

Optimisation of Beetroot Juice Encapsulation by Freeze-Drying

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Sensitivity of natural antioxidants could be improved using encapsulation technologies. In this study, encapsulation of beetroot juice (BJ) with soy proteins has been optimized in terms of the wall:core ratio, BJ dilution and mixing time of both components, maximizing DPPH radical scavenging activity (SA) and the encapsulation efficiency (EE) based on the total phenolics content. Multi response optimization has indicated that the optimal encapsulation is accomplished when soy protein is mixed with undiluted BJ at a wall:core ratio of 50 g/L for 9.8 min. Applying these conditions, the optimal encapsulate has been produced, where the contents of total phenolics, flavonoids and betalains were 150.71 mg GAE/100 g, 9.31 mg RE/100 g, and 521.28 mg/100 g, respectively, while EE and SA were in accordance with the values obtained by optimization, *i.e.* 92.48% and 1.01 mmol TE/100 g, respectively, confirming the validity of the optimization process. The resulting encapsulates have favorable physicochemical and functional characteristics and can be potentially applied as natural color additives.

LIST OF ABBREVIATIONS

a_w – water activity; BE – betanin equivalents; BJ – beetroot juice; CPC – core phenolic compounds; DW – dry weight; EE – encapsulation efficiency; GAE – gallic acid equivalents; OE – optimal encapsulate; RE – rutin equivalents; RP – reducing power; RSM – response surface methodology; SA – DPPH radical scavenging activity; SPC – surface phenolic compounds; SPI – soy protein isolate; TE – Trolox equivalents; VE – vulgaxanthin-I equivalents.

INTRODUCTION

Human body produces reactive oxygen and nitrogen species which, when imbalanced with antioxidants as protective mechanisms, can contribute to cellular aging and endanger health [Poljsak *et al.*, 2013; Valko *et al.*, 2007]. Overproduction of these reactive species results in oxidative stress, which is known to cause damage to biological molecules such as lipids, proteins and nucleic acids [McCord, 2000]. Antioxidants in cells scavenge excessively produced damaging species.

Fruit and vegetables are a rich source of antioxidant compounds such as ascorbic acid, carotenoids, flavonoids, and other phenolics [Rouanet *et al.*, 2010]. Research has shown that many chronic diseases are diet-induced [López-Varela *et al.*, 2002]. It has been suggested that a diet rich in fruit and vegetables might strengthen the antioxidant de-

fense network endogenously, and contribute to protection from oxidative damage.

Beetroot (*Beta vulgaris* L.) is a vegetable cultivated for its roots rich in betalains, water-soluble nitrogenous pigments which include betacyanins colored from purple to violet and betaxanthins with colors from yellow to orange formed in the root [Chevallier, 1996]. Betalain profile in a plant depends on its maturity degree, variety, and climatic conditions [Ampletil *et al.*, 2015]. Betalains are commonly used as natural food dye (E162), but also have potential health benefits due to their antioxidant and anti-inflammatory activities [Georgiev *et al.*, 2010; Zielinska-Przyjemaska *et al.*, 2009]. Beetroot contains also phenolic acids such as *p*-coumaric, protocatechuic, ferulic, vanillic, *p*-hydroxybenzoic, and syringic [Kujala *et al.*, 2000].

The sensitivity of natural compounds, such as betalains and phenolics, to environmental or technological process conditions (temperature, pH, oxygen content, water activity, light, radiation, presence of metal ions and redox enzymes) could be improved by microencapsulation [Krajka-Kuzniak *et al.*, 2012; Ravichandran *et al.*, 2013]. Pitalua *et al.* [2010] have found that betalain content, color, antioxidant activity, and redox potential of beetroot juice encapsulated in gum Arabic obtained were stable during storage for 44 days at $a_w < 0.521$. Also, betalains from *Lampranthus productus* have been stable during six months of storage when spray dried with maltodextrin or chitosan [Gandía-Herrero *et al.*, 2013]. This technology presents packing target molecules in capsules which release the content at controlled rates and conditions, protecting them from deterioration [Shahidi & Han,

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1993]. One of the most widely used techniques of microencapsulation is freeze-drying which is based on the phenomenon of sublimation. Its main advantage is that the most of the favorable properties of raw material, e.g. shape, dimensions, appearance, taste, color, flavor, texture, and bioactivity, remain preserved [Ceballos *et al.*, 2012]. Saikia *et al.* [2015] found a higher efficiency of the encapsulation process and retention of phenolics in the freeze-dried samples compared to the spray-dried ones, and explained that by the large surfaces area exposed to air and high temperatures used in the spray drying technique.

The materials used as the wall of an encapsulate in food usually include protein isolates, gum Arabic, pectin, skim milk powder, non-fat dry milk solids, soy, modified starch, maltodextrin and sugars, which are food-grade and biodegradable [de Vos *et al.*, 2010; Nedovic *et al.*, 2011]. A soy protein isolate (SPI) is a well-known food carrier, due to its high purity, low cost, availability, high nutritional value, and functional properties such as solubility; the ability to absorb water and oil, the ability to stabilize emulsions; the ability to form gels, foams and films; as well as its fine organoleptic properties [Franzen & Kinsella, 1976; Maltais *et al.*, 2009; 2010].

