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Total phenolics content and antioxidant capacities of microencapsulated blueberry anthocyanins during *in vitro* digestion

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ABSTRACT

The goal of this research was to investigate the change in phenolics content and antioxidant capacity of microencapsulated anthocyanins (ACNs) digested *in vitro*. Blueberry ACN microcapsules were prepared from two wall materials (whey protein isolate and gum arabic) and ACN powder, previously extracted with three solvent systems (acetonic, ethanolic, methanolic); this was then spray-dried. The physicochemical properties and release characteristics of the microcapsules were evaluated. Rehydrated gum arabic microcapsules retained more total ACNs but less ferric reducing power than did whey protein microcapsules. Ethanolic extracts retained most of the total ACNs while methanolic extracts possessed the highest antioxidant capacity. During *in vitro* digestion, gum arabic microcapsules had high release rates of phenolics with high antioxidant activity during the gastric phase. Whey protein microcapsules had comparably lower release rates but high antioxidant activity throughout digestion.

1. Introduction

Food breakdown and absorption are crucial factors in the development of several chronic diseases. The postprandial state represents a dynamic phase of catabolism and synthesis, leading to short-term disturbances (Burton-Freeman, 2010). For instance, the gastric epithelium is a major production site of reactive oxygen species (ROS) arising from various physical, chemical and microbiological activity in the lumen. In turn, ROS are implicated in the pathogenesis of gastrointestinal ulcers and cancers (Alvarez-Suarez et al., 2011). Physiological antioxidant mechanisms occur naturally, but dietary interventions that complement these mechanisms and alleviate oxidative stress may still be needed (Burton-Freeman, 2010).

Studies involving human subjects revealed a direct relationship between consumption of foods such as blueberries and improvement in markers of antioxidant activity. Consumption of freeze-dried wild blueberries caused an increase of hydrophilic and lipophilic plasma antioxidants (Prior et al., 2007). In another report, consumption of fresh blueberries was associated with a significant decrease of lipid hydroperoxides among chain smokers (McAnulty et al., 2005) and lower plasma oxidised low-density lipoprotein (LDL), serum malondialdehyde and hydroxynonenal concentrations for individuals diagnosed with metabolic syndrome (Basu et al., 2010).

Anthocyanins (ACNs), common bioactives present in most berries, possess antioxidant capacities *in vivo*. Bilberry anthocyanidins significantly reduced HCl/ethanol-induced gastric ulcers by scavenging superoxide and hydroxyl radicals (Ogawa, Oyagi, Tanaka, Kobayashi, & Hara, 2011). Use of ACN-enriched strawberry extracts reduced ethanol-induced gastric lesions, with concomitant increases in catalase and superoxide dismutase activities (Alvarez-Suarez et al., 2011). The efficacy of dietary ACNs is attained with high initial concentrations. However, isolated ACNs are labile and degrade under thermal stress, neutral pH and the presence of oxygen, metal ions and enzymes (Frank et al., 2012). Microencapsulation, using food-grade matrices, is an option that stabilizes ACNs and controls their release at potential absorption sites (Betz & Kulozik, 2011).

Among microencapsulation techniques, spray-drying is a cost-effective method that can result in the formation of a stable, free-flowing powder. Structurally, the bioactives are trapped in a glassy polysaccharide or proteinaceous matrix that forms when the atomized liquid slurry comes into contact with hot air. Compared with conventional drying methods, the operating temperatures employed in spray-drying are higher but the drying time is generally shorter. Published reports on spray-drying of ACNs involve the effect of drying conditions, wall materials (such as polysaccharides or proteins) on encapsulation efficiency and, recently, the antioxidant capacity of microencapsulated ACNs. Wall materials could affect the morphology of the resulting spray-dried powder, product yield, as well as retention and associated antioxidant activities of the core material (Fang & Bhandari, 2012; Tonon, Brabet, Pallet, Brat, & Hubinger, 2009). Yet, the effect of gastrointestinal digestion on the antioxidant capacity of encapsulated ACNs has not been studied. The choice and characteristics of the wall materials also affect the stability of the encapsulated ACNs during digestion (Betz et al., 2012). Previously, we compared the characteristics of blueberry ACNs extracted with selected solvent systems (Flores, Singh, Kerr,

