

## ENHANCED STABILITY OF POLYPHENOLIC EXTRACTS FROM GRAPE POMACE ACHIEVED BY EMBEDDING INTO MESOPOROUS SILICA-TYPE MATRICES

Ana-Maria BREZOIU <sup>1</sup>, Daniel LINCU <sup>1,2</sup>, Mihaela DEACONU <sup>1</sup>, Raul-Augustin MITRAN <sup>2</sup>, Daniela BERGER <sup>1</sup>, Cristian MATEI <sup>1</sup>

*We report the embedding of Cabernet Sauvignon polyphenolic ethanolic extracts prepared by conventional or microwave-assisted (MW) extraction into MCM-41-type mesoporous silica (modified with MgO, ZnO or CeO<sub>2</sub>) with enhanced stability and good radical scavenger activity. The chemical profiling of polyphenolic extract was assessed through RP-HPLC. Polyphenolic extract prepared by MW extraction exhibited higher radical scavenger activity (717.69 and 812.14 µmol TE/g<sub>extract</sub>) in comparison with the conventional one (465.76 and 326.74 µmol TE/g<sub>extract</sub>) determined by DPPH method and ABTS assay, respectively. The embedded extracts into mesoporous silica-type matrices showed enhanced radical scavenger properties than the free ones after six months storage in refrigerator.*

**Keywords:** grape pomace, microwave-assisted extraction, polyphenolic extract, embedded extract, mesoporous silica

### 1. Introduction

Grape pomace is an important residue from the winery industry representing about 20-25% of processed grapes [1]. The exploiting of this by-product could reduce environmental impact of its incineration during the harvesting season [2]. Due to a high content of polyphenols, it can be valorized in polyphenolic extracts with applications in several domains like nutraceuticals, pharmaceutical or cosmetic products, as well as in the food industry [3-4]. However, the use of extracts is related to the preservation of their stability, bioactivity and bioavailability [5].

Firstly, it is important to obtain polyphenolic extracts with high amount of valuable compounds, which can be obtained either by conventional extraction [6-8] or processes assisted by auxiliary energies like microwaves (MW) [6, 9, 10] or ultrasounds [11-13]. The polyphenols supercritical extraction is not feasible because of the high cost and non-polar nature of common supercritical solvents

<sup>1</sup> Dept. of Inorganic Chemistry, Physical Chemistry and Electrochemistry, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: anamaria\_brezoiu@yahoo.com; danaberger01@yahoo.com;

<sup>2</sup> "Ilie Murgulescu" Institute of Physical Chemistry, Romanian Academy, Romania;

[14]. However, some papers reported the supercritical  $\text{CO}_2$  extraction adding ethanolic-water mixture to increase the solvent polarity [15- 19]. Another possibility is to use pomace as a source of seeds from which valuable compounds such as oils and fatty acids can be extracted in the presence of non-polar solvents like hexane [20].

The polyphenols stability is drastically affected by epimerization, auto-oxidation or modification of polyphenols hydroxyl group (e.g. esterification or alkylation reactions) [21]. Epimerization products, which occur during heat treatments, can present either higher or lower radical scavenging activity than the compounds from which they come depending on the studied systems [22]. Polyphenolic compounds are prone to oxidation reactions in presence of oxygen when the hydroxyl groups in ketone form are not able to donate hydrogen atoms, thus the polyphenolic compound stability decreases with the increase of hydroxyl groups number [23].

The stability of different polyphenols could be assessed through several accelerated degradation methods based on thermal treatment, microwave irradiation or pH variation. For example, rutin solution is degraded 10% and 50% weight loss when heated at 70 °C and 90 °C, respectively, or completely at 130 °C [24]. Gallic and vanillic acid solutions are stable up to 80 °C, catechin solution starts to decompose at 60 °C [25], while protocatechuic acid is very stable when heated in water at high temperature (up to 200°C) [26]. MW-irradiation of polyphenols led to more pronounced degradation of flavonoids in comparison with phenolic acids. For instance, the synaptic and ferulic acid methanolic solutions content decreased with 21-23% and 31-35%, respectively, when MW power is in the range of 460 - 700 W [27]. Myricetin and *trans*-resveratrol solutions undergo first-order degradation in base medium [28]. However, myricetin is stable under UV irradiation in the presence of other antioxidants, which makes it suitable for topical applications [29].

To increase the stability of polyphenolic extracts, several nanocarriers could be used for their encapsulation. Recently, we reported enhanced stability of grape pomace extract embedded in mesoporous MCM-41-type silica pristine and modified with  $\text{ZnO}$  or  $\text{MgO}$  [30]. Herein, we report the chemical profile of ethanolic polyphenolic extracts from Cabernet Sauvignon grape pomace prepared by both MW and conventional treatment for each extraction stage. For the first time, a polyphenolic extract from grape pomace prepared by MW irradiation was embedding in mesoporous silica-type matrices. We compared the stability of embedded and free extracts prepared by both methods. For encapsulation of polyphenolic extracts, we chose mesoporous MCM-41-type silica modified with  $\text{ZnO}$ ,  $\text{MgO}$  and  $\text{CeO}_2$ .

## 2. Experimental

### 2.1. Materials

All the reagents, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), potassium persulphate ( $\text{K}_2\text{S}_2\text{O}_8$ ), 36.5–38% (wt) hydrochloric acid (Sigma), ethanol (Sigma-Aldrich), Folin-Ciocalteu reagent were used as received. For chromatographic analyses, the following standard HPLC-grade compounds were used: gallic acid (Alfa Aesar, 98%), protocatechuic acid (TCI, >98%, HPLC-grade), catechin hydrate (Sigma, >98%, HPLC-grade), vanillic acid (TCI, >98%, GC-grade), caffeic acid (Sigma, 98%, HPLC-grade), syringic acid (Molekula, >98.5%), (–) epicatechin (TCI, >98%, HPLC-grade), quercetin (Sigma, >95%, HPLC-grade), rutin hydrate (Sigma, 95%, HPLC-grade), chlorogenic acid (HWI group, primary reference standard), *trans*-*p*-coumaric acid (Sigma Aldrich, analytical standard), myricetin (Sigma, >96%, HPLC-grade), rosmarinic acid (Sigma, >98%, HPLC-grade), *trans*-resveratrol (Sigma Aldrich, certified reference material), kaempferol (Sigma, >97%, HPLC-grade), cyanidin chloride (Sigma, >95%, HPLC-grade), malvidin chloride (Sigma Aldrich, >95%, HPLC), pelargonidin chloride (Aldrich) and delphinidin chloride (Sigma Aldrich, analytical standard), solvents like ethanol, acetonitrile (ACN), formic acid, and for radical scavenger activity determination, 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Aldrich, 97%) and 2,2'-azino-bis (3 ethylbenzothiazoline-6-sulphonic acid) (ABTS, Sigma Aldrich). The Cabernet Sauvignon grape pomace is from the Black Sea Region (Murtfatlar) and ascorbic acid powder was purchased from local vendors. Ultrapure water (Millipore Direct- Q3 UV water purification system with Biopack UF cartridge) was used for all solutions and experiments.

### 2.2. Preparation of polyphenolic extracts from Cabernet Sauvignon grape pomace

The ethanolic polyphenolic extracts were prepared from a fermented red grape pomace from the Black Sea region (Cabernet Sauvignon, CS), obtained after the winemaking process in 2017, using either microwave-assisted (MW) or conventional (Conv) extraction. The polyphenols conventional extraction was performed by mixing the vegetal material with absolute ethanol at room temperature overnight, followed by a reflux heating in three stages of 1h (vegetal material/ethanol ratio of 1/6 (w/v) in each extraction stage), under constant magnetic stirring, intermediate filtration and solvent replacement and then ethanolic extracts were stored separately.

