University of Modena and Reggio Emilia

Ph.D. SCHOOL OF AGRI-FOOD SCIENCES, TECHNOLOGIES AND BIOTECHNOLOGIES

CYCLE XXXII

Knowledge transfer between research and ice-cream machines industry: development and validation of new tools

Ph.D Candidate: **Dr. Antonio Biscotti**

Tutor **Prof. Maddalena Rossi**

Co-Tutor **Ing. Roberto Lazzarini**

Dean of Ph.D School **Prof. Alessandro Ulrici**

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Abstract (English)

For the food industry, it is of primary importance to ensure quality control and approved products. Measurement of food quality parameters, including microbiological ones associated to a safety risk, is necessary to characterize the product and to avoid adulteration or contamination. A microbial population associated to the environment of production and to the ingredients of the product itself is normally present in food matrices. Dairy products, due to their composition characteristics, are well suited as an ideal growth substrate capable of promoting bacterial growth. Ice-cream is not a sterile product, albeit ice-cream machines generally perform a heat treatment cycle that reduces the load of microorganisms, in order to lay below the limits of pathogenic bacteria imposed by law.

The first part of the research project was aimed to develop a biosensor system for bacterial concentration measurement in a milk-based mix for ice-cream, that exploits the impedance technique. The measurement of the impedance has been done applying to the samples a sinusoidal voltage signal. Ice-cream mix samples inoculated with increasing concentration of *E. coli* ATC11229, ranging from 10^1 to 10^{10} CFU/ml, have been monitored over the time to obtain a calibration curve. As a comparison value, the ice-cream mix milk-based without inoculum was used as control in the tests. The samples were analyzed in parallel by both measurement with the biosensor and by plate count enumeration, in order to compare the results. The Detect Time (DT) was calculated for each sample at the different concentration of inoculum. The ice-cream mixes not inoculated didn't show any change in electrical parameters measured over the time. Where the inoculum was added to the ice-cream mix, change in the electrical parameters was recorded with an increase of the DT decreasing the bacterial load.

The second part of the research work was aimed to develop equipment and processing methods to control the microbiological safety of ice-cream machines in a centralized laboratory. In recent years there has been a growth in the phenomenon of the relocation of ice-cream mixes production centers that prepare the product to be sent directly to the sales points. Ice-cream mixes can be produced and packaged in a production center, then stored and transported to sales points. Once the sales points are reached, the ice-cream mixes are transformed into ice-cream. In this perspective, a shelf-life study of ice-cream mixes and a packaging process has been performed, in order to develop an optimal protocol to offer to the customers for a standardized quality in a multi-location gelato retail context. Preliminary experiments evaluated the microbiological stability of the products processed and stored at 4 °C. Bacteriological analysis and pH measurements confirmed the stability of

the product. In order to limit the possibility to contaminate the product, a professional bag filling machine was developed. Using the bag filling system, the microbiological stability for up to one month of the ice-cream mix was confirmed. Sensory analysis was performed to evaluate the quality of the product during one month of shelf-life at 4 °C. Every 7 days, a bag containing ice-cream mix was opened and the ice-cream was tasted. The analysis demonstrated good process reliability in the sensorial quality of the ice-cream produced with the mixes during all the shelf-life period tested.

Abstract (Italiano)

Per l'industria alimentare è di primaria importanza garantire il controllo della qualità ed avere prodotti approvati. La misura dei parametri di qualità degli alimenti, compresi quelli microbiologici associati ad un rischio per la sicurezza, è necessaria per caratterizzare il prodotto ed evitare adulterazione o contaminazione. Una popolazione microbica associata all'ambiente di produzione e agli ingredienti del prodotto stesso è normalmente presente nelle matrici alimentari. I prodotti lattiero-caseari, grazie alle loro caratteristiche di composizione, sono adatti come substrato di crescita ideale in grado di favorire la crescita batterica. Il gelato non è un prodotto sterile, sebbene le macchine per gelato eseguano in genere un ciclo di trattamento termico che riduce la carica di microrganismi, al fine di porla al di sotto dei limiti per batteri patogeni imposti dalla legge.

La prima parte del progetto di ricerca mirava a sviluppare un sistema biosensore per la misura della concentrazione batterica in miscela gelato a base di latte, sfruttando la tecnica dell'impedenza. La misura dell'impedenza è stata effettuata applicando ai campioni un segnale di tensione sinusoidale. I campioni di miscele gelato, inoculati con concentrazione crescente di *E. coli* ATC11229 compresi tra 10^1 e 10^{10} CFU/ml, sono stati monitorati nel tempo per ottenere una curva di calibrazione. Come valore di confronto, miscela per gelato a base di latte senza inoculo è stata utilizzata come controllo nei test. I campioni sono stati analizzati in parallelo sia mediante il sistema biosensore che enumerazione di conte in piastra, al fine di confrontare i risultati. Il tempo di rilevazione (DT) è stato calcolato per ciascun campione alle diverse concentrazioni di inoculo. Le miscele per gelato non inoculate non hanno mostrato alcun cambiamento nei parametri elettrici misurati nel tempo. Laddove l'inoculo è stato aggiunto alla miscela per gelato, è stato registrato un cambiamento nei parametri elettrici con un aumento del DT al diminuire della carica batterica.

La seconda parte del lavoro di ricerca era finalizzata allo sviluppo di attrezzature e

metodi di elaborazione per controllare la sicurezza microbiologica delle macchine per gelato in un laboratorio centralizzato. Negli ultimi anni, si è verificato un aumento del fenomeno di rilocazione di centri di produzione di miscele per gelato che preparano il prodotto da inviare direttamente ai punti vendita. Le miscele per gelato possono essere prodotte e confezionate in un centro di produzione, quindi conservate e trasportate nei punti vendita. Una volta raggiunti i punti vendita, le miscele per gelato vengono trasformate in gelato. In questa prospettiva, è stato condotto uno studio sulla shelf-life delle miscele per gelato e sul processo di confezionamento, al fine di sviluppare un protocollo ottimale da offrire ai clienti per una qualità standardizzata in un contesto di multi-location per la vendita al dettaglio di gelato. Esperimenti preliminari hanno valutato la stabilità microbiologica dei prodotti trattati e conservati a 4 °C. L'analisi batteriologica e le misurazioni del pH hanno confermato la stabilità del prodotto. Al fine di limitare la possibilità di contaminare il prodotto, è stata sviluppata una macchina professionale per il riempimento dei bag. Utilizzando il sistema di riempimento del bag, è stata confermata la stabilità microbiologica delle miscele per gelato per un massimo di un mese. È stata eseguita un'analisi sensoriale per valutare la qualità del prodotto durante il mese di conservazione a 4 °C. Ogni 7 giorni un bag contenente la miscela per gelato è stato analizzato e il gelato ottenuto con questo prodotto è stato assaggiato. L'analisi ha dimostrato una buona affidabilità del processo nella qualità sensoriale del gelato prodotto con le miscele testate, durante tutta la durata del periodo di shelf-life testato.

1. Introduction

1.1 Ice-cream

Ice-cream presence is noted since ancient times. In fact, since the 12th century BC, in Mesopotamia snow and ice were used to cool the drinks consumed during royal banquets and religious ceremonies. The Arabs, instead, created “sherb” (sugar syrup) and in Palermo they cultivated numerous different types of flowers to make sorbets. In Roman times, the ice-creams were made of snow softened with honey and were flavored with fruit juice or with must. However, the real ice-cream was officially born in Florence in the 16th century, thanks to Buontalenti and then spread to France and later all over the world. From a chemical-physical point of view, ice-cream is a pasty product (foam), where three phases coexist: liquid, solid and aeriform.
The ice-cream, in fact, is composed of four fundamental elements:

- water, useful for forming ice crystals;
- sugar, indispensable for creating small ice crystals and giving the characteristic sweet taste;
- air, essential because ice-cream is not an ice lolly;
- cold, allows to transform the liquid mix into ice-cream.

The formulation of the ice-cream, however, is a very complex mix of different substances with a high nutritional content (milk, cream, sugar, eggs, fruit) that allow to make an infinite amount of tastes. Within the category of ice-cream there are numerous variations depending on ingredients, sweeteners, stabilizers and emulsifiers, flavors, methods of freezing, sizes, shapes, techniques for dispensing. Each of these elements characterizes the type of final product obtained.

Ice-cream is a term that refers to frozen product. Among the frozen product can be a distinction that characterizes the different types of frozen products based on the characteristics of the product and on how to store and sell them. One type of ice-cream is gelato. Gelato can be described by two categories, artisanal and industrial.

Artisanal gelato differs from industrial gelato because of its exclusive features:

- ingredients: always fresh;
- production: in small quantities;
- gelato: continuously fresh;
- tastes: in great variety and originality;
- sale: direct, from the laboratory to the shop.

Moreover, ice-cream can be classified by how it is produced and stored:

- traditional gelato: produced by batch freezer and stored in small containers;
- soft or espresso ice-cream: produced at the time of consumption and stored in the machine.

Traditional gelato is extruded at a temperature between -7.5 °C and -10.5 °C with an increase in volume from 25 to 40%. Soft ice-cream is generally extruded at a temperature between -6 °C and -7.5 °C with an increase in volume of between 40 and 80%.
During the thesis work the term ice-cream is used to talk about of frozen products, unless the cases in which the type of product is explicitly indicated.

1.2 Carpigiani factory timeline

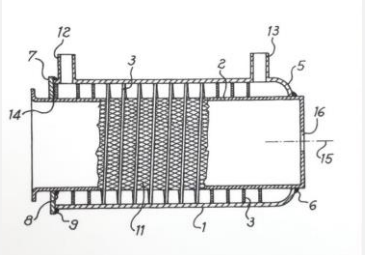
In 1944 the Carpigiani brothers, Brutus and Poerio Carlo, invented the first automatic machine for the production of ice-cream: "The Auto-ice-cream maker". The success was such that, in 1945, the Carpigiani company was founded. At present Carpigiani, with about 50% market share for artisanal gelato and just over 20% for soft, can be considered leader of the world market in its sector and one of the major standard-bearers of "Made in Italy" in the world.

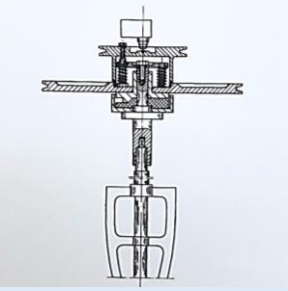
In 1969, Carpigiani moved its headquarters to Anzola dell' Emilia and in those years with the spread of the Italian ice-cream culture in the world, it began to expand internationally, both through the creation of the first foreign branches and with the development of a sales network of exclusive dealers that has always ensured widespread distribution and continuous and timely assistance to end users. Since 1989, Carpigiani has been part of the Ali group in Milan, one of the top three companies in the world in the production and distribution of equipment for professional catering. The new management has brought an additional incentive to technological and organizational growth, which

resulted in the creation of the Carpigiani Group, headed by Carpigiani and including numerous other companies in the sector. Over the years new independent companies and brands have been acquired and created, such as GBG, Cattabriga, Coldelite, Promag, Ott Freezer, Sencotel and Frizital. Carpigiani has also created an internal department (Key Accounts) to manage large customer chains such as McDonald's, Burger King, Quick, Hennig-Olsen, KFC and others. Then in 1995, the "Customer Quality Service" was born: the Carpigiani brand post-sales assistance, which is at the complete disposal of the customer both with timely interventions and practical telematic assistance from the headquarters.

With the beginning of 2000 and the introduction of a renewed quality control system, Carpigiani was the first company in the sector to obtain the new ISO 9001 quality certifications. In 2003, the first University of ice-cream was founded: "Carpigiani Gelato University", an international training center at the service of those who already work or want to operate in the field of ice-cream and artisanal pastry. "Technology for a sweeter world" is the motto of the company, which is strongly committed to the design and development of innovative and, above all, high quality machines. The constant commitment to research and technological excellence is accompanied, in a global vision strategic, from a coordinated and complementary development of the technical-commercial organization that ensures, both in Italy and abroad, a widespread distribution and a continuous and timely assistance to customers.

The impressive sales network, direct network and affiliated companies, subsidiaries and licensees, allows Carpigiani to be present in over 100 countries around the world, even in the most remote places, confirming itself day after day as a leading brand for laboratory machines typical in the ice-cream and artisanal pastry sector, but not only. Over time, his leadership position was constantly strengthened with regard to the production of "SOFT" ice-cream machines, frozen yogurt, shakes, granitas and whipped cream. In keeping with the challenges of the global market, today the Carpigiani Group allows one hundred million people to consume an excellent "Italian ice-cream" every day.

Business Development Milestones	Years	Technical Development Milestones
 **FOUNDED** By the Carpigiani brothers Bruto and Poerio	**1946**	 **AUTOGELATIERA** The first gelato machine
 **THE FIRST SOFT MACHINE WITH THE UNIQUE GEAR PUMP**	**1955**	
 **DIRECT EXPANSION**	**1961**	**THE FIRST INTERNATIONAL BRANCH** 



1962

HARD-O-MATIC

1968

Labo choc
The first gelato machine with a horizontal cylinder

1980

SELF PASTERUZING SYSTEM
A guaranteed and automatic sanitation process for the soft serve range

1982

Cylinder exchange pump

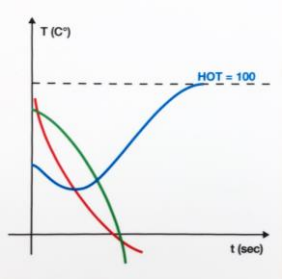
1989

Carpigiani joined the Ali Group

1992

HARD-O-TRONIC
Unique electronic gelato, ice-cream and soft serve quality control

2003



HARD-O-DYNAMIC
Unique electronic gelato, ice-cream and soft serve quality control

The first worldwide training program to create and support gelato and frozen dessert entrepreneurs

2006

SEMI AUTOMATIC SOFT SERVE MACHINE

2007

GELATO, ICE-CREAM AND SOFT SERVE INTERNET OF THINGS

2012

GELATO MUSEUM
Dedicated to 14.000 years of gelato history and gelato artisanal

2013

EVO TECHNOLOGY
The most efficient soft serve quality control

GELATO WORLD TOUR

2016

IDEATRE SYSTEM
The brakethrough solution for gelato, ice-cream and pastry

THE SPECIALIZED UNIVERSITY
The first training program for pastry chefs to fuse gelato and frozen desserts with high level pastry

2018

1.3 Ice-cream ingredients

An important requirement to obtain a quality ice-cream product is to select first quality ingredients. These are dosed and balanced to obtain a final mix which is then freezed. The chemical, organoleptic and microbiological properties of ingredients contribute in equal measure to determining the final quality of the product. Also, the storage of the ingredients and their use are other parameters to take into consideration to obtain a quality product. The quality of an ice-cream must not be understood only from a hedonic point of view but must also refer to an evaluation of the texture of the frozen product. To better understand how to obtain a quality ice-cream product it is important to know the individual ingredients that make it up. Water, sugar and air are the essential ingredients for making ice-cream. The water, under the action of cold, turns into ice crystals. Sugar softens the water, preventing it from freezing, lowering the freezing point. The air, freezing, does not harden and mixing with the mix (water and sugar), makes it increase in volume, making it at the same time soft and mellow. The more air is incorporated into the ice-cream, the more it increases in volume and becomes soft. Fats and aromas, although not indispensable ingredients of ice-cream, are fundamental to improve quality and give a specific flavor. The concept of interchangeability of ingredients in ice-cream recipes is of fundamental importance. In an ice-cream mix, the components of each ingredient are important and not the ingredients themselves. The same recipe can be made with different ingredients, as long as the basic components are the same. In a recipe, milk must be considered as a natural set of water, solid residues and fat: in case of need, or convenience, it can be replaced with water, whole milk powder or even water and condensed milk, obtaining an identical ice-cream, at least in the structure, on condition that the proportions of liquid, solid and fat are respected. So, the evaluation of a recipe for ice-cream mix does not only take place on the basis of the ingredients but above all on the type of components.

The components that affect the final features of the ice-cream are:

- water;
- sugar;
- fat;
- milk solid not fat (MSNF) (proteins + lactose + mineral salts);
- stabilizers and emulsifiers;
- others ingredients;
- air.

1.3.1 Water

Water is present in most of the ingredients used in the production of ice-cream. It constitutes almost 90% of milk and 60% of cream. Moreover, it is the main ingredient for the production of water-based fruit ice-creams, often called sorbets. The water must be pure and drinkable. Some ice-cream makers also prefer to use natural mineral water.

1.3.2 Sugars

In addition to lactose, naturally present in milk, other types of sugars are added to the basic ice-cream mixes. They are essential for the taste, the freezing-point depression and the consistency. Sugars act on the viscosity of ice-cream mixes increasing it. An absence of sugar could determine a flat taste, also due to the greater sensation of cold on the palate. An excessive presence of sugar in the ice-cream mix, however, also has negative effects. From a taste point of view the ice-cream with high sugars level can be sandy, while from a structural point of view it can result with a reduced whippability. Different types of sweeteners are used for ice-cream preparation. The sugar most commonly used is sucrose. Others are corn sweeteners, honey, sugar alcohols and nonnutritive sweeteners.

- **Sucrose**

Sucrose ($C_{12}H_{22}O_{11}$) is a disaccharide composed of glucose and fructose (Fig. 1). These two monosaccharides are linked via an ether bond, a glycosidic linkage, between C1 on the glucosyl subunit and C2 on the fructosyl unit.

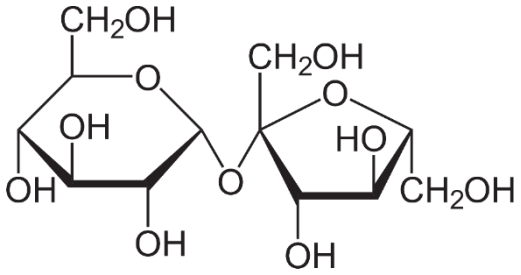


Fig. 1: Sucrose chemical structure.

Sucrose appears as white odorless crystalline or powdery solid. Sucrose comes from plants, being extracted from cane or sugar beets. Sucrose is generally provided in crystalline form but it can also be sold as a syrup thanks to its high solubility. The quantity of sugar

dissolved in a liquid is determined with a brix degrees measure. One degree Brix (°Bx) is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. However, ice-cream mixes contain other dissolved solids. For this reason, the °Bx only approximates the dissolved solid content.

● Corn sweeteners

In ice-cream mix recipes, in addition to sucrose, corn starch hydrolysate syrup is also used. Starting from corn starch or other starch sources such as potato, rice, tapioca is possible obtain substitute sugars. Starch is the major source of carbohydrate in the human diet (Ratnayake & Jackson, 2008). Chemically starches are polysaccharides composed of alpha-D-glucopyranosyl unit linked together with alpha-D-(1-4) and/or alpha-D-(1-6) linkages. Starch is composed by two fractions. One is the amylose, which is a linear polymer with glucose residues linked with alpha-D-(1-4) linkages. The other fraction is amylopectin which is the larger branched molecule with alpha-D-(1-4) and alpha-D-(1-6) linkages (British Nutrition Foundation, 1990; Sharma et al., 2008). Amylose and amylopectin can be hydrolyzed into simpler carbohydrates by acids, various enzymes or a combination of the two acting on the glycosidic bond. The enzymes alpha-amylase are used to reduce total molecular weight, while glucoamylase to produce dextrose or beta-amylase to produce maltose.

The products of digestion result in a mix of oligosaccharides of medium molecular weight and molecules of low molecular weight (dextrose, maltose). Dextrose equivalent (DE) is a parameter used to measure the degree of hydrolysis of carbohydrates, in particular of glucose polymers derived from starch, based on the length of the polymer chains from which they are formed. Increasing the DE, increase also the sweetness and the freezing point decrease and the contribution to viscosity and chewiness in the mouth decrease. Starch hydrolysate products having 20 to 70% of the glycosidic linkages broken are known as corn syrup. They are classified based on degree of conversion (Goff et al., 1990a and 1990b).

Generally, DE conversion varies from 28-38 DE for a low conversion, to 59-68 DE for high conversion, passing through a regular (39-48 DE) and intermediate (49-58 DE) conversion. Maltodextrins are an example of water-soluble complex carbohydrate obtained by hydrolysis. Their DE can vary from 4 to 20, resulting in little sweets for this. Their use finds main application in the textural aspects like cohesivity and meltdown of the ice-cream.

● Sugar alcohols

Polyols or poly-alcohols are hydrogenated carbohydrates used as sweeteners to replace simple sugars. This group includes both monosaccharides (mannitol, sorbitol and xylitol) and disaccharides (maltitol, lactitol) but characterized by a hydroxyl function instead of the ketonic or aldeic one. They have a sweetening power equal to or slightly higher than sucrose (Table 1, Wilson, 2007):

Sweeteners	Sweetening power
Sucrose	1
Lactitol	0.3-0.4
Maltitol	0.8-0.9
Mannitol	0.5-0.6
Sorbitol	0.8-1
Xylitol	0.5-0.6

Table 1: Sweeteners and sweetening power.

Polyols have different sweetness, solubility, freezing point depression, stability, caloric content and cost. However, they are combined with the intense sweeteners to satisfy the needs of taste and optimal structure of the frozen product. Polyols are generally not cariogenic and are therefore used in products, such as sweets or chewing gum "without sugar". The metabolism of polyols is independent of insulin: therefore, they are indicated in diabetic patients (Whelan et al., 2008). Monosaccharide polyols are slowly absorbed (compared to glucose) and the di- and polysaccharides are partially or not completely digested and absorbed in the small intestine and ferment in the large intestine. Due to the fact that they are not completely metabolized, they provide less calories per gram than completely digestible carbohydrates.

Their energy content (Table 2) is lower than that of food sugars: about 2.4 kcal/g (compared to 4 kcal/g of sugar).

Polyols	kcal/g
Erythritol	0-0.2
Mannitol	1.6
Sorbitol	2.6
Xylitol	2.4
Isomalt	2.0
Lactitol	2.0
Maltitol	2.1

Table 2: FDA (2018) reference values for polyols.

Due to the incomplete absorption and fermentation in the large intestine, consumption of polyols, especially in large quantities at once or when consumed by sensitive individuals, can lead to gastrointestinal or laxative effects (Grabitske and Slavin, 2009). The Academy of Nutrition and Dietetics (formerly the American Dietetic Association, ADA) does not recommend an excessive polyol load, if it is higher than 50 grams/day of sorbitol or more than 20 grams/day of mannitol (Fig. 2), it can cause diarrhea (Grembecka, 2015). Effects of the bulk sweeteners used as partial substitutes of sucrose on the rheological properties of ice-cream mixes at 4 °C have been analyzed. Thermal melting profile, ice-cream hardness and meltdown rate were all affected by variation in sugar alcohol. All the sensory characteristics of the product are also affected. For this reason, it is important to evaluate the effects of the different mix formulations that are developed (Soukoulis et al., 2010).

1.3.3 Fat

The major contribution to fat in the formulations of ice-cream mixes is given by cream and milk.

- **Cream**

According to the EC Regulation n. 1308/2013, the cream of milk or cream is the product obtained from milk, in the form of an emulsion of fat in water, with a minimum fat content by weight 10% dairy farmers. Compared to milk, it contains ten times more of fat content. In general, fresh cream contains 35% fat but it is still possible to find products with a different concentration of fat on the market. The cream is obtained or by separation of the fat phase of milk followed by spontaneous surfacing in tank or by centrifugation. However, the cream is not only rich in fats but also has a percentage (85.7%) of solid lean milk residues (lactose) and proteins. With regard to the raw material used instead the cream is differentiated into:

- cream coming from fresh milk;
- centrifuge whey cream: the whey from the cheese processing is centrifuged. The "centrifuge serum breakouts" are acidic and rich in lactic bacteria and have a concentration of very variable fat;
- regenerated cream: different butters are melted giving rise to the so-called "regenerated creams". After the fusion the plasma is removed and the fat is re-suspended in milk.

As for the heat treatments undergone it is possible to distinguish the cream in:

- pasteurized cream, to be kept in the fridge for a short time, maintains a thick consistency but liquid and has a neutral aroma;
- UHT sterilized cream, can be stored at room temperature. It has more consistency creamy and an aroma altered by heat treatment.

