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## How the Addition of Spices and Herbs to Virgin Olive Oil to Produce Flavored Oils Affects Consumer Acceptance

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With the aim to expand the olive oil market to a larger number of consumers who are not familiar with the sensory characteristics of virgin olive oil, the use of novel products known as "flavored olive oils", obtained by adding different kind of spices and aromatic herbs, is spreading in many countries. In order to test consumer acceptability of this type of product, in a country (Tunisia) in which virgin olive oil is regularly consumed, flavored olive oils were prepared by adding aromatic extracts of thyme, oregano, a mix of herbs (used as pizza seasoning), rosemary, and basil to a monovarietal Chemlali virgin olive oil and a consumer test on 206 subjects was performed. Selected quality parameters (free acidity, peroxide number, oxidative stability, specific absorption at  $K_{232}$  nm and  $K_{270}$  nm) were also measured and no significant variations were detected. Slight differences were found concerning the content of minor compounds (chlorophylls, carotenoids and total phenols). On the other hand, notable differences were seen in the profiles of volatile compounds, which appeared to be responsible for the observed variability in consumer acceptance. Although the unflavored oil was more appreciated than the flavored ones, among the latter, thyme flavored olive oil was the most appreciated.

Keywords: Flavored olive oil, Aromatic extracts, Physical-chemical composition, Consumer acceptance, Volatile compounds.

Spices and herbs are widely used in Mediterranean cuisine for their palatability. Moreover, they provide some healthy effects, extend the shelf life, and improve the safety of prepared food. These effects are mainly due to well-known antioxidant and antimicrobial properties of herbs and spices [1-3]. Virgin olive oil is a basic culinary ingredient of the Mediterranean diet and is generally highly appreciated for its characteristic taste and odor [4]. Wholesome and healthy effects of virgin olive oil have also been reported [5] as well as their cosmetic uses as an oil phase ingredient and odor fixative of perfumes and essential oils. Recently, a new set of products known as "flavored olive oils", with many different tastes, has been introduced into the market [6]. The main strategy is to expand the olive oil market to a larger number of consumers by acquiring those not yet familiar with the uses and properties of olive oil, who might be tempted by an olive oil enriched with other ingredients of the Mediterranean diet [7].

According to the definition of the European Union Commission [8-9], an extra virgin olive oil must be extracted "only from olives with a superior quality, cannot undergo any treatment other than washing the fruits, and decanting, centrifuging and filtering the extracted olive oil. It excludes oils obtained from seeds by chemical or mechanical methods or the use of solvent extraction or reesterification methods, and those mixed with oils from other sources". Based on this clarification, a flavored olive oil obtained using an extra virgin olive oil (EVOO) cannot be called "extra virgin olive oil" on the label, but can be defined [10] as an olive oil that has been processed with vegetables, herbs, spices, or other fruits to improve its nutritional value, enrich the sensory characteristics and increase its shelf-life. Flavored olive oils are very popular in the US, UK, and Australia, none of which is an IOC member and there are no laws that forbid the commercialization of these kind of products in those countries. Because of the success of these oil dressings, the California Olive Oil Council (COOC) is trying to establish a meaningful labeling standard [10]. A strong demand for flavored oils in the UK and other not heavy consumer countries was noted for the last decade; this may be explained by the attitude of flavor from species, plants, and essential oils to camouflage the strong attribute of olive oil that can be unpleasant for those who are unfamiliar with it [10].

Many procedures of oil flavored production are available and the choice is fundamental since the extraction method affects both acceptability and oxidative stability of the oil preparation. Maceration is the oldest method of oil aromatization: herbs, spices, and fruits are mixed with oil and left at room temperature for a defined time. The mixture is then filtered to remove turbidity and solid parts [7, 10, 11]. Co-milling the olives with herbs, spices, or fruits such as lemons and bergamots [12] is a new approach for preparing clear and safe flavored olive oils [10]. Recently, another approach used is the addition of essential oil to the EVOO [10] that presents advantages in terms of high safety. In fact, many spices and herbs can carry spores produced by *Clostridium botulinum* [10] and this latter procedure permits more flexibility of production because it is not necessary to have the added flavor (herbs, spices, or fruits) available during milling.

