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Flavored olive oils: focus on their acceptability and thermal stability

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SUMMARY: The presence of flavored olive oils (FOO) on the market represents an answer to an increasing consumer demand for novel and healthy food. This work aims to compare the sensory acceptability and the thermal stability of FOO prepared by mixing different flavors (lemon, onion, garlic, paprika) to an extra virgin olive oil (EVOO) also used as the control sample. 96 Tunisian citizens were involved in a consumer test and the lemon flavored oil was the most liked whereas the least liked was the oil with onion. Samples were subjected to different heat treatments (60 °C, 100 °C, 200 °C for 1, 2, 4, 8 hours) and the flavor addition did not influence the EVOO stability when samples were heated at 60 °C, whereas at 200 °C the FOO with onion and garlic showed higher oxidative stability. The thermo-oxidation process at 60 °C and at 100 °C of the FOOs was not detrimental for the volatile compound markers but the effect was noticeable for all these markers at 200 °C.

KEY WORDS: *Acceptance test; Flavored olive oil; Heating treatments; Oxidative stability*

RESUMEN: ***Aceites de oliva aromatizados: enfoque sobre su aceptabilidad y estabilidad térmica.*** La presencia de aceites de oliva con sabor (FOO) en el mercado representa una respuesta a la demanda cada vez mayor de los consumidores de alimentos novedosos y saludables. Este trabajo tiene como objetivo comparar la aceptabilidad sensorial y la estabilidad térmica de FOO preparado mediante la mezcla de diferentes sabores (limón, cebolla, ajo, pimentón) a un aceite de oliva virgen extra (AOVE), utilizado como muestra de control. 96 ciudadanos tunecinos participaron en una prueba de consumo: el aceite con sabor a limón fue el que más gustó, mientras que el que menos gustó fue el de la cebolla. Las muestras se sometieron a diferentes tratamientos térmicos (60 °C, 100 °C y 200 °C durante 1, 2, 4, 8 horas). La adición de saborizantes no influyó en la estabilidad del AOVE cuando las muestras se calentaron a 60 ° C mientras que a 200 °C el FOO con cebolla y ajo mostraron una mayor estabilidad oxidativa. El proceso de termooxidación a 60 °C y a 100 °C de los FOO no fue perjudicial para el marcador de compuestos volátiles, en oposición al efecto a 200 °C que resultó notable para todos estos marcadores.

PALABRAS CLAVE: *Aceite de oliva aromatizado; Estabilidad oxidativa; Pruebas de aceptación; Tratamientos térmicos*

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1. INTRODUCTION

Increasing the antioxidant status in people's diets is a promising solution to contrast the development and progression of many diseases such as coronary artery disease (Servili *et al*., 2009; Frankel, 2011), cancer (BiasiniC *et al*., 2015), neurodegenerative conditions and others (Nakbi *et al*., 2012). Nowadays, consumers are more conscious and informed about food products following the development of quality standards and regulations. Consumers' perceptions regarding food quality are related to their conception of healthy, safe, secure, nutritional and innovative products.

Extra virgin olive oil (EVOO), represents one of the most commonly studied antioxidant food sources (Saleh and Saleh, 2011; Nakbi *et al*., 2012; Abdallah *et al*., 2018). Ample research has supported the healthy benefits resulting from the adoption of the Mediterranean diet (Grossi *et al*., 2013; Martinez Gonzalez *et al*., 2014; Grosso *et al*., 2017). EVOO is the founding fat of Mediterranean diet and it is highly appreciated for its peculiar flavor and its nutritional proprieties. Compared with other fats, EVOO has been shown to be more resistant to oxidation due to its high contents in monounsaturated fatty acids and antioxidant compounds (Condelli *et al*., 2015).

To attract more consumers to a large spectrum of fat products, the industry aimed at enriching EVOO with new antioxidant compound from other food sources, such as thyme, rosemary, oregano and so on (Moldao-Martins *et al*., 2004; Issaoui *et al*., 2011). The objective was to improve the radical scavenging activity of EVOO, to enhance its shelf life and to give it original sensory notes. Mixtures of VOO and other typically Mediterranean ingredients are marketed as "flavored olive oil" (FOO), "aromatized olive oil" or "gourmet olive oil" and represent a possible answer for olive oil producers and industries to the increasing demand of consumers for a novel and healthy food. Different strategies for producing FOO were cited in the literature (Moldao-Martins *et al*., 2004; Gambacorta *et al*., 2007; Issaoui *et al*., 2011; Sousa *et al*., 2015; Sacchi *et al.*, 2017). In order to develop an effective and efficient way to produce FOO without mitigating the nutritional quality value and without compromising the chemical characteristics of VOO, researchers from the University of Bari in Italy, studied three different processes: infusion of olive oil with ground herbs, adding herbs to crushed olives before malaxation and the use of ultrasound technology. At the end of the study, in order to produce FOO with a higher concentration of polyphenols and important radical scavenging activity, the authors recommended the addition of herbs to olive paste before malaxation to obtain an increase in total polyphenols three times greater than the two other processes. They have also explained that in this phenomenon, water in the olive paste may act as a solvent and enhance the extraction of organic acids into the oil. Moreover, the continuous mixing of olive paste may play a crucial role in boosting the release of polyphenols from herbs added herbs to the VOO (Clodoveo *et al*., 2016).

Concerning the nature and the kind of the ingredients used to prepare gourmet olive oil, industrial experts and researchers have used dried and⁄or fresh herbs, whole spices, ground spices, essential oils or as oleoresin, vegetables, fruits, mushrooms and nuts (Issaoui *et al.*, 2011; Sousa *et al*., 2015; Sacchi *et al*., 2017). Regardless of the method used to aromatize olive oil, the addition of ingredients was found to have a positive influence on the final product due to an increase in total polyphenols, which enhances antiradical and antioxidant activity (Gambacorta *et al*., 2007; Clodoveo *et al*., 2016) and improves its sensory profile (Sacchi *et al.*, 2017). Negative effects were also determined, such as the possible presence and survival of some microorganisms, as previously reported by Ciafardini *et al*., 2004.