In a cream the main physical properties that depend on cooling are viscosity and stability. The viscosity of the cream is also influenced by factors related to the raw material (triglyceride composition of fat) and many others related to his production process (fat content, degree of homogenization, intensity and type of treatment temperature, duration of storage).

- **Milk**

Milk is one of the most important ingredients used for the production of ice-cream. Whole cow milk is the one used mainly in ice-cream. Sometimes whole milk powder is also used as a source of protein and fat. Instead, an alternative to whole milk powder and widely used in ice-cream is skim milk powder. The use of skim milk powder greatly improves the structure of the ice-cream. Its percentage composition highlights the rich content of proteins that have a fundamental role in giving the ice-cream a smooth and velvety structure, increasing the incorporation of air (overrun). Due to these important characteristics, skimmed milk powder is widely used by ice-cream makers, not as a substitute for liquid milk, but as a highly functional ingredient in the recipe.

From a structural point of view (Fig. 2), milk is a matrix composed of three phases in equilibrium (Jensen, 1995):

- a solution of lactose, soluble proteins, mineral salts and vitamins;
- a colloidal suspension of casein organized in micelles, globular proteins and lipoproteins;
- an emulsion of oil in water, in which the fat globules are dispersed in a continuous serous phase.

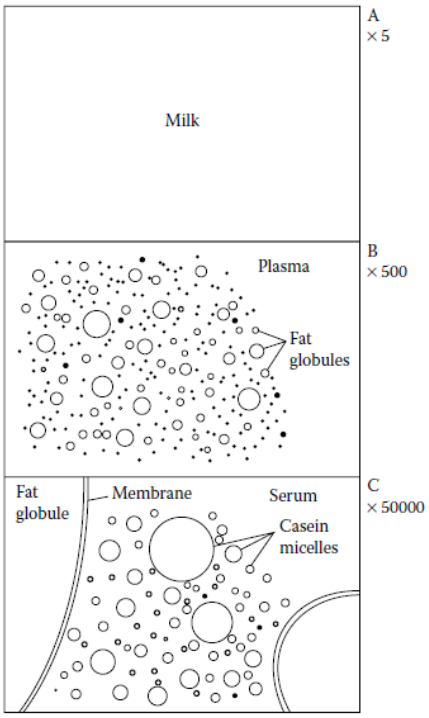


Fig. 2: Milk viewed at different magnifications, showing the relative size of structural elements. (A) Uniform liquid. However, the liquid is turbid and thus cannot be homogeneous.

(B) Spherical droplets, consisting of fat. These globules float in a liquid (plasma), which is still turbid. (C) The plasma contains proteinaceous particles, which are casein micelles. The remaining liquid (serum) is still opalescent, so it must contain other particles. The fat globules have a thin outer layer (membrane) of different constitution (figure adapted from Mulder and Walstra, 1974).

The total content of all substances except water is called the content of dry matter. Furthermore, one distinguishes solids not fat and the content of fat in the dry matter. The chemical composition of milk largely determines its nutritional value, the extent to which microorganisms can grow in it, its flavor and the chemical reactions that can occur in milk. The latter include reactions that can cause off-flavour. The nutritional characteristics of milk differ according to the species of mammal of origin, the constituents in any case are the same. The proportion between the milk constituents determines therefore different characteristics not only nutritional but also from a structural point of view (Goff, 2003).

A classification of the principal constituents of milk is given in Fig. 3:

Component	Average Content in Milk (% w/w)	Range[a] (% w/w)	Average Content in Dry Matter (% w/w)
Water	87.1	85.3–88.7	—
Solids-not-fat	8.9	7.9–10.0	—
Fat in dry matter	31	22–38	—
Lactose	4.6	3.8–5.3	36
Fat	4.0	2.5–5.5	31
Protein[b]	3.3	2.3–4.4	25
casein	2.6	1.7–3.5	20
Mineral substances	0.7	0.57–0.83	5.4
Organic acids	0.17	0.12–0.21	1.3
Miscellaneous	0.15	—	1.2

Note: Typical for milks of lowland breeds.

[a] These values will rarely be exceeded, e.g., in 1 to 2% of samples of separate milkings of healthy individual cows, excluding colostrum and milk drawn shortly before parturition.

[b] Nonprotein nitrogen compounds not included.

Fig. 3: Approximate composition of milk and properties of the main structural elements of milk.

● Milk fat

Milk is an oil-in-water emulsion, in which the fat globules are dispersed in a continuous serous phase. Milk lipids are mostly represented by triglycerides (Fig.5, 6). From a structural point of view, triglycerides consist of glycerol backbone binding up to three different fatty acids. The fatty acids are composed of a hydrocarbon chain and a carboxyl group. Triglycerides differ from each other for chain length and saturation (Walstra et al., 2006).

● Milk fat structure: fat globules

Milk lipids can be different for composition, size, distribution among the principal breeds. This variation can affect rheological properties, color, chemical stability and nutritionals properties (Fox et al., 2015). Nearly all of the fat in milk is in separate small globules. The milk fat globules vary in diameter from about 0.1 to 20 μm. They are surrounded by a membrane (milk fat globule membrane – MFGM) that works as emulsifier. The membrane prevents flocculation phenomena, the fat coalescence and therefore the separation of the fat phase from the lean one. About 90% of the MFGM is composed by polar lipids and proteins, whereas the remaining part includes glycoproteins, cholesterol, enzymes and other bioactive components (Keenan and Mather, 2006).

● Milk fat crystallization

Crystallization is a supramolecular process where randomly organized molecules in a fluid come together and form an ordered three-dimensional molecular structure: a crystal (Davey and Garside, 2000). Milk fat crystallization is affected by chemical and physical parameters, such as the fatty acid (FA) composition, presence of milk fat globules, temperature conditions and shear forces. The quality of the raw material and the parameters used during product processing are fundamental for the crystallization process, thus for the final quality of the product. At temperatures below 35°C, part of the fat in the globules can crystallize. Milk minus fat globules is called *milk plasma*, the liquid in which the fat globules float (Walstra et al., 2006). The crystals in a fat globule cannot grow larger than the globule diameter. If there are sufficient crystals in a globule, they can aggregate into a space-filling network that provides a certain rigidity to the globule. As crystallization proceeds (by

cooling), the tangentially oriented crystals may grow into a solid layer. Crystallization of fat in the globules is of great importance for their stability. Globules containing crystals cannot be called droplets, and they can generally not show coalescence but partial coalescence.

● Milk fat instability

The presence in the milk of fat globules, casein micelles and particles of colloidal dimensions, ranging between 10 nm and 100 μm in diameter, has different consequences:

- some substances are present in separate particles. This means that interactions with compounds in the serum have to occur via the surface of the particles, which may act as a barrier. Moreover, compounds in one fat globule generally cannot reach another globule;
- several physical properties depend on the state of dispersion; for instance, turbidity, color and viscosity. If aggregation of particles occurs, this will further affect viscosity and often a gel will eventually result;
- the particles can be subject to various instabilities. Most of these instabilities eventually lead to an inhomogeneous product with greatly altered properties.

It is possible distinguish various kinds of fat instability (Fig. 4). These instabilities can occur simultaneously and influence each other.

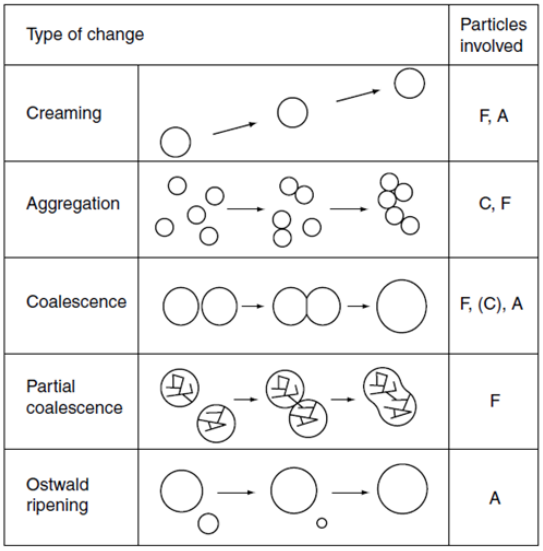


Fig. 4: Illustration of the various changes that can occur with colloidal particles. (A) is air bubbles (diameter, in example 50 μm). (C) casein micelles (0.1 μm) and (F) fat globules (3 μm). The solid lines in the fat globules (partial coalescence) denote fat crystals.

All changes that cause an increase in particle size promote creaming and thereby de-mixing. Aggregation of fat globules rarely leads to formation of a particle gel because the aggregates soon start creaming before a gel can be formed. Coalescence can only occur if the droplets are close, be it due to aggregation or creaming. If coalescence goes on, it will lead to phase separation: an oil layer on top of an emulsion layer of decreased oil content.

Fat globules can aggregate in various ways (Fig. 5):

- in floccules, attractive forces between globules are weak, and stirring disrupts the floccules. Flocculation does not happen normally with milk fat globules because electrostatic and steric repulsion prevents it. Milk fat globules do not flocculate even at their isoelectric pH, which is approximately 3.7;
- in clusters, two globules share part of the membrane material, generally micellar casein. Clusters usually cannot be disrupted by stirring;
- in granules, that occur if the fat globules contain a network of fat crystals, giving the globules a certain rigidity. Granules usually cannot be disrupted.

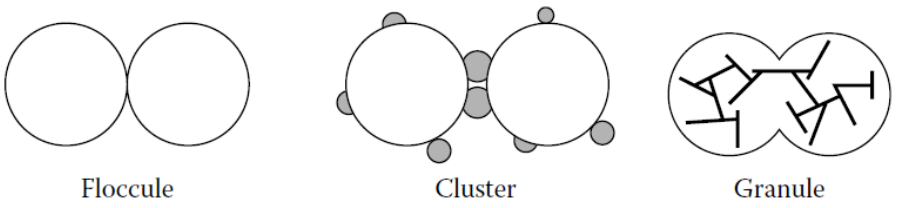


Fig. 5: Schematic examples of different types of aggregates of fat globules (figure adapted from Walstra et al., 2006)

1.3.4 Milk Solids Not Fat

Milk solids not fat (MSNF) are important components of milk because they improve the texture of the ice-cream. Among MSNF, there are lactose, caseins, proteins and minerals.

● Glucidic fraction

Lactose is the distinctive carbohydrate of milk. Lactose is a disaccharide composed of D-glucose and D-galactose (Fig. 6). The aldehyde group of galactose is linked to the C-4 group of glucose through a β-1, 4-glycosidic linkage.

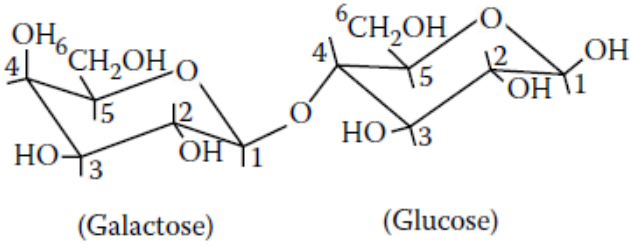


Fig. 6: β-lactose chemical structure.

● Nitrogen fraction

The nitrogen component of milk is made up of proteins and non-protein nitrogenous substances. About 95% of the nitrogen in milk is in the form of proteins (Fig. 7). More important is the presence of nonprotein nitrogen components, comprising about 5% of the total nitrogen in fresh milk. The values generally given for the 'protein content of milk' are thus too high by 5 to 6%, or nearly 0.2 percentage units.

Protein	mmol/m³ Milk	g/kg Milk	g/100 g Protein	Molar Mass	g Protein/g N	Remarks
Casein	1120	26	78.3		6.36	IEP ≈ 4.6
α_{S1}-Casein	450	10.7	32	~23600	—	Phosphoprotein
α_{S2}-Casein	110	2.8	8.4	~25200	—	Same, contains –S–S–
β-Casein	360	8.6	26	23983	—	Phosphoprotein
κ-Casein	160	3.1	9.3	~19550	—	"Glycoprotein"
γ-Casein	40	0.8	2.4	~20500	—	Part of β-casein
Serum proteins	~320	6.3	19	—	~6.3	Soluble at IEP
β-Lactoglobulin	180	3.2	9.8	18283	6.29	Contains cysteine
α-Lactalbumin	90	1.2	3.7	14176	6.25	Part of lactose synthase
Serum albumin	6	0.4	1.2	66267	6.07	Blood protein
Proteose peptone	~40	0.8	2.4	4000–40000	~6.54	Heterogeneous
Immunoglobulins	~4	0.8	2.4	—	~6.20	Glycoproteins
IgGl, IgG2	—	0.65	1.8	~150000	—	Several types
IgA	—	0.14	0.4	~385000	—	
IgM	—	0.05	0.2	~900000	—	Part is cryoglobulin
Miscellaneous	—	0.9	2.7	—	—	
Lactoferrin	~1	0.1	—	86000	6.14	Glycoprotein, binds Fe
Transferrin	~1	0.01	—	76000	6.21	Glycoprotein, binds Fe
Membrane proteins	—	0.7	2	—	~7.1	Glycoproteins, etc.
Enzymes	—	—	—	—	—	

Note: Approximate composition. IEP = isoelectric pH.

Fig. 7: Protein composition of milk (figure adapted from Walstra et al., 2006).

The caseins represent about 75% of the nitrogenous substances in milk. Almost all caseins in fresh milk are present in roughly spherical particles, mostly 40 to 300 nm in diameter. They do not form anything more than short lengths of α-helix and have little tertiary structure. According to the genetically determined primary structures, it is possible distinguish αS1-, αS2-, β- and κ-casein, but each of these occurs in a number of variants. Most of the κ-casein molecules are glycosylated to various extents. Part of the β-casein is split by proteolytic enzymes into γ-casein and proteose peptone. The αS- and β-caseins are phosphoproteins that have a number of phosphate groups esterified to serine residues. They precipitate with Ca^{2+} ions but κ-casein protects them from precipitation (Martin et al., 1999).

Caseins are hydrophobic and they have many prolines and few cysteine residues. Many hydrophobic groups are exposed, so that the molecules readily form hydrophobic bonds. Caseins have a relatively high charge. This is important to be in solution. The various caseins tend to associate into small aggregates (4 to 25 molecules), according to casein species (Walstra et al., 2006). These aggregates are called micelles (Fig. 8). The casein micelles also contain inorganic matter, mainly calcium phosphate, about 8 g per 100 g for casein. They contain also small quantities of some other proteins, such as part of the proteose peptone and certain enzymes. The micelles are voluminous, holding more water than dry matter.

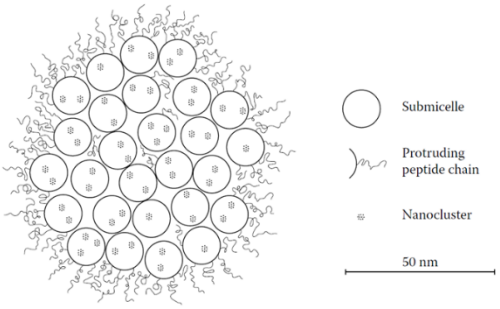


Fig. 8: Cross section through a tentative model of casein micelle (figure adapted from Walstra et al., 2006).

Caseins resist to high temperatures. They are not denatured but heating at temperatures above approximately 120 °C, the casein to slowly become insoluble due to chemical changes. Temperature resistance is higher for small micelles. The presence of caseins in the form of micelles and their physical characteristics determine the physical stability of milk products during heat treatment and storage. The interaction of casein micelles with oil–water interfaces is of importance with respect to properties of milk products.

In the serum, proteins are present in a dissolved form. They are often called whey proteins, although they are not precisely identical to the proteins of rennet whey, which also contains the peptides split off from κ-casein. Most serum proteins typically are globular proteins: they have relatively high hydrophobicity and compactly folded peptide chains. Most contain an appreciable proportion of α-helix and β-sheet, the charge distribution is rather homogeneous. They become insoluble at pH values below 6.5 if milk is heated. This change is related to the denaturation of the proteins involved. An example of α-protein is the α-lactalbumin. The biological function of this protein is coenzyme in the synthesis of lactose. The protein is a small, compactly folded and spherical. An example of β-protein is the β-lactoglobulin. It is the major serum protein and its properties tend to dominate the properties of whey protein preparations, especially the reactions occurring upon heat treatment.

- **Enzymes**

Milk contains several enzymes. The native enzymes can be present at different locations in the milk. Many of them are associated with the fat globule membrane, others are in solution, dispersed in the serum and some (such as lipoprotein lipase) are generally associated with the casein micelles (Walstra et al., 2006).

- **Salts**

Milk contains inorganic and organic salts. The salt composition varies but the different components do not vary independently of each other. Not all of the salts are dissolved and not all of the dissolved salts are ionized. The casein micelles contain the undissolved salts. In addition to counterions of the negatively charged casein (mainly Ca, Mg, K, and Na), the micelles contain the called colloidal calcium phosphate, which also contains some citrate. The colloidal phosphate is amorphous, can vary in composition and may have ion exchange properties.

1.3.5 Stabilizers

Stabilizers are one of the ingredients of preparations for ice-cream mixes present in small quantities but assigned to perform many tasks. Stabilizers in general represent the 0.1-0.5% of a recipe for an ice-cream mix. The stabilizers are a group of compounds, usually polysaccharide food gums, that are responsible for adding viscosity to the mix and the unfrozen phase of the ice-cream, due to the fact that they are able to interact with water through hydration (water holding).

Stabilizers in ice-cream are aimed to (Bahrampavar and Tehrani, 2011):

- produce smoothness in body and texture;
- increase ice-cream volume;
- retard or reduce ice and lactose crystal growth during storage;
- provide uniformity to the product;
- stabilize the protein in the mix to avoid wheying off;
- help in suspension of flavoring particles;
- create a stable foam with easy cutoff and stiffness at the barrel freezer for packaging.

Interacting with water molecules the rheological characteristics of the mix change. Stabilizers affect the mechanical and stress relaxation properties of ice-cream mixes. Other characteristics of stabilizers are a neutral flavor, not bind to other ice-cream flavors, contribute to acceptable meltdown of the ice-cream and provide desirable texture upon consumption (Goff and Sahagian, 1996). European laws treat stabilizers as food additives, for this reason they are associated E numbers (Clarke, 2004).

Different hydrocolloids, also of natural origin, are used as stabilizers.

Proteins:

- Gelatin (E441).

Plant Exudates:

- Locust Bean Gum (carob bean gum) (LBG) (E410);
- Guar Gum (E412).

Cellulosic hydrocolloids:

- Sodium carboxymethyl cellulose (CMC) (E466).

Microbial gums:

- Xanthan (E415).

Seaweed extracts:

- Alginates;
- Agar;
- Carragean (Irish moss) (E407).

The amount and kind of stabilizer required in ice-cream depend on its properties, mix composition, ingredients used, processing times, temperatures and pressures, storage temperature, time and many other factors. Excessive use of stabilizers may have undesirable effects on melting characteristics and excessive mix viscosity, producing an heavy product with a low increase in volume.

1.3.6 Emulsifiers

An emulsifier produces a stable emulsion of two liquids that do not mix naturally, as oil and water. Usually 0.2-0.5% of the blend stabilizers/emulsifiers is used in the ice-cream mix (Goff and Sahagian, 1996). They are used to:

- promote nucleation of fat during aging, thus reducing aging time;
- improve the whipping quality of the mix, due to their function at the air interface, resulting in reduced air cell sizes and homogenous distribution of air in the ice-cream;
- provide smooth texture in the finished product, due to fat structuring and the interaction of fat agglomerates with the mouth during consumption.

Emulsifiers are also involved in the fat destabilization process. They lower the fat/water interfacial tension in the mix, resulting in protein displacement from the fat globule surface. In this way, the stability of the fat globules is reduced, with next partial coalescence during the whipping and freezing process. The presence of this fat structure in the frozen product contributes to texture and meltdown properties (Goff and Jordan, 1989). Different emulsifiers are used in foods:

- egg yolk;
- egg protein;
- mono and diglycerides;
- polysorbates.

The most used emulsifiers are mono and diglycerides. Usually, they are used in combination with sorbitan esters (polysorbates). Mono and diglycerides turn out to be more functional at air interface, while polysorbates are more functional at the fat interface.

1.3.7 Other ingredients

- **Fruit**

Fruit is the ingredient that gives taste to all sorbet preparations but also in milk-based ice-creams with fruit taste. Thanks to the sugars naturally present in the fruit, this ingredient plays an important role in the depress of the freezing point. However, natural sugar content in the fruit is not enough to guarantee a good structure and a quality texture. For this reason fruit-based recipes are balanced with the addition of other sugars.
Fruits are available as:

- fresh fruit: it must always be ripe, with the maturation cycle completed on the plant, when this is possible, because it is tastier. It is necessary to handle it correctly, washing it carefully. It must also be peeled, if necessary, or deprived of all those inedible parts such as cogs, pits or excessively large and/or hard seeds.
- frozen fruit: it is fresh fruit that has undergone a freezing process, in example a rapid reduction in temperature, after having been washed and cleaned. Compared to fresh fruit, it has the advantage of being available all year round and arriving in the lab already washed and cleaned, saving time and labor. The ice-cream maker will have to check its quality, especially in terms of maturation;
- frozen fruit pulp: it offers good guarantees of coming from fruit at the highest degree of ripeness and is already blended, a state in which it must find itself to become frozen. This frozen fruit pulp contains added sugar, usually 10%, to maintain its fragrance over time. This peculiarity is indicated on the package and the ice-cream maker must take this into account when formulating the recipe;
- fruit ice-cream pastes: they can be an alternative to fresh or frozen fruit, or a useful reinforcement of taste and color.

1.4 Ice-cream: production processes and Carpigiani machines

Heat treatment, aging and freezing represent three very important steps for obtaining a quality ice-cream product. During these three moments, the ice-cream mix goes through physical-chemical changes. The supporting technology therefore becomes of primary importance. Carpigiani offers a wide range of solutions for each of the phases mentioned above.

1.4.1 Heat treatment and aging

Pasteurization is the production phase of the hygienically safe ice-cream mix, in which all the ingredients are finely blended. The process involves the combined action of a correct heat treatment cycle and a strong mechanical mixing/emulsion action of the ingredients. This process is achieved through the use of a pasteurizer. It is possible to use different heat treatment cycles, depending on the various types of mix to be treated. Here are some examples:

- high heat treatment (85-4 °C), generally used for fior di latte base;
- high heat treatment (90-4 °C), generally used for chocolate base;
- low heat treatment (65-4 °C), generally used for custard cream base;
- intermediate heat treatment (65/85-4 °C), for custom mixes.

During the heat treatment cycle the ingredients are poured according to a correct sequence that guarantees the perfect solubilization of all the dry components (sugars, powdered milk derivatives, stabilizers, etc.). The heat treatment cycle is considered finished once the mix reaches the storage temperature (4 °C). At this point the aging phase begin. Aging is an essential process to improve the quality of the ice-cream mix. Aging means a rest time of the mix at the end of heat treatment. A good aging phase lasts at least 4 hours, up to the ideal 12 hours. During aging, the ice-cream mix is stored at 4 °C. The aging can be dynamic or static, depending on whether the mix is subjected to stirring (continuous or intermittent) or not.

Aging can be carried out:

- in the pasteurizer at the end of the automatic heat treatment cycle;
- inside a maturation tank, mainly a machine dedicated to this function;
- inside a batch freezer equipped with this function;
- statically in cold storage, a 4 °C.

The aging phase is important due to the chemical-physical processes that take place in the ice-cream mix during this phase:

- the solids, and in particular the milk proteins, hydrate by binding part of the free water contained in the ice-cream mix;
- water, having broken down into tiny droplets thanks to the bond with proteins, will freeze in tiny crystals instead of large ice particles;
- the viscosity of the mix increases. Subjected to stirring in the batch freezer, the aged mix will retain more air and the ice-cream will be softer, less cold and more resistant to temperature changes;
- the fats present in the mix homogeneously have time to be distributed finely: the ice-cream will be more palatable. The aromas are fixed to the fats in a uniform manner. The flavor of the ice-cream will be more intense.

The Carpigiani solution for heat treatment and aging is represented by Pastomaster (Fig. 9).

Fig. 9: Carpigiani pasteurizer Pastomaster HE.

