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Improved functional properties for soy–wheat doughs due to modification of the size distribution of polymeric proteins

E. Maforimbo [a], G. Skurray [a], S. Uthayakumaran [b], C.W. Wrigley [b,*]

[a] *School of Science Food and Horticulture, University of Western Sydney, Hawkesbury Campus, Building M8, Locked Bag 1797, Penrith South, DC, NSW 1797, Australia*

[b] *Food Science Australia and Wheat CRC, Riverside Life Science Centre, 11 Julius Avenue, Riverside Corporate Park, North Ryde, NSW 2113, Australia*

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Abstract

Physical modification of soy flour was shown to greatly improve the dough and baking qualities of soy–wheat (1:1) composite doughs, compared to raw soy flour, giving better stability and R_{max}, although extensibility was still below that of the wheat dough.

Reasons for improvements caused by the physical-modification process were sought by determining the relative size distribution of proteins in the soy–wheat composite doughs by size-exclusion high-performance liquid chromatography (SE-HPLC). Results were expressed as the proportion of 'unextractable polymeric protein' (%UPP)—the proportion of the protein that is over 100,000 Da and only extractable after sonication. Protein extracts from the soy–wheat dough were sampled at different stages of dough mixing and fermentation, and their molecular-size distributions evaluated.

Unextractable soy proteins were lower in raw soy flour (only 8% UPP) than in two physically-modified soy flours (19 and 34% UPP, respectively). Unextractable polymeric protein was much greater for wheat flour (57% UPP). After mixing a 1:1 soy–wheat composite dough, the %UPP was 36 and 22 (for the two types) when made from physically modified soy flours, compared to 8 for a composite dough using raw soy flour, and 43 for a wheat-only dough. The higher proportion of UPP for the wheat-modified soy doughs was taken as a reason for this composite dough providing better dough and baking qualities. Prolonged fermentation time caused a decrease in UPP percentages for all composite doughs and for the wheat-only dough.

Keywords: Protein size distribution; Unextractable polymeric proteins; Composite dough; Dough strength

1. Introduction

High levels of soy flour in wheat dough are known to cause deleterious effects on rheological and bread-making properties (D'Appolonia, 1977; Lorimer et al., 1991; Pomeranz et al., 1969), but the use of soy–wheat composite doughs is desirable in regions where wheat flour is expensive, and where soy protein is nutritionally desirable. Accordingly, the studies described employed wheat-soy bread as a vehicle for soy proteins in an attempt to address Protein Energy Malnutrition in developing countries. The use of physically-modified soy flour made from optimal thermal treatment of the beans or meal is a practical strategy for implementation in developing countries, as this process also destroys lipoxygenase while retaining functional and nutritional properties.

Understanding soy- and wheat-protein interactions should give an insight into possible ways of minimising the dough-weakening effect of soy flour in wheat doughs. Soy–wheat composite doughs offer an unusual contrast of differing protein classes. Most soy proteins are globulins, insoluble in water at their iso-electric points (pH 4.2–4.6), but are extractable in water and dilute salt solutions (Hou and Chang, 2004; KeShun, 1997). They consist of four major fractions (2S, 7S, 11S and 15S globulins), of which the 7S and 11S fractions are the major components comprising about 70% of the storage proteins. The soy proteins are not suitable for pan-bread making.

On the other hand, wheat-flour proteins are divided into four main classes, of which the albumins and globulins are minor

Abbreviations: Da, Daltons; *E*, extensibility; EPP, extractable polymeric protein; EP, extractable protein; kDa, kilo Dalton; M_r, molecular weight; PMSF, physically modified soy flour; RSF, raw soy flour; R_{max}, resistance to extension; SDS, sodium dodecyl sulphate; SE-HPLC, size-exclusion high performance liquid chromatography; SS/SH, disulphide/sulphydryl; UPP, unextractable polymeric protein; UP, unextractable protein.