In order for olive oil to be classified as virgin olive oil, it must comply with the criteria established in the trade standard of IOC (IOC/T.15NC N0 3/ Rev. 11, 2016). Therefore, such infused or flavored oils can only be commercialized as "aromatized" or "flavored" olive oil.

However, EVOO and FOO are used as seasonings not only for uncooked dishes, but also for cooking or for frying food. For this reason, it is important to evaluate their thermal stability. The main goals of this study were: i) to evaluate whether FOOs prepared by mixing lemon, onion, garlic, and paprika essential oils with EVOO attract Tunisian consumers; ii) to check if the addition of lemon, onion, garlic, and paprika essential oils improve the oxidative stability of olive oil samples under the tested thermal conditions (60 °C, 100 °C, 200 °C); iii) to determine if the volatile compounds found in flavored oils are significantly changed by heat treatments.

2. MATERIALS AND METHODS

2.1. Samples

FOOs were obtained by mixing oil preparations of different flavours (onion, garlic, paprika and lemon) to an EVOO (according to Regulation EEC 2568/91 and subsequent amendments) produced from Chemlali olives in the mills of "Huilerie Loued" located in Monastir (Tunisia). Olive (*Olea europaea* L.) fruits were collected from the center of Tunisia with a maturity index of 4 based on the degree of skin and pulp pigmentation according to the method developed by the Agronomic Station of Jaén (Uceda *et al*., 1998). Before the extraction process, the olive fruits were sorted and the damaged fruits were removed. A rinsing step preceded the grinding operation. Olives were crushed to a fine paste. The obtained paste was then malaxed for 45–50 min. The paste was pumped into a three-phase

decanter and, finally, the extra virgin olive oil (T) was filtered.

Commercial oil preparations with onion, garlic and paprika were first mixed with organic sunflower oil (OSO) and then with the EVOO (T) used as the control sample. Onion–flavored olive oil was prepared by mixing 1.5% onion dissolved in OSO (the sample was coded S2), garlic–flavored olive oil was prepared by adding 1.0% garlic dissolved in OSO (the sample was coded S3) and paprika-flavored olive oil was prepared by mixing 0.2% paprika dissolved in OSO (the sample was coded S4). Only the lemon-flavored olive oil was prepared by mixing an aliquot of about 0.8% lemon essential oil directly into the EVOO (S1).

2.2. Heat treatment

The heat treatment was carried out (dark conditions and presence of air) at three different temperatures: 60 °C (low frying process), 100 °C (medium frying process) and 200 °C (deep frying process) for 1, 2, 4 and 8 hours. The deep frying (DF) temperature represents the temperature of the smoke point as defined by AOCS (1997). At the end of the treatments, the samples were stored at -20 °C before analysis.

2.3. Chemical, physical and sensory analyses

Basic quality parameters. The Determination of the basic quality parameters of EVOO (T) such as free acidity (FA), peroxide value (PV) and spectrophotometric indices (K_{232}, K_{270}), were evaluated according to official methods (Regulation EEC 2568/91 and subsequent amendments). All analyses were performed in three replicates for each sample under heating conditions (from 0 to 8 hours at 60 °C, 100 °C and 200 °C, respectively) and the results are reported in Table 1.

Fatty acid composition. Fatty acid methyl esters (FAMEs) were prepared as described by Issaoui *et al*., (2011). Individual FAMEs were separated and quantified by gas chromatography using a Model 5890 Series II instrument (Hewlett–Packard, Palo Alto, CA) equipped with a flame ionization detector, and a fused silica capillary column (HP-Innowax; 30 m 0.25 mm 0.25 μm). The results were expressed as relative percent of total area and are reported in Table 2.

Extraction of phenolic compounds and determination of total phenols. The method reported by Montedoro *et al.,* (1992) was used to obtain the phenolic extract. An amount of sample mixed with a solution of methanol/water (80:20, v/v) and an aliquot of Tween 20 (2%, v/w) were homogenized using an Ultra- Turrax T25 apparatus (IKA Labortechnik, Janke & Kunkel, Staufen, Germany). After being homogenized, a step of centrifugation at 5000 rpm for 10 min at 4 °C was carried out. The extraction process was repeated twice. In order to eliminate oil droplets, the methanol extract was conserved at –20 °C for one day. Total phenols were determined colorimetrically at 765 nm and the results were expressed as mg of hydroxytyrosol per kg of oil for each sample under the different heating conditions (from 0 to 8 hours at 60 °C, 100 °C and 200 °C, respectively) (Table 1).

Oxidative stability evaluation. The Rancimat apparatus (Mod. 743, Metrohm Ω, Switzerland), was applied for this analysis. Briefly, a stream of purified air was passed through a sample of 3 g of oil which was held at a constant temperature (120 °C) and air flow (20 $L \cdot h^{-1}$). The stability was expressed as hours (induction time) needed to reach the maximum change in conductivity of deionized water produced by volatile organic acids obtained from the oxidation process. The results for each sample under heating conditions (from 0 to 8 hours at 60 °C, 100 °C and 200 °C, respectively) are reported in Table 1.

Volatile compound analyses. In the present study, the analytical conditions, and identification and quantification of the constituents were designed according to the procedure described by Issaoui *et al.,* (2011). In detail, a Supelco solid phase micro extraction (SPME) fiber coated with polydimethylsiloxane (PDMS, 100 μm) was used and an aliquot of sample was placed into a glass vial. A half hour was required for the equilibration of the fiber, which was then exposed in the headspace of the sample at room temperature. After 50 min the fiber was withdrawn into the needle, transferred and desorbed in the injection port of the GC-MS system.

GC-EIMS analyses were performed with a Varian CP 3800 gas-chromatograph equipped with a DB-5 Capillary column (30 m x 0.25 mm, 0.25 μm coating thickness) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperature were 250 °C and 240 °C, respectively; oven temperature was programmed from 60 °C to 240 °C at 3 $°C \cdot min^{-1}$; carrier gas was helium at 1 $mL \cdot min^{-1}$; splitless injection. The identification of compounds was based on comparisons of the retention times with those of pure standards, comparing their linear retention indices relative to the series of *n*-hydrocarbons, using the information from the National Institute of Standards and Technology library (NIST 98 and ADAMS) and homemade library mass spectra built from pure substances and components of known mixtures and MS literature data. Molecular weights of identified substances were confirmed by GC-CIMS using MeOH as CI ionizing gas. The relative proportions of the volatile constituents were expressed as percentage (%) by peak-area normalization. The analysis was carried by SPME/GC-MS.