The MW extraction was carried out on a Sairen Miniflow 200SS microwave reactor using the grape marc and absolute ethanol in the same vegetal

material/solvent ratio as in the case of the conventional extraction, in three stages at 80°C/15 min. using a MW power of 35 W (average reflected power of 1 W) with intermediate filtration, solvent replacement and then extracts were stored separately. The polyphenolic extracts or fractions were dried under vacuum until a constant mass, and then re-dissolved in absolute ethanol.

### **2.3. *Characterization of phenolic extracts***

Polyphenolic extracts were characterized by several spectrophotometric methods (Shimadzu UV-1800) to determine total polyphenols, ascorbic acid, flavonoids, as well as anthocyanin contents, while the extract composition was assessed using high performance liquid chromatography with photodiode array detector, HPLC-PDA (Shimadzu Nexera 2). The description of the spectrophotometric methods and HPLC analysis were previously reported in our paper [6].

### **2.4. *Encapsulation of polyphenolic extract into mesoporous silica-type nanocarriers***

As supports for encapsulation of polyphenolic extracts, MCM-41-silica materials modified with either ZnO, MgO or CeO<sub>2</sub> were used. We reported the synthesis and the features of employed supports elsewhere [30].

The materials containing polyphenolic extract were prepared by incipient wetness impregnation method using overall extracts obtained from CS grape pomace by either conventional extraction or MW irradiation. In brief, the ethanolic polyphenolic extract was mixed with silica-type matrix, previously outgassed at 110 °C for 12 h and the resulted suspension was dried under vacuum for 6 h. The embedded extracts were denoted extract@carrier.

### **2.5. *Characterization of materials containing polyphenolic extract***

The samples containing embedded polyphenolic extract were characterized by infrared spectroscopy, thermogravimetric analysis coupled with differential thermal analysis, nitrogen adsorption-desorption isotherms, scanning differential calorimetry, and assessment of radical scavenger properties using DPPH assay. The FTIR spectra were recorded in 4000–400 cm<sup>-1</sup> range on a Bruker Tensor 27 spectrophotometer (KBr pellets technique) to confirm the organic molecules adsorption. The thermogravimetric analyses (TG) was performed to determine the number of polyphenolic compounds embedded into silica-type matrices using a Mettler Toledo GA/SDTA851e at a scan rate of 10 °C/min, under synthetic air flow. Differential scanning calorimetry analyses (DSC), heating-cooling cycles, were performed to assess the free extracts stability on Mettler Toledo DSC 3+ at a scan rate of 5°C/min under nitrogen flow, using pierced crimped aluminum pans.

Nitrogen adsorption-desorption isotherms were recorded at the 77 K on a QuantachromeAutosorb iQ<sub>2</sub> gas sorption analyzer. Prior to the isotherms recording, the materials containing embedded extract were outgassed at 35 °C for 17 h. The specific surface area values,  $S_{BET}$ , were computed based on the Brunauer-Emmett-Teller method in the relative pressure range of 0.05–0.25 and the total pore volume was determined for a relative pressure,  $p/p_0$ , of 0.99.

## 2.6. *Determination of free radical scavenging activity*

The radical scavenger activity (RSA) of polyphenolic extracts was assessed by both DPPH and ABTS assays and that of materials containing embedded extract through DPPH method. The detailed procedures for each determination are presented elsewhere [6]. Briefly, the material containing embedded extract was tested in comparison with the corresponding support and the free extract in the same concentration as in the composite material, having as control the inherent degradation of DPPH free radical solution after 24 h. The experiments were carried out in closed containers and kept on an orbital shaker in dark conditions. Afterwards, aliquots of samples containing composite material, support, extract or DPPH-free radical solution were withdrawn, centrifugated for 10-15 minutes in the case of suspensions and then the solution absorbance was measured at 517 nm.

## 3. Results and discussion

### 3.1. *Characterization of polyphenolic extracts*

Lately, it was observed an interest in using by-products from industry, these raw materials being cheap and valuable for developing products containing phytochemicals. Regarding grape pomace, it was intensively studied the recovery of polyphenols [6, 14, 16, 31-36], flavonoids [6, 31, 33, 34] or anthocyanin monomeric pigments [6, 32, 33] by extraction. In this study, the Cabernet Sauvignon grape marc from the Black Sea region (Murfatlar, Romania) was used to prepare polyphenolic extracts either by conventional or MW-assisted extraction.

A comparison between the number of polyphenols recovered in each stage of MW-treatment (Fig. 1-A) and conventional extraction can be observed in Fig. 1A and B. The microwave treatment enhanced the polyphenols content obtained in the first extraction stage, while the conventional treatment led to a higher amount of polyphenolic extract compared to the dry weight of grape marc.

The ethanolic extracts were analyzed using spectrophotometric methods like total polyphenols (Fig. 2A) and ascorbic acid (Fig. 2B) using Folin-Ciocalteu reagent, total flavonoids by AlCl<sub>3</sub> complexation (Fig. 2C), as well as total anthocyanin monomeric pigment content (Fig. 2D).

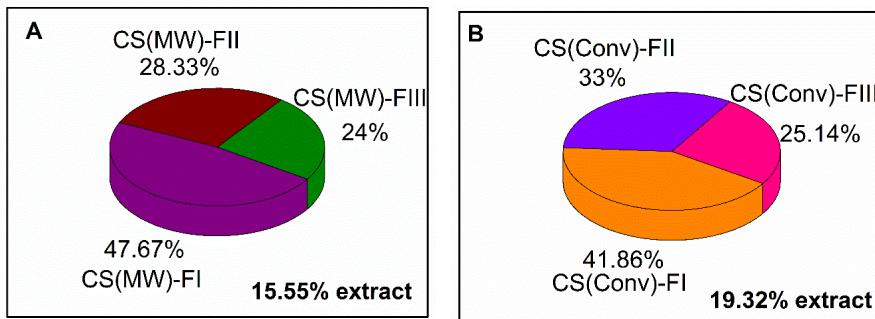


Fig. 1. Polyphenols amount in each extraction stage for A- MW and B-conventional extracts