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Pegg, & Kong, 2013). The objective of this paper was to determine the changes in the physicochemical and *in vitro* release properties of spray-dried blueberry ACNs as affected by extraction methods (acetonic, ethanolic and methanolic) and wall materials (gum arabic and whey protein isolate).

2. Materials and methods

2.1. Materials

Blueberries (Sunnyridge) were purchased from commercial supermarkets in Athens, Georgia and prepared as described in a previous study (Flores et al., 2013). Freeze-dried ACNs, extracted from whole blueberries and blueberry pomace using three different solvent systems (acetonic, ethanolic and methanolic), were employed. Briefly, blueberries were extracted with each solvent system, subjected to solid–liquid fractionation using an Amberlite XAD16N column, concentrated *in vacuo* and freeze-dried. The resulting powder is referred to as ACN powder in Section 2.2.3. Spray-dried whey protein isolate (*Bi*PRO®), which has a minimum protein content of 95%, was a kind donation from Davisco Foods International (Eden Prairie, MN). Cold water-soluble spray-dried gum arabic (Pretested® FT) was a gift from TIC Gums (White Marsh, MD). Chemicals, digestive enzymes and adjuncts used were reagent-grade and obtained from the Sigma–Aldrich Chemical Company (St. Louis, MO). Porcine pancreatin (Cat. No. P1750) converts not less than 25 times its weight of potato starch into soluble carbohydrates (within 5 min in water at 40 °C), digests not less than 25 times its weight of casein (within 1 h at pH 7.5 and 40 °C) and releases not less than 2 μequiv. of acid per min per mg of pancreatin from olive oil (at pH 9.0 and 37 °C). Porcine pepsin (Cat. No. P7000), from gastric mucosa, has a unit activity defined as 0.001 change in absorbance units per min measured at λ of 280 nm (at pH 2.0 and 37 °C), measured as TCA-soluble products and haemoglobin as substrate. A unit of porcine pancreatic lipase (Cat. No. L3126) hydrolyzes 1.0 μequiv. of fatty acid from triacetin (within 1 h at pH 7.4 at 37 °C). Type VI-B α-amylase from porcine pancreas (Cat. No. A3176) hydrolyzes 1.0 mg of maltose from starch (within 3 min at pH 6.9 at 20 °C). Type II mucin from porcine stomach (Cat. No. M2378) has 1% of bound sialic acid. Porcine bile extract (Cat. No. B8631) contains glycine and taurine conjugates of hyodeoxycholic acid among other bile salts.

2.2. Methods

2.2.1. Spray-drying

Aqueous solutions of whey protein isolate (W) or gum arabic (G) were prepared at 30% (w/v) with deionized water at room temperature. Acetonic (A), ethanolic (E), or methanolic (M) powder extracts were stirred in at a ratio of 0.0025:1 (ACN extract:wall material) or for a total of 0.075% (w/v), resulting in six combinations: GA, GE, GM, WA, WE and WM. Each sample was pumped at 6 ml/min (20% pump speed) via a peristaltic pump to the atomizer of a Model B-290 mini spray-dryer (Büchi Corporation, Flawil, Switzerland) under the following process conditions: 160 °C inlet air temperature; 80 °C outlet air temperature; 100% aspirator rate (corresponding to a maximum air flow of 35 m^3/h), actual air flow rate of 0.667–1.744 m^3/h (40–60 mm Q flow) and a nozzle setting of 2 cleaning cycles/min. The resultant powders were stored in polypropylene bottles at −20 °C.