In the present study, a set of five flavored olive oils obtained using thyme, oregano, herbs (a mixture used as pizza seasoning), rosemary, and basil was prepared and compared with an unflavored one. The main aims were to: (i) study the possible influence of the aromatization process on the quality of the product, (ii) characterize the volatile fraction of different samples, and (iii) test consumer acceptance.

The addition of oily flavored preparations (S1-S5) to EVOO (T) had no effect on its basic quality parameters. These results were in good agreement with our previous study [11] and other recent investigations [13]. The oxidative stability index (OSI) of EVOO (T) was 5.0 hours (Table 1). The addition of oily flavored preparations did not significantly increase oxidative stability, in agreement with previous observations [11]. However, a slight ability to counteract oxidation better was seen for samples flavored with oregano (S2), partially confirming the results of Sousa *et al.* [14].

**Table 1:** Mean values of oxidative stability (OSI), pigments (chlorophylls and carotenoids), and total phenols of extra virgin olive oil (EVOO: T) and flavored olive oils (EVOO + thyme: S1; EVOO + oregano: S2; EVOO + mix of herbs: S3; EVOO + rosemary: S4; EVOO + basil: S5). Values in the same row with different subscript letters represent significant differences between samples at p < 0.05 by Tukey's test (n = 3).

	Т	<b>S1</b>	S2	<b>S</b> 3	<b>S4</b>	<b>S</b> 5
OSI (hours)	5.0 <sup>a</sup>	5.7 <sup>a</sup>	6.0 <sup>a</sup>	5.6 <sup>a</sup>	5.3 <sup>a</sup>	5.8 <sup>a</sup>
Chlorophylls (mg/kg)	8.4 <sup>b</sup>	8.1 <sup>b</sup>	8.7 <sup>b</sup>	8.3 <sup>b</sup>	8.1 <sup>b</sup>	9.2 ª
Carotenoids (mg/kg)	5.5 <sup>a</sup>	5.2 <sup>a</sup>	5.2 ª	4.9 <sup>a</sup>	5.1 <sup>a</sup>	5.2 ª
Phenols (mg/kg)	452.3°	518.6 <sup>b</sup>	651.4ª	427.6°	418.7°	477.9°

The concentrations of chlorophylls and carotenoids of Chemlali EVOO (T) are reported in Table 1. Values of 8.4 and 5.5 mg kg<sup>-1</sup> were obtained for chlorophylls and carotenoids, respectively. The addition of oily flavored preparations (S1-S5) had no appreciable effect on the content of chlorophylls, with the exception of a slight increase (p<0.05) in the case of the basil-flavored olive oil (S5). For carotenoids, no significant variations were noted among samples. In Chemlali EVOO (T), a concentration of 452.3 mg kg<sup>-1</sup> of total phenols was determined and the addition of oily flavored preparations induced a slightly significant increase in the case of oregano (S2) and thyme (S1)-flavored oils (Table 1).

The aromatic substances identified in the headspace of oily flavored preparations, EVOO, and flavored olive oils were studied (data not shown). Thymus essential oil and extracts are widely used in pharmaceutical preparations and for flavoring and preservation of several food products. *Thymus* species, widespread in the Mediterranean area, are well known as medicinal plants due to their biological and pharmacological properties [12]. In the case of the commercial thyme oily preparation, 25 components, which represented 99.6% of total volatiles, were identified. Typically high percentages of the constituents derived from the biosynthetic pathway of thymol/carvacrol, such as *p*-cymene (46.6%) and  $\gamma$ -terpinene (18.8%), were seen, even if the chemical composition can markedly vary in relation to different seasons and species of *Thymus* L. (Lamiaceae) [12].