* Corresponding author. Address: P.O. Box 52, North Ryde, NSW 1670, Australia. Tel.: +61 2 9490 8401; fax: +61 2 9490 8499.

E-mail address: colin.wrigley@csiro.au (C.W. Wrigley).

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fractions, compared to the gluten-forming monomeric gliadins and the polymeric glutenins. These very large polymeric glutenins proteins are composed of high-molecular weight (high M_r) and low-molecular weight (low M_r) subunits linked together by disulphide bridges (Bietz and Wall, 1972; Fisichella et al., 2003; Grosch and Wieser, 1999; Schofield, 1986). When hydrated, the gliadin fraction behaves as a viscous liquid and the glutenin fraction contributes cohesion and elasticity (Schofield, 1986); in balance, their visco-elastic properties make wheat dough uniquely suited to bread making.

Attempts to use legume proteins for bread making have generally been unsuccessful. Lorimer et al. (1991) reported that the addition of non-gluten-forming proteins (e.g. bean-seed proteins) causes a dilution effect and consequent weakening of wheat dough. They suggested several factors that cause weakening, namely, competition between the legume proteins and gluten for water molecules, the disruption of starch–protein complexes by the foreign proteins and disruption of SS interchange by the non-gluten proteins. However, they did not produce sufficient evidence to conclude that globular proteins disrupt the disulphide-interchange system of dough.

Ryan et al. (2002) offered the hypothesis that gluten- and soy–protein interactions have the potential to provide dough improvement. They claimed that the sulphydryl groups of the soy proteins may even contribute to dough development through SS–SH interchange, and that negative effects associated with soy–wheat breads are primarily due to lack of interaction between soy and gluten proteins. Hyder et al. (1974) demonstrated, by gel electrophoresis, the interaction between soy–protein fractions, sucrose esters and a pH 6.1 gluten-insoluble fraction. Similar interactions to improve soy protein functionality in wheat bread have been reported, between sucrose esters and soy proteins (Pomeranz et al., 1969), e.g. the binding of sodium stearoyl-2-lactylate to wheat and soy proteins (Chung et al., 1981).

Contrasting differences between soy and gluten proteins are their water-solubilities, the associated differences in amino-acid composition and their size distributions, and the consequent visco-elastic properties which are unique to wheat gluten proteins, enabling the gluten proteins to stretch and retain gas bubbles during baking. The dough-making quality of gluten has been attributed to the high proportion of very large proteins with molecular weights up into the tens of millions (Southan and MacRitchie, 1999; Stephenson and Preston, 1996). This set of observations raises the possibility that soy proteins might be better suited to dough forming if the proportion of high molecular-weight protein could be increased considerably.

In this paper we explore the hypothesis that a process of physical modification of soy flour by moist heat treatment causes an increase in the proportion of high molecular-weight protein of the soy proteins, thus making them more suitable for dough formation. A 1:1 soy–wheat dough incorporating heat treated soy protein exhibits higher resistance to extension (R_{max}), greater tolerance to mixing, better mixing stability, higher water uptake and better water absorption than a 1:1 soy–wheat composite dough made from raw soy flour (Maforimbo et al., 2005). Size-exclusion high-performance liquid chromatography (SE-HPLC) was used to determine the protein compositions of these composite doughs during mixing and resting, and thus to gain a better understanding of size distribution and possible interactions at the molecular level.

