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ORIGINAL ARTICLE

Encapsulation of grape seed extract phenolics using whey protein concentrate, maltodextrin and gum arabica blends

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Abstract Grape seed extract (GSE) contain phenolic compounds that decrease the proclivity to various chronic diseases such as several types of cancer and cardiovascular diseases. The objective of the present study was to investigate the encapsulation of GSE polyphenols and their characterization. For this study, whey protein concentrate (WPC), maltodextrin (MD) and gum arabic (GA) were evaluated as encapsulating materials. For the preparation of stable microcapsules different WPC:MD/GA (5:0, 4:1, 3:2 and 0:5) ratios were assessed using ultrasonication for different time periods (20–40 min) followed by freeze drying. Encapsulation efficiency, antioxidant activity, particle size, surface morphology and release mechanism were determined. The GSE microcapsules coated with WPC:MD/GA ratio of 4:1 and 3:2 with core to coat ratio of 1:5 and prepared by sonication for 30 min were found to have highest encapsulation efficiency (87.90–91.13%) and the smallest particle size with maximum retention of antioxidant activity. Under optimized conditions, the low level release (43–49%) of phenolic compounds resulted under simulated gastric condition and significantly ($p < 0.05$) increased (88–92%) under simulated intestinal condition. Thus the results indicated blending of MD or GA with WPC improved the microencapsulation of GSE.

Keywords Microcapsules · Maltodextrin · Gum arabic · Encapsulation efficiency · Antioxidant activity · Freeze drying

Introduction

Addition of antioxidants is a developing trend for growth of functional foods. Among important ingredient groups that can be used for the development of functional foods, polyphenols are preferred as a natural source of antioxidants. Polyphenols in most fruits (such as Grapes) are recognized as the major class of phytochemicals with antioxidant activity (Seeram and Heber 2006). The antioxidant activity of phenolics in fruits is mainly due to their redox properties, which allow them to act as reducing agent and recognized by their free radical scavenging activity. It has been established that consumption of fruits polyphenols provides protection against several type of cancer and cardiovascular diseases (Rice-Evans et al. 1997).

Grape seeds are produced as a byproduct from wine and juice production industries. The most important polyphenols exist in grape seed includes gallic acid, the monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin 3-*O*-gallate, anthocyanins, procyanidin dimers, trimers and more highly polymerized procyanidins (Kammerer et al. 2004). However, the major challenge associated with the application of GSE polyphenols during food processing is related to strong bitterness and astringency (McRae and Kennedy 2011) together with the sensitivity to oxidation, epimerization and polymerization at high temperature (Davidov-Pardo et al. 2011). In this respect, microencapsulation is one of the best techniques to minimize some of these problems

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related to their incorporation in food stuffs. Microencapsulation may be defined as the packaging technology of solids, liquid or gaseous material with thin polymeric coating materials, forming small particles called microcapsules. The polymeric coating materials act as a protective film, isolating the core and avoiding the effect of its inadequate exposure. This membrane dissolves itself through a specific stimulus, releasing the core material in the ideal place or at the ideal time (Gharsallaoui et al. 2007).

Various kinds of coating materials have been used for microencapsulation of polyphenols, including polysaccharides (maltodextrin, gum arabic, starches, and corn syrups), lipids (mono and diglycerides) and proteins (casein, whey protein, gelatin and soy protein) (Estevez et al. 2019; Farrag et al. 2018; Gibis et al. 2014; Drusch and Schwarz 2006). Whey protein exhibits excellent emulsifying properties and provides a protection against oxidation as an encapsulating material and enhances the encapsulation efficiency (Young et al. 1993). Similarly, maltodextrin also protects the encapsulated material from oxidation (Ersus and Yurdagel 2007) by forming amorphous glassy matrices during the encapsulation process. It also provides the properties of stabilizers, emulsifiers and thickeners to the product. Correspondingly, gum arabic is preferred in microencapsulation, as an encapsulating agent due to its good emulsifying (Gabasa et al. 2007) and stabilizing capacity (Krishnan et al. 2005). For obtaining greater encapsulation efficiency, blends of different wall materials (WPC-MD or WPC-GA) can be used, as no single encapsulating material can meet all of the desired properties or characteristics in the microcapsule (Perez-Alonso et al. 2009). It has been reported that on heating GSE at 100 °C for 60 min, a significant loss of phenolic components takes place corresponding to 70% of gallocatechin, 61% of catechin, 65% of epicatechin, 75% of procyanidin B1 and 73% of procyanidin B2 (Chamorro et al. 2012). Therefore in order to protect the phenolic profile and antioxidant activity of GSE against commonly used thermal treatments in food processing like pasteurization and sterilization, it essentially requires the encapsulation. Among various encapsulation techniques, freeze drying is one of the most common techniques of choice for microencapsulation of all heat sensitive materials (Desai and Park 2005). Mild ultrasonication method used for encapsulation under controlled conditions is based on the phenomenon of acoustic cavitations. It has an advantage as over processing is not observed (Jafari et al. 2007a). There are limited studies available on microencapsulation of GSE polyphenols using milk proteins- polysaccharide complexes (Estevez et al. 2019; Farrag et al. 2018) for their utilization in food products. Therefore the present study is designed to optimize the encapsulation of GSE polyphenols (core to coat ratio, coating materials and ultrasonication time) and their characterization (encapsulation efficiency, particle size, SEM and in vitro release).

