View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by TUGraz OPEN Library

Journal of Food and Nutrition Research (ISSN 1336-8672) Vol. *58*, 2019, No. 1, pp. 51–62

Utilization of olive pomace as a source of polyphenols: Optimization of microwave-assisted extraction and characterization of spray-dried extract

SANJA JURMANOVIĆ – MARIO JUG – TONI SAFNER – KRISTINA RADIĆ – ANA-MARIJA DOMIJAN – SANDRA PEDISIĆ – SANJA ŠIMIĆ – JASNA JABLAN – DUBRAVKA VITALI ČEPO

Summary

Olive pomace lags behind in significant amounts after production of olive oil. Although it contains various biologically active compounds, its potential as a valuable source of functional ingredients has not been fully utilized. The main reasons are shortages of existing extraction and drying methods that result in poor quality extracts, unsuitable for further testing and processing. In this study, optimal conditions for sustainable, fast and efficient microwave-assisted extraction of pomace phenols has been proposed. The proposed procedure is based on applying the power of 700 W for 10 min in a closed vessel system, using 20% ethanol as the extraction solvent. For ensuring satisfactory yields and powder characteristics during spray drying, hydroxypropyl-β-cyclodextrin was used and inlet temperature was set to 130 °C with the aspirator set at 100 %. Dry extracts obtained under optimized conditions contained significantly higher amounts of hydroxytyrosol (1 216 $mg\cdot kg^{-1}$) and showed improved antioxidant activity in β-carotene model system (214.7 $g\cdot kg^{-1}$) and DNA-scisson model system (minimal inhibitory concentration was 52.9 $mg\cdot l^{-1}$) in comparison to conventionally obtained extracts. Presented results enable objective assessment of the advantages of microwave extraction in processing olive waste into a functional food ingredient.

Keywords

olive pomace; microwave-assisted extraction; spray drying; scanning electron microscopy; antioxidant activity

The well known health benefits of olive oil-rich diet can, at least partially, be attributed to potent anti-inflammatory, antioxidative and antimicrobial effects of olive polyphenols. Oleuropein, tyrosol and hydroxytyrosol are among the most prevalent polyphenolic active constituents of olive oil [1]. Their potential as biologically active compounds with potential health benefits was recognized by competent authorities, such as European Food Safety Authority [2]. Therefore, investigating the possibilities of their recovery from alternative sources and subsequent incorporation in functional food, food supplements or cosmetic products is of considerable interest [3].

Given the fact that only 2% of phenolic compounds are transferred to the oil during olive oil production [3], olive pomace that remains after oil production has recently been targeted as a valuable source of phenolic compounds, in particular hydroxytyrosol and tyrosol. Extraction of phenolic compounds from olive pomace is usually carried out by conventional solvent extraction (CE) after removal of residual oil [3]. Conventional extraction procedures are often time-consum-

Sanja Jurmanović, Kristina Radić, Dubravka Vitali Čepo, Department of Food Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia.

Mario Jug, Department of Pharmaceutical Technology, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia.

Ana-Marija Domijan, Jasna Jablan, Department of Analytical Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia.

Toni Safner, Department of Plant Breeding, Genetics and Biometrics, Faculty of Agronomy, University of Zagreb, Svetošimunska 1, 10000 Zagreb, Croatia.

Sandra Pedisić, Department of Food Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Pijerottijeva 6, 10000, Zagreb, Croatia.

Sanja Šimić, Graz Centre for Electron Microscopy, Steyrergasse 17, 8010 Graz, Austria.

Correspondence author

Dubravka Vitali Čepo, e-mail: dvitali@pharma.hr

© 2019 National Agricultural and Food Centre (Slovakia)

ing, require large amounts of (organic) solvents, lack selectivity and result in thermal decomposition of thermolabile compounds.

Microwave-assisted extraction (ME) is a relatively novel extraction technique that combines microwaves and traditional solvent extraction resulting in shorter extraction times, less solvent consumption, higher extraction rate and lower cost, in comparison to the traditional method of extraction. Due to mentioned advantages, it was used for efficient and sustainable extraction of various compounds from various matrices, in particular natural products [4]. However, until now it has not been applied for the extraction of polyphenols from olive pomace. Various factors can influence efficiency of ME, therefore optimization of the extraction process parameters is required to retain the maximum amount of target compounds.

Even in the case of availability of efficient extraction procedures, further utilization of plant or food extracts as food additives, nutraceuticals or functional food ingredients is often limited due to poor stability and organoleptic properties of obtained extracts that appear as sticky, highly hygroscopic materials often with penetrating smell. In addition, high hydrophilicity, loss of the constituents due to evaporation and degradation or oxidation processes taking place during the storage period may significantly impair the nutritional or therapeutical value of the extracts prepared [5]. Careful selection of drying technique as well as the type and the amount of the excipient can be crucial for achieving target characteristics of the final product. Spray drying is a widely used drying technique that is also successfully applied for encapsulation of valuable food or plant components into stable functional food ingredients ensuring adequate powder consistency and protecting bioactive compounds of the extracts against the damage during drying and during the storage period. In this view, spray drying is a technique particularly appropriate for heat-sensitive components such as polyphenols [6].

In this work, an efficient and sustainable ME method for the extraction of polyphenols from olive pomace was developed by using multifactorial optimization design. The content of hydroxytyrosol derivatives (*HTDC*), calculated as the sum of the major olive pomace polyphenols hydroxytyrosol and tyrosol, antioxidant activity and total reducing capacity (*TRC*) were used as the response variables and the yields were compared to those obtained by CE. Spray drying procedure was optimized in terms of the type of the carrier material, inlet temperature and aspirator power in order to achieve the highest polyphenol yields and antioxidant activity, the lowest moisture content and optimal physico-chemical characteristics of the final product. To additionally assess the advantages of ME, functional characterization of obtained powders was conducted in terms of the content of particular biologically active compounds and antioxidant activity in different biological model systems.

MATERIALS AND METHODS

Plant material

Frozen olive pomace was obtained from several two-phase olive mills in Croatia during seasons 2016/2017 and 2017/2018. It was thawed and dried for 36 h at 50 °C in heat oven sterilizer (Inkolab, Zagreb, Croatia). The dried material was then mulched, kernels being removed by sieving (pore size 1.25 mm) and the remaining material was milled to powder. Remaining pomace oil was removed by using petroleum ether in a Soxhlet apparatus. Defatted, dry olive pomace was used for the development of ME and CE.

Chemicals

Ethanol (96 %; Gram-Mol, Zagreb, Croatia) was mixed with distilled water in order to obtain ethanol-water mixtures for the optimization of ME. Acetonitrile (≥ 99.9 %), methanol (≥ 99.9 %) and formic acid used for the preparation of chromatographic analysis were from Sigma-Aldrich (St. Louis, Missouri, USA). Reference standards of phenolic compounds (2-(4-hydroxyphenyl) ethanol (tyrosol, > 99.5 %), 3-hydroxytyrosol (≥ 98 %) and oleuropein (≥ 98 %) were purchased from Sigma-Aldrich. Folin-Ciocalteu reagent, sodium carbonate, gallic acid, butyl hydroxy anisole (BHA), phosphate buffer saline (PBS), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), randomly methylated β-cyclodextrin (RAMEB), hydroxypropyl-β-cyclodextrin (HPβCD), 3',6'-dihydroxyspiro[isobenzofuran-1[3H],9'[9H]-xanthen]-3-one (fluorescein), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and pBR322 plasmid DNA from *Escherichia coli* RRI were purchased from Sigma-Aldrich. GelRed dye for gel electrophoresis was obtained from Biotium (Fremont, California, USA). All other chemicals were from Kemika (Zagreb, Croatia). Deionized water (electrical resistivity 18 mΩ) used throughout the experiments was obtained from Millipore-MiliQ water purification system (Merck, Darmstadt, Germany).

