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Olive leaves extract encapsulated by spray-drying in vacuum fried starch–gluten doughs

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ABSTRACT

Olive leaves extract (OLE) was microencapsulated with inulin (OLE-IN) by spray-drying using a central composite design. Oleuropein encapsulation efficiency and recovery values were over 87% in the OLE-IN microparticles obtained under optimal conditions. OLE or OLE microparticles were added into starch–gluten fried matrices with the aim of studying the effect of microencapsulation and the frying method on the polyphenols content and antioxidant activity, fat content and crispness. Vacuum starch–gluten fried samples absorbed slightly, but significantly, less oil than the atmospheric fried ones (except for the dough with 350 mg GAE/kg), but crispness was higher in the atmospheric fried products. Although vacuum fried matrices showed significantly higher content of polyphenols than atmospheric fried matrices, both types of matrices showed similar antioxidant activity, suggesting the formation of antioxidant metabolites derived from the Maillard reaction during the atmospheric frying. The results highlighted the importance of the microencapsulation of OLE to preserve the beneficial effects of polyphenols in processed food.

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1. Introduction

Epidemiological evidence has demonstrated that diets rich in fruits and vegetables promote healthy effects, preventing oxidative stress-related diseases (Fraga et al., 2010), which has been associated to the content of bioactive compounds such as polyphenols. In the fast modern lifestyle, more processed foods are consumed instead of fresh fruits and vegetables, leading to the development of healthy ready-for-eat food products. Polyphenols have been extracted from several sources such as fruits, vegetables, seeds, flowers, leaves, and recently from agroindustrial wastes (Moure et al., 2001). A large amount of wastes are generated from olive oil industry, being the olive leaves (*Olea europaea* L.) one of the main by-products (Nunes et al., 2016). A wide profile of polyphenols has been described in olive leaves, where the oleuropein (OE) is the major compound. Moreover, beneficial effects of OE on human health, such as anti-atherogenic, anti-carcinogenic and anti-inflammatory have been reported (Bouaziz and Sayadi, 2005); therefore OE could play an important role as a food supplement.

The inclusion of polyphenol extracts from several natural sources as healthy/functional ingredients in food matrices has gained considerable attention in recent times (Robert et al., 2017). Regarding olive leave extracts (OLE), they have been used in different technological and functional applications such as biodegradable films for food packaging (Moudache et al., 2017), and food fortification (Nunes et al., 2016). Some examples of application of OLE in food matrices are enrichment of edible oils (Sahin et al., 2017), frying oils (Chiou et al., 2007, 2009), table olives, meat and meat products and fruit and fruit derivatives (Nunes et al., 2016). Furthermore, olive leave extracts have been included in biscuit formulations as a strategy for mitigation of dietary advanced glycation endproducts (Navarro and Morales, 2017).

Snack categories with the greatest growth are those that offer a wide range of product alternatives and declare healthy effects. Major

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salty snack manufacturing technologies are baking, extrusion, drying and frying; using doughs based on gluten and starch as starting material. Particularly frying, involves exposing the food to oxygen and high temperatures (usually, between 160 °C to 180 °C), processing conditions that may induce degradation of polyphenols (Dueik and Bouchon, 2011a, 2016). The stability of polyphenols during frying can be improved by modifying the process conditions. Several researchers have shown that vacuum frying is a technology able to retain polyphenols adequately (Dueik and Bouchon, 2011b; Da Silva and Moreira, 2008; Fang et al., 2011). Vacuum frying is an emerging frying technology carried out at pressure well below atmospheric conditions, which causes a great reduction of water boiling point, allowing frying at low temperatures (Garayo and Moreira, 2002; Dueik and Bouchon, 2011a).

Another useful technology to stabilize phenolic compounds is microencapsulation, wherein the active compounds are entrapped in a polymeric matrix to protect them and control their release at specific conditions (time and/or place) (Fang and Bhandari, 2010). Different encapsulation methods for OLE have been described, such as freeze-drying (Taghvaei et al., 2014; Mourtzinos et al., 2007; Ganje et al., 2016), W/O nanoemulsions and W/O/W double emulsion (Mohammadi et al., 2016a), electrostatic extrusion (Belščak-Cvitanovicí et al., 2011), spray-drying (Kosaraju et al., 2006, 2008). However, studies focused on the incorporation of encapsulated OLE in functional and/or healthy foods are scarce (Mohammadi et al., 2016b; Ganje et al., 2016), and none of these studies used the technology of spray-drying to encapsulate the extract. Spray-drying is a useful technique for the encapsulation of heat-sensitive materials because of its short drying times (5–30 s) (Desai and Park, 2005). Furthermore, this is simple, low-cost, reproducible and easy to scale-up, and the microparticles obtained by spray-drying have low water activity, simplifying storage, handling and transport (Gharsallaoui et al., 2007).

The objective of this work was to study the effect of both microencapsulation and frying method on the polyphenol content and quality parameters of starch–gluten fried doughs added with OLE or spray-dried OLE microparticles.

2. Materials and methods

2.1. Materials

Olive leaves (*O. europaea* L.) cv. Arbequina were collected at Melipilla (Chile, 2013). Inulin HP (DP > 23) Raftilina® was purchased from Alfa Chile S.A. (Chile). Matrices were formulated using wheat gluten (Asitec S.A., Chile) and native wheat starch (Blumos S.A., Chile). High oleic sunflower oil (HOSO) was donated by Camilo Ferrón Chile S.A. (Chile). Oleuropein (OE) and gallic acid standards were purchased from Sigma–Aldrich (Chile).

