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ORIGINAL ARTICLE

Effects of different carrier materials on physicochemical properties of microencapsulated grape skin extract

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Abstract The goal of this study was to investigate the characteristics of grape skin extract (GSE) spray dried with different carriers: maltodextrin (MD), gum Arabic (GA) and skim milk powder (SMP). The grape skin extract was obtained from winery by-product of red grape variety *Prokupac* (*Vitis vinifera* L.). The morphology of the powders, their thermal, chemical and physical properties (water activity, bulk and tapped densities, solubility), as well as release studies in different pH conditions were analyzed. Total anthocyanin content and total phenolic content were determined by spectrophotometric methods. MD and GA-based microparticles were non-porous and spherical, while SMP-based ones were irregularly shaped. The process of spray drying *Prokupac* GSE using these three carriers produced powders with low water activity (0.24–0.28), good powder characteristics, high yields, and solubility higher than 90%. The obtained dissolution/release profiles indicated prolonged release of anthocyanins and phenolic compounds in different mediums, especially from GSE/GA microparticles. These results have shown that grape skin as the main by-product of wine production could be used as a source of natural colorants and bioactive compounds, and microencapsulation as a promising technique for the protection of these compounds, their stabilization in longer periods and prolonged release.

Keywords Grape skin · Spray drying · Prokupac · Powder · Anthocyanins · Microparticles

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Introduction

Grape, as one of the most commonly cultured fruit worldwide, generates a significant amount of by-products during processing. Those by-products are mainly grape skin, grape pomace and seeds (about 20% of the processed grapes weight) (Llobera and Cañellas 2007; Maier et al. 2009). Recently, various technologies have been developed in order to achieve maximum utilization of by-products of wine production as well as higher productivity. Grape skin as a by-product of red grape processing may be considered as an interesting source of fibers, as well as anthocyanins, and could therefore be used in the production of natural colorants (Llobera and Cañellas 2007; Maier et al. 2009).

Anthocyanins are compounds belonging to the flavonoid group, and the most abundant group of pigments responsible for the coloring of fruits, vegetables and flowers. Besides their colorant characteristics, the importance of anthocyanins was further investigated due to their potential role in promoting health by reducing the risk of different diseases like atherosclerosis, cancer, diabetes and neurodegenerative disorders (Wrolstad 2004). Therefore, the research into anthocyanins stabilization has been the main focus of recent studies due to their high potential and beneficial health effects (Castañeda-Ovando et al. 2009). These compounds have found their application in

numerous fields: food industry, cosmetic and pharmaceutical products. However, the stability of these compounds during processing and storage may be affected by several factors such as: storage temperature, light exposure, oxygen level, environment pH and the presence of solvents, metal ions, etc.

Generally, one of the most suitable preservation methods for fruit or grape juices and extracts is the process of drying them into powder. However, these powders (of sugar-rich foods) have some functional characteristics that make handling fairly inconvenient. The stickiness and high hygroscopicity make packaging, storage, as well as usage quite difficult (Cano-Chauca et al. 2005).

In relation to the aforementioned issues, microencapsulation by spray drying has been confirmed as a useful tool from multiple perspectives;

First of all, the encapsulation technology is an optimal solution for the protection of polyphenol compounds, such as anthocyanins, and the formulation of stable dosage forms for food purposes since it enables the protection of sensitive ingredients, such as polyphenols, from degradation caused by the above factors. Moreover, encapsulation of actives can be used to improve their bioavailability and modify time and/or place of their release upon administration (Nedović et al. 2013).

Secondly, spray drying is a simple, continuous, and widely used process where an active dissolved in a dispersion of the carrier material can be rapidly transformed into powder. This technique enables the drying of heat-sensitive compounds since the atomization of the liquid mixture in a flow of hot air (lasts only a few (milli)seconds) keeps the temperature of the particles relatively low (Đorđević et al. 2015).

Finally, the development of powders with improved physical properties, as a highly organized structure, and the possibility of reaching higher yields are enabled, thanks to the addition of numerous carrier materials. In the case of sugar-rich solutions such as grape skin extracts, stickiness phenomena is reduced during the spray drying process due to the addition of carrier materials and their ability to produce low molecular weight sugars and organic acids which can enter the safe drying regime and act as an effective drying aid (Adhikari et al. 2004; Cano-Chauca et al. 2005).

The formation of liquid products (such as juices, extracts) with the addition of carrier materials (e.g. polymers, gums) before spray drying solves several problems, like hygroscopicity, stickiness, thermoplasticity and solubility. Some of these materials are maltodextrin, gum Arabic and skim milk powder (Bayram et al. 2008; Kanakdande et al. 2007; Krishnan et al. 2005; Loksuwan 2007).

