Food Chemistry 264 (2018) 180–188

Contents lists available at ScienceDirect

Food Chemistry

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

Effect of the degree of esterification and blockiness on the complex coacervation of pea protein isolate and commercial pectic polysaccharides

Sumudu Warnakulasuriya, Prasanth K.S. Pillai, Andrea K. Stone, Michael T. Nickerson*

Department of Food and Bioproduct Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada

ARTICLE INFO

Keywords:
Coacervation
Pea protein isolate
Pectin
Degree of esterification
Blockiness
Surface charge
Protein solubility

ABSTRACT

The complex coacervation of pea protein isolate (PPI) with commercial pectic polysaccharides [high methoxy citrus pectin (P90, 90 representing DE), apple pectin (P78) sugar beet pectin (P62), low methoxy citrus pectin (P29)] of different degrees of esterification (DE) [and galacturonic acid content (GalA)] and blockiness (DB), was investigated. The maximum amount of coacervates formed at a biopolymer weight mixing ratio of 4:1 for all PPI-pectin mixtures, with the exception of PPI-P29 where maximum coacervation occurred at the 10:1 mixing ratio. The pH at which maximum interactions occurred was pH 3.4–3.5 (PPI: P90/P78) and 3.7–3.8 (PPI: P62/P29). PPI complexed with pectins with high levels of DE (low levels of GalA) and DB displayed greater interactions at optimal mixing conditions compared to pectin having lower levels of esterification and blockiness. The addition of P78 to PPI greatly increased protein solubility at pH 4.5.

1. Introduction

Admixtures of proteins and polysaccharides have a broad spectrum of applications, such as micro and nano encapsulation, designing multilayer structures, controlled delivery, food emulsions, protein purification from industrial by-products etc. (Dickinson, 2008; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). The controlled addition of polysaccharide enhances the functionality and the surface chemistry of proteins under select complexing conditions; furthermore the admixture forms a polymer rich (coacervate) phase through electrostatic attractive forces between proteins and polysaccharides, and a polymer depleted phase (solvent rich) (Schmitt and Turgeon, 2011; Schmitt et al., 1998; Tolstoguzov, 1991). Consequently, the compatibility of these biopolymers is critical to the formation of the coacervates and is highly dependent on various characteristics such as, biopolymer mixing ratio, molecular weight, conformation, charge density, pH, ionic strength, solvent quality and mixing conditions (Aryee and Nickerson, 2012; Elmer, Karaca, Low, & Nickerson, 2011; Niu et al., 2014; Schmitt et al., 1998; Stone, Teymurova, Chang, Cheung, & Nickerson, 2015). The interaction of proteins and polysaccharides proceeds with two structure-forming events: the initial formation of soluble complexes through non-covalent interactions which occurs at pH_c and the subsequent development of insoluble complexes at $pH_{\phi 1}$ (De Kruif, Weinbreck, & de Vries, 2004; Doublier, Garnier, Renard, & Sanchez, 2000). Maximum coacervation (pH_{opt}) is observed at the electrical neutrality point (stoichiometric charge equivalence) of these biopolymers, which on further protonation results in the progressive breakdown and ensuing dissolution ($pH_{\phi 2}$) of the complexes.

The selection of protein and polysaccharide is of great consequence for the commercial viability of protein-polysaccharide complexes. There is a move towards decreasing the use of animal based proteins due to growing concerns surrounding environmental impact and consumer dietary restrictions or choices; pea protein is an emerging alternative to animal protein as well as soy protein (Klassen, Elmer, & Nickerson, 2011; Klemmer, Waldner, Stone, Low, & Nickerson, 2012; Liu, Elmer, Low, & Nickerson, 2010; Mession, Assifaoui, Lafarge, Saurel, & Cayot, 2012). Pea is mainly comprised of salt-soluble globulins and water-soluble albumin proteins, representing ~70–80% and ~10–20% to the total proteins, respectively (Liang & Tang, 2013). The globulin proteins are dominated by the hexameric protein, legumin (11 S, S is a Svedberg Unit) with a molecular mass of 350–400 kDa, and the trimeric protein, vicilin (7S) with a molecular mass of 150–170 kDa (Gatehouse, Lycett, Croy, & Boulter, 1982). A third minor globulin protein is known as convicilin (7S) which has a molecular mass of 210–290 kDa, and is comprised of 3 sub-units (70 kDa) (Reinkensmeier, Bußler, Schlüter, Rohn, & Rawel, 2015).