Materials and methods

Materials

Grape seed extract was obtained from Mehta pharmaceutical, Ahmedabad, (Gujarat). Whey protein concentrate (WPC-70%) was purchased from the Modern Dairies, Karnal. Maltodextrin and Gum arabic (acacia powder) powders were provided by Hi Media Laboratories Pvt. Ltd (Mumbai, India). All other reagents including gallic acid, sodium carbonate, Folin–Ciocalteu's reagent and DPPH (2, 2-diphenyl-1-picrylhydrazyl) were of analytical grade.

Methods

Preparations of grape seed extract (GSE) microcapsule

Whey protein concentrate, maltodextrin and gum arabic were used as coating materials and mixed using magnetic stirrer for corresponding ratios (WPC:MD/GA) 5:0, 4:1, 3:2 and 0:5 on dry weight basis. GSE polyphenol powder was mixed at 1:1, 1:3 and 1:5 core to coat ratio using the magnetic stirrer (2 h). The prepared mixtures were subjected to ultrasonication (Sonics, Vibra Cell, Model VC × 750, Sonics and Materials Inc., New Town, USA) at 150 W power and 20 kHz frequency having 50% pulse rate for 20–40 min via titanium probe (3.8 mm diameter), while keeping the samples in ice bath. Finally the treated samples were subjected to freeze drying (Freeze dryer; Hanil Science Industrial Co. Ltd., South Korea) to obtain dried GSE microcapsules.

Grape seed extract powder and microcapsules analysis

Estimation of Total phenolic content (TPC) of GSE powder

TPC of the GSE powder was assessed using the Folin–Ciocalteu method proposed by Zhang et al. (2006) using Multiplate reader (Infinite 200; Tecan, Mannedorf, Switzerland) by measuring absorbance at 750 nm. The standard curve (R^2 = 0.99) of gallic acid (0–120 µg/ml) was prepared and the results were expressed as mg gallic acid equivalent (GAE) per gram of sample.

Estimation of surface phenolic content (SPC) and TPC of GSE microcapsules

SPC and TPC level of GSE microcapsules was determined following the methodology as described above. Unlike for GSE powder the microcapsules were dispersed in 5 ml of 10% methanol for 1 min initially to measure the amount of phenolics present on the outer surface which remained non-encapsulated. While the determination of TPC was done by dispersion of GSE microcapsules in 10% methanol containing 25% salt, at room temperature for 180 min and the supernatant was collected after centrifugation at 5000*g* for 10 min.

Estimation of encapsulation efficiency of GSE microcapsules

The encapsulation efficiency (EE) of the prepared GSE microcapsules was analyzed using the method given by Cilek et al. (2012) with slight modifications. EE is expressed as the ratio of encapsulated phenolic content (EPC) to total phenolic content (TPC) based on the initial TPC of GSE powder. EPC is measured by calculating the difference between TPC and SPC. The EE of the prepared GSE microcapsules was calculated based on following equation:

$$\mathrm{EE}(\%) = \frac{\mathrm{EPC}}{\mathrm{TPC}} * 100 = \frac{\mathrm{TPC} - \mathrm{SPC}}{\mathrm{TPC}} * 100$$