Carpigiani Pastomaster has a processing tank in which all the ingredients of the mix are inserted and heat treated. Depending on the expected production volumes, the processing tank is available in versions useful to contain different volumes. The available heat treatment cycles and processing speeds can be chosen independently by the operator according to his needs and the mix to be treated. Pastomaster has a display that suggest the correct sequence of ingredients for the selected processing cycle. The machine also integrates the heat treatment function with the aging one. In this case the display works as an electronic register, keeping time and temperature monitored throughout the entire aging phase.

1.4.2 Freezing

Freezing is the transformation phase of the liquid mix into ice-cream. Through the stirring and the administration of cold, air is incorporated and at the same time very fine ice crystals are formed. This process takes place in a machine called batch freezer. Most of the structure of the final product depends on how the cooling and stirring process takes place. Cold and stirring must be in the right relationship.

From the combination of these elements it is possible to obtain ice-cream products with different characteristics between them, like:

- gelato;
- soft ice-cream;
- milk shake.

● Gelato

The classic gelato production cycle includes the following phases:

- choice of ingredients;
- ingredients balance;
- mix ingredients;
- heat treatment;
- aging;
- freezing;
- hardening and storage.

The Carpigiani batch freezer, Maestro HE (Fig. 10), is the machine dedicated to the production of gelato. This machine has important technical features that adapt to the needs of the operator. Once assured of the correct assembly of the components and door closing, the operator pours the mix directly into the whipping chamber through the top hopper. Depending on the type of mix, fruit or milk base, it is possible select the dedicated cycle. The cold is managed by the machine according to the quantity and type of mix. Maestro HE is flexible to automatically produce minimum and maximum quantities. The stirrer scrapes and spread, forming very fine ice crystals. Maestro produces large quantities of ice-cream in a short time, washing is quick and versatile. This guarantees hygiene and safety.

Fig. 10: Carpigiani batch freezer Maestro HE.

● Soft ice-cream

Soft ice-cream (Fig. 11) differs from traditional artisanal gelato, due to the production and sales process. While with the traditional batch freezer, the production takes place entirely inside the laboratory and the ice-cream is then brought to the sales counter, the production of soft ice-cream takes place directly in the sales area and immediately served. Soft ice-cream is characterized by the greater softness compared to traditional gelato, due to the greater incorporation of air that the product undergoes during the production phase.

Fig. 11: Soft ice-cream.

Carpigiani soft ice-cream machines (Fig. 12) have storage tanks of the already characterized mixes, which continuously feed the production cylinders. It is therefore possible to have a dedicated freezing cylinder for each flavor of soft ice-cream. The extraction of product is done by lowering the dispensing lever. A soft ice-cream machine can have a single or double tank and therefore able to offer one or two flavors of ice-cream simultaneously. The feeding system works by means of a pump. The ice-cream mix is pressurized with air, so as to constantly generate a soft and creamy ice-cream. A variant is gravity feeding, the ice-cream mix falls into the freezing cylinders only by exploiting the force of gravity.

Fig. 12: Carpigiani 193 Soft ice-cream machine.

● Milkshake

Among soft-frozen dessert there is milkshake. This product is usually used in fast-food outlets. The product is prepared in a principal factory, packaged and shipped. The milkshake product differs from gelato and soft ice-cream in consistency, temperature and overrun. The product is a soft-frozen dessert to drink, with a temperature higher than gelato and soft ice-cream. Also overrun differs from the other typology of products resulting lower.

Carpigiani solution for milkshake product is K3 machine (Fig. 13). This machine is able to produce simultaneously soft-ice-cream (right side) and milkshake (left side). The machine has storage tanks and feeding system with a pump. Milkshake production takes place inside a freezing cylinder with larger dimensions than a soft product cylinder. The beater scrapes the product from the cylinder walls with a dedicated speed, allowing the formation and growth of ice crystals typical of the milkshake product.

Fig. 13: K3 Carpigiani machine.

1.5 Microbiological food safety

The quality and safety of a food must always be guaranteed. To safeguard the health and the safety of the consumers, specific laws limit the presence of pathogenic or potentially pathogenic microorganisms in foods. Food products must not contain microorganisms or their toxins or metabolites, in quantities that represent an unacceptable risk to human health. Flavor and textural quality can vary but microbiological quality of the food must be guaranteed. Microbial contamination of foods is of particular concern for consumers that are more susceptible to illness. Dairy products, in particular ice-cream, reach a wide range of consumers.

They can also be consumed by children and the elderly, therefore more fragile populations from the point of view of the immune system and of the general state of health. The microorganisms are ubiquitous, they can contaminate both the raw materials and the finished product. Pathogenic bacteria and toxins are the most frequent biological dangers. Dairy products due to their composition characteristics are well suited as an ideal growth substrate capable of promoting bacterial growth. In terms of food safety, it is important to distinguish preventive and control actions from the regulations that set microbiological limits. Limits must help to establish a level of acceptability for food safety and related processing, handling and distribution processes.

1.5.1 General food law regulation

The regulations are constantly evolving and have undergone changes and adjustments over the last few years, regulations may also vary between countries. Into the Union European are applied the same requirements in all the Member States. In order to guarantee high quality level for consumer health protection and allow the free circulation of food and feed produced or placed on the market within the European Union, a Community legislation has been adopted. Furthermore, the EC Regulation 178/2002 of the European Parliament and of the Council (GUCE L 31 of 1 February 2002) proposed the general principles and requirements of food legislation and established the European Food Safety Authority (European Food Safety Authority, EFSA), based in Parma, that handles procedures in the field of food safety with regard to risk management and communication. The EFSA institute has the task of providing scientific and technical advice and assistance. It collects and analyzes data used for risk surveillance, formulates scientific opinions for the development and implementation of risk management measures.

● Preventive actions

In 2004, Regulations 852 and 853 established additional specific hygiene rules for food of animal origin. These regulations are mainly aimed at:

- empowering food business operators;
- trace and validate production and processing plants;
- define bacteriological limits;
- guarantee food safety from production to transport up to the sale of food;
- define the specific conditions of food conditions;
- apply the procedures based on the HACCP system;
- creation by the member states and the European community of manuals of good hygiene practices;
- ensure that imported foods meet the same hygiene standards established by the community.

Article 5 of Regulation (EC) No 852/2004 of the European Parliament and of the Food Hygiene Council requires sector operators to prepare, implement and maintain a permanent procedure based on principles of the HACCP system (Hazard Analysis and Critical Control Points). HACCP represents an approach for the management of biological, chemical and physical hazards that could affect food safety. The probability that the danger will result in harm to the consumer is called risk. The HACCP system is a tool that helps food business operators to achieve a higher level of food safety. The objective of the HACCP system is the drawing up of a specific manual, which includes all the actions and verifications necessary to correctly manage the different phases like production, food and drink administration, food sales. This self-control manual must always be present in the company, appropriately drafted and updated, because this is the documentation to be provided to the control bodies for any check.

HACCP is based on seven principles:

1. conduct a hazard analysis;
2. identify critical control points (CCP) for the phases in which it is possible to prevent, reduce or eliminate the risk;
3. establish control limits for each control point that differentiate acceptability from unacceptability;

4. establish monitoring procedures effective at critical control points;
5. establish corrective actions if a critical point is not under control (exceeding the established critical limits);
6. establish record keeping procedures to verify the effective functioning of the measures adopted;
7. establish record verification procedures.

In order to apply the principles of the hazard analysis system and critical control points (HACCP), the person in charge of the self-control plan must prepare the Self-Control Plan. The plan must be preventing the causes of the occurrence of non-compliance and must provide for appropriate corrective actions to minimize the risks in the event of non-compliance. The main objective is to establish a documented system with which the company/organization is able to demonstrate that it has operated in a way that minimizes the risk.

● Microbiological limits

In 2005, after the Regulation (EC) No 852/2004, the Regulation (EC) n. 2073 of the Commission, established the microbiological limits for specific microorganisms and implemented the rules that food industry operators must comply. The regulation (EC) n. 2073/2005 established that food business operators must ensure that food products comply with the relevant microbiological criteria set out in Annex I of that regulation and respect of other hygiene control measures. Food safety criteria set acceptable limits for the presence of *Listeria, Salmonella,* staphylococcal toxins, *Enterobacter sakazaki.* An important change made by this regulation is to apply the food safety criteria to the product up to the end of its shelf-life and no longer only after leaving the production plant. Process hygiene criteria are defined imposing limits for *E. coli, Enterobacteriaceae* and coagulase positive staphylococci. These criteria, on the other hand, do not apply to the finished product but are used to evaluate a manufacturing process. This serves to evaluate the entire production chain, identifying the critical phases in which the product could lose the required characteristics of conformity. The Commission Regulation (EC) No. 1441/2007 of December 5 2007 amending regulation (EC) n. 2073/2005 established microbiological criteria applicable to food products.

1.5.2 Food information to consumers

Regulation (EU) n. 1169/2011 of the European Parliament and of the Council, of 25 October 2011, rules the free circulation of safe and healthy foods. To obtain a high level of protection of the health of the consumers and ensure their right to information, it is appropriate to ensure that consumers are adequately informed about the foods they consume. The general labeling requirements are supplemented by a set of provisions applicable to all foods in particular circumstances or to certain categories of food. There are also several specific rules applicable to specific foods. In accordance to EU Regulation 1169/2011 products on the market must have the indication on the label of the "minimum term of conservation" (often referred to by the acronym TMC) or "Expiration date", defined as follows:

- *"Minimum term for storing a food":* the date up to which such product retains its specific properties in adequate storage conditions;
- *"Expiry date":* in the case of highly perishable foods from the point of microbiological view that could therefore constitute, after a short time, an immediate danger to human health, the minimum term of storage is replaced by the date of deadline. After the expiry date a food is considered at risk pursuant to Article 14 of the regulation (CE) n. 178/2002.

Labeling phase is very important to report data of possible perishability of a food, in order to give all the information for the safeguarding of consumers. The legislation on the labeling of food products was born in 1978 with Directive 79/112/EEC, which was implemented in the various Member States (in Italy by means of Legislative Decree 109/1992). In 2003, Directive 79/112/EEC was repealed by Directive 2000/13/EEC. In Italy, the legislator, instead of issuing a new law, continued to make changes to Legislative Decree 109/1992. After a complex process in which legislation was issued and repealed, on 25 October 2011, the European Parliament and the Council adopted Regulation (EU) 1169 "concerning the supply of food information to consumers", which to date it is the community reference containing the discipline to label food products.

The label is "any trademark or factory mark, sign, image or other graphic representation written, printed, stamped, marked, embossed or imprinted on the packaging or on the container of a food or accompanying such packaging or container." (Art. 1 Reg. 1169/2011). The art. 44 of Regulation n. 1169/2011 establishes that, if the food is offered

for sale to the final consumer or to the community without pre-packaging or is packaged on the sales premises at the consumer's request or pre-packaged for direct sale, the supply of any ingredient or adjuvant is mandatory, technological or derived from a substance or product that causes allergies or intolerances used in the manufacture or preparation of a food and still present in the finished product, even if in an altered form.

At least the following information must be included:

a) the name of the food;

b) the list of ingredients from which the indications must appear the substances or products that cause allergies or intolerances;

c) the storage methods for rapidly perishable food products, where necessary;

d) the expiry date for fresh pasta and stuffed fresh pasta;

e) the actual alcoholic strength by volume for beverages with an alcohol content exceeding 1.2 percent by volume;

f) the percentage of glazing, considered tare, for frozen frosted products;

g) "defrosted" designation.

For the products of ice-cream parlors, pastry shops, bakeries, fresh pasta and delicatessen, including food preparations, the list of ingredients can be reported on a single, well-marked sign (the so-called “single sign of the ingredients ") or, for individual products, on a special register or other equivalent system, also digital, to be kept clearly visible, available to the purchaser, near the display counters of the products themselves, provided that the indications relating to the substances or products referred to in Annex II of the Regulation n. 1169/2011 (substances or products that cause allergies or intolerances) are attributable to the individual foods offered for sale.

For who produce semi-finished products for ice-cream and confectionery destined for sale to the professional user (not intended for the final consumer) Legislative Decree 109/1992, art. 17 provides the following mandatory information for products not intended for the final consumer on packaging, label or commercial document:

1. food name;
2. net quantity;
3. allergens;
4. name or business name and full address of the manufacturer or packager or seller;
5. a wording that identifies the lot.

The microbiological quality of the samples and the evaluation of the processes were determined with reference to Chapter 1 of Regulation (EC) n. 1441/2007 that describes food safety criteria and Chapter 2 of Regulation (EC) n. 1441/2007 describes process hygiene criteria (specifically chapter 2.2 is about milk and dairy products).

2. Aims

New tool for microbiological safety and product quality of ice-cream mixes have been developed: a portable biosensor system for detection of bacterial presence in milk-based ice-cream mix and a novel packaging process for the ice-cream mixes distribution. The biosensor exploits the impedance technique (IT). IT is based on monitoring the changes of the electrical featured determined by metabolism of bacteria, since the time needed for the electrical parameter to deviate from the baseline value, called Detect Time (DT), is linearly related to the logarithm of initial bacterial concentration. The measurement of the impedance has been done applying to the samples a sinusoidal voltage signal. Ice-cream mix samples inoculated with increasing concentration of *E. coli* ATC11229, ranging from 10^1 to 10^{10} CFU/ml, have been monitored over the time to obtain a calibration curve. The ice-cream mix without inoculum was used as control in the tests. The samples were analyzed in parallel by both measurements with the biosensor and by plate count enumeration, in order to compare the results. The DT was calculated for each sample at the different concentration of inoculum and in control samples.

In order to meet the requests of the ice-cream mix distribution, and in particular of the centralized production laboratories, a shelf-life study of ice-cream mixes has been performed and a novel packaging process has been developed, with the aim to provide the customers a standardized quality in a multi-location ice-cream retail context. In recent years, the relocation of ice-cream mixes production centers that prepare the product to be sent directly to the sales points constantly grew. Ice-cream mixes can be produced and packaged in a production center, then stored and transported to sales points. Once the sale points are reached, the ice-cream mixes are transformed into ice-cream. Equipment and processing methods to control the microbiological safety of ice-cream mixes in a centralized laboratory were developed. Preliminary experiments evaluated the microbiological stability of the products processed and stored at 4 °C. Bacteriological analysis and pH measurements confirmed the stability of the product. In order to limit the possibility to contaminate the product, a professional bag filling machine was developed. Using the bag filling system, the microbiological stability of the ice-cream mix was confirmed for up to one month. Sensory analysis was performed to evaluate the quality of the product during one month of shelf-life at 4 °C.

3. Biosensor

For the food industry, it is of primary importance to ensure quality control and approved products. Measurements of food quality parameters, either physical, chemical, microbiological or sensorial, are necessary to characterize the product and avoid possible adulteration or contamination. Quality control of product is important at every stage, from the production to the sale, passing through storage and distribution, because food contaminants can derive from different sources. A microbial population associated to the production environment and to the ingredients is normally present in food matrices. Furthermore, pathogenic bacteria can be transferred through feces, mouth, nose and skin. Within the foods, they survive and multiply if conditions are favorable. It is necessary to respect the hygiene rules (see paragraph 1.5) during working operations and carry out the planned controls.

Ice-cream is not a sterile product, although ice-cream machines are able to perform a heat treatment cycle that reduces the load of microorganisms, in order to lay below the limits of pathogenic bacteria imposed by law at the end of this process. Ice-cream machines keep the product at a temperature of 4 °C or lower between a heat treatment cycle and another. This limits bacterial growth. However, *L. monocytogenes* charge in ice-cream can remain constant during freezing, due to the cryoprotective effect of the solutes creating an unfrozen phase which holds and protects the cells (Dean and Zottola, 1996). One of the classic methods of bacterial identification consists in the enumeration of cultivable bacteria in agar plates. Selective media are used to obtain the growth of different microbial groups. The standard plate approach is an “off-line” analysis that has a high accuracy but needs a long detection time (24-48 hours). Agar plate count analysis is performed in a central laboratory, with advantages in terms of know-how and equipment. The preparation of the sample, the equipment and the technique are chosen in function of the type of sample. The principal disadvantage of this “off-line” analysis is represented by the time between sampling and the response of the analysis. The analysis described so far are carried out downstream of the production process or directly on the finished product. However, in order to carry out a preventive action it is important to perform screening tests at higher throughput of samples, lower costs and reduced operator training, so larger numbers of samples can be analyzed (McGrath et al., 2012). The use of biosensors in food microbiology provides a preventive action. The term biosensor was coined by Cammann in 1977 and its definition (Thevenot et al., 2001) was introduced by the International Union of Pure and

Applied Chemistry (IUPAC) as a complete and integrated device capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element in direct spatial contact with a transducer. The biosensor, as a particular type of transducer, consists of a biologically active sensitive element called bioreceptor (enzymes, cells, antibodies, etc.) and of an electronic part.

The materials used in biosensors are categorized into three groups based on their mechanisms:

- biocatalytic group, comprising enzymes;
- bioaffinity group, including antibodies and nucleic acids;
- microbe based, containing microorganisms.

A transducer is generally composed of 3 elements (Fig.14):

1. input interface;
2. sensor;
3. output interface.

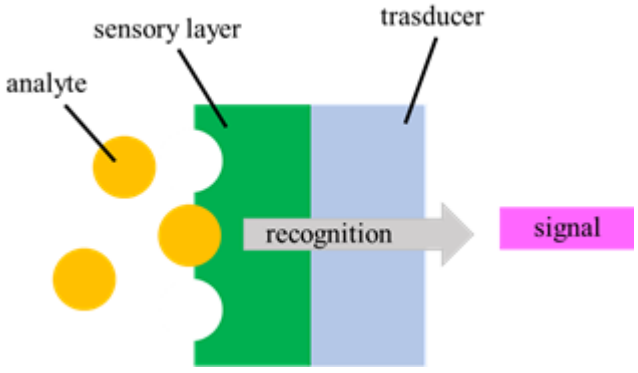


Fig. 14: Transducer graphical representation.

The sensor is the element that converts the physical input quantity into a physical output quantity that can be easily acquired by electrical means. The interaction between the bioreceptor and the analyzed sample modifies one or more chemical-physical parameters. Then, the transducer captures the biochemical variation in the system and produces an electrical signal that can be amplified, processed and read by the operator (Thevenot et al., 1999).

Biosensors are responses to the demand of simple, real-time, selective and inexpensive microbiological analysis. In the last thirty years many different types of biosensors, based on different methods, have been proposed with this purpose. Detection of microbes is based on bioluminescence (Stanley, 1989), amperometry (Pérez et al., 2001), turbidity (Koch, 1970), piezoelectricity (Plomer et al., 1992), optical waveguide (Zourob et al., 2007) and flow cytometry (Gunasekera et al., 2000). The detection time of these methods is faster (from 20 minutes to 2 hours) than traditional method like standard plate count but they all require a series of operations that only a skilled technician may be able to complete in a laboratory.

A method highly competitive with standard plate count is the impedance method (Firstenberg-Eden and Eden, 1984; Suehiro et al., 2003; Gomez et al., 2003). It is fast, since the time required to obtain a reliable measurement is between 3 to 12 hours. It is accurate, suitable to be developed in automated forms and easy to use. It can be implemented in systems able to perform measurements “on-line”. Monitoring parameters in “on-line” mode reduces the time between sampling, delivery and output of the analysis. On-line analysis allows to set up corrective actions immediately, when required.

3.1 The impedance technique (IT)

The Impedance Technique (IT) is based on monitoring the changes of the electrical features determined by metabolism of bacteria (Firstenberg-Eden and Eden, 1984). Impedance (Z) can be defined as the resistance to flow of an alternating current as it passes through a conducting material (Kell and Davey, 1990). The concept of impedance generalizes Ohm's law extending it to circuits operating in a sinusoidal regime (commonly called alternating current): in fact, in a continuous current regime it represents the electrical resistance.

“Z” is described mathematically by a complex number, whose real part represents the dissipative phenomenon and corresponds to the electrical resistance, “R”, in the schematization with elements in series. The imaginary part, called reactance, “X”, is associated with energy storage phenomena and “j” is the imaginary unit:

$$Z = R + jX$$

Z is a vector. The impedance module is indicated with |Z| and is obtained from the following formula:

$$|Z| = \sqrt{R^2 + (jX)^2}$$

The angle between R and X is defined Arg (Z) (Fig. 15).

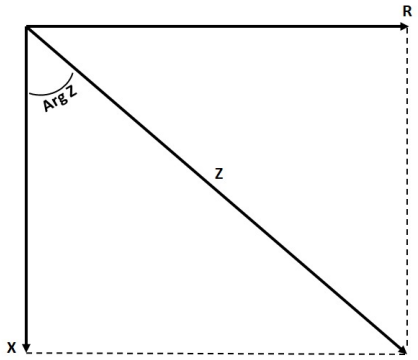


Fig. 15: Vector representation of resistive component (R) and reactive component (X) of impedance (Z).

When the system is represented by a series combination, the application of an alternating sinusoidal potential will produce a resultant current which depends from impedance (Z) of the system, that is a function of resistance (R), capacitance (C) and applied frequency (F).

In this case, Z is represented by:

$$Z = \sqrt{R^2 + (1/2\pi FC^2)}$$

Microbial metabolism affects the electrical parameters, usually resulting in an increase in capacity and a decrease in resistance and impedance. The IT works as follows: the sample is incubated at a temperature which fosters bacterial growth (for mesophilic bacteria in the range 30 - 40 °C). At regular intervals, the impedance parameters (|Z|, R, X) are measured (Cady et al., 1978; Silley and Forsythe, 1996). The user sets a detection criteria and when the rate of change of impedance exceeds this predetermined value the system will detect growth. Since the time needed for the electrical parameter to deviate from the baseline value, hereafter called Detect Time (DT), is linearly related to the logarithm of initial bacterial concentration and this value can be easily worked out. The main drawback of the

IT is that, since bacterial concentration is calculated by measuring the time needed for the microbial population to reach a threshold concentration, the measurements time depends on the time needed for a species of interest to duplicate. It is necessary to correctly choose the incubation temperature in order to minimize differences in generation times among different microorganisms possibly present in the sample under test. The threshold level has been identified as a concentration of 10^6 CFU/ml of *E. coli* (Cady et al., 1978; Silley and Forsythe, 1996). When this bacterial concentration is reached in the sample, Z and R begin to decrease.

3.2 Carpigiani biosensor - background

In order to provide a tool that can be used in various processing stages of ice-cream mixes, Carpigiani developed a biosensor system based on impedance measurement (Grossi et al., 2008, 2009, 2010). The system combines the Sample Under Test (hereafter called SUT) and a couple of electrodes stimulated with a low frequency sinusoidal voltage. The SUT is kept at a constant temperature (generally in the range 37 - 40 °C) suitable for efficient bacterial growth. At regular intervals of 300 seconds, the electrical parameters, Impedance (Z) and (Arg Z), are measured by a couple of electrodes immersed in the mix.

"Z" and Arg(Z) remain almost constant until the bacterial concentration reaches a threshold level which depends on the species and on the substrate (Grossi et al., 2008, 2009, 2010). The threshold level has been identified as a concentration of 10^7 CFU/ml of *E. coli*, the model microorganisms used to validate the device. When this bacterial concentration is reached in the sample, Z and R begin to decrease. The choice to monitor the resistive or reactive components depends on the chemical composition of the sample, since normally one of the components provides more reliable results than the other.

3.3 Project research goal

The objective of the project was to develop an optimized biosensor and to realize a new embedded portable biosensor system for bacterial concentration measurement in milk-based mix for ice-cream, starting from the tool already available (Grossi et al. 2008, 2009, 2010).

Furthermore, the biosensor was integrated in the production line of the new machines in Carpigiani factory, in order to verify the conformity of the machines for heat treatment efficacy.

3.4 Material and methods

3.4.1 Experimental design

In order to obtain a calibration curve for the biosensor instrument, ice-cream mix samples were inoculated with increasing concentration (% vol/vol) of *E. coli.* As a comparison value, the ice-cream mix without inoculum was used as white in each of the tests performed. The samples were analyzed in parallel by both measurement with the biosensor developed during thesis research and plate count enumeration, in order to compare the results. For the plate count technique, the samples were serially diluted in saline solution and the opportune dilutions were plated in duplicate on MacConkey Agar and Plate Count Agar. Plates were incubated for 24-36 hours at 37 °C. For the biosensor, 4 simultaneous measurements were carried out in parallel: three directed to measure inoculated samples and one for the control sample. For each sample, a value of DT was registered. The value of DT assigned for each concentration was obtained from the average of the various detection times signed.

