Evaluation of extracts prepared from olive oil by-products using microwave-assisted enzymatic extraction - Effect of encapsulation on the stability of final products

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Abstract

The feasibility of microwave-assisted extraction (MAE) and microwave-assisted enzymatic extraction (MAEE) for the polyphenols recovery from olive kernel and leaves was examined. The study revealed that applying MAEE at 60 °C for 30min, extracts with phenolic content 10.37 and 29.52 mg GAE/g raw material (dw) were obtained from olive kernel and leaves, respectively and with antioxidant activity 25.47 and 6.46 g raw material (dw)/ g DPPH, respectively. Similarly, applying conventional extraction (CE) at 60 °C for 1h, extracts with phenolic content 11.41 and 34.53 mg GAE/ g raw material (dw) were obtained from olive kernel and leaves respectively, and with antioxidant activity 22.23 and 5.66 g raw material (dw)/ g DPPH, respectively. HPLC analyses of phenolic compounds of extracts proved that the use of enzyme on extraction process leaded to valuable recovery of phenolic compounds. The predominant phenolic compounds in olive kernel and leaves extracts were oleuropein, hydroxytyrosol and rutin. Moreover, in order to protect the phenolic compounds' properties, extracts of olive kernel and leaves with high antioxidant activity were encapsulated in maltodextrin by applying freeze-drying technique. The final encapsulated polyphenolics' products were evaluated in terms of hygroscopicity, solubility, moisture content and microencapsulation efficiency (MEE %). The MEE % varied from 82.39 to 92.12 % and from 87.98 to 91.06 % for olive kernel and leaves formulations, respectively. The encapsulation improved the moisture content, solubility and hygroscopicity of the encapsulated products. Concluding, the MAEE method being environment-friendly was efficient for the polyphenols recovery, while the polyphenols having significant antioxidant activity can be used as a source of potential antioxidant. Importantly, the use of olive kernel and leaves for such polyphenolic products reduces the impact of olive oil production on the environment.

Keywords: olive kernel, olive leaves, microwave-assisted enzymatic extraction, encapsulation

1. Introduction

Olive oil production is an important agricultural activity and one of the primary driving forces of the economy of Greece involving by-products such as olive kernel and olive leaves. Olive kernel and olive leaves can reach up to 30% of olive mill manufacturing and 5% of the total weight of processed olives, respectively [1,2]. Additionally, olive leaves are by-products after pruning of olive trees. It is well known that these residues are rich in polyphenols, since only 2% of the phenolic compounds are transferred to the oil and as much as 98% remained in the residue. These by-products are considered valuable sources of polyphenolic compounds. Nevertheless, some specific issues of olive oil production industry, such as scattering of small sized mills over the territory and production seasonality, introduce economical, technical and organizational constraints that make difficult the adoption of environment-compatible approaches for a sustainable waste disposal. More specifically, the usual practices in Greece are the production of olive kernel oil, as additives in animal feeding, as combustible biomass and mainly the directly disposal into environmental systems without any pretreatment [3].

The direct disposal of olive kernel and leaves constitute a major environmental problem due to their high organic and polyphenol content, the latter being toxic to water and soil ecosystems [4, 5]. However, polyphenols are one of the most important groups of natural antioxidants and may act, by different mechanisms, to confer an effective defense system against free radical attack. Recent studies demonstrated that the containing bioactive components in the leaves and olive kernel such as oleuropein, hydroxytyrosol, verbascoside, lutein and rutin have showed antiviral, antimicrobial, antioxidant, anti-inflammatory and anti-carcinogenic activities [6]. Therefore, ways to valorize these by-products are needed and most welcome.

Different extraction techniques have been used to extract bioactive compounds from olive leaves and olive kernel; among them, conventional extraction (CE) with ethanol: water. Considering the importance of the extraction process as a way to recover valuable compounds from natural raw materials, investigating of different extraction methods is necessary. Water and aqueous-based solvent system has represented an increasingly crucial choice for the replacement of conventional organic solvents in the food industry [7]. Currently, microwave-assisted extraction (MAE) is a novel and green extraction technique that can offer simplified manipulation, reduced solvent consumption and lower energy input [8]. Therefore, compared to the convectional extraction (CE) methods, MAE is known as a more environmental-friendly process with economic advantages.

Enzyme-assisted extraction (EAE) technology with the environmental friendly, cost and safe advantages can be used as an alternative to the release of bioactive compounds from plant materials [9]. Hydrolytic enzymes, including cellulase and pectinase, are commonly utilized to hydrolyze and degrade cell wall constituents and improve the release of intracellular contents [10]. Furthermore, cell wall degrading-

enzymes have been proved to be efficient for the extraction of polyphenols from various species of plant materials such as peanut shells, Pinus taiwanensis, Pinus morrisonicola and Geranium sibiricum Linne [11-13]. Microwave irradiation has been widely utilized in accelerated enzyme-catalyzed reactions for natural products and oils extraction [13-15]. These modern extraction techniques can be regarded as a possible tool not only from a laboratory point of view but also for industrial application. To our knowledge, the combined use of enzymes and microwave-assisted extraction of polyphenols from olive oil by-products, such as olive kernel and leaves, has not been previously reported.

