Food and Chemical Toxicology 133 (2019) 110787

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Polyphenols extract from grape pomace. Characterization and valorisation through encapsulation into mesoporous silica-type matrices

Ana-Maria Brezoiu[a], Cristian Matei[a], Mihaela Deaconu[a], Ana-Maria Stanciuc[b], Adrian Trifan[a], Alexandra Gaspar-Pintiliescu[b], Daniela Berger[a,*]

[a] University "Politehnica" of Bucharest, Faculty of Applied Chemistry and Material Sciences, 1-7 Gheorghe Polizu Street, Bucharest, 011061, Romania
[b] National Institute of Research and Development for Biological Sciences, 296 Splaiul Independetei, Bucharest, 060031, Romania

ARTICLE INFO

Keywords:
Grape pomace extract
Microwave-assisted extraction
Phenolic extract
Encapsulated extract
Mesoporous silica

ABSTRACT

We report the encapsulation of two grape pomace polyphenolic extracts into mesoporous MCM-41-type silica matrices (pristine and Zn or Mg heteroatom modified) to reduce the extract sensitivity and enhance its stability, while preserving the radical scavenger activity. Various grapes marc (Cabernet Saugvinon and Feteasca Neagra from the Black Sea region and commercially available grape skins powder) were used to prepare ethanolic extracts either through conventional extraction, or microwave-assisted procedure. The polyphenolic extracts composition was analysed by reversed phase-high pressure liquid chromatography and spectrometric determination of total polyphenols and ascorbic acid (using Folin Ciocalteu reagent), total flavonoids (by $AlCl_3$ complexation), as well as total anthocyanin monomeric pigments content.

The encapsulated extract into MCM-41 silica, as well as Zn-MCM-41 and Mg-MCM-41 matrices showed an enhanced radical scavenger activity assessed by DPPH procedure developed for solid samples. The cytocompatibility tests performed on HaCaT keratinocyte human cells demonstrated a good cytocompatibility for the Cabernet Saugvinon and grape skins extracts free and encapsulated into MCM-41-type matrices.

1. Introduction

Grape pomace, a mixture of skins, seed and stems resulted after pressing grapes in the winemaking process is an abundant by-product, representing about 20%wt of the processed grapes (Schieber et al., 2001). Considering that the winemaking process lead to an incomplete extraction of valuable compounds, grape pomace is a by-product with relatively high concentration of various and inhomogeneous distributed polyphenolic compounds (Fontana et al., 2013; Rockenbach et al., 2011). This by-product can be easily turned into cheap valuable raw compounds for cosmetics, nutraceuticals, pharmaceutical and food industries, thus reducing the environmental impact related to waste disposal in the region of wine factories (Teixeira et al., 2014).

Grape polyphenolic extracts showed cardioprotective effects, antifungal, antimicrobial, anti-inflammatory and anti-cancer properties (Ky et al., 2014). For example, polyphenolic extracts from Feteasca Neagra grape pomace were investigated in a seven-day pre-treatment on the isoprenaline-induced infarct-like lesion in rats (e.g., heart rate reduction, respiratory rate modifications, which allowed the consecutive loss of cell membrane potential in the injured myocardium because of the oxidative stress), assessed by ECG monitoring, serum levels of creatine kinase, aspartate transaminase, and alanine transaminase and demonstrated *in vivo* cardioprotective effect against isoprenaline-induced myocardial ischemia due to reducing oxidative stress (Balea et al., 2018). Also, *in vivo* studies carried out on spontaneously hypertensive rats proved the efficiency of Grenache seed pomace and Alicante skin pomace extracts to regulate blood pressure (Ky et al., 2014).

It is well known that the solvent used for the extraction and experimental conditions greatly influence the polyphenols composition, which in turn determines the features of the extracts. For instance, when ethanolic extracts from Cabernet Sauvignon, Carmenere and Syrah grape pomace from Chile (Misiones de Rengo Vineyard), were sequentially extracted with hexane, chloroform and ethyl acetate, and were analysed *in vivo* on mycelial growth of *Botrytis cinereal* fungus (isolated from naturally infected grapes), the extraction fractions showed either prooxidant and inhibition effect on mycelial growth, or antioxidant activity depending on the solvent nature (Cotoras et al., 2014).

Hydroalcoholic extracts obtained from Uruguayan species grape pomace proved to have *in vitro* anti-inflammatory properties due to

* Corresponding author.
E-mail address: danaberger01@yahoo.com (D. Berger).

https://doi.org/10.1016/j.fct.2019.110787
Received 3 June 2019; Received in revised form 19 August 2019; Accepted 22 August 2019
Available online 23 August 2019
0278-6915/

high content of polyphenols. Cyclooxygenases, COX-1 and COX-2 are involved in the inflammatory mechanism and the grape extracts exhibited inhibition activity in COX-1 and COX-2 similar to the propolis extract and commercially available drug, Colecoxib (Paulino et al., 2016). Even if the phenolic compounds are less concentrated in grape marc extract than in the propolis, the grape extract compounds showed higher activity. Another benefit of grape pomace extract could be related to its antitumoral activity, the grape seed petroleum ether extract showed beneficial anti-cancer properties against skin cancer A431 cell line when it is used in conjunction with doxorubicin (Mohansrinivasan et al., 2015).

Concerning the antimicrobial activity of grape marc extracts, the reported minimum inhibitory concentration values were in the range of 4.69–18.8 mg/mL and 40.6–250 mg/mL for *Listeria monocytogenes* and *S. aureus,* respectively, which proved that they could exhibit bactericidal effect against Gramm-positive strains, but at very high concentration (Xu et al., 2016).

However, the effectiveness of phenolic extracts is directly related to the preservation of the stability, bioactivity, and bioavailability of their compounds (Fang and Bhandari, 2010). The unsaturated bonds in the polyphenols molecular structure make them sensitive to oxidizers, water, light, heat, pH, or enzymes (Cilek et al., 2012). As polyphenols are unstable either during food processing, distribution and storage, or in the gastrointestinal tract, their activity and health benefits are limited. Unfortunately, they easily oxidize, leading to the progressive appearance of a brown colour and/or unwanted odours with a considerable antioxidant activity loss (Munin and Edwards-Lévy, 2011).

The stability of different polyphenols was assessed through several accelerated degradation methods, like heating, microwave irradiation or various pH testing. For example, the thermal degradation of rutin is complete at 130 °C in 45 min or it suffers a 10% and 50% content loss when heated at 70 °C for 2 h and 90 °C, respectively (Chaaban et al., 2017). Gallic acid and vanillic acid are stable up to 80 °C, while catechin starts to decompose at 60 °C (Volf et al., 2014). Myricetin undergoes first-order degradation in basic medium, but it is stable under UV irradiation in the presence of other antioxidants, which makes it suitable for topical applications (Franklin and Myrdal, 2015). Like myricetin, *trans*-resveratrol is also unstable in basic medium (Robinson et al., 2015).

An increased stabilization of polyphenolic extracts could be achieved by encapsulation in various nanocarriers. The embedding matrices act as a physical barrier reducing the permeability of oxygen and other molecules and therefore, the shelf-life of the extraction compounds could be prolonged (Wang et al., 2009).

The encapsulation of polyphenolic extracts in different matrices was reported for the development of preservation systems, mainly for the food industry. For example, procyanidin extracts from grape seeds encapsulated in a mixture of maltodextrin and Arabic gum remained unchanged during the encapsulation process and consequently, they exhibited an increased stability (Zhang et al., 2007). Kosaraju et al. reported that the grape seed extracts embedded in a matrix based on sodium caseinate-soy lecithin proved significant prolonged antioxidant activity in comparison with placebo (Kosaraju et al., 2008). Also, the polyphenolic extracts encapsulated in a mixture of Arabic gum, partially hydrolysed guar gum, and polydextrose with a retention factor of polyphenolic compounds of 80%, exhibited high antioxidant properties, being proposed as potential dyes in functional foods (Kuck and Noreña, 2016). Recently, a faster degradation of the free grape pomace extract, prepared by MW-assisted extraction, than that of the extract encapsulated in maltodextrin-based microcapsules was noticed (Tsali and Goula, 2018).

Regarding the use of inorganic carriers, Khan et al. reported the encapsulation of polyphenolic flavonoids in titania-functionalized mesoporous silica, quercetin being bidentate bonded on TiO_2 nanoparticles via catechol molecule. The natural compounds recovery in a 20% citric acid solution in ethanol was possible because citric acid bound on TiO_2 and therefore led to the flavonoids release. The radical scavenger activity of encapsulated flavonoids was similar to that of the free extract (Khan et al., 2017). Recently, it was reported that tannic acid, a polyphenolic compound, could form supramolecular network with silica-type species, being applied as template agent for synthesis of mesoporous silica nanoparticles for bio-medical applications (Luo et al., 2019). Among nanocarriers employed for the encapsulation of biomolecules (enzymes, peptides, genes, vitamins, etc.), mesoporous silica nanoparticles (MSN) were widely studied in order to design nanostructures as versatile platforms for various medical applications (Doadrio et al., 2015; Argyo et al., 2014; Tang and LiChen, 2012). The tailoring of MSN surface properties by functionalization either with organic groups/molecules (Bouchoucha et al., 2016; Choi et al., 2014), or metallic oxides (An et al., 2016) is a major advantage for achieving the desired biomolecules-carrier interactions, sensitive to stimuli produced by biological systems, which could facilitate the delivery of the cargo molecules in a targeted tissue, leading to an enhanced therapeutic efficiency (Zhou et al., 2018).

