ENHANCING SOY-WHEAT BREAD-MAKING PROPERTIES USING PHYSICALLY-MODIFIED SOY FLOUR

A thesis submitted to the University of Western Sydney, Hawkesbury in fulfilment of the requirements for the degree of Doctor of Philosophy

By

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Declaration

The work presented in this thesis is original and has been completed by the author, a PhD candidate in the School of Science, Food and Horticulture, University of Western Sydney, Hawkesbury Campus, NSW, Australia, under the supervision of Associate Professor, Geoff Skurray; Dr Minh Nguyen (University of Western Sydney, Hawkesbury Campus); Dr Surjani Uthayakumaran and Dr. Colin Wrigley (Food Science Australia and Wheat CRC).

I certify that the work presented in this thesis is original except as acknowledged in the text and I also declare that the material, either in part or in full, has not been submitted to any other University or Institution for any degree or qualifications. All resources used for the preparation of this thesis have been acknowledged.

Elizabeth Maforimbo

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Publications from this work

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1. Maforimbo, E.; Nguyen, M.; Skurray, G. R. The effect L-ascorbic acid on the rheological properties of soy-wheat dough: a comparison of raw and physically modified soy flours. *Journal of Food Engineering* **2006**, *72*, 339-345.
2. Maforimbo, E.; Nguyen, M.; Skurray, G. R. Effect of L-Ascorbic acid on sulphydryl concentration in soy-wheat dough during resting at room temperature. *LWT Food Science and Technology.* ***Article in press***

3 Maforimbo, E.; Skurray, G.; Uthayakumaran, S.; Wrigley, C. Improved functional properties for soy-wheat doughs due to modification of the size distribution of polymeric proteins. *J. Cereal Science* **2006***,* 43, 223-229.

4 Maforimbo, E.; Skurray, G.; Nguyen, M.; Uthayakumaran, S.; Wrigley, C. Effect of physical modification of soy flour in modulating the bread making performance of soy-wheat dough. Pages 144-145 in: Innovation Conference **2005** proceedings, College of Science, Technology and Environment; UWS, NSW, Australia

5 Maforimbo, E., Skurray, G., Uthayakumaran, S., and Wrigley, C. 2005. Size distribution of polymeric protein in soy-wheat doughs during fermentation. Pages 155-159 in: Cereals **2005.** Proc. 55th Aust. Cereal Chem. Conf. C.L.Blanchard, H.Truong, H.M.Allen, A.B.Blakeney and L.O'Brien, Eds. Royal Aust. Chem. Instit., Melbourne

6. Maforimbo, E., Skurray, G., Nguyen, M., Uthayakumaran, S., and Wrigley, C. Size distribution of polymeric protein in soy-wheat dough during fermentation, a comparison of raw and physically modified soy flour. Poster presentation at the 38th Annual AIFST Conference, Sydney. 10 -13 **July, 2005.**

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List of Abbreviations

AACC	: American Association of Cereal Chemists
ANOVA	: Analysis of variance
AOAC	: Association of Analytical Chemists
BEC	: Biomass Energy Conservation
BRI	: Bread Research Institute
BU	: Brabender units
CIMMYT	: The International Maize and Wheat Improvement Centre
cm^3/g	: Cubic centimetres per gram
CSH	: Reduced cysteine
CSSC	:Oxidised cysteine
C_p	: Change in heat capacity
Da	: Dalton
DDT	: Dough development time
DHAA	: Dehydro-ascorbic acid
DNP	: Dinitrophenol
DSC	: Differential scanning calorimetry
DTNB	: 5, 5'-dithiobis 2-nitrobenzoic acid
DTT	: Dithiothreitol
E	: Extensibility
EP	: Extractable proteins
FAO	: Food and Agricultural Organisation
FDA	: Food and Drug Administration
FDNB	: Flouro-2-4-dinitrobenzene
$g\ kg^{-1}$	: Grams per kilogram
$g\ s^{-1}\cdot mm^{-1}$	: Grams per second per millimetre
GSH	: Reduced glutathione
GSH-DH	: Glutathione dehydrogenase
GSSG	: Oxidised glutathione
? *H*	: Enthalpy change
? H_{vap}	: Enthalpy of vaporisation
HCl	: Hydrochloric acid
HMW	: High molecular weight
HPI	: Human poverty index
HPMC	: Hydroxypropylmethylcellulose

J/g	: Joules per gram
kDa	: kilo Dalton
KIO_3	: Potassium iodate
L-AA	: L-ascorbic acid
LMW	: Low molecular weight
LOX	: Lipoxygenase
LSD	: Least significant difference
M	: Molar (Moles per litre)
mM	: Millimole
mM / L	: Millimoles per litre
M^{-1} cm^{-1}	: Per mole per centimetre
mL	: Millilitre
mm	: Millimetre
mg/g	: milligrams per gram
mg/kg	: Milligrams per kilogram
mg mL^{-1}	: Milligrams per millilitre
MMW	: Medium molecular weight
mJ	: Millijoules
MTI	: Mixing tolerance Index
N	: Normal
$NaHCO_3$	: Sodium hydrogen carbonate
NCHS	: National Centre for Health Statistics
NEMI	: N-ethyl maleimide
NGO	: Non-governmental organizations
nm	: nanometres
nmol	: nanomole
NSI	: Nitrogen Solubility Index
NTB^{2-}	: 2-nitro-5-thiobenzoate anion
$NTSB^{2}$	: 2- nitro-5-thiosulphobenzoate
PDCAAS	: Protein Digestibility Corrected Amino Acid Score
PDI	: Protein dispersibility index
PEM	: Protein energy malnutrition
PMSF	: Physically modified soy flour
PMSF-W	: Physically modified soy flour and wheat
ppm	: Parts per million
PRA	: Participatory rural appraisals
PSH	: Protein thiols

PSSC	: mixed disulphide of protein and cysteine
PSSG	: mixed disulphide of protein and glutathione
PSSP	: Protein disulphides
r^2	: Correlation coefficient
RDA	: Required daily amount
R_m	: Maximum resistance to extension
rpm	: Revolutions per minute
RSM	: Response surface methodology
RSF	: Raw soy flour
RSF-W	: Raw soy flour and wheat
SD	: Standard deviation
SDS	: Sodium dodecyl sulphate
SDS-PAGE	: Sodium dodecyl sulphate – Polyacrylamide gel electrophoresis
SE-HPLC	: Size-exclusion High-Performance Liquid Chromatography
Sf	: Soy flour
SH	: Sulfhydryl
SMT	: Sucrose mono tallowate
SPC	: Soy protein concentrate
SPI	: Soy protein isolate
SPTF	: Soybean Promotion Task Force
SPRL	: Soil Productivity Research Laboratory
SPSS	: Statistical Package for Social Sciences
SS	: Disulphide
SSL	: Sodium stearoyl-2-lactylate
TFA	: Tetra fluoro-acetic acid
TGA	: Thermal gravimetric analysis
T_g	: Glass transition
T_o	: Onset temperature
T_p	: Peak temperature
U/g	: Units per gram
UNICEF	: United Nations Children Emergency Fund
UNO	: United Nations Organisation
UPP	: Unextractable polymeric proteins
UV	: Ultra violet
v/v	: Volume for volume
W/g	: Watts per gram
WHO	: World Health Organisation

w/v	: Weight for volume
w/w	: Weight for weight
μg	: Microgram
μg/L	: Micrograms per litre
μL	: Microlitre
μm	: Micrometre
μmol	: Micromole

General Summary

Soy enhances the protein quality of wheat bread because of its lysine content which is deficient in wheat. The aim of this work was to use high levels of soy flour in wheat bread in order to maximise the potential of soy flour protein in an attempt to address Protein Energy Malnutrition in developing counties. Raw soy flour (RSF) and physically modified soy flours (PMSF1 and PMSF2) were used for the preparation of the composite dough with wheat flour. The two physically modified soy flours were prepared by steam flushing (PMSF2) and water boiling (PMSF1) of raw soy beans before flour preparation. Physical modification of soy flour was chosen over chemical modification because of its practical significance in developing countries.

The Farinograph and Extensograph were used to study the effect of L-ascorbic acid and physical modification of soy flour on the rheological properties of soy-wheat composite doughs at various ratios up to 50% soy flour. Soy-wheat composite doughs made from physically modified soy flour (PMSF) exhibited higher resistance to extension (R_m), greater tolerance to mixing, better mixing stability, higher water uptake rate and water absorption than the soy-wheat composite doughs made from raw soy flour (RSF) *($P<0.05$)*. The combination of L-ascorbic acid (0.05% w/w) and PMSF2 (0-50% w/w) in wheat flour significantly improved resistance to extension (R_m), as it also improved loaf volumes and crumb texture of soy-wheat bread *($P<0.05$)*.

Size-exclusion high performance liquid chromatography (SE-HPLC), used to evaluate the reasons for these improvements, indicated that the soy flour physical modification process altered the relative protein size distribution, showing the PMSF2-wheat dough SE-HPLC profile to be much closer to that of the wheat dough compared to the SE-HPLC profile given by RSF-wheat dough. SE-HPLC profiles during Farinograph mixing of composite doughs revealed higher numbers of polymeric (HMW) proteins compared to those of control composite doughs, suggesting interactions of soy and wheat proteins.

The results indicated that soy-wheat composite doughs made from PMSF2 form stronger doughs with potentially better baking qualities compared to RSF. 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 and where wheat grain is imported.

Differential scanning calorimetry (DSC) was used to study the thermal properties of soy-wheat doughs at various ratios and to study the interactive and main effects of

L-ascorbic acid, soy flour concentration and water amount on soy-wheat dough DSC endotherms. Water had highly significant and positive linear effects on peak temperature (T_p) onset temperature (T_o) and enthalpy of vaporisation (? H_{vap}) (*P <0.005*) indicating that water increased T_p T_o and ?*H* of soy-wheat dough evaporation endotherms. Increases in the levels of soy flour proportionally decreased DSC water evaporation enthalpies of soy-wheat doughs (*(P<0.05)*. Using physically modified soy flour (PMSF2) to prepare soy-wheat dough, a mathematical model was developed from estimated regression coefficients of L-ascorbic acid and water percentages (30% w/w soy flour) on soy-wheat dough DSC water evaporation enthalpies (? *H* J/g). The model was successfully used for the prediction of loaf volumes and for the formulation of soy-wheat breads. The optimal formulation was estimated to contain 13.6% (w/w) protein (*N x 6.25*) and 0.68 % available lysine. A daily serving of 100 to 200 grams of this bread was calculated to provide 60 -100% of the lysine and protein requirements (FAO/WHO) of children and adults.

The results from descriptive sensory analysis revealed that soy-wheat bread, using at least 30% physically modified soy flour (PMSF#2) could be prepared with acceptable organoleptic properties (9/15 average acceptability score) , improved loaf volume (750 -760 cm^3) and crumb texture (14.7 to 16.3g /sec·/mm). There was a significant and positive influence of L-AA on masking the bean flavour *(P<0.05)* in the soy-wheat bread formulations (r^2 of 0.83). The resultant breads developed in this project thus offer an attractive and sustainable food that is nutritionally superior.

Chapter 1

Literature review

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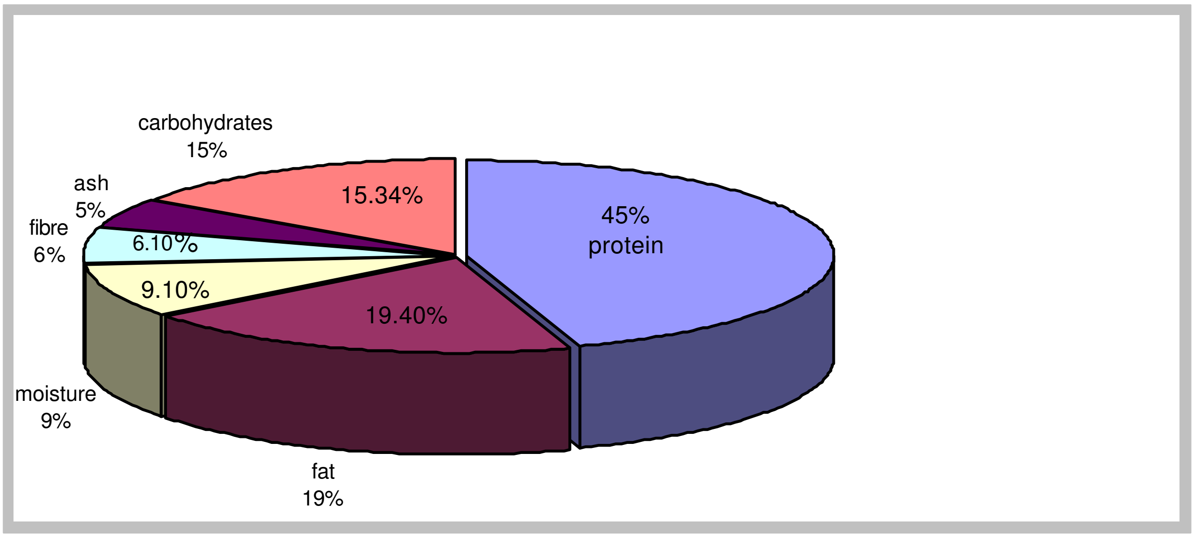
1.1 The nutritional value of soybean

Soy bean (*Glycine max)* is an important source of oil (17-25%) and protein (35-45%). It contains large amounts of Vitamin B1 and B2 but it is rather low in vitamin C (Neilson, Wei and Steinberg, 1979). The crude protein of soybean meal ranges from 41 to 50% (dry matter basis) depending on the amount of hull that is removed, and the processing method used (Liener, 1994). Results from proximate analysis show the nutritional profile for soybean meal (Figure 1.1).

Soybean has the highest quality protein in meeting the human physiological needs. The press cake remaining after the oil has been expressed from the soy meal contains about 60% protein of relatively good biological value. Soybean's high protein content is characterised by a good balance of amino acids, which is better than for any other common plant and approaches the FAO dietary standards (Neilson et al., 1979). Proteins of high quality are those which are fully digested and whose amino acid composition closely matches the amino-acid pattern for humans and animals given by FAO and WHO standards (Liu, 1997). Of the 21 essential amino acids (building blocks of protein), 9 must be supplied in our diet. Soybean provides all the nine amino acids, making it a complete protein. It is the only vegetable that offers a complete protein profile. However, methionine (an essential amino-acid) is limiting in soy but it is plentiful in certain cereals.

Cereals are a good source of sulphur containing amino acids but they are limiting in lysine. On the other hand, soybean is rich in lysine and limiting in methionine (sulphur bearing amino acid) (Coultate, 1996; Nielson et al., 1979). Cereals can therefore be blended with soybean to give a combined protein that meets the FAO standards. This is why soy-cereal combinations make better products, because protein quality, as well as flavour and functionality are also improved, thus enhancing acceptability (Fleming and Sosulski, 1978; Yousseff et al., 1976; Sharma et al., 1999; Dhingra and Jood, 2002). The addition of methionine to cowpea protein has been shown to increase protein quality significantly thus demonstrating the nutritional value of increasing the sulphur amino-acid content in legume protein (Bressani, 1985). Results in Table1.1 show the effect of sulphur amino-acid supplementation to cowpea on its protein quality.

Figure 1.1



Maforimbo 2001 (unpublished)

Figure1.1.The nutritional value of soybean seed (Values calculated on dry matter basis)

Table 1.1 Sulphur amino-acid supplementation: effect on protein quality

Cowpea	*Biological value %*	*Net protein utilization*
Meal	58.2	50.6
Meal + cystine	80.3	72.7
Meal +cystine +methionine	94.6	82.1
Meal + methionine	95.8	81.5
Albumin	101.7	99.5

Bressani, 1985

Table 1.2 Essential amino acids contained in soybean and other foods (g/100g food)

Food	*valine*	*Leucine*	*Isoleuc*	*Threonine*	*Phenala*	*Trypt*	*Meth*	*Lysin*
Soybeans	1.30	3.67	1.61	1.65	1.80	0.46	0.41	2.29
Lean-beef	1.04	1.46	0.77	0.93	0.70	0.21	0.51	1.44
Chicken	1.20	1.84	0.96	1.18	0.90	0.27	0.65	1..84
FAO/WHO	**1.10**	**1.40**	**0.82**	**0.80**	**1.21**	**0.22**	**0.73**	**1.14**

Chen, 1999

Results in Table 1.2 show the amino acid profiles for the essential amino acids contained in soybeans and other foods. From the essential amino-acids shown, lysine is highest in soybean followed by chicken meat. Methionine on the other hand is limiting in soybean which has the least amount (0.41g %), well below the recommended (0.73g %) per day.

1.2 Nutritional and health value of soybean oil

Soybean is a leading edible oil widely used in various foods including cooking, salad, shortening, margarine and mayonnaise. Soy oil provides energy, essential fatty acids and fat-soluble vitamins. Of the 20% oil content in soybean, 85% of it is unsaturated fat (61% polyunsaturated fat and 24% mono unsaturated fat) and this is highly desirable in the human diet for lowering serum cholesterol (FAO, 1993). It has also been revealed in a meta -analysis that a consumption of 17-25 grams of soy protein per day could have a meaningful effect in lowering serum cholesterol (Anderson et al., 1995). Soybean is also claimed to contain nutrient anti oxidants and non-nutrient anti - oxidants (phenolic acids) which are free radical quenchers, and fight products of lipid peroxidation in biological systems (Diplock, 1998).

Table 1.3 Percentages of unsaturated fatty acids in some common fats

	Soya	*Sunflower*	*Maize*	*Groundnut*	*Coconut*	*Palm*	*Lard*	*Beef*
Oleic	23.3	24.7	32.3	53.0	7.0	38.0	42.8	40.5
Linoleic	52.3	63.2	49.3	27.0	2.0	10.0	7.3	2.5
Linolenic	7.2	---	1.0	---	---	---	0.8	0.8

Bressani et al. (1982)

Linoleic and linolenic (polyunsaturated fatty acids) are effective in lowering serum cholesterol and also have a critical role in membrane structure and as precursors of eicosanoids (FAO, 1993). From the table above, soy oil has the highest content of linolenic acid, (omega-3 fatty acid) and thus provides the nutritional profile demanded by today's health conscious consumers.

1.3. The minor components in soybean

The minor components in soybean include minerals, vitamins, phytin and phenolics. The ash content of soybean (about 5%), include sulphates, phosphates and carbonates (Liu, 1997). The vitamins in soybean are the water-soluble vitamins, including, thiamine, riboflavin, niacin, pantothenic acid and folic acid. These remain with the meal after oil extraction, but most of them are lost during tofu making where water is lost in whey. Vitamin C is very insignificant in soybean seed except in germinating soybean.

The oil soluble vitamins present in soybean are Vitamin A, which exists in the form of provitamin β-carotene and vitamin E (tocopherol) which exists in four isomers, namely α, β, δ and τ tocopherol. α-tocopherol is significant in soybean and is extracted during oil extraction. It is considered an important constituent because it is the most effective natural anti - oxidant (Liu, 1997).

1.4. Protein digestibility

While the amino acid profile pattern is probably the main determinant of protein nutritional quality, the digestibility of a protein and bio-availability of its constituent amino acids are the next important factors (Oshodi et. al., 1995). This is true because proteins are digested, absorbed and utilised to different extents. Protein digestibility is defined as the percentage protein absorbed after ingestion of a certain amount of protein by humans or animals (Oshodi et. al., 1995; Liu, 1997; Maforimbo, 2001). It is closely related to amino-acid availability. Protein digestibility is a major index of protein quality because a certain amount of amino-acids may be present in a food and it may not necessarily be available to the organism for nourishment. This means proteins cannot be utilized unless they are digested (Liu, 1997). The differences in protein digestibility are brought about by the susceptibility of a protein to enzymatic hydrolysis in the digestive system and this is directly related to the primary, secondary and tertiary structure of the protein (Barbara, 1994).

The problem of relatively low protein digestibility in legumes deserves attention (Bressani, 1985). Although proteins of plant origin offer a considerable protein source for alleviating the shortages of food protein facing the greater part of the population Liener (1982) pointed out that many plants contain anti - nutritional factors which affect protein quality, and Bressani et.al. (1982) also implicated legume seeds to contain proteins that inhibit the proteases of the mammalian digestive tract, notably trypsin and chymotrypsin.

Table 1.4 Protein digestibilty of various soybean foods in humans

Soy food	*Protein digestibilty (%)*
Roasted soy meal	78
Fermented whole soybean (natto)	90
Deep fried soy curd (tofu)	91
Boiled whole soybean	92
Freeze-dried soy curd (tofu)	93
Soy film (yuba)	100

Liu, 1997

Different soy products have different protein digestibility as shown in Table 1.4. Processing and storage conditions can alter the protein structure thus improving or lessening its susceptibility to enzymes. Poor protein digestibility has been reported in developing countries due to the use of less refined cereals and pulses (Oshodi et al., 1995). In order to improve the digestibility of soybean and address the protein needs for the target groups, dehulling of soybeans should be encouraged before secondary processing so as to improve protein utilisation efficiency (Maforimbo, 2001). Table 1.5 shows the trend for relative protein digestibility for steamed whole soybean and dehulled soybean meals.

Table 1.5 In vitro relative protein digestibility for heat treated (steamed for 1 h) soybean meal of five varieties

*Whole seeds**	*% Relative Digestibility*	*Dehulled** seeds*	*% Relative Digestibility*
A1	69.9	B1	80.4
A2	61.5	B2	69.5
A3	68.2	B3	75.5
A4	78.0	B4	87.0
A5	66.7	B5	77.7
Mean	68.9	Mean	78.0
S.D,	5.4	S.D.	5.8
CV%	7.8	CV%	7.4

Source: Maforimbo (2001)

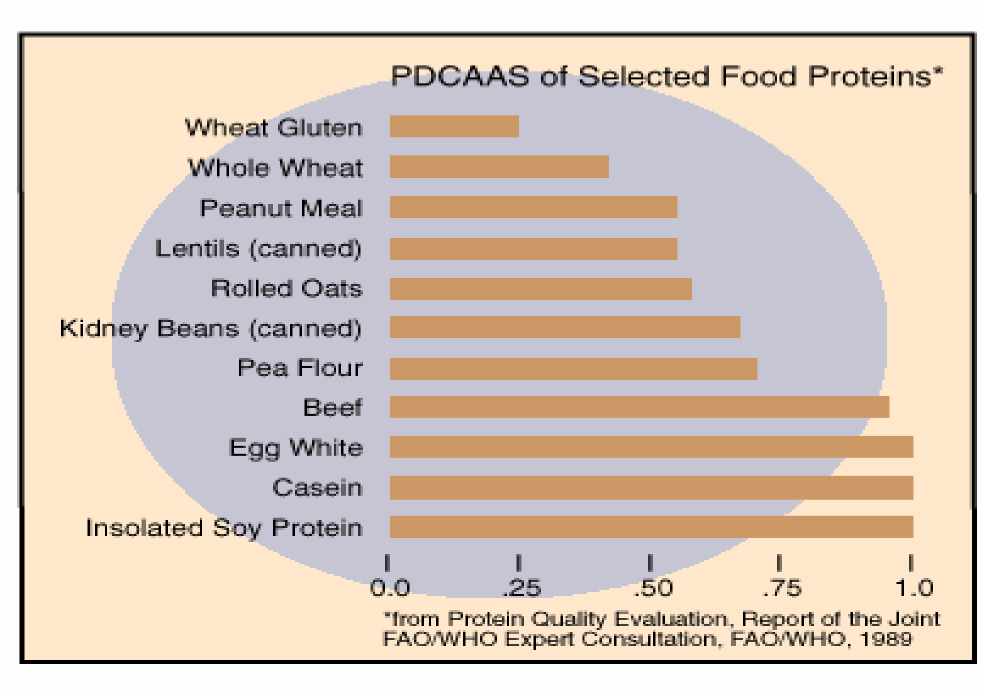
* A = Whole seeds; **B = dehulled seeds

1-- Roan; 2 -- Soma; 3-- Duiker; 4-- Nyala; 5-- Nondo

Relative in vitro protein digestibility, as illustrated in Table 1.5 varied in both whole and dehulled seed, ranging from 61.5% to 78.0% for the whole and 69.5 to 87.0% for the dehulled seed. The higher mean values for the dehulled seeds indicate that dehulled seeds have better digestibilities. This was also confirmed by the t-test which indicated that dehulling significantly improves digestibility *($P < 0.05$).*

Evaluation of protein quality for adults using the Protein Digestibility Corrected Amino Acid Score (PDCAAS) has recently been found to be more accurate and has been adopted as an official measurement by the World Health Organization and the US Food and Drug Administration (Messina and Messina, 2001). The lowest uncorrected amino - acid score is multiplied by the protein digestibility to obtain the PDCAAS value. Figure 1.2 shows the comparison of PDCAAS values for various protein foods.

Figure 1.2



Source: http://www.talksoy.com/Health/tProtein.htm

Figure 1.2 compares PDCAAS values across various protein foods. From the protein quality evaluation shown in Figure1.2, PDCAAS for soy protein matches that of casein and egg white, indicating that soy protein is a high quality protein in terms of digestibility.

1.5. Forms of soy protein

Soy protein products that are currently manufactured include concentrates, isolates and textured products. These are derived from defatted soybean meal or flour (Wolf and Cowan, 1975; Liu, 1997; Stauffer, 2002).

1.5.1. Defatted soy flakes and meal

Soybeans are dried, cleaned, cracked and dehulled. They are later conditioned with moisture up to 60-75% and flaked using small rolls. Solvent extraction, usually with hexane, is used to defat the flakes. A desolventisation process is used to remove the residual solvent. Soy meal is prepared by milling these flakes into meal that contains about 50% protein, 30-35% carbohydrates and 1% lipids. The desolventisation process is optimised because it affects protein solubility, moisture and the usefulness of these flakes in the product (Wolf and Cowan, 1975).

1.5.2. Flours and grits

These are obtained from grinding of the defatted flakes into the required coarse grits or fine flour.

1.5.3 Soy protein concentrates (SPC)

These are produced from defatted soy meal/ flakes/ flour after the removal of the non-proteinace soluble fraction, usually sugars. The remaining major protein fractions are removed by denaturation with aqueous alcohol (20-80%), moist heat, or isoelectric precipitation at pH 4.2-4.5 with dilute mineral acid, usually HCl (Liu, 1997). High NSI (Nitrogen Solubility Index) flakes are preferred for the preparation of SPC since the percentage of soluble sugars in these is higher than in toasted meal. Toasting renders sugars less soluble by binding them to proteins during Maillard reaction or caramelisation. Because of these reactions, sugars are not extractable by the solvent and they remain in the product, lowering the protein content of the product. The overheated meal also gives a darker colour to the product and the nutritional value is lower due to lysine destruction (Liu, 1997). SPC contain a minimum of 70% protein on a dry basis. Concentrates made by aqueous alcohol wash and a heat treatment/water extraction process have low nitrogen solubility because of protein denaturation. Those made from aqueous acid leaching or by steam injection/jet cooking have a higher solubility if they are neutralised prior to drying (Liu, 1997).

1.5.4. Soy protein isolates (SPI)

Soy protein isolates have a high protein concentration of 90-95% on a dry basis because soluble and insoluble carbohydrates have been removed. Soy protein isolates have little of the carbohydrate that is related to off flavour problems and are more versatile across a wider range of applications than soy flour (Marsili, 1993). A high protein dispersibility index (PDI) is required for protein isolates (usually 85-95) and low PDI is required for flours (Liu, 1997; Stauffer, 2002). This is desirable, because many of the food applications for soy protein depend on its ability to bind water. The emulsion capacity of soy protein isolates varies from 10-35 milliliters of oil per 100 milligrams of protein.

1.5.5 Textured soy proteins

These are produced by thermoplastic extrusion, starting with soy flour, soy concentrates or isolates. Their texture can be modified by varying the extrusion mix. They absorb water and fat and therefore have a physical function apart from providing meat - like textural properties. They can be incorporated in food formulations in dry, partially hydrated or fully hydrated form and they require less water than the concentrates (Liu, 1997).

1.6. Functional properties of soybean proteins

The relationship between protein structure and its functional properties is well established (Wolf and Cowan, 1975; Kilara and Sharkasi, 1986; Liu, 1997; Utsumi and Kinsella, 1985; Hoover, 1979; Marsili, 1993; Stauffer, 2002; Puppo et al., 2003). Functionality, as applied to food ingredients, is any property that influences the ingredient's usefulness in food (Nnyanyelugo, 1997). The diversity of physio-chemical properties, brought about by the protein's nature and flexibility, defines the functionality of proteins in food systems. Apart from their nutritional and economic uses, soy protein products are used primarily for their functional characteristics (Kilara and Sharkasi 1986; Liu, 1997; Utsumi and Kinsella, 1985). The presence of both lipophilic and hydrophilic groups in the same polymer chain facilitates association of the protein with both fat and water, and this forms the basis for formation and stabilisation of emulsions in food systems. Solubility, size, arrangement of charged groups, hydrophobic groupings, water - binding properties and interactive regions all affect functionality, and these properties can be altered by chemical, physical and enzymatic modifications, (Marsili, 1993). Understanding how proteins interact with other food components and how proteins can be physically and chemically altered for improved functionality is important for the future of soy proteins (Nnyanyelugo, 1997). Table 1.6 shows the trend beteween physical properties of proteins and protein functionality.

Table 1.6 Protein properties responsible for specific functionalities in food systems

Physical Properties	*Functionality*
Organoleptic	Colour, flavour and odour
Kinesthetic	Texture and chewiness
Hydration	Water absorption, solubility, gelling and swelling
Surface action	Emulsion, foaming and fat binding
Rheological	Viscosity, elasticity, adhesiveness, cohesiveness, gelation, fiber formation
Other	Enzymatic activity, antioxidant, hyper-cholesterolemic

Source: Liu, 1997

1.6.1. Solubility

For optimum functional properties, a solubility index above 90% is required. Good protein solubility generally correlates with optimum gelation, emulsifying and foaming ability of the protein (Lakemond et al., 2000). Solubility of proteins decreases at ionic concentrations of less than 0.1M and increase above this concentration. This is caused by the decreased electrostatic repulsion and enhanced hydrophobic interaction of protein molecules as a result of electrostatic shielding of charge groups in proteins by ions. Solubility increases sharply below and above their iso-electric point (4.2- 4.6 pH) (Liu, 1997).