Nevertheless, polyphenols, containing unsaturated bonds in their molecular structure, are not chemically stable as they are susceptible to oxidative deterioration, when exposed to oxygen, light, moisture, and temperature [16]. Oxidation of polyphenols leads to unpleasant flavor taste, affecting thus the quality and limiting the shelf-life of the products in which they incorporated [17]. Due to the above, polyphenols needs to be masked before incorporation into food products and microencapsulation is considered as a relevant technique [18].

The microencapsulation process basically consists of the preparation of an emulsion or dispersion that contains the encapsulating agent and the core material (polyphenols), which is then dried, commonly using spray or freeze-drying techniques [19]. Freeze-drying technique has proved to be the most efficient technique for the encapsulation of phytochemicals due to its low processing temperature and the absence of water, minimizing the deteriorative reactions as well as offering a final product of excellent quality. For the encapsulation different types of encapsulating agents have been used including polysaccharides (maltodextrins), lipids (mono and diglycerides), and proteins (gelatin, casein and soy); maltodextrins are also widely used agents [20, 21]. Maltodextrins are hydrolyzed starches produced by partially hydrolysis of starch with acid or enzymes, possessing a good compromise between cost and effectiveness [22].

Therefore, the objective of this study was to investigate the feasibility of microwave-assisted extraction (MAE) and microwave-assisted enzymatic extraction (MAEE) for the polyphenols' recovery from olive kernel and leaves. An enzyme mixture comprised of pectinase and polygalacturonase was used. The antioxidant potential of extracts prepared from both olive oil by-products was evaluated in terms of their total phenolic content and their antioxidant radical scavenging. The analysis of phenolic profile and the quantification in individual compounds of the two extracts was carried out by HPLC. Moreover, the extracted polyphenols that showed high antioxidant activity were encapsulated in matrices of maltodextrin by freeze-drying technique. Encapsulation efficiency and antioxidant activity of the encapsulated polyphenols products were determined. The results of the present study may be helpful to further exploit and utilize these resources.

2. Materials and methods

2.1.1 Raw Materials

Olive kernel and olive leaves were used as raw materials for their phenolic compounds recovery. Olive kernel (initial moisture 45.0% w/w) obtained from a local olive oil mill using a continuous three-phase centrifugation system and olive leaves (initial moisture 49% w/w) were collected from the region of Thiva (Voiotia, Greece). Both raw materials were air dried at 35 °C for 24 h by an airstream (final moisture 5% w/w) and were ground in 1mm with a cutting mill (FRITSCH, cutting mill, pulverisette 15). The pretreated raw materials were kept at 4°C.

2.1.2 Chemicals and reagents

Folin–Ciocalteu's reagent, citric acid, gallic acid, 1,1-diphenyl-2 picrylhydrazyl (DPPH), methanol (HPLC grade), ethanol, water (HPLC grade), acetonitrile (HPLC grade), sodium carbonate, sodium sulfate, sodium citrate and sodium acetate were purchased from Sigma Aldrich Chemical Co. (St Louis, MO). Maltodextrin from waxy maize (19DE) was obtained from Chemicotechnica S.A. (Athens, Greece). Phenolic standards: hydroxytyrosol, caffeic acid, vanillin, rutin and luteolin were procured from Sigma- Aldrich (St. Louis, MO), oleuropein was purchased from Extrasynthese (Genay, France), while enzyme was obtained from Novozymes (Novozym 33095).

2.2.1 Extraction of phenolic compounds from olive kernel and leaves

The phenolic compounds from olive kernel and leaves were conventionally extracted under reflux condenser (CE) with stirring (200rpm) at different combinations of temperature (40°C, 60°C) and time (1h, 4h) using citric buffer pH=4.5 as solvent at ratio 1 g/12,5 mL. Conventional enzymatic extraction (CEE) was also carried out using 1% enzyme solution in buffer volume (v/v). Microwave assisted extraction (MAE) additionally was performed using a laboratory microwave equipment (Nanjing Xianou Instruments Manufacture CO., LTD, China) at 400 W; citric buffer pH=4.5 as solvent was also used at at different combinations of temperature (40°C, 60°C) and time (5min, 30min). Microwave enzymatic assisted extraction (MAEE) was also performed with the same enzyme solution. Experimental conditions of extractions conducted are presented in *Table 1.* All extraction experiments were carried out in duplicate. The extracts were separated by centrifugation (10000rpm/10min) and their total phenol content was determined. The antioxidant activity of extracts was evaluated and their phenolic compounds profiles were analyzed using HPLC-DAD.