Acceptance test. In the present study 96 habitual Tunisian consumers of olive oil ranging in age from 10 to 90 were randomly recruited. No information regarding tasted oils was made available at this

TABLE 1. Acidity (%), peroxide value (meq O_2/kg), K_{232}, K_{270}, Rancimat test (h) and total polyphenols (mg/kg) of EVOO (T) and FOOs (lemon S1, onion S2, garlic S3, paprika S4) under heating conditions (from 0 to 8 hours at 60 °C, 100 °C and 200 °C, respectively). Values in the same row with different subscript letters (a-c) represent significant differences among samples at $p < 0.05$ by Duncan's test (n = 3). Values in the same column with different subscript letters (A-C) represent significant differences among treatments at $p < 0.05$ by Tukey's test (n = 3).

Heating treatments		**T**	**S1**	**S2**	**S3**	**S4**	**T**	**S1**	**S2**	**S3**	**S4**	**T**	**S1**	**S2**	**S3**	**S4**
T°C	***h***	**Acidity (%)**					**Peroxyde value (meq O_2/kg)**					**Total polyphenols (mg/kg)**				
60°C	0	$0.34^{a,B}$	$0.32^{a,C}$	$0.30^{a,C}$	$0.30^{a,C}$	$0.34^{a,B}$	$16^{a,C}$	$16^{a,B}$	$15^{a,B}$	$16^{a,B}$	$15^{a,B}$	$452.3^{bc,A}$	$467.5^{b,A}$	$505.7^{a,B}$	$427.8^{c,AB}$	$461.3^{b,A}$
	1	$0.34^{a,B}$	$0.33^{a,C}$	$0.32^{a,C}$	$0.30^{a,C}$	$0.34^{a,B}$	$15^{a,C}$	$17^{a,B}$	$12^{b,C}$	$13^{b,C}$	$15^{a,B}$	$425.9^{c,B}$	$421.0^{c,B}$	$520.5^{a,B}$	$490.0^{b,A}$	$420.2^{c,B}$
	2	$0.34^{a,B}$	$0.34^{a,C}$	$0.34^{a,C}$	$0.36^{a,B}$	$0.36^{a,B}$	$16^{a,C}$	$17^{a,B}$	$15^{b,B}$	$14^{b,C}$	$16^{a,B}$	$429.9^{c,B}$	$415.7^{d,B}$	$525.4^{a,B}$	$482.7^{b,A}$	$480.0^{b,A}$
	4	$0.34^{b,B}$	$0.34^{b,C}$	$0.36^{ab,C}$	$0.40^{a,A}$	$0.36^{ab,B}$	$15^{b,C}$	$18^{a,B}$	$17^{a,B}$	$17^{b,B}$	$18^{a,B}$	$419.6^{d,B}$	$423.2^{c,B}$	$595.3^{a,B}$	$478.1^{b,A}$	$470.6^{b,A}$
	8	$0.34^{ab,B}$	$0.34^{ab,C}$	$0.36^{a,C}$	$0.30^{b,C}$	$0.36^{a,B}$	$14^{b,D}$	$18^{a,B}$	$17^{a,B}$	$17^{a,B}$	$9^{c,C}$	$409.4^{c,C}$	$395.1^{d,C}$	$597.9^{a,A}$	$475.2^{b,A}$	$463.7^{b,A}$
100°C	1	$0.34^{b,B}$	$0.40^{a,B}$	$0.36^{ab,C}$	$0.34^{b,B}$	$0.36^{ab,B}$	$18^{c,B}$	$18^{c,B}$	$23^{a,A}$	$20^{b,A}$	$18^{c,B}$	$416.3^{c,B}$	$420.5^{c,B}$	$590.0^{a,A}$	$380.7^{d,B}$	$461.3^{b,A}$
	2	$0.34^{b,B}$	$0.40^{a,B}$	$0.38^{a,B}$	$0.36^{ab,B}$	$0.36^{ab,B}$	$19^{a,B}$	$19^{a,B}$	$20^{a,A}$	$20^{a,A}$	$18^{a,B}$	$408.4^{b,C}$	$425.7^{a,B}$	$357.0^{c,D}$	$320.1^{d,B}$	$220.4^{e,C}$
	4	$0.38^{a,B}$	$0.40^{a,B}$	$0.38^{a,B}$	$0.36^{b,B}$	$0.36^{b,B}$	$25^{a,A}$	$25^{a,A}$	$20^{b,A}$	$21^{b,A}$	$23^{b,A}$	$410.1^{c,B}$	$455.3^{b,A}$	$470.1^{a,C}$	$310.2^{d,B}$	$239.1^{e,C}$
	8	$0.36^{c,B}$	$0.44^{a,B}$	$0.40^{ab,B}$	$0.38^{b,B}$	$0.38^{b,B}$	$24^{a,A}$	$24^{a,A}$	$21^{b,A}$	$22^{b,A}$	$23^{a,A}$	$394.1^{a,C}$	$310.3^{e,CD}$	$380.5^{b,D}$	$307.2^{c,BC}$	$210.7^{d,C}$
200°C	1	$0.36^{b,B}$	$0.38^{b,B}$	$0.38^{b,B}$	$0.38^{b,B}$	$0.60^{a,A}$	$10^{a,D}$	$8^{a,D}$	$10^{a,C}$	$10^{a,C}$	$9^{a,C}$	$301.6^{b,D}$	$305.8^{b,CD}$	$378.7^{a,D}$	$300.9^{b,BC}$	$239.1^{c,C}$
	2	$0.38^{c,B}$	$0.43^{b,B}$	$0.46^{b,B}$	$0.42^{bc,A}$	$0.56^{a,A}$	$12^{a,D}$	$11^{b,D}$	$11^{b,C}$	$13^{a,C}$	$11^{b,C}$	$289.1^{c,E}$	$310.9^{b,CD}$	$378.7^{a,D}$	$300.9^{b,BC}$	$239.1^{d,C}$