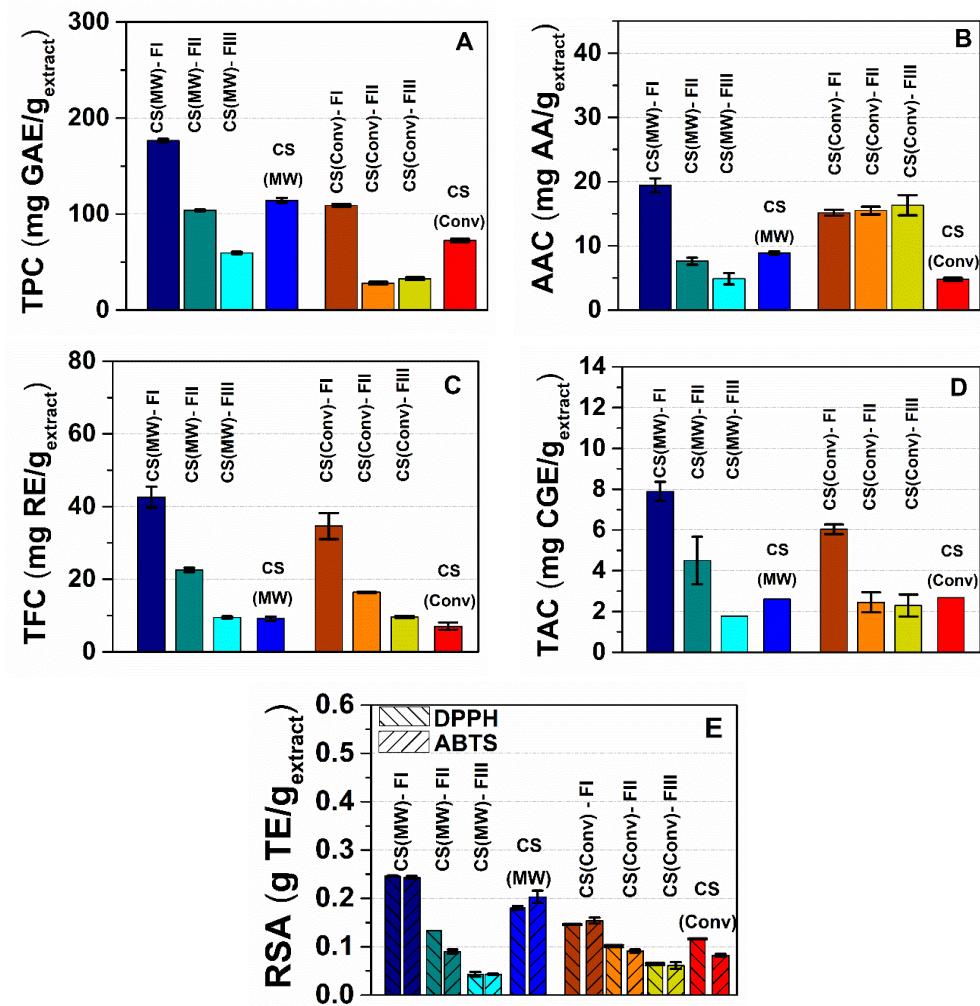


Fig. 2. Spectrophotometric determination of A- total polyphenols (as gallic acid equivalents, GAE), B- ascorbic acid, C- total flavonoids (as rutin hydrate equivalents, RE), D- total anthocyanins (by extinction of cyanidin-3-glycoside method, CGE) and E- radical scavenger activity (as Trolox equivalents, TE)

The total polyphenols (TP) content was evaluated as gallic acid equivalent at both 765 nm ( $y=0.00945*x+0.017$ ;  $R^2=0.9995$ ) and 650 nm ( $y=0.00965*x+0.013$ ;  $R^2=0.9998$ ) wavelengths and provided as an average value of four replicates (Fig. 2A). Using the standard curve for gallic acid, the TP content was determined, and from the obtained values were subtracted the amount of ascorbic acid (Fig. 2B) to obtain the corrected values for total polyphenols content, considering that ascorbic acid is a very well-known interfering compound in the Folin-Ciocalteu assay [37]. From the values for TP content for both microwave and conventional extracts, one can observe that the highest amount is obtained in the first stage of extraction. Also, it can be noticed that the overall extract obtained by microwave-assisted extraction present higher amount of TP in comparison with the conventional one.

The total flavonoids (TF) content was determined as rutin hydrate equivalents (RE) in duplicate being assessed based on a standard curve established at 430 nm ( $y=0.04074*x$ ,  $R^2=0.9991$ ). For extracts (0.5 mg/mL), the solution absorbance was read at the maximum absorption wavelength, at around 420 nm, and the obtained values are presented in Fig. 2C. A higher content of TF was identified for the extract prepared by MW irradiation either in each extraction stage or in overall extract in comparison with that of the conventional one.

The total anthocyanin monomeric pigment (TA) content was assessed according to the procedure described by Lee [38] based on the molar extinction coefficient of cyanidin-3-glycoside (denoted CGE) and its corresponding molar weight. The TA values are presented in Fig. 2D as an average of two measurements, being similar for both overall extracts.

Concerning the radical scavenging activity (RSA) of the extracts, both DPPH assay and ABTS method were applied using the calibration curves for Trolox in 0.010-0.300 mg/mL concentration domain by plotting the RSA (%) against Trolox concentration ( $y=178.2*x+1.69$ ,  $R^2=0.9983$  - DPPH method and  $y = 79.298*x + 3.413$   $R^2=0.9984$  - ABTS assay). The RSA values as Trolox equivalents per gram of extract, for both DPPH and ABTS assays are presented in Fig. 2E. The ABTS method led to lower values for RSA, but the same trend among samples was observed that is consistent with literature. Previously, ABTS assay was considered a better method than DPPH to estimate antioxidant capacity [39]. Higher values of RSA by both ABTS and DPPH were observed in the case of MW extract in comparison with the conventional one (Fig. 2E).

The RP-HPLC-PDA analysis was performed for phenolic compounds identification and quantification in extracts considering their retention time and UV spectra against that of standard substances. For HPLC analysis, a C18 stationary phase (reverse phase) was chosen being already proven to be efficient for the separation of polyphenolic compounds. Up to nine substances of the available standard compounds were identified in the extracts (Table 1). The

chromatograms of CS(MW) and CS(Conv) extracts are presented in Fig. 3 and Fig. 4, respectively.