Table 1: Experimental conditions of extractions of olive kernel and leaves.

	Solvent	Time	Temperature
Convectional extraction (CE)/ Convectional enzymatic extraction (CEE)	Buffer	1h, 4h	40°C, 60°C
	Buffer + 1% enzyme solution	1h, 4h	40°C, 60°C
Microwave assisted extraction (MAE) / Microwave enzymatic assisted extraction (MAEE)	Buffer	5min, 30min	40°C, 60°C
	Buffer + 1% enzyme solution	5min, 30min	40°C, 60°C

2.2.2 Encapsulation of extracts

The extracts with high antioxidant activity were selected for encapsulation after their freeze drying. For the encapsulation, the following procedure was carried out: 1g of freeze dried polyphenol extract was mixed with 20g maltodextrin (19 DE), the mixture was homogenized at 11000 rpm for 5min using an homogenizer (CAT, Unidrive 1000) and freeze dried (Alpha 1-4LD plus, Christ, Osterode, Germany) at -52°C. The encapsulated products were stored in an airtight container at 2-4°C until further analysis. The encapsulating efficiency (MEE%) was estimated after determination of the phenolic content in the core and surface of the encapsulated products. Also, they were evaluated for their moisture, water activity (a_w), solubility, hygroscopicity and antioxidant activity.

2.3.1 Total phenolic content (TPC)

Total phenolic content was determined by the Folin–Ciocalteu method, as described by Waterhouse [23], using gallic acid as the standard. In 0.1mL of extract, 7.9mL distillate water and 0.5mL of Folin–Ciocalteu reagent were added and the mixtures were vortexed. Then, 1.5mL of supersaturated Na_2CO_3 was added, the mixture was revortexed and allowed for 2h in darkness. The absorbance of the solution was then measured using a spectrophotometer (Hitachi, U-2900 UV/Vis, 200V) at 765 nm. The results were expressed as mg gallic acid equivalents (GAE) per g of dry weight of raw materials (mg GAE/ g raw material (dw)).

2.3.2 Antioxidant activity

Antioxidant activity was determined as described by Brand-Williams [24], using the DPPH• assay. 0.1mL of the extract was added to 3.9mL of DPPH radical solution (0.0025g/100mL methanol) and after 20 min remaining in darkness the absorbance of the mixture was measured at 515 nm. Results were expressed as

IC50 (g raw material (dw)/g DPPH). IC50 is determined as the concentration of extract that declines 50% the initial concentration of DPPH radical; the less IC50 express maximum antioxidant activity. In the case of the encapsulatied products, the antioxidant activity expressed as % DPPH remaining.

% DPPH remaining $=100*A_{sample}/ A_{control}$

where $A_{control}$ is the absorbance without extract and A_{sample} is the absorbance of sample.

2.3.3 HPLC - DAD analysis

HPLC-analysis was performed on a HP 1100 Series gradient HPLC system (Agilent Technologies, USA) equipped with Class VP chromatography data station software, a SIL-10AF autosampler, a CTO-10AS column oven (251C), an SPD-10AV UV Visible detector and a diode array detector (DAD) (Hewlett-Pachard, Waldbronn, Germany). A column (250 × 4.6 mm) packed with 5 μm particles Hypersil C18 (MZ 156 Analysentechnik, Mainz, Germany) was used. The elution solvents consisted of aqueous 0.1% acetic acid and 2mM sodium acetic (solvent A) and acetonitrile (solvent B). Gradient elution: 0–25min, 100–50% A και 0–50% B, flow rate 0.8mL/min; 25–26min, 50–0% A και 50–100% B, flow rate 0.8mL/min; 26–27min, 0% A και 100% B, flow rate 0.8–1.2mL/min; 27–40min, 0% A και 100% B, flow rate 1.2mL/min; 40–41min, 0–100% A και 100–0% B, flow rate 1.2–0.8mL/min, 41–45min, 100% A και 0% B, flow rate 0.8mL/min The injection volume was 20μL. Polyphenols were identified and quantified using reference curves of standards. The level of polyphenols was expressed in μg/g of raw material (dw).

2.3.4 Micro-encapsulation efficiency (MEE%)

Micro-encapsulation efficiency (MEE%) of phenolic encapsulation products was determined by means of the phenolic content inside the core and the surface of the encapsulate following the procedure described by Saenz et al. [25]. 100mg of sample was dispersed in 1mL ethanol, acetic acid and water (50:8:42) for core phenolic compounds or in 1mL of ethanol and methanol (1:1) mixture for surface phenolic compounds. In both cases the mixtures were vortexed for 1min and filtered through 0.45μm filter and their phenolic content was determined by Folin–Ciocalteu method. The micro-encapsulation efficiency was expressed as:

$MEE\ (\%) = (TPC_{core} - TPC_{surface})*100/ TPC_{core}$

2.3.5 Moisture Content and water activity

The moisture content of the encapsulated product was determined gravimetrically by oven drying at 100 °C to a constant weight [26]. The water activity (a_w) was measured using a measured using an AquaLab 4TEV (Decagon Devices Inc., Pullman, Wash., U.S.A.).