1.6.2. Emulsification

Soybean proteins form and stabilise emulsions. An emulsion is a dispersion of oil droplets in a continuous aqueous phase as this is brought about by the surface action of soy proteins, which lower the surface tension between the oil and water interface, bringing about an emulsion. This emulsion is stabilised by a collection of proteins at the surface of droplets to form a protective barrier that prevents coalescence and emulsion breakdown. Hydrophobicity is an important factor in emulsifying properties. The hydrophobic sites of a protein are provided by the non - polar amino acids and covalently attached hydrophobic groups. The hydrophobicity of a protein can be changed by partially denaturing the protein and exposing the interior primary structure that contains most of the hydrophobic amino acids (Rosenberg, 1996; Sikorski, 2001; Wagner and Anon, 1990). The surface properties of a protein depend on the conformational stability – the more unstable, the higher the emulsifying properties. Emulsion by soy protein is applied in bologna, sausages, bread, cakes, soups, whipped toppings. Soy protein in meat - based products improves emulsion and stabilisation formation, enhances binding in meat and vegetables and improves moisture holding, mouth feel and palatability, (Wolf, 1970; Kinsella, 1979; Liu, 1997; Matlani, 2004).

1.6.3. Fat absorption

Soy proteins promote fat absorption or fat binding in ground meats. This is achieved by the formation and stabilisation of an emulsion and gel matrix formation that hinders migration of fat to the surface. In doughnuts or pancakes, addition of soy protein flour prevents excessive fat absorption during frying. The high NSI (nitrogen solubility index) value in soy flours imparts the bean flavours in doughnuts. NSI values from 50-65 are generally used to compromise between bean flavour and functionality (Liu, 1997; Matlani, 2004).

1.6.4. Water absorption

Soy proteins contain polar groups along their peptide bonds, therefore are hydrophilic and tend to absorb water and retain it in the finished product (Wolf and Cowan, 1975; Liu, 1997). The polarity of these groups, like carbonyl and amino can be varied with pH and therefore pH alteration can vary the water absorption properties of soy flour. In baked goods, where soy flour is substituted for non-fat milk powders, more water is needed to increase dough yield and to improve dough handling. In cakes, soy flour minimises shrinkage (Marsili, 1993). The rate of water absorption depends on particle size and distribution of soy flour or grits. Water absorption in baked products helps improve moisture retention freshness for longer periods. In crunchy textures of dry extruded soy flour products, hydration improves the fibrous chewy texture resembling that of meat (Wolf, 1970; Kilara and Sharkasi, 1986; Utsumi and Kinsella, 1985; Liu, 1997: Endres, 2001).

1.6.5. Texture

Soy proteins have the ability to provide texture to other dishes, including traditional dishes and cereals. Soy flour can thicken soups, gravies, impart gelling properties in ground meats, and soy flour can be thermally extruded into fibre - like proteins to produce meat imitations (Wolf and Cowan, 1975; Liu, 1997; Endres, 2001; Matlani, 2004).

1.6.6. Colour control

Soy flours control colour by the bleaching of white bread during lipoxygenase activity in which wheat flour carotenoids are bleached to a colourless form (Coultate, 1996; Wolf and Cowan, 1975). Secondly, soy proteins contribute to crust colour formation in bread that is brought about by the reaction of soy protein’s lysine with the reducing sugars in wheat. Therefore when soy flour is added to bread mixes, like pancakes and waffles, it improves browning characteristics as well as retarding fat absorption during frying (Wolf and Cowan, 1975).

1.6.7. Aeration

Soy proteins are surface active, therefore they can foam and this phenomenon is employed in whipped toppings and deserts (Wolf and Cowan, 1975; Liu, 1997; Endres, 2001).

1.7. Anti - nutritional factors in soybean

The anti-nutritional factors in soybean are often associated with the low acceptance of soybean products as they also inhibit protein digestibility. These mainly consist of the heat labile (trypsin inhibitors, lectins, goitrogens, phytates) and heat stable (oligosaccharides) factors. In order for the nutritional value of soybean meal to be maximised, these anti-nutritional factors need to be inactivated or minimised (Liener, 1982; Liener, 1994; Liu, 1997).

1.7.1 Heat labile factors

Trypsin inhibitors are the most notable and studied protease inhibitors as they inhibit the action of digestive enzymes (*trypsin and chymotrypsin*), thereby decreasing protein digestibility. Elimination of trypsin inhibitors involves heat treatment to inactivate the trypsin inhibitor. These include; steam, boiling in water, dry roasting, di-electric heating, microwave radiation, micronisation and extrusion cooking. In addition to heating time and temperature, moisture condition prior to cooking is important for significant destruction of the trypsin inhibitor (Liu, 1997).

Lectins (haemagglutinins) bind specifically to saccharides on the surface of the cells (components of the intestinal epithelial cells), and this can result in a decreased nutrient absorption (Neilson et al., 1979; Campbell, 1979; Liu, 1997).

Goitrogens are composed of 2 or 3 amino acid residues linked to a sugar moiety. They may prevent intestinal reabsorption of thyroxine, resulting in hypertrophy of the thyroid gland (Neilson et al., 1979; Campbell, 1979; Liu, 1997).

Phytates are the principal sources of phosphorus in soybean. The phytate content in soybean ranges from 1 – 1.4% on dry matter basis. Phytates affect mineral absorption by binding with elements like calcium, magnesium, iron, copper etc. Phytates affect protein solubility, the iso-electric point of proteins and functionality for proteins because they are capable of forming complexes with negatively charged proteins at alkaline pH, as they also bind with the positively charged proteins at the pH below their iso-electric points (Neilson et al., 1979; Liu, 1997).

1.7.2. Heat - stable factors

Oligosaccharides (flatulence factors)

Soy carbohydrates, which total up to 35 % in a mature seeds include the soluble and insoluble ones. The oligosaccharides in soybean are non-reducing sugars containing fructose, glucose and galactose linked by a β-fructosidic and α - galactosidic linkages. Of the most important factors limiting the use of legumes in the human diet is the production of flatulence associated with their consumption. The low molecular weight and stubborn oligosaccharides containing α-galactosidic and β-fructosidic linkages, namely raffinose and stachyose have received a lot of attention because of their link to flatulence and abdominal discomfort (Liener, 1994; Liu, 1997).

Soybean is a good source of dietary fibre, up to 30% pectin, 50% hemi-cellulose and 20% cellulose. Because of certain physiological responses associated with dietary fibre, including increase in faecal bulk, reduction in plasma cholesterol, decrease of nutrient bioavailability, soybean carbohydrates are now receiving attention.

1.7.3. Phytoestrogens (Isoflavones)

Isoflavones in soybean have generated a lot of interest among researchers. Soybean and soy foods are the only natural dietary sources that provide nutritionally relevant amounts of isoflavones, up to 3 mg/g of dry weight, (Messina, 2001). The physiological effects of isoflavones on humans and animals have been highlighted by Kuduo et al. (1991), Wang and Murphy (1994), Kenneth and Setchell (1999), with claims that isoflavones possess estrogenic activity because these bioactive non-nutrients are similar in chemical structure to estradiol, the main female hormone. Isoflavones in soy foods fight cancer in a variety of ways including inhibition of certain enzymes and hormones that promote cancer (Liu, 1997; Messina, 2001).

1.7.4. Lipoxygenase (LOX)

LOX catalyses the hydroperoxidation of linoleic acid and other polyunsaturated lipids that contain a cis-cis –1,4 – pentadiene moiety, in the presence of molecular oxygen. Accumulation of these peroxides leads to the formation of aldehydes and ketones, which damage seed meal and oil flavour (Coultate, 1996). The volatiles (from aldehydes and ketones) are responsible for most of the objectionable off flavors associated with soy products. LOX also catalyses the oxidation of pigments and carotenoids, a phenomenon applied in the bleaching of wheat flour using enzyme - active full - fat soy flour in bread making (Coultate, 1996; Cumbee et al., 1997; Hoseney et al., 1980).

Most methods of LOX elimination in food are centered on the use heat sensitivity. When the tissue is damaged (when beans are broken, milled or ground), this enzyme is exposed to the active sites (Neilson et al., 1979; Bressani, 1982; Liu, 1997). Addition of water to the broken tissue brings the enzyme and the active sites together and they react and produce instant, off flavors. Therefore this enzyme must be timely deactivated before processing the soy products (Bressani, 1982).

1.8. Soy flour proteins in baking applications

Most soy flour for baking is derived from white flakes that have been lightly cooked using steam. This process inactivates lipoxygenase and improves the storage stability of the flour. A longer heating time increases protein denaturation, lowering PDI, which is desirable in certain bakery applications. The protein dispersibility index (PDI) expresses the amount of water - dispersible protein expressed as a percentage. It is negatively correlated with the extent of heating. The protein solubility index is easily determined by extracting the soy products with water and analysing them using the Kjeldahl method (Wolf and Cowan, 1975).

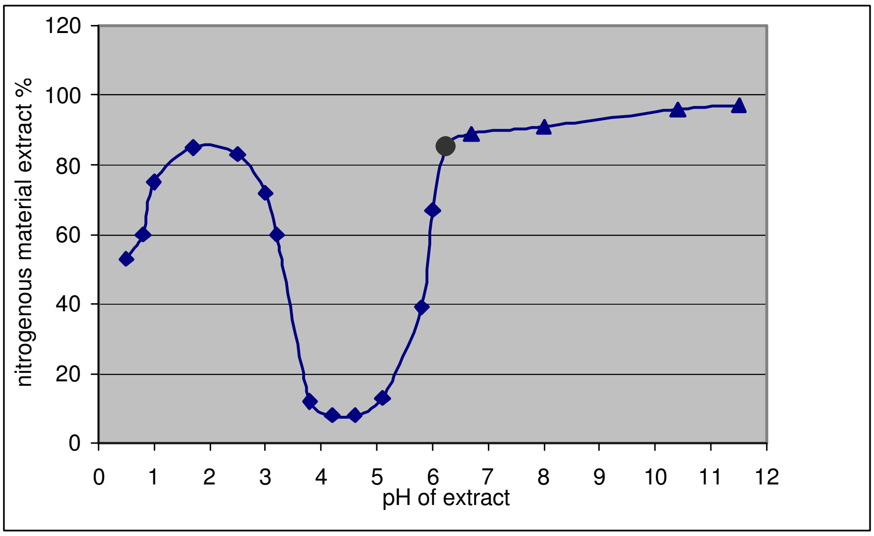
Soy proteins are insolubilised by moist heat, therefore protein solubility measurements have been used to determine the extent of heat treatment to soy products. An example of commercial soy flours in order of the increases in heat treatment include; lightly heated/steamed (PDI 65-80); Moderately steamed (PDI 35-45); Toasted (PDI 8-20). Lightly heated (70 PDI) soy flour is used for most bakery applications, where soluble proteins are desired for their functionality, particularly water binding (United Nations University Press, 1980). Toasted soy flour is used in applications where soluble protein is not desired, e.g. in cookies or biscuits (Stauffer, 2002).

Solubility is one of the most basic physical properties and prime requirements in functional food systems (Liu, 1997). The hydrodynamic behaviour and protein –protein interactions depend basically on the degree of dissociation, denaturation and aggregation of the major storage soy protein globulins, 7S and 11S. Many workers have modified specific functional properties of soy proteins and these modifications have included thermal and chemical treatments (Kinsella, 1979; Sorgentini, Wagner and Anon, 1995; Wagner, Sorgentini and Anon, 2000; Ryan et al., 2002; Ryan and Brewer, 2005). Mild acid or alkali treatment on soy proteins has been frequently used and the inclusion of acid to heat treatment has resulted in other modifications such as hydrolysis and deamidation of proteins. Deamination usually leads to accumulation of ammonia and this elevates pH. Wagner et al. (2000) classified soy protein in different groups according to different degrees of denaturation and aggregation, highlighting that the relationship between solubility and surface hydrophobicity reinforced the importance of hydrophobic interactions in the aggregation process (insolubilisation) of soy proteins. The solubility of protein isolates was analysed as a function of the surface hydrophobicity of their proteins. Three well defined groups of laboratory protein isolates were identified: native, partially or totally denatured isolates with high solubility and surface hydrophobicity, through to totally denatured isolates with low solubility and surface hydrophobicity.

Highly soluble protein, as measured by the nitrogen solubility index (NSI), is desirable for optimum functionality in most foods, but denatured isolates with low solubility and high surface hydrophobicity would be desirable in baking. The solubility of soy proteins is dependent on pH, (Wolf and Cowan, 1975; Liu, 1997; Wagner et al., 2000). Soy proteins are least soluble at their iso-electic point (4.2-4.6). A slurry of native soy flour in water has a pH from 6.5 - 6.8 and at this pH range, 85% of the proteins are soluble. Soy-wheat dough from 10 –50% soy flour has a pH range from 5.5 –6.5 (Maforimbo, unpublished). These observations technically imply that in general, >85% of soy proteins are soluble in a soy –wheat dough. Use of partially denatured protein isolates with low solubility, high surface hydrophobicity with a combination of organic acids (L- ascorbic acid or citric acid) to adjust dough pH would theoretically improve interaction between soy and wheat proteins.

Figure 1.3 shows the solubility pattern for soy proteins as given by Liu (1997) and Sikorski (2001). Up to pH 3.5 soy protein is very extractable; between pH 3.8 – 5.2 the proteins are very insoluble and above pH 5.6, soy proteins become extractable. Above pH 6.5 when the solvent is water, soy proteins are >85% soluble.

Figure 1.3



Source: Liu (1997), Sikorski (2001)

Figure 1.3 A typical soy protein solubility curve highlighting pH of solution versus the protein extract (shown as nitrogenous material) percentage.

Symbol ¦ denotes HCl; symbol? denotes H_2O; while symbol? denotes NaOH in solution

1.8.1. History of composite bread

Supplementation of wheat flour with high protein plant sources to improve protein quality and amino-acid balance of the resultant products has been of great interest to nutritional scientists. Using formula alterations, acceptable bread has been produced from concentrated plant proteins from soy, sunflower, faba bean and field pea flours using vital gluten (2%) and emulsifiers as effective conditioners (Fleming and Sosulski, 1978). Cowpea flour has been used with surfactants to produce acceptable bread, cookies and *chappatis** from cowpea/wheat – flour blend (Sharma et al., 1999). Barley flour has been incorporated in wheat bread (Gujral et al., 2002) with wet gluten and ascorbic acid used to improve loaf volumes and crumb texture. Barley flour and defatted soybean flour (15%) have been substituted in wheat bread (Dhingra and Jood, 2001), and the composite breads have been organoleptically and nutritionally acceptable. The deleterious effects of legume flours on bread have been minimised by the use of surfactants and oxidising improvers (D'Appolonia, 1977), as these treatments were claimed to strengthen the dough as well as the addition of extra water to give larger loaf volumes (Shogren et al., 2003). These authors also reported that nutritious bread containing up to 40% defatted soy flour could be made using high quality active yeast to mask the bean flavour.

Acceptable rice flour breads have been formulated to provide for people with coeliac disease (Nishita et al., 1976). Rice flour yeast breads have also been formulated using surface response methodology (Ylimaki et al., 1988) and several optimum combinations of gums and water were found to produce rice breads with specific volumes, and crumb and crust properties comparable to those of reference wheat bread. Gluten - free breads require polymeric substances that mimic the visco-elastic properties of gluten in bread. Response surface methodology (RSM) has been successfully applied to find optimum levels of gums and egg white for the formulation of gluten free pocket - type flat bread from pre-gelatinised rice and corn starch, (Toufeili et al., 1994), from corn starch and cassava starch (Sanchez et al., 2002). Interactive effects of water, hydroxypropylmethylcellulose (HPMC) and egg white on thermal properties of dough consisting of corn and cassava starches were successfully studied using RSM (Kobylanski et al., 2004).

1.8.2. Functional properties of soy flour in bread

Soy is very popular in the Asian diet and has also found increasing acceptance in Western diets due to its health claims from the Food and Drug Administration in 1999 and its link with lower risks of heart diseases (Vittadini and Vodovotz, 2003). However, it is generally known that products made from soy flour substitution for wheat flour (> 15% soy flour) are generally not acceptable in terms of organoleptic properties and baking qualities, as it is also known that products of lipoxygenase activity are responsible for the off flavour in the final products. The effects of adding soy ingredients to bread have been characterised by sensory analysis (Surana, 1973; Yousseff et al., 1976; Dhingra and Jood, 2001); objective measurements (Fleming and Sosulski, 1997; Shogren et al., 2003); physiochemical properties of soy-wheat bread (Vittadini and Vodovotz, 2003) and in most cases, significant decreases of loaf volumes were observed with the addition of soy flour.

The negative effects associated with soy-wheat bread making quality have been attributed to lack of interaction between soy and gluten proteins (Ryan et al., 2002; D'Appolonia, 1977). They hypothesised that non-gluten protein networks formed are unable to stretch and retain carbon dioxide formed during fermentation. Ryan and colleagues (2002) also reported that gluten and soy protein interactions provide improved dough characteristics. They also claimed that the addition of emulsifiers to the low gluten wheat breads improved gas retention and crumb texture. They demonstrated that sucrose esters increased interactions between texturised soy flour proteins and wheat gluten adding that the process of texturisation improved the functionality of soy proteins in wheat dough through enhancement of non - covalent interactions. Earlier on, Chung et al. (1981) reported the effects of surfactants on interactions among native lipids and proteins or starch in optimally mixed wheat dough. They demonstrated that a small amount of polar lipids or lipid - related surfactants could counteract the adverse effects of high protein non-wheat products like soy flour on functional properties in bread making. Improved loaf volumes, as a result of interaction of soy flour proteins, glycolipids and wheat flour proteins, have also been reported by Hyder et al. (1974) who attributed the loaf volume response to soy flour and wheat protein interactions in the presence of sucrose mono tallowate (SMT). On the other hand, Lorimer et al. (1991) highlighted the importance of sulphydryl and disulphide interchange in controlling bread quality and loaf volume, adding that the non-gluten forming protein could disrupt the SH /SS interchange. His work however, could not conclude that globular proteins (from legumes) disrupted SH/SS interchange. Sahan et al. (1986) studied the effects of soy flour and bread improvers on wheat bread quality and revealed that the relative volume of bread was decreased with increasing additions of soy flour (up to 10%) to wheat flour in blends.

However, the use of enzymically active soy flour at very low levels (1% w/w) with wheat dough has been reported to have the following positive effects: bleaching of wheat carotenoids (Coultate, 1996; Cumbee et al., 1997; Liu, 1997), oxidative improvement of the rheological properties, increased mixing tolerance and improvement of the loaf volume (Hoseney et al., 1980; Grosch, 1986). Soy flour in bread has enhanced bread crust colour and improved browning in bread mixes, pancakes and waffles when used at 2% to15% (Matlani, 2002). Soy bread has been made from a combination of full fat and defatted soy flour and barley flour (10 - 15%) substituted for wheat flour with acceptable organoleptic results (Dhingra and Jood, 2001).

Despite the adverse organoleptic properties from the soy flours, the protein rich soy flour facilitates greater water absorption, improves moisture retention, cake tenderness, crumb texture and colour. It improves dough handling and machineability in cookie dough, retards fat absorption in doughnuts (Matlani, 2002). Nutritional quality is improved with the addition of soy flour in most of these studies (Yousseff et al., 1976; Surana, 1973 and Dhingra and Jood, 2001). The lypoxygenase activity results in whiter bread. Currently, enzymically active soy flour is widely employed in the commercial production of white wheat breads, where LOX is presumably catalysing the oxidation of pigments in wheat flours (Cumbee et al., 1997).

Furthermore, the introduction of soy-fortified flour into bakery products requires very little change in bakery technology and equipment. The increased water absorption of soy flour proteins requires more water for optimum dough development and less mixing than normal dough for optimum bread quality (Hoover, 1979). This phenomenon is a blessing because most bakers in underdeveloped countries use manual mixing. Soy - fortified doughs require shorter fermentation times and cakes need less shortening when using soy fortified flour. Baking techniques for composite flours using the latest methods, like, mechanical dough development, chemical dough improvers and use of additives, have all made a breakthrough in the improvement of composite bread quality (Saxena and Rao, 2003). All these factors, coupled with the fact that bread is a convenient food eaten by large numbers of the population, makes the future of soy fortified bakery products seem bright (Hoover, 1979).

1.8.3. Dough rheology, chemistry and processing

Rheology is the mechanical response of materials to stress or strain. It can be defined as the study of the deformation and flow of matter (Bushuk, 1985).
Measurement of the rheological properties gives valuable information on the quality of raw materials, the machining properties of the dough and the textural characteristics of the finished product (Faridi, 1985). In describing the two basic types of rheological measurements as fundamental and empirical, Faridi (1985) explained fundamental test results to be basic physical quantities such as stress, strain and rate of strain, whilst the empirical tests are easily performed and results could be correlated to baking performance.

The rheological properties of gluten depend on its main proteins, gliadin and glutenin. Gliadin (single-chain molecules) which forms a highly viscous mass when mixed with water about the same amount used to make dough. This property is assumed to contribute to the viscous properties of gluten. Glutenin (a heterogeneous mixture of proteins with several polypeptide chains cross-linked with di-sulphide (S-S) bonds) forms a highly elastic mass on hydration and this is presumed to contribute to the elastic properties of dough (Bushuk, 1985; Schofield and Chen, 1995).

During dough development, the giant glutenin molecules are stretched out into linear chains, which interact to form elastic sheets under the gas bubbles formed from carbon dioxide. During the early stages of mixing of the dough, the polypeptide chains of both gliadins and glutenins tend to align alongside each other and this gives an opportunity for hydrogen bond formation, and the resistance of the dough to mixing shows a sharp increase (Coultate, 1996). At peak development, the fibrils (polypeptide chains) attain maximum dimensions for optimum opposition to mixing force and thus have optimum gas retention properties (Preston, 1984).

During mechanical development, the mobile SH groups reduce rheologically active S-S groups and thereby initiate S-S interchanges which facilitate re-orientation of the protein chains required for the formation of a developed dough structure (Grosch, 1986; Preston and Kilborn, 1984; Bushuk, 1985). At break time, the dough weakens due to disruption of SS bonds from continous mixing, and this is shown by the drop of the Farinograph trace from the 500 Brabender unit (BU) line.

1.8.4. Fundamental rheological tests

Addo et al. (2001) briefly reviewed the traditional methods of dough testing highlighting that analysis using these methods involves large deformation of dough samples providing practical information that can be empirically correlated to the true viscoelastic properties of dough, while dynamic measurements are useful in measuring short time or high rate rheological behaviour at very low deformations and strains. Because they determine the fundamental viscoelastic properties of the dough, these techniques have increasingly become popular.

Khatkar and Schofield (2002b) concluded that fundamental rheological tests on gluten could be used to predict the bread-making qualities in wheat flours as they also demonstrated that the starch component had a dominating influence, masking the differences in the protein phase that is associated with flour strength. They used small amplitude frequency sweep experiments with wheat flour. Uthayakumaran et al. (2002) have also demonstrated that starch causes the lower linear viscoelastic strain limits of wheat flour doughs.

The visco-elastic properties of wheat dough are predominantly controlled by gluten proteins (Bushuk, 1985; D'Appolonia, 1977; Khatkar and Schofield, 2002a; Uthayakumaran et al., 2002) even though both starch and gluten are said to contribute to the viscoelastic response (Agyare et al., 2004). The minor components of flour also contribute to the rheological properties of wheat dough Uthayakumaran et al. (2002). Keentok et al. (2002) also emphasised the dependence of dough composition on rheological properties, highlighting that current dough rheological measurements provide further insight into molecular structure. Fundamental tests of specific parameters have also been shown to reflect structural properties of material on the molecular and microscopic levels (Loh, 1985).

The Farinograph instrument is dynamic in that its plot represents stress and strain factors during mixing and these basic factors can be fundamentally translated (Bushuk, 1985). Secondly, the mixing action imparted to the dough in Farinograph testing is reproducible and the differences in rheological properties due to flour type or composition can be determined. This method was thus found suitable to evaluate the rheological properties of soy-wheat composite doughs for bread-making.

1.8.5. The rheology of legume-wheat products

The causes of dough weakening by soy flour addition to wheat flour are still quite obscure. Surana (1973) fortified breads using 5,10,15, 20% partially defatted soy flour (PSF), and observed decreased quality scores of bread especially at higher levels of soy flour. Loaf volume was also greatly diminished. Yousseff et al. (1976) investigated the effects of supplementation levels of 0-25% chick-pea flours and 0-15% defatted soybean flour to wheat flour. On addition of soy and parboiled chick-pea flours to wheat flours, water absorption was little affected by soy flour; dough-mixing time, stability, valorimetric values increased, and the mixing tolerance decreased.

Farinogram dough development time and stability decreased with increased legume flour levels, and incorporation of stearoyl-2-lactylate (SSL) restored dough strength (D'Appolonia, 1977). The Navy bean globulins (phaseolins and G2 lectins) increased Farinograph absorption and arrival time while decreasing the departure time and dough stability. The increase in arrival time indicated a delay in the rate of hydration for the composite flour but there was not sufficient evidence to conclude that these globular proteins disrupted disulphide interchange (Lorimer et al., 1991).
Lorimer et al. (1991) also attributed the weakening of dough with addition of non-gluten forming proteins to the dilution effect, to competition of water between bean seed and wheat protein, to disruption of protein-starch complex and to S-S interchange by the foreign proteins.

Cumbee et al. (1997) reported purified isozymes (*LOX* 2) to have their greatest impact on dough extensibility and strength, whilst mutant isolines in soy meal and linoleic acid substrate caused reductions in dough strength and extensibility, indicating that products of oxidation in soy meal are detrimental to dough strength. But defatted soy flour with added LOX 2 (no linoleic acid) weakened wheat dough, and the addition of linoleic acid to the defatted soy flour (with LOX) restored the effect of lipoxygenase and this led to the conclusion that free lipids are required for the lipoxygenase effect (Hoseney et al., 1980)

1.8.6. Rheologically important thiols and disulphides

The physical and chemical properties of dough are affected by its components. In a soy-wheat dough system, proteins containing SH (thiol) and SS (disulphide) groups, lipids containing linoleic and linolenic acids are all suitable to undergo redox reactions. The vital contribution of disulphides to dough stability has been shown by previous workers in rheological studies (Jones et al., 1974; Grosch, 1986; Schofield, 1986; Lorimer et al., 1991; Grosch and Wieser, 1999). Jones et al. (1974) reported that only 25 – 30% of the total thiols contribute to dough development and tolerance to mixing, and 4% of total disulphide groups contribute to dough development time, while 11 –13% are involved in resistance to mixing. They concluded that disulphide groups stabilise the structure of dough at three levels, within protein molecules, within multi-molecular aggregates of protein and between aggregates of protein.

Thiol groups were also found to be distributed among three locations, buried in the interior of protein molecules, on the surfaces of individual molecules within the interior of large multi-molecular aggregates and exposed at the surface of protein aggregates. Those thiol groups buried within molecules would not be available under mild mixing conditions of short duration except when exposed to denaturing agents, (chemical or physical). On the other hand, thiol groups exposed on surfaces would be free to participate in exchange reactions, particularly during mixing (Jones et al., 1974).

Intermolecular disulphide bonds lead to insolubility, turbidity and increased viscosity. Addition of reducing agents like mercaptoethanol, sodium sulphite, cysteine and dithiothreitol reduces the disulphide linkages to SH groups (Kilara and Sharkasi, 1986; Schofield, 1986; Schofield and Chen, 1995). The addition of SH compounds like cysteine and dithiothreitol also increase the extensibility of wheat dough (Jones et al., 1974; Lorimer et al., 1991). Similarly the addition of oxidising agents like N-ethyl maleiImide, potassium bromate or iodate, L-ascorbic acid can oxidise the SH groups to SS bonds (Grosch, 1986; Andrews et al., 1995; Every et al., 1999; Grosch and Wieser, 1999). These SH blocking agents like N-ethyl maleimide (NEMI), L-ascorbic acid have rheological effects in blocking the endogenous SH groups which would otherwise continue to weaken the dough through SH/SS interchange (Grosch and Wieser, 1999; Every et al., 1999). Jones at al. (1974) had reported weakening of wheat dough and less tolerance to mixing from the action of NEMI adding that only strong oxidising agents like iodates increased mixing resistance and restored dough stability. Andrews et al. (1995) could not link the decrease of free SH groups to potassium bromate reaction in wheat dough. These theories would suggest that oxidation of the SH groups in dough is still not clear.

The SS-SH interchange between gluten protein (Pr-SS-Pr) and the low molecular weight thiol compounds like glutathione and cysteine are represented thus:

P-SS-P + GSH ? P- SH + G-SS-P (1) OR

P-SS-P + CSH ? P- SH + C-SS-P (2) (Schofield and Chen, 1995)

When disulphides are cleaved in this reaction, the depolymerisation of gluten proteins decrease the resistance to extension and increase the extensibility of the dough (Schofield and Chen, 1995). In soy-wheat composite flour, reaction 2 would theoretically be more dominant due to the presence of soy flour cysteine. Cleavage of protein disulphides (P-S-S-P) result in either complete reduction or single SH/SS interchange forming mixed disulphides, P-S-S-R, that can react with further protein sulphydryl, P-SH (3) and this provides an ongoing mechanism for stress relaxation during dough mixing (Schofield and Chen, 1995).

P-S-S-R, + P-SH ? P-S-S-P + R,-SH (3) (Schofield and Chen, 1995)

A distinction between rheologically important and total thiols and disulphides was shown by Jones at al. (1974), highlighting that modification of only a portion of the thiol groups produces a maximum effect on the rheological properties of the dough. Lorimer et al. (1991) reported that 19.8% of SH and 7.4% of SS were involved during mixing. When globular proteins from lama beans (10%) were added to wheat flour, they did not affect the ratios of SH and SS during mixing. Because the current work will use high concentration of soy flour (> 20 %), soy flour globular proteins will likely interfere with SS/SH interchange and affect rheological properties of wheat dough.

1.8.7. Classification of wheat proteins

Schofield (1986) reported wheat gluten proteins to represent about 80-90% of total protein in wheat flour and emphasised the importance of these proteins in bread making. In his review on flour proteins and their functionality in bread making, Schofield classified gliadins as proteins that are not extracted by aqueous salt solutions but extractable by concentrated aqueous aliphatic alcohol solutions; whilst glutenins are extractable neither in salt nor alcohol solutions, but extractable in dissociating solvents such as dilute acids, chaotropic agents (denaturating), soaps and ionic detergents. He also reported that glutenin polypeptides may be extracted in concentrated aqueous alcohol solutions in the presence of reducing agents and dilute acid particularly at elevated temperatures up to 80℃. Gliadins and glutenin are storage proteins and have a similar amino-acid composition, rich in proline, glutamine and amide nitrogen and are poor in charged residues.

Other workers have classified these proteins into two main fractions according to their solubility in aqueous alcohol, the soluble gliadins and insoluble glutenins (Grosch and Wieser, 1999). The gliadins are mainly monomeric proteins whilst glutenins are the polymeric proteins 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; Grosch and Wieser, 1999; Fisichella et al., 2003). After reduction of the disulphide bonds with reducing agents, the glutenin subunits exhibit similar solubility in aqueous alcohols to the gliadins. Based on their amino-acid composition and molecular weights, Grosch and Wieser (1999) classified them into three groups namely, 1) the high molecular weight (HMW) subunits which include the x and y type of glutenin subunits; 2) the medium molecular weight (MMW) subunits consisting of ? 5- and ? 1, 2-type gliadins and 3) a low molecular weight group containing 3 types of protein, LMW glutenin subunit, a- type and ?-type gliadins. The HMW subunits have molecular masses of 90-140 kDa and the LMW have an apparent molecular weight of 30-50kDa (Verbruggen et al., 1998; Grosch and Wieser, 1999).