2. Experimental

2.1. Preparation of soy flours

Whole-seed soybeans (Meriram Pty Ltd, Everton Hills, NSW, Australia) were used to produce physically-modified soy flour no. 1 (PMSF1) by immersion of the soybeans in boiling water for 3 min. The beans were then spread on stainless-steel trays and blow-dried with hot air (80 °C) to constant weight in an oven for 5–6 h. The beans were later milled to fine flour through a 0.8 mm sieve, using a hammer mill (Newport Scientific Cereal Mill 6000 model, Warriewood, NSW, Australia). Using the same whole soybeans as above, a second physically-modified soy flour no. 2 (PMSF2) was made by mechanically dehulling soybeans and flush-steaming them for 3 min at atmospheric pressure to inactivate enzymes. The beans were spread on stainless-steel trays and blow-dried with hot air (80 °C) to constant weight in an oven for 3 h. The cooled beans were later milled as above. The raw soy flour (RSF) was full fat, enzyme-active whole soy flour (Meriram Pty Ltd), made by milling the raw soy beans, using the hammer mill with a 0.8 mm sieve, as for PMSF1. Commercial strong-wheat baking flour was obtained from Centerion Milling Company, Pty Ltd, Melbourne. All flours were stored in the cold room at 4 °C. The moisture content for these flours (in the sequence RSF, PMSF1, PMSF2 and the wheat flour) was 6.0, 7.3, 5.8 and 11.57 g/100 g and protein content (as is basis) was 33.8, 33.1, 36.8 and 12.5 g/100 g, respectively. Conversion factors were $N\times6.25$ and $N\times5.7$ for soy and wheat proteins, respectively.

2.2. Rheological tests

For Farinograph and Extensograph testing, the soy–wheat doughs were prepared by mixing wheat and soy flour in a 1:1 weight ratio, using all three soy-flour types. Farinographs were performed following methods from Preston and Kilborn (1984), using the Do-Corder Brabender OHG (Duisburg, Germany). Extensibility (Ext) and maximum resistance (R_{max}) were determined by the Extensograph method of Rasper (1991) using the Brabender Duisburg mod Exek/7, No. 779, (Germany).

2.3. Dough preparation for protein extraction

The wheat flour (20 g) was mixed in turn with an equal mass of one of the three types of soy flour. To each of the composite flour mixes, distilled water (70 ml or 80 ml per 100 g of flour for RSF or PMSF (nos 1 and 2), respectively) was added, and the dough was mixed for 7, 5 and 5 min to maximum consistency for RSF or PMSF (nos 1 and 2), respectively. The amounts of water used were selected as the optimum to

provide doughs whose resistances reached to the 500 Brabender-Unit mark on the Farinograph. The pH values of the doughs were 5.2, 6.4 and 6.5 for wheat dough, RSF–wheat and PMSF–wheat doughs (nos 1 and 2), respectively. After mixing ('time zero'), about 2 g of dough were removed, and the rest of the dough was allowed to ferment (without yeast) in an incubator at 37 °C. Sampling was repeated after 1 and 2 h fermentation. Samples were placed in sterile plastic bottles after each sampling; nitrogen was quickly flushed into the bottles before freezing to avoid further reaction. The samples were freeze dried and later ground using the hammer mill (0.8 mm sieve).

2.4. Extraction and fractionation of proteins

Flour or freeze-dried dough samples were extracted following the method of Gupta et al. (1993). Sample (10 mg) was suspended in 1 mL 0.5% sodium dodecyl sulphate (SDS) in phosphate buffer (pH 6.9) and mixed, initially in a vortex-mixer and later shaken at 30 °C on a Thermomixer Compact (Eppendorf) for 10 min at 2000 rpm. The suspension was then centrifuged for 10 min at 17,000 *g* to obtain supernatant ('extractable' or 'SDS-soluble' protein).

The resulting residue was extracted with 0.9 ml 0.5% SDS-phosphate buffer by sonication for 30 s using a Microson Ultrasonic cell distributor, ensuring that the sample was completely dispersed within the first 5 s. The supernatant from centrifugation for 10 min at 17,000 *g* was termed 'unextractable' protein. All extracts were filtered through a 0.45 μm PVDF filter prior to SE-HPLC analysis.