2.2. Preparation of the olive leaves extract

Polyphenols were extracted from olive leaves, previously blanched at 95 °C for 4.5 min, dried at 45 °C for 18 h in a forced-air oven (WTE, Germany). The dried olive leaves (400 g) were ground in a windmill (Fuchs-Müllen, Masch. Kom. N° 18791, Kriens, Switzerland) and macerated in ethanol:water (50:50 v/v, 1.6 L) for 24 h. The mixture was vacuum filtered (Whatman n°1) and the solid residue was reextracted twice and filtered. The extracts were combined and the volume was reduced in a rotatory evaporator (Buchi R-3, Switzerland) at 40 °C. The aqueous olive leaves extract (OLE) was filled up 1 L with distilled water and frozen at −20 °C in dark bottles.

2.3. Characterization of the olive leaves extract

Moisture and soluble solids (°Brix) of OLE were determined according to AOAC methods (1996).

2.3.1. Total polyphenol content

Total polyphenol content of OLE was determined by Folin Ciocalteau (Singleton and Rossi, 1965). The determination was carried out using a spectrophotometer (Unicam UV/vis ATI UNICAM, Cambridge, UK) at 765 nm, and the results were expressed as gallic acid equivalent (GAE) according to a calibration curve obtained using gallic acid solutions ranging from 0.1 to 1 mg/mL ($R^2 = 0.99$).

2.3.2. OLE analysis by HPLC

The OE content in the OLE was determined by HPLC at 280 nm according to Al-Rimawi (2014), using a Merck Hitachi L-6200 pump, a Waters 996 photodiode-array detector and a C18 column (5 μm × 4.6 mm i.d. × 250 mm, Symetry®, Waters, Ireland). An isocratic mobile phase of water:acetonitrile (80:20 v/v) containing 0.1% glacial acetic acid was used at a flow rate of 1 mL/min. OE was quantified using an OE standard calibration curve (20–200 μg/mL; $R^2 = 0.99$). Before injecting into the HPLC, the OLE was filtered (0.22 μm) and an aliquot (0.5 mL) was transferred to a volumetric flask filled up 25 mL with the mobile phase.

2.3.3. Antioxidant capacity

The antioxidant capacity of OLE was determined by 2.2-diphenyl-1-picryhydrazyl (DPPH) (Brand-Williams et al., 1995), ferric reducing ability of plasma (FRAP) (Benzie and Strain, 1996) and oxygen radical absorbance capacity (ORAC) (Dávalos et al., 2004) methods. DPPH was expressed as EC_{50}, whereas FRAP and ORAC were expressed as trolox equivalent (TE).

2.4. Preparation of olive leaves extract microparticles

The encapsulation of OLE was performed by spray drying, using inulin (IN) as encapsulating agent, according to a central composite experimental design with 12 runs. The OLE:IN ratio (1:0.34–1:2.15) and inlet air temperature (135–184 °C) were evaluated as independent variables. The dependent variables were oleuropein encapsulation efficiency (EE), recovery (R) and yield (Y). The data were fitted to a second-order regression model, according to Eq. (1). All of the experiments were conducted randomly to avoid systematic bials.

$$Y = \beta o + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{1} \sum_{j=i+1}^{2} \beta_{ij} X_i X_j \quad (1)$$

where Y was the response; subscript i and j ranged from 1 to the number of variables (n = 2); β_o was the intercept term; β_i values were linear coefficients; β_{ii} values were the quadratic coefficients; β_{ij} values were the cross-product coefficient; and X_i and X_j were the levels of independent variables.

The analysis of variance (ANOVA), test of lack of fit, and determination of regression coefficients were performed with the software Statgraphics (5.0 program, Manugistics Inc., Rockville, MA). Response surface methodology (RSM) was used to determine the optimal conditions for each independent variable. Optimisation was performed using the desirability function (DF), where all the independent variables were maximized.

The OLE-IN feed solution (100 g) was prepared as follows: inulin (2.5–10.79 g) was dissolved in distilled water (92.5–84.21 g) and heated to 65–70 °C with constant stirring, then cooled to 25 °C, mixed with OLE (5 g). The resulting solution was homogenized at 11,000 rpm for 5 min with a Polytron PT 2100 homogenizer (Kinematica A.G, Switzerland) and fed into a laboratory scale spray-dryer (Mini Buchi-290, Switzerland), co-current equipment where hot air and the feed atomization flows through the drying chamber at the same direction. Compressed air was used to disperse the infeed solution (held at room temperature 25 ± 1 °C) into fine droplets. The spray-dryer was equipped with an inlet air heater at the desired temperature, a dual fluid nozzle of 0.7 mm diameter for droplet formation, a drying chamber for conductive heat exchange between drying gas (air) and sample droplets, a cyclone separator for separation of dried product from the air. The outlet air temperature resulted from the combination of inlet air temperature, pump setting and aspirator setting. The air flow, rate of feeding and atomization pressure were 600 L/h, 3 mL/min (5%) and 20 psi, respectively. The microparticles obtained were stored in the dark at −20 °C.

2.5. Characterization of olive leaves extract microparticles

2.5.1. Total OE

OLE-IN microparticles (100 mg) were dispersed in water (2 mL) in a test tube, then put into a water bath at 65 ± 5 °C until a translucent solution was observed. The resultant solution was transferred to a volumetric flask, filled up 10 mL with water:acetonitrile (80:20: v/v) containing 0.1% glacial acetic acid. OE analysis was performed by HPLC as described in Section 2.3.2.

2.5.2. Surface OE

OLE-IN microparticles (100 mg) were dispersed in ethanol (2 mL) and centrifuged at 2906 g for 10 min. This process was performed twice from the pellet obtained. The supernatants were transferred to a volumetric flask filled up 10 mL with water:acetonitrile (80:20: v/v) containing 0.1% glacial acetic acid. OE analysis was performed by HPLC as described in Section 2.3.2.