Investigation design

Investigation was conducted in three phases. During the first phase, ME procedure was optimized for efficient extraction of polyphenols from olive pomace. All analyses were conducted with freshly prepared liquid extracts within 24 h. During the second phase, spray-drying procedure was optimized for obtaining dry extracts with satisfactory yields and physico-chemical characteristics. In the last phase of the investigation, two olive pomace extracts were prepared by previously optimized ME and CE, spray dried under optimized conditions and additionally characterized.

Extraction of olive pomace polyphenols

CE was conducted according to the previously optimized method [7] using a closed-vessel microwave extraction system (Milestone, Sorisole, Italy). The aim was to determine optimal operational parameters for obtaining the highest possible *TRC*, antioxidant capacity equivalent to Trolox (*TEAC)* and the highest possible *HTDC*, since hydroxytyrosol derivatives (HTD) are known to be associated with the majority of health-promoting properties of olive-derived food products [2]. For that purpose, the influence of the extraction solvent (20% ethanol, 58% ethanol and 96% ethanol), microwave power (400–700 W) and extraction time (from 1 min to 10 min) were investigated. Ethanol-water mixtures were used as safe and efficient "green" solvents. Ranges of experimental domains were based on preliminary experiments (our unpublished data). All experiments were conducted under a constant liquid-to-solid ratio (50 ml to 1 g).

The experiment was designed as full factorial with two replicates of 2^3 runs consisting of combinations of minimum and maximum levels of each of three factors (*TRC*, *TEAC* and *HTDC*) using JMP version 13 software (SAS Institute, New York, New York, USA). To provide an estimate of variability within the design space and to allow the testing for curvature between or within factors, four replicates of central point were included in the design with the aim of minimizing the risk of non-linear relationship in the middle of intervals and determination of confidence intervals. In total, 20 runs under 9 experimental conditions were performed to collect the data (Tab. 1).

Tab. 1. Experimental design with the observed responses using microwave-assisted extraction and responses obtained for conventional extraction.

Run	Independent variable			Responses		
	A	*B*	*C*	Y_1	Y_2	Y_3
	Microwave power [W]	Extraction time [min]	Ethanol concentration [%]	*TRC* [g·kg⁻¹]	*TEAC* [g·kg⁻¹]	*HTDC* [g·kg⁻¹]
1	700	1	96	11.85	15.08	0.41
2	700	1	96	10.90	13.46	0.34
3	550	5.5	58	20.73	20.64	1.68
4	550	5.5	58	19.93	20.40	1.60
5	550	5.5	58	19.86	20.70	1.64
6	550	5.5	58	19.38	21.32	1.68
7	400	10	20	21.39	22.87	1.33
8	400	10	20	20.05	21.11	1.20
9	400	1	96	11.29	12.25	0.38
10	400	1	96	9.87	11.30	0.34
11	400	1	20	11.96	13.69	0.35
12	400	1	20	12.07	13.15	0.38
13	400	10	96	14.00	15.56	1.06
14	400	10	96	14.58	14.05	1.10
15	700	10	20	48.43	44.70	3.76
16	700	10	20	50.18	45.42	3.79
17	700	10	96	20.96	15.98	1.51
18	700	10	96	19.90	16.56	1.39
19	700	1	20	14.18	17.60	0.47
20	700	1	20	14.17	17.78	0.47
Conventional extraction				21.12	17.11	2.25

TRC – total reducing capacity (expressed as gallic acid equivalents), *TEAC* – Trolox-equivalent antioxidant activity (expressed as Trolox equivalents), *HTDC* – hydroxytyrosol derivatives content.

Spray-drying procedure

Aliquots of 130 ml of olive pomace extracts prepared with either HPβCD or RAMEB were subjected to spray drying (Büchi, Flavil, Switzerland), the instrument being equipped with a standard 0.5 mm two-fluid nozzle and a high-performance cyclone. The compressed air flow rate used for atomization was maintained at 600 normal litres per hour and the sample flow rate was set to 6.5 ± 0.5 ml·min^{-1}. Those parameters were kept constant in all preparations, while temperature of the drying air and the aspirator rate varied as follows: inlet air temperature was 130 °C or 160 °C and aspiration rate was 75 % or 100 %. Optimal drying conditions were chosen based on maximal product yields, *HTDC*, *TEAC* and the lowest moisture content. Product yield was expressed as the mass of powder obtained by spray drying relative to the mass of powder obtained by vacuum drying (maximal yield). Furthermore, impact of the drying process on powder characteristics, namely, particle size, morphology and bulk as well as tapped density, was also investigated.

Powder characterization

Moisture content in the obtained spray-dried products was determined gravimetrically using moisture analyser MLS (Kern, Balingen, Germany).

For the determination of particle mean diameter and particle size distribution, sample powders were dispersed on glass slides and observed using an Olympus BH-2 optical microscope (Olympus, Tokyo, Japan) equipped with a CCD camera (Ikegami Tsushinki, Tokyo, Japan) and computer-controlled image analysis system Optomax V (Optomax, Cambridge, United Kingdom). The microscopical field was scanned by video camera and the images of the scanned fields were digitalized and analysed by the Optomax V software. In all measurements, at least 10 000 particles were examined in order to obtain data reproducibility.

For scanning electron microscopic (SEM) analysis, Zeiss Ultra 55 electron microscope (Carl Zeiss, Oberkochen, Germany) equipped with energy selective backscattered detector was used. Samples were fixed on a SEM sample stub using double-sided tape and made electrically conductive by sputtering with a thin layer of gold and palladium. The images were taken operating in a second electron mode at an accelerating voltage of 5 kV. Image width ranged from 2.934 mm to 7.622 μm.

Bulk and tap densities of the spray-dried powders were measured by modifying the Pharmacopoeia test, as reported in literature [6]. The bulk and tap densities were calculated from the difference between the net weight of the plastic syringe content divided by the volume in the syringe before and after tapping, respectively. The experiments were performed in triplicate for each spray-dried replicate ($n = 6$) and results were expressed as mean ± standard deviation.

Chromatographic determination of hydroxytyrosol, tyrosol and oleuropein

Hydroxytyrosol and tyrosol were determined by HPLC using Waters 2695 system (Waters, Milford, Massachusetts, USA) coupled to a 2475 multi fluorescence detector according to the method of Tan et al. [8] with some modifications. Zorbax Eclipse Plus C18 column (250 mm × 4.6 mm, particle size 5 μm, Agilent Technologies, Santa Clara, California, USA) was used for the separation of phenolic compounds. Mobile phase A (sodium acetate buffer 0.05 mol·l^{-1} sodium carbonate/0.05 mol·l^{-1} acetic acid 1:1 adjusted to pH 5.0 with 0.1 mol·l^{-1} acetic acid) and mobile phase B (acetonitrile) were used for elution at a flow rate of 1.0 ml·min^{-1}. The fluorescence detector was set at the excitation wavelength of 281 nm and emission wavelength of 316 nm. Identification of hydroxytyrosol and tyrosol was carried out by the comparison of the retention times of pure standards. Analytes were quantified using calibration curves obtained with mixtures of standards in the concentration range of 1–81 mg·l^{-1} at five concentration points.

