Spray drying of *Gentiana asclepiadea* L. root extract: successful encapsulation into powders with preserved stability of bioactive compounds

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Abstract

Willow gentian as a source of bitter compounds is traditionally used for digestive disorders. *Gentiana* root extract was spray dried using five different carriers (maltodextrin, whey protein, pectin, starch, gelatine) at various concentrations. Powders were characterized in terms of physical properties and encapsulation efficiency of bioactive compounds. The moisture content of all powders was between 1.78 and 3.46%, and bulk density from 0.23 to 0.32 g/mL. Powders produced with maltodextrin and whey protein provided the highest yield (around 75 and 70%, respectively) and the lowest hygroscopicity (6 and 7%, respectively). Gelatin and pectin provided powders with the highest encapsulation efficiency of total phenolic as well as the individual compounds. The stability of encapsulated bioactive compounds was studied after 6 months, and the most stable in all samples were gentiopicrin and sweroside with their content decreased by 10% only. This study has shown that spray drying of gentian root extract produces powders with good physical properties and encapsulation of bioactives.

Keywords: *Gentiana*; microencapsulation; carriers; secoiridoids; phenolics

Abbreviations:

LGE-liquid gentian extract; SGE-spray-dried gentian extract; FGE-freeze-dried gentian extract; MD-Maltodextrin; WP-whey protein; TP-Total phenolic content; EE-encapsulation efficiency; ZP-Zeta potential; FTIR-Fourier-transform infrared analysis.

1. Introduction

Gentiana genus comprises about 400 plant species that are widespread in Europe, Asia, and America. The roots of these plants have been used extensively in traditional medicine since ancient times. Phytochemical studies indicate that plants of this genus are source of more than 500 secondary metabolites including iridoids, xanthones, flavonoids, alkaloids, triterpenoids, and other chemical compounds (Pan et al., 2016). The main compounds found in willow gentian (*Gentiana asclepiadea* L.) roots are secoiridoids gentiopicrin, sweroside, and swertiamarin, flavonoids isoorientin and isovitexin, and xanthones gentisin and gentioside (Olennikov et al., 2019). Willow gentian is traditionally used for digestive disorders (dyspepsia), as a bitter tonic and appetite stimulant, and for hepatitis A virus infections (Sarić, 1989; Menković et al., 2010). The bitter compounds of this plant irritate sensory nerve endings on the tongue and reflexively stimulate the secretion of saliva and digestive juices. Recent pharmacological studies have shown that other compounds such as xantones (mangiferin, gentioside), flavonoides (isoorientin, isovitexin), and triterpens (ursolic acid, beta-sitosterol, squalene) manifest a synergistic effect in exerting hepatoprotective, gastroprotective, antimicrobial, antioxidant, and DNA repair activity of this plant species (Hudecová et al.,2012; Mihailović et al. 2011; Mihailović et al. 2013).

The stability of these valuable bioactive compounds can be preserved by encapsulation technique which entraps them inside a coating material (Ćujić et al. 2016). Such formed product protects bioactive ingredients from harmful environmental influences (oxygen, light, water), improves their bioavailability, and masks undesirable organoleptic characteristics. During extraction process, encapsulating compounds are derived in liquid form and they have to be converted into dry powder by various drying technologies like spray drying, freeze drying or extrusion (Desai and Park, 2005). Spray drying has been widely utilized due to the short time and

controlled operation conditions (Đorđević et al., 2015). It represents a relatively simple, efficient, high capacity, and cost-effective conventional method, which convert liquid extract into a powder in a stream of heated air. The obtained powder is suitable for further use in pharmaceutical or food industry, or it can be used in obtained form due to its instant properties making it convenient for users. Encapsulating agents commonly used for spray drying are natural biopolymers such as polysaccharides (starch, maltodextrin, chitosan, gum arabic, pectin, cyclodextrin) and proteins (skim milk, whey protein, soy protein isolate) (Coimbra et al., 2020). Selection of appropriate wall materials depends on the properties of coating material, nature of spray-dried (core) material, and intended usage of the final powder. The most commonly used polysaccharides are starch and maltodextrin due to their low viscosity at high solids content, good solubility in water, neutral aroma and flavor, and low-cost (Gharsallaoui et al., 2007). The main disadvantage is their hydrophilic nature and therefore they have limited emulsifying capacity. Pectin is an interesting alternative which produce stable emulsions, and it can be used in combination with other encapsulants. Proteins and protein-containing isolates are able to absorb hydrophobic compounds, and thus have excellent emulsification capabilities. Milk proteins, soy protein, and gelatin are common protein-based carriers due to their film-forming properties, high retention efficiency, and easy access. Their main drawback is that they are animal proteins which may cause intolerance and allergenic reactions.

There are many reports on applications of spray drying in food industries, especially for the production of fruit extracts microparticles, but regarding medicinal and aromatic plants the number of studies is limited. Maltodextrin, gum arabic, β-cyclodextrin, pectin, and whey protein have been reported as microencapsulating agents for sage, rosemary, mountain tea, lemon balm, and winter savory (Bušić et al., 2018; Şahin-Nadeem et al., 2011; Şahin-Nadeem et al., 2013;

90 Sansone et al., 2011; Vidović et al., 2014). No studies have been conducted to investigate the
91 feasibility of spray drying of any gentian species.

92 The aim of the present research is to develop and examine microencapsulation system for
93 *G. asclepiadea* roots extract using different carriers such as maltodextrin, corn starch, pectin,
94 whey protein, and gelatin. The obtained microcapsules were analyzed for powder yield, particle
95 size distribution, moisture content, hygroscopicity, caking, zeta potential, and encapsulation
96 efficiency of bioactive compounds (secoiridoids, flavonoids, and xanthone). In addition, the
97 powders were stored under a normal condition for 6 months, and stability of encapsulated
98 compounds was monitored.

99 **2. Materials and methods**

100 *2.1. Chemicals*

101 Maltodextrin (MD) ($DE_{16\text{-}19.9}$) was provided from Davisco Foods International (Le Sueur,
102 MN, USA), whey protein (WP) was provided from Polmlek (Raciąż, Poland), pectin was
103 provided from CPKelco (Großenbrode, Germany), and corn starch was supplied from Production
104 sector of the Institute for Medicinal Plants Research Dr. Josif Pančić. Gelatin was produced by
105 Aleva, Novi Kneževac. Folin-Ciocalteu reagent, gallic acid, orthophosphoric acid, and sodium
106 carbonate were purchased from Sigma–Aldrich Chemie GmbH (Munich, Germany). Ultra-pure
107 water was prepared using a Milli-Q purification system (Millipore, France), and HPLC-grade
108 acetonitrile was obtained from Merck (Darmstadt, Germany). Standards swertiamarin,
109 sweroside, and gentiopicroside were purchased from ChromaDex, USA, isoorientin and
110 isovitexin were from Extrasynthese (Cedex, France), and isogentisin was purchased from
111 Phytolab (Germany).

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113

114 *2.2. Plant material and preparation of extract*

115 Dried roots of *G. asclepiadea* were purchased from the Institute for Medicinal Plants
116 Research "Dr. Josif Pančić" (Belgrade, Serbia; batch: 01540120). Plant material was grounded in
117 laboratory mill and subjected to percolation process, using ethanol-water mixture (50:50) for 12
118 h, while solid to solvent ratio was 1:2. After the percolation process, ethanol was evaporated
119 under vacuum by rotary evaporator (Buchi rotavapor R-114), at 50°C. Obtained liquid gentian
120 extract (LGE) was collected and used for future experiments.

121 *2.3. Spray drying process*

122 The prepared LGE was spray dried with and without carrier addition. Five different
123 biopolymers in three concentrations were used: MD and WP in concentrations 20, 40, 60%, w/w,
124 and pectin, corn starch, and gelatin in concentrations 2.5, 5.0, 7.5%, w/w. Each biopolymer was
125 separately dissolved in a previously produced LGE, and the concentrations used in experiments
126 were calculated based on the dry weight of the LGE. The prepared solutions were heated at 40°C
127 and mixed using magnetic stirrer to completely homogenization, before the spray drying process.
128 The liquid feed was spray dried in a Labtex ESDTi spray dryer (Labtex, Huddersfield, UK) with
129 0.5 mm standard diameter nozzle under following conditions: inlet temperature 130 ± 5°C, outlet
130 temperature 80 ± 5°C, spraying air flow rate (75 m^3/h), liquid feed (10.8 mL/min rate),
131 atomization pressure (3 bar). Experimental drying conditions such as inlet and outlet
132 temperature, flow rate and rate of liquid feed were fixed during the experiments. Due to the
133 different used carriers with wide viscosity ranges, one set of spray-drying operating conditions
134 needed to be selected in order to enable the comparison of the product yield, encapsulation
135 efficiency and other parameters of each sample.

136 The obtained spray-dried gentian extract (SGE) was separated from the air by a cyclone.
137 Free-flowing powders were obtained and transferred to high-density glass bottles before
138 analyses. They were stored in the dark, in desiccator at room temperature, and these conditions
139 ensured physical stability and active compounds preservation.

140 *2.4. Preparation of the freeze-dried gentian extract (FGE*)

141 One portion of LGE was frozen at -80^{o}C for 1 h and freeze-dried (Beta 1-8 Freeze Dryer,
142 Martin Christ, GmbH, Osteroide am Harz, Germany) at -60^{o}C (pressure of 0.011 mbar) for 24h,
143 and at -60^{o}C (pressure of 0.0012 mbar) for an additional hour in order to remove the capillary
144 water residues. After lyophilization process, FGE was disintegrated into powder for the further
145 study of bioactive content, and stored under the same conditions as SGE.

