Food Research International 43 (2010) 1684–1691

Contents lists available at ScienceDirect

Food Research International

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

Assessment of the effects of soy protein isolates with different protein compositions on gluten thermosetting gelation

Eduarda M. Bainy [a,b], Milena Corredig [b], Vaino Poysa [c], Lorna Woodrow [c], Susan Tosh [a,*]

[a] Food Research Program, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada N1G 5C9
[b] Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[c] Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, Harrow, Ontario, Canada N0R 1G0

ARTICLE INFO

Article history:
Received 18 January 2010
Accepted 15 May 2010

Keywords:
Soy protein isolate
Gluten
Thermoset gel
Beta-conglycinin
Glycinin
Viscosity

ABSTRACT

Although soy proteins are known to have a deleterious effect on gluten thermosetting gelation, the causes are still poorly understood. Different sources of soy protein isolates (SPI) were used to investigate the interactions between gluten and soy proteins during hydro-thermal treatments. Commercial SPI and isolates prepared from soybean lines with different subunit composition were used to study the influence of protein denaturation and subunit composition on thermoset gel formation. Rapid Visco Analyser analysis showed that replacement of gluten with more than 1% SPI decreased the peak viscosity and interfered with formation of thermoset gels. However, peak viscosity was higher for 11% gluten + 2% SPI than for 11% gluten alone, suggesting a cooperative effect. After heating and cooling, 11% gluten + 2% SPI rich in A1 and A2 subunits formed a coherent thermoset gel suggesting that the cysteine residue content of soy proteins can affect gel formation.

1. Introduction

Gluten is a very heterogeneous protein mainly composed of gliadin and glutenin storage proteins (Wrigley, Békés, & Bushuk, 2006) found in wheat flours. The higher molecular weight fraction, glutenin, is responsible for elasticity while the extensibility is conferred by the lower molecular weight protein, gliadin (Wrigley et al., 2006). Soy protein isolate (SPI) is a refined soy protein product produced from defatted flour with most of the carbohydrates removed.

Soy and gluten proteins are complementary in terms of amino acid content: soy proteins contain small amounts of sulphur-containing amino acids, which are relatively abundant in wheat gluten. On the other hand, soy proteins are rich in lysine, a limiting essential amino acid in gluten proteins (Bloksma, & Bushuk, 1988; Erdman, 1988). Therefore, formulations containing both proteins yield products with high protein quality in terms of nutrition. Blends of wheat gluten and soy protein are commonly used to produce texturized vegetable protein products such as meat analogs.

The major soybean storage proteins are the globulins, β-conglycinin (often referred to as 7S) and glycinin (11S). β-Conglycinin is composed of α', α, and β subunits while glycinin is composed of an acidic peptide linked by a single disulphide bond with a basic peptide (Liu, 1999). Glycinin's temperature of denaturation is higher (85–95 °C) than that of β-conglycinin (65–75 °C) (German, Damodaran, & Kinsella, 1982; Utsumi, Matsumura, & Mori, 1997). The higher temperature of denaturation of glycinin is attributed to the three or four disulphide bonds present in the protein structure which can dissociate upon heating to temperatures above 85 °C (German et al., 1982; Lakemond, Jongh, Gruppen, & Voragen, 2002). Previous studies (Bainy, Tosh, Corredig, Woodrow, & Poysa, 2008; Renkema, & van Vliet, 2002; Van Kleef, 1986) reported that thermal denaturation is a pre-requisite for soy protein gelation and that gel stiffening occurs during cooling.

Wheat gluten is a heterogeneous mixture of protein subunits containing high amounts of proline, glutamic acid and glutamine (Byers, Miflin, & Smith, 1983). When mixing wheat flour and water, gliadin and glutenin proteins interact, and cysteine residues, which form intermolecular disulphide interactions, play a determinant role in the formation and development of hydrated viscoelastic dough (Tanaka, & Bushuk, 1973). The covalent network is reinforced by hydrogen bonds. The gliadin subunits of wheat gluten are monomeric proteins, most of which are soluble in 70% ethanol (Wrigley et al., 2006). The glutelin fraction of gluten is not soluble in water or ethanol but is soluble in 0.05 M acetic acid (Chen, & Bushuk, 1970). The glutelins are polymeric proteins made up of high molecular weight subunits and low molecular weight subunits.

It has been previously reported that the presence of soy protein weakens the formation of gluten gels, but that a ratio of 80% gluten and 20% soy protein caused the elastic modulus to be larger than that of heat-set gels prepared with either protein in isolation (Apichartsrangkoon, 2002).

* Corresponding author. Tel.: +1 519 780 8019; fax: +1 519 829 2600.
E-mail address: Susan.Tosh@agr.gc.ca (S. Tosh).

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doi:10.1016/j.foodres.2010.05.010

The development of intermolecular disulphide bonds can occur during mixing of gluten at ambient temperatures (Bloksma, & Bushuk, 1988). However, soy proteins unfold exposing reactive cysteine residues only above 70 °C, and subsequently with cooling, a network develops strengthened by hydrogen bonds. Therefore, covalent interactions between gluten and soy proteins and the development of a gel network would occur during heating or cooking. The development of a mixed gluten/soy thermoset gel would be of interest as it occurs not only during baking of bread but also during the processing of texturized vegetable protein products. For this reason, a more detailed study of the properties of thermoset gluten/soy gels was carried out.

Very little information is available on the mechanisms related to the weakening effect of incorporating soy proteins on the thermosetting gelation of gluten. Consequently, a better understanding of the effect of the addition of soy protein on the processing functionality of gluten needs to be developed. By using protein isolates from soybean lines null for different protein subunits it was possible to investigate the role of different protein subunits in gelation. Some differences between the functionality of the protein subunits in these null lines have already been established (Bainy et al., 2008; Malaki Nik, Tosh, Woodrow, Poysa, & Corredig, 2009).