Oleuropein was analysed by ultra-performance liquid chromatography-electrospray-ionization-tandem mass-spectrometry, using Agilent series 1290 coupled to an triple quadrupole mass spectrometer (6430) with electrospray ionization ion source (Agilent Technologies). Zorbax Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 μm particle size) was used and the solvent composition and gradient conditions used were as described previously by Lopez de las Hazas et al. [9] with some modifications: instead of 0.2% acetic acid as solvent A, 0.1% formic acid was used, and flow rate was reduced to 0.2 ml·min^{-1}. The mass detector parameters were as follows: capillary voltage 4 000 V in positive and 3 500 V in negative acquisitions, drying gas temperature 300 °C, gas flow rate 11 l·min^{-1}. Nitrogen was used as the nebulizing and collision gas. Oleuropein was identified based on mass spectra and retention time compared with a standard. Quantification was performed using the external standard method.

Determination of antioxidant activity

For determination of antioxidant activity,

spray-dried samples were analysed in two replicates (n = 4) and results were expressed as mean ± standard deviation. One-way analysis of variance or Student t-test were used for the comparison of the obtained data. Differences were considered significant if $p < 0.05$.

TRC was determined by a colorimetric assay based on a modified procedure initially described by SINGLETON and ROSSI [10]. The reaction mixture consisted of 200 μl of adequately diluted extract or deionized water (blank), 1.35 ml of deionized water and 150 μl of Folin-Ciocalteu reagent. Reaction mixture was vortexed, 1.5 ml of 6% Na_2CO_3 was added and shaken in a water bath for 30 min at 50 °C. After cooling to room temperature, absorbance was measured at 725 nm. Calibration curve was prepared with gallic acid (1–150 mg·l^{-1}) and *TRC* was expressed as gallic acid equivalents (GAE).

TEAC was determined by a colorimetric assay based on a modified ABTS cation radical ($ABTS^{+\bullet}$) assay initially described by RE et al. [11]. $ABTS^{+\bullet}$ solution was prepared by 7 mmol·l^{-1} aqueous ABTS solution reacting with 2.45 mmol·l^{-1} potassium persulfate solution in the dark at 4 °C for 12 h. After reaction, $ABTS^{+\bullet}$ solution was diluted with distilled water to give an absorbance of (0.70 ± 0.02) at 732 nm. The reaction mixture consisted of 100 μl of adequately diluted extract and 1167 μl $ABTS^{+\bullet}$ solution. Absorbance of the samples was measured at 732 nm, after 3 min of reaction. The percentage of quenching the absorbance was calculated according to the equation [1]:

$$\Delta A = \frac{A_0 - A_3}{A_0} \times 100 \quad (1)$$

where ΔA is the percentage of absorbance quenching, A_0 is the initial absorbance and A_3 is absorbance of the reaction mixture after 3 min.

Calibration curve was generated by plotting different Trolox concentrations (0–40 g·l^{-1}) against respective absorbance quenching percentages (R^2 = 0.9993). Antiradical efficiency was expressed as Trolox equivalents (TE).

Oxygen radical absorbance capacity (*ORAC*) was determined according to the procedure described by OU et al. [12] using fluorescein as the fluorescent probe. The assay was performed in a 96-well microplate and measured by Victor X3 Multilabel Plate Reader (Perkin Elmer, Waltham, Massachusetts, USA). Briefly, 150 ml of 5 μmol·l^{-1} fluorescein was added to each well of a black microplate and mixed with 25 μl of phosphate buffer (75 mmol·l^{-1}, pH 7.0; blank), Trolox standard (6.25–100 μmol·l^{-1}) or adequately diluted sample. Reaction mixture was incubated at 37 °C for 10 min and oxidation reaction was initiated by addition of 25 μl of AAPH (150 mmol·l^{-1} in phosphate buffer). The fluorescence of the reaction mixtures (excitation at a wavelength of 493 nm, emission excitation at a wavelength of 515 nm) was plotted against time (60 min) and the average area under the curve (AUC) was calculated for each sample by integrating the relative fluorescence curve. The net AUC of the sample was calculated by subtracting AUC of the blank. Using Trolox solutions of known concentration, a standard curve was generated by plotting the net AUC and the corresponding Trolox concentration. Antioxidant activity of the samples was expressed as TE.

Antioxidant activity of extracts in β-carotene emulsion model system was determined by the method of PRIETO et al. [13]. For the preparation of β-carotene–linoleic acid emulsion, β-carotene (4 mg), linoleic acid (0.5 ml) and Tween 40 (4 g) were dispersed in 20 ml of chloroform in a round-bottomed flask, with vigorous shaking of the suspension. Chloroform was evaporated in a rotary evaporator at a temperature lower than 50 °C in a short period of time to avoid initiation of the lipid oxidation process as much as possible. A volume of 1 ml of the oily residue was mixed with 30 ml of 100 mmol·l^{-1} Briton buffer (pH 6.5, pre-heated at 45 °C) to prepare emulsion. The procedure was performed in a 96-well microplate with flat-bottom wells by combining 50 μl of the extract (sample), BHA (control) or buffer (blank) and 250 μl of emulsion. The reader device was heated to 45 °C and agitated (660 sections per minute, 2 mm amplitude). The reaction mixture was incubated for 2 h and absorption was measured at 470 nm using Victor X3 Multilabel Plate Reader. Linear regression curve was generated by plotting absorbance inhibition ratios with respective Trolox concentrations. Results were expressed as TE.

Inhibition of DNA scission in plasmid pBR322 was measured according to the procedure modified from GU et al. [14]. Briefly, 4 μl of pBR322 plasmid DNA solution (50 μg·l^{-1}), was mixed with 2 μl of extract solution (different dilutions, designated as sample), PBS (10 mmol·l^{-1}; pH 7.4; blank) or Trolox (different concentrations; control); 2 μl of AAPH solution (50 mmol·l^{-1}) or PBS (control). The final volume of the reaction mixture was brought to 10 μl with PBS. Following incubation at 37 °C for 120 min, 4 μl of loading dye solution (2.5 g·l^{-1} of bromophenol blue; 40 g·l^{-1} saccharose; 10 ml·l^{-1} GelRed dye was added to the reaction mixture and loaded onto agarose gel (7 g·l^{-1}) prepared by dissolving agarose in electrophoresis buffer (0.04 mol·l^{-1} Tris HCl, 5μmol·l^{-1}

sodium acetate, 0.04 μmol·l⁻¹ EDTA; pH 7.9). After electrophoresis, DNA bands were visualized and quantified with Image Quant LAS 500 (GE Healthcare Life Sciences, Chicago, Illinois, USA). Activity was assessed based on the percentage of DNA remaining in its native form after incubation with AAPH radical. Based on regression equation calculated by plotting the sample concentration in the reaction mixture (10–250 mg·l⁻¹) against the percentage of intact DNA, half-maximal inhibitory concentration (EC_{50}) was established for each analysed sample.