Maltodextrin, a hydrolyzed starch, has been frequently used as a carrier material in microencapsulation of various functional by-products by spray drying (de Souza et al. 2015). It offers numerous advantages: low cost, neutral aroma and taste (moderately sweet), high solubility, low viscosity, easy digestibility, and protection against oxidation (Kanakdande et al. 2007; Loksuwan 2007; Wandrey et al. 2010).

Gum Arabic has been one of the most frequently used materials for spray drying of different infusions, emulsions and extracts (Belščak-Cvitanović et al. 2015; Daza et al. 2016; Krishnan et al. 2005). Despite many desirable characteristics of gum Arabic (e.g. high solubility and low viscosity, neutral to slightly acidic substance), supply problems and the increasing cost are the reasons behind the search for alternative carriers (Krishnan et al. 2005; Wandrey et al. 2010).

In contrast to gum Arabic, skim milk has been widely available around the world. Skim milk powder is not commonly used as a carrier material for anthocyanin-rich extracts, but it provides very high yield, non-stick properties, well shaped particles, high encapsulation efficiency, and it is very convenient for application (Bayram et al. 2008). Besides, as a source of protein, carbohydrate (lactose), vitamins and minerals, this material provides additional nutritional value (Jing and Giusti 2005). Thus, skim milk powder as a carrier could be very convenient for future application in numerous food products (dairy products, bakery products, special liquors, breakfast mixes, etc.). In addition, the study of Marchiani et al. (2016) has already demonstrated that grape pomace powder (non-encapsulated form) is a functional ingredient that increases polyphenol content in cheese. However, microencapsulated form (10–100 times smaller particles) could be even more suitable for this and similar applications in novel products.

For this purpose, *Prokupac* variety was used as raw material. *Prokupac* is not only one of the most widely grown red grape varieties in the region of Southeastern Europe, but also an autochthonous Serbian variety often compared to the French ones (*Cabernet Sauvingon, Pinot Noir* and *Gamay*) due to its very intensive dark-blue skin, sugar level, and acidity (Menkovic et al. 2014; Radovanović et al. 2009).

The objective of this study was to evaluate the potential of using grape skin as a by-product and different natural biopolymers as carriers for spray drying in order to define a formulation that provides maximum process yield, desirable physical powder properties, and release of anthocyanins and total phenolic compounds.

Materials and methods

Materials

Grape skin used in this study was obtained from the red grape variety *Prokupac* grown at the Faculty of Agriculture

Experimental School Estate Radmilovac (Serbia). Maltodextrin with dextrose equivalent (DE = 16–19.9) produced by Cargill was kindly provided by Palco (Serbia). Gum Arabic (Gum Acacia, powder) was purchased from Himedia (India). Skim milk powder was generously donated by dairy Subotička mlekara (Serbia). All other chemicals used for the experimental procedures were of analytical grade and used as such without further purification: Folin–Ciocalteu reagent and gallic acid (Merck, Germany); potassium chloride, sodium carbonate and sodium acetate (Centrohem, Serbia); hydrochloric acid (Sigma Aldrich GmbH, Germany); acetic acid and sodium hydrogen phosphate (Hemos, Serbia); potassium dihydrogen phosphate (Fisher Chemical, UK); ethanol (Vrenje Spiritana, Serbia).

Extraction of grape skin anthocyanins

Milled grape skin of *Prokupac* was extracted with 70%v/v ethanol/water solutions during the period of 2 h. The samples were extracted for two hours at ambient temperature by shaking (200 rpm) with the application of ultrasound in the ultrasonic bath Elmasonic S15H (Elma, Germany) at 95 W and the frequency 40 kHz for 15 min intervals. Ethanol was chosen as the solvent due to its environmentally friendly characteristics and ability to enhance the extraction of phenolic constituents as anthocyanins from grape skins, compared to mono-component systems. Extraction was followed by evaporation of GSE (6 Brix) in rotary evaporator with vacuum ($p = 40$ mbar) at 45 °C (Elektromedicina, Slovenia) in order to eliminate ethanol from the extract. Extracts were diluted by distilled water to adjust previous volume.

Preparation of feed mixture for spray drying

The feed mixture was prepared by mixing maltodextrin (MD), gum Arabic (GA) and skim milk powder (SMP) into grape skin extract (GSE) in the quantity of 10 g/100 g, 5 g/100 g, and 10 g/100 g of the solution, respectively. The composition of feed mixtures was established in accordance with specific physicochemical properties of carriers (Wandrey et al. 2010). The applied carrier concentrations ensured appropriate liquid atomization during spray drying and minimized production losses. The homogenous mixtures were prepared under continuous magnetic stirring at ambient temperature for approximately 20 min.

Microencapsulation by spray drying

A spray dryer (Büchi mini B-290, Büchi Labortechnik AG, Switzerland) equipped with the standard 0.7 mm diameter nozzle and co-current flow was used to prepare the spray dried powders. The inlet and outlet temperatures were 140 and 65 ± 2 °C, respectively. The air flow rate, liquid feed rate and atomization pressure were 600 L/h, 8 mL/min and 0.55 bar, respectively.

