Article

Influence of Drying and Storage Conditions on the Volatile Organic Compounds Profile of Spirulina Platensis

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Abstract: *Spirulina platensis* (SP) has gained popularity over the last few years, owing to its remarkable nutritional properties and high potential across various industrial sectors. In this study, we analyzed the volatile profile of eight SP samples from the same strain subjected to different drying (oven-drying, air-drying, and spray-drying) and storing conditions ("freshly prepared" and after 12 months of storage) using HS-SPME-GC-MS. Principal component analysis (PCA) was used as a multivariate technique to discern similarities and differences among the samples. The main aim was to assess the impact of the drying technique on the aroma profile and storage life of SP samples. Air-drying leads to the less pronounced formation of by-products related to heat treatment, such as Maillard and Strecker degradation compounds, but promotes oxidative and fermentative phenomena, with the formation of organic acids and esters, especially during storage. Thermal treatment, essential for limiting degradation and fermentation during storage and extending shelf life, alters the aroma profile through the formation of volatile compounds, such as Strecker aldehydes and linear aldehydes, from amino acid and lipid degradation. High temperatures in spray-drying favor the formation of pyrazines. The findings underscore the trade-offs inherent in choosing an appropriate drying method, thereby informing decision-making processes in industrial settings aimed at optimizing both product quality and efficiency.

Keywords: SPME-GC-MS; VOCs; *Spirulina platensis*; drying methods; storage conditions; aroma profile

Citation: Ughetti, A.; D'Eusanio, V.; Strani, L.; Russo, A.L.; Roncaglia, F. Influence of Drying and Storage Conditions on the Volatile Organic Compounds Profile of Spirulina Platensis. *Separations* **2024**, *11*, 180. https://doi.org/10.3390/separations11060180

Academic Editor: Lina Raudonė

Received: 20 May 2024
Revised: 5 June 2024
Accepted: 7 June 2024
Published: 10 June 2024

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1. Introduction

Spirulina platensis (SP), a planktonic blue–green algae, has gained widespread recognition as a natural superfood. A superfood is a nutrient-dense food that is especially beneficial for health owing to its high concentration of essential vitamins, minerals, antioxidants, and other beneficial compounds. Superfoods, often derived from fruits, vegetables, and other natural sources, provide numerous health benefits beyond those of regular foods [1]. SP is incredibly nutrient-dense, as it contains high levels of proteins [2], polyunsaturated fatty acids [3], β-carotene and other carotenoids [4], antioxidants [5], essential minerals [6], sulfated polysaccharides (antivirals [7]), and sterols (antimicrobials [8]). Moreover, SP is the world's richest natural source of vitamin B12 and contains vitamin C and E [9–11]. Therefore, SP is commonly used as a dietary supplement or functional ingredient.

The applications of SP have been extensively studied in non-food industrial sectors, such as biofuel production [12–14], wastewater treatment [15], animal feed [16], cosmetics [17], and pharmaceuticals [18]. Moreover, because of the photosynthetic efficiency of SP algae and the minimal competition with land-based crops, SP strains show potential for the biofixation of significant amounts of CO_2 [19]. This strategy is particularly interesting

because it combines CO_2 sequestration with the production of versatile biomass, thereby offering multiple benefits from an operational perspective.

It is well known that the chemical composition of microalgae depends heavily on several factors, including the nutrient solution content, culture conditions (temperature, pH, and illumination), and growth phase [20–22]. For example, a higher CO_2 concentration in cultures of SP leads to an increase in the carbohydrate content and a decrease in proteins and pigments [23], while nitrogen deficiency usually leads to an increase in the fatty acid content [24]. Microalgae adapt to these factors by altering their metabolism, consequently modifying the profile of the volatile organic compounds (VOCs) produced during their growth [25]. VOCs are secondary metabolites produced by cyanobacteria and are easily released into the atmosphere owing to their low molecular weight and high vapor pressure. Several studies have reported the presence of alkanes, alkenes, oxygenated VOCs, and volatile sulfide chemicals in the headspace composition of algae and microalgae [26]. These molecules are known to contribute significantly to the distinctive marine taste and odor associated with microalgae. Consequently, when SP is incorporated into consumables, such as food, beverages, and supplements, VOCs affect the sensory attributes of the final product, potentially resulting in undesirable taste and flavor profiles. Understanding and characterizing VOCs in SP-based consumables is crucial for maintaining product quality and consumer acceptance. Furthermore, Reese et al. [27] demonstrated that VOCs can be used as diagnostic biomarkers to evaluate the overall status of algal crops. Therefore, it is important to monitor VOC emissions from SP, especially after biomass-altering treatments, such as drying and storage for long periods.

Dehydration is one of the most important long-term preservation techniques of food products since it limits microbial growth and minimizes deteriorative reactions. Drying SP powder is a crucial step in its processing, which is necessary to preserve its nutritional value, extend its shelf life, and facilitate storage and transportation [28–30]. In fact, freshly harvested SP typically has a dry residue level of approximately 10% [28]. Because of its high water content, a drying treatment following the harvesting process is necessary to ensure the production of a stable, usable, and safe SP powder.

Although drying effectively extends the shelf life of agricultural products, the significant degradation of nutritional value occurs owing to undesirable structural, textural, and biochemical changes [28,29,31,32]. There are various methods for drying SP powder, including air-drying, spray-drying, and oven-drying, each with its advantages and considerations.

Air-drying involves spreading the harvested SP slurry into thin layers and allowing it to dry naturally in open air. Although this method is simple and cost-effective [31], it may not always guarantee uniform drying and can be susceptible to contamination from environmental factors such as microorganisms [33,34]. Moreover, air-drying may not be suitable for large-scale production because of its slow drying rate and potential space requirements.

Spray-drying involves spraying SP biomass into a hot airstream, which quickly evaporates moisture and leaves behind dried powder particles [35,36]. This method offers greater control over the drying process and produces a fine and consistent powder. Spray-drying is highly scalable and suitable for large-scale production because it can efficiently handle large volumes of SP biomass [34]. However, it requires specialized equipment and can be energy- and cost-intensive.

Oven-drying is another common method used to dry SP powder. In this process, SP biomass is spread out in a thin layer on trays and placed inside an oven set to a specific temperature. The warm air circulating within the oven evaporates moisture from the SP and gradually dries it out. Compared to more specialized drying methods such as spray-drying, oven-drying is generally more affordable. However, oven-drying typically has a slow drying rate and can be energy-intensive [34]. This method can also cause the greatest deterioration, particularly if a high temperature is used for an extended period [33,34,37].