This study was designed to investigate the optimal conditions for beetroot juice (BJ) encapsulation using soybean proteins, using the freeze-drying method. The optimal conditions for obtaining the encapsulates with the highest encapsulation efficiency (based on the total phenolic compound content) and the highest antioxidant activity against the stable DPPH radicals were defined with the response surface methodology (RSM). The optimal encapsulate (OE) was characterized regarding its physicochemical characteristics; total phenolics, flavonoid and betalain contents; antioxidant activity against DPPH radicals; and reducing power.

MATERIAL AND METHODS

Reagents, chemicals and instruments

The Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and trichloroacetic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA); ferric chloride was obtained from J.T. Baker (Deventer, Holland); and sodium nitrite from LACH-NER (Brno, Czech Republic). Other chemicals and solvents used were of the highest analytical grade. Distilled water was produced using the DESA 0081 Water Still destilator water purification system (POBEL, Madrid, Spain). Soy protein isolate was purchased from "Macrobioic Prom" company (Belgrade, Serbia). Absorbances in spectrophotometrical assays were measured using a Multiskan GO microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA) and a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

Beetroot as plant material

Beetroot (*Beta vulgaris* L.) was obtained at a local supermarket. Roots of the beet were washed, cut into pieces, and blended in a laboratory blender (model Neo SK-400, TCL King Electrical Appliances Co., Ltd., Huizhou, China).

Beetroot juice (BJ) was separated from pomace by vacuum filtration and stored at -20°C until analysis.

Encapsulation procedure

The BJ (initial pH 5.45) was encapsulated *via* freeze-drying. BJ (core) was mixed with a soy protein isolate (wall) according to the experimental design (Table 1). The samples were frozen at -40°C for 2 h in a Martin Crist Alpha 2-4 (Osterode, Germany) freeze-drier. The main drying process was performed at pressure of 0.01 mbar and temperatures from -40 to 20°C for 59.5 h. The final drying lasted 5.5 h at pressure of 0.005 mbar and temperatures from 20 to 30°C. The collected freeze-dried samples were stored at -20°C until further use. Freeze-drying experiments were executed in a laboratory freeze-drier (model Alpha 2-4 LSC, Martin Christ, Osterode, Germany), under the conditions described above.

Optimization of encapsulation

Parameters of encapsulation were optimized using the response surface methodology (RSM). The adopted experimental model was a Box-Behnken design for three variables at three levels. Three independent variables (parameters of encapsulation) were: the ratio of wall:core (X_1), dilution of BJ (X_2), and mixing time (X_3). The coded values of the independent variables were -1, 0, and 1. The actual values, selected from the preliminary study, based on literature survey [Cheng *et al.*, 2012; Ezhilarasi *et al.*, 2013; Ramírez *et al.*, 2015; Roopchand *et al.*, 2013; Saikia *et al.*, 2015; Tumbas Šaponjac *et al.*, 2016] by the corresponding coded values of three independent variables are given in Table 1. The complete model consisted of 15 experiments with three replicates.

For process optimization, encapsulation efficiencies (EE) and DPPH radical scavenging activity (SA) of encapsulates were chosen as the responses. Single response as well as multi response optimization were performed to enable the selection of optimal parameters for the production of the optimal encapsulate (OE), which will be further examined.

Spectrophotometric determination of the total phenolics content of beetroot juice and its encapsulates

The content of total phenolics in BJ, in the core of encapsulates (CPC), and in the surface of encapsulates (SPC) was determined by the method with the Folin-Ciocalteu reagent [González-Molina *et al.*, 2008]. Briefly, the reaction mixture was prepared in a 96-well microplate by mixing 170 μ L of distilled water, 15 μ L of BJ/encapsulate extract, 12 μ L of the Folin-Ciocalteu's reagent, and 30 μ L of 20% sodium carbonate. The mixture was incubated in the dark at room temperature for 1 h. After incubation, the absorbance was read at 750 nm. The corrections for interfering substances originating from beetroot have been made by simultaneous preparation of control samples with a matching concentration of BJ/encapsulate extract in the same way. Results were expressed as gallic acid equivalents (GAE) per 100 mL of BJ or per 100 g of encapsulate.