3.4.2 Instrument description

The Fig. 16 reports a schematic representation of the biosensor system developed during this project. The system is composed of an impedance measurement board, a thermoregulation board and an incubation chamber containing the sample under test. The instrument is housed into a suitcase, developed to contain all the elements cited above. The instrument is powered by a transformer 12 Volt/20 Ampere.

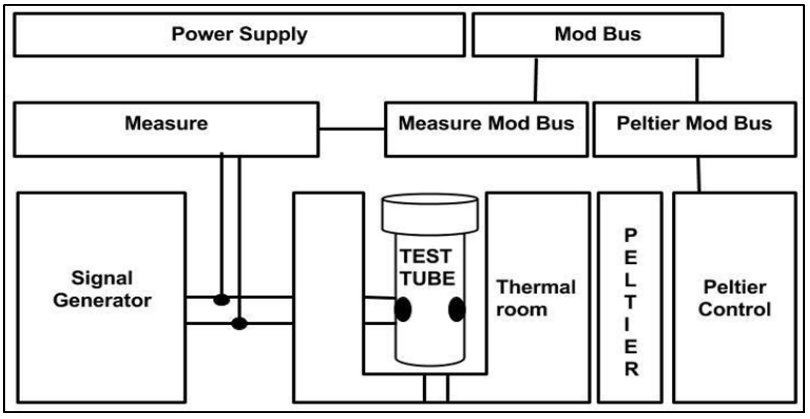


Fig. 16: Schematic representation of the instrument.

- **Test tube**

The test tube contains the SUT (Fig. 17, 18, 19). The containment volume for the test is 5 ml. The material used for the production of test tubes is polycarbonate. It is a thermoplastic polymer, widely used for mechanical properties, such as elongation, tensile strength, impact and bending strength.

Fig. 17: Test tube.

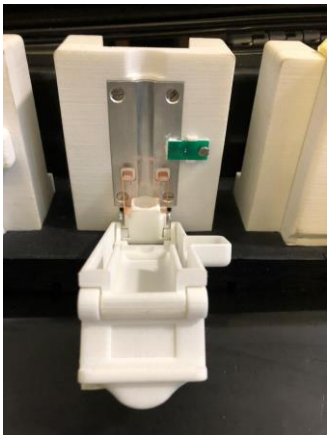


Fig. 18: Thermal chamber.

Fig. 19: Test tube in position in the thermal chamber.

● The Peltier cell

The Peltier cell is a solid-state active heat pump able to generate or extract heat, depending on the direction of the current applied. The electronic board which manages the Peltier cell is able to maintain the temperature of the thermal chamber to the set value with an accuracy of ±1 °C. This is possible by using two PT1000 temperature sensors, which measure respectively the temperature of the thermal chamber and the ambient temperature.

The control of the Peltier cell is done by a bridge of 4 metal oxide semiconductor (MOS) transistors, driven directly by the microprocessor through pulse-width modulation (PWM). The control algorithm is a proportional-integral-derivative (p.i.d.), which uses as feedback signal the temperature of the heating chamber, while the ambient temperature is used to prevent any thermal shock that might impact the structure of the measuring chamber (Fig. 20).

Fig. 20: The Peltier Cell assembled on the measuring chamber.

● Suitcase and software

A design study phase was carried out to develop a in a suitcase containing the biosensor system (Fig. 21).

Fig. 21: External view for biosensor suitcase.

The suitcase has been designed in order to allow the operator to carry out instant measurements. The suitcase is portable and makes possible the analysis of the sample directly next to the ice-cream machine or even at a distance. The data can be displayed in real time. The upper part of the suitcase is occupied by the screen (7 inches, 800x480 pixels) (Fig. 22).

Fig. 22: Touch screen display in the upper part of the suitcase.

In the lower part of the suitcase (Fig. 23), four thermal chambers and the power button are located. The device is switched on after connecting the power cable to the power supply.

Fig. 23: Thermal chamber in the lower part of the suitcase.

Through the touch screen (Fig. 24, 25), four different independent analysis can be carried out simultaneously, selecting the chamber and pressing the “Start” button.

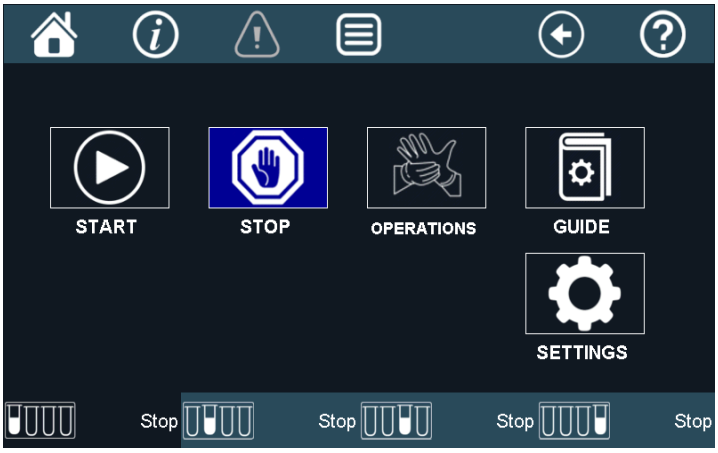


Fig. 24: Screen user biosensor interface.

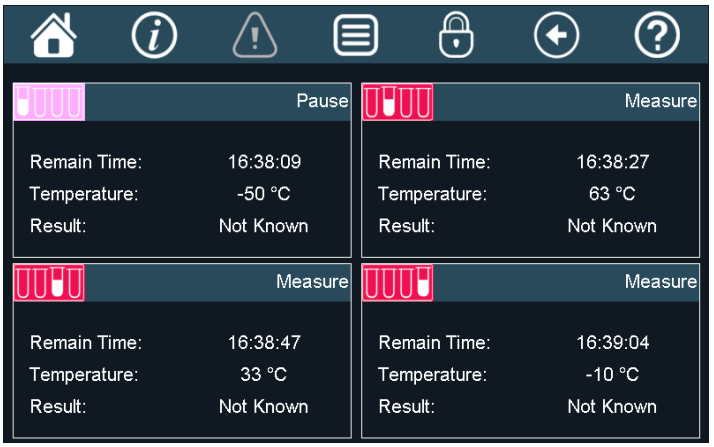


Fig. 25: Screen biosensor interface measurement mode.

Once the instrument has performed the measure, the result is displayed. Clicking on the corresponding chamber on the home screen, the result is represented by a traffic light: the red light indicates that the result is not acceptable (>10^1 CFU/ml *E. coli*), while with the green light the result is acceptable (<10^1 CFU/ml *E. coli*). This is an easy and intuitive way to make the results immediate to an inexperienced operator.

3.4.3 Measurement method

The measurement of the impedance of the ice-cream mix has been done applying to the test tube a sinusoidal voltage signal. The signal is digitally generated by the microprocessor, supplied externally through a digital analog converter and then filtered to delete from the spectrum the high frequency components typical of the digitally generated signals. The signal chosen for the measurement is a sine wave with a frequency of 200 Hz and its amplitude is controlled by the microprocessor in order to optimize the measurement error, which is not possible to avoid. The choice of an alternating current and of a high frequency is aimed to reduce the possible electrolytic effects potentially due to the direct current. The type of the signal and the frequency may be set from the device that controls the entire system through the Mod bus interface.

● Measure phase shift

The phase displacement (PHI- Φ) is the angle between voltage “V” and current “I”. The measurement of the impedance of the sample is obtained by the Ohm's law. The system applies a voltage signal at known frequency to one of the electrodes in direct contact with the ice-cream mix. On the other electrode, the current is measured (Fig. 26).

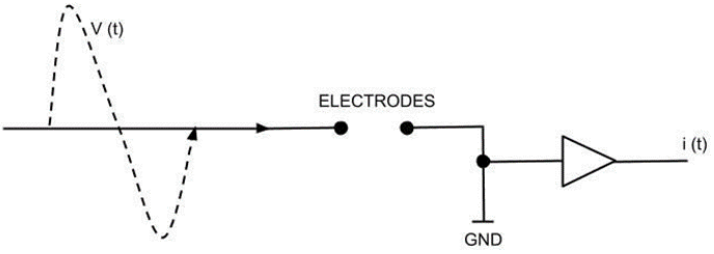


Fig. 26: Schematic representation of impedance measure between the two electrodes.

This process allows the system to calculate the impedance value (Kaspar and Tartera, 1990) as follows:

$$Z = Vin(t)/ Iout(t)$$

When the values are measured, the value absolute of impedance can be estimated, while the phase shift (Φ) is calculated as the time difference between the zero-crossing of the input voltage and the zero-crossing of the output current (Fig. 27). The quality of the microprocessor which measures this lapse of time allows the estimation of the Φ with great accuracy.

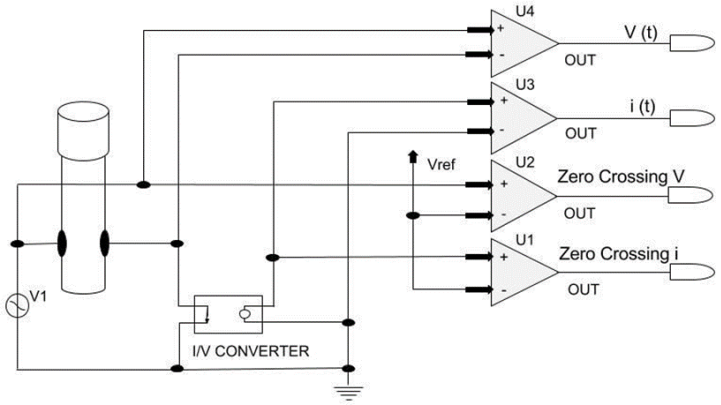


Fig. 27: Schematic representation of the electronic used to manage the measurement.

In a circuit the sinusoidal alternating voltage and the consequent sinusoidal alternating current have the same frequency but normally they are out of phase. Between the two quantities there is a constant phase shift angle (Φ) that is measured in degrees (considering the value Φ = 360 ° for the entire period). At regular intervals, typically every 300 seconds, a small sinusoidal current (Fig. 28) is supplied through the sample and the current-voltage phase shift is calculated.

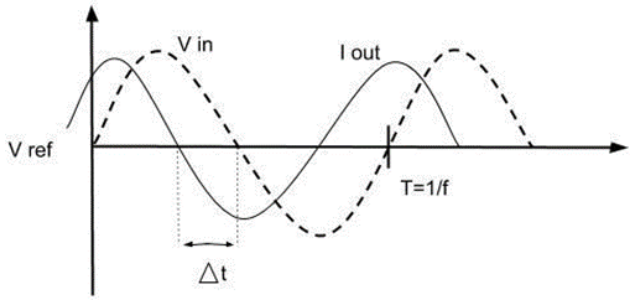


Fig. 28: Representation of voltage and current trends.

3.4.4 Method validation

The evaluations on the curves obtained were performed by combining all the measured points approximated with a polynomial interpolation function. To minimize the error of the curve with the polynomial approximation, a high degree polynomial was used (typically 9^{th}). If "n" is the degree of the polynomial, interpolation will only start if at least "n+1" points are collected. For each new sample, the system calculates the coefficients of the polynomial of degree "n" that best approximates the linear curve that would result from the union of the acquired points. By calculating the derivative of the polynomial point by point, the system can find the shape of the curve over time and automatically evaluate when it changes the slope.

When the decrease of the slope exceeds a threshold value, the system recognizes the DT, which in fact corresponds to the time when the electrical parameters begin to deviate from the baseline value. Given that there a linear relationship correlates the DT value calculated and the logarithm of bacterial concentration, it is possible to determine the level of bacterial concentration present in the sample. More precisely, the DT is inversely

proportional to the logarithm of the initial microbial concentration. The higher is the microbial concentration, the shorter is the DT.

For each sample, the measurement is repeated in three different tests in order to validate the data. However, for each test an initial time of about 45 minutes is required for the stabilization of the temperature.

3.4.5 Media and organism

Escherichia coli ATC11229 was sub-cultured aerobically at 37 °C for 24 hours in Tryptic Soy Broth. MacConkey Agar and Plate Count Agar were used for isolation and enumeration of *E. coli* and total bacteria, respectively. All the media were provided by BD Difco, Sparks, USA.

3.4.6 Inoculum preparation

A known volume of ice-cream mix UHT was inoculated with cultures of *E. coli* diluted in saline water. Increasing concentrations of *E. coli* were obtained by adding a known quantity of bacterial culture volume to a known volume of ice-cream mix (% v/v). The dilutions were prepared to obtain concentrations of *E. coli* from 10^1 to 10^{10} CFU/ml in the ice-cream mix. Ice-cream mix without *E. coli* was also analyzed and used as control sample.

3.4.7 Ice-cream mix

The mix used for the test was UHT ice-cream mix milk-based, containing: 7% fat, 18% carbohydrates, 8% MSNF, 0.5% other solids.

3.5 Results

The biosensor system based on impedance measurement developed during thesis research was used to determine the bacterial load in ice-cream mix. The instrument is able to simultaneously record the parameters of resistance (R), capacity (C) and phase-shift (Φ). Electrical parameters were monitored and recorded over a time span ranging between 2 h and 16 h, applying the same voltage signal at known frequency. Data were collected with a sampling time of ten minutes, then recorded and saved by the software.

The ice-cream mix without inoculum and the samples inoculated with known bacterial loads of *E. coli* ATC11229 ranging from 10^1 to 10^{10} CFU/ml were analyzed with the portable biosensor in order to check the electric response of the samples.

In Fig. 29, 30, 31 are presented the trends of all the electrical parameters registered for different initial inocula of *E. coli* and for the sample control. The values reported are the average of 3 independent experiments, each one performed three times. Standard deviation resulted always less than 5%.

The trend of resistance values (μΩ) measured for each inoculum concentration are shown in Fig. 29. The parameter R drop immediately after the first three hours of measurements. Then, the values tend to stabilize and remain constant throughout the rest of the measurements. The ice-cream samples with concentration 10^3, 10^4 and 10^5 show an initial value of resistance lower than the other concentrations tested. These samples show a less marked decrease in resistance values within the first three hours of measurements but still stable over times until the end of measurements.

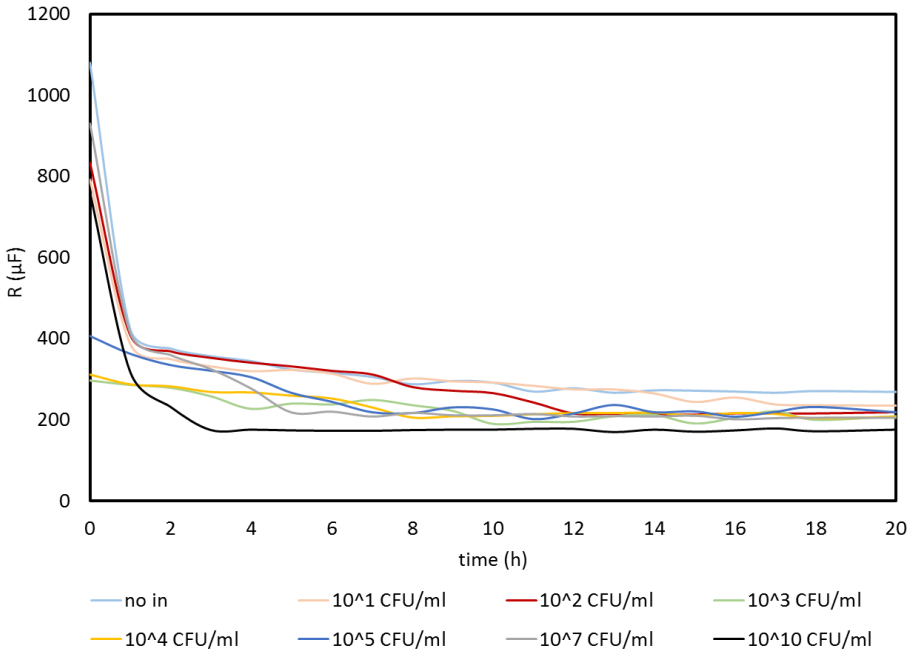


Fig. 29: Time trend of the measured R value for each analyzed concentration of *E. coli*.

Fig. 30 shows the trend of the C (µF) for each inoculum concentration of *E. coli*. Different ice-cream mix samples, inoculated with increasing concentration of *E. coli*, showed a marked variation in measured values over time. Increasing the initial load, the deviation from the baseline value of the capacity appeared gradually in less time.

Once the change in slope of the observed curves occurred, the samples reached a peak in capacity and then stabilized at a value that was lower than the peak itself (Fig. 30). The capacity value reached in the peak did not show correlation with the concentration of the samples themselves. The maximum peak height was very similar among all the tested samples, reaching 6000 µF. Only the sample inoculated with a concentration of 10^3 CFU/ml of *E. coli* reached a lower peak (4000 µF).

The sample of ice-cream mix without inoculum showed a trend almost linear over the entire measurement time.

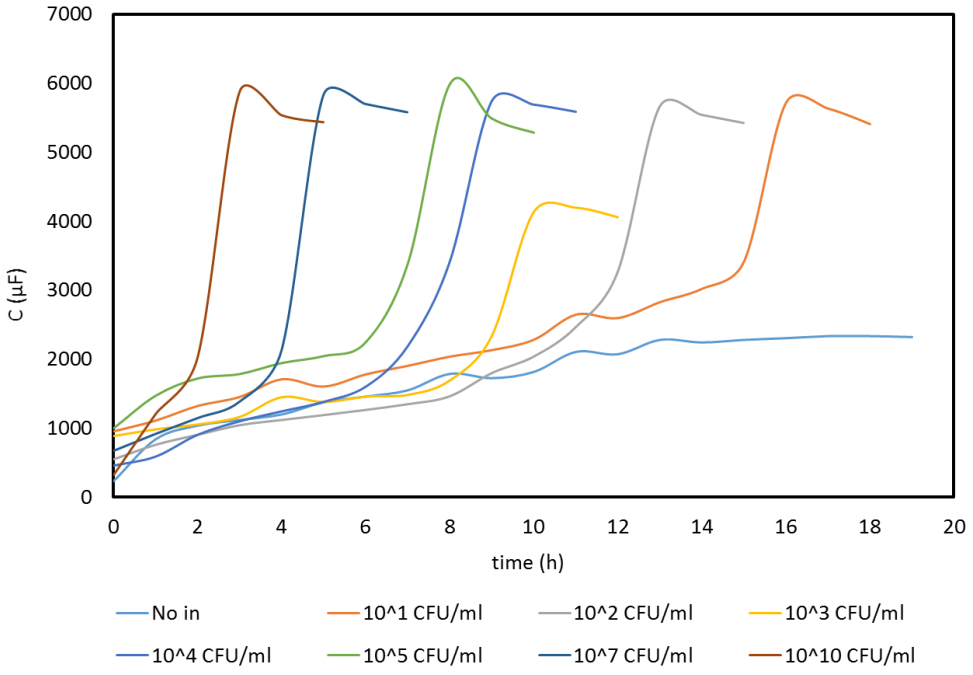


Fig. 30: Time trend of the measured C value for each analyzed concentration of *E. coli*.

The trend of the Φ (mrad) for each inoculum concentration of *E. coli* revealed that *E. coli* inoculum determined a drop of the curve, with an increase of DT decreasing the bacterial load (Fig. 31). DT ranged between 2 hours for 10^{10} CFU/ml and 16 hours for 10^{1} CFU/ml, without taking into account the 45 minutes necessary to reach the constant temperature required.

The ice-cream mixes not inoculated didn't show any change in the shift value over the whole time of the measurements. Once reached the DT, data acquisition continued until a constant value was reached.

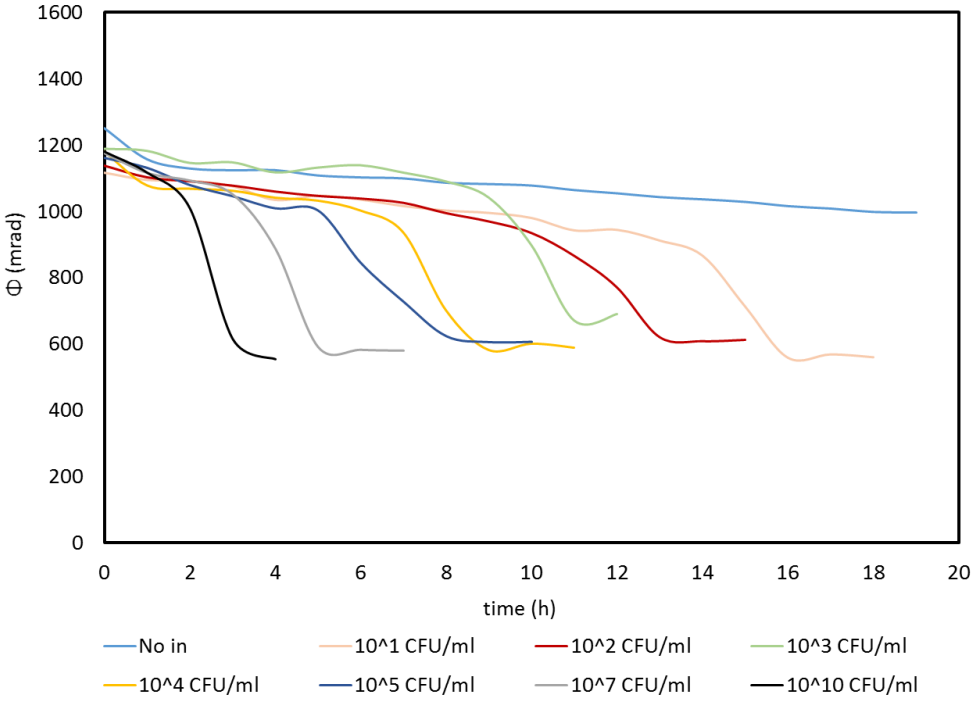


Fig. 31: Time trend of the measured Φ value for each analyzed concentration of *E. coli*.

A linear relationship between the DT value calculated and the logarithm of the bacterial concentration can be extrapolated (Fig. 32), making possible to determine the order of magnitude of bacterial concentration present in a test sample. Indeed, DT was inversely proportional to the logarithm of the initial microbial concentration. Since the bacterial concentration was calculated by measuring the time required for the microbial population to reach the threshold concentration. The measurement time depends also on how long it takes a bacterial cell to replicate.

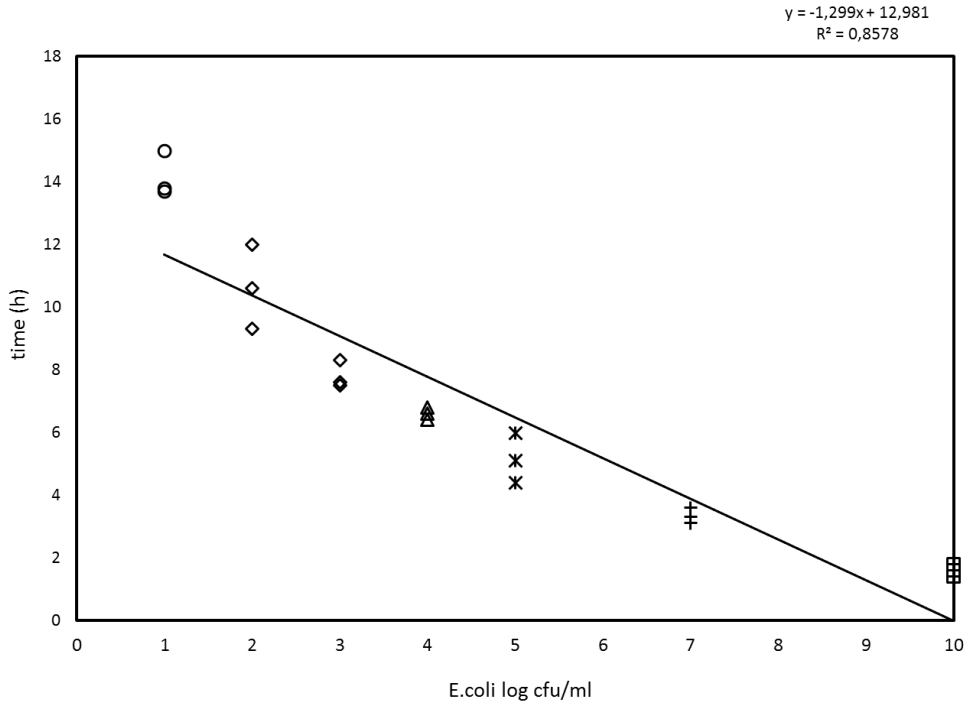


Fig. 32: DT measured by the instrument for each concentration of *E. coli* in the UHT milk-based mix for ice-cream.