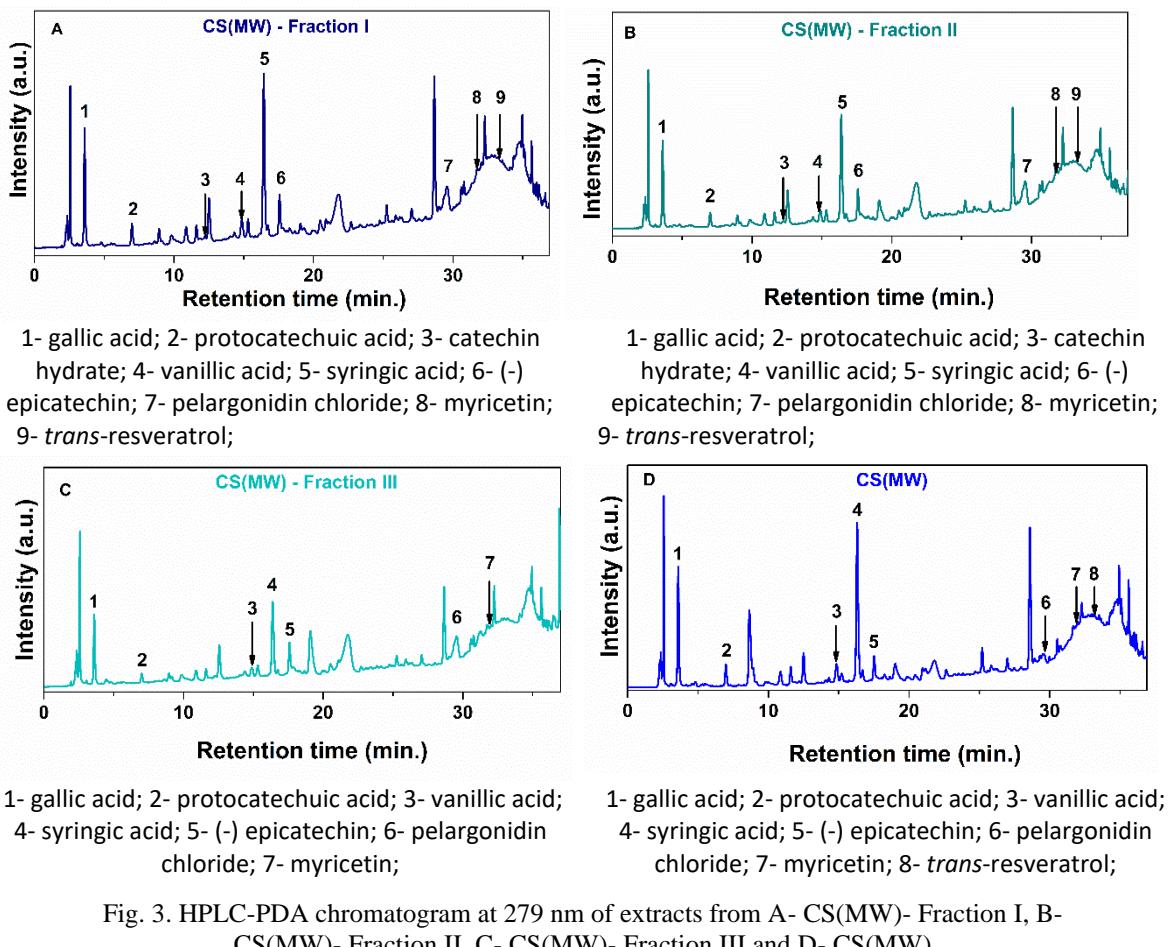


Fig. 3. HPLC-PDA chromatogram at 279 nm of extracts from A- CS(MW)- Fraction I, B- CS(MW)- Fraction II, C- CS(MW)- Fraction III and D- CS(MW)

Table 1.

Phenolic compounds identification and quantification by reverse-phase - HPLC-PDA

Concentration in extract (mg/g)	Standard substances	CS(MW) - Fr I	CS(MW) - Fr II	CS(MW) - Fr III	CS (MW)	CS(Conv) – Fr I	CS(Conv) – Fr II	CS(Conv) – Fr III	CS (Conv)
Gallic acid	0.927 ± 0.000	0.544 ± 0.004	0.220 ± 0.005	<b>0.719 ± 0.002</b>	0.706 ± 0.001	0.327 ± 0.001	0.247 ± 0.000	<b>0.447 ± 0.006</b>	
Protocatechuic acid	0.400 ± 0.001	0.211 ± 0.000	0.073 ± 0.001	<b>0.297 ± 0.003</b>	0.313 ± 0.001	0.131 ± 0.001	0.089 ± 0.000	<b>0.192 ± 0.001</b>	
Catechin hydrate	0.136 ± 0.005	0.084 ± 0.001	n.d.	<b>n.d.</b>	0.072 ± 0.001	n.d.	n.d.	<b>n.d.</b>	
Vanillic acid	0.315 ± 0.001	0.175 ± 0.000	0.067 ± 0.002	<b>0.232 ± 0.002</b>	0.237 ± 0.001	0.106 ± 0.001	0.072 ± 0.000	<b>0.146 ± 0.001</b>	
Syringic acid	1.768 ±	1.004 ±	0.349 ±	<b>1.374 ±</b>	1.399 ±	0.605 ±	0.389 ±	<b>0.843 ±</b>	

	0.002	0.001	0.001	<b>0.022</b>	0.001	0.001	0.001	<b>0.021</b>
(-) Epicatechin	1.555 ± 0.002	0.962 ± 0.001	0.479 ± 0.001	<b>0.913 ± 0.006</b>	0.941 ± 0.001	0.487 ± 0.002	0.396 ± 0.002	<b>0.491 ± 0.003</b>
Pelargonidin chloride	0.836 ± 0.001	0.609 ± 0.002	0.281 ± 0.004	<b>0.256 ± 0.004</b>	0.433 ± 0.001	0.193 ± 0.001	0.095 ± 0.007	<b>0.049 ± 0.001</b>
Myricetin	0.132 ± 0.000	0.090 ± 0.000	0.047 ± 0.001	<b>0.107 ± 0.003</b>	0.094 ± 0.000	0.051 ± 0.001	0.046 ± 0.000	<b>0.063 ± 0.002</b>
<i>trans</i> -resveratrol	0.020 ± 0.000	0.015 ± 0.000	n.d.	<b>0.010 ± 0.000</b>	0.020 ± 0.001	0.014 ± 0.001	0.020 ± 0.000	<b>0.013 ± 0.001</b>

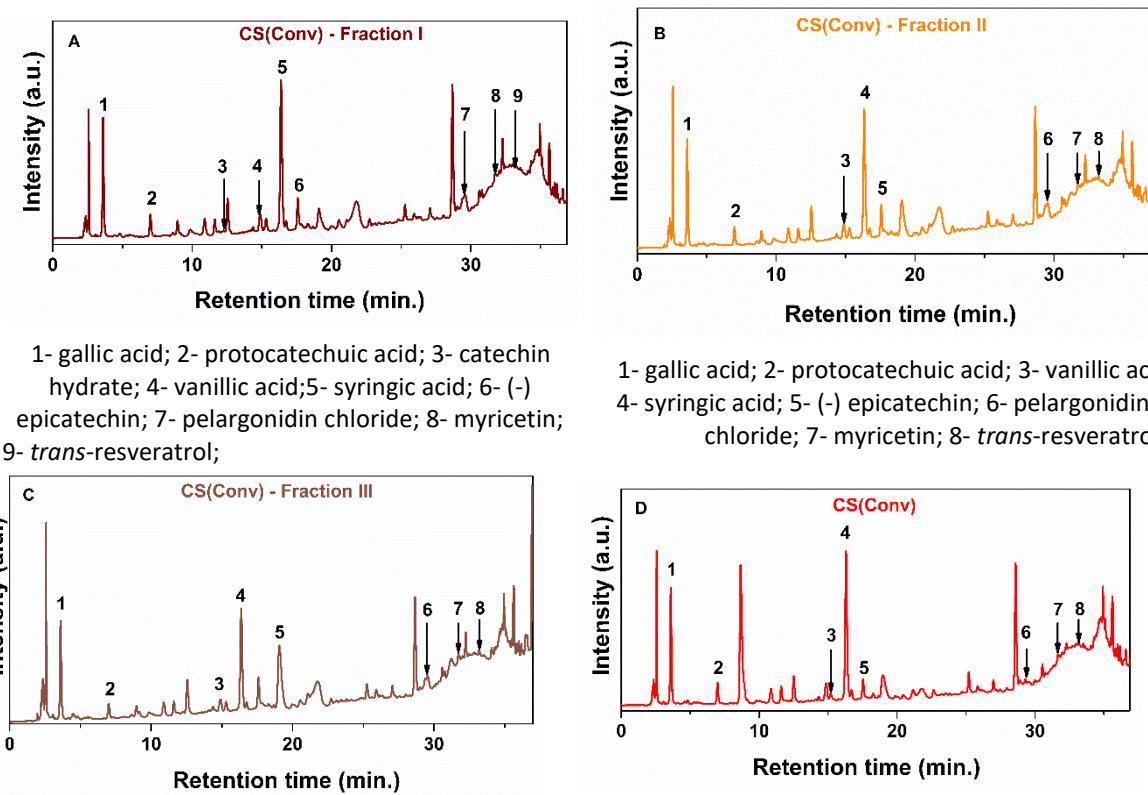


Fig. 4. HPLC-PDA chromatogram at 279 nm of extracts from **A**- CS(Conv)- Fraction I, **B**- CS(Conv)- Fraction II, **C**- CS(Conv)- Fraction III, and **D**- CS(Conv)

Considering the distribution of polyphenolic compounds in the extracts from different stages, one can observe that the first fraction presents the highest polyphenolic content. The microwave-assisted extraction determined a higher polyphenolic amount of the overall extract in comparison with the conventional treatment, except *trans*-resveratrol, which was more in the conventional extract. Catechin hydrate was identified only in the first two fractions obtained by MW

irradiation and in the first fraction prepared by conventional method. Also, one can notice a decrease of anthocyanidin content in both overall extracts in comparison with each fraction, which proved the free anthocyanin instability [40,41].

