Food Research International 44 (2011) 911–916

Contents lists available at ScienceDirect

Food Research International

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

Effect of pH on the properties of soy protein–pectin complexes

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ARTICLE INFO

Article history:
Received 5 July 2010
Accepted 26 January 2011

Keywords:
Soy protein
Pectin
Solubility
pH

ABSTRACT

The interactions of a commercial soy protein isolate (SPI) and a 2:1 SPI:high methoxy pectin (PEC) complex were evaluated over a range of pH values (3–7). The SPI formed very large (>50 μm) and largely insoluble aggregates (<10%) close to its isoelectric point (IEP, pH 4 and 5) and smaller, more soluble (>80%) particles at higher and lower pH values. The addition of PEC increased the solubility of SPI close to its IEP (pH 4 and 5) and prevented the formation of very large aggregates. However, PEC reduced the solubility of SPI at higher and lower pH values presumably via a depletion mechanism. The ζ-potential of diluted SPI dispersions decreased from positive to negative with increasing pH, passing through zero at pH 4.6, the isoelectric point (IEP) of the protein. At pH<6, the addition of PEC reduced the charge of the protein suggesting the formation of a complex while at pH 6 or 7 there was no evidence of complex formation. The increased SPI solubility in the IEP in the presence of PEC is probably due to the formation of charged complex which do not aggregate while the decreased solubility of protein in the presence at high and low PEC is probably due to the formation of insoluble complexes and a depletion interaction respectively. Thermal treatment (30 min, 90 °C) enhanced the solubility of the SPI:PEC complexes close to the IEP (pH 4 and 5), but reduces it at low pH (pH 3). The SPI:PEC complexes could be manufactured in the form of a beverage at pilot scale where their solubility was enhanced by homogenization.

1. Introduction

After extraction of oil from soybeans, the remaining defatted soy flakes remain as a co-product and are typically ground into meal for animal feed. Alternatively, the soy flakes can be further processed to obtain high protein ingredients (e.g., concentrates and isolates) that can be used in human food. Soy protein isolate (SPI) is a high (>90%) protein product from soy flakes. It is largely a mixture of the globulin proteins glycinin and β-conglycinin, (also known as the 11S and 7S fractions) which together represent more than 85% of storage proteins in the soybean. Commercial soy protein is often denatured during extraction and may be partly hydrolyzed by the manufacturer to improve its solubility.

Foods containing sufficient soy protein (6.25 g of soy protein per reference amount customarily consumed) are permitted in the United States to carry a health claim on their labels based on evidence that consumption of soy protein can reduce the risk of coronary heart disease (FDA, 1999). Furthermore, epidemiological and clinical studies suggest that a low incidence of certain cancers is associated with a diet rich in soy (Badger, Ronis, Simmen, & Simmen, 2005; Messina, Nagata, & Wu, 2006; Sharp et al., 2005; Hamilton-Reeves, Rebello, Thomas, Kurzer, & Slaton, 2008). These observations are prompting the development of new applications and technologies to produce soy protein ingredients that can be used in a wider range of human foods.

SPI has poor solubility around its isoelectric point (IEP, pH ~ 4.6), which can limit its application in acid foods (Malhotra & Coupland, 2004). The solubility of proteins around their isoelectric point can be improved by complexing with charged polysaccharides (Ye, 2008). For example, pectin [largely a polymer of D-galacturonic acid (pK ~ 3.5) partially esterified with methyl groups and with branches of various other sugars (McFeeters, 1985)] is used to prevent precipitation and whey separation from acidified milk drinks (Sejersen et al., 2007). Previous studies have evaluated the interactions between pectin with specific milk proteins and protein fractions (casein, whey protein, and β-lactoglobulin) (Maroziene & De Kruif, 2000; Kazmierski, Wicker, & Corredig, 2003; Bédié, Turgeon, & Makhlouf, 2008). These studies illustrate that protein–polysaccharide interactions are mainly electrostatic; even at pH<IEP where positive patches on the protein can bind negatively charged pectin.

Pectin also forms complexes with soy proteins. Lam, Paulsen, and Corredig (2008) prepared pectin complexes with SPI and 11S and 7S soy protein fractions at pH 3.8 and 7.2 (i.e., pH<pI and pH>pI respectively). At low pH the negatively charged pectin bound to the positively charged soy protein particles, reducing their ζ-potential. At high pH mixing the ingredients caused no change in the ζ-potential of

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doi:10.1016/j.foodres.2011.01.057

the protein. Binding small amounts of pectin tended to cause protein aggregation via a bridging mechanism and subsequent precipitation while larger amount of pectin stabilized the solution. Furthermore, high methoxy pectin formed more stable complexes with soy protein than low methoxy pectin and better stabilized the solution against precipitation (Lam, Shen, Paulsen, & Corredig, 2007). Lam et al. (2007) showed that commercial SPI was less soluble at pH 3.8 than the native SPI but it too could be stabilized by pectin.

This work builds on the findings of Lam et al. (2007, 2008). We consider the interactions between high methoxy pectin and a commercial SPI sample over a wider range of pH values (3–7) and show how the nature of protein–polysaccharide complex changes as it is titrated through its IEP and the relative charges on the polymers change. We also consider the effects of thermal processing on these complexes. Some previous reports have documented that protein–pectin complexes may be stabilized by heat (Gentes, St-Gelais, & Turgeon, 2010), however no description is available for soy protein–pectin complexes. Finally, we demonstrate similar functionality can be achieved in SPI:PEC complexes at product produced at pilot scale.