	4	$0.42^{c,A}$	$0.58^{a,A}$	$0.59^{a,A}$	$0.48^{b,A}$	$0.42^{c,B}$	$11^{b,D}$	$10^{b,D}$	$11^{b,C}$	$15^{a,B}$	$10^{b,C}$	$253.4^{c,EF}$	$230.6^{d,D}$	$348.3^{a,D}$	$297.2^{b,BC}$	$210.7^{e,C}$
	8	$0.47^{c,A}$	$0.64^{a,A}$	$0.68^{a,A}$	$0.56^{b,A}$	$0.38^{d,B}$	$12^{a,D}$	$11^{a,D}$	$12^{a,C}$	$12^{a,C}$	$12^{a,C}$	$169.8^{d,F}$	$198.6^{c,E}$	$344.7^{a,D}$	$210.5^{b,C}$	$193.6^{c,D}$
T°C	***h***	**K_{232}**					**K_{270}**					**Rancimat (*h*)**				
60°C	0	$2.28^{c,C}$	$2.62^{a,BC}$	$2.55^{a,C}$	$2.37^{b,C}$	$2.27^{c,C}$	$0.15^{b,C}$	$0.14^{b,C}$	$0.16^{b,C}$	$0.16^{b,}$	$0.20^{a,C}$	$5.0^{b,B}$	$5.9^{b,B}$	$6.4^{a,B}$	$5.4^{b,B}$	$5.4^{b,B}$
	1	$2.55^{b,BC}$	$2.55^{b,BC}$	$2.57^{b,C}$	$2.66^{a,BC}$	$2.60^{a,BC}$	$0.16^{a,C}$	$0.16^{a,C}$	$0.15^{a,C}$	$0.16^{a,C}$	$0.15^{a,C}$	$4.8^{b,B}$	$5.3^{b,B}$	$6.3^{a,B}$	$5.2^{b,B}$	$5.3^{b,B}$
	2	$2.41^{c,C}$	$2.41^{c,C}$	$2.66^{b,BC}$	$2.69^{b,BC}$	$3.21^{a,A}$	$0.15^{b,C}$	$0.16^{ab,C}$	$0.13^{b,C}$	$0.16^{ab,C}$	$0.18^{a,C}$	$4.9^{b,B}$	$5.6^{b,B}$	$6.2^{a,B}$	$5.3^{b,B}$	$5.3^{b,B}$
	4	$2.21^{b,C}$	$2.21^{b,C}$	$2.72^{a,B}$	$2.72^{a,B}$	$2.72^{a,BC}$	$0.13^{b,C}$	$0.17^{a,C}$	$0.13^{b,C}$	$0.16^{a,C}$	$0.17^{a,C}$	$4.8^{b,B}$	$5.7^{b,B}$	$6.2^{a,B}$	$5.3^{b,B}$	$5.3^{b,B}$
	8	$2.26^{c,C}$	$2.26^{c,C}$	$2.44^{b,C}$	$2.81^{a,B}$	$2.76^{a,BC}$	$0.13^{c,C}$	$0.19^{a,C}$	$0.17^{b,C}$	$0.17^{b,C}$	$0.17^{b,C}$	$4.8^{b,B}$	$5.7^{b,B}$	$6.3^{a,B}$	$5.3^{b,B}$	$5.3^{b,B}$
100°C	1	$2.89^{b,B}$	$3.12^{a,A}$	$2.88^{b,B}$	$2.99^{ab,B}$	$3.02^{a,B}$	$0.17^{a,C}$	$0.17^{a,C}$	$0.17^{a,C}$	$0.18^{a,C}$	$0.18^{a,C}$	$4.9^{b,B}$	$2.8^{c,C}$	$3.2^{b,C}$	$3.5^{c,C}$	$2.8^{c,C}$
	2	$3.05^{b,AB}$	$3.06^{b,AB}$	$3.11^{b,A}$	$3.50^{a,A}$	$2.95^{c,B}$	$0.19^{c,D}$	$0.18^{c,C}$	$0.20^{b,C}$	$0.24^{a,C}$	$0.17^{c,C}$	$4.8^{a,B}$	$1.9^{c,C}$	$2.9^{b,C}$	$3.0^{b,C}$	$2.5^{b,C}$
	4	$3.10^{b,A}$	$3.34^{a,A}$	$3.07^{b,AB}$	$3.19^{b,AB}$	$3.25^{a,A}$	$0.22^{a,D}$	$0.18^{b,C}$	$0.19^{b,C}$	$0.19^{b,C}$	$0.19^{b,C}$	$4.2^{a,C}$	$1.1^{b,D}$	$2.2^{b,C}$	$2.2^{b,D}$	$1.6^{b,D}$
	8	$3.22^{a,A}$	$3.28^{a,A}$	$3.12^{b,A}$	$3.25^{a,A}$	$3.10^{b,AB}$	$0.27^{a,D}$	$0.22^{c,C}$	$0.24^{b,C}$	$0.23^{b,C}$	$0.22^{c,C}$	$3.2^{a,C}$	$0.9^{b,D}$	$1.6^{b,D}$	$1.5^{b,D}$	$1.1^{b,D}$
200°C	1	$2.89^{b,B}$	$3.09^{a,AB}$	$3.08^{a,AB}$	$3.07^{a,AB}$	$3.07^{a,AB}$	$0.14^{d,C}$	$0.95^{c,B}$	$0.97^{c,B}$	$1.81^{a,B}$	$1.62^{b,B}$	$7.9^{a,A}$	$7.7^{a,A}$	$8.5^{a,A}$	$8.2^{a,A}$	$7.4^{a,A}$
	2	$2.99^{c,B}$	$3.32^{b,A}$	$2.97^{c,B}$	$3.56^{a,A}$	$3.56^{a,A}$	$0.44^{d,C}$	$1.54^{b,AB}$	$1.15^{c,B}$	$2.03^{a,B}$	$1.53^{b,B}$	$6.7^{a,A}$	$6.3^{a,A}$	$7.3^{a,A}$	$7.3^{a,A}$	$6.4^{a,A}$
	4	$3.21^{b,A}$	$3.21^{b,A}$	$2.98^{c,B}$	$3.45^{a,A}$	$3.45^{a,A}$	$0.98^{d,B}$	$1.38^{c,AB}$	$1.56^{b,AB}$	$2.35^{a,AB}$	$1.64^{b,B}$	$5.6^{b,B}$	$4.2^{b,B}$	$6.5^{a,B}$	$6.6^{a,B}$	$4.7^{b,B}$
	8	$3.45^{a,A}$	$2.98^{c,B}$	$3.51^{a,A}$	$3.22^{b,A}$	$3.22^{b,A}$	$1.49^{d,A}$	$1.92^{b,A}$	$1.84^{c,A}$	$2.87^{a,A}$	$1.86^{c,A}$	$4.5^{b,B}$	$4.8^{b,B}$	$5.2^{a,B}$	$5.5^{a,B}$	$3.8^{b,C}$