Herein, we report for the first time the encapsulation of two grape pomace extracts into mesoporous MCM-type silica matrices (pristine, and Zn or Mg heteroatom modified) to improve their chemical stability and preserve antioxidant properties, as well as the development of a procedure for the evaluation of radical scavenger activity for materials containing encapsulated phytochemicals. In our study, the modified mesoporous silica supports were specially designed to have the inner pores surface decorated with amorphous zinc or magnesium oxide nanoparticles, to minimise their effects on living cells.

Nowadays, zinc oxide is one of the most popular ingredients found in protection sunscreen creams, being a good UV absorber due to its large band gap, but cheaper than TiO_2 (Threes and Stanislav, 2011). This property could be exploited in the preservation of encapsulated polyphenolic extracts in order to extend their protection to UV radiations. On the other hand, once they entered in the blood system, it was proved that ZnO nanoparticles exhibit increased toxicity versus bulk oxide (Mohammed et al., 2019), affecting especially liver and kidney (Srivastav et al., 2016). It was demonstrated that ZnO toxicity is dose dependent, the IC50 values for human pancreatic cell lines, Panc-1 and AsPG-1 being 40 μM and 30 μM, respectively, while for human normal fibroblast HuO_2 cell line, 80 μM (Zhang et al., 2018). Our recent method developed for the obtaining of heteroatom modified mesoporous silica, based on ion-exchange at the level of pores inner surface, leads to the formation of metal oxide inside the silica pores (Brezoiu et al., 2019), ensuring a minimum exposure of ZnO to the leaving cells. Lately, MgO nanoparticles has drawn attention for biomedical applications, like MRI contrast agent, nano-cryosurgery, as potential bactericidal material, etc. Experiments performed on HeLa cells exposed to MgO nanoparticles showed that cell viability is dependent on MgO concentration, a loss of approximately 14% of viable cells being observed for a concentration of 250 μg/mL (Akram et al., 2018).

2. Experimental

2.1. Materials

All the reagents, sodium carbonate (Na_2CO_3), potassium persulphate ($K_2S_2O_8$), 36.5–38%wt hydrochloric acid (Sigma), ethanol (Sigma-Aldrich), MCM-41 (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), vanilic acid (TCI, > 98%, GC-grade), caffeic acid (Sigma, 98%, HPLC-grade), syringic acid (Molecula, > 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 grape pomace came from the Black Sea Region and the grape skin powder and ascorbic acid powder were 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 grape pomace and grape skins

The polyphenolic extracts in absolute ethanol were prepared from two different red grape pomaces from the Black Sea region (Cabernet Saugvinon, CS, and Feteasca Neagra, FN), as well as a commercially available grape skins powder (GS) as vegetal materials using either conventional (Conv) or microwave-assisted (MW) extraction. The polyphenols conventional extraction was performed by refluxing the vegetal material with absolute ethanol (vegetal material/ethanol ratio of 1/6 (w/v) for three times for 1 h), under constant magnetic stirring, intermediate filtration and solvent replacement in the same volume and then the ethanolic extracts were mixed.

The MW extraction was carried out on a Sairen Miniflow 200SS microwave reactor using the grape skins powder and absolute ethanol in the same vegetal material/solvent ratio as in the case of conventional extraction, in three stages at 80 °C/15 min using a MW power of 75 W (average reflected power of 6 W) with intermediate filtration and solvent replacement.

The obtained polyphenolic extracts were dried under vacuum until reached a constant mass and then re-dissolved appropriately for preparation of alcoholic solutions of certain concentration.

2.3. Characterization of phenolic extracts

The polyphenolic extracts were characterized through several spectrophotometric methods (Shimadzu UV-1800) to determine total ascorbic acid, polyphenols, flavonoids and anthocyanin pigments content.

The ascorbic acid content was assessed based on a calibration curve of commercially available ascorbic acid in the 10–500 μg/mL domain established by mixing 1 mL standard ethanolic solution with 0.5 mL Folin-Ciocalteu reagent, previously diluted with 4.5 mL ultrapure water, thoroughly homogenized for 3 min at room temperature, protected from the light and afterwards measured the solution absorbance at 765 nm wavelength. In the case of phenolic extracts, the standard ascorbic acid solution was replaced with 1 mL extract with the concentration of 1 mg/mL. The measurements of ascorbic acid content (AA) were made in replicated three experiments.

The total polyphenolic content was determined based on the standard curve for gallic acid (50–450 μg/mL) established by mixing 1 mL standard ethanolic solution with 0.5 mL Folin-Ciocalteu reagent, previously diluted with 4.5 mL ultrapure water. The mixture was homogenized for 3 min at room temperature and then 4 mL Na_2CO_3 solution (75 g/L) were added, followed by another homogenization, incubated at room temperature in dark conditions, for 30 min. The solution absorbance values were measured at both 765 nm and 650 nm wavelengths. For the extracts analysis, the standard solution was replaced with the extract of 1 mg/mL concentration and the incubation time was increased at 1.5 h considering the literature in which the incubation time is 1 h (Nayak et al., 2018), 1.5 h (Tsali and Goula, 2018; Nayak et al., 2011) or 2 h (Sanchez-Rangel et al., 2013). Also, it was observed that between 1.5 h and 2 h incubation time, there was no difference of the solution absorbance, hence, 1.5 h was enough to obtain the proper results.

The total flavonoids content was assessed by adapting a reported procedure (Pekal and Pypzynska, 2014). Briefly, 1 mL tested solution was mixed with 0.5 mL of 2% aqueous solution of aluminium chloride and 0.5 mL ultrapure water. After 15 min of incubation in dark conditions, at room temperature, the formed complex was analysed at maximum wavelength (in the range of 410–430 nm). In the case of extracts, the incubation time was increased at 30 min and a concentration of 0.5 mg/mL was used. For the calibration curve, an ethanolic solution of quercetin (5–50 μg/mL) was used and its absorbance was measured at 430 nm wavelength.

The content of total anthocyanin pigments was assessed according to the procedure described by Lee (2005), considering that anthocyanin pigments are coloured at pH 1 due to the flavylium cation (oxonium form) and become colourless at pH 4.5 in the hemiketal form (carbinol pseudo-base). Therefore, plant extracts with a concentration of about 1 mg/mL were diluted with buffer solution pH 1 until the solution absorbance was in the spectrophotometer range and the dilution factor was determined. Using the same dilution factor, the plant extract was diluted with buffer solution of pH 4.5 and the corresponding absorbance was measured at 520 nm and 900 nm having as reference the corresponding buffer solution.

Total content of monomeric anthocyanin pigments expressed as cyanidin-3-glucoside equivalents was determined using equation. (1):

$$\text{Anthocyanin pigment (cyanidin} - 3 - \text{glucoside equivalents, mg/L)} = \frac{A * M * f * 10^3}{\varepsilon * 1},$$

where $A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5}$, where M (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu), *f*, dilution factor, l = pathlength in cm, ε = 26 900-M extinction coefficient, in L/mol/cm for cyd-3-glu and 10^3 = mass conversion factor from grams to milligrams.

The polyphenols composition was evaluated using HPLC (Shimadzu Nexera 2) with photodiode array detector (SPD-M30A) on a Nucleoshell C18 column (2.7 μm*2.7 μm*100 mm), using a gradient elution at a constant flow (0.4 mL/min). Two mobile phases were used A-H_2O/HCOOH = 100/2.5 (v/v) and B-ACN/H_2O/HCOOH = 90/10/2.5 (v/v/v) and the B mobile phase concentration was 5% at 1.5 min, then gradually increased at 7.5% in 6 min, further increased at 13.5% in 6 min and maintained for another 2.5 min, then gradually increased at 18.5% in 5 min and maintained for 1 min, gradually increased to 22.5% in 3.5 min, further increased to 35% in 4.5 min, followed by the wash stage, which comprised the concentration increase to 100% in 5 min and maintained for 5 min, then gradually decreased to 5% in 10 min.

The identification of each component in the extract samples was done considering the retention times, as well as the spectrum similarity in comparison with the standard substances (HPLC-grade) and the quantification was performed using the calibration curve at the maximum absorption wavelength for each reference substances.

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

As supports for encapsulation of polyphenolic extracts, commercially available MCM-41 silica and Zn- and Mg-modified MCM-41 silica were considered. The synthesis of Zn- and Mg-modified MCM-41 carriers and their properties were reported in our previous paper (Brezoiu et al., 2019).

The materials containing polyphenolic extract were prepared by incipient wetness impregnation method using either the extract

obtained from grape skin powder by conventional method with a 48.2% wt extract content in MCM-41 silica matrix, or Cabernet Saugvinon extract with 46.5% and 49.7%wt content in Mg-MCM-41 and Zn-MCM-41, respectively. 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 4–6 h. The materials resulted after the extract encapsulation into the mesoporous support were denoted extract@support.

2.5. Characterization of materials containing polyphenolic extract

The samples containing encapsulated polyphenolic extract were characterized by thermogravimetric analysis, infrared spectroscopy, nitrogen adsorption–desorption isotherms, as well as DPPH-antioxidant activity. The FTIR spectra were recorded in 4000–400 cm^{-1} range on a Bruker Tensor 27 spectrophotometer (KBr pellet technique) in order to confirm the extract encapsulation. The thermogravimetric analyses (TG) were performed to determine the amount of polyphenolic compounds in materials containing encapsulated extract using a Netzsch STA 449 F3 Jupiter equipment at a scan rate of 10 °C/min, under synthetic air flow. Nitrogen adsorption-desorption isotherms were recorded at the 77 K using a Quantachrome Autosorb iQ_2 gas sorption analyser. Prior to the isotherms recording, the materials containing encapsulated extract were outgassed at 35 °C, 17 h. The specific surface area values, S_{BET}, were computed through the Brunauer-Emmett-Teller method in the relative pressure range of 0.05–0.25 and the total pore volume was determined for relative pressure of 0.99.