De Falco *et al.* [15] reported that oregano essential oils have been shown to have antioxidant, antibacterial, antifungal, diaphoretic, carminative, antispasmodic, and analgesic activities and, among these, the antimicrobial potential is of special interest. In the oily preparation of oregano used in the present investigation, 23 constituents, which represented 99.7% of the total volatiles, were identified. Among the main constituents of the aroma, we detected high amounts 1,8-cineole (36.1%), *p*-cymene (15.6%),  $\beta$ -pinene (6.3%), and  $\gamma$ -terpinene (5.8%). Fifteen compounds, accounting for 99.5% of total volatiles, were identified in the oily flavored

preparations of the mix of herbs. More than 40% was represented by  $\alpha$ -pinene (42.3%). Other monoterpene hydrocarbons such as  $\beta$ -pinene (19.7%) and  $\gamma$ -phellandrene (11.6%) were detected in high percentages.

Globally, 18 constituents, accounting for 99.7% of total volatiles were identified in the rosemary oily preparation. Its main components were: 1,8-cineole (47.8%),  $\alpha$ -pinene (16.9%),  $\beta$ -pinene (15.1%), and camphor (5.0%). It can thus be stated that flavoring of this commercial solution was obtained from a 1,8-cineole chemotype [16]. Jiang *et al.* [17] demonstrated that the essential oil of rosemary, particularly rich in 1,8-cineole, showed pronounced antibacterial and antifungal activity.

In the flavored oily preparation of basil, 28 volatiles were identified, which accounted for 99.8% of the total composition. The three main constituents were typical compounds of basil essential oil: 1,8-cineole (27.5%), linalool (21.8%), and methyl chavicol (21.0%) [18]. Hussain *et al.* [19] reported that the essential oil of basil had antioxidant and antimicrobial activities, mainly due to the presence of linalool, a typical component of basil.

In the headspace of the EVOO (T), several constituents were identified (Table 2). It was characterized above all by  $C_6$  aldehydes, mainly represented by (*E*)-2-hexenal (41.4%), a volatile with green, sweet, and fruity sensory notes and, secondly, by hexanal (4.1%). Other representative compounds were esters such as (*Z*)-3-hexenyl acetate (3.6%) and 1-hexyl acetate (1.6%). The presence of (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, and 1-hexyl acetate is usually correlated with freshness of virgin olive oil and normally has a positive effect on consumer preference [20].

In the thyme-flavored olive oil (S1), more than 40% was constituted by *p*-cymene, followed by appreciable amounts of other monoterpenes, such as  $\gamma$ -terpinene (17.9%), thymol (8.3%), and linalool (4.2%). Some of the compounds deriving from the EVOO were still detectable, such as *(E)*-2-hexenal (2.5%), 1-hexanol (0.9%), and *(Z)*-3-hexenyl acetate (0.3%) (Table 2).

Around 27 compounds were identified in the oregano-flavored olive oil (S2) and the main components were 1,8-cineole (36.1%), *p*-cymene (15.6%),  $\alpha$ -pinene (6.9%), and  $\beta$ -pinene (6.3%). Among the volatiles of EVOO, *(E)*-2-hexenal (1.0%) was identified (Table 2).

In the herb-flavored olive oil (S3), the resulting aroma was dominated by  $\alpha$ -pinene (27.5%),  $\beta$ -pinene (15.3%),  $\gamma$ -phellandrene (11.6%), carvone (8.2%), and linalool (7.4%). However, some of the aromatic compounds of EVOO were still detectable, even if in lower amounts, such as *(E)*-2-hexenal (7.5%) (Table 2).

In the rosemary-flavored olive oil (S4), 20 compounds were detected. More than 60% of the total aromatic compounds were represented by 1,8-cineole (61.3%), followed by  $\alpha$ -pinene (8.9%),  $\beta$ -pinene (8.6%), and camphor (8.3%). The typical constituents of EVOO were present in lower amounts: *(E)*-2-hexenal (0.9%) and *(Z)*-3-hexenyl acetate (0.1%) (Table 2).

