Composition and sensory aspects of cold-pressed avocado (*Persea americana* Mill.) oils

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Cold-pressed avocado oil is gaining attention for its nutritional and sensory properties, and, given its commercial value, it is therefore particularly subject to fraud. The risk is also higher given the lack of robust, recognized and harmonized quality and authenticity standards. This study aims to make a contribution in this regard by characterizing the compositional and sensory attributes of 5 commercial cold-pressed avocado oils. Samples were analyzed for key quality parameters and minor components using ISO and UNI standard methods, and underwent sensory evaluation through Flash and Free Choice Profiling. Free acidity values ranged from 1.85 to 26.27 mg KOH/g, with three samples exceeding the limit established by the Codex Alimentarius (4.0 mg KOH/g), indicating the possibility of strong hydrolytic degradation. Peroxide values remained within acceptable limits (3.67–9.57 mEq O2 /kg), while K232 values ranged from 2.04 to 4.34, with only one sample below the olive oil threshold of 2.50. Oxidative stability (Rancimat test) varied widely, from 3.59 to 16.99 h, confirming wide differences in oxidative state and shelf-life potential. Tocopherol contents ranged from 103.01 to 147.14 mg/kg, with α-tocopherol being predominant. Chlorophyll levels were between 40.70 and 111.40 mg/kg, and carotenes between 25.95 and 47.03 mg/kg, with the highest pigment levels found in oils probably extracted with peel. Fatty acid profiles were rich in monounsaturated fatty acids (MUFA: 62.54–72.56%), primarily oleic acid (52.79–67.14%), while total saturated and polyunsaturated fatty acids ranged from 17.60–23.62% and 9.73–13.85%, respectively. Sensory data revealed heterogeneity among samples, with most (4 out of 5) characterized by negative attributes (e.g., oxidized, rancid), and only one sample exhibiting fruity and fresh notes. The findings, which even for the only five oils sampled highlight significant quality problems among cold-pressed avocado oils, support the urgent need for quality standards and authentication criteria in the European market.

Keywords: cold-pressed, avocado oil, authenticity, quality

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Received: November 25, 2025
Accepted: February 23, 2026

1. INTRODUCTION

The production and global market of cold-pressed avocado oil (*Persea americana* Mil.) intended for food use are steadily growing, as it represents a relatively new product in this sector, probably perceived as healthy and less processed with respect to other more common and refined oils [1]. According to data from the Food and Agriculture Organization (FAO), updated to 2024, global avocado production has steadily increased from 716,000 tonnes in 1961 to approximately 11 million tonnes in 2023, with particularly pronounced growth observed over the last decade. The Americas represent the leading avocado-producing region worldwide, accounting for 72.6% of global production, followed by Africa (12.6%) and Asia (11.8%). Avocado production in fact originated in Central America, in Puebla, a central-eastern

state of Mexico, later spreading to a large area of Central America, and then all over the world. To date, there are also important crops in the Mediterranean basin, such as in Spain and Sicily, and production of this fruit is constantly growing in Asian countries too. Mexico is the world's leading producer, while the largest other producers are New Zealand, Peru, the Dominican Republic, Guatemala, the United States, South Africa and Chile [1]. Europe contributes only 1.7% of total production, and Italy is currently not included among avocado-producing countries. Nevertheless, in response to climate change and the associated improvement in pedoclimatic conditions, new avocado production initiatives are emerging in Southern Italy, particularly in Sicily (FAOSTAT).

Avocado fruits are widely used as a raw material in the food industry and for the extraction of oil. The fruit is a drupe, about 15 cm wide in length, and it consists of 65% pulp (mesocarp), 20% seed (stone) and 15% peel (exocarp). However, all these characteristics also depend on the cultivar, the harvest period and the season [2], together with the oil content in the fruit of commercially ripe avocado, which can vary between 10% and 32%, also based on the fresh weight of the pulp. The total oil content in the fruit increases during the growth of the fruit on the tree [3]. The fruit takes on a green color on the outside, or brown, with different shades depending on the variety and the stage of ripeness, while the pulp is yellow/green with a buttery consistency [4].

Over the years, several different varieties have been selected and the plants that have better characteristics and produce higher quality fruits, which are now more marketed, are the *Fuerte* (green color) and the *Hass* (brown color). The latter has a higher lipid content and is therefore more suitable for oil extraction [4].

Avocado is a fruit characterized by the accumulation of significant amounts of lipids. During fruit development on the tree, a progressive increase in both fruit size and lipid content in the pulp tissue is observed. The oil content of avocado fruits is influenced by, and may vary depending on, cultivar, pedoclimatic conditions, harvest time, and post-harvest storage conditions. The literature reports oil contents ranging from 7.6% to 30% [5,6,7,8,9,10].

In recent years, the demand for this fruit has tripled and its use has become a trend, as consumers' attention is increasingly oriented towards vegetable products, which are considered healthy, rich in bioactive compounds, such as vitamins and antioxidants and, in the case of avocado, highly palatable and well-liked [11]. Avocado oil is mainly made up of monounsaturated fatty acids, which are associated with cardiovascular benefits and anti-inflammatory effects [12,13]; its triacylglycerols are mainly composed of monounsaturated fatty acids [14].

Thanks to its composition, it can be considered somewhat similar to olive oil in terms of nutritional properties, a basic product of the Mediterranean diet [15].

Avocado oil can be extracted using different methods, some of which have higher yields (pressure/ultrasonic extraction, solvent extraction and biological extraction using enzymatic formulations), and are generally followed by standard refining steps (e.g. deacidification, decolorization and deodorization); others, such as supercritical CO2, are more expensive and therefore used for specific applications. Over the last few years, the system of cold-pressing the avocado fruit has been approached using technologies similar to those used to produce virgin olive oil [15]. This development was led by New Zealand and its commercialization began in 2000. Using only mechanical methods, the oil can retain some nutritional and sensory properties of the drupes and the extraction does not require the use of solvents [3].

Furthermore, any virgin or cold-pressed oil that has not undergone refining may contain relatively high concentrations of minor components, such as tocopherols, chlorophylls, phenolic compounds and sterols [16,17]. However, it should be remembered that an unrefined oil is not free, for instance, from oxidation products, which may form if the fruit, or the oil itself, is of poor quality or improperly stored, nor from possible contaminants [18,19].

Avocado fruit shows the highest levels of plant sterols of any other fruit. The main plant sterol found in avocado oil is β-sitosterol, but other sterols have also been identified in the oil, including Δ-5-avenasterol, campesterol and stigmasterol [16]. It is well known that plant sterols may have a beneficial effect on lowering blood cholesterol levels in humans, reducing the risk of heart disease [20,21]. In addition, other characteristic compounds, recovered from the fruit flesh containing a furan (or cyclic keto) moiety and characterized by double-bond positions in the side chain, identical to those of linoleic acid, often referred to as avocatins or avocadofurans, were first described in avocado by Frega et al. (1991) [22].

