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HEALTH PROMOTING OLIVE LEAF AS A FOOD INGREDIENT: INDUSTRIAL STUDY ON A CONFECTIONERY PRODUCT

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Abstract

The chemical composition and functional properties of olive (*Olea europaea* L.) leaf powder (OL) as an ingredient to be used in green and sustainable food processing were determined. OL contained low moisture (8% of dry weight) and fat (1.6%), moderate rates of proteins (10.8%) and high amounts of carbohydrates (70.8 %) composed mainly of insoluble fibers (57.9%). Due to its high content in insoluble fibers, OL displayed high fat absorption power and moderate water solubility index and holding capacity. Therefore, OL can be an appropriate ingredient to stabilize lipid rich food formulations. OL was incorporated at an industrial scale in a pistachio paste based confectionery product. This addition enabled to protect the oil from oxidation during cooking, drying and storage of the pistachio paste mixture. Moreover, the microbiological quality, textural properties, sensory attributes, and overall acceptance of the product were improved. This work promotes the use of OL as a health promoting multi-functional food ingredient.

Keywords: health promoting, olive leaf, food ingredient, confectionery product, pistachio paste

Introduction

In response to consumers' demand for more healthy food, manufacturers search to replace synthetic ingredients with natural and nutritive additives. Recently, natural

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plants have received much attention as cheap and abundant sources of bioactive compounds including natural antioxidants and nutrients such as dietary fibers which are known for their functional properties. Plant derived polysaccharides and extracts have been widely described as a source of anti-oxidants and anti-microbial products for food preservation (Ayadi *et al.*, 2009). Cactus cladodes were used to increase fibers and minerals content and to improve oil colloidal and oxidative stability in cakes and cookies (Ayadi et al. 2009). To reduce oil oxidation in food preparations, antioxidants are widely used. Chemically synthesized antioxidants were described to be harmful to human health. An attractive alternative to their use is natural antioxidants present at high yields in fruits and vegetables such as olive leaves considered an olive oil by-product. Using green natural products is an emerging innovative food processing technique that plays a key role in promoting a sustainable food industry (Chemat *et al*., 2017). Olive leaf is widely studied for its fruits and oil consumed worldwide and known for its health benefits. The antioxidant activity of olive leaves is mainly due to phenolic compounds but also to polysaccharides (Kiritsakis *et al*., 2017). The olive oil polyphenolic content and anti-oxidant activity were increased by carrying out oil extraction using olive leaf aqueous extract or olive mill wastewater (Kiritsakis *et al.*, 2017). Olive leaf extract was reported to lower blood pressure in animals and stage-1 hypertension in humans, increase blood flow in the coronary, relieve arrhythmia and prevent intestinal muscle spasms (Benavente-García *et al*., 2000; Susalit *et al*., 2011). Furthermore, olive leaves bioactive compounds displayed antioxidant, anti-atherogenic, anti-inflammatory, hypoglycemic, and hypo-cholesterolemic properties. These properties are attributed to their phenolic compounds (mainly oleuropein) (6–9% of dry matter, vitamins (essentially α-tocopherol), sterols (β-sitosterol), carotenoids and chlorophylls. The leaves are important for their secondary metabolites like secoiridoid compounds such as oleacein and oleuropein described to display hypotensive and hypoglycemic activities. Experimental evidence obtained from human and animal studies demonstrates that *Olea europaea* leaf phenolic compounds display biological activities that may be important to reducing the risk and severity of certain chronic diseases. Phenolic compounds confer to the olive leaves interesting anti-carcinogenic, antimicrobial (Lee and Lee, 2010) and antioxidant activities. Moreover, dietary fibers, abundant in plant leaves, are known for their nutritive value as well as for their good fat holding capacity and could be a cheap substitute for commercial oil stabilizers (Elleuch *et al*., 2011).