Our study focused on analyzing the volatile fingerprints of eight SP samples. These specimens originated from the same SP strain and were cultivated under the same conditions but differed only in their drying and/or storage methods. By analyzing the distinct volatile profiles of these samples, we aimed to discern the advantages and disadvantages of the various drying and storage techniques examined in this study. The volatile fraction analysis was performed using Headspace–Solid-Phase Microextraction–Gas Chromatography–Mass Spectrometry (HS-SPME-GC-MS). HS-SPME is a simple, useful, and sensitive method for analyzing volatile fractions, and when coupled with GC-MS, it serves as a crucial tool for understanding the effects of different drying methods and storage behaviors of SP samples.

However, due to the dynamic nature of microalgae cultivation, variations in environmental conditions can lead to fluctuations in the composition of SP biomass. Changes in nutrient availability, light intensity, and temperature gradients within the cultivation system can significantly influence the biochemical composition of SP. Therefore, it is challenging to generalize our observations to all SP batches. Despite these limitations, our research provides valuable insights into the industrial applications of SP, particularly in terms of selecting appropriate drying methods to preserve its quality and sensory attributes. Previous studies have dealt with the analysis of bioactive compounds in SP after storage [38,39], but as far as we know, there is no evidence regarding the effect on the volatile profile after storage. Volatile compounds serve as indicators of chemical composition and sensory characteristics, providing valuable insights into quality, stability, and processing conditions. Notably, different degradative processes originating from the native constituent compounds can lead to the release of volatile compounds. Therefore, this analytical method sheds light on the underlying chemistry of SP samples. Moreover, the analysis of the volatile fraction is closely related to the aromatic profile, which is particularly significant in the food and cosmeceutical industries. Ultimately, our goal was to assess the impact of different drying methods on the shelf life of SP and to determine the best compromise in terms of cost-effectiveness, drying time, and nutrient preservation. Such information enables the more efficient and effective utilization of SP in diverse contexts, ranging from food and cosmeceuticals to pharmaceuticals and biotechnology.

2. Materials and Methods

2.1. Microalgae Strain and Culture Conditions

The food-grade reagents were used as received. The nutrient solution was a modified version of Zarrouk's medium [40], specific for industrial use. The SP strain used in this study was SAG 85.79 *Arthrospira platensis*, a strain kindly donated by Algae S.p.A., a company devoted to the development and supply of industrial photobioreactors. To confirm the absence of contamination in the SP batch, we obtained light microscope images of the strain, which are available in the Supplementary Materials of our previous study [19]. Algae S.p.A.'s plant was used for industrial-scale experiments. The plant for SP production consisted of two production lines, each including 34 PBRs with 1.4 m^3 of working volume. SP was grown continuously at a temperature of 30 $\pm$ 2 °C using a patented illumination setup (350 W illuminating power per PBR). The culture was kept stirred using an air pump. Every day, approximately 33% of the volume of a single production line was harvested (approximately 15 m^3) and filtered using a vibrating screen. The depleted water and nutrients were then reintegrated. The filtered slurry was stored in food-grade plastic containers at 4 °C for further use. For the laboratory experiments, the same custom-made PBR used in our previous study was used [19]. Briefly, it consists of a thermostatic recirculated water glass tank that can host eight biological glass tubes. SP was grown inside these test tubes under the same culture conditions used in the industrial PBRs. After 5 days, the culture reached the same OD680 as the industrial culture and was then harvested and filtered using a polyamide membrane (Sartolon®, 0.45 μm pore size; Sartorius AG, Göttingen, Germany). The filtered biomass was stored at 4 °C.

2.2. Sample Preparation

One-third of the algal slurry obtained from the vibrating screen underwent drying at ambient temperature (T °C) inside the industrial plant (T °C = 25 °C) for 96 h. Another third of the slurry was dried in a large heater maintained at 50 °C for 48 h. The remaining third part was processed using a spray-dryer (T °C = 120 °C), with an evaporation rate of 19 L/h and a dry production rate of 1 kg/h. Following drying, the samples were placed in plastic food-grade containers and stored in the dark at room temperature. One batch of dried samples was promptly analyzed, whereas another batch was stored for 12 months prior to analysis. Moreover, the filtered biomass derived from the laboratory experiments, serving as the control, was dried under the same conditions as the industrially produced biomass.

We purchased one sample of store SP from Amazon.it (https://www.amazon.it/, accessed on 12 September 2023) and used it as a benchmark for our analyses, aiming to identify the primary differences between our samples and a representative sample found in the market.

This series of drying and storage methods resulted in eight distinct samples: Freshly Air-Dried (FAD), Freshly Oven-Dried (FOD), Freshly Spray-Dried (FSD), Stored Air-Dried (SAD), Stored Oven-Dried (SOD), Stored Spray-Dried (SSD), Control Air-Dried (CAD), Control Oven-Dried (COD), Control Spray-Dried (CSD), and Commercially Available (CA).

2.3. HS-SPME

A solid-phase micro-extraction (SPME) holder (Supelco Inc., Bellefonte, PA, USA) was used to conduct the SPME headspace (HS) analysis. Volatiles were extracted using a SPME fiber composed of PDMS/CAR (Supelco, Bellefonte, PA, USA). Sample extraction was performed using an autosampler (MPS2XL, Gerstel, Mühlheim a.d. Ruhr, Germany) equipped with a sample tray holder, a needle heater, and an agitator/stirrer (all components from Gerstel) to heat the vials. The autosampler control was managed using Gerstel Maestro software. The fiber underwent a bakeout process in a needle heater for 10 min at 270 °C before each extraction. Following a 15 min equilibration period in the agitator at 35 °C, the fiber was inserted into the HS of the vial for a 15 min extraction at 35 °C. Subsequently, the SPME needle was removed from the HS vial and inserted into the GC-MS inlet for analyte desorption over a 2 min period.

The reproducibility of the experimental procedure was ensured by analyzing three replicates of the same sample. After the chromatographic analysis of the three samples, blank tests were conducted.

2.4. GC-MS

GC-MS analysis of the extracted volatile compounds was conducted using an Agilent 6890 Network gas chromatograph system coupled with a 5973N mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). A DB-5MS UI column (60 m × 0.25 mm i.d., 1.00 µm film thickness; J&W Scientific, Folsom, CA, USA) was used, and He was employed as the carrier gas at a flow rate of 1.0 mL/Min. The detector began to operate immediately after each injection. The column temperature was increased from 40 °C to 280 °C at a rate of 6 °C/min. The transfer line was heated to 270 °C.