To determine the encapsulation efficiency, the total contents of phenolic compounds in the core (CPC) and surface (SPC) were evaluated using the encapsulate extraction procedure described by Vergara *et al.* [2014]. For CPC, 100 mg

of the sample were suspended in 1 mL of an ethanol:acetic acid:water mixture (50:8:42, v/v/v), vortexed for 1 min, centrifuged for 2 min at 14,000×g (model Rotilabo-mini-centrifuge, Carl Roth, Karlsruhe, Germany), and then the supernatant was separated. For SPC, 100 mg of the sample were suspended in 1 mL of an ethanol:methanol mixture (1:1, v/v). The mixture was vortexed for 1 min, centrifuged at 3018.6×g for 2 min, and then supernatant was separated. CPC and SPC were determined with the Folin-Ciocalteu method [González-Molina *et al.*, 2008]. The encapsulation efficiency (EE) was determined by using the equation:

$$EE (\%) = ((CPC-SPC)/CPC) \times 100 \quad (\text{Eq. 1})$$

Spectrophotometric determination of the total flavonoid contents in beetroot juice and its optimal encapsulate

The total content of flavonoids in BJ and OE was determined spectrophotometrically with a modified method described by Markham [1989]. The reaction mixture was prepared in a 96-well microplate by mixing 125 μL of BJ/OE extract obtained using ethanol:acetic acid:water (50:8:42, v/v/v), 25 μL of distilled water, and 62.50 μL of AlCl_3 . Blank sample was prepared in the same manner replacing AlCl_3 with water in the reaction mixture. The absorbance was measured immediately at 430 nm. Absorbance of the experimental samples was corrected for the absorbance of control samples with the appropriate concentration of BJ or extract encapsulated without the reagent. The total content of flavonoids was expressed as rutin equivalents (RE) per 100 mL of BJ or per 100 g of OE.

Determination of betalain content in beetroot juice and its optimal encapsulate

Contents of betalain pigments (betacyanins and betaxanthin) in OE and BJ were determined as described by von Elbe [2003] in a 96-well microplate by mixing 240 μL or 250 μL of a phosphate buffer (0.05 M, pH 6.5) and BJ/ethanol:acetic acid:water (50:8:42, v/v/v) OE. Phosphate buffer was used as a blank. Wavelengths of 545 nm and 476 nm were used for the analysis of betacyanins and betaxanthin, while the wavelength of 600 nm was used for correction. Total betalains content was calculated as the sum of betacyanin and betaxanthin contents. Content of betacyanins was expressed as mg betanin equivalents (BE) per 100 mL of juice or per 100 g of encapsulate, while the content of betaxanthins was expressed as mg vulgaxanthin-I equivalents (VE) per 100 mL of BJ or per 100 g of OE.

Determination of radical scavenging activity against DPPH radical

The ability of BJ and OE to scavenge DPPH radicals was determined using the spectrophotometric method which is based on monitoring changes in solution color from purple-colored stable nitrogen DPPH radicals to yellow-colored reduced non-radical form DPPH-H [Brand-Williams *et al.*, 1995]. Samples of the encapsulate were prepared in the same way as for determination of CPC. Briefly, 250 μL of a DPPH solution in methanol was mixed with 10 μL of the sample (BJ or encapsulate extract) in the microplate well and left in dark

at room temperature for 50 min. Afterwards, absorbance was read at 515 nm. The samples containing corresponding concentrations of BJ and encapsulate extracts without DPPH radicals were analyzed in parallel. Their absorbance was used to correct the absorbance of experimental samples. The following equation was used to calculate the DPPH radical scavenging activity:

$$SA (\%) = (A_C - A_S) / A_C \times 100 \quad (\text{Eq. 2})$$

where: A_C is the absorbance of the control (without antioxidant) and A_S is the absorbance in the presence of the sample (BJ or encapsulate extract) after correction. The calibration curve was made with Trolox and results were expressed as mmol of Trolox equivalents (TE) per 100 mL of BJ or per 100 g of encapsulate.

Determination of the reducing power of beetroot juice and its optimal encapsulate

The method of Oyaizu [1986] was used to determine the reducing power (RP) of BJ and OE. OE was prepared in the same way as for determination of CPC: 25 μL of the sample (BJ/OE extract), or 25 μL water as a blank, 25 μL of sodium phosphate buffer (pH 6.6), and 25 μL of 1% potassium ferricyanide, were mixed and incubated in a water bath for 20 min at 50°C. When the solution was cooled, 25 μL of 10% trichloroacetic acid was added and solutions were centrifuged at 3018.6×g for 10 min and afterwards 50 μL of the supernatant with 50 μL of distilled water and 10 μL of 0.1% ferric chloride were mixed in a microplate well. Absorbance was measured immediately at 700 nm. The control samples with matching concentration of BJ and encapsulate extracts were prepared in the same way. The calibration curve for this test was made with Trolox and results were expressed as mmol of Trolox equivalents (TE) per 100 mL of BJ or per 100 g of OE.

Water activity

Water activity (a_w) was determined by placing approximately 3 g of OE in a sample holder of a LabSwift a_w -meter "Novasina" (Lachen, Switzerland) at 25°C. The a_w values were recorded after equilibration.

Moisture content

The moisture content of OE was determined according to the procedure described by Şahin Nadeem *et al.* [2011] by drying in an oven at 70°C until constant weight.

