NO, it is not useful for addressing the problem of planetary malnutrition'.

5 Conclusions

This study allowed to establish the composition of the aroma of the fresh and candied mesocarp, obtained from Crimson Sweet watermelon. The compounds identified belong to 9 major chemical groups, such as acids, esters, furans, aldehydes, alcohols, phenols, ketones, terpenoids and alkanes. In addition, we have mentioned another class (OTH) collecting some minor analytes not pertaining to any of the previously mentioned groups. The initial matrix has a tough texture, generally considered very poor from a nutritional point of view, inedible and intended for disposal as waste. On the contrary, the candying process makes the mesocarp particularly pleasant, soft and with a consistency similar to that of all other candied fruits. The composition of the aroma of the candied mesocarp reflects the complexity of the enzymatic processes and other reactions that occur during the period of treatment of the fruit in the presence of a high concentration of sucrose. Our study can be intended for exploratory purposes considering the nature of the starting pre-waste material.

We will continue to indicate as "pre-waste" the conservation status of these rinds, at least until they will not come into contact with other materials already in the phase of high bacterial and/or fungal contamination, degraded by molds and by the presence of spores activities of any kind. The pre-waste condition means that the material has not yet been eliminated but is in the pre-conclusive phase of its life cycle, when it can still be recovered with minimal effort and destined for subsequent applications, according to the principles of the Circular Economy. When it is decided to recover something, the life cycle of the natural material is obviously extended for a further time window. That raw material will certainly be transformed in such a way to allow its reintegration into the value-chain of sustainable foods. This happens exactly for any other artifact that comes back to life in any other context compatible with the Circular Economy models.

What we propose may have a symbolic value for the domestic recovery of a product generally intended not edible and produced in very small quantities.

However, it can represent a real industrial challenge and opportunity for the recovery of large quantities of watermelon rinds, regardless of the production methodology of the candying process.

Of course, industrial processing times are not compatible with the transformation time scale proposed with the WET and DRY methodologies of this research.

The candying process is certainly expensive but, considering that it ennobles a fresh and zero-value matrix, mainly made of hard cellulose and with very poor nutritional properties, it allows to extend the mass of edible foods for human consumption. This way helps reducing losses, waste and the need for interventions for composting operations before dispersion on agricultural land.

It is important to emphasize that, after the candying process, we obtain a highly energetic and hypercaloric product, probably suitable for fighting hunger in the world, even if with a small and modest massive contribution.

On the other hand, this is one of the primary objectives of the 2030 ONU Agenda, signed in the form of a programmatic agreement by 193 UN Countries and with the FAO auspices [41].

We would like to point out that the WET and DRY candying processes we have carried out, are free from any treatment that may leave food contaminants. In fact, they are respectful of the naturalness of the raw material and of the finished products, since it is not necessary to introduce any other preservative and anti-fermentative ingredient, as it happens, on the contrary, with the industrial sulphitation for the production of candied fruit. Finally, even to our great surprise, it seems fair to remember that the panel of tasters approved the candied products obtained from a very poor matrix, indicating that to attribute added value and improve food sustainability, just a little sugar would be enough, and the right time to wait for the transformation [42].

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CHAPTER 12

12 - CONCLUSIONS

The medium-term research activities underlying the results framework, allowed us to achieve some goals setted at the beginning of the PhD project: to recover some agro-industrial waste materials according to the principles of biorefinery and circular economy for giving them new life.
Drawing inspiration from the latest and most innovative ideas that seek to implement the 'bioeconomy concept' with increasing effectiveness, putting into practice biorefinery transformations, some dietary fibers have been obtained starting from the pomace of fruits belonging to the *Cucurbitaceae* family: i) *Cucumis Melo* L. var. *Inodorus*, commonly known as winter melon, and ii) *Citrullus Lanatus* L. var. Gavina, Asahi Miyako and Crimson Sweet, namely watermelon.
This project provides some tentative answers to some increasingly topical and insistent questions from public opinion regarding overproduction and relevant waste in the agri-food sector.
The idea of obtaining dietary fiber from winter melon pomace depends on the fact that its sensory characteristics are almost neutral, both for the color and for the rather weak aroma.

