Food Hydrocolloids 87 (2019) 61–70

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Study of the interactions between pectin in a blueberry puree and whey proteins: Functionality and application

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ARTICLE INFO

Keywords:
Blueberry
Functionality
Beverage
Pectin
Protein-pectin interactions
Whey proteins

ABSTRACT

Protein and fiber, especially pectin, can form complexes at acidic pH. Studies on these complexes under actual food conditions are scarce. The aim of this work was to study interactions between whey proteins and blueberry puree, in particular its pectin, and to evaluate the impact on the functionality of the puree alone or incorporated into a model beverage. After the addition of a whey protein isolate (WPI) into purees at pH 3.5 or 6.5, the soluble pectin and protein contents and the viscosity of the resulting mixtures were determined. The decrease in the solubility of pectin (80%) and proteins (94%) indicated the formation of protein-pectin complexes by electrostatic interactions at pH 3.5, contributing to increase the mixture viscosity. The amount of soluble pectin in blueberry limited the formation of complexes when more WPI was added (5%). Heating the puree prior to the WPI addition solubilized pectin, which limited the formation of insoluble complexes and reduced the viscosity increase. The solubility of the blueberry polyphenols did not decrease after WPI addition. Finally, the non-heated puree enriched in WPI was used to prepare smoothies. This time, the protein-pectin complexation, probably reinforced by the final pasteurization of the smoothies, contributed to reduce the smoothie viscosity and can be explained in particular by particles of smaller sizes. Although the smoothie stability can be improved, the interactions between blueberry pectin in a puree and whey proteins allowed to design a novel functional ingredient that may be helpful in formulating beverages rich in fiber and protein.

1. Introduction

Protein and fiber are two food constituents of growing interest to health-conscious consumers. In recent years, the molecular interactions between these two macromolecules and the behavior of mixed systems have received increasing research attention in order to extend their food applications (Turgeon & Laneuville, 2009). Indeed, some fiber present in plants, in particular pectin, are anionic polysaccharides which can form complexes with proteins, most often through electrostatic interactions when both have opposite charges. In addition to their nutritional contribution, protein-polysaccharide (P-PS) complexes have shown interesting functional properties, such as decreased aggregation and precipitation of proteins, improved thermal stability of proteins, and modification of the viscosity of mixed systems (Schmitt & Turgeon, 2011; Turgeon & Laneuville, 2009). These properties could favor the incorporation of both macromolecules into food products to develop new functional foods, high in fiber and protein. However, P-PS interactions depend on the molecular characteristics of the two macromolecules (e.g. charge density, structural characteristics, and molecular weight), their concentration and ratios, as well as the environmental conditions (e.g. pH, temperature, ionic strength, and the presence of other molecules). All these factors may affect the properties of the P-PS complex and limit their use in food formulations.

Although there is a significant amount of publications on P-PS mixed systems, studies have generally been conducted in diluted and aqueous systems, using purified macromolecules, far from the complex conditions of a real food matrix. The present study focused on the formation of complexes between proteins from a whey protein isolate (WPI) and a blueberry puree, a matrix that can easily be incorporated into a food product. Whey proteins are a mixture of globular proteins of which β-lactoglobulin is the most abundant and has been thoroughly studied for P-PS complexation. Whey proteins are soluble at acidic pH and have an isoelectric point (IEP) around pH 5.0–5.2 (Edwards, Creamer, & Jameson, 2008; Ju & Kilara, 1998; Pritchard & Kailasapathy, 2011). Blueberry, a very popular fruit in Canada, contains more than 2% fiber and about 0.5% pectin (Chevalier, Rioux, Angers, &

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https://doi.org/10.1016/j.foodhyd.2018.07.038
Received 5 February 2018; Received in revised form 19 July 2018; Accepted 22 July 2018
Available online 24 July 2018

Turgeon, 2017). Pectin is an anionic polysaccharide and is the only fiber in fruit that carries charges. Its structure consists mainly of linear chains of galacturonic acid. Its pKa is in the range of 2.9–3.3 (Brejnholt, 2010), and therefore pectin is typically negatively charged in most food systems. The global charge density of pectin is governed by the amount of methyl-esterified carboxylic groups, referred as the degree of methylation (DM). Recently, it has been shown that a low-temperature blanching (1 h at 60 °C) resulted in decreased pectin DM in a blueberry puree, likely due to increased endogenous pectin methyl-esterase activity (Chevalier et al., 2017). A lower DM value results in a higher global charge density, which enhances the interactions of pectin with proteins (Girard, Turgeon, & Gauthier, 2002). Therefore, we hypothesized that pectin within a blueberry puree can form complexes with an added protein (WPI), and the application of such a heat treatment on the puree, prior to protein addition, could improve protein-pectin complexation.

On the other hand, other components in blueberry may interfere in protein-pectin complex formation. Indeed, blueberry is rich in phenolic compounds (0.2–0.3%) (Chevalier et al., 2017; Prior et al., 1998), which can interact with proteins and polysaccharides through hydrogen bonding and hydrophobic interactions. For example, protein-polyphenol interactions are well known, particularly for their contribution to astringent perception and haze formation in juices and wines. These polymers may form insoluble complexes, and several recent reviews have described these interactions in more detail (Le Bourvellec & Renard, 2012; Ozdal, Capanoglu, & Altay, 2013).

The aim of the present work was to study the interactions and the formation of complexes between added whey proteins and pectin present in blueberry purees. Two pH conditions have been tested in order to favor or limit complex formation and the effect of a pre-heating step of the puree has been studied. Functionality of the complexes has been assessed using a model smoothie to demonstrate their potential to enrich beverages in both fiber and protein.