Moreover, cold-pressed avocado oil contains significant levels of pigments such as carotenes, xanthophylls, and substantial amounts of chlorophyll, which are reflected in its rich green color. Chlorophyll acts as a sensitizer in photooxidative processes; however, under dark storage conditions, it has been reported to have no effect or may even exhibit antioxidant properties [23,24].

Compared to other oils, relatively little is known about the polyphenolic content of avocado oil. However, since phenolic compounds are present in avocado fruit, they are likely to be present in avocado oil as well. The phenolic compounds present in the oil are still being studied, compared to those found in the pulp of the mesocarp [25].

Cold-pressed avocado oil is aimed at consumers looking for a mild, buttery flavor without the pungent notes of extra virgin or virgin olive oil. Another selling point is its reportedly high smoke point of over 250°C [21]. Although this measurement is empirical and not very indicative of the actual performance of an oil at high temperatures, it is, unfortunately, very popular in the increasingly influential world of kitchens and chefs.

Avocado oil is a relatively new product and, as such, it can face problems of authenticity, quality and genuineness. Some products labelled as "cold-pressed" may exhibit a very light color, suggesting a high likelihood of processing treatments such as bleaching or refining, which are not declared on the label. There may be fraud related to dilution or partial or total substitution to increase the available quantity of cold-pressed avocado oils, or oils produced from poor-quality raw materials, which should absolutely be refined before being marketed [3,26].

The commonly used values for avocado oil before 2024 were those recommended for olive oil, and the quality and authenticity standards for olive oil and olive pomace oil are, for the countries that recognize them, those proposed by the International Olive Council (IOC) [27]. More in general, according to the CODEX standard CXS 210-1999, updated in 2024 [28], the method of extraction of cold-pressed edible vegetable oils is defined by physical-mechanical procedures, e.g. extrusion or pressing, without the application of heat, and the obtained oil can only be purified by washing, sedimentation, filtration and centrifugation, while the other three categories reported are edible vegetable oils, virgin oils and cold-pressed oils, according to the processing method and quality parameters. Although Codex Alimentarius Standard CXS 197-1995 [29] (revised in 2013) classifies avocado fruits into three quality categories (Extra, Class I, and Class II), the updated Codex Standard CXS 210-1999 (2024) [28], which establishes benchmarks for avocado oil, defines it as "derived from the mesocarp (i.e., the fleshy part) of the avocado fruit (*Persea Americana* Mill.) or obtained by processing the whole avocado fruit," without providing any categorization based on the fruit's type or quality. Moreover, current literature offers little or no research comparing oil extracted from the whole fruit with that obtained solely from the mesocarp. There are some classification proposals in literature, for example, Woolf et al. [3] proposed a classification of avocado oil based on the extraction method and the quality of the fruit, suggesting that "extra virgin" avocado oil should refer to a premium-quality product obtained from carefully selected fruits, extracted solely through mechanical processes at temperatures below 50 °C and without the use of chemical solvents. Avocado oil referred to as "virgin" was, on the other hand, defined as obtained from inferior fruits, extracted exclusively by mechanical methods, at a temperature below 50 °C and without the use of chemical solvents. Lastly, "pure" avocado oil refers to a type of oil where the quality of the raw fruit is not a priority; thus, it should certainly be bleached and deodorized and can be used, in particular, for cosmetic applications.

To check these possible classifications, to date, no specific markers or measures, together with limits or ranges, have been proposed to assess the quality and authenticity of cold-pressed avocado oil on which a detailed standard, in respect of the general proposal by Codex in 2024, could be based.

Thus, this research work has aimed to search, select and compare data on the composition of cold-pressed avocado oil from the literature with those obtained in our laboratories in order to define possible quality and authenticity parameters, considering that, at present, there is no specific legislation for cold-pressed avocado oil at a national level. For this reason, the references and the data discussed in this work can be useful to draft a UNI trade standard, entitled "Cold-pressed avocado oil obtained from the fruits of *Persea americana Mill*. - Characteristics and methods of analysis". In addition, considering that, regardless of the unclear product definition of the oils analyzed (some were labelled as virgin and not cold-pressed and, more generally, it is not reported whether or not they have been refined), the subject of this study is oils extracted by mechanical means, a sensory evaluation was also carried out.

2. MATERIALS AND METHODS

2.1. SAMPLES

All samples analyzed were purchased from the market and labelled as cold-pressed avocado oil, or in the case of samples 1 and 3, as "virgin avocado oil". Furthermore, samples 2, 3 and 4 were also claimed as organic. For sample 4, the label indicated that the oil was cold-pressed using only organic mesocarp, while for sample 5, the producer declared that only the Hass cultivar was used, along with a small amount of fruits with peel.

2.2. ANALYTICAL DETERMINATIONS

All the methods applied to carry out the analytical determinations are listed in Table I.

All reported results are obtained from the average of three analytical replicates. In addition to the full set of analyses, two samples (sample 1 and 5) were also subjected to further testing, i.e. sterol compositions (%) and stigmastadienes content (%), following the methods reported in Table I.

Table I – Methods used for analytical determinations carried out on cold-pressed avocado oil

Chemical parameters	Methods of method
Acidity	ISO 660:2020. Animal and Vegetable Fats and Oils. Determination of Acid Value and Acidity [30].
Peroxide value	ISO (2017) 3960. Animal and vegetable fats and oils. Determination of peroxide value. Iodometric (visual) endpoint determination [31].
K_{232}, K_{268}, ΔK	ISO (2017) 3656. Animal and vegetable fats and oils. Determination of ultraviolet absorbance expressed as specific UV extinction [32].
Fatty acid composition	ISO 12966-2:2017; Animal and Vegetable Fats and Oils. Gas Chromatography of Fatty Acid Methyl Esters-2: Preparation of Methyl Esters of Fatty Acids. International Organization for Standardization: Geneva, Switzerland, 2011 [33]. ISO 12966-4:2015; Animal and Vegetable Fats and Oils. Gas Chromatography of Fatty Acid Methyl Esters-4: Determination by Capillary Gas Chromatography. International Organization for Standardization: Geneva, Switzerland, 2015 [34].
Total sterols, sterol composition, erythrodiol, uvaol and aliphatic alcohols	NGD C71 -1989 [35]. NGD C72 -1989 [36].
Stigmastadienes	COI/T.20/Doc.n.11/rev.4 2021 [37].
Induction Time	ISO 6886:2016 Animal and vegetable fats and oils. Determination of oxidative stability (accelerated oxidation test) [38].
Tocopherols	UNI/TS 11825:2021 Vegetable oils and fats - Determination of Tocopherols and Tocotrienols by high-pressure liquid chromatography (RP-HPLC) and fluorimetric detection [39].
Chlorophylls	Minguez-Mosquera et al., 1991 -Spectrophotometric method. [40]
Carotenes	Minguez-Mosquera et al., 1991 -Spectrophotometric method [40]

2.3. SENSORY EVALUATION

Descriptive sensory analysis has been widely used to characterize the sensory attributes of food products, and conventional descriptive analysis is the most commonly applied sensory profiling method. Alternatively, to overcome some limitations of this approach, faster methods, amply used for both scientific research and industrial applications, such as Free-Choice Profiling Williams & Langron (1984) [41] and Flash Profile [42], have been applied here.