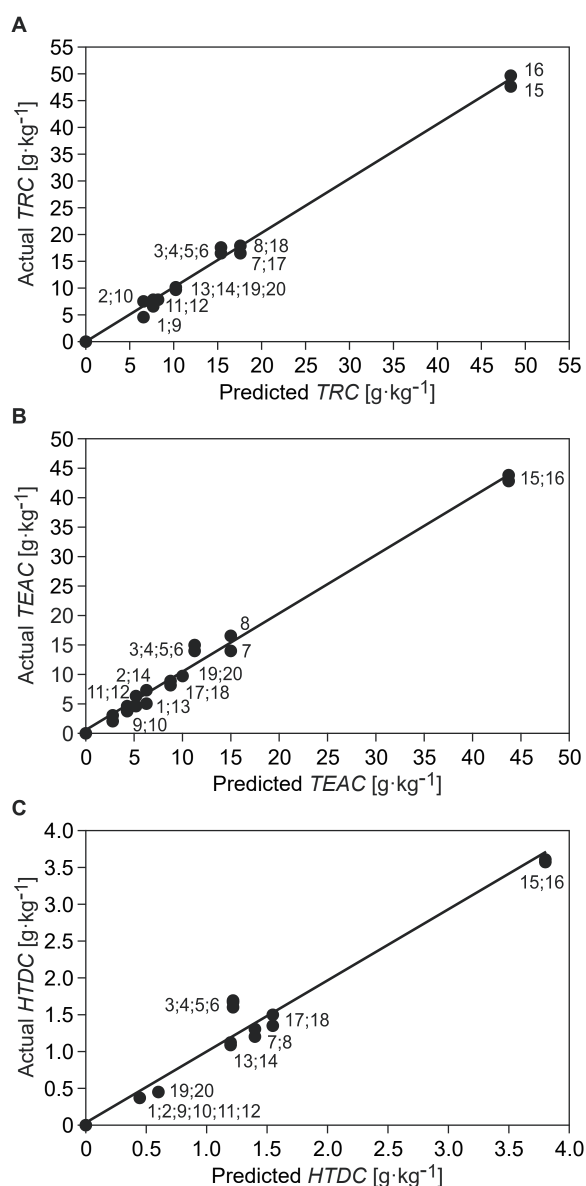

Fig. 1. Actual vs predicted responses obtained by multivariate linear regression.

A – total reducing capacity, B – Trolox-equivalent antioxidant activity, C – hydroxytyrosol derivatives content.
Numbers near particular values indicate particular runs (as presented in Tab. 1).
TRC – total reducing capacity (expressed as gallic acid equivalents), *TEAC* – Trolox-equivalent reducing capacity (expressed as Trolox equivalents), *HTDC* – hydroxytyrosol derivatives content.

RESULTS AND DISCUSSION

Optimization of microwave-assisted extraction

The most widespread technique currently used for extraction of biologically active compounds from plant matrices is CE, mainly due to its simplicity and widely available equipment. The major expected advantages of applying novel techniques are shortened duration of extraction, reduced use of toxic solvents, reduced energy consumption and increased yields. ME is a long known extraction technique that combines traditional solvent extraction with using microwaves for heating the solvents and plant tissues in the extraction process and improving the kinetics of extraction. However, until now it has not been applied to the extraction of polyphenols from olive pomace.

Analysis of data obtained after experimental testing of ME process showed that all analysed factors and their interactions had significant influence on *TRC*, *TEAC* and *HTDC*. Applying higher microwave power and longer extraction time had a positive effect on the extraction yield. Similar effect was observed regarding a higher water-to-ethanol ratio in the extraction solvent. The highest yields were obtained with the microwave power of 700 W, after 10 min of extraction using 20% ethanol (Fig. 1, Tab. 1).

Regression models for all analysed responses described high proportions of variability in the data (adjusted R^2 for *TEAC* was 0.989, for *TRC* was 0.994 and for *HTDC* was 0.931). Increasing ratio of ethanol in the extraction solvent and all interactions that included it showed a negative effect on all analysed responses ($p < 0.01$). This was not in concordance with the results of our previous investigations that focused on optimization of CE and ultrasound-assisted extraction of polyphenols from olive pomace where the use of 60% ethanol resulted in optimal extraction yields [8, 15]. The observed discrepancies can be explained by the fact that the ability of the material to absorb microwave energy is defined by its dissipation factor (ratio of dielectric loss and dielectric constant) and, therefore, the optimal extraction solvents for ME need not always be reasoned directly from those used in conventional methods [16]. Microwave power and time, as well as all interactions that did not include the extraction solvent, had positive effects on all analysed responses (Fig. 1,

Tab. 1). The statistical significance of two-way interaction power × extraction time × extraction solvent suggested that an optimal combination of tested factors existed for each of the responses.

Analyses of responses showed that the highly effective combination (700 W, 10 min and 20% ethanol) resulted in the highest *HTDC*, *TEAC* and *TRC* of obtained extracts (Tab. 1). Although it was found that higher microwave power and longer extraction time had a positive effect on the extraction yield, due to technical limitations of ME such as evaporation of extraction solvent and partial carbonization of the sample, the next step of optimization that might imply using more harsh extraction conditions was not conducted.

In comparison to CE, all responses (*TRC*, *TEAC* and *HTDC*) were significantly higher in ME extracts obtained under optimized ME conditions (132 %, 163 % and 68 %, respectively). Although both extractions were performed with the same solid-to-solvent ratio (1 g per 50 ml), ME extraction was significantly shorter (10 min vs 120 min). The observed advantages of ME over CE exceeded those obtained previously by using ultrasound-assisted extraction at extraction of polyphenols from olive pomace [15].

Optimization of spray-drying procedure

In order to optimize the quality of the spray-dried product, the velocity of compressed air used for atomization and the sample flow rate were set as constant values, while the type of cyclodextrin used as an extraction additive, inlet temperature of the drying air and the aspirator rate were varied. Total product yield, *HTDC*, *TEAC* and moisture were taken as the major quality indicators. Furthermore, powder characteristics, such as particle size, morphology and bulk as well as tapped density were measured (Tab. 2).

The outlet temperature of the drying air has the highest impact on the moisture content and the content of thermosensitive bioactive compounds in the spray-dried product. In our experiments, the outlet temperature was proportional to the inlet temperature of the drying air, air flow rate and inversely proportional to the feed spraying rate. However, as this last parameter was kept constant, variation in the inlet temperature and the aspirator setup led to the gradual increase in the outlet temperature (Tab. 2). Surprisingly, significant differences ($p < 0.01$) in the outlet temperatures obtained at the same dryer setup were observed while drying the extracts prepared with different cyclodextrins. As concentration of HPβCD and RAMEB, as well as the ethanol

Tab. 2. Influence of spray-drying parameters on quality attributes and powder characteristics of the prepared product.