146 *2.5. Powder yield*

147 The yield (Y) of drying process was calculated as the ratio between mass (g) of the SGE
148 and the expected mass:

149 $Y\ (\%) = m_{extract} / m_{expected} \times 100$ (1)

150 Expected mass was calculated as the sum of share of dry residue in LGE multiplied with
151 a mass of LGE used for drying process and mass of the used carrier:

152 $m_{expected}\ (g) = m_{carrier} + m_{dry\ residue} \times m_{LGE}$ (2)

153 *2.6. Physical characterization of powders*

154 *2.6.1. Particle size distribution*

155 The particle size distribution for each powder was defined and quantified by Mastersizer
156 2000 analyzer (Malvern Instruments, Worcestershire, UK). The parameters of d10, d50, d90,
157 which represent the sizes where 10%, 50%, and 90% of the particles are smaller than the

158 remaining particles, were determined. Span was taken as the indicator of the width of size
159 distribution, and was expressed through SPAN value calculated as (d90-d10)/d50.

160 *2.6.2. Bulk density*

161 Bulk density was performed according to the method described previously by Vidović et
162 al. (2014) with slight modifications. One gram of each powder was placed into a 10 mL
163 graduated glass cylinder. The glass cylinder was held on a shaker for 5 minutes (Unimax 1010,
164 Heidolph, Germany), with agitation fixed at 300 rpm, ambient temperature of 25^{o}C. After
165 exposition of 5 min vibration, volumes of dry powders in glass cylinder were measured. Bulk
166 density was calculated as the ratio of powder mass and measured dry powder volume, and
167 expressed in milligram of dry powder per milliliter (mg/mL).

168 *2.6.3. Moisture content*

169 The moisture content of each sample was analyzed thermogravimetrically. The obtained
170 extracts (SGEs and FGE) were dried until they achieved constant weight using Halogen Moisture
171 Analyzer HB43-S by Mettler Toledo. Results were expressed in percent (%).

172 *2.6.4. Hygroscopicity*

173 Hygroscopicity of powders was determined according to the modified method of Cai and
174 Corke (2000). Approximately 1 g of obtained powder was placed at room temperature in stability
175 chamber (Memmert, Schwabach, Germany), filled with NaCl saturated solution (70% RH).
176 Hygroscopicity was monitored during 7 days. Results were expressed in percent (%), and
177 calculated as gram of absorbed water (moisture) per 100 g of powders (g/100 g).

178 *2.6.5. Rehydration*

179 Rehydration time of powders is a period during the dry extract is completely dissolved in
180 water at room temperature. Tests were carried out on magnetic stirrer, and it has been measured

the time taken to fully reconstitute 1 gram of powder in 50 mL of water, expressed in seconds (s) (Goula and Adamopoulos, 2010).

2.6.6. Caking

Caking tests were carried out by using the method described by Goula and Adamopoulos (2010), with slight modification. The powders were placed in a thin layer in Petri dish and stored in stability chamber with high relativity humidifies. These conditions were induced with saturated salt solution under controlled temperature conditions (25^0 C) for 90 minutes. The samples were then placed in a vacuum oven at 50°C for 2 h and after cooling the dried sample was sieved through 750 μm size for 5 minutes. The result was calculated according to Eq. 3:

$$DC = c / d \times 100, \quad (3)$$

where DC is caking degree (%), *c* is the amount of powder remaining in the sieve, and *d* is the initial amount of powder.

2.6.7. Zeta potential

After the spray drying process, zeta potential was determined by Malvern Zetasizer Nano Series (Malvern Instruments, Worcestershire, UK) in order to examine the powders physical stability. The measurements of each sample were repeated in triplicate using deionized water for suspension preparation, at room temperature. The results were presented as average values.

2.6.8. Microparticles composition analysis by FTIR spectroscopy

Fourier-transform infrared (FTIR) spectra of the obtained samples (encapsulated extracts with carriers, pure dried extract, and pure carriers) was recorded in the range mode between 400 and 4000 cm-1 using a Nicolet iS10 (Thermo Scientific, Sweden) spectrometer.

2.7. Chemical characterization of powders

2.7.1. Total phenolic content (TP)

For TP determination, Folin-Ciocalteu assay with slight modifications was applied (Waterman and Mole, 1994). An amount of 25 mg of SGEs or FGE were dissolved in 10 mL of distilled water, while 15 mg of LGE was diluted in 10 mL of distilled water. The reaction mixture was prepared by mixing 200 μL of each sample and 1000 μL of 10% Folin-Ciocalteu reagent and after four minutes 800 μL of 7.5% Na_2CO_3 was added. The mixture was incubated for 2 hours. Distilled water was used as blank, while control was prepared to contain distilled water instead of sample. Absorbance was recorded at 740 nm after two hours incubation at room temperature. Obtained results were presented as milligrams of gallic acid equivalent per gram of powders (mg GAE/g).

2.7.2. HPLC analysis

The concentration of individual components in LGE, FGE, and SGEs was determined using the HPLC method. Analyses were carried out on Agilent series 1200 RR HPLC instrument (Agilent, Waldbronn, Germany), using DAD detector, on a reverse phase Zorbax SB-C18 (Agilent), analytical column (150 mm × 4.6 mm i.d.; 5 μm particle size) according to the previously described method (Balijagić et al., 2012). The amounts of the investigated compounds (swertiamarin, gentiopicrin, sweroside, isoorientin, isovitexin, isogentisin) were calculated using calibration curves and the results are presented as milligrams per gram of powders (mg/g).

2.7.3. Encapsulation efficiency of GE bioactive compounds

The encapsulation efficiency (EE%) for all microencapsulated powders were calculated according to the equation:

$$EE\ (\%) = E/E_{total} \times 100, \quad (4)$$

where E represents quantity of TP or individual compounds microencapsulated in the powders, and E_{total} presents quantity of TP or individual components and their respective amount in the LGE.

2.7.4. Storage stability

The dried extracts (SGEs and FGE) were stored in brown glass tubes for 6 months under room temperature. Changes in the content of individual compounds were analyzed by HPLC in order to determine the effect of storage on their stability.

2.8. Statistical analysis

All experiments were executed in triplicates determinations. Results were presented as mean value ± standard deviation. One-way ANOVA was conducted to test the individual factors influence on observed property and Duncan *post hoc* test was used for differences between the mean values detection. Significant levels were considered at $p \leq 0.05$ (STATISTICA v.7.0.3). Statistical analysis was performed using the MS Office Excel v. 2010.

3. Results and Discussion

3.1. Powder yield

The carrier-free SGE achieved 55% yield, and the type of added carriers showed significant effect on the powders yield (Table 1). The highest yield, around 75%, was achieved using MD with increase by 37% at all applied concentrations. High yield (61.25 - 73.03%) of powders was also obtained when WP was used as a carrier and it increased with increasing the WP concentration. Addition of gelatin gave 58.93-65.48% powder yield, and better results were obtained using lower (2.5%) concentration. Decreasing of yield with increasing the concentration of gelatin was also reported for spray drying of saffron (Rajabi et al., 2015). Samples obtained with starch as a biopolymer exhibited yields with increase by 3-10%, whereas the lowest yield

248 compared with other carriers was noticed in pectin powders (49.51-62.12%). In general, it can be
249 noted that all types of carriers reached powder yield above 50%, which is regarded as a reference
250 value for successful drying process (Bhandari et al., 1997).

251 Obtained results showed that MD was the most effective carbohydrate-based carrier and
252 WP was the most effective protein-based carrier, increasing the yield by 37% and 31%,
253 respectively. It has been reported previously that MD enabled high powder yield during spray
254 drying of mountain tea, sage and willow bark (Şahin-Nadeem et al., 2011; Şahin-Nadeem et al.,
255 2013; Vidović et al., 2014), and WP was effective carrier for encapsulation of green tea
256 (Belščak-Cvitanović, et al., 2015). In some cases, carrier concentration also influenced the
257 powder production, as it was shown for sage, mountain tea, and saffron (Rajabi et al., 2015;
258 Şahin-Nadeem et al., 2011; Şahin-Nadeem et al., 2013). In this study, the addition of 20% WP
259 increased the yield by 10%, and increasing the WP to 40% and 60% significantly increased the
260 yield by 28% and 31%, respectively. On the contrary, concentration of MD had no significant
261 influence on the powder yield, which is in accordance with the findings of Vidović et al. (2014)
262 for spray drying of willow bark.

263 **Instert Table 1**

264 *3.2. Moisture content*

265 The moisture content is determined by different factors including type and concentration
266 of carrier and the inlet air temperature (Goula and Adamopoulos, 2008). Extract with moisture
267 content lower than 5% can be marked as a stable product in terms of microbiological and
268 physical properties (Amidon and Houghton, 1995). In the presented study, the moisture content
269 of microencapsulated powders has demonstrated satisfactory values between 1.78 and 3.46%,
270 with significant difference between powders produced using different carriers (Table 1). Samples

encapsulated with MD and WP had higher moisture content (3.14 and 3.46%, respectively) in comparison to those encapsulated with other carriers. Similar results were reported for the moisture content of the MD encapsulated mountain tea and sage (Şahin-Nadeem et al., 2011; Şahin-Nadeem et al., 2013), whereas Vidović et al. (2014) reported higher values for MD willow bark powders (4.69 - 4.97%). The obtained results for starch and pectin as carriers were similar as values reported for green tea powders (Belščak-Cvitanović, et al., 2015). Increased MD concentration resulted in lower moisture content, but without statistical significance among 40 and 60% MD. Spray-dried extracts with WP, pectin, starch, and gelatin showed different trends but usually with increased carrier concentration, the moisture content decreased.