2. Materials and methods

2.1. Materials

Soy protein isolate (SPI) was provided by Archer Daniels Midland Company (Pro-Fam® 922, Decatur, IL). According to the manufacturer's specifications, Pro-Fam 922 has a minimum protein content of 85% (calculated as N × 6.25), maximum moisture of 6%, 4% fat and 12% ash. Preliminary thermal analysis of these samples showed no detectable denaturation peaks suggesting this sample was already completely denatured. This is unsurprising as most commercial soy protein products are acid washed and jet-cooked as part of processing and these steps denature the proteins (Egbert, 2004). Unstandardized high methoxy pectin (PEC) from citrus peels with a degree of esterification between 69 and 77% was provided by TIC Gums (TIC Pretested® Pectin 1400, Belcamp, MD). This pectin was selected because it is recommended by the manufacturer for protein stabilization in acidic environments. Hydrochloric acid solution (N/10), sodium hydroxide solution (N/10), NF/FCC grade hydrochloric acid (HCl) and sodium azide were obtained from Fischer Scientific (Fair Lawn, NJ). Sodium hydroxide (purum grade, pellets) was purchased from Acros Organics (Geel, Belgium). Sodium nitrate ($NaNO_3$) was purchased from J.T Baker (Phillipsburg, NJ) and rhodamine B isothiocyanate was obtained from Sigma (St. Louis, MO).

2.2. Preparation of SPI:PEC dispersions

Stock solutions of 3 wt.% SPI, and 2 wt.% PEC were prepared in ultrapure water (NanoPure Barnstead/Thermolyne, Dubuque, IA, USA) with 0.04 wt.% sodium azide to limit microbial growth, by stirring at room temperature for at least 2 h. SPI dissolution was facilitated by adjusting the pH to 11 using small volumes of 5 N NaOH to enhance protein solubility. Solutions were then stored at 4 °C overnight to allow complete hydration. Aliquots of the SPI and PEC stock solutions were brought to room temperature, combined and stirred to obtain dispersions with SPI:PEC at a 2:1 mass ratio. Similar experiments were conducted using a 4:1 mass ratio that gave very similar results and are not discussed further here. These ratios were considered as they allowed the solubilization of reasonable concentrations of SPI without adding so much pectin that the viscosity became unreasonable for a beverage. The mixture had a final concentration of 1.5% SPI and 0.75% PEC. A sample of the SPI stock solution diluted in water was used as a control. The pH of the mixtures was adjusted to values 3, 4, 5, 6 and 7 with small volumes of 5 N HCl or 5 N NaOH. Some samples were thermally treated by heating to 90 °C then holding for 30 min in a sealed beaker. Following heating, the samples were quickly cooled to 20 °C in iced water.

2.3. Solubility measurements

The apparent solubility of SPI was assessed as a function of pH by the procedure described by Malhotra and Coupland (2004). Samples were gently centrifuged at room temperature (1000 rpm, 5 min) to induce partial precipitation and protein solubility was calculated as the ratio of the nitrogen content of the supernatant to the nitrogen content of the original sample prior to centrifugation. Nitrogen was determined by combustion analysis (FP-528 LECO Corporation, St. Joseph, MI). The protein content of the PEC powder was very low ($0.53 \pm 0.01\%$) so that the presence of pectin in the samples did not interfere with the analysis. Solubility assessed by this method is an empirical measurement of the proportion of soy protein that did not precipitate under these specific conditions.

2.4. ζ-potential measurements

The ζ-potential of the SPI:PEC dispersions was determined by dynamic light scattering measurements in an oscillating electrical field (ZetaPALS, Brookhaven instruments, Holtsville, NY). SPI:PEC dispersions prepared at pH 3–7 were diluted (150 μL in 9.5 mL) in 5 mM $NaNO_3$ solution previously adjusted with 0.1 N NaOH or 0.1 N HCl to the same pH (Croak & Corredig, 2006). Measurements in the ZetaPALS were the average of 10 complete runs (20 cycles each) performed at 25 °C. The ζ-potential was calculated by the ZetaPALS software based in the Smoulokowski model.

2.5. Particle size measurements

Particle size distributions of the SPI:PEC dispersions were determined by static light scattering (LA-920, Horiba, Irvine, CA) one day after preparation of the mixtures. Pectin solutions did not scatter light in this instrument and so their size could not be measured. Because some samples started to show phase separation, all samples were gently stirred until complete suspension before analysis to allow comparisons between treatments. An adequate volume of the SPI:PEC dispersions was diluted directly in the measuring chamber filled with 5 mM $NaNO_3$ solution adjusted to the sample pH and at a circulation speed of 5 (≈33% of the maximum pumping speed). Sample was added to the unit until the through transmission value reached between 85 and 90% of the water blank. A relative refractive index of 1.06 was used for the protein particles (Lam et al., 2007). The measurements reported are the average of three separate analyses.

2.6. Confocal microscopy

SPI dispersions were stained with a solution of Rhodamine B in water (0.2 wt.%) prior to the addition of PEC and adjustment of pH. Microscope images were obtained of freshly prepared samples using a laser scanning confocal microscope (Olympus America Inc., Melville, NY), equipped with an inverted microscope (Olympus IX-70), and a 60× oil-immersion objective. A green HeNe laser (wavelength 543 nm) was used to excite the dye (568 nm) and fluorescence emission was collected between 565 and 570 nm. Multiple fields of view were examined for each sample and a representative image selected. The stains selected showed the protein as red against a black background. To allow clearer presentation in gray scale the images were inverted to show the protein as dark particles against a white background. Contrast and brightness are adjusted to more clearly show the structures present while taking care to create artifacts. Gamma values were not modified.