2.3.1 Flash Profile

To determine the sensory profile of the avocado oil samples, a rapid descriptive sensory test was carried out using the Flash Profile method. Eight volunteers (25-65 y.o.) were recruited for the test from among the staff of the Department of Agricultural and Food Science at the University of Bologna. The oils were served in portions of approximately 15 mL at room temperature in white disposable plastic cups. Each sample was identified with a three-digit numerical code and presented simultaneously to the panelists. Evaluations were conducted at room temperature in the sensory booths, allowing each panelist to assess the samples individually. The panelists participated in three tasting sessions. The first session, which served as the qualitative analysis phase, included the evaluation of all the samples simultaneously across each sensory modality. Each panelist developed an individual vocabulary, noting on the sensory sheet the visual, olfactory, taste, and texture attributes that they considered useful for discriminating the samples, while avoiding the use of hedonic terms. At the end of this phase, a group discussion was held, during which participants reviewed and revised their attribute lists by adding or removing descriptors. During the second and third tasting sessions, participants were asked to rank the samples according to the perceived intensity of each identified attribute. These two sessions served as replicates of the same test phase — the quantitative analysis — to ensure data reliability. Statistical analysis has been performed on the mean of the results from the second and third testing sessions; data collection was carried out manually.

2.3.2 Free Choice Profile

To determine the sensory profile of avocado oil sample 5, which arrived later, directly from the producer during the fruit harvest, in February 2025, a rapid descriptive sensory test was conducted using the Free-Choice Profiling method. Eight volunteers (25-65 y.o.) were selected for the test from among the staff of the Department of Agricultural and Food Science at the University of Bologna, as before. The oil was served in portions of approximately 15 mL at room temperature (around 20°C) in white disposable plastic cups. The sample

was identified by a three-digit numerical code. Evaluations were carried out at room temperature in individual sensory booths, allowing each panelist to conduct the assessment independently. The panelists participated in two tasting sessions. In the first session, each participant was asked to individually generate the sensory attributes that best described the sample and was instructed to avoid using hedonic terms. Each judge developed a personal vocabulary, recording on the sensory sheet the visual, olfactory, taste, and texture attributes they considered relevant for describing the sample. In the second session, panelists were asked to rate the intensity of the generated descriptors using a linear scale anchored at the left end with "low intensity" and at the right end with "high intensity". Data collection was performed manually.

2.4. DATA ANALYSIS

Data related to peroxide values, free acidity, spectrophotometric extinction in the UV, induction time, tocopherols, chlorophylls and carotenes were elaborated by one-way ANOVA (Tukey's HSD, $p<0.05$) (XLSTAT, Version 2024.4.2; Lumivero, USA). The data related to Flash Profile and Free Choice Profile were processed using the Sensory package of the XLSTAT software (Version 2024.4.2; Lumivero, USA), applying the appropriate data analysis methods.

3. RESULTS AND DISCUSSION

3.1. ACIDIC VALUE

Free acidity, which, as is well known, cannot be perceived as "acid taste", given the low pKa of long-chain fatty acids, offers an assessment of the degree of hydrolytic alteration of an oil and serves as a representative indicator mainly of the "freshness" and conservation of the fruits, before the extraction. It reflects the amount of free fatty acids present in the oil due to the hydrolysis of triglycerides into glycerol and fatty acids. A high acidity index mainly indicates that the fruits extracted are of bad quality. The Codex Alimentarius sets a maximum of 4 mg KOH/g for cold-pressed oils; as reported in Table II, three out of five samples exceeded this limit, surpassing that value [28]. Literature reports that in Fuerte avocados, triglyceride degradation increases free fatty acids during maturation [43]. High free acidity values were also reported by Werman and Neeman (1987) [14], who found a value equal to 8.35 mg KOH/g of oil in an avocado oil obtained through centrifugation. Green and Wang (2023) [44] found free acidity values between 0.09 and 0.13 (% of oleic acid) in cold-pressed avocado oils, and between 0.12 and 1.71 (% of oleic acid) for virgin and extra virgin avocado oil samples (defined as such by the authors). Nasri et al. (2021) reported significant differences ($p < 0.05$) in acid values among avocado varieties, with values of 1.4, 2.6, 1.9, and 2.4 mg KOH/g oil for Ettinger, Fuerte, Hass, and Reed pulp oils, respectively. These values were higher than those previously reported for the Fuerte variety (1.23 mg KOH/g oil) and for avocados grown in Mexico (1.46 mg KOH/g oil) [23], while Jorge et al. (2015) reported free acidity values of 3.64 mg KOH/g and 1.58 mg KOH/g for avocado oil from the Margarida and Hass variety, respectively [45]. The values observed in the present study are partially consistent with those reported in the literature (samples 1 to 4), while sample 5 shows significantly higher levels of free acidity (Table II). This may be related to the fact that the fruits used for oil production were of very low and poor quality. High acidity can occur both on and off the tree — for example, due to the use of overripe or insect-damaged fruits, or improper handling and excessive heat during processing. These conditions can cause fatty acids to break away from the triacylglycerols — the main components of oil — and become "free" within the oil [30].

