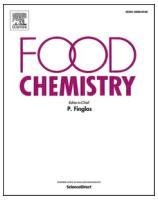
Food Chemistry 428 (2023) 136778

Contents lists available at ScienceDirect

Food Chemistry

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



Phenolic profile of micro- and nano-encapsulated olive leaf extract in biscuits during *in vitro* gastrointestinal digestion

Călina Ciont [a,b,1], Graziana Difonzo [c,*], Antonella Pasqualone [c], Maria Simona Chis [d,1], Florica Ranga [a], Katalin Szabo [b], Elemer Simon [b], Anca Naghiu [e], Lucian Barbu-Tudoran [f], Francesco Caponio [c], Oana Lelia Pop [a,b,*], Dan Cristian Vodnar [a]

[a] Department of Food Science, University of Agricultural Sciences and Veterinary Medicine, 400372, Cluj-Napoca, Romania
[b] Molecular Nutrition and Proteomics Laboratory, Institute of Life Sciences, University of Agricultural Sciences and Veterinary Medicine, 400372, Cluj-Napoca, Romania
[c] Department of Soil, Plant and Food Science (DISSPA), University of Bari Aldo Moro, I-70126 Bari, Italy
[d] Department Food Technology, University of Agricultural Sciences and Veterinary Medicine, 400372, Cluj-Napoca, Romania
[e] Research Institute for Analytical Instrumentation, National Institute of Research and Development for Optoelectronics INOE 2000, 67 Donath Street, 400293 Cluj-Napoca, Romania
[f] Electron Microscopy Center, Faculty of Biology and Geology, Babes-Bolyai University, Clinicilor 5-7, 400006 Cluj-Napoca, Romania

ARTICLE INFO

Keywords:
Olive leaf
Phenolic
Microencapsulation
Nanoencapsulation
Evolution
In vitro digestion

ABSTRACT

Olive leaf was characterized by a high content of phenols and flavonoids (oleuropein, luteolin, and their derivatives), presenting functional and health-related properties. The chemical instability of phenolics through technological processes and their degradation in the digestive system may negatively impact them, leading to lower absorption. This study evaluates the phenolic profile of micro- and nano-encapsulated olive leaf extract in biscuits during the INFOGEST static *in vitro* digestion, aiming to enhance stability and sensorial properties. Ultrasound-assisted extraction and chromatography characterized the extract, while spray drying (maltodextrin-glucose) and nano-encapsulation (maltodextrin, whey protein isolate, and arabic gum) techniques were used with specific solutions. Encapsulated formulations underwent microscopy (TEM, SEM) and encapsulation efficiency analysis. Micro- and nano-encapsulation improved biscuit functionality by enhancing phenolic stability during digestion. However, the highest concentration adversely affected sensory and textural parameters. These findings contribute to developing functional food products enriched with bioactive compounds, providing improved health benefits while maintaining sensory attributes.

1. Introduction

Recently, food development with bioactive compounds has gained significant interest in market expansion (de Gennaro et al., 2022; Man et al., 2021). Among the wide range of bioactive found naturally in food, polyphenols, which are secondary metabolites of plants, have received substantial attention for their capacity to scavenge free radicals and promote health effects (Hoskin et al., 2019). Several epidemiological studies showed that a diet rich in polyphenols reduced the risk of carcinogenesis, cardiovascular disease, and vision-related disorders (Amini et al., 2017; Hoskin et al., 2019; Pacheco et al., 2018).

Olive leaves, pomace, and wastewater constitutes a significant by-product of olive oil production (Ghasemi et al., 2018; Urzúa et al., 2017; Vergara-Barberan et al., 2015). Leaves are considered a low-cost, rich source of valuable substances that may be extracted by relatively cheap processes (Ghanem et al., 2019). Spain is estimated to generate around half of olive leaf waste each year, around 1.25 million tons (Espeso et al., 2021). These by-products have the potential to support the resource efficiency action plan, which encourages the recovery and integration of nutrients from alternate sources for a more sustainable bio-economy (Espeso et al., 2021; González-Ortega et al., 2020). The olive leaves are well-known for their high content of phenols, which may be subdivided into the categories of secoiridoids (oleuropein, verbascoside, and derivatives), flavonoids (luteolin, rutin, apigenin, and products), and substituted phenols (hydroxytyrosol, tyrosol, elenolic acid, and vanillic acid) (Ghanem et al., 2019).

* Corresponding authors.
E-mail addresses: graziana.difonzo@uniba.it (G. Difonzo), oana.pop@usamvcluj.ro (O. Lelia Pop).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.foodchem.2023.136778
Received 4 April 2023; Received in revised form 16 June 2023; Accepted 29 June 2023
Available online 6 July 2023

From a techno-functional perspective, incorporating olive leaf extract (OLE) into food products is challenging due to its herbal aroma and bitter and astringent taste (Moghaddam et al., 2020). Also, the chemical instability of phenolics through technological processes (acidity, light, heat, oxidation, or moisture) and their bioavailability may negatively impact them, leading to lower absorption (Pacheco et al., 2018). To accomplish these claims, the literature proposes encapsulation as a promising technique. Various procedures, such as cross-linking gelation, freeze drying, microemulsion, spray drying, and different nano-formulations (nanoliposomes), have been used to encapsulate bioactive core components with diverse polymers as wall materials (González-Ortega et al., 2020; Pacheco et al., 2018; Paciulli et al., 2023). Of these methods, spray drying and freeze-drying have been more commonly preferred for their low cost, higher rate, industrial flexibility, and minimal thermal and oxidative degradation (El-Messery et al., 2020; Gonzalez et al., 2019). The term "microencapsulation" refers to the methods used to generate particles or capsules ranging in size from 1 to 1000 μm, while "nanoencapsulation" describes the strategies for producing particles or capsules ranging in size from 1 to 1000 nm (Ștefănescu et al., 2022; Veronica Cardoso de Souza et al., 2022). The capsules are designed to stabilize the bioactive molecule during production, storage, and consumption (Muhammad et al., 2021). With regard to previous researchers (González-Ortega et al., 2020; Gonzalez et al., 2019; Gonzalez et al., 2020; Paciulli et al., 2023; Urzúa et al., 2017), the present study takes the investigation one step further by comparing the effects of microencapsulation and nano-encapsulation using maltodextrin and whey protein isolate as encapsulating materials, due to their good glass-forming properties, preservation, and controlled release.

Following the circular economy concept, the research aims to recover the main bioactive components from OLE, effectively valorize them by producing microspheres and nano-capsules, and assess their stability by incorporating them into biscuits matrix and by subjecting the samples to the static *in vitro* digestion model. Individual phenolic content was traced by high-performance liquid chromatography coupled with diode-array detection (HPLC/DAD) from the OLE to the simulated intestinal phase of the biscuits.

2. Materials and methods

2.1. Materials

Food-grade maltodextrin (DE 17.00) was provided by Roquette (Frankfurt, Germany), and Arabic gum (Art. No. 51198), as well as pepsin from porcine gastric mucosa (Art. No. P6887), pancreatin from porcine pancreas (Art. No. P7545), and bile extract porcine (Art. No. B8631) were acquired from Sigma-Aldrich (Taufkirchen, Germany). Linseed oil and whey protein isolate (80% protein) was purchased from a food store (Cluj-Napoca, Romania).

The ingredients used for biscuit manufacturing, such as wheat flour (2.5% fat and 13% protein), butter (82% fat), sugar, salt, and sodium bicarbonate, were acquired from local stores in Cluj-Napoca, Romanian. For the HPLC system, acetonitrile, HPLC-gradient, was provided by Merck (Germany), and water was purified with a Direct-Q UV system by Millipore (USA). The pure standard of oleuropein, luteolin, rutin, and chlorogenic acid (purity 99% HPLC) was purchased from Sigma (USA).

2.2. Extraction

The olive leaves were collected, stored at 4 °C, and processed in<24 h. After washing with tap water at room temperature, the olive leaves were dried at 120 °C for 8 min in a ventilated oven (Argolab, Carpi, Italy) to reach a moisture content < 1%, and then milled with a blender (Waring-Commercial, Torrington, CT, USA). The extraction process was ultrasound-assisted (CEIA, Viciomaggio, Italy), and water was added in a ratio of 1/20 (w/v). After three washings, each performed for 30 min at a temperature of 35 ± 5 °C, the extracts were filtered through Whatman filter paper (GE Healthcare, Milan, Italy), freeze-dried, and stored at −20 °C.

2.3. Extract encapsulation and characterization

The OLE (1 mg/mL) was dissolved in distilled water and ultrasonically treated in an ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) at 37 kHz for 30 min. The aqueous extract was characterized using the HPLC-DAD-ESI-MS method described by Călinoiu et al. (Calinoiu & Vodnar, 2019). The sample was centrifuged at 12298 × g for 10 min, and the polyphenols were extracted into the supernatant. The supernatant was then filtered through a 0.45 μm nylon filter, and 20 μL was injected into the HPLC system. The positive ionization mode was applied to detect the phenolic compounds; different fragmentor in the 50–100 V range was applied. The column was a Kinetex XB-C18 (5 μm; 4.5x150 mm i.d.) from Phenomenex, USA. The mobile phase was (A) water acidified by formic acid 0.1 % and (B) acetonitrile acidified by formic acid 0.1 %. The following multistep linear gradient was applied: start with 5% B for 2 min; from 5% to 90% of B in 20 min; hold for 4 min at 90% B; then 6 min to arrive at 5% B. The total analysis time was 30 min, the flow rate 0.5 mL/min, and the oven temperature was 25 ± 0.5 °C. Mass spectrometric detection of positively charged ions was performed using the Scan mode. The applied experimental conditions were: gas temperature 350 °C, nitrogen flow 7 l/min, nebulizer pressure 35 psi, capillary voltage 3000 V, fragmentor 100 V, and *m/z* 120–1500. Chromatograms were recorded at wavelengths λ = 280 nm and λ = 350 nm. The data acquisition was made with the Agilent ChemStation software.