EE, R and Y, were calculated according to Eqs. (2)–(4), respectively:

$$EE = \frac{\text{Total oleuropein content} - \text{Surface oleuropein content}}{\text{Total oleuropein content}} \times 100 \quad (2)$$

$$R = \frac{\text{Total oleuropein content}}{\text{Theoretical content of oleuropein in the feed solution}} \times 100 \quad (3)$$

$$Y = \frac{\text{Microparticles powder (g)}}{\text{Initial encapsulant agent (g)} + \text{Soluble solids of the extract (g)}} \times 100 \quad (4)$$

2.5.3. Antioxidant activity

OLE polyphenols were extracted from OLE-IN microparticles according to the procedure described for total OE (Section 2.5.1). The antioxidant capacity of the OLE-IN microparticles was determined by DPPH (Brand-Williams et al., 1995) and FRAP (Benzie and Strain, 1996) methods. DPPH and FRAP were expressed as TE.

2.6. Starch–gluten matrices formulation

Dough matrices based on wheat gluten and starch were prepared ensuring the same final moisture content (40%). The doughs were prepared by mixing gluten (12% dry base, db) and starch (88% db) at 40 rpm for 2 min using a mixer (5K5SS, Kitchen aid, USA). Water was added in two steps: half of the amount of distilled water was added at 20 °C, mixing for 1 min; and the other half was added at 90 °C, mixing for 1 min. Then, the dough was left inside a plastic film for 1 h and sheeting (LSB516, Doyon, Canada) to obtain sheets of 2 mm thickness. The sheets were cut into squares (4 × 4 cm), ensuring a constant weight (2.9 ± 0.2 g), and maintained in plastic films before frying to prevent dehydration. OLE or OLE-IN microparticles were incorporated to dough at three concentrations (200, 350 and 500 mg gallic acid equivalent GAE/kg raw dough).

2.7. Starch–gluten matrices processing

Atmospheric and vacuum frying experiments were performed in the frying equipment described by Dueik and Bouchon (2011b) using an equivalent thermal driving force of 70 °C, defined as the difference between oil temperature and the boiling point of water at the working pressure (100 °C for atmospheric conditions and 46 °C for vacuum frying) (Mariscal and Bouchon, 2008), which resulted in frying temperatures of 170 °C for atmospheric frying and 116 °C for vacuum frying. In both set of experiments, the fryer was kept closed during frying. HOSO was preheated to 160 °C for 1 h prior to frying and discarded after 3 h of frying.

2.7.1. Atmospheric frying experiments

Once the oil reached the frying temperature, about 8 squares (~20 g) were placed inside the frying basket and covered with a grid, to prevent floating of the samples while allowing natural expansion. The basket was immersed in the oil for 5 min, until the samples reached 2% moisture. The basket was lifted out and samples were removed from the fryer and cooled at room temperature.

2.7.2. Vacuum frying experiments

Once the oil reached the frying temperature, about 8 squares (~20 g) were placed inside the frying basket and covered with a grid. The vessel lid was fastened and the vessel was depressurized. When the pressure inside the vessel reached −27 pulg Hg, the basket was immersed in the frying oil for 3 min until the doughs reached 2% moisture. Then, the basket was risen and the vessel pressurized. The samples were removed from the fryer and cooled at room temperature.

2.8. Characterization of the starch–gluten fried matrices

2.8.1. Oil and moisture contents

Total oil content of ground fried matrices was determined gravimetrically by Soxhlet extraction with petroleum ether (AOAC, 1996). Moisture content was determined on defatted fried samples in a forced air oven at 105 °C to constant weight (AOAC, 1996).

2.8.2. Texture analysis

Texture of the fried matrices was determined according to Da Silva and Moreira (2008), using a three-point bending test in a texturometer (TA.TX2 Texture Analyzer, Stable Microsystems, UK). The force (N) at the breaking point (highest value in the plot) was used as the resistance to breakage.

2.8.3. Total polyphenol content

Total polyphenol content was measured by Folin Ciocalteau (Singleton and Rossi, 1965). Briefly, polyphenols from ground defatted fried samples (1 g) were extracted using 5 mL of an aqueous solution containing 50% acetone and 15% acetic acid, and mixed using a vortex, sonicated for 5 min and stirred for 45 min. Afterwards, the samples were centrifuged at 1860 g for 5 min and the supernatant was collected. The procedure was repeated twice. The supernatants were vacuum evaporated until dried. The residue was suspended in 3 mL of distilled water.

2.8.4. Antioxidant capacity assays

The antioxidant capacity of the fried samples was determined by DPPH (Brand-Williams et al., 1995), and the results were expressed as TE.

2.9. Statistical analysis

The reported results correspond to the arithmetic mean of three batches ± standard deviation. One-way variance analyses were carried out to establish significant differences between values obtained for the same sample. The differences of means between samples were resolved by confidence intervals using a Bonferroni test (Statgraphics 5.0 program, Manugistics Inc., Rockville, MA). The level of significance was set for $p \leq 0.05$.

3. Results and discussion

3.1. Characterization of the olive leaves extract

Table 1 shows the chemical and physical properties of the OLE. Total polyphenols in the OLE was 25.7 mg GAE/mL or 64.3 mg GAE/g dry leaves. Total polyphenols reported in OLE (cv. Arbequina) using ethanol:water as extracting solvent were in the range 5.3–11.6 mg caffeic acid equivalents/g dry leaves (Jiménez et al., 2011). The OLE had an OE content of 28.4 mg OE/mL (78.8 mg OE/g dry leaves), being the main polyphenol found in the OLE. This content was higher than those reported by Talhaoui et al. (2014) (17.08 mg OE/g dry leaves) or Japón-Luján and Luque de Castro (2008) (12.9 mg OE/mL) for OLE obtained from cv. Arbequina with methanol–water and ethanol, respectively. In other studies, higher OE contents have been reported. Thus, Altiok et al. (2008) obtained 134.4 mg OE/g dry leaves using ethanol:water (70:30) as extracting solvent, whereas Lee-Huang et al. (2003) reported OE contents of 128 mg/g dry leave in OLE obtained with water. However, olive leaves cultivar was not specified in any of the studies.