2.5. SE-HPLC analysis

The method of Batey et al. (1991) was used to determine the amounts of very large protein ('Peak 1') in the extractable and unextractable protein samples, using a Phenomenex BIOSEP-SEC 4000 column. Analytes were eluted with a 10-min isocratic separation at a flow rate of 2 ml/min with 50% aqueous acetonitrile solution, containing 0.05% trifluoroacetic acid. Proteins were detected at a wavelength of 214 nm. Areas for the glutenin peak (>100,000 Da) and the gliadin peak (<100,000 Da) were measured by Gold Nouveau software (Beckman Instruments, Inc., Fullerton, CA, USA). The proportion of 'unextractable' polymeric protein (%UPP) was determined as the ratio of Peak 1 of the 'unextractable glutenin' extract to the sum of Peak-1 protein of both unextractable and extractable extracts (Gupta et al., 1993). %UPP was used as a measure of protein size distribution for both soy and wheat samples.

2.6. Determination of free SH groups

Ellman's reagent, DTNB (5,5′-dithiobis(2-nitrobenzoic acid)), which reacts with SH groups to generate 5-mercapto-2-nitrobenzoic acid or 2-nitro-5-thiobenzoate (NTB^{2-}) was used for colorimetric determination of free SH groups in soy and wheat flours following the methods of Kin-Yu and Bruce (1993); Wagner and Anon (1990).

3. Results and discussion

3.1. Rheological results of soy–wheat doughs at 1:1 ratio

The rheological parameters, shown in Table 1, indicate the considerable decreases in maximum resistance to extension (R_{max}) and stability in the RSF–wheat dough, compared to the wheat dough. These changes, also seen in the actual Farinograms and Extensograms (Fig. 1), explain the difficulties in making bread using raw soy flour. In contrast, the PMSF–wheat dough has better stability and R_{max}, although its extensibility is still below that of the wheat dough. The differences in dough properties between these various doughs were so great that they are unlikely to be explained by the relatively small differences in protein or moisture contents for the soy preparations.

3.2. SE-HPLC of wheat and soy flours

The elution profiles for extractable protein from wheat flour (Fig. 2a) show a polymeric-protein peak of glutenin at the extreme left of the profile (Peak 1, >100,000 Da), followed by a large peak of monomeric gliadin proteins (<100,000 Da) and finally small peaks of albumins and globulins (Carceller and Aussenac, 2001). In contrast, the profile of the unextractable wheat proteins shows a much greater proportion of protein in the first peak (Fig. 2b), in accordance with Gupta

Table 1
Comparison of %UPP, loaf volumes and rheological properties of wheat dough and soy–wheat doughs

Dough type	(R_{max})[a] at 45 and 90 min	Ext[b] at 45 and 90 min	Stability (min)[c]	Loaf volume (cm^3)	UPP% of dough after 0 and 1 h rest
Wheat dough	280; 320	18; 16.5	16	790	51; 40
PMSF no. 1–wheat dough 1:1	380; 470	12.5; 11.5	6	700	36; 36
PMSF no. 2–wheat dough 1:1	400; 400	3.5; 10	8	710	25; 22
RSF–wheat dough 1:1	200; 180	4; 14	3	630	8; 4

All values shown are the means of duplicate analysis, error ±2% of the mean.
[a] Dough resistance to extension.
[b] Dough extensibility.
[c] Farinograph index for mixing tolerance.

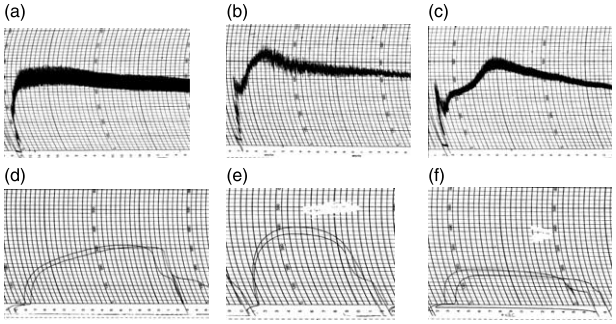

Fig. 1. Quality of wheat and composite doughs. Parts a–c show Farinograms. Parts d–f show Extensograms. Parts a and d are wheat doughs. Parts b and e are wheat–PMSF no. 2 doughs. Parts c and f are wheat–RSF doughs.

et al. (1993); Carceller and Aussenac (2001). The percentage of unextractable polymeric protein (%UPP) in the total wheat-flour protein was calculated according to Batey et al. (1991).