However, to increase the degree of whiteness and further reduce its sensory characteristics close to neutrality, we have chosen to treat the fiber with hydrogen peroxide, a perfectly biocompatible chemical agent. This procedure led to the expected results: the dietary fiber reached an excellent white point, confirmed by the UV-Vis analysis. In fact, the spectral absorption bands of the analytes which contribute to the initial color of the fiber "as it is" (flavonoids, flavones, flavonols, among other greenish-yellowish molecules, such as xanthophylls and chlorophylls) are completely absent in the bleached material, and the natural pigments originally present in pomace have been successfully degraded.
As far as the preparation of the dry fiber concern, we have applied two alternative techniques: i) “hot method”, in an oven at 40 °C; ii) “cold method”, by vacuum-freeze dryer.
Both drying conditions adopted allowed us to obtain extremely satisfying whiteness degrees, but the vacuum-freeze drying technique has led to slight better results than the oven-drying one. This difference may be due to interaction phenomena between different components. These processes are favored by the drying temperature in the oven at 40 °C, which leads to the formation of brown-colored compounds, such as the products of Maillard reactions (albeit to a modest extent).

HS-SPME-GC-MS analysis shows that also the aromatic profile is strongly modified after treatment with H_2O_2. From the analysis of chromatograms, a decrease in the number of sampled analytes and in their concentration is detected, compared to those identified in the run relating to the natural and untreated fiber. Furthermore, the bleaching procedure involves increasing the quantitative ratios of oxidized molecules, such as acids, to the detriment of alcohols and ketones. Among the alcohols, a class of molecules particularly abundant in the HS of the untreated sample, the most important are the aliphatic C9 ones, i.e. the main characteristic odorants of melon fruits.
These analytes are completely absent in the bleached fiber samples; therefore, H_2O_2 treatment leads to a substantial modification of the aromatic profile typical of the initial matrix.

Furthermore, we studied the thermal stability of both fiber samples, i.e. both that of natural melon "as it is" (or TDF) and that of bleached melon (IDF), to define their behavioral profile towards thermal stress.
This information is very important as it helps to optimize uses and applications of the different fibers. The main difference between the two analyzed samples lies in the presence of the soluble fraction (monosaccharides, oligosaccharides, mineral salts, etc.) in the natural TDF "as it is" material.
Therefore, the TGA/DTG/DTA curves are rather complex and some peaks, quite absent in the analogous plots related to the bleached sample, underlie structural deformations compatible with the presence of the soluble compounds. The latter components, during the heating process, favors the plasticization of the mass and the lowering of the melting temperature. In conclusion, the winter melon fiber treated with H_2O_2 is much more

stable than the corresponding untreated one. Hence, it is particularly recommended as a formulation ingredient, since it can better withstand thermal stress.

The idea of recovering the dietary fiber from watermelon is related to the presence in its pomace of numerous bioactive species with different beneficial properties for human health. Among these compounds, the most important is lycopene, a carotene abundantly present in the fruit pulp, with a strong antioxidant power and capable of imparting the characteristic red color to *Citrullus Lanatus* endocarp. Unfortunately, this compound is very unstable. The presence of this carotene was confirmed by UV-Vis spectroscopy, a technique that also allowed us to monitor its degradation over time. In fact, we have studied the ageing process and the stability of the fiber over time, as well as making a semi-quantitative estimate of lycopene content. Regardless of the initial treatments of the pomace, the drying methods and storage conditions, all the fibers undergo a chromatic variation over time. The final color point tends to be almost the same for samples which differ only in the storage conditions (room or low temperature, dark or daylight exposure, inert or oxidant atmosphere). Among them, the raw samples undergo an alteration with slower lycopene degradation kinetics. On the contrary, the washed ones reach the plateau faster and show a more marked variation in the color point parameters compared to the initial values.

The HS-SPME-GC-MS analysis shows that the presence of lycopene also affects the aroma of fibers, which is characterized by volatile molecules also originating from the degradation of this carotene. These molecules, however, in turn undergo oxidative processes which lead to their progressive decrease during storage. Some characteristic compounds of the watermelon aroma, among these the C9 aldehydes are the main species, follow this trend. Their decrease over time involves the progressive loss of the aromatic bouquet typical of the starting matrix. In addition, the main differences in the aromatic profile of the samples obtained from the three cultivars examined were evaluated.
The HS-SPME-GC-MS analysis of the fiber recovered from the pomace of Gavina cultivar is characterized by a high number of terpene species, including those deriving from lycopene degradation. The Crimson Sweet one shows the simplest chromatographic profile, with fewer peaks and fewer analytes. Dietary fiber from Miyako cultivar displays a great abundance of fatty acid degradation compounds, which mainly impart herbaceous fragrances and green notes to the aromatic bouquet. Finally, the high amount of C9 aldehydes and other terpene species found in the fiber of Gavina variety, allowed us to state that this sample better preserves the aromatic qualities of the initial matrix.

