Journal of Functional Foods 41 (2018) 112–117

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Retention and pre-colon bioaccessibility of oleuropein in starchy food matrices, and the effect of microencapsulation by using inulin

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ARTICLE INFO

Keywords:
Oleuropein
Microparticles
Retention
Digestion *in vitro*
Bioaccessibility

ABSTRACT

Oleuropein is a phenolic compound that is present in fruits and olive tree leaves, which has shown enormous health benefits. This study aimed to assess the effects of the baking and boiling cooking methods and the inclusion of extract of olive leaf and inulin microparticles on oleuropein retention and bioaccessibility in a food matrix. Retention was measured after cooking, and bioaccessibility was measured in cooked samples subjected to *in vitro* digestion. Our findings showed that oven cooking generated matrices that possess oleuropein retention 15% higher than those presented by food cooked in boiling water, while bioaccessibility at small intestine conditions was 27.5% lower when microparticles, rather than an extract in the starchy matrix, were included, which proves the benefits of using inulin microcapsules to enhance the amount of oleuropein that reaches the colon.

1. Introduction

Currently, the design of functional and/or healthy foods has attracted increasing interest. Hence, the addition of bioactive compounds to normally consumed food has in recent years become increasingly common (Boroski et al., 2011). The olive tree is well-known for its high content of biophenols, which are present both in the fruits and leaves (Al-Rimawi, 2014). Hydroalcoholic olive leaf extract (OLE) is a dark, bitter liquid that is derived from leaves of the olive tree (*Olea europaea* L., Oleaceae). This extract has high potential as a functional ingredient due its cardio-protective and chemo-protective characteristics, in addition to its proven capacity to reduce colonization by the micro-organisms *H. pylori* and *C. jejuni*, which are both related to some diseases that affect the digestive system and can even trigger the development of cancer (Sudjana et al., 2009). Additionally, it has also been used to treat malaria and malaria-associated fever (Ahmadvand, Noori, Dehnoo, Bagheri, & Cheraghi, 2014). Its effect as a modulator of digestive metabolism has been established as it stimulates pepsin enzymatic activity, diminishes lipase enzymatic activity, slows triacylglycerol metabolism and inhibits its absorption (Polzonetti, Natalini, Vincensetti, Vita, & Pucciarelli, 2010, chap. 148). All of these beneficial effects are conferred by the major bioactive compound that is present in OLE, the oleuropein (OE). This molecule is composed by a 4-(2-hydroxyethyl)-1,2 benzenediol (Hydroxytyrosol), an elenolic acid and one glucose unit (Omar, 2010).

Regarding nutrient bioaccessibility, which is defined as the amount of compound liberated from the matrix after digestion and available for absorption (Carbonell-Capella, Buniowska, Barba, Esteve, & Frigola, 2014), studies carried out in olive polyphenols established that the concentration of phenolic compounds rapidly increases after the ingestion of olive oil and reaches a maximum in the plasma and urine after approximately 1 and 2 h, respectively (Weinbrenner et al., 2004).

In contrast, starch is known to be one of the most common components of the human diet, as food based on this biopolymer is easy to prepare and obtain (Boroski et al., 2011). This makes the possibility of including OLE in starchy foods fairly attractive; nonetheless, to overcome the unwanted organoleptic characteristics presented by extracts of olive oil, such as bitterness and colour, micro-encapsulation may represent a practical improvement. In this manner, those unwanted characteristics might be mitigated. Additionally, most processes used in food elaboration, such as cooking, influence the matrix and present the possibility that those compounds into food could be degraded (Boroski et al., 2011), thus influencing the amount of compound that remains in a matrix and modifying the bioaccessible fraction of such compound.

Micro-encapsulation represents the action of generating a “cover” for a specific compound, which acts to mitigate compound deterioration and/or loss, hiding any unwanted organoleptic characteristics, providing stability and introducing the option to achieve controlled

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https://doi.org/10.1016/j.jff.2017.12.037
Received 26 March 2017; Received in revised form 23 November 2017; Accepted 18 December 2017

release in a specific site (Ray, Raychaudhuri, & Chakraborty, 2016). Inexpensive, flexible, continuous and capable of being produced in powdered particles, spray drying represents the most widely used mechanism in the food industry for encapsulation (Fang & Bhandari, 2010; Ray et al., 2016). Inulin, which belongs to the fructan group, is a polymer that is currently used in the food industry as an encapsulant material due its beneficial prebiotic properties (Nazzaro, Orlando, Fratianni, & Coppola, 2012; Ronkart et al., 2007), and is specially abundant in chicory (*Chicorium intybus* L., *var. Sativum*), which is one of the most important sources of this compound (Beirao-Da-Costa et al., 2013). Several reports have suggested the beneficial effects of both inulin and OE, supporting the idea of include these compounds into common, easily accessible foods, such as wheat flour-based food (Ahmadvand et al., 2014; Beirao-Da-Costa et al., 2013; Lee & Lee, 2010; Polzonetti et al., 2010; Ronkart et al., 2007; Sudjana et al., 2009; Vissers, Zock, Roodenburg, Leenen, & Katan, 2002). The present study aimed to assess the effect of including OLE and encapsulated OLE in a food matrix, and comparing the effects of the final cooking method (baking vs boiling) on the retention and bioaccessibility of OE.