2.3.1. Spray drying

Based on previous works, a BÜCHI Mini Spray Dryer B-290 Swiss-made (Switzerland) was used for microencapsulation (Dobrinčić et al., 2020; Szabo et al., 2021; Urzúa et al., 2017). Stock solutions, maltodextrin-glucose solution (24% maltodextrin and 4% glucose, w/v) as the encapsulating agents, was prepared in distilled water (50 °C) during stirring for 30 min with a heater-stirrer (RH Basic, IKA, Germany) (Dobrinčić et al., 2020; Szabo et al., 2021). Under vortex agitation, 1.5% OLE was added to the maltodextrin + glucose solution (after cooling, 25 °C) in a steady but slow stream to produce the feed solutions for spray-drying. The feed solution was homogenized during spray drying by constant magnet stirring at room temperature. Spray-drying process parameters included: inlet air temperature of 126 ± 3 °C, outlet air temperature of 66 ± 3 °C, capacity flow rate of 15 mL/min, air pressure of 6 bars, drying air flow rate of 0.04 m^3/h, and the spraying nozzle diameter of 0.7 mm. The resulting microspheres were packed in plastic bags, hermetically sealed, stored at 25 ± 2 °C, and protected from light until further characterization and analysis.

2.3.2. Freeze drying

Firstly, W/O emulsion was produced according to the method (Szabo et al., 2021) with slight modifications. Briefly, 10% maltodextrin, 40% whey protein isolate, and 10% arabic gum solutions were prepared by constant magnet stirring at ambient temperature in distilled water. The W/O emulsions were obtained by adding 2% linseed oil and 1.5% OLE, drop by drop, to the biopolymer solution while homogenizing with a rotor–stator homogenizer (Ultra-Turrax IKA T18 Basic, Wilmington, USA) at 12298 × g for 15 min in an ice bath to avoid thermal degradation. Secondly, ultrasonic emulsification was performed using a 20 kHz Sonicator (Ultrasonics, USA) to 750 W power. Energy input was given through a 14 × 100 mm (diameter length) titanium sonotrode, plunged into the liquid at a predetermined amplitude with an on: off cycle per second. The samples were cooled in an ice bath to reduce the temperature from the ultrasound. Then, the formulated nanoemulsion was put at 1:3 into a 1000 mL capacity flask and immediately frozen at target temperatures (-80 °C), according to a previous study of González

et al. (González et al., 2020). Further, the frozen samples were dried in a lyophilizer (Telstar LyoQuest). The ice condenser was cooled to −55 °C, and the pressure was set to 0.001 mBar. After 72 h of freeze-drying, the powdered product was collected and kept in an airtight container for further characterization and analysis.

2.4. Characterization of the nanoemulsion

2.4.1. Size and zeta potential

Nanoemulsion was characterized according to their size distribution and Zeta–potential using the Zetasizer Nano ZS (Malvern Instruments, United Kingdom). Zeta potential was measured by an electrophoretic light scattering technique at 25 ºC using the conductivity at 45 ± 2 μS/cm.

2.4.2. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) analysis was used to determine the shape of the oil droplets in the nanoemulsions and to assess whether the extract precipitated after the aqueous phase was added. The nanoemulsion was diluted 100 times, and a drop was put on a copper grid with 300-mesh holes. The grid was examined using JEOL JEM1010 transmission electron microscope operated at 60–80 kV and equipped with a MegaViewIII CCD camera.

2.5. Determination of the physicochemical properties of the encapsulation extract

2.5.1. Scanning electron microscopy (SEM)

A scanning electron microscope observed the size and surface morphologies of microspheres and nanocapsules (SEM VEGAS 3 SBU, Tescan, Brno-Kohoutovice, Czech Republic). The samples were analyzed on aluminum stubs with double-sided conductive carbon tape. SEM images were taken at a magnification of 20,000 times at an accelerating voltage of 20 kV.

2.5.2. Encapsulation efficiency (EE)

For the EE, the relation of the phenolic compounds content was used according to earlier research (El-Messery et al., 2020). For this purpose, the phenolic compound content was determined on the surface of the particles and in the particles after drying. Briefly, 0.15 g for each sample type, initial solutions (spray drying and freeze drying) before drying, and powders (microencapsulated/nanoencapsulated) were dissolved in 10 mL water. The mixtures were vortexed for 1 min, followed by 30 min ultrasonic treatment at room temperature, and centrifuged at 12298 × g for 10 min at room temperature. The phenolic compounds were quantified using the HPLC-DAD-ESI-MS technique, similar to the procedure mentioned in Chapter 2.3.

The following equation (1) was used to determine the EE:

$$\text{Encapsulation efficiency}\ (\%) = \frac{\text{Actual amount of polyphenols}}{\text{Theoretical amount of polyphenols}} x100 \quad (1)$$

2.6. Biscuits formulation

Biscuits were manufactured according to (Man et al., 2021). Before mixing, butter was left at room temperature for one hour to change its solid consistency and then homogenized (KitchenAid® Precise Heat Mixing Bowl, Greenville, OH, USA) with sugar to obtain a soft cream. The extracts were mixed with water until a solution was produced that had concentrations of 10%, 20%, and 30%, as shown in Supplementary file 1 (Table S1). All the ingredients were finally mixed for 3 min at 50 RPM and for 5 min at 100 RPM. The obtained doughs were left to rest for 1 h at a temperature of 4 °C and laminated (Flamic SF 600, laminator, Vinecza, Italia) until a final thickness of 0.4 cm was achieved. After that, the laminated dough was molded into cylindrical shapes with a diameter of 5.5 cm and baked in an electric oven (Zanolli, Verona, Italy) at 180 °C for 15 min. The final baked goods were cooled to room temperature and packed in polypropylene bags until further analysis. The recipes were calculated as the same total individual polyphenols extract concentration in dried extracts, microspheres, and nano-capsules samples.

2.7. Sensory analyses

A quantitative descriptive analysis (QDA) of biscuits was performed by a sensory panel composed of eight trained judges at the University of Bari (Italy). All the panelists had neither allergies nor food intolerances and were regular consumers of bakery products. The sensory analysis followed the ethical guidelines of the laboratory, and written informed consent was obtained from each panelist. The samples were labeled with an alphanumeric code and distributed in random order. The sensory evaluation was performed on biscuits with no extract added (B0), biscuits with 40% non-encapsulated extract (E40), biscuits with 40% microencapsulated extract (M40), and biscuits with 40% nano-encapsulated extract (N40). Fourteen sensory descriptors were considered to indicate the intensity of olfactory, taste, and textural attributes using a 9-point scale. The olfactometric descriptors were evaluated indicating the general odor intensity, the intensity of the typical odor, and caramel odor notes. In contrast, sweetness, saltiness, acidity, bitterness, astringency, off taste, and aftertaste were evaluated by tasting. Finally, textural attributes were evaluated in terms of hardness, dryness, graininess and chewiness.

2.8. Texture analysis

Biscuits' texture parameters (hardness and brittleness) were determined through a three-point bending test according to (de Gennaro et al., 2022), providing some modifications. Texture analyzer Z1.0 TN (Zwick/Roell, Ulm, Germany) was equipped with a 1 KN load cell. The biscuits were placed in the middle of the support, setting the distance of the bar at 60 mm and probe speed of 3 mm/s. The maximum force required to break the biscuit (N) and the distance crossed by the blade before the biscuits broke (mm) were measured, representing the hardness and brittleness, respectively. The analysis was carried out in triplicate.

2.9. Nutritional values determination

The nutritional properties of the biscuits were obtained using the AACC (2000) methods (AACC, 2000). Moisture (AACC 44–15.02, 2000), fat (AACC 30–25.01, 2000), ash (AACC 08–01.01, 2000), and total fiber (AACC 32–07.01, 2000) content were estimated by employing the standard method of analysis. The protein content was detected according to the Kjeldahl method (AACC 46–11.02, 2000), employing a factor of 5.7 for the conversion of nitrogen to protein. Total carbohydrate (%) content was calculated according to Equation (2) (Man et al., 2021).

$$\begin{aligned}\text{Total carbohydrate}\ (\%) = 100 - [&\text{moisture}(\%) + \text{ash}(\%) + \text{proteins}(\%) \\ &+ \text{lipids}(\%) + \text{total fiber}(\%)]\end{aligned} \quad (2)$$

2.10. Phenolic content of the biscuits

The biscuits were dissolved in distilled water (1:5 w/v) to quantify polyphenolic content, following the method described in section 2.3. The mixture was magnetically stirred, followed by 30 min ultrasonic treatment at room temperature. After centrifugation (12298 × g for 10 min at 24 °C), the supernatant was filtered and analyzed in the HPLC-DAD-MS system.

2.11. In vitro gastrointestinal assay

The phenolic profile of the non-encapsulated extract, the microspheres, and the nano-capsules was investigated from the biscuits,

following the updated static *in vitro* digestion method developed by the INFOGEST working group (Brodkorb et al., 2019).

2.11.1. Oral digestion

Briefly, each type of biscuit (2 g) was mixed with 3 mL water to obtain a paste-like consistency and further diluted 1:1 (wt/wt) with simulated oral fluid (SOF) to achieve a swallowable bolus. The SOF was composed of electrolyte solutions KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2{\cdot}6H_2O$, $(NH_4)_2CO_3$, alongside $CaCl_2(H_2O)_2$ and water. Subsequently, α-amylase was added to reach an activity of 75 U/mL, and the mixture was incubated in a shaker instrument (New Brunswick Innova 44, Eppendorf AG, Hamburg, Germany) at 37 °C and 95 RPM for 2 min.