PEROXIDE VALUE

The peroxide index, which provides a relative assessment of primary oxidation compounds in vegetable oils according to the Codex Alimentarius (CODEX STAN 210-1999) [28], is set at a maximum of 15 $mEqO_2$/kg. The determinations carried out on the five samples examined yielded values between 3.67±0.18 $mEqO_2$/kg and 9.57±0.23 $mEqO_2$/kg, indicating that all samples were below the limit established by the Codex, as shown in Table II. The peroxide value indicates the extent of oil oxidation by measuring the concentration of peroxides, which can form through

Table II – Quality parameter - Free Acidity (mg KOH/g), POV Peroxide value (mEq O_2/kg), Spectrophotometric indices K_{232}, K_{268}, ΔK, Induction Time (h). Different letters in columns indicate statistically significant differences (one-way ANOVA, $p<0.05$)

Sample	POV	Free Acidity	K_{232}	K_{268}	ΔK	Induction Time
1	6.94±0.02	1.85±0.06^{e}	3.81^{b}	0.77^{a}	0.02	16.99±0.30^{a}
2	8.25±0.18	4.90±0.14^{b}	2.04^{d}	0.32^{d}	0.01	11.64±0.12^{b}
3	9.57±0.23	4.61±3.06^{c}	3.81^{b}	0.43^{c}	0.01	9.09±0.51^{d}
4	3.67±0.18	2.12±0.02^{d}	2.68^{c}	0.34^{d}	0.02	10.25±0.34^{c}
5	8.67±0.24	26.27±0.42^{a}	4.34^{a}	0.63^{b}	0.02	3.59±0.09^{e}

photooxidation, autoxidation, thermal oxidation, and prolonged exposure to oxygen, such as when the bottle is opened [44]. Jorge et al. (2015) [45] reported PV values of 4.98 for Margarida avocado oil and 5.54 for Hass variety avocado oil; while Green and Wang (2022) [46] demonstrated that a peroxide value (PV) limit of 10.0 mEq O_2/kg is both appropriate and achievable for virgin and extra virgin avocado oils. The same authors also demonstrated in a subsequent study that all the virgin and extra virgin samples that they analyzed complied with the proposed PV limit of ≤10.0 mEq O_2/kg [44]. In accordance with this, PVs of the samples analyzed here were below 10 mEq $O2_2$/kg as reported in Table II; while Flores et al. (2014) [47] found a higher value for a sample of avocado oil from the market. It is important to bear in mind that peroxides are highly unstable, that they represent only the primary oxidation compounds, and that they provide merely a partial indication of the oxidative status of an oil [48].

3.2. SPECTROPHOTOMETRIC INVESTIGATION IN ULTRAVIOLET UV

Spectrophotometric analysis measures the conjugated diene and triene systems present in oils, thus determining parameters used as indicators of PUFA (polyunsaturated fatty acids) oxidation. In particular, K232 is another indicator of primary oxidation products and is often regarded as more reliable than peroxide value (PV), as it relies on spectrophotometric analysis rather than visually assessed titration. Moreover, K232 can detect a wider spectrum of primary oxidation compounds, such as linoleic hydroperoxide and conjugated dienes. However, its use is less widespread than that of PV and, as a result, it is not currently included in the proposed CODEX standards for avocado oil [46]. K268 is another measure of oxidation, specifically targeting secondary oxidation products such as carbonyls, aldehydes, and conjugated trienes. Prolonged storage or excessive heat during refining are known to elevate K268 values [46]. Although no specific values are established for avocado oil, those of olive oil can be used as a reference, given that it has a very similar fatty acid composition. Specifically, according to EU Regulation 2105/2022 [49], the limit values are ≤ 2.50 for K232 and ≤ 0.01 for ΔK. In our samples, high K232 values were observed; however, only sample 4 had a K232 value ≤ 2.50.

3.3. RESISTANCE TO FORCED OXIDATION (RANCIMAT TEST)

The Rancimat test is a commonly used method to evaluate oxidative stability, and it can be somewhat useful to predict an oil's shelf life [50], in particular in comparison with another, so mainly as a relative value. Heating (110 °C) and air flow (9 l/h) promote the oxidation of an oil sample and, when the oxidation phenomenon becomes incipient, formic and acetic acids are produced and distilled from the reaction vessel into the measurement vessel, where a dramatic increase in conductivity of deionized water is recorded, which determines the induction time as an inflection point of the oxidation curve [50,51]. A longer induction time indicates better oxidative stability; conversely, a low value suggests that oxidative degradation reactions are already underway, compromising shelf life. Using the Rancimat test, sample 1 showed the highest stability with a value of 16.99±0.30 h, while sample 5 exhibited a high degree of degradation, with a very low induction time of 3.59±0.09 h, considering the matrix type. As shown in Table II, the sample with the highest induction time also had the lowest levels of free acidity and low levels of peroxides. All these parameters highlight a very advanced oxidative state. Some authors have reported that the stability of avocado oil was similar to that of olive oil [15] or reported forced oxidation resistance values of 14 and 20 h [47].

3.4. TOCOPHEROL CONTENT

The nalysis of tocopherols showed their total contents ranging from 103.01±1.24 mg/kg to 147.14±0.97 mg/kg. α-tocopherol was the most represented with concentrations from 75.31±3.45 mg/kg to 123.87±6.33 mg/kg, followed by γ-tocopherol with concentrations from 20.90±1.01 mg/kg to 34.44±0.40 mg/kg, while δ-tocopherol was present only in traces, well below the quantification limit in some samples, or not detected. Fernandes et al. (2018) [16] reported marked

Table III – Minor bioactive compound – Tocopherols (mg/kg), Chlorophylls (mg/kg), Carotenes (mg/kg). Different letters in columns indicate statistically significant differences (one-way ANOVA, p<0.05)

Sample	α-Tocopherol	γ-Tocopherol	Total Tocopherols	Chlorophylls	Carotenes
1	112.70±1.33^{b}	34.44±0.40^{a}	147.14±0.97^{a}	59.11±1.05^{c}	36.24±0.42^{c}
2	75.31±3.45^{c}	34.34±1.42^{a}	109.65±4.87^{b}	40.70±1.78^{d}	31.63±0.18^{d}
3	79.45±0.59^{c}	23.56±0.68^{b}	103.01±1.24^{b}	91.60±2.78^{b}	43.64±1.48^{b}
4	123.87±6.33a	21.28±1.03^{c}	145.15±7.36^{a}	60.15±0.94^{c}	47.03±0.84^{a}
5	121.65±6.15^{a}	20.90±1.01^{c}	142.55±7.16^{a}	111.40±4.39^{a}	25.95±1.37^{e}

variability in total tocopherol content among avocado oils, with values of 138.5 mg/kg for the Bacon variety, 141.5 mg/kg for Fuerte, 125.98 mg/kg for Hass, and 107.39 mg/kg for Pinkerton. Lower tocopherol levels were reported for avocado oils from different geographical origins, including 9.30 mg/kg for samples from Brazil, 31.14 and 44.94 mg/kg for two samples from Chile, and 4.99 mg/kg for a sample from New Zealand; however, it may be assumed that, for these latter oils, which were commercially purchased rather than obtained by laboratory extraction as in the previous cases, the markedly low tocopherol contents - lower than those reported by the Codex for avocado oil (50–450 mg/kg) [28] - could be attributable to extensive refining processes or to a lack of oil authenticity. The results obtained, as shown in Table III agreed with what was found in the literature, and with what was also published by Flores et al. (2019) [15], Woolf et al. (2009) [3] for cold-pressed avocado oils, who indicate a range for α-tocopherol between 70 mg/kg and 190 mg/kg. In the samples examined here, no tocotrienols were found.