2. Materials and methods

2.1. Chemicals

MeOH (certified ACS) was purchased from Fisher Chemicals (Fair Lawn, NJ), 3-phenylphenol and Folin & Ciocalteu's phenol reagent were obtained from Sigma-Aldrich Inc. (Milwaukee, WI), and deuterated MeOH (d3-MeOH) was from CDN Isotopes (Pointe-Claire, QC, Canada).

2.2. Material

Highbush blueberries (*Vaccinium corymbosum* L.) were obtained from Agridor (Beaumont, QC, Canada). Two cultivars, “Patriot” and “Polaris”, were harvested at their physiological maturity (July/August 2015), frozen at −30 °C, vacuum-packed, and stored at −30 °C until being pureed and heat treated or not (March 2017). Whey protein isolate (WPI) was obtained from Davisco Foods International Inc. (BiPRO®, Le Sueur, MN).

2.3. Blueberry puree preparation and characterization

Blueberries from each cultivar were thawed at 4 °C overnight then mashed with a food processor for 15 min at speed 6 (Thermomix TM31, Vorwerk, Germany) to obtain a puree. Then, for each cultivar, half of the puree was heated at 60 °C for 1 h directly in the same food processor, under gentle stirring (speed 3), then pasteurized at 90–95 °C for 5 min, and finally cooled at 20 °C by transferring the puree in an ice bath. This puree was referred as the “heated” puree. The “non-heated" puree was obtained with the other half of the puree, stirred at the same speed, in the same food processor, and for the same total time as the heated puree, but at 4 °C to prevent enzymatic activity. At the end of both treatments (heated or not), the purees were aliquoted, frozen and stored at −30 °C.

Moisture content of the purees was determined by drying samples (3 g) at 102 °C for 24 h in an air oven. The alcohol-insoluble residue (AIR) was extracted from purees as follows: puree sample (10 g) was mixed with 95% EtOH (30 mL) for 30 min then centrifuged (6000 g for 10 min). The pellet was washed thrice with 75% EtOH (20 mL) then once with acetone (20 mL). Pellet was transferred in aluminum dishes and dried overnight at 37 °C in an air oven. The AIR was then ground using a mortar and pestle then used for total pectin content determination. The total pectin content was estimated as galacturonic acid content (GalA) by colorimetric assay with 3-phenylphenol reagent (Blumenkrantz & Asboe-Hansen, 1973), after acid hydrolysis of the AIR (Melton & Smith, 2001). The degree of methylation (DM) of pectin in AIR was determined by headspace GC-MS after saponification of AIR as described by Chevalier et al. (2017). The results were expressed as the molar ratio of methanol to GalA (%). Puree samples were freeze-dried to determine protein content by the Dumas method (IDF standards 185:2002) (N x 6.25) using a LECO FP-528 (LECO Corporation, Saint Joseph, MI) and total phenolic content by Folin-Ciocalteu method as described by Chevalier et al. (2017). Phenolic compounds will be referred as polyphenols for the rest of this article. The characteristics of these initial purees are presented in Table 1.

2.4. WPI-blueberry mixture preparation and analysis

Aliquots from blueberry purees were thawed at 4 °C overnight. The pH of the puree was adjusted by the addition of droplets of appropriate solutions of HCl and NaOH to reach pH 3.5 (chosen to correspond to the mean pH of blueberry purees (Chevalier et al., 2017)) or 6.5, in order to be in associative or dissociative conditions, respectively. Then, WPI powder (0, 0.5, or 5%, w/w) was added into the puree and the mixture was stirred at 20 °C under moderate magnetic agitation. The WPI concentration of 0.5% was chosen to have a whey protein:pectin ratio of about 1:1, since the total amount of pectin in the blueberry purees was reported to be about 0.5 g/100 g (wet basis) (Chevalier et al., 2017). The concentration of 5% was used to study the effect of WPI concentration. After 1 h of stirring, the pH of the mixture was re-adjusted at pH 3.5 or 6.5. The total number of HCl or NaOH droplets was counted and if necessary, droplets of water were added to the mixture to

Table 1
Composition (g/100 g dry weight) and pectin degree of methylation of the initial purees, and global charge (Zeta-potential) of the supernatants at pH 3.5.

Purees	Moisture	Protein	GalA[a]	Polyphenols[b]	DM (%)	Zeta-potential[c] (mV)
PaNH	88.2 ± 0.1^{b}	3.4 ± 0.0^{b}	2.4 ± 0.3^{b}	1.5 ± 0.04^{b}	54 ± 5^{b}	−16.8 ± 3.4^{a}
PaH	88.2 ± 0.1^{b}	3.4 ± 0.1^{b}	2.3 ± 0.2^{b}	1.7 ± 0.01^{a}	73 ± 1^{a}	−8.0 ± 1.9^{b}
PoNH	89.4 ± 0.2^{a}	5.2 ± 0.3^{a}	3.3 ± 0.6^{a}	1.8 ± 0.02^{a}	59 ± 5^{b}	−9.7 ± 2.0^{ab}
PoH	89.2 ± 0.1^{a}	5.1 ± 0.0^{a}	3.1 ± 0.4^{a}	1.7 ± 0.02^{a}	72 ± 5^{a}	−7.6 ± 1.6^{b}

Mean value ± standard deviation. Means with the same letter were not significantly different according to Tukey's test (α = 0.01). Pa: Patriot. Po: Polaris. NH: non-heated. H: heated. DM: degree of methylation.

[a] Galacturonic acid from AIR, as an estimate of pectin content.

[b] Total phenolic compounds, as gallic acid equivalent.

[c] Determined on the supernatants after centrifugation.