2.11.2. Gastric digestion

The oral phase sample (10 mL) was diluted 1:1 (v/v) with simulated gastric fluid (SGF) composed of electrolyte solutions KCl, KH_2PO_4, $NaHCO_3$, $MgCl_2{\cdot}6H_2O$, $(NH_4)_2CO_3$, alongside $CaCl_2(H_2O)_2$ solution (0.3 M), porcine pepsin (2000 U/mL in the final digestion mixture), and water. The pH of the samples was adjusted to 3 by adding HCl (1 M), and the mixture was homogenized (95 RPM) and incubated at 37 °C for 2 h in a shaking incubator (New Brunswick Innova 44, Eppendorf AG, Hamburg, Germany).

2.11.3. Intestinal digestion

The stomach phase solution (20 mL) was mixed with 20 mL of pre-warmed simulated intestinal fluid (SIF) to achieve a final ratio of 1:1 (v/v). The SIF was composed of electrolyte solutions KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2{\cdot}6H_2O$, alongside $CaCl_2(H_2O)_2$, bile extract solution (10 mM in total digesta), pancreatic enzymes (100 U/mL) and double-distilled water. The pH was set to 7 using NaOH (1 M), and the mixture was homogenized and incubated at 37 °C for 2 h in a shaking incubator (95 RPM).

Following incubation, samples were centrifuged (Eppendorf 5810 R, Eppendorf AG, Hamburg, Germany) for 30 min at 12298 × g, 4 °C to remove undigested material. The supernatant was filtered through a nylon filter (pore size 0.45 μm) to separate the bioaccessible fraction and analyzed by the HPLC system as described below.

2.12. Statistical analysis

All measurements and analyses were made on three prepared samples, and the results are presented as means ± standard deviations (SD). One-way analysis of variance (ANOVA) and Tukey's comparison test via Minitab statistical software (version 16.1.0; LEAD Technologies, Inc., Charlotte, NC, USA) were applied to analyze the differences among samples with significance levels of $p < 0.05$. Statistical significance was assumed at the 95% confidence level for differences in mean values.

3. Results and discussions

3.1. Biologically active content of OLE

Phenolics were recovered from OLE using ultrasound-assisted extraction. To obtain a higher yield of total phenolic, ultrasound was applied to shorten the extraction time and reduce solvent requirements, as reported previously (Calinoiu et al., 2019; Rashid et al., 2022). Freeze-drying and spray-drying processes were selected for drying the extract. The phenolic content of the dried extract and its encapsulated form (microspheres (spray drying) and nano-capsules (freeze drying)) were evaluated qualitatively and quantitatively by HPLC-DAD-ESI-MS (Table 1). The compounds were identified based on their retention times, UV–Vis absorption spectra, and mass spectra of analyzed molecules. Moreover, the representative chromatograms were included in the Supplementary file 1 (Figure S1).

In total, 13 compounds were separated and identified, achieving a total phenolic concentration of 280.089 mg/g in the dried extract. Hydroxyoleuropein (56.95%) was the principal phenolic component in the dried extract, followed by hydroxytyrosol-glucoside (12.58%). Other isomers of oleuropein (oleuropein-aglycone and demethyloleuropein) were recorded at a lower concentration, 6.42% and 2.98%, respectively. The presence of the flavonoids category represented by luteolin-glucoside (1.84%), quercetin-rutinoside (1.78%), and luteolin-glucuronide (1.11%) compounds should be noted as well. Earlier research on OLE total phenolic concentration reported values ranging between 42.35 ± 0.002 and 190.65 ± 0.03 mg/g for dried extract (Ghasemi et al., 2018). This wider variation can be attributed to many factors like cultivation area, ecological conditions, agronomical practices, cultivar, tree age, leaf growth stage, and other abiotic and biotic stress factors (Çetinkaya & Kulak, 2016; Ghasemi et al., 2018). All these factors may affect and produce a different phenolic profile available for separation and identification.

Following exposure to encapsulation processes, the total phenolic content in both matrices, microencapsulated and nanoencapsulated, reported different values, 15.136 ± 0.08 and 13.092 ± 0.10 mg/g, respectively. The similar values can be attributed to the phenolic compound's property to exhibit favorable encapsulation characteristics. The phenolic compounds may form associations or interact with encapsulating materials through various processes, including hydrogen bonding, electrostatic, and hydrophobic interactions. Regardless of particle size, these interactions could be beneficial in retaining polyphenols inside the encapsulation systems (Veronica Cardoso de Souza et al., 2022). Also, the high temperature of spray drying process can impact the phenolic content. Heat exposure during spray drying can lead to the degradation or alteration of sensitive compounds, including phenolic compounds (Aniesrani Delfiya et al., 2015; Dobrinčić et al., 2020).

Table 1
The content of phenolic compounds in olive leaf extract, microspheres, and nano-capsules detected by HPLC-DAD-ESI-MS.

Phenolic Compounds (mg/g)		**Rt** (min)	**λ_{max}** (nm)	**Dried extract** (mg/g)	**Microspheres** (mg/g)	**Nano-capsules** (mg/g)
1	Hydroxytyrosol-glucoside	8.96	280	35.25 ± 0.14	3.14^{A} ± 0.28	2.13^{B} ± 0.12
2	Demethyloleuropein	11.13	330	8.34 ± 0.16	0.58^{A} ± 0.19	0.37^{B} ± 0.17
3	Oleoside dimethylester	12.91	320	15.03 ± 0.24	0.51^{A} ± 0.10	0.51^{A} ± 0.20
4	Oleoside 11-methylester	13.38	320	11.16 ± 0.20	0.43^{A} ± 0.20	0.42^{B} ± 0.11
5	Luteolin-diglucoside	13.68	340	0.84 ± 0.18	0.02^{A} ± 0.10	0.02^{A} ± 0.23
6	Quercetin-rutinoside (Rutin)	15.38	360	5.00 ± 0.10	0.23^{A} ± 0.14	0.20^{B} ± 0.21
7	Verbascoside	15.74	332	13.33 ± 0.09	0.65^{A} ± 0.14	0.57^{B} ± 0.17
8	Luteolin-glucoside	16.02	340	5.17 ± 0.25	0.14^{A} ± 0.27	0.09^{A} ± 0.16
9	Apigenin-rutinoside	16.91	341	1.54 ± 0.12	0.03^{A} ± 0.21	0.03^{A} ± 0.14
10	Apigenin-glucoside	17.39	341	3.74 ± 0.10	0.07^{A} ± 0.21	0.05^{B} ± 0.17
11	Luteolin-glucuronide	17.70	340	3.12 ± 0.26	0.09^{A} ± 0.19	0.03^{B} ± 0.23
12	Hydroxyoleuropein	18.31	280	159.53 ± 0.30	8.12^{A} ± 0.14	7.71^{B} ± 0.29
13	Oleuropein-aglycone	18.93	280	17.989 ± 0.19	1.07^{A} ± 0.28	0.91^{B} ± 0.34
	Total Phenolics			**280.089** ± 0.28	**15.136**^{A} ± 0.08	**13.092**^{B} ± 0.10

All data are the mean ± SD of three independent determinations. The mean followed by different letters in the same row differs significantly ($p < 0.05$).

The presence of all 13 phenolic compounds identified in the dried extract in the powders should be noted, showing low degradation through encapsulation. When comparing protection behavior through encapsulation, it can be observed that the microspheres present significant covering ($p < 0.05$). Before and after encapsulation, the highest phenolic compound was represented by hydroxyoleuropein, emphasizing its thermal stability. For these reasons, it has gained attention in the food industry to develop functional products with higher nutritional properties or extended shelf-life via antibacterial properties. Similar results were reported for olive leaf microparticles in the study conducted by Gonzalez et al. (Gonzalez et al., 2019).

On the other hand, significant resilience was observed regarding luteolin-diglucoside, luteolin-glucoside, and apigenin-rutinoside, which essentially remained at similar values in both powders. One possible explanation is that phenolic chemicals behave differently to various environmental variables, such as high temperature and pressure (Amini et al., 2017). An *in vitro* investigation on blueberries conducted by Hoskin et al. suggests that spray-dried protein–polyphenol particles exhibited higher biochemical activity than freeze-dried protein–polyphenol particles (Hoskin et al., 2019). Also, the results of Rezende et al. suggest that spray-drying led to higher concentrations of bioactive compounds than freeze-drying (Rezende et al., 2018). As the powdered phenolic microspheres and nano-capsules were manufactured by high-speed homogenization and different technological factors, mechanical stress could have damaged the phenolic compounds by breaking up the interfaces between the phenolic extract and cover materials.

3.2. Physical-chemical characterization of nanoemulsion and extract encapsulation

For the freeze-drying encapsulation, a nanoemulsion was prepared using the ultra-sonication process. The nanoparticle size and stability of the nanoemulsion were determined by dynamic light scattering (DLS). The mean size of the nanoparticles was 214.67 ± 52 nm. The nano-scale reduction of particles causes significant changes in their physicochemical characteristics and enhances their biological activity (Faridi Esfanjani & Jafari, 2016). Increasing their surface area to volume ratio makes these structures more bioavailable and may be carried across cell membranes (Tavakoli et al., 2018). Production of nanoemulsions requires careful consideration of formulation to meet rigorous particle-size requirements (nanometer range: 20–200 nm diameter) (Parvez et al., 2022). The larger size of the particles can be attributed to the SEM images microspheres; 1.2. SEM images nanocapsules; 1.2.a.b. TEM images nano-capsules; images were taken in different magnifications low temperature required for the freeze-drying process and the absence of pressures that would split up the frozen liquid into tiny droplets or significantly alter the topology of surfaces during drying (Parvez et al., 2022). Furthermore, particle size is essential since it determines the product's stability, aesthetics, bioavailability, and texture. Similar results for size (218.80 ± 0.8 nm) were reported by using a complex with maltodextrin, whey protein isolate, and arabic gum (Rashid et al., 2022).