Total antioxidant activity using DPPH radical scavenging method

Antioxidant activity of GSE powder and microcapsules was assessed using the DPPH method given by Williams et al. (1995) with slight modifications. The GSE powder/microcapsules samples (100 µl) were mixed with 3.9 ml of freshly prepared DPPH solution (6.925 mg/l) in methanol and the absorbance was measured at 515 nm (UV-2700, SHIMADZU, JAPAN) after incubation in dark for 30 min at 37 °C. For blank determination, 100 µL methanol was taken instead of sample. A calibration curve was prepared using 100 µL of Trolox standard solution (100–1000 µM) along with DPPH solution similar as samples. The results were measured as % DPPH scavenging activity = [(A515 nm blank − A515 nm sample)/A515 nm blank] × 100 and expressed as trolox equivalent (TE) values i.e. mM TE per g of sample on dry weight basis.

Particle size distribution

The particle size distribution of freeze dried GSE microcapsules was measured by using particle size analyzer (Malvern Instruments Ltd., UK). It calculates mean particle diameter (average Z-value) and particle distribution width, by means of photon correlation spectroscopy. During measurement disposable four-side plain cuvettes were used under an operating temperature of 25 °C along with 85% humidity. The whole assay was carried out in triplicate (Fernandes and Botrel 2014).

Surface morphology analysis

GSE powder and microcapsules were assessed for particle structures using scanning electron microscopy (Carl Zeiss EV018, 18th edition, Cambridge, UK). For particle structure evaluation, samples were attached on scanning electron microscopy (SEM) stub and coated with gold (ion coater) having thickness 20 nm, at 0.05–0.07 torr for 4 min keeping the ion current at 6 mA. Prepared samples were evaluated by SEM at voltage of 15 kV along with vacuum $9.0*10^{-5}$ and SEM images were recorded at 1000× magnification.

Release characteristics of the microcapsules

The release mechanism of the polyphenols from GSE microcapsules was assessed using the method given by Hur et al. (2009) with slight modifications. Prepared GSE microcapsules (3.5 g) were placed in stoppered Erlenmeyer flasks (125 ml) and incubated at 37 °C in a water bath, at orbital agitation of 200 rpm. The GSE microcapsule samples were processed sequentially as follows: mouth-addition of salivary juice (6 ml) followed by mixing for 5 min, stomach- addition of gastric juice (12 ml) and mixed for 2 h, and intestines-addition of duodenal juice (12 ml) and bile juices (6 ml) followed by mixing for 2 h. Aliquots of processed samples (1.5 ml) were collected after every 30 min intervals for a total period of 4 h and the supernatant was collected for total phenolic content analysis after centrifugation (at 5000*g* for 10 min).

Statistical analysis

The statistical analysis was assessed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison test using Graphpad prism 5.0 software. In statistical analysis significant difference was considered at $p < 0.05$.

Result and discussion

Preliminary evaluation of conditions for preparation of GSE microcapsules

GSE microcapsules were prepared using mixtures of WPC: MD and WPC: GA as encapsulating material having

different concentrations of core material (GSE: 0.5–10%) and coat material (WPC: MD/GA; 1–30%) in order to optimize the combinations for stable GSE microcapsules. Among various combinations evaluated, only a few combinations were selected for preparation of suitable GSE microcapsules. The GSE microparticles prepared at 0.5 and 1% as core concentration having core: coat ratio (CCR) of 1:20 and 1:10 by using blends of WPC: MD or WPC: GA as coating material resulted higher encapsulation efficiency (data not shown). However, due to lower encapsulation yield, these combinations were not considered for further optimization of microcapsules. In the same way microcapsules prepared at 10% core concentration along with CCR of 1:1 and 1:3 by using blends of WPC: MD or WPC: GA as coating material were also not taken into account due to very high viscosity problems during preparation of microcapsules and lower encapsulation efficiency (data not shown). According to Jafari et al. (2007b) large amount of core material at the surface led to higher particle size and lower encapsulation efficiency. Among different combinations (0.5%, 1%, 5% and 10%), the microcapsules containing 5% core material resulted in maximum encapsulation yield together with high encapsulation efficiency (EE). Therefore, microcapsules containing 5% GSE polyphenol (as core material concentration) was selected for further study and the samples were prepared by varying the coat material at core to coat ratio (CCR) of 1:5 for optimization (Table 1).