The mass spectrometer operated in electron impact (EI) ionization mode at 70 eV, using the full scan acquisition mode with an m/z scanning range from 25 to 450. The chromatograms and mass spectra were analyzed using Enhanced ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The identification of volatile compounds was achieved by comparing the mass spectra with the data system library (NIST14/NIST05/WILEY275/NBS75K) and by using databases accessible via the web, such as the National Institute for Standards and Technology (NIST database https://webbook.nist.gov/ accessed on 21 February 2024) and the Mass Bank of North America (https://mona.fiehnlab.ucdavis.edu/ accessed on 21 February 2024).

The amount of each volatile identified in the SPME-GC-MS analysis is expressed as the total ion current (TIC) peak area.

All the data presented in Table S1 correspond to the values obtained from the analysis performed in triplicate. The reproducibility of the results is expressed as the standard deviation (SD) in Table S1.

2.5. Statistical Analysis

Principal Component Analysis (PCA) [41] was used for the exploratory data analysis of the TIC peak areas. Equation (1) represents the decomposition process.

$$\mathbf{X} = \mathbf{T}\mathbf{P}^{\mathrm{T}} + \mathbf{E} \quad (1)$$

X represents a data matrix with m rows (samples) and n columns (variables). The matrix **T** is the scores matrix, which describes the interrelationships among the samples, while the loading matrix **P** captures insights into the impact of measured variables on the model and their correlation structure. **E** denotes the residual matrix, containing unexplained variability, and shares the same dimensions as **X**. It is obtained by subtracting the reconstructed data ($\mathbf{TP^T}$), obtained from the PCA model, from **X**. Consequently, the original dataset is condensed into a reduced number of independent variables, namely, principal components (PCs), which are mutually orthogonal. This process generates a novel projection space, smaller in dimensionality, with its coordinates defined by the PCs. Consequently, the dimension of the scores matrix **T** is $m \times R$, whereas the dimension of the loading matrix **P** is $R \times n$, where R is the number of PCs used to build the model.

In the present study, the TIC peak areas have been arranged in a dataset (27×97). Before performing PCA, the data were preprocessed using Autoscaling to remove any impact on the overall variance exclusively attributable to variations in the units of measurement or scales of the variables. The number of PCs used to build the PCA models was chosen by inspecting the scree plot, which shows the eigenvalues, i.e., the total amount of variance explained, of the first 20 PCs. However, since PCA is used as an exploratory data analysis tool, only scores and loading plots that provided relevant information for the current study were displayed. The eigenvalues and explained variance of the first 20 PCs for the two conducted PCA can be found in the Supplementary Materials (Table S2).

3. Results and Discussion

3.1. HS-SPME-GC-MS Results

The choice of the drying method significantly influences the aroma profile of food matrices or plant powders, including SP. During processing, as the temperature increased, a sequence of chemical reactions occurred, reshaping the composition and concentration of volatile aroma compounds. Thermal processing can trigger degradation reactions, oxidation, or the rearrangement of temperature-sensitive compounds, consequently altering sample aroma characteristics. In our study, employing HS-SPME-GC-MS analysis, we predominantly identified compounds associated with the degradation of the two major constituents of SP: amino acids (ranging from 50 to 65% of the total weight) and fatty acids (ranging from 7 to 15% of the total weight), as well as a minor component, carotenoids. The results are presented in Table S1, which includes all identified analytes along with the total ion chromatogram (TIC) area of the individual chromatographic peaks. The chemical classes of the detected VOCs mainly included hydrocarbons, ketones, aldehydes, alcohols, pyrazines, furans, esters, and organic acids, which is comparable to the results of similar studies found in the literature [25,42]. Figure 1 shows the formation pathways of the main VOCs in Spirulina.

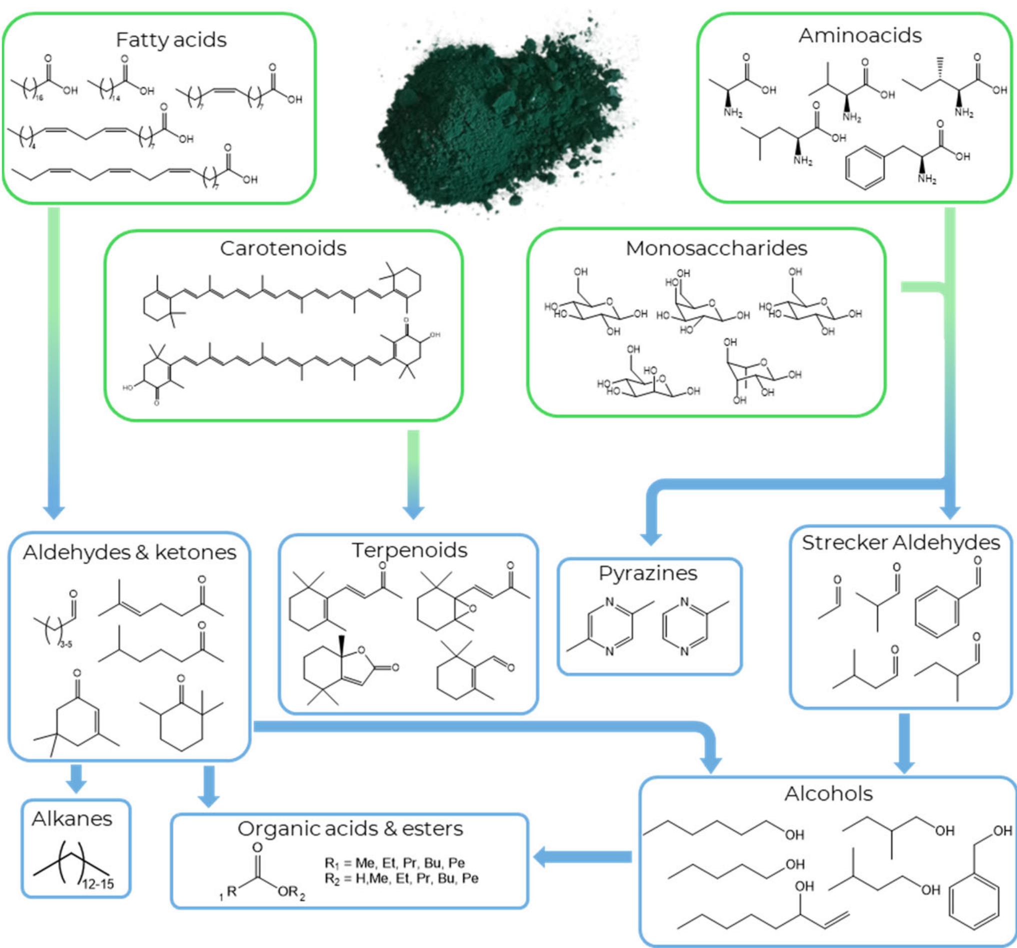

Figure 1. Formation pathways of the main volatile organic compounds (VOCs) found in Spirulina.