For all examined samples low moisture content was accomplished, providing powders with good shelf-life, and possible low microbiological contamination.

3.3. Bulk density

Bulk density values (Table 1) were in the range from 0.23 g/mL (for 2.5% gelatin) to 0.32 g/mL (for 5% pectin). All powders with the lowest concentration of each carrier (MD 20%, WP 20%, pectin 2.5%, starch 2.5%, and gelatin 2.5%), as well as powder without carrier addition, showed no statistically significant difference in the value of bulk density. In the case of powders produced by using protein carriers (WP and gelatin), the concentration dependence of the bulk density was observed - increased carrier fraction led to increase the bulk density. Obtained values for WP, starch, and pectin as carriers were similar to those reported for encapsulated green tea extract (Belščak-Cvitanović, et al., 2015). Powder bulk density is one of important factors that determine the quality of final product in pharmaceutical process. High bulk density provides ease of packing and transportation, but increase in the bulk density increase the

293 tablet mass, hardness, and dissolution performance (Singh et al., 2015), which influence the
294 effectiveness of the product.

295 *3.4. Rehydration*

296 Rehydration (synonym for powder reconstitution) is expressed as the time required for
297 completely dissolving a certain amount of powder in a solvent. Unlike solubility, which
298 represents dissolution capacity, rehydration represents dissolution kinetics. Rehydration time of
299 powders with different carriers were between 31.30 s (WP 20%) and 417.64 s (pectin 5%), as
300 shown in Table 1. Powders encapsulated with MD and WP at lowest applied concentration,
301 starch at all used concentrations, as well as dry extract without carrier showed the shortest
302 rehydration time, with no statistical differences among them. Powders obtained with pectin were
303 characterized with significantly longer rehydration time (124-417 s), followed by particles
304 produced with gelatin (91-217 s). Pectin and its salts have a great ability to bind water. In
305 aqueous medium, particles on their surface create a pectin-gel coating which is poorly permeable
306 and difficult to disperse. Due to this characteristic, pectin is often used as a carrier for prolonged
307 drug delivery (Liu et al., 2007). Also, long rehydration time of gelatin powders is probably due
308 to the poor solubility of gelatin in cold water (Ashford et al., 1993).

309 Measuring the time required for powder rehydration has practical importance in the
310 formulation of instant dried products or reconstituted beverages.

311 *3.5. Hygroscopicity*

312 Water absorption of the SGEs was monitored after storing for one week, and results are
313 shown in Fig. 1. The hygroscopicity of all obtained powders was less than 10% during the
314 monitored period, and powders produced with MD and WP showed the lowest hygroscopicity (6
315 and 7%, respectively). Pectin and starch proved to be inferior as carriers, they exhibited the

316 highest hygroscopicity of 9 and 8.3%, respectively. Regarding the effect of carrier concentration,
317 the lowest hygroscopicity was noticed when the highest concentration of all carriers was applied,
318 except for WP where addition of 40% gave powders with lower water absorption than 60% WP.
319 In general, hygroscopicity of the powder depends of the nature of the carrier, the type and
320 concentration of carrier, and the particle size (Tontul and Topuz, 2017). Powders with
321 hygroscopicity more than 20% are considered as a very hygroscopic (Nurhadi et al., 2012), and
322 high level of hygroscopicity cause stickiness which contribute to the decrease of powder stability
323 during storage. In this study, MD showed the best properties followed by WP. Similar results
324 were obtained by Du et al. (2014), where powders obtained with MD and WP showed lower
325 hygroscopicity than powders obtained with gum arabic, starch, sodium octenyl succinate, and
326 egg albumen.

327 **Insert Figure 1**

328 *3.6. Caking*

329 Degree of caking is also a parameter that reflects the quality of powder, and is important
330 for its storage and handling. According to the literature data, slightly caking powders have
331 degree of caking below 20%, and desired values for foodstuff powders are between 9 and 34%
332 (Jaya and Das, 2004; Jaya et al., 2006). The values of caking degree in collected samples varied
333 from 12.9 to 56.9% (Table 1). Extract without any carrier addition showed poor caking
334 properties (56.1%), while pectin, gelatin, and medium concentrations of MD and starch
335 improved the quality of dry extract. The best caking properties, with the lowest degree of caking
336 (12.92%) had SGE with 7.5% pectin. The addition of gelatin also gave powders with lower
337 degree of caking (21.9-34.7%), which falls in the range reported in the literature. Powders
338 obtained by using MD and starch in medium concentrations (40 and 5%, respectively) had

caking degree of 27.2 and 35.6%. Applied WP concentrations of 20 and 40% showed an influence on caking, with values of 56.99 and 56.95%, respectively, while increasing WP concentration to 60% improved degree of caking (30.78%) compared with extract without carrier addition.

In general, addition of pectin and gelatin decreased caking degree of the gentian powder, thus enhanced its handling and storage ability.

3.7. Particle size distribution

The obtained powders contained particles with diameter ranging from 0.82 (d10 for 2.5% starch) to 22.46 μm (d90 for 7.5% pectin), indicating that spray drying of willow gentian promoted the formation of small particles (Table 2). According to the literature data, the mean size of spray-dried particles was up to 50 μm, and although smaller particles were considered as fine, larger particles provided better protection of sensitive compounds (Ferrari et al., 2013; Zhiqing et al., 2007). The mean diameter over the volume distribution (D (4,3)) varied between 4.20 to 13.52 μm. The highest mean particle diameter was obtained using 7.5% and 5% pectin and 7.5% starch (13.52, 10.95 and 11.80 μm, respectively), whereas no statistical difference in values between the other used carriers was noticed. The influence of MD and WP was statistically insignificant, which is in contrast to the other reports where these carriers affected the particle size of spray-dried mulberry juice and blackberry (Ferrari et al., 2013; Wang et al., 2020). The bimodal distribution of particle size for all studied powders was observed (Fig. 2), with two distinct peaks representing predominant sizes. Smaller size peak had lower volume (<1.5%) and smaller particle sizes (0.5-0.9 μm), and the main peak with larger volume (about 6-9%) had larger particles (around 5-9 μm).

Insert Figure 2

362 The results for span varied between 1.74 (40% MD) and 3.63 (7.5% pectin). Powders
363 produced with 7.5% and 5% pectin and 7.5% starch exhibited span values >2.1, therefore they
364 were less homogeneous than powders produced with other carriers (span values approx. 1.8). A
365 smaller span value indicates a narrower size variation, which offers various options for the
366 desired applications in pharmaceutical or food industry.

367 **Insert Table 2**

368 *3.8. Zeta potential*

369 Zeta potential (ZP) is measured as a function of the microparticles surface potential, and
370 is important indicator for their long-term stability. The results of ZP determined on obtained
371 spray-dried powders are presented in Table 3. The absolute values of ZP ranged from 2.5 to 14.7
372 mV. All examined powders except those encapsulated with gelatin had negative ZP value,
373 indicating the nature of particles surface charge. Pectin demonstrated the highest absolute values
374 (11.3-14.7 mV) among all examined microencapsulated powders, and maltodextrin also
375 improved ZP (10.4-12.8 mV) of powders. Powders encapsulated with starch, WP, and gelatin
376 displayed ZP below 10 mV, pointed out poor extract stability. When ZP values are close to zero,
377 electrostatic repulsion between particles decreased which allows particle aggregation, leading to
378 powders instability. A ZP value of about ±30 mV could be considered as a value required for a
379 highly stable system (Bhattacharjee, 2016). In this respect, powders encapsulated with pectin and
380 MD in this study can be classified as a relatively stable, whereas those encapsulated with starch,
381 WP, and gelatin are highly unstable.

382 **Insert Table 3**

383 *3.9. Fourier-transform infrared analysis*

384 The FTIR spectra of SGEs demonstrated several relevant peaks, originating from SGE
385 and biopolymers (Fig. 3). The FTIR spectra analysis was used to assess the relative ratio of
386 extract and carriers in the final product, as well as to see if there were significant differences
387 between samples with different amount of carrier used in the drying process. The FTIR spectrum
388 of the extract showed the presence of different chemical groups. The bands of the highest
389 intensity are overlapping bands in the region between 1200 cm^{-1} and 800 cm^{-1}, mostly associated
390 with C-O stretching vibrations. These bands may originate from structures containing C-OH
391 bonds, such as polyphenols from SGE or sugars (Ćujić-Nikolić et al. 2019; Espinosa-Andrews et
392 al., 2010). A weak band at 1508 cm^{-1} is characteristic for aromatic C=C bonds, and may be
393 associated with xanthones and flavonoids present in the extract. Spectral region between 1500
394 and 1600 cm^{-1} originated from proteins existence when protein type of biopolymers was used.
395 In the FTIR spectra of all the samples, no bands or interactions were detected that would
396 suggest that extract compounds form covalent or other types of strong chemical bonds with
397 carriers. Therefore, the analysis indicates that extract compounds remain stable during the
398 process of drying. Since dominant picks are evident across all examined spectra, it should be
399 attributed to the successfully incorporated GE in biopolymers. In general, all examined
400 biopolymers were compatible materials for GE microencapsulation according to the FTIR
401 analysis.
402 **Insert Figure 3**
403 *3.10. TP and EE*
404 All examined powders showed high holding rates of microencapsulated phenolics with
405 EE from 42.55 to 71.32%, affirming spray drying as adequate microencapsulation technique
406 (Table 4). Samples prepared with starch and gelatin had the highest EE (64.78-71.32%), while

MD gave lowest values of EE (42.55-52.06%). Belščak-Cvitanović et al. (2015) also reported significantly better EE of green tea polyphenols in modified starch comparing with other carriers. Obtained results also indicated that lowest concentrations of all carriers provided better EE, which is in accordance with the findings of Şahin-Nadeem et al. (2013) for sage TP encapsulated in MD, gum Arabic, and β-cyclodextrin.