3.6 Discussion

Starting from the first studies on impedance applied to the ice-cream mix and from the prototype device developed by the company (Grossi et al., 2008, 2009, 2010), the new biosensor improved the measurement technology exploiting a new interpolation data of Φ value. The measurement of the Φ value resulted a reliable tool to determine the microbial load in milk-based ice-cream mixes.

The curves did not show any background noise. The algorithm applied removed the noise and produced more reliable and reproducible measures. The use of Φ value as parameter related to the bacterial load allowed analysis of ice-cream mix samples within a wide range of *E. coli* bacterial concentrations. Previous studies reported two different DT values calculated for the capacity curve and for the resistance curve, respectively, and only in a second time these values were compared to each other (Grossi et al., 2008, 2009, 2010). The possibility to work with a single curve resulting from the two variables considered in the system allowed a consistent response and facilitated the final measurement operations.

An intuitive software allowed the operator to manage the measurement using a touchscreen display to see and verify the measurement results. Also, concentrations of *E. coli* higher than 10^7 CFU/ml have been analyzed thank to the new measurement system taking into account the parameters of capacity, resistance, impedance and phase shift angle. The DT was calculated for ice-cream mix samples inoculated with a dosed bacterial load of *E. coli*, ranging from 10^1 to 10^{10} CFU/ml, applying the voltage signal at known frequency.

The ice-cream mixes not inoculated didn't show any change in the values of R, C and Φ over the time. Where the inoculum was added, a drop of the Φ curve was detected, as well as for resistance curve, with an increase of the DT decreasing the bacterial load. Instead, capacity curves recorded showed an increase in values of detection time. DT ranged between 2 h for 10^{10} CFU/ml and 16 hours for 10^1 CFU/ml, ruling out the 45 minutes necessary to reach the constant temperature required. Once reached the DT, data acquisition continued until a constant value was reached.

The instrument does not aim to be as precise as the plate count enumeration but it describes the microbiological status of the sample, showing the presence of potentially harmful bacteria. Furthermore, it can be integrated with existing systems. The device requires a small sample of ice-cream mix, it is easy to use, portable and potentially it can be exploited also for other dairy products, improving the quality control for food industries and environmental monitoring, scalable for distributed control within the food supply chain. It

can offer low cost analysis useful for the customer to plan cleaning operations of the ice-cream machine on the basis of bacterial charge.

3.7 Application of the portable biosensor system in the internal testing process of Carpigiani production line

Once assessed the measurement reliability of the upgraded biosensor, established a calibration curve, the biosensor has been integrated in the production line of the new machines in Carpigiani factory, where after machine assembling, they are tested. The process of assembling new ice-cream machines involves the calibration of the probes of temperature, tests of production processes and of heat treatment cycles. At the end of the tests, the machines are cleaned and sanitized, then placed in storage rooms until the sale. Within this context, the exploitation of the biosensor is integrated between the production and the final sale of the machine (Fig. 33). With the aim of verifying the conformity of the machines in terms of heat treatment efficacy and make line operators more aware of their key role in the company during the test of new machines, kaizen flow has been implemented with this further step.

Every three weeks, a pasteurizer machine is randomly selected among the machines in stock. Specialized technicians check all the operating parameters of the machine. Once the correct functioning has been assessed, the heat treatment efficacy test is performed through a challenge test using the biosensor.

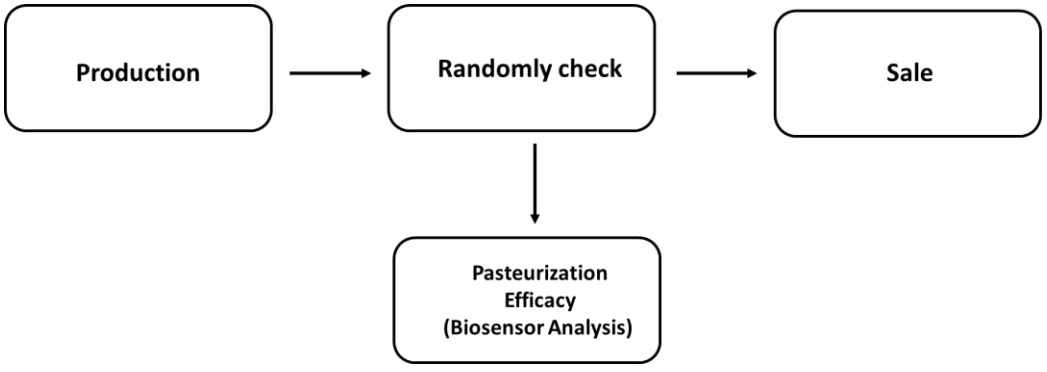


Fig. 33: Kaizen flow and biosensor check point.

The test flow continues only if the machine is compliant to heat treatment efficacy. Conversely, if the result is not compliant, the test to determine heat treatment efficacy is repeated, in order to understand if there is a problem on the machine.
Test procedure for line operator requires the following steps:

1. pick up the machine from stock;
2. clean and sanitize the machine following Carpigiani manual instruction;
3. fill the machine with fresh and clean ice-cream mix;
4. inoculate a known volume of ice-cream mix with *E. coli* suspension at the concentration of 10^6 CFU/ml, according to NSF International Standard /American National Standard NSF/ANSI 6 – 2018 Dispensing Freezers_Point 6.5 (NSF food equipment standards include requirements for material safety, design, construction and product performance. It is used by the company internally as a reference standard).
5. perform a heat treatment cycle;
6. upon completion of one heat treatment cycle, collect 4 samples of ice-cream from the machine. One from the tank and three from the production cylinder.
7. analyze the samples using the biosensor:

   7.1 insert the samples to be tested (5 mL) in the tubes;

   7.2 switch on the biosensor and starting the measurement acquisition;

   7.3 check the test result.

Future applications of the biosensor will be aimed to test the use inoculating bacterial strains other than E. *coli* and validating the use of the suitcase even in contexts outside the company, such as at directly at the ice-cream machine owners.

4. Novel approach to extend the shelf-life of ice-cream mixes for a centralized production laboratory

In recent years there has been a growth in the phenomenon of the relocation of one or few ice-cream mixes production centers who prepare the product to be sent directly to many sales points (Fig. 34). This work approach is placed halfway between artisanal and industrial production. Ice-cream mixes can be produced and packaged in a production center, stored and transported to sales points. Once the sales points are reached, the ice-cream mixes are transformed into ice-cream. This working method has the advantage of standardizing a type of product, allowing the sale in points located throughout the territory.

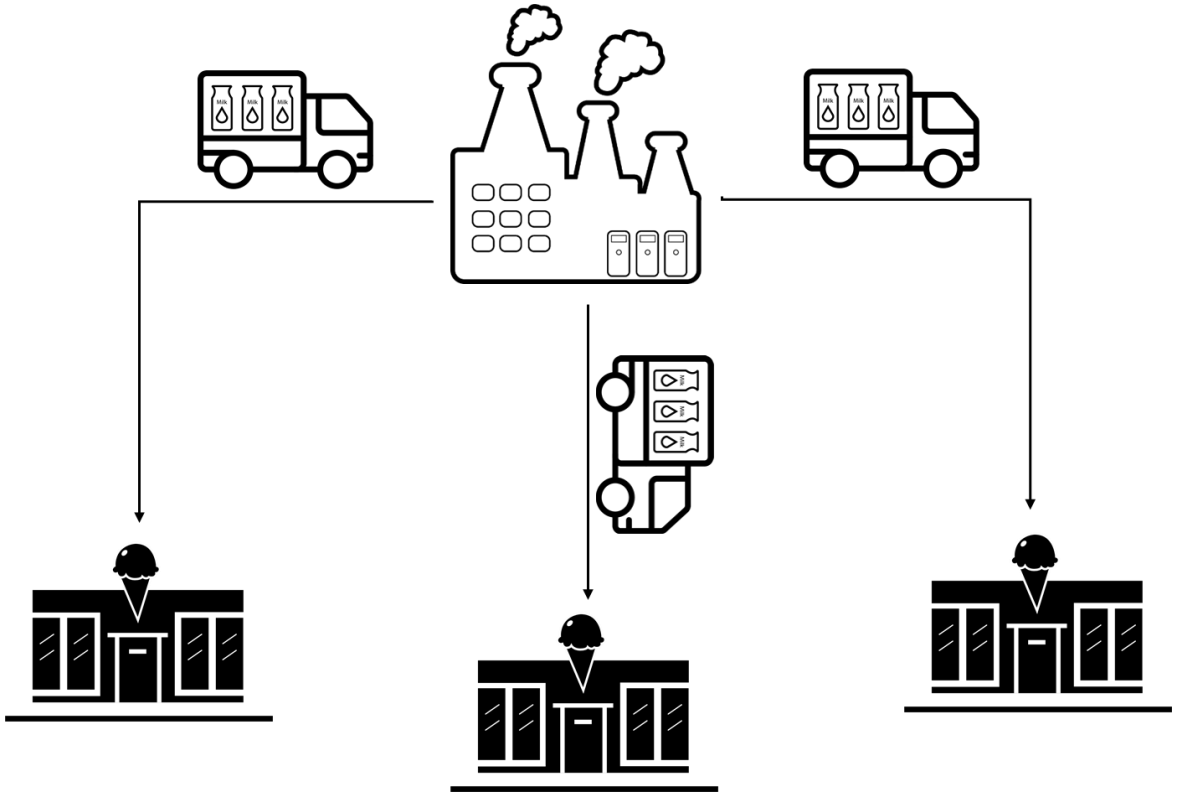


Fig. 34: Example of ice-cream mix distribution in the case of a centralized production laboratory.

The choice to set up an ice-cream centralized laboratory involves the development and management of various factors that allow the production of a guaranteed quality product to different sales points:

- production equipment must be able to handle volumes that are adequate for higher production than single ice-cream shop;
- the container for storing the product must be adequate;
- the transport methods must be defined and respect the cold chain where required.

Replicating quality and recipes in multiple shop is very difficult. The quality of a product is defined by food safety criteria but also by the whole sensorial and nutritional features that characterize a food. For these reasons it is important to know the shelf-life of a product.

4.1 Shelf-life

The Institute of Food Technologists (IFT) in the United States has defined shelf-life as "the period between the manufacture and the retail purchase of a food product, during which time the product is in a state of satisfactory quality in terms of nutritional value, taste, texture and appearance" (Anon., 1974). Not all the conditions of use of a product provide for instantaneous consumption. It is therefore possible that a package with product inside is opened, not completely consumed and re-maintained. The period after pack opening during which a food product maintains an acceptable quality level is defined as "secondary shelf-life" (Nicoli, 2012). The shelf-life envisaged for the product in intact packaging certainly cannot remain valid for the product after opening; it is the manufacturer's responsibility to supply indications on the label on the methods and on the storage times in this phase. This is an important aspect both for products intended for domestic use and for large-capacity products intended for fractionated sales within the gastronomy departments of large-scale retail trade or catering services. In the open product the degradation processes are accelerated and the risks due to the handling of the product increase. The private product of the protection due to the packaging system, in particular for vacuum-packed products and in modified atmosphere, comes into contact with the external environment and with new possible contaminating and altering agents. When a period of conservation is assumed after the opening must be taken into consideration these aspects, in some cases exactly as if it were a new shelf-life study.

A shelf-life study must include a preliminary phase of evaluation of all aspects and characteristics of the product which may have an influence on conservation. It is therefore important to identify:

- the raw materials that make up a particular food;
- the chemical, physical characteristics of the food;
- the processing processes to which the food is subjected;
- storage conditions.

To define a shelf-life product it is necessary considering the interaction between different factors. Some of these are directly related to the product and therefore defined as intrinsic. Others, instead, do not depend directly on the product but condition its shelf-life, also called extrinsic. The commercially required shelf-life of a product is given by the sum of intrinsic and extrinsic characteristics.

Among the intrinsic parameters to be considered, there are:

- formulation of the foods;
- ingredients;
- modalities of degradation.

Among the extrinsic parameters to be considered, there are:

- environmental conditions (temperature, humidity, etc.);
- work equipment;
- packaging.

Another aspect to consider is that the shelf-life of a product can be conditioned also by external factors and mainly dependent on human causes. This is the case of “human environment” where abnormal behavior on the part of buyers may result in a acceleration of the degradative reactions and therefore a decrease in the shelf-life. Transport, movements, replenishment, consumer manipulation, cold chain interruption are other factors that can reduce the shelf-life of a product (Koutsoumanis et al., 2005).

The information collected have the function of making a first estimate on which dangers and degradation phenomena to expect. These considerations may be supported by additional information collected in bibliography or analytical tests.

It is not easy to establish the limits that define the acceptability of a food product. A food can be bacteriologically compliant but poor in quality following a preservation with loss of aromas. A food may not show visible changes at the time of consumption but may not be bacteriologically compliant.

The shelf-life of a food is given by a balance of the different characteristics:

- legal limits;
- sensory attributes;
- market demands.

The preliminary study of the product is useful for formulating a first hypothesis on the shelf-life and to plan possible tests that can confirm or not this hypothesis. However, the microbiological conditions are not the only ones to determine the quality and edibility of a product. It becomes important to guarantee the persistence of qualitative or nutritional characteristics. For this reason, a shelf-life analysis is coupled with sensory analysis.

4.1.1 Bacteriological analysis

Food packets and containers have to preserve the quality of food in microbiological terms. Some foods, as ice-cream mixes, represent a good growth medium. Bacteria can growth also during storage conditions at low temperatures. The shelf-life in this way ends, as well as if the organoleptic characteristics change, when the product became unacceptable due to the growth of undesirable microorganisms. Growth of pathogenic organisms should be ever prevented or controlled in order to reduce potential risks for the consumers. The critical limits for a food can vary among countries, since consumers in different geographical areas can assign different values to the acceptable quality of a food, due to different cultures and different taste.

4.1.2 pH analysis

The pH is the index of the degree of acidity. The pH range for ice-cream mixes varies from 3 to 7. Generally, for milk-based mixes the average measured pH is around 6.5 (Silvestroni, 1996). This value may vary depending on the type of ingredients used to prepare the mix. For fruit-based mixes and sorbets, the pH is mainly influenced by the characteristics of the fruit used and the mean value is around 3.5. In yogurt-based ice-cream mixes the pH is around 4.5.

4.1.3 Sensory analysis

Sensory analysis has been defined as "a scientific method used to awaken, measure, analyze and interpret those responses to products that are the result of perception through the senses of sight, smell, touch, taste and hearing" (Stone and Sidel, 1993). Sensory analysis in the strict sense is a scientific method, born in the mid-twentieth century and regulated internationally by ISO standards (see paragraph 6) through which reliable and comprehensive measurements can be derived from human senses. It is possible to speak of sensory analysis only when tasting:

- blindly: without knowing what you are tasting or at least without specific information that may influence the judgment;
- as a group: a minimum number of tasters is provided for each type of assessment in order to provide statistically significant results;
- with a method: each taster performs the same operation, aimed at obtaining information that responds to specific questions.

A valuable sensory evaluation requires:

- repeatability: similar evaluations are given to the same products, even if tasted at different times;
- discrimination: different evaluations are given to different products;
- collimation: a judgment in substantial agreement with other tasters who carry out the same assessment is given.

- **Sensory analysis of the ice-cream**

Everyone is able to express personal evaluation of the ice-cream tasting experience. Ice-cream testing combines chemical and physical properties. Ingredients and manufacturing processes affect sensory properties. Only few experts can detect defined flavor, texture defects or other specifications. There are three different approach to evaluate frozen desserts for sensory quality:

- industry expert analysis;
- trained expert analysis;
- untrained consumer analysis.

Among consumers the judgments and impressions can vary a lot even evaluating the same taste. They do not have the training or the sensitivity to make informed evaluations. To obtain a reliable result, the number of consumer panels would be greater than that of expert panelists.

Sensory evaluation is perceived mainly through the human senses. The stimuli generated by the environment are collected by the sensory system through the sense organs, each designed to detect a particular type of physical or chemical stimulus. When the external agent (distal stimulus, be it physical or chemical) comes into contact with the receptors, nerve cells of which every sense organ is endowed in large numbers, transform it into an electrical signal (proximal stimulus) through transduction. This impulse runs through the nerves, which behave similarly to electrical cables, reaching the brain that decodes it and organizes it through basic cognitive processes and dynamic psychological processes that result in perception. This regulates the behavior and the response to the stimulus. This explains why different tasters can provide different evaluations.

In the evaluation of any product it is customary to start from sight, although it is now established that visual stimuli often arrive together or after others, for example, the smells and noises that occur when entering the ice-cream parlor. Sight is a physical sense that allows the perception of the external environment through light, a form of electromagnetic energy. The sense organ in charge of detecting signals is the visual system whose primary element are eyes. Color is the way our brain encodes the wavelength of energy reflected from the surface of the ice-cream. The white light is given by the set of electromagnetic waves included in the visible field: these have a variable length between 400 and 760 nanometers. Depending on the wavelength of the energy reflected by the ice-cream on the retina, the perception of color vary from red to violet. It is possible to visually perceive the grain of the ice-cream because the particles that make up the structure reflect light in different ways and measures: ice crystals will therefore be brighter than other components.

Another sense used for the analysis of ice-cream is certainly the tactile one. Tactile can be expressed by different types of bodily sensitivity, also called somesthetic system. Tactile sensations are essential to define artisanal gelato.

In order to conduct an accurate and uniform analysis within the panel test it is important to define conditions, tools and assessment procedures to be respected:

- present samples randomly without revealing sample identity to analysts;
- evaluate all the sample at the same light;
- analyze the samples at the same room temperature (20 - 24 °C), limiting the differences in the melting rates;
- taste the ice-cream not too cold, because the cold tends to numb the mouth and tongue of the analyst and in order to avoid difficulties with the spoon;
- taste the ice-cream not too warm to properly judge body and texture attributes;
- taste the ice-cream as soon as served at a temperature between -7 ° and -13 °C.

In order to obtain a targeted technical evaluation, the sensory analysis aimed at understanding the characteristics to be evaluated by the consumer should also reproduce the consumption gestures.

The ice-cream can be evaluated either in cone or cup with an ideal scoop:

- the cone is a wafer edible container that avoids the tactile evaluation in the mouth. The influence of the wafer on the perception of ice-cream must be considered and the wafer needs a sensory analysis by itself.
- the cup is used in combination with the scoop. In order not to alter the visual perception of the ice-cream surface, the cup should be white at least inside. The ideal cup thermally insulates the ice-cream for as long as possible. The cup should have a shape that balances the contact surface with the air (subject to melting) compared to the thickness of the ice-cream; it should also allow a handle that does not heat the walls in contact with the ice-cream.
- ideal scoop is of smooth plastic and it does not interfere with the sensation of cold on the palate. It should be easy to handle, with a good grip, flat and thin.

Ice-cream can therefore be evaluated by:

1. visual analysis;
2. olfactory analysis;
3. tactile and gustatory analysis.

For each of these analysis methods it is important to standardize and define the main characteristics taken into consideration for each sample.

Ice-cream – visual analysis

Parameters useful for the visual definition of ice-cream are:

- color intensity defined as chromatic intensity of the ice-cream, given by the concentration of the coloring pigments present in the ice-cream and visible on its surface. Color saturation in ice-cream depends on type of coloring pigments, abundance of coloring pigments in raw materials, overrun or quantity of air incorporated in the ice-cream, evolutionary and conservation stage, in relation to the stability of the ice-cream;
- color tone is the type of color that ice-cream assumes in the color spectrum, produced by the set of wavelengths of light reflected by the surface itself. The color tone depends on the color of the primary ingredients used, processing phases that can change the color scheme of the raw materials or ice-cream, evolution and conservation stage of ice-cream, especially with raw materials that tend to oxidize;
- lucidity/homogeneity surface is the characteristic of the surface to reflect light in a single direction without deviating or absorbing it. It is understood as the opposite of opacity. The perfectly creamy, spatulated and served ice-cream at the right temperature tends to be ideally opaque on the surface. Any lucidity may be due to: % of sugars, % of lean milk solids, unbalancing in the recipe or incorrect processing factors that lead to the presence of a lot of free water in the ice-cream or temperature changes during storage, which lead to the gradual release of free water.

Ice-cream – olfactory analysis

The olfactory analysis expresses the measure of the quantity of perception obtained by direct olfactory sense, that is the odors perceived through the nose, both negative and positive.

Parameters useful for the olfactory definition of ice-cream are:

- intensity allows us to express an opinion on how a certain typical taste is smelled;
- aromatic persistence measures the intensity and duration of aromatic sensations after swallowing, whether they are positive or negative.

Ice-cream - tactile and gustatory analysis

Tactile sensitivity is given by different types of perceptions:

- chemical: volume, viscosity shape;
- physical: astringent, prickly, spicy, metallic.

4.2 Project research goal

A shelf-life study of ice-cream mixes has been performed. This should allow the preparation on the mix in production centers and the use to prepare the ice-cream in the sales points. An improved packaging process has been developed. It provides a standardized quality in a multi-location gelato retail concept, with the aim to make available equipment and processing methods to work in a centralized laboratory.

The project developed within the Carpigiani company is based on a work process that results in:

- ice-cream mix processed with a pasteurizer machine;
- storage at positive temperature 4 °C until the whipping stage;
- with a shelf-life of 28/30 days.

The volume of product to pack was identified in 3 L, the minimum quantity useful to obtain ice-cream, preferably gelato, ready to sell. Single-use portions containing ice-cream mixes are prepared, that cannot be stored after opening. Then, secondary shelf-life studies have not been performed.

The shelf-life study focused on the quality of the product from different points of views. For each product, ice-cream obtained with the mixes stored into the bags have been analyzed and tested throughout the expected storage period, evaluating bacterial load, pH trend over time, and sensory features.

4.3 Materials and methods

4.3.1 Experimental design

Recipes and equipment for the shelf-life study of ice-cream mixes has been set. The stability of the products processed and stored according to the tested method was assessed, monitoring microbial charge and pH value. The stability tests were performed during storage at 4 °C for a month. Assessed the stability of the ice-cream mixes over the shelf-life, a professional bag filling machine was developed. It allowed the transfer of the mix after heat treatment from the pasteurizer machine into the chosen container, in this case a bag. The development of this tool was carried out to reduce the manual operations and limit the risk of contamination. A pH analysis and a bacteriological shelf-life study of ice-cream mixes, packaged using the bag filling system and stored at 4 °C for a month, has been carried out. Once the microbiological stability of these has been confirmed, a sensory analysis was performed.

4.3.2 Process description and analysis of critical control point

One of the main objectives of the HACCP system is the prevention (see paragraph 1.5). HACCP system serves the food business operator to identify hazards, manage products and operate in a controlled way. Within a production process it is therefore possible to establish in production manuals codified production procedures and good manufacturing procedures (GMP). The shelf-life study is directly correlated to the working process. The evaluations carried out during this study were focused to the development of a method easy to perform and able to give to our customers a standardized quality in a multi-location gelato retail concept. Within the project, an analysis of possible critical points was carried out.

As a first step, a list of working operations to be performed during ice-cream mix production with a particular attention to packaging phase was drawn up, in compliance with the requirements of the HACCP system.