Although the high molecular weight glutenin subunits form a relatively minor component of flour, while the a and ? gliadins and the LMW subunits form the major components of flour (Grosch and Wieser, 1999), the high molecular weight glutenin subunits are implicated as an important quality indicator for bread-making qualities (Schofield, 1986; Gupta et al., 1993; Weegles et al., 1995; Stephenson and Preston, 1996; Verbruggen et al., 1998; Southan and MacRitchie, 1999).

The wheat proteins play a major role in baking, affecting water absorption, redox reactions, rheology, gas retention and product quality. Because the major proteins in flour (glutenin and gliadin) possess similar amino-acid composition, few of the carboxyl groups are able to ionise. And also because of the low levels of lysine, histidine and arginine, which are capable of acquiring positive charges in solution, these proteins have low solubility. High levels of non-polar groups facilitate hydrophobic interactions, glutamine content facilitates hydrogen bonding and the presence of proline discourages a-helix formation. The stability of gluten proteins can be attributed to extensive hydrogen bonding that occurs between polypeptides of glutamine formed from highly ordered ß-structures, as this is facilitated by the carboxamide groups of glutamine side chains that can act as hydrogen bond donors or acceptors. The viscoelastic properties of gluten proteins are impaired if the side chain carboxamide groups are converted into carboxy – groups by mild acid hydrolysis or into esters by methanolysis (Schofield, 1986).

Because the gliadin fraction behaves as a viscous liquid and the glutenin fraction as cohesive elastic solid when hydrated, the glutenins and gliadins are said to be responsible for the visco-elastic properties of wheat bread (Schofield, 1986; Verbruggen et al., 1998). A linear glutenin hypothesis has been proposed by Schofield (1986), in which glutenin molecules are considered as linear chains of polypeptide subunits which are joined head to tail by disulphide bonds. When tension is applied to the ends of subunit chains, the conformation can be stretched out of its native form and when tension is removed, the structure recoils to its original conformation.

Because of high levels of non-polar groups that favour hydrophobic interactions, wheat proteins have a high tendency towards aggregation. The existence of glutamine residues and the solubility of a proportion of glutenin in urea, guanidine or detergents indicate that both hydrogen bonding and hydrophobic interactions are also involved in the association of glutenins. Intramolecular disulphide bonds are responsible for conferring stability on the folded random coil structures of gliadin, whilst glutenin subunits are formed from intra and inter – polypeptide disulphide bonds. When the temperature of gluten is raised, fluidity increases (elastic modulus decrease) until the starch begins to gelatinise and there is a dramatic increase in elastic modulus. This increase occurs at 80°C in the absence of (or with little) starch. At this temperature, additional protein-protein aggregation occurs and a network is formed due to disulphide linkages from cysteine (Davies, 1986).

1.8.8. Classification of soy proteins

The majority of soy proteins are reported to be globulins and these are extractable in water and dilute salt solutions, but are insoluble in water at their iso-electric point, pH 4.2 - 4.6 (Wolf and Cowan, 1975; Liu, 1997; Stauffer, 2002; Hou and Chang, 2004). This phenomenon is used during the preparation of protein concentrates, isolates and curd making from soy milk. Soy protein globulins are further divided into two classes, legumin and vacilin, which have been given trivial names, namely glycinin (11S) for the legumin and conglycinin (7S) for the vacilins. These names have been derived from the genus name for soybean plant, *Glycine*.

Hou and Chang (2004) reported a typical ultracentrifuge pattern of water extractable soy proteins to consist of four major fractions designated 2S, 7S, 11S and 15S, adding that 7S and 11S fractions are the major soy proteins comprising 70% of storage proteins. The 7S fraction consists of at least three major proteins, β-conglycinin, ?-conglycinin and basic 7S. Hou and Chang (2004) reported β-conglycinin to be the most prevalent of the three, accounting for 30-35% of the total seed protein and it is used interchangeably with 7S because it is the major 7S protein (trimer protein with a molecular mass of 150-200 kDa). According to Liu (1997), β-conglycinin (7S) globulins consists of three subunit proteins labeled as α^1, α and β. The α^1 and α subunits have molecular weights of 57 kDa each and the β subunit has a molecular weight of 42 kDa. The suggested combinations of native 7S globulins are as follows, $\alpha\alpha\alpha$, $\alpha\alpha\alpha^1$, $\alpha\alpha\beta$, $\alpha\alpha^1\beta$, $\alpha\beta\beta$, $\alpha^1\beta\beta$. All three major subunits are rich in aspartate /asparagine, glutamate/glutamine, leucine and arginine. α^1 and α are devoid in cysteine, and have low levels of methionine whereas the β subunit contains no methionine.

Apichartsrangkoon (2002) classified the two major storage proteins in soybean seeds as the 7S (β-conglycinin) with a molecular mass of 180 kDa, and 11S glycinin with a molecular mass of 360 kDa; as he also reported the 11S fraction to be the largest single fraction of total seed protein, comprising from 31 to 52% of the soluble soy protein. The 11S globulin was considered a hexamer model with a molecular weight of about 360 kDa (Liu, 1997) or 300-380 kDa (Hou and Chang, 2004). Each subunit was said to be composed of an acidic polypeptide (A_n) with a molecular mass of ~35 kDa and the basic polypeptide (B_n) with a molecular mass of ~20 kDa (Hou and Chang 2004). Liu (1997) reported the 11S subunits to be represented with A-S-S-B structure, where A represents an acidic polypeptide of 34-44 kDa; B is a basic polypeptide of about 20 kDa; and S-S is a single disulphide linkage between the two polypeptides. On complete dissociation, fractions with molecular weights of 34.8 and 19.6 kDa are recovered. The acidic subunits have been shown to contain three proteins with different N-terminal amino acids, leucine, isoleucine and phenylalanine. The basic subunits all contain a terminal glycine. The 11S exists as a hexamer complex at pH 7.6 and at an ionic strength of 0.5, but dissociates from a hexamer into a trimer (7S) at pH 3.8 and an ionic strength of 0.03. The 7S fraction also contains minor proteins namely, hemagglutinins, lipoxygenases, β-amylase (Liu, 1997; Hou and Chang, 2004), whilst 11S consists of 11S globulin as its only component (Liu,1997).

The soy protein subunits can be separated by the use of urea plus a disulphide reductant such as β mercaptoethanol. The reduced and denatured polypeptide units can be resolved by chromatography with DEAE-Sephadex. Basic components can be separated by chromatography with CM-Sephadex (Liu, 1997). In a mixed system, glycinin is responsible for the gel matrix structure and it is also related to the hardness and strength of gels, whereas β-conglycinin mainly contributes to the elasticity of the gels (Hou and Chang, 2004).

The functional properties of soy proteins that are relevant to baked goods include water absorption and binding, through hydrogen bonding of water molecules; cohesion and adhesion, where protein acts as an adhesive material in baked materials; elasticity through disulphide linkages (Wolf, 1975; Kinsella, 1979; Liu, 1997; Stauffer, 2002).

A summary of wheat and soy protein classification is shown in Table 1.7

Table 1.7 Summary of wheat and soy protein classification, with molecular weight ranges in kDa

WHEAT POLYPEPTIDES							SOY PROTEINS	
HMW		MMW		LMW			7S globulins 180 kDa	11S globulins 360 kDa
high M_r glutenin subunits k Da		? gliadins		a gliadins.	? gliadins	low M_r subunits		
x	y	? 5	?1,2				α^1 α β	A B
104-124	90-102	66-79	55-65	32	38-42	36-44	57 57 42	34-44 20

Grosch and Wieser (1999); Liu (1997)

1.8.8.1. Differences between 7S and 11S fractions

These two proteins exhibit differences in both nutritional and functional properties because of their different structures and composition. 11S (glycinin) globulins contain 3-4 times more methionine and cysteine per unit protein than the 7S (conglycinin) protein. The 11S protein has better gel formation ability than 7S; and 7S has greater emulsifying properties than the 11S proteins. Both forms are capable of forming gel when induced by a coagulant or heat as in tofu making (Liu, 1997). Glycinin (11S) has a larger molecular size, is less soluble in salt solutions, and has a higher thermal stability than conglycinin (7S) (Liu, 1997).

Extremes of acid or alkali denature the proteins as this process disrupts the subunit structures of 7S and 11S and other globulins. However if pH is adjusted back to neutral, this process is not reversed and half of the proteins precipitate. In extremes of acid conditions, the 7S globulins are more stable than the 11S, as the 11S globulins undergo dissociation reactions that are irreversible on neutralisation (Wolf and Cowan, 1975). In a critical review on soy proteins Kilara and Sharkasi (1986) reported that protein - protein interactions occur in soy proteins adding that 7S and 11S proteins are capable of forming intermolecular disulphide bonds leading to insolubility, turbidity and increased viscosity. However, these disulphide linkages can be readily reversed in the presence of reducing agents such as mercaptoethanol, sodium sulphite and cysteine.

1.8.9. Soy protein and wheat protein interaction in a dough system

Protein – protein association involves the specific complementary recognition of two macromolecules to form a stable assembly. Hydrophobic interactions are important to the stabilization of these associated molecules (Kinsella, 1979; Wagner and Anon, 1990; Sorgentini et al., 1995; Liu, 1997; Ryan and Brewer, 2005). Because most proteins in the soy-wheat dough system are denatured following the flour processing conditions used, interactions under these conditions may not be as specific as in biological systems, but will depend on physio-chemical forces. The fact that dough is a complex food matrix, protein interaction will also depend on the concentration of other ingredients in the dough system. Protein-protein interactions are generally favoured under conditions which reduce the net charge on the molecules (near the iso-electric point, pH 4.2 - 4.7) (Sikorski, 2001). The dough pH studied in this work ranges from 5.0 to 5.4 (wheat dough) and from 5.5 to 6.5

(soy-wheat dough up to 50% soy flour). This means that the interaction of soy and wheat proteins will depend on the soy flour concentration in the wheat dough.

Although the mode of interaction between soy proteins and wheat proteins in dough is still obscure, Ryan et al. (2002) hypothesised that soy protein and gluten interactions provided the characteristics for dough improvement and attributed the negative effects associated with soy-wheat bread to the lack of interaction between soy protein and gluten, the non-retention of carbon dioxide during fermentation from non-gluten protein, and an inability of the non-gluten protein network to temporarily bind water required during starch gelatinisation. They documented enhancement of non-covalent interactions from texturized soy flour proteins and wheat proteins after their study on protein-protein interaction in the dough matrix, in which they used lipid extracted soy flour, texturized soy flour and wheat gluten fractions on electrophoretic gels of the wheat-soy flour mixtures and found that these proteins interacted. This interaction was also increased with addition of sucrose esters. Because there was no change in the SH values from texturized soy flours and from dough containing equal amounts of wheat and soy flour, this led them to conclude that the protein-protein interaction was non-covalent.

Hyder et al. (1974) demonstrated by starch gel electrophoresis the interaction between soy protein isolate, sucrose esters and 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 (SSL) to wheat and soy proteins (Chung et al., 1981). Lorimer et al. (1991) reported that the addition of non-gluten forming proteins causes a dilution effect and a weakening of wheat dough, suggesting that several factors could cause dough weakening including; competition of water molecules between the bean seed and wheat protein, the disruption of starch-protein complex by the foreign protein and the disruption of SS interchange by the non-gluten proteins, although their investigations could not produce sufficient evidence to conclude that globular proteins disrupted the disulphide interchange system.

Lampart-Szczapa and Jankiewicz (1983) explained the role played by 11S soybean protein which they treated as a model soy protein in modifying the gluten matrix of wheat dough. Their results presented the effects of 11S globulin on the fractional distribution of the dough protein complex and average molecular weights of the fractions. Evidence that soy globulin mostly interacted with gluten proteins of dough, forming glutenin –like complexes of high molecular weight was shown by the fluorescence of proteins extracted from doughs. They suggested that the formation of soy globulin - gluten protein aggregates was counter-balanced by disaggregation of part of the gluten proteins.

Ryan and Brewer (2004) also documented the disruption of starch-protein interactions with non-wheat proteins, attributing this disruption to a lack of interaction among proteins and that soy proteins exhibit extreme hydrophilicity, which interrupts the formation of starch-protein complexes. They quantified interaction characteristics of wheat starch proteins, puroindoline, gliadin and glutenin and protein containing soy fractions including soy flour isolate, soy flour 7S and 11S protein fractions. They concluded that textured soy protein fractions displayed a higher solubility and surface hydrophobicity than their non-textured counterparts. The textured soy protein and their corresponding 11S fractions had a greater affinity for puroindoline than the non-textured soy proteins, and 11S dictated these characteristics. In their conclusion, they reported the possibility of protein-protein interaction between soy proteins and puroindoline through hydrophobic interactions and suggested that heat and sucrose addition enhanced these interactions.

The use of soy protein as a functional ingredient primarily depends on physicochemical properties that are determined by the structural and conformational attributes of the proteins (Wolf and Cowan, 1975; Kinsella, 1979; Liu, 1997). Wheat proteins have low solubility and favour hydrophobic interactions, whereas soy proteins are highly soluble and exhibit hydrophilic characteristics in a soy-wheat composite dough system within a pH range from 5.5 – 6.5. Because unextractable polymeric proteins (UPP) in wheat form part of the quality criteria for baking (Gupta et al., 1993; Grosch and Wieser, 1999; Fisichella et al., 2003), soy proteins could be modified to become more hydrophobic (less water extractable) so as to enhance their interaction with wheat protein and possibly contribute more to the rheological and baking performance in soy-wheat flour blends.

1.9. Cysteine distribution in wheat and soy flour

Cysteine amounts to about 2.5g per 100 grams of wheat flour protein, corresponding to 20-25 μmoles per gram of flour with a protein content of 10-12% (Grosch and Wieser, 1999). The well-established effects of oxidising or reducing agents on the rheological properties of dough are undoubtedly due to changes in the thiol/disulphide structure of gluten proteins, and this is brought about by the presence of cysteine disulphides (CS-SC) which represent 95 % of the total cysteines in wheat flour. Only 5% of the total cysteine exists as free SH groups and only 10% of these SH groups are present in low molecular weight compounds (mainly GSH and CSH), while the rest exist mainly in flour proteins and 99% of the total SS are present in flour proteins. Most of the a - and ? - type gliadins have only intramolecular disulphide bonds located at the C-terminal, whilst the LMW and HMW glutenin subunits are formed by intra- and inter-molecular disulphide bonds to form aggregated glutenin polymers (Grosch and Wieser, 1999; Antes and Wieser, 2000).

To explain the mechanistic basis of dough weakening by GSH, Grosch and Wieser (1999) estimated a proportion of 9000 nmol of PSSP of gluten proteins to about 50 nmol of GSH in 1 gram of flour. Based on this proportion of reactants, they hypothesised that the dough - weakening effect caused by the GSH on PSSP of gluten proteins is strongly directed. In the presence of L-AA, most GSH in the flour is oxidised to GSSG rapidly, while the SS/SH interchange reactions of GSH with gluten proteins is minimised. This is how L-AA is capable of strengthening the wheat dough (Antes and Wieser, 2000).

Soy protein contains 23 to 25 mg/g of cysteine, and 9.6 to10.7mg /g of methionine (Liu, 1997). The sulphur atom of cysteine is very reactive and involved in the formation of sulphydryl groups, making it a strong reducing agent. On the other hand, the sulphur atom of methionine is attached to a methyl group and therefore methionine is more hydrophobic, sterically larger and much less reactive than cysteine. Non-covalent forces from amino-acid side chains including electrostatic, hydrogen bonding, hydrophobic and covalent disulphide links between thiol groups of cysteine residues are expected in the soy-wheat dough system.

1.10. L-AA as an oxidising improver in wheat bread

Ewart (1990) reported that baking quality may partly depend on sulphydryl groups (SH) in flour since they lower dough strength and reduce loaf volumes, adding that improvers were capable of removing SH groups to prevent the breaking of glutenin molecules. The improving effects of L-*threo*-ascorbic acid on the rheological and bread making properties have been well substantiated and research efforts have been directed towards understanding its role and mechanism of action (Every et al., 1999). The general understanding is that L-AA is rapidly oxidised to DHAA (dehydro-ascorbic acid) in the presence of air and that DHAA is the actual improver. As revealed by Every et al. (1999), there have been many postulates as to the improving mechanism of DHAA on bread, the first being that AA inhibited proteases which were detrimental to dough. Later it was discovered that the improver effect of

LAA was stereo-specific for L-AA or L-DHAA and the enzyme dehydro-ascorbate reductase {glutathione *dehydrogenase, (*GSH-DH, EC 1.8.5.1)} was implicated in this improver effect (Elkassabany and Hoseney, 1980; Grosch, 1986; Sarwin et al., 1993; Grosch and Wieser, 1999; Koehler, 2003). In a major hypothesis, Grosch and Wieser (1999) proposed that GSH-DH catalysed the oxidation of GSH (reduced glutathione) to GSSG (oxidised glutathione) by DHAA during the mixing of wheat dough. In this review, they highlighted a sequence of reactions leading to the rapid removal of endogenous GSH that would otherwise cause dough weakening by promoting SH/SS interchange with gluten proteins.

L-*threo*-AA + ½ O_2 $\longrightarrow$ L-*threo*-DA + H_2O (1) Grosch and Wieser (1999)

L-*threo*-DA + 2 GSH $\xrightarrow{\text{GSH-DH}}$ L *threo* -AA + GSSG (2) Grosch and Wieser (1999)

During further mixing of the dough, GSSG reacts with protein thiols and cysteine residues (reactions 3 and 4).

GSSG + PSH $\longrightarrow$ PSSG + GSH (3) Grosch and Wieser (1999)

GSSG + 2CSH $\longrightarrow$ CSSC + 2 GSH (4) Grosch and Wieser (1999)

The oxidation of S-H to S-S bonds induces important rheological properties in dough including an increase in free lipids, increase in mixing tolerance as well as an improvement in the relaxation times of the dough (Pomeranz et al., 1969; Hoseney et al., 1980; Chung and Tsen, 1981). The rheological effects of SH-blocking agents like N-ethyl maleimide (NEMI) and L-ascorbic acid have also led authors to conclude that the mechanism based on this improving effect involves the removal of endogenous SH groups, which would otherwise continue to weaken the dough through SH/SS interchange. The SS - SH interchange between gluten protein (P-SS-P) and the low molecular weight thiol compounds like glutathione and cysteine can be represented thus;

P-SS-P + GSH $\longrightarrow$ P- SH + G-SS-P (5) OR

P-SS-P + CSH $\longrightarrow$ P- SH + C-SS-P (6) Sarwin et al. (1993).

When protein disulphides are cleaved in this reaction, the depolymerisation of gluten proteins decreases the resistance to extension and increases the extensibility of the dough (Sarwin et al.,1993). It is also important to understand that the ratio of GSH to the thiols that are not substrates of GSH-DH affects the postulated mechanism of dough strengthening by L-AA. Grosch and Wieser (1999) pointed out that doughs, which contain too much of the low molecular weight thiols may be negatively affected with L-AA addition. The soy-wheat dough is one such system to contain high levels of SH groups which are not substrates of GSH-DH. The presence of soy flour in a wheat dough system is also likely to induce reactive radicals from lipoxygenase activity. These radicals are likely to participate in the redox reactions in the soy-wheat dough.

1.11. Structural changes during baking

The gelatinisation of starch during baking is important as it explains the transformation of viscous dough or batter into a solid baked product. Wheat flour is favoured for bread making because of the capacity of its protein to combine with water to form gluten. During baking the heat causes the gluten protein to coagulate and release the bound water to starch for gelatinisation. The extent of gelatinization depends upon the amount of water available, the presence of salts, sugar, fats and emulsifiers. The gelatinization process starts at lower temperatures and progresses depending on the water available. The mode of release of water is critical; that is, the more evenly the water is released to the starch, the finer and more even the porosity and the crumb texture of the bread (Kumar, 2002).

The rheological properties affect the transformation of the dough during baking with the polymerisation of proteins through SS/SH interchange enhancing the transformation of the viscous dough into an elastic baked product. The viscosity of the dough decreases at the first stages of baking. When the temperature rises above 60°C, viscosity rises sharply (Bloksma, 1986). Expansion of the dough is caused mainly by the production of gas from the yeast or chemical-leavening agents. This expansion can be hindered by the resistance to viscous flow of the material between the gas cells. The breaking strength of dough between gas cells determines the extent to which gas cells combine and this affects the expansion of the dough, the fineness and homogeneity of crumb structure. Gas production will increase pressure and speed up gaseous diffusion to the outside so as to keep pace with evaporation outside and this, together with changes in rheological properties leads to loss of gas retention.

The original structure with separate gas cells is transformed into a sponge-like structure (Bloksma, 1986).

1.11.1. Differential scanning calorimetry (DSC) application in dough systems

The importance of starch gelatinisation in cereal-based products has been highlighted by many workers (Zeleznak and Hoseney, 1987; White and Lauer, 1990; Biliaderis, 1992; Chinachoti, 1994; Addo et al., 2001; Agyare et al., 2004). Thermally induced interactions among wheat flour components have also received attention (Addo et al., 2001; Vittadini and Vodovotz, 2003; Kobylanski et al., 2004; Agyare et al., 2004). In all these reviews, applications of thermal analysis in studying heat related phenomena in foods and their constituents have used the differential scanning calorimetry (DSC) approach. This technique detects the heat-flow changes associated with both first order transition (melting) and second order (glass transition) of polymeric materials (Billiaderis et al., 1986; Biliaderis, 1992). DSC reveals the endothermic event at temperatures similar to those at which structural changes are observed at a molecular level.

Research has been focused on testing the physical properties of fresh dough and the relationship of the rheological behaviour of wheat dough to the physical properties of the final product. Fundamental rheological studies have also been conducted to monitor specific changes in the visco-elastic properties of wheat dough during heating and subsequent cooling (Addo et al., 2001; Agyare et al., 2004). Of particular interests are studies from White and Lauer (1990) where starch gelatinization in baked foods was studied using DSC and the relationship between starch gelatinisation temperatures and percentages of flour, water and fructose or sucrose was determined. They successfully developed a useful model equation which was used to predict the onset of starch gelatinisation in cake formulations. Kobylanski et al. (2004) used DSC to study the interactive effects of water, hydroxypropyl-methylcellulose (HPMC) and egg white on the thermal properties of gluten-free bread dough consisting of a blend of corn and cassava starches. Their findings revealed interactions between each of the components on the thermal properties of the dough and successful dough formulation was achieved through optimisation of the variables. Agyare et al. (2004) studied the effects of substituting shortening with structured lipid on the rheological and thermal properties of soft wheat flour doughs using DSC. The thermal properties of wheat flour and of individual flour components were also studied using DSC (Adoo et al., 2001).

The current work would be similar to the work done by Okechukwu and Rao (1997) who studied thermal transition properties of dispersions of corn starch and cowpea protein (10% solids) using the differential scanning calorimeter. Blends of protein and corn starch (90% moisture) produced a single DSC endotherm peak which gradually broadened into a bimodal peak as the protein/starch ratio increased. They attributed this thermogram to an overlap of two transition processes namely, protein denaturation and starch gelatinisation. The transition onset and peak temperatures for the protein-cowpea mixtures increased from that of starch while endotherm enthalpy decreased proportionally with increase of levels of cowpea protein. They concluded that the trend in starch transition temperatures was an indication of hindrance to starch gelatinisation by the cowpea protein.

Soy proteins form the major component in soy flour (> 40% w/w) on dry basis. The diversity of physio-chemical properties brought about by soy protein's nature and flexibility define the functionality of these proteins in food systems (Wolf and Cowan, 1975; Kinsella, 1979; Liu, 1997; Kilara and Sharkasi, 2002). In cereal based products, the structural components responsible for thermal events such as glass transition and melting are starch and gluten, as these are also the key factors to determine the desired textural qualities (Chinachoti, 1994; Addo et al., 2001; Kobylanski et al., 2004). Thermal gelation of globular proteins involves partial unfolding of protein molecular strands at temperatures higher than the transition onset, and subsequent aggregation, association and cross-linking to form a gel network. The gelation of 7S and 11S proteins by heat has shown that gelation rate is first order {(latent heat of transformation) absorbs or releases heat while melting} and this rate increases with heating temperature (Nagano et al., 1992). Starch gels are composites in which intact and ruptured swollen granules are embedded in a continuous gel network composed of amylose or a mixture of amylose and amylopectin depending on the type of starch and pasting properties (Okechukwu and Rao, 1997). During a review on important factors influencing protein-starch interactions, Okechukwu and Rao reported that in starch-protein systems, the component with the lower transition temperature would form a continuous gel network first, adding that if a starch network is formed before protein gelation, the two structures would supplement each other without any specific interaction to form a complex system with two continuous networks. On the other hand, if a protein network is formed first, the diffusion and aggregation of amylose and the swelling of starch granules is hindered. In this case, starch will not form a continuous network but will act as filler and reinforce the protein network. This theory would be inconsistent with their finding on delayed onset and peak temperatures and on decreased enthalpies for starch gelatinisation during the heating of starch and cowpea mixtures. Soy protein denaturation (published values) exist in two phases, 70 -75ºC and 80-90ºC for 7S 11S soy protein respectively (Wagner and Anon,1990; Puppo et al., 2004), while wheat starch gelatinisation ranges from 54-86 °C (Michelle, 2002; Blanshard, 1987) or 61 to 76 °C (White and Lauer, 1990; Blanshard, 1986; Kobylanski et al., 2004). Therefore, it may be theoretically anticipated that during the heating of soy-wheat dough, 7S soy protein and wheat starch gelatinisation would undergo a melting transition concurrently while the melting of 11S soy protein may exist at a higher temperature (>80 °C).

1.11.2. Starch gelatinisation

Starch is a polymeric mixture of essentially linear (amylose) and branched (amylopectin) a-D-glucan molecules, as it is also a major structure – forming food hydrocolloid (Billiaderis, 1992), who also highlighted that small amounts of non carbohydrate constituents in native starch contribute to its functionality in food systems. The amylopectin and amylose (inside the starch granule) are believed to be crystalline while the surface granule is thought to be amorphous (Olkku and Rha, 1978).

Starch gelatinisation in wheat flour dough undergoing baking has been reported as an important transition that determines the texture of the final product, the baking process as well as the thermally induced interactions among the wheat fractions (Addo et al., 2001; Kobylanski et al., 2004). Starch gelatinisation has been described by many theories. It has been described as a property of starch granule mainly in cereals, roots and tubers and it is the result of heat induced interaction between water and starch granules which results in gelatinisation and disintegration (Olkku and Rha, 1978; Iwe et al., 1998). Blanshard (1987) and Michelle (2002) explained the term gelatinisation as the loss of order, which can be seen as loss of birefringence and X-ray crystallinity and also as the irreversible swelling of the starch granules. Cooke and Gidley (1992) described starch gelatinisation as the collapse or disruption of molecular orders within the starch granule, which in turn cause the swelling of these granules. The starch transformation sequence has been described (Liu et al., 2002; Sopade et al., 2004) as granule disruption followed by hydration, swelling and solubilisation of starch molecules produced by the application of heat to starch-water slurry. Gelatinisation is believed to result from the formation of a three-dimensional network which binds the swollen granules, (Olkku and Rha, 1978). Bread baking, pasta cooking and thickening of sauces are some of processes which depend on proper starch gelatinisation for the desirable end product texture and consistency (Olkku and Rha, 1978). The process of gelatinisation in wheat flour is enhanced when the gluten phase is transformed from a viscous dough to an elastic material, during which the process of protein denaturation releases water from the gluten proteins, to be used for starch gelatinisation (Bloksma, 1986; Therdthai et al., 2004). Factors influencing starch gelatinisation properties include starch molecular size, amylase to amylopectin ratio, granular size, surface-active agents and inorganic salts. The gelatinisation temperature of native starch is well documented in literature, 54-86 °C, 58-95 °C, 66-100 °C, 68-96 °C, and 52-70 °C for wheat, potato, rice, maize and cassava, respectively (Blanshard, 1987; Michelle, 2002).

Chapter 2

Background information and survey results

2: Table of Contents

Page

Abstract

This chapter explores the demographic and socio-economic status of areas, including rural populations of Zimbabwe (and those from other developing countries), that are likely to be the main beneficiaries of this project. It also covers the background information including findings from baseline and follow-up surveys (conducted by the author of this thesis) during the year 2000 and 2004 in an attempt to assess the nutritional status for children of 5 years or less, general dietary patterns, soybean production and consumption patterns, and general household information for two communal areas in Zimbabwe: firstly, the soybean consumer area and secondly, the non-soybean consumer area. Methods used for data collection during surveys included, secondary data collection from clinics (growth monitoring cards), anthropometric measurements and participatory rural appraisals (PRA), including household interviews and structured questionnaires for food production and consumption patterns.

Results from surveys revealed that soybean consumption was mostly in the form of bakery products and that constraints to the adoption of soybean processing have been attributed to lack of production skills and increased input costs for the production of soybean grain, unavailability of low-cost small-scale processing equipment and inadequate research infrastructure to address parameters for quality soybean products. Results for the nutritional status of children initially suggested that the prevalence of stunting and wasting in children increased from 19 to 24 % in the soybean consumer area and from 9 to 10% in the non-soybean consumer area. The comparison of the impact of soybean utilisation on the nutritional status of soybean and non-soybean consumer children was unsuccessful due to the food crisis in Zimbabwe, as non-soybean consumers benefited from food-handout programs {corn-soy porridge from Non-Governmental Organizations, United Nations Children Emergency Fund (UNICEF)}. Because of this development, the nutrition for non-soybean consumer children was not adversely affected (as expected), compared to that of the soybean consumer children.

In order to address some of the constraints to soybean processing and utilisation revealed from the surveys, the broad objective of this PhD study (to enhance protein functionality in soy-wheat bread by making use of physically modified soy flour that would suit small-scale farmers and village users) is defined at the end of this chapter.

2.1. Introduction

In developing countries, women and children suffer from infections associated with malnutrition due to insufficient food and nutrient intakes; one of the most common health problems is protein energy malnutrition. The use of local agricultural products, in particular the less costly and available plant protein in alleviating malnutrition and the use of low-cost processing technology, has been highlighted by several workers (Bressani et al., 1982; Neilson et al., 1979; Nnanyelugo et al.,1997; Sanginga, 1999; Maforimbo, 2001; Obatulu, 2003).