Insert Table 4

The value of TP content in SGE using different polymers varied between 21.77 and 36.49 mg GAE/g (Table 3). Compared with TP content in SGE without carrier addition (32.92 mg GAE/g), only samples encapsulated using starch and gelatin provided significantly higher TP content. The lowest TP content was achieved in the case of microencapsulated extract using MD (21.77 - 26.64 mg GAE/g) and WP (22.93 - 29.01 mg GAE/g), which is probably associated with a higher carrier concentration compared with other ones. The higher biopolymer concentration probably led to the dilution of the compounds in the dried extract. Decrease in TP content with increase in concentration of used carriers was reported by other authors for willow bark, sage, and yarrow (Şahin-Nadeem et al., 2013; Vidović et al., 2014; Vladić et al., 2016).

The recorded high content of polyphenols for FGE is comparable with SGEs without carriers and with gelatin and starch as carriers. Taking into account technological, economical, and time-consuming parameters, the recorded results showed that spray drying represented more suitable drying method for willow gentian extract.

3.12. HPLC analysis of individual compounds

The quantification of individual bioactive components in samples SGEs, FGE as well as in LGE, was carried out using an HPLC method, and results are shown in Table 5. Important compounds such as three secoiridoids (swertiamarin, gentiopicrin, sweroside), two flavonoids

(isoorientin, isovitexin), and one xanthone (isogentisine) were found in all examined samples. As expected, gentiopicrin was the most dominant compound in the tested samples. Among dried powders, the highest gentiopicrin content was found in powders prepared with pectin, starch, and gelatin, as well in dried extract without carrier addition (125 – 136 mg/g), with no significant difference between these samples. High EE of gentiopicrin indicated that process of spray drying and certain carriers (pectin, starch, and gelatin) were suitable for gentiopicrin stabilization. Powders obtained with MD and WP as carriers exhibited lower gentiopicrin content (61-110 mg/g), which is probably due to the higher carrier concentrations (20-60%). Sweroside showed similar pattern as gentiopicrin – it was also less sensitive to the spray drying process, and higher amounts (3.6 - 4.2 mg/g) were obtained with the same carriers (pectin, starch, and gelatin). The addition of MD or WP gave powders with decreased sweroside content. Unlike gentiopicrin and sweroside, swertiamarin was sensitive to the spray drying process, its content was much lower in SGE without carrier addition (3.60 mg/g) than in LGE (21.50 mg/g), indicating that elevated temperature during drying process had a major impact on the swertiamarin stability. However, obtained results demonstrated carrier's protective effect. Powders prepared with pectin, starch, and gelatin in all concentrations and WP at 20% contained 2.4-fold higher level of swertiamarin than plain SGE, whereas samples prepared with MD contained lowest amounts of swertiamarin compared with the other carriers, but still higher than the extract without carrier.

Insert Table 5

Powders produced with pectin, starch, and gelatin had significantly higher content of flavonoids isoorientin and isovitexin, and xanthone isogentisine than samples prepared with WP and MD (Table 5). Gelatin at 7.5% had the greatest EE of all three compounds (81-98%), followed by 5% pectin (81-93%), and they differed significantly from the sample without carrier

addition. Starch at all concentrations also showed good EE of isoorientin and isogentisine (84-90%), with lower EE of isovitexin (around 80%) compared with the plain SGE (92%). Regarding samples microencapsulated with MD and WP, only those prepared with 20% WP showed no significant differences in the content of observed compounds with SGE without carrier, while other samples contained the lowest level of detected compounds. These findings are similar to the total phenolic content measurements, where gelatin, starch, and pectin were better encapsulating agents than WP and MD. In general, obtained results indicated that type of carrier had significant influence on the content of individual compounds in the spray-dried root extracts of *G. asclepiadea*.

Since the attached results showed that swertiamarin was sensitive to the spray drying process, it was not surprising that its content in the FGE was higher (8.19 mg/g) than in plain SGE (3.60 mg/g). However, levels of other compounds except isoorientin were lower in FGE than in SGE, thus favoring spray drying process over lyophilization.

3.13. Storage stability of individual compounds

The stability of individual compounds in powders was evaluated after 6 months, and results are shown in Table 5. A general reduction in the amount of all compounds was observed, and the effect was most pronounced in swertiamarin level. Storage caused the decrease of swertiamarin content up to 73%, and the greatest loss was noticed in powders produced with gelatin and starch. The most stable in all samples were gentiopicrin and sweroside, with their content declined by 10% only, while the content of isoorientin, isovitexin, and isogentisine decreased by 20%. Obtained results showed that spray drying process could save valuable phytochemicals in willow gentian root extract.

Conclusion

476 The feasibility of spray drying of *Gentiana asclepiadea* root extract to obtain powder
477 with optimal physical properties and high retention of individual bioactive compounds was
478 studied. Addition of maltodextrin and whey protein provided powders with higher yield, the
479 lowest hygroscopicity, and short rehydration time, whereas addition of pectin, gelatin, and starch
480 improved powder degree of caking and retention of total phenolics and individual secoiridoids,
481 flavonoids, and xanthone compounds. Storage of powders at room temperature for 6 months
482 revealed that secoiridoids gentiopicrin and sweroside, and phenolic compounds were stable with
483 their content decreased by 10 and 20%, respectively, indicating that carriers exhibited protective
484 effect on these valuable compounds.

485 The results obtained in the presented study showed that *G. asclepiadea* root extract was
486 successfully encapsulated into powders with preserved stability of bioactive compounds. This
487 could be important for the further use of willow gentian in pharmaceutical and food industry due
488 to its confirmed health benefits.

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492 **References**

493 Amidon, G.E., Houghton, M.E., 1995. The effect of moisture on the mechanical and powder
494 flow properties of microcrystalline cellulose. Pharm. Res. 12, 923–929.
495 https://doi.org/10.1023/A:1016233725612.

496 Ashford, M., Fell, J., Attwood, D., Sharma, H., Woodhead, P., 1993. An evaluation of pectin as a
497 carrier for drug targeting to the colon. J. Control. Release. 26(3), 213–220.
498 https://doi.org/10.1016/0168-3659(93)90188-B.

499 Balijagić, J., Janković, T., Zdunić, G., Bošković, J., Šavikin, K., Gođevac, D., Stanojković, T.,
500 Jovančević, M., Menković, N., 2012. Chemical profile, radical scavenging and cytotoxic activity
501 of yellow Gentian leaves (Genitaneae luteae folium) grown in northern regions of Montenegro.
502 Nat. Prod. Commun. 7(11), 1487–1490. https://doi.org/10.1177/1934578X1200701119.
503 Belščak-Cvitanović, A., Lević, S., Kalušević, A., Špoljarić, I., Đorđević, V., Komes, D., Mršić,
504 G., Nedović, V., 2015. Efficiency Assessment of Natural Biopolymers as Encapsulants of Green
505 Tea (Camellia sinensis L.) Bioactive Compounds by Spray Drying. Food Bioproc. Tech. 8,
506 2444–2460. https://doi.org/10.1007/s11947-015-1592-y.
507 Bhandari, B.R., Datta, N., Crooks, R., Howes, T., Rigby, S., 1997. A semi-empirical approach to
508 optimize the quantity of drying aids required to spray dry sugar-rich foods. Dry. Technol. 15(10),
509 2509–2525. https://doi.org/10.1080/07373939708917373.
510 Bhattacharjee, S., 2016. DLS and zeta potential – What they are and what they are not?. J.
511 Control. Release. 235, 337–351. https://doi.org/10.1016/j.jconrel.2016.06.017.
512 Bušić, A., Komes, D., Belščak-Cvitanović, A., Vojvodić Cebin, A., Špoljarić, I., Mršić, G.,
513 Miao, S., 2018. The potential of combined emulsification and spray drying techniques for
514 encapsulation of polyphenols from rosemary (Rosmarinus officinalis L.) leaves. Food Technol.
515 Biotechnol. 56(4), 494–505. https://doi.org/10.17113/ftb.56.04.18.5680.
516 Cai, Y.Z., Corke, H., 2000. Production and properties of spray dried Amaranthus Betacyanin
517 pigments. J. Food Sci. 65, 1248–1252. https://doi.org/10.1111/j.1365-2621.2000.tb10273.x.
518 Coimbra, P.P.S., Cardoso, F.S.N., Gonçalves, É.C.B.A., 2020. Spray drying wall materials:
519 relationship with bioactive compounds. Crit. Rev. Food Sci. Nutr. 2, 1–18.
520 https://doi.org/10.1080/10408398.2020.1786354.