Table 1 – Characterization of olive leaves extract.

Parameter	OLE (X ± DS)
Moisture (%)	90.11 ± 0.03
Soluble solids (°Brix at 20 °C)	11.30 ± 0.10
Total polyphenols	
mg GAE/mL extract	25.7 ± 0.8
mg GAE/g dry leaf[a]	64.3 ± 2.0
Oleuropein content	
Oleuropein (mg OE/mL extract)	28.4 ± 0.3
Oleuropein (mg OE/g dry leaf)	78.8 ± 1.1
Antioxidant capacity	
DPPH EC_{50} (mg GAE/mL extract)	0.15 ± 0.00
ORAC (μmol TE/mL extract)	442 ± 21
FRAP (μmol TE/mL extract)	109 ± 3

OE: oleuropein; GAE: gallic acid equivalent; TE: trolox equivalent; X: mean; DS: standard deviation (n = 3).
[a] Expressed as dry matter.

Regarding OLE antioxidant capacity, EC_{50} value for DPPH was 0.15 mg GAE/mL extract. Scognamiglio et al. (2012) studied the antioxidant capacity of several OLEs, ranged from 0.037 to 0.18 mg GAE/mL extract. FRAP and ORAC assays gave results of 109 y 442 μmol TE/mL of extract. Hayes et al. (2011) reported similar FRAP values (120.3 μmol TE/mL) but lower ORAC values (69.6 μmol TE/mL) for an OLE obtained with methanol. Several factors, such as the olive cultivar, geographic location, leave pre-treatment, extraction method, total solid content of the extract and expression of the results, have been reported to influence both the antioxidant activity and the polyphenol composition and/or content of OLE (Scognamiglio et al., 2012), which would explain the differences found between this study and the scientific literature.

3.2. OLE microencapsulation

A central composite design was applied to evaluate the effect of the process (air inlet temperature) and the formulation (OLE/IN ratio) variables. Table 2 shows the experimental design for the microencapsulation of OLE by spray-drying as well as the values for both the independent and response variables. RSM was used to optimize each response variable (EE, R and Y), considering linear, quadratic and cross-product forms of the independent variables at 95% confidence level. Table 3 shows the analysis of variance (ANOVA) for the microencapsulation of OLE by spray-drying. Non-significant terms were removed from the equation, but when the linear forms of temperature and OLE/IN ratio were not significant, they were considered in the quadratic equation because they are fundamental elements of the mathematical model.

The encapsulation efficiency of OE represents the OE-polymer interaction, which ranged from 80 to 89% (Table 2). The linear and quadratic forms of OLE/IN ratio were significant for the EE of OE, whereas the linear temperature, quadratic temperature and the interaction between temperature and OLE/IN ratio forms were not significant. The model explained 80.8% of the variability (R^2 adj. for d.f.) in EE of OE (Table 3). The quadratic regression equation describing the effect of the

Table 2 – Experimental design for the microencapsulation of OLE by spray-drying.

Run	Temperature (°C)	OLE/IN ratio	EE (%)	R (%)	Y (%)
1	140 (−1)	1/0.50 (−1)	82.0 ± 0.8	91.4 ± 1.6	52.0 ± 1.1
2	180 (+1)	1/0.50 (−1)	80.0 ± 1.0	95.0 ± 1.4	48.0 ± 1.0
3	140 (−1)	1/2.00 (+1)	87.0 ± 1.2	97.0 ± 1.2	73,1 ± 1.5
4	180 (+1)	1/2.00 (+1)	86.0 ± 0.9	92.8 ± 1.3	58.0 ± 2.0
5	135 (−1.21)	1/1.25 (0)	89.0 ± 1.1	98.0 ± 1.7	71.0 ± 1.5
6	184 (+1.21)	1/1.25 (0)	87.0 ± 0.9	92.0 ± 1.8	59.0 ± 1.9
7	160 (0)	1/0.34 (−1.21)	80.0 ± 1.4	90.0 ± 0.9	38.0 ± 2.1
8	160 (0)	1/2.15 (+1.21)	85.0 ± 1.8	85.0 ± 1.4	66.0 ± 1.8
9	160 (0)	1/1.25 (0)	86.0 ± 1.0	85.6 ± 1.5	64.0 ± 1.1
10	160 (0)	1/1.25 (0)	85.0 ± 1.0	86.8 ± 1.6	63.0 ± 1.0
11	160 (0)	1/1.25 (0)	84.0 ± 1.1	85.0 ± 1.4	59.7 ± 1.4
12	160 (0)	1/1.25 (0)	85.3 ± 1.2	84.0 ± 1.4	67.0 ± 0.9

EE: encapsulation efficiency; R: recovery; Y: yield; OLE: olive leaves extract; IN: inulin.

Table 3 – Analysis of variance (ANOVA) for the microencapsulation of OLE by spray-drying.