Optimization of conditions for preparation of stable GSE microparticles

Effect of different CCR, coat material ratio and ultrasonication time on encapsulation efficiency (using 5% GSE polyphenols)

Effect of varying CCR The initial total phenolic content of GSE was determined to be 640.24 ± 0.80 mg GAE per g of sample. It was observed that the GSE microcapsules prepared using WPC: MD/GA blends having core to coat ratio of 1:5 (Table 1) had higher encapsulation efficiency as compared to CCR 1:1 and 1:3 (data not shown) at 5% core concentration on the basis of total phenol content. For different WPC:MD ratios, efficiency of capsules having CCR of 1:5 varied between 52.46 and 89.07% while it fall between 25.19 and 44.14% for 1:1 and 42.49–66.84% for CCR of 1:3. Similarly, for different WPC:GA ratios, efficiency of capsules having CCR of 1:5 varied between 54.96 and 91.13% (Table 1) while it ranged between 28.41 and 48.48% for 1:1 and 43.73–72.86% for CCR of 1:3 (data not shown). Higher efficiency results were observed for GSE microcapsules coated with WPC: MD blends or WPC: GA blends as coating material having CCR of 1:5 instead of 1:1 and 1:3, since better encapsulation could be performed by using more coating material relative to core material. Cilek et al. (2012) also found that sour cherry pomace microcapsules using different MD/GA ratios corresponding to CCR of 1:20 had higher EE (78–92%) than those with a CCR of 1:10 (EE 70–85%) by using more coating material concentration relative to core material.

Effect of varying coat material ratio The results on EE of different coating material combinations WPC:MD or WPC:GA (w/w) ratios including 5:0, 4:1, 3:2 and 0:5 at 1:5 CCR are shown in Table 1. The GSE microcapsules prepared with WPC:MD or WPC:GA blends at ratio of 4:1 and 3:2 as coating material showed higher EE than that observed at ratio of 5:0 and 0:5 having CCR of 1:5. However, no significant difference ($p > 0.05$) was observed between WPC:MD/GA ratio of 4:1 and 3:2 on EE (Table 1). Although, the EE of GSE microcapsules coated with WPC: GA blends was non-significantly ($p > 0.05$) higher than WPC: MD blends coated microcapsules having CCR of 1:5. The blending of WPC-MD or WPC-GA as a wall material resulted in lower SPC and higher EE in comparison to using WPC, MD and GA alone (Table 1). Whey proteins in combination with carbohydrates have been used as encapsulating material for encapsulation of volatile material (Young et al. 1993; Sheu and Rosenberg 1995). As whey proteins served as emulsifying agents and carbohydrate (maltodextrin and corn syrup solids) formed the matrix structure (Sheu and Rosenberg 1998).

Thus, EE of capsules increased with the blending of WPC with GA or MD as a coating material (Table 1). This can be explained by emulsifying effect of WPC and stabilizing effect of GA on encapsulation process (Alftren et al. 2012). Gum arabic has the ability of forming a dried matrix around core material which prevents contact of core material with air (Thevenet 1988) whereas MD forms amorphous glassy matrices during the encapsulation process (Ersus and Yurdagel 2007). The surface active characteristic of GA has increased its intended use as an encapsulation material for protection of chemically reactive and volatile compounds (Kaushik and Roos 2007) whereas whey proteins have been found to exhibit excellent encapsulation properties and are superior to commonly used agents (Young et al. 1993). Whey protein-polysaccharide elctrostatic complexes have been reported to maintain the barrier properties of whey protein, even at reduced concentration of proteins in the complex (Berendsen et al. 2015). Ferrari et al. (2012) found 78.2% retention of anthocyanins in blackberry extract encapsulated with 7% GA, whereas Souza et al. (2015) found 88.3–93.8% retention of anthocyanins when 10% maltodextrin was used for encapsulating grape skin aqueous extract.