Taking into consideration the last sample (S5, basil-flavored olive oil), 33 compounds were identified, the most abundant of which was linalool (30.6%), followed by methyl chavicol (26.5%), and 1,8-cineole (22.6%). The aromatic substances of the EVOO were found in very low amounts: (*E*)-2-hexenal (1.3%) and (*Z*)-3-hexenyl acetate (0.3%) (Table 2).

**Table 2:** Volatile compounds<sup>a</sup> of the extra virgin olive oil (EVOO: T) and the flavored olive oils EVOO + Thyme: S1; EVOO + Oregano: S2; EVOO + Herbs: S3; EVOO + Rosemary: S4; EVOO + Basil: S5. Values in the same row with different subscript letters represent significant differences between samples at p < 0.05 by Tukey's test, (n = 3). Percentages obtained by FID peak area normalization (HP-5 column). \*Linear retention indices (DB-5 column). nd: not detected.

Volatile compounds (%)	I.r.i*	Т	S1	S2	S3	S4	S5
Aldehydes from LOX							
Hexanal	800	4.1 <sup>a</sup>	nd <sup>b</sup>				
(E)-2-Hexenal	851	41.4 <sup>a</sup>	2.5 °	1.0 <sup>d</sup>	7.5 <sup>b</sup>	0.9 <sup>d</sup>	1.3 <sup>cd</sup>
Alcohols from LOX							
1-Hexanol	871	0.7 <sup>a</sup>	0.9 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Esters from LOX							
(Z)-3-Hexenyl acetate	1007	3.6 <sup>a</sup>	0.3 °	nd <sup>c</sup>	1.1 <sup>b</sup>	0.1 <sup>c</sup>	0.3°
1-Hexyl acetate	1009	1.6 <sup>a</sup>	nd <sup>b</sup>				
Terpenic compounds							
α-Thujene	932	nd <sup>c</sup>	2.0 <sup>a</sup>	0.8 <sup>b</sup>	0.2 °	0.2 °	nd <sup>c</sup>
α-Pinene	940	nd <sup>d</sup>	3.2 °	6.9 <sup>b</sup>	27.5 <sup>a</sup>	8.9 <sup>b</sup>	0.8 <sup>cd</sup>
Camphene	955	nd <sup>d</sup>	1.7 °	2.6 <sup>b</sup>	nd <sup>d</sup>	5.1 <sup>a</sup>	0.2 <sup>d</sup>
Sabinene	977	nd <sup>c</sup>	nd°	0.7 <sup>a</sup>	nd <sup>c</sup>	nd <sup>c</sup>	0.3 <sup>b</sup>
β-Pinene	980	nd <sup>e</sup>	1.5 <sup>d</sup>	6.3 °	15.3 <sup>a</sup>	8.6 <sup>b</sup>	1.5 <sup>d</sup>
Myrcene	993	nd <sup>a</sup>	5.0 <sup>a</sup>	3.2 <sup>b</sup>	5.0 <sup>a</sup>	0.3 <sup> d</sup>	1.1 °
α-Phellandrene	1006	ndb	nd <sup>b</sup>	0.2 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
δ-3-Carene	1012	nd <sup>b</sup>	0.2 <sup>b</sup>	1.7 <sup>a</sup>	nd <sup>b</sup>	0.2 <sup>b</sup>	nd
α-Terpinene	1019	nd <sup>b</sup>	0.3 <sup>b</sup>	1.5 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<i>p</i> -Cymene	1027	nd <sup>c</sup>	40.5 <sup>a</sup>	15.6 <sup>b</sup>	0.3 °	0.3 <sup>c</sup>	nd <sup>c</sup>
Limonene	1032	nd <sup>c</sup>	nd <sup>c</sup>	2.0 <sup>a</sup>	nd <sup>c</sup>	0.2 <sup>b</sup>	0.3 <sup>b</sup>
γ-Phellandrene	1033	nd <sup>b</sup>	nd <sup>®</sup>	nd <sup>b</sup>	11.6 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>
1,8-Cineole	1034	nd <sup>d</sup>	nd <sup>d</sup>	36.1 <sup>b</sup>	nd <sup>d</sup>	61.3 <sup>a</sup>	22.6 <sup>c</sup>
(E)-β-Ocimene	1051	nd <sup>c</sup>	0.2 <sup>b</sup>	nd	0.3 <sup>b</sup>	nd <sup>c</sup>	0.8 <sup>a</sup>
γ-Terpinene	1062	0.8 °	17.9 <sup>a</sup>	5.8 <sup>b</sup>	0.1 °	nd <sup>d</sup>	nd <sup>d</sup>
Fenchone	1080	nd <sup>b</sup>	0.3 <sup>a</sup>				