Also, the TEM images (Fig. 1.2.a. and Fig. 1.2.b.) of the nano-formulation showed nanosized droplets, which were relative with the results found using DLS. As can be seen in the images, the samples were well-prepared and evenly distributed. Nanoparticles often appeared as round, smooth-surfaced globules about 300 nm in size.

The zeta potential was used to determine the electrokinetic potential of the formulation. The results showed a value of −31.2 mV, indicating the stability of the preparation. The distribution of the nanoemulsion was identified in the negative charge zone, proving that Van der Waals electrostatic repulsion is strong enough to maintain w/o system stability. These results align with previous studies conducted on nanoemulsion of *Nigella sativa* oil and *Woodfordia fruticosa* extract nanoemulsion, respectively (Mohammed et al., 2020; Najda et al., 2022). For nanoemulsions, a zeta potential of ± 30 mV was proposed as the optimal value for preventing the coalescence of the droplets due to electrostatic repulsion (Mohammed et al., 2020). Therefore, the nanoemulsion may stay disseminated throughout the system longer.

After the drying processes, the obtained powders (microencapsulated and nanoencapsulated) were examined by SEM (Fig. 1) to determine their morphology and appearance (the presence of cracks, fractures, or surface degradation). Most microspheres (Fig. 1.1) ranged from 2.88 μm to 3.96 μm and presented a spherical shape and smooth surfaces. Moreover, the matrix material's low permeability and good protection may be confirmed by their continuous surface. As a result of the low drying temperature (140 °C) and the viscoelastic qualities of linseed oil, no cracks can be observed on the surface of the microspheres. This allows for greater phenolic retention as superior physical protection (Navarro-Flores et al., 2020). The irregular ellipsoidal shape of the microparticles could be caused by their resistance to thermal denaturation (Muhammad et al., 2021). On the other hand, certain agglomeration of the particles can be observed. These agglomerations can be attributed to the feed atomization pressure, which led to smaller droplets/particles forming. Previous studies also reported smaller particles' affinity to larger particles' surfaces for the mixtures with gum arabic and maltodextrin as wall materials (Silva et al., 2013; Szabo et al., 2021). This structural configuration can make the microsphere more resistant to oxidation and water penetration, extending powder shelf life (Szabo et al., 2021).

Regarding the nano-capsules (Fig. 1.2.), a porous cake was produced following freeze-drying as a result of ice sublimation, giving rise to a structure composed of a glassy matrix, including air cells, whose size and shape are a function of the processing conditions and the composition of the starting system. The grinding process from the freeze-drying system generated a powder with irregularly shaped particles, some of whose original cell-like structure and wall properties are preserved. On the surface of samples, cracks and fissures can also be seen. This indicates low protection of the biological compounds. In previous research by El-Messery et al., freeze-dried nano-capsules had similar morphological characteristics (El-Messery et al., 2020). Moreover, powders containing encapsulated OLE have been reported with a rougher surface with bud-like spherical domains (González-Ortega et al., 2020).

3.3. Encapsulation efficiency

In this stage, the effectiveness of the different drying methods (spray-drying vs. freeze-drying) and coatings to encapsulate the phenolic compounds extracted from olive leaf was evaluated by the HPLC-DAD-ESI-MS method (Table 1). The encapsulation effectiveness of the capsules was established by the matrix material's ability to retain a bioactive compounds and confirms process success. EE of each powder was assessed by measuring the total amount of phenolics. Both drying procedures successfully retained the powder encapsulates' bioactive components, with up to 80% retention efficiencies. The findings showed that the EE was significantly different ($p \leq 0.05$) between the microspheres and nano-capsules. Therefore, the best results of EE (71.42 ± 0.034%) were obtained for the microspheres, where the maltodextrin wall was dispersed completely on the surface of polyphenols. The encapsulation cover was essential in protecting the phenolic compounds inside the matrix. For the freeze-drying method, EE was 68.09 ± 0.024%. The results of microspheres were consistent with those reported by González et al. (74.96 ± 0.26%) and Kiritsakis et al. (73%) (González-Ortega et al., 2020; Kiritsakis et al., 2017). Some essential factors in ensuring the bioactive compounds stability are the matrix material's nature and quality. Thus, the matrix materials could play a role in the variations in encapsulation efficiency in OLE preparations. Therefore, the results show that incorporating oil into the wall structures of nanocapsules decreased the EE. Similar observations were made by El-Messery et al. (El-Messery et al., 2020). However, because no one matrix material supplies all the characteristics needed in an efficient encapsulating

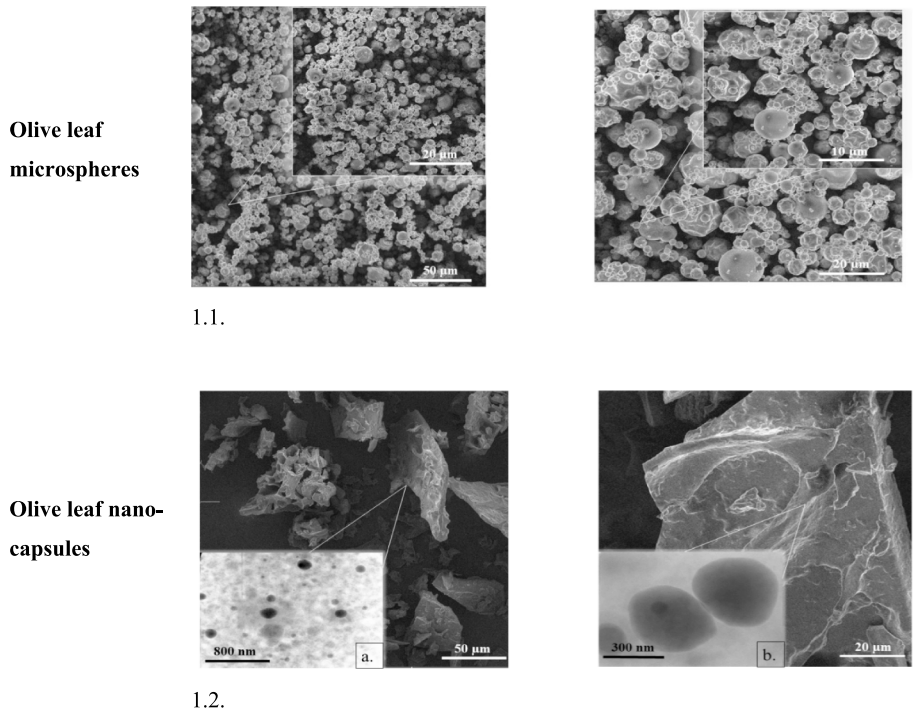


Fig. 1. Scanning electron microscopy and transmission electron microscopy images;

agent, a combination of matrix materials might enhance the powder's features (Mahdi et al., 2022). Also, the encapsulation method can impact the EE. According to the results of Elik et al., in terms of EE, spray drying (89.8%) encapsulates the bioactive material more efficiently than the freeze-drying method (70.9%) (Elik et al., 2021). Similarly, in encapsulating krill oil in the whey protein concentrate, maltodextrin, and arabic gum matrix, the EE was higher by spray drying method than the freeze-drying method (El-Messery et al., 2020).

3.4. Sensory evaluation

After the biscuits manufacturing, they were subjected to sensorial analysis. Sensory evaluation is important for the development of innovative and functional foods due to the low acceptability that these products may have. Fig. 2 summarizes the sensory analysis results of the biscuits B0, E40, N40, and M40. Among the olfactometric descriptors only the caramel odor was significantly more perceived in samples E40 and N40, whereas no significant differences were found for the other attributes. The acidity was perceived in the samples added with the OLE, both free and encapsulated; the astringency was perceived with the highest score in the sample M40 and the off-taste was perceived in M40 and N40. The addition of the extracts also affected some perceived textural parameters, specifically, the addition of the free extract increased the dryness, and the addition of the encapsulated extracts the hardness.

3.5. Texture analysis

Hardness and brittleness were common parameters considered for evaluating the textural properties of baked snacks, closely related to their freshness and wholesomeness. As reported in Table 2, the highest values of hardness were found in M40, M30 (biscuits with 30% microencapsulated extract), M20 (biscuits with 20% microencapsulated extract) and N40 (biscuits with 40% nanoencapsulated extract). As regards brittleness, it refers to the distance crossed by the tool before the breadsticks broke, thus suggesting the fracturability of the biscuit. The highest values of brittleness were found in M40 and N40.

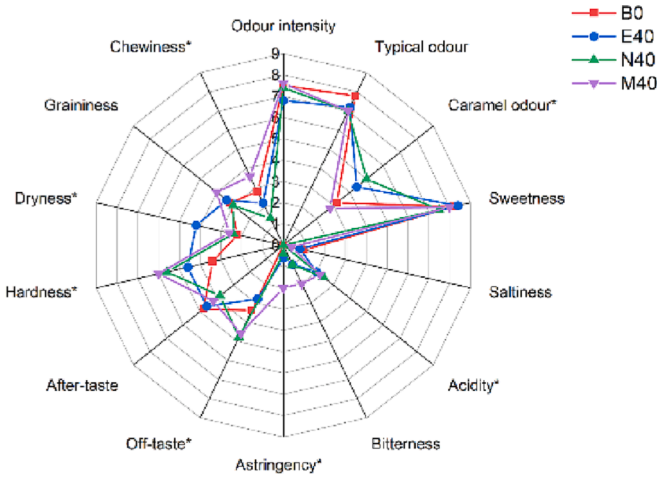


Fig. 2. Sensory evaluation of the biscuits. Means ± standard deviation followed by * indicate statistically significant differences between the types of biscuits tested according to Tukey's test ($p < 0.05$). B0- biscuits with no extract added; E40- biscuits with 40% non-encapsulated extract; M40- biscuits with 40% microencapsulated extract; N40- biscuits with 40% nano-encapsulated extract;

According to other authors, adding the encapsulated extracts affects both the hardness and the brittleness (Mosafa et al., 2017; Ovalle-Magallanes et al., 2017). Mosafa et al. (2017) state that adding maltodextrin can increase biscuits hardness and fracturability. It is because maltodextrin can form a gel in water. Some water will immobilize and significantly reduce the water available to the gluten for hydration, increasing the hardness and breaking strength of the resulting biscuit (Mosafa et al., 2017). Meanwhile, adding gum arabic by 0.3–0.5% can also increase the hardness and fracturability of biscuits because of its ability to bind water and other components producing a stronger and harder biscuit (Mosafa et al., 2017; Ujong et al., 2020).