### 3.2. Characterization of materials-containing encapsulated extract

Based on silica widely use in food industry (as a flavor carrier or a clarifying agent) or in cosmetics (as nanoparticles in products for skin, nails, hair or lips) [42], we proposed three types of mesoporous silica-type materials, MCM-41 decorated with amorphous ZnO (14.6 %wt; Zn-MCM-41) or MgO (6 %wt; Mg-MCM-41), as well as crystalline nanoparticles of CeO<sub>2</sub> (9.9 %wt; Ce-MCM-41) as supports for embedding the polyphenolic ethanolic extracts. The mesoporous matrices have high capacity to accommodate organic molecules due to their large pore volume (0.47-0.88 cm<sup>3</sup>/g) and specific surface area (510 - 796 m<sup>2</sup>/g). The textural parameters determined from nitrogen adsorption-desorption isotherms for the mesoporous supports were listed in Table 2. For encapsulation were used both CS(Conv) and CS(MW) overall extracts to study the influence of extraction technique on the properties of embedded extracts.

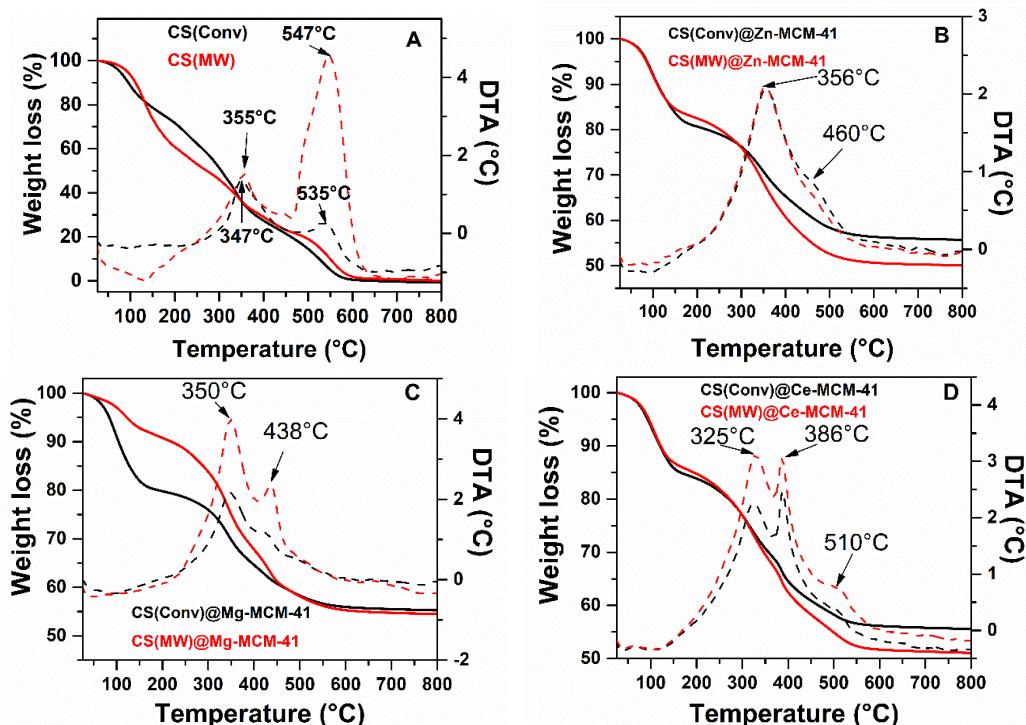


Fig. 5. TG-DTA analyses for CS(Conv) and CS(MW) free extracts dried in vacuum (A) and embedded in Zn-MCM-41 support (B), Mg-MCM-41 (C) and Ce-MCM-41 matrices (D)

The samples containing extract were characterized by thermal analysis (TG-DTA) for determination of the phenolic compounds content, FTIR spectroscopy, which emphasized the presence of organic compounds into silica-type supports and nitrogen adsorption-desorption isotherms to verify if the mesopores of silica support are completely filled with extract components. Based on thermogravimetric analyses of materials containing embedded extracts (Fig. 5), the phenolic compounds content was determined by considering the total weight loss up to 600°C, after subtraction of physically adsorbed water molecules, corresponded first endothermic event (Fig. 6). In the case of conventional CS extract, the polyphenolic content was in the range of 30.5–32.3 %wt, the highest value being obtained in the case of CS(Conv)@Ce-MCM-41 sample, while for the materials containing MW extract, the phytocompounds amount ranged from 37.5 to 40.0 %wt (Table 2).

The differential scanning calorimetry recorded on vacuum dried extracts showed stability up to 150°C. The residual solvent evaporation can be noticed during the first heating cycle for both extracts between 25 – 100 °C.

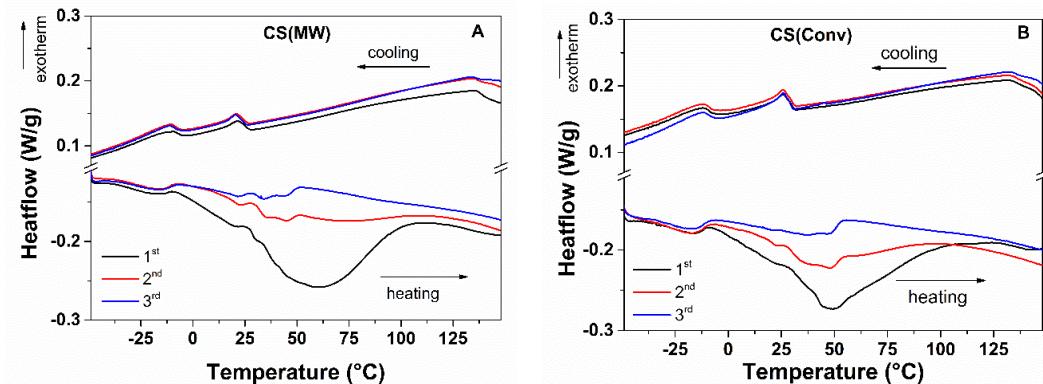


Fig. 6. DSC analysis for CS(MW) (A) and CS(Conv) (B) free extracts dried in vacuum, showing the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> heating – cooling cycle

This endothermic effect is greatly reduced during the 2<sup>nd</sup> heating run, and it is absent during the 3<sup>rd</sup> cycle, indicating complete solvent removal. Two types of reversible transformations can also be noticed at -7 °C and between 15 – 50 °C, which do not affect the extracts stability.

The nitrogen adsorption-desorption isotherms confirmed the complete filling of support mesopores with organic compounds, the remained pore volume being negligible (Table 2).

In the FTIR spectra of embedded extract (Fig. 6f-h), one can notice vibrations specific to both carrier and extract.