Table 1 Conditions optimization for preparation of GSE microparticles using whey protein concentrate (WPC), maltodextrin (MD) and gum arabic (GA) as coating material at 5% GSE concentration (WPC:MD/GA; 1:5)

Coat ratio	Ultrasonication time					
	20 min		30 min		40 min	
	WPC:MD	WPC:GA	WPC:MD	WPC:GA	WPC:MD	WPC:GA
Surface phenolic content*						
5:0	263.49 ± 2.02[a]	263.49 ± 1.02[a]	138.18 ± 1.36[a]	138.18 ± 1.36[a]	148.87 ± 2.60[a]	148.87 ± 2.60[a]
4:1	249.48 ± 6.01[b]	204.95 ± 4.25[b]	82.05 ± 3.79[b]	72.93 ± 5.11[b]	93.47 ± 4.80[b]	83.67 ± 4.88[b]
3:2	234.95 ± 2.02[b]	196.32 ± 4.78[b]	61.59 ± 3.72[b]	50.82 ± 4.91[b]	79.15 ± 3.69[b]	62.68 ± 2.96[b]
0:5	312.55 ± 2.91[a]	299.70 ± 2.48[a]	165.34 ± 4.72[a]	144.37 ± 2.86[a]	174.34 ± 0.91[a]	164.35 ± 2.15[a]
Encapsulation efficiency (%)						
5:0	58.25 ± 2.16[b]	58.25 ± 2.16[b]	84.88 ± 1.43[b]	84.88 ± 1.11[b]	82.57 ± 1.44[b]	82.57 ± 1.44[b]
4:1	60.85 ± 1.02[a]	67.60 ± 1.75[a]	87.90 ± 0.90[a]	89.96 ± 0.80[a]	85.09 ± 0.53[a]	87.09 ± 0.75[a]
3:2	63.35 ± 1.99[a]	69.60 ± 1.25[a]	89.07 ± 1.94[a]	91.13 ± 1.54[a]	87.48 ± 0.35[a]	89.71 ± 0.15[a]
0:5	52.46 ± 3.95[b]	54.96 ± 0.45[b]	81.17 ± 1.39[b]	82.99 ± 1.55[b]	79.18 ± 0.64[b]	81.39 ± 1.35[b]
Antioxidant activity** (DPPH method)						
5:0	72.11 ± 2.68[b]	72.11 ± 2.68[b]	40.11 ± 3.84[b]	40.11 ± 3.84[b]	48.11 ± 3.32[b]	48.11 ± 3.32[b]
4:1	67.47 ± 7.69[c]	45.27 ± 3.24[c]	37.78 ± 0.46[c]	26.58 ± 2.60[c]	42.46 ± 0.53[c]	32.58 ± 1.43[c]
3:2	64.44 ± 8.78[c]	43.45 ± 4.22[c]	32.22 ± 0.44[c]	24.14 ± 2.60[c]	38.24 ± 1.01[c]	29.14 ± 3.24[c]
0:5	89.15 ± 0.25[a]	78.12 ± 1.12[a]	47.88 ± 7.98[a]	45.34 ± 2.49[a]	57.82 ± 7.98[a]	52.34 ± 2.49[a]
Particle size (Z-average, nm)						
5:0	546.9 ± 138.4[b]	546.9 ± 138.4[b]	483.2 ± 124.4[a]	483.2 ± 124.4[a]	453.5 ± 105.2[a]	433.5 ± 105.2[a]
4:1	536.4 ± 106.15[c]	526.4 ± 146.5[c]	423.3 ± 83.6[b]	419.3 ± 133.6[b]	422.9 ± 79.44[b]	412.9 ± 89.4[b]
3:2	532.3 ± 78.92[c]	509.3 ± 98.9[c]	408.7 ± 44.6[b]	394.7 ± 94.6[b]	403.2 ± 93.12[b]	396.2 ± 94.2[b]
0:5	673.0 ± 96.23[a]	644.0 ± 106.2[a]	504.2 ± 86.62[a]	489.2 ± 96.6[a]	490.9 ± 104.42[a]	480.9 ± 114.4[a]

Data are presented as mean ± SD, In each group different letters indicates significant differences ($p < 0.05$)

*Results expressed as mg GAE/g sample, **mM TE/g sample on dry basis (The weight of the encapsulating agents was discounted)

Effect of varying ultrasonication time Results as presented in Table 1 showed that the ultrasonication time (20–40 min) had a significant ($p < 0.05$) effect on EE for GSE microcapsules prepared using blends of WPC: MD or WPC: GA as coating material having CCR of 1:5. Further the EE was significantly different ($p < 0.05$) for samples prepared by ultrasonication for 20 and 30 min whereas non-significant difference ($p > 0.05$) was observed between samples treated for ultrasonication period of 30 and 40 min. The lower EE of all the samples prepared using ultrasonication time of 20 min as compared to 30 and 40 min may be due to the inefficiency of ultrasonication time to encapsulate the treated samples. On comparison of ultrasonication treatment for 30 and 40 min, non-significant ($p > 0.05$) difference was observed in terms of SPC and EE (Table 1). However, ultrasonication treatment for 40 min resulted in increase of surface phenolic content corresponding to lowering of EE. Higher ultrasonication treatment might have resulted in higher energy density which caused the degradation of phenolic components. Consequently, the optimum ultrasonication time could be chosen as the 30 min based on EE (Table 1).