stage (blind conditions). Plastic cups with around 20 mL of oil were offered to the consumers for the olfactory and gustatory phases. All samples were anonymized and presented in a randomized order. Unsalted bread, apples or water were used to clean the oral cavity between samples.

As acceptance test, the hedonic rating method was applied to provide an indication of the magnitude of acceptability of products (Kemp *et al*., 2009).

Participants were presented with the five samples (T and S1-S4) and were asked to evaluate (by smell and teste) each sample and rate it in terms of overall liking using a 9-point structured hedonic scales ranging from extreme dislike (1) to extreme liking (9) (Peryam *et al.,* 1952). Sensory data were evaluated by analysis of variance (ANOVA) to determine whether significant differences in mean degree of overall liking scores existed among the results for the different samples.

2.4. Statistical analysis

All chemical analyses were carried out in triplicate and the results were reported as mean values. Significant differences among samples and heating treatments were determined by analysis of variance with a 95% significant level ($P < 0.05$), using the SPSS program, release 11.0 for Windows. The student's test was used to compare the fatty acid profile of EVOO (T) with the flavored samples (S1-S4) ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Quality grade parameters, total phenols and oxidative stability

The mixing of the studied ingredients with the control sample did not affect the basic quality parameters significantly and the compositional characteristics in terms of fatty acid profile (Tables 1 and 2). Based on free acidity, peroxide number and extinction coefficients, the control sample (T) was classified as EVOO; it was characterized by the oleic and linoleic ratio equal to 4.0 and a medium-high amount of polar phenols (452.3 mg of hydroxytyrosol per kg of oil) (Table 1). These results were in agreement with our previous ones (Issaoui *et al*., 2011) and with those of Ayadi, *et al*., 2009 and Clodoveo *et al*., 2016. However, we have noticed the slight increase in the K232 for lemon and onion flavored olive oils (2.62 and 2.55, respectively vs 2.28 in the control sample). Sacchi *et al.,* (2017) have explained this increase by the presence of terpenes, such as citral and ß-mircene, from lemons which may have an impact on the absorbance at 232 nm of hydroperoxydienes.

The concentration of total polyphenols in FOOs was influenced by the kind of ingredient added to the EVOO, specifically total polyphenols of S2 (onion FOO) increased markedly (505.7 vs 452.3 mg/kg), whereas a tendency to decrease (427.82 vs 452.31 mg/kg) was observed in S3 (garlic FOO). This behavior was also verified by Sousa *et al.,* (2015) who detected a loss of around 20 mg/kg in the case of olive oil added with garlic.

Table 2. Fatty acid composition of EVOO (T) and FOOs (lemon S1, onion S2, garlic S3, paprika S4). No significant differences between T and each flavored sample were found at $p < 0.05$ by the Student's test (n = 3).

Samples	EVOO	FOOs			
Fatty acid composition (%)	T	S1	S2	S3	S4
C16:0	15.96	15.89	15.83	15.82	15.79
C16:1	2.21	2.09	2.13	2.12	1.96
C17:0	0.07	0.04	0.04	0.04	0.04
C17:1	0.07	0.07	0.07	0.07	0.08
C18:0	2.74	2.40	2.65	2.67	2.53
C18:1	61.57	62.11	61.87	61.85	61.83
C18:2	16.02	16.13	16.12	16.12	16.03
C18:3	0.72	0.72	0.68	0.70	0.66
C20:0	0.42	0.35	0.41	0.42	0.43
C22:0	0.22	0.20	0.21	0.21	0.22

Compared to EVOO, the tested FOOs showed a tendency toward slightly higher oxidative stability which was significant only for S2 (Onion – FOO) (Table 1).

During the heating tests, a significant increase in free acidity mainly at 200 °C for 8 hours (Table 1) was observed for all samples with the only exception of S4 (paprika FOO). However, this value was under the limit fixed for EVOO established by EU regulations. The heating at 60 °C for 1, 2, 4 and 8 hours had no significant effect on the peroxide value (Table 1). However, the heating at 100 °C conducted for more than 2 hours, produced a marked increase in the peroxide value which exceeded the limit established by EU regulations for edible virgin olive oils. On the other hand, the heating process at 200 °C caused a decrease in the peroxide values until 8 $meqO_2/kg$ (in the case of S1). This mechanism can be explained by the transformation of primary to secondary oxidation products. At high heating conditions (100 and 200 °C) an increase in both K_{232} and K_{270} extinction coefficients, was also seen. As expected, high temperature treatment (from 60 °C to 200 °C) caused a marked decrease in the polar phenol contents in all the tested samples. However, S2 and S3 (onion and garlic FOO, respectively) showed values which were not significantly different from 1 to 8 h of heating at 200 °C.

S2 was the only flavored sample which showed a higher oxidative stability than the control sample T after heating (6.4 vs 5.0 h, respectively). The oxidative stability monitored by the Rancimat instrument exhibited no significant variation for all samples during the 8 hours under the heating treatment at 60 °C (Table 1). However, after 8 hours, a decrease

was noticed at 100 °C for all samples which was less dramatic for T. A particular behavior, in agreement with our previous study (Issaoui *et al*., 2011), was observed at 200 °C. In fact, after 2 hours of heating, higher values were obtained. After 4 and 8 hours of heat treatment at 200 °C, S2 and S3 resulted more stable to accelerated oxidation than the other samples.