2.6. Determination of free radical scavenging activity

The radical scavenger activity (RSA) of polyphenolic extracts was assessed by DPPH and ABTS assays. DPPH method consists in the free radical DPPH absorbance reduction when mixed with a substance with antioxidant activity with the formation of DPPH-H and free radical of the antioxidant species. To assess the RSA, 50 μL of polyphenolic extract was mixed with 2950 μL ethanolic DPPH solution (0.025 g/L), vigorously shaken and incubated in dark conditions for 30 min and then the solution absorbance was measured at 517 nm. To determine the linearity domain, several extract concentrations were tested (from 0.001 to 10 mg/mL). All the experiments were performed in triplicate for each concentration. The RSA was determined having as reference the initial DPPH solution absorbance by the following equation: $RSA(\%) = 100*(A_{DPPH}-A_{Extract})/A_{DPPH}$, where A_{DPPH} is the solution absorbance of DPPH free radical and $A_{Extract}$ is the one of extract-DPPH mixture at 517 nm. From the linearity domain, the concentration of extract that inhibits 50% of DPPH free radicals (IC 50%) was determined.

In the case of ABTS assay, the ABTS carbocation free radical was generated by the chemical reaction between 10 mL of 7 mM aqueous solution of ABTS and 176 μL of 2.45 mM aqueous solution of potassium persulfate, the prepared solution being left in the dark conditions, at room temperature, for at least 12 h before the use. Afterwards, the ABTS free radical solution was diluted with absolute ethanol to have the absorbance in the spectrophotometer range (at 753 nm) and was incubated at 30 °C. 980 μL ABTS carbocation free radical ethanolic solution were mixed with 20 μL of Trolox standard solution or extract solutions in a 1 cm cuvette, homogenize for about 1 min and the solution absorbance was measured at 753 nm prior and after the addition of the antioxidant sample. All determinations were made in triplicate and for each extract, and the calibration curve was determined in the range of 0.05–1.25 mM Trolox. The radical scavenger activity was calculated using the following equation: $RSA(\%) = 100*(A_{ABTS}-A_{Extract})/A_{ABTS}$, where A_{ABTS} is the solution absorbance of ABTS free radical at 753 nm and $A_{Extract}$ is that of extract-ABTS mixture at the same wavelength.

The radical scavenger activity of materials containing encapsulated extract (DPPH assay) was assessed after determination of IC 50% values for the free extract. Considering the linearity domain of DPPH assay, a lower concentration than IC 50% of the free extract was chosen because of the inherent degradation of DPPH solution during 24 h experiment. Then, the corresponding mass of encapsulated extract (containing the same extract amount as in 50 μL extract of chosen concentration per 2.95 mL DPPH free radical solution) was considered. Also, the same quantity of support as in the encapsulated extract was tested in the same conditions for evaluation of its influence on the radical scavenger activity. After the addition of DPPH solution on solid samples or extracts, the experiments were carried out in closed containers, and kept 24 h under magnetic stirring, using DPPH solution as control. Afterwards, aliquots of samples containing suspensions of encapsulated extract or support, as well as extracts and DPPH-free radical solution were withdrawn, centrifugated for 10–15 min and then the solution absorbance was measured at 517 nm.

2.7. In vitro cytocompatibility and cell morphology assessment

The cytocompatibility of Cabernet Saugvinon and grape skin extracts prepared by conventional extraction, as well as encapsulated extracts in Zn-MCM-41, Mg-MCM-41 and MCM-41 matrices in comparison with the corresponding mesoporous supports, was assessed *in vitro* on human normal keratynocytes - HaCaT spontaneously immortalized cell line (AddexBio, USA). MTT assay was used to evaluate the metabolic activity of human keratynocytes after 24 h incubation in the presence of the samples. Briefly, human keratynocytes were cultured in RPMI (Roswell Park Memorial Institute) 1640 (Millipore, USA) supplemented with 10% faetal bovine serum and 1% penicillin/streptomycin till confluence (80–90%) was reached. For experiments, cells were seeded 10×10^3 cells/well in 96 wells plate and incubated at 37 °C in humide atmosphere (5% CO_2). The control sample was considered the culture media without ethanol content. The stock solution or suspension of samples were sterilized by UV exposure for 2 h. Prior the evaluation, 100 μg/ml of each sample were incubated with the cells. After 24 h, cells were washed with phosphate-buffer saline solution and further incubated with MTT solution (5 mg/mL) for 3 h, at 37 °C, in dark conditions. Afterwards, the intracellular formazan crystals were solubilized with isopropanol and the absorbance was measured at 570 nm with background subtraction at 690 nm. The cell viability data were reported as mean value ± standard deviation of three replicated experiments. Statistical analysis of the data was performed using Student's t-test on each pair of interest. Differences were considered statistically significant for which $p < 0.05$.

For cell morphology assessment, prior Giemsa staining, the cell culture medium was removed, cell monolayer was washed with phosphate buffer solution, fixed with cold methanol (−20 °C) for 5 min and stained with Giemsa solution for 20 min. Giemsa stained morphological features were observed with an optical microscope Zeiss Axio Observer, 20X.

3. Results and discussion

3.1. Characterization of polyphenolic extracts

Wine-making industry by-products, grape pomace and grape skins or seeds, are widely studied for recovery of their polyphenols, flavonoids or anthocyanin monomeric pigments by extraction. Two grapes marc, Cabernet Saugvinon and Feteasca Neagra from the Black Sea region (Romania), as well as commercially available grape skin powder were used to obtain polyphenolic extracts either by conventional extraction, or MW treatment.

The alcoholic extracts were analysed by spectrometric determination of total polyphenols and ascorbic acid using Folin Ciocalteu reagent, total flavonoids by $AlCl_3$ complexation, as well as total anthocyanin monomeric pigments content, and the data were listed in

Table 1
Spectrophotometric determination of ascorbic acid (AA), total polyphenols (as gallic acid equivalents, GAE), total flavonoids (as quercetin equivalents, QE) and total anthocyanin pigments content (by extinction of cyanidin-3-glicoside method, CGE) and radical scavenger activity, RSA (as Trolox equivalents, TE) for extracts.

Extract	Extract (%wt)	AA (mg AA/g)	TP (mg GAE/g)	TF (mg QE/g)	TA (mg CGE/g)	RSA_{DPPH} (mg TE/g)	RSA_{ABTS} (mg TE/g)
CS (Conv)	9.17	45.54 ± 0.14	265.21 ± 4.97	14.11 ± 0.87	13.06 ± 0.86	344 ± 4	230 ± 7
FN (Conv)	9.69	33.79 ± 0.56	279.64 ± 4.52	16.72 ± 0.00	12.38 ± 2.17	119 ± 1	105 ± 2
GS (Conv)	23.2	37.78 ± 0.18	212.21 ± 0.85	18.96 ± 0.02	3.51 ± 0.71	125 ± 6	106 ± 9
GS (MW)	22.2	28.13 ± 0.33	239.38 ± 1.28	11.52 ± 0.01	3.40 ± 0.25	193 ± 3	137 ± 5

CS-Cabernet Saugvinon; FN-Feteasca Neagra; GS-grape skin. All values are expressed per gram of extract.

Table 1. One can notice a higher extract content when grape skin powder was used (22.2–23.2%) in comparison with the grape pomace (9.17% for Cabernet Saugvinon and 9.69% for Feteasca Neagra, respectively), which suggests that the grape skin powder has a higher amount of ethanol soluble compounds, probably because the grape marc contains besides skins, seeds and stems with a lower polyphenols content. These values are higher than that reported by Sagdic et al. (2011) (1.14–4.59% extract) for ethanolic grape pomace extracts obtained through a conventional extraction, or by Otero-Pareja et al. (2015) for grape pomace extracts prepared by supercritical carbon dioxide extraction with 20% ethanol (3.50–7.00% extract).

The ascorbic acid content varied between 3.27 and 4.17 mg AA per gram of vegetal material for grape pomace extracts and 6.24–8.76 mg AA/g of vegetal material for grape skin powder extracts (Table 1), which are higher than that reported by Abdrabba et al. (Abdrabba and Hussein, 2015) for grape pulps, seeds and skins (0.049–0.122 mg AA/g of vegetal material), or by Sousa et al. (2014) for grape pomace flour (0.2625 ± 0.0001 mg AA/g of vegetal material). It can be noticed that MW treatment diminishes the content of ascorbic acid.

The total polyphenolics (TP) content was evaluated as gallic acid equivalent at two wavelengths (765 nm and 650 nm) and an average of four replicates (Table 1). Using the standard curve for gallic acid, the TP content was determined at 765 nm and 650 nm, and from obtained value was subtracted the ascorbic acid, a very well-known interfering substance in the Folin-Ciocalteu assay (Sanchez-Rangel et al., 2013). A higher TP content was obtained for extracts from grape pomace (Cabernet Saugvinon and Feteasca Neagra) than from grape skins powder. One can observe that the MW treatment enhanced slightly the TP amount when compared with conventional extraction. The TP values were in the range of 24.33–53.14 mg GAE per gram of vegetal material and are consistent with that reported for red grape pomace extract prepared by MW treatment using 24% ethanolic solution (26.44 mg GAE/g of vegetal material) (Tsali and Goula, 2018) and red grape pomace extract obtained in 80% ethanol acidified with 0.5% 0.1 N HCl (41.9 ± 0.4 mg GAE/g vegetal material) (Negro et al., 2003). Sagdic et al. evidenced that up to 2.7 times higher TP content is obtained from red grape pomace in comparison with white grape pomace (Sagdic et al., 2011). For instance, for acetone-water extract from Cabernet Saugvignon grape skin, Deng et al. (2011) reported a TP content of 26.7 ± 1.8 mg GAE/g vegetal material, a slightly higher than our value, which could not be attributed only to the influence of solvent, but also the fact that grape pomace also contains seeds and stems leading to an overall decrease of TP content. For 70% ethanolic extracts from Feteasca Neagra grape pomace, a TP index in the range of 114.71–161.58 mg catechin equivalent/g vegetal material was reported (Balea et al., 2018). Ky et al. measured for several grape skin extracts, a TP content in the range of 34.8–52.3 mg GAE/g vegetal material, which are consistent with our values (Ky et al., 2014).