Olive tree leaves have been widely used in traditional remedies in European and Mediterranean countries. Olive tree leaves are used as a traditional herbal drug like an extract, herbal tea, or powder and are well known for their beneficial effects on metabolism (El and Karakaya, 2009). Olive polyphenols are already used as antioxidants and antimicrobials in functional foods and beverages, as well as in several cosmetic products (Ciriminna *et al*., 2016). Recently, a plastic film containing olive leaf extracts was successfully used to improve the oxidative stability of fresh minced pork meat (Moudache *et al*., 2017). In animal feed, the addition of olive leaves to the pork diet (10g/kg) inhibited lipid oxidation in refrigerated stored n-3-enriched meat (Botsoglou *et al*., 2014). The olive leaf powder was successfully used to protect minced Atlantic horse mackerel stored at 4°C against oxidation (Albertos *et al*., 2018). Few reports described the industrial use of olive leaves as a source of dietary fibers and antioxidants in the human diet (Bouaziz *et al*., 2008). Confectionery products prepared out of oleaginous dried fruits (pistachio, almond) or seeds pastes are importantly consumed worldwide. Major concerns of these products are oil and microbial stability during storage. The objective of the present work was to highlight the technological potential of olive leaf (*Olea europaea* L.) powder (OL) as a food ingredient by studying its functional properties. The capability of health-protective OL to reduce the oil oxidation and enhance the product's organoleptic characteristics of a pistachio-based confectionery product was addressed.

Material and methods

Olive leaf powder (OL)

The powder of olive leaf was prepared from dried and grinded leaves collected from local olive trees in Sfax city (Tunisia) and then stored in a sealed container at room temperature, in the dark.

Confectionery product preparation

The confectionery products were produced at the "Pâtisserie Masmoudi" which is a fine pastry manufacturer located in the Sfax region (Tunisia).

Pistachio paste was prepared, on an industrial scale, by mixing shelled and grinded pistachio seeds, sugar, eggs, and water. 0.5% or 1.5% of OL were added at this step. After baking at 120°C for 15 min and cooling, the paste was introduced into molds and covered by royal icing (a mixture of icing sugar, egg white, Arabic gum, lemon, rose extract and food coloring) prepared at time. The pastry product was then incubated in a warm room at 51°C for a day to dry the royal icing layer. The finished products were then stored at 4°C for subsequent experimental analysis. A commercial emulsifier (EM) E 472 (mixture of di-monoglycerides (90%) was used to improve oil retention in the product under storage. Two different levels of EM, namely 0.5% or 1.5%, were added at the mixing step instead of OL. Five samples were prepared. Samples containing 0.5% OL, 1.5% OL, 0.5% EM and 1.5% EM of the pistachio paste were named OL0.5, OL1.5, EM0.5, EM1.5, respectively. The control sample (C) was prepared without the addition of OL or a commercial emulsifier.

Characterization of OL

Chemical analysis

OL was analyzed for moisture, protein, fat, ash and dietary fiber contents as previously described (Prosky *et al*., 1987). Nitrogen content was estimated by the Kjeldhal method and was converted to protein using factor 6.25.

Phenolic compounds' concentration was estimated in leaves' methanolic extract by the spectrophotometric method (wavelength 725 nm) of the Folin–Ciocalteu method (Gutfinger, 1981). Chlorophylls (a and b) and carotenoids contents were determined according to the method previously described (Wrolstad *et al*., 2005).

Physical properties

Fat absorption capacity (FAC), water holding capacity (WHC) and water solubility index (WSI) were estimated for OL (Ayadi et al. 2009). Color determination was executed with CIE Lab color space using a Konica Minolta Chroma Meter CR-410 (Japan). The CIE color value included L* (lightness), a* (redness), and b* (yellowness).

Radical scavenging activity

This was evaluated by the previously reported DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Kirby and Schmidt, 1997). The percentage of DPPH radical scavenging activity was plotted against the olive leaf methanolic extract concentration to determine the amount of extract necessary to decrease the DPPH radical concentration by 50% (IC50).

Characterization of pastry product enriched with OL

Oil retention

Oil retention during storage was estimated by measuring oil separation using a modified version of the previously described method (Ereifej *et al*., 2005). Twenty grams of pistachio paste were covered with perforated aluminum foil and then placed on a petri dish containing three filter papers (Whatman #4) (weight A) to absorb the oil passing through the perforations in the aluminum foil. The weight of the petri dish with absorbed oil by filter paper was taken during storage time to determine the amount of oil separation (weight B). Oil separation from the product was evaluated at room temperature (eq.1).

$$Oil\ separation\ (\%) = \frac{\mathrm{B-A}}{\mathrm{sample\ weight}} \times 100 \qquad (1)$$

Oxidative stability

Peroxide value (PV) was measured for pistachio paste oil extracted by chloroform at different times during its preparation and storage according to the AOAC Official Methods number 965.33 and 939.05, respectively (AOAC, 1990).

Sensory evaluation

The sensory profiles of pastry product samples were evaluated by trained panelists, using a five-point scale scoring test on the aspects of odor, color, texture, and taste characteristics of pastry products (1: extremely bad; 5: excellent). Pieces of pastry products were wrapped with aluminum foil, coded with two-digit numbers, and presented to the panelists in a randomized serving sequence.