3.1.1. Maillard-Derived VOCs

Strecker aldehydes, such as acetaldehyde, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal, commonly present in the volatile fraction of processed plant foods, can indicate the occurrence of the Maillard reaction during drying operations. These compounds, with their low odor thresholds, significantly contribute to the development of flavor compounds in processed plant foods [43,44]. Strecker aldehydes are generated through the Strecker degradation of some amino acids (alanine, valine, isoleucine, and leucine). This degradation pathway involves the oxidative deamination and decarboxylation of α-amino acids in the presence of α-dicarbonyl compounds formed during the Maillard reaction. The presence of Strecker aldehydes in the SP samples is not surprising because they contain a high quantity of proteins. Strecker aldehydes were detected in almost all the analyzed samples, particularly in those subjected to thermal treatment. Furthermore, the quantities of 3-methylbutanal and 2-methylbutanal tended to increase during storage. This increase was more pronounced in the oven-dried (OD) samples, whereas only a slight increase was observed in the spray-dried (SD) samples. Air-dried (AD) samples exhibited lower or absent levels of Strecker aldehydes, suggesting the pronounced influence of thermal treatment on the volatile profile and amino acid stability of the samples. There were no statistically significant differences ($p > 0.05$) between the 3-methybutanal content in the control samples (COD and CAD) and the corresponding industrially prepared SOD and SAD samples. The same was true for 2-methylbutanal, but only for the COD and SOD samples, whereas it was completely absent in the CAD samples. 2-methylbutanal and 3-methylbutanal impart apple-like and malt aroma [45]. Once formed, Strecker aldehydes undergo further reactions to produce their corresponding alcohols, 3-methylbutanol and 2-methylbutanol, as detected in our SP samples. These alcohols were found in the same

sample, where the corresponding aldehydes were detected. Another amino acid-derived volatile compound is benzaldehyde, which is derived from the degradation of phenylalanine together with its corresponding alcohol (benzyl alcohol) [25]. It was detected only in the stored samples and in higher quantities in the OD samples. Benzaldehyde is known for its characteristic bitter, almond-like odor, and its presence in SP samples has been reported previously [20,46]. All these Strecker-related compounds were also found in commercially available samples (CA).

Pyrazines contribute to nutty and/or roasted notes and are formed through the Maillard reaction or fermentation processes occurring in matrices containing amino acids and sugars. Several pyrazines were identified in the SP samples, in line with previous studies [25,42,47]. Their content was higher in the heat-treated samples, particularly in the stored samples. Some of them, such as 2,5-dimethylpyrazine, were also detected in the stored air-dried samples, indicating that both thermal treatment and fermentation processes led to their formation.

3.1.2. Lipid-Derived VOCs

Lipid oxidation significantly affects the quality of natural matrices through various mechanisms, manifesting in undesirable effects such as the generation of off-odors, the alteration of color, taste, and texture [48], as well as a reduction in functional properties and the bioavailability of essential nutrients. It reduces the shelf life and causes food spoilage, which is an important factor in food security. The susceptibility of fatty acids to lipid oxidation increases with the degree of unsaturation [48], and a matrix with a high content of polyunsaturated fatty acids (PUFAs), such as SP, severely decreases food acceptability and shelf life. For example, in water supplies, the presence of algal polyunsaturated fatty acid derivatives results in noticeable changes in odor, often perceived as unpleasant, reminiscent of fishiness and rancidity [49]. The presence of off-odors in lipid-containing samples undergoing oxidation is primarily attributed to linear aldehydes, notably pentanal, hexanal, and heptanal. These aldehydes are formed as a result of lipid peroxidation, a process initiated by exposure to oxygen, light, and high temperatures. The major aldehyde detected in the SP samples was hexanal, a volatile compound known for its characteristic grassy odor. This observation is in line with previous studies regarding SP [25,42,47] and other microalgae species [50]. Among the fresh samples, those subjected to air-drying exhibited higher levels of hexanal, indicating heightened oxidative stress on the lipids due to increased air contact. Furthermore, hexanal levels tended to increase over time during storage in spray-dried (SD) and oven-dried (OD) samples. Specifically, SSD and SOD samples exhibited higher levels of hexanal than their fresh counterparts (FSD and FOD) and the SAD samples. Interestingly, after one year of storage, hexanal was no longer detectable in the air-dried (AD) samples, likely due to complete volatilization, whereas its levels continued to rise in the SSD and SOD samples. Hexanal was also detected in the control samples (COD and CAD). Pentanal and heptanal followed a trend similar to that of hexanal, albeit with a lower abundance. However, unlike hexanal, they were also detected in SAD samples. In addition to aldehydes, ketones and alcohols are also produced during lipid oxidation [42]. Many of the identified ketones are related to fatty acid oxidation because enzymes cleave fatty acids to produce these compounds through a sequence of additional reactions [25]. Among these, 3,5,5-trimethylcyclohex-2-en-1-one, isophorone (3,5,5-trimethylcyclohex-2-en-1-one), sulcatone (6-methylhept-5-en-2-one), and 6-methylheptan-2-one have been previously found in cyanobacteria and algae [26–28]. Acyclic alcohols, such as 1-pentanol, 1-hexanol, and oct-1-en-3-ol, are also formed because of lipid peroxidation. 1-hexanol is characterized by a herbal odor, whereas C8 alcohols, such as oct-1-en-3-ol, contribute to a mushroom-like odor. They were mainly found in stored samples, except for the FAD samples, where both 1-pentanol and 1-hexanol were present. These volatile compounds contribute to the overall flavor profile of lipid-containing samples. However, their impact on flavor is often less pronounced than that of aldehydes, owing to their higher odor threshold concentrations. Almost all of these lipid-related

volatile compounds were also detected in the CA samples. Another class of molecules associated with lipid degradation are alkanes and alkenes. Tetradecane, pentadecane, hexadecane, and heptadecane were the main alkanes that were detected. These findings are consistent with earlier reports where medium-chain alkanes were found in high quantities in SP samples [20]. Higher chain hydrocarbons may also be present in our samples but were not detected because of the low temperature (35 °C) used during headspace sampling. They are associated with a two-step conversion of fatty acids, first to fatty aldehydes and then to alkanes. Alkanes were more abundant in the SAD sample but were detected across all analyzed samples.

