Physical and storage properties of spray-dried blueberry pomace extract with whey protein isolate as wall material

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ABSTRACT

In the food industry, attempts have been made to extract and encapsulate bioactives from pomace using organic solvents, e.g., ethanol. Ethanolic extracts contain high concentrations of bioactives, but encapsulation of them with whey proteins presents challenges arising from ternary phase equilibrium. This study aimed to prepare and characterize spray-dried powders made from blueberry pomace extract and whey proteins. The resulting microcapsules measured 48.5 μm in diameter, had 5% moisture content, and contained 1.32 mg cyanidin-3-O-glucoside (C3G), 2.83 mg gallic acid equivalents (GAE) and 48.52 nmol Fe (II) equivalents per gram powder. Sorption data obeyed Guggenheim–Anderson–De Boer isotherm. Storage tests revealed first-order degradation kinetics for monomeric anthocyanins, a two-fold increase in total phenolics and slight increase in antioxidant capacity. Exposure to light was comparable to storage at 37 °C, but slightly more severe in decomposing monomeric anthocyanins. The spray-dried encapsulated powder could be used as a suitable health-promoting food ingredient.

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1. Introduction

The health-promoting properties of blueberries (Vaccinium sp.) have generated considerable interest after a report was published about its high antioxidant activity among 42 fruits and vegetables (Lee and Wrolstad, 2004). Blueberries were found to be a rich source of bioactive compounds such as anthocyanins and other flavonoids. Anthocyanins can be considered “signature” health compounds because of their greater abundance than the other flavonoids found in blueberries, and their capability to cross the blood–brain barrier (Kalt et al., 2008). Higher concentrations of anthocyanins and phenolics are found in the pomace, which is a common by-product of juice processing (Khanal et al., 2012; Lee and Wrolstad, 2004). Pomace was used as an ingredient in extruded products with health benefits in vivo, such as reduction of plasma cholesterol and abdominal fat (Khanal et al., 2009, 2012). Purified blueberry anthocyanin extracts were found to be more effective than whole berries in altering the development of obesity (Prior et al., 2010) and this could also be true with pomace. We previously compared several solvent systems in the extraction of anthocyanins from whole blueberries and pomace. Results showed that the ethanolic pomace extract possessed the highest amount of total monomeric anthocyanins and total phenolics (Flores et al., 2013).

Purified anthocyanins are labile compounds and susceptible to degradation in the presence of high pH, oxygen, heat, light and metallic ions, among others (Castañeda-Ovando et al., 2009). Hence, microencapsulation, such as by spray drying, can be carried out to impart protection and facilitate targeted release (Betz and Kulozik, 2011). Both ethanolic and aqueous extracts from blueberry pomace were successfully spray-dried, but the anthocyanin content from the aqueous extract was 10-fold lower than that of the alcoholic extract (Jiménez-Aguilar et al., 2011; Ma and Dolan, 2011). Elsewhere, ethanolic extracts were also used in spray drying of anthocyanin-rich extracts from other botanical sources (Burin et al., 2011; Ersus and Yurdagel, 2007). Food-grade ethanolic extracts may be further processed for human consumption.

Whey proteins are by-products of cheese manufacturing with significant commercial potential. They possess superior gelling and emulsification properties, and an amino acid profile suitable for protein fortification in beverages. Other health benefits associated with whey proteins include antimicrobial activity, inhibition of angiotensin-converting enzyme, and anticarcinogenic activity, among others (Chatterton et al., 2006). They can also be processed into pH-sensitive hydrogels or nanoparticles for the controlled release of bioactive compounds such as anthocyanins. Whey proteins can thus be used as alternatives to polysaccharide-based wall