2.3.6 Solubility

A 1g sample of the encapsulated product was added to recipients containing 100mL of distilled water, stirred at 110rpm for 30min, and centrifuged at 4000rpm for 5min. Aliquot of the supernatant was then removed, transferred to previously weighed porcelain dishes, and dried to constant weight in an oven at 100°C. The dishes were weighed and the solubility was estimated from the difference in weight [27].

2.3.7 Hygroscopicity

Approximately 1g of the encapsulated product was placed in hermetic pot containing a saturated sodium sulphate solution (RH of 81 %) and weighed again after 7 days remaining. The hermetic pot was kept at 25˚C in an incubator with controlled temperature. The hygroscopicity was expressed as g of water absorbed by 100g of sample [28].

All the analyses were carried out in triplicate and mean values are presented.

2.4 Statistical Analysis

The experimental data were assessed by analysis of variance (ANOVA) using STATISTICA 7 (Statsoft Inc., Tulsa, USA), while significant differences of mean values were estimated at the probability level $P<0.05$.

3. Results and Discussion

3.1 Effect of temperature and time extraction on phenolic compounds recovery and their antioxidant activity

The effect of temperature and time by conventional (CE) and microwave extraction (MAE) and/or with enzyme (CEE, MAEE) of olive kernel and leaves on the phenolic compounds recovery and the antioxidant activity is shown in *Fig. 1 - 4*, respectively. In both phenolic sources, the total phenolic content of the extracts showed significant increase ($P<0.05$) with temperature (40 and 60°C). Higher temperature may promote an increase in solubility of phenolic compounds and an increase of their diffusion rate into the solvent bulk, increasing the mass transfer rate [29, 30]. Also, the antioxidant activity values of both extracts

affected significantly (P<0.05) by the extraction temperature as well as by the use of enzyme; using enzyme in extraction higher antioxidant activity values result due to cell wall constituents hydrolysis and degradation, improving thus the release of phenolic compounds [10].

The total phenolic compound content of the olive kernel extracts ranged from 6.35 to 9.92mg GAE/g olive kernel (dw) in CE and from 8.41 to 11.41mg GAE/g olive kernel (dw) in CEE; respectively, from 8.96 to 10.61mg GAE/g olive kernel (dw) in MAE, and from 4.98 to 10.47mg GAE/g olive kernel (dw) in MAEE. Applying CEE at 60°C, the maximum total phenolic content was obtained (11.41mg GAE/g olive kernel (dw)) with maximum antioxidant activity (22.23 g olive kernel (dw)/ g DPPH) (*Fig.1 and 3*).

Similarly, the values of the total phenolic content of olive leaves extracts ranged from 25.36 to 30.15mg GAE/g olive leaves (dw) in CE and from 23.98 to 34.53mg GAE/g olive leaves (dw) in CEE, while from 22.67 to 28.00mg GAE/g olive leaves (dw) in MAE and from 26.43 to 29.52mg GAE/g olive leaves (dw) in MAEE. Applying CEE at 60°C, the maximum total phenolic content was obtained (34.53mg GAE/g olive leaves (dw)) with maximum antioxidant activity (5.66g olive leaves (dw)/ g DPPH) (*Fig. 2 and 4*).

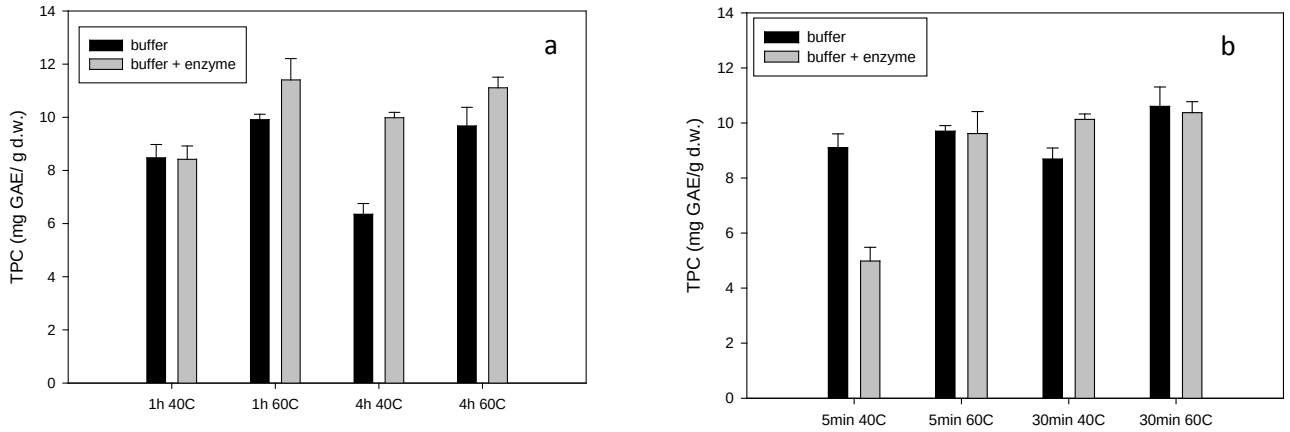

Fig. 1: Effect of extraction temperature and time on total phenolic content of olive kernel extracts using CE/CEE (a) and MAE/MAEE (b).