Source	Sum of squares	d.f.	Mean square	F-ratio	p-Value	R^2	R^2 adj. d.f.
Encapsulation efficiency							
A:temperature	4.24012	1	4.24012	6.15	0.0892	92.5	88.2
B:ratio	41.9593	1	41.9593	60.88	0.0044*		
AA	8.1769	1	8.1769	11.86	0.0411*		
BB	24.1931	1	24.1931	35.10	0.0096*		
Lack-of-fit	4.31228	4	1.07807	1.56	0.3715		
Pure error	2.0675	3	0.689167				
Total (corr.)	84.9492	11					
Recovery							
A:temperature	8.91712	1	8.91712	6.51	0.0839	78.9	71.1
B:ratio	1.01361	1	1.01361	0.74	0.4530		
AA	200.791	1	200.791	146.56	0.0012*		
Lack-of-fit	52.0045	5	10.4009	7.59	0.0629		
Pure error	4.11	3	1.37				
Total (corr.)	266.837	11					
Yield							
A:temperature	163.145	1	163.145	18.02	0.0240*	91.5	88.3
B:ratio	609.451	1	609.451	67.30	0.0038*		
BB	233.401	1	233.401	25.77	0.0148*		
Lack-of-fit	66.4144	5	13.2829	1.47	0.3998		
Pure error	27.1675	3	9.05583				
Total (corr.)	1099.58	11					

d.f.: degrees of freedom.
* Statistically significant.

independent variables on the EE is the following:

$$EE = 81.451 - 0.0391155 \times \text{Temperature} + 14.318 \times OLE/IN\,ratio - 4.41468\,(OLE/IN\,ratio)^2$$

As can be seen in surface graph (Fig. 1), the EE was higher for microparticles with higher IN. As expected, the higher the IN content, the higher the EE of OE, since a higher content of IN provides larger number of hydroxyl groups available to form hydrogen bonds with OE (Gharsallaoui et al., 2007). Furthermore, the EE slightly decreased with the increase of the inlet air temperature (Fig. 1), which may explained because high inlet air temperatures lead to an increase in the total polyphenols and therefore to a decrease in the EE of OE.

The OE recovery corresponds to the loss of OE during the spray-drying process. In this study, despite the high drying temperatures, the recovery of OE from OE-IN microparticles was high, ranging from 84 to 98% (Table 2). Although only the quadratic form of temperature was significant for OE recovery with a positive effect, the linear form of the temperature and the OLE/IN ratio were considered in the quadratic equation.

$$R = 523.822 - 5.40235 \times \text{Temperature} - 0.509994 \times OLE/IN\,ratio + 0.0167051 \times Temperature^2$$

The model explained 71% of the variability (R^2 adj. for d.f.) in recovery of OE (Table 3). The surface graph shows (Fig. 1) that the OE recovery was higher for microparticles with higher IN content. In this case, the higher the IN content, the higher the R of OE. A high inlet air temperature (140–160 °C) quickly forms a dry crust at the droplet surface, making that the residual moisture diffuses through the outside of the particles, but retaining the OE (Gharsallaoui et al., 2007).

The yield is the ratio between the solids content in the feed solution before spray-drying and the solids content obtained after spray-drying. The yield ranged from 38 to 73% (Table 2), and the linear and quadratic forms of OLE/IN ratio and lin-

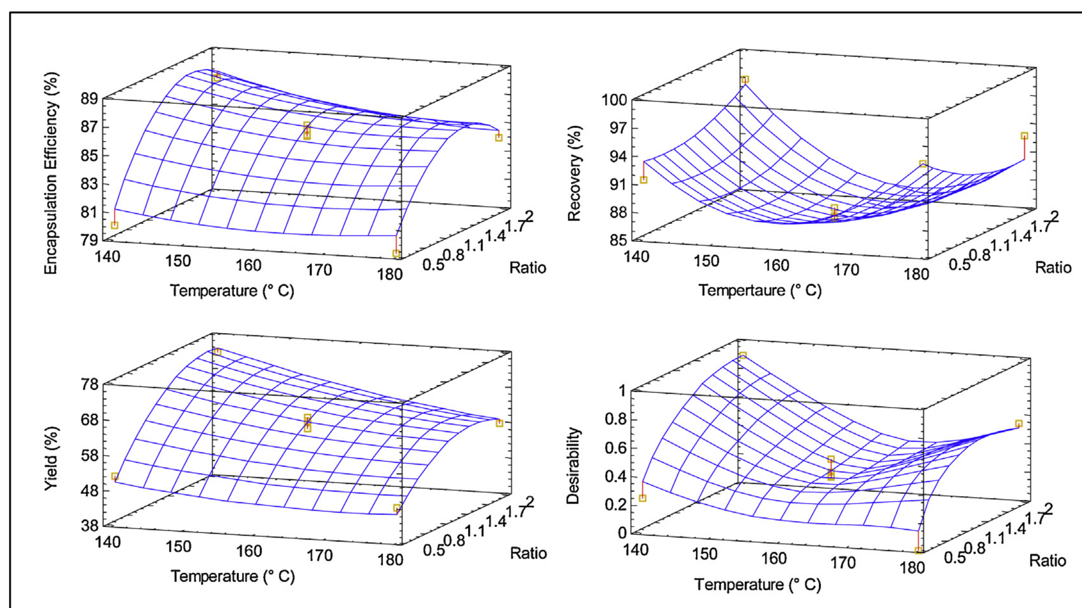

Fig. 1 – Response surface graphs for encapsulation efficiency, recovery, yield and desirability function for OLE-IN microparticles.

ear form of temperature were significant for yield. The model explained 88% of the variability (R^2 adj. for d.f.) in yield (Table 3).

The quadratic regression equation describing the effect of the independent variables on the yield is the following:

$$Y = 66.8535 \times 0.242632 \times \text{Temperature} + 45.2985 \times OLE/IN\,ratio - 13.1172 \times (OLE/IN\,ratio)^2$$

The response surface graph (Fig. 1) shows that the yield was higher for microparticles with higher OE/In ratio and lower inlet air temperatures. The higher the IN content, the higher the yield of powdered microparticles, which can be attributed to the rapid formation of the dry crust avoiding that the powder sticks in the drying chamber (Robert et al., 2017). However, the higher inlet air temperature, the lower the yield of powder, which could be explained because low temperatures lead to microparticles with higher moisture, where water would act as plasticizer obtaining spherical microparticles with a more uniform flow with minimum loss.