Determination of total anthocyanin content (TAC)

The TAC was determined for the two types of mixtures: before microencapsulation and in the powders dissolved in different mediums by a pH differential method. The anthocyanin extracts and powders were diluted with potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5). The absorbance of the solutions was measured at 520 and 700 nm using a UV–Vis double beam spectrophotometer (HALO-DB/2S, Dynamica, Switzerland) according to Lee et al. (2005) method. The concentration was expressed as cyanidin-3-glucoside equivalents according to the following formula (Eq 1):

$$\begin{aligned} &\textit{Anthocyanin Content}\,(\textit{cyanidin-3-glucoside equivalents},\ \mathrm{mg/L}) \\ &\quad = A \times MW \times DF \times 10^3/\varepsilon \times l, \end{aligned} \tag{1}$$

where $A = (A_{520\mathrm{nm}} - A_{700\mathrm{nm}})_{\mathrm{pH1.0}} - (A_{520\mathrm{nm}} - A_{700\mathrm{nm}})_{\mathrm{pH4.5}}$; MW = molecular weight of cyanidin-3-glucoside; DF = dilution factor; l = path length; ε = molar extinction coefficient, 10^3 = conversion factor.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) in samples was determined according to the Folin–Ciocalteu method. Briefly, 0.25 mL of diluted samples were mixed with 1.25 mL of tenfold diluted Folin–Ciocalteu's phenol reagent and allowed to react for 5 min. One milliliter of sodium carbonate solution (75 g/L) was added to the mixture. After 2 h of reaction at room temperature (20 °C), the absorbance at 760 nm was measured using UV–Vis double beam spectrophotometer (HALO-DB/2S, Dynamica, Switzerland). The calibration curve was prepared with the gallic acid solution according to Singleton and Rossi (1965) method.

Scanning electronic microscopy (SEM)

SEM analysis was performed using a JEOL JSM 6390LV microscope (JEOL, Japan). The samples were attached to stubs using a two-sided adhesive tape and sputter coated with gold (50 nm) for 100 s at 30 mA (Sputter Coater BAL-TEC SCD 005). The SEM was operated at 15 kV with a magnification of 1900×. The microparticle size was analyzed with SEM micrographs using ImageJ analysis software (National Institute of Health, USA). The particle

diameter for each formulation was calculated as the average value of the size of 100 spray dried microparticles.

Fourier-transform infrared (FTIR) spectroscopy

Attenuated Total Reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) analysis of grape skin extract, plain carriers and spray dried powders obtained was performed using the IRAffinity-1 FTIR spectrophotometer (Schimadzu, Japan). The wavelength range was from 4000 to 600 cm^{-1}, while the resolution was 4 cm^{-1}.

Differential scanning calorimetry (DSC)

The DSC experiments were performed using a DSC 1 instrument (Mettler-Toledo, Switzerland). Dried samples were crimped in standard pierced 40 μL aluminum pans and analyses were performed from 25 and 300 °C, at a heating rate of 10 °C/min under constant nitrogen purge of 50 mL/min. The empty sealed pan was used as a reference.

Bulk and tapped density

Bulk and tapped density of the samples were determined using the method described in the European Pharmacopeia (Council of Europe 2010). Of each sample, 30 g was poured into a 100 mL measuring cylinder and the volume occupied by the sample was recorded. Bulk density (g/mL) was determined by dividing the net weight of the sample with the volume occupied by the sample in the cylinder and calculated as an average value of 3 measurements.

The same samples in cylinders were used for mechanical tapping on volumeter STAV 2003 (Gemini B.V. Apeldoorn, Netherlands) in order to calculate tapping density (g/mL). The reading of volumes was done after 10, 500 and 1250 taps on the same powders. V_{1250} is tapped volume if the difference between V_{500} and V_{1250} is less than 2 mL.

Powder compressibility

Powder compressibility was estimated by calculating and comparing the values of the microparticles' compressibility index (CI) and Hausner ratio (HR). The CI and HR depend on the difference between the bulk volume (V_0) and the final tapped volume (V_f) of the microencapsulate, and according to the European Pharmacopeia (Council of Europe 2010) can be used as a measure of the propensity of a material to be compressed. The CI and HR are calculated as follows (Eqs. 2 and 3):

$$CI = \left[\left(V_0 - V_f\right)/V_o\right] \times 100 \quad (2)$$

$$HR = V_o/V_f \quad (3)$$

Solubility and water activity

Powder solubility was determined using the method of de Souza and coworkers (de Souza et al. 2015). The powder (0.5 g) was dissolved in 50 mL of distilled water and continuously stirred for 30 min at 100 rpm followed by centrifugation at 3000 rpm (Boeco U-320, Germany) for 5 min. The supernatant was dried in the drying oven (Memmert UF-55, Germany) at 105 °C until constant weight. The dried weight of the soluble solid was measured and used to calculate the solubility as a percentage.