The SE-HPLC elution profiles for raw soy flour showed several peaks, the greatest proportion being proteins of intermediate size, in both the extractable and unextractable preparations (Fig. 2c). The profiles for the physically-modified soy flour (PMSF no. 2) had higher proportions of large proteins than those for raw soy flour. In fact, the profile for unextractable PMSF protein (Fig. 2f) was similar to that for unextractable wheat-flour protein. It was thus evident that the physical modification process had caused a major upward shift in protein size distribution. The profiles for PMSF no. 1 were similar to those shown for PMSF no. 2, indicating that the two modification processes had caused similar alterations in protein size distribution (results not shown). Based on the areas of the elution profiles, it did not appear that the treatments had altered the ability of the combined sonication–extraction processes to provide the bulk of the soy protein into solution for analysis by SE-HPLC.

The extractability of soy proteins was greater for raw soy flour (only 8% UPP) than for physically-modified soy flour (25% UPP and 34% UPP for PMSF nos 2 and 1, respectively). Nevertheless, the level of unextractable polymeric protein was much greater for the wheat flour (57% UPP).

3.3. SE-HPLC profiles for wheat and soy–wheat composite doughs

Fig. 3a, c and e shows the SE-HPLC profiles for extractable proteins in wheat and in soy–wheat doughs. The elution profiles are essentially similar for the soy–wheat doughs, irrespective of whether they were made with raw or modified soy flour. However, there were dramatic differences between these profiles for the unextractable proteins (Fig. 3b, d and f). The profile for unextractable protein from the composite dough made from physically modified soy was predominantly Peak 1—containing the largest proteins. The profile was similar to