Pectin is an anionic structural polysaccharide (molecular mass of ~60–130,000 g/mol) that can be used with pea protein to modify the physicochemical behaviour of the biopolymers. It is extracted from the cell wall of plant materials and is widely used by the food industry as a thickener and gelling agent (Niture and Refai, 2013; Ovodov, 2009; Thakur, Singh, Handa, & Rao, 1997). Pectin is comprised of dominant

* Corresponding author.
E-mail address: Michael.Nickerson@usask.ca (M.T. Nickerson).

https://doi.org/10.1016/j.foodchem.2018.05.036
Received 26 December 2017; Received in revised form 1 May 2018; Accepted 5 May 2018
Available online 06 May 2018
0308-8146/

linear α-(1–4)-linked D-galacturonic acid units with an approximate degree of polymerization (DP) of 100 (Cameron and Grohmann, 2005). The dominant α-(1–4)-linked D-galacturonic acid is called homogalacturonan (HG), which is substituted by complex sugar rich regions such as, rhamnogalacturonan-l (RGI), rhamnogalacturonan-ll (RGII), xylogalacturonan (XGA) and apiogalacturonan (AGA) (Sila et al., 2009). The HG backbone represents ~65% of all pectin and is methyl esterified at the C-6 carboxyl and/or, depending on the plant source, partially *O*-acetylated at O-2 or O-3 (Mohnen 2008; Pelloux, Rustérucci and Mellerowicz, 2007; Ridley, O'Neill, & Mohnen, 2001; Sila et al., 2009). The different commercial pectins are classified according to the degree of methyl esterification (DE), which is defined as the amount of methyl ester (mol) present in 100 mol of total galacturonic acid (GalA). Generally, pectins with higher than 50% DE are considered as high methoxy (HM) pectin and those with less than 50% DE are known as low methoxy (LM) pectin (Sila et al., 2009). Apart from the number of methyl ester groups in pectin, the pattern of esterification (orientation of methyl ester groups in the linear HG chain) can affect the complexation to protein significantly (Fraeye et al., 2009). This can be addressed by another parameter called degree of blockiness (DB), which is defined as the ratio of the number of non-methyl esterified GalA moieties to the total non-methyl GalA moieties in the pectin after its endo-polygalacturonase digestion (Daas, Meyer-Hansen, Schols, De Ruiter, & Voragen, 1999; Fraeye et al., 2009). The leading HG region of pectin with varied methyl esterified segments can be tailored to modify the gelling, thickening and stabilizing properties of pectin for various food applications. Thus, the important parameters such as DE, DB, GalA content etc., have significant impact in determining the functional properties of these natural polymers.

The present work is intended to investigate the effect of DE, GalA content and DB of four commercial pectic polysaccharides on coacervate formation behaviour with PPI under different biopolymer mixing ratios, and additionally determine the solubility of complexed PPI-pectin relative to PPI alone. Obtaining a better understanding of PPI interactions with pectin may lead to the development of new food ingredients where the pea protein surface is modified with pectin to give new functional properties (e.g., solubility, emulsifying, foaming, etc).

2. Materials and methods

2.1. Materials

Green peas (*Pisum sativum L.*), cultivar CDC Meadow, of Certified seed grade, grown in North Battleford, SK, Canada, during the 2014 growing season were used for this project. High methoxy citrus pectin (P90), and apple pectin (P78) were purchased from Sigma-Aldrich, Canada Ltd. (Oakville, ON, Canada). Sugar beet pectin (P62) and low methoxy citrus pectin (P29) were obtained from CP Kelco U.S., Inc. (Atlanta, GA, USA) and TIC Gums, Inc. (Belcamp, MD, USA), respectively. Endo-polygalacturonanase from *Aspergillus aculeatus* was purchased from Megazyme (Wicklow, Ireland). Moisture, ash, crude protein and lipid content of the biopolymers were determined according to AOAC Official Methods 925.10, 923.03, 920.87 and 920.85, respectively (AOAC, 2003). Carbohydrate content was determined on the basis of percent differential from 100%. The respective moisture, ash, and carbohydrate contents of the pectins were as follows: P90: 11.4%, 0.6% and 87.9%; P78: 7.7%, 3.7% and 88.6%; P62: 7.2%, 6.4%, and 86.5%; and P29: 10.4%, 2.1% and 87.5%; protein and lipid contents of the pectins were considered to be negligible. The PPI was comprised of 88.87% protein, 0.51% fat, 5.32% ash, 0.76% carbohydrate and 4.54% moisture.