Table 2
Nutritional composition and textural properties of olive leaf extracts enriched biscuits.

Nutritional parameters	Biscuit Samples B0	E20	E30	E40	M20	M30	M40	N20	N30	N40
Moisture (%)	$3.73^{E} \pm 0.05$	$4.2^{DE} \pm 0.06$	$3.79^{E} \pm 0.09$	$3.82^{E} \pm 0.03$	$6.37^{A} \pm 0.23$	$4.35^{CD} \pm 0.14$	$4.73^{C} \pm 0.11$	$4.00^{DE} \pm 0.08$	$5.60^{B} \pm 0.70$	$5.46^{B} \pm 0.05$
Protein (%)	$9.00^{E} \pm 0.50$	$9.17^{CDE} \pm 0.30$	$9.17^{CDE} \pm 0.9$	$9.25^{BCDE} \pm 0.30$	$9.15^{DE} \pm 0.14$	$9.37^{BCD} \pm 0.10$	$9.50^{BC} \pm 0.20$	$9.55^{AB} \pm 0.10$	$9.57^{A} \pm 0.30$	$9.52^{AB} \pm 0.10$
Fat (%)	$16.39^{B} \pm 0.50$	$16.60^{B} \pm 0.30$	$16.85^{B} \pm 0.10$	$17.04^{B} \pm 0.10$	$16.56^{B} \pm 0.15$	$16.78^{B} \pm 0.2$	$16.83^{B} \pm 0.40$	$18.58^{A} \pm 0.50$	$18.69^{A} \pm 0.20$	$18.75^{A} \pm 0.30$
Ash (%)	$1.95^{E} \pm 0.06$	$2.29^{D} \pm 0.03$	$2.36^{CD} \pm 0.03$	$2.45^{BC} \pm 0.04$	$2.54^{AB} \pm 0.10$	$2.61^{A} \pm 0.08$	$2.65^{A} \pm 0.08$	$2.59^{AB} \pm 0.09$	$2.60^{AB} \pm 0.10$	$2.62^{A} \pm 0.15$
Total fiber (%)	$1.09^{H} \pm 0.02$	$1.24^{G} \pm 0.07$	$1.31^{FG} \pm 0.04$	$1.33^{F} \pm 0.10$	$1.91^{E} \pm 0.09$	$2.12^{D} \pm 0.05$	$2.35^{C} \pm 0.07$	$2.66^{B} \pm 0.10$	$2.75^{A} \pm 0.11$	$2.8^{A} \pm 0.09$
Total carbohydrate (%)	$68.19^{A} \pm 0.57$	$66.47^{B} \pm 0.15$	$66.50^{B} \pm 0.08$	$66.12^{B} \pm 0.23$	$63.46^{D} \pm 0.22$	$64.76^{BC} \pm 0.43$	$63.92^{CD} \pm 0.05$	$62.61^{D} \pm 0.07$	$60.78^{E} \pm 0.33$	$60.82^{E} \pm 0.02$
Textural parameters										
Hardness (N)	$14.51^{CD} \pm 1.06$	$15.02^{BCD} \pm 1.35$	$15.81^{BCD} \pm 0.79$	$14.11^{CD} \pm 0.67$	$17.17^{ABC} \pm 1.64$	$18.14^{AB} \pm 0.75$	$19.21^{A} \pm 1.37$	$13.24^{D} \pm 1.19$	$15.04^{BCD} \pm 0.27$	$17.60^{AB} \pm 1.10$
Brittleness (mm)	$0.94^{AB} \pm 0.02$	$0.92^{AB} \pm 0.04$	$0.97^{AB} \pm 0.04$	$0.89^{AB} \pm 0.03$	$0.87^{AB} \pm 0.08$	$0.91^{AB} \pm 0.07$	$1.27^{A} \pm 0.30$	$0.80^{B} \pm 0.06$	$0.75^{B} \pm 0.03$	$1.22^{A} \pm 0.29$

All data are the mean ± SD of three independent determinations. Mean followed by different letters in the same row differs significantly ($p < 0.05$). B0- biscuits with no extract added; E20- biscuits with 20% non-encapsulated extract; E30- biscuits with 30% non-encapsulated extract; E40- biscuits with 40% non-encapsulated extract; M20- biscuits with 20% microencapsulated extract; M30- biscuits with 30% microencapsulated extract; M40- biscuits with 40% microencapsulated extract; N20- biscuits with 20% nanoencapsulated extract; N30- biscuits with 30% nanoencapsulated extract; N40- biscuits with 40% nanoencapsulated extract;

3.6. Nutritional values determination

The nutritional composition of the biscuits with OLE micro-encapsulated, and nanoencapsulated is presented in Table 2. The extract's addition to the biscuits significantly influences the main nutritional components ($p < 0.05$).

The addition of microencapsulated and nanoencapsulaed OLE significantly increases the moisture content ($p < 0.05$). This could be attributed partly to the powder fiber content, which enables more water absorption than the other ingredients. The results agree with other research on bread with OLE, which reports that adding micro-encapsulated extract (10%) retained the bread's moisture more efficiently (Moghaddam et al., 2020). The fiber content was enhanced significantly with the increase of encapsulated powder in the biscuits compared with the control. This could be associated with the encapsulating materials' high fiber content (maltodextrin 85%). For protein level, the proportion ranged from 9.0% in the control biscuits to 9.57% in the supplemented biscuits; however, no significant differences were obtained when compared to the control. Since OLE was reported to have low protein content (0.032%) (Vergara-Barberan et al., 2015), biscuits' protein concentrations were quite close. As expected, the addition of nanoencapsulated OLE (20–40%) has shown good enhancement in fat content in biscuits when compared to control and microencapsulated OLE ($p < 0.05$). The addition of linseed oil can justify the high-fat content of the biscuit. More than that, the increase of extract proportion (20, 30, 40%) in the biscuits significantly influence the ash content ($p < 0.05$). Earlier research related to the potential of OLEs to improve the mineral content of bakery products (Ghanem et al., 2019). The total carbohydrate content was significantly decreased ($p < 0.05$) in biscuits enriched with OLE from 66.50% to 60.78% compared to control biscuits (68.19%) (Man et al., 2021). These results are similar to the other research. According to these results, the potential of OLE can be observed to improve the biscuits' nutritional values.

3.7. Evaluation of bioactive polyphenols profile from biscuits

In order to evaluate the stability of the bioactive polyphenols in the baking process, the non-encapsulated and encapsulated extract was added, in different concentrations, as an ingredient in biscuits. HPLD/DAD system was used to quantify phenols content after cooking the biscuits in an oven at 180 °C. The results, reported in Table 3, were calculated by subtracting the contribution of the negative control biscuit (formulated without OLE) and expressed in μg/g biscuits. Chromatography confirmed the presence of all 13 biological compounds identified in the dried extract in the enriched biscuits samples. The total phenolic content of the biscuits ranged from 199.314 μg/g to 668.933 μg/g. As expected, the lowest value was registered for E20, E30, and E40 (the biscuits with non-encapsulated extract), while the highest values were obtained for biscuits with microspheres. The drying methods of the powders significantly impact the protection of the phenolic compounds in the baking process ($p < 0.05$). Spray-drying was more efficient than freeze-drying, which was also reflected in the EE. The differences in values between these methods were attributed mostly to the structural configuration of the capsules. (Veronica Cardoso de Souza et al., 2022) suggested that it may be due to the difficulty in extracting some polyphenols from the nano-capsules, which are physically held by the matrix and by hydrogen bonds.

After baking, the biscuit's phenolic content varied according to the powder administered. The increase in extract proportion had a significant enhancement in almost all the phenolic content. Compared to the content in the corresponding powders, verbascoside displayed a similar concentration in M20 and N30 ($p > 0.05$), regardless of the percentage of powder added. In addition, the same observations can be made for quercetin-rutinoside, oleoside 11-methylester, apigenin-glucoside, and luteolin-glucuronide. Based on this observation, it may be assumed that the encapsulation process can influence the phenolic content in the biscuits more than the powder proportion increase. The encapsulation has also been previously reported to have a significant impact on the concentration of phenolic content in the bakery samples (Budryn et al., 2016; Paciulli et al., 2023).

The data showed that the highest significant loss was observed for luteolin class (77–98%), apigenin-glucoside (75–88%), and demethyloleuropein (74–81%). On the other hand, oleuropein-aglycone (4–21%) and hydroxyoleuropein (16–31%) exhibited the highest stabilities in bakery conditions. In general, flavonoids were the most unstable polyphenols during the baking process of the biscuits, as also occurs during the encapsulation, because these molecules are more susceptible to heat treatments (Chaaban et al., 2017). Also, other authors have noticed that both free and encapsulated phenolic compounds disintegrate after the biscuits are baked (Budryn et al., 2016; Paciulli et al., 2023). They

Table 3
The content of phenolic compounds in biscuits with olive leaf extract.