3.1.3. Carotenoid-Derived VOCs

The degradation of carotenoids, specifically β-carotene, plays a significant role in shaping the aroma profile of the SP samples. Carotenoid breakdown leads to the release of several VOCs, owing to the high instability of their conjugated double-bound structure. Carotenoids play a key role as photosynthetic pigments that harvest light and prevent photo-oxidative damage [51]. In the later stages of carotenoid degradation, the longer chain intermediates are oxidized, forming short-chain mono- and deoxygenated compounds, such as sulcatone, ionones, dihydroactinidiolide, and β-cyclocitral. Sulcatone formation is associated with lipid oxidation [50] and carotenoid degradation [52]. It was detected across almost all analyzed samples, except for the SSD and COD samples. Its abundance seems to be slightly higher in the freshly prepared samples than in the stored samples. Moreover, β-ionone and β-ionone epoxides were detected, in agreement with previous studies [25]. Ionones are produced through the oxidation of carotenoids, are naturally found in foods containing β-carotene, and are responsible for the characteristic flowery violet and woody odors [53,54]. In particular, β-ionone contributes to floral and seaweed odors, whereas β-ionone epoxide provides a fruity odor. The origin of dihydroactinidiolide((S)-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one) is attributed to the oxidation of β-ionone, suggesting that its high abundance is associated with the high β-ionone content in the SP samples. It has a hay-like, pungent, and violet-like odor. Ionones and dihydroactinidiolide were detected in all the stored samples and in the FAD samples but were absent in the control samples. β-Cyclocitral (1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-) is a volatile substance related to the enzymatic degradation of β-carotene that imparts a fresh aroma [55]. Its production is linked to *Microxystic* cyanobacterial strains, and its presence can indicate the death of cyanobacterial cells [20,56]. It was found across all analyzed samples, except for the control samples (COD and CAD) and FSD. All these carotenoid-related volatile compounds were also detected in the CA samples.

3.1.4. Fermentative-Related VOCs

One of the major distinctions observed among the examined samples was the high acetic acid content in the stored samples. Its formation is strictly related to fermentation processes involving yeasts and bacteria, such as acetic acid bacteria. The concentration of acetic acid was significantly higher in the stored, air-dried samples (SAD), indicating that heat treatment inhibited fermentation. Moreover, other organic acids, including propanoic acid, butanoic acid, pentanoic acid, and hexanoic acid, were exclusively detected in the SAD samples, imparting pungent and unpleasant odor notes. High concentrations of organic acids are associated with the presence of esters, particularly methyl butanoate, pentyl acetate, butyl butanoate, and pentyl butanoate. These were formed because of the esterification of organic acids through reactions with alcohols, and they were detected only in the SAD samples. The absence of organic acids and other fermentative compounds in thermally treated samples underscores the importance of a thermal treatment to ensure a long shelf life of Spirulina powders. This aspect is certainly crucial for industrial applications and large-scale productions. While air-dried samples are a more cost-effective and readily available option, their shorter shelf life can restrict their applicability.

3.2. Principal Component Analysis (PCA)

PCA is a powerful statistical technique used to simplify complex datasets by reducing the dimensionality of data while retaining their essential features. In the analysis of volatile compounds using HS-SPME-GC-MS, PCA may play an important role in uncovering patterns and trends within the vast array of chemical compounds present in the samples. PCA can facilitate the identification of the characteristic volatile compounds associated with specific drying methods or storage conditions. These biomarkers serve as indicators of quality, allowing for the assessment of the effectiveness of different processing techniques in preserving desirable volatile compounds. Although the score and loading plots for up to 8 PCs were investigated, they are not presented here, as they do not provide relevant information for this study. However, in Table S1, the eigenvalues and explained variance of the first 20 PCs are reported.

Two distinct versions of the analysis were performed. The first version revealed that PC1 accounted for 37.04% of the total variance, describing the difference between commercially available (CA) samples and other samples, as shown in Figure 2A. On the other hand, PC2 (21.12% of the total variance) shows the difference between the stored, air-dried (SAD) samples and the others, which formed a cluster close to the axes' intersection. In the loading plot (Figure 2B), these differences translate into higher quantities of organic acids and esters in the SAD samples and hydrocarbons and carotenoids-related compounds in the CA samples, such as limonene, carene, and pinene. This difference can be attributed to the different strains of SP as well as the growing conditions [57].