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materials with relatively greater functionality (Betz and Kulozik, 2011; Betz et al., 2012; Gunasekaran et al., 2007; Oldtmann et al., 2012). Consequently, we compared the in vitro release properties of anthocyanin extracts spray-dried with either gum arabic or whey protein isolates (Flores et al., 2014). Results showed a rapid increase in phenolics content and antioxidant activity with gum arabic particles during simulated gastric digestion, followed by a drastic decrease in antioxidant activity after simulated intestinal digestion. In contrast, whey protein microcapsules promoted a gradual increase in phenolics content and maintained a high level of antioxidant activity throughout the entire digestion process. Thus, whey protein microcapsules could be utilized as an encapsulant for sustained-release of phenolics with high antioxidant activity. However, to the best of our knowledge, whey protein isolates have yet to be used as wall material in the spray drying of aqueous, alcoholic anthocyanin extracts. This could be due to the complex temperature and pH dependence of a ternary mixture of alcohol, water, and β-lactoglobulin, the major protein in whey (Abascal and Lencki, 2004).

The economic potential of encapsulating aqueous, alcoholic anthocyanin extracts with whey protein isolates includes greater concentration of bioactives and reduced energy costs due to the absence of a lyophilization step to remove the solvent. In this study, our main goal was to prepare spray-dried powder made with whey protein isolate and an aqueous, ethanolic extract from blueberry pomace. We also characterized the powder properties including particle size, moisture sorption isotherm, and encapsulation efficiency, and investigated physicochemical properties including total monomeric anthocyanin content, total phenolics content and antioxidant capacity as affected by storage conditions. Results of this study can be used in the development of health-promoting food ingredients.

2. Materials and methods

2.1. Materials

Ripe rabbiteye ("Powderblue" cultivar) blueberries were harvested from the Horticulture Research Farm of the University of Georgia in July 2013 and immediately frozen at −20 °C prior to processing. The berries were processed within two months. Whey protein isolate, containing at least 95% protein (BiPro™) was a kind gift from Davisco Foods International (Eden Prairie, MN). Chemicals used were reagent-grade and obtained from the Sigma–Aldrich Chemical Company (St. Louis, MO).

2.2. Methods

2.2.1. Processing of anthocyanin-rich extract

The berries were thawed between 4 and 6 °C and blanched in boiling water for 3 min. The juice was expressed using a commercial 2.5-L centrifugal Kuvings NJ-9310U juicer (Elk Grove Village, IL). The pomace was collected and extracted with 80% (v/v) aqueous ethanol at a mass:volume ratio of 1:10 (pomace:solvent). Extraction was performed at room temperature (22 °C) for 24 h and at an agitation rate of 300 rpm. The extraction flasks were covered with aluminum foil to protect against photodegradation. The mixture was filtered and the supernate was collected. The alcohol content of the supernate was measured using a Fisherbrand Model 11-590 alcohol hydrometer (Fisher Scientific, Pittsburgh, PA) and was found to be 67% (v/v). Next, the residual solvent was evaporated in vacuum for 30 min (Rotavapor R-124, Büchi Corp., New castle, DE) under the following conditions: 10 kPa total pressure, 40 °C bath temperature, 5 °C cooling water temperature, and 180 rpm agitation rate. The alcohol content of the resulting concentrate was found to be 12% (v/v) and the pH was 3.4.

2.2.2. Spray drying of the blueberry extracts (BBE)

Examination of the ternary diagrams developed for mixtures of ethanol–water–β-lactoglobulin showed that at a pH of 3.0 and temperature of 20 °C, a transparent liquid could be obtained at low ethanol and low-to-moderate β-lactoglobulin concentrations (Abascal and Lencki, 2004). Changes in pH during addition of whey protein were also considered. Results of our preliminary trials revealed an optimum mass ratio of 8:67:25 of ethanol:water:whey protein isolate to maximize the amount of both BBE and whey protein at 22 °C. The final pH of the ternary mixture was 6.8. Consequently, the ternary mixture was spray dried using a Model B-290 mini spray dryer (Büchi Corporation, Flawil, Switzerland) under the following process conditions: 6 mL/min peristaltic pump speed (corresponding to 20% pump rate), 160 °C inlet air temperature; 86–90 °C outlet air temperature; 100% aspirator rate (corresponding to a maximum air flow of 35 m³/h), actual air flow rate of 0.667 m³/h (40 mm Q flow), and a nozzle setting of 1 cleaning cycle/min. The powders were collected and stored in polypropylene bottles at −20 °C.