For the multiple optimization (Desirability Function), all the response variables had coefficients of determination over 70% (R^2 adj for d.f.) and residual below 6.0; therefore, they were considered in the model. Moreover, the lack-of-fit was not significant, indicating that the mathematical model fitted well with the experimental data ($R^2 = 0.82$). The response variables were maximized (EE, R and Y) and the surface response graphic is showed in Fig. 1.

The optimal inlet air temperature (136 °C) was below the lower limit of the range studied (140–180 °C) and the optimal OLE/IN ratio (1:1.8) was near to the upper limit of the range studied. It is remarkable that the optimal conditions obtained for OLE-IN microparticles according to statistical design are specific for this system and this cannot be applied when other biopolymers are used as encapsulating agents. In this context, the nature and properties of the biopolymers play an important role in the encapsulation parameters (mainly inlet air temperature) and the stability of the active compounds.

Table 4 – Characterization of olive leaves extract (OLE) microparticles with inulin obtained under optimal conditions.

Parameter	OLE-IN (X ± SD)
OLE/IN ratio	1:1.8
Inlet air temperature (°C)	136
Encapsulation efficiency OE (%)	87.1 ± 0.27
Recovery OE (%)	90.1 ± 2.30
Yield (%)	64.3 ± 3.75
Surface OE (mg/g powder)	1.7 ± 0.07
Total OE (mg/g powder)	13.2 ± 0.41
DPPH (μmol TE/g microparticle)	32.8 ± 0.5
FRAP (μmol TE/g microparticle)	52.3 ± 0.9

OLE: olive leaves extract; IN: inulin; OE: expressed as oleuropein equivalent; X: mean; SD: standard deviation (n = 3)

OLE has been encapsulated by spray-drying with chitosan (Kosaraju et al., 2006) and casein-lecithin (Kosaraju et al., 2008) as encapsulating agents. The inlet air temperature was 180 °C in both studies, but these authors do not discuss how the temperature was selected.

Table 4 shows the characteristics of OLE-IN microparticles obtained under optimal conditions. A high R (90.1%) and EE (87.1%) of OE were found, in agreement with values predicted by the model. In this study, total phenolic compounds were 13.2 mg GAE/g for the OLE-IN microparticles, which corresponded to load of 80%, whereas the amount of OLE loaded in OLE-chitosan microparticles by spray-drying was 27% (Kosaraju et al., 2006). Moreover, the OLE microparticles showed antioxidant capacity measured by FRAP and DPPH (Table 2), as was also reported for OLE spray-dried lipid-protein emulsions (Kosaraju et al., 2008).

3.3. *Starch–gluten fried matrices*

3.3.1. *Oil content*

As can be seen in Fig. 2, vacuum starch–gluten fried samples absorbed slightly, but significantly ($p < 0.05$), less oil than the atmospheric fried ones, except for the samples with 350 mg

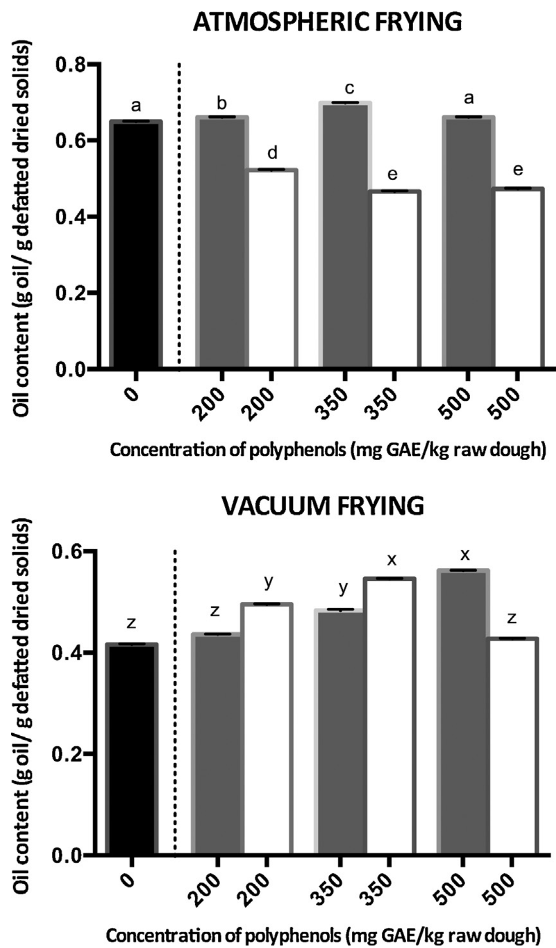

Fig. 2 – Oil content for atmospheric and vacuum fried doughs added with OLE or OLE-IN microparticles at different polyphenol concentrations. ▬ Control dough, ▬ Dough + OLE, ▭ Dough + OLE-IN microparticles.