Pastry product quality is characterized by many response variables (odor, color, texture, and taste). Thus, to optimize product quality and select the most appreciated pastry product, the desirability function approach was used to maximize odor, color, texture and taste simultaneously. Desirability is a statistical method used for simultaneous optimizing of multiple properties (called responses (Y_i)).

It is based on the transformation of all the responses (Y_i) from different scales at the same unitless desirability scale, individual desirability function: d_i according to equation 2 (Derringer and Suich, 1980).

$$d_i = \begin{cases} 0 & Y_i \leq Y_{i-\min} \\ \left[\dfrac{Y_i - Y_{i-\min}}{Y_{i-\min} - Y_{i-\max}} \right]^r & Y_{i-\min} < Y_i < Y_{i-\max} \\ 1 & Y_i \geq Y_{i-\max} \end{cases} \qquad (2)$$

where $Y_{i\text{-min}}$ and $Y_{i\text{-max}}$ are the minimum and the maximum values of the response Yi, respectively. Also, r is a weight used to determine the scale of desirability and equals 1 in this study. The values of the desirability functions (d_i) are between 0 and 1, with $d_i = 0$ representing a completely undesirable value of y_i and $d_i = 1$ representing a completely desirable or ideal response value.

The next step is to combine these functions into a single global desirability D equal to the geometric mean of the individual desirability functions (d_i):

$$D = [d_1 (y_1) \text{ x } d_2 (y_2) \text{ x} \ldots \text{ x } d_k (y_k)]^{1/k} \qquad (3)$$

where k is the number of responses (Derringer and Suich, 1980).

The optimum corresponds to the maximum of the global desirability function D. An experimental designer, Design Expert 7.0.0 (Stat-Ease, USA), was used to transform the responses and calculate the optimum points. In the present study, the criteria of the desirability function correspond to maximal scores of the sensory evaluation parameters: odor, color, texture, and taste.

Texture analysis

The TPA (Texture profile analysis) of pistachio paste was performed using a texture analyzer (LLOYD instruments, England) equipped with a 1000 N load cell. Hardness, cohesiveness, adhesiveness, and elasticity were calculated from curves generated by the software of the texture analyzer NEXYGEN™ MT version 4.5 (AMETEK, USA). Results were the average of three triplicate determinations.

Microbiological analysis

Microbial counts for aerobic plate count (APC) were determined according to NF V 08–051 (1999) (NF V 08–051. 1999. Microbiology of Food and Animal Feeding

Stuffs – Enumeration of Microorganisms by Colony-Count Technique Obtained at 30°C – Routine Method. AFNOR).

Statistical analysis

All analytical determinations were performed at least in triplicates. Means and standard deviations calculations were carried out with SPSS version 20.0 (IBM, USA). Significant differences between means were verified by Duncan's test at $p < 0.05$. For the sensory evaluation, Design Expert 7.0.0 software (Stat-Ease, USA) was used to apply the desirability function and calculate the optimum point.

Results and discussion

OL characterization

Chemical composition and DPPH radical scavenging activity

The approximate chemical composition of dried and grinded Tunisian olive leaves (OL) was determined (Table 1). This powder had low moisture (8.6%), which could prevent microbiological development and limit the biochemical alteration during storage. The carbohydrates were the major compound of the dry matter (DM) (70.84%) and were mainly composed of insoluble fibers (56.50%). The fat content in leaf powder was low (1.6% of DM) whereas important protein rates (10.80% of DM) were measured. Olive leaves can be considered an important source of nutrients.

Several studies were devoted to find natural antioxidants in cheap raw material by-products. Olive leaves are attracting resources since they are considered as unused olive tree by-products during fruit harvesting. They were studied as a source of natural antioxidants such as phenolic compounds. Total polyphenols content in OL was 1.13g/100g DM (Table 1). Chlorophylls and carotenoids are the most abundant lipid-soluble pigments within the plant kingdom, known also for their antioxidant capacity (Wrolstad *et al*., 2005). Chlorophylls and carotenoids contents of OL were 3.6 mg /g DM and 1.36 mg/g DM, respectively (Table 1). Antioxidant capacity can be assessed using many tests such as the radical scavenging activity. OL extract displayed an important antioxidant activity. The amount of OL methanol extract required to scavenge 50% of DPPH radicals present in the reaction mixture (IC50), was 20µg/ml. OL seems to display an important DPPH radical scavenging activity. This antioxidant activity is probably due to the high content of OL in phenolic compounds and their high hydrogen atom donating abilities.