It is well known that the essential oils from onion and garlic are characterized by sulfur-containing compounds, particularly allyl polysulfides (including diallyl sulfide, diallyl disulfide, diallyl trisulfide, allyl methyl disulfide, allyl methyl trisulfide) which are responsible for sensory, healthy and antioxidant properties (Mnayer *et al*., 2014). It is possible to suppose that at 200 °C the lower availability of oxygen for the oxidation reactions causes a preferential formation of evolution oxidation products (characterized by high molecular weight such as dimers and polymers of fatty acids and triglycerides) instead of demolition oxidation products (characterized by low molecular weight such as saturated and unsaturated aldheydes). Under this condition, the Rancimat test, which measures the concentration of volatile oxidation molecules, cannot be considered a suitable analytical approach to evaluate the oxidative stability; whereas the estimation of the total polar materials (TPM) would be more appropriate.

3.2. Volatile compounds

It is well known that the main volatile compounds in VOO are aldehydes (*E*-2-hexenal (41.1%) and hexanal (4.1%)), followed by esters (*Z*–3-hexenyl acetate (3.6%), hexyl acetate (1.6%)) and alcohols (hexanol (0.7%)). All of them arise from the lipoxygenase pathway (LOX). The impact of thermo-oxidation on these compounds was studied and their evolution is reported in Table 3. In particular, it is possible to appreciate the clear decrease in (*E*)-2-hexenal at 200 °C, one of the main LOX compounds responsible for the positive notes of EVOO, and the increase in the markers of the oxidation process, such as nonanal and (*E,E*)-2,4 decadienal. Nonanal showed a significant increase during the heat treatment at 100 °C and 200 °C. In fact, the treatment at the smoke point seems to be detrimental for the percentage of (*E*)-2-hexenal, hence at only one hour of thermo-oxidation the level was reduced to 97.6%. However, the percentage of reduction in (*E*)-2-hexenal at a temperature of 100 °C for 8 hours of processing was only around 36%. In contrast, it seems that the treatment at a smoke point promotes the production of nonanal with a level of genesis around 10 times more than the initial percentage (from 2.6 to 27.2%). The impact of termo-oxidation at 100 °C is slightly remarkable (from 2.6 to 9.8 at 8 hours) and is negligible at 60 °C (Table 3). The (*E,E*)-2,4-decadienal (as well as the (*E,Z*)-2,4-decadienal isomer), was found only in the oil treated at 200 °C (Table 3), as well as other new volatiles such as (*E*)-2-decenal, 1-dodecene, dodecane, (*E*)-2-undecenal, undecane, 3-nonen-2-one, etc. These volatile molecules represent well-known off-flavor compounds, responsible for the unpleasant notes in oxidized oils (Aparicio *et al*., 1996).

TABLE 3. Behavior of (*E*)-2-hexenal, hexanal, nonanal and (*E,E*)-2,4-decadienal of EVOO (T) and behavior of limonene, dipropyl disulfide, diallyl disulfide, and eugenol of FOOs with lemon (S1), onion (S2), garlic (S3) and paprika (S4) under different heating conditions. Values in the same column with different subscript letters (a,b,c) represent significant differences among samples at $p < 0.05$ by Duncan test (n = 3). Nd, not detected.

Heating treatments		Volatile of unflavored EVOO				Volatile of FOOs			
		T				S1	S2	S3	S4
T°C	h	(*E*)-2-hexenal	hexanal	nonanal	(*E,E*)-2,4-decadienal	limonene	dipropyl disulfide	diallyl disulfide	eugenol
60°C	0	41.6^{a}	4.4^{c}	2.6^{d}	0.2^{c}	64.1^{a}	45.8^{a}	38.0^{a}	12.6^{b}
	1	40.9^{a}	3.9^{c}	2.8^{d}	nd	65.0^{a}	43.7^{a}	35.7^{a}	15.5^{b}
	2	43.1^{a}	4.2^{c}	2.6^{d}	nd	65.5^{a}	40.7^{ab}	35.5^{a}	15.9^{b}
	4	38.9^{b}	3.5^{c}	2.7^{d}	0.4^{c}	64.6^{a}	41.3^{a}	35.8^{a}	14.9^{b}
	8	nd	nd	nd	nd	64.9^{a}	41.9^{a}	35.2^{a}	18.9^{a}
100°C	1	44.5^{a}	5.3^{c}	3.6^{d}	nd	65.2^{a}	42.3^{a}	37.0^{a}	18.8^{a}
	2	35.7^{b}	5.3^{c}	4.0^{cd}	nd	65.8^{a}	39.6^{b}	36.3^{a}	18.3^{a}
	4	38.7^{b}	7.8^{b}	7.2^{c}	nd	66.6^{a}	41.2^{a}	38.1^{a}	17.7^{a}
	8	26.7^{b}	9.6^{b}	9.8^{c}	0.1^{c}	64.2^{a}	38.5^{b}	38.2^{a}	13.9^{b}
200°C	1	5.0^{c}	18.3^{a}	16.2^{b}	13.4^{a}	65.8^{a}	20.8^{c}	nd	3.8^{c}
	2	1.7^{cd}	16.1^{a}	19.6^{b}	10.6^{a}	63.8^{ab}	12.0^{d}	4.0^{b}	2.0^{c}
	4	0.8^{d}	14.1^{a}	21.7^{a}	9.1^{b}	61.5^{ab}	5.7^{e}	nd	1.3^{cd}
	8	0.1^{d}	4.5^{c}	27.2^{a}	8.6^{b}	55.0^{b}	0.5^{f}	nd	0.8^{d}

TABLE 4. Volatile compounds of flavored solutions (lemon F1, onion F2, garlic F3; paprika F4) and FOOs (lemon S1, onion S2, garlic S3, paprika S4). Percentages obtained by FID peak area normalization.