Sample code	Inlet temperature [°C]	Aspirator [%]	Outlet temperature [°C]	Cyclodextrin	Product yield [%]	Moisture content [%]	*TRC* [g·kg^{-1}]	*TEAC* [g·kg^{-1}]	*HTDC* [g·kg^{-1}]	Mean spherical diameter [μm]	Bulk density [kg·l^{-1}]	Tap density [kg·l^{-1}]
S1	130	75	67 ± 1	HPβCD	78.7 ± 2.0^{ac}	6.7 ± 0.2^{a}	13.93 ± 0.56^{a}	16.93 ± 0.16^{a}	0.30 ± 0.01^{a}	3.44 ± 1.22^{a}	0.200 ± 0.001^{a}	0.363 ± 0.012^{ad}
S2			63 ± 3	RAMEB	76.4 ± 2.5^{ab}	6.2 ± 1.1^{b}	13.51 ± 0.52^{a}	16.54 ± 1.85^{a}	0.32 ± 0.01^{a}	3.58 ± 1.30^{a}	0.250 ± 0.006^{b}	0.413 ± 0.013^{b}
S3	160		76 ± 1	HPβCD	76.7 ± 4.6^{ab}	5.6 ± 0.1^{c}	13.41 ± 0.25^{a}	14.96 ± 0.22^{b}	0.32 ± 0.02^{a}	3.62 ± 1.44^{a}	0.238 ± 0.003^{c}	0.384 ± 0.007^{a}
S4			68 ± 2	RAMEB	70.2 ± 4.3^{b}	6.2 ± 0.1^{b}	12.85 ± 0.03^{b}	16.73 ± 1.09^{a}	0.32 ± 0.02^{a}	3.72 ± 1.34^{a}	0.194 ± 0.004^{a}	0.303 ± 0.021^{c}
S5	130	100	88 ± 1	HPβCD	82.4 ± 2.6^{c}	6.7 ± 0.8^{a}	13.57 ± 0.69^{a}	17.85 ± 0.57^{c}	0.31 ± 0.01^{a}	3.66 ± 1.34^{a}	0.274 ± 0.005^{b}	0.479 ± 0.026^{d}
S6			84 ± 2	RAMEB	81.6 ± 4.8^{c}	4.7 ± 0.4^{d}	12.76 ± 0.63^{b}	14.61 ± 0.03^{b}	0.17 ± 0.01^{b}	3.76 ± 1.31^{a}	0.216 ± 0.007^{a}	0.394 ± 0.038^{ab}
S7	160		91 ± 3	HPβCD	80.6 ± 3.8^{c}	6.0 ± 0.8^{b}	13.18 ± 1.15^{ab}	16.77 ± 0.21^{a}	0.29 ± 0.01^{a}	3.42 ± 1.24^{a}	0.197 ± 0.003^{a}	0.320 ± 0.028^{c}
S8			86 ± 2	RAMEB	82.6 ± 3.6^{c}	4.5 ± 0.4^{d}	13.31 ± 0.05	15.09 ± 0.50	0.21 ± 0.03	3.69 ± 1.35	0.204 ± 0.005^{a}	0.343 ± 0.008^{cd}

Results are presented as mean ± standard deviation. Different letters in the same column indicate statistically significant difference ($p < 0.05$).
TRC – total reducing capacity (expressed as gallic acid equivalents), *TEAC* – Trolox-equivalent antioxidant activity (expressed as Trolox equivalents), *HTDC* – hydroxytyrosol derivatives content, HPβCD – hydroxypropyl-β-cyclodextrin, RAMEB – randomly methylated-β-cyclodextrin.

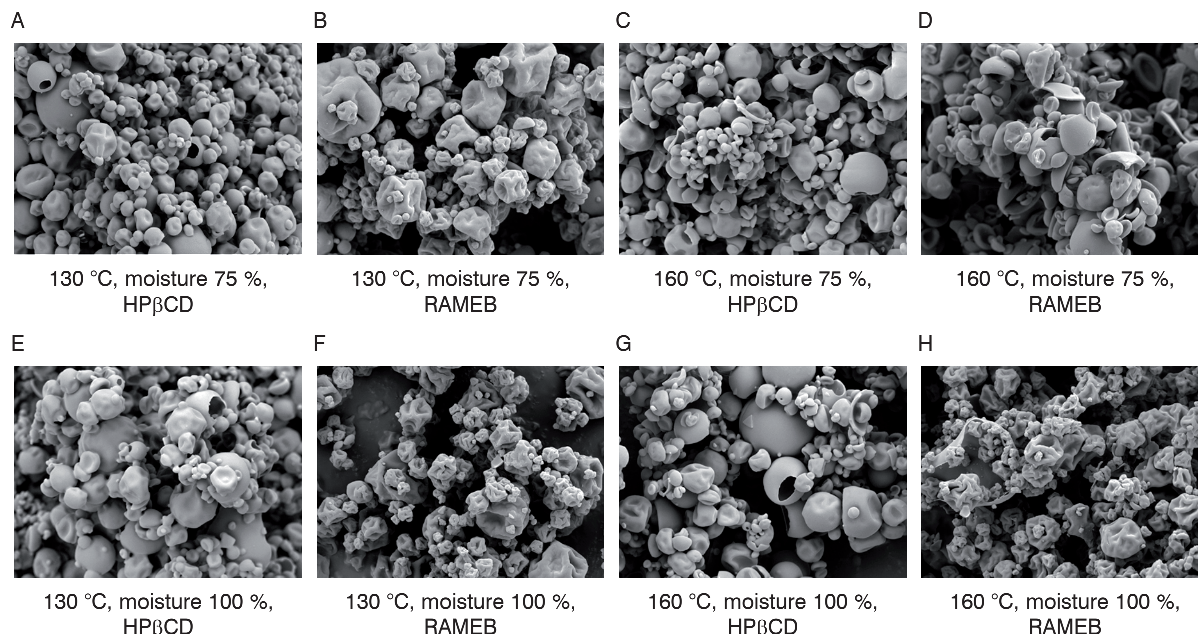

Fig. 2. Scanning electron micrographs of olive pomace extracts.

HPβCD – hydroxypropyl-β-cyclodextrin; RAMEB – randomly methylated β-cyclodextrin.

content in the spray-drying feed were equal in all cases, the observed difference could be attributed to the different thermal capacity of the carriers used.

Besides the outlet temperature, air flow rate controlled by the aspirator setup is another parameter regulating the drying process. Presented results (Tab. 2) clearly showed that the interplay of the drying temperature and drying time had a major impact on the moisture content as well as on the content of active compounds and corresponding antioxidative activity of collected samples. Surprisingly, for RAMEB-containing extracts dried at 130 °C and 160 °C with the aspirator set at 100 %, the moisture content and the content of bioactive compounds was found to be significantly lower ($p < 0.05$ in both cases) than that of the other extract. Regarding the moisture content, it should be noted that the drying air and the atmosphere in the laboratory were not conditioned. Therefore, the drying capacity of the air was not constant and the product could absorb the moisture from the atmosphere during its collection phase. However, the obtained moisture content ranging from 45.4 $g\cdot kg^{-1}$ to 66.8 $g\cdot kg^{-1}$ is favourable to maintain physical, chemical and microbiological stability of the dried product [17]. On the contrary, lower *HTDC*, *TRC* and *TEAC* indicated that RAMEB was less effective in the protection of the bioactive compounds, in particular when the drying was performed at higher temperatures of the drying medium (i.e. outlet temperatures above 80 °C).