*Table 2.*  
**Textural features of embedded extracts and for the corresponding supports**

Support	$d_{BJH}$ (nm)	$S_{BET}$ ( $m^2/g$ )	$V_p$ ( $cm^3/g$ )	Embedded extract	extract (%wt)	$V_p$ ( $cm^3/g$ )
Zn-MCM-41	2.66	796	0.88	CS(MW)@Zn-MCM-41	37.5	0.035
				CS(Conv)@Zn-MCM-41	31.5	-
Ce-MCM-41	2.66	510	0.47	CS(MW)@Ce-MCM-41	38.4	-
				CS(Conv)@Ce-MCM-41	32.3	-
Mg-MCM-41	2.67	718	0.79	CS(MW)@Mg-MCM-41	40.0	0.080
				CS(Conv)@Mg-MCM-41	30.5	0.080

The stretching vibrations of C-H bonds ( $\nu_{C-H}$ ) in the 2840-2940  $cm^{-1}$  region, the stretching vibrations of C-O bond (1733-1734  $cm^{-1}$  for polyphenolic extracts and 1733-1754  $cm^{-1}$  for extract-loaded materials), skeletal  $=C-O-C$  vibrations (1522  $cm^{-1}$  and 1529  $cm^{-1}$  in the extracts and 1530  $cm^{-1}$  for encapsulated extracts) specific to flavonoids [7], as well as C-N stretching vibrations, were assigned to polyphenolic compounds (Fig. 7 a and b) and the bands from 1096  $cm^{-1}$  and 815  $cm^{-1}$  were attributed to the asymmetrical and symmetrical stretching vibrations of Si-O-Si bonds and the bands from 972  $cm^{-1}$  and 470  $cm^{-1}$  to  $\nu_{asSi-OH}$  and  $\delta_{Si-O-Si}$ , respectively, that belong to the silica-type carriers (Fig. 7 c-e).

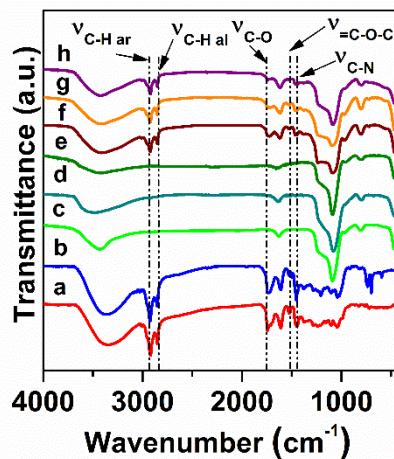


Fig.7. FTIR spectra of CS(Conv)(a), CS(MW)(b), Ce-MCM-41(c), Zn-MCM-41(d), Mg-MCM-41(e), CS@Ce-MCM-41(f), CS@Zn-MCM-41(g) and CS@Mg-MCM-41(h)

### 3.3. Determination of free radical scavenger activity

The radical scavenger activity (RSA) of free and embedded extracts was assessed *via* DPPH method. Three concentrations from the linearity domain,

which allowed the setting of a correlation equation for each tested sample and *IC50%* values (50% of DPPH free radical inhibition) were established and the data are listed in Table 3. The MW extract exhibited the highest radical scavenging capacity (Table 3).

The radical scavenger capacity of overall extracts determined by DPPH and ABTS methods was in the range of 465.76–717.69  $\mu\text{mol TE/g extract}$  (89.86–111.93  $\mu\text{mol TE/g marc}$ ) and 326.74–812.14  $\mu\text{mol TE/g extract}$  (63.04–126.66  $\mu\text{mol TE/g marc}$ ), respectively, similar to the ones reported by Ben Aziz *et al.* for grape pomace extract from CS, determined by DPPH assay (120  $\mu\text{mol TE/g marc}$ ) [43] and lower than that reported by Melo *et al.* for Chenin Blanc, Petit Verdot and Syrah cultivars (191–540  $\mu\text{mol TE/g marc}$  and 218–653  $\mu\text{mol TE/g marc}$  through DPPH and ABTS assays, respectively) [35], or reported by Xu *et al.* by ABTS assay (951–1013  $\mu\text{mol TE/g extract}$ ) for extracts prepared from Viognier and Cabernet Franc grape pomace [33]. Rockenbach *et al.* reported RSA values ranging from 188.02 to 505.52  $\mu\text{mol TE/g marc}$  and from 193.36 to 485.42  $\mu\text{mol TE/g marc}$  using DPPH method and ABTS assay, respectively, the best activity being obtained for Cabernet Sauvignon extract [34].

The radical scavenger activity of embedded extracts was assessed after 24 h of incubation in DPPH free radical solution, in duplicate, and compared to that of the free extract and corresponding support in the same amount as in the sample-containing embedded extract, using as control the degradation in time of the DPPH free radical solution.

Table 3.

**Radical scavenging activity of phenolic extracts (DPPH assay).**

Sample	IC50% (mg/mL)	Correlation equation	R <sup>2</sup>
CS(Conv)	<b>2.325</b>	<b>y=21.060*x+1.024</b>	<b>0.9950</b>
CS(Conv)- Fr I	1.858	y=25.863*x+2.002	0.9999
CS(Conv)- Fr II	2.683	y=15.355*x + 8.794	0.9940
CS(Conv)- Fr III	4.248	y=11.182*x + 2.498	0.9971
<b>CS(MW)</b>	<b>1.509</b>	<b>y=32.030*x + 1.660</b>	<b>0.9930</b>
CS(MW)- Fr I	1.110	y=43.900*x + 1.670	0.9999
CS(MW)- Fr II	2.043	y=24.035*x + 0.880	0.9940
CS(MW)- Fr III	6.285	y=6.675*x + 8.045	0.9931

The radical scavenger capacity determined simultaneously for free and embedded phenolic extract (conventional or microwave CS extract free and embedded in Zn-MCM-41, Mg-MCM-41, and Ce-MCM-41) and along with that of corresponding mesoporous support in the presence of control are shown in Fig. 8.

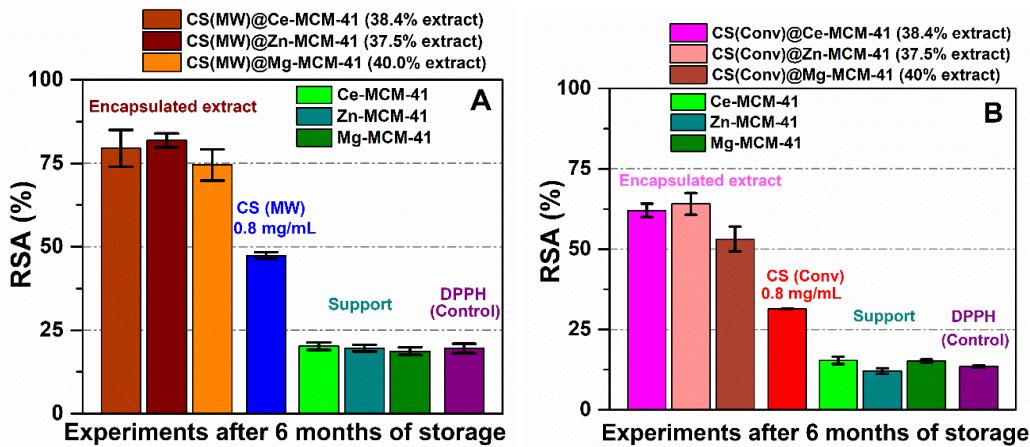


Fig. 8. Radical scavenger activity for: (A) free and embedded CS(MW) extract in Zn-MCM-41, Mg-MCM-41 and Ce-MCM-41 matrices and (B) free and entrapped CS(Conv) extract in Zn-MCM-41, Mg-MCM-41 and Ce-MCM-41 supports; after 24 h incubation in comparison with the corresponding MCM-41-type support using DPPH free radical solution degradation as control

One can notice that embedded extracts preserved their radical scavenging activity almost constant, while that of the free extracts decreased in time (Fig. 8), probably because they are prone to a faster degradation. Also, it can be observed that the MW extract both free and embedded in silica-type supports present higher radical scavenger activity than that of conventional extract.