The thermal analysis carried out on the “as it is” sample show a plasticization of the mass during the heating phase. The presence of polysaccharide and other soluble analytes, which tend to caramelize, leads to the formation of a plastic and pitchy matrix. Therefore, the fiber subjected to preliminary washing, devoid of its soluble fraction, is much more stable towards high temperature heating. Consequently, this sample is particularly recommended as a formulation ingredient, since it can withstand the thermal stress without undergoing significant structural deformations and thermally activated decompositions.

The research study also turned to other subfractions from *Cucurbitaceae*, including the melon’s yellow skin and the watermelon’s fresh rind. The yellow peel has been subjected to extraction processes with alcoholic, hydroalcoholic and aqueous solutions, in order to obtain samples containing the bioactive compounds responsible for its intense yellow color. The differences between the various extraction conditions used have been studied through UV-Vis spectroscopy. The spectra related to the methanol-water binary solvent show a slightly higher absorbance than those in pure methanol. The extraction in NaOH 3 M solution is more effective than that in half-saturated Na_2CO_3, given the higher absorbance values recorded at the three absorption bands.

The HPLC-MS-ESI-IT technique allowed us to obtain more compositional information about melon skin extracts. In particular, we have identified: luteolin-6-glycoside and naringenin (two flavonoids), gibberellin A7 (a hormones of plant origin and diterpenic nature), hydroxybutanoic acid ethyl ester-hexoside as well as caffeic acid-O-hexoside, species reported in the literature in similar matrices.

The technological properties, or functional properties (WHC, WSC, OHC, among many others), express important characteristics for the specific applications to which the by-products obtained from the fractionation of the pre-waste material could be destined. These properties describe the mechanical behavior of samples when subjected to the action of solvents, fats, or other compounds with which the fibers come into contact when inserted into formulation products. All the above mentioned technological properties of TDFs, IDFs and SDFs make it possible to modulate the gelling, texturizing, emulsifying properties, i.e. the typical macroscopic properties of a generic multicomponent foodstuff product. The best results have been obtained with melon fibers, especially if they have undergone some washing or whitening treatment with H_2O_2.

Finally, we tentatively carried out a laboratory-scale experiment, for an eventual and potential technology transfer. We carried out the candying of fresh watermelon rinds, working with 2 different operational strategies, wet- and dry-method, respectively.
For this experiment to enhance the pre-waste material, we used only the mesocarp of the fruit, a typical material to be eliminated as it is considered inedible. The initial matrix has a tough texture, generally considered very poor from a nutritional point of view. On the contrary, the candying process makes the mesocarp particularly pleasant, soft and with a consistency similar to that of other candied fruits. We have studied the aromatic profile of the fresh rinds and the candied ones using HS-SPME-GC-MS technique. The composition of the candied mesocarp aroma reflects the complexity of the enzymatic processes and other reactions which occur during the treatment period, in presence of a high concentration of sucrose.
Terpenes and some terpenoid compounds are present in quite high concentration in the fresh rinds, although this fraction is not particularly aromatic from the start. Unfortunately, these analytes are not persistent, and are strongly reduced in the candied samples.

This research project paves the way for a huge number of other possible studies, extended both to the already extensively analyzed fibers from the pomace of *Cucurbitaceae* fruits and to other subfractions not examined during the PhD period, such as seeds, juice or watermelon peel.
For example, the polyphenolic content and the antioxidant power of the bioactive compounds present in dietary fibers can be studied. As far as the watermelon fibers concern, it is possible to make quantitative measurements of the lycopene content, using HPLC techniques. It would also be interesting to examine the heat treatments and fermentation process of the juice subfraction, i.e. its acetification process. It is also possible to study the compositional characteristics and the technological-applicative qualities of the flour obtained by grinding the seeds.
And many other studies can still be done, other experimental activities, more technological tests, and so on.