Water activity (a_w values) of obtained powders was measured by using a water activity meter (Testo 650 Water Activity System, Cole-Parmer, USA). All measurements were done in triplicate.

Yield and efficiency of the microencapsulation process

The process yield was calculated from Eq. (4) shown below, as the ratio of the total dry weight of the microencapsulated powder obtained (W_2) and the dry weight of the material in its own feed mixture (W_1). The process yield was expressed as a percentage (%). The encapsulation efficiency (EE) was calculated using Eq. (5) shown below, as the ratio of the total phenolic content of the microparticles obtained (TPC_2) and the total phenolic content of the material in its own feed mixture (TPC_1). The EE was expressed as a percentage (%).

$$Yield\,(\%) = W_2 \times 100/W_1 \quad (4)$$

$$EE\,(\%) = TPC_2 \times 100/TPC_1 \quad (5)$$

In vitro release of TAC and TPC from microparticles

The release of TAC and TPC from microparticles was performed using a DFZ 60 flow-through cell dissolution tester (Erweka, Germany) in the open loop configuration under a constant medium flow rate at 1 mL/min. The release studies were observed in distilled water (2 h) and in media simulating pH conditions of the gastrointestinal tract: 0.1 mol/dm^3 HCl solution (simulated gastric fluid (SGF), pH 1.2, 3 h); phosphate buffer (simulated small intestinal fluid (SIF), pH 6.8, 2 h) (Čalija et al. 2013). At scheduled time intervals, samples were withdrawn and assayed spectrophotometrically by the above-described pH differential and Folin–Ciocolteu methods. All the assays were carried out at 37 °C. The average percent of released anthocyanins versus time was plotted.

Statistical analysis

The experimental data were subjected to a One-way analysis of variance (ANOVA), and Tukey's test was used to detect difference ($p \leq 0.05$) between the mean values. Statistical analyses were performed with the statistical program STATISTICA 12 (Data Analysis Software System, Stat-Soft, Inc., USA).

Results and discussion

Properties and characterization of spray dried microparticles

Morphology

As shown in Fig. 1, microparticles were poly-dispersed in size, regardless of the carrier material. Microparticle diameters ranged from 1 to 20 μm with the average value of 5.2 ± 3.3 μm.

SEM analysis showed well-formed spherically shaped, smoothed surfaced microparticles of spray dried GSE/MD (grape skin extract/maltodextrin) with no visible cracks or pores. However, those microparticles had a high diameter distribution 4.6 ± 3.8 μm (Fig. 1a), already noticed by other authors (Loksuwan 2007). The smoother surface of MD-based microparticles compared to other carriers could be attributed to the difference in sugar composition. MD used in this study consisted of a greater amount of low molecular weight sugar which can act as a plasticizer preventing surface shrinkage during spray drying (Loksuwan 2007).

The acceptable, but not ideal spherical shape of GSE/GA (grape skin extract/gum Arabic) microparticles with diameters of 5.6 ± 2.4 μm (Fig. 1b) could be ascribed to gum characteristics and conclusions of the study reported by Kanakdande et al. (2007).

The microparticles of GSE/SMP (grape skin extract/skim milk powder) sized 5.3 ± 3.7 μm had collapsed forms (Fig. 1c) which is typical for spray dried microparticles and attributable, to rapid drying. However, the absence of ideal sphericity and surface roughness could be attributed to the composition of this carrier material, primarily lactose and casein. It has been reported that such problems for spray dried SMP powders could be attributed to the effect of conditions of atomization and drying on casein (Bylaitë et al. 2001), as well as the pH value of used extract (Bayram et al. 2008). Laokuldilok and Kanha (2015) reported shriveled surface on the microparticles of milk powder produced by spray drying with a lower inlet air temperature.

FTIR spectroscopy and DSC analysis

Spray dried samples were analyzed using FTIR spectroscopy in order to investigate possible chemical interactions between carrier materials and GSE. FTIR spectra of all samples (Fig. 2) showed, broad bands in the spectral region 3500–3000 cm^{-1} correspond to –OH groups, while the bands at ~2900 cm^{-1} resulted from the stretching vibration of C–H bond (de Souza et al. 2015; Espinosa-Andrews et al. 2010; Krishnaiah et al. 2012). Spray dried GSE (Fig. 2a) exhibited a strong band that could be assigned to carbohydrates (peak at 1022 cm^{-1}), while small bands around this peak could appear due to the presence of organic acids. Also, numerous bands in the spectral region 1600–1200 cm^{-1} were most likely related to phenolic compounds in the GSE (de Souza et al. 2015).