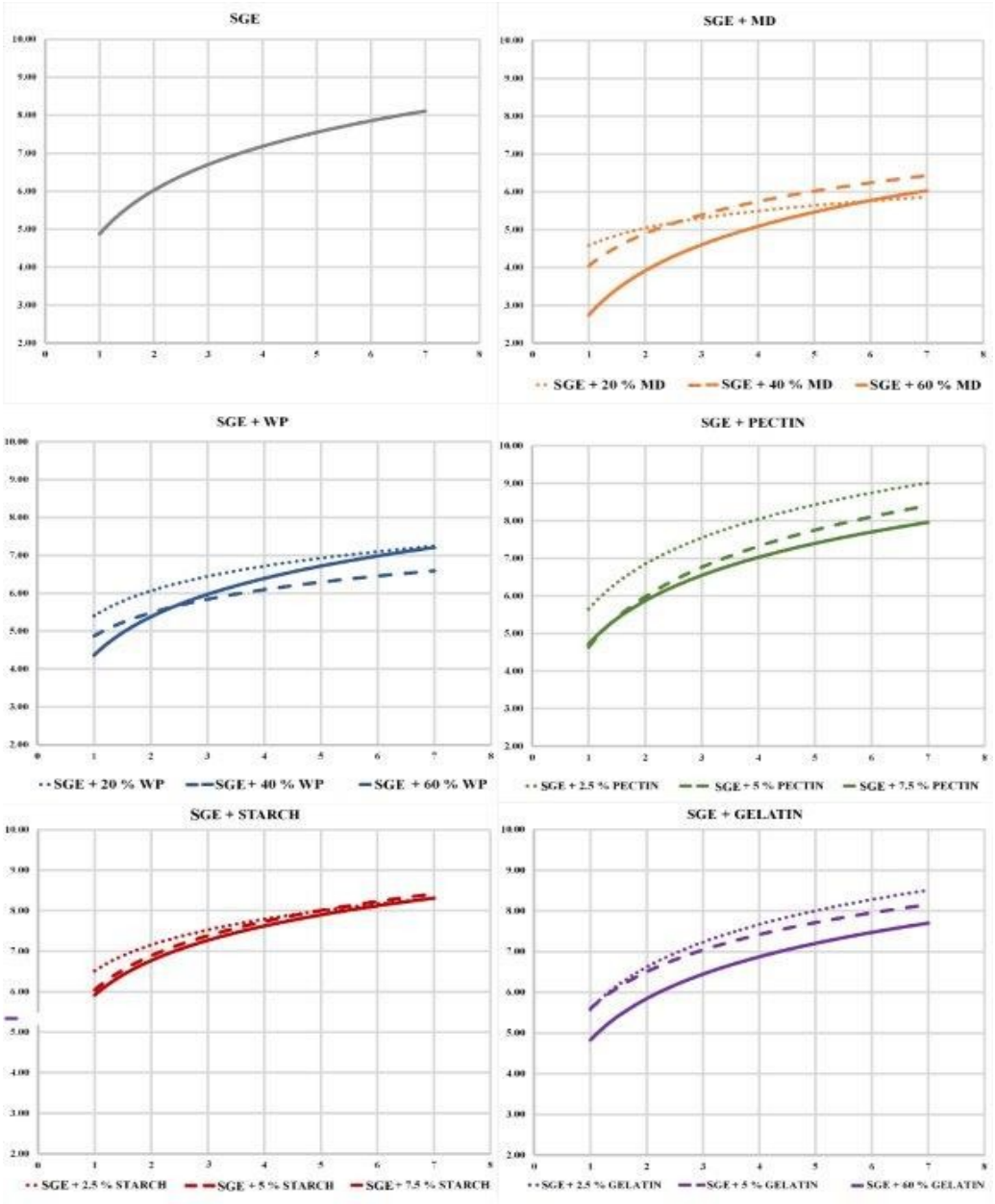
521 Ćujić, N., Trifković, K., Bugarski, B., Ibrić, S., Pljevljakušić, D., Šavikin, K., 2016. Chokeberry
522 (Aronia melanocarpa L.) extract loaded in alginate and alginate/inulin system. Ind. Crops Prod.
523 86, 120–131. https://doi.org/10.1016/j.indcrop.2016.03.045.
524 Ćujić-Nikolić, N., Stanisavljević, N., Šavikin, K., Kalušević, A., Nedović, V., Samardžić, J.,
525 Janković, T., 2019. Chokeberry polyphenols preservation using spray drying: effect of
526 encapsulation using maltodextrin and skimmed milk on their recovery following in vitro
527 digestion. J. Microencapsul. 36(8), 693–703. https://doi.org/10.1080/02652048.2019.1667448.
528 Desai, K.G.H., Park, H.J., 2005. Recent developments in microencapsulation of food ingredients.
529 Dry. Technol. 23, 1361–1394. https://doi.org/10.1081/DRT-200063478.
530 Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., Kostić,
531 I., Komes, D., Bugarski, B., Nedović, V., 2015. Trends in encapsulation technologies for
532 delivery of food bioactive compounds. Food Eng. Rev. 7, 452–490.
533 https://doi.org/10.1007/s12393-014-9106-7.
534 Du, J., Ge, Z.-Z., Xu, Z., Zou, B., Zhang, Y., Li, C.-M., 2014. Comparison of the Efficiency of
535 Five Different Drying Carriers on the Spray Drying of Persimmon Pulp Powders. Dry. Technol.
536 32(10), 1157–1166. https://doi.org/10.1080/07373937.2014.886259.
537 Espinosa-Andrews, H., Sandoval-Castilla, O., Vazquez-Torres, H., Vernon-Carter, E. J., Lobato-
538 Calleros, C., 2010. Determination of the gum Arabic–chitosan interactions by fourier transform
539 infrared spectroscopy and characterization of the microstructure and rheological features of their
540 coacervates. Carbohydr. Polym. 79(3), 541–546. https://doi.org/10.1016/j.carbpol.2009.08.040.
541 Ferrari, C.C., Germer, S.P.M., Alvim, I.D., de Aguirre, J.M., 2013. Storage stability of spray-
542 dried blackberry powder produced with maltodextrin or gum Arabic. Dry. Technol. 31, 470–478.
543 https://doi.org/10.1080/07373937.2012.742103.

544 Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R., 2007. Applications of spray-
545 drying in microencapsulation of food ingredients: An overview. Food Res. Int. 40, 1107–1121.
546 https://doi.org/10.1016/j.foodres.2007.07.004.
547 Goula, A.M., Adamopoulos, K.G., 2008. Effect of maltodextrin addition during spray drying of
548 tomato pulp in dehumidified air: II. Powder properties. Dry. Technol. 26, 726–737.
549 https://doi.org/10.1080/07373930802046377.
550 Goula, A.M., Adamopoulos, K.G., 2010. A new technique for spray drying orange juice
551 concentrate. Innov. Food Sci. Emerg. Technol. 11(2), 342–351.
552 https://doi.org/10.1016/j.ifset.2009.12.001.
553 Hudecová, A., Kusznierewicz, B., Hašplová, K., Huk, A., Magdolenová, Z., Miadoková, E.,
554 Gálová, E., Dušinská, M., 2012. Gentiana asclepiadea exerts antioxidant activity and enhances
555 DNA repair of hydrogen peroxide- and silver nanoparticles-induced DNA damage. Food Chem.
556 Toxicol. 50, 3352–3359. https://doi.org/10.1016/j.fct.2012.06.017.
557 Jaya, S., Das, H., 2004. Effect of maltodextrin, glycerol monostearate and tricalcium phosphate
558 on vacuum dried mango powder properties. J. Food Eng. 63, 125–134.
559 https://doi.org/10.1016/S0260-8774(03)00135-3.
560 Jaya, S., Das, H., Mani, S., 2006. Optimization of maltodextrin and tricalcium phosphate for
561 producing vacuum dried mango powder. Int. J. Food Prop. 9, 13–24.
562 https://doi.org/10.1080/10942910500217666.
563 Liu, L., Fishman, M.L., Hicks, K.B., 2007. Pectin in controlled drug delivery – a review.
564 Cellulose. 14, 15–24. https://doi.org/10.1007/s10570-006-9095-7.

565 Menković, N., Šavikin, K., Tasić, S., Zdunić, G., Stešević, S., Milosavljević, S., Vincek, D.,
566 2010. Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains
567 (Montenegro). J. Ethnopharmacol. 133(1), 97–107. https://doi.org/10.1016/j.jep.2010.09.008.
568 Mihailović, V., Mihailović, M., Uskoković, A., Arambašić, J., Mišić, D., Stanković, V., Katanić,
569 J., Mladenović, M., Solujić, S., Matić, S., 2013. Hepatoprotective effects of Gentiana asclepiadea
570 L. extracts against carbon tetrachloride induced liver injury in rats. Food Chem. Toxicol. 52, 83–
571 90. https://doi.org/10.1016/j.fct.2012.10.034.
572 Mihailović, V., Vuković, N., Solujić, S., Mladenović, M., Mašković, P., Stanković, M., 2011.
573 Studies on the antimicrobial activity and chemical composition of the essential oils and alcoholic
574 extracts of Gentiana asclepiadea L. J. Med. Plant Res. 5(7), 1164–1174.
575 https://doi.org/10.5897/JMPR.9001155.
576 Nurhadi, B., Andoyo, R., Indiarto, R., 2012. Study the properties of honey powder produced
577 from spray drying and vacuum drying method. Int. Food Res. J. 19(3), 907–912.
578 Olennikov, D., Gadimli, A., Isaev, J., Kashchenko, N., Prokopyev, A., Kataeva, T., Chirikova,
579 N., Vennos, C., 2019. Caucasian Gentiana species: untargeted LC-MS metabolic profiling,
580 antioxidant and digestive enzyme inhibiting activity of six plants. Metabolites. 9(11), 271.
581 https://doi.org/10.3390/metabo9110271.
582 Pan, Y., Zhaoa, Y.-L., Zhang, J., Li, W.-Y., Wang, Y.-Z., 2016. Phytochemistry and
583 pharmacological activities of the genus Gentiana (Gentianaceae). Chem. Biodivers. 13(2), 107–
584 150. https://doi.org/10.1002/cbdv.201500333.
585 Rajabi, H., Ghorbani, M., Jafari, S.M., Mahoonak, A.S., Rajabzadeh, G., 2015. Retention of
586 saffron bioactive components by spray drying encapsulation using maltodextrin, gum Arabic and