The total flavonoids (TF) content made in duplicate and expressed as quercetin equivalents (QE) was assessed based on a standard curve established at 430 nm. For extracts (0.5 mg/mL), the solution absorbance was read at the maximum absorption wavelength, 420 nm. It can be observed higher TF values in the case of conventional grape skin extract than the one obtained by MW treatment (Table 1). The values for the grape pomace and grape skin extracts are in the range of 11.52–18.96 mg QE/g extract, which are higher than the ones reported by Kuru et al. for both young and mature grape seeds (1.75–5.72 mg QE/g extract), but lower than for young and mature grape leaves (66.62–107.21 mg QE/g extract) (Kuru et al., 2017). Wang et al. reported the obtaining of an extract from muscadine pomace with a TF content of 3 ± 0.3 mg QE/g extract (Wang et al., 2010), which is at least four-times lower than the values of TF content determined for our extracts.

The total anthocyanin monomeric pigments (TA) content was assessed based on the procedure described by Lee (2005) considering the molar extinction coefficient of cyanidin-3-glicoside (denoted CGE) and its corresponding molar weight. The TA values are listed in Table 1 as an average of two measurements. Low values for anthocyanin content for extracts obtained from grape skin (3.51 ± 0.71 mg CGE/g extract) and even smaller for the extract prepared by MW treatment (3.40 ± 0.25 mg CGE/g extract) were determined. However, the anthocyanin content of grape marc extracts was higher than those reported by Xu et al. for white and red grape pomace (0.02–0.06 mg and 1.38–10.7 mg CGE/g extract, respectively) (Xu et al., 2016).

The reverse phase HPLC-PDA analysis was carried out for identification and quantification of phenolic compounds in extracts based on their retention times and comparison with UV spectra. For HPLC analysis, a C18 stationary phase was used that had been proved to be efficient for the separation of polyphenolic compounds. The separation of polyphenolic standard mixture is provided in Supplementary Material (Fig. S1), along with the calibration curve, retention time and detection wavelength for each reference compound: gallic acid, protocatechuic acid, catechin hydrate, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, (−) epicatechin, delphinidin chloride, *p*-coumaric acid, cyanidin chloride rutin hydrate, pelargonidin chloride, malvidin chloride, myricetin, rosmarinic acid, quercetin and kaempferol (see Supplementary Material, Table S1). Thirteen substances of the available standard solutions were identified in the prepared extracts or in the extracts analysed after 4 months and their concentrations are listed in Table 2. The chromatograms of prepared extracts are presented in Fig. S2. The limit of detection (LOD), limit of quantification (LOQ) and the linear range of calibration are presented in Table S1. LOD and LOQ values were determined in accordance with European Pharmacopeia considering a signal/noise ratio of at least 3 for LOD determination and more than 10 for LOQ, respectively.

High amounts (0.462–1.171 mg/g extract) of gallic acid, vanillic acid (0.368–1.088 mg/g extract), syringic acid (0.339–2.031 mg/g extract) and protocatechuic acid (0.104–0.489 mg/g extract), as well as low concentrations (0.019–0.083 mg/g extract) in *trans*-resveratrol were found in all samples. The MW treatment decreased the content of gallic acid, protocatechuic acid, vanilic acid, syringic acid and *p*-coumaric acid (not detected), but enhanced concentration of rutin hydrate, (−) epicatechin, and *trans*-resveratrol (Table 2). For the last compound, the concentration was in the range of 0.019–0.083 mg/g extract, the highest amount being obtained for MW treatment (21.31 mg/kg of vegetal material), similar value with that reported by Geana et al. (18.7 mg/kg). For methanolic extracts prepared from two grape pomace of the same cultivars, Feteasca Neagra and Cabernet Saugvinon, the concentration of *trans*-resveratrol was 18.7 mg/kg and 2.77 mg/kg, respectively (Geana et al., 2015), different than ours for ethanolic extracts

Table 2
Phenolic compounds identification and quantification by reverse phase HPLC-DAD.

Concentration in extract (mg/g)									
Standard substances	RT (min.)	CS (Conv)	CS (Conv)*	FN (Conv)	FN (Conv)*	GS (Conv)	GS (Conv)*	GS (MW)	GS (MW)*
Gallic acid	3.545	1.171 ± 0.004	1.191 ± 0.001	1.069 ± 0.001	1.060 ± 0.020	0.625 ± 0.001	0.647 ± 0.000	0.462 ± 0.008	0.477 ± 0.002
Protocatechuic acid	6.982	0.451 ± 0.003	0.482 ± 0.001	0.489 ± 0.001	0.513 ± 0.000	0.127 ± 0.000	0.146 ± 0.001	0.104 ± 0.000	0.113 ± 0.001
Catechin hydrate	12.382	0.184 ± 0.005	-nd	-nd	-nd	-nd	-nd	-nd	-nd
Chlorogenic acid	13.016	-nd	-nd	-nd	-nd	-nd	0.121 ± 0.000	-nd	-nd
Vanillic acid	14.825	0.368 ± 0.001	0.386 ± 0.002	1.088 ± 0.001	1.097 ± 0.047	0.743 ± 0.000	0.802 ± 0.000	0.568 ± 0.001	0.543 ± 0.001
Syringic acid	16.418	2.031 ± 0.008	2.135 ± 0.002	1.917 ± 0.001	2.314 ± 0.007	0.480 ± 0.000	0.491 ± 0.000	0.339 ± 0.002	0.312 ± 0.001
(−) Epicatechin	17.554	2.666 ± 0.018	2.962 ± 0.002	2.270 ± 0.005	1.507 ± 0.006	-nd	0.612 ± 0.004	1.983 ± 0.010	1.653 ± 0.003
Trans-p-coumaric acid	21.674	-nd	-nd	-nd	0.044 ± 0.001	0.022 ± 0.001	0.022 ± 0.001	-nd	-nd
Rutin hydrate	25.964	0.532 ± 0.001	0.637 ± 0.000	-nd	-nd	0.382 ± 0.000	0.394 ± 0.000	4.944 ± 0.007	0.213 ± 0.001
Pelargonidin chloride	29.140	0.606 ± 0.001	0.209 ± 0.002	-nd	-nd	-nd	-nd	-nd	-nd
Myricetin	31.646	0.172 ± 0.001	0.166 ± 0.000	0.055 ± 0.001	-nd	-nd	-nd	-nd	-nd
Trans- resveratrol	33.129	0.033 ± 0.001	-nd	0.049 ± 0.001	0.048 ± 0.001	0.019 ± 0.005	0.021 ± 0.000	0.083 ± 0.001	0.086 ± 0.001
Quercetin	34.919	-nd	-nd	-nd	0.370 ± 0.001	-nd	-nd	-nd	-nd

RT-retention time, nd – not detected, *- extracts analysed after 4 months. All concentrations are given in mg compound per gram of extract.

(4.74 mg/kg and 3.02 mg/kg, respectively). The *trans*-resveratrol content in Feteasca Neagra extracts obtained in 50% ethanol and 5.25% potassium metabisulfite aqueous solution was in the range of 0.57–2.92 mg/kg (Muncaciu et al., 2017), which suggests the influence of either solvent and extraction conditions, or winery by-product composition, the small amount of resveratrol could be attributed to its low stability in basic medium (Robinson et al., 2015).

Sagdic et al. reported for pomace extracts obtained in 95% ethanol from five grape cultivars, a high content of gallic acid in the range of 0.675–1.525 mg/g extract, slightly higher than in our extracts (0.462–1.171 mg/g extract), an amount of *p*-coumaric acid between 0.020 and 0.065 mg/g extract, similarly to our GS (Conv) extract (0.022 ± 0.001 mg/g extract), and a resveratrol concentration in the range of 0.0031–0.0146 mg/g extract lower than our extract content in resveratrol (0.019–0.083 mg/g extract) (Sagdic et al., 2011).

The stability of the free extracts was assessed based on chemical profiling determined by RP-HPLC (in the same conditions as for freshly prepared extracts) after 4 months of storage in refrigerator in tightly closed brown glass bottles. Some changes in the chemical composition of extracts stored in refrigerator for four months were observed. For example, in the case of CS extract, there is a slight increase in gallic acid, protocatechuic acid, vanillic acid, syringic acid, (−) epicatechin and rutin hydrate amounts and a significant decrease in pelargonidin chloride and *trans*-resveratrol concentrations, while myricetin content suffers a slight reduction.

For GS (Conv) extract, similar concentrations of all components (gallic acid, protocatechuic acid, vanilic acid, syringic acid, rutin hydrate, *trans-p*-coumaric acid and resveratrol) were observed. Also, one can notice the appearance of chlorogenic acid and (−) epicatechin. Unlike the GS extract obtained by conventional extraction, in the case of GS extract prepared by MW treatment, the same compounds were found in the old extract, but the rutin hydrate content dropped approximately 23 times. Also, the total phenolic compounds content of GS (MW) sharply diminished from 239.38 ± 1.28 mg GAE/g extract to 119.39 ± 3.82 mg GAE/g extract.