After six months of storage in the refrigerator (4-6 °C), all embedded extracts showed higher radical scavenging activity than that of free extract (Fig. 8), probably due to better stability when confined in mesopores of silica-type matrix, although all supports did not exhibit significant activity. The best results, in terms of preserved radical scavenger properties, were obtained for CS(MW)@Zn-MCM-41 and CS(Conventional)@Zn-MCM-41 materials. Hence, the embedding of extract into a mesoporous silica-type matrix helps to preserve the radical scavenger activity of phytocomponents.

#### 4. Conclusions

Two ethanolic polyphenolic extracts from Cabernet Sauvignon cultivar were prepared by either MW-assisted extraction or conventional treatment. Regarding the chemical profiling of extracts, high amounts of polyphenols and flavonoids were identified, an enhanced amount being obtained in the case of MW irradiation. As expected, the highest number of polyphenols for both extracts was identified in the first extraction stage. The MW extract exhibited better radical scavenger activity explained by its higher content of polyphenols than that of the conventional extract.

The embedding of MW extract prepared into mesoporous silica-type supports led to a better preservation of phytocomponents radical scavenger activity than that of free extracts stored for six months in the same conditions. The best results for radical scavenging activity were obtained for extracts embedded in Zn-MCM-41 support, which can be used for developing of nutraceutical formulations with good stability.

### Acknowledgements

This work was supported by UEFISCDI - Romanian project PCCDI no. 85/2018. The authors thank to Murtfatlar Research Station for Viticulture and Oenology, Romania, for grape pomace vegetal material. The authors are grateful to dr. Adrian Trifan for his help in preparing the microwave extract.

### R E F E R E N C E S

- [1] A. Schieber, F. C. Stintzing and R. Carle, “By-products of plant food processing as a source of functional compounds -recent developments”, in Trends Food Sci. Technol., **vol. 12**, 2001, pp. 401–413.
- [2] E. Kalli, I. Lappa, P. Bouchagier, P. A. Tarantili and E. Skotti, “Novel application and industrial exploitation of winery by-products”, in Bioresour. Bioprocess., **vol. 5**, Nov. 2018, pp. 46-67.
- [3] J. Garcia-Lomillo and M. L. Gonzales-SanJose, “Applications of Wine Pomace in the Food Industry: Approaches and Functions”, in Compr. Rev. Food Sci. Food Saf., **vol. 16**, no. 1, Ian. 2017, pp. 1-16.
- [4] A. Teixeira, N. Baenas, R. Dominguez-Perles, A. Barros, E. Rosa, D. A. Moreno and C. Garcia-Viguera, “Natural Bioactive Compounds from Winery By-Products as Health Promoters: A Review”, in Int. J. Mol. Sci., **vol. 15**, no. 9, Sep. 2014, pp. 15638–15678.
- [5] Z. Fang and B. Bhandari, “Encapsulation of polyphenols-a review”, in Trends Food Sci. Technol., **vol. 21**, no. 10, Oct. 2010, pp. 510–523.
- [6] A.-M. Brezoiu, C. Matei, M. Deaconu, A.-M. Stanciu, A. Trifan, A. Gaspar-Pintilieescu and D. Berger, “Polyphenols extract from grape pomace. Characterization and valorization through encapsulation into mesoporous silica-type matrices”, in Food Chem. Toxicol., **vol. 133**, Nov. 2019, pp. 110787.
- [7] L. I. L Favaro, V. M. Balcao, L. K. M. Rocha, E. C. Silva, J. M. Oliviera Jr, M. M. D. C. Vila and M. Turbino, “Physicochemical Characterization of a Crude Anthocyanin Extract from the Fruits of Jussara (Euterpe edulis Martius): Potential for Food and Pharmaceutical Applications”, in J. Braz. Chem. Soc., **vol. 29**, no. 10, 2018, pp. 2072-2088.
- [8] A. O. Matei, F. Gatea, A. Alecu and G. L. Radu, “Total polyphenolic content and antioxidant activity from anthocyanin extracts”, in U.P.B. Sci. Bull., Series B, **vol. 77**, no. 2, 2015, pp. 53-62.
- [9] I. Asofieie, I. Calinescu, A. Trifan. and A. I. Gavrla, “A Semi-Continuous Process For Polyphenols Extraction From Sea Buckthorn Leaves”, in Sci. Rep., **vol. 9**, Aug. 2019, pp. 12044.

[10] *A. Liazid, R. F. Guerrero, E. Cantos, M. Palma and G. Barroso*, “Microwave assisted extraction of anthocyanins from grape skins”, in *Food Chem.*, **vol. 124**, no. 3, Feb. 2011, pp. 1238–1243.

[11] *S. G. Jahromi, M. Soto-Hernández, R. García-Mateos and M. Palma-Tenango*, “Extraction Techniques of Phenolic Compounds from Plants, Plant Physiological Aspects of Phenolic Compounds”, in *IntechOpen*, 2019, DOI: 10.5772/intechopen.84705.

[12] *H. Bagheri and M. Esmaiili*, “Ultrasound-Assisted Extraction of Phenolic Compounds from Unripe Grape (Qora)”, in *Erwerbs-Obstbau*, **vol. 59**, Jan. 2017, pp. 221.

[13] *C. C. Crisan, I. Calinescu, C. Zalaru and Z. Moldovan*, “Techniques for extracting polyphenols from coreopsis tinctoria nutt. Fruits”, in *U.P.B. Sci. Bull., Series B*, **vol. 75**, no. 4, Iss.4, 2013, pp. 169-178.

[14] *A. Tsali, and A. M. Goula*, “Valorization of grape pomace: Encapsulation and storage stability of its phenolic extract”, in *Powder Technol.*, **vol. 340**, Dec. 2018, pp. 194–207.

[15] *K. Tyskiewicz, M. Konkol and E. Roj*, “The Application of Supercritical Fluid Extraction in Phenolic Compounds Isolation from Natural Plant Materials”, in *Molecules*, **vol. 23**, Oct. 2018, pp. 2625-2642.

[16] *C. Da Porto and A. Natolino*, “Supercritical fluid extraction of polyphenols from grape seed (*Vitis vinifera*): Study on process variables and kinetics”, in *J. Supercrit. Fluid.*, **vol. 130**, Dec. 2017, pp. 239-245.

[17] *A. M. Farías-Campomanes, M. A. Rostagno, J. J. Coaquira-Quispe and M. A. A. Meireles*, “Supercritical fluid extraction of polyphenols from lees: overall extraction curve, kinetic data and composition of the extracts”, in *Bioresour. Bioprocess.* **vol. 2**, 2015, pp. 45-58.

[18] *A. Peralbo-Molina, F. Priego-Capote, and M. D. Luque de Castro*, “Comparison of extraction methods for exploitation of grape skin residues from ethanol distillation”, in *Talanta*, **vol. 101**, Nov. 2012, pp. 292–298.