587 gelatin as wall materials. Food Hydrocoll. 51, 327–337.
588 https://doi.org/10.1016/j.foodhyd.2015.05.033.
589 Şahin-Nadeem, H., Dinçer, C., Torun, M., Topuz, A., Özdemir, F., 2013. Influence of inlet air
590 temperature and carrier material on the production of instant soluble sage (Salvia fruticosa
591 Miller) by spray drying. LWT. 52, 31–38. https://doi.org/10.1016/j.lwt.2013.01.007.
592 Şahin-Nadeem, H., Torun, M., Özdemir, F., 2011. Spray drying of the mountain tea (Sideritis
593 stricta) water extract by using different hydrocolloid carriers. LWT. 44, 1626–1635.
594 https://doi.org/10.1016/j.lwt.2011.02.009.
595 Sansone, F., Mencherini, T., Picerno, P., d'Amore, M., Aquino, R.P., Lauro, M.R., 2011.
596 Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts. J. Food
597 Eng. 105, 468–476. https://doi.org/10.1016/j.jfoodeng.2011.03.004.
598 Sarić, M., 1989. Lekovite biljke SR Srbije. SANU, Belgrade, Serbia, pp. 278-279 (in Serbian).
599 Singh, R., Román-Ospino, A.D., Romañach, R.J., Ierapetritou, M., Ramachandran, R., 2015.
600 Real time monitoring of powder blend bulk density for coupled feed-forward/feed-back control
601 of a continuous direct compaction tablet manufacturing process. Int. J. Pharm. 495, 612–625.
602 https://doi.org/10.1016/j.ijpharm.2015.09.029.
603 Tontul, I., Topuz, A., 2017. Spray-drying of fruit and vegetable juices: Effect of drying
604 conditions on the product yield and physical properties. Trends Food Sci. Technol. 63, 91–102.
605 https://doi.org/10.1016/j.tifs.2017.03.009.
606 Vidović, S., Vladić, J., Vaštag, Ž., Zeković, Z., Popović, Lj., 2014. Maltodextrin as a carrier of
607 health benefit compounds in Satureja montana dry powder extract obtained by spray drying
608 technique. Powder Technol. 258, 209–215. https://doi.org/10.1016/j.powtec.2014.03.038.

609 Vladić, J., Ambrus, R., Szabó-Révész, P., Vasić, A., Cvejin, A., Pavlić, B., Vidović, S., 2016.
610 Recycling of filter tea industry by-products: Production of A. millefolium powder using spray
611 drying technique. Ind. Crops Prod. 80, 197–206. https://doi.org/10.1016/j.indcrop.2015.11.085.
612 Wang, R., Zhao, Y., Zhu, L., Fang, Z., Shi, Q., 2020. Effect of carrier types on the
613 physicochemical and antioxidant properties of spray-dried black mulberry juice powders. J. Food
614 Meas. Charact. 14, 1201–1212. https://doi.org/10.1007/s11694-019-00369-0.
615 Waterman, P.G., Mole, S., 1994. Analysis of phenolic plant metabolites. Blackwell Scientific
616 Publication, Oxford.
617 Zhiqing, G., Min, Z., Arun, S. M., Jincai, S., 2007. Spray Drying and Agglomeration of Instant
618 Bayberry Powder. Dry. Technol. 26(1), 116–121. https://doi.org/10.1080/07373930701781751.
619
620 Figure 1. Hygroscopicity of spray dry gentian extracts (SGE) with different carriers
621 (maltodextrin (MD), whey protein (WP), starch, pectin and gelatin)
622 Figure 2. Particle size distribution of spray dry gentian extracts (SGE) obtained with 20%
623 maltdextrin (MD), 20% whey protein (WP), 7.5% starch, 7.5% pectin and 7.5% gelatin
624 Figure 3. Fourier-transform infrared spectra of spray dry gentian extracts (SGE) with different
625 carriers (maltodextrin (MD), whey protein (WP), starch, pectin and gelatin)
626
627 Figure 1.



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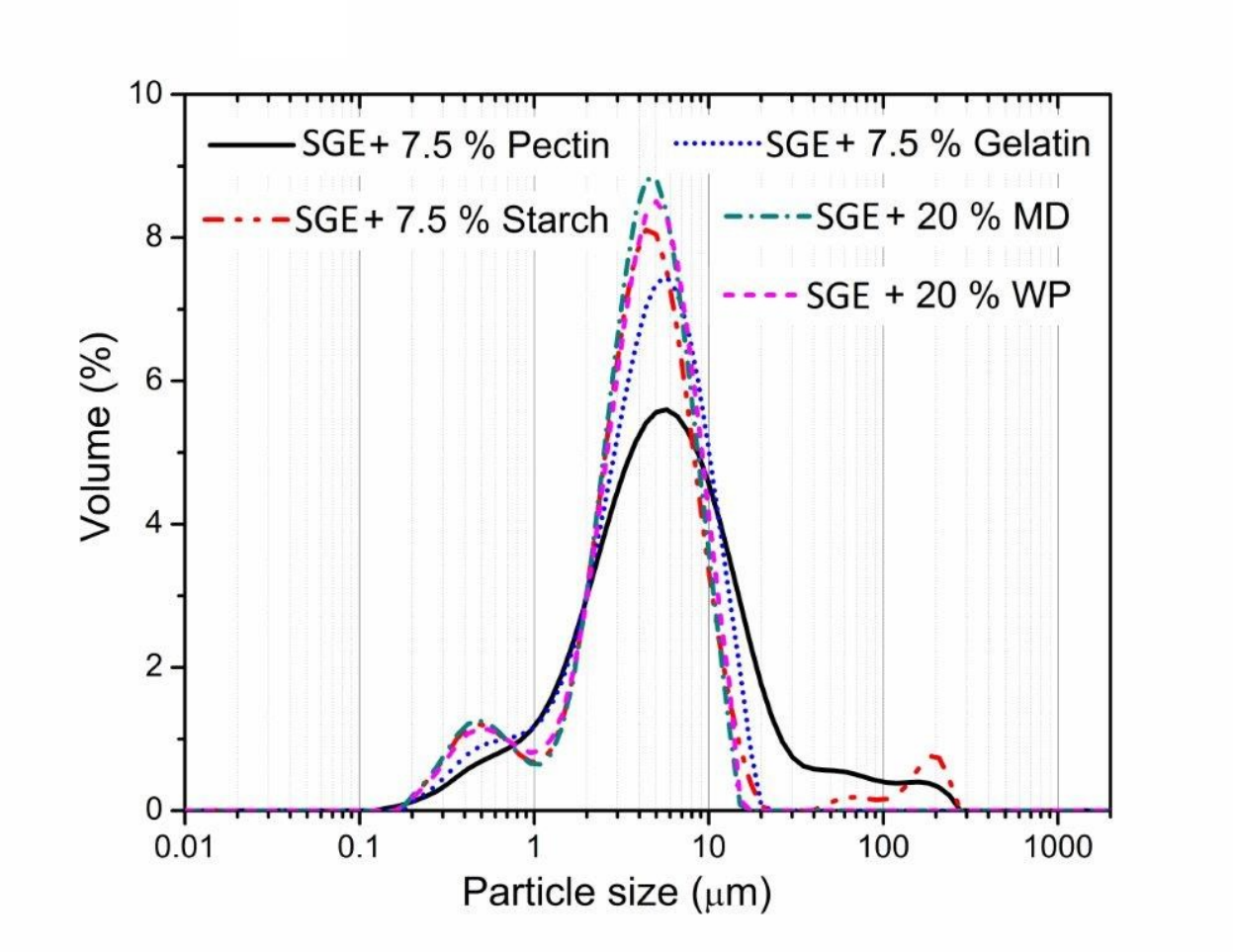
629 Figure 2.