Particle size distribution

Particle size distribution of OE was determined using the Mastersizer 2000 laser diffraction particle size analyzer (Malvern Instruments, Malvern, England). The Scirocco dispersion unit was used for dispersing encapsulate in the air. The sample was added at ambient temperature until an adequate obscuration was obtained (5–10%). The results were quantified as the volume-based particle size distribution by means of the Mastersizer 2000 software.

Statistical analysis

All experiments were performed in triplicate and the results are presented as means \pm standard deviation (\pm SD, $n=3$). Data were analyzed by one-way analysis of variance (ANOVA) and t -test, where applicable, and the least significant difference (LSD) test ($p<0.05$). Statistical analysis was performed using Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA). Optimization of experiments was conducted using Design-Expert® Version 7.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA, 2005).

RESULTS AND DISCUSSION

Beetroot encapsulation

Optimization of the BJ encapsulation parameters (wall:core ratio, BJ dilution and mixing time), in terms of encapsulation efficiency (EE) and DPPH radical scavenging activity (SA), was conducted using RSM with the Box-Behnken design (Table 1). The experimental model included 15 different combinations of encapsulation parameters for the measurements of EE and SA as responses. The actual values were chosen from the preliminary studies. Encapsulation efficiency of BJ with soy protein based on total phenolics content ranged from 47.79% (experiment 13) to 92.47% (experiment 2), while the experimental values for SA were in the range from 0.03 mmol TE/100 g (experiment 12) to 1.05 mmol TE/100 g (experiment 1). Microencapsulation of phenolics highly depends on the carrier and the technique employed. Figure 1 shows the influence of independent variables (X_1 , X_2 , X_3) on EE and SA of encapsulates obtained in experiments 1–15. According to the response surfaces, it can be concluded that increasing the wall:core ratio caused a slight decrease in SA and EE values. Mixing time had the same effect on the responses, while increasing dilution decreased SA and increased EE of the encapsulates.

The results of single and multi-response optimization are reported in the Table 2. When using single response optimization it was found that a high wall:core ratio (149.50 g/L), low extract dilution (0.2), and medium mixing time (18.9 min) are needed to ensure the maximal encapsulation efficiency (93.44%). The single response optimization was used to optimize the response SA as well. In this case, the optimal sample, with maximum SA (1.01 mmol TE/100 g) may be obtained by using relatively low wall:core ratio (50.0 g/L), no BJ dilution (0.0) and medium mixing time (12.9 min). The conditions for producing the optimal encapsulate sample (OE) were found by using a multi response optimization, where the EE and SA values are considered at the same time, and represent

TABLE 1. Experimental design, encapsulation efficiency (EE), and DPPH radical scavenging activity (SA) of beetroot juice encapsulates.

Exp	X_1 (g/L)	X_2	X_3 (min)	EE (%) ^a	SA (mmolTE/100g) ^a
1	50 (-1)	0 (-1)	15 (0)	89.07 \pm 0.40	1.05 \pm 0.04
2	150 (+1)	0 (-1)	15 (0)	92.47 \pm 1.67	0.99 \pm 0.05
3	50 (-1)	4 (+1)	15 (0)	91.87 \pm 0.53	0.21 \pm 0.02
4	150 (+1)	4 (+1)	15 (0)	87.40 \pm 3.27	0.14 \pm 0.00
5	50 (-1)	2 (0)	5 (-1)	91.07 \pm 0.17	0.34 \pm 0.07
6	150 (+1)	2 (0)	5 (-1)	89.97 \pm 3.34	0.10 \pm 0.00
7	50 (-1)	2 (0)	25 (+1)	84.59 \pm 4.41	0.22 \pm 0.00
8	150 (+1)	2 (0)	25 (+1)	90.70 \pm 2.57	0.07 \pm 0.02
9	100 (0)	0 (-1)	5 (-1)	86.29 \pm 3.55	0.58 \pm 0.03
10	100 (0)	4 (+1)	5 (-1)	79.73 \pm 12.51	0.08 \pm 0.01
11	100 (0)	0 (-1)	25 (+1)	89.57 \pm 2.07	0.56 \pm 0.03
12	100 (0)	4 (+1)	25 (+1)	91.25 \pm 6.18	0.03 \pm 0.00
13	100 (0)	2 (0)	15 (0)	47.79 \pm 3.03	0.08 \pm 0.01
14	100 (0)	2 (0)	15 (0)	58.02 \pm 0.28	0.11 \pm 0.00
15	100 (0)	2 (0)	15 (0)	58.83 \pm 3.44	0.10 \pm 0.00

^aResults are presented as mean values of three replications \pm SD. The ratio of wall:core (X_1), dilution of beetroot juice (X_2) and mixing time (X_3); TE – Trolox equivalent.

the optimal conditions that provide maximum values of both responses (Table 2).