2.2. Preparation of pea protein isolate (PPI)

The PPI was extracted from pea flour by alkaline extraction followed by isoelectric precipitation (Karaca, Low, & Nickerson, 2011; L'hocine et al., 2006). The seeds were dehulled using a Satake mill (Satake, Penrith, NSW, Australia) and then ground into coarse flour by a disc mill (Glen Mills Inc., Clifton, NJ, USA), before processing it into finer flour by means of a UDY cyclone sample mill (UDY Corp., Fort Collins, CO, USA). The flour was defatted by stirring with hexane (1:3, w/v) for 10 min followed by vacuum filtration through No.1 Whatman filter paper (Whatman International Ltd., Maidstone, UK). The defatting was continued thrice and the resulting flour was dried in order to remove the solvent. After which the defatted flour was dispersed in water (1:10, w/v) and the solution pH was adjusted to pH 9.0 using 1 M NaOH. The solution was stirred for 1 h at room temperature (22–23 °C) and then centrifuged for 10 min at 4500 ×*g* at 4 °C (Sorvall RC-6 Plus centrifuge, Thermo Scientific, Ashville, NC, USA). The supernatant was decanted with the aid of glass wool filtration to remove insolubles and the soluble supernatant pH was adjusted to 4.5 using 1 M HCl. The solution was kept overnight at 4 °C to promote the precipitation of proteins, and further decanted to concentrate the protein slurry. The resultant mixture was centrifuged to obtain protein rich pellets, which were washed and then diluted with 200 mL of ddH_20. The pH of the washed solution was adjusted to 7.0 by adding 1 M NaOH before freeze drying (Labconco Free Zone 6 freeze-dryer, Labconco Corp., Kansas City, MO, USA). The resulting PPI was stored at 4 °C.

2.3. Determination of degree of methyl esterification (DE)

DE of the commercial pectin samples was determined by the titration method of Mizote, Odagiri, Tôei and Tanaka (1975). To 5 g of pectin sample, a mixture of 5 mL of HCl (2.7 M) and 100 mL of 60% (v/v) ethanol was added and stirred (900 rpm) for 10 min at room temperature. The mixture was further washed with the same solvent mixture (6 × 15 mL) and then with 60% ethanol until the filtrate was free of any chlorides. The sample was then rinsed with 20 mL of anhydrous ethanol and oven dried for 1 h at 105 °C. To the dried pectin sample (0.5g) in an Erlenmeyer flask, 2 mL of ethanol and 100 mL of CO_2 free water was added and the resultant solution was titrated against 0.1 M NaOH using phenolphthalein indicator. The initial titer value was recorded as V_1 (ml). To the titrated reaction mixture, a 20.0 mL aliquot of 0.5 M NaOH was added, then stirred vigorously for 15 min. Subsequently, 20.0 mL of 0.5 M HCl acid was added and stirred until the pink color disappeared. The resultant solution after the addition of 0.5 M HCl was then titrated against 0.1 N NaOH to get the titer value V_s (ml). DE was calculated using Eq. (1), which is shown below.

$$DE\% = [V_s/(V_1 + V_s)] \times 100 \quad (1)$$

2.4. Determination of galacturonic acid (GalA)

Galacturonic acid (GalA) content was obtained by a colorimetric method (Yoo, Fishman, Hotchkiss, & Lee 2006). To 0.4 mL of pectin sample (100 μg/mL), 4 M ammonium sulfamate (40 μL) and 2.5 mL of sodium tetraborate (0.075 M made in conc. H_2SO_4) was added. The reaction mixture was mixed vigorously and kept in an ice bath to cool it to room temperature. After cooling, the mixture was heated in a boiling water bath for 15 min after which it was submersed in an ice bath for ~ 2 min to bring it to room temperature. Lastly, 80 μL of 0.15% 3-phenylphenol in 0.5% NaOH (w/v) was mixed with the reaction mixture before reading its absorption at 525 nm. The standardization curves for galacturonic acids at different concentrations (10, 25, 50, 75 and 100 μg/mL) were produced and GalA concentration of the commercial pectin was obtained from their initial sample concentration with the utilization of the standardization curve.

2.5. Endopolygalacturonase (Endo PG) degradation of commercial pectins

Endo-PG of *A. aculeatus* was used to degrade pectin in order to