	Phenolic Compound (μg/g)	Biscuit Samples B0	E20	E30	E40	M20	M30	M40	N20	N30	N40
1	Hydroxytyrosol-glucoside	0	$67.02^{I} \pm 0.2$	$65.62^{H} \pm 0.08$	$93.61^{F} \pm 0.28$	$169.96^{C} \pm 0.33$	$190.48^{B} \pm 0.37$	$215.98^{A} \pm 0.33$	$79.77^{G} \pm 0.18$	$96.72^{E} \pm 0.28$	$127.82^{D} \pm 0.17$
2	Demethyloleuropein	0	$1.40^{I} \pm 0.35$	$2.18^{F} \pm 0.07$	$4.67^{C} \pm 0.12$	$2.80^{D} \pm 0.17$	$4.67^{C} \pm 0.17$	$6.84^{A} \pm 0.15$	$1.56^{G} \pm 0.16$	$2.49^{E} \pm 0.24$	$4.98^{B} \pm 0.20$
3	Oleoside dimethylester	0	$5.76^{I} \pm 0.35$	$11.20^{G} \pm 0.28$	$15.86^{D} \pm 0.08$	$15.24^{E} \pm 0.14$	$22.24^{B} \pm 0.35$	$25.97^{A} \pm 0.19$	$6.53^{H} \pm 0.12$	$12.29^{F} \pm 0.07$	$17.42^{C} \pm 0.16$
4	Oleoside 11-methylester	0	$4.36^{H} \pm 0.14$	$9.64^{G} \pm 0.17$	$12.75^{E} \pm 0.09$	$13.53^{D} \pm 0.18$	$15.71^{B} \pm 0.22$	$28.30^{A} \pm 0.14$	$4.98^{H} \pm 0.23$	$9.95^{F} \pm 0.12$	$14.15^{C} \pm 0.34$
5	Luteolin-diglucoside	0	$0.01^{H} \pm 0.14$	$0.32^{G} \pm 0.07$	$0.78^{E} \pm 0.10$	$1.10^{C} \pm 0.11$	$1.71^{B} \pm 0.12$	$2.32^{A} \pm 0.23$	$0.04^{H} \pm 0.30$	$0.43^{F} \pm 0.22$	$0.91^{D} \pm 0.28$
6	Quercetin-rutinoside (Rutin)	0	$3.49^{H} \pm 0.07$	$4.66^{F} \pm 0.10$	$6.61^{C} \pm 0.30$	$4.61^{F} \pm 0.14$	$5.72^{D} \pm 0.17$	$8.06^{A} \pm 0.15$	$3.60^{G} \pm 0.19$	$4.77^{E} \pm 0.20$	$6.94^{B} \pm 0.16$
7	Verbascoside	0	$9.08^{F} \pm 0.13$	$10.40^{E} \pm 0.11$	$13.73^{C} \pm 0.33$	$11.14^{D} \pm 0.28$	$14.12^{C} \pm 0.08$	$20.50^{A} \pm 0.06$	$10.01^{E} \pm 0.18$	$11.73^{D} \pm 0.35$	$15.05^{B} \pm 0.14$
8	Luteolin-glucoside	0	$1.02^{I} \pm 0.07$	$1.84^{F} \pm 0.07$	$2.50^{D} \pm 0.12$	$1.58^{G} \pm 0.14$	$2.67^{C} \pm 0.21$	$4.11^{A} \pm 0.35$	$1.19^{H} \pm 0.11$	$2.06^{E} \pm 0.23$	$2.93^{B} \pm 0.14$
9	Apigenin-rutinoside	0	$1.84^{G} \pm 0.17$	$1.89^{G} \pm 0.26$	$2.76^{C} \pm 0.11$	$2.54^{D} \pm 0.09$	$3.11^{B} \pm 0.34$	$4.04^{A} \pm 0.36$	$2.00^{F} \pm 0.10$	$2.24^{E} \pm 0.12$	$3.20^{B} \pm 0.10$
10	Apigenin-glucoside	0	$0.433^{E} \pm 0.12$	$0.978^{D} \pm 0.14$	$1.217^{C} \pm 0.05$	$1.261^{C} \pm 0.17$	$1.544^{B} \pm 0.10$	$2.307^{A} \pm 0.11$	$0.542^{E} \pm 0.15$	$1.109^{CD} \pm 0.13$	$1.501^{B} \pm 0.24$
11	Luteolin-glucuronide	0	$0.04^{G} \pm 0.11$	$0.21^{F} \pm 0.22$	$0.67^{D} \pm 0.19$	$0.80^{C} \pm 0.28$	$1.06^{B} \pm 0.30$	$1.34^{A} \pm 0.32$	$0.06^{G} \pm 0.24$	$0.32^{E} \pm 0.23$	$0.78^{CD} \pm 0.10$
12	Hydroxyoleuropein	0	$93.61^{I} \pm 0.24$	$215.21^{F} \pm 0.17$	$236.66^{D} \pm 0.04$	$185.20^{G} \pm 0.15$	$269.16^{B} \pm 0.35$	$295.13^{A} \pm 0.25$	$100.76^{H} \pm 0.12$	$228.27^{E} \pm 0.11$	$250.50^{C} \pm 0.21$
13	Oleuropein-aglycone	0	$11.20^{H} \pm 0.31$	$18.97^{F} \pm 0.08$	$37.79^{C} \pm 0.09$	$33.42^{D} \pm 0.14$	$33.59^{D} \pm 0.31$	$53.96^{A} \pm 0.34$	$12.75^{G} \pm 0.18$	$20.53^{E} \pm 0.15$	$45.87^{B} \pm 0.13$
	Total Phenolics	0	$199.31^{I} \pm 0.35$	$343.18^{G} \pm 0.23$	$429.65^{D} \pm 0.13$	$413.23^{E} \pm 0.06$	$565.84^{B} \pm 0.20$	$668.93^{A} \pm 0.18$	$223.84^{H} \pm 0.25$	$392.96^{F} \pm 0.14$	$492.11^{C} \pm 0.24$

All data are the mean ± SD of three independent determinations. Mean followed by different letters in the same row differs significantly ($p < 0.05$). B0- biscuits with no extract added; E20- biscuits with 20% non-encapsulated extract; E30- biscuits with 30% non-encapsulated extract; E40- biscuits with 40% non-encapsulated extract; M20- biscuits with 20% microencapsulated extract; M30- biscuits with 30% microencapsulated extract; M40- biscuits with 40% microencapsulated extract; N20- biscuits with 20% nanoencapsulated extract; N30- biscuits with 30% nanoencapsulated extract; N40- biscuits with 40% nanoencapsulated extract;

hypothesized that the polyphenols in cookies were responsible for this phenomenon because of their interaction with other substances, such as sugar molecules released during caramelization and Maillard reactions.

Among the encapsulated powders, M40 indicates the greatest phenolic content and lowest decrease during baking. It may be proposed as a suitable component for formulating enhanced biscuits and other baked products.

Experiments were conducted mimicking the conditions of the mouth, stomach, and small intestine following the INFOGEST protocol extensively described by Brodkorb et al. (Brodkorb et al., 2019). *In vitro* digestion of the biscuits was performed to investigate the phenolic profile and the stability changes of the bioactive components recovered after simulated gastrointestinal conditions. The digestive process composed of sequential oral, gastric, and intestinal digestion of the biscuits was followed by sampling; samples were taken at the end of the intestinal phase and further analyzed, qualitatively and quantitatively, by HPLC-DAD-ESI-MS system. Fig. 3 shows the effects of the *in vitro* digestion on the total phenolic compound content. The phenolic compounds for the olive leaf extract were decomposed under gastric and intestinal conditions, leading to elenolic acid. Once polyphenols are removed from their natural structure, they are very vulnerable to being decomposed by factors like temperature and oxygen, as well as by elements in food and the digestive tract (like pH and the action of enzymes), which can make it more difficult for them to be absorbed (Pacheco et al., 2018).

Given the high water solubility of maltodextrin, oleuropein, the principal phenolic component from the biscuits, was rapidly released and exposed to the gastric condition. Oleuropein has been reported to cause enzymatic hydrolysis in gastric conditions, giving hydroxytyrosol and elenolic acid (Yuan et al., 2015). In our study, hydroxytyrosol was not detected after the intestinal stage, which may be related to its high instability under alkaline conditions. Similar results were reported by González et al., which evaluated the stability of OLE polyphenols spray-dried in sodium alginate capsules. They reported that encapsulating OLE prevented oleuropein from degrading through gastric digestion. During intestinal digestion, sodium alginate was dissolved, and oleuropein was discharged from the capsules, which were released in elenolic acid (Gonzalez et al., 2019). Accordingly, a more significant release of polyphenols was detected after intestinal digestion, indicating increased bioaccessibility.

At the endpoint of the simulated intestinal phase, the concentration of the elenolic acid ranged between 144.817 μg/mL (E20) and 193.205 μg/ml (M40). A significant difference was observed between the samples with encapsulated and non-encapsulated extract in the biscuits ($p < 0.05$). The lowest concentration was found for the non-encapsulated samples (144.817–161.591 μg/mL), followed by nanoencapsulated samples (164.208–178.513 μg/mL), and the highest values for microencapsulated samples (168.788–193.205 μg/mL). Significant protection was confirmed for microspheres after digestion compared to nanoencapsulated and non-encapsulated.

Rising awareness of the possible health advantages of specific - phenolic compounds has prompted the food industry to develop functional food items containing phenolics. Thus, the encapsulation matrix significantly affects the release process (Gonzalez et al., 2019; Ștefănescu et al., 2022). Gonzalez et al. reported that bioaccessibility was ten times higher ($p \leq 0.05$) for OLE encapsulated than non-encapsulated (Gonzalez et al., 2020). Aniesrani et al. results indicated that spray-dried microencapsulation prolongs polyphenol (curcumin) release in gastrointestinal circumstances (Aniesrani Delfiya et al., 2015). Also, the bioaccessibility and antioxidant capacity was increased after the adsorbed olive phenolics from leaf infusions into microorganism like *Saccharomyces cerevisiae* (Jilani et al., 2016).