3.5. CHLOROPHYLLS AND CAROTENES

Chlorophylls, together with carotenes, are fat-soluble pigments responsible for the yellow-green color of oil. Nasri et al. (2015) [23] reported varietal differences in pigment composition of avocado oils, with chlorophyll contents of 0.54, 1.63, 1.04, and 0.83 mg/kg for Ettinger, Fuerte, Hass, and Reed varieties, respectively, and corresponding carotenoid contents of 0.77, 0.40, 0.56, and 0.35 mg/kg. Jorge et al. (2015) [45] reported a chlorophyll content of 3.26 and 10.07 mg/kg for the Margarida and Hass varieties, respectively. In the samples evaluated in our laboratories, the chlorophyll contents were very different and varied from a minimum of 40.70±1.78 mg/kg to a maximum of 111.40±4.39 mg/kg. This last high value is probably due to the fact that the whole fruits were pressed. In fact, high chlorophyll contents could derive from the inclusion of the peel during pressing, even if this is not specified on the label. In one study [52], the authors found that chlorophyll levels in oil extracted from 100% fruit peel were three times higher than those in oil extracted without the peel. Literature reports chlorophyll contents that vary greatly from 1.23 to 69.8 mg/kg [14,47,52,53,54,55]. There are many factors that can influence this parameter, such as the cultivar and the ripening of the fruit, as well as the methods used to extract and conserve the oil. The presence of chlorophylls can compromise the oxidative stability of oils, due to their pro-oxidant and photosensitizing effects [56]. For this reason, they are generally undesirable among the minor cold-extracted compounds, as they can reduce their shelf life [57]. With reference to carotene content, values between 25.95±1.37 mg/kg and 47.03±0.84 mg/kg were found. In other scientific studies, carotenes in avocado oil have been found from 72 to 105 mg/kg and from 0.9 to 3.5 mg/kg [3,55]. Many factors, as before, influence the pigment content in avocado fruits, including the cultivar, the harvest time, and post-harvest storage. Additionally, factors such as temperature, exposure to light and oxygen, and the already cited inclusion or exclusion of the peel during the extraction process, as well as proper storage during shelf life, can impact the pigment levels in the oils. With reference to the stability of minor compounds present in avocado oil, Resende et al. (2019) [58] found that chlorophylls were the most sensitive to temperature increases, followed by carotenes and sterols. Many scientific studies have investigated the pigment content and the ratios between the fraction of chlorophyll and that of carotenes in vegetable oils and olive oil. The results indicate that these parameters, with some limitations, can be used for the definition of authenticity, geographical and/or varietal classification and quality assessment [52,59,60,61,62].

3.6. FATTY ACIDS

The results obtained for avocado oil shown in Table 4 highlight a fatty acid profile in which monounsaturated fatty acids (MUFA) are predominant, ranging from 62.54±0.01% to 72.56±0.10%, followed by saturated fatty acids (SFA), which range from 17.60±0.10% to 23.62±0.01%, and polyunsaturated fatty acids (PUFA), ranging from 9.73±0.03% to 13.85±0.02%. There are many data referring to the total fatty acid content of cold-pressed avocado oil [16,63,64,65,66], which indicate percentages of monounsaturated fatty acids (MUFA) ranging from 65.29% to 78.61%, saturated fatty acids (SFA) from 12.65% to 21.78% and polyunsaturated fatty acids (PUFA) from 5.43% to 15.96%. In cold-pressed and virgin avocado oil, the Codex Alimentarius (CODEX STAN 210-1999) [28] indicates, for the most monounsaturated representative fatty acids oleic and palmitoleic acids, a range of 42.0-75.0% and 4.0-17.1%, respectively, palmitic and stearic acids among the saturated fats in a range of 11.0-26.0% and 0.1-1.3%, and linolenic and linoleic acids among the polyunsaturated fats in a range of 0.5-2.1% and 7.8-19.0%. The qualitative and quantitative composition of total fatty acids is specific and closely linked to the botanical species of origin and also to the geographical origin of the avocado. For example, Tan et al. (2017) reported that Hass avocado pulp is characterized by a high lipid content and that its oil is predominantly composed of unsaturated fatty acids and triacylglycerols. However, the relative distribution of these unsaturated components was found to vary according to geographical origin. In particular, compared with Hass avocado oils from Mexico, Australia, and the United States, samples originating from New Zealand exhibited higher levels of unsaturated

Table IV – Fatty acid compositions (%)

Compound	1	2	3	4	5
Fatty acid					
Lauric	0.01±0.00	0.01±0.00	0.01±0.00	< 0.01	< 0.01
Myristic	0.07±0.00	0.03±0.00	0.04±0.00	0.03±0.00	0.05±0.00
Pentadecanoic	0.02±0.01	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Palmitic	22.54±0.00	16.50±0.10	18.60±0.03	16.93±0.08	17.07±0.09
Palmitoleic*	9.51±0.01	5.19±0.00	7.98±0.01	6.20±0.03	7.34±0.05
Margaric	0.03±0.00	0.04±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Margaroleic	0.07±0.00	0.08±0.00	0.08±0.00	0.08±0.00	0.09±0.00
Stearic	0.79±0.01	0.80±0.00	0.64±0.01	0.72±0.01	0.46±0.00
trans-Oleic*	0.03±0.01	< 0.01	< 0.01	< 0.01	< 0.01
cis-Oleic	52.79±0.02	67.14±0.09	61.70±0.05	65.97±0.11	62.48±0.13
cis/trans Linoleic*	0.01±0.00	0.34±0.01	0.22±0.00	0.23±0.00	< 0.01
trans/cis Linoleic*	0.00±0.00	0.10±0.00	0.02±0.00	0.03±0.00	0.01±0.00
Linoleic	12.91±0.01	8.82±0.01	9.73±0.02	8.86±0.02	11.42±0.02
Arachidic	0.09±0.01	0.14±0.00	0.07±0.00	0.07±0.00	0.06±0.00
Eicosaenoic	0.15±0.00	0.15±0.00	0.15±0.00	0.16±0.00	0.18±0.00
Linolenic	0.93±0.01	0.58±0.00	0.67±0.00	0.62±0.00	0.75±0.00
Behenic	0.03±0.00	0.03±0.00	0.02±0.00	0.02±0.00	0.03±0.00
Lignoceric	0.05±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00
Monounsaturated Fatty Acids (MUFA)	62.54±0.01	72.56±0.10	69.91±0.04	72.41±0.09	70.08±0.08
Polyunsaturated Fatty Acids (PUFA)	13.85±0.02	9.83±0.01	10.64±0.02	9.73±0.03	12.18±0.02
Saturated Fatty Acids (SFA)	23.62±0.01	17.60±0.10	19.45±0.04	17.85±0.08	17.73±0.09
Σ*-transoleic isomers	0.03±0.01	< 0.01	< 0.01	< 0.01	< 0.01
Σ*-translinoleic +translinolenic isomers	0.03±0.01	0.44±0.01	0.25±0.00	0.25±0.00	0.01±0.00