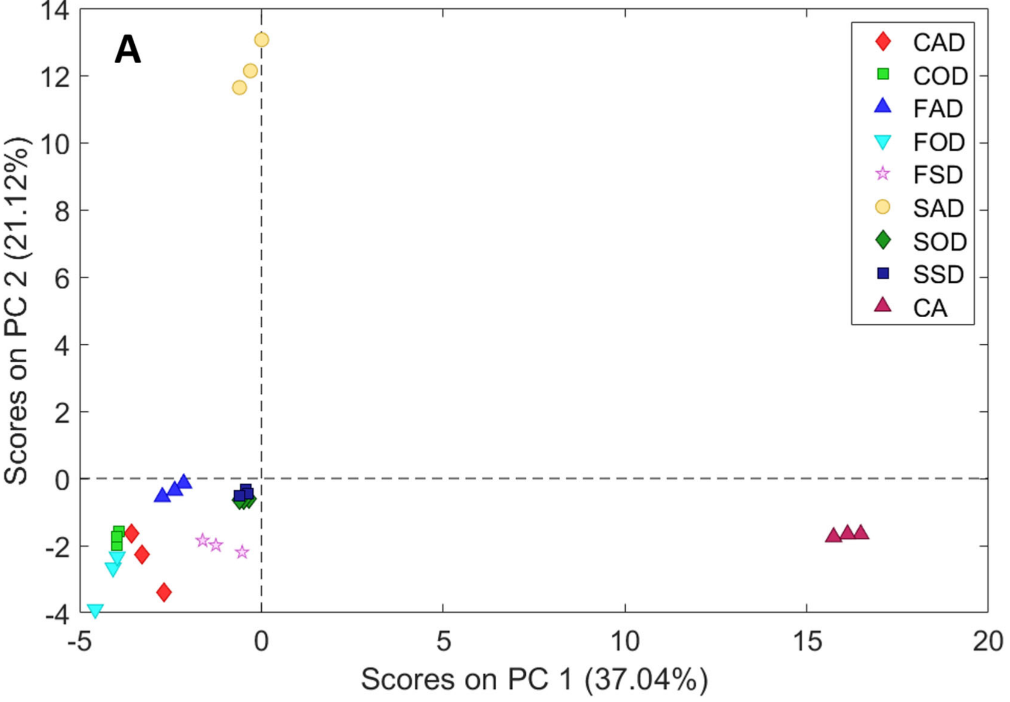

Figure 2. *Cont.*

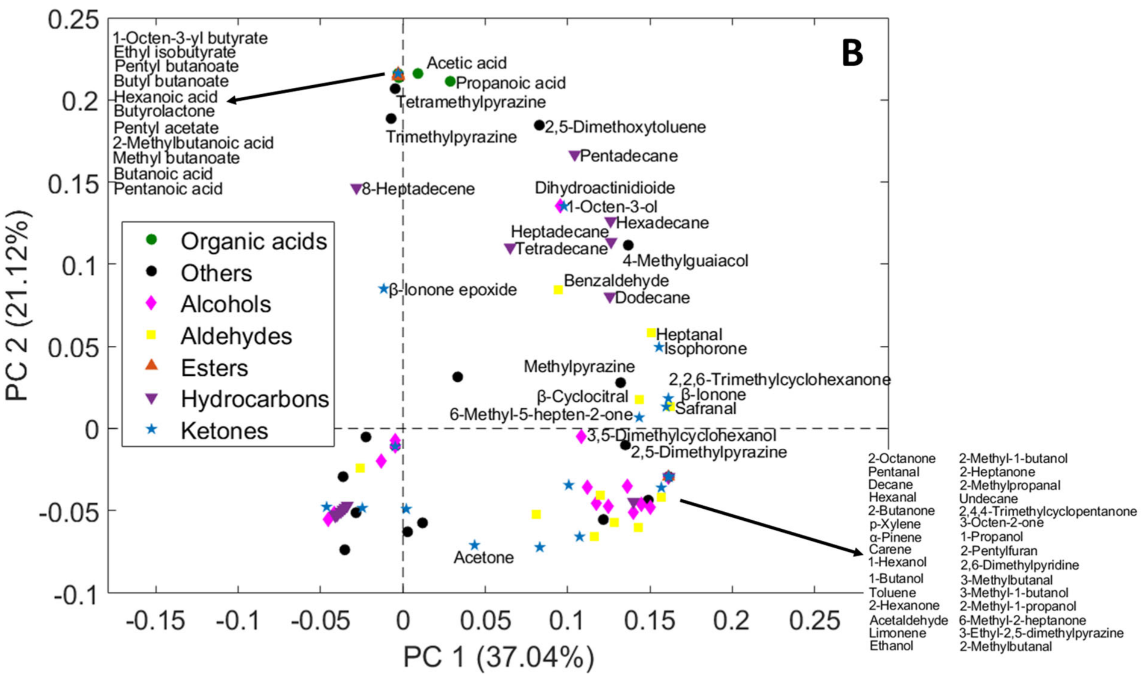

Figure 2. PCA performed on all samples. PC1 vs. PC2 scores (**A**) and loadings (**B**) plots.

Nonetheless, to better discern the smaller differences among the remaining samples derived from the same strain, we conducted another PCA excluding the CA samples.

This approach yields a more detailed perspective on certain aspects.

For this analysis, four PCs were examined, accounting for over 75% of the total variance. PC1 and PC2 explained 31% and 19% of the total variance, respectively. From the PCA analysis of Figure 3A, which shows the PC1 vs. PC2 score plot, a cluster emerges containing two fresh samples (FSD and FOD) and the control samples (COD and CAD). A slight deviation from this cluster was observed in the FAD samples, indicating that air-drying yields a volatile profile distinct from those obtained through spray-drying and oven-drying. Furthermore, differences were highlighted between air-dried control samples (CAD) and their fresh counterparts (FAD), primarily observed in the loading plot of Figure 3B. The CAD sample was characterized by higher quantities of ketones, such as acetone, 2-pentanone, 2-octanone, and 2-nonanone. Obtaining air-dried samples under industrial plant conditions appears to entail the more pronounced oxidation of carotenoids and lipids, consequently altering the aromatic profile. Furthermore, in the PC1 versus PC2 plot, there were some differences between the FSD and FOD samples. In particular, the FOD samples showed higher quantities of compounds, such as 2-ethyl-4,5-dimethylphenol, 2-methylfuran, and 6-methyl-2-heptanone, whereas FSD was characterized by higher quantities of some pyrazines (trimethylpyrazine, 2,5-dimethylpyrazine, and 3-ethyl-2,5-dimethylpyrazine). These differences can be attributed to the higher temperatures of the spray-drying technique, as pyrazine formation is known to occur through Maillard reactions at high temperatures. The samples that stood out the most in the PC1 versus PC2 score plot were the stored samples. The SSD and SOD samples exhibited more similar aromatic profiles, as they are closer in the plot shown in Figure 3A. Conversely, the SAD sample displayed an aromatic profile that deviated significantly from that of the others. The SSD and SOD samples showed more pronounced contents of pyrazines, linear and Strecker aldehydes, branched and linear alcohols, and carotenoid degradation compounds. This is not surprising, as the use of high temperatures for drying promotes Maillard reactions, lipid degradation, and amino acid degradation, leading to alterations in the aromatic profile, favoring the aforementioned compound classes. On the other hand, the SAD samples showed more pronounced organic acid and ester contents, indicating that the absence of the thermal treatment of the matrix results in more pronounced fermentative

phenomena, leading to alterations in the aromatic profile favoring sharper and more acidic and pungent notes.