The FTIR spectrum of samples based on MD (Fig. 2b, c) showed several bands in the spectral region 1100–900 cm^{-1} that could be associated with the typical C–O–C bonds (de Souza et al. 2015). The band at 1411 cm^{-1} is most likely due to $–CH_2$ bending (Krishnaiah et al. 2012). Several prominent bands were observed in the FTIR spectrum of GA (Fig. 2d, e). The band at 1020 cm^{-1} is due to C–O stretching vibration, while two bands at 1420 and 1600 cm^{-1} were also identified in the FTIR spectrum of this natural gum (Espinosa-Andrews et al. 2010). The bands at 1650 cm^{-1} and at 1540 cm^{-1} in the FTIR

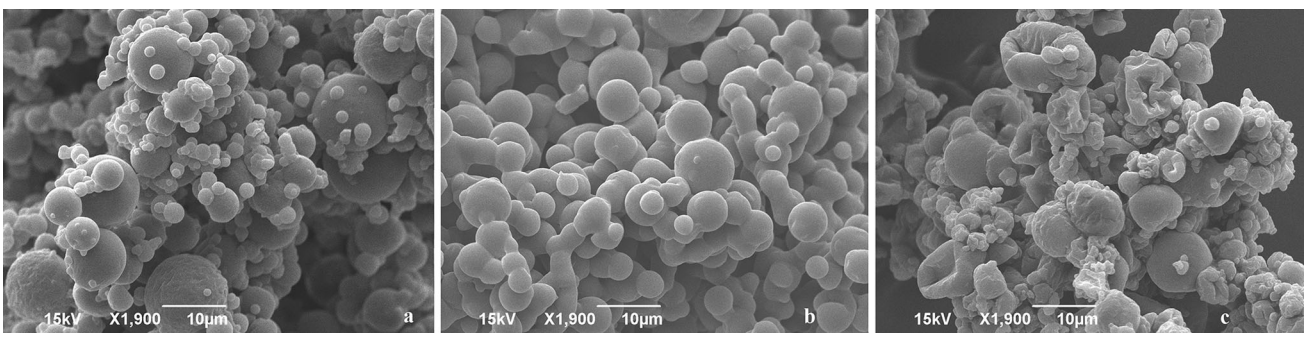

Fig. 1 SEM micrographs of a spray dried GSE with MD (**a**), GA (**b**), SMP (**c**)

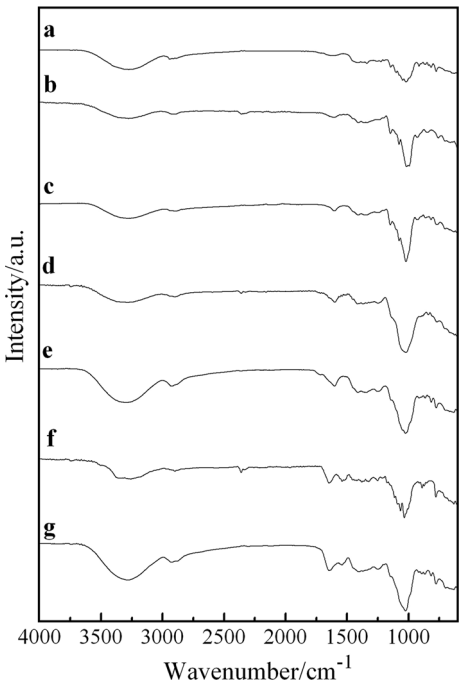

Fig. 2 FTIR spectra of grape skin extract GSE (**a**), pure carrier materials MD, GA, SMP (**b**, **d**, **f**), and microencapsulated GSE in the corresponding carriers (**c**, **e**, **g**)

spectrum of SMP (Fig. 2f, g) are related to amide I and II, respectively. The bands between 1150 and 1000 cm^{-1} are due to the presence of lactose in milk (Nicolaou et al. 2010). The FTIR analysis showed the absence of new chemical interactions between carrier materials and GSE during spray drying process.

The DSC study of GSE, carriers (MD, GA, SMP) and microparticles was performed to investigate possible interactions between individual components and the physical stability of microparticles (Fig. 3).

In general, the DSC curves of individual carriers are characterized with initial broad endotherm pick which could be ascribed to the loss of absorbed water. Differences in position and intensities of these endotherm peaks are related to the different amounts of water and its binding modes in certain samples. The second endothermic events followed by exothermic events correspond to the melting and subsequent degradation of polymers. These findings are in complete agreement with the results of the thermogravimetric analysis of MD and GA reported by Klein and coworkers (Klein et al. 2015), and the SMP analysis reported by Choudhary and coworkers (Choudhary et al. 2012).

The DSC curve of non-encapsulated GSE exhibited an endothermic event in the range between 125 and 200 °C due to the crystalline structure loss of D-glucose and D-fructose, as previously reported (Lee et al. 2010). Similar DSC thermogram of the anthocyanin extract of jabuticaba skin was obtained by Santos et al. (2013).