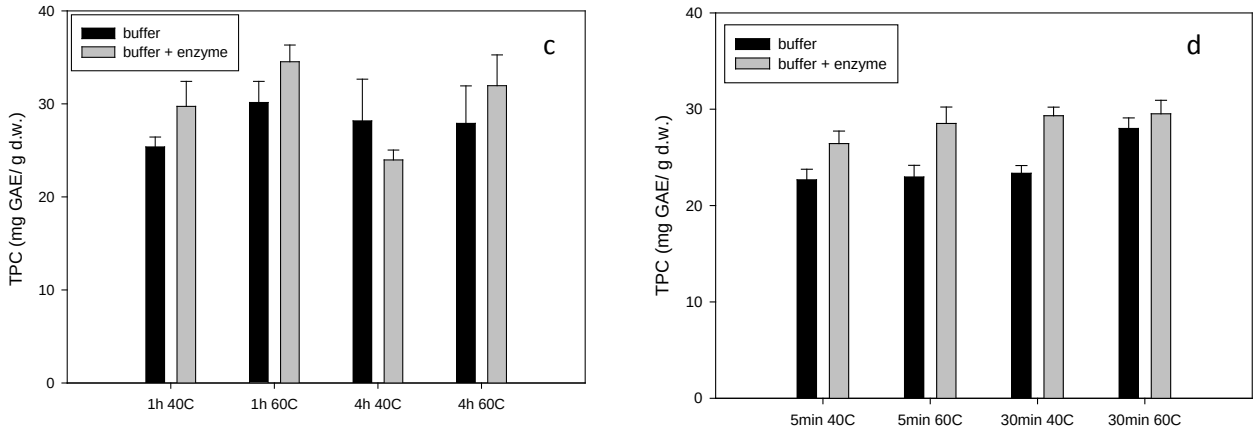

Fig. 2: Effect of extraction temperature and time on total phenolic content of olive leaves extracts using CE/CEE (c) and MAE/MAEE (d).

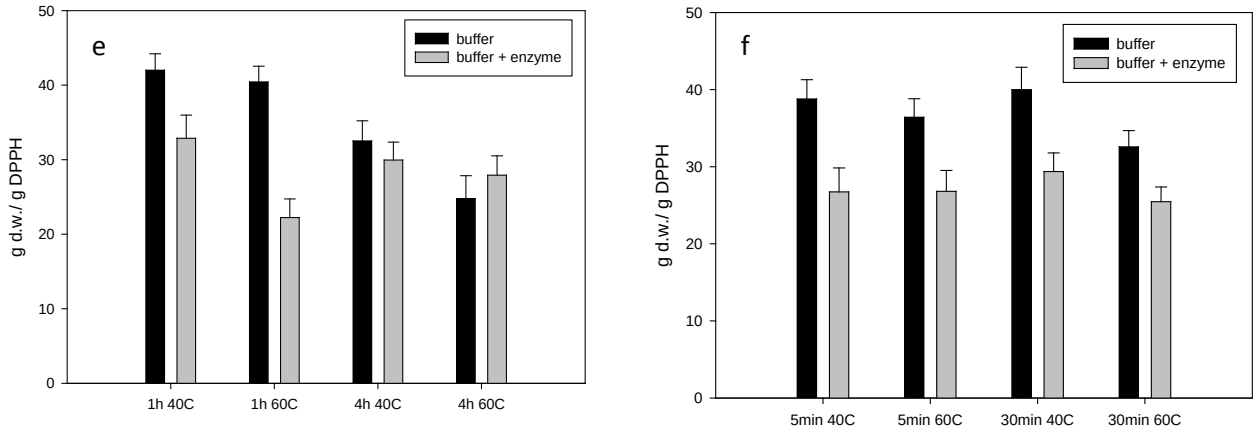

Fig. 3: Effect of extraction temperature and time on antioxidant activity of olive kernel extracts using CE/CEE (e) and MAE/MAEE (f).