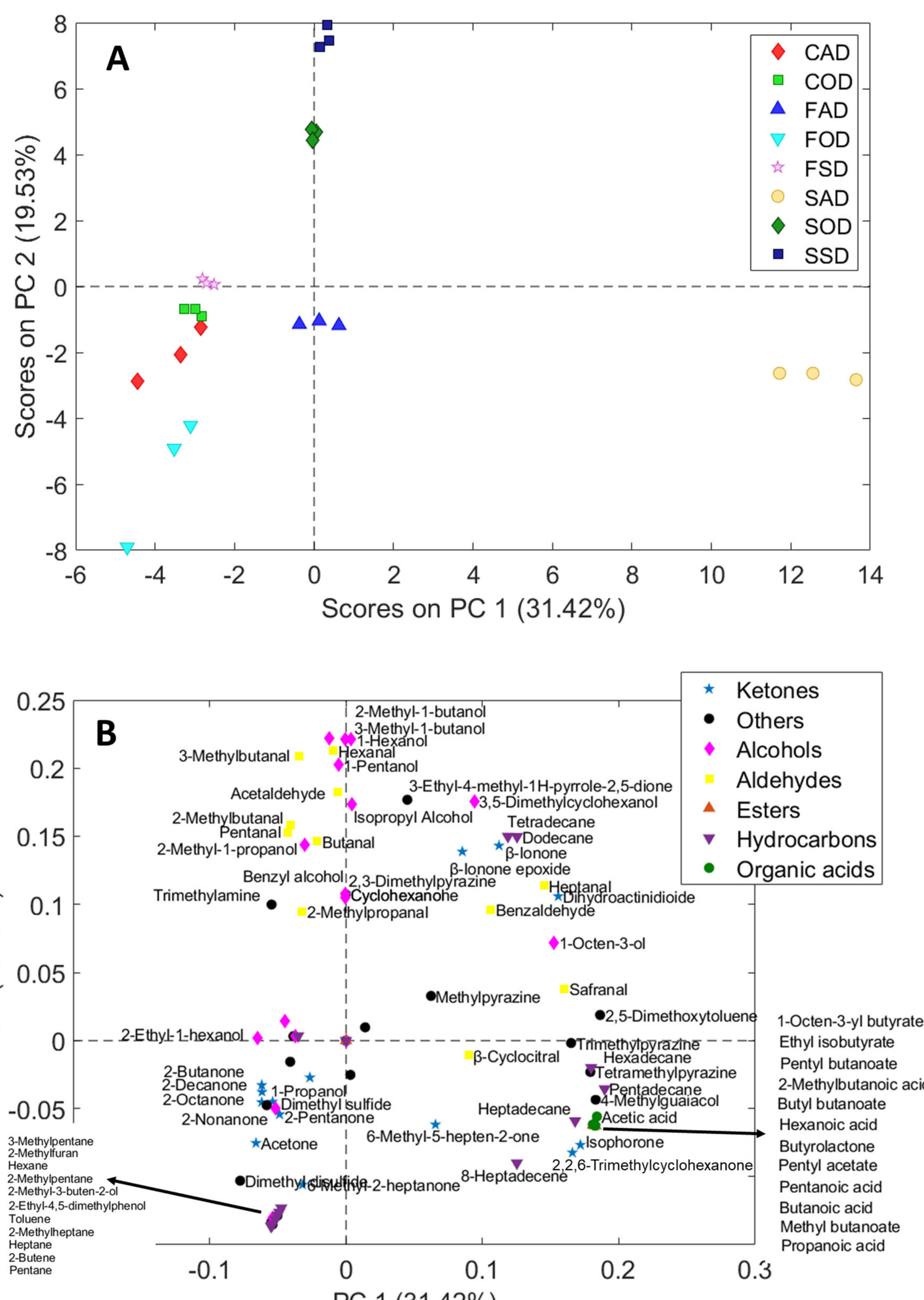

Figure 3. PCA performed without CA samples. PC1 vs. PC2 scores (**A**) and loadings (**B**) plots.

PC3 and PC4 explain 13.25% and 11.11% of the total variance, respectively. From the PC3 vs. PC4 score plot (Figure 4A), it was challenging to highlight distinct clusters because of increased variability, even among replicates of individual samples. However, as shown in Figure 4B, the differentiation of the FSD mainly stems from their higher pyrazine content, whereas the SOD samples differentiated mainly because of their higher contents of benzyl alcohol, benzaldehyde, 3-methylbutanal, and some ketones (including 2,2,6-trimethylcyclohexanonea and 6-methyl-5-cylohepten-2-one). Furthermore, FOD samples differed from the others mainly because of a cluster comprising compounds such as branched hydrocarbons (2-methylheptane, 3-methylpentane, and 2-methylpentane) and small hydrocarbons (pentane, hexane, and heptane). The absence of Strecker compounds and lipid degradation products in the FOD samples, in contrast to their presence in the SOD samples, demonstrates that oven-drying does not lead to the substantial degradation of amino acids and fatty acids in fresh samples. Instead, this degradation occurs only during storage. The SAD, FAD, and COD samples form a relatively large cluster characterized by a higher content of heavier linear hydrocarbons (including tetradecane, dodecane, and 8-heptadecene) and ketones (including 2-butanone, 2-pentanone, and 2-decanone). CAD samples are characterized by a higher variability among replicates and by higher quantities of some linear ketones (acetone, 2-pentanone, 2-nonanone, and 2-decanone) related to lipid degradation. This differentiation distinguishes the control samples from the industrially prepared (FAD) ones. This phenomenon could be attributed to differences in the drying conditions because, despite efforts to maintain consistency in those conditions, some variations may have occurred, particularly considering light exposure.