By comparing DSC curves of all investigated microparticles, it can be concluded that the endothermic peak of GSE was the most noticeable on the GSE/GA thermogram (Fig. 3b). This can be explained by lower GSE/carrier ratio in GSE/GA microparticles in comparison with the other two samples. A slight shift of this peak toward higher temperatures suggests faintly higher thermal stability of microparticles. Contrary to this sample, the endothermic peaks of GSE/MD were insignificantly moved to lower temperatures (Fig. 3a) with overlapped peacks of individual components similar to GSE/SMP (Fig. 3c).

The results of the DSC study pointed out that the obtained powders indicated good thermal stability in the temperature region important for food processes.

Physical properties of microparticles

The comparison of MD, GA and SMP-based microparticles' bulk and tapped density, CI and HR, water activity, solubility yield and encapsulation efficiency are given in Table 1.

In comparison with the bulk density (0.39–0.68 g/mL) and tapped density (0.50–0.86 g/mL) of carrier materials, the bulk and tapped density of the obtained powders (0.16–0.30 g/mL) and (0.21–0.36 g/mL) were lower by approximately 57% due to the increase in voids. Also, the addition of GSE to carriers affected their own values of HR and CI (Table 1). According to CI and HR values, the obtained microparticles could be defined as powders with the following flow characteristics: SCE/GA (good) > SCE/SMP (fair) > SCE/MD (poor). In general, a decrease in bulk density implies a decrease in flowability. To be more specific, HR and CI decreased by 9.6 and 41.3% in the case of GSE/GA, with values that define good powder flowability, while the GSE/SMP powder, with the lowest difference in comparison with pure SMP, had fair flow characteristics. However, for GSE/MD those values increased by 6.0 and 16.3%, suggesting poor flow characteristics of this powder in terms of the values given by European Pharmacopeia (Council of Europe 2010).

The water activity of microparticles was low (0.24–0.28), suggesting forms desirable from the microbial stability point of view.

Solubility as the ability of powders to form a solution in water is an important physicochemical property that influences functional characteristics of powders in food systems. High solubility was determined in all samples (Table 1): GSE/SMP < GSE/GA < GSE/MD. Similar results for grape skin extract microencapsulated with maltodextrin were reported by de Souza et al. (2015). Also, Cano-Chauca et al. (2005) reported the solubility of over 90% for spray dried mango powder using maltodextrin as the carrier. Daza et al. (2016) found very similar solubility

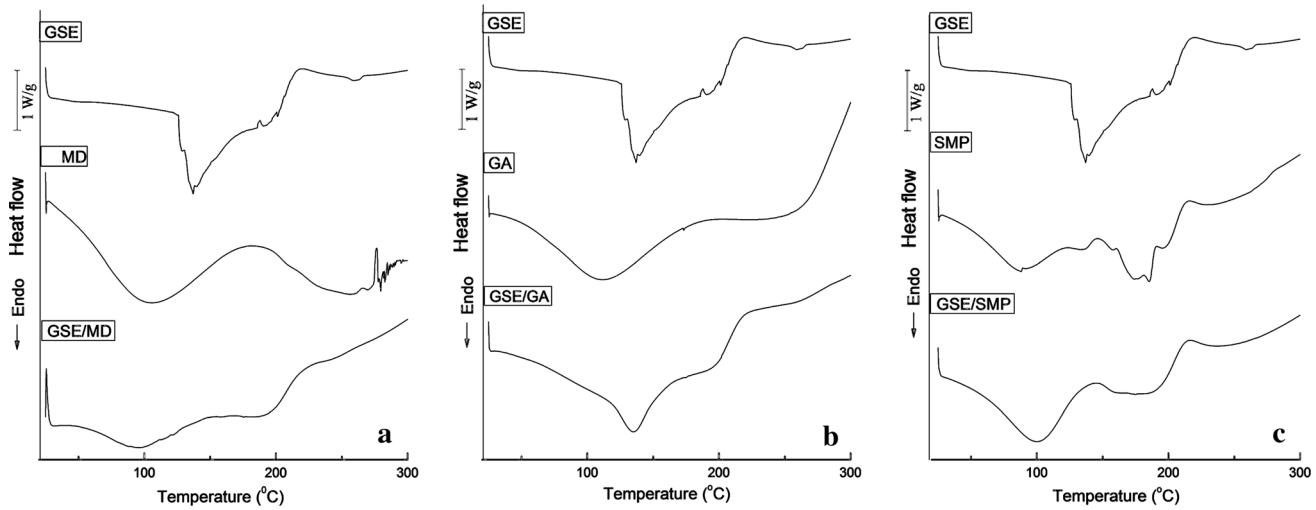

Fig. 3 DSC thermograms of free GSE, pure carrier materials and the microparticles

Table 1 Physical properties, yield and encapsulation efficiency of GSE/MD, GSE/GA and GSE/SMP microparticles