2.2.2. Scanning electron microscopy (SEM)

Surface morphology of the spray-dried microcapsules was analysed using a Zeiss 1450 EP scanning electron microscope (Carl Zeiss Micromanaging, Inc., Thornwood, NY). An acceleration potential of 10 kV was used, along with a probe current of 400 pA and a backscatter electron detector. A layer of gold coating (15 nm) was applied to each of the mounted samples prior to analysis.

2.2.3. Sample preparation

2.2.3.1. General. All sample powders and controls were dissolved at 0.1 g/ml for 3–4 h in deionized water at room temperature prior to tests.

2.2.3.2. Total monomeric anthocyanin content (TMAC). The pH differential method was used to estimate the TMAC of the spray-dried powders (Giusti & Wrolstad, 2001). Absorbance at λ_{max} of 510 nm (corresponding to cyanidin-3-*O*-glucoside [C3G], molar mass = 449.2, ε = 26 900) was measured in a 1 cm borosilicate cuvette with an Evolution 300 spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI). Samples prepared with whey protein isolates were centrifuged for 5 min at 6238×*g* prior to measurement. Values are reported as mg C3G/g of spray-dried product and mg C3G/mg of lyophilized ACN powder.

2.2.3.3. Total phenolics content (TPC). The Folin–Ciocalteu method was employed with modifications (Srivastava et al., 2010). Powders that contain whey protein isolate were diluted 10-fold. Absorbance was measured at λ of 765 nm. The TPC was calculated from a standard curve prepared using gallic acid. Results are presented as mg of gallic acid equiv. (GAE)/g of spray-dried product and mg of GAE/mg of lyophilized ACN powder.

2.2.3.4. Ferric reducing antioxidant power (FRAP) assay. A modified FRAP assay (Benzie & Strain, 1996) was conducted. The FRAP reagent consisted of 2.5 ml of 2,4,6-tri(2-pyridyl)-*s*-triazine solution (10 mM in 40 mM HCl), 2.5 ml of ferric chloride hexahydrate (20 mM) and 25 ml of acetate buffer (300 mM, pH 3.6). The reagent was prepared fresh daily and held in a water bath kept at 37 °C. Each sample (30 μl) was mixed with 1 ml of the reagent in a 1 cm borosilicate cuvette and allowed to react for 4 min before absorbance was measured at λ of 593 nm. FRAP values were calculated from a standard curve generated with 100 μl of various ferrous sulfate heptahydrate solutions (0.1–1 mM). Values are presented as nmol of Fe(II) equiv./g of spray-dried product and nmol of Fe(II) equiv./mg of lyophilized ACN powder.

2.2.4. Static in vitro digestion

A static model that simulates digestion in the mouth, stomach and intestines, was adapted (Hur, Decker, & McClements, 2009). The compositions of the simulated saliva, gastric, duodenal, and bile juices are shown on Table 1. The pH of the simulated digestive juices was adjusted with 1 M HCl or 1 M NaOH. Powder samples (3.5 g) were placed in stoppered 125 ml Erlenmeyer flasks and incubated at 37 °C in a Model 290400S water bath (Boekel Scientific, Feasterville, PA), equipped with orbital agitation, set at 200 rpm. The samples were digested sequentially as follows: mouth – addition of 6 ml of salivary juice and mixing for 5 min; stomach – addition of 12 ml of gastric juice and mixing for 2 h; and intestines – addition of 12 ml of duodenal and 6 ml of bile juices and mixing for 2 h. Aliquots (1.5 ml) were collected at 1 h intervals for a total of 4 h, centrifuged at 6238×*g* for 5 min and stored at −20 °C prior to further analyses.

2.2.5. Release properties

The frozen aliquots were thawed at room temperature, centrifuged again at 6238×*g* for 5 min and the supernatant fluid was analysed for TPC and FRAP. Results are presented as described previously (see Sections 2.2.3.3 and 2.2.3.4).