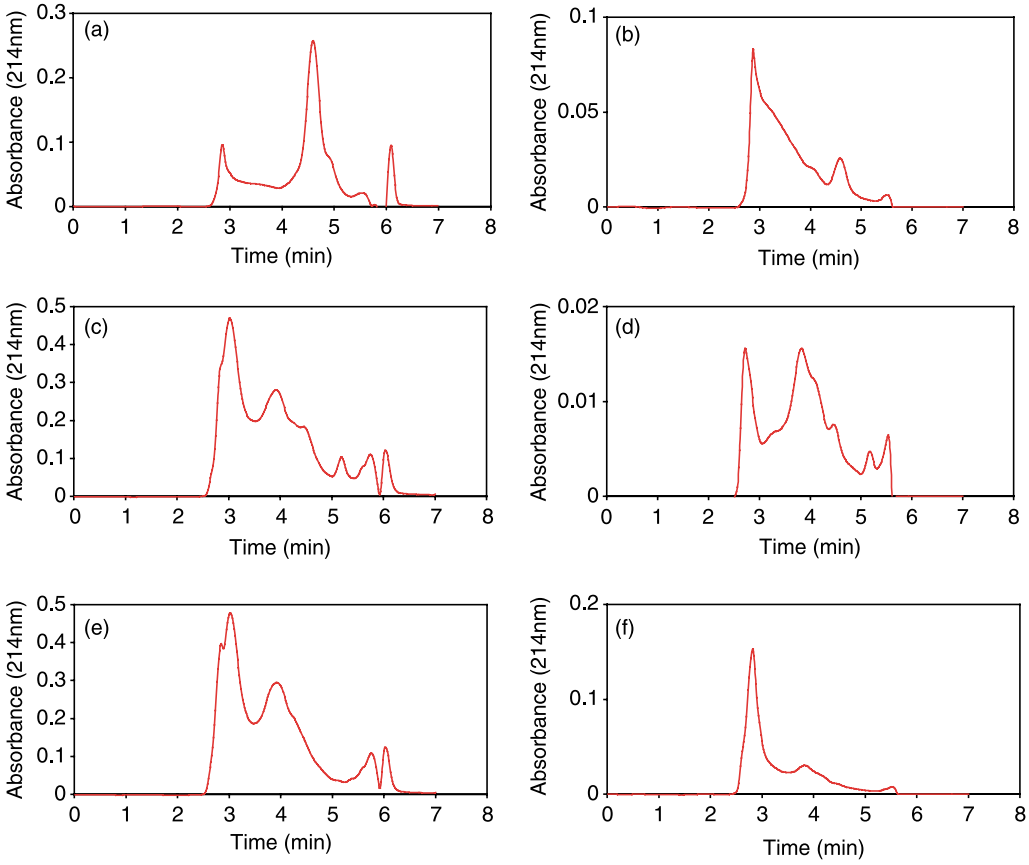

Fig. 2. SE-HPLC elution profiles for extractable protein (EP) and for unextractable protein (UP) of wheat flour, raw soy flour (RSF) and physically-modified soy flour (PMSF no. 2).