	Bulk density (g/mL)	Tapped density (g/mL)	CI (%)	HR	a_w	Solubility (%)	Yield (%)	EE (%)
GSE/MD	0.21 ± 0.02^{a}*	0.31 ± 0.01^{a}	30.0 ± 1.0^{a}	1.43 ± 0.02^{a}	0.26 ± 0.00^{a}	94.81 ± 0.18^{a}	75.3 ± 2.5^{a}	62.4 ± 4.9^{a}
GSE/GA	0.30 ± 0.03^{b}	0.36 ± 0.03^{a}	12.0 ± 1.7^{b}	1.14 ± 0.02^{b}	0.24 ± 0.00^{b}	91.90 ± 0.12^{b}	65.9 ± 1.6^{b}	85.2 ± 5.6^{b}
GSE/SMP	0.16 ± 0.02^{a}	0.21 ± 0.02^{b}	20.0 ± 2.0^{c}	1.25 ± 0.02^{c}	0.28 ± 0.00^{c}	89.40 ± 0.19^{c}	80.9 ± 1.2^{c}	63.7 ± 8.3^{a}

*Values are shown as mean ± SD (n = 3). Different letters within columns indicate a significant difference at $p < 0.05$

and water activity for spray dried Cagaita fruit extract with GA. In the same study authors reported a fairly high and comparable process yield. However, the highest and remarkably better yield was provided by SMP as the carrier. Also, Loksuwan (2007) found high and similar solubility for MD in spray dried β-carotene powder with water activity around of 0.22.

The process yield of all microparticles ranged from 65.9 to 80.9%. The results of MD-based microencapsulates yield (75.3%) are in high correlation with the yields of spray dried anthocyanin-rich extract with MD reported by Laokuldilok and Kanha (2015). The highest yield was achieved for SMP-based microparticles. Similar results were reported in a study that compared milk protein based microparticles with other carrier materials (Belščak-Cvitanović et al. 2015). Regarding powder properties and microparticles' yield, SMP was found to be a promising carrier for anthocyanins encapsulation.

The highest EE was achieved using GA as the carrier (Table 1). This could be explained by hydrogen bonding of hydroxyl groups from GSE phenolics with nonstarch polysaccharide such as GA that has a high proportion of anion fraction contributed by glucuronic arabinogalactan (Bordenave et al. 2014). In this respect, GA could actively link to GSE phenolics during contact and be able to retain phenolics extract throughout the spray drying (Wu et al. 2014).

In vitro release of TAC and TPC from microparticles

The dissolution/release profiles of total anthocyanins from the spray dried powders in SGF, SIF, and water are reported in Fig. 4. The dissolution tests were performed and designed in the gastric and small intestine conditions (pH and temperature) to simulate the places where anthocyanins are metabolized and absorbed. According to the results obtained in SGF (Fig. 4a), in the first 60 min GSE/SMP microparticles showed the slowest release of anthocyanins (45%). GSE/MD and GSE/GA microparticles released 65% of anthocyanin content in 100 min. From that moment, GSE/SMP equated to GSE/GA, despite of MD that released approximately 90% of anthocyanins in 3 h.

During the first 30 min in SIF, the release profiles of anthocyanins from spray dried powders were almost identical, reaching a cumulative value of up to 50%

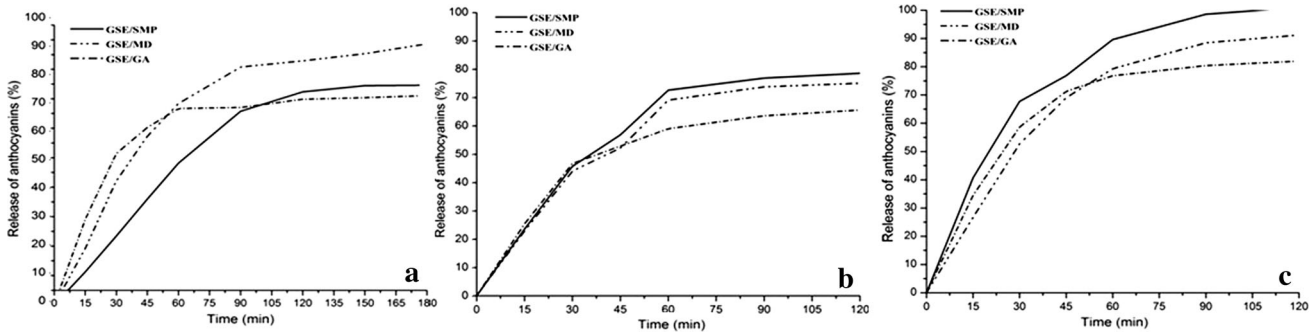

Fig. 4 Anthocyanins release profiles from microparticles in SGF (**a**), SIF (**b**) and water (**c**)