2.2.3. Particle size distribution

Particle size distribution was measured using a laser diffraction analyzer (Model LS 13 320, Beckman Coulter Inc., Fullerton, CA) under the following conditions: 30% pump speed, 10% obscuration rate, 10 s wait before the first run, 10 s sonication at power setting of 2 before the first run, and 50 s run time. Polarization intensity differential scanning (PIDS) was turned on. An optical model was developed with refractive indexes of 1.333 for the fluid (water) and 1.473 for the solid particles. Volume mean diameters (D₃,₃), and cumulative mean diameter values corresponding to 10th and 90th percentile of the distribution (D₁₀ and D₉₀) were reported.

2.2.4. Chemical analyses

All powders were dissolved at 0.01 g/mL for about 1 h in deionized water prior to the tests. Whey protein isolate served as control.

2.2.4.1. Total monomeric anthocyanin content (TMAC), total phenolics content (TPC) and ferric reducing antioxidant power (FRAP). The tests were conducted according to the procedures in our previous study (Flores et al., 2013). The pH differential method was used to measure the total monomeric anthocyanins. Results were calculated as mg of total cyanidin-3-O-glucoside (C3G) per gram of powder. The total phenolics content was measured using the Folin–Ciocalteu method and calculated as mg gallic acid equivalent (GAE) per gram of powder. Antioxidant activity was measured by FRAP and computed as nmol Fe²⁺ equivalents per gram powder.

2.2.4.2. Encapsulation efficiency.

The method of Idham et al. (2012) was employed with modifications. Fifty milligrams of the spray-dried powder was dissolved in 3 mL of 95% (v/v) ethanol in test tubes, agitated for 1 min with a vortex mixer and centrifuged for 10 min at 3823 g. The supernate was assayed for surface TMAC as described earlier and reported as mg surface C3G/g powder. The encapsulation efficiency is defined as follows:

\[
% \text{Encapsulation efficiency} = \frac{\text{Total C3G/g} - \text{Surface C3G/g}}{\text{Total C3G/g}} \times 100
\]
vials and placed in 5 chambers equilibrated at different % relative humidities (%RH). The chambers were subsequently stored away from light. Saturated salt solutions were used to maintain different %RH: lithium chloride (11%), potassium acetate (22.5%), magnesium chloride (32%), magnesium nitrate (57%) and sodium chloride (75%). The samples were equilibrated for two weeks at room temperature and weighed, and then for another week and reweighed. Equilibrium was assumed when the difference between consecutive weights was less than 1 mg. The initial moisture content of the samples was obtained by vacuum drying at 65 °C for 24 h. Moisture content was computed as the ratio between the mass lost during dehydration over the initial mass.

Equilibrium moisture content was plotted against equilibrium relative humidity and data were modeled using Brunauer-Emmett-Teller (BET) and Guggenheim-Anderson-de Boer (GAB) isotherm equations.

\[
\text{BET equation: } \frac{a_m}{(1 - a_m)X_m} = \frac{1}{X_m C} + \frac{a_m (C - 1)}{X_m C} \tag{2}
\]

\[
\text{GAB equation: } \frac{a_m}{X_m} = 2a_m^\gamma + \beta a_m + \gamma \tag{3}
\]

where \(a_m\) is water activity or equilibrium relative humidity/100, \(X_m\) is equilibrium moisture content (dry basis), \(X_m\) is the monolayer moisture constant (dry basis), \(C\) is an empirical constant and \(k\) is related to the heat of sorption (Saravacos, 2005).