Regarding FN (Conv) extract, it was observed a similar content of gallic acid, protocatechuic acid, vanilic acid, syringic acid and *trans*-resveratrol and the disappearance of myricetin, which is unstable, mainly because of its several hydroxyl groups (Maini et al., 2012), a diminished content of (−) epicatechin, as well as the appearance of *trans-p*-coumaric acid and quercetin.

3.2. Characterization of materials-containing encapsulated extract

Silica is listed as food additive, E551, in European Union and the United States Food and Drug Administration considers it an anticaking agent. Therefore, silica is widely used in food industry as flavour carrier, clarifying agent in brewery and oils, or in cosmetics as nanoparticles in products for skin, nails, hair or lips (Mebert et al., 2017).

In our study, to increase the extracts stability, they were encapsulated in mesoporous silica matrices. As nanocarriers for polyphenolic extracts encapsulation, MCM-41 silica and MCM-41 silica decorated with ZnO (Zn-MCM-41) or MgO (Mg-MCM-41) nanoparticles were chosen due to their high capacity to accommodate cargo molecules related to the large pore volume (ranging in 0.79–0.88 cm^3/g) and specific surface area (in the range of 718–976 m^2/g).

Both Zn- and Mg-modified MCM-41 silica materials employed in this work are amorphous, and the silica inner pore surface are decorated with ZnO (14.6 %wt) and MgO (6 %wt), respectively (Brezoiu et al., 2019). For encapsulation, the extract which exhibited the best radical scavenger activity, Cabernet Sauvignon grape pomace extract, and the grape skins extract, obtained in the same conditions, were chosen.

The materials containing encapsulated extract were characterized by thermogravimetric analysis, FTIR spectroscopy and nitrogen adsorption-desorption isotherms. The thermogravimetric analysis was applied to determine the content of polyphenolic compounds encapsulated into mesoporous silica-type matrices by considering the weight loss up to 600 °C (Fig. 1). Based on thermogravimetric analyses of encapsulated extracts, subtracting the physically adsorbed water determined from mass loss suffered during samples outgassing in vacuum, the phenolic compounds content was in the range of 46.5–49.7% wt, the highest value being for CS@Zn-MCM-41 sample (46.5%wt for CS@Mg-MCM-41 and 48.2%wt for GS@MCM-41). The extract content is well correlated with the total pore volume of the corresponding MCM-41 silica-type matrix.

The nitrogen adsorption-desorption isotherms (Fig. 2) confirmed the complete filling of support mesopores with polyphenolic compounds, e.g., the remained pore volume of GS@MCM-41 sample being 0.08 cm^3/g from 0.85 cm^3/g of the corresponding MCM-41 support and for CS@Mg-MCM-41 total pore volume being 0.08 cm^3/g from 0.79 cm^3/g for Mg-MCM-41.

In the FTIR spectra of encapsulated extract (Fig. 3), one can notice the vibration bands of both carrier and extract. The stretching vibrations of C–H bonds ($\nu_{C\text{-}H}$) in the 2850-2900 cm^{-1} region, the stretching vibrations of C–O bond (1742 - 1744 cm^{-1} for polyphenolic extracts and 1722 - 1746 cm^{-1} for extract-loaded materials), skeletal = C–O–C vibrations (1522 cm^{-1} and 1520 cm^{-1} for extracts and 1531 cm^{-1} for encapsulated extracts) specific to flavonoids (Favaro et al., 2018), as well as C–N stretching vibrations were assigned to polyphenolic compounds (Fig. 3 a, b, c and d) and the bands from 1096 cm^{-1}, 815 cm^{-1}, 972 cm^{-1}, and 470 cm^{-1} were attributed to the asymmetrical and symmetrical stretching vibrations of Si–O–Si bonds, stretching

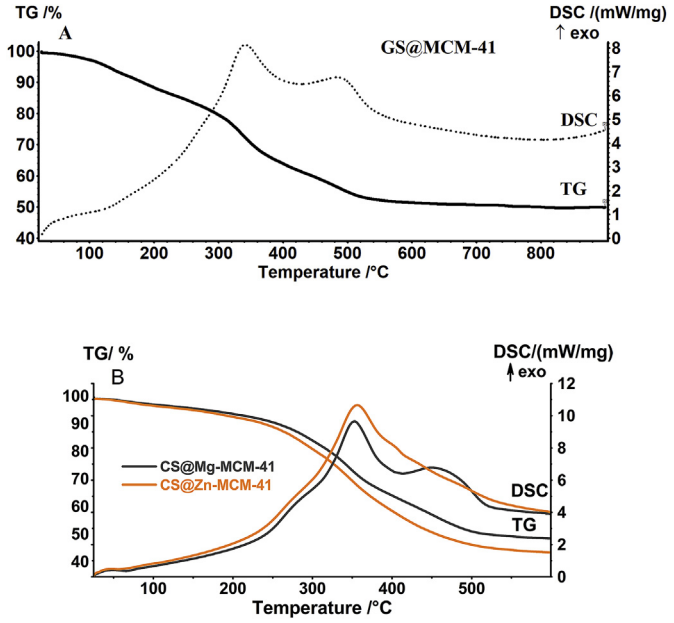


Fig. 1. DSC-TG analysis for: A-GS@MCM-41 and B-CS@Mg-MCM-41 and CS@Zn-MCM-41 samples.

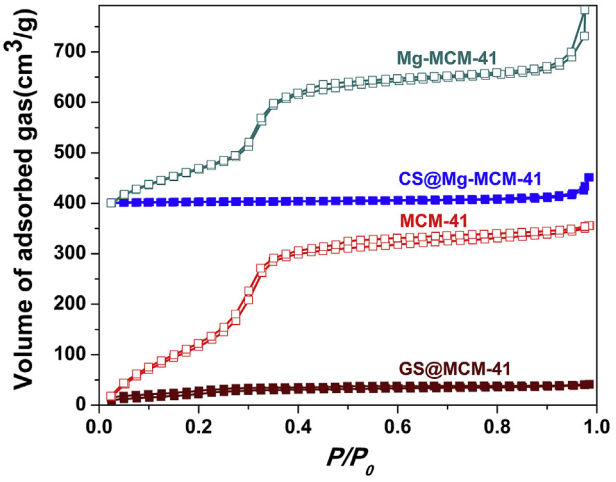


Fig. 2. N_2 adsorption-desorption isotherms for GS@MCM-41 and CS@Mg-MCM-41 samples in comparison with that of corresponding supports.

vibration, $\nu_{asSi\text{-}OH}$, and bending vibration ($\delta_{Si\text{-}O\text{-}Si}$) of MCM-41 silica-type carriers (Fig. 3 e and f).

3.3. Determination of free radical scavenger activity

The radical scavenger activity (RSA) of free and encapsulated extracts was assessed *via* DPPH method and compared with several standard substances, which are found in all extracts, like, gallic acid and *trans*-resveratrol. Three concentrations from the linearity domain, which allowed the setting of a correlation equation for each tested sample and IC 50% values (50% of DPPH free radical inhibition) were established and the data are gathered in Table 3. Among extracts, CS (Conv) exhibited the highest antioxidant capacity. It can be also seen that the MW enhanced the antioxidant activity of grape skin extract when compared with conventional extraction. However, all extracts had lower radical scavenging capacity than tested standard compounds (HPLC-grade), the best properties having quercetin (Table 3). To compare the results obtained through DPPH assay and ABTS method, calibration curves for Trolox in 0.05–1.25 mM domain

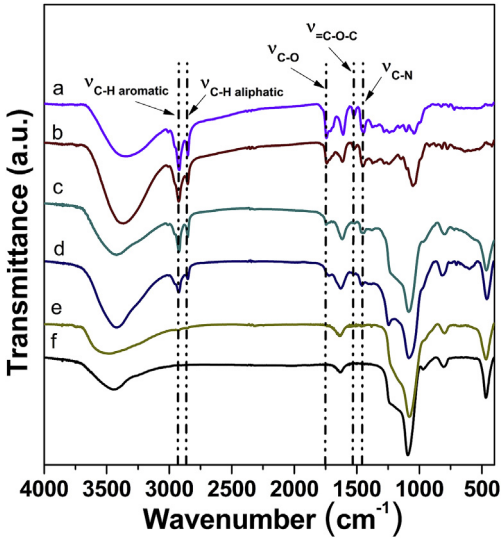


Fig. 3. FTIR spectra of CS extract (a), GS extract (Conv) (b), CS@Zn-MCM-41 (c), GS@MCM-41 (d); Zn-MCM-41 carrier (e) and MCM-41 support (f).

Table 3
Radical scavenging activity of phenolic extracts in comparison with that of standard substances (DPPH assay).