Soybean food acceptance and popularity have increased over the past few years due to the health benefits of soy foods. Consumers have become aware of the link between diet and health. Published papers, articles, and ongoing studies are proving that soy foods are not only nutritious, but have the potential to prevent and treat the world's deadliest and debilitating chronic diseases (Malnutrition matters, 2001). To date, soybean has found its worldwide uses in livestock nutrition, soy derivatives and soy foods. Soy protein product acceptance has grown because of its diverse functional properties, its abundance and low cost. In formulated foods, Food and Drug Administration (FDA) standards do not inhibit the use of soy protein ingredients in the development of non-meat foods, including ready-to-eat cereals, soups, cooking sauces and condiments, cookies, snacks and non-standard breads (Endres, 2001), but labelling is important to highlight its nutritional and health benefits.

Before 1980, soybean production in Zimbabwe had been marginalized to commercial farmers. The seed was mainly exported or used for oil extraction by the national food manufacturers. The remaining cake after oil extraction was used as a livestock feed until 1980, when this defatted cake was used to produce soy mince by some national food companies. This soy mince product was however not superior to the textured soy protein produced in developed countries, where isolated soy protein is used for the production of texturised vegetable products. Production scientists dominated Zimbabwe's agricultural development planning, and as such, investment in the creation of high yielding, disease resistant and drought tolerant crop varieties was the priority. The post-harvest production activities, such as handling, storage, processing, preservation and marketing, did not form part of these programs. Up until 1996, a lot of research has been carried out and the multiple effects of soybean for the small-holder farmer have been realised in Zimbabwe. Soybean has shown great potential to alleviate poverty through soil fertility improvement, improved human nutrition and enhanced farm incomes for the rural populace. Sanginga (1999) provided a strong case for the promotion of soybean as a cheap solution for malnutrition and a means of poverty alleviation for poor people. In his *ex-post* impact case study on the introduction of soybean production and utilisation technologies to Nigeria, Sanginga and others revealed that the nutritional status of children was significantly better in soybean consumer households than in non-soy consumer households.

Research work on soybean production has been set up by a National Soybean Promotion Task Force (SPTF) team, University of Zimbabwe and Soil Productivity Research Laboratory (SPRL), a branch under the Ministry of Agriculture. The research was mandated to extend soybean to small-holder farmers in an effort to boost food security. Soybean production and utilisation have since taken a leading role among other developing projects in upgrading the living standards of the rural people. Since then, Zimbabwean rural communities have realised the nutritional and economic benefits in the processing and utilisation of soybean and as such, soybean has been successfully incorporated into the diets of most people among several communities in Zimbabwe through the efforts from SPTF, SPRL and other non-governmental organisations (NGOs).

2.2. The demographic and socio-economic status of Zimbabwe

According to the Zimbabwe National Population Report for 1992, Zimbabwe had a current projected population of about 14 million people. Females were reported to constitute 52% of the population in Zimbabwe, whilst 68% of this population lives in the rural areas (Zimbabwe National Population Policy, 1998). Infant and child mortality rates were shown to increase from statistics done in 1988 to 1994.This was attributed to the HIV/AIDS pandemic, droughts and deteriorating social conditions (Zimbabwe National Population Policy 1998). Because of the increased rate of malnutrition that has been reported to increase from 13% in 1988 to 17% in 1994 {Zimbabwe Demographic Health Survey (ZDHS) 1994}, programs have also been put in place to address malnourishment in children in Zimbabwe.

A Poverty Assessment Study Survey in 1995 showed that over 65% of households in Zimbabwe were living below the Total Consumption Poverty Line. The poverty levels are likely to have increased since then, given the prevailing economic hardships. Women are over-represented in agriculture and unpaid family work, after the males migrate to urban areas in search for jobs in the formal sector.

This and other social factors make women marginalised in most development aspects. The Government, on realising this, has put in place a gender policy to try to address these issues and to ensure that women are included in the country's mainstream of development.

Figure 2.1a

Figure 2.1b

Figures 2.1a and b show women participants (farmers) in the soybean utilisation program during a field day in Zimbabwe (2001).

Because the Zimbabwe economy is based largely on agriculture, food security at the household and national levels plays an important role. There has been a decline in the production of the staple food, maize, mainly due to climate, declining soil fertility and increasing costs of inputs. This has resulted in food insecurity in most communities. This situation has forced many households to engage in informal income-generating activities. The need to improve the position of small-scale farmers and their families, particularly the communal and resettlement areas of Zimbabwe, is a top priority in the Government's policy (Zimbabwe National Population Policy 1998). Because of the increasing levels of unemployment and economic hardships, the informal sector of employment will assume an even greater role to meet the livelihood requirements of the people (Cavendish, 1999; Sanginga, 1999).

Malnutrition continues to inflict mortality in Zimbabwean communities due to poverty and the AIDS pandemic. Results in Table 2.1 highlight the poverty status across a number of communities in Zimbabwe.

Table 2.1 Poverty indicators across some rural and urban sites in Zimbabwe

District	Under-weight Children (%)	Living Standard Deprivation (%)	Human Poverty Index
Guruve	17.0	21.9	21.3
Plumtree	12.8	20.8	21.0
Goromonzi	6.4	18.5	19.5
Chipinge	27.9	21.6	18.3
Harare *	5.9	2.7	12.8
Bulawayo *	5.4	4.9	12.1
Mutare *	9.3	2.0	11.7
Bindura	4.5	2.0	11.7

** Urban centres*

Source: Human Development Report, United Nations Development Program, 1999

Table 2.1 compares two poverty indicators, the percentage of underweight children and that of living-standard deprivation, to human poverty indices (HPI) in Zimbabwe (United Nations Zimbabwe Humanitarian Report, 2005). The urban centres in the study sites had low HPI values whilst the rural areas had high indices, indicating that the rural communities are poorer than those in urban sites.

2.3. Baseline and follow-up surveys

Reports on this section are findings from the surveys conducted by the author of this thesis, who was the principal investigator for a project on soybean processing and utilization for the rural households in Zimbabwe (2000-2002). With the assistance from Agricultural extension officers in the rural farming areas surveyed, baseline and a follow-up surveys were conducted in the year 2000 and 2004 respectively, to assess the nutritional status for children of 5 years or less, general dietary patterns, soybean production and consumption patterns. General household information of participating households was also collected. The baseline survey study compared the growth patterns of children (1-5 years) from soybean-consumer households (Mrewa area) and non-soybean consumer households (Mhondoro area), while the follow up survey was done to follow up the nutritional status for the same children, for soybean production and consumption patterns, and for general household information from the same participants surveyed in the year 2000. There was a decline in numbers of participants during follow-up surveys and this was attributed to the migration of rural farmers to resettlement areas as part of the government's land-redistribution program. As a result, measurements were done with the available number of children and, children from those families who started soybean utilisation at a later stage after 2001 (in Mrewa) were included. Similarly in Mhondoro, children from pre-schools were picked in order to supplement the total number of controls (non-soy consumers).

2.3.1 Field sites for surveys

The survey population comprised small-scale communal farmers from two districts in Zimbabwe. The first study was conducted in Mrewa District of the Mashonaland East Province, which is about 80 km east of Harare in Zimbabwe. The area is a high rainfall area receiving about 750-1000 mm per year, and it lies in Natural Region IIa, which is an intensive farming area. It has a total land area of about 5694 square kilometers. The district comprises Mangwende Communal Land, Chitowa Small Scale Commercial Farming Area (SSCFA) and Macheke, Large Scale Commercial Farming Area (LSCFA), each with total land area of 2033, 113 and 3548 square kilometers, respectively. Soils are generally poor especially in the communal areas. They are mainly grained sands and sandy clay loams derived from granite. Patches of red clay soils are found especially in the SSCFA and LSCFA. According to the 1992 population census, Mrewa district has 152 505 people, which accounts for 14.7 per cent of the provincial population. The population growth rate was reported to be 2.2% compared to the national rate of 3.2%. At the time of the study, the population was estimated to be 200,000. The district is characterized by very high population density, 43 persons per square kilometre. The average Zimbabwean population density is 27 persons per square kilometre. Mrewa district is among the country’s bread baskets (Mauricio and Bellon, 1999).

The second study was done in Mhondoro communal area which is located in Mashonaland West Province of Zimbabwe. Mhondoro is relatively close to Harare (100-120km) and thus farmers have relatively important farm and non-farm opportunities. Most of the soils in the area are sands of granite origin although there are some patches of red clay soils which are mainly found in the small scale areas. Some research on soil fertility has been conducted in the area by the Department of Research and Extension Specialist Services, CIMMYT and the University of Zimbabwe. The soil fertility work in the area is mainly centred on liming and cereal-legume rotations (Mauricio and Bellon, 1999).

2.3.2 Data collection and analysis

Several methods of data collection were used including secondary data collection from clinics, anthropometric measurements and participatory rural appraisals (PRA) including household interviews, structured questionnaires for food production and food consumption patterns (Appendix 2.1).

2.3.2.1 Secondary data from growth monitoring cards

A review of secondary data was conducted to elicit background information on the nutritional aspects of children under the age of five years in the two districts from 1996 to 2002. The growth-monitoring master card from the two district clinics (Mrewa and Mhondoro) was used to capture secondary data for nutritional information. The growth-monitoring cards, as the name suggests, provide a simple and practical method to monitor children's growth as they also provide the easiest and quickest method to detect disease, developmental and nutritional problems for children from birth up to five years. Weight is the most sensitive indicator of a child's growth, while most workers have also highlighted growth as the main indicator for health in childhood (Mascarenhas et al., 1998; Reinhard and Wijayaratne, 2002: Cogill, 2003; Obatulu 2003).

The growth-monitoring card reveals the child's weight in kilograms against the age in months (Figure 2.2). The centiles, which are solid lines drawn on the card, are symmetrical above and below the median (50th centile) curve. A child with normal growth, (average height and weight) would follow the 50th centile throughout life (Secker, 2004; Mascarenhas et al., 1998; www.doh.gov.za). In order to obtain a normal range of weights, upper (97th) and lower (3rd) centile-reference curves are also plotted (above and below the 50th centile curve) on this card as cut off points. If a child's weight falls above (97th) or below (3rd) centiles, the child is classified overweight or underweight. However, if a child's weight is near or below the fourth centile reference (or below 60% of the average weight) the child is likely to be seriously malnourished (www.doh.gov.za). The National Centre for Health Statistics (NCHS) growth chart uses the 5th and 95th centiles as cut-offs, whilst the WHO's international reference uses the standard deviation scores (SDS) also known as z scores (Secker, 2004).

Figure 2.2

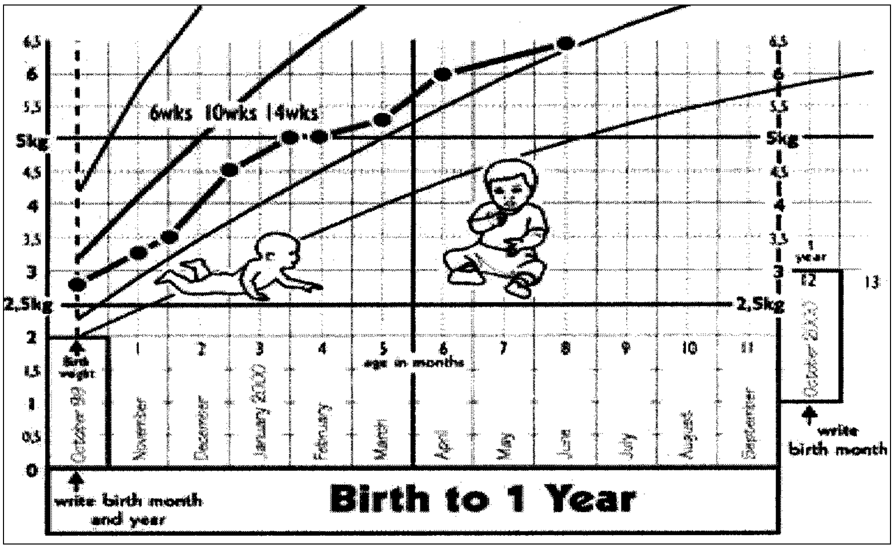


Figure 2.2 shows an example of a growth monitoring card highlighting four centiles and a child's growth curve (bold with dots).
Source:www.doh.gov.za (2005)

2.3.2.2 Anthropometric measurements

In order to supplement the secondary data, anthropometric measurements (height, age and weight) of children (1-5 years) were carried out. 72 children from soybean consumer households (Mrewa area) and 72 children from non-soybean consumer households (Mhondoro area) were sampled. During participatory rural appraisals, consent was obtained from parents of participating children and village authorities in accordance with the Ethics values. In the Mrewa communal area, purposive sampling was done to ensure that all sampled children were from households participating on a soybean program.

In anthropometry, the three common indicators of nutritional status of children <5 years are stunting, wasting and underweight (Reinhard and Wijayaratne, 2002; Mamabolo, 2004; Steyn et al., 2005). A common classification applied in child nutrition is the deviation from the median reference weight, where children below or above 2 standard deviations from the median reference (taken as cut-off point) are considered malnourished. Alternatively if children's weights fall below 70% of the median weight (3^{rd} centile), they are classified as malnourished, whilst those below 60% (4^{th} centile) are severely malnourished (Cogill, B., 2003; Reinhard and Wijayaratne, 2002). For anthropometric data in this study, stunting and wasting in children were classified using information from the National Centre for Health and Statistics, also as recommended by World Health Organization (NCHS/WHO) tables.

Figure 2.3 Figure 2.4

Figure 2.3, Collection of data (anthropometric measurements) in Zimbabwe (2004). Figure 2.4, Participatory rural appraisals with one of the groups in Zimbabwe (2001).

2.3.2.3 Participatory Rural Appraisals (PRA)

Participatory Rural Appraisal tools were also used in data collection. A total of 6 focus group discussions from 6 rural communities were held with farmers to elicit information on dietary patterns, crop production patterns, soybean and other crop consumption patterns. Pictures taken during surveys are shown (Figures 2.3 and 2.4).

2.3.2.4 Structured questionnaire

In addition to the above tools, a structured questionnaire was administered to 145 purposely sampled farmers in the 6 communal areas of Mrewa and Mhondoro. Purposive sampling was employed because of the need to include some soybean growing farmers in the sample. The questionnaire collected information on the demographic status of the households, soybean production and consumption, and food-consumption patterns. Information on general crop production patterns was also collected but was not used for the current work.

2.3.2.5 Statistical Analysis

Demographic data was subjected to descriptive analysis to obtain frequencies, means and percentages. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) (1994). National Centre for Health Statistics (NCHS) percentiles were used to classify children into categories of nutritional status and this was done using standard deviations (SD) ± 2 SD of the NCHS 50th percentiles of height for age, weight-for-age and weight-for-height recommended by James and Schofield (1990), Cogill (2003) and Steyn et al. (2005).

2.4. Survey results

This section deals with the nutritional status for children from the two district communities in Zimbabwe. Information given includes data which was gathered by the author during baseline and follow up surveys in the year 2000 and 2004.

2.4.1. Nutritional information from secondary data

Table 2.2 Growth-monitoring master card (0- 5 yrs); Mrewa District Hospital

Year	Total no. of children weighed	Number of malnourished children	Percentage of malnourished children
1996	61649	4825	7.8
1997	76735	5433	7.1
1998	67880	4609	6.8
1999	44520	3165	7.1
2000	46875	2905	6.2

Source; Mrewa District Hospital (2000)

Table 2.3 Growth-monitoring master card (0- 5 years), Mhondoro District Clinic

Year	Total number of children weighed	No. of malnourished children	Percentage malnourished
1996	4375	186	4.2
1997	5007	315	6.3
1998	4389	267	6.1
1999	5975	316	5.2
2000	4327	249	5.7

Source; Mhondoro District Clinic (2000)

Results revealed in Tables 2.2 and 2.3 are taken from secondary data collection (2000) from the two district hospitals of two communal areas studied. Although the figures for malnourished children were higher in Mrewa, figures of malnourished children (classified from the growth monitoring master card) decreased with time in the two communal areas. These figures reflect the numbers of children who were weighed at clinics or community health centres. The numbers of children given in these tables may not reflect the true numbers of undernourished children because most children could not come to clinics because of the distance they have to travel and the costs required for clinical treatments.

2.4.2. Anthropometric data

Figure.2.5

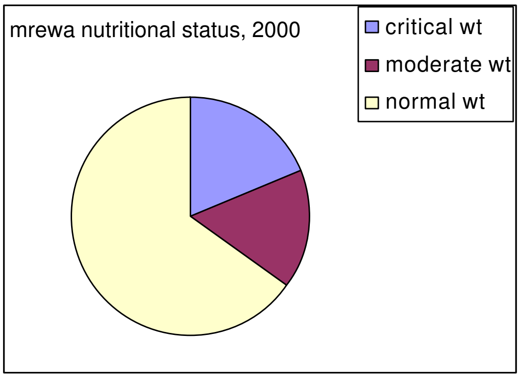


Figure 2.6

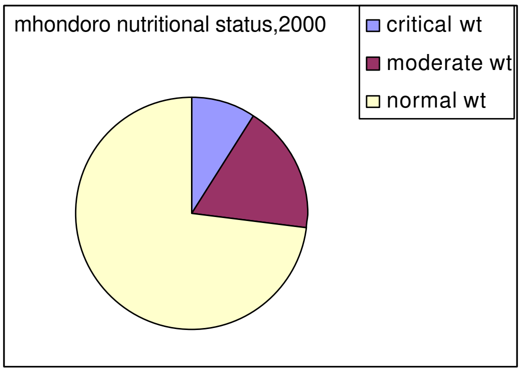


Figure 2.7

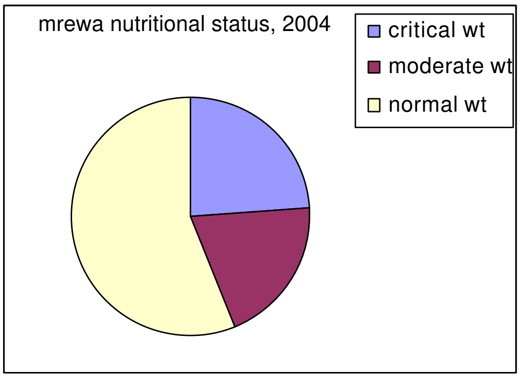
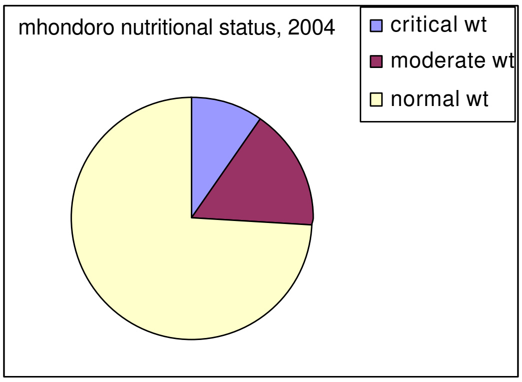


Figure 2.8



Figures 2.5 and 2.6 show anthropometric results from baseline surveys (2001) Figures 2.7 and 2.8 show anthropometric results for follow up surveys (2004).
For the baseline survey, N was 72 for Mrewa and 72 for Mhondoro; while for the follow up survey; N was 74 for Mrewa and 74 for Mhondoro (N is the number of children studied).

The pie charts, (Figures 2.5 and 2.6) compare the nutritional status of soybean consumers (Mrewa) and non-consumers (Mhondoro) studied during the year 2001. Charts 2.7 and 2.8 compare the same for the year 2004. Anthropometric measurements on weights, age and height for the children showed percentages of children with critical, moderate and normal weights in the two groups of children. The majority of the children's weights were above 10^{th} centile (> -1 SD). Children who were deficient in height for their age (= -2 standard deviations NCHS 50^{th} percentile) were classified as stunted, those who were deficient in weight for their height (= -2 standard deviations NCHS 50^{th} percentile) were classified as wasted and those who were both stunted and wasted were considered deficient in age-to-weight and therefore malnourished (James and Schofied, 1990). Percentages of children shown with critical weights (Figures 2.5 to 2.8) were stunted and wasted

[(= -2 standard deviations NCHS 50th percentile) also referred as < 3rd centile]; these children were regarded to be deficient in age-to-weight and therefore undernourished (James and Schofield, 1990). Those children below the 10th centile (< -1 > -2 SD) were classified as moderately undernourished while weights (> -1 SD) were regarded as normal.

Observations from pie charts showed that prevalence of stunting and wasting in children increased from 19 to 24 % in Mrewa and from 9 to 10% in Mhondoro, while those with moderate malnutrition (< 10th centile) increased from 16 to 20 % in Mrewa and dropped from 18 to 16% in the Mhondoro area from 2000 to 2004, respectively. In 2004, the total number of children with weights below the 10th centile in Mrewa was at 44 % and out of these children, 5.2 % were wasted, 11.2% were stunted and 27.6 % were both stunted and wasted. In the Mhondoro area, the figure for children with weights below the 10th centile was at 26 % with 1.4 % wasted, 8.1 % stunted and 16.2 % both wasted and stunted. It is clear from these figures that levels of stunting in both areas were higher compared to those for wasting suggesting that malnutrition is more of a chronic nature than acute (Immelman and Bamford, 2000) in both communal areas. It is also apparent from these results that figures of children with under nutrition have increased significantly in Mrewa (soybean consumers) compared to Mhondoro (non-consumers). This was also an unexpected development.

Figure 2.9

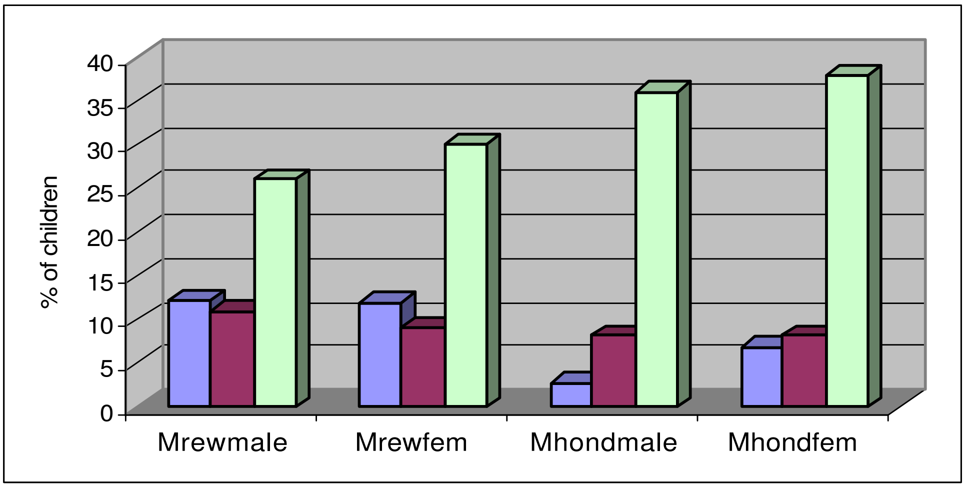


Figure 2.9 Male and female children nutritional status from soybean non-consumers (Mhondoro) and soybean consumers (Mrewa) as at 2004. Mrewmale and Mrewfem = male and female children in Mrewa; Mhondmale and Mhondfem = male and female children in Mhondoro respectively. Critical weights are represented by ¦ moderate weights represented by ¦ and normal weights = ¦

Figure 2.9 compares the nutritional status of female and male children in the two areas. Numbers of malnourished children were greater in soybean consumer children compared to non-consumer children has already been described. During the follow up survey in 2004, it was learnt that children in Mhondoro (non-soy consumers) communities were consuming more corn-soy porridge than the children in the Mrewa (soy consumers) community. Although Mhondoro was a non-soy consumer community, corn-soy porridge has been generously donated by the Non Governmental Organizations including the United Nations Children Emergency Fund (UNICEF) from 2002 to date. The composite meal has been distributed to pre-schools in most rural communities in Zimbabwe, and Mhondoro is one such area where donations have been very successful, such that children in Mhondoro have benefited from this development. This explained why nutritional status has been better in the non-soy consumer communal area. On the other hand, we learnt that children in Mrewa have not received corn-soy porridge effectively and as a result, the nutritional status of children there has been adversely affected by the national food and economic crisis. For this reason, results turned out unexpectedly but still explained the impact of soy-corn porridge on the non-consumer children who were not adversely affected by the national food crisis. Because of this development, statistical analysis to evaluate whether soybean consumption played a significant role on the nutritional status of children could not be carried out. But observations from anthropometric charts serve to highlight the impact of corn-soy porridge in controlling the numbers of malnourished children to low numbers in Mhondoro. The economic crisis in Zimbabwe has brought about constraints to the adoption of soy processing and utilisation as it has also affected the production of most staple food crops. According to the Zimbabwe Humanitarian Report released on March (2005), commercial farms averaging over 10 districts had alarmingly high stunting (47%) and high underweight (23.5%) among children 6-59 months. All three nutrition indicators were worse for orphans compared to non-orphans. Information in Table 2.4 gives a summary for the nutritional status of children studied in 2000 and 2004 with the classifications used. Z-scores represent the number of standard deviations a control is from the mean.

Table 2.4 Percentages of children and the corresponding nutritional index (z-scores) for soybean and non-soybean consumer children studied (2000 to 2004).

*N = number of children sampled, two figures given in each cell are for 2000 to 2004 respectively

Average z-scores	*Soybean consumers* **N = 74*	*Soybean non-consumers* **N = 74*
= -1.00 (normal)	65 to 56%	73 to 74%
< -1.00 >-2.00 (moderately undernourished)	16 to 20%	18 to 16.4%
Z< -2.00 (undernourished)	19 to 24%	9 to 9.6%
Z < -3.00 (severely undernourished)	0	0

The existing dietary patterns in the developing countries mainly comprise staple cereals. These cereal diets do not meet the required daily amount (RDA) of protein. As gathered from the baseline surveys, meat and legume consumption, which could provide the calorific protein required was only occasional in their diets.

Results from the questionnaire survey revealed that all respondents from the two communities studied (Appendix 2.1) utilise maize *sadza* (maize thick porridge) and maize porridge as a staple food. This observation justifies the need to promote the utilization of soy-maize meal as a staple food, thereby substituting maize meal which offers very little protein. Legume utilisation in all communities was mainly in the form of cowpeas, *bambara* nuts and groundnuts. Beef was an occasional dish for dinner as chicken seemed to be more popular for meals. These communities breed chickens as part of their livestock and therefore chicken was found to be more accessible than beef meat.

Observations from baseline surveys indicated that most people may not meet the average daily protein intake required by FAO/WHO standards, which is 0.8 g/kg body weight and 1g/ kg body weight for adults and children, respectively. Generally supplementation of protein has been achieved through the use of legumes and therefore, incorporation of soybean in their diets would be easy. Because soybean has the required amino-acid profile, this makes it a better source of quality protein compared to other legumes. Growth remains the most sensitive index of health in childhood. Obatulu (2003) pointed out that most children in developing countries experience loss of weight and impaired growth because most of the energy foods they consume do not cover children's nutritional requirements. Obatulu and several workers have also emphasised the need to use protein-rich food of plant origin that is less costly and affordable to the people with less purchasing power.

2.4.3. Structured questionnaire and Participatory Rural Appraisals

Figures 2.10 and 2.11 serve to illustrate the soybean consumption patterns in the two communal areas of Zimbabwe. The information was obtained during the baseline surveys conducted on the sites where pilot centres for soybean processing have been established.

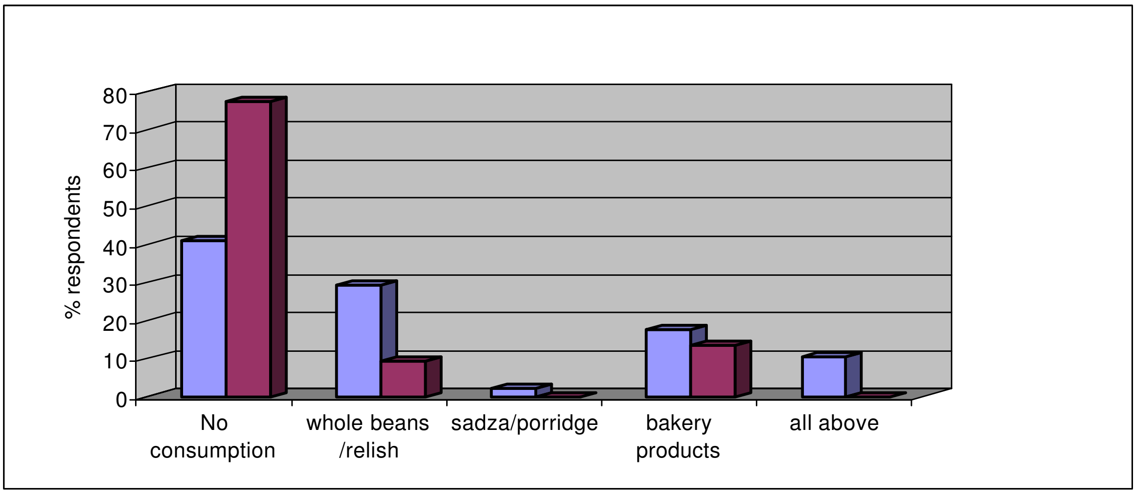


Figure 2.10, Soy consumption form versus percentage of respondents in two districts in 2000; ¦ represents Mhondoro ¦ represents Mrewa (N (number of respondents.) = 92 in Mrewa and 89 in Mhondoro.

Results in Figure 2.10 indicate that the highest percentage of respondents did not consume soybean, and that consumption of soybeans as relish and bakery products was more popular than other forms of soybean consumption as at year 2000.

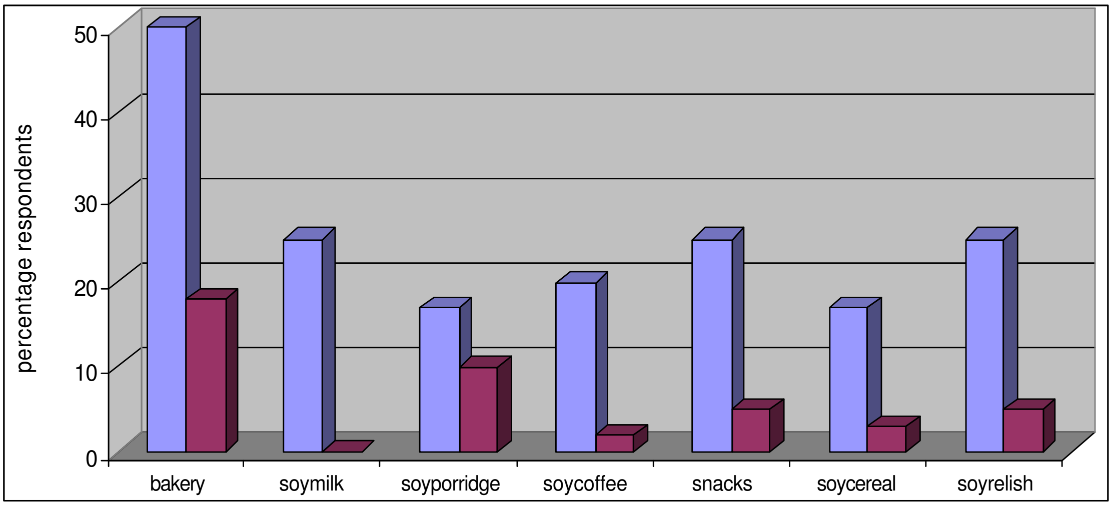


Figure 2.11, Soybean consumption forms versus the percentages of respondents as at 2004. N = 60 for Mrewa (soybean consumers) and 61 for Mhondoro (nonsoy consumers); ¦ represents Mrewa; ¦ represents Mhondoro

Results in Figure 2.11 indicate that consumption of soybean has increased tremendously between the year 2000 and 2004 in Mrewa. There has been a wider scope of soybean utilisation from the soybean consumer area in 2004 compared to year 2000, with the use of bakery products on the lead (Appendix 2.2).