2.2.6. Storage tests

Three different temperatures (45, 37, and 22 °C) and a light source (40 W, 260 lumens, and color temperature of 2650 K) were used for this study. Control samples were kept at −20 °C. A sampling schedule that spanned a total of 6 weeks was made and physicochemical tests (TMC, TPC and FRAP) were conducted at each storage condition. Temperature-dependent kinetics was investigated by comparing test results at each storage time–temperature combination. Samples (1 g) were placed in 7-mL screw-capped borosilicate vials, sealed with paraffin, and stored at each temperature. Light-dependent kinetics was evaluated by conducting the same tests for samples exposed to light and samples kept in the dark. Samples (0.5 g) were placed in clear polystyrene covered dishes measuring 35 mm diameter and 10 mm deep and sealed with paraffin. Results are presented as fraction of original C3G and ratios of mg GAE or nmol Fe²⁺ equivalents of sample to that of the control.

2.3. Statistical analysis

Extractions, spray-drying, and sampling were performed in duplicate, while assays were conducted in triplicate. The Proc GLM and Proc REG functions of SAS 9.3 (SAS Inst., Cary, NC) were used to analyze one-way design data and lack-of-fit tests, respectively. Tukey’s honestly significant difference was employed as posthoc test and means were considered significantly different at \(p < 0.05\).

3. Results and discussion

Generally, processes to microencapsulate extracts vary depending on solvents and wall materials. Aqueous extracts were used because of the simplicity of the process, but the resulting spray-dried powders contained less bioactive compounds (Fang and Bhandari, 2012; Ma and Dolan, 2011). Ethanol-based extraction methods have been used in several papers, usually followed by rotary evaporation to achieve a desired solids concentration. Polysaccharides are preferred over proteins as encapsulating materials because of higher retention of phenolics content and antioxidant activity upon storage (Burin et al., 2011; Idham et al., 2012; Jiménez-Aguilar et al., 2011; Tonon et al., 2009). However, whey protein was a better encapsulant than gum arabic in terms of sustaining a high level of antioxidant activity in vitro (Flores et al., 2014). Whey protein isolate and maltodextrin were compared as wall materials for spray drying of bayberry juice and results showed that on a mass basis, less proteins were required to encapsulate a known amount of extracts (Fang and Bhandari, 2012). Whey proteins were previously used in encapsulating anthocyanin extracts prepared as emulsions (Betz et al., 2012; Oidtmann et al., 2012). A process to microencapsulate alcoholic extracts with whey protein, however, has not yet been reported. This is possibly due to a complex temperature- and pH-dependent ternary phase equilibrium that exists among whey proteins, water and ethanol (Abascal and Lencki, 2004).

In this study, we were able to demonstrate a process to recover bioactive compounds from blueberry pomace, encapsulate the extracts, and monitor the changes in physicochemical properties with time. In contrast to published methods that measured solids content (Idham et al., 2012; Jiménez-Aguilar et al., 2011), we controlled the concentration of alcohol in the mixture prior to addition of whey protein isolate, so that a stable, homogeneous mixture could be spray-dried. The final alcohol concentration prior to spray drying employed in one study (Burin et al., 2011) was too high to permit significant addition of whey protein isolate. The anthocyanin content of our spray-dried extract from blueberry pomace was of the same magnitude to that reported for spray-dried juice extract from cull blueberries without the use of ethanol (Ma and Dolan, 2011). We also used a relatively more dilute concentration of ethanol (80% by volume) compared to that used in other studies (Idham et al., 2012; Jiménez-Aguilar et al., 2011).

3.1. Powder characteristics

Fig. 1 shows the mean particle size distributions of the spray-dried BBE and the whey protein isolate. The volumetric mean diameters \((D_{50})\) of the spray-dried powder and the whey protein isolate control are 48.5 and 86.8 μm, respectively. Both distributions possessed comparable cumulative mean diameters for 10% of the distribution \((d_{10})\) [BBE = 11.9 μm, WPI = 13.2 μm] but varied in the 90th percentiles [BBE = 82.8 μm, WPI = 175.2 μm]. The size distribution within the 90th percentile was unimodal for spray-dried BBE, while multiple peaks were found in the WPI control.
The particle size of the spray-dried BBE (48.5 μm) was comparable to a range (32–63 μm) reported for another spray-dried blueberry powder (Ma and Dolan, 2011) and smaller than either the microencapsulated emulsions (200 μm) made with anthocyanin extracts and whey protein isolates or the spray-dried powder made (250–500 μm) with maltodextrin and pectin (Oudtmann et al., 2012).