In all cases, the observed product yields were relatively high, slightly over the typical range of 50–70 % observed for spray-drying process. It could be primarily related to the use of high efficiency cyclone and the aspirator setup. Higher drying air flow rate led to more efficient particle separation in the cyclone and consequent higher production yield (Tab. 2), while effect of the drying temperature was not statistically significant. On the contrary, Urzúa et al. [18] showed that, when inulin was used as an additive in spray-drying process of olive leafs extracts, the yield ranged from 38 % to 73 % and was significantly influenced by the drying temperature. This clearly underlines the benefits provided by the use of cyclodextrins as the spray-drying additives and is consistent with our previous findings indicating protective effects of β-cyclodextrin on carob polyphenols, during spray drying [19]. Such protective role could explain the insignificant changes in the *HTDC* and *TRC* in the samples prepared with HPβCD. Less efficient protection observed in case of RAMEB-based products could be attributed to the preferential steric compatibility and higher complex stability with HPβCD over RAMEB for tyrosol and probably also with other antioxidants present in the olive pomace [20]. All samples presented a relatively narrow log-normal particle size distribution, with 82–95 % of the particles in the size ranges between 2.0 μm and 5.0 μm. The

extent of variation in particle size is also evident from SEM results (Fig. 2). The mean spherical diameter of the particles (Tab. 2) in the prepared systems did not vary significantly ($p < 0.05$), since the solid concentration in the spray-drying feed and pressured air flow used for atomization and feed rate, which are recognized as the main parameters affecting the size of the drying droplets, were kept constant in all preparations. In all cases, SEM presented irregularly spherical hollow particles, having many shrinkages and dents on their surface (Fig. 2). That effect was pronounced in case of smaller particles and especially in case of particles prepared using RAMEB as a spray-drying additive. The observed morphology of the prepared particles indicated that the drying process was very rapid, leading to concentration of the solute at the solvent gas interface and an initial shell formation [21]. The wrinkled morphology of particles suggests that the shell formed during the early stages of the droplet drying was not rigid, probably due to high solubility of cyclodextrin derivatives in the solvent system. Due to fast drying, solvent evaporation generated vapour pressure inside the particles resulting in their inflation and possible rupture. Further drying process decreased the vapour pressure inside the forming particles, resulting in their crumpling and typical wrinkled morphology of completely dry particles. Similar particle morphology was already reported for different spray-dried extracts, such as that of sumac (*Rhus coriaria* L.) [22] or for by spray-drying encapsulated phenolic compounds from olive pomace [23], both prepared using a maltodextrin as a spray-drying additive. The crumpling phenomenon was especially pronounced in case of particles containing surface-active compounds, such as RAMEB [23].

The prepared powders presented rather low bulk density, ranging from 0.197 $mg \cdot cm^{-3}$ to 0.274 $mg \cdot cm^{-3}$ (Tab. 2), owing to the hollow structure of the formed particles. The variation in applied parameters of the spray-drying process did not affect significantly the bulk density of the prepared powder ($p < 0.05$). A similar range of bulk densities was previously observed in case of polyphenols extracted from the olive mill wastewater [24].

Based on the presented results, it appears that the most satisfactory conditions to perform the drying of the olive pomace extracts were obtained when HPβCD was used as an additive and inlet temperature was set to 130 °C with aspirator at 100 %. The obtained results clearly demonstrate the benefits of cyclodextrin application in the spray-drying process. This is in line with results of Wilkowska et al. [25] showing that the use of HPβCD yielded a lower moisture content and higher encapsulation efficiency of polyphenols of the spray-dried black chokeberry juice and wine, compared to those obtained by the use of maltodextrin or Arabic gum as spray-drying additives.

Characterization of olive pomace extract obtained under optimal extraction conditions

Significant differences in *TRC* and *TEAC* were observed between ME and CE showing twice as high values in ME for both tests (44.2 $g \cdot kg^{-1}$ GAE vs 105.9 $g \cdot kg^{-1}$ GAE and 14.0 $g \cdot kg^{-1}$ TE vs 36.0 $g \cdot kg^{-1}$ TE, respectively). These observations are in consistence with the results obtained during ME optimization (Tab. 1). Both *TRC* and *TEAC* measurements are indirect approaches that measure the reduction of inorganic oxidizing species or the reduction of a persistent radical, respectively. On the other hand, a direct method for measuring antioxidant activity is determination of *ORAC*. It is based on competitive bleaching of a probe and measures the ability of a particular sample to inhibit the consumption of a target molecule [26]. As shown in Fig. 3A, *ORAC* values in analysed samples obtained by CE and ME were 84.0 $g \cdot kg^{-1}$ TE and 90.0 $g \cdot kg^{-1}$ TE, respectively. Although *ORAC* was higher in the sample obtained by ME, observed differences were not statistically significant and certainly not so prominent as in the case of *TRC* and *TEAC*.

The capacity of a particular antioxidant to reach the place where radicals are formed is also an important aspect to consider. Therefore, antioxidants with a high antioxidant capacity in aqueous media are not necessarily efficient in compartmentalized systems [26]. This aspect of antioxidant activity can be tested by β-carotene-linoleate method, which investigates the potential of the sample to protect the β-carotene molecule in an emulsion system. It is therefore considered as more relevant approach for screening of potential natural antioxidants to be used in complex food matrices. In β-carotene-linoleate emulsion system, ME extracts showed significantly higher antioxidant activity in comparison to CE samples (214.7 $g \cdot kg^{-1}$ TE vs 166.0 $g \cdot kg^{-1}$ TE). However, the observed differences were not as dramatic as in the case of *TRC* or *TEAC* (Fig. 3B). This is in consistence with the results of Chandrasekara and Sahidi [27] who showed that roasting resulted in a dramatic increase in *TRC* of cashew nuts (by approximately 290 %) but resulted in insignificant changes of antioxidant activity in the β-carotene-linoleate model system.

Antioxidant activity of samples prepared by

ME and CE was also investigated in a model that measures their protective effects against AAPH peroxyl radical-induced DNA strand scission. DNA scission can be monitored by electrophoresis since it results in occurrence of additional circular or linear nicked DNA forms in addition to the native supercoiled form. Each form is characterized by different electrophoretic mobility and the content of a particular form can be determined based on the intensity of the particular band. As explained previously, antioxidant activity of tested extracts was determined based on the percentage of DNA remaining in its native form after incubation with peroxyl radical. Analysed extracts (at 10–250 mg·l^{-1}) produced visible protective effects against DNA scission in all investigated concentrations. EC_{50} values of CE and ME extracts were 52.9 mg·l^{-1} and 64.8 mg·l^{-1}, respectively, indicating a similar protective effect that was significantly lower but still comparable to Trolox (its EC_{50} was 3.26 mg·l^{-1}). Obtained results are comparable with recently published literature data on DNA strand scission inhibitory effect of *Camelina sativa* seeds phenolics [28].