Ramírez *et al.* [2015] optimized the encapsulation of model fruit juice in gum Arabic and/or maltodextrin using spray- or freeze-drying, following the concentration of gallic acid as a response. RSM optimization showed that the freeze-dried encapsulates achieved with a wall blend ratio close to 100% gum Arabic and core concentration from 10 to 20% had higher contents of gallic acid. Maltodextrin concentration of 15% was found to be the optimal for encapsulation of beetroot juice using spray-drying [Bazaria & Kumar, 2018]. The wall concentration in these studies was much higher than in our study (5%), although the wall material in our study was protein, not polysaccharide. Robert *et al.* [2010] optimized the encapsulation of polyphenols and anthocyanins from pomegranate using maltodextrin and soy protein isolate by spray drying. Encapsulation efficiency was significantly better upon the use

TABLE 2. Single (EE and SA) and multi response (EE+SA) optimization of encapsulation parameters using response surface methodology.

Optimization	Variable codes			Variable values			Optimal responses	
	X_1	X_2	X_3	X_1	X_2	X_3	EE (%)	SA (mmol TE/100 g)
EE	0.99	-0.90	0.39	149.50	0.20	18.9	93.44	–
SA	-1	-1	-0.21	50.00	0.0	12.9	–	1.01
EE+SA	-1	-1	-0.52	50.00	0.0	9.80	92.48	1.01

The ratio of wall:core (X_1), dilution of BJ (X_2) and mixing time (X_3); EE – encapsulation efficiency; SA – DPPH radical scavenging activity.

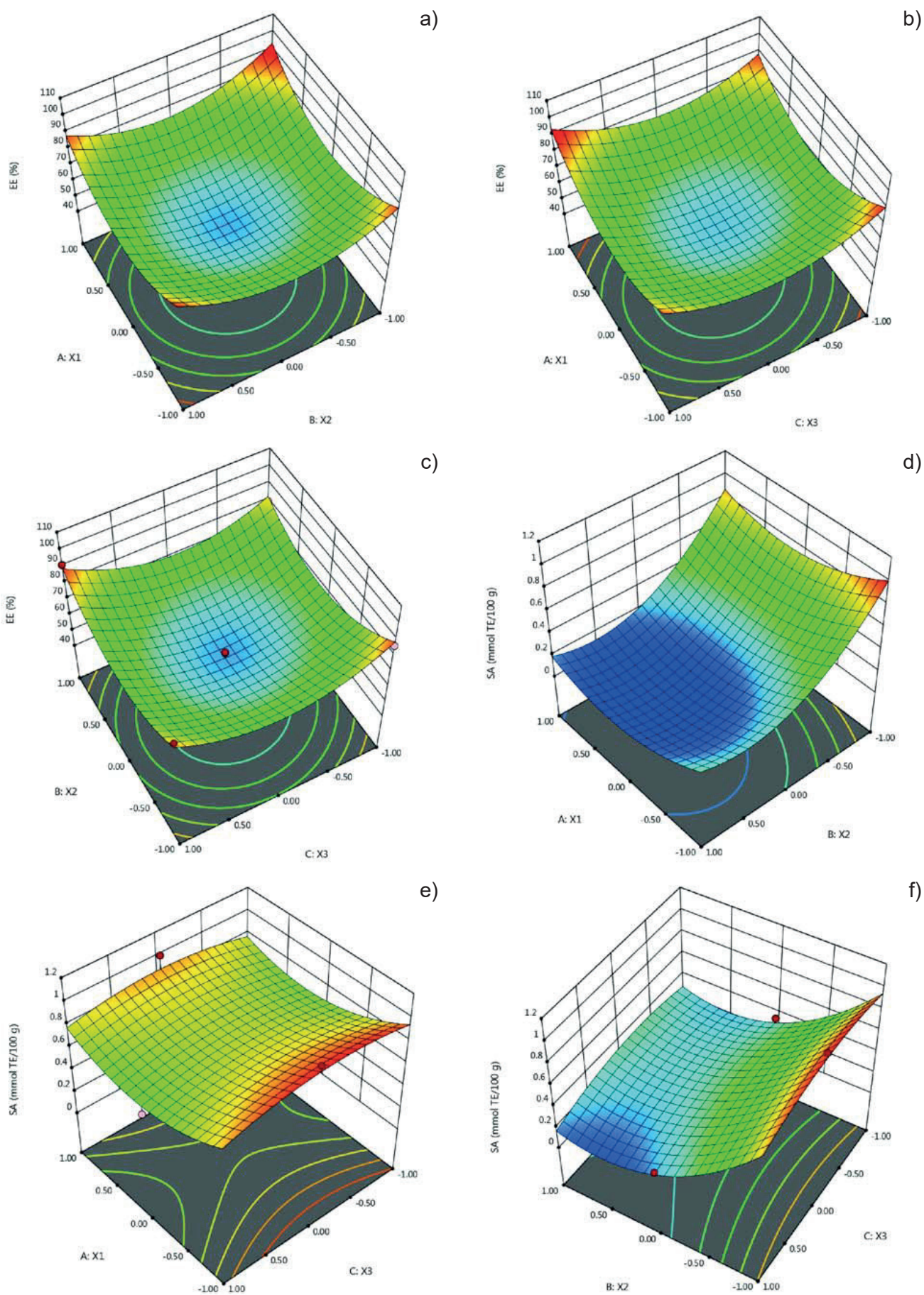


FIGURE 1. The influence of wall:core ratio (X_1), juice dilution (X_2), and mixing time (X_3) on encapsulation efficiency (EE) of beetroot juice with soy protein based on total phenolics content (a, b and c) and DPPH radical scavenging activity (SA) of encapsulates (d, e and f).