* Σ of isomers

fatty acids and tri-unsaturated triacylglycerols [67]. Therefore, along with other parameters, it contributes to the assessment of the oil's authenticity. In a recent study, Green et al. (2023) [26] compared the fatty acid profiles of avocado oil samples with the current CODEX standards. Most samples fall within the proposed ranges, especially for oleic acid, which shows a natural variability. The study highlights the importance of setting appropriate standards to distinguish genuine avocado oil from adulterated products, considering factors like cultivar, region, and processing methods. Adjusting certain limits, such as for stearic acid, could improve the detection of adulteration while accounting for natural differences. In the samples examined here, only in sample 1 was the presence of *trans* oleic isomers 0.03±0.01%, while in all samples the presence of trans linoleic and/or *trans* linolenic isomers was detected in the range from 0.01±0.00% to 0.44±0.01%. Regarding cold-pressed vegetable oils, the UNI (Ente Italiano di Normazione) standards referring to cold-pressed oils indicated values of 0.05% and 0.1% for Σ-*trans* oleic isomers and Σ-*trans* linoleic + *trans* linolenic isomers, respectively. The IOC International Olive Council (COI/T.15/NC No 3/Rev. 20 November 2024) commercial standard, referring to the various categories of olive oils, indicates limits for Σ-*trans* oleic isomers values from 0.05% to 0.4% and 0.05% to 0.35% for Σ-*trans* linoleic + *trans* linolenic

isomers. In particular, the limit for Σ-*trans* linoleic + *trans* linolenic isomers in virgin and extra virgin olive oil is ≤0.05% [68], whereas many UNI standards (ref) set the limit for the same parameter at ≤0.10% for cold-pressed hemp seed oil [69]. *Trans* fatty acid (TFA) is formed as a result of high temperature treatments, for example in the presence of bleaching earths, such as in refining processes. Wagner et al. (2000) [70] and Wolff (1993) [71] reported TFA contents in refined oils between 0.1% and 0.8%, while in cold-pressed oils the maximum was 0.1%. TFA formation could also result from thermal treatments applied to dry the seeds before extraction [72]. For example, Berasategi et al. (2012) [63] reported that the *trans* fatty acid content in avocado oil was 0.52%, slightly higher than the level detected in olive oil (0.33%). Similarly, Ortiz-Moreno et al. (2003) [73] observed total trans fatty acid values in avocado oil ranging from 0.33% to 0.87%, depending on the extraction method employed. In their study, microwave-assisted extraction and mechanical pressing yielded the lowest trans fatty acid levels, whereas extraction with acetone resulted in the highest value, reaching 0.87%. These analytical parameters are very relevant for verifying adulteration of cold-pressed oils with refined oils.

3.7. STEROLS AND STIGMASTADIENES

Analysis of minor compounds, in particular sterol components, is commonly applied to lipid matrices to verify their authenticity. In fact, the sterol profile is characteristic of each botanical species and can therefore be used as an indicator to identify and authenticate the origin of an oil. The determinations carried out here on the two samples highlighted that the most represented compound was β-sitosterol followed by campesterol and Δ5-avenasterol, while the total sterols were 4904.70±437.56 mg/kg and 4248.67±64.07 mg/kg for samples 1 and 5, respectively. The details of the results are reported in table V. The values found are consistent with what is reported in the literature, where the proposed values of β-sitosterol were reported in ranges from 81.71% to 85.87%, of campesterol from 4.85% to 9.75% and of Δ5-avenasterol from 3.80% to 8.45% [26,74]. However, in some samples other authors have found a greater presence of Δ5-avenasterol compared to campesterol [16]. The contents of total sterols were included in the ranges between 2859 mg/kg and 7612 mg/kg. Fernandes et al. (2018) [16] reported substantial variability in total sterol content among avocado oils, with values of 5,376.33 ± 47.86 mg·kg^{-1} for the Bacon variety, 6,798.42 ± 3.88 mg·kg^{-1} for Fuerte, 7,611.88 ± 0.91 mg·kg^{-1} for Hass, and 6,723.05 ± 62.06 mg·kg^{-1} for Pinkerton. Lower sterol contents were observed in oils from different geographical origins, including Brazil (4,889.06 ± 41.72 mg·kg^{-1}), Chile A (3,828.78 ± 11.67 mg·kg^{-1}), Chile B (5,490.61 ± 56.95 mg·kg^{-1}), Ecuador (545.14 ± 10.64 mg·kg^{-1}), and New Zealand (656.95 ± 9.94 mg·kg^{-1}). However, as noted previously, some of these samples were commercially purchased, and therefore there is no certainty that they were unrefined or even fully authentic avocado oils. Moreover, as reported by Lozano et al. (1993) [17], the total sterols content is higher in mature than immature fruits.

3,5-Stigmastadienes are compounds that are not naturally present in cold-pressed vegetable oils. Their formation occurs following refining or deodorization processes as a result of the dehydration of β-sitosterol. This parameter can be considered an indicator of oils that have undergone refining processes and/or possible adulteration with refined oils. Generally, the standards for reference levels in cold-pressed vegetable oils specify a maximum value of 0.10 mg/kg for 3,5-stigmastadienes. In our study, the values found in the two samples analyzed were well below this limit for sample 5, while they were above for sample 1, as shown in Table V, and this result could be attributable to mild refining. Additionally, Flores et al. (2014) [47] analyzed stigmastadiene levels in virgin avocado oil samples of the Hass variety found in Chilean markets. In one sample no stigmastadienes were detected, while in another, the presence of 45.6 mg/kg of 3,5-stigmastadiene was found, likely related to high-temperature treatments during refining and the resulting dehydration of thermosensitive sterols.