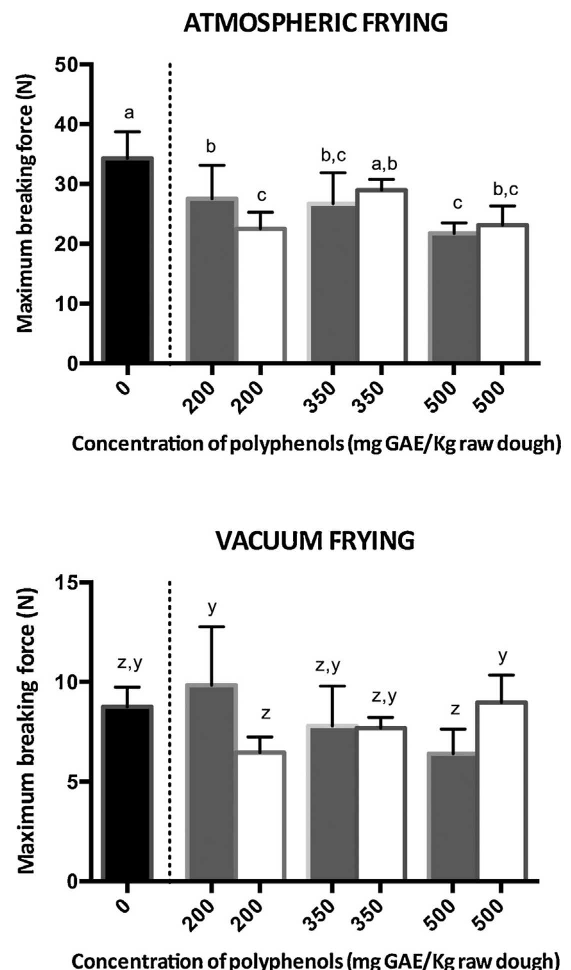

Fig. 3 – Maximum breaking force for atmospheric and vacuum fried doughs added with OLE or OLE-IN microparticles at different polyphenol concentrations. ▬ Control dough, ▬ Dough + OLE, ▭ Dough + OLE-IN microparticles.

GAE/kg raw dough. Garayo and Moreira (2002) suggested that oil reduction may be a consequence of the fast air diffusion into the porous space when the vessel is pressurized, which may obstruct the oil passage.

The starch–gluten systems with OLE-IN microparticles showed significantly lower ($p \leq 0.05$) oil content than those doughs with OLE at all the polyphenol concentrations for atmospheric frying conditions. Conversely, there was not a clear tendency in the case of the vacuum fried ones. In this case, the oil content increased significantly in doughs with OLE-IN microparticles at 200 and 350 mg GAE/kg raw dough, with respect to doughs with OLE at the same concentrations; whereas the oil content decreased significantly ($p \leq 0.05$) in the case of OLE-IN microparticles at 500 mg GAE/kg raw dough. The reduction of oil content could be explained by the formation of a coating layer of inulin over intact starch granules, which reduces oil absorption.

3.3.2. *Texture*

When analyzing a force-time curve, the crispness corresponds to the maximal force produced upon compression of the sample. This characterization is similar to the masticatory action that takes place during eating (Rizvi and Tong, 1997). The maximum breaking force of both vacuum and atmospheric fried matrices is shown in Fig. 3. As can be seen for all the samples, the maximum breaking force was significantly ($p \leq 0.05$) higher for atmospheric fried products than for vacuum fried ones, which can be attributed to the lack of starch gelatinization, a process that requires enough water and heating. During vacuum frying, water evaporation occurs at lower temperature limiting the gelatinization process, resulting in an unbound weaker structure (Sobukola et al., 2012).

Furthermore, the addition of polyphenols (OLE or OLE-IN microparticles) reduced significantly ($p \leq 0.05$) the maximum breaking force in the atmospheric fried matrices. This may be a result of the interaction between polyphenols or microparticles and any of the components of the starch–gluten network, producing a weaker structure. The effect of polyphenol addition was not observed in the vacuum fried matrices, likely as a result of the weak structure developed during vacuum frying.

3.3.3. *Total polyphenol content in starch–gluten fried matrices*

Polyphenols are susceptible to degradation during food processing. High temperature and the presence of oxygen are the most detrimental factors, severely affecting beneficial compounds such as polyphenols in fried foods (Dueik and Bouchon, 2011a,b). Fig. 4 shows the polyphenol content for both atmospheric and vacuum fried matrices. Doughs added with OLE-IN microparticles had the highest polyphenol content at atmospheric frying, showing the protective role of encapsulation on polyphenol degradation (Fang and Bhandari,

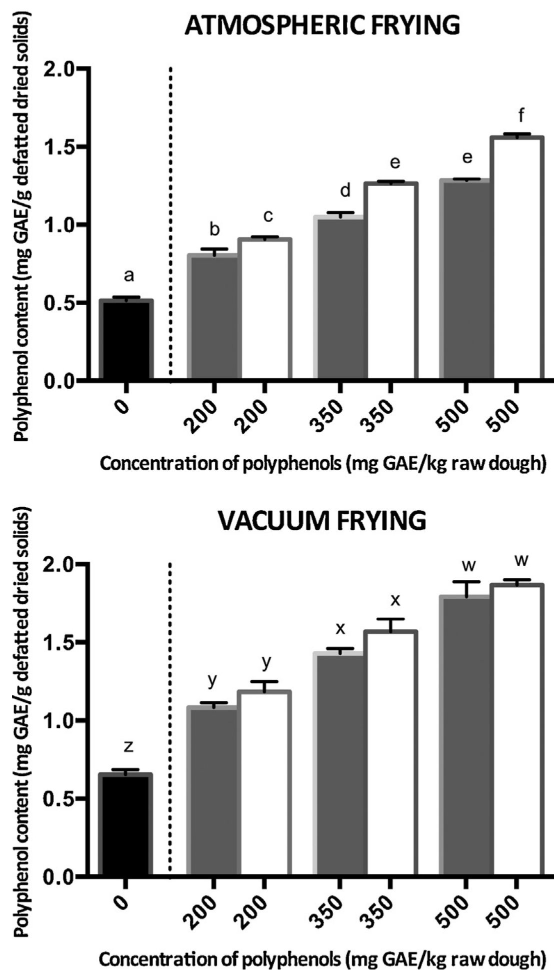

Fig. 4 – Polyphenol content for atmospheric and vacuum fried doughs added with OLE or OLE-IN microparticles at different polyphenol concentrations. ▬ Control dough, ▬ Dough + OLE, ▭ Dough + OLE-IN microparticles.