p-Mentha-2,4(8)-diene	1088	nd <sup>b</sup>	nd <sup>b</sup>	0.3 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<i>p</i> -Cymenene	1090	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	3.0 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Terpinolene	1090	nd <sup>b</sup>	0.2 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Linalool	1101	nd <sup>d</sup>	4.2 °	1.7 <sup>d</sup>	7.4 <sup>b</sup>	$0.4^{de}$	30.6 <sup>a</sup>
1,3,8-p-Menthatriene	1112	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	2.5 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Camphor	1147	nd <sup>e</sup>	1.4 °	4.0 <sup>b</sup>	0.3 <sup>de</sup>	8.3 <sup>a</sup>	1.1 <sup>cd</sup>
Menthone	1154	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	0.3 <sup>b</sup>	nd <sup>c</sup>	1.2 <sup>a</sup>
Isomenthone	1165	nd <sup>b</sup>	$0.8^{a}$				
Borneol	1170	nd <sup>c</sup>	1.0 <sup>b</sup>	0.7 <sup>b</sup>	nd <sup>c</sup>	1.4 <sup>a</sup>	nd <sup>c</sup>
Menthol	1174	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	0.1 <sup>b</sup>	nd <sup>b</sup>	1.0 <sup>a</sup>
4-Terpineol	1179	nd <sup>e</sup>	1.5 <sup>a</sup>	0.6 <sup>b</sup>	0.1 <sup>de</sup>	0.4 °	0.2 <sup>d</sup>
p-Cymen-8-ol	1184	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	0.2 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>
γ-Terpineol	1191	nd <sup>c</sup>	0.2 <sup>bc</sup>	0.4 <sup>ab</sup>	0.2 <sup>bc</sup>	0.6 <sup>a</sup>	0.3 <sup>abc</sup>
Methyl chavicol	1198	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	4.1 <sup>b</sup>	nd <sup>c</sup>	26.5 <sup>a</sup>
endo-Fenchyl acetate	1221	nd	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	$0.2^{a}$
Methylcarvacrol	1245	nd <sup>b</sup>	0.3 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Carvone	1245	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	8.2 <sup>a</sup>	nd <sup>c</sup>	3.6 <sup>b</sup>
Geranial	1271	nd <sup>b</sup>	0.2 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Isobornyl acetate	1287	nd <sup>b</sup>	0.4 <sup>b</sup>	0.4 <sup>b</sup>	0.1 <sup>b</sup>	1.0 °	0.9 <sup>a</sup>
Thymol	1292	nd <sup>c</sup>	8.3 <sup>a</sup>	1.5 <sup>b</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>
Carvacrol	1301	nd	0.6 <sup>a</sup>	3.0 <sup>b</sup>	nd <sup>b</sup>	nd	nd
iso-Dihydrocarveol acetate	1330	nd	nd	nd	0.2 <sup>a</sup>	nd	0.1 6
Eugenol	1361	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	0.4 <sup>a</sup>	nd <sup>b</sup>	0.4ª
α-Copaene	1377	1.2 <sup>a</sup>	0.1 <sup>b</sup>	0.2 b	0.1 b	0.1 b	nd <sup>b</sup>
β-Elemene	1392	nd <sup>®</sup>	nd <sup>®</sup>	nd <sup>®</sup>	nd <sup>®</sup>	nd <sup>o</sup>	0.5 <sup>a</sup>
β-Caryophyllene	1418	0.7 °	1.5 <sup>a</sup>	1.7 <sup>a</sup>	nd <sup>c</sup>	0.7°	0.1 °
(E)-α-Bergamotene	1437	nd <sup>o</sup>	nd <sup>o</sup>	nd <sup>o</sup>	nd	nd <sup>o</sup>	1.3ª
Germacrene D	1483	nd	nd	nd	nd	nd	0.2ª
Valencene	1494	0.5 <sup>a</sup>	nd <sup>o</sup>	nd	nd°	nd°	nd <sup>o</sup>
$(E,E)$ - $\alpha$ -farnesene	1505	3.4 <sup>a</sup>	0.5 °	0.2 °	0.2 °	0.1 <sup>b</sup>	nd <sup>®</sup>
(E)-γ-Cadinene	1513	nd°	0.2ª	nd <sup>o</sup>	nd <sup>o</sup>	nd°	0.2 <sup>a</sup>