Analysis of total and surface anthocyanins revealed that the spray drying process was 70% efficient in encapsulating monomeric anthocyanins. In addition, the spray-dried BBE was found to contain an average of 1.32 mg C3G, 2.83 mg GAE, and 48.52 nmol Fe (II) per g spray-dried powder. Our spray-dried BBE averaged 5% moisture content dry basis, which is comparable to that reported in other papers (Fang and Bhandari, 2012; Pitalua et al., 2010). In contrast, the spray-dried blueberry extract reported in another paper contained much higher moisture (15–20%) and consequently higher monolayer values (Jiménez-Aguilar et al., 2011). The final moisture content of the spray-dried product influences the extent of water diffusion, and values less than 7% are generally considered to reduce moisture migration (Pitalua et al., 2010). Due to the variety of characterization methods and units of measurement available in the literature, it was difficult to make a comprehensive comparison of other powder characteristics, such as phenolics content and antioxidant capacity.

The sorption isotherms of spray-dried BBE and corresponding models are shown in Fig. 2. Lack-of-fit tests were conducted for the entire water activity range. Results showed that both GAB and BET isotherms have comparable values of coefficient of determination, $R^2$ (0.92 and 0.96, respectively) but the GAB is the adequate model ($p > 0.05$). Further, one coefficient (the y-intercept) of the BET equation was found to be statistically insignificant. Data were also truncated to remove the values at the highest tested water activity (0.75). On the basis of higher $R^2$ of the linear model ($R^2 = 0.99$) and the lack-of-fit test ($R^2 = 0.99$), the BET equation was more adequate than the GAB equation. Consequently, the parameters of the GAB equation for the entire data range are as follows: $C = 9.35$, $k = 0.84$ and $X_m$ (monolayer value) = 7.42 g water/100 g dry solids. For the BET equation, the values of the parameters are as follows: $C = 13.85$ and $X_m = 5.92$ g water/100 g dry solids. In both cases, the calculated monolayer values were greater than the moisture content of the spray-dried BBE.

### 3.2. Changes in TMAC, TPC and FRAP during storage at different conditions

The rate of anthocyanin degradation as a function of time is shown in Fig. 3. Anthocyanin loss followed the first-order kinetics and the following rate constants were calculated: $k_{C>2} = 0.0182$ d$^{-1}$, $k_{light} = 0.0083$ d$^{-1}$, $k_{22C} = 0.0069$ d$^{-1}$ and $k_{37C} = 0.0031$ d$^{-1}$. The rate of monomeric anthocyanin loss under various storage conditions was in the order: $45^\circ C > light > 37^\circ C > 22^\circ C$. The disadvantage of using whey protein as a wall material in spray drying arises from significantly higher rates of anthocyanin degradation compared with polysaccharide-based wall materials (Idham et al., 2012; Tonon et al., 2009). The temperature-dependence of the rate constants was modeled by Arrhenius equation (Fig. 4). The calculated energy of activation (Ea) was equal to 62.46 kJ/mol, while the preexponential factor A was equivalent to $2.81 \times 10^9$ d$^{-1}$. The parameters of the modeled Arrhenius equation were statistically significant and the equation was adequate based on lack-of-fit tests ($R^2 = 0.82$, $p = 0.19$). Higher values of Ea are usually associated with resistance to thermal degradation (Fischer et al., 2013).