		Flavored solutions				Flavored olive oils			
Volatile compounds (%)	**I.r.i***	**F1**	**F2**	**F3**	**F4**	**S1**	**S2**	**S3**	**S4**
Aldehydes from LOX									
Hexanal	800	nd	nd	nd	nd	nd	nd	nd	0.2
(*E*)-2-hexenal	851	nd	nd	nd	nd	0.1	**6.1**	**7.2**	**25.5**
Esters from LOX									
(*Z*)-3-hexenyl acetate	1007	nd	nd	nd	nd	nd	1.0	0.9	2.7
1-hexyl acetate	1009	nd	nd	nd	nd	nd	0.6	0.4	1.2
Terpenic compounds									
α-thujene	932	0.4	nd	nd	1.5	0.4	nd	nd	nd
α-pinene	940	2.0	nd	4.5	nd	3.3	nd	nd	2.7
β-pinene	980	**13.1**	nd	nd	0.7	**19.7**	nd	nd	nd
Myrcene	993	1.7	nd	nd	1.7	1.3	nd	nd	nd
a-phellandrene	1006	0.1	nd	nd	3.0	nd	nd	nd	nd
δ-3-carene	1012	nd	nd	nd	1.0	nd	nd	nd	nd
p-cymene	1027	0.7	nd	nd	**5.8**	0.8	nd	nd	1.4
Limonene	1032	**62.3**	1.1	nd	**10.5**	**64.1**	0.6	0.7	**21.8**
1,8-cineole	1034	nd	nd	nd	2.2	nd	0.5	nd	0.8
(*E*)-β-ocimene	1051	0.1	nd	nd	1.4	0.1	nd	0.4	0.7
γ-terpinene	1062	**10.1**	nd	nd	2.1	**7.4**	nd	nd	1.2
Terpinolene	1090	0.5	nd	nd	1.9	0.2	nd	nd	nd
Linalool	1101	nd	nd	nd	2.2	nd	nd	nd	nd
(*E*)-limonene oxide	1141	nd	nd	nd	nd	0.1	nd	nd	nd
Camphor	1147	nd	nd	nd	1.6	nd	nd	nd	0.6
Neral	1240	1.2	nd	nd	nd	nd	nd	nd	nd
Geranial	1271	1.9	nd	nd	nd	1.1	nd	nd	nd
Eugenol	1361	nd	nd	nd	**40.1**	nd	nd	nd	**12.6**
α-copaene	1377	nd	nd	nd	0.3	nd	nd	nd	0.8
Methyl eugenol	1405	nd	nd	nd	2.7	nd	nd	nd	1.1
β-caryophyllene	1418	0.7	nd	nd	**8.2**	0.1	nd	nd	2.5
(*E*)-α-bergamotene	1437	0.5	nd	nd	nd	nd	nd	nd	nd
α-humulene	1456	nd	nd	nd	1.0	nd	nd	nd	nd
Valencene	1494	nd	nd	nd	nd	nd	nd	nd	0.2
β-bisabolene	1508	0.9	nd	nd	nd	0.1	nd	nd	nd
(*E,E*)-α-farnesene	1505	nd	nd	nd	nd	nd	0.7	0.3	1.2
Organosulfur compounds									
Diallyl sulfide	866	nd	nd	**25.5**	nd	nd	nd	nd	nd
2,3-dimethylthiophene	899	nd	2.4	0.1	nd	nd	0.2	nd	nd
Methyl allyl disulfide	918	nd	0.7	**20.8**	nd	nd	nd	**8.5**	nd
Methyl propyl disulfide	937	nd	**13.6**	nd	nd	nd	4.0	nd	nd
(*Z*)-1-propenyl methyl disulfide	948	nd	**8.9**	2.2	nd	nd	1.6	2.6	nd
(*E*)-1-propenyl methyl disulfide	952	nd	nd	0.2	nd	nd	1.4	0.3	nd
Dimethyl trisulfide	973	nd	4.3	3.5	nd	nd	1.1	1.6	nd
Diallyl disulfide,	1082	nd	nd	**26.6**	nd	nd	nd	**38.4**	nd
(*Z*)-1-propenyl propyl disulfide	1093	nd	0.9	3.0	nd	nd	nd	3.9	nd
(*E*)-1-propenyl propyl disulfide	1099	nd	nd	4.2	nd	nd	**5.2**	**5.3**	nd
Dipropyl disulfide	1105	nd	**40.3**	nd	nd	nd	**45.8**	nd	nd
(*E*)-1-propenyl propyl disulfide	1117	nd	**8.8**	nd	nd	nd	nd	nd	nd
Methyl allyl trisulfide	1150	nd	**9.2**	**8.8**	nd	nd	**8.7**	**12.8**	nd
Diallyl trisulfide	1298	nd	nd	nd	nd	nd	nd	**9.8**	nd
Dipropyl trisulfide	1325	nd	3.9	0.1	nd	nd	**11.8**	nd	nd
3,5-diethyl-1,2,4-trithiolane	1336	nd	0.6	nd	nd	nd	0.8	nd	nd

*Linear retention indices (DB-5 column). Nd, not detected. Other compounds under 3.5% (heptanal, (*Z*)-2-heptenal, nonanal, methyl octanoate, decanal, (*E*)-2-decenal, methyl salicylate, ethyl salicylate, 2-undecanone) were not inserted in the table.

Some typical compounds such as limonene, β-pinene, eugenol, dipropyl disulfide, diallyl disulfide were found in the studied flavored solutions (Table 4). It is possible to observe the main markers of the lemon essential oil in S1 (limonene, β-pinene), of onion and garlic essential oils in S2 and S4, both characterized by organo-sulfur compounds (mainly dipropyl disulphide and diallyl disulphide, respectively) and of paprika essential oil in S4 (limonene and eugenol).

The addition of lemon essential oil to EVOO caused obvious changes in the volatile aroma of olive oils, not only with the occurrence of terpene compounds but also through their impact on the LOX pathway. The addition of lemon leads to an increase in limonene concentration to the detriment of the percentage of (*E*)-2-hexenal, which is the most abundant VOO volatile compound (64.1 vs 0.1%, respectively in S1). Our results are in agreement with Sacchi *et al*., (2017) on lemon - FOO volatile composition.