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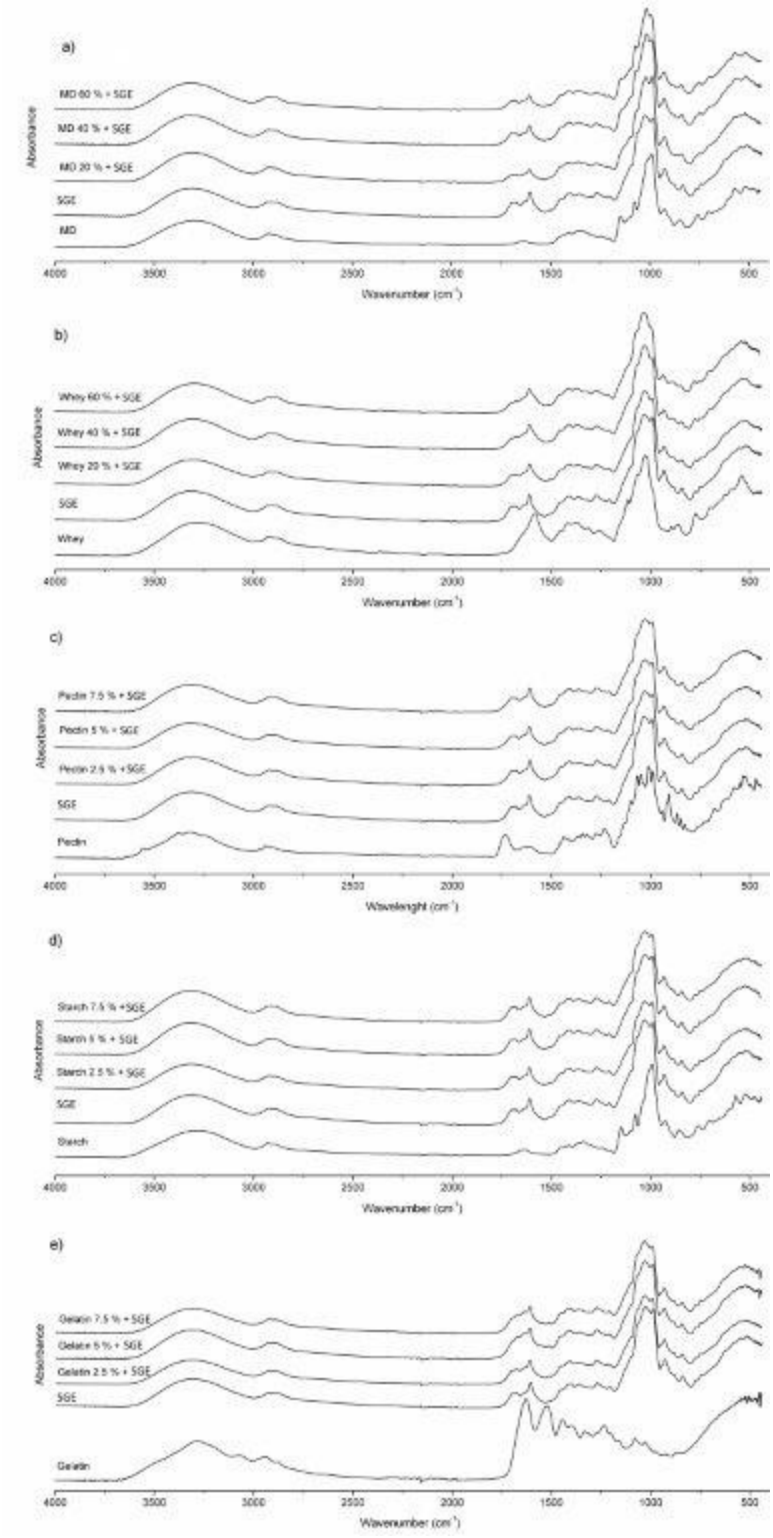
631 Figure 3.


a)
MD 60 % + SGE
MD 40 % + SGE
MD 20 % + SGE
SGE
MD
Absorbance
Wavenumber (cm-1)
b)
Whey 60 % + SGE
Whey 40 % + SGE
Whey 20 % + SGE
SGE
Whey
Absorbance
Wavenumber (cm-1)
c)
Pectin 7.5 % + SGE
Pectin 5 % + SGE
Pectin 2.5 % + SGE
SGE
Pectin
Absorbance
Wavelenght (cm-1)
d)
Starch 7.5 % +SGE
Starch 5 % + SGE
Starch 2.5 % + SGE
SGE
Starch
Absorbance
Wavenumber (cm-1)
e)
Gelatin 7.5 % + SGE
Gelatin 5 % + SGE
Gelatin 2.5 % + SGE
SGE
Gelatin
Absorbance
Wavenumber (cm-1)

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Graphical abstract

Highlights

- Willow gentian root extract powders were obtained by spray drying method.
- Powders produced with five different carriers were characterized.
- Powders had good physical properties and encapsulation of bioactive compounds.
- Gentiopicrin and sweroside were the most stabile after six months in all samples.

Table 1. Yield, moisture content, bulk density, rehydration and caking degree of obtained spray-dried *Gentiana asclepiadea* extracts (SGE)

	Yield (%)	Moisture content [a] (%)	Bulk density (g/mL)	Rehydration (s)	Caking degree (%)
SGE without carriers	55.61	1.81 ± 0.01 hi	0.25 ± 0.01 d	44.86 ± 1.67 g	56.13
SGE + 20% MD [b]	75.65	3.14 ± 0.09 b	0.25 ± 0.00 d	31.40 ± 2.18 g	48.31
SGE + 40% MD	76.63	2.83 ± 0.09 c	0.24 ± 0.01 d	67.08 ± 4.10 f	27.19
SGE + 60% MD	76.14	2.83 ± 0.23 c	0.26 ± 0.01 cd	74.09 ± 7.53 ef	36.63
SGE + 20% WP [c]	61.25	2.38 ± 0.01 de	0.25 ± 0.01 d	31.30 ± 4.26 g	56.99
SGE + 40% WP	71.46	3.46 ± 0.07 a	0.29 ± 0.02 abc	90.51 ± 12.47 e	56.95
SGE + 60% WP	73.03	2.17 ± 0.02 ef	0.30 ± 0.02 ab	80.08 ± 11.85 ef	30.78
SGE + 2.5% Pectin	57.37	2.49 ± 0.14 d	0.27 ± 0.01 bcd	124.87 ± 5.79 d	35.07

SGE + 5.0% Pectin	62.12	1.97 ± 0.00 f-i	0.32 ± 0.02 a	417.64 ± 3.67 a	48.08
SGE + 7.5% Pectin	49.51	2.14 ± 0.06 efg	0.26 ± 0.01 cd	389.49 ± 6.12 b	12.92
SGE + 2.5% Starch	60.23	2.09 ± 0.05 e-h	0.26 ± 0.02 cd	41.84 ± 1.32 g	48.73
SGE + 5.0% Starch	57.96	2.13 ± 0.09 efg	0.25 ± 0.02 d	47.34 ± 0.17 g	35.66
SGE + 7.5% Starch	61.70	1.78 ± 0.06 i	0.27 ± 0.01 bcd	34.26 ± 1.91 g	51.65
SGE + 2.5% Gelatin	65.48	2.47 ± 0.15 d	0.23 ± 0.01 d	124.02 ± 8.22 d	32.30
SGE + 5.0% Gelatin	64.16	2.59 ± 0.14 cd	0.25 ± 0.01 d	91.42 ± 0.75 e	21.88
SGE + 7.5% Gelatin	58.93	1.87 ± 0.02 ghi	0.29 ± 0.01 abc	217.60 ± 2.14 c	34.70

[a] Means followed by different letters are significantly different according to the post hoc Duncan's test al level $p \leq 0.05$
[b] MD – stands for maltodextrin
[c] WP – stands for whey protein

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Table 2. Particle size of spray-dried *Gentiana asclepiadea* extracts (SGE)

Samples	d10 [a,d] (μm)	d50 [a] (μm)	d90 [a] (μm)	SPAN [b]	D [4.3] [c] (μm)
SGE	0.95 ± 0.09 cd	4.13 ± 0.51 bc	9.43 ± 0.72 cd	2.05 ± 0.2 bc	13.48 ± 1.62 a
SGE + 20% MD [e]	1.03 ± 0.11 bcd	4.60 ± 0.50 abc	9.65 ± 0.80 cd	1.87 ± 0.25 c	5.10 ± 0.52 c
SGE + 40% MD	0.97 ± 0.11 cd	4.08 ± 0.25 bc	8.08 ± 0.93 cd	1.74 ± 0.16 c	4.41 ± 0.49 c
SGE + 60% MD	1.05 ± 0.14 bcd	4.20 ± 0.57 bc	8.39 ± 1.09 cd	1.75 ± 0.16 c	4.57 ± 0.37 c
SGE + 20% WP[f]	1.12 ± 0.12 a-d	4.65 ± 0.28 abc	9.70 ± 0.69 cd	1.85 ± 0.23 c	5.15 ± 0.68 c
SGE + 40% WP	1.00 ± 0.13 bcd	3.99 ± 0.59 bc	8.26 ± 0.94 cd	1.82 ± 0.24 c	4.41 ± 0.43 c
SGE + 60% WP	1.06 ± 0.12 bcd	4.01 ± 0.23 bc	8.71 ± 0.44 cd	1.87 ± 0.21 c	4.59 ± 0.28 c
SGE + 2.5% pectin	0.99 ± 0.09 bcd	4.02 ± 0.47 bc	8.61 ± 1.06 cd	1.90 ± 0.14 c	4.52 ± 0.56 c
SGE + 5.0% pectin	1.33 ± 0.10 ab	5.28 ± 0.68 ab	15.44 ± 1.06 b	2.67 ± 0.24 b	10.95 ± 0.84 b
SGE + 7.5% pectin	1.41 ± 0.07 a	5.80 ± 0.30 a	22.46 ± 2.76 a	3.63 ± 0.42 a	13.52 ± 1.39 a
SGE + 2.5% starch	0.82 ± 0.06 d	3.87 ± 0.32 c	7.72 ± 1.07 d	1.78 ± 0.13 c	4.20 ± 0.36 c
SGE + 5.0% starch	1.01 ± 0.08 bcd	4.49 ± 0.39 abc	9.20 ± 0.50 cd	1.82 ± 0.22 c	4.94 ± 0.61 c
SGE + 7.5% starch	1.08 ± 0.14 a-d	4.62 ± 0.35 abc	11.17 ± 1.18 cd	2.19 ± 0.12 bc	11.17 ± 0.67 ab
SGE + 2.5% gelatin	0.94 ± 0.14 cd	3.80 ± 0.49 c	8.08 ± 1.20 cd	1.88 ± 0.13 c	4.25 ± 0.60 c
SGE + 5.0 % gelatin	1.05 ± 0.15 bcd	4.23 ± 0.26 bc	9.59 ± 1.25 cd	2.02 ± 0.28 bc	4.87 ± 0.25 c
SGE + 7.5 % gelatin	1.24 ± 0.18 abc	4.98 ± 0.59 abc	11.43 ± 1.55 c	2.04 ± 0.13 bc	5.78 ± 0.51 c