2.5. Statement of the problem

2.5.1. Relevance of soybean utilisation to the Zimbabwean communities

The prevailing hardships, aggravated by the economic structural adjustment program in 1990s have made it difficult for the low-income groups, particularly those in rural areas, to afford foods from animal production due to their expense (Cavendish, 1999). This has resulted in serious imbalances in nutrient intakes, as evidenced by the prevalence of malnutrition in children and nursing mothers. Plant sources of food have assumed an even greater role in the diets of most rural households. Soybean has the potential to alleviate food insecurity and malnutrition problems due to its high protein content, characterised by a good amino-acid balance. Protein energy malnutrition (PEM) causes severe malnutrition in children and adults, and it prevents normal gains in body weight and it delays chemical maturation of the body in children. It is an important factor in economic development as it contributes to the mortality of children who do not survive for more than 5 years in Zimbabwe (Tagwirei, 1994).

The adoption of soybeans has had a positive impact on the nutritional status of children, on household income generation and distribution, on material welfare, on human capital development, on gender relations, on social equity and on other social-impact processes in Nigeria (Sanginga, 1999), with the adoption of many innovations in soybean utilisation to the extent that soybean has become a staple food in Nigeria. Zimbabwe, being a developing country has paid less attention in terms of research that is directed to the chain through which food reaches the consumer. Food technologists and scientists have to accept the challenges of inadequate knowledge, skills and new technologies in our post-harvest systems, Zimbabwe must develop adaptive, low-cost means for processing and utilisation of various cereals, legumes, fruits and vegetables for use at the village level, making use of locally-available raw materials and energy. As such, processing and utilisation of soybean must be used as a driving force to maintain and sustain the soybean-production system.

2.5.2. Relevance to women and food security

Because of the position of the woman in Zimbabwe, there are few opportunities for them, and as such, very few women are integrated into the mainstream of development, particularly in the rural situation. These communal farmers, who are mostly women, lack institutional support and have high labour requirements for production and processing. As pointed out by Gomez (1988), traditionally women carry out all the processing of crops either for the communal market or for home consumption. Primary-stage processes, like dehulling, milling, sieving and winnowing, are done by hand. The lengthy process involved, further aggravates the work-load situation of women. The introduction of a wider utilisation scope for soybean and other grains through product development, preservation and marketing will empower the women in Zimbabwe. Zimbabwe currently process only 2 % of the total crop while the rest is exported unprocessed (Dr. Muchena, 11-12 May 1999 China visit). Stimulating communal farmers in the post-harvest production process may be the key to releasing the potential, locked up in the natural resources, which must be realised if we are to avert a food crisis. Soybean utilisation will improve food security and nutrition for women and children who are the targets for undernourishment due to the hard labour and poverty. The production of soybean by women has had significant effects on long-term food security and better nutritional status of children in Nigeria because women's control of soybean production was associated with the household consumption of soybean (Figure 2.12, 2.13), thereby improving child nutrition (Sanginga, 1999).

Figure 2.12

Figure 2.13

Figures 2.12 and 2.13 highlight women during a soybean field day in Zimbabwe, (2001)

Results from surveys revealed that soybean consumption was mostly in the form of bakery products, and that constraints to the adoption of soybean processing have been attributed to the following reasons: lack of production skills to produce adequate soybean for utilisation, increased input costs for the production of soybean grain, unavailability of low-cost small-scale processing equipment and inadequate research infrastructure to address parameters for quality products.

2.5.3. Constraints to soybean processing

As highlighted by Sanginga and others (1999), the most important constraint to the adoption of soybean innovations in Nigeria related to a lack of awareness of the processing methods. This was also revealed as the most important constraint in Zimbabwe, from baseline survey results.

The common drawback to soybean utilisation was the off flavour given by the soybean products and this was mainly due to processing problems. In order to combat the off flavours given from soybean products (mainly due to the lipoxygenase activity), consumers sometimes have to pre-treat the soybeans by boiling and drying in the sun and by milling the soybeans using traditional methods or mechanical grinding mills. Bakery products that are made from this meal usually result in a heavy crumb texture, although the bean flavours from the products are successfully minimised. The resultant heavy texture in bread is possibly due to excessive and harsh pre-treatment to the soybeans which in turn compromise the baking quality of the soybean flour.

The hard-to-cook phenomenon of soybeans prepared for relish has been another area of concern to soybean consumers. This cooking process has severe implications on protein quality and energy costs. Bicarbonate of soda has been added to the boiling water before cooking the soybeans; and this has greatly reduced the cooking times, also providing great relief in terms of energy consumption. Other measures that have been used to tenderise soybeans include soaking beans in plain cold water for at least six hours before cooking. Soaking of beans in water for a longer period before cooking has been found more acceptable by consumers who found bicarbonate of soda to impart a negative flavour to the cooked soybeans.

The other major problem encountered has been poor yield and quality (shelf life) from soy-milk production. This problem has also been compounded by the unfavourable storage conditions for soy milk in rural communities and this has caused serious limitations in the production of soy milk by the small scale-farmers. Research work is needed on soy protein functionality, including the development of functional soy-protein products (at the village level) to meet the quality criteria and shelf life needed for the rural communities. To date, the production of soy curd (soy-protein precipitate) using citric acid or lemon juice has already been achieved in some of the communities in Zimbabwe. The curd has been accepted as a meat extender and some local recipes have been adopted to prepare soybean curd to suit local taste.

From the nutritional point of view, one of the drawbacks of soybean processing in developing countries would be the lack of appropriate equipment for optimisation of processing parameters. Optimal processing conditions are difficult to achieve at the village and household levels and this will remain the challenge for future work. Optimum processing parameters ensure the destruction of undesirable enzymes and microorganisms, whilst retaining essential nutrients in the processed food (Fennema, 1985). Protein quality, which is the most important nutritional aspect in soybean, is compromised with excessive thermal treatment to destroy anti-nutritional factors and during the process, essential amino acids (in particular lysine) in soybean are destroyed.

A number of training workshops have been carried out with the Department of Agricultural and Extension Services in Zimbabwe with support from non -

governmental organisations and these workshops were targeted to train women on soybean processing. This training has emphasised the removal of anti-nutritional factors including minimisation of bean flavours during soybean processing. Simple soybean recipes have been developed.

2.6. Broad objectives for this study

Although recommendations cited above may enhance soybean processing and utilisation in Zimbabwe, research is of prime importance on product and process control to exploit the full potential of soy protein, to enhance soy-protein functionality and maximize the nutritional and sensory properties for soy-based products. The success of soybean in both the urban and rural communities of Zimbabwe is going to depend on its compatibility as an ingredient in composite products, in particular in the soy-cereal bakery products which have been found to be the most popular. Preliminary sensory evaluation tests, performed during product-development surveys with rural communities (2001 to 2002), revealed that physical modification of soy flours (from thermal treatment) enhanced the sensory properties of the bakery products made from the soy flour.

In order to promote soybean processing and utilisation and to address some of the problems, this PhD study was aimed at enhancing protein functionality in soy-wheat bread by making use of physically modified soy flour in the formulation of soy-wheat bread that would suit small-scale farmers and village users. Physical modification of soy flour was chosen over chemical modification since the former would be more practical for developing countries. Currently, the rural population is using soy flour substitution at high levels in bread because of hardships in obtaining wheat flour. This work will therefore focus on high levels of the physically modified soy flour in wheat bread in order to exploit the potential of soy flour protein and to use bread as a vehicle for soy proteins in an attempt to address Protein Energy Malnutrition. The study will employ the use of L-ascorbic acid as a bread improver. L-ascorbic acid has been chosen to strengthen soy-wheat dough because of its availability and mostly because it is an accepted food ingredient.

Chapter 3

Rheological properties of raw and physically modified soy flours

3: Table of Contents

Abstract

Farinograph and Extensograph studies were conducted to evaluate the effects of physical modification of soy flour and L-ascorbic acid on the rheological properties of soy-wheat composite doughs at various ratios up to 50% soy flour. The role played by disulphide bonds in determining dough development time and resistance to mixing of soy-wheat composite doughs (rheologically important disulphides) was determined using dithiothreitol (DTT) titrations during Farinograph mixing. The rheological properties of soy-wheat doughs were correlated with bread making qualities using regression coefficients (MINITAB software). Raw soy flour (RSF) and physically modified soy flours (PMSF1 and PMSF2) were used for the preparation of composite doughs with wheat flour. For the preparation of two physically modified soy flours, PMSF1 was prepared by steam water boiling of raw soy beans for 3 minutes to partially deactivate enzymes and micro-organisms before flour preparation; and (PMSF2) by steam flushing of raw soy beans for 3 minutes at atmospheric pressure before flour preparation.

Soy-wheat composite doughs made from physically modified soy flours (PMSF1 and 2) exhibited higher resistance to extension (R_m), greater tolerance to mixing, better mixing stability, higher water uptake and water absorption than the soy-wheat composite doughs made from raw soy flour (RSF). Rheologically important disulphides were higher (88 µmoles / 100g) in the PMSF2-wheat dough compared to RSF-wheat dough (70 µmoles / 100g), suggesting that physical modification of soy flour resulted in greater mixing resistance through modification of its disulphide content. L-Ascorbic acid at 250 and 500 ppm improved R_m ($P < 0.05$) of the dough made from the RSF and PMSF1 and 2 at 50% soy flour substitution for wheat but L-ascorbic acid decreased the extensibility of the soy-wheat dough after 135 minutes of resting ($P < 0.05$). The Farinograph stability of soy-wheat dough was a reliable predictor of loaf volumes, while Farinograph water absorption was considered a reliable predictor of crumb texture and specific loaf volume ($P<0.05$).

3.1. Introduction

The use of soy flour to improve the nutritional value of wheat bread has been well recognised and it has received the attention from previous researchers. Soy provides high protein quality bread because of its essential amino-acid content; in particular for lysine content that is deficient in wheat. On the other hand, wheat flour contains which when gluten when blended with water, forms elastic dough which is capable of gas retention, hence maintaining loaf volume (Lorimer et al., 1991).

The use of non-glutenous legume flours to make composite flours with wheat flour has however resulted in adverse effects on the physical dough properties and the bread quality (Pomaranz et al., 1969; Yousseff et al., 1976; D'Appolonia, 1977; Cumbee et al., 1997; Dhingra and Jood, 2001). Although the general understanding is that legume flours weaken wheat dough, the strengthening capacity of soy flour in wheat dough has been reported by some workers and this strengthening capacity has been mainly observed at low levels of soy flour (<5%) in wheat dough. The role of soy flour lipoxygenase in improving dough mixing tolerance has been highlighted by several workers (Jocelyn 1972; Hoseney et al.1980; Grosch 1986), with the use of enzymically active soy flour at 1% (w/w) increasing the mixing tolerance of wheat dough (Grosch, 1986; Grosch and Wieser, 1999). Cumbee et al. (1997) also concluded that the purified L2 isozyme had the greatest effect among lipoxygenase isozymes on dough strength and extensibility.

The current study was designed to evaluate the effect of full-fat enzymically active, (raw soy flour) and partially enzymically inactive (physically modified soy flour) on the mixing tolerance, dough strength and stability at different levels of soy flour concentration in wheat dough. The work was also intended to get an insight into the rheological properties of the two systems and to open up an in-depth study of the interaction of soy and wheat proteins in the soy-wheat dough systems. Lipoxygenase activity is the most important quality in the use of enzymically active soy flours, while in the use of enzymically inactive soy flours, the functional and nutritional properties of soy proteins take precedence (Deneck, 1974).

Statement of the problem

The largest commercial and domestic food use of soy flour in developed and developing countries is in bakery products, yet the use of blended bakery products have not been popular in most developed countries due to the beany flavours imparted by soy flour, especially at levels above 15% soy flour substitution for wheat (Hettiarachchy and Kalapathy, 1997). Previous studies done on soy-wheat bread have concentrated their efforts on soy-flour substitution for wheat up to 20%. Results from these have indicated that organoleptic results were poor although the nutritional benefits were high (Surana et al., 1973; D'Appolonia, 1977; Grosch, 1986; Schofield, 1986; Dhingra and Jood, 2001). The deleterious effects of legume flours on bread are generally minimised by the use of surfactants (Yousseff et al., 1976; Chung et al., 1981) and oxidising improvers (Elkassabany and Hoseney, 1980; Grosch and Wieser, 1999) which improve the dough strength of soy-wheat bread but do not minimise the beany flavours.

The use of physically modified soy flour made from optimal thermal treatment of the beans, to destroy the lipoxygenase and retain the functional and nutritional properties, may be another way to get around the problem of soy product acceptance. Lipoxygenase activity leads to the accumulation of peroxides which break down during a series of biochemical reactions to form aldehydes and ketones. These volatiles in turn spoil the flavour in soybean products (Coultate, 1996; Cumbee et al., 1997). Soybean is currently being popularised in developing countries to address Food Security and Nutrition; as such, soy-wheat and soy-maize bread are slowly becoming staple foods for the under privileged communities.

L-threo ascorbic acid (Vitamin C) is widely used as a bread improver in wheat breads. Improver interaction is achieved by the oxidation of L-ascorbic acid to dehydro-ascorbic acid (DHAA) which becomes the oxidising agent (Elkassabany and Hoseney, 1980; Bushuk, 1986; Grosch, 1986; Sarwin et al., 1993; Grosch and Wieser, 1999; Every et al., 1999; Koehler, 2003). Because previous studies using L-ascorbic acid were based on wheat flour dough only, this study seeks to alleviate the deleterious bread making performance of soy-flour at high levels (> 15%) in substitution for wheat-flour, by using physically modified soy flour to alleviate the bean flavour and L-AA to strengthen the dough. L-AA was chosen for this study because of its availability, permittence as a food ingredient and mainly because it does not leave toxic compounds on the final product. From previous reports mentioned earlier, there has not been any detailed study on the use of L-ascorbic acid on soy-wheat bread. In addition, rheological properties have not been studied for soy-wheat doughs with soy flour concentrations up to 50%, and with physically modified soy flours. Therefore this study evaluated the effect of L-ascorbic acid on rheological parameters of raw and physically modified soy flours in soy-wheat composite doughs.

Firstly, the Extensograph technique was used to evaluate and compare the effect of L-ascorbic acid on the maximum resistance to extension and extensibility of the soy-wheat doughs made from the two soy-flour types. Secondly, the Farinograph technique was used to evaluate the effect of physical modification of soy flour and soy flour composition on the rheological parameters of soy-wheat doughs. Finally, because of the importance of disulphides in strengthening dough (Jones et al., 1974; Bushuk, 1985; Schofield, 1986; Lorimer et al., 1991; Chen and Schofield, 1995), rheologically important disulphides were evaluated in soy-wheat doughs based on resistance to mixing in the Farinograph. Soy flour substitution for wheat in this study was high (up to 50%). This was done to cater for the protein and amino-acid requirements which cannot be met otherwise due to domination of cereal diets in developing countries. It is estimated that high soy flour content (>30%) in soy-wheat bread would provide more than 50% of the estimated safe intakes of protein requirements from FAO/WHO. Estimated safe intakes (RDA) for protein are 10 -20g for children from 10 to 20 kg body weight; and 40 - 48 g for adults from 50 to 60 kg body weight; while those for total amino acids are 260-300 mg / kg body weight for children and 84 mg/kg body weight for adults (FAO/WHO, 1985).

Objectives

- To evaluate the effect of physical modification of soy flour, soy flour composition and L-ascorbic acid on rheological parameters of soy-wheat composite dough using the Farinograph and Extensograph.
- To estimate rheologically important disulphides in soy-wheat dough using dithiothreitol (DTT).
- To correlate rheological properties of soy-wheat doughs to their bread making qualities using regression coefficients.
- To use the rheological results to develop a more suitable soy flour for soy-wheat bread making.

Hypothesis

Physical modification of soy flour (moist heat treatment) will enhance the rheological and bread making performance of soy-wheat dough through modification of disulphide groups.

3.2. Principles of methods used

This part describes the principles of the methods used in this Chapter

3.2.1. Physical modification of soy flour

Physical modification of soy flour is better achieved through physical treatment of soybeans before they are dried and milled into flour (Kordylas, 1991). The soybeans are subjected to a controlled thermal treatment (specific temperature, humidity and pressure) either exposed to dry heat in thermostat-controlled ovens or beans are moist heated using steam flushing or wet blanching before they are rapidly dried to a constant weight or moisture. The beans are later cooled and milled to fine flour using standard sieves. Modification of soy flour results in the conformation of major soy components including soy proteins, lipids and polysaccharides. Modification of soy proteins usually results in changes in the secondary and tertiary structure of protein molecules. This process is referred to as denaturation, a phenomenon resulting in the loss of solubility for proteins, the increase in viscosity and the loss of biological activity (Wagner and Anon, 1990; Liu, 1997; Wagner et al., 2000). The process of denaturation often involves dissociation and unfolding of proteins, and is often accompanied by the formation of disulphide linkages and exposure of hydrophobic amino acids on the surface. This is why soy protein modification methods affect the functional behaviour of the proteins (Utsumi and Kinsella, 1985; Liu, 1997, Wagner et al., 2000; Puppo et al., 2003; Puppo et al., 2004).

3.2.2. Evaluation of dough rheology

3.2.2.1. Farinograph

Shuey (1984) described the Farinograph as a physical dough-testing instrument, by which the dough resistance given to the mixing blades during mixing is transmitted to a dynamometer. The dynamometer is connected to the lever, a scale system and a pen that traces a curve on a Kymograph chart. Using this technique, mixing and stretching properties of the dough can be obtained. Zaidul et al. (2004) described the Farinograph to be a dynamic physical dough testing instrument involving the measurement of a torque.

The Farinograph is most widely used to test the quality and strength of bread flours using a simple flour-water dough system. It is a dynamic instrument and a useful tool with which to study gluten, carbohydrate functionality, enzymic activity in flours, composite flour properties and special flour treatments. The resultant Farinograph curve is basically a plot of dough resistance against time as this plot also represents stress and strain factors during mixing (Preston and Kilborn, 1984). The Farinograph has proved to be highly useful in dough rheology studies because the factors measured from the Farinograph curve (water absorption, dough development times, stability) have proved to be good predictors of baking qualities. Secondly, this form of analysis is useful because the mixing action imparted to the dough is reproducible and the differences in rheological properties due to flour type or composition can be shown (Preston and Kilborn, 1984; Othira et al., 2004).

3.2.2.2. Interpretation of Farinograms

Dough development time (DDT) also defined as the peak time, is the time to the nearest half a minute between the first addition of water and the development of the dough's maximum consistency, the point immediately before the first indication of weakening (Shuey, 1984; Bushuk, 1985; Zaidul et al., 2004). Arrival Time is the time required for the top of the curve to reach the 500 Brabender units (BU) line after the mixer has been started and the water introduced (Zaidul et al., 2004). Departure Time, to the nearest half a minute is the time from the addition of water until the top of the curve leaves the 500 BU line. Stability is the difference in time between the arrival and the departure time. This value gives some indication of the flour's tolerance to mixing which is described as Mixing Tolerance Index (MTI) defined as the difference in Brabender units between the top of the curve at the peak and the top the curve measured 5 minutes after the peak is reached (Shuey, 1984; Bushuk, 1985).

3.2.2.3. Extensograph

This technique is used to test the stretching properties of flour- wheat -salt dough prepared without yeast, but it has also been used by researchers for fermented dough (Rasper, 1991). The Brabender Extensograph records the load extension for a test piece of dough stretched until it breaks. The characteristics of load-extension curves or extensograms are used to assess general quality of flour and its response to improving ingredients (Preston and Hoseney, 1991; Rasper, 1991).

Evaluation is by the use of common measurements made on load-extension charts. These include; R_m (the maximum resistance, maximum height of the curve in

BU/ cm on the kymograph), R_5 (the resistance at constant extension of 5cm,-a different constant can also be used), *E* (extensibility,- the total length of curve in centimeters), and lastly the area under the curve (A) measured with a planimeter in square centimeters. The ratio of maximum resistance to extension, (viscoelastic ratio) is given by R_m / E (Preston and Hoseney, 1991). As flour strength increases, the resistance to extension increases correspondingly. Wheat flours with a low extensibility (high R_m/E ratio) viscoelastic ratio are classified as short or bucky,while those with a low viscoelastic ratio are classified as extensible or pliable. Higher protein content wheat flours are usually associated with larger Extensogram areas and greater resistance to extension; the inverse is true (Preston and Hoseney, 1991).

3.2.2.4. Evaluation of the rheologically important thiols and disulphides

Attempts have been made to classify wheat-flour sulphur groups into reactive and non-reactive based on their chemical activity in denaturing and non-denaturing solvents. Such determinations were however reported to have poor correlation with the rheological effectiveness of thiols and disulphide groups. This was attributed to the difference in environment between the chemical environments and the natural environment in the dough system (Jones et al., 1974).

The method developed by Jones et al. (1974) to determine rheologically effective thiol and disulphide groups in wheat dough estimates the role of thiol and disulphide bonds in determining dough development time and resistance to mixing. N-ethylmaleimide (NEMI) which blocks the thiol (S-H) groups to S-S was used to estimate thiol groups. Dithiothreitol (DTT) is a reducing agent that lowers the resistance to mixing, by promoting cleavage of the S-S bonds to S-H mobile bonds and this was used to estimate disulphides.

The resistance to mixing of dough during Farinograph mixing was plotted against concentration of DTT (μmoles) added. The resultant plots show two distinct straight lines, the one on which resistance falls rapidly to a steady value and the one on which resistance remains stable. When these distinct lines are extrapolated, the point at which they meet indicates the dithiothreitol concentration (in μmoles) equal to (estimate) the rheologically important disulphides involved in the mixing resistance of this dough. The reaction between disulphides and the DTT is stoichiometric.

For the determination of thiols (SH), the loss of resistance of the dough during mixing is plotted against the concentration of NEMI. The loss of resistance is the difference between maximum resistance and the resistance at 30 minutes of mixing. On plotting, there are two straight lines, one for which the loss of resistance increases to a steady value and one on which loss of resistance remains stable. Extrapolation of the two lines gives a point of meeting that indicates the concentration of NEMI equivalent to rheologically important thiols during dough mixing. The reaction between thiol groups and NEMI is also stoichiometric.

3.3. Methods and materials

The materials and methods employed to evaluate the effect of physically modified soy flour compared to raw soy flour substitution in wheat dough on rheological characteristics of wheat dough, including the use of L-ascorbic acid in modifying the rheological properties of soy-wheat dough, are described in this part.

3.3.1 Materials

Soybeans were purchased from Meriram Pty Ltd, Everton Hills, NSW Australia. Commercial strong baker's wheat flour was obtained from Centerion Milling Company Pty Ltd. Melbourne. L-ascorbic acid, N-ethylmaleimide, dithiothreitol used were analytical grade and purchased from Sigma Chemicals.

3.3.2. Methods

3.3.2.1 Preparation of soy flours

The raw soy flour (RSF) was full fat soy flour made by milling the whole soy beans using a 0.8 mm sieve and Newport Scientific Cereal Mill 6000, Australia. The physically modified soy flour #1 (PMSF 1) was par-boiled full fat soy flour. Soybeans were dry cleaned, plunged in steam boiling water for 3 minutes to partially deactivate lipoxygenase enzyme and to denature the soy proteins. The beans were later spread on a stainless steel racks and blow dried in an oven (WTC binder model) for 5-6 hours at a temperature of 80°C for 5-6 hours to a constant weight. The dried beans were later milled to fine flour through a 0.8 mm sieve using a hammer mill, Newport Scientific Cereal Mill 6000 model, Australia. Physically-modified soy flour #2 (PMSF 2) was made by dehulling (mechanically) soybeans and flush-steaming them for 3 minutes at atmospheric pressure to deactivate 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 hours. The cooled beans were later milled as above. All flours were stored in the cold room at 4°C in paper bags placed in plastic containers. The moisture content for these flours was 6.0%, 7.3%, 5,8% and 11.57% for RSF, PMSF l, PMSF2 and wheat flour respectively Protein content (as is basis) was 33.8, 33.1, 36.1 and 12.5 g /100 g, respectively. Protein conversion factors were N ? 6.25 and N ? 5.7 for soy flour and wheat flour, respectively.

3.3.2.2: The effect of physical modification and soy flour composition on Farinograph analysis

The Farinograph was used to evaluate the effect of physically modified and raw soy flour substitution on rheological characteristics of wheat doughs. Soy flour was substituted for wheat flour from 10% to 50% (Appendix 3.1) using 3 types of soy flour, namely, raw soy flour (RSF) and physically modified soy flours (PMSF 1 and PMSF 2). The rheological parameters of doughs prepared from controls and flour blends were determined following the AACC method (54 -21). The Farinograph thermostat was adjusted to maintain temperature of 30°C. The speed of the driving shaft of the Farinograph was set to 60 revolutions per minute. 300 grams of the composite flours were mixed in a Farinograph bowl for 3 minutes before water addition to ensure a homogeneous blend before starting. As dough was forming, the sides of the bowl were scraped to remove stuck dough, and water was continuously added until the Farinograph curve leveled at the 500 BU (Brabender units) line. Titrations were repeated to obtain curve peaks that straddled the 500 BU line, as this point is considered the maximum water absorption peak. Water absorption was determined as the volume of water expressed as a percentage of the weight of flour used. A factor of 1.8 -2.2 mL of water for 20 BU was used for the water absorption correction. The average titration value for two runs was reported. Parameters for each Farinogram curve were determined according to Preston and Kilborn (1984).

3.3.2.3. The effect of L-ascorbic acid and physical modification of soy flour on the resistance to mixing during Farinograph analysis

The effect of L-ascorbic acid on the resistance to mixing during Farinograph was evaluated for soy-wheat composite dough made from RSF and PMSF 2. Soy flour was substituted from 10% up to 50% for wheat flour. 300 grams of composite flours were mixed in a Farinograph bowl for 3 minutes before water addition to ensure a homogeneous blend before start. Farinographs were run up to dough development time, after which point serial additions of L-ascorbic acid 50 mg were added at four minutes intervals up to 700mg. The final concentration of L-AA in the composite dough was 2300ppm. The resistance to mixing was recorded for each addition of L-ascorbic acid. The loss in resistance was estimated as the difference between maximum resistance at dough development point and each recorded resistance. Loss in resistance versus time and L-ascorbic acid addition curves were obtained from each soy-wheat composite type.

For the evaluation of the effect of physical modification of soy flour on resistance to mixing during farinograph, the Farinograph was run as above up to dough development time, after which the resistance to mixing was recorded every 4 minutes, (without addition of L-AA). This procedure was repeated for each soy-wheat composite type.

3.3.2.4. Evaluation of rheologically important disulphides in soy-wheat doughs during mixing using dithiothreitol (DTT)

Several workers have emphasised the important contribution of disulphides to dough stability in rheological studies (Jones et al., 1974; Grosch, 1986; Schofield, 1986; Lorimer et al., 1991; Andrews et al., 1995; Grosch and Wieser, 1999). This experiment was therefore done to evaluate rheologically important disulphides in composite doughs using enzymically active (RSF) and partially enzymically inactive (PMSF) soy. Methods from Lorimer et al. (1991) and Jones et al. (1974) were adapted to estimate disulphides important to mixing resistance in a Farinograph bowl. For the estimation of disulphides, the dough was mixed to full development and as mixing continued, 50 μmoles of DTT was serially added at four-minute intervals up to 350μmoles. The resistance to mixing was recorded for each addition of DTT.

3.3.2.5: The effect of L-ascorbic acid on resistance to extension of soy-wheat dough using the Extensograph

The experiment was done to investigate the effect of L-AA addition on the Extensograms for the soy-wheat flour blends. The soy-wheat flour blend of 1:1 ratio was mixed with the optimum water required for Farinograph curve to centre on the 500 BU line, except that water absorption was reduced by 4% for the dough containing RSF. This reduction in the water used was to facilitate handling of the sticky dough. No salt was added to the doughs; only water and flour were used.

The method after kneading was followed from the Extensograph Handbook by Preston and Hoseney (1991). Manual kneading of the doughs was done for five minutes after which the dough was divided into two pieces of 150 grams each. The pieces of dough were gently rolled into balls. They were then pressed into a mould, rested in a fermentation cabin at a temperature of 30°C and later tested on an Extensograph after 45, 90,135 minutes of rest. For the Extensograph measurement, the dough rolls were clamped onto a holder and stretched at a constant speed (1.45 ± 0.05 cm /sec). Maximum Resistance, (R_m) and Extensibility, (E) were measured. For each flour blend used, the first dough sample did not contain L-ascorbic acid, while the second and third samples contained 0.025% and 0.05% L-ascorbic acid respectively. The average of two Extensograph measurements was recorded for each sample.

3.3.2.6: Procedure for bread making

The bread making qualities of soy-wheat bread were to be correlated to the rheological properties of the breads made from RSF and PMSF flours. Composite flours were prepared from 10, 20, 30, 40 and 50% soy flour substitutions for wheat flour using three soy flour types, raw soy flour (RSF); physically modified soy flours 1 and 2 (PMSF1 and 2). Bread-making procedures were followed from D'Appolonia (1977) with some alterations. The bread-making formula included 200 g flour, 1.8 g dry yeast, sugar 10g, salt 2 g, margarine [55% vegetable oils (canola, palm, cottonseed), water, salt, emulsifiers and antioxidants] 6 g, L-ascorbic acid 50mg. Because micro baking was done for these experiments, the dough was mixed at maximum speed (Kenwood Chef Mixer) for 3 minutes and allowed to proof in a proofer at 32°C for 1 hour. The dough was taken out and further kneaded for 2 minutes and later put back in the fermentation proofer (WTC binder 78532 Tuttlingen Germany) at 40°C for 30 minutes to proof. Because L-AA is a slow oxidising agent being used as the sole oxidising agent, the fermentation time was selected to allow adequate structural development of the dough. After the second proofing, the doughs were baked in an oven (CPC Rational GmbH, D86899) at 180°C for 10 minutes. Baking tests were duplicated.