2. Materials and methods

2.1. Materials and enzymes

Olive leaves (O. *europaea* cv Arbequina) were collected from "El Oliveto" farm (Cholqui Valley, Melipilla, Metropolitan Region, Chile). Wheat flour Collico was purchased at Kunstmann Mill (Valdivia, Chile); Coumarin (≥99% HPLC), Oleuropein (OE, ≥98% HPLC) and Digestive enzymes and bile salts (α-amylase 300–1000 U mg^{-1} protein, A1031-5KU; pepsin from porcine gastric mucosa ≥250 U mg^{-1} solid, P7000-100 G; and Pancreatin 4 × USP, P1750-100 G; Bile extract porcine, B8631-100 G) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Inulin HP (IN, DP > 23, Raftilina) was purchased from the Alfa-Chilena S.A. (Santiago, Chile).

2.2. Obtaining of olive leaves extract (OLE)

The olive leaves (7 kg) were scalded at 95 °C for 4.5 min and then was quickly cooled in cold water. Next, leaves were dried at 45 °C in an air forced oven (WTE, Germany) at 8% final moisture (18 h). Dry olive leaves (760 g) were ground and macerated in ethanol:water (50:50 v/v; 3 L) for 24 h at room temperature. Subsequently, extract was separated by filtration in Buchner funnel; this procedure was carried out twice with 2.5 L. Extracts were combined, and the volume was reduced by using a rotary evaporator (Büchi R-205, Switzerland) at 40 °C until it reached a final volume of 1500 mL. The resulting extract was frozen at −20 °C. Physical and chemical characteristics of OLE are shown in Table 1.

2.3. Microencapsulation of olive leaves extract

The encapsulation of OLE was performed by spray drying, using inulin (IN) as encapsulating agent. The OLE-IN infeed solution (400 g) was prepared as follows: Inulin (36 g) was dissolved in distilled water (344 mL) and heated to 65–70 °C with stirring, then cooled to 30 °C, and mixed with OLE (20 mL, 31.05 mg OE/mL OLE). 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 B-290 mini spray-dryer (Büchi, Switzerland). The parameters used were as follows: inlet air temperature, 136 °C; air flux, 600 L/h; feeding rate, 2 mL/min; and atomization pressure, 0.5 MPa. At the end of the process, 23.09 g of microparticles (MP) were obtained.

Table 1
Physical and chemical characteristics of olive leave extract (OLE).

Parameter	Olive leave extract (X ± SD)
Moisture (%)	90.1 ± 0.03
Soluble solids (°Brix at 20 °C)	11.3 ± 0.1
Oleuropein content (mg OE/mL extract)	31.1 ± 0.9
Total polyphenols (mg gallic acid equivalent/mL extract)	25.7 ± 0.82
Antioxidant capacity EC_{50} (mg gallic acid equivalent/mL extract)	0.15 ± 0.01

2.4. Microparticles powder analysis

2.4.1. Encapsulation efficiency and recovery

2.4.1.1. Total OE. OLE-IN microparticles (100 mg) were dispersed in water (2 mL), then put into a water bath at 65 ± 5 °C. 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. An aliquot was injected into the HPLC.

2.4.1.2. Surface OE. OLE-IN microparticles (100 mg) were dispersed in ethanol (2 mL) with soft stirring. The dispersion was centrifuged at 2906*g* for 10 min. The supernatant was transferred to a volumetric flask filled up 10 mL with water:acetonitrile (80:20 v/v) containing 0.1% glacial acetic acid. An aliquot was injected into the HPLC.

Encapsulation efficiency (EE) and recovery (R), were calculated according to Eqs. (1) and (2), respectively:

$$EE(\%) = \frac{OE_{Total} - OE_{Surface}}{OE_{Total}} \times 100 \quad (1)$$

$$R(\%) = \frac{OE_{Total}}{OE_{Total\ theoretical}} \times 100 \quad (2)$$

where OE_{Total} corresponds to total OE content in the MP powder (mg/g), $OE_{Surface}$ corresponds to the OE content in the surface of MP powder (mg/g), and $OE_{Total\ theoretical}$ corresponds to the theoretical content of OE in the feed solution (mg/g).

2.4.2. Scanning electron microscopy

Morphological descriptions of the structure of MP were carried out according to the methodology used by Robert, García, Reyes, Chávez, and Santos (2012) and Palma, García, Márquez-Ruiz, Vergara, and Robert (2014), which consists of using a scanning electron microscope (SEM). This approach was used to analyse the superficial structure of the OLE-IN microparticles. The samples were covered with gold/palladium using a vacuum evaporator and analysed with an electronic microscope (LEO Electron Microscopy Ltd., Cambridge, UK). Digital images (1024 × 768 pixels) were saved as 8 bits TIFF image file, without compression (EDS INCA × sight, Oxford Instruments, Oxford, UK).

2.5. Preparation of starchy matrix with OLE inclusion

Dough matrices were prepared by mixing sifted wheat flour (200 g) and distilled water (120 mL) for 12 min using a semi-industrial mixer (Kitchenaid 4.3 Liters 5 QT). The dough was sheeting (ATLAS, 150 Chrome line Marcato design) to obtain sheets 2 mm thickness. Finally, the sheets were cut with an iron circular mould 50 mm diameter. Cooking was performed in the following two ways: in a water bath (distilled boiling water for 20 min) or in an oven (air forced oven at 190 °C for 12 min). After cooking, samples were cooled at room temperature for 20 min. For water cooking, samples were placed over an absorbent paper to eliminate water excess (Aravena, Garcia, Muñoz, Pérez-Correa, & Parada, 2016). OLE (2 mL OLE, 31.05 mg OE/mL) or OLE-IN (5 g MP, 11.45 mg OE/g MP) were incorporated to dough during mixing.