[a] d10, d50, d90 represent the sizes where 10%, 50%, and 90% of the particles are smaller than the remaining particles
[b] Calculated as (d90-d10)/d50
[c] Mean diameter
[d] Means followed by different letters are significantly different according to the post hoc Duncan's test al level $p \leq 0.05$
[e] MD – stands for maltodextrin
[f] WP – stands for whey protein

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Table 3. Zeta potential of used carriers and freeze-dried (FGE) and spray-dried (SGE) *Gentiana asclepiadea* extracts

Samples	ZP [a] (mV)
MD [b]	-6.89 ± 0.57 de
WP [c]	-6.10 ± 0.44 d
Gelatin	2.08 ± 0.52 ab
Pectin	-13.63 ± 1.19 k
Starch	-0.72 ± 0.32 c
FGE	-7.34 ± 0.27 def
SGE without carriers	-8.24 ± 1.14 efg
SGE + 20% MD	-12.80 ± 1.01 jk
SGE + 40% MD	-10.38 ± 0.93 hi
SGE + 60% MD	-10.41 ± 0.42 hi
SGE + 20% WP	-6.36 ± 0.24 de
SGE + 40% WP	-5.86 ± 0.66 d
SGE + 60% WP	-6.76 ± 0.18 de
SGE + 2.5% Pectin	-11.27 ± 0.35 ij
SGE + 5.0% Pectin	-14.20 ± 0.46 k
SGE + 7.5% Pectin	-14.67 ± 0.46 k
SGE + 2.5% Starch	-9.00 ± 0.75 fgh
SGE + 5.0% Starch	-6.74 ± 0.11 de
SGE + 7.5% Starch	-10.06 ± 0.57 ghi
SGE + 2.5% Gelatin	2.46 ± 0.14 a
SGE + 5.0 % Gelatin	2.77 ± 0.17 a
SGE + 7.5% Gelatin	2.66 ± 0.22 a

[a] Means followed by different letters are significantly different according to the post hoc Duncan's test al level $p \leq 0.05$
[b] MD - stands for maltodextrin
[c] WP - stands for whey protein

Table 5. Content of individual compounds of liquid (LGE), freeze-dried (FGE) and spray-dried (SGE) *Gentiana asclepiadea* extracts

	Swertiamarin [a]		Gentiopicrin		Sweroside		Isoorientin		Isovitexin		Isogentisine	
		after 6 months		after 6 months		after 6 months		after 6 months		after 6 months		after 6 months
LGE	21.50±1.07 a		147.26±7.36 a		4.28±0.21 a		3.92±0.20 a		15.59±0.78 a		2.05±0.10 a	
FGE	8.19±0.41 bc	3.80±0.19 bc	122.58±6.13 bc	118.83±5.94 abc	4.01±0.20 abc	2.96±0.15 e	3.38±0.17 bcd	3.20±0.16 ab	11.55±0.58 def	11.27±0.56 bcd	1.84±0.07 ab	1.24±0.06 cd
SGE	3.60±0.18 f [a]	1.46±0.07 g	135.91±6.88 ab	135.07±6.80 a	4.17±0.19 abc	3.91±0.20 a	3.10±0.20 c-f	2.88±0.14 abc	14.44±0.72 ab	12.72±0.64 abc	1.85±0.13 ab	1.47±0.10 ab
SGE + 20% MD [b]	tr	tr	61.71±3.09 g	59.03±2.95 f	tr	tr	1.95±0.10 hi	1.93±0.10 ef	7.67±0.38 h	6.64±0.33 h	tr	tr
SGE + 40% MD	7.12±0.36 cd	3.47±0.17 c	102.75±5.14 de	102.57±5.13 cd	3.63±0.18 cde	3.28±0.16 de	2.73±0.14 fg	2.27±0.11 de	11.25±0.56 ef	9.39±0.47 ef	1.20±0.08 d	1.16±0.06 cd
SGE + 60% MD	5.73±0.29 e	4.00±0.20 b	86.07±4.30 ef	81.74±4.09 e	tr	tr	2.03±0.10 hi	1.82±0.09 f	8.42±0.42 gh	7.66±0.38 gh	1.41±0.07 cd	1.05±0.05 de
SGE + 20% WP [c]	8.25±0.42 bc	4.26±0.21 ab	110.69±5.53 cd	108.89±5.44 bc	tr	tr	2.97±0.15 def	2.84±0.14 abc	12.73±0.64 b-e	12.13±0.61 a-d	1.66±0.08 bc	1.55±0.08 ab
SGE + 40% WP	6.51±0.33 de	2.78±0.14 ef	89.67±4.48 ef	86.28±4.31 de	3.40±0.17 de	3.38±0.17 b-e	2.41±0.12 gh	2.30±0.11 de	9.71±0.49 fg	8.90±0.45 fg	1.33±0.07 d	1.09±0.05 de
SGE + 60% WP	5.54±0.28 e	2.93±0.15 de	77.40±3.87 fg	73.30±3.66 ef	3.19±0.16 e	3.13±0.16 e	1.79±0.09 i	1.67±0.08 f	8.22±0.41 gh	7.10±0.36 h	1.18±0.06 d	0.93±0.05 e
SGE + 2.5% Pectin	8.81±0.44 b	4.28±0.21 ab	136.39±6.80 ab	133.77±6.69 a	4.15±0.21 abc	3.84±0.19 abc	3.18±0.16 c-f	3.09±0.15 abc	14.21±0.71 abc	12.25±0.61 a-d	1.77±0.09 b	1.54±0.08 ab
SGE + 5.0% Pectin	8.92±0.45 b	3.79±0.19 bc	125.58±6.28 bc	125.35±6.27 ab	3.91±0.20 a-d	3.74±0.19 a-d	3.26±0.16 cd	2.95±0.15 abc	14.64±0.73 ab	13.21±0.66 a	1.83±0.09 ab	1.63±0.08 a
SGE + 7.5% Pectin	8.41±0.42 bc	4.55±0.23 a	131.18±6.56 ab	126.60±6.33 a	4.21±0.21 ab	3.82±0.19 abc	2.78±0.14 efg	2.68±0.13 cd	12.49±0.62 cde	11.05±0.55 cde	1.85±0.09 ab	1.34±0.07 bc

SGE + 2.5% Starch	8.79±0.44 b	2.40±0.12 f	132.65±6.6 3 ab	131.49±6.5 7 a	4.07±0.21 abc	3.85±0.19 ab	3.45±0.17 abc	2.82±0.14 bc	12.48±0.6 2 cde	12.38±0.6 2 a-d	1.76±0.09 b	1.60±0.08 a
SGE + 5.0% Starch	8.37±0.42 bc	2.74±0.14 ef	131.00±6.5 5 ab	126.21±6.3 1 ab	3.69±0.18 b-e	3.36±0.17 cde	3.42±0.17 bcd	2.75±0.14 c	12.89±0.6 4 b-e	10.81±0.5 4 de	1.73±0.10 b	1.55±0.07 ab
SGE + 7.5% Starch	8.51±0.43 b	2.65±0.13 ef	134.34±6.7 2 ab	128.48±6.4 2 a	4.04±0.20 abc	3.28±0.16 de	3.55±0.18 abc	3.23±0.16 ab	13.29±0.6 6 bcd	12.05±0.6 0 a-d	1.75±0.09 b	1.61±0.08 a
SGE + 2.5% Gelatin	8.72±0.44 b	3.47±0.17 c	136.07±7.3 2 ab	132.20±6.6 1 a	3.83±0.19 a-d	3.32±0.17 de	3.41±0.17 bcd	3.24±0.16 a	13.90±0.7 4 abc	12.08±0.6 0 a-d	1.81±0.09 ab	1.58±0.06 a
SGE + 5.0 % Gelatin	8.99±0.45 b	2.90±0.15 def	129.69±6.4 8 ab	120.42±6.0 2 ab	3.99±0.20 abc	3.30±0.17 de	3.22±0.16 cde	2.98±0.15 abc	13.32±0.6 7 bcd	12.93±0.6 2 ab	1.83±0.09 ab	1.60±0.08 a
SGE + 7.5% Gelatin	9.08±0.45 b	3.37±0.17 cd	128.64±6.4 3 bc	125.65±6.2 8 ab	4.03±0.20 abc	3.28±0.16 de	3.84±0.19 ab	3.20±0.16 ab	14.60±0.7 3 ab	13.64±0.6 8 a	1.89±0.03 ab	1.63±0.08 a

[a] Means followed by different letters are significantly different according to the post hoc Duncan's test al level $p \leq 0.05$

[b] MD – stands for maltodextrin

[c] WP – stands for whey protein

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