Olive leaves are a natural source of valuable nutriments such as carbohydrates, proteins, and minerals, as it was previously suggested (Boudhrioua *et al.*, 2009). This fact justifies their use in animal feeding. OL's high content in insoluble fibers is in line with the fact that leaves are rich in cellulose (the most-used food fiber), and lignin, known as insoluble fibers, present in the pecto-cellulose walls of plant cells (Chau and Huang, 2004). The fiber content in dried OL (57.90 % of DM) is higher than that reported for guduchi (*Tinospora cordifolia*) leaves, cactus cladodes (*Opuntia ficus-indica*) or curry (*Murraya koenigii*) leaf powders (16.10%;

28.84% and 39.90% of dry matter, respectively) (Drisya *et al.*, 2015; Msaddak *et al.*, 2015). The incorporation of curry latter leaf powders in the cookies improved the nutritional quality and physical properties of the prepared dough and the final product (Drisya *et al.*, 2015).

Table 1. Chemical characteristics and physical properties of dried OL.

Parameters	Value
Moisture (%)	8.60 ± 0.64
Fat (% of dry matter (DM))	1.60 ± 0.16
Proteins (% DM)	10.80 ± 0.39
Ash (% DM)	6.40 ± 0.00
Carbohydrates (% DM)	70.84 ± 0.38
Total fibers (% DM)	57.90 ± 0.25
Soluble fibers (% DM)	1.40 ± 0.06
Insoluble fibers (% DM)	56.50 ± 0.18
Total chlorophylls (mg/g DM)	3.65 ± 0.08
Chlorophyll a (mg/g DM)	1.60 ± 0.05
Chlorophyll b (mg/g DM)	2.05 ± 0.03
Total carotenoids (mg/g DM)	1.36 ± 0.05
Total polyphenols (g GAE/100g DM)	1.13 ± 0.11
L*	54.94 ± 0.18
a*	-5.33 ± 0.01
b*	24.51 ± 0.18
WSI (%)	16.63 ± 0.26
WHC (g water/g DM)	3.15 ± 0.11
FAC (g fat/g DM)	2.20 ± 0.07

OL chlorophylls content was in the same range previously described (Boudhrioua *et al.*, 2009). The phenolic content of OL (1.13g/100g DM) is in the same range of values reported for other sources of natural antioxidants like apple pomace (1.02g/100g DM) and cactus cladodes (0.90g/100g DM) (Ayadi *et al*., 2009). Due to its rich chemical composition (natural polyphenols and carotenoids, proteins, ash, dietary fibers, antioxidants), the olive leaf could be considered an interesting cheap raw material to be used as a natural antioxidant and stabilizer in food preparations.

Physical characteristics

The study of OL physical characteristics (Table 1) is an important step to evaluate its functional properties before its incorporation in a food product. CieLab indexes (L*, a* and b*) measured for OL are presented in Table 1. As compared with fresh olive leaf, the powder has a higher lightness value (L*) (54.94 *vs* 32.51) but a similar a* index (-5.33 vs -5.69) (Boudhrioua *et al.*, 2009). Its greenness remained however lower than that of other green materials such as spineless cladodes and honeybush herbal (a* values are -8.45 and -7.08, respectively) (Ayadi *et al*., 2009). The green tint is mainly due to chlorophylls, a natural pigment present in green plant material especially leaves. The use of OL in aqueous preparations requires a high solubility index. The water solubility index is related to the presence of soluble molecules in the powder. Fat absorption capacity (FAC) of OL was 2.2g/g DM (Table 1). OL displayed a water solubility index of 16.63% and a water holding capacity value of 3.15g/g DM. This property enhanced by the presence of hydrophobic constituents is required for the stabilization of oil-in-water emulsions to avoid oil separation in the product and to preserve its texture. OL seems to display interesting water and fat retention properties that are useful to stabilize a food product during manufacturing and storage.

The OL water solubility index (16.63%) is lower than those described for Stevia leaf (36.5%) and for spineless cladodes (27.8%) (Ayadi *et al*., 2009). OL showed a water holding capacity value (3.15g/g of Dry Matter) comparable to that of spineless cladodes but that remained lower than those reported for lime residues and outer leaves of cabbage (10.28–12.49 g/g of dry matter) (Jongaroontaprangsee *et al.*, 2007). Hydration properties results might be explained by a high insoluble fibers content in OL and reduced amounts of soluble fibers.