The operations, internally defined for the project, are:

1) wash and sanitize the equipment, to work in a clean environment;

2) clean and sanitize the hands, wearing appropriate work apparel, respecting all prevention measures to avoid possible contamination;

3) clean and sanitize the components of the machine according to the procedures recommended by the manufacturer;

4) insert the ingredients inside the machine and start the heat treatment cycle;

5) wash and sanitize all the components of the mix transport system;

6) connect the conveyor tube to the base of the pasteurizer, avoiding to contaminate the far end of the tube by placing it in a clean bucket;

7) place the end of the conveyor tube near the bag filling hole, open the pasteurizer, and handle dispensing;

8) transfer the ice-cream mix into the bag at the end of the selected heat treatment cycle;

9) start the stirring function on the machine, fill the bag up to the maximum capacity;

10) check that the area around the bag inlet, the hole and the cap are clean and close the cap;

11) record the bags with the label;

12) store at a positive temperature (4 °C) all the bags prepared;

13) control the temperature recorded in rooms or equipment used for store the product.

Notes:

- The time required to fill a 3 liters bag is about 30 seconds;
- The time required to empty a production batch of 60 liters of ice-cream mixes is about 15 minutes.

Table 3 reports the analysis of critical control point (CCP) for the process described immediately above:

Step/ Element	Type of danger and risk	Critical limits	Preventive measures	Check	Corrective actions	Recordings
Use of a mix trasport system	- Damage and wear of the trasport system - Risks of contamination (chemical, physical, microbiological)	- Physical: absence of foreign bodies - Chemical: sanitizing absence	- Correct storage - Correct sanitizing use - Proper cleaning and sanitizing before use - Correct use of work equipment according to the manufacturer's instruction	With each use	- Component replacement in case of breakage - Proper cleaning and sanitizing of system trasport mix	Equipmenet maintenance Log
Bags	- Physical alterations of the bags - Risks of contamination (chemical, physical, microbiological)	Absence of migration from the bag to the mix	- Correct storage - Check product conformity on receipt (check specific purchase techniques)	With each use		Product compliance with every purchase

Table 3: Analysis of CCP.

4.3.3 Pastomaster 60 RTL

The pasteurizer machine Pastomaster 60 RTL (Fig. 35) was used for the test:

- it has a large capacity of the processing tank. It can work with a minimum of 15 liters to a maximum of 60 liters;
- it is designed to work the mixes even at different speeds, allowing the mix of solid ingredients with the liquid ones;
- it preserves the characteristics of the mix during the storage phase;
- it can age the mixes during the storage.

The technical information of the machine are:

- rated 400 V, 50 Hz, 3 ph;
- water cooling;
- code/matr.: IC89120.

Fig. 35: Carpigiani Pastomaster 60 RTL.

One of the possible applications of this machine involves the use of a mix transport system. The machine extraction tap (Fig. 36) can be coupled to a conveyor tube which makes the extraction of the mix more comfortable.

Fig. 36: Pastomaster 60 RTL extraction tap.

The Fig.37, 38, 39 show in detail the pieces that make up the transport system.

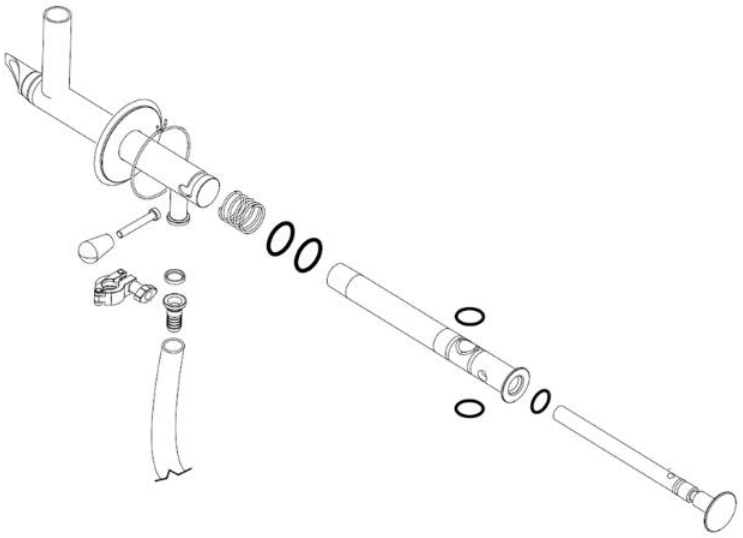


Fig. 37: Exploded connection components and extraction tap Pastomaster 60 RTL.

Fig. 38: Extraction tube, o-ring and locking ring.

Fig.39: Pastomaster 60 RTL extraction tap connected to extraction tube.

4.3.4 Maestro HE ***

Ice-cream mix storage in bags has been frozen and ice-cream obtained was compared with ice-cream prepared with freshly mix. The machine used to freeze the product was Maestro HE ***.

The batch freezer machine Maestro HE *** (Fig. 40) was used for the versatility:

- it can work with a minimum of 3 liters to a maximum of 15 liters;
- freezing cycles are available depending on the recipe and the quantity of product available.

The technical information relating to the machine used is shown below:

- rated 400 V, 50 Hz, 3 ph;
- water cooling;
- code/matr.: IC134643.

Fig. 40: Carpigiani Maestro HE***.

4.3.5 The recipes

The ice-cream mixes are formulated with ingredients that have specific chemical-physical characteristics that give the typical taste to each recipe. The choice of mixes to be tested was aimed to analyze milk-based mixes with diverse and opposite features, including a higher fat content than regular milk ones (chocolate) and fruit ice-cream mixes that present lower pH, sugar and fat content. The recipes tested were:

- fior di latte;
- hazelnut;
- chocolate;
- custard cream;
- strawberry.

The recipes are presented in Table 4:

	fior di latte	hazelnut	chocolate	custard cream	strawberry
Whole milk	1 liter	1 liter	1 liter	1 liter	-
Low-fat milk powder	75 g	10 g	20 g	35 g	-
Cream	385 g	40 g	135 g	200 g	-
Sucrose	270 g	260 g	220 g	280 g	0.73 g
Glucose syrup dry	50 g	40 g	35 g	50 g	0.22 g
Dextrose	41 g	55 g	77 g	45 g	0.11 g
Stabilizer SE30	9 g	8 g	8 g	9 g	-
Hazelnut pasta	-	145 g	-	-	-
Cocoa 22-24%	-	-	95 g	-	-
Chocolate 70%	-	-	80 g	-	-
Yolk pasteurized egg	-	-	-	150 g	-
Water	-	-	-	-	1 liter
Stabilizer SL24	-	-	-	-	0.02 g

Table 4: Ingredients for recipes tested.

4.3.6 Heat treatment cycle

The heat treatment cycles available on the Pastomaster machine are different. Those mainly used are the low and high heat treatment cycle (see paragraph 1.4). In addition to these two cycles, a specific cycle for chocolate is available. The table 5 shows the heat treatment cycles of the machine Pastomaster RTL 60 and the corresponding trend of temperatures.

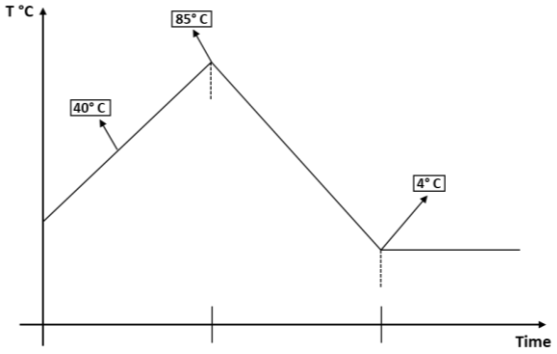
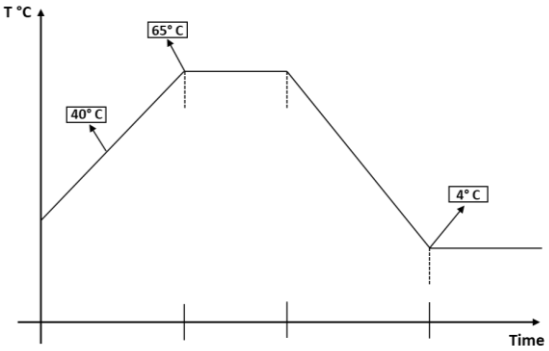
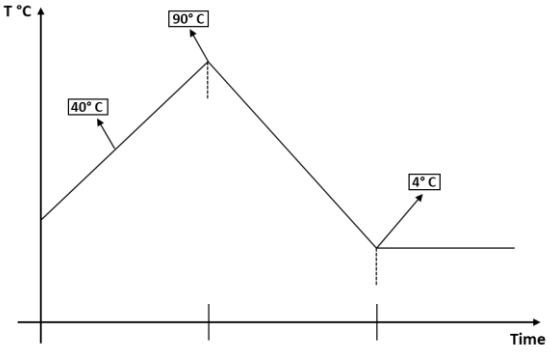
Cycle	Temp. °C	Graphics	Internal taxonomy
High heat treatment	**85 °C**		**Cycle A**
Low heat treatment	**65 °C**		**Cycle B**
Heat treatment for chocolate	**90 °C**		**Cycle C**

Table 5: Pastomaster 60 RTL heat treatment cycle representation.

In order to enhance the final taste of ice-cream mixes, a specific processing heat treatment was defined for each recipe (Tab. 6)

Recipe	Heat treatment (internal taxonomy)
fior di latte	cycle A
hazelnut	cycle A
chocolate	cycle C
custard cream	cycle B
strawberry	cycle A

Table 6: Combination recipe/heat treatment cycle used

4.3.7 Packaging

The temperature represents the largest environmental variable that affects ice-cream shelf-life. It can change mostly in the distribution channel of a product, then it must be controlled and kept within the limits set for each product. Conditions of temperature, relativity humidity, and exposure to light are factors that affect the choice of packaging and the shelf-life of the product. Relative humidity is the second environmental variable and it is not always controlled. In the air, oxygen represent the principal factor of degradation. Exposure to light affects shelf-life by accelerating the degradation of the product. The choice of the package and its characteristics are very important to limit as much as possible the degradation phenomena that would otherwise alter the characteristics of the product. Oxidation, loss of aromas, acquisitions of "off-flavors", development of aerobic microorganisms, loss of effervescence, color changes, and many others defects may be the consequences of an exchange of gas through the packaging. For tests it was therefore of primary importance to identify the right container to store the product, on the basis of shape and material. The shape and the dimension of the package influence the shelf-life. Testing the shelf-life of a product using different size packages, made with the same material, results in a shorter shelf-life results for the smaller package.

This effect is due to surface/volume area in contact with the container that is larger for smaller package (as reported in Fig. 41, Robertson 2009).

Surface Areas of Different Package Shapes, All with a Volume of ~450 mL

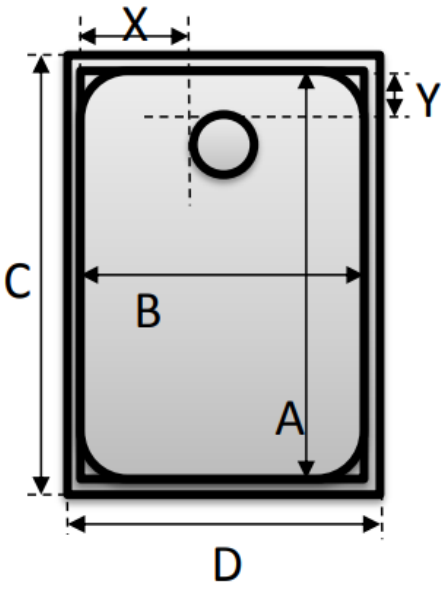
Shape	Dimensions cm	Surface Area		Increase %	Surface Area: Volume Ratio
		cm^2	m^2		
Sphere	Diameter 9.52	285	0.0285	0	0.63
Cylinder	Diameter 7.3 Height 10.8	331	0.0331	16	0.73
Cube	Sides 7.67	353	0.0353	24	0.78
Tetrahedron	Sides 15.65	424	0.0424	49	0.94
Rectangular pack	Height 3 Length 15 Width 10	450	0.0450	58	1.0
Thin rectangular pack	Height 1 Length 20 Width 22.5	985	0.0985	246	2.18

Fig. 41: Surface areas of different package shapes for volume of~450 ml.

Food packaging can be produced starting from different materials such as metals, aluminum, glass, plastic, paper, each with its proper characteristics and performances (Lee et al., 2008; Piringer and Baner, 2008). These materials can be little permeable to small molecules such as gases, water vapor, organic vapors and other low molecular weight compounds, with the exception of plastic polymers. For plastic polymers permeability is affected by the ambient condition, with temperature as the main factor.

Containment and protection are the functions of packaging. Sometimes a single material does not meet all the food protection requirements. Multilayer solutions are therefore increasingly used. Different materials are used in combination, in order to enclose in a single structure all, the positive features that each material can offer. An external and an internal film part can be distinguished.

In this study, bags have been provided by Liquid box SPAIN, S.L.U. A representation of the structure of the bags is reported in Fig. 42, 43.



Length int. (A)	380
Width int. (B)	295
Lenght ext. (C)	396
Width ext. (D)	311
Spout (X)	129
Spout (Y)	30

Fig. 42: Dimension of the bag.

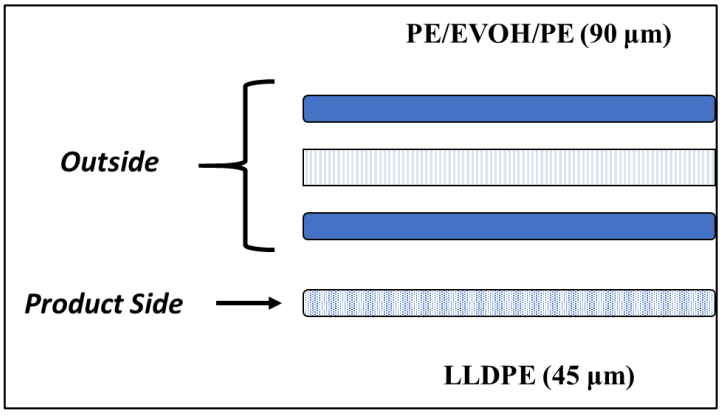


Fig. 43: Bag multilayer solution adopted for the tests.

The external side of the bag is a multilayer of polyethylene (PE) and Ethylene-vinyl-alcohol (EVOH).

PE is one of the most commonly used polymers (Fig. 44). It is commonly obtained from the copolymerization of ethene with longer chain alkenes.

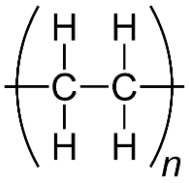


Fig. 44: Polyethylene chemical structure representation.

EVOH is an excellent gas-barrier with very good chemical resistance (Fig. 45). It is largely used as intermediate barrier layer in multilayer structures. It delays the ingress of oxygen reducing deteriorations process (Lopez–Rubio et al., 2018).

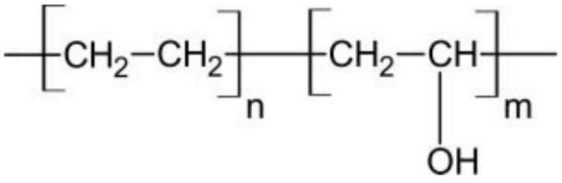


Fig. 45: EVOH chemical structure representation.

The internal side of the bag is in Linear Low Density Polyethylene (LLDPE, Fig. 46). LLDPE is often used as an intermediate layer in multilayer structures based on polyolefins (PE or PP) that are not very sensitive to humidity. This material is largely diffused for the excellent impermeability to oxygen, carbon dioxide and aromas, provided that it is protected from the effects of humidity. Low density linear polyethylene is structurally different from the common low density polyethylene (LDPE, low-density polyethylene), due to the absence of long branches (long chain branching).

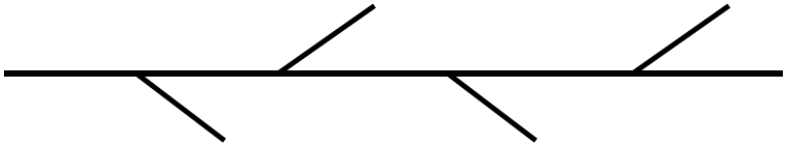


Fig. 46: LDPE structure representation.

In addition to the dimensions and materials, the definition of the closure cap is important. The cap chosen (Fig. 47) is single-position, hand-removable cap designed primarily for use in the dairy industry.

Fig. 47: Bags used during the tests.

The color of the bags was chosen transparent for a communication reason. In this way the color of the ice-cream mix can directly communicate the contents of the bag. This solution is also dedicated to store layouts with a laboratory and exposed cold rooms. In this way the customer is able to follow the different stages of production.

- # Labeling

The bags were identified by labeling (Fig. 48), showing the main characteristics (flavor, ingredients, Lot.N.).

Ice-cream mix flavor:			
Ingredients	***grams***	***supplier***	***Lot.N.***
Milk	-	-	-
Cream	-	-	-
Sucrose	-	-	-
Dextrose	-	-	-
Glucose Dry	-	-	-
Skimmed milk powder	-	-	-
x	-	-	-
x	-	-	-
x	-	-	-
x	-	-	-
Date of production			
Expiration date			

Fig. 48: Example of label used during the tests.

4.3.8 Microbiological analysis

Microbiological analysis for each recipe of ice-cream-mix were performed at the end of the heat treatment cycle (4 °C), taking a sample directly from the tank of the machine. Subsequently, every seven days starting from the first upon the achievement of 28 days, the packaged samples were analyzed taking a sample from the bag.

MacConkey Agar and Plate Count Agar were used for isolation and enumeration of *E. coli* and total bacteria, respectively.

Mannitol Salt Agar, was used to the isolation of Micrococcaceae (presumptive *Staphylococcus aureus*).

Potato Dextrose Agar, was used to isolation of yeasts and molds.

All the media were provided by BD Difco, Sparks, USA.

For each sample, acceptance criteria were evaluated according to Regulation (EC) n. 1441/2007:

- the total bacterial count (PCA): $\leq 10^4$ CFU/ml;
- the coliforms count (MCA): ≤ 10 CFU/ml;
- the count for Gram-positive bacteria and Micrococcaceae (presumptive *Staphylococcus aureus*) (MSA): $\leq 10^2$ CFU/ml;
- yeasts and molds (PDA): molds $< 10^2$ CFU/ml and yeasts $\leq 10^4$ CFU/ml. This limit is not established by law but reference can be made to the experience or guidelines in the bibliography.

4.3.9 pH analysis

Dispersion of pH measurements of ice-cream mixes stored in bags at 4 °C was used to evaluate the samples. The pH of the ice-cream mix was evaluated by pH meter EUTECH_pH700 every 7 days during the shelf-life tests. The value of 6.5 has been chosen as freshness reference value for ice-cream mixes milk-based. The value of 3.5 has been chosen as freshness reference value for ice-cream mixes fruit based. A decrease or increase of 2.0 points respect the reference value has been considered not acceptable.

4.3.10 Statistical analysis

One-way analysis of variants (ANOVA), followed by Tukey's *post hoc test,* was used to evaluate differences in microbiological counts and pH across the different time points. Differences were considered significant for $P < 0.05$.

4.3.11 Improvement of work equipment

A design study was started to improve the bag filling system in order to make it more manageable and safer for the operator. The tool developed was designed to improve the user's manual operations, the food safety of the process and the filling speed of the bag itself. In the Fig. 49 the 3D study of the bag filling machine is shown.

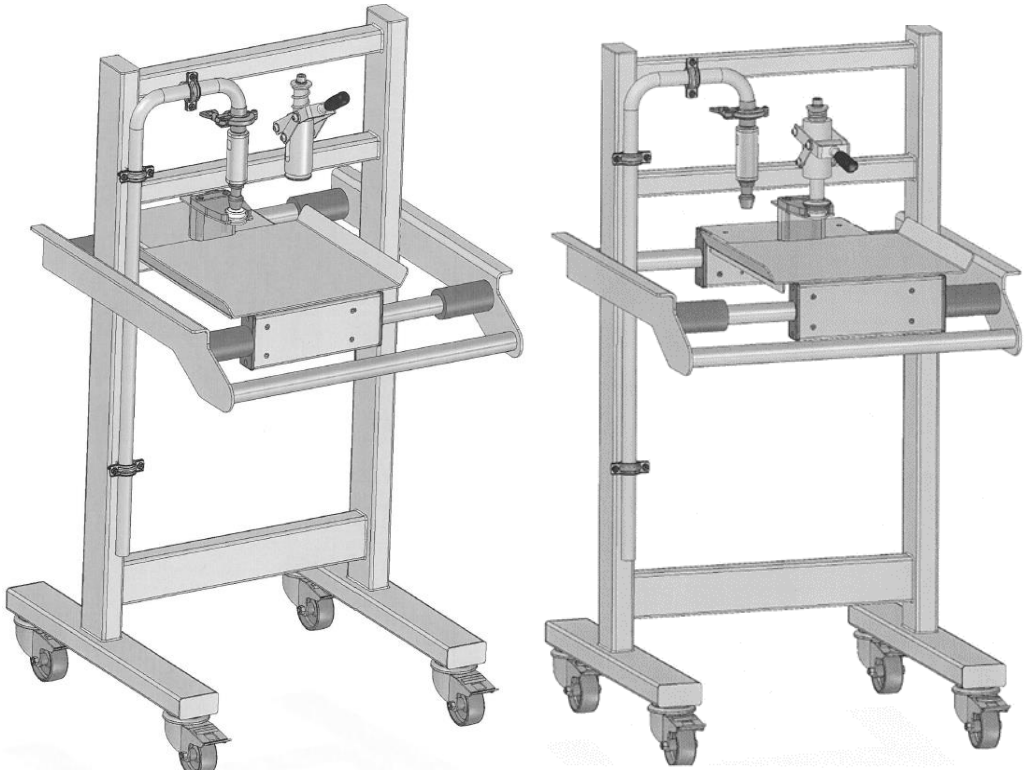


Fig. 49: Carpigiani bag filling - 3D representation.

The main elements of the bag filling machine are shown in the Fig. 50, 51, 52:

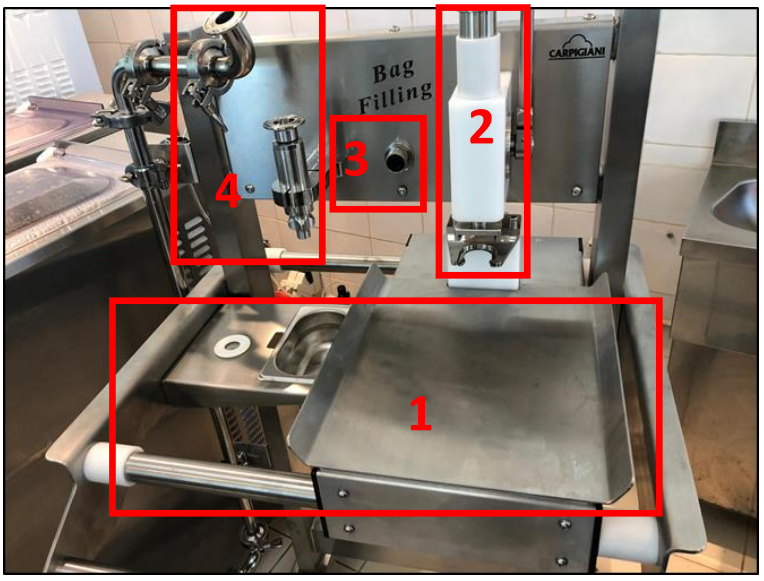


Fig. 50: Main components of work on bag filling machine.

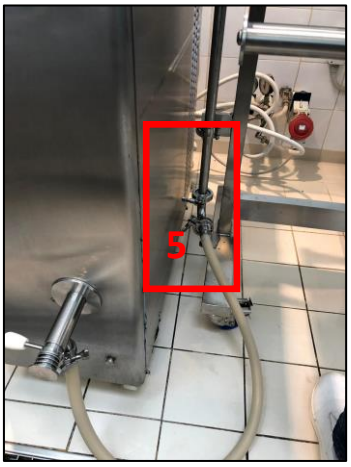


Fig. 51: Connection point between Pastomaster 60 RTL-Bag filling machine.

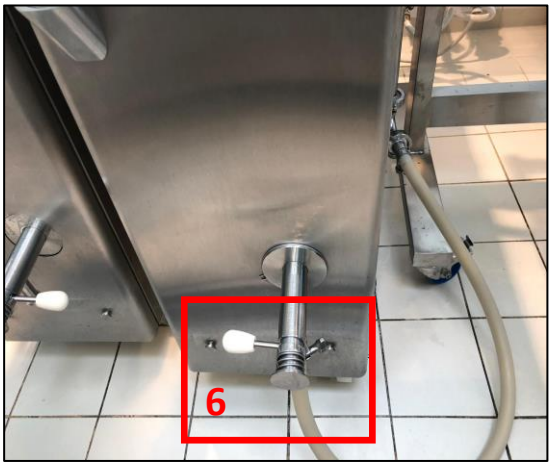


Fig. 52: Extraction tap connected to the bag filling transport tube.