Sample	IC 50% (μg/mL)	Correlation equation	R^2
CS (Conv) extract	812.67	$y = 0.058*x + 3.022$	0.9976
FN (Conv) extract	2271.69	$y = 0.012*x + 23.210$	0.9963
GS (Conv) extract	2176.07	$y = 0.014*x + 18.608$	0.9996
GS (MW) extract	1402.99	$y = 0.027*x + 11.861$	0.9916
Ascorbic acid	184.12	$y = 0.251*x + 2.006$	0.9917
Quercetin	105.02	$y = 0.457*x + 2.008$	0.9976
Gallic acid	175.34	$y = 0.637*x + 2.008$	0.9891
trans-Resveratrol	584.01	$y = 0.078*x + 4.447$	0.9982

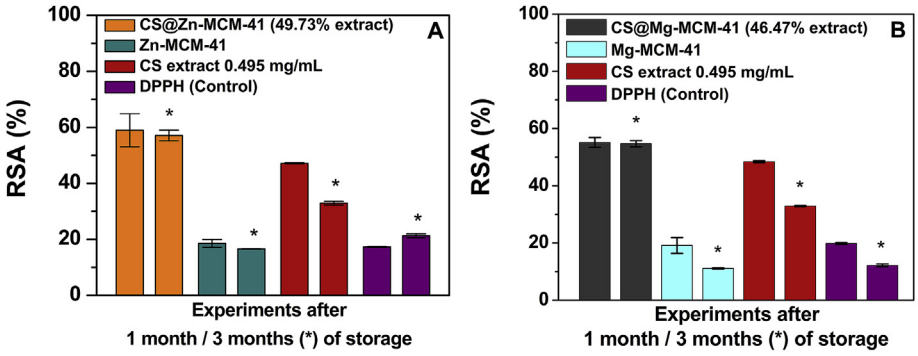
($y = 51.701*x + 1.69$, $R^2 = 0.9983$ - DPPH method and $y = 79.298*x + 3.413R^2 = 0.9984$ – ABTS assay) were settled. The RSA values, expressed as Trolox equivalents per gram of extract, for both DPPH and ABTS assays are listed in Table 1. The ABTS method led to lower values for RSA for extracts, but the same trend among samples was observed that could be explained by different mechanism of reactive oxygen species generating in the DPPH and ABTS assays.

The antioxidant activity of extracts established by DPPH and ABTS methods was in the range of 476.8–1332.8 μmol TE/g extract and 419.51–918.9 μmol TE/g extract, respectively (Table 1), higher than that reported by Melo et al. for Chenin Blanc, Petit Verdot and Syrah cultivars (191–540 μmol TE/g extract and 218–653 μmol TE/g extract through DPPH and ABTS, respectively, assays) (Melo et al., 2015). Rockenbach et al. reported RSA values in the range of 188.02–505.52 μmol TE/g extract and 193.36–485.42 μmol TE/g extract using DPPH method and ABTS assay, respectively, the best activity being obtained for Cabernet Saugvinon extract, which is lower than that of our extract prepared from the same cultivar (Rockenbach et al., 2011). Higher values for antioxidant activity by ABTS assay (951–1013 μmol TE/g extract), comparing to ours, were obtained by Xu et al. for Viognier and Cabernet Franc grape pomace extracts (Xu et al., 2016).

Before the extract encapsulation in mesoporous silica-type matrices, the antioxidant activity (DPPH assay) and total phenolics content were re-evaluated because the properties of free extracts can be altered, though the extracts were stored in the refrigerator. A decrease of the total phenolics content (Table 4), mainly because of the reduction of ascorbic acid content, which is known to be unstable in ethanolic solutions (Hsu et al., 2012). Unexpected, the antioxidant activity of

Table 4
Total phenolic and ascorbic acid contents, as well as antioxidant activity (DPPH assay) of chosen extracts before their encapsulation in silica-type matrices.

Extract	TP (mg EAG/g extract)	AA (mg AA/g extract)	IC 50% (mg/mL)	Correlation equation	R^2
CS (Conv.)	242.07 ± 5.83	23.46 ± 0.86	0.530	$y = 23.287 + 50.429*x$	0.9974
GS (Conv.)	181.63 ± 2.24	15.30 ± 0.03	2.176	$y = 18.608 + 14.426*x$	0.9996



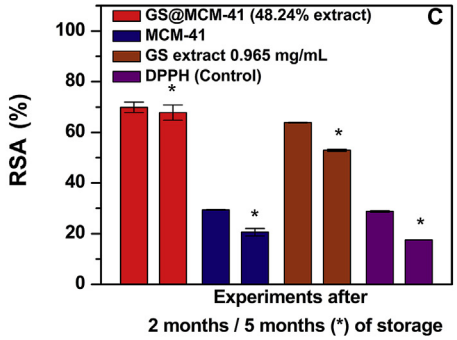


Fig. 4. Radical scavenger activity for: free and encapsulated CS extract in Zn-MCM-41 carrier (A); free and encapsulated CS extract in Mg-MCM-41 support (B); free and entrapped GS extract in MCM-41 matrix (C) after 24 h incubation, in dark conditions, in comparison with the corresponding MCM-41-type support and using DPPH degradation as control.

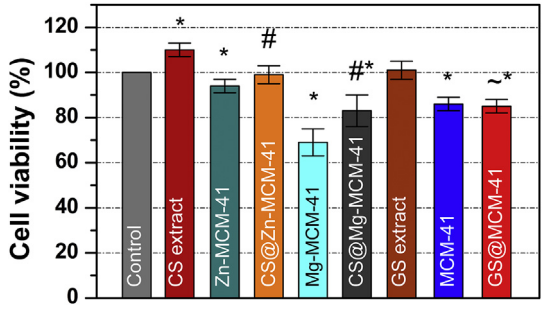


Fig. 5. Cell viability at 24 h on HaCaT. Data are presented as mean ± SD (n = 3). * - $p < 0.05$ compared to control (untreated cells); #$p < 0.05$ compared to CS and ~$p < 0.05$ compared to GS free extract, respectively.

Cabernet Saugvinon extract increased in time, probably due to the transformation of some compounds (by hydrolysis) in substances with higher radical scavenger capacity. As result of degradation or polymerization processes, other phenolic compounds with high antioxidant activity could be formed and thus compensate the decrease or loss of some initial substances (Flores et al., 2014). Ascorbic acid might be involved in generation of other phenolic compounds (for example quercetin, catechin, etc.) with higher antioxidant capacity (Samra et al., 2011). Although, the total polyphenols content decreased in time (Tsali and Goula, 2018), it has generally found that the antioxidant capacity could remain unchanged or increases slightly during storage in the case of anthocyanin-based extracts (Flores et al., 2014; Bakowska-Barczaka and Kolodziejczyk, 2011; Nayak et al., 2011; Hager et al., 2008).

The radical scavenger activity of materials containing encapsulated extract (DPPH assay) was evaluated. Considering that mesoporous silica exhibits a high porosity, being able to adsorb organic molecules (DPPH radicals) and the weak interaction between a solid sample and radicals from solution, firstly, the DPPH method was tested on commercial MCM-41 silica. Thus, at set time intervals, 0.5 h, 2 h, 8 h and 24 h, the contribution to the antioxidant capacity of MCM-41 silica was evaluated by UV–vis spectrometry having as control the degradation of DPPH free radical ethanolic solution in the same conditions. It can conclude that MCM-41 material has no radical scavenger activity when compared with DPPH free radical degradation. For more details see Supplementary Material (Fig. S3).

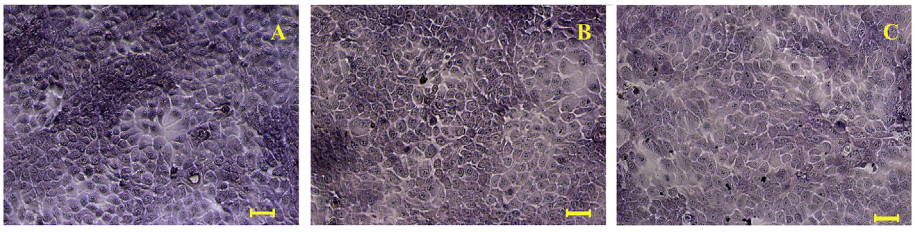


Fig. 6. Giemsa staining of the human keratinocytes: A – Control, B – Cabernet Saugvinon extract, C – encapsulated extract (CS@Zn-MCM-41 sample). Scale bar: 50 μm.

Based on the conditions established for MCM-41 support, the antioxidant activity of encapsulated extracts was assessed after 24 h of incubation in DPPH ethanolic solution, in duplicate, and compared to that of the free extract and corresponding support in the same amount as in the sample-containing extract, using as control the degradation in time of DPPH free radical solution. The radical scavenger activity determined simultaneously for free and encapsulated phenolic extract (free CS and encapsulated extract in Zn-MCM-41 and Mg-MCM-41, as well as free GS extract, obtained by conventional extraction, and encapsulated in MCM-41 carrier) and along with that of corresponding MCM-41 silica-type support in the presence of control are presented in Fig. 4. One can notice that the encapsulated extracts preserved their antioxidant activity almost constant in comparison with the free extracts whose radical scavenger capacity decrease in time (Fig. 4), probably because they are prone to a faster degradation.

After at least one-month storage in the refrigerator (4 °C), all encapsulated extracts showed higher radical scavenging activity than the free extracts (Fig. 4), probably due to better stability when confined in mesopores of silica-type matrix, although all supports did not exhibit antioxidant capacity. The best results, in terms of enhanced radical scavenger properties when compared encapsulated and free extract, were obtained for CS@Zn-MCM-41 sample. Hence, the extract encapsulation helps to preserve beneficial properties of phytochemicals.

3.4. *In vitro* cytocompatibility evaluation

The *in vitro* cytocompatibility of CS and GS extracts free and encapsulated in mesoporous silica matrices modified with zinc oxide, Zn-MCM-41, or magnesium oxide, Mg-MCM-41 was tested on human normal keratynocytes, HaCaT cells, using MTT assay to assess the metabolic activity of cells after 24 h. Human keratinocytes represent a clinically relevant type of cell line due to the potential applications of proposed systems, e.g., dermato-cosmetics or nutraceuticals. In order to design cosmetics formulation, the direct contact with the skin cells represents a first step in assessing their safety and biocompatibility.

MTT results showed high cytocompatibility, similar (GS) or higher (CS) when compared to control, in correlation with the antioxidant capacity. The cell viability was dependent on type of functionalization of the MCM-41 carrier. While ZnO modification of MCM-41 support slightly reduced percentage of viable cells (94 ± 3%) when compared to control, MgO functionalization of silica significantly lowered the percentage of viable cells (69 ± 6%) (Fig. 5). Nevertheless, the encapsulation of the CS extract into silica-type matrices showed beneficial effect on keratinocytes viability, 99 ± 4% for CS@Zn-MCM-41 and 83 ± 7% in the case CS@Mg-MCM-41. When compared to the commercial MCM-41, Zn-MCM-41 exhibited an increase in cell viability, while GS extract encapsulation in MCM-41 maintained a good cytocompatibility (85 ± 3%).