Effect of varying coat material ratio and ultrasonication time on antioxidant activity (using 5% GSE polyphenols)

Effect of varying coat material ratio Based on DPPH assay, initial antioxidant activity of GSE powder was determined to be 48.18 ± 0.10 mM Trolox Equivalents (TE)/g of sample on dry matter basis. The antioxidant activity of WPC: MD blends coated GSE microcapsules was ranged between 32.22 and 51.88 mM (TE)/g of sample (after discounting the weights of coating materials), and the corresponding values for WPC: GA blends coated GSE microcapsules ranged between 24.14 and 45.34 mM (TE)/g. The results indicated a direct relationship between antioxidant activity and SPC of the GSE microcapsules. The GSE microcapsules prepared with WPC:MD or WPC:GA blends at ratio of 4:1 and 3:2 as coating material showed maximum retention of antioxidant activity than at ratio of 5:0 and 0:5 having CCR of 1:5. However, there was

no significant difference ($p > 0.05$) between WPC:MD/GA ratio of 4:1 and 3:2 on retention of antioxidant activity (Table 1).

Effect of varying ultrasonication time It was observed that the ultrasonication treatment (20–40 min) had significant effect ($p < 0.05$) on antioxidant activity for GSE microcapsules prepared using blends of WPC: MD or WPC: GA as coating material having CCR of 1:5 (Table 1).The maximum retention of antioxidant activity was observed for the WPC: MD/GA ratio of 4:1 and 3:2 with CCR of 1:5. The antioxidant activity was significantly different ($p < 0.05$) for samples prepared by ultrasonication treatment for 20 and 30 min whereas non-significant ($p > 0.05$) difference observed between samples treated for ultrasonication time of 30 and 40 min. The minimum retention of antioxidant activity results were observed for the GSE capsules prepared using ultrasonication time of 20 min (Table 1). While no significant effect ($p > 0.05$) on retention of antioxidant activity was observed corresponding to ultrasonication time of 30 and 40 min, respectively. However, ultrasonication treatment for 40 min resulted in increased surface antioxidant activity along with surface phenolic content. Therefore, the optimum ultrasonication time as 30 min was selected based on maximum retention of antioxidant activity and total phenolic content.

Effect of varying coat material ratio and ultrasonication time on particle size (using 5% GSE polyphenols)

Effect of varying coat material ratio The GSE microcapsules prepared with coating material WPC: MD or WPC: GA blends at a ratio of 4:1 and 3:2 showed smaller particle size in comparison to WPC (5:0), MD (0:5) and GA (0:5) individually. However, no significant difference ($p > 0.05$) was observed between WPC: GA blends at ratio 4:1 and 3:2 on particle size (Table 1). Further, the particle size was significantly lower ($p < 0.05$) in case of GSE microcapsules prepared using WPC: GA (394.7 ± 94.6 nm) blends as compared to WPC: MD blends (408.7 ± 104.6 nm) having coating material ratio 3:2 (Table 1). Thus the results confirmed that encapsulation using whey protein–polysaccharide blend as wall material at similar CCR of 1:5 had no effect on particle size diameter. McClements (2005) also reported that the mean droplet size increased as the ratio of core to wall material increased.

Effect of varying ultrasonication time To investigate the effect of ultrasonication time on particle size, the measurement was performed for samples treated for 20, 30 and 40 min (Table 1). The results indicated that sonication time of 20 and 30 min had significant ($p < 0.05$) effect on mean particle diameter whereas no significant difference ($p > 0.05$) was observed between samples treated at 30 and 40 min. It can be described by increasing energy density along with sonication time that leads to formation of smaller particles and extra disruption; therefore, particle diameter slightly decreases and then becomes stabilized (Delmas et al. 2011).