Table V – Sterol compositions (%) and stigmastadienes content (mg/kg)

Compound	1	5
Sterol		
Cholesterol	0.10±0.00	0.17±0.01
Brasicasterol	<0.01	< 0.01
24-methylene-cholesterol	0.60±0.04	0.49±0.06
Campesterol	7.31±0.16	5.85±0.19
Campestanol	0.05±0.01	0.10±0.01
Stigmasterol	0.66±0.00	0.36±0.01
Δ7-campesterol	< 0.01	< 0.01
Δ5,23-stigmastadienol	< 0.01	< 0.01
Clerosterol	1.97±0.04	1.75±0.06
β-Sitosterol	83.65±0.38	85.75±0.45
Sitostanol	0.52±0.03	0.40±0.23
Δ5-avenasterol	4.72±0.06	4.76±0.33
Δ5,24-stigmastadienol	0.16±0.04	0.19±0.01
Δ7-Stigmastenol	0.09±0.00	0.05±0.00
Δ7-avenasterol	0.18±0.00	0.14±0.01
Compounds	**mg/kg**	**mg/kg**
Total sterols	4904.70±437.56	4248.67±64.07
Stigmastadienes (sum of isomers)	0.80±0.05	0.01±0.00

3.8. SENSORY ANALYSIS

3.8.1 Flash profile

The first Flash Profile tasting session yielded a high number of descriptors (19 in total); however, not all adjectives were used by the panelists in the subsequent sessions, as each panelist employed their individual vocabulary, in accordance with the method. The reported descriptors were: i) appearance attributes (yellow and green); ii) texture attributes (cloudy/cloudiness, clearness and oily); iii) taste attribute (bitter); iv) olfactory notes (sunflower seeds, olive, avocado, rancid, oxidized, paint, cooked vegetables, smoky, brined olives, winey-vinegary, animal/stable and brine). From the second and third tasting sessions, a rank score was calculated for each attribute, based on the ordering of samples according to perceived intensity. The results from the second and third sessions were treated as independent replicates of the same test phase. After averaging the data, they were analyzed using GPA (Generalized Procrustes Analysis). The data were processed using Commandeur's algorithm to obtain a consensus configuration reflecting the judges' collective perception of the samples. This analysis allows for the identification of common patterns among the samples and their sensory attributes. Figure 1 displays the avocado oil samples, labelled with numerical codes and represented as blue dots, on a two-dimensional plot. The axes represent the first two principal components, which explain the majority of the data variance (84.52%). The position of each sample on the plot reflects the similarity of its sensory characteristics. The relationship between sample positions and the location of the variables helps determine whether samples are correlated with each other or with specific attributes. If samples appear close to one another on the plot, it can be concluded that they share similar sensory profiles based on the 19 attributes considered and vice versa. Notably, as seen in Figure 1, the sensory evaluation produced linear results, as the replicate evaluations for the same sample appear close to each other, indicating consistency and reproducibility among the judges.

In particular, sample 3 was most strongly associated with negative sensory attributes such as "*oxidized*" and "*winey*". Figure 1 highlights how the direction of the vectors, which represent the variables, helps to identify the correlation between each sensory attribute and the different samples. The longer the vector, the greater the influence of that attribute in differentiating the samples. For instance, the attribute "*clearness*" is positively correlated with sample 4, while the attribute "*olive*" is positively associated with sample 2.

The results of the Flash Profile sensory evaluation revealed a heterogeneous profile across all samples, as they are distributed across different quadrants of the graph. However, as shown in Figure 1, most samples were primarily characterized by negative sensory attributes such as winey, fermented, oxidized, and rancid. Only sample 2 was distinguished by fruity sensory attributes, including olive and avocado notes.

As shown in Figure 2, the flash profile results reveal the formation of two distinct clusters, each consisting of two samples: the first cluster includes the two organic (BIO) samples, while the second comprises the two virgin samples.

3.8.2 Free Choice Profile

Sample 5 was analyzed using the Free-Choice Profiling method, as it was obtained at a later stage compared to the other samples. Therefore, it was not possible to conduct a comparative sensory analysis with this sample; however, its main sensory characteristics, as perceived by the panelists, were still highlighted. The descriptor common to all judges, and rated with the highest intensity, was "winey" or "vinegary/acetic acid note", related to olfactory perception; this was accompanied by "green" or "green color", associated with visual perception. In particular, "winey" or "vinegary/acetic acid" sensory notes are generally associated with the growth of yeasts causing aerobic fermentation and characterized by a high concentration of acetic acid [75,76]. Most judges also detected rancid notes in the sample, while only two panelists perceived "fruity" or "avocado notes". The presence of rancid notes can be attributed to the nonenzymatic oxidation of unsaturated fatty acids, which occurs mainly through the process of autoxidation. This radical-induced chain reaction leads to the depletion of antioxidants and the formation of toxic free radicals and hydroperoxides. The latter subsequently decompose into numerous secondary products, including several aroma-active volatile organic compounds (mainly aldehydes) responsible for sensory defects, primarily rancid. The susceptibility of an oil to this process is also influenced by the presence of minor compounds with pro- or antioxidant activity [64,65]. Other frequently mentioned descriptors included "brine" and "greasiness". Bitterness and pungency were reported by only a few judges, both at low intensity.