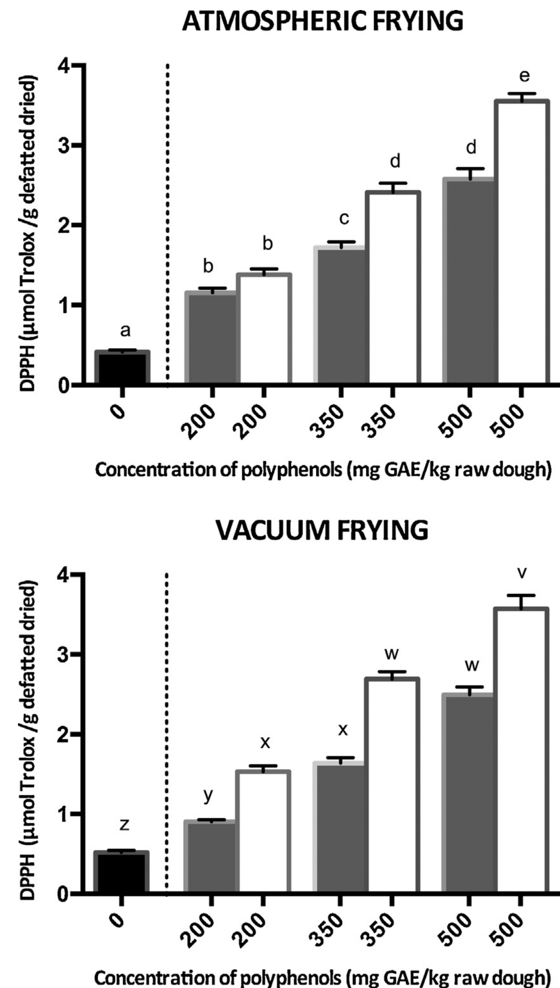

Fig. 5 – Antioxidant capacity by DPPH for atmospheric and vacuum fried doughs added with OLE or OLE-IN microparticles at different polyphenol concentrations. ▬ Control dough, ▬ Dough + OLE, ▭ Dough + OLE-IN microparticles.

2010). However, there were not significant differences in the polyphenol content between doughs added with OLE or OLE-IN microparticles during vacuum frying. This result could be attributed to the milder frying conditions (lower temperature and absence of oxygen) reached in vacuum frying experiences. Dueik and Bouchon (2016) added olive leaf polyphenol extract to starchy matrices at a level of 5 mg GAE/g dried solids, and fried them under vacuum and atmospheric conditions. These authors found that vacuum frying adequately protected polyphenols (70% retention) when a driving force of 60 °C (frying oil temperature of 106 °C) was used. However, polyphenol retention decreased to 42% (frying oil temperature of 160 °C) when frying under atmospheric conditions.

3.3.4. *Antioxidant capacity of fried matrices*

Fig. 5 shows the antioxidant capacity measured by the DPPH assay for both atmospheric and vacuum fried matrices enriched with OLE or OLE-IN microparticles. The higher the concentration of polyphenols in OLE and OLE-IN microparticles added doughs, the greater the antioxidant capacity at both atmospheric frying and vacuum frying. Matrices added with OLE-IN microparticles showed the highest antioxidant capacity at both atmospheric frying and vacuum frying. This effect could be attributed to the lower degradation of polyphenols when added as OLE-IN microparticles, as well as the protective effect of the microparticle against interactions with other components of the food matrix that may reduce their antioxidant capacity.

Interestingly, the vacuum fried matrices did not show significantly higher antioxidant capacity than the atmospheric fried ones ($p > 0.05$), despite their higher polyphenol content. Several authors have reported strong antioxidant capacity associated with browning and formation of melanoidins in model systems (Lingnert and Eriksson, 1980) and foods (Nicoli et al., 1999) during Maillard reaction that takes place during the atmospheric frying. Dueik and Bouchon (2011b) measured the browning development of melanoidins during frying of apples and potatoes, and a linear relationship was reported between the development of melanoidins and antioxidant capacity ($R^2 = 0.88$ for potatoes and $R^2 = 0.70$ for apples). The lower correlation found in apples was attributed to the higher concentration of compounds with antioxidant activity. In this study, a linear relationship was stablished between antioxidant capacity and polyphenol content for both vacuum and atmospheric fried matrices ($R^2 = 0.87$ and $R^2 = 0.99$, respectively).

4. Conclusions

Microencapsulation of OLE had a protector role on polyphenol degradation at atmospheric frying, whereas this effect was not

found in vacuum fried doughs due to the lower frying temperature used in this technology. Moreover, starch–gluten matrices with OLE-IN microparticles showed the highest antioxidant activity at both frying technologies. In the case of atmospheric frying this higher antioxidant activity may be mainly attributed to the effect of microencapsulation, leading to greater retention of polyphenols and lower polyphenol–matrix interactions that may impair the antioxidant activity; together with the formation of Maillard compounds due to the high frying temperatures. However, in the case of vacuum frying the minimization of polyphenol–matrix interactions would explain the highest antioxidant activity of the starch–gluten matrices with OLE-IN microparticles. Nevertheless, crispness of the starch–gluten fried matrices was lower in the case of vacuum frying, which may decrease consumer acceptability of the product. Thus, further studies have to be carried out to improve also the crispness of the product in order to have the same sensory properties of the atmospheric fried products. The results obtained in this study highlight the relevance of using a processing technology such as vacuum frying, able to preserve polyphenols and/or microencapsulation, a technology able to provide protection during food processing, in the design of polyphenol-rich foods with potential beneficial effects on human health.

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