As expected, the transfer of aromatic compounds to the EVOO used as a lipid matrix depended on the type of aroma profile of the added oily preparation. However, it should be noted that some of the aromas (rosemary, thyme, oregano, and basil) had strong flavoring properties, while the mixture of herbs used for pizza affected the aroma less and allowed for perception of the typical aroma notes in EVOO. In terms of series, our studies showed that aldehydes dominated the total volatile fraction of EVOO, while the headspace fraction of flavored olive oils was dominated by the terpenoid fraction, as expected.

The present study is in agreement with a previous report [11] which showed that the majority of volatiles belonging to thyme and oregano, such as carvacrol and limonene, were efficiently incorporated into an EVOO matrix. It has to be considered that the percentage of each volatile molecule incorporated into the EVOO depends mainly on the concentration of spices and herbs used to prepare the flavored olive oil.

Sensory analysis plays a major role in market product acceptability [21, 22]. In order to optimize a product, the industry usually applies many sensory methods, mainly affective ones. Among these, the acceptance test allows for assessment of the consumer's overall liking [23]. Results of the 9-point hedonic scale are summarized in Figure 1. It was highlighted that EVOO (T, mean score 7.5) was significantly more liked than flavored olive oils. In fact, for EVOO, about 73% of consumers attributed the highest score for values belonged to the range 7-9 (Figure 1). There were also significant differences regarding overall-liking among flavored samples: the thyme-flavored one (S1) was the most appreciated (6.6 mean scores) and 63.6% of consumers gave it a positive score (7-9 range), whereas only 9.7% disliked it and assigned lower values of overall



**Figure 1:** Percentages of overall-liking for EVOO (T) and flavored olive oils EVOO + thyme: S1; EVOO + oregano: S2; EVOO + mix of herbs: S3; EVOO + rosemary: S4; EVOO + basil: S5) assessed by 206 consumers. Values in the same row with different subscript letters (a-c) represent significant differences (for 7-9 range) between samples at p < 0.05 by Duncan's test with F value (23.45) > F critical (2.21).



**Figure 2:** Percentages of overall-liking for EVOO (T) and flavored olive oils EVOO + thyme: S1; EVOO + oregano: S2; EVOO + mix of herbs: S3; EVOO + rosemary: S4; EVOO + basil: S5) assessed by 103 females (graphic at the top) and 103 males (graphic at the bottom) consumers. Values in the same row with different subscript letters (a-c) represent significant differences (for 7-9 range) between samples at p < 0.05 by Duncan's test with F value (9.3) > F critical (2.2) for the case of females and F value (18.5) > F critical (2.2) for the case of males. The subscript letters x and y are the differences between males and females for the same sample at p < 0.05 by Duncan's test.

liking (Figure 1). For the other samples (S2, S3, S4, and S5), there were no significant differences, but considering the consumers' scores, the rosemary-flavored olive oil (S4) had the least negative judgements among these four oils, with 10.7% of consumers in the range of 1-3. Preference of consumers appeared to correlate with the aromatic fractions of olive oil samples.