of the soy protein isolate (36.60–81.50%). However, the encapsulation efficiency using soy protein isolate in that study was lower than in our study (47.79–92.47%). Storage capacity of soy protein isolate was also better than that of maltodextrin, where polyphenol and anthocyanin retention increased during storage, while in the maltodextrin-based microcapsules the retention of these compounds diminished. Spray- and freeze-drying techniques require different temperature regimes and can lead to different physicochemical characteristics of encapsulates. Many factors such as light, temperature, and oxygen are considered to influence their quality.

Functional characteristics of beetroot and its optimal encapsulate

Janiszewska [2014] reported that the drying of beetroot juice is a method to obtain a pure and easy to use pigment in powder form. However, it is not possible to dry the beetroot juice without addition of carriers, because of its low glass transition temperature (measured Tg of juice $43 \pm 4^\circ\text{C}$). In this study, we decided to use freeze-drying due to its favorable mild process parameters (low temperature) to avoid degradation of bioactive compounds from BJ. Before drying, BJ was characterized in terms of contents of total phenolics, total flavonoids, total betacyanins, and total betaxanthins, as well as free radical scavenging activity against DPPH \cdot (SA) and reducing power (RP) (Table 3).

Beetroots have a high content of betalains (betacyanins and betaxanthins) that serve as color pigments [Delgado-Vargas *et al.*, 2000]. From Table 3 it can be observed that betalains were the dominant bioactive compounds in BJ with their concentration reaching 236.51 mg/100 mL, which is higher than the value of 767–1309 mg/L reported by Wruss *et al.* [2015]. The ratio between betacyanins and betaxanthins was from 1.75 to 1, which is close to the value obtained in this study (0.99). However, the total phenolics content obtained in this study was much lower compared to the report of Wruss *et al.* [2015] (0.85–1.29 g/L). Wootton-Beard & Ryan [2011] determined also a higher total content of phenolic compounds in the beetroot juice (101.50 mg GAE/70 mL) than that presented in this study. Flavonoids had a small share in total phenolics content found in the BJ. Kujala *et al.* [2002] reported the presence of betagarin, betavulgarin, cochliophilin A, and dihydroisorhamnetin, in extracts of beetroot peel. The differences in the physicochemical characteristics of beetroot could be due to the environmental characteristics, period of harvesting, cultivar variability, and maturity stage.

Free radical scavenging activity of BJ against DPPH \cdot was determined to be 1.93 mmol TE/100 mL and reducing power to be 107.80 mmol TE/100 mL. Wootton-Beard & Ryan [2011] analyzed 23 vegetable juices and found that beetroot juices displayed the highest ferric reducing antioxidant power (FRAP) values (8355–9500 $\mu\text{mol/L}$) and antioxidant capacity (100% DPPH \cdot inhibition and 92.1–92.3% ABTS $^{+\cdot}$ inhibition) compared to the other juices analyzed (tomato, carrot, mixed vegetable, mixed fruit and vegetable). Wruss *et al.* [2015] found that the oxygen radical absorbance capacity (ORAC) and FRAP of beetroot juices ranged from 19.70 to 37.90 mmol TE/L, and from 17.40 to 37.10 mmol TE/L, respectively.

TABLE 3. Physicochemical characteristics of beetroot juice (BJ) and its optimal encapsulate (OE).

Determination	BJ	OE
Total phenolics content ^a	56.65 \pm 1.00	150.71 \pm 3.07
Total flavonoids content ^b	7.33 \pm 8.52	9.31 \pm 0.64
Total betalains content	236.51 \pm 10.95	521.28 \pm 25.52
Betacyanins ^c	117.61 \pm 10.16	259.73 \pm 2.03
Betaxanthins ^d	118.90 \pm 10.27	261.55 \pm 2.05
SA ^e	1.93 \pm 0.04	1.02 \pm 0.08
RP ^e	107.80 \pm 5.12	1.81 \pm 0.04
Moisture content (%)	–	2.61 \pm 0.12
a _w	–	0.028 \pm 0.001
Particle size (μm)	–	174.57 \pm 8.51

Data represent mean value of three replicates \pm SD. Results are expressed as: ^a mg GAE/100 mL BJ or 100 g OE, ^b mg RE/100 mL BJ or 100 g OE, ^c mg BE/100 mL BJ or 100 g OE, ^d mg VE/100 mL BJ or 100 g OE, ^e mmol TE/100 mL BJ or 100 g OE; GAE – gallic acid equivalent; RE – rutin equivalent; BE – betanin equivalent; VE – vulgaxanthin equivalent; TE – Trolox equivalent; SA – DPPH radical scavenging activity; RP – reducing power; a_w – water activity.