Presented results point out that significantly higher antioxidant activity of ME extracts in comparison to CE extracts. as shown by *TRC* and *TEAC*, was not confirmed by other performed antioxidant assays. Such discrepancies between antioxidant activities obtained using different chemical methods are common and are partially due to different reaction mechanisms, reaction conditions and the variable nature of free radicals and target molecules involved. This is the reason why the combination of three or more different tests is usually recommended for more realistic antioxidant activity assessment [26]. On the other hand, in complex plant matrices, different

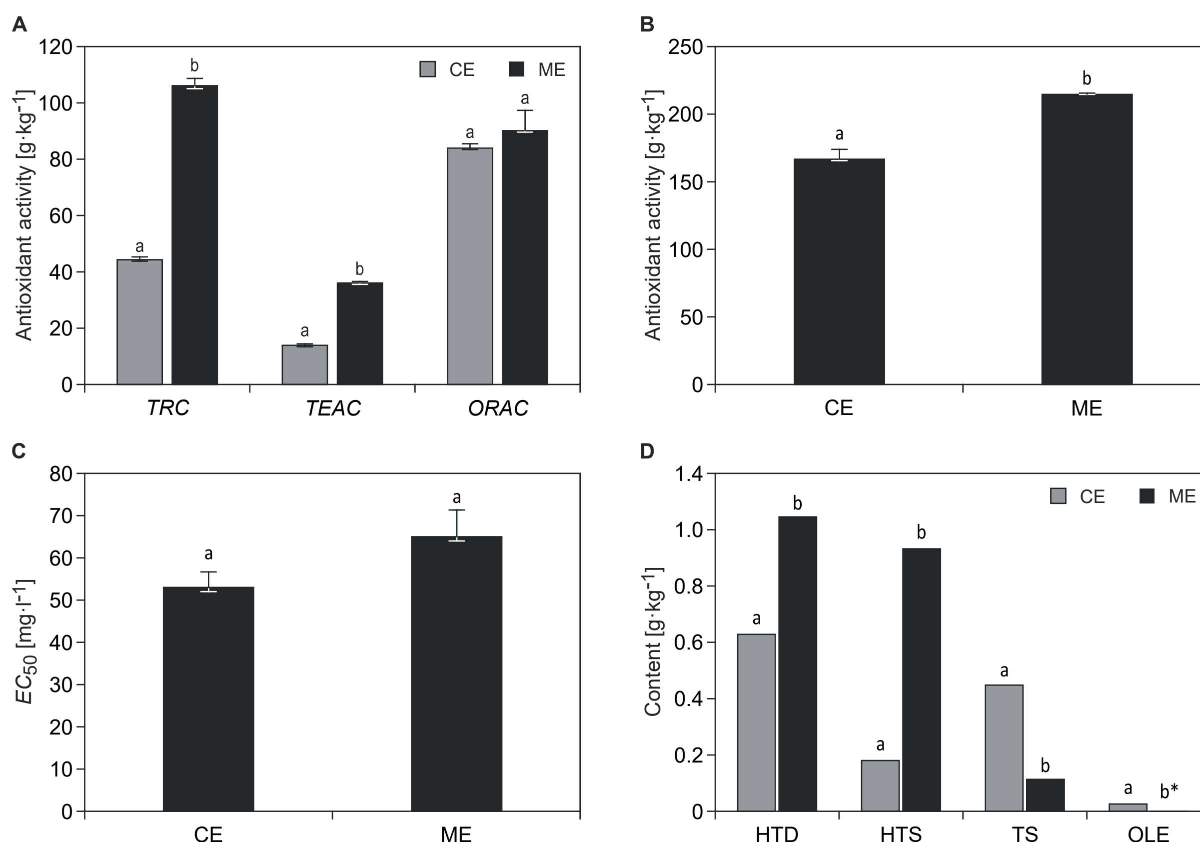

Fig. 3. Antioxidant activity of olive pomace extracts.

A – antioxidant activity in chemical-based assays, B – antioxidant activity in β-carotene-linoleate model system, C – antioxidant activity in DNA strand scission model, D – hydroxytyrosol derivatives content.
Results are expressed as mean ± standard deviation. Columns belonging to the same group of data marked with different letters belong to different statistical groups ($p < 0.05$). * – values were below the limit of detection of the applied method.
Antioxidant activity is expressed as grams of Trolox equivalents except total reducing capacity.
CE – conventional solvent extraction; ME – microwave-assisted extraction, *TRC* – total reducing capacity (expressed as grams of gallic acid equivalents), *TEAC* – Trolox-equivalent reducing capacity; *ORAC* – oxygen radical absorbance capacity, EC_{50} – half-maximal inhibitory concentration, HTD – hydroxytyrosol derivatives, HTS – hydroxytyrosol, TS – tyrosol, OLE – oleuropein.

chemical entities can be responsible for particular aspects of antioxidant activity and specific synergistic effects are also possible. Therefore, variable results obtained by using different antioxidant tests can at least partially be attributed to different chemical composition of CE and ME extracts. Typical and the most representative polyphenols present in olive pomace were hydroxytyrosol and tyrosol, while oleuropein was also present in significant concentrations, depending on the type of pomace (Fig. 3D).

It is obvious from presented data that ME extracts contained a significantly higher content of hydroxytyrosol (1216 $mg\cdot kg^{-1}$ vs 734 $mg\cdot kg^{-1}$), significantly lower content of tyrosol (131 $mg\cdot kg^{-1}$ vs 531 $mg\cdot kg^{-1}$) and only traces of oleuropein (27 $mg\cdot kg^{-1}$) in comparison to CE extracts. The observed differences can probably be attributed to more aggressive extraction conditions in the case of ME, which led to degradation of tyrosol and oleuropein, as well as probably to partial conversion of oleuropein to hydroxytyrosol [29]. However, the significantly higher hydroxytyrosol content did not contribute significantly to antioxidant activity of ME extracts in particular antioxidant tests (*ORAC*, β-carotene linoleate emulsion test, inhibition of DNA scission) indicating that antioxidant activity of complex natural extracts must be critically assessed with respect to the applied method of analysis and the targeted area of application.

Acknowledgements

This work was fully supported by Croatian Science Foundation (UIP-2014-09-9143).