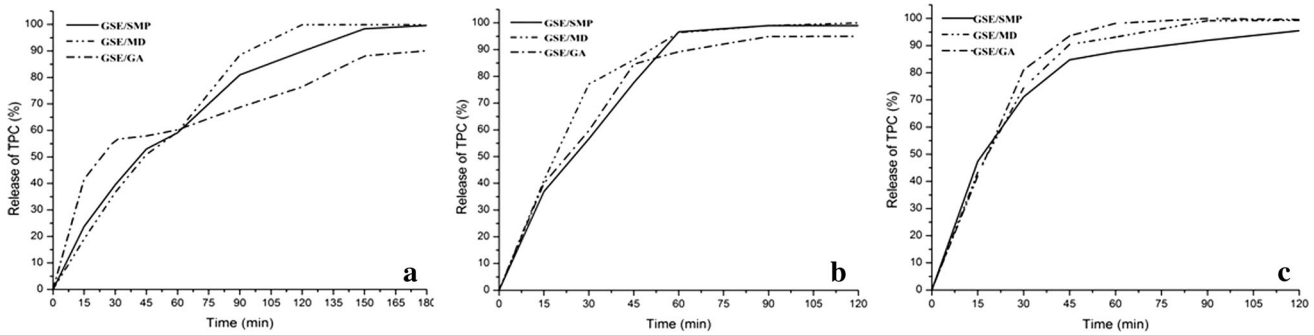

Fig. 5 TPC release profiles from microparticles in SGF (**a**), SIF (**b**) and water (**c**)

(Fig. 4b). From that point, the release was slower from GA in comparison with the two other samples.

Similarly, when compared to the MD and SMP, GA showed a slower release of anthocyanins in water (Fig. 4c). Based on these findings, it can be observed that gum as the carrier provides the most suitable release pattern in both SIF and water.

The dissolution/release profiles of TPC from spray dried powders in SGF, SIF, and water are reported in Fig. 5.

Although GSE/GA demonstrated the fastest release in the first 30 min, further release during the following 2 h was noticeably slower than in the other two samples (Fig. 5a). However, both profiles in SGF showed a similar tendency.

According to the results obtained in SIF (Fig. 5b) in the first 15 min all microparticles released almost 40% of TPC, whereas GSE/MD released even 75% in next 15 min. Two other samples showed a slower release with a small preference for GSE/GA. Contrasting with anthocyanins, the release profiles of TPC in water were quite similar for all three samples. They showed the same trends, especially in the first 30 min.

As can be seen from Figs. 4 and 5, of all the investigated microparticles, the SMP-based microparticles exhibited the most significant difference between dissolution profiles in acidic and neutral conditions, suggesting that pH value affects the release of TAC and TPC (Figs. 4, 5). More precisely, the solubility of these particles at lower pH values was significantly lower, thus affecting the release rates of microencapsulated actives. It can be concluded that the exposure of SMP-based microparticles to extremely acidic conditions, similarly to those in the upper part of GIT, could result in a somewhat prolonged release of microencapsulated actives despite the fact that particles are highly soluble in water (neutral pH).

However, based on all findings, it can be observed that gum Arabic as the carrier provides the most suitable release pattern in SGF, SIF and water. This is especially related to anthocyanin release profiles.

Conclusion

This study explored the use of microencapsulation of GSE with different natural carrier materials.

The results confirmed spray drying at 140 °C/65 °C as an efficient technique to produce anthocyanin-rich powders with very good physicochemical properties: water activity (0.24–0.28), bulk and tapped density (0.16–0.30 and 0.21–0.36 g/mL), compressibility (12–30%), solubility (89–95 g/100 g) and yield (65.9–81.9%) with the average diameter of microparticles of about 5 μm. GSE microparticles had a regular spherical shape in the case of MD and GA as carriers, and irregular in the case of SMP. The FTIR

and DSC analyses showed chemical and thermal stability of microparticles. The obtained dissolution/release profiles indicated a prolonged release of anthocyanins in different pH conditions that can be achieved primarily using GA. On the other hand, the fastest and complete release in water and slightly alkaline conditions was achieved with MD. Considering the above-mentioned results, GSE/MD and GSE/GA powders demonstrated the desirable characteristics. However, potential employment of SMP as the carrier for this type of actives should be more often considered due to its high yield, nutritional value and fair flowability and dissolution.

The process described in this study can be of interest for the food industry. Certainly, regardless of cultivar and vintage influences, grape skin has a significant content of anthocyanins and other phenolic compounds that make the utilization of such material worthwhile.

The starting materials are relatively cheap and readily available, making the described process both inexpensive and easy to apply. These carriers containing natural colorants are a promising alternative for artificial ones in various food products. Depending on the carrier, these powders can find application in beverages, dairy products, bakery products, ice-creams and various novel products. Further studies are required in order to determine accurate sensorial and beneficial effects of these powders as food ingredients on consumer acceptance and/or health.

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