Fig. 5 shows the variation of the total phenolics as a function of time. The total phenolics increased across all test conditions and eventually the ratio of final to initial GAE ranged between 2 and 2.5. The initial increase in total phenolics was greater at elevated temperatures compared to lower temperatures. The effect of photodegradation was comparable to storage at $37^\circ C$. Similarly, the antioxidant capacity of the samples increased upon storage (Fig. 6). Interestingly, however, the final ferric reducing antioxidant power varied proportionally with increasing temperatures. Furthermore, the effect of photodegradation was again comparable to storage at $37^\circ C$. The final concentration of Fe (II) equivalents ranged from unity ($22^\circ C$) to a peak of 1.29 ($45^\circ C$).

Generally, it is accepted that the concentration of monomeric anthocyanins decreases with an increase in temperature. However, thermal effects on phenolics content and antioxidant activity are not clear. The phenolics content and antioxidant activities of spray-dried açai and blueberry both decreased under prolonged storage (Jiménez-Aguilar et al., 2011; Tonon et al., 2009). The rate of decrease in phenolics content and antioxidant activity appeared to be linear for the non-encapsulated control and non-linear for the encapsulated extracts, but mathematical information regarding the observed trends was not discussed (Jiménez-Aguilar et al., 2011). With our samples, there was a slight increase in antioxidant activity and a two-fold increase in phenolics content. The plots in Fig. 5 suggest that the phenolics content ratio stabilizes at a value between 2 and 2.5. A linear relationship may be proposed for $22^\circ C$ but may need prolonged storage conditions longer than that normally considered in the literature. Similarly, the considerably higher standard deviations in antioxidant activity observed for samples stored at $37^\circ C$ and $22^\circ C$ (Fig. 6) seem to support the conclusion that bioactivity may be conserved because an increase in

![Fig. 2. Modeling of the experimental sorption isotherm based on the BET and GAB equations.](image)

![Fig. 3. Rate of monomeric anthocyanin loss modeled after first-order kinetics. Legend: ● $45^\circ C$, □ $37^\circ C$, △ $22^\circ C$, X light.](image)
heat, anthocyanins were found to decompose to phloroglucinoldehyde and benzoic acid derivatives such as syringic acid or 4-hydroxybenzoic acid (De Villiers et al., 2009; Patras et al., 2010). However, the rate-limiting step, which is usually used for kinetic modeling, has not yet been identified. Further, anthocyanins have varying sensitivity to temperature increase, with cyanidins being more sensitive to thermal decomposition than delphinidin. The use of FRAP to measure antioxidant capacity may also lead to a redox reaction between ferric ions and some anthocyanins and accelerate degradation of delphinidin but not cyanidin (Xiong et al., 2006). This could impact processing of blueberries from different cultivars. Besides decomposition, anthocyanins may also polymerize upon heating or prolonged storage (Hager et al., 2008). Thermally produced phenolic compounds from degradation or polymerization may partially or fully compensate for loss in antioxidant activity arising from decreased monomeric anthocyanins (Fischer et al., 2013; Sadilova et al., 2007). In our case, the increase in both phenolics content and antioxidant capacity of the spray-dried BBE upon storage implies that the powdered BBE can be used as a food ingredient with health-promoting properties. Fang and Bhandari (2012) evaluated the surface atomic composition of the encapsulated, spray-dried whey protein-blueberry extracts and concluded that the encapsulated extracts promoted the surface migration of proteins compared to the whey protein control. Using the same technique, our preliminary results (data not shown) revealed no significant variation between samples and control, even after accelerated shelf life tests. Hence, the extent of thermal- and photo-degradation on surface phenolic compounds and the encapsulated fraction remains unclear and may be the subject of future investigations.

4. Conclusions

Microencapsulated blueberry powder was successfully prepared from an aqueous, ethanolic pomace extract and whey protein isolate. Anthocyanin degradation followed the first-order kinetics. Photodegradation was comparable to storage at 37°C in terms of rate of phenolic increase and antioxidant activity, but was more severe in the rate of loss of monomeric anthocyanins. Upon storage, the total phenolics concentration increased approximately two-fold across all storage conditions, while the antioxidant capacity increased only slightly. Based on these results, the spray-dried product may be further studied in developing a food ingredient that promotes health.

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