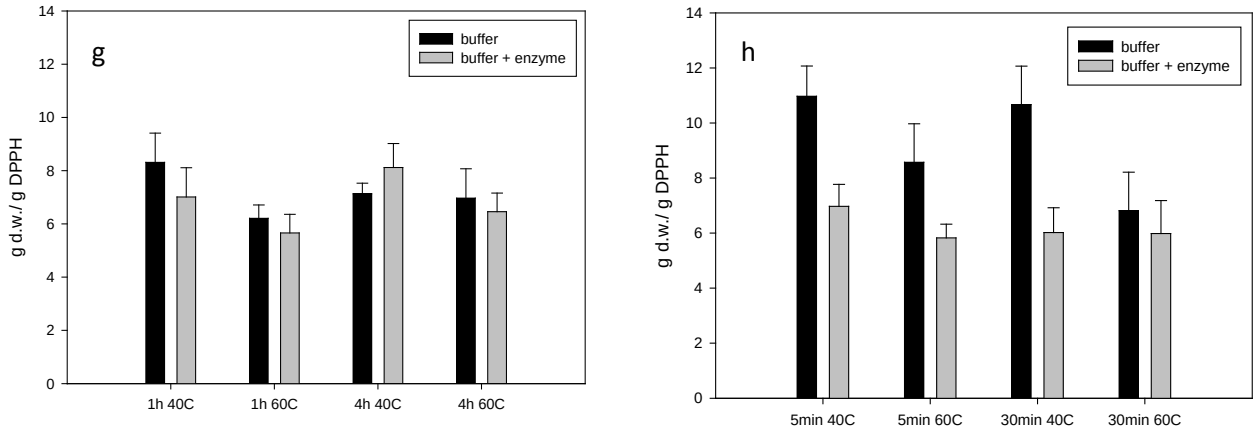

Fig. 4: Effect of extraction temperature and time on antioxidant activity of olive leaves extracts using CE/CEE (g) and MAE/MAEE (e).

3.2 HPLC analysis of phenolic compounds of olive kernel and leaves extracts

Tables 2-5 show the HPLC phenolic compounds profile of extracts of olive kernel and leaves using CE/CEE and MAE/MAEE. The phenolic profiles of extracts either from olive kernel or leaves were almost similar independently the extraction method applied. In all extraction methods, increasing the temperature and time of extraction the phenolic recovery yield increased for both phenolic sources. It must be noted that the use of enzyme contributed to more enriched phenolic profiles of the extracts.

In olive kernel extracts, the predominant phenolic compounds were hydroxytyrosol, oleuropein, luteolin and rutin, while a very small amount of caffeic acid and vanillin was also present. Noticed that oleuropein was not detected in CEE extracts. In olive leaves extracts, the predominant phenolic compounds were oleuropein, hydroxytyrosol, vanillin and rutin, while a very small amount of caffeic acid was present. Moreover, in the extracts obtained by conventional or microwave extractions without enzyme using (CE and MAE), luteolin was not detected.

3.3 Characterization of encapsulated products

Olive kernel and leaves' phenolic extracts showed high antioxidant activity were encapsulated in maltodextrin by freeze-drying technique and the results are presented in *Table 6 and 7*. The microencapsulation efficiency (MEE %) varied from 82.39 to 92.12% and from 87.98 to 91.06% for olive kernel and leaves encapsulated products, respectively. The extracts of olive kernel and leaves obtained by CE, exhibited the highest 92.12 and 91.06% values of MEE, respectively indicating thus a better protection offered by the encapsulating agent.

Finally, the properties of the phenolic encapsulated products which are of interesting for their incorporation in food systems were examined. The moisture content values were within the expected range for such polyphenols powdered products (0.39 - 0.58% for olive kernel extracts and 0.99 - 1.88 % for olive leaves products), as reported by other researchers [25]. Regarding the hygroscopicity, it was reduced after encapsulation, enhancing the stability of the encapsulated products (*Table 7 and 7*). The solubility of the encapsulated products was reinforced; in particular, arriving from 80.58 to 61.13% and from 71.52 to 64.93%, for olive kernel and leaves polyphenols, respectively, reached 98.43 to 90.60% and 97.41 to 91.28% after encapsulation. Similar observations of improved moisture content, hygroscopicity and solubility values have been reported for encapsulated products by our previous work [31].

4. Conclusions

By conventional (CE) or microwave (MAE) extraction of olive kernel and leaves extracts with or without enzyme (CEE, MAEE) the maximum phenolic content with high antioxidant activity was obtained applying CEE at 60°C for 1h. It was observed that the higher the temperature, the higher the phenolic content and antioxidant activity of both olive kernel and leaves extracts by CE/CEE or by MAE/MAEE. HPLC phenolic profiles revealed that the use of enzyme enriched the phenolic content of the extracts. The predominant phenolic compounds in both olive kernel and leaves extracts were oleuropein, hydroxytyrosol and rutin. Although CE requires simple and inexpensive equipment, MAE is a promising environmental-friendly technology for extraction in the food industry involving shorter time as well as reducing solvent consumption and energy input. Finally, by encapsulation of the phenolic extracts permit us to assume the development of stable polyphenols products from olive kernel and leaves phenolic extracts. Microencapsulation products will enable high polyphenols MEE%, and facilitate their incorporation in food products. This study also revealed that olive kernel and leaves can be successfully used as natural, inexpensive sources of phenolic compounds and antioxidant activity and can be used in pharmaceutical, nutraceutical, and functional food products. Importantly, the use of olive kernel and leaves for such products reduces the impact of olive oil production on the environment.