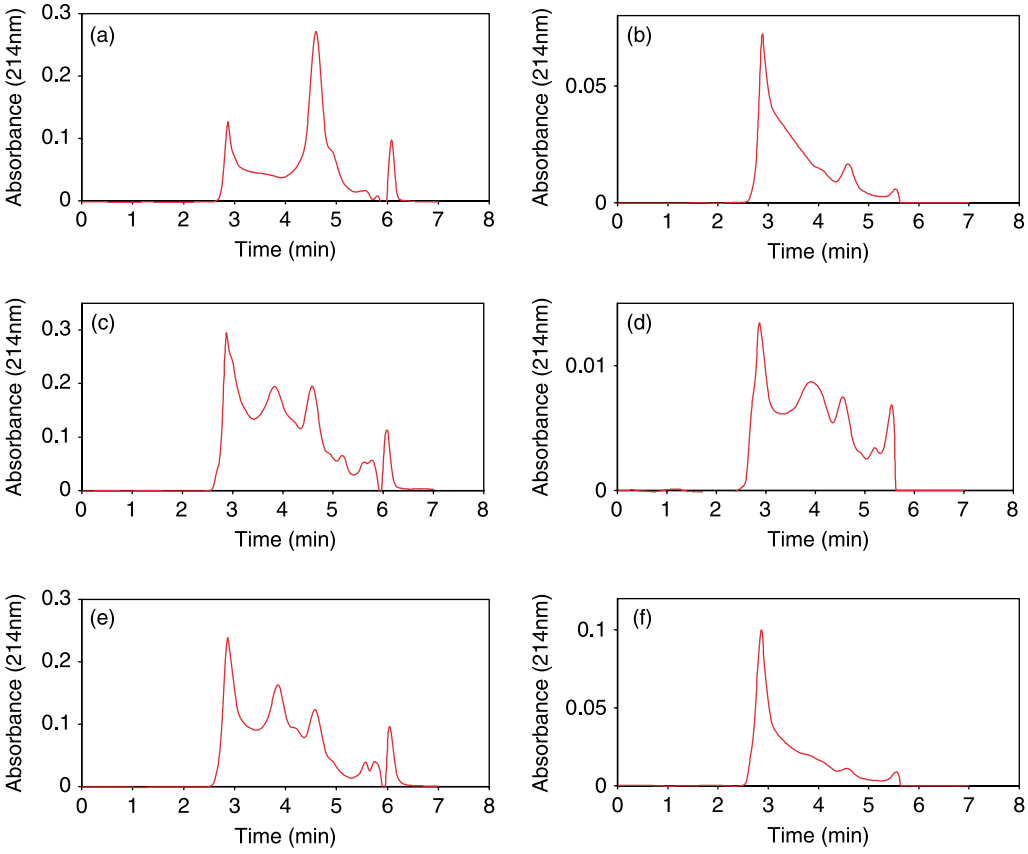

Fig. 3. SE-HPLC elution profiles for extractable protein (EP) and for unextractable protein (UP) from doughs (fermented for 1 h), made from wheat flour, wheat mixed with raw soy flour 1:1 (W-RSF) and wheat mixed with physically-modified soy flour no. 2 1:1 (W-PMSF).

the corresponding profile for the wheat-only dough. On the other hand, the raw-soy composite dough showed most of medium-sized proteins, and was similar to the corresponding profile for extractable proteins.

Unextractable polymeric protein continued to decrease during the fermentation period for all doughs (Fig. 4), indicating that the polymeric proteins were being gradually broken down during dough fermentation. The percentage of unextractable polymeric protein in total polymeric proteins in soy–wheat dough is summarized in Fig. 4. The rate of decrease in %UPP was greatest during fermentation for the dough made from wheat flour only.

Wheat glutenin polymers are linked by inter-chain disulphide bonds and the addition of reducing agents to glutenin results in cleavage of these bonds, causing a drastic decrease in molecular size distribution to smaller components (Schofield and Chen, 1995), with consequent loss of rheological and bread-making performance. It is thus essential to the baking quality of wheat dough that these disulphide bonds remain intact, thereby preserving the large polymer structure of glutenin. It is likely that dough weakening in RSF–wheat dough may be due to disruption of SH/SS interchange caused by oxidation products from lipoxygenase activity, in which case the heat treatment in PMSF would have inactivated lipoxygenase activity. By analogy, the presence of very large protein species in the composite dough made from physically-modified soy is presumed to explain why it is so much better than the raw soy for dough formation and for baking. The physical modification treatment may cause the formation of additional disulphide bonding, leading to the aggregation of the soy flour proteins into larger polymers (as evidenced from the SE-HPLC elution profiles).

Modification of soy proteins by heat usually results in changes in secondary and tertiary structure of the protein molecules (denaturation); this phenomenon results in loss of solubility for proteins, increased viscosity and loss of biological activity (KeShun, 1997; Wagner and Anon, 1990).

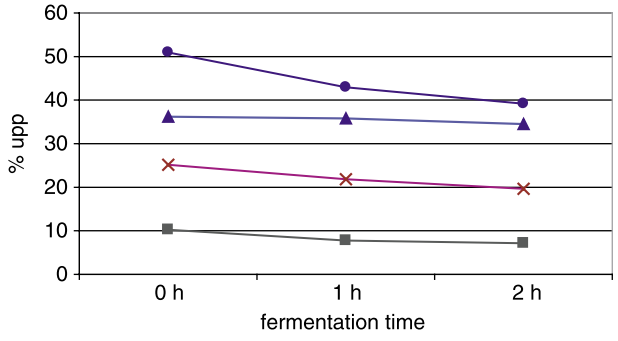

Fig. 4. Changes in unextractable polymeric protein (%UPP) in total polymeric proteins in a soy–wheat dough (1:1 ratio) during fermentation. Points on the figures represent the mean of duplicate values; error was within ±3% of the mean. ●, wheat dough; ▲, PMSF 1-wheat dough; ×, PMSF 2–wheat dough; ■, RSF–wheat dough.

Table 2
Effect of physical modification of soy flour on free SH groups and UPP% of the wheat and soy flours

Sample/flour	SH concentration (μmol/g of protein)	UPP%
Wheat flour	7.04	57
PMSF no. 1	11.93	34
PMSF no. 2	4.48	19
RSF	21.21	8

The process of denaturation involves dissociation and unfolding of proteins, and is often accompanied by formation of disulphide linkages and the exposure of hydrophobic amino-acids on the surface. These are all potential explanations of why modification methods affect the functional behaviour of the soy proteins (KeShun, 1997; Puppo et al., 2004; Wagner and Anon, 1990). Indications that disulphide bonds are involved in these processes for the soy proteins have been obtained from preliminary experiments with size-based capillary electrophoresis on extracts after breaking SS bonds.