Fat absorption capacity (FAC) of OL (2.2g/g DM) was higher than those reported for sesame and spineless cladodes flours (1.30 and 1.31g/g of DM, respectively) (Ayadi *et al*., 2009). This could be explained by the high content of OL in insoluble fibers. The high oil absorption capacity of OL makes them useful additives in ground meat formulations such as sausages, soups and baked goods. They might be also used in oil-rich pastry products. Msaddak *et al*. (2015) reported that cactus cladodes powder (rich in insoluble fibers) incorporation in cookies reduced significantly ($p<0.05$) oil loss from 3.39% to 2.28% during storage.

Effect of OL incorporation on the product characteristics

The OL is incorporated into the pistachio paste used to formulate the final product before cooking. The effects of this addition on the characteristics of the paste were studied.

Oil retention and Oxidative stability

The pistachio paste has a relatively high-fat content (about 22%). Oil oxidation and release were the main problems that could occur during storage and deteriorate the product quality. Oil release from the pistachio paste at various stages of processing was recorded. Oil exudation from the pistachio layer of the final product was also assessed during 3 months of storage at room temperature. Oil exudation increased with storage time. Exuded oil rates found for OL enriched samples were not significantly different from values found for OL free samples (Figure 1).

To check if the oil retention rate can be improved through an emulsification process rather than oil retention exerted by olive leaf powder, control samples containing 0.5% or 1.5% of a commercial emulsifier instead of OL were prepared. Samples containing an emulsifier displayed also similar oil retention rates (Figure 1) as compared to pastry with or without OL.

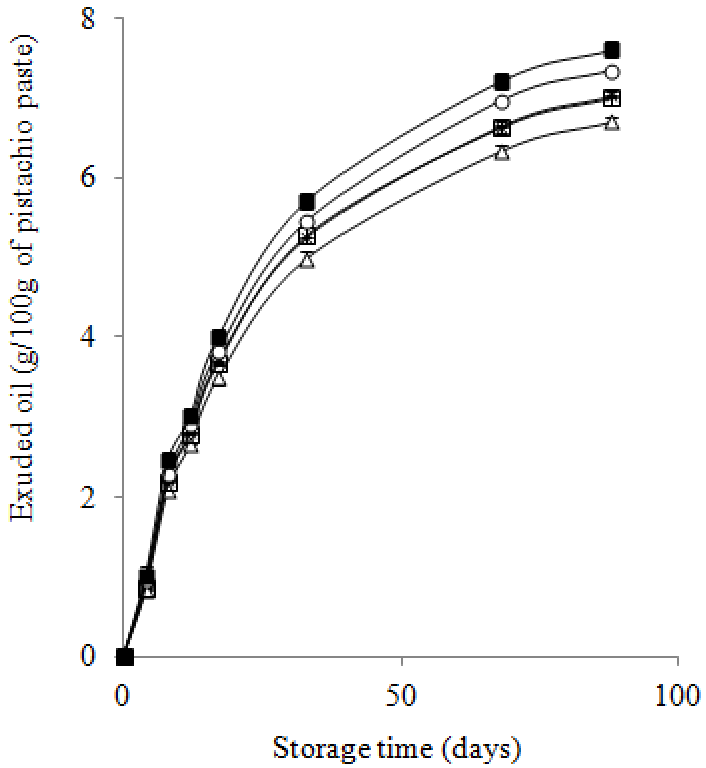

Figure 1. Evolution of exuded oil from pistachio paste during storage at room temperature. (■) pistachio paste containing 1.5% of OL, (○) pistachio paste containing 0.5% of OL, (Δ) pistachio paste containing 1.5% of EM, (x) pistachio paste containing 0.5% of EM, (□) standard pistachio paste. OL: Olive Leaves, EM: Emulsifier.

Despite its good fat absorption capacity, likely due to the presence of insoluble fibers, incorporating OL powder in the pastry product did not increase the oil retention capacity during storage, as compared to the control samples (figure 1). This might be explained by the fact that fibers' oil retention effectiveness usually is highly dependent on the food matrix. Replacing the OL with an industrial emulsifier did not improve oil holding. The exuded oil rates reached after three months of storage (7-8% of the pistachio paste) remain acceptable with regards to the high lipid content of the paste (22%). In fact, an oil release rate of 16% was reported for fat-rich sesame-based halva upon storage for three months (Aloui *et al.*, 2016).

Pistachio oil is rich in unsaturated fatty acids (C18:1 and C18:2) (Ghrab *et al.*, 2010) that increase the oil oxidation risk. Oil was extracted from pistachio paste to estimate its oxidative state by measuring the peroxide value (PV) (Figure 2). PVs increased slightly during the manufacturing process for all oil samples.