Recently, Yadav et al. (2018) reported the major phenolic compounds present in GSE as gallic acid, epicatechin, procyanidin B2, procyanidin B1 and catechin, respectively. The optimization of GSE encapsulation as a function of coat material, core to coat material ratio and ultrasonication time following freeze drying showed that GSE microcapsules coated with WPC:MD/GA ratio of 4:1 and 3:2 with core to coat ratio of 1:5 (ultrasonication treatment for 30 min) exhibit maximum encapsulation efficiency (87.90–91.13%). GSE encapsulation using whey protein–polysaccharide complexes resulted in higher retention of phenolic compounds and antioxidant activity.

Release of phenolic compounds from GSE microcapsules under simulated gastric fluid and simulated intestinal fluid

The release of phenolic compounds from WPC: MD or WPC: GA blends coated GSE microcapsules in the simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) is presented in Table 2. The results showed that the in vitro release rate of GSE polyphenols from WPC: GA blends coated microcapsules was significantly ($p < 0.05$) lower than that for WPC: MD under SGF at pH 1.8 after 120 min. Further the release rate significantly ($p < 0.05$) increased up to 92.60% in WPC: GA coated microcapsules and 90.64% in WPC: MD blends coated microcapsules under SIF at pH 8 after 240 min (Table 2).

The release of GSE polyphenols from WPC: GA blends coated microcapsule was significantly ($p < 0.05$) higher than that of WPC: MD blends coated microcapsules under SIF. The low level release of GSE phenolic compounds in SGF showed that the encapsulating material type was gastric-insoluble material. Therefore, encapsulating material acted as a barrier against the gastric medium. Thus, the results indicated that encapsulation had a significant effect on the retention of the GSE phenolic compounds. Relatively small amount of phenolic compounds was released at low pH and their release increased in SIF, hence the encapsulated GSE phenolic compounds could be effectively absorbed in the small intestine. This behavior could be described by increase in water interaction, wettability and solubility of the microcapsules at the higher pH of the SIF (Sansone et al. 2011). Seok et al. (2003) performed a similar study in order to determine the stability of isoflavone microencapsulated by polyglycerol monostearate in

Table 2 Release of phenolic compounds from GSE microcapsules in simulated gastric fluid and simulated intestinal fluid

Sample type	% Release	
	SGF (0–120 min)	SIF (120–240 min)
WPC:MD		
5:0	38.99 ± 0.76^{c}	86.61 ± 1.32^{b}
4:1	46.42 ± 0.55^{b}	88.14 ± 0.84^{a}
3:2	49.03 ± 0.64^{b}	90.64 ± 0.21^{a}
0:5	55.95 ± 0.83^{a}	80.41 ± 0.96^{c}
WPC:GA		
5:0	38.99 ± 0.76^{c}	86.61 ± 1.32^{b}
4:1	43.42 ± 0.55^{b}	89.10 ± 1.84^{a}
3:2	45.03 ± 0.64^{b}	92.60 ± 1.01^{a}
0:5	51.95 ± 0.83^{a}	84.01 ± 2.96^{c}

Data are presented as mean ± SD, In each group different letters indicates significant differences ($p < 0.05$)

simulated gastrointestinal fluid in media with different pH and found that most of the isoflavones were released at pH 7 and 8.

Surface morphology of GSE microcapsules

As no significant difference ($p > 0.05$) was observed between microcapsules coated with WPC: MD/GA ratio of 4:1 and 3:2, therefore, WPC: MD/GA (3:2) blends were assessed for surface morphology. GSE microcapsules coated with WPC: MD blends or WPC: GA blends having CCR of 1:5 using freeze drying method were illustrated in Fig. 1. It could be observed that phenolic powder and GSE microcapsules coated with WPC: MD blends or WPC: GA blends exhibited larger size and resembled broken glass or flake-like structure (irregular shape) for all formulations. It was observed that the surface morphology of GSE microcapsules is different from non-encapsulated GSE phenolic powder. The results clearly showed that the addition of wall materials affects the structure of core material. However, the outer surface of the GSE microcapsules coated with WPC: MD and WPC: GA blends at ratio of 3:2 were similar because formulation had no effect on appearance of capsules. Many studies have reported a similar morphology for freeze dried microcapsules. During freeze-drying, ice supported the frozen structure and once ice was removed by sublimation, encapsulates retained the porous structure. Krishnan et al. (2005) reported that blends of GA, MD and modified starch in ratio of 4:1:1 produced spherical microencapsules suitable for encapsulation.