Volatile compounds released from onion solutions in the olive oil samples showed the dominance of dipropyl disulfide (45,8%) compared to (*E*)-2-hexenal (6.1%). Diallyl disulfide took the place of (*E*)-2-hexenal in the aromatic profile of garlic – FOO (38.41 vs 7.2%). However, paprika – FOO preserved (*E*)-2-hexenal as the most abundant volatile compound (25.5%), with a remarkable presence of limonene (21.8%) followed by eugenol (12.6%).

The thermo-oxidation process at 60 °C as well as at 100 °C of the FOOs was not detrimental for the volatile compound markers, as depicted in Table 3. For example, limonene, dipropyl disulfide and diallyl disulfide showed a great resistance to thermo-oxidation at 60 °C (64.9, 41.9 and 38.2% respectively) and 100 °C (64.2, 38.5 and 35.2% respectively) whereas moderate variations during heating at these temperatures were shown by eugenol. On the contrary, the effect of deep-frying at 200 °C was noticeable for all these markers. Limonene seemed to be the most resistant volatile compound among those studied. Hence, even under the most severe conditions (200 °C for 8 hours) the rate of degradation did not exceed 15%. We can classify the order of resistance of each volatile compound marker based on its rate of degradation as follows: limonene (14.20%) > eugenol (93.65%) > dipropyl disulfide> (99.77%) diallyl disulfide > (100%).

The incorporation of oil preparations with different flavors to the EVOO has provided new aromatic compounds characterized by specific sensory perceptions. Sacchi *et al*., (2017) have demonstrated that the incorporation of the lemon with olive to produce lemon flavored olive oil led to the appearance of new volatile compounds able to mask the negative attributes by conferring strong notes of lemon leaf, albedo and lemon juice. At the same time, it had a negative impact on the positive attributes of VOO obtained from fresh olives by decreasing its intensity of fruity, bitter and pungent.

3.3. Hedonic sensory evaluation by consumers

Hedonic sensory methods, mainly acceptance tests, are usually applied to study the factors that can affect the liking of and consumers' behavior toward foods. The overall liking (9-point hedonic scale) of the 96 interviewed subjects is summarized in Figure 1. Observing the mean of the overall liking scores given by the consumers, 72% of them provided values on the hedonic scale ranging from 7 to 9 fors ample T, which clearly indicated the unflavored oil as the most liked: only 6% of consumers gave the lowest score (sum of 1-3 scores).

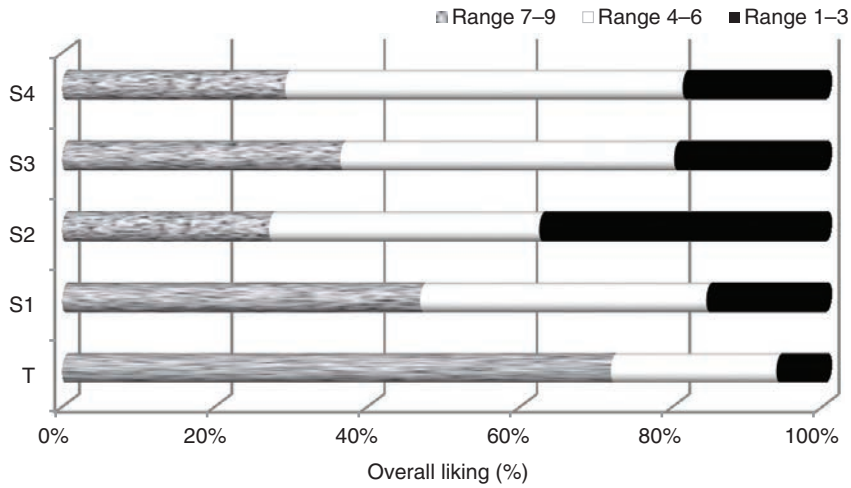

FIGURE 1. Percentages of overall liking for EVOO (T) and FOOs (lemon S1, onion S2, garlic S3, paprika S4) evaluated by 96 consumers.

Among the flavored olive oils, S1 (with lemon essential oil) was significantly more liked than S2, which was the least liked (Figure 1). In fact, 47% of consumers expressed their overall liking using values of the scale ranging from 7 to 9, whereas S2 showed the highest percentage (38%) of consumers who indicated values in the range of 1-3. Considering the results registered by the garlic flavored (S3) and the paprika flavored oil (S4), there were no significant differences among the mean values of liking. For both these samples, the majority of people interviewed showed a medium degree of appreciation: 44% (for S3) and 52% (for S4) of consumers provided values of overall liking between 4 and 6 on the hedonic scale.

4. CONCLUSIONS

This study confirmed that consumer appreciation is affected mainly by familiarity and habits; in fact, the overall liking scores are rewarded to the EVOO which is part of Tunisian culinary tradition.

Among the four flavored oils (lemon, onion, garlic and paprika essential oils), the most liked by consumers was the one with lemon.

In recent years, some spices and herbs with important nutritional and health properties have become more prominent in our diet. In this study, the hedonic test did not take into account possible flavored olive oil pairing. This concept should be considered for further investigation of flavored oils in combination with specific food preparations/dishes (raw and cooked) to determine if modifications in the sensory properties of foods induced by oils affect consumer hedonic responses in terms of liking.

Interestingly, the oxidative stability measured by the Rancimat instrument of flavored oils with onion and garlic subjected to deep-frying (200 °C) resulted in an improvement; while the flavor addition did not influence EVOO stability when samples were heated at 60 °C or 100 °C.

Further studies using different analytical approaches should be undertaken to clarify whether the characteristics of sulfur-containing compounds in onion and garlic can effectively exert antioxidant activity. The mixing of oil preparations with different flavors with EVOO clearly modified its volatile profile enriching it in the volatile compounds of the added flavored solution and therefore in sensory notes.

Among the heat treatments, only the thermo-oxidation at 60 °C and at 100 °C of the FOOs was not detrimental for the volatile compound markers of the flavored solution under study.

Limonene seemed to be the most resistant volatile compound among all the studied ones; even under the most severe conditions (200 °C for 8 hours) its rate of degradation did not exceed 15%.

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