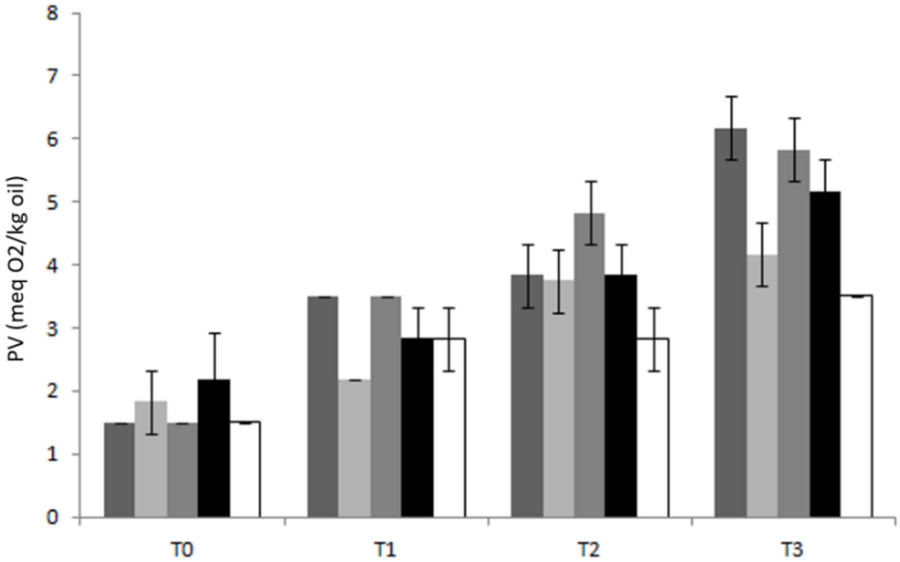

Figure 2. Changes in peroxide values (PV) after the kneading (T0), after the cooking (T1), after the drying (T2) and after 15 days of storage at room temperature (T3) of pistachio oil extracted from: (■) C (without additives), (■) EM0.5, (■) EM1.5, (■) OL0.5 and (□) OL1.5, OL: Olive Leaves, EM: Emulsifier.

The PVs increased also during the first 15 days of storage at room temperature and reached 6.2 meq O_2/kg. The incorporation of a commercial emulsifier instead of OL did not significantly affect the oxidative stability of pistachio pastry oil (Figure 2). For all studied samples, the PVs seem however to be relatively low (ranging from 1.5 meq O2/kg to 6.2 meq O_2/kg). Peroxide values (PVs) increased slightly during the manufacturing process for all oil samples which can be due to the high temperature (120°C) during paste cooking and drying of the finished product (51°C) indicating that the oxidation process continued likely under the influence of air oxygen. This phenomenon often occurred for other food products with high-fat content and intensifies with the increase in the storage temperature (Msaddak *et al.*, 2015). Adding OL resulted in reducing the PV during storage. These results recall those of Bouaziz *et al*. (2008) who showed that the addition of 400 ppm of olive leaf phenolic compounds improved oxidative stability of refined husk and olive oils during storage at 50°C. Moreover, in animal feed, the incorporation of dietary olive leaves (10g/kg) decreased lipid oxidation of refrigerated stored n-3-enriched pork (Botsoglou *et al*., 2014). Increasing oil resistance to oxidation by using OL powder can be explained by the high radical scavenging activity measured in the powder's extract (IC50= 20µg/ml). This effect is attributable to a high content of antioxidants such as polyphenols (1.13 ± 0.11 mg /100g DM) and alkaloids (1.36 g GAE/ 100g of dry matter). The stabilizing capacity depends greatly on the phenolic composition. Benavente-García *et al.* (2000) showed that the flavonols, flavans-3-ols and flavones constituents of olive leaves were very efficient antioxidant compounds, and their ability was greater as more free hydroxyl groups were present in the flavonoid structure. These authors also reported that the most active flavonoids such as rutin and luteolin had antioxidant activities almost 2.5 times those of C and E vitamins. The phenolic compound Oleuropein has been extensively studied and described as having human health benefits and important antioxidant activity. PVs values of pistachio oil remain relatively low which can be explained by the presence of natural antioxidants identified in the pistachio such as tocopherols, squalene, phytosterols, polyphenols and flavonoids.