[19] *M. R. M. Junior, A. V. Leite and N. R. V. Dragano*, “Supercritical Fluid Extraction and Stabilization of Phenolic Compounds From Natural Sources – Review (Supercritical Extraction and Stabilization of Phenolic Compounds)”, in *TOCENGJ*, **vol. 4**, 2010, pp. 51-60.

[20] *G. Isopencu, M. Stroescu, A. Brosteanu, N. Chira, O. C. Pârvulescu, C. Busuioc and A. Stoica-Guzun*, “Optimization of ultrasound and microwave assisted oil extraction from sea buckthorn seeds by response surface methodology”, in *J. Food Process. Eng.*, **vol. 42**, no.1, Feb. 2019, e12947.

[21] *J. Deng, H. Yang, E. Capanoglu, H. Cao and J. Xiao*, “Technological aspects and stability of polyphenols. In “Polyphenols: Properties, Recovery, and Applications”, 2018, pp. 295-323.

[22] *T. Theppakorn*, “Stability and chemical changes of phenolic compounds during Oolong tea processing”, in *Int. Food Res. J.*, **vol. 23**, no. 2, Sep. 2016, pp. 564-574.

[23] *S. Maini, H. L. Hodgson and E. S. Krol*, “The UVA and Aqueous Stability of Flavonoids Is Dependent on B-Ring Substitution” in *J. Agric. Food Chem.*, **vol. 60**, Jul. 2012, pp. 6966–6976.

[24] *H. Chaaban, I. Ioannou, L. Chebil, M. Slimane, S. Gerardin, C. Paris, C. Charbonnel, L. Chekir and M. Ghoul*, “Effect of heat processing on thermal stability and antioxidant activity of six flavonoids”, in *J. Food Process. Preserv.*, **vol. 00**, e13203, Aug. 2018, pp. 1-12.

[25] *I. Volf, I. Ignat, M. Neamtu and V. I. Popa*, “Thermal stability, antioxidant activity, and photo oxidation of natural polyphenols”, in *Chem. Pap.*, **vol. 68**, no. 1, 2014, pp. 121–129.

[26] *Y. Cheng, Q. Xu, J. Liu, C. Zhao, F. Xue and Y. Zhao*, “Decomposition of Five Phenolic Compounds in High Temperature Water”, in *J. Brazil. Chem. Soc.* (2014), **vol. 25**, no. 11, Sep. 2008, pp. 1609-1619.

[27] *E. Nakilcioglu-Tas and S. Otles*, “Degradation kinetics of bioactive compounds and antioxidant capacity of Brussels sprouts during microwave processing”, in *Int. J. Food Prop.*, **vol. 20**, S3, Jan. 2017, pp. S2798–S2809.

[28] *K. Robinson, C. Mock, and D. Liang*, “Pre-formulation studies of resveratrol”, in *Drug Dev. Ind. Pharm.* **vol. 41**, no. 9, Sep. 2015, pp. 1464-1469.

[29] *S. J. Franklin and P.B. Myrdal*, “Solid-State and Solution Characterization of Myricetin”, in *AAPS Pharm. Sci.Tech.*, **vol. 16**, no. 6, Dec. 2015, pp. 1400-1408.

[30] *A.-M. Brezoiu, M. Deaconu, I. Nicu, E. Vasile, R.-A. Mitran, C. Matei and D. Berger*, “Heteroatom modified MCM-41-silica carriers for Lomefloxacin delivery systems”, in *Micropor. Mesopor. Mater.*, **vol. 275**, Feb. 2019, pp. 241-222.

[31] *A. Nayak, B. Bhushan, A. Rosales, L. Rodriguez Turienzo and J. L. Cortina*, “Valorisation potential of Cabernet grape pomace for the recovery of polyphenols: process intensification, optimisation and study of kinetics”, in *Food Bioprod. Process.*, **vol. 109**, Mar. 2018, pp. 74–85.

[32] *R. Fontana, A. Antonioli and R. Bottini*, “Grape pomace as a sustainable source of bioactive compounds: extraction, characterization, and biotechnological applications of phenolics”, in *J. Agric. Food Chem.*, **vol. 61**, no. 38, Sep. 2013, pp. 8987–9003.

[33] *Y. Xu, S. Burton, C. Kim and E. Sismour*, “Phenolic compounds, antioxidant, and antibacterial properties of pomace extracts from four Virginia-grown grape varieties”, in *Food Sci. Nutr.*, **vol. 4**, no. 1, 2016, pp. 125–133.

[34] *I. I. Rockenbach, L. V Gonzaga, V. M. Rizelio, A. E. D. S. S. Gonçalves, M. I. Genovese and R. Fett*, “Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking”, in *Food Res Int.*, **vol. 44**, no. 1, Jul. 2011, pp. 897–901.

[35] *P. S. Melo, A. P. Massarioli, C. Denny and L. Ferracini dos Santos*, “Winery by-products: Extraction optimization, phenolic composition and cytotoxic evaluation to act as a new source of scavenging of reactive oxygen species”, in *Food Chem.*, **vol. 181**, Aug. 2015, pp. 160–169.

[36] *A. A. Casazza, B. Aliakbarian, S. Mantegna, G. Cravotto and P. Perego*, “Extraction of phenolics from *Vitis vinifera* wastes using non-conventional techniques”, in *J. Food Eng.*, **vol. 100**, Mar. 2010, pp. 50-55.

[37] *J. C. Sanchez-Rangel, J. Benavides, J. B. Heredia, L. Cisneros-Zevallos and D. A. Jacobo-Velazquez*, “The Folin-Ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination”, in *Anal. Methods*, **vol. 5**, no. 21, 2013, pp. 5990-5999.

[38] *J. Lee*, “Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural colorants and Wines by the pH- differential method: Collaborative Study”, in *J. AOAC Int.*, **vol. 88**, no. 5, 2005, pp. 1269-1278.

[39] *A. Floegel, D.-O. Kim, S.-J. Chung, S. I. Koo and O. K. Chun*, “Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods”, in *J. Food Comp. Anal.*, **vol. 24**, no. 7, Nov. 2011, pp. 1043–1048.

[40] *B. M. Muche, R. A. Speers and H. P. V. Rupasinghe*, “Storage Temperature Impacts on Anthocyanins Degradation, Color Changes and Haze Development in Juice of “Merlot” and “Ruby” Grapes (*Vitis vinifera*)”, in *Front. Nutr.*, **vol. 5**, Oct. 2018, pp. 1-9.

- [41] *P. Loypimai, A. Moongngarm and P. Chottanom*, “Thermal and pH degradation kinetics of anthocyanins in natural food colorant prepared from black rice bran”, in *J. Food Sci. Technol.*, **vol. 53**, no. 1, Jan. 2016, pp. 461–470.
- [42] *A. M. Mebert, C. J. Baglole, F. Martin, M. F. Desimone and D. Maysinger*, “Nanoengineered silica: Properties, applications and toxicity”, in *Food Chem. Toxicol.*, **vol. 109**, part. 1, Nov. 2017, pp. 753-770.
- [43] *M. Ben Aziz, M. Garcia, L. Moulis, H. Fulcrand and H. Hajjaj*, “Proanthocyanidins and anthocyanins contents, chromatic and antioxidant properties of red grape pomaces from morocco”, in *J. Food Meas. Charact.*, **vol. 13**, Apr. 2019, pp. 2051-2061.