The objectives of this work were to confirm whether addition of SPI to gluten-rich mixes affects the gluten thermosetting gelation and to investigate whether the effects derive from particular subunit of SPI or depend on the level of protein denaturation. The addition of gluten to SPI-rich mixes was also conducted to understand how SPI-rich systems behave alone and in the presence of low gluten protein content.

2. Materials and methods

2.1. Materials

Two different sources of defatted soybean flours were used in this study. The Solae Company (St. Louis, MO) donated a commercially available soy protein isolate (referred to as COM) and defatted soy flakes to prepare SPI in the laboratory (referred to as LAB–SPI or LAB). Defatted flours were prepared from the parent Harovinton variety and three derived lines with different protein composition grown in 2005 in Harrow, Ontario at the Greenhouse and Processing Crops Research Centre (GPCRC).

The defatted flours were prepared by grinding soybean seeds (30–35 g) to a fine powder using a Knifetec Mill (Foss, Eden Prairie, MN) equipped with a sharp blade and water-cooled to prevent samples from being heat degraded. The ground sample (10 g) was mixed with 100 ml hexane using an orbital shaker (DS-500, VWR, Scientific) at 70 rpm for 1 h in a fume hood. Hexane was decanted, discarded and the previous operation was performed two more times. Defatted flours were dried in the fume hood overnight and refrigerated.

Harovinton is an established Canadian tofu-type soybean variety and the three lines were developed by conventional breeding in order to obtain cultivars with null-genotypes (Poysa, Woodrow, & Yu, 2006; Zarkadas et al., 2007), where expression of certain protein subunits was suppressed. Isolates from the four lines were prepared in the laboratory and are referred as HAROV, A3-null line, multi-null line and 11S-null line. Harovinton is the parent line containing all the 7S and 11S subunits in a 1.30 11S/7S ratio; A3-null line lacks the A3 subunit of 11S and has a 0.86 11S/7S ratio; multi-null line lacks the α' subunit of 7S and the A1–A3 subunits of 11S and has a 0.73 11S/7S ratio; and 11S-null line lacks all the 11S subunits and has a 0.27 11S/7S ratio. The 11S/7S ratio was calculated. These different lines were used to investigate the effect of subunit composition on the interactions of soy proteins with gluten proteins during the gluten–SPI thermosetting gelation.

Wheat gluten was extracted from commercial all purpose hard wheat flour without additives (Hockley Valley, Orangeville, ON).

2.2. Preparation of soy protein isolate (SPI)

Soy protein isolate (SPI) was prepared by acid precipitation as previously reported (Renkema, & van Vliet, 2002), with slight modifications. The flour was suspended in 100 mM Tris–HCl buffer at pH 8.0 in a 1:10 ratio (w/v), and stirred at room temperature for 1 h. The insoluble fraction was separated by centrifugation (12,000 × g, 30 min, 10 °C) using a Beckman Coulter Model J2-21 centrifuge (Follerton, CA) and recovered using a porcelain filter with a filter paper (Qualitative P8, Fisher Scientific, Pittsburgh, PA). The supernatant was adjusted to pH 4.8 with 2 M HCl to induce protein precipitation. After 2 h at 4 °C the dispersion was centrifuged as described above. The precipitate was washed with 10 mM sodium acetate buffer at pH 4.8 (1:8 ratio (w/v)) and centrifuged as described above. The final precipitate (SPI) was resuspended with ultrapure water, adjusted to pH 7.5, dialyzed overnight against ultrapure water using regenerated cellulose dialysis tubing with molecular weight cutoff 6000–8000, (Fisherbrand, Fisher Scientific, Nepean, Canada) and subsequently freeze-dried.

2.3. Preparation of vital gluten

Gluten protein was extracted using the gluten washing method (AACC Method 38-10.01, 1999) with slight modifications. Initially, a firm dough ball was formed with flour and cold distilled water, and then soaked in water for 1 h. The dough was then washed and kneaded continuously under a slow constant flow of water until wash water ran clear. This cohesive mass of wet gluten was soaked for 30 min in water. After a further washing step to remove any presence of trapped soluble matter, the gluten was freeze-dried. Freeze-dried gluten was ground in a Planetary Ball Mill PM 100 (Retsch PM, Newton, PA) to a powder with a final maximum particle size of 350 μm.

2.4. Protein analysis

Protein content was determined by Dumas combustion method (Leco FP-528 Mississauga, ON) using 6.25 as the conversion factor for soy protein and 5.70 as the conversion factor for gluten. SPI and gluten protein contained minimum 90% (N = 14.4) and 80% protein (N = 14.1) on a dry matter basis, respectively.

2.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (TA 2920 modulated, Newcastle, DE) was used to investigate the effect of ethanol addition on the thermal properties of gluten and LAB–SPI. Control samples were dispersed in sufficient ultrapure water to make 13% protein (total weight of ~50 mg). Test samples were dispersed in 95% ethanol and diluted with 50 mM sodium acetate buffer (pH 5.5, 0.2 M NaCl) (total weight of ~50 mg) to attain the same ratio of components as in the RVA experiments. Samples were sealed in aluminum pans and heated at 2 °C/min from 30 to 115 °C. An empty aluminum pan was used as a reference. Denaturation temperature (T_D in °C) and thermal denaturation enthalpies (ΔH in Joules per gram) were calculated from the endothermic curves. T_D is the point of maximum heat flow for the denaturation temperature; ΔH is the area under the endothermic curve using the Universal Analysis 2000 software supplied by TA instruments. Determinations were performed in triplicate.