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Development and evaluation of a novel alginate-based *in situ* gelling system to modulate the release of anthocyanins

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ABSTRACT

Several health benefits have been associated with the consumption of anthocyanins (ACNs). These compounds are absorbed in the upper digestive tract (stomach and initial sections of the small intestine). However, an insufficient residence time of ACNs in these organs could result in limited absorption and contribute to degradation. In this paper, a novel alginate-based *in situ* gastroretentive gelling system is described as a platform to modulate the release and increase the retention time of ACNs from haskap berries in sites where their absorption and stability are favored. Thirteen formulations were assessed, where their composition differed by the concentrations of alginate, sodium bicarbonate, and calcium carbonate. Freeze-dried ACN-rich extract from haskap berries was incorporated into selected formulations. The ACN release profile indicated that the Peppas (power law) equation had the best fit to the experimental data and diffusion was most likely the dominant release mechanism.

1. Introduction

Anthocyanins (ACNs) are an important group of hydrophilic pigments in higher plants (Delgado-Vargas, Jiménez, & Paredes-López, 2000). These compounds have been associated with reduced risk of degenerative diseases (Cassidy et al., 2013; Pascual-Teresa, Moreno, & García-Viguera, 2010; Wallace, 2011; Wang & Stoner, 2008), including type 2 diabetes (T2D) (Guo & Ling, 2015; van Dam, Naidoo, & Landberg, 2013). For instance, recent reports have indicated that ACNs from haskap berries (*Lonicera caerulea* L.) could contribute to the management of T2D by controlling weight gain (Wu et al., 2013) and reducing postprandial blood glucose levels (Takahashi et al., 2014). Due to the high levels of ACNs in these fruits, they could be used as a source of bioactive compounds for the development of value-added products with health-promoting benefits (Celli, Ghanem, & Brooks, 2014).

Research has shown that ACNs are absorbed in the upper gastrointestinal (GI) tract (stomach and initial part of the small intestine) (Felgines et al., 2007; Passamonti, Vrhovsek, Vanzo, & Mattivi, 2003). An insufficient residence time of ACNs in these organs could result in limited absorption and contribute to degradation at high pHs found in the intestines (Liu et al., 2014; McDougall, Dobson, Smith, Blake, & Stewart, 2005) and due to metabolism (Czank et al., 2013; Ferrars et al., 2014; Fleschhut, Kratzer, Rechkemmer, & Kulling, 2006). In order to modulate the release and absorption of ACNs, the use of gastroretentive systems (GRS) could be a promising strategy to increase their retention time in portions parts of the GI tract where they are absorbed, as outlined in a recent review paper by Celli, Kalt, and Brooks (2016). Among the different GRS platforms that could be used by the food industry (Sharma & Khan, 2014), intra-gastric floating *in situ* gelling systems (or raft systems) are advantageous as the product could be commercialized in a powder form and reconstituted immediately before consumption, in a simple user-controlled preparation.

In situ gelling systems are often used for the treatment and amelioration of heartburn symptoms and as reflux suppressants (Ibrahim, 2009; Rajinikanth, Balasubramaniam, & Mishra, 2007; Rao & Shelar, 2015; Strugala, Dettmar, & Thomas, 2012; Tytgat & Simoneau, 2006). More recently, these systems have been proposed as drug delivery platforms (Prajapati, Mehta, Modhia, & Patel, 2012; Rajinikanth et al., 2007). They consist of polymers (e.g., alginates) that gel when in contact with body fluids or due to changes in pH (Kubo, Konno, Miyazaki, & Attwood, 2004), combined with gas-generating compounds (e.g., carbonate). Alginates (pKa 3.4–3.65) often require a divalent cation for the sol to gel transformation or low gastric pH to precipitate as alginic acid (Hampson et al., 2010). The gas released is entrapped in the gel matrix (forming a foamy gel structure), which reduces the density

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of the systems and contributes to its flotation above the gastric content (Tang, Alvani, & Tester, 2010). Reports have indicated that these gels can remain in the stomach for longer periods of time in comparison to a meal (Davies, Farr, Kellaway, Taylor, & Thomas, 1994; Dettmar, Little, & Baxter, 2005; Strugala et al., 2012; Washington, Greaves, & Wilson, 1990) and increase the efficiency of drugs that are absorbed and/or act locally (Chevrel, 1980; Rajinikanth et al., 2007).

To the best of our knowledge, this is the first time that an alginate-based *in situ* gelling system is described as a platform for the delivery of ACNs. Thus, the objective of the present study was to develop an *in situ* gelling formulation consisting of ACN-rich extract from haskap berries and determine its characteristics, such as the gelling capacity, gel weight, volume, density, thickness, resilience, and release profile. Thirteen base formulations were initially assessed and their composition differed by the concentrations of sodium alginate, sodium bicarbonate, and calcium carbonate. The best formulations were selected based on their gelling capacity and resilience at 0.1 and 0.05 N HCl. From this, freeze-dried fractionated haskap berry extract was incorporated and the ACN release profile under acidic conditions was measured over time.