3.3.2.7. Objective evaluation

Bread crumb sample was subjected to texture profile analysis using the Texture Expert Exceed, Stable Micro Systems. Three equal pieces of bread were cut (8.3 x 5 x 1.5cm) and placed on the platform of the testing machine. A circular plate of 4 cm diameter, attached to a 25 kg load cell, was used to compress the bread crumb to 10mm distance at a speed of 1mm per second. The force in grams per unit time in seconds (g/sec) was plotted and recorded. Texture analysis was done 24 hours after baking to compare crumb softness among the three flour bread types.
Weights and volumes for the breads were determined 2 hours after cooling. Loaf volume was determined using a rice displacement method (Fleming and Sosulski, 1978). Specific loaf volumes were calculated using loaf volumes and mass, and results were expressed as cm^3/ g.

3.3.2.8. Proximate analysis

Preparation of the flour blends for proximate analysis was carried out as follows;
Each soy flour type was blended with wheat flour at 1:1 ratio and thoroughly mixed on a mechanical mixer to obtain a homogeneous mixture. Proximate analysis to estimate protein, fat, ash and moisture was done by employing standard methods (AOAC, 1995). All determinations were carried out in replicate and the results were reported as the mean ± SD.

3.3.2.9. Statistical analysis

Data was subjected to two way ANOVA (Analysis of variance) to calculate significant differences between flours and treatments. LSD (least significant difference) was calculated and used to compare the means and to find the effect of physical modification of soy flour, soy flour concentration and L-ascorbic acid addition on rheological properties. Regression coefficients for rheological properties on the bread making qualities of the soy-wheat doughs made from the three soy flour types (RSF, PMSF1 and PMSF2) were performed to determine correlations.

3.4. Results and discussion

This part describes the results, with discussion, of studying physical modification of soy flour, soy flour composition and L-AA addition on rheological properties of soy-wheat dough. The effect of physical modification of soy flour on its proximate composition is also described.

Table 3.1 Proximate composition for soy-wheat flour composites, 1:1 ratio.
± values given are standard deviations.

Sample	Moisture %	Crude protein % (N x 6.25)	Crude fat %	Minerals/ash %
Wheat	10.3± 0.2	12.0 ± 0.1	1.8 ± 0.1	0.5 ± 0.1
RSF-wheat	8.4 ± 0.2	23.9 ± 0.4	10.9 ± 0.1	2.5 ± 0.1
PMSF1-wheat	8.3 ± 0.3	23.8 ± 0.4	11.1 ± 0.1	2.4 ± 0.1
PMSF2-wheat	7.7 ± 0.2	25.8 ± 0.4	11.2 ± 0.3	2.8 ± 0.1
Mean	8.1	24.5	11.1	2.6
S.D.	0.30	0.92	0.12	0.17
CV %	3.7	3.75	1.1	6.54

RSF-wheat = Raw soy flour and wheat dough; PMSF1- wheat = physically modified soy flour 1 and wheat dough; PMSF2-wheat = physically modified soy flour 2 and wheat blend

3.4.1 Proximate composition for soy-wheat composite flours

Information in Table 3.1 shows that physical modification of soy flour did not alter the chemical composition of flours significantly. There was an inter–model similarity in chemical composition as attested by the low co-efficient of variances. There was no significant difference between the three soy flours ($P< 0.05$) in protein, fat, moisture and ash content, suggesting that physical modification did not alter the chemical composition of the soy flour significantly.

3.4.2 Effect of physical modification and concentration of soy flour on Farinograph parameters

Table 3.2 A comparison of important rheological parameters of raw soy flour, (RSF) physically modified soy flour (PMSF1) and (PMSF2), using soy-wheat composite dough. Flour composition is from 10-50% soy flour in wheat flour. Values given are means of 3 replicates, ± are SD values.

	Water abs. %			Dough Dev. Time/ mins			Arrival time/mins			Stability/mins		
Soy fl.%	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
10	67±1	67±1	67±1	5.5±0.5	6.0±0.5	5.0±0.5	2.0±0.2	1.5±0	2.0±0.3	14±1	12.5±0.7	14±1
15	68±1	70±0	70±0	7.5±0.3	7.0±0.5	6.5±0.5	5.1±0.3	4.5±0.5	2.5±0.3	10.5±0.5	8.0±0.5	12±1
20	70±1	71±0	71±0	6.5±0.5	6.5±0.8	5.0±0.5	4.5±0.3	4.5±0.3	2.5±0.3	10±1	9.5±1.5	11±1
25	70±1	73±0	73±1	8.5±0.5	7.0±0.8	5.5±0.3	6.5±0.5	5.0±0.5	2.5±0.2	9.5±0.5	7.0±0.5	10±1
30	70±0	73±1	74±1	8.0±0.5	6.0±0.5	6.1±0.3	6.5±0.5	3.5±0.5	2.5±0.2	8.0±0.5	9.0±0.5	11±1
35	69.5±0.5	74±0	76±1	11±1	6.0±0.8	5.0±0.3	9.5±0.7	4.5±0.3	2.5±0	5.1±0.3	6.5±0.5	9±1
40	69±1	75±1	77±1	10±1	6.0±0.5	5.5±0.5	7.0±0.5	5.0±0.5	3.5±0.5	5.0±0.5	6.5±0.4	7.5±0.5
45	64±1	76±0	78±0	10.5±0.6	6.5±0.3	5.5±0.3	8.5±0.5	5.5±0.5	3.5±0.3	4.0±0.2	7.5±0.3	7.5±0.5
50	63±1	75±0	80±1	7.0±0.5	6.5±0.5	4.5±0.3	5.6±0.3	5.5±0.5	3.5±0.5	3.0±0.5	5.5±0.8	8±1
mean*	68[a]	73[b]	74[b]	8.5[a]	6.4[b]	5.4[b]	6.2[a]	4.4[b]	2.8[c]	7.7[a]	8[a]	10[b]
wheat	65±1			5.5±0.5			1.5±0.5			16.0±1.5		

1 = RSF, 2 = PMSF1 and 3 = PMSF2. *mean values followed by the same letter for each parameter are not significantly different (P<0.05).

Results in Table 3.2 indicated that water absorption was generally increasing with soy flour concentration in soy-wheat dough made from PMSF1 and PMSF2. F-statistic value from two way Analysis of variance revealed that there was significant difference between the flours (P< 0.05). Farinograph absorption for RSF-wheat dough was significantly different from the other two composite doughs (P<0.05). Using LSD to separate the means, the PMSF1-W and PMSF2-W doughs were not significantly different, but the RSF-W dough was significantly different from the other two physically modified soy flours (P<0.05). This meant that the Farinograph absorption pattern was similar for the physically modified soy flours.

ANOVA results for dough development time (DDT) revealed that dough development times were significantly different among the three flour types (P<0.05), yet PMSF1-W and PMSF2-W doughs were not significantly different according to the LSD test. RSF-W dough had a significantly higher DDT compared to the other flours (P<0.05). The F-statistic value from two-way ANOVA also revealed that arrival times were significantly different among the flours (P <0.05), with the PMSF2-W dough having the value closest to that of the wheat dough (2.8 and 1.5 for PMSF2-W and wheat dough respectively). Dough stability, which is an indication of dough mixing tolerance, decreased with increases in soy flour concentrations and this was also attested by the highly significant F-statistic value (P<0.005). The LSD test indicated that the PMSF2-W dough was significantly greater in stability compared to PMSF1-W and RSF-W dough (P<0.05). Observations from the rheological data in Table 3.2 suggested that soy-wheat doughs made from PMSF2 gave rheological parameters closer to the wheat dough, except for the water absorption which was higher than that of wheat. This high water absorption is however considered to be an advantage in improving the loaf volumes during bread making.

The results on Farinograph absorption were in line with results from previous workers who have reported Farinograph absorption to increase with protein increases in wheat-legume doughs (D'Appolonia, 1977), using navy and pinto bean flours; (Lorenz, 1984; Preston and Kilborn,1984; Matlani, 2004; Zaidul et al., 2004) using sago-wheat flours, (Othira et al., 2004) using cowpea, soybean and sunflower.

Zaidul et al. (2004) described water absorption percentage as the amount of water needed to centre the Farinograph curve at the 500 BU line for a flour/water dough. They also pointed out that water absorption is one of the most important physical factors affecting a Farinogram. Generally the increase in Farinograph absorption has been attributed to the increase in starch content of composite flours (Othira et al., 2004) and increases in the fibre /hull content of the meals (Sharma et al., 1990). Preston and Kilborn (1984) reported the major factors contributing to

Farinograph absorption to be protein content, starch, in particular damaged starch, pentosans and gluten strength, adding that rheological properties of wheat dough depended primarily on the structure of gluten proteins. They attributed the control of rheological properties to the sulfhydryl and disulphide bonds of gluten proteins, adding that rheological properties were also affected by the flour treatments or flour composition. According to Lorenz (1984) Farinograph absorption decreases with increasing starch concentration in dough until a minimum is reached; beyond this, further addition of starch results in progressive increases in Farinograph absorption and this rheological phenomenon is attributed to an increase in surface area of the dispersed starch phase and to a dilution of the continuous gluten phase. Insignificant increase in water absorption with increased soy flour substitution was observed in this experiment for the raw soy flour as also reported by Yousseff et al. (1976) who revealed that water absorption was little affected by soy flour up to a 20% level of substitution. They also attributed this to the stronger water-imbibing capacity of gluten compared to the soy proteins. The relationship between Farinograph water absorption and soy flour concentration for the soy-wheat composite dough using RSF and PMSF2 is shown on Figure 3.1

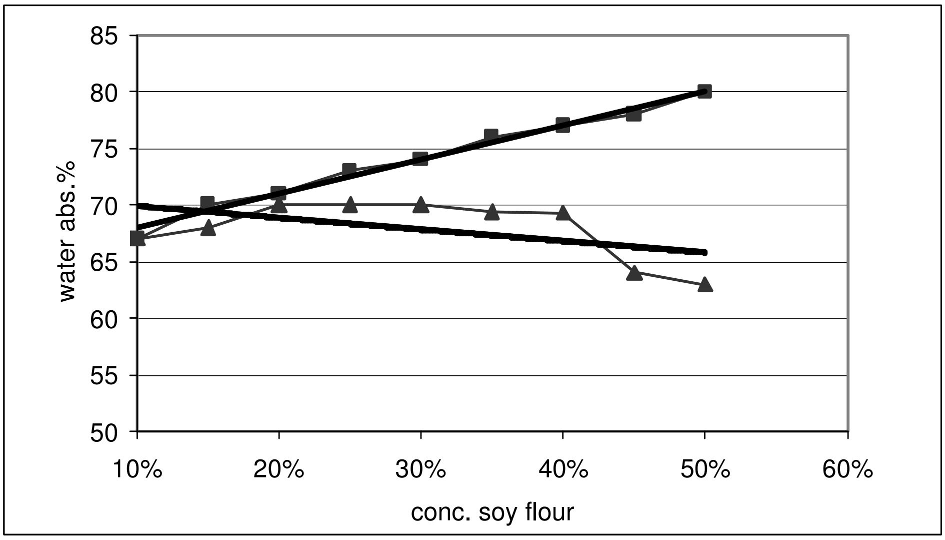


Figure 3.1, The relationship between water absorption (mL) and soy flour concentration (%) for RSF-W dough ? , y = -10x + 70.86; PMSF2-W dough ¦ , y = 30.33x + 64.9. Points on the figures represent the mean of duplicate values. Lines of best fit are included.

Results in Figure 3.1 indicate that water absorption was a function of soy flour concentration in PMSF2 -W dough, with higher water absorption and higher correlation between water absorption and soy flour composition (r^2 = 0.99). Soy flour concentration could be used to predict water absorption up to 50% for this flour. There was no correlation between soy flour concentration and water absorption for the RSF-W dough. The important influence of physical modification on soy flour would be denaturation of soy proteins due to the heat treatment. Water absorption for the PMSF1 and 2 was high. This observation agrees with results from Wagner and Anon (1990) who reported that isolates with highly denatured proteins and high surface hydrophobicity had low-SH contents and these exhibited high water absorption capacity.

The arrival time is the time required for the top of the Farinograph curve to reach the 500 BU (Brabender units) line as it is also a measure of the rate of water uptake by the flour (Zaidul et al., 2004). Arrival time was observed to increase with the increase in soy flour content for soy-wheat doughs, (Figure 3.2, Table 3.2.). This was in agreement with previous reports that higher protein content increased the arrival time (Seyam and Kidman, 1975; Zaidul et al., 2004).

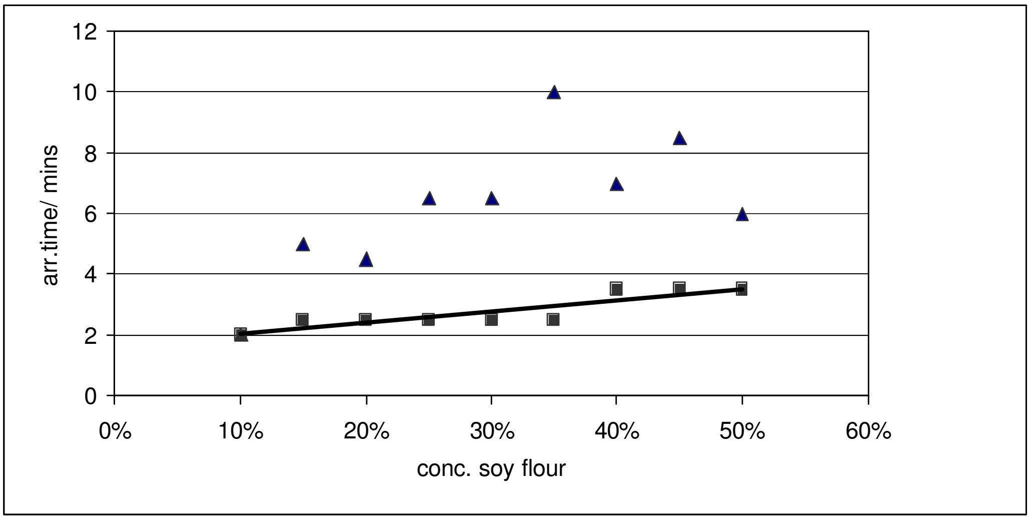


Figure 3.2, The relationship between water uptake rate in minutes and soy flour concentration (%) is shown for RSF-W dough ? ; PMSF2-W dough ¦ . Points on the figures represent the mean of duplicate values. Line of best fit is given for PMSF2-W dough, y = 3.67x + 1.68.

For the PMSF2-W dough, there was a higher water uptake rate and a higher correlation between arrival time and soy flour composition, (r^2 = 0.79), with the arrival time increasing steadily up to 50% concentration of soy flour. There was no correlation between arrival time and soy flour concentration with the RSF-W, (r^2= 0.48).

Results in Figure 3.3 indicate that stability has a negative correlation with the concentration of soy flour for both soy flour types. As expected, there was a negative correlation between soy flour concentration and stability (r^2 = -0.9003 and -0.9521 for PMSF2-W and RSF-W doughs, respectively). The decrease in stability (mixing tolerance index) is attributed to the dilution of gluten proteins by the non-wheat proteins which are slightly cohesive and entirely inelastic (Sharma et al., 1999; Othira et al., 2004).

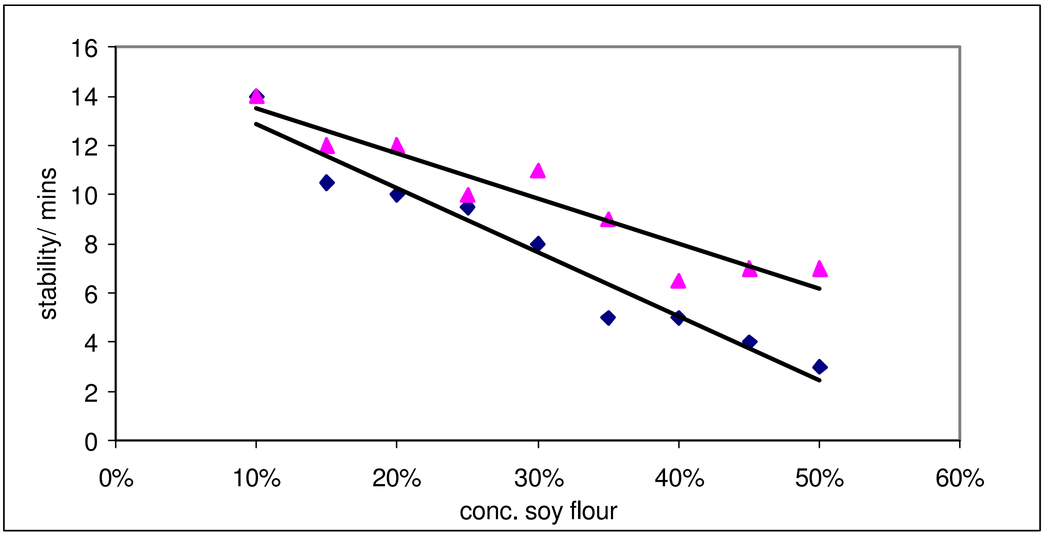


Figure 3.3, Soy flour concentration versus stability (minutes) in soy-wheat dough made from the two soy flours; ? = PMSF2-W dough; ¦ = RSF-W dough. Lines of best fit are included.

The increase in dough development time with increased soy flour content (Table 3.2) was observed in RSF-W dough only. This might have been due to the weakening effect of raw soy flour on wheat dough in contrast to the physically modified soy flours which maintained the composite dough strength. Increased stability given by the physically modified soy flours, possibly due to reformed disulphide bonds during heating, is in accordance with reports from Jones et al. (1974), Lorimer et al. (1991), Andrews et al. (1995) and Bugusu et al. (2001) that reformation of disulphide bonds contribute to dough strength and stability.

3.4.3. Effect of physical modification and L-ascorbic acid addition on resistance to mixing in the Farinograph

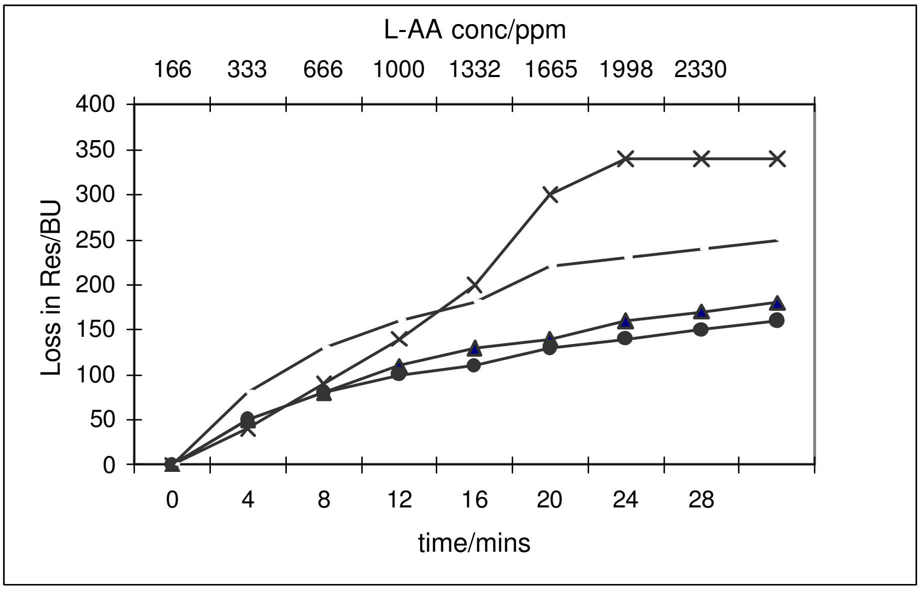


Figure 3.4a, The loss in resistance in Brabender Units (BU) as a function of time in minutes and L-ascorbic acid concentration (mg) is shown for 10, 20 and 50% soy flour substitution for wheat flour during mixing of dough in the RSF. Data points on all figures represent means of duplicate determinations. ? = loss in resistance for reference; ? = loss in resistance for 10% soy flour; ---- = loss in resistance for 20% soy flour; and x = loss in resistance for 50% soy flour.

Results in Figure 3.4a show the effect of soy flour composition and L-ascorbic acid serial additions on the resistance to mixing in the Farinograph for RSF-W dough. The loss in resistance to mixing increased with higher RSF substituted (50% soy flour) dough showing a sharp increase in the loss of resistance compared to low soy flour doughs (10 and 20% soy flour). During mixing, the dough undergoes extreme deformation in every phase of the process (Bushuk, 1985). In liquids or doughs, this deformation increases continuously under the action of finite forces. The non-linear behaviour of soy-wheat doughs at about 22 minutes of mixing could indicate that deformation has reached its maximum for this particular dough. Because deformation in liquids is called flow which is a rate process (Bushuk, 1985), it is best expressed as a rate of strain. The loss in resistance curve for the composite doughs would fundamentally be translated into the rate of strain. Raw soy flour increases the rate of strain in wheat dough as indicated by the sharp increase in the loss in resistance for the soy-wheat dough at 50% soy flour as compared to that of reference wheat flour. This rate of strain was slower for the low soy flour dough

(10% soy flour) compared to that of the reference, indicating that raw soy flour at 10% was capable of strengthening the wheat dough. This observation is consistent with results from Hoseney et al. (1980) and Grosch (1986), who reported low levels of soy flour to strengthen soy-wheat dough in the presence of oxygen as this strength was attributed to lipoxygenase.

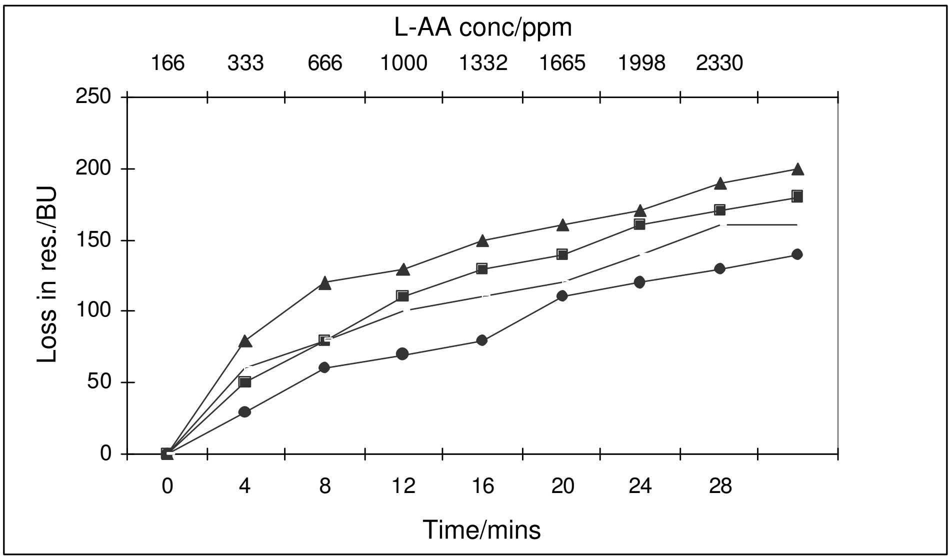


Figure 3.4b, The loss in resistance (BU) as a function of time in minutes and L-ascorbic acid concentration (mg) is shown for 10, 20 and 50% soy flour substitutions for wheat flour during mixing of dough in PMSF2. ¦ = loss in resistance for reference; ? = loss in resistance for 10% soy flour; ? = loss in resistance for 20% soy flour; and --- = loss in resistance for 50% soy flour.

The loss in resistance for the PMSF2-W composite doughs, effect of L-ascorbic acid serial additions and soy wheat composition is shown in Figure 3.4b. The loss in resistance during mixing for the 10% soy flour dough was lower than that of the wheat reference; with the 20% and 50% soy flour comparing well with the wheat plot. There was a decrease in the rate of strain from the 10% and 50% soy flour composite doughs. PMSF, up to 50% concentration, decreased the rate of strain in wheat dough, indicating that physical modification of soy flour imparted greater mixing tolerance compared to RSF in the Farinograph.

Because raw soy flour (>10%) decreased mixing tolerance in wheat dough, an explanation for this would be the high concentration of charged species from the lipoxygenase activity which possibly disrupted SH/SS interchange and caused weakening of the dough. Soy flour cysteine may cause depolymerisation of glutenin proteins, which are responsible for dough strengthening.

The following interchange reactions (1) – (4) were presumed to contribute to weakening of the dough in the Farinograph.

GSSG + 2CSH	?	CSSC + 2GSH	(1)
DHAA + 2 GSH	(GSH-DH) ?	AA + GSSG	(2)
P-SS-G + CSH	?	P-SS-C + GSH	(3)
P-SS-C + GSH	?	P-SS-G + CSH	(4)

GSSG is oxidised glutathione; GSH is reduced glutathione; CSH is reduced cysteine; CSSC is oxidized cysteine; CSSG is cysteine and glutathione mixed disulphide; P-SS-G is protein and glutathione mixed disulphide; P-SS-C is protein and cysteine mixed disulphide; AA and DHAA is ascorbic acid and dehydro-ascorbic acid, respectively.

The interchange reactions between gluten protein and protein cysteine mixed sulphides (3 and 4) presumably overwhelmed the enzymatic oxidation of GSH to GSSG by L-AA (2).

There was no significant change in pH in all the doughs tested in the Farinograph, with the wheat reference ranging from 5.1 to 4.8; PMSF – wheat dough from 6.5 to 6.3 and RSF – wheat dough from 6.2 to 6.2 from 50 ppm and 2300 ppm L-AA, respectively. Although the pH of dough did not change significantly, L-AA addition at these levels was likely to interact with dough proteins and destabilise proteins, by disrupting hydrophobic interactions between proteins. Suryaprakash et al. (2000) reported destabilisation of the multimeric structure of helianthinin (sunflower seed protein), from the interaction of organic acids with the protein. In the current Farinograph results, L-ascorbic acid failed to improve the resistance to mixing but improved dough strength during resting of soy-wheat dough. These results suggest that dough mixing caused gluten to stretch points on disulphide bonds causing them to break (Andrews et al., 1995). These findings, together with findings from Jones et al. (1974), suggest that the stretching of disulphides during dough mixing can overwhelm the effect of the oxidising agents.

The relative rates of reaction for L-AA and potassium bromate were classified as slow (Fitchett and Frazier, 1986), but the oxidative improvement from a combination of these two has been observed in today's bakery practice. Grosch and Wieser (1999) found that the amount of oxygen kneaded into the dough is the rate - limiting step of L-AA oxidation. The mixing speed applied in this study was low (60 rpm), and this could also have contributed to the slow effect of L-AA.

Composite flours used in these experiments had high soy flour concentrations and the resulting interferences were anticipated to be high.

Therefore, L-ascorbic acid used in this study was higher (up to 2300 ppm) than the recommended concentration used to strengthen bread, 20-30 ppm (Grosch and Wieser, 1999). Other countries also use large amounts of L-ascorbic acid as an improver for the redox reactions in dough preparation, 20,000–100,000ppm (Koehler, 2002). L-AA is water-soluble and its anti-oxidant properties include scavenging of free radicals, (Lambelet et al., 1985). Because of the likelihood of high levels of free radicals from the oxidation of free fatty acids in soy flour, L-AA is also capable of participating in scavenging of these free radicals. From theoretical considerations, such a dough system would consume more L-AA than a wheat-only dough. The fact that L-ascorbic acid is easily available, a permitted food ingredient and does not leave toxic compounds on the final product; it is superior to other oxidising agents.

Table 3.3 Effect of L-AA on resistance to mixing during Farinograph analysis; a comparison of RSF-wheat and PMSF2-wheat dough (10 – 50 % soy flour)

	Resistance (Brabender Units) after 30 minutes of mixing, L-AA addition up to 2300 ppm.			
Conc.Soy flour	RSF & L-AA	RSF no L-AA	PMSF& L-AA	PMSF no L-AA
10%	440^{b}	500^{b}	430	450
20%	340^{b}	380^{ab}	390	430^{a}
30%	320^{b}	380^{ab}	390	410^{a}
40%	220^{b}	300^{ab}	390	390^{a}
50%	180	180^{a}	400	400^{a}
Reference	400			

Values shown are the means of duplicate analysis; error was ± 2% of the mean for all values
Values marked with the same letter, on the same row are significantly different at (P <0.05),

The summary in Table 3.3 compares the resistance to mixing after 30 minutes of mixing (with L-AA serial additions up to 2300 ppm) on Farinograph between RSF-W and PMSF2-W dough (10-50% soy flour). The resistance to mixing after 30 minutes was generally higher in the soy-wheat dough without L-AA. A paired t-test to compare means (L-AA and non L-AA resistance) indicated that L-AA significantly decreased the resistance to mixing (P <0.05) in the RSF-W dough. The decrease in resistance to mixing was however not significant in PMSF2-W dough (P <0.05). The effect of physical modification of soy flour on improving the resistance to mixing was significant from 20 to 50% soy flour concentration (marked with [a]). The weakening effect of dough from an oxidising agent (L-AA) was also highlighted by Jones et al. (1974) where dough to which N-ethylmaleimide (NEMI) was added decreased in resistance to mixing after 30 minutes. NEMI is an oxidising agent that can be used to block SH bonds.

3.4.4. Evaluation of rheologically important disulphides in soy-wheat dough using dithiothreitol

Because rheological results for soy-wheat dough made from PMSF2 were closer to the reference (Table 3.2), estimation of disulphides was done to evaluate the role played by physical modification of soy flour during mixing of soy-wheat dough made from this soy flour. It was also emphasised (Jones et al., 1974) that modification of only a portion of thiol groups would produce a maximum effect on rheological properties of dough. This theory was tested using RSF-W and PMSF2-W dough. The results highlighted on Figure 3.5 show how rheologically important disulphides were estimated during dough mixing using dithiothreitol (DTT) titration for the raw soy flour and physically modified soy flour (PMSF2) composite dough with wheat flour.