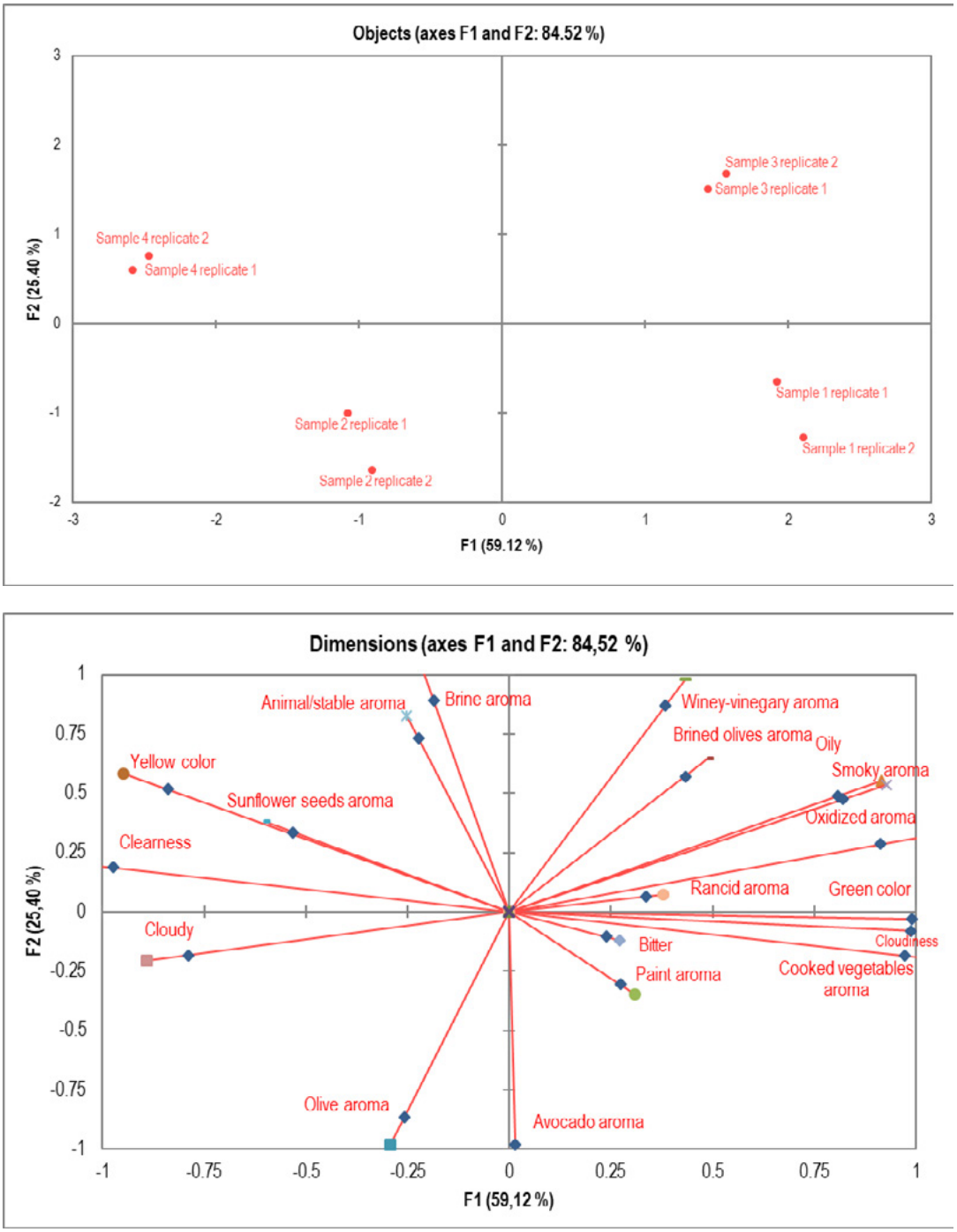


Figure 1 - Biplot representing the samples (first one) and the sensory attributes (second one) resulted from the Flash Profile of the avocado oils.

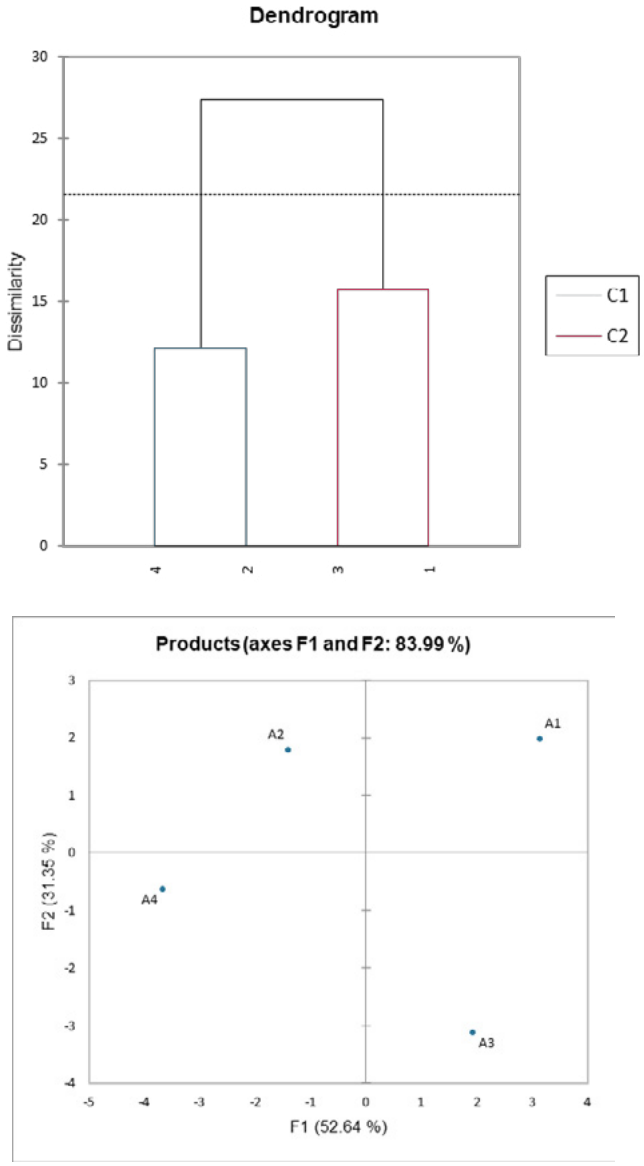


Figure 2 - Results of the Agglomerative Hierarchical Clustering (AHC) performed on Flash Profile data.

CONCLUSIONS

Cold-pressed avocado oil has been gaining growing interest from both consumers and industry, largely because it is perceived as a high-quality product that is not subjected to refining processes. This perception, which, as is evident from this paper, may or may not be confirmed by the real quality and authenticity of what is found on the market, is reinforced by possible distinctive culinary and sensory attributes, as well as by the presence of minor bioactive compounds such as phytosterols and tocopherols, contributing to possible nutritional value and functional properties. These features make it trendy not only in the food sector but also for cosmetic and nutraceutical applications. Given the growing demand, cold-pressed avocado oil is also highly vulnerable to adulteration, which can compromise both its authenticity and consumer trust. For this reason, work is currently underway to establish a dedicated UNI standard, aimed at defining at least basic quality parameters for the quality control of this product. The intention is to try to harmonize the quality criteria with the Codex Alimentarius to ensure international consistency and wide diffusion and adoption by the market. Among the compositional parameters under consideration, the fatty acid profile (in particular, fatty acid composition and amount of *trans* fatty acids) and the sterol composition (notably □-sitosterol) show strong potential for authentication purposes. Furthermore, the determination of steradienes provides a reliable means of detecting the presence of refined oil. In addition, sensory evaluation — although currently

only recognized officially by EU regulations for olive oil — would be a fundamental inclusion for the quality assessment of cold-pressed avocado oil, essential, e.g., to confirm the absence of perceptible rancid notes or fermentation defects such as winey-vinegary. Proposing and applying quality and authenticity parameters is vital for identifying potential adulterations or fraud relating to a product that is present and growing on the market, and which can currently be subject to very few controls, in order to safeguard consumers' trust and health.

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