The most promising samples, CS free and encapsulated into Zn-MCM-41 matrix, were also evaluated with regards to the Giemsa stained cell morphology. Normal cuboidal morphological phenotype was maintained for the cell monolayer treated with free or encapsulated extract, similar to the control, untreated keratinocytes (Fig. 6). The enhancement of cell proliferation could be linked to the polyphenolic content, beneficial effects being already reported, mainly due to their antioxidant properties (Dzialo et al., 2016). Phenolic compounds showed skin cell renewal potential on dermal fibroblasts, while grape pomace phenolic compounds (gallic and caftaric acid, *trans*-resveratrol) were recommended to stimulate extracellular matrix synthesis. Wittenauer et al. proposed the crude pomace phenolic content as useful in cosmetic formulations based on the inhibitory effect on matrix metalloproteinases, with the gallic acid as the strongest effector (Wittenauer et al., 2015). Moreover, ascorbic acid was described as cell proliferation promoter of mesenchymal stem cells and fibroblasts proliferation (Phillips et al., 1994; Fujisawa et al., 2018), endorsing *in vitro* cell growth and collagen synthesis.

4. Conclusions

Several polyphenolic extracts in absolute ethanol using grape pomace of two cultivars, Cabernet Sauvignon and Feteasca Neagra from Black See region (Romania), as well as commercially available grape skin powder were prepared by conventional or MW extraction at 80 °C. Concerning the chemical profiling, myricetin was identified in both extracts prepared from grape pomace, pelargonidin chloride in CS extract while gallic acid, vanillic acid, syringic acid, protocatechuic acid, (−) epicatechin, and *trans*-resveratrol were found in all extracts. Rutin hydrate is also present in all extracts, except the FN extract. The highest radical scavenger activity was determined for CS extract, possibly because of the highest ascorbic acid content and total flavonoids amount expressed as quercetin equivalents. Also, a higher antioxidant capacity of GS extract was observed when MW extraction was applied in comparison with that of the extract prepared from the same source through conventional extraction.

To mitigate the extracts degradation, they were encapsulated in MCM-41-type silica matrices. The encapsulated CS extract into Zn-MCM-41 and Mg-MCM-41 mesoporous silica carriers and GS extract in commercial MCM-41 silica matrix showed an enhanced radical scavenger activity than the corresponding free extract, assessed by DPPH method developed for solid samples. The stability of free and encapsulated extracts was evaluated by determination of radical scavenger activity in time. The encapsulated extracts exhibited almost the same antioxidant capacity up to 5 months, while the free extracts showed a decrease of their activity in time, which could be seen as a degradation. A comparison of chemical profiling for free extracts freshly prepared and stored for several months was done using HPLC analysis.

The cytocompatibility tests performed on HaCaT keratinocyte human cell line demonstrated a very good cytocompatibility for the CS and GS extracts, especially for CS that stimulated cell proliferation. The best results were obtained for encapsulated extract into Zn-MCM-41 matrix, which exhibited good cytocompatibility making it a good candidate for cosmetic or nutraceutical formulations.

Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The work was supported by UEFISCDI Romanian project PCCDI no. 85/2018.

Acknowledgements

This work was supported by Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI), Romania - Romanian Complex Projects Completed in Consortia CDI (PCCDI) no. 85/2018. The authors thank to Murtfatlar Research Station for Viticulture and Oenology, Romania, for grape pomace vegetal material. Also, the authors are grateful to Prof. Ovidiu Oprea for performing of TG analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110787.

References

Abdrabba, S., Hussein, S., 2015. Chemical composition of pulp, seed and peel of red grape from Libya. GJSR 3 (2), 6–11.

Akram, M.W., Fakhar-e-Alam, M., Atif, M., Alvina Rafique Butt, A.R., Asghar, A., Jamil, Y., Alimgeer, K.S., Wang, Z.M., 2018. *In vitro* evaluation of the toxic effects of MgO nanostructure in Hela cell line. Sci. Rep. 8, 4576. https://doi.org/10.1038/s41598-018-23105-y.