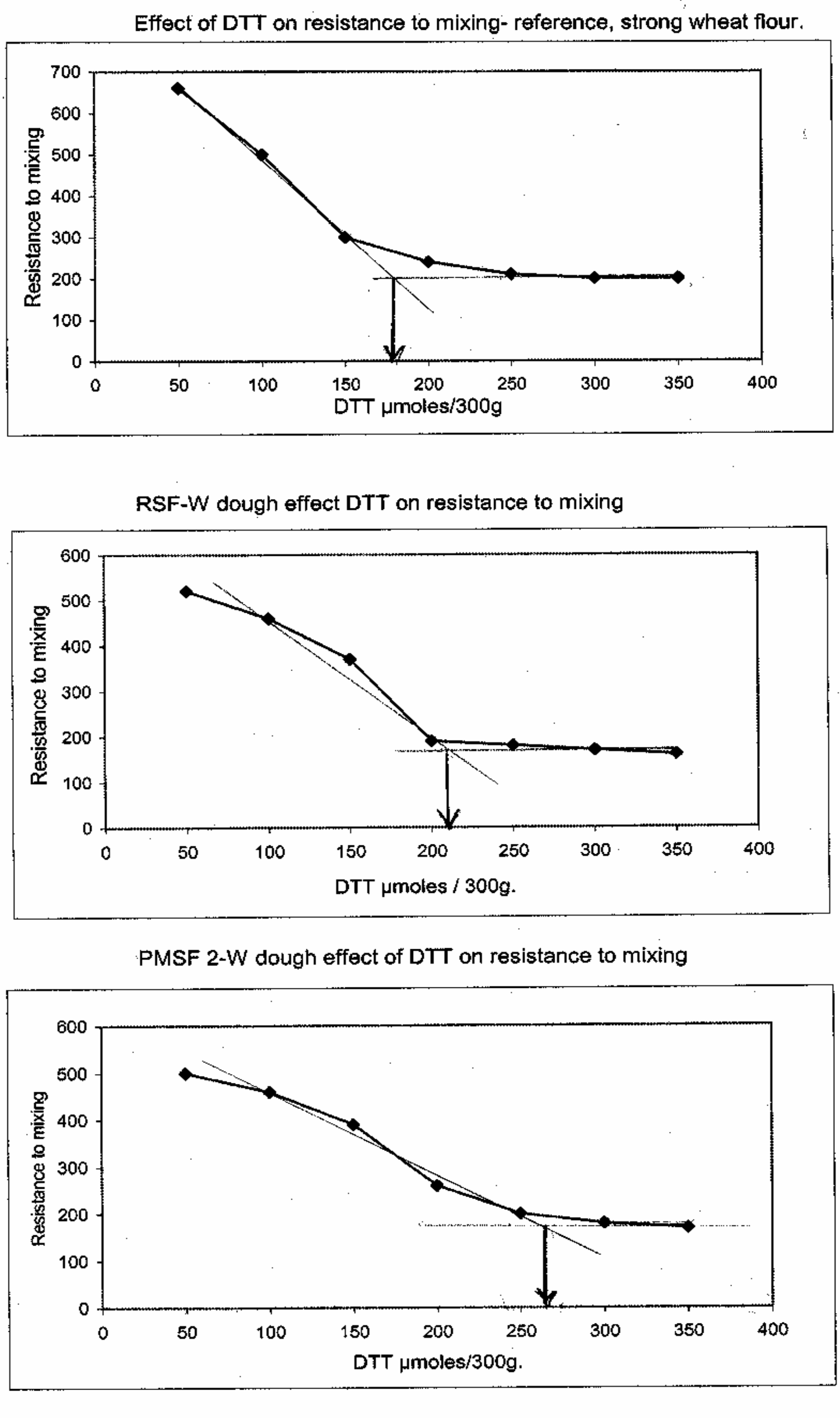


Figure 3.5. The effect of dithiothreitol (DTT) on resistance to mixing in the Farinograph in soy-wheat flour doughs at 1:1 ratio. DTT (x-axis) against resistance to mixing (y-axis) in Brabender units (BU) for wheat dough; RSF-W and PMSF2-W dough

Each plot on Figure 3.5 shows two distinct straight lines, the one on which resistance falls rapidly to a steady value and the one on which resistance remain stable. The point of intersection (from extrapolation of the straight lines) is the function of the amount of dithiothreitol used and is equivalent to the disulphides important to mixing in the Farinograph (Jones et al., 1974; Lorimer et al., 1991). The approximate disulphide concentrations (from extrapolations) are 58, 70 and 88 μmoles per 100 grams of flour for wheat dough, RSF-W and PMSF2-W dough, respectively. These results indicate higher numbers of disulphides involved in resistance to mixing in PMSF2-W than in RSF-W dough. The results indicated that the physical modification of soy flour resulted in greater mixing resistance through modification of its disulphide content. The higher figures for rheologically important disulphides in the soy-wheat doughs (70 and 88 μmoles /100g) compared to the reference wheat dough (58 μmoles /100g) suggest the participation of soy proteins in the SS/SH interchange during mixing. Higher resistance to extension (R_{max}) was also shown in this physically modified flour in the Extensograph in the earlier stages of this work.

Rheologically important disulphides varied between 56 to 87 μmoles/ 50 g in wheat dough for three varieties of wheat flour tested using the same method (Jones et al., 1974). The difference in values of disulphides in the wheat flour dough used in these experiments could be attributed to different types of wheat flours and also to the different experimental conditions used. Jones et al. used 50 grams of wheat flour for the determination of rheologically important disulphides compared to 300 grams used in the current experiment.

3.4.5. Effect of L-ascorbic acid on the Extensograms of soy-wheat doughs made from RSF and PMSF2 (1:1 ratio)

The results in Table 3.4 show the effects of L-AA on the dough strength and extensibility for the soy-wheat dough. RSF and PMSF2 were used to make composite doughs with wheat flour. Water and flour doughs were used. No salt was added to the doughs as it was suggested (Grosch and Wieser, 1999) that the activity of GSH-DH (glutathione dehydrogenase) may be affected with the addition of salt.

Table 3.4: Effect of L-ascorbic acid (L-AA) addition to soy-wheat dough at 1:1 ratio on Extensograms

Extensogram time	45 mins		90 mins		135 mins	
	Max Res. R_m in BU	Extensib E in cm	Max Res. R_m in BU	Extensib E in cm	Max Res. R_m in BU	Extensib E in cm
Reference dough						
Reference no LAA	280	18.0	320	16.5	290	17.5
Reference + LAA 250ppm	680^{8}	16.0	$>1000^{88}$	14.5	$>1000^{88}$	11.0
Reference + LAA 500ppm	600^{8}	14.5	$>1000^{88}$	15.5	$>1000^{88}$	12.5
RSF-W dough						
RSF-W no LAA	200	4.0^{8}	180	14.0^{88}	160	14.5^{88}
RSF-W + LAA 250ppm	250^{8}	3.5^{8}	280^{88}	11.5^{88}	320^{888}	12.5^{888}
RSF-W+ LAA 500ppm	290^{8}	10.0^{8}	340^{88}	11.0^{88}	400^{888}	12.5^{888}
PMSF2-W dough						
PMSF2 -W no LAA	400	3.5^{8}	400	10.0^{88}	320	10.5^{88}
PMSF2 -W + LAA 250ppm	580^{8}	9.5	600^{88}	10.0	600^{88}	9.5
PMSF2 -W+ LAA 500ppm	560^{8}	7.5^{8}	600^{88}	9.5^{88}	620^{888}	9.0^{88}

All values shown are the means of duplicate analysis, error ± 2% of the mean. Significant increase of R_m and extensibility (*E*) with time due to L-ascorbic acid is marked with increasing superscript stars across the row P <0.05) for each model.The absence of superscripts across the rows indicates that there was no increase in *E* and R_m with time. Reference is the strong wheat flour.

Table 3.4 shows results of the effects of L-ascorbic acid on the dough strength and extensibility for the soy-wheat dough using the raw, physically modified and strong wheat flour. Physical modification of soy flour significantly improved the maximum resistance to extension (R_m) P <0.05. This was shown by the higher R_m values at 400, 400 and 320 Brabender units (BU) for PMSF2 compared to 200,180,160 BU for the RSF and this was observed at 45, 90,135 minutes of resting, respectively. Physical modification decreased extensibility (P <0.05) from 4 to 3.5 cm; 14 to10 cm and 14.5 to10.5 cm in the RSF and PMSF2 composite dough respectively. This decrease was shown at 45, 90 and 135 minutes after resting the dough. Thermal treatment, which was applied to PMSF2, denatures protein. Because this process involves S-S bond formation, this tends to immobilise parts of the protein chains. This could have resulted in the decrease in dough elasticity in this composite dough.

L-ascorbic acid increased the R_m of the composite dough ($P < 0.05$) from 250 to 320 BU with 250ppm L-AA and from 290 to 400 BU with 500ppm L-AA in RSF, from 580 to 600 BU with 250ppm L-AA and from 560-620 BU with 500ppm L-AA in PMSF2. This suggested that the dough was strengthening with L-AA in both soy-wheat doughs. L-ascorbic acid improved extensibility in the composite dough at 45 minutes, with values of 4.0 to10 cm and 3.5 to7.5 cm from 0 to 500ppm L-AA in RSF and PMSF2 respectively. L-AA at the same concentration decreased extensibility with values of 14.5 to11 cm and 14.5 to12.5 cm in RSF; 10 to 9.5 cm and10.5 to 9.0 cm in PMSF2 at 90 and 135 minutes respectively. The composite dough for both flours showed weakening without L-AA during the resting period, with values of 200, 180, 160 BU for RSF and 400, 400, 320 BU for PMSF2.

Preston and Hoseney (1991) substituted soy flour up to 5% for wheat flour and showed that peroxides could hasten the process of oxidation of S-H groups to S-S groups thus stabilising the soy-wheat dough. In the present experiment, however, 10-50% soy flour was substituted for wheat and the dough showed weakening with time suggesting high levels of S-H groups in the system.

L-ascorbic acid at 250 ppm and 500 ppm improved the dough strength, suggesting that L-AA interacted with the S-S/S-H system. L -ascorbic acid had the greatest effect on improving R_m in the reference dough with BU values of 280 to 600, 320 to >1000, and 290 to >1000 at 45, 90 and 135 minutes respectively. This is in agreement with results obtained by Grosch and Wieser (1999), where L-AA was effective in strengthening wheat dough in Extensogragh measurements. However, extensibility in the reference dough was decreased with L-AA addition with values from 18.0 to 14.5cm at 45 minutes; 16.5 to 15.1cm at 90 minutes; 17.5 to 12.5cm at 135 minutes. Physical modification and L-AA treatments have improved viscosity of the soy-wheat dough but L-AA has reduced the elastic behaviour (*E*) of the soy-wheat and wheat dough. The highly elastic polypeptide mass, formed by the disulphide bond cross-links, contributes to the elastic behaviour of gluten in wheat dough (Bushuk, 1985). L-AA and cysteine (in soy flour) possibly disrupted the elastic mass by reducing the S-S bonds. Observations from this work have suggested that the high levels of SH may improve viscosity but reduce elasticity in soy-wheat doughs. An increase in spread ratio and wheat dough mobility due to the addition of cysteine (CSH) and glutathione (GSH) was observed by Elkassabany and Hoseney (1980) during dough fermentation as they attributed this to SS/SH interchange reactions. But dehydro-ascorbic acid (DHAA) was effective in overcoming this thiol effect, thus restoring the stiffening effect on the dough (decreasing spread). A decrease in dough spread would physically imply an increase in the resistance to extension (R_m). The results observed (Elkassabany and Hoseney, 1980) are in accordance with the results from this study, when RSF and PMSF decreased R_m in the soy - wheat composite dough during resting and L-AA restoring strength (stiffening) by increasing R_m.

The dough pH in the Extensograph ranged from pH 5.1 to 5.4 in the wheat reference dough without L-AA; from 5.09 to 5.1 in the dough with 500ppm L-AA, from 45 to 135 minutes, respectively. In the PMSF2-wheat dough, the pH ranged from 6.5 to 6.3 and 6.5 to 6.5 between 45 and 135 minutes at 0 and 500ppm respectively. In the RSF-wheat dough, pH ranged from 6.4 to 6.5 and 6.2 to 6.5, between 45 minutes and 135 minutes with 0 and 500ppm of L-AA, respectively. These results show an insignificant effect of L-AA (up to 500ppm) on the pH of dough.

Analysis of Variance (ANOVA) applied to find if physical modification had an effect on $R_{m.}$ (resistance to extension), revealed that there was significant difference between RSF and PMSF2 wheat dough ($P< 0.05$). Results from ANOVA showed that physical modification did not have any significant effect on extensibility ($P< 0.05$). The least significant difference (LSD) was used to compare the means and the effect of L-AA at different concentrations. The results indicated that R_m for both flour doughs changed significantly from 0 to 0.25% and from 0 to 0.05% concentration of L-AA. L-AA significantly decreased extensibility at 0.05% concentration after 135 minutes of dough resting.

3.4.6. Effect of physical modification of soy flour and L-ascorbic acid addition on bread making qualities of soy-wheat bread

Table 3.5: Effect of L -AA (0.05% w/w) addition on bread crumb texture ($g\ s^{-1}\cdot mm^{-1}$) for the three types of soy-wheat dough.

Values are means of duplicate analysis, ± values are error of the mean values

Soy flour (%)	RSF-W Compression		PMSF1-W Compression		PMSF2-W Compression	
	L-AA	no L-AA	L-AA	no L-AA	L-AA	no L-AA
Ref	18.5±1.5	20.9±1.9	18.5±1.5	20.9±1.6	18.5±1.5	20.9±0.8
10	19.4±1.4	21.7±2	17.3±1.2	20.4±2.3	20.4±1.8	22.8±2.2
20	22.2±2.1	24.0±1.6	16.3±1.0	18.9±2.0	19.2±1.1	18.2±1.8
30	24.1±1.6	31.0±2.2	20.7±2.0	23.2±2.5	13.7±0.7	15.7±1.1
40	22.5±1.8	31.7±2.5	18.6±1.8	20.1±2.3	14.9±0.9	20.2±2.4
50	19.7±1.7	32.9±3.1	18.3±2.1	17.5±1.6	17.1±1.2	20.9±2.6

RSF-W, PMSF1-W and PMSF2-W represent RSF-wheat dough, PMSF1- wheat dough and PMSF2-wheat dough respectively.

Table 3.6: Effect of L-AA (0.05% w/w) addition on soy-wheat loaf volumes;

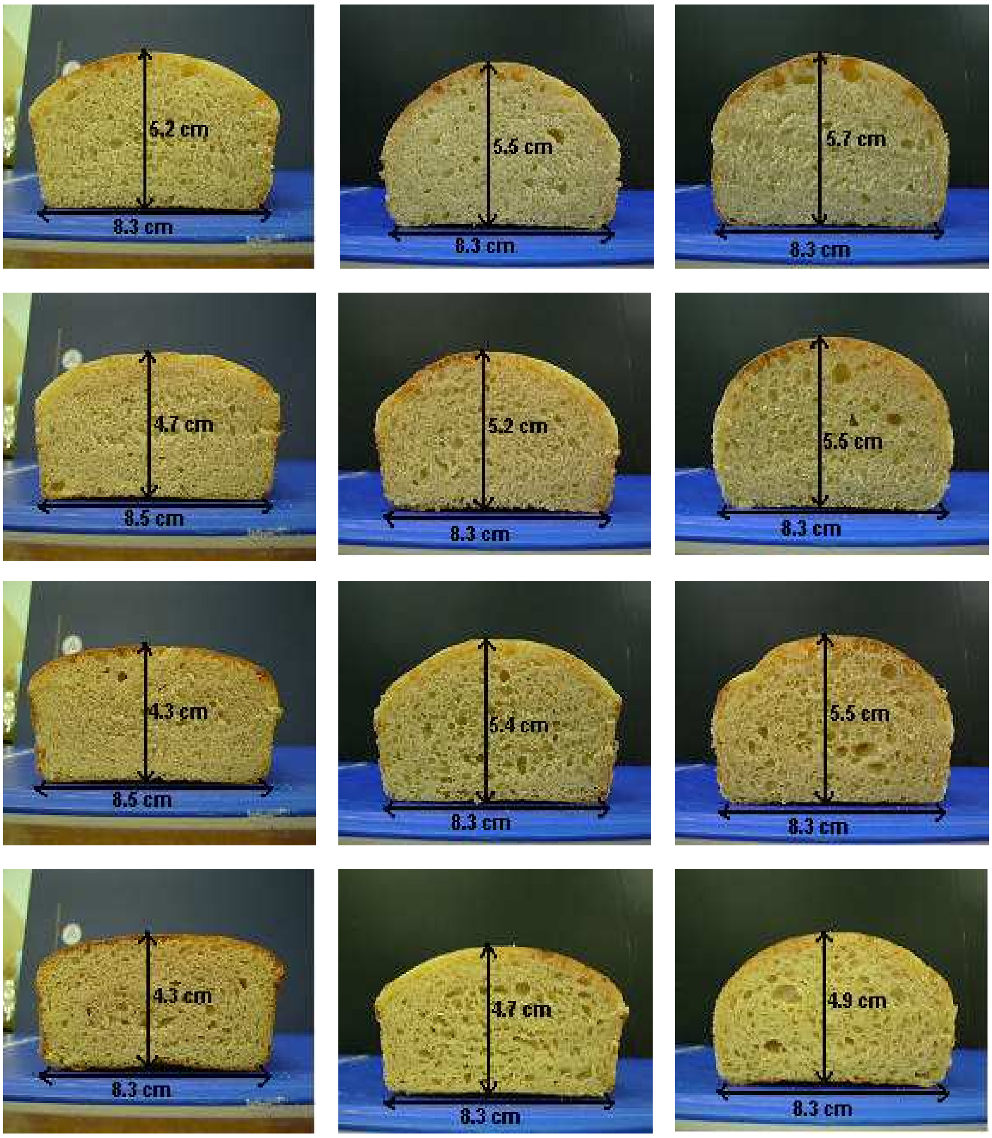
Values are the mean of duplicate analysis, error ± 3% for all values

Soy flour (%)	RSF-W loaf volume /cm^3		PMSF1-W loaf volume /cm^3		PMSF2-W loaf volume /cm^3	
	L-AA[a]	no L-AA[b]	L-AA	no L-AA	L-AA	No L-AA
0	790	760	780	760	780	760
10	760	720	760	750	760	720
20	720	680	720	700	730	700
30	680	650	700	680	720	710
40	670	640	650	640	710	680
50	670	640	640	640	690	680

[a] symbol represents loaf volumes with L-ascorbic acid and [b] represents loaf volumes without L-ascorbic acid. RSF-W, PMSF1-W and PMSF2-W is RSF, PMSF1 and PMSF2 and wheat flour bread respectively

Results in Tables 3.5 and 3.6 highlighted the positive effects of L-AA on the bread making qualities of soy-wheat breads. These observations confirm reports by previous workers on wheat bread (Grosch, 1986; Elkassabany and Hoseney, 1980; Koehler, 2003). L-AA at 0.05% significantly improved loaf volumes and crumb texture (P<0.05) in soy-wheat bread (10-50% soy flour). The force (grams) required to compress through the bread slices was greater for the bread without L-AA compared to that with L-AA (Table 3.5). The observations at this stage may indicate that soy flour components do not interfere with the improver effect of L-AA on wheat bread. Analysis of variance (ANOVA) revealed positive and significant effects of L-AA and physical modification on the crumb texture and loaf volumes of soy-wheat breads (P<0.05). Observations in Table 3.6 also indicated that loaves made from PMSF2-W flour were significantly larger compared to those made from RSF-W and PMSF1-W flour. Results in Figure 3.6 also confirm the changes in bread volumes as improved by physical modification of soy flour (column 2) and by the addition of 0.05% L-AA (Column 3). The loaf heights are decreasing in the order of Column 3, Column 2 and Column 1, indicating that use of physically modified soy flour and L-ascorbic acid (Column 3) resulted in the largest loaf volumes and that composite bread made from raw soy (Column 1) had the smallest loaves.

Column 1 RSF-W (no L-AA) | Column 2 PMSF2-W (no L-AA) | Column 3 PMSF2-W (+L-AA)



Reference (wheat bread)

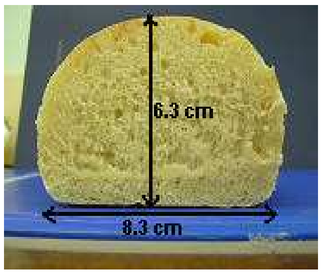


Figure 3.6, The effect of physically modified soy flour (Column 2), and the effect of L-AA (0.05% w/w) addition (Column 3) on soy-wheat bread loaf volumes. Column 1 represents RSF-W bread; Column 2 is PMSF2-W bread, and Column 3 is PMSF2-W bread with L-AA (0.05%) from 10, 20, 30, and 50% soy flour (down the column). Wheat bread (reference) is shown at the bottom.

3.4.7. The relationship between the rheological parameters and bread making properties of soy-wheat bread

Table 3.7. Regression coefficients for rheological properties on bread making qualities of soy-wheat dough from the three soy-flour types

	Loaf volume	Crumb texture	Spec loaf vol	Moisture content
Water abs.	- 0.227	- 0.584*	- 0.017*	- 0.645
Dough dev time	-1.31	- 0.060	- 0.011	3.19
Arrival time	- 4.39	1.61	0.052	- 1.72
Stability	5.61*	- 0.315	0.038*	- 0.069
R^2	87%	71%	85%	21%

Values followed by * are significant *(P <0.05);* while those without are non significant.

Linear regression analysis to evaluate the relationship between rheological properties and baking qualities was done. Results in Table 3.7 show results on regression coefficients for each rheological property on bread making qualities of soy-wheat bread made from all three soy flour types. Using the F-statistical values (Analysis of variance), water absorption, dough development time, arrival time and stability were all significantly related to loaf volume, crumb texture and specific loaf volume in soy-wheat bread (P< 0.05). These rheological parameters were however not related to the moisture content of the bread (P< 0.05). The negative regression coefficients for water absorption, dough development time and arrival time on loaf volume indicated that higher values of these rheological parameters resulted in smaller loaf volumes. However these coefficients were non significant (P< 0.05). Stability was the most important parameter in predicting loaf volumes in soy-wheat bread, as attested by the positive and significant coefficient (P<0.05).

Correlation coefficient (R^2) value for stability on loaf volume was high, 0.82 (P<0.05), while the R^2 value to explain the relationship of all given rheological parameters on loaf volume was 0.87.

Water absorption was the only significant factor (Table 3.7) related to crumb texture as attested by its significant coefficient ($P < 0.05$). The negative coefficient means that increases in water absorption by the soy-wheat dough results in smaller compression force as measured by the texture analyzer (softer bread). Dough development time, arrival time and stability were not significantly correlated with crumb texture ($P < 0.05$). The R^2 value for coefficients on crumb texture was 71%.

Water absorption and stability had significant coefficients on specific loaf volume ($P < 0.05$). The negative coefficient of water absorption indicated that high water absorption gave low specific loaf volumes. This is true because high water absorption would be caused by the hydrophilic soy flour proteins which on the other hand decreased loaf volumes because soy proteins do not have the expansion and gas retention properties desirable for bread making compared to wheat gluten proteins. This means that technically, soy flour proteins absorbed more water and still reduced loaf volumes. The regression coefficient of water absorption on loaf volume was also negative for the same reason. Stability had a positive coefficient on specific loaf volume ($P < 0.05$) meaning that stability was a reliable predictor of specific loaf volume in soy-wheat dough. Dough development time and arrival time had non significant correlation with specific loaf volume. The R^2 value to explain the relationship of the given rheological parameters on specific loaf volume was 85%.

Coefficients of rheological parameters on the moisture content of soy-wheat bread were all non significant ($P < 0.05$) and the R^2 for this equation was low (21%) suggesting that these rheological factors were not related to the water retention capacity of the final bread product (as determined by the moisture content).

3.5. Conclusions

Physical modification of soy flour did not alter the proximate composition of soy flour but the treatment altered the physical properties of soy-wheat dough as attested by the differences in rheological behaviour of the composite doughs. Soy-wheat composite doughs made from physically modified soy flour (PMSF2) exhibited higher resistance to extension (R_m), greater tolerance to mixing, better mixing stability, higher water uptake and water absorption compared to soy-wheat doughs made from raw soy flour (RSF). Rheological results indicated that soy-wheat doughs made from PMSF2 was significantly closer to the reference *($P<0.05$)* than dough made from RSF-W and PMSF1-W flour. Higher water absorption of PMSF2-W dough compared to wheat dough was considered an advantage in improving the loaf volumes during bread making. Therefore steam blanching of soybeans prior to the preparation of soy flour was considered a better process than boil blanching of soybeans.

Rheologically important disulphides were higher (88 µmoles / 100g) in the PMSF2-W dough compared to RSF-W dough (70 µmoles / 100g) suggesting that physical modification of soy flour resulted in greater mixing resistance through modification of its disulphide content

The stability of the dough (during Farinograph mixing) was observed to be the most important parameter in predicting loaf volumes in soy-wheat bread, while Farinograph water absorption was considered a reliable predictor of crumb texture and specific loaf volume in soy-wheat bread making ($P<0.05$).

The addition of L-AA (0.05% w/w) to soy-wheat doughs significantly improved resistance to extension (R_m), as it also improved loaf volumes and crumb texture of soy-wheat bread from 10 to 50% soy flour ($P<0.05$). The combination of L-ascorbic acid and physically modified soy flour was considered effective in strengthening soy-wheat dough and improving the bread making properties of this composite dough. This development was considered a positive step towards the promotion of higher levels of soy flour in wheat bread.

Chapter 4

Interaction of soy and wheat gluten proteins in soy-wheat dough

4. Table of contents

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Abstract

The work on this chapter explores the hypothesis that physical modification of soy flour using moist heat treatment causes an increase in the molecular-weight distribution of the soy proteins, thus making soy flour proteins more suitable for dough formation. Rheological results reported from the preceding chapter indicated that soy-wheat dough made from physically modified soy flour (PMSF) exhibited better dough strength properties compared to that made from raw soy flour (RSF). Because L-ascorbic acid (0.05% w/w) improved the resistance to extension (R_m) in soy-wheat dough, a follow-up study to find the role of L-ascorbic acid on protein interaction, including the role of soy protein on S-H/S-S interchange in soy-wheat dough, was undertaken using UV/visible spectrophotometry.

Although rheological results indicated that there were possible interactions between soy and wheat proteins in dough during mixing and resting, it was important to gain a better understanding of protein size distribution and possible interactions at the molecular level. SE-HPLC and Lab-on-a-chip capillary electrophoresis were used to study these changes in protein composition. SE-HPLC elution profiles demonstrated that the improved contribution to dough properties by physical modification of soy flour was due to changes in the molecular size distribution of the soy proteins, where PMSF-wheat dough gave a SE-HPLC profile closer to that of wheat dough. Although capillary electrophoresis (using Lab-on-a-chip methodology) did not reveal the presence of very large proteins, capillary electrophoregrams revealed that partial reduction and reoxidation of composite doughs facilitated interaction of medium molecular weight wheat proteins (40-60kDa) and soy proteins (11S acidic subunits) through oxidation of sulphydryl groups (-SH); this study also indicated that interactions were covalent. These interactions were more evident in soy-wheat dough made from PMSF than from RSF. L-AA (0.05% w/w) reduced polymeric proteins in soy-wheat dough, but partial reduction and re-oxidation of soy-wheat dough using dithiothreitol and potassium iodate increased polymeric proteins in these doughs. Results from SE-HPLC elution profiles and capillary electrophoregrams from soy-wheat dough protein extracts have provided an insight into possible methods to study the contribution of soy proteins to dough chemical properties.

4.1. Introduction

High levels of soy flour in wheat dough are known to cause deleterious effects on rheological and bread-making properties (Pomeranz et al., 1969; D'Appolonia, 1977; Lorimer et al., 1991), 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 in this project 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 nutritional properties and improving functional 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 dough. Soy-wheat composite dough offers an unusual contrast of differing protein classes. Functional properties of the soy proteins are defined by their interactions with wheat proteins and other components in the dough system (Liu, 1997). Wheat proteins have low solubility and favour hydrophobic interactions. Unextractable polymeric proteins (UPP) in wheat are the large glutenin polymers (>100,000 Da) and these form part of the quality criteria for baking (Gupta et al., 1993; Gupta et al., 1995; Grosch and Wieser, 1999; Fisichella et al., 2003). On the other hand, soy proteins are highly soluble and exhibit hydrophilic characteristics in a soy-wheat composite dough system (p H 5.5 – 6.5). The soluble soy proteins are desired for functional properties like water absorption and water binding in bread whilst the water insoluble proteins are likely to be the large molecular weight polymers which would interact with the hydrophobic wheat proteins and contribute to bread making performance.

Although the polymeric distribution of proteins has been studied in wheat flour varieties, no such work has been published on protein size distribution in wheat dough during fermentation. Neither have the effects of wheat flour substitution with legume flours been reported, as they affect protein size distribution in composite doughs. The work described in this chapter sought to define the relative size distribution of proteins in soy-wheat composite dough and to relate this to the dough-weakening properties of soy-wheat doughs.

Statement of the problem

Attempts to use legume proteins for bread making have generally been unsuccessful. Several factors have been suggested to cause dough weakening in wheat flour dough, namely, competition for water molecules between legume proteins and gluten, and disruption of starch-protein complexes by the foreign proteins (Lorimer et al., 1991). The hypothesis that gluten- and soy-protein interactions have the potential to provide dough improvement and that soy-protein sulphydryl groups may contribute to dough development through SS-SH interchange has been put forward by Ryan et al. (2002). On the other hand, Lorimer et al. (1991) suggested that dough weakening would occur from the interruption of SS/SH interchange by the non-gluten proteins. Glycolipids and sucrose esters (Pomeranz et al., 1969; Chung et al., 1981) and gums that mimic the visco-elastic properties of wheat proteins (Nishita et al., 1976; Toufeili et al., 1994; Kobylanski et al., 2004) have been used to improve the bread-making properties of composite doughs.
The oxidising capacity of soy flour on wheat dough has been illustrated at low levels of soy flour where air-mixed doughs containing enzymically active soy flour have improved rheological properties. This improvement is more effective than without air. This oxidising capacity has been attributed to the effect of lipoxygenase in strengthening wheat flour dough (Hoseney et al., 1980; Cumbee et al., 1997). However, the causes of dough weakening in wheat doughs composited with legume flours are still not clear.

Important differences between soy and gluten proteins are the contrasts between them in their water-solubilities, the associated differences in amino-acid composition and in size distribution, 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 (Stevenson et al., 1996; Southan and MacRitchie, 1999). This set of observations raises the possibility that soy proteins might be better suited to dough forming if their molecular-weight distribution could be increased considerably.

The work in this chapter explores the hypothesis that a process of physical modification of soy flour (moist heat treatment) causes an increase in the molecular-weight distribution of the soy proteins, thus making them more suitable for dough formation. This treatment has been reported to produce a 1:1 soy-wheat dough that exhibits higher resistance to extension (R_m), 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 (Chapter 3). Although rheological studies indicated that there was possible interaction between soy and wheat proteins in dough during mixing and resting, size-exclusion high-performance liquid chromatography (SE-HPLC) was used to determine the protein compositions of these composite doughs during mixing and resting (App. 4.1), thus to gain a better understanding of size distribution and of possible interactions at the molecular level. Because L-ascorbic acid increased the resistance to mixing as well as the maximum resistance to extension (R_m) of soy-wheat dough during resting, (preceding chapter) the first part of this work studied the effects of L-ascorbic acid on protein interactions in soy-wheat composite doughs during resting using Visible Spectrophotometry. In order to further understand the soy and wheat protein interaction, the role of soy flour protein in SS/SH interchange had to be understood and this was evaluated using a kinetic cell for the production of L-AA from the oxidation of GSH to GSSG by dehydro-ascorbic acid (DHAA).