We would have liked to do all this too, but ... *tempus fugit* ... (Virgilio).

LIST OF SCIENTIFIC PUBLICATIONS

Scientific publications in international journals with impact factor

1) C. Baraldi, G. Foca, L. Maletti, A. Marchetti, F. Roncaglia, S. Sighinolfi, L. Tassi: Red horse-chestnut of Aesculus X Carnea: a new way for health and food design? in "Nuts and Seeds in Health and Disease Prevention – II Edn", V. R. Preedy and R.R. Watson (Ed.s), chpt 3, p. 27-43, Academic Press, London (2020). ISBN: 978-01-2818-553-7

2) F. Roncaglia, L. Forti, S. D'Anna, L. Maletti: An expedient catalytic process to obtain Solketal from biobased glycerol, *Processes*, 2021, 9, 141.

3) C. Durante, M. Cocchi, L. Lancellotti, L. Maletti, A. Marchetti, F. Roncaglia, S. Sighinolfi and L. Tassi: Analytical concentrations of some elements in seeds and crude extracts from Aesculus hippocastanum, by ICP-OES technique, *Agronomy*, 2021,11,47, doi.org/10.3390/agronomy11010047.

4) R. Arletti, G. Confalonieri, G. Vezzalini, L. Maletti, F. Di Renzo, V. Gozzoli: Ion exchange capacity of LTL zeolite: a promising way for cerium recovery, *Microporous and Mesoporous Materials*, 2021, Submitted.

5) A. Marchetti, L. Lancellotti, S. Sighinolfi, C. Durante, L. Tassi, L. Maletti, A. Ulrici: Multi-isotope and multi-element pattern to discriminate wines of different geographical origin, *Current Research in Food Science*, 2021, 4, 807-824, doi.org/10.1016/j.crfs.2021.11.001

6) L. Maletti, V. D'eusanio, L. Lancellotti, A. Marchetti, L. Pincelli, L. Strani and L. Tassi: Effective valorization of pre-waste residues from primary agricultural production of watermelons: a new challenge for food sustainability, *Future Foods*, 2021, In press.

7) L. Maletti, V. D'Eusanio, C. Durante, A. Marchetti, L. Pincelli and L. Tassi: Comparative analysis of VOCs from winter melon pomace fibers before and after bleaching treatment with H_2O_2, *Molecules*, 2022, In press.

8) L. Maletti, C. Durante, V. D'Eusanio, A. Marchetti, L. Tassi, «Thermal characterization of dietary fibers from Cucurbitaceae», *Thermochimica Acta*, 2022, Submitted.

9) L. Maletti, M. Cocchi, C. Durante, L. Strani, L. Tassi. «Evaluation of the chromatic stability of dietary fibers from watermelon», *Bioactive carbohydrates and dietary fibre*, 2022, Submitted.

Scientific publications in journals without impact factor

1) V. D'Eusanio, L. Lancellotti, L. Maletti, S. Sighinolfi, L. Tassi: Valorization of Cucurbitaceae residues from primary agronomic productions, Summer School: Making business with new technologies within green chemistry & sustainable energy, Sarteano (SI), 22-26 luglio 2019.

2) L. Maletti, V. D'Eusanio, L. Lancellotti, L. Lancellotti, L. Tassi: Valorization of agri-food residues from industrial processes, Report of Conference Proceedings, XIX Day of Chemistry of Emilia Romagna, University of Modena and Reggio Emilia, Modena, 06 December 2019.

3) L. Lancellotti, L. Maletti, F. Roncaglia, L. Tassi, M. Borsari: electrochemical organic synthesis of hydrovanilloin, a new non-toxic bioavailable substitute of bis phenol A, Report Conference Proceedings, XIX Day of Chemistry of Emilia Romagna, University of Modena and Reggio Emilia, Modena, 06 December.

4) C. Durante, L. Lancellotti, L. Maletti and L. Tassi: Stability and aging of dietary fiber from waste watermelon, Academia.edu 2021, doi.org/10.20935/AL2386.

5) C. Durante, L. Lancellotti, L. Maletti, L. Strani: Stability and aging of dietary fiber from waste watermelon, Report of Conference Proceedings, XXVII National Congress of the Italian Chemical Society, 14-23 September 2021.

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