- The ***box 1*** in Fig. 50 shows the mobile trolley on which the bag is placed during the filling phase. The bag is positioned and held in place. in this way the operator limits direct contact with the bag cap;
- The ***box 2*** in Fig. 50 at the bottom shows the fixing area of the bag cap. At the top of the same box is present the handle for opening and closing the bag cap;
- The ***box 3*** in Fig. 50 shows the button that opens the mix retention valve of the bag filling machine;

- The ***box 4*** in Fig. 50 shows the connection between the mixture transport tube and the delivery point placed immediately above the cap of the bag;
- The ***box 5*** in Fig. 51 shows the connection between the bag filling machine and the transport system of the Pastomaster 60 RTL;
- The ***box 6*** in Fig. 52 shows the connection between machine extraction tap and the conveyor tube that transfer the mix after heat treatment from the pasteurizer.

The operations for use the bag filling machine are organized as follow:

1) Wear disposable gloves. Clean and sanitize all equipment provided;

2) Open the extraction tap of Pastomaster 60 RTL turning the white knob counterclockwise (Fig. 53):

Fig. 53: Pastomaster 60 RTL extraction tap in open position**.**

3) Place the bag cap, in the appropriate seat provided (Fig. 54):

Fig. 54: Empty bag placement.

4) Pull the handle upwards to open the cap of the bag (Fig. 55);

Fig. 55: Bag cap opening.

5) Place the bag below the bag filling dispensing point, moving the cart to the left, push the extraction button (Fig. 56).

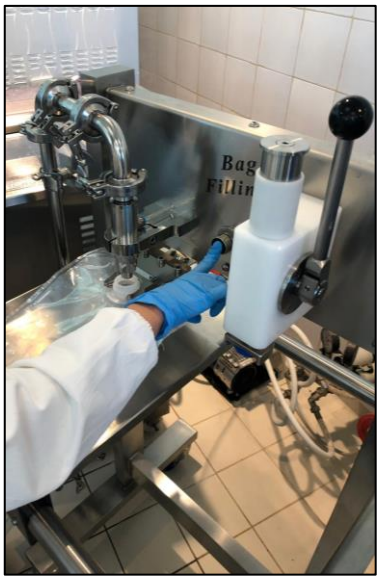


Fig. 56: Start filling bag.

6) Keep the extraction button pressed until the bag is full (Fig. 57).

Fig. 57: Filling bag.

4) Move the cart to the right and pull the handle downward (Fig. 58).

Fig. 58: Bag closing.

5) Label the bags (paragraph 4.3.7);

6) Store the bags at 4°C.

4.3.12 Ice-cream sensory analysis - method and score

The use of a protocol is very important in order to standardize the results of a sensory analysis. Bodyfelt et al. (1988) developed a scorecard and a scoring guides for ice-cream, that has been the starting point to develop the evaluation protocol for panel tests of ice-creams carried out in Carpigiani. The method adopted was:

1. Hold the cup correctly, taking care not to touch the walls in direct contact with the ice-cream it contains, which could accelerate the heating and the consequent fusion;
2. Observe the color, the way and the extent to which the surface reflects light and its structure, noting the texture of the ice-cream and the possible presence of visible solid particles. Take care to do this just after the service and not to prolong it much, because the surface of the ice-cream will tend to melt quickly and become more glossy. In this case remove the molten surface layer with a clear gesture, and observe how the underlying structure looks;
3. Bring the cup close to your nose. Inhale for about 3 seconds;
4. Use the scoop to check the resistance of the product. Observe the mechanics with which the ice-cream behaves during and after picking and pulling;
5. Examine the body and the texture;
6. Take ice-cream by sinking the scoop into the cup (5-10 grams) and place it in the mouth, leaving it to the tongue, until it spreads over the entire surface of the tongue and swallowing, without make any artificial movement;
7. Evaluate the flavors (sweet, possibly sour, bitter, sometimes salty) and tactile stimuli: consistency, cold sensation, melting speed, crystallization, tactile homogeneity and any residual oiliness after swallowing;
8. Check the meltdown dishes for melting qualities;
9. Record accurately and clearly the results of the analyses.

The judges for each parameter express an opinion among those reported in tab to which corresponds a numerical score.

The parameters evaluated during the sensory analysis carried out in Carpigiani were:

- consistency: the tactile perception of mechanical resistance given by the first bite of ice-cream to the compression between tongue and palate, which defines the gradation from soft to hard;

- cold: the sensation during ice-cream contact with the oral cavity surface. The thermal perception is not from the temperature of the ice-cream itself but from the speed with which it absorbs heat;
- melting speed: the measure of the speed with which the ice-cream passes from the solid to the fluid state;
- crystallization: the presence of ice crystals embedded in the ice-cream paste, perceptible by pressure and rubbing from the tactile receptors placed on the oral cavity mucosa;
- homogeneity: the ability of ice-cream to melt completely, without leaving lumps, sand or floury on the surface of the oral cavity;
- greasiness: the sensation of perceived fatness;
- sweet: the intensity of the flavor given by sugars;
- acid: the measure with which the taste of acid in ice-cream is perceived, which is revealed during the first seconds of its dissolution on the tongue with a contraction of the salivary ducts on the sides of the tongue. Many relate this sensation to freshness, in the case of fruit ice-creams;
- bitter: the measure with which the bitter taste is perceived, which begins to reveal itself after the first seconds of contact of the ice-cream with the tongue and can persist for a long time after swallowing. Bitterness is also called the sentinel of taste in that it indicates the presence of compounds with a suspicious effect on the organism, which is why its level of perceptibility and tolerability is very variable in the population;
- equilibrium: the value of gustatory and tactile pleasure of an ice-cream.

Regarding on the score phase, for each chosen attribute in relation to the preestablished sensory specification the evaluation was organized as follow (Table 7):

Judgment	***Score***
Absent	0
Low	3
Medium	6
High	9

Table 7: Score system adopted for sensorial evaluation.

4.4 Results and discussion

4.4.1 Microbiological analysis results

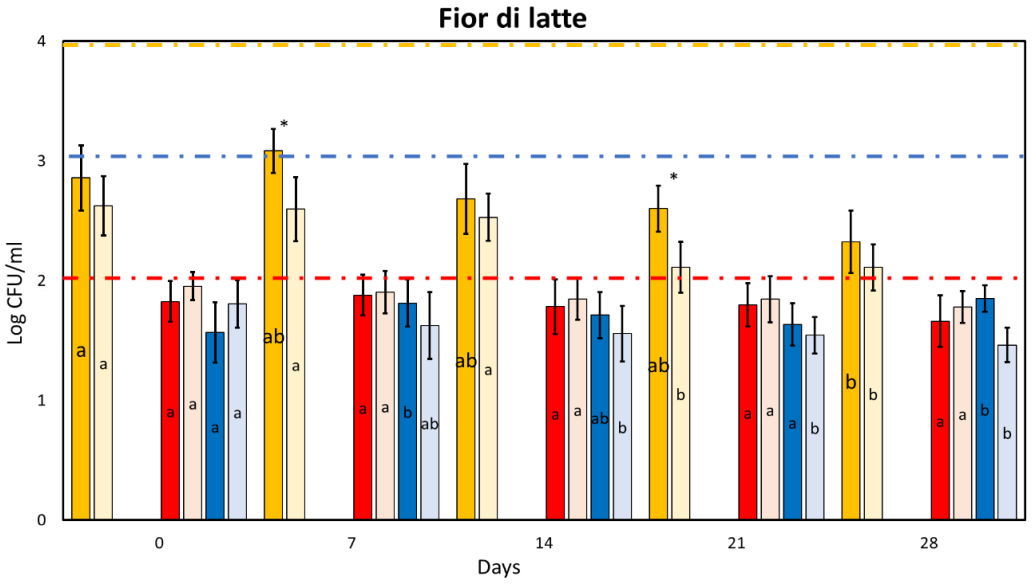
Microbiological stability of the product packaged with bag filling indicate compliance to the requirements (Regulation (EC) n. 1441/2007) (Fig. 59 A, B. C, D, E). The number of microorganisms must not exceed the set limits imposed by the law.

At the end of the shelf-life (28 days at 4 °C), ice-cream mix samples were below the set limits in both tests, aimed to verify: 1) the stability of the product itself; 2) the stability of the product packaged with bag filling machine. No coliforms were detected in any sample (legal limit: < 10 CFU/ml). This result was taken as marker of the hygienic process conditions.

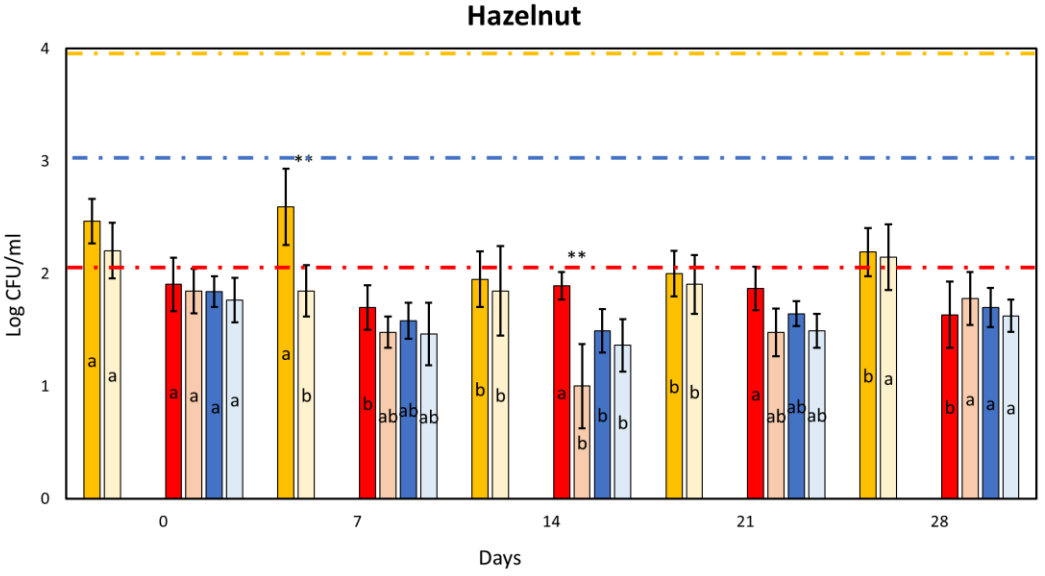
Micrococcaceae (presumptive *Staphylococcus aureus*) resulted in all the samples below the permitted legal limit (10^2 CFU/ml).

The search of yeasts and molds is necessary in food samples, where they cause deterioration from an organoleptic point of view (smell, taste, color and texture) and can be visible (the limit of these contaminants is not established by law but reference can be made based on the experience or by guidelines in the bibliography. Generally, the limit for molds is less than 100 CFU/g, while for yeasts less than 10.000 CFU/g). Ice-cream mix samples analyzed were below the set limits in both tests.

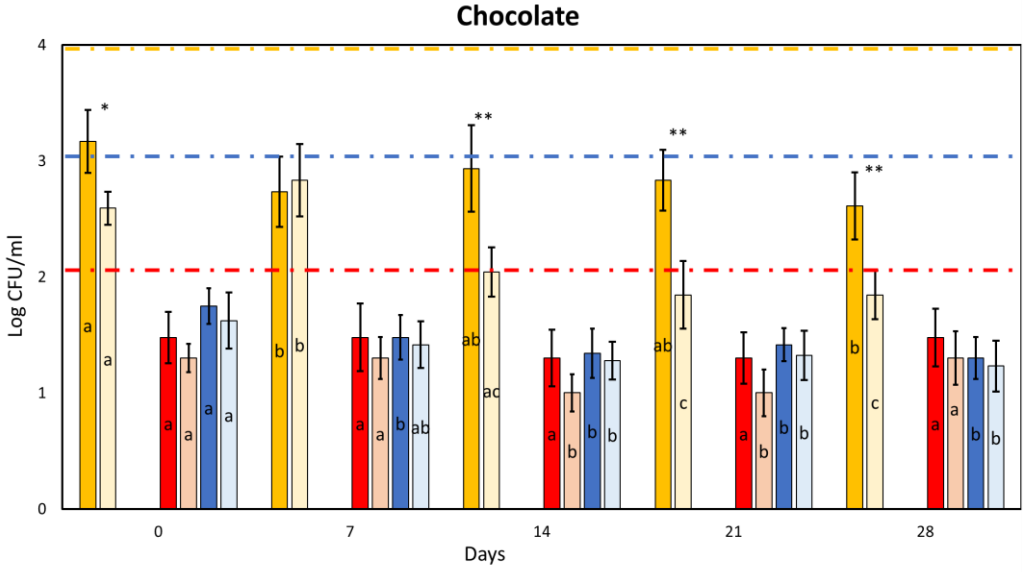
A


Fior di latte
Log CFU/ml
Days
0
7
14
21
28

B


Hazelnut
Log CFU/ml
Days
0
7
14
21
28

C


Chocolate
Log CFU/ml
Days
0
7
14
21
28

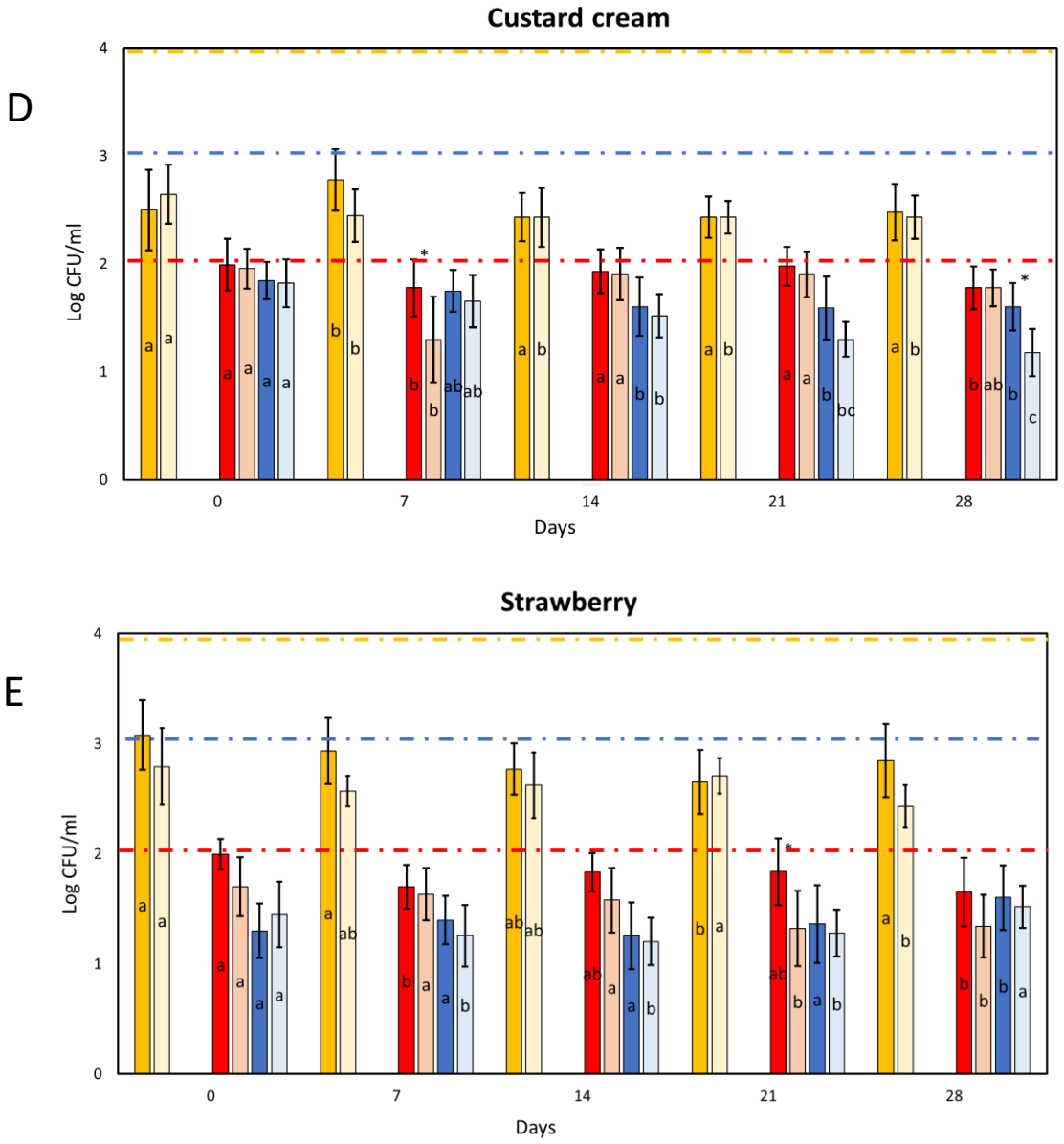


Fig. 59: Microbiological analysis of fior di latte (A), hazelnut (B), chocolate (C), custard cream (D), and strawberry (E) ice-cream mixes. Colors: total bacteria (■, ■), micrococcaceae (■, ■), and yeasts and molds (■, ■). Dark shades indicate samples packaged with the conveyor tube from Pastomaster 60 machine, light shades indicate samples packaged with bag filling machine. Values are means ± SD, n=3. Within each series, means sharing the same letter did not significantly differ ($p > 0.05$). Dash-dotted lines are the limit values for total bacteria (yellow), micrococcaceae (red), and yeasts and molds (blue), according to European Regulation.

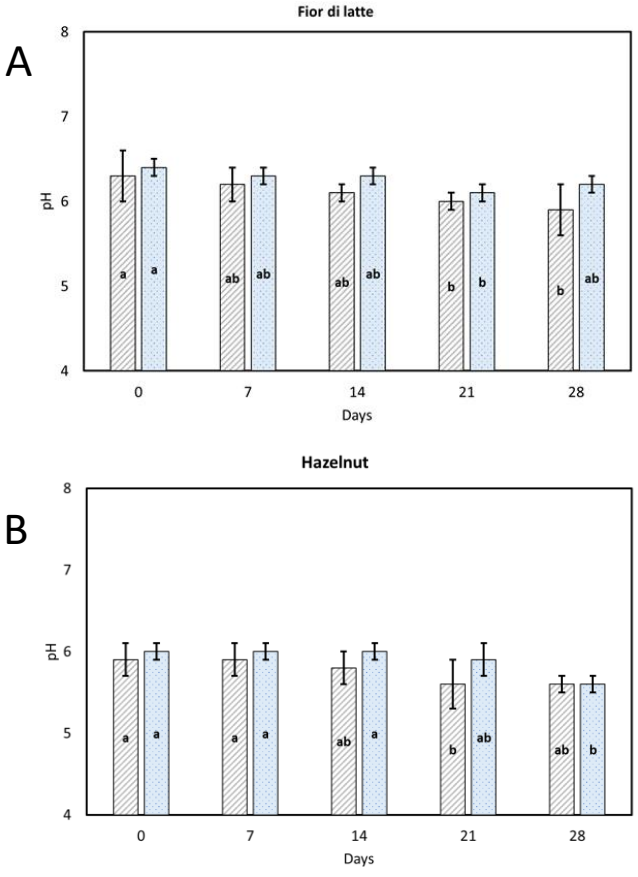
4.4.2 pH analysis results

The pH of ice-cream mix is related to the composition. The normal pH of milk-based mixes is 6.3, and it is taken as a reference for freshness. pH analysis was used to assess the degree of freshness of the ice-cream mixes. During the shelf-life test, milk-based ice-cream mixes showed a pH decrease (Fig. 60, panel A, B, C, D, E).

The starting value of mixes fior di latte and chocolate were 6.4, according to the freshness parameter. During the shelf-life, pH showed a trend of acidification not consistent with a change of the organoleptic properties of the mix. This difference was less marked in products packaged using a bag filling system, albeit significant differences between the two packaging systems were not found.

The mixes custard cream and hazelnut showed a lower initial pH, because of the ingredient composition. Custard cream ice-cream packaged through the bag filler presented the lowest change of pH during the shelf-life, without significant differences over the time in the pH of the bag filling system.

pH of hazelnut ice-cream tended to decrease during the shelf-life. For ice-cream fruit mix, pH value of the starting reference can vary between 2 and 5. This depend essentially on the type of fruit used. Albeit strawberry ice-cream mix showed an initial value of 2.7-2.8, significant acidification was detected at the end of the shelf-life.



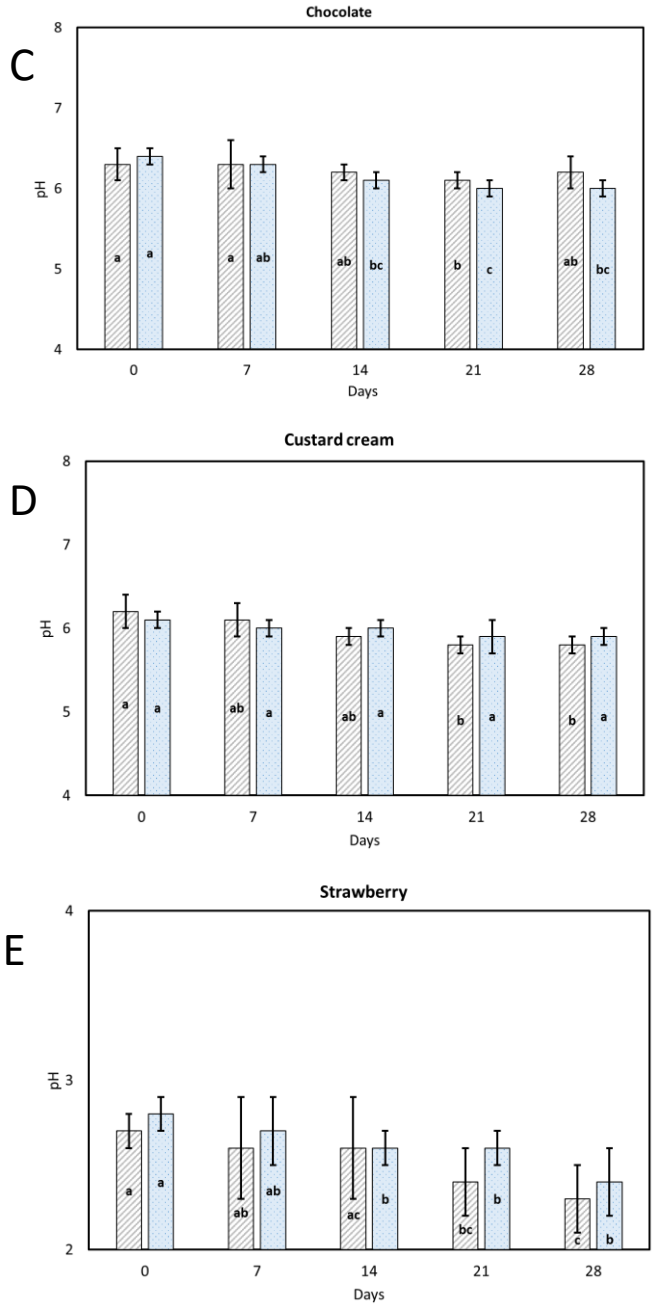


Fig. 60: pH of fior di latte (A), hazelnut (B), chocolate (C), custard cream (D), and strawberry (E) ice-cream mixes. Colors: grey bars are samples packaged with the conveyor tube from Pastomaster 60 machine, blue bars are samples packaged with bag filling machine. Values are means ± SD, n=3. Within each series, means sharing the same letter did not significantly differ ($p > 0.05$).

4.4.3 Sensory analysis results

Assessed the microbiological quality of the products and the stability in terms of pH, ice-cream mixes stored in the bags have been whipped, and ice-cream sensory analysis has been performed through panel tests carried out in Carpigiani during the shelf life,. A specific method and a grid of parameters was defined to evaluate the products with a relative score. Every week, for each analysis, the samples were compared with the same ice-cream prepared from a fresh mix. Ice-cream samples were evaluated by:

1. visual analysis;
2. olfactory analysis;
3. tactile and gustatory analysis.