The OE was produced using the encapsulation parameters from multi response optimization (Table 2). Water is an important basic element in food. OE had a low moisture content (2.61%), which corresponds to the powdered product of good stability, effective packaging and storage [do Carmo *et al.*, 2018; Sinija *et al.*, 2007]. Higher moisture content in products enables microbial growth and caking [do Carmo *et al.*, 2018]. In the study of do Carmo *et al.* [2018], beetroot extract encapsulated in whey protein isolate alone and mixtures with maltodextrin and inulin presented higher moisture values (3.81–4.24%) than the powders obtained with maltodextrin and/or inulin (3.33–3.58%) because of the greater ability of proteins to maintain moisture trapped in the particles [Jayasundera *et al.*, 2009].

The water activity (a_w) measurement, often used as a critical control point for dry and dehydrated products, provides important information about the quality of a product such as the possibility of microbiological growth on the surface and sample stability and shelf-life. The control of the water activity allows preserving its structure, texture, stability, density, and the possibility of reconstitution. Water activity of the freeze-dried OE sample was found to be 0.028, which is more than acceptable to ensure microbiological, chemical, and physical stability of the powders. Jafari *et al.* [2016] reported that saffron petals extract encapsulated by freeze-drying with different combinations of maltodextrin, Arabic gum, and seed cress gum had their a_w values in the range from 0.07 to 0.29 and moisture content from 1.88 to 3.13%. Red wine encapsulated with maltodextrin and gum Arabic, employing freeze-drying as well, had a_w value of 0.11 [Rocha-Parra *et al.*, 2016]. The results of this study indicate that water activity was a key factor affecting phenolics stability during storage. Do Carmo *et al.* [2018] reported color

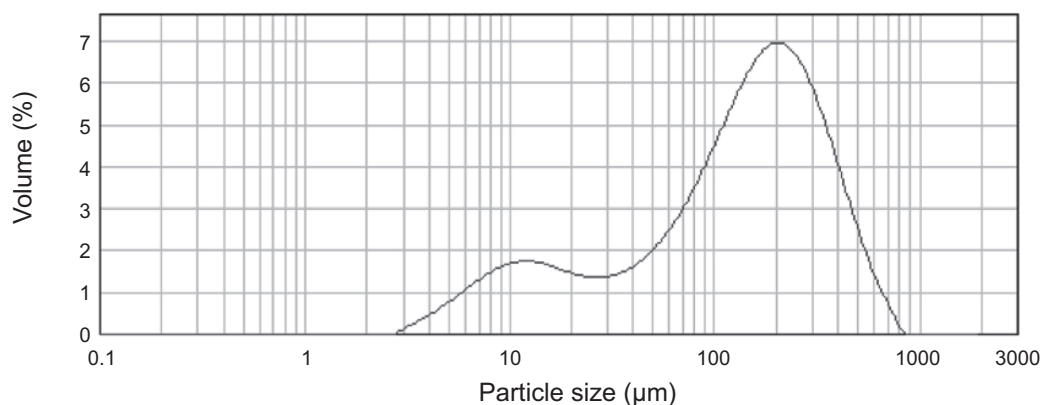


FIGURE 2. Particle size distribution of optimal encapsulate of beetroot juice.

change of the beetroot juice powders at high water activity ($a_w=0.843$), resulting from high moisture adsorption which may have facilitated betalains hydrolysis to yellow colored betalamic acid [Herbach *et al.*, 2006]. Pitalua *et al.* [2010] also concluded that the stability of betalains in microcapsules and scavenging activity depend on the a_w value. They found that when a_w values were in the range of 0.11–0.52 there were no significant differences in betalains content, color, scavenging activity and reducing power, during 45 days of storage. However, during storage of encapsulates with a_w values of 0.75 and 0.90, the concentration of betalains decreased, while scavenging activity increased significantly. It is speculated that the degradation of betalains occurs through hydrolytic reaction, due to the increase of moisture in the dry product, which leads to the diffusion of oxygen within encapsulated material, causing oxidation.

The mean particle size of the OE powder was determined to be 174.57 μm (Table 3, Figure 2), which is not in the range of fine powders ($<5 \mu\text{m}$), as reported by Medina-Torres *et al.* [2013]. From the particle size distribution of OE presented in Figure 2 it is evident that there are two distinct peaks representing predominant sizes, the largest volume of the OE being of the particles with diameters ranging from 100 to 300 μm . Kuck & Noreña [2016] obtained similar sizes (104.30–684.90 μm) of particles of a grape skin phenolic extract encapsulated by freeze-drying. Ezhilarasi *et al.* [2013] reported the particle size of *Garcinia* fruit extract encapsulated with different wall materials, employing freeze-drying, in the range of 15–100 μm . Janiszewska [2014] determined that the average diameter of spray-dried beetroot juice encapsulated in maltodextrin and gum Arabic and their mixture was in the range of 7.60–12.80 μm . Man *et al.* [1999] reported that the particle size of spray-dried powders is usually in the range of 1–15 μm , while freeze-dried powder particles can reach 300 μm . According to Chen *et al.* [2012], the larger size of the particles obtained by freeze-drying could be caused by low temperatures used in this process and lack of forces for breaking up the frozen liquid into droplets or to substantially alter their surface topology during the drying process. Ramírez *et al.* [2015] reported that in freeze-drying the product's structure is mainly developed during the previous freezing step, where fast freezing rates generate small ice crystals and slow cooling rate generates large crystals. Kuck