The protection of phenolic compounds also occurs through nano-encapsulation due to the second layer of nanoparticles improving

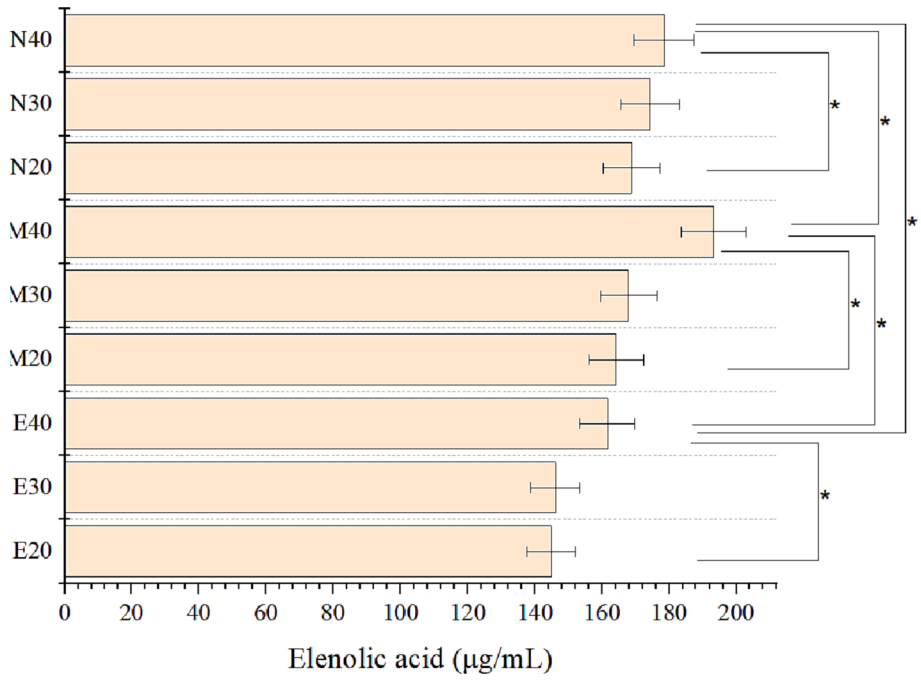


Fig. 3. Changes in the total phenolic content of biscuits *in vitro* digestion; *: $p < 0.05$.

physicochemical stability and inhibiting polyphenol migration from the matrix. Regarding the controlled release, nanoparticles' capacity to delay the release of polyphenols is advantageous since it prevents an uncontrolled release burst (Mahdi et al., 2022). Therefore, the method of encapsulation and the encapsulating agent used may influence the release of the molecules at a specific area and their impact on human health.

4. Conclusion

Olive leaves are an inexpensive source of natural phenolics. The recovery and integration of phenolics into functional food products are consistent with the circular economy and sustainability. Due to their bitter taste and physicochemical instability, phenolics integration into different food matrices, especially in bakery food products, can be challenging. Thus, encapsulation might be a suitable strategy for overcoming these limitations. TEM and SEM analyses confirmed the encapsulation, and the generated particles had a spherical morphology. Enriching biscuits with OLE-loaded microparticles and nanoparticles effectively improved their functional properties. In terms of EE, spray drying (71.42 ± 0.034%) encapsulates the bioactive material more efficiently than the freeze-drying method (68.09 ± 0.024%). The sample with M40 displayed a significantly higher protection compared to other sample conditions, indicating its ability to effectively retain the phenolic compounds within the encapsulation system. However, the highest content of OLE (40%) in biscuits affected the sensory and textural parameters. Thus, the selected encapsulating methodology could not cover or regulate the bitterness perception, leading to the conclusion that futher investigations are required in terms of encapsulation techniques and biscuits formulations to minimize these issues. During digestion, encapsulation materials were dissolved, and phenolic compounds were discharged from the capsules, which were released in elenolic acid. The present study has the opportunity to contribute significantly to the vast field of functional foods. It provides new options for producing healthier foods while optimizing the valorization of olive by-products.

Funding: This work was supported by a grant granted by the Ministry of Research and Innovation, C.N.C.S.—UEFISCDI, project number PN-III-*P*4-ID-PCE-2020–2306 and PN-III-*P*4-IDPCE-2020–2126 PNCDI III.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.136778.

References

AACC. (2000). Method 38-12. Approved Methods of the American Association of Cereal Chemists, 11th ed.; American Association of Cereal Chemists: St. Paul, MN, USA.

Amini, A., Liu, M., & Ahmad, Z. (2017). Understanding the link between antimicrobial properties of dietary olive phenolics and bacterial ATP synthase. *International Journal of Biological Macromolecules, 101*, 153–164. https://doi.org/10.1016/j.ijbiomac.2017.03.087

Aniesrani Delfiya, D. S., Thangavel, K., Natarajan, N., Kasthuri, R., & Kailappan, R. (2015). Microencapsulation of Turmeric Oleoresin by Spray Drying and *In Vitro* Release Studies of Microcapsules. *Journal of Food Process Engineering, 38*(1), 37–48. https://doi.org/10.1111/jfpe.12124

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assuncao, R., Ballance, S., et al. (2019). INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nat Protoc, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Budryn, G., Zaczyńska, D., & Oracz, J. (2016). Effect of addition of green coffee extract and nanoencapsulated chlorogenic acids on aroma of different food products. *Lwt, 73*, 197–204. https://doi.org/10.1016/j.lwt.2016.06.019

Calinoiu, L. F., & Vodnar, D. C. (2019). Thermal Processing for the Release of Phenolic Compounds from Wheat and Oat Bran. *Biomolecules, 10*(1). https://doi.org/10.3390/biom10010021

Çetinkaya, H., & Kulak, M. (2016). Relationship between total phenolic, total flavonoid and oleuropein in different aged olive (*Olea europaea* l.) Cultivar leaves. *African Journal of Traditional, Complementary and Alternative Medicines, 13*(2), 81. https://doi.org/10.4314/ajtcam.v13i2.10

Chaaban, H., Ioannou, I., Chebil, L., Slimane, M., Gérardin, C., Paris, C., et al. (2017). Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. *Journal of Food Processing and Preservation, 41*(5). https://doi.org/10.1111/jfpp.13203

de Gennaro, G., Difonzo, G., Summo, C., Pasqualone, A., & Caponio, F. (2022). *Olive Cake Powder as Functional Ingredient to Improve the Quality of Gluten-Free Breadsticks., 11* (4), 552. https://www.mdpi.com/2304-8158/11/4/552.

Dobrinčić, A., Tuđen, L., Repajić, M., Garofulić, I. E., Zorić, Z., Dragović-Uzelac, V., et al. (2020). Microencapsulation of Olive Leaf Extract by Spray Drying. *Acta Alimentaria, 49*(4), 475–482. https://doi.org/10.1556/066.2020.49.4.13

El-Messery, T. M., Altuntas, U., Altin, G., & Ozcelik, B. (2020). The effect of spray-drying and freeze-drying on encapsulation efficiency, *in vitro* bioaccessibility and oxidative stability of krill oil nanoemulsion system. *Food Hydrocolloids, 106*, Article 105890. https://10.1016/j.foodhyd.2020.105890.

Elik, A., Koçak Yanık, D., & Göğüş, F. (2021). A comparative study of encapsulation of carotenoid enriched-flaxseed oil and flaxseed oil by spray freeze-drying and spray drying techniques. *Lwt, 143*. https://doi.org/10.1016/j.lwt.2021.111153

Espeso, J., Isaza, A., Lee, J. Y., Sörensen, P. M., Jurado, P., Avena-Bustillos, R., et al. (2021). Olive Leaf Waste Management. *Frontiers in Sustainable Food Systems, 5*, Article 660582. https://doi.org/10.3389/fsufs.2021.660582

Faridi Esfanjani, A., & Jafari, S. M. (2016). Biopolymer nano-particles and natural nano-carriers for nano-encapsulation of phenolic compounds. *Colloids Surf B Biointerfaces, 146*, 532–543. https://doi.org/10.1016/j.colsurfb.2016.06.053

Ghanem, M. T. M., Tawfik, W. A., Mandy, E. S. M., Abdelgawad, M. E., Abdel-Azim, N. S., & El-Missiry, M. M. (2019). Chemical and biological evaluation of olive leaves as a waste by-product of olive oil industry. *Egyptian Pharmaceutical Journal, 18*(2), 172–177. https://doi.org/10.4103/epj.epj_6_19

Ghasemi, S., Koohi, D. E., Emmamzadehhashemi, M. S. B., Khamas, S. S., Moazen, M., Hashemi, A. K., et al. (2018). Investigation of phenolic compounds and antioxidant activity of leaves extracts from seventeen cultivars of Iranian olive (*Olea europaea* L.). *J Food Sci Technol, 55*(11), 4600–4607. https://doi.org/10.1007/s13197-018-3398-1

González-Ortega, R., Faieta, M., Di Mattia, C. D., Valbonetti, L., & Pittia, P. (2020). Microencapsulation of olive leaf extract by freeze-drying: Effect of carrier composition on process efficiency and technological properties of the powders. *Journal of Food Engineering, 285*, Article 110089. https://doi.org/10.1016/j.jfoodeng.2020.110089

González, C. M., Llorca, E., Quiles, A., Hernando, I., & Moraga, G. (2020). Water sorption and glass transition in freeze-dried persimmon slices. *Effect on physical properties and bioactive compounds. Lwt, 130*, Article 109633. https://doi.org/10.1016/j.lwt.2020.109633