Color, olfactory intensity, and gustatory features of all the ice-creams, regardless of the type (fior di latte, chocolate, hazelnut, custard cream, strawberry), obtained a progressively lower score over the time with, until the end of the month of storage. However, the popularity rate was different among the different types during the shelf-life period. For example, for fior di latte no critical issues were recorded over the duration of the test, while for strawberry more changes were recorded, when compared to the reference product. The color became darker progressively, with discordant evaluations of the taste.

Color intensity of fior di latte ice-cream did not change. Surface homogeneity (Fig. 61, panel A, B, C) was better than the corresponding reference at the end of the shelf-life. A drop in olfactory intensity and an increase in sweetness and acidity was recorded. During the time emerged the taste of baked and of milk, but the product tended to lose the feature of creaminess.

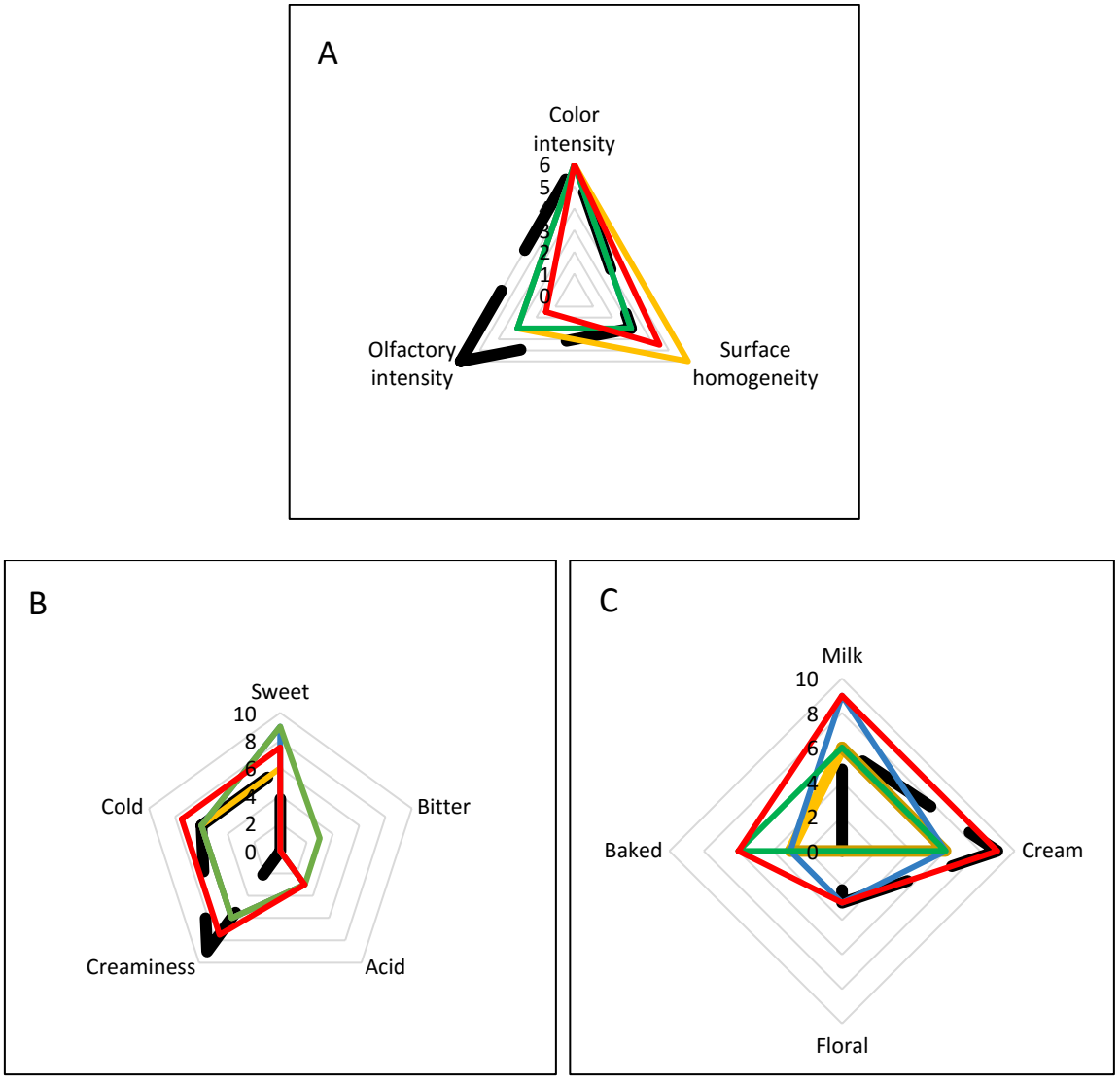


Fig. 61: Radar charts of sensorial properties of fior di latte: visual (A), tactile and gustatory (B), and olfactory (C) analysis. Colors: fresh ice-cream (black), ice-cream produced after 7 (yellow), 14 (blue), 21 (green), and 28 (red) days of shelf-life.

In hazelnut ice-cream (Fig. 62, panel A, B, C), during the 28 days of shelf-life, a slight decrease in color and olfactory intensity were observed. At the end of the shelf-life, the flavors of milk, cream and the toasted flavor were more pronounced. For this specific type of the ice cream, after 28 days, a group of panelists perceived an excessive taste of hazelnut and rancid, and another did not notice this feature. However, a drop in the popularity rating occurred.

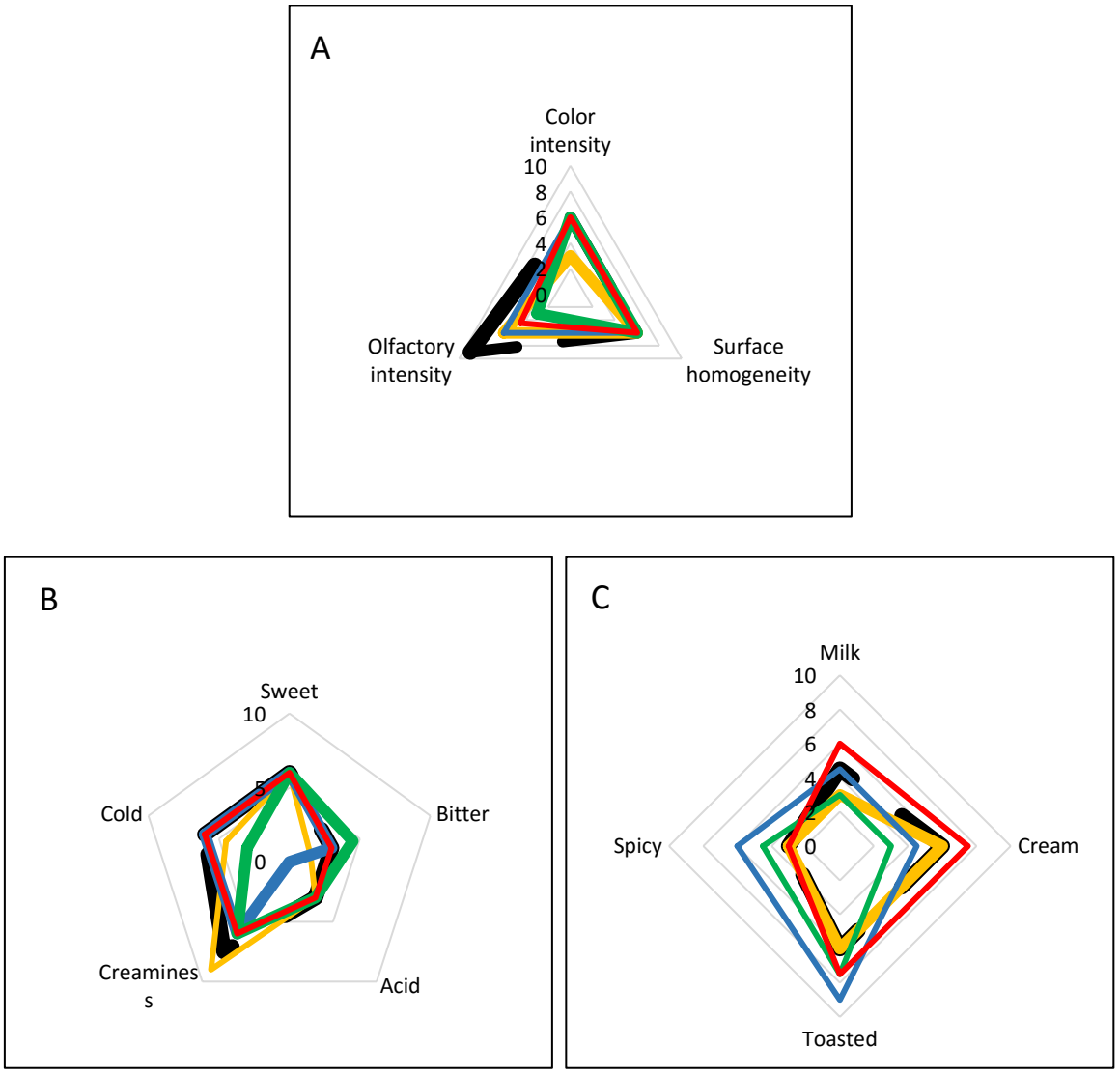


Fig. 62: Radar charts of sensorial properties of hazelnut: visual (A), tactile and gustatory (B), and olfactory (C) analysis. Colors: fresh ice-cream (black), ice-cream produced after 7 (yellow), 14 (blue), 21 (green), and 28 (red) days of shelf-life.

The panel test of chocolate ice-cream (Fig. 63, panel A, B, C), during the 28 days of shelf-life, assessed a decrease in olfactory and surface homogeneity. Furthermore, the perception of cold and bitterness increased, while the creaminess and sweetness decreased. The taste changed, decreasing the spicy, toasted, dried fruit, and the empireumatic features of the product. Chocolate ice-cream, over time, enhanced on the palate the flavor of cocoa rather than chocolate, and the taste resulted very astringent. As a whole, the ice-cream was not appreciated, and after 21 days the approval rate dropped.

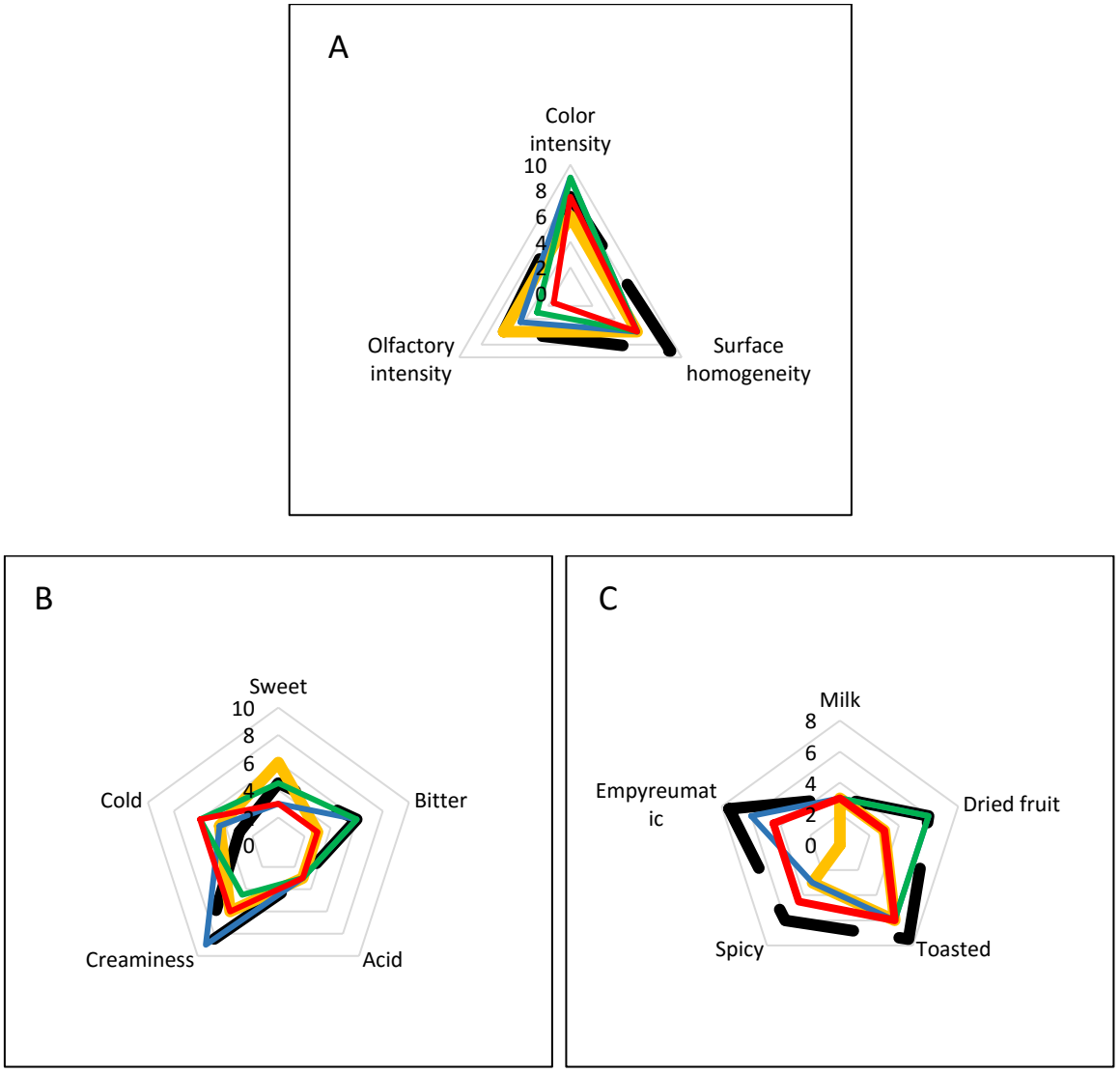


Fig. 63: Radar charts of sensorial properties of chocolate: visual (A), tactile and gustatory (B), and olfactory (C) analysis. Colors: fresh ice-cream (black), ice-cream produced after 7 (yellow), 14 (blue), 21 (green), and 28 (red) days of shelf-life.

For custard cream ice-cream (Fig. 64, panel A, B, C), surface homogeneity and color intensity were positively evaluated until 14 days of shelf-life, then a reduction of the score was recorded. Olfactory intensity did not change over the time. A reduction in egg taste, acidity, bitterness, and the taste of cooked was determined at the end of the shelf-life. However, the product was evaluated better than the reference one after 14 days, since the perception of egg taste was less. This peculiar taste came back in the product toward the end of the shelf-life.

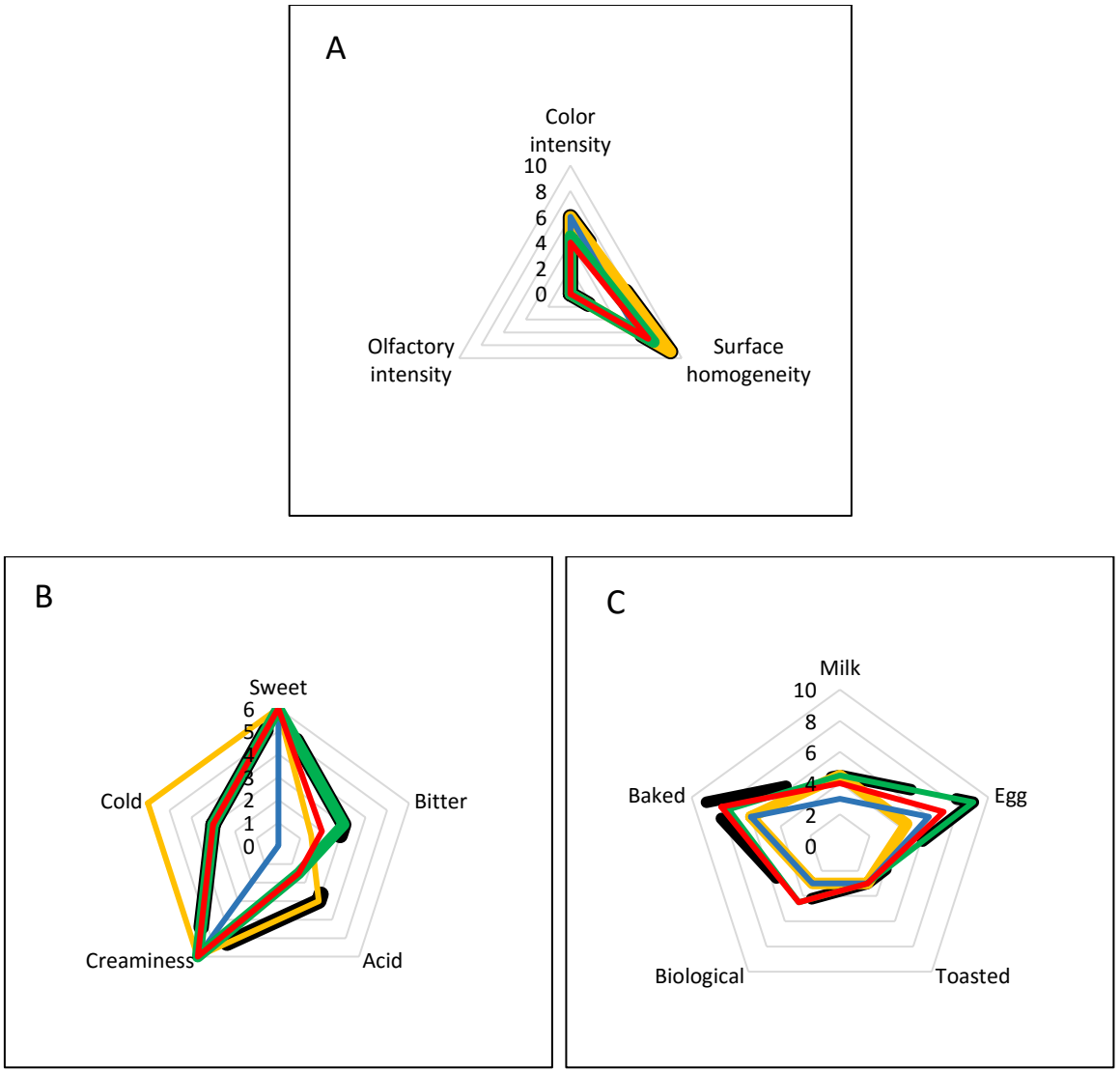


Fig. 64: Radar charts of sensorial properties of custard cream: visual (A), tactile and gustatory (B), and olfactory (C) analysis. Colors: fresh ice-cream (black), ice-cream produced after 7 (yellow), 14 (blue), 21 (green), and 28 (red) days of shelf-life.

In strawberry ice-cream (Fig. 65, panel A, B, C), during the 28 days of shelf-life, a decrease in both olfactory intensity and homogeneity of the surface were perceived. The taste changed: the perception of cold, acid, bitterness, and sweetness increased. The creaminess, the floral taste and the taste of fresh fruit decreased. On the other hand, the flavor of processed fruit was more intense. For this taste the flavor changed considerably (even the color of the ice-cream was darker than the reference just creamed), with consistent negative evaluation of the taste.

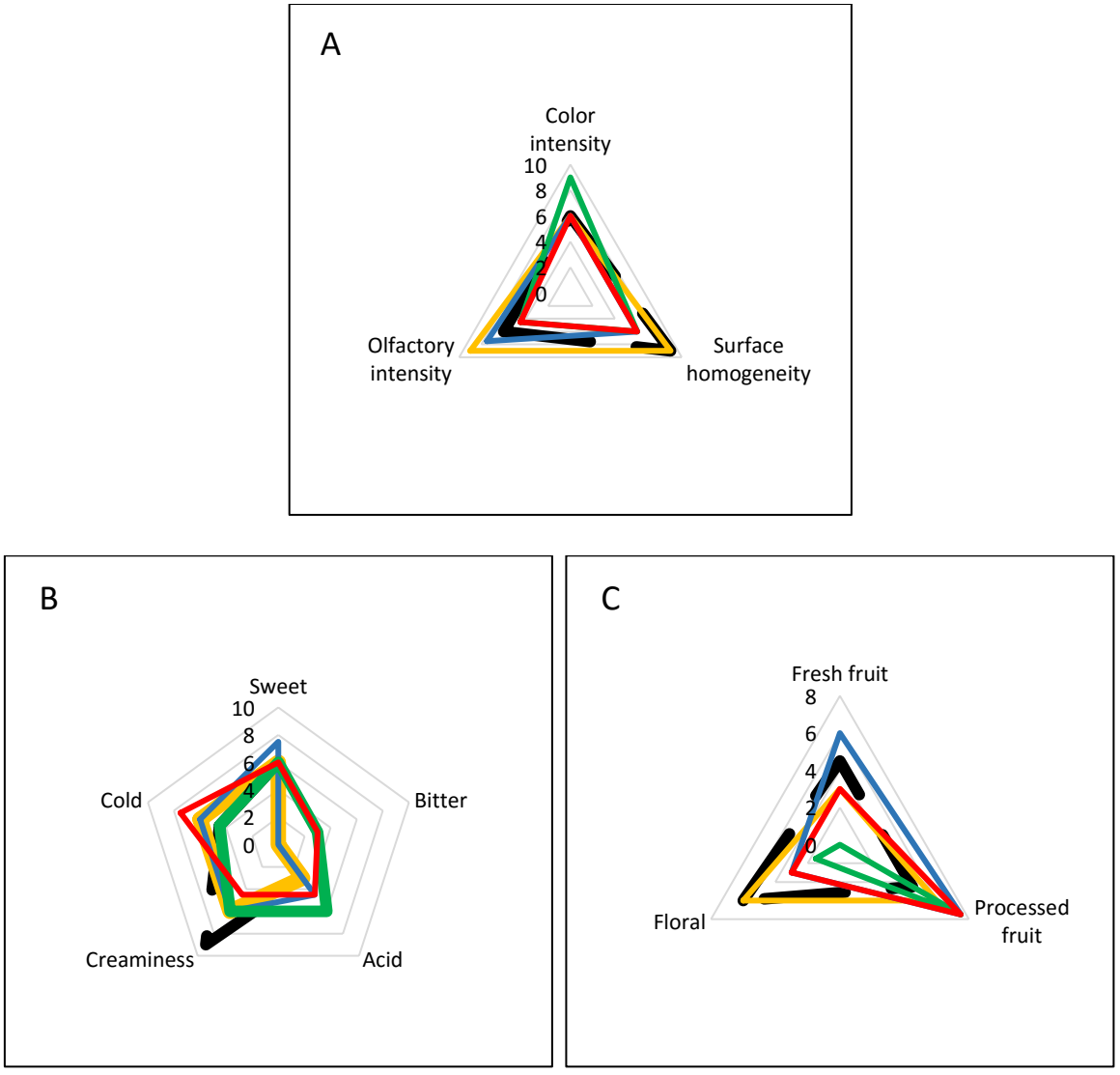


Fig. 65: Radar charts of sensorial properties of strawberry: visual (A), tactile and gustatory (B), and olfactory (C) analysis. Colors: fresh ice-cream (black), ice-cream produced after 7 (yellow), 14 (blue), 21 (green), and 28 (red) days of shelf-life.

5. Conclusions

The outcome of this project is relevant for the industry of the ice-cream machines. It provided a new biosensor implemented in terms of accuracy of the measurement of the bacterial load, that has been included in the pipeline of quality control of the new machines. The possibility to evaluate the efficacy of the pasteurization step in the new machines at the end of the assembling, when the ice-cream machine is ready for sale, increases the control on the products and concurs to prevent the release of products that do not properly perform the thermic cycle aimed to reduce the bacterial load of the mix. In a system always more competitive, the performance of the machine in terms of safety of the ice-cream offers competitiveness and becomes an important instrument against the competitors. On the other hand, the biosensor can find several other possibilities of exploitation, being user-friendly, portable, precise and having the possibility to be calibrated on diverse matrices for different targets of bacteria.

The development of the bag filling system represents a main contribution to the new proposals of Carpigiani. The possibility to produce stable products, in terms of both safety and sensorial properties, in an ice-cream centralized laboratory is a main advantage for the novel economy of the ice-cream markets. In fact, several trademarks prepare artisanal products that are sold in different stores. The possibility to obtain, also with the help of the new bag filler, stable products in a centralized laboratory and to deliver then to the ice-cream stores improves the management of this economy and is an advantage in terms of reproducibility and quality.

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