& Noreña [2016] highlighted that due to the larger particle size freeze-dried powders have lower hygroscopicity compared to the spray-dried powders, because larger particles have smaller exposed surface area and lower water absorption rate [Tonon *et al.*, 2010].

The content of bioactive compounds (total phenolic compounds, total flavonoids, and total betalains), and biological activity (reducing power and DPPH radical scavenging activity) were determined to characterize the OE (Table 3). According to the results obtained, flavonoids had a small contribution (6.18%) in total phenolics content of the encapsulate as determined by the Folin-Ciocalteu assay. The content of betaxanthins (261.55 mg VE/100 g) did not differ significantly ($p>0.05$) from the content of betacyanins (259.73 mg BE/100 g). It is evident that the content of betalains in BJ is high, which indicates a good potential of the carrier to trap betalains. Encapsulation efficiency of OE, based on the total phenolics content, was 75.91%, which is lower than the result obtained in the multi response optimization (Table 2). On the other hand, predicted SA values (Table 2) were in accordance with the values obtained for OE as well, without any significant difference ($p>0.05$).

Bazaria & Kumar [2018] optimized the spray-drying of beetroot juice with whey protein concentrate. Total phenolics content of the obtained powders was in the range of 16.69–25.89 mg GAE/100 g, which is significantly lower than the value obtained in our study. It was suggested that lower inlet temperatures as well as higher addition of whey protein (30%) could contribute to the higher preservation of phenolics during spray-drying [Bhusari *et al.*, 2014]. Janiszewska [2014] investigated the most effective carrier that would preserve the stability of beetroot pigments. They have examined microencapsulation process using the spray-drying method and gum Arabic, maltodextrin and mixtures thereof as wall materials. The results showed that the highest yield of purple but the lowest yield of yellow pigments was obtained using gum Arabic as a carrier (42.60 mg/100 g DW and 35.10 mg/100 g DW, respectively). The content of betacyanins (109–129 mg/100 g) and betaxanthins (34–61 mg/100 g) in this study was much lower than in our study (259.73 and 261.55 mg/100 g). The results reported by do Carmo *et al.* [2018] demonstrate slightly lower contents for betaxanthins and betacyanins (136.86–155.37 and

211.93–230.10 mg/100 g) in spray-dried beetroot extract powders as well. This could be attributed to the high temperatures used in spray-drying, different carriers and wall:core ratios used in the experiments as well as the differences in beetroot juice composition. Otálora *et al.* [2015] improved betalain content (49.7 mg/100 g) as well as the a_w value (0.18) and moisture content (2.90%) in spray-dried cactus fruit juice by adding cactus cladode mucilage to maltodextrin as encapsulating agents. Pitalua *et al.* [2010] encapsulated beetroot juice using gum Arabic as a carrier and spray-drying as a method. Total betalain content in these encapsulates was even lower (11.98 mg/100 g), which is due to a much lower wall:core ratio (1:3) than we used in our study (50 g/L, or 1:20), apart from above mentioned differences in experimental conditions. Ahmed *et al.* [2010] have reported that a higher core:wall ratio increases the content of biologically-active components in the encapsulated system.

It is generally known that the DPPH radical scavenging activity of food is closely related to the content of bioactive compounds, such as phenolics and betalains, which are present in OE. Spray-dried beetroot extract powders obtained with whey proteins and their mixtures with inulin or maltodextrin exhibited higher antioxidant activities than the inulin- and/or maltodextrin-based powders [do Carmo *et al.*, 2018]. Authors suggest that in the case of protein-based encapsulates, antioxidant activity could be attributed not only to the bioactive compounds from beetroot extract but also to Maillard reactions occurring between sugars from beetroot extract and whey proteins.

CONCLUSION

Beetroot juice is a potential source of bioactive compounds and thus it can be used in the development of functional food. RSM was used to find the optimal conditions for producing the encapsulates of beetroot juice with soy proteins using the freeze-drying process with the highest content of phenolic compounds and the highest antioxidant activity. The optimization process was confirmed by applying the optimal conditions resulting in the sample with bioactive characteristics similar ($p > 0.05$) to the predicted one. Based on these results, the optimized conditions obtained could be used for the encapsulation of bioactive compounds from beetroot juice with soybean proteins. The resulting encapsulates, with favorable physicochemical characteristics, have a potential to be used in the food industry as food colorants and as components of functional foods.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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