Textural analysis

The textural parameters of the OL enriched pistachio paste layer were determined and compared to those of the standard sample (Table 2). No differences in the cohesiveness and elasticity were noticed ($p>0.05$) indicating that incorporating OL did not affect these properties. The adhesiveness and hardness were the highest for the sample containing 1.5% of OL ($p<0.05$). OL could be used as a functional ingredient in food preparation thanks to its texture modifying capacity and potential health benefits. The incorporation of OL did not have a marked effect on the pistachio paste texture parameters except for the adhesiveness and hardness that were higher as compared to the OL free samples ($p<0.05$). This could be explained by the effect of insoluble fibers, contained in OL, that strengthen the product structure (Jridi *et al.*, 2015) and by the lower water availability due to the OL water holding capacity mentioned above (3.15 ± 0.11 g/g DM). Similarly, it was reported that the addition of plant leaf flours with high fiber content (guduchi or curry) resulted in harder cookies (Drisya *et al.*, 2015). Likewise, the addition of 2.5% of cladodes powder caused an increase in the cookie's hardness from 59.28 to 67.07 N (Msaddak *et al.*, 2015).

Table 2. Quality parameters of pistachio paste layer of pastry product C (control without additives), OL0.5, OL1.5, EM0.5, and EM1.5.

	C	**OL0.5**	**OL1.5**	**EM0.5**	**EM1.5**
L*	$39.04{\pm}0.31^{ab}$	$40.51{\pm}2.32^{b}$	$37.95{\pm}0.88^{a}$	$39.01{\pm}0.89^{ab}$	$43.43{\pm}0.41^{c}$
a*	$3.70{\pm}0.17^{cd}$	$2.47{\pm}0.01^{ab}$	$1.99{\pm}0.25^{a}$	$3.98{\pm}0.55^{d}$	$3.14{\pm}0.05^{bc}$
b*	$20.03{\pm}0.09^{a}$	$23.62{\pm}3.30^{bc}$	$19.53{\pm}1.19^{a}$	$21.27{\pm}0.95^{ab}$	$25.44{\pm}0.23^{c}$
Hardness (N)	$5.52{\pm}1.19^{b}$	$5.30{\pm}0.33^{b}$	$8.73{\pm}1.08^{c}$	$4.47{\pm}0.74^{ab}$	$3.12{\pm}0.32^{a}$
Cohesiveness	$0.24{\pm}0.04^{a}$	$0.23{\pm}0.01^{a}$	$0.26{\pm}0.09^{a}$	$0.23{\pm}0.02^{a}$	$0.28{\pm}0.04^{a}$
Elasticity (mm)	$0.93{\pm}0.02^{a}$	$1.12{\pm}0.21^{a}$	$1.18{\pm}0.43^{a}$	$0.86{\pm}0.07^{a}$	$0.97{\pm}0.27^{a}$
Adhesiveness (N)	$1.30{\pm}0.07^{a}$	$1.21{\pm}0.14^{a}$	$2.31{\pm}0.06^{b}$	$1.04{\pm}0.26^{a}$	$0.88{\pm}0.02^{a}$
Odor	$3.09{\pm}0.21^{a}$	$3.36{\pm}0.20^{a}$	$3.27{\pm}0.27^{a}$	$3.09{\pm}0.18^{a}$	$3.18{\pm}0.24^{a}$
Color	$3.45{\pm}0.23^{a}$	$3.73{\pm}0.30^{ab}$	$4.00{\pm}0.21^{b}$	$3.08{\pm}0.28^{a}$	$3.14{\pm}0.25^{a}$
Texture	$3.64{\pm}0.19^{a}$	$3.18{\pm}0.30^{a}$	$3.64{\pm}0.15^{a}$	$3.27{\pm}0.34^{a}$	$3.45{\pm}0.36^{a}$
Taste	$3.27{\pm}0.27^{a}$	$3.10{\pm}0.37^{a}$	$3.18{\pm}0.37^{a}$	$3.45{\pm}0.26^{a}$	$3.27{\pm}0.48^{a}$
Desirability D	0.51^{a}	0.52^{a}	0.56^{b}	0.47^{c}	0.48^{c}
Aerobic plate count (APC) (cfu/g)	$1.13 \times 10^{4\ a}$	$2 \times 10^{3\ b}$	$2.7 \times 10^{3\ b}$	$1.17 \times 10^{4\ a}$	$1.09 \times 10^{4\ a}$

Data with different letters (a, b, c or d) for each line represent significant difference at $p<0.05$.