Table 2. Phenolic compounds content (assayed using HPLC) of olive kernel extracts using CE.[a]

Phenolic compound	Buffer pH=4.5				Buffer pH=4.5 and enzyme			
	1h 40°C[b]	1h 60°C[b]	4h 40°C[b]	4h 60°C[b]	1h 40°C[b]	1h 60°C[b]	4h 40°C[b]	4h 60°C[b]
Oleuropein	159.8±30,2	232.6±12.0	158.9±32.2	345.2±42.5	n.d.	n.d.	n.d.	157.3±27.8
Hydroxytyrosol	261.3±4.6	273.79±2.82	250.60±4.17	337.34±0.45	296,69±3.48	234.04±1.28	305.52±4.78	314.70±3.95
Caffeic acid	2.0±0.1	6.3±0.1	2.9±0.1	12.9±0.1	7.6±0.1	8.8±0.1	10.0±0.1	18.0±0.1
Vanillin	12.6±0.2	18.4±2.2	12.9±0.7	25.3±0.5	14.9±0.8	16.5±0.5	23.4±2.33	28.2±2.5
Rutin	48.3±3.2	56.2±1.8	90.2±9.9	91.1±4.9	140.7±20.9	141.6±3.4	176.8±9.7	216.6±7.8
Luteolin	513.4±0.3	530.6±11.1	565.6±2.2	973.1±27.7	809.1±40,4	526.4±53.6	678.7±18.1	667.1±3.6

[a] Mean value of three replicates ± standard deviation.

[b] µg/g d.w.

n.d.: not detected.

Table 3. Phenolic compounds content (assayed using HPLC) of olive kernel extracts using MAE.[a]

Phenolic compound	Buffer pH=4.5				Buffer pH=4.5 and enzyme			
	5min 40°C[b]	5min 60°C[b]	30min 40°C[b]	30min 60°C[b]	5min 40°C[b]	5min 60°C[b]	30min 40°C[b]	30min 60°C[b]
Oleuropein	157.7±26.3	160.0±25.0	193.6±9,9	180.6±2.8	n.d.	138.1±4.7	182.5±17.3	193.5±34.7
Hydroxytyrosol	203.9±0.9	224.6±1.1	255.6±6.3	202.9±0.2	281.2±15.5	223.5±8.1	328.5±8.1	374.8±19.2
Caffeic acid	2.1±0.1	2.1±0.0	3.8±0.7	1.6±0.0	7.2±0.8	6.3±0.2	8.1±0.4	10.8±1.1
Vanillin	14.3±4.1	10.8±0.8	14.4±4.1	12.1±3.1	12.2±2.6	9.1±2.0	15,4±4.8	16,3±4.2
Rutin	89.0±3.1	80.3±1.7	85.5±1.2	85.8±3.6	164.0±5.2	116.6±2.7	135,8±0.6	200.9±11.1
Luteolin	750.1±7.6	571,4±7.7	870.2±15.8	731.7±2.4	948.0±1.4	1324.9±28.6	1204.9±87.6	1754.9±181.9

[a] Mean value of three replicates ± standard deviation.

[b] µg/g d.w.

n.d.: not detected.

Table 4. Phenolic compounds content (assayed using HPLC) of olive leaves extracts using CE.[a]

Phenolic compound	Buffer pH=4.5				Buffer pH=4.5 and enzyme			
	1h 40°C[b]	1h 60°C[b]	4h 40°C[b]	4h 60°C[b]	1h 40°C[b]	1h 60°C[b]	4h 40°C[b]	4h 60°C[b]
Oleuropein	n.d.	13649.6±171.1	n.d.	8908.5±88.7	749.2±1059.6	15427.2±154.3	3421.5±45.3	11290.3±280.2
Hydroxytyrosol	878.2±41.67	915.0±354.6	735.6±25.2	645.3±35.2	646.5±84.7	1218,2±363.1	1267.3±5.1	1295.4±278.1
Caffeic acid	53.8±0.26	62.9±6.6	30.1±1.1	19,6±2.9	17.1±3.2	18.1±9.1	27.7±4.4	21.5±3.0
Vanillin	119.6±8.35	614.4±2.8	163,5±1,8	522.7±7.0	n.d.	861.6±2.7	749.5±13.3	448.4±25.3
Rutin	2244.6±82.96	3824.1±152.8	1806,7±1.4	3465.0±34.5	1767.4±61.6	3110.7±38,8	706.4±31.4	2193.0±40.3
Luteolin	n.d.	n.d.	56,8±6.1	41.1±8.0	73.6±7.3	140.9±1.2	110.1±6.5	113.0±10.4

[a] Mean value of three replicates ± standard deviation.
[b] µg/g d.w.
n.d.: not detected.