Specifically, some typical aromatic compounds of EVOO were responsible for the highest appreciation of EVOO. In fact, a close positive statistical correlation was found between (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, and overall-liking (r=0.80 and r=0.77, respectively; p<0.05). On the other hand, some aroma compounds belonging to oily flavored preparations appeared to have a negative impact.

Our results are in agreement with various studies reporting on the impact of the incorporation of aromatic preparations on consumer acceptance. Acceptability is not only dependent on the incorporation level, but also on the essential oil composition. In this regard, Antoun and Tsimidou [24] prepared oregano and rosemary gourmet olive oils at varying percentages (from 1 to 5%, w/w). They found that consumers (32 untrained people randomly depicted) were able to differentiate between levels of addition and preferred samples with the low to moderate odor and flavor, and also claimed that all flavorings were sensory accepted by consumers. In addition, Gambacorta et al. [13] evaluated the sensory acceptability of EVOO flavored with oregano and rosemary (prepared by infusion of 10-40 g of herbs and species into one liter of virgin olive oil). According to their studies and as demonstrated by 30 tasters, the addition of herbs and species enhanced the sensory characteristics of the EVOO used as lipid matrix. Observing the overall liking scores given by the judges with different gender (Figure 2), it is possible to affirm that males agree with the general overall liking, but they liked EVOO (T) and the sample flavored with thyme (S1) more than females. On the other hand, females preferred the oil flavored with oregano (S2).

Volatile molecules and overall liking were elaborated by principal component analysis and shown as vectors in a plane composed of four quadrants to highlight possible correlations (Figure 3). The first two components were responsible for 58.0 % of variance (30.8 % for F1 and 27.2 % for F2). In Figure 3 it can be observed that there was a clear discrimination between unflavored and flavored olive oils; in particular, EVOO (T) was located in the second quadrant and was characterized by the highest overall liking score as well as by the highest percentage of aroma compounds given by the lipoxygenase pathway; a group characterized by the flavored olive oils with the taste of thyme (S1) and oregano (S2) were placed in the third quadrant and were characterized by the presence of 1,8cineole, sabinene, y-terpineol, and myrcene. The rosemary-flavored olive oil (S4) was located between the third and the fourth quadrant, probably because they are affected by variables which characterize both quadrants. Finally, the last group is represented by basil- and herb-flavored olive oils (S3 and S5), which showed similar characteristics in terms of volatile profile.

The results of our study demonstrate that the addition of oily flavored preparations to EVOO at the percentages used does not generally influence the stability and the concentration of some minor compounds (phenols, chlorophylls, carotenoids) in a significant manner. However, marked changes in the aroma bouquet were noticed. One of the aims of this study was to determine if the addition of spices and herbs to an EVOO used as lipid matrix to produce flavored oils can meet a satisfactory level of consumer acceptability. Tunisian consumers seemed to prefer the smell and taste of the unflavored olive oil over flavoured ones. Considering the different flavors of olive oils, the presence of thyme essential oil was well accepted, whereas the incorporation of oregano, a mix of herbs (used for pizza seasoning), rosemary, and basil oily preparations into the EVOO matrix did not meet an adequate level of liking.



Figure 3: Principal component analysis (PCA) obtained for extra virgin olive oil (EVOO: T) and flavored olive oils EVOO + thyme: S1; EVOO + oregano: S2; EVOO + mix of herbs: S3; EVOO + rosemary: S4; EVOO + basil: S5).