Sensory evaluation and microbial stability

We studied the color parameters of the pistachio layer of the pastry product (Table 2). OL incorporation did not significantly affect the pistachio layer lightness (L*) (p>0.05). It enhances however its green color (a* decreased) (p<0.05). Olive leaves could be used as a natural green food coloring agent and replace chemically synthetic food coloring which was considered harmful to human health and environmental security.

According to the sensory evaluation of the pastry products (Table 2), color scores were significantly higher for samples containing 1.5% OL (p<0.05) than for the control. Overall sensory scores for the various samples were close. Despite improving color and texture scores, the addition of a commercial emulsifier or OL powder did not significantly affect the taste and odor characteristics (p>0.05) (Figure 3). We used the desirability function to search for conditions giving the best product quality. The sample enriched with 1.5% OL provided the most desirable response value (0.56) (Table 2). This sample provided the optimal compromise for maximizing odor, color, texture, and taste scores. Adding OL did not affect the pistachio layer lightness but enhanced the green taint likely due to the high content of OL in chlorophylls. This result is consistent with previous studies which investigated food supplementation with different green plant leaves (Drisya *et al.*, 2015).

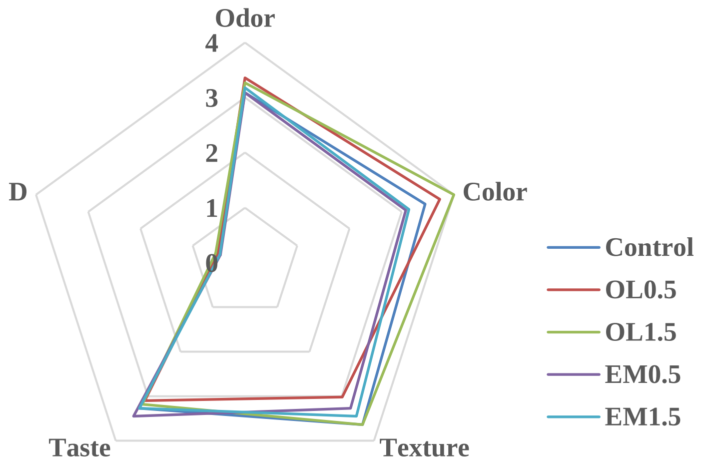

Figure 3. Sensory scores of odor, color, texture and taste attributes for pistachio paste samples OL0.5, OL1.5, EM0.5 and EM1.5 containing 0.5% OL, 1.5% OL, 0.5% EM or 1.5% EM, respectively. D: Desirability function value, OL: Olive Leaves, EM: Emulsifier. The control sample (C) did not contain OL or EM.

Overall sensory scores of the pastry product were not affected by incorporating OL. Similar findings were reported for meat products where the addition of olive leaves at 10g/kg feed exerted a positive effect on the sensory attributes of cooked n-3-enriched chops (Botsoglou *et al*., 2014). Nevertheless, it was described that pastry enrichment with other plant leaves flours decreased their sensory scores and consumer overall acceptability (Ayadi *et al*., 2009; Drisya *et al*., 2015). The sample enriched with 1.5% OL provided the most desirable response value (0.56) and displayed optimal compromise for maximizing odor, color, texture and taste scores. Hence it could be concluded that pistachio-based pastry with the most acceptable quality can be prepared by using incorporating OL at a rate of 1.5%.

OL addition also improved the microbial stability of the product. Aerobic mesophilic bacteria are involved in food contamination and degradation as well as in infections in humans. The aerobic plate count (APC) for the standard pistachio layer and for samples containing commercial emulsifiers ranged from 1.13 104 to 1.29 104 cfu/g (Table 2). These levels are lower than those reported for pistachio nuts (105 cfu/g) (Al-Moghazy *et al.*, 2014). This could be explained by the intense heat treatment (120 ° C, 15 min) during cooking. For the pistachio layer enriched with OL (0.5% or 1.5%), the APC was considerably lower than for the other samples ($p<0.05$). This is likely to be explained by the potential antibacterial activity of olive leaves. The anti-microbial activity of olive leaf extracts against a wide range of microorganisms was linked to the presence of phenolic compounds such as hydroxytyrosol and oleuropein (Lee and Lee, 2010).

Conclusions

The enrichment of pistachio paste with OL as a source of dietary fibers and antioxidants improved the oil oxidative stability in a pistachio paste-based product under processing and storage. This incorporation improved the texture, sensory attributes, and microbiological quality of the pistachio paste. In addition to its high content in antioxidant and health promoting natural biomolecules, OL displayed interesting functional properties such as high lipid holding capacity. It may be a promising functional ingredient to improve food products' quality and nutritional value.

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Conflicts of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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