Table 5. Phenolic compounds content (assayed using HPLC) of olive leave extracts using MAE.[a]

Phenolic compound	Buffer pH=4.5				Buffer pH=4.5 and enzyme			
	5min 40°C[b]	5min 60°C[b]	30min 40°C[b]	30min 60°C[b]	5min 40°C[b]	5min 60°C[b]	30min 40°C[b]	30min 60°C[b]
Oleuropein	950.8±1344.7	1872.8±1181.5	1625.7±460.4	10602.2±563.9	8905,0±2351.7	14354.3±1598.4	8469.7±5802.9	12607.2±1631.7
Hydroxytyrosol	953.7±26.4	1063.0±29.0	1052.2±67.9	555.9±786.2	660.0±933.3	680.0±960.5	1394.9±136.0	770.0±1082.0
Caffeic acid	18.8±0.6	23.9±0.0	20.7±2.3	23.5±3.4	91.0±9.4	51.4±10.9	72.9±36.3	52.8±11.9
Vanillin	54.9±77.7	150.7±26.5	121.2±9.1	397.7±28.3	469.5±78.7	762.3±18.9	746.9±69.6	756.0±30.7
Rutin	2102.9±75.1	2906.6±253.3	2434.6±182.2	3768.5±292.5	2324,1±155,3	2672.7±12.5	2406.2±103.7	1839.3±17.3
Luteolin	n.d.	n.d.	n.d.	n.d.	148.0±5.4	164.2±22.3	165.8±29.4	160.8±40.9

[a] Mean value of three replicates ± standard deviation.
[b] µg/g d.w.
n.d.: not detected.

Table 6. Properties of the encapsulated products of olive kernel extracts.

Sample[a]	MEE%[b]	Moisture	a_w[c]	Solubility	Hygroscopicity	%DPPH rem
KCE	90.12±1.87	0.99±0.01	0.16±0.00	97.41±0.11	22.88±0.13	79.87±3.21
KCEE	88.89±1.46	1.85±0.04	0.17±0.00	96.07±0.10	22.83±0.14	82.51±3.02
KME	87.58±1.59	1.88±0.02	0.18±0.00	95.32±0.09	23.19±0.09	85.14±2.78
KMEE	82.39±1.53	1.49±0.04	0.16±0.00	91.28±0.07	6.69±0.05	89.45±2.49
MD	-	6.28 ± 0.03	0.98±0.00	88.21 ± 0.10	24.95 ± 0.09	-
A	-	3.58±0.06	0.36±0.00	71.52±0.13	67.28±0.12	-
B	-	4.58±0.08	0.36±0.00	64.93±0.09	65.02±0.13	-
C	-	4.46±0.09	0.39±0.00	68.16±0.10	64.59±0.04	-
D	-	3.89±0.07	0.37±0.00	71.58±0.12	64.87±0.07	-

[a] un-encapsulated extracts: A:CE, B: CEE, C: MAE, D: MAEE
[b] MEE%: microencapsulation efficiency (%)
[c] a_w: water activity at 25°C.

Table 7. Properties of the encapsulated products of olive leaves extracts.

Sample[a]	MEE%[b]	Moisture	a_w[c]	Solubility	Hygroscopicity	%DPPH rem
LCE	91.06±1.40	0.78±0.04	0.16±0.02	98.19±0.12	26.59±0.07	79.27±2.45
LCEE	87.98±1.35	0.39±0.02	0.18±0.01	98.43±0.10	25.80±0.08	80.46±3.21
LME	90.45±1.87	0.56±0.07	0.16±0.01	90.60±0.09	39.91±0.13	65.08±2.74
LMEE	88.27±1.45	1.47±0.02	0.19±0.02	97.43±0.12	8.30±0.12	59.96±2.58
MD	-	6.28 ± 0.03	0.98±0.00	88.21 ± 0.10	24.95 ± 0.09	-
A	-	3.64±0.08	0.36±0.00	61.13±0.09	59.06±0.09	-
B	-	2.84±0.07	0.31±0.01	75.44±0.07	67.29±0.08	-
C	-	2.98±0.07	0.35±0.00	79.14±0.10	68.29±0.09	-
D	-	3.58±0.04	0.29±0.00	80.58±0.09	64.55±0.12	-

[a] un-encapsulated extracts: A:CE, BCEE, C: MAE, D: MAEE
[b] MEE%: microencapsulation efficiency (%)
[c] a_w: water activity at 25°C.

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