#### Experimental

*Samples:* EVOO produced from Chemlali olives by a three-phase continuous extraction system was used for the preparation of flavored oils. It belonged to the EVOO commercial class according to the basic parameters [8,9]. Flavored olive oils and oily preparations of flavours used in this study were produced by the Mills of "Rivière d'or" localised in Monastir, Tunisia. Specifically, oily preparations of flavors were obtained by mixing the essential oils into an organic sunflower oil. The flavored olive oils were prepared by mixing the EVOO and a percentage of five commercial oily preparations of flavors (thyme, S1; oregano, S2; mix of herbs for pizza, S3; rosemary, S4; basil, S5). According to the appropriate intensity of the flavor (preliminarily tested), we used 0.7% of the flavor of rosemary and 1% of the other flavors. All chemical and sensory tests were also carried out on the EVOO control sample (T).

**Physical-chemical parameters:** Basic quality parameters of EVOO such as free acidity (FA), peroxide value (PV) and spectrophotometric indices ( $K_{232}$ ,  $K_{270}$ ) were evaluated according to official methods [8,9] and subsequent amendments. All analyses were performed in triplicate for each sample.

**Oxidative stability evaluation:** The sensitivity to oxidative phenomena was evaluated by the Rancimat apparatus (Mod. 743, Metrohm  $\Omega$ , Switzerland). Briefly, 3 g of each sample was heated to 120°C and submitted to an air flow of 20 L h<sup>-1</sup>. Stability was expressed as induction time (h).

*Pigment determination:* Amounts of carotenes and chlorophylls were determined as described by Minguez-Mosquera *et al.* [25]. Results were expressed as mg of pheophytin "a" and lutein per kg of oil, respectively [25].

*Extraction of phenolic compounds and determination of total phenols:* The phenolic extract was obtained as previously reported by Montedoro *et al.* [26]. Total phenols were determined colorimetrically and the results were expressed as mg of hydroxytyrosol per kg of oil.

*Volatile compound analysis:* Analytical conditions applied for solid phase micro extraction (SPME) coupled with gas mass spectrometry (GC-MS) were according to Issaoui *et al.* [11] The relative proportions of the volatile constituents were expressed as percentage (%) by peak-area normalization.

Acceptance test: A total of 206 subjects participated in the study. Specifically, 103 females and 103 males were interviewed to investigate the overall liking of samples. The group of participants came from different regions of Tunisia and was selected using predetermined screening criteria based on level of education (high or incomplete), purchasing and consumption frequency as well as familiarity with the typical EVOO of Tunisia.

Participants were asked to evaluate, according to their preference, the 6 samples (T and S1-S5) by smell and taste and to express their degree of liking using a 9-point hedonic scale (scores: like extremely: 9; like very much: 8; like moderately: 7; like slightly: 6; neither like nor dislike: 5; dislike slightly 4; dislike moderately: 3; dislike very much: 2; dislike extremely: 1).

The test was realized in blinded conditions and each consumer had to complete a questionnaire on personal data and other information (age, gender, region of origin, socio-professional category, and consumption frequency of EVOO). Samples were served at room temperature in plastic glasses coded with three-digit numbers and presented to consumers by randomization. The amount of each sample served was 20 mL with no obligation to finish the glass. During the test, unsalted bread, apples, and water were provided to each judge.

*Statistical analysis:* All parameters were determined in triplicate for each sample. Data were processed by SPSS statistical package (Version 12.00 for Window, SPSS Inc. Chicago, Illinois, 2003). The significance of differences at a 5% level among means was determined by one-way ANOVA, using Tukey's test. For the acceptance sensory test, in order to check if a difference between samples existed, we used ANOVA and the F-test. Duncan's multiple range test was used to obtain all pair wise comparisons among sample means. Correlation analysis was performed employing Person's test and principal components analysis (PCA) with XLSTAT for Windows release 6.0 (Addinsoft, New York, NY).

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