REFERENCES

1. Alagna, F. – Mariotti, R. – Panara, F. – Caporali, S. – Urbani, S. – Veneziani, G. – Esposto, S. – Taticchi, A. – Rosati, A. – Rao, R. – Perrotta, G. – Servili, M. – Baldoni, L.: Olive phenolic compounds: metabolic and transcriptional profiling during fruit development. BMC Plant Biology, *12*, 2012, article 162. DOI: 10.1186/1471-2229-12-162.
2. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA): Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL-cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "anti-inflammatory properties" (ID 1882), "contributes to the upper respiratory tract health" (ID 3468), "can help to maintain a normal function of gastrointestinal tract" (3779), and "contributes to body defences against external agents" (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal, *9*, 2011, article 2033. DOI: 10.2903/j.efsa.2011.2033.
3. Araujo, M. L. – Pimentel, F. B. – Alves, R. C. – Oliveira, M. B. P. P.: Phenolic compounds from olive mill wastes: Health effects, analytical approach and application as food antioxidants. Trends in Food Science and Technology, *45*, 2015, pp. 200–211. DOI: 10.1016/j.tifs.2015.06.010.
4. Spigno, G. – De Faveri, D. M.: Microwave-assisted extraction of tea phenols: A phenomenological study. Journal of Food Engineering, *93*, 2009, pp. 210–217. DOI: 10.1016/j.jfoodeng.2009.01.006.
5. Thakur, L. – Ghodasra, U. – Patel, N. – Dabhi, M.: Novel approaches for stability improvement in natural medicines. Pharmacognosy Review, *5*, 2011, pp. 48–54. DOI: 10.4103/0973-7847.79099.
6. Mourtzinos, I. – Makris, D. P. – Yannakopoulou, K. – Kalogeropoulos, N. – Michali, I. – Karathanos, V. T.: Thermal stability of anthocyanin extract of *Hibiscus sabdariffa* L. in the presence of β-cyclodextrin. Journal of Agricultural and Food Chemistry, *56*, 2008, pp. 10303–10310. DOI: 10.1021/jf801389j.
7. Vitali Čepo, D. – Albahari, P. – Zovko Končić, M. – Radić, K. – Jurmanović, S. – Jug, M.: Solvent extraction and chromatographic determination of polyphenols in olive pomace. Food in Health and Disease, *6*, 2017, pp. 7–14. ISSN: 2233-1220. <https://hrcak.srce.hr/file/269677>
8. Tan, H. W. – Tuck, K. L. – Stupans, I. – Hayball, P. J.: Simultaneous determination of oleuropein and hydroxytyrosol in rat plasma using liquid chromatography with fluorescence detection. Journal of Chromatography B, *785*, 2003, pp. 187–191. DOI: 10.1016/S1570-0232(02)00855-3.
9. López de las Hazas, M. C. – Piñol, C. – Macià, A. – Romero, M. P. – Pedret, A. – Solà, R. – Rubió, L. – Motilva, M. J.: Differential absorption and metabolism of hydroxytyrosol and its precursors oleuropein and secoiridoids. Journal of Functional Foods, *22*, 2006, pp. 52–63. DOI: 10.1016/j.jff.2016.01.030.
10. Singleton, V. L. – Rossi, J. A.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, *16*, 1965, pp. 144–158. ISSN: 0002-9254. <http://www.ajevonline.org/content/ajev/16/3/144.full.pdf>
11. Re, R. – Pellegrini, N. – Proteggente, A. – Pannala, A. – Yang, M. – Rice-Evans, C.: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, *26*, 1999, pp. 1231–1237. DOI: 10.1016/S0891-5849(98)00315-3.
12. Ou, B. – Hampsch-Woodill, M. – Prior, R. M.: Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 4619–4626. DOI: 10.1021/jf010586o.
13. Prieto, M. A. – Rodríguez-Amado, I. – Vázquez, J. A. – Murado, M. A. J.: β-Carotene assay revisited, application to characterize and quantify

antioxidant and prooxidant activities in a microplate. Journal of Agricultural and Food Chemistry, *60*, 2012, pp. 8983–8993. DOI: 10.1021/jf302218g.
14. Gu, J. – Zawistowski, J. – Ling, W. – Kitts, D. D.: Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 5271–5277. DOI: 10.1021/jf034466n.
15. Albahari, P. – Jug, M. – Radić, K. – Jurmanović, S. – Brnčić, M. – Rimac Brnčić, S. – Vitali Čepo, D.: Characterization of olive pomace extract obtained by cyclodextrin-enhanced pulsed ultrasound assisted extraction. LWT – Food Science and Technology, *92*, 2018, pp. 22–31. DOI: 10.1016/j.lwt.2018.02.011.
16. Shu, Y. Y. – Ko, M. Y. – Chang, Y. S.: Microwave-assisted extraction of ginsenosides from ginseng root. Microchemical Journal, *74*, 2003, pp. 131–139. DOI: 10.1016/S0026-265X(02)00180-7.
17. Cortés-Rojas, D. F. – Souza, C. R. F. – Oliveira, W. P.: Optimization of spray drying conditions for production of *Bidens pilosa* L. dried extract. Chemical Engineering Research and Design, *93*, 2015, pp. 366–376. DOI: 10.1016/j.cherd.2014.06.010.
18. Urzúa, C. – González, E. – Dueik, V. – Bouchon, P. – Giménez, B. – Robert, P.: Olive leaves extract encapsulated by spray-drying in vacuum fried starch–gluten doughs. Food and Bioproducts Processing, *106*, 2017, pp. 171–180. DOI: 10.1016/j.fbp.2017.10.001.
19. Vitali Čepo, D. – Jug, M. – Grdić Rajković, M. – Jablan, J.: Formulation of a nutraceutical derived from carob: β-cyclodextrin encapsulation of antioxidants from carob pod. Journal of Food and Nutrition Research, *56*, 2017, pp. 48–60. ISSN: 1336-8672 (print), 1338-4260 (online). <http://www.vup.sk/download.php?bulID=1924>
20. García-Padial, M. – Martínez-Ohárriz, M. C. – Isasi, J. R. – Vélaz, I. – Zornoza, A.: Complexation of tyrosol with cyclodextrins. Journal of Inclusion Phenomena and Macrocyclic Chemistry, *75*, 2013, pp. 241–246. DOI: 10.1007/s10847-011-0095-3.
21. Walters, R. H. – Bhatnagar, B. – Tchessalov, S. – Izutsu, K. I. – Tsumoto, K. – Ohtake, S.: Next generation drying technologies for pharmaceutical applications. Journal of Pharmaceutical Sciences, *103*, 2014, pp. 2673–2695. DOI: 10.1002/jps.23998.
22. Caliskan, G. – Nur Dirim, S.: The effects of the different drying conditions and the amounts of maltodextrin addition during spray drying of sumac extract. Food and Bioproducts Processing, *91*, 2013, pp. 539–548. DOI: 10.1016/j.fbp.2013.06.004.
23. Paini, M. – Aliakbarian, B. – Casazza, A. A. – Lagazzo, A. – Botter, R. – Perego, P.: Microencapsulation of phenolic compounds from olive pomace using spray drying: A study of operative parameters. LWT – Food Science and Technology, *62*, 2015, pp. 177–186. DOI: 10.1016/j.lwt.2015.01.022.
24. Chasekioglou, A. N. – Goula, A. M. – Adamopoulos, K. G. – Lazarides, H. N. : An approach to turn olive mill wastewater into a valuable food by-product based on spray drying in dehumidified air using drying aids. Powder Technology, *311*, 2017, pp. 376–389. DOI: 10.1016/j.powtec.2017.02.008.
25. Wilkowska, A. – Ambroziak, W. – Adamiec, J. – Czyżowska, A.: Preservation of antioxidant activity and polyphenols in chokeberry juice and wine with the use of microencapsulation. Journal of Food Processing and Preservation, *41*, 2017, e12924. DOI: 10.1111/jfpp.12924.
26. López-Alarcón, C. – Denicola, A.: Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. Analytica Chimica Acta, *763*, 2013, pp. 1–10. DOI: 10.1016/j.aca.2012.11.051.
27. Chandrasekara, N. – Shahidi, F.: Antioxidative potential of cashew phenolics in food and biological model systems as affected by roasting. Food Chemistry, *129*, 2011, pp. 1388–1396. DOI: 10.1016/j.foodchem.2011.05.075.
28. Rahman, J. – Ambigaipalan, P. – Shahidi, F.: Biological activities of camelina and sophia seeds phenolics: Inhibition of LDL oxidation, DNA damage, and pancreatic lipase and α-glucosidase activities. Journal of Food Science, *83*, 2018, pp. 237–245. DOI: 10.1111/1750-3841.14007.
29. Liu, M. – Yong, Q. – Yu, S.: Efficient bioconversion of oleuropein from olive leaf extract to antioxidant hydroxytyrosol by enzymatic hydrolysis and high temperature degradation. Biotechnology and Applied Biochemistry, 65, 2018, pp. 680–689. DOI: 10.1002/bab.1651.

Received 27 June 2018; 1st revised 21 August 2018; accepted 28 August 2018; published online 27 January 2019.