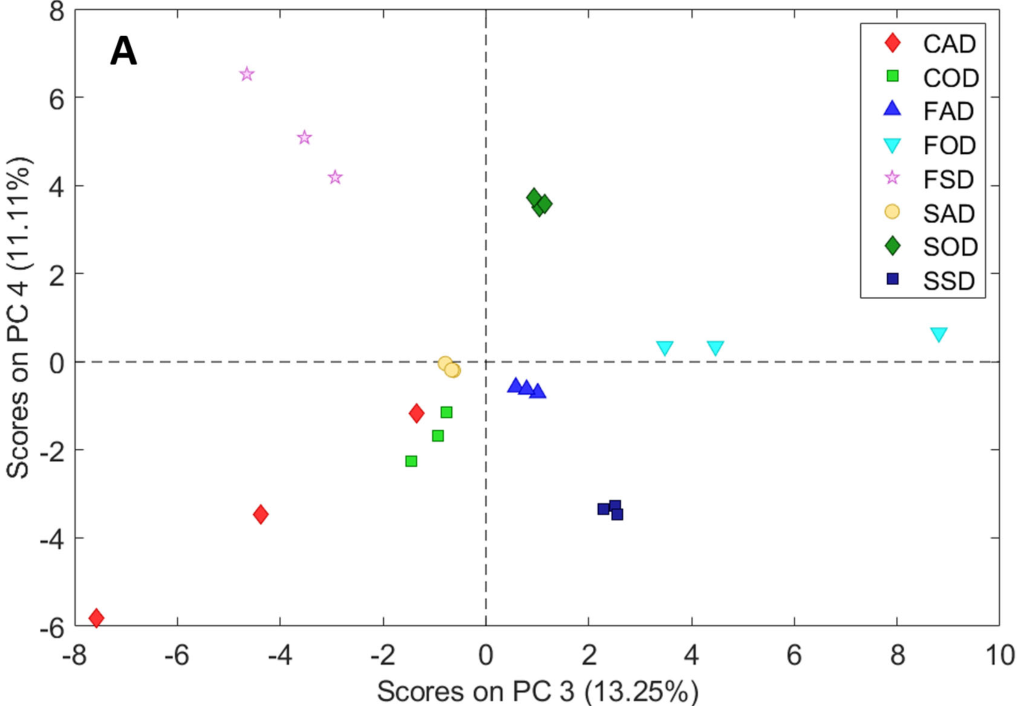

Figure 4. *Cont.*

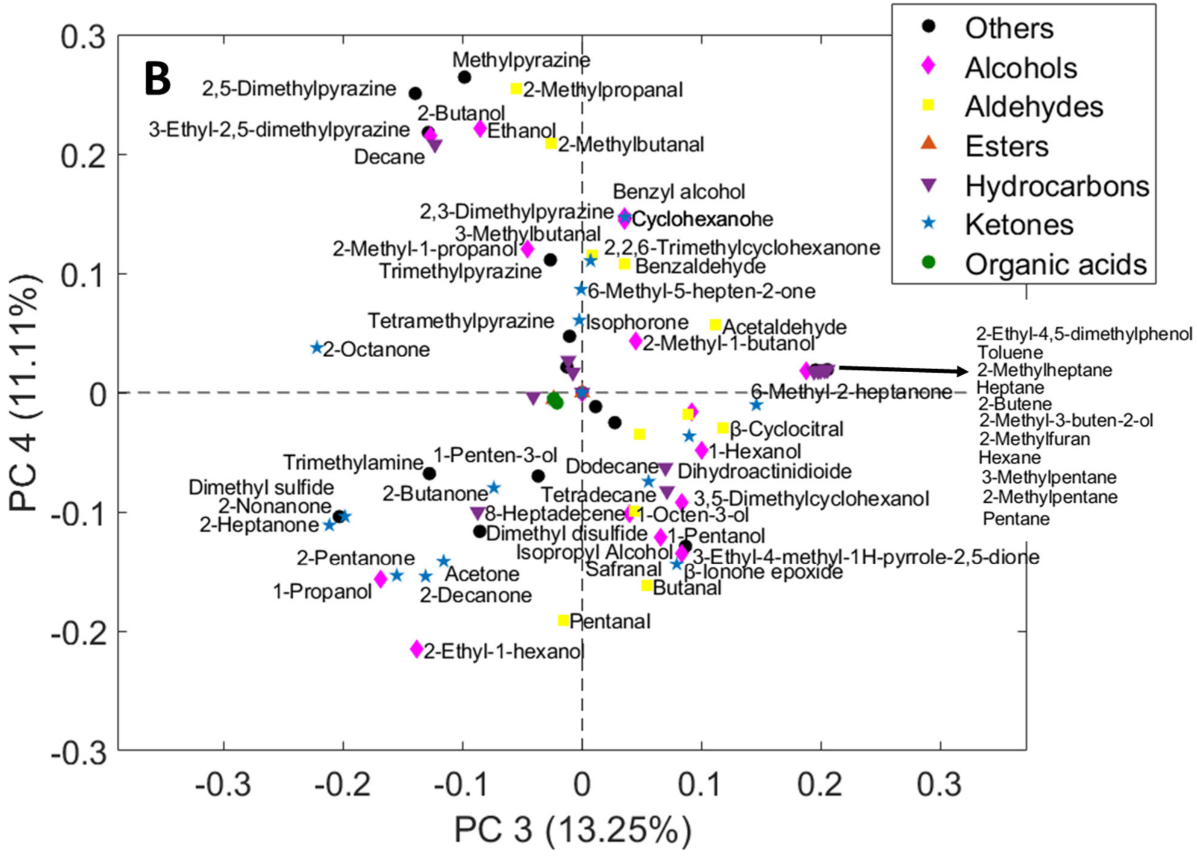

Figure 4. PCA performed without CA samples. PC3 vs. PC4 scores (**A**) and loadings (**B**) plots.

4. Conclusions

In this study, various samples of SP algae belonging to the same strain originating from an industrial plant were examined. These samples were dried using three distinct techniques (spray-drying, oven-drying, and air-drying) and aged in two different steps (freshly prepared and stored for one year). Additionally, comparisons were made with the samples from the same strain but prepared under laboratory control conditions. For the latter, the comparison was limited to freshly prepared, oven-dried, and air-dried samples. Furthermore, a comparison with a commercially available SP supplement was performed.

This study highlights the compositional complexity of SP algae, which results in an elaborate and varied volatile profile. Although air-drying leads to the less pronounced formation of compounds related to Maillard and Strecker degradation and is generally more cost-effective than other methods, this process is significantly slower. Air-drying also leads to the formation of compounds related to oxidative and fermentative phenomena, such as organic acids and esters, particularly during storage (as observed in the SAD sample). These phenomena can lead to off flavors, resulting in a shorter shelf life. Carotenoid degradation was more evident in the stored samples, although β-cyclocitral was also found in the FAD samples, likely due to the prolonged contact of the matrix with atmospheric oxygen.

Thermal treatment is crucial for limiting the degradation and fermentation phenomena during storage and extending the shelf life. However, the associated costs are higher owing to the energy requirements and, in the case of spray-drying, the need for sophisticated equipment. Moreover, the aroma profile is altered because of the formation of volatile compounds such as Strecker and linear aldehydes, which result from the degradation of amino acids and lipids. In the spray-dried samples, the formation of pyrazines was favored by the higher temperatures. Hence, when choosing a drying method, it is necessary to consider the significance of all the intervening factors. These results underscore the importance of employing a systematic analysis to select the appropriate drying method.

There is no better drying method; rather, it is essential to find a suitable compromise depending on the desired application. This approach ensures that the drying method aligns with the specific goals and constraints of the application, optimizing both quality and efficiency.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations11060180/s1, Table S1: HS-SPME-GC-MS results expressed as the TIC Area × 10^6. Table S2: Eigenvalues and explained variance of the first 20 PCs for the two conducted PCA.

Author Contributions: Conceptualization, A.U. and V.D.; methodology, V.D. and F.R.; software, L.S. and A.L.R.; validation, V.D., A.U. and F.R.; formal analysis, V.D. and L.S.; investigation, A.U. and V.D.; resources, A.L.R. and F.R.; data curation, V.D., A.U. and L.S.; writing—original draft preparation, V.D. and A.U.; writing—review and editing, V.D., A.U., F.R. and L.S.; visualization, V.D. and A.U.; supervision, A.L.R. and F.R.; project administration, F.R.; funding acquisition, F.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by Algae S.p.A. under the PON Ph.D. MUR program (DM 1061, 2021).

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: Author Andrea Luca Russo was employed by the company Algae S.p.A. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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