An, N., Lin, H., Yang, C., Zhang, T., Tong, R., Chen, Y., Qu, F., 2016. Gated magnetic mesoporous silica nanoparticles for intracellular enzyme-triggered drug delivery. Mater. Sci. Eng. C Mater. Biol. Appl. 69, 292–300.
Argyo, C., Weiss, V., Bräuchle, C., Bein, T., 2014. Multifunctional mesoporous silica nanoparticles as a universal platform for drug delivery. Chem. Mater. 26, 435–451.
Bakowska-Barczaka, A.M., Kolodziejczyk, P.P., 2011. Black currant polyphenols: their storage stability and microencapsulation. Ind. Crops Prod. 34, 1301–1309.
Balea, S.S., Parvu, A.E., Pop, N., Marín, F.Z., Pârvu, M., 2018. Polyphenolic compounds, antioxidant, and cardioprotective effects of pomace extracts from feteasc㠠neagr㠠cultivar. Oxid. Med. Cell. Longev., 8194721. https://doi.org/10.1155/2018/8194721. https://doi.org/10.1155/2018/8194721.
Bouchoucha, M., Côté, M.-F., Gaudreault, R.C., Fortin, M.-A., Kleitz, F., 2016. Size-Controlled functionalized mesoporous silica nanoparticles for tunable drug release and enhanced anti-tumoral activity. Chem. Mater. 28, 4243–4258.
Brezoiu, A.-M., Deaconu, M., Nicu, I., Vasile, E., Mitran, R.-A., Matei, C., Berger, D., 2019. Heteroatom modified MCM-41-silica carriers for Lomefloxacin delivery systems. Microporous Mesoporous Mater. 275 241-222.
Chaaban, H., Ioannou, I., Chebil, L., Slimane, M., Gerardin, S., Paris, C., Charbonnel, C., Chekir, L., Ghoul, M., 2017. Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. J. Food Process. Preserv., e13203. https://doi.org/10.1111/jfpp.13203. 1-12.
Choi, S.R., Jang, D.-J., Kim, S., An, S., Lee, J., Oh, E., Kim, J., 2014. Polymer-coated spherical mesoporous silica for pH-controlled delivery of insulin. J. Mater. Chem. B. 2, 616–619.
Cilek, B., Luca, A., Hasirci, V., Sahin, S., Sumnu, G., 2012. Microencapsulation of phenolic compounds extracted from sour cherry pomace: effect of formulation, ultrasonication time and core to coating ratio. Eur. Food Res. Technol. 235, 587–596.
Cotoras, M., Vivanco, H., Melo, R., Aguirre, M., Silva, E., Mendoza, L., 2014. In vitro and in vivo evaluation of the antioxidant and prooxidant activity of phenolic compounds obtained from grape (Vitis vinifera) pomace. Molecules 19, 21154–21167.
Doadrio, A., Salinas, A., Sánchez-Montero, J., Vallet-Regí, M., 2015. Drug release from ordered mesoporous silicas. Curr. Pharmaceut. Des. 21, 6213–6819.
Deng, Q., Penner, M.H., Zhao, Y., 2011. Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. Food Res. Int. 44, 2712–2720.
Dzialo, M., Mierziak, J., Korzun, U., Preisner, M., Szopa, J., Kulma, A., 2016. The potential of plant phenolics in prevention and therapy of skin disorders. Int. J. Mol. Sci. 17 (2), 160.
Fang, Z., Bhandari, B., 2010. Encapsulation of polyphenols-a review. Trends Food Sci. Technol. 21, 510–523.
Favaro, L.I.L., Balcao, V.M., Rocha, L.K.M., Silva, E.C., Oliviera Jr., J.M., Vila, M.M.D.C., Turbino, M., 2018. Physicochemical characterization of a crude anthocyanin extract from the fruits of jussara (euterpe edulis martius): potential for food and pharmaceutical applications. J. Braz. Chem. Soc. 29 (10), 2072–2088.
Flores, F.P., Singh, R.K., Kong, F., 2014. Physical and storage properties of spray-dried blueberry pomace extract with whey protein isolate as wall material. J. Food Eng. 137, 1–6.
Fontana, R., Antoniolli, A., Bottini, R., 2013. Grape pomace as a sustainable source of bioactive compounds: extraction, characterization, and biotechnological applications of phenolics. J. Agric. Food Chem. 61, 8987–9003.
Franklin, S.J., Myrdal, P.B., 2015. Solid-state and solution characterization of myricetin. AAPS PharmSciTech 16 (6), 1400–1408.
Fujisawa, K., Hara, K., Takami, T., Okada, S., Matsumoto, T., Yamamoto, N., Sakaida, I., 2018. Evaluation of the effects of ascorbic acid on metabolism of human mesenchymal stem cells. Stem Cell Res. Ther. 9, 93.
Geana, E.I., Dinca, O.,R., Ionete, R.E., Artem, V., Niculescu, C., 2015. Monitoring trans-resveratrol in grape berry skins during ripening and in corresponding wines by HPLC. Food Technol. Biotechnol. 53 (1), 73–80.
Hager, T.J., Howard, L.R., Prior, R.L., 2008. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blackberry products. J. Agric. Food Chem. 56, 689–695.
Hsu, H.-Y., Tsai, Y.-C., Fu, C.C., Wu, J.S.-B., 2012. Degradation of ascorbic acid in ethanolic solutions. J. Agric. Food Chem. 60, 10696–10701.
Khan, M.A., Wallace, W.T., Islam, S.Z., Nagpure, S., Strzalka, J., Littleton, J.M., Rankin, S.E., Knutson, B.L., 2017. Adsorption and recovery of polyphenolic flavonoids using TiO_2-functionalized mesoporous silica nanoparticles. ACS Appl. Mater. Interfaces 9 (37), 32114–32125.
Kosaraju, S.L., Labbett, D., Emin, M., Konczak, I., Lundin, L., 2008. Delivering polyphenols for healthy ageing. Nutr. Diet. 65, S48–S52.
Kuck, L.S., Noreña, C.P.Z., 2016. Microencapsulation of grape (Vitis labrusca var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. Food Chem. 194, 569–576.
Kuru, I.S., Karakus, P., Akbas, F., Isikalan, C., Namli, S., 2017. The evaluation of total phenolic, flavonoid, sugar contents and antioxidant activity of tayfi grape in Turkey. Batman Univ. J. Life Sci. 7 (2/2), 14–22.
Ky, I., Crozierb, A., Cros, G., Teissedrea, P.L., 2014. Polyphenols composition of wine and grape sub-products and potential effects on chronic diseases. Nutr. Aging 2, 165–177.
Lee, J., 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants and wines by the pH- differential method: collaborative study. J. AOAC Int. 88 (5), 1269–1278.
Luo, J., Panzarasa, G., Osypova, A., Sorin, F., Spano, F., Rossi, R.M., Sadeghpour, A., Boesel, L.F., 2019. Polyphenols as morphogenetic agents for the controlled synthesis of mesoporous silica nanoparticles. Chem. Mater. 319, 3192–3200.
Maini, S., Hodgson, H.L., Krol, E.S., 2012. The UVA and aqueous stability of flavonoids is dependent on B-ring substitution. J. Agric. Food Chem. 60, 6966–6976.
Mebert, A.M., Baglole, C.J., Martin, F., Desimone, M.F., Maysinger, D., 2017. Nanoengineered silica: properties, applications and toxicity. Food Chem. Toxicol. 109, 753–770.
Melo, P.S., Prado Massarioli, A., Denny, C., Ferracini dos Santos, L., Franchin, M., Elias Pereira, G., Ferreira de Souza Vieira, T.M., Rosalen, P.L., Matias de Alencar, S., 2015. Winery by-products: extraction optimization, phenolic composition and cytotoxic evaluation to act as a new source of scavenging of reactive oxygen species. Food Chem. 181, 160–169.
Mohansrinivasan, V., Devi, S.C., Deori, M., Biswas, A., Naine, J.S., 2015. Exploring the anticancer activity of grape seed extract on skin cancer cell lines A431. Braz. Arch. Biol. Technol. 58 (4), 540–546.
Mohammed, Y.H., Holmes, A., Haridass, I.N., Sanchez, W.Y., Studier, H., Grice, J.E., Benson, H.A.E., Roberts, M.S., 2019. Support for the safe use of zinc oxide nanoparticle sunscreens: lack of skin penetration or cellular toxicity after repeated application in volunteers. J. Investig. Dermatol. 139 (2), 308–315.
Muncaciu, M.L., Zamora Marian, F., Pop, N., Babeş, A.,C., 2017. Comparative polyphenolic content of grape pomace flours from 'feteasc㠠neagr㠠' and 'Italian riesling' cultivars. Not. Bot. Horti. Agrobo. 45, 532–539.
Munin, A., Edwards-Lévy, F., 2011. Encapsulation of natural polyphenolic compounds; a review. Pharmaceutics 3, 793–829.
Nayak, B., Liu, R.H., De J Berrios, J., Tang, J., Derito, C., 2011. Bioactivity of antioxidants in extruded products prepared from purple potato and dry pea flours. J. Agric. Food Chem. 59, 8233–8243.
Nayak, A., Bhushan, B., Rosales, A., Rodriguez Turienzo, L., Cortina, J.L., 2018. Valorisation potential of Cabernet grape pomace for the recovery of polyphenols: process intensification, optimisation and study of kinetics. Food Bioprod. Process. 109, 74–85.
Negro, C., Tommasi, L., Miceli, A., 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour. Technol. 87, 41–44.
Otero-Pareja, M.J., Casas, L., Fernández-Ponce, M.T., Mantell, C., Martínez de la Ossa, E.J., 2015. Green extraction of antioxidants from different varieties of red grape pomace. Molecules 20, 9686–9702.
Paulino, Z.M., Alvareda, E., Iribarne, F., Miranda, P., Espinosa, F.V., Aguilera, S., Pardo, H., 2016. Toward the understanding of the molecular basis for the inhibition of COX-1 and COX-2 by phenolic compounds present in Uruguayan propolis and grape pomace. J. Biomol. Struct. Dyn. 34 (12), 2643–2657. https://doi.org/10.1080/07391102.2015.1124808.
Pekal, A., Pypzynska, K., 2014. Evaluation of aluminum complexation reaction of flavonoid content assay. Food Anal. Methods 7, 1776–1782.
Phillips, C.L., Combs, S.B., Pinnell, S.R., 1994. Effects of ascorbic acid on proliferation and collagen synthesis in relation to the donor age of human dermal fibroblasts. J. Investig. Dermatol. 103 (2), 228–232.
Robinson, K., Mock, C., Liang, D., 2015. Pre-formulation studies of resveratrol. Drug Dev. Ind. Pharm. 41 (9), 1464–1469.
Rockenbach, I.I., Gonzaga, L.V., Rizelio, V.M., De Sauza Schmidt Gonçalves, A.E., Genovese, M.I., Fett, R., 2011. Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (Vitis vinifera and Vitis labrusca) pomace from Brazilian winemaking. Food Res. Int. 44, 897–901.
Sagdic, O., Ozturk, I., Ozkan, G., Yetim, H., Ekici, L., Yilmaz, M.E., 2011. RP-HPLC–DAD analysis of phenolic compounds in pomace extracts from five grape cultivars: evaluation of their antioxidant, antiradical and antifungal activities in orange and apple juices. Food Chem. 126, 1749–1758.
Samra, M.A., Chedea, V.S., Economou, A., Calokerinos, A., Kefalas, P., 2011. Antioxidant/prooxidant properties of model phenolic compounds: Part I. Studies on equimolar mixtures by chemiluminescence and cyclic voltammetry. Food Chem. 125, 622–629.
Sanchez-Rangel, J.C., et al., 2013. The Folin-Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. Anal. Methods 5, 5990–5999.
Sousa, E.C., et al., 2014. Chemical composition and bioactive compounds of grape pomace (Vitis vinifera L.), Benitaka variety, grown in the semiarid region of Northeast Brazil. Food Sci. Technol. 34, 135–142.
Schieber, A., Stintzing, F.C., Carle, R., 2001. By-products of plant food processing as a source of functional compounds -recent developments. Trends Food Sci. Technol. 12, 401–413.
Srivastav, A.K., Kumar, M., Ansari, N.G., Jain, A.K., Shankar, J., Arjaria, N., Jagdale, P., Singh, D., 2016. A comprehensive toxicity study of zinc oxide nanoparticles versus their bulk in Wistar rats: toxicity study of zinc oxide nanoparticles. Hum. Exp. Toxicol. 35, 1286–1304.
Tang, F., Li, L., Chen, D., 2012. Mesoporous silica nanoparticles: synthesis, biocompatibility and drug delivery. Adv. Mater. 24, 1504–1534.
Teixeira, A., Baenas, N., Dominguez-Perles, R., Barros, A., Rosa, E., Moreno, D.A., Garcia-Viguera, C., 2014. Natural bioactive compounds from winery by-products as health promoters: a review. Int. J. Mol. Sci. 15 (9), 15638–15678.
Threes, G.S., Stanislav, P., 2011. Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness. Nanotechnol. Sci. Appl. 4, 95–112.
Tsali, A., Goula, A.M., 2018. Valorization of grape pomace: encapsulation and storage stability of its phenolic extract. Powder Technol. 340, 194–207.
Volf, I., Ignat, I., Neamtu, M., Popa, V.I., 2014. Thermal stability, antioxidant activity, and photo oxidation of natural polyphenols. Chem. Pap. 68, 121–129.
Wang, X., Tong, H., Chen, F., Gangemi, J.D., 2010. Chemical characterization and antioxidant evaluation of muscadine grape pomace extract. Food Chem. 123, 1156–1162.
Wang, Y., Lu, Z., Lv, F., Bie, X., 2009. Study on microencapsulation of curcumin pigments by spray drying. Eur. Food Res. Technol. 229, 391–396.
Wittenauer, J., Mackle, S., Sussmann, D., Schweiggert-Weisz, U., Carle, R., 2015. Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity. Fitoterapia 101, 179–187.
Xu, Y., Burton, S., Kim, C., Sismour, E., 2016. Phenolic compounds, antioxidant, and antibacterial properties of pomace extracts from four Virginia-grown grape varieties.

Food Sci. Nutr. 4 (1), 125–133.
Zhang, L., Mou, D., Du, Y., 2007. Procyanidins: extraction and microencapsulation. J. Sci. Food Agric. 87, 2192–2197.
Zhang, C., Li, Y., Liu, L., Gong, Y., Xie, Y., Cao, Y., 2018. Chemical structures of polyphenols that critically influence the toxicity of ZnO nanoparticles. J. Agric. Food Chem. 66, 1714–1722.
Zhou, J., Wang, M., Ying, H., Su, D., Zhang, H., Lu, G., Chen, J., 2018. Extracellular matrix component shelled nanoparticles as dual enzyme-responsive drug delivery vehicles for cancer therapy. ACS Biomater. Sci. Eng. 4, 2404–2411.