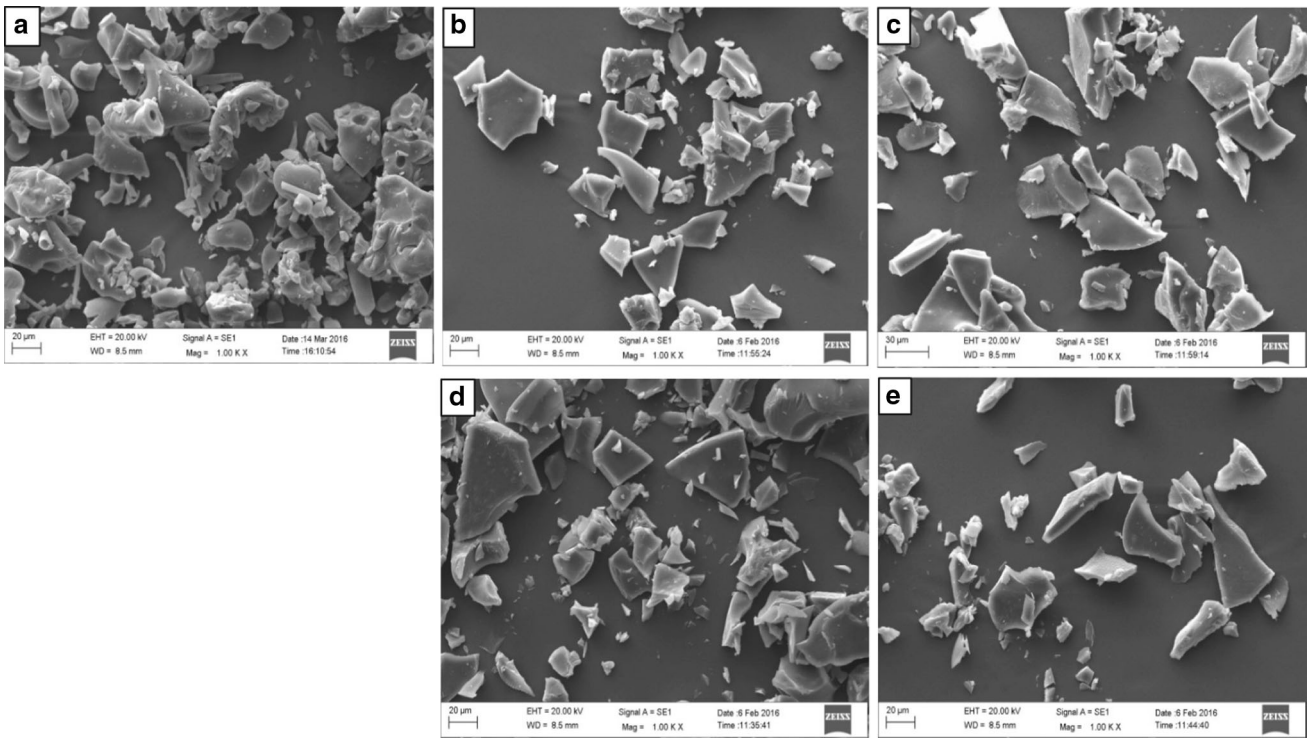

Fig. 1 SEM image of GSE powder and microcapsules prepared by freeze drying method with a core to coat ratio of 1:5 and ultrasonication time of 30 min having WPC:GA/MD ratios: 3:2 at 5% core material **a** GSE powder, **b** WPC:GA (control), **c** GSE + WPC:GA (microcapsule), **d** WPC:MD (control), **e** GSE + WPC: MD (microcapsule)

Conclusion

Freeze dried, GSE microcapsules coated with WPC: MD/GA ratio of 4:1 and 3:2 with core to coat ratio of 1:5 and prepared by sonication for 30 min resulted highest EE and the smallest particle size with maximum retention of antioxidant activity as compare to microcapsules coated with WPC: MD/GA blends at 5:0 and 0:5 ratios. The release of phenolic compounds from the WPC: MD/GA blends coated (4:1 and 3:2 ratios) microcapsules were significantly ($p < 0.05$) lower under SGF (43–49%) and increased significantly (88–92%) under SIF condition in comparison to WPC: MD/GA blends coated (5:0 and 0:5 ratios) microcapsules. Thus the results indicated blending of MD or GA with WPC improved the microencapsulation of GSE.

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