The degree of heat treatment would result in differences in the extent of denaturation and the functionality of the proteins. The more the hydrophobic amino groups are exposed, the more insoluble the soy proteins become. It is expected that modification using moist heat would be less intense than the use of immersion boiling; therefore, PMSF2 (moist heated) would have undergone less denaturation than PMSF1 (boiled) and changed properties would be in the order: raw (native) soy flour, PMSF2 and PMSF1.

Results in Table 2 indicate the effect of physical modification of soy flour on free SH groups. The lower content of SH groups in the physically-modified soy flours compared to that of raw soy flour suggests that the process of physical modification has converted many of the free SH groups into disulphide bonds. As a result, presumably, cross links have formed that have contributed to the higher molecular-weight distribution of the modified soy flours, indicated by their higher values of unextractable polymeric proteins compared to those of raw soy flour. The formation of disulphide bonds, and of consequent increases in molecular size distribution and in insolubility, have been reviewed by Wrigley and Bekes (1999) for situations involving moist heat such as bread baking, extrusion, gluten drying and even hot storage of grain.

3.4. Relation between UPP of composite doughs and baking quality

Evidence that baking performance is positively related to the disulphide-linked glutenin polymers, and that the highest proportion of these polymers are unextractable under certain conditions has been reviewed by Schofield (1986), who pointed out that the amount of unextractable protein varies according to the extracting solvent used and the wheat sample. Later studies confirmed that the polymeric protein fraction governs the technological parameters of wheat flours. The unextractable glutenin proteins have a high proportion of the HMW-glutenin subunits, and these contribute in a major way to dough strength and baking quality (Fisichella et al., 2003; Grosch and Wieser, 1999). Significant correlations were also obtained between the relative quantity of unextractable polymeric protein (large glutenin polymers) in total polymeric protein and dough strength parameters over a range of wheat genotypes (Gupta et al., 1993). If this quality criterion were to relate to soy flour, physically modified soy flours would be regarded as making better contributions to dough strength than raw soy flour.

Soy flour is acknowledged to weaken wheat dough and reduce loaf volume and extensibility in wheat flour (Bushuk, 1985; D'Appolonia, 1977; Surana et al., 1973). Results from our work now demonstrate that molecular-size distribution (shown by %UPP) is reduced by the RSF in soy–wheat doughs during fermentation. From the trends in Table 1, we could conclude that in soy–wheat doughs the higher relative size distribution of proteins (UPP%) contributed to stronger doughs that were capable of producing larger loaf volumes. The PMSF–wheat dough contributed higher resistance to extension (R_{max}) and greater mixing tolerance than the RSF–wheat dough, although these values were still weaker (in stability and extensibility) than for a wheat dough.

4. Conclusion

SE-HPLC was used to demonstrate that the improved contribution to dough properties of physical modification of soy proteins is due to changes in the molecular size distribution of the soy proteins. SE-HPLC profiles showed that raw-soy–wheat dough has much of its protein in an intermediate size range, compared to the profile for the wheat dough. The physical modification process appears to have altered this size distribution, giving the PMSF–wheat dough a SE-HPLC profile much closer to that of the wheat dough. The results indicate that a soy–wheat composite dough, made from PMSF, forms a stronger dough with potentially better baking qualities. However, further research with a larger range of soy-flour types may be justified. Nevertheless, the physical modification process provides a relatively simple method for improving the baking quality of soy flour, in combination with wheat flour, for use at the village level in regions where soy can be grown but where wheat grain must be imported.

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