Gonzalez, E., Gomez-Caravaca, A. M., Gimenez, B., Cebrian, R., Maqueda, M., Martinez-Ferez, A., et al. (2019). Evolution of the phenolic compounds profile of olive leaf extract encapsulated by spray-drying during *in vitro* gastrointestinal digestion. *Food Chem, 279*, 40–48. https://doi.org/10.1016/j.foodchem.2018.11.127

Gonzalez, E., Gomez-Caravaca, A. M., Gimenez, B., Cebrian, R., Maqueda, M., Parada, J., et al. (2020). Role of maltodextrin and inulin as encapsulating agents on the protection of oleuropein during *in vitro* gastrointestinal digestion. *Food Chem, 310*, Article 125976. https://doi.org/10.1016/j.foodchem.2019.125976

Hoskin, R. T., Xiong, J., Esposito, D. A., & Lila, M. A. (2019). Blueberry polyphenol-protein food ingredients: The impact of spray drying on the *in vitro* antioxidant activity, anti-inflammatory markers, glucose metabolism and fibroblast migration. *Food Chem, 280*, 187–194. https://doi.org/10.1016/j.foodchem.2018.12.046

Jilani, H., Cilla, A., Barberá, R., & Hamdi, M. (2016). Improved bioaccessibility and antioxidant capacity of olive leaf (Olea europaea L.) polyphenols through biosorption on Saccharomyces cerevisiae. *Industrial Crops and Products, 84*, 131–138. https://doi.org/10.1016/j.indcrop.2016.02.002

Kiritsakis, K., Goula, A. M., Adamopoulos, K. G., & Gerasopoulos, D. (2017). Valorization of Olive Leaves: Spray Drying of Olive Leaf Extract. *Waste and Biomass Valorization, 9* (4), 619–633. https://doi.org/10.1007/s12649-017-0023-x

Mahdi, A. A., Al-Maqtari, Q. A., Mohammed, J. K., Al-Ansi, W., Aqeel, S. M., Cui, H., et al. (2022). Nanoencapsulation of Mandarin Essential Oil: Fabrication. *Characterization, and Storage Stability., 11*(1), 54. https://www.mdpi.com/2304-8158/11/1/54.

Man, S. M., Stan, L., Paucean, A., Chis, M. S., Muresan, V., Socaci, S. A., et al. (2021). Nutritional, Sensory, Texture Properties and Volatile Compounds Profile of Biscuits with Roasted Flaxseed Flour Partially Substituting for Wheat Flour. *Applied Sciences-Basel, 11*(11), 4791. https://10.3390/app11114791.

Moghaddam, M. F. T., Jalali, H., Nafchi, A. M., & Nouri, L. (2020). Evaluating the effects of lactic acid bacteria and olive leaf extract on the quality of gluten-free bread. *Gene Reports, 21*, Article 100771. https://10.1016/j.genrep.2020.100771.

Mohammed, N. K., Muhialdin, B. J., & Meor Hussin, A. S. (2020). Characterization of nanoemulsion of *Nigella sativa* oil and its application in ice cream. *Food Sci Nutr, 8* (6), 2608–2618. https://doi.org/10.1002/fsn3.1500

Mosafa, L., Nadian, N., & Hojatoleslami, M. (2017). Investigating the effect of whole oat flour, maltodextrin and isomalt on textural and sensory characteristics of biscuits using response surface methodology. *Journal of Food Measurement and Characterization, 11*(4), 1780–1786. https://doi.org/10.1007/s11694-017-9559-5

Muhammad, Z., Ramzan, R., Zhang, R. F., & Zhang, M. W. (2021). Resistant Starch-Based Edible Coating Composites for Spray-Dried Microencapsulation of *Lactobacillus acidophilus,* Comparative Assessment of Thermal Protection, *In Vitro* Digestion and Physicochemical Characteristics. *Coatings, 11*(5), 587. https://10.3390/coatings11050587.

Najda, A., Bains, A., Klepacka, J., & Chawla, P. (2022). *Woodfordia fruticosa* extract nanoemulsion: Influence of processing treatment on droplet size and its assessment for *in vitro* antimicrobial and anti-inflammatory activity. *Front Nutr, 9*, Article 944856. https://doi.org/10.3389/fnut.2022.944856

Navarro-Flores, M. J., Ventura-Canseco, L. M. C., Meza-Gordillo, R., Ayora-Talavera, T. D. R., & Abud-Archila, M. (2020). Spray drying encapsulation of a native plant extract rich in phenolic compounds with combinations of maltodextrin and non-conventional wall materials. *J Food Sci Technol, 57*(11), 4111–4122. https://doi.org/10.1007/s13197-020-04447-w

Ovalle-Magallanes, B., Eugenio-Perez, D., & Pedraza-Chaverri, J. (2017). Medicinal properties of mangosteen (*Garcinia mangostana* L.): A comprehensive update. *Food Chem Toxicol, 109*(Pt 1), 102–122. https://doi.org/10.1016/j.fct.2017.08.021

Pacheco, C., González, E., Robert, P., & Parada, J. (2018). Retention and pre-colon bioaccessibility of oleuropein in starchy food matrices, and the effect of microencapsulation by using inulin. *Journal of Functional Foods, 41*, 112–117. https://doi.org/10.1016/j.jff.2017.12.037

Paciulli, M., Grimaldi, M., Rinaldi, M., Cavazza, A., Flamminii, F., Mattia, C. D., et al. (2023). Microencapsulated olive leaf extract enhances physicochemical stability of biscuits. *Future Foods, 7*, Article 100209. https://doi.org/10.1016/j.fufo.2022.100209

Parvez, S., Ahmed Wani, I., & Masoodi, F. A. (2022). Nanoencapsulation of green tea extract using maltodextrin and its characterisation. *Food Chem, 384*, Article 132579. https://doi.org/10.1016/j.foodchem.2022.132579

Rashid, R., Wani, S. M., Manzoor, S., Masoodi, F. A., & Dar, M. M. (2022). Improving oxidative stability of edible oils with nanoencapsulated orange peel extract powder during accelerated shelf life storage. *Food Bioscience, 49*, Article 101917. https://doi.org/10.1016/j.fbio.2022.101917

Rezende, Y., Nogueira, J. P., & Narain, N. (2018). Microencapsulation of extracts of bioactive compounds obtained from acerola (*Malpighia emarginata* DC) pulp and residue by spray and freeze drying: Chemical, morphological and chemometric characterization. *Food Chem, 254*, 281–291. https://doi.org/10.1016/j.foodchem.2018.02.026

Silva, P. I., Stringheta, P. C., Teofilo, R. F., & de Oliveira, I. R. N. (2013). Parameter optimization for spray-drying microencapsulation of jaboticaba (*Myrciaria jaboticaba*) peel extracts using simultaneous analysis of responses. *Journal of Food Engineering, 117*(4), 538–544. https://doi.org/10.1016/j.jfoodeng.2012.08.039

Ştefănescu, B. E., Nemes, S.-A., Teleky, B.-E., Călinoiu, L. F., Mitrea, L., Martău, G. A., et al. (2022). *Microencapsulation and Bioaccessibility of Phenolic Compounds of Vaccinium Leaf Extracts., 11*(4), 674. https://www.mdpi.com/2076-3921/11/4/674.

Szabo, K., Teleky, B. E., Ranga, F., Simon, E., Pop, O. L., Babalau-Fuss, V., et al. (2021). Bioaccessibility of microencapsulated carotenoids, recovered from tomato processing industrial by-products, using *in vitro* digestion model. *Lwt-Food. Science and Technology, 152*. https://10.1016/j.lwt.2021.112285.

Tavakoli, H., Hosseini, O., Jafari, S. M., & Katouzian, I. (2018). Evaluation of Physicochemical and Antioxidant Properties of Yogurt Enriched by Olive Leaf Phenolics within Nanoliposomes. *J Agric Food Chem, 66*(35), 9231–9240. https://doi.org/10.1021/acs.jafc.8b02759

Ujong, A. E., Oguzor, U. C., & China, M. A. H. (2020). Effect of Gum Arabic Incorporation on the Proximate Composition and Sensory Properties of Biscuits Produced from Flour Blends of Wheat and Water Yam. *Asian Food Science Journal, 18*, 1–11. https://doi.org/10.9734/afsj/2020/v18i130201

Urzúa, C., González, E., Dueik, V., Bouchon, P., Giménez, B., & Robert, P. (2017). Olive leaves extract encapsulated by spray-drying in vacuum fried starch–gluten doughs. *Food and Bioproducts Processing, 106*, 171–180. https://doi.org/10.1016/j.fbp.2017.10.001

Vergara-Barberan, M., Lerma-Garcia, M. J., Herrero-Martinez, J. M., & Simo-Alfonso, E. F. (2015). Use of an enzyme-assisted method to improve protein extraction from olive leaves. *Food Chem, 169*, 28–33. https://doi.org/10.1016/j.foodchem.2014.07.116

Veronica Cardoso de Souza, B., de Morais Sousa, M., Augusto Gasparotto Sattler, J., Cristina Sousa Gramoza Vilarinho Santana, A., Bruno Fonseca de Carvalho, R., de Sousa Lima Neto, J., et al. (2022). Nanoencapsulation and bioaccessibility of polyphenols of aqueous extracts from *Bauhinia forficata* link. *Food Chem (Oxf), 5*, 100144. 10.1016/j.fochms.2022.100144.

Yuan, J. J., Wang, C. Z., Ye, J. Z., Tao, R., & Zhang, Y. S. (2015). Enzymatic hydrolysis of oleuropein from Olea europea (olive) leaf extract and antioxidant activities. *Molecules, 20*(2), 2903–2921. https://doi.org/10.3390/molecules20022903