2. Material and methods

2.1. Materials

Formic acid, hydrochloric acid, reagent ethanol, calcium carbonate, potassium chloride, and sodium acetate anhydrous were purchased from Fisher Scientific (Ottawa, ON, Canada). Low-viscosity sodium alginate Protanal LFR5/60 (65–75% guluronic acid) from brown seaweed was kindly donated by FMC BioPolymer (Philadelphia, PA, USA). Sodium bicarbonate was acquired from Church & Dwight Co., Inc. (Princeton, NJ, USA).

2.2. Plant material

Haskap berries (var. *Indigo Gem*) grown in Saskatchewan, Canada, were used in this study. Berries were harvested, packed, and shipped frozen to Nova Scotia. The berries were kept at −35 °C prior to chopping in half and freeze-drying in a Labconco FreeZone 2.5 L Bench-top Freeze Dry System (Labconco, Kansas City, MO, USA) until the final moisture content was below 5% (w/w). The freeze-dried samples were stored in a desiccator at −18 °C until use.

2.3. Preparation of anthocyanin-rich freeze-dried haskap extract

The extraction of ACNs from freeze-dried haskap berries was conducted according to Celli, Ghanem, and Brooks (2015). Briefly, 80% ethanol acidified with 0.5% formic acid was added to freeze-dried samples in a solid to solvent ratio of 1:25 (w/v) and stirred at 600 rpm for 10 min at room temperature using a magnetic stirrer. Then, the mixture was filtered through a Whatman filter paper no. 1 and the extract was kept at −18 °C in the absence of light until further use.

Prior to fractionation, the organic solvent was removed using a rotary evaporator model RE-51 (Yamato Scientific America Inc., Santa Clara, CA, USA) kept at $T < 50$ °C, equipped with a vacuum pump V-700 (Büchi Labortechnik AG, Flawal, Switzerland) and a water circulation cooler WKL 230 (Lauda, Lauda-Königshofen, Germany). The resultant fraction was kept at −18 °C and protected from light until further use.

The fractionation of the evaporated extract was performed according to Kalt et al. (2008) with some modifications. A column consisting of approximately 300 g of C18 bulk packing material (Waters, Missisauga, ON, Canada) was previously washed with water. The evaporated extract was added to the column and washed with approximately two volumes of water to remove sugars. The bound components were eluted with 1.5 volume of reagent ethanol (acidified with 0.5% formic acid). The solvent of the resultant fraction was removed by rotary evaporation and freeze drying, and the powder was stored at −18 °C prior to use.

2.4. Preparation of in situ gelling system

The *in situ* gelling base formulations investigated in this study are presented in Table 1 and consisted of different concentrations of sodium alginate (x_1, 1.5–3.5%, w/v), sodium bicarbonate (x_2, 1.5–2.5%, w/v), and calcium carbonate (x_3, 0.5–2%, w/v). The range of concentrations was determined in preliminary experiments, which indicated that these formulations could be easily dispersed and gelled within seconds when in contact with acidic solutions. The powder mixtures were dissolved/dispersed with distilled water immediately prior to use by vigorous manual agitation for 30 s in a Falcon tube at room temperature, to simulate preparation by the consumer, and further evaluated for their properties. Gel characteristics were evaluated (gelling capacity, weight, volume, density, thickness, and resilience) and the most appropriate formulations were selected for further incorporation of the ACN-rich powdered extract.

2.5. In vitro evaluation of the in situ gelling system

2.5.1. Gelling capacity (raft formation)

The raft-forming ability of the formulations (as measured by assessing the gelling capacity of each formulation) was evaluated according to the method described by Rao and Shelar (2015). Briefly, 5 mL of the gelation solution consisting of 0.1 N or 0.05 N HCl was transferred to a 15-mL borosilicate tube and maintained at 37 ± 0.5 °C in water bath. Then, 1 mL of the gelling solution was added by placing the pipette tip at the surface of the gelation solution and slowly releasing its contents into the test tube. The formulations were assessed visually for the time required to complete the gelation and any dispersion of the gel up to 24 h after preparation.

2.5.2. Raft volume, weight, density, and thickness

Raft weight and volume were determined according to the procedure by Hampson et al. (2005) and used to calculate the density and measure the thickness of the gels formed. Briefly, each beaker used for raft formation was pre-weighed (**W1**). Then, 10 mL of the formulation were added to 150 mL of 0.1 N or 0.05 N HCl maintained at 37 ± 0.5 °C and the gel was allowed to develop for 30 min. The outside of the beaker was marked on the position to which the top of the gel reached and the beaker (and its content) was weighed (**W2**). The subnatant liquid was carefully decanted. The gel was let to stand for 30 s in the beaker, the excess liquid was drained off, and the gel was weighed (**W3**). The last step consisted of removing any liquid from the inside of the beaker, refilling with water to the marked position, and weighing (**W4**). For this experiment, it was assumed that the density of the gelation solution was similar to the water (i.e. 1 g/mL). All weights were expressed in g. The raft volume was calculated as follows:

$$\textit{Raft volume}\ (mL) = (W4 - W1) - (W2 - W1 - W3) \quad (1)$$

The raft density was calculated by dividing the raft weight (in g) by its volume (in mL). Each gel was measured in three places using a caliper ruler to determine gel thickness and account for differences in gel formation (Johnson, Craig, Mercer, & Chauhan, 1997), and the average was reported.