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

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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.

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## 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

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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 doughweakening 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.

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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 aminoacid 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  $(R_{\text{max}})$ , 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  $N \times 6.25$  and  $N \times 5.7$  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 Download English Version:

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