Objectives

1 To evaluate the effects of L-ascorbic acid on protein interactions in soy-wheat dough during resting using Visible Spectrophotometry.
2 To establish the role of soy protein in SS/SH interchange using a kinetic study (UV/Vis Spectrophotometry) for the oxidation of GSH to GSSG by DHAA in the presence / absence of soy cysteine.
3 To use size exclusion high performance liquid chromatography (SE-HPLC) to evaluate the size distribution of proteins and possible protein interactions in soy-wheat dough at the molecular level.
4 To evaluate if protein - protein interactions in soy-wheat doughs (using partial reduction and oxidation during mixing) are covalent or non-covalent using Lab-on-a-chip capillary electrophoresis.

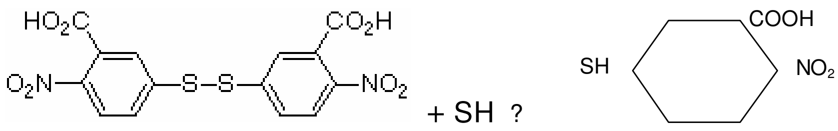
Hypothesis

The present study explores the hypothesis that a process of physical modification of soy flour (moist heat treatment) causes an increase in the molecular-weight distribution of the soy proteins, thus making them more suitable for dough formation.

4.2 Principles of methods used

4.2.1 Determination of thiols (SH) and using Ellman's reagent

Ellman's reagent {DTNB (5, 5'-dithiobis (2-nitrobenzoic acid)} reacts with SH groups to generate 5-mercapto-2-nitrobenzoic acid or 2-nitro-5-thiobenzoate (NTB^{2-}); and is used for the colorimetric determination of SH groups. It is reasonably soluble in water and very soluble in ethanol. Because 5-mercapto-2-nitrobenzoic acid has an absorption maximum at 412nm, the absorption spectrum of DTNB does not interfere with the determination of thiols.



5 mercapto-2 nitro-benzoic acid

Ellman's reagent reacts specifically with thiol groups, (Chan and Wasserman, 1993; Wagner and Anon, 1990). The key feature in this reaction is that the chromophore NTB^{2-} produced is soluble in aqueous solution and gives a yellow colour with an extinction coefficient of 13,600 M^{-1} cm^{-1} at 412nm. The reaction mixture of urea and SDS maximises the reactivity of any thiols and disulphides that may be buried within the hydrophobic protein matrix (Chan and Wasserman, 1993).

Extraction of proteins was done using urea and SDS (sodium dodecyl sulphate) and because this may lead to only partial solubilisation of proteins, the whole flour sample is preferably suspended in urea, SDS, and the colour reagent {5, 5' –dithiobis (2-nitrobenzoic acid) (DTNB)}. This method allows simultaneous reaction of both soluble and insoluble proteins and releases a soluble chromophore NTB^{2-} (2-nitro-5-thiobenzoate anion) which absorbs UV light at 412 nm.

To determine disulphide content, the sample is reacted with 8 M urea buffer, 0.1 M sodium sulphite and 10m M disodium 2- nitro-5-thiosulphobenzoate, $NTSB^{2-}$ made by reacting DTNB and sodium sulphite in the presence of oxygen. $NTSB^{2-}$ reacts with cysteine and thiol groups formed after reduction of disulphide bonds with sodium sulphite. Disulphide content is calculated as the difference between thiols before reduction and the total thiols after reduction. Total cysteine is calculated as SH concentration + 2 x SS concentration (Chan and Wasserman, 1993).

4.2.2 Size Exclusion High Performance Liquid Chromatography (SE-HPLC)

Size Exclusion High Performance Liquid Chromatography (SE-HPLC) gives information on the molecular weight of a polymer based on the time of elution from the column. The SE-HPLC columns have a silica-based support with a surface coating of a hydrophilic bonded phase to reduce interaction of the packing material with proteins (Batey et al., 1991). A calibrated size-exclusion chromatograph gives information on the molecular weight distribution of a polymer based on its elution time through the column. Molecular weight can be calculated by calibrating the column using proteins of known size. The elution volume is thus calibrated for molecular weight on the x-axis; protein quantity is indicated on the y-axis. Bigger molecules pass through the column faster than smaller molecules meaning that the higher the molecular weight, the shorter time it takes to pass through the column.

This technique was used to measure the relative size distribution of protein polymers and the total polymeric protein in the composite dough extracts in order to evaluate protein-protein interactions between soy and wheat proteins during mixing and fermentation of the dough. The methods for SDS extraction and SE-HPLC separation of proteins have been reviewed by Batey et al. (1991), who reported that the amount of SDS used affected the pore size and column separation since SDS binds to the column support, adding that the amount of SDS passing through the column caused a loss of resolution with time. They recommended 0.5% SDS in 0.05*M* phosphate buffer, pH 6.9 to extract protein from flour and 50% (v/v) aqueous acetonitrile containing 0.1% tetra fluoro-acetic acid (TFA) as the elution solvent.

4.2.3 Partial reduction and oxidation of soy-wheat dough proteins during farinograph mixing

The effects of added glutenin subunits on dough properties have been reported by Uthayakumaran et al. (2000), and this has been achieved by chemical incorporation of the glutenin subunits into the glutenin network of the base flour. Techniques that allow partial reduction and reoxidation of glutenin without changing its functionality have been described to be the key factor for such studies (Bekes et al., 1994). The simple addition of glutenin subunits to dough is unlikely to show any effect since, in this case, the added glutenin polypeptides would not be incorporated covalently into the gluten matrix. The concept of partial reduction and oxidation was applied in soy-wheat dough to allow for the possibility that soy proteins may be incorporated into a glutenin-soy complex during the oxidation process. Estimates of polymeric glutenin proteins and UPP% from the treated (partially reduced and oxidised) and non-treated (controls) doughs were used to evaluate incorporation and the possible contribution of soy proteins to dough properties.

4.2.4 Lab-on-a-chip capillary electrophoresis

The fractionation of charged macromolecules in an electric field is called electrophoresis. The net charge of proteins is determined by the pH of the medium in which the proteins are suspended. Below its iso-electric point, a protein is positively charged and migrates towards the cathode; above the iso-electric point, the proteins have net negative charge and they migrate towards the anode. At a given pH and under non-denaturing conditions, the electrophoretic separation of proteins is determined by both size and charge of molecules, depending on the extent of the molecular-sieving effect of the electrophoresis medium. Protein separation by SDS-PAGE is used to determine the relative abundance of major proteins in a sample, their approximate molecular weights and to identify what class they belong to. This is a useful technique because it separates the proteins according to a single molecular parameter, namely apparent size. SDS-PAGE can therefore be used for molecular characterisation using molecular standards to calibrate the system.

On the other hand, the capillary electrophoresis technique has many advantages over the traditional SDS-PAGE; including high separating power, shorter analysis time, moderate cost of operation and very low sample consumption. Capillary electrophoresis complements HPLC since the methods are based on different chemical and physical properties. While SDS-PAGE separates proteins mainly according to their apparent molecular weights, separation in capillary electrophoresis may be based on either size or charge differences. The Lab-on-a-chip system adopted fractionates mainly on size differences. Molecular weight determinations are made by comparison with standards (Robert et al., 1991). Lab-on-a-chip, a size-based capillary electrophoresis system, has been used for the identification of grain varieties, the determination of wheat flour composition, the determination of glutenin-subunit composition for wheat and the analysis of protein composition for other grains (Uthayakumaran et al., 2005).

4.3 Methods and Materials

4.3.1. List of chemicals

Ellman's reagent {DTNB (5,5'- dithiobis 2-nitrobenzoic acid)}, urea, SDS (sodium dodecyl sulphate), L-*threo* - Ascorbic acid, meta-phosphoric acid, potassium hydrogen phosphate, disodium hydrogen phosphate, dithiothreitol (DTT); potassium iodate, acetonitrile, triflouroacetic acid, glutathione (GSH), L - dehydroascorbic acid (L-DHAA). All were of analytical grade and purchased from Sigma Chemicals.

4.3.2. Preparation of flours

Whole-seed soybeans (Meriram Pty Ltd, Everton Hills, NSW Australia) were used to produce physically-modified soy flour #1 (PMSF1) by immersion of the soybeans in boiling water for three minutes and, a second physically-modified soy flour #2 (PMSF 2) which was made by dehulling (mechanically) soybeans and flush-steaming them for 3 minutes at atmospheric pressure to deactivate enzymes. The full method for the soy flour preparation is described in section 3.3.2 on Chapter 3. The raw soy flour (RSF) was full fat, enzymically active whole soy flour (Meriram Pty Ltd), made by milling the raw soy beans (also described on section 3.3.2 of Chapter 3). Commercial strong-wheat baking flour was obtained from the 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, PMSF 2 and the wheat flour) was 6.0, 7.3, 7.4 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. Protein conversion factors were N x 6.25 and N x 5.7 for soy flour and wheat flour, respectively.

4.3.3. Sampling for sulfhydryl and L-ascorbic acid analysis

Doughs were formulated from wheat flour, soy flour and water. 100 grams of flour consisting of 100 - 500 (g kg^{-1}) or 10-50% (w/w) soy flour substitution for wheat flour was weighed and transferred into a mixing bowl (flour composition as listed in Table 4.1). The amount of water added to the flour was the water absorption equivalent of 500 Brabender units for each sample. The preparation of RSF- wheat dough; PMSF1- wheat dough; PMSF2 - wheat dough is illustrated in Table 4.1. Dough blends were manually mixed using a stainless-steel dough mixer at an approximate speed of 60 rpm. The speed and time of mixing was monitored using a clock. The optimum time used for dough development was followed from Farinograph results of soy-wheat doughs at various concentrations (Chapter 3). At optimal dough development time, approximately 0.5g of sample was taken for SH determination. To the remaining dough, L-AA (0.05g / 100g flour) was added and this was mixed thoroughly for a further minute, using the same manual speed (60 rpm). The dough samples were covered (not tightly) and allowed to rest for another hour at ambient temperature (23ºC). Further sampling was done (as before) after resting the dough for I hour and this sample was used for the determination of free SH and L-AA consumed during resting. Control samples (without L-ascorbic acid treatment) were also mixed and sampled after resting the dough for I hour. The dough samples were frozen under N_2, freeze dried, ground to powder and stored in a cold room at 4ºC for further analysis of L-AA and SH.

Table 4.1.Preparation of soy-wheat doughs for sulfhydryl and L-ascorbic acid analysis

Soy flour (% w/w)	*Amount of water used for dough (mL water/100g flour)*		
	RSF-W dough	PMSF1-W dough	PMSF2-W dough
0	65	65	65
10	67	67	67
20	70	71	71
30	70	73	74
40	69	74	77
50	68	75	78

4.3.4. Determination of free SH (thiol) groups using Ellman's reagent

Extraction of proteins was done using urea and SDS, but this led to only partial solubilisation of proteins. To overcome this problem, the whole flour sample was suspended in urea, SDS and the colour reagent, 5, 5'– dithiobis (2-nitrobenzoic acid) (DTNB). This allowed simultaneous reaction of both soluble and insoluble proteins and the release of a soluble chromophore NTB^{2-} (2-nitro-5-thiobenzoate anion) which absorbed visible light at 412 nm. The method was modified following the reports from Wagner and Anon (1990), Chan and Wasserman (1993) and Borrelli et al. (2003).

40 mg of dried sample (from above) was suspended in 0.9 mL of 8M urea and 1% (w/v) SDS (sodium dodecyl sulphate) buffer. To make up to 1 mL, 0.1 mL of a 10m*M* Ellman's reagent, DTNB (5,5'-dithiobis 2-nitrobenzoic acid), was added. This reagent reacts with both soluble and insoluble proteins to release a soluble chromophore (NTB^{2-}). The samples were allowed to stand at room temperature for an hour with shaking of samples every 15 minutes to allow solubilisation of proteins. For clarification of the samples before reading the absorbance, micro-centrifugation for 15 minutes at 13,600 x g using a 12-24 Hettich Zentrifugen, Germany, model was used. After centrifugation, 0.1 mL of sample was diluted to 1 mL with 8M urea buffer and 1% (w/v) SDS solution. The absorbance was then read at 412nm using a UV/VIS spectrophotometer, Shimadzu mini 1240 model. To ensure the procedure was carried out under minimal oxidation conditions, nitrogen was flushed through the reacting buffer and colour reagents. The experiments were also conducted under minimum light since the chromophore formed (NTB^{2-}) is light sensitive (Borrelli et al., 2003). The molar absorption coefficient, 13,600M^{-1} cm^{-1} was used to calculate the concentration of thiols according to the equation:

$e = A/ C \times L$

where e is the molar absorptivity coefficient,

A = Absorbance

C = concentration in moles per litre

L = length of cell in cm

4.3.5. Estimation of L-ascorbic acid by UV/Vis Spectrophotometry

This method was adapted and modified from an HPLC method to determine L-AA and dehydro-ascorbic acid (DHAA) in vegetable products (Trifiro et al., 2003).

100 mg of dried sample from above were extracted with 1mL of a chilled 2% (w/v) meta-phosphoric acid solution in micro-test tubes. The extracting solution was chilled to minimise oxidation of LAA. After shaking for 5 minutes, the samples were centrifuged at 14,000 x *g* on a micro centrifuge 12-24 Hettich Zentrifugen for clarification. For the estimation of L-AA, an aliquot of 0.3 mL of sample was diluted to 3 mL using the 2% (w/v) meta-phosphoric acid solution in a test tube. The absorbance of this solution was read at 265 nm using a UV/VIS Spectophotometer, Pharmacia Biochrom system 4060.

For estimation of total L-AA, a second aliquot of 0.3mL was added to a separate test tube, together with 0.6mL of 10m*M* dithiothreitol solution in phosphate buffer at pH 7.6 and 2.1mL of phosphate buffer pH 7.6. These pH conditions were to optimise the reduction of DHAA to L-AA. The concentration of DHAA was then calculated as the difference between the total L-AA and the initial L-AA. To determine the concentration of L-AA from the UV absorbances, standards from 5, 10, 20, 30, 40 and 50 ppm were used to plot a calibration curve.

4.3.6. Isolation of soy proteins from raw and physically modified soy flour

Soy protein isolates were prepared following the methods from Yao et al. (1988), Liu (1997) and Puppo et al. (2004).

Protein slurries were made using defatted soy flours and distilled water at a ratio of 1:10 for grams of flour to mL of water. The slurry was adjusted to pH 9.0 using 6N sodium hydroxide. This was placed on a magnetic stirrer and allowed to stir for 1 hour at room temperature. The slurry was poured into 300 mL centrifuge bottles and separated on a 6 K15 B Braun Biotech International centrifuge (9600 x g for 30 minutes at 20°C). Following separation, the supernatant was decanted into a clean beaker and adjusted to pH 4.5 using 6N HCl and stirring gently. The iso-electric precipitate was allowed to stand for 1 hour in a refrigerator at 4°C after which, the suspension was placed in centrifuge bottles and centrifuged as above at 5°C. The supernatant was discarded and the precipitate was rinsed with distilled water and freeze dried. This was regarded as the iso-electric soy protein isolate.

The protein content for the soy protein isolates (SPI) was 77% and 87% for SPI made from RSF and PMSF#2 respectively (N x 6.25 for protein conversion factor).

4.3.7. Separation of 7S and 11S soy protein

Isolation of 7S and 11S soy protein was done following a fractionation method from Hou and Chang (2004). Soy flour was defatted by *n*-hexane (1:5, v/v = soy flour: hexane) by magnetic stirring for 1 hour at room temperature. Centrifugation followed (9,600*g*, 15 min) after which the supernatant was discarded and the residue was extracted once more. The defatted soy flour was allowed to dry overnight under a fume extract fan. Defatted soy flour was extracted with10 fold distilled water at pH 7.8 for 1 hour at room temperature. The solution was centrifuged (9,600*g*, 40 min at 4ºC) to separate the pellet, which was discarded. The supernatant was adjusted to pH 6.5 with 0.1 N HCl and allowed to stand overnight at 4ºC. This solution was centrifuged (9,600*g*, 40 min at 4ºC) to obtain the pellet (11S), which was washed with distilled water and freeze dried. The supernatant was adjusted to pH 5.5 with 0.1 N HCl to obtain a precipitate which was centrifuged (9600*g*, 40 min at 4ºC). The pellet was discarded and the supernatant was adjusted to pH 4.8 with 0.1 N HCl to obtain a precipitate which was centrifuged as above to obtain a pellet (7S). This pellet was washed with distilled water and freeze dried. The freeze dried pellets (11S and 7 S) were later ground to powder using a motor and pestle, and sieved through 0.8 mm sieve before analysis.

4.3.8. Extraction of glutathione dehydrogenase (GSH-DH) from wheat flour

A simple and rapid method to evaluate the role of soy protein (cysteine) was developed by modifying the glutathione dehydrogenase (GSH-DH) activity method of Every et al. (1999). The extraction of GSH-DH followed the method of Verbruggen et al. (1998). 20 grams of defatted wheat flour was pre-extracted three times with 100 mL of (50% v/v) propan-1-ol at room temperature for 30 minutes. Soluble gliadins were decanted after centrifugation of suspensions at 2500 x g for 15 minutes. After 3 extractions of gliadin, the glutenin residue was extracted in a water bath at 60°C for 30 minutes with 100 mL of 50% (v/v) propan-1-ol containing 1 % (w/v) dithiothreitol (DTT). After the extraction, the suspension was centrifuged at 10,000 x g for 30 minutes at 20°C. The resultant supernatant was adjusted to 60 %

(v/v) propan-1-ol by adding 1% DTT propan-1-ol. This solution was allowed to stand at 7°C ± 1°C for 1 hour to precipitate HMW glutenin subunits. The suspension was centrifuged at 10,000 x g for 30 minutes at 20 °C to separate the HMW glutenin subunit pellet, which was freeze-dried under nitrogen.

For the preparation of GSH-DH, 10 mg of the freeze-dried HMW glutenin was dissolved in1 mL (50% v/v) propanol in water and shaken to allow solubilisation. The solution was micro-centrifuged for 15 minutes at 13,600 x g to obtain a clear solution. The clear supernatant (crude GSH-DH) was decanted and used in the next experiment. This 1:1 propanol/ water - soluble fraction was considered to be effective (GSH-DH activity) for this experiment. Significant and non-enzymic reduction of DHAA by GSH has been reported (Kaid et al., 1997), therefore crude GSH-DH was found suitable for this work. Methods requiring GSH-DH of high purity and activity (320 U/g) have been reported for kinetic studies of wheat GSH-DHA oxido-reductase activity (Kaid et al., 1997) and kinetics of GSH-DH activity (Every et al., 1999).

4.3.9. Extraction of soy cysteine (CSH) from soy protein

Soy cysteine was extracted by dissolving 60mg soy protein isolate in 1 mL of 8M urea and 1% SDS buffer. The solution was shaken for 15 minutes and later micro-centrifuged for 15 minutes at 13,600 x g using a 12-24 Hettich Zentrifugen model to obtain a clear solution. This solution was used as crude soy protein cysteine (PSH).

4.3.10. Oxidation of glutathione (GSH) to oxidized glutathione (GSSG) using dehydro ascorbic acid (DHAA); A kinetic study

10µL of GSH-DH solution (from above) was mixed with 940 µL of 0.05 M disodium hydrogen phosphate-citric acid buffer (p H 6.2) and 25 µL of GSH solution (60mg/mL distilled water) in a UV cell (ambient temperature). The UV absorbance was adjusted to zero at 265 nm and 25 µL of L-DHAA solution (4mg/mL distilled water) was quickly added into the mixture (total solution of 1mL). From this point, the rate of UV absorbance change to L-AA production was recorded for the first 15 minutes using the UV/Visible spectrophotometer (Shimadzu mini 1240 Pharmacia Biochrom 4060) kinetic cell at ambient temperature (23ºC). The concentration of L-AA produced was calculated from the increase in absorbance at 265 nm using a molar extinction coefficient of 16 500 for L-AA (Davies et al., 1991).

To determine the effect of soy protein (PSH) on the production of L-AA, 25 µL of soy PSH solution was added to the mixture containing all the above solutions, except that the disodium hydrogen phosphate citric acid buffer was adjusted to 915 µL. PSH and GSH solutions were added to the cell before the UV absorbance was adjusted to zero at 265nm and 25 µL of L-DHAA solution (4mg/mL) was quickly added into the mixture. The rate of absorbance change to L-AA production was recorded for 15 minutes using the UV/Visible spectrophotometer as above.

A control model was carried out to evaluate the rate of L-AA production by substituting GSH with soy PSH and evaluating the effect of PSH and possible oxidation of PSH from DHAA in the presence of GSH-DH. This system contained; 940 µL buffer; 10 µL GSH-DH; 25 µL soy CSH and 25 µL DHAA solution.

Lastly, for non-enzymatic oxidation of GSH by DHAA; 25 µL of GSH, 25 µL of DHAA and 950 µL of buffer were tested without GSH-DH. L-AA absorbance curves were obtained and compared to find the effects on each system and mostly, to evaluate the role played by soy protein (CSH) on the rate of oxidation of GSH by DHAA. All experiments were run in 3 replicates and the mean values were used.

4.3.11. SE-HPLC

4.3.11.1. Dough preparation for protein extraction (during fermentation)

Dough was formulated from wheat flour, soy flour and water. The wheat flour was mixed in turn with an equal mass of one of the three types of soy flours (RSF, PMSF #1 and #2). To each of the composite flour mixes, amounts of water used were selected as the optimum to provide doughs whose resistances reached to the 500 Brabender-Unit mark on the Farinograph (Table 4.3.1). The mixing procedure and optimum time for dough development was followed from soy-wheat dough preparation on Section 4.3.3. The pH values of the composite doughs (1:1) were 5.1, 6.4, 6.3 and 6.5 for wheat dough, RSF-wheat and PMSF-wheat doughs (#1 and #2), respectively. After mixing, (“time zero minutes”), about 2 grams of dough was removed, and the rest of the dough was allowed to ferment (without yeast) in an incubator at 37ºC. Sampling was repeated after 1 and 2 hours of fermentation. Samples were placed in sterile plastic bottles and 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 a Newport hammer mill (0.8 mm sieve).

4.3.11.2. Dough preparation (partial reduction and oxidation)

Farinograph analysis was performed using a composite flour (equal amounts of wheat and soy flours) as base flour (total of 300g). To each of the composite flour mixes (1:1, wheat :soy flour), distilled water (70 mL or 80 mL per 100 g of flour for RSF or PMSF#2 wheat flour, 500 µL of dithiothreitol {(100mg / mL) ending up with 324 µ moles DTT in 300g of flour blend} was added to a 300-gram Farinograph bowl. This was mixed for 60 seconds, and allowed to react without mixing for 4 minutes. The partially reduced dough was treated with 500 µL of potassium iodate solution {(200 mg / mL) ending up with 462 µmoles KIO_3 /300g flour blend} and the dough was mixed for 1 minute and allowed to rest and react without mixing for 5 minutes. The dough was then mixed for a further 10 minutes. Dough samples were taken at peak time and break time in the Farinograph and these were freeze dried for SE-HPLC analysis and capillary electrophoresis (Lab-on-a-chip).

4.3.11.3. Extraction and fractionation of proteins for HPLC

Flour or freeze-dried dough samples were extracted following the method of Gupta et al. (1993). 10 mg sample was suspended in 1 mL 0.5 % SDS in phosphate buffer (p H 6.9) and mixed, initially by vortex-mixer and later shaken at 30°C on a Thermomixer Compact (Eppendorf) for 20 minutes at 2000 rpm. The suspension was then centrifuged for 10 minutes at 17,000 x 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 seconds using a Microson Ultrasonic cell distributor, ensuring that the sample was completely dispersed within the first five seconds. The supernatant from centrifugation for 10 minutes at 17,000 x g was termed “unextractable” protein. All extracts were filtered through a 0.45 µm PVDF filter prior to SE-HPLC analysis.

4.3.11.4. 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 ten-minute isocratic separation at a flow rate of 2 mL/minute 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 Daltons) and the gliadin peak (<100,000 Daltons) were measured by Gold Nouveau software (Beckman Instruments, Inc., Fullerton, CA USA). The proportion of "unextractable" polymeric protein (%UPP) was determined as a 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 very large proteins in the general size distribution for both soy and wheat samples.

4.3.12. Lab-on-a-chip capillary electrophoresis

In order to understand further the type of interactions between wheat and soy proteins during mixing of the dough, Lab-on-a-chip capillary electrophoresis was carried out on the dough powder samples (partially reduced and oxidized) following methods from Uthayakumaran et al. (2005). 20 mg of wheat flour, soy flour and freeze dried wheat - dough samples (1:1) were extracted in 0.5 mL 1% SDS solution [containing1% dithiothreitol (DTT) to provide reducing conditions] by vortex-mixing (5 sec) and shaking for 3 minutes at 65 ºC. The extracts were clarified by brief centrifugation. Extracts were applied with Agilent buffer to each of the 10 wells of a Protein 200 + Lab-on-a-chip for analysis in Agilent 2100 Bioanalyser (Agilent Technologies, Palo Alto, Ca).

4.3.13. Statistical Analysis

All analyses were done in triplicate. The data for SH groups were subjected to ANOVA (Analysis of Variance) using the Excel software package. Fisher's least significant difference procedure (LSD) was used to compare means and to determine the effect of L-AA on SH groups in the 3 flour types. Sample means were considered to be significantly different ($P < 0.05$).

HPLC and capillary electrophoresis analysis was done in duplicate for all samples and averages were used for results.

4. 4. Results and Discussion

4.4.1. Free SH (thiol) groups in soy-wheat dough

This section summarises results for the effects of L-AA on free SH content in soy-wheat dough during resting of the dough.

4.4.1.1 Effect of L-ascorbic acid on free SH groups in soy - wheat dough

Figure 4.1a

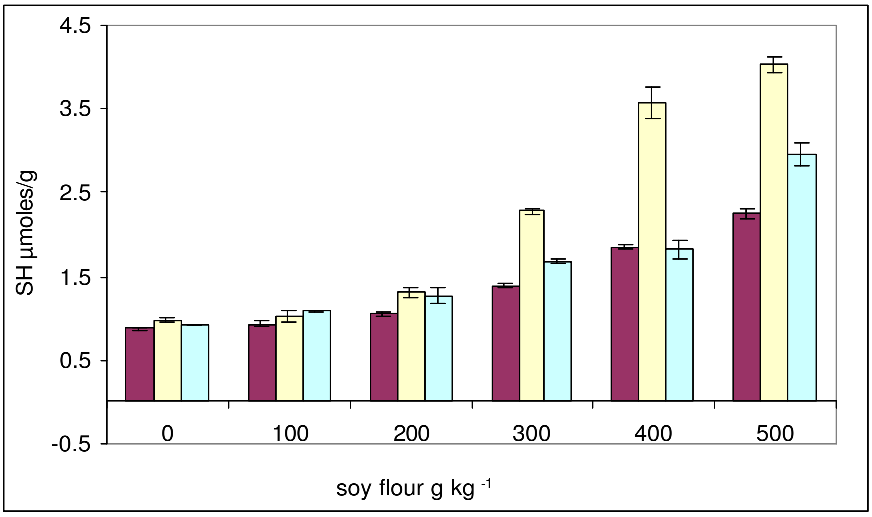


Figure 4.1b

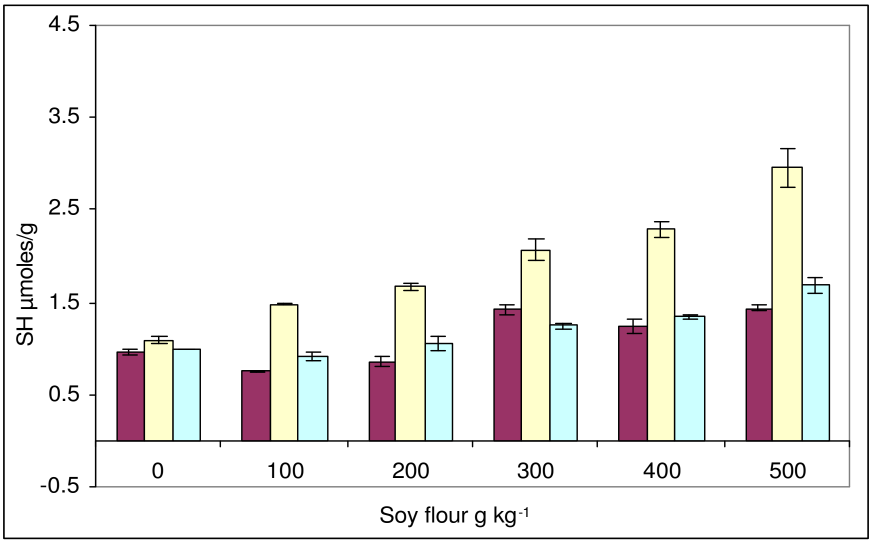


Figure 4.1c

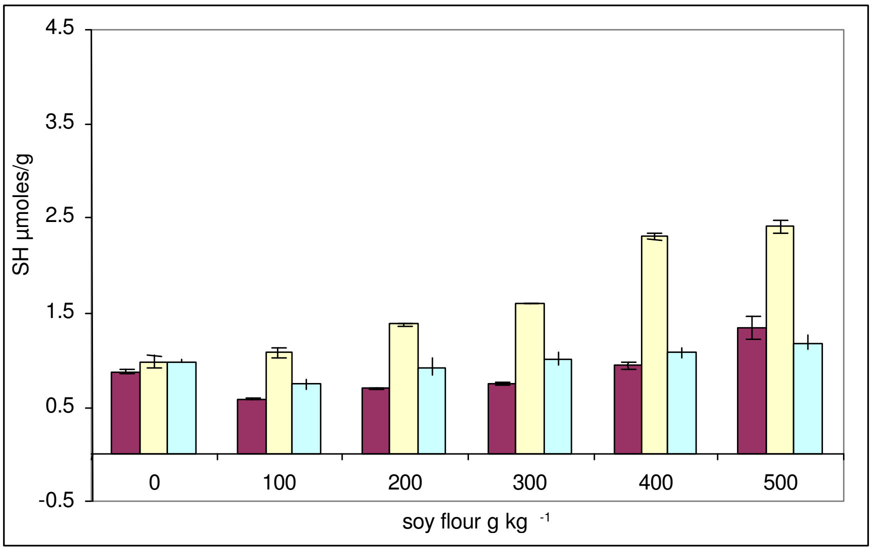


Figure 4.1a, 4.1b and 4.1c, SH concentration (µmoles/g) plotted against soy flour concentration (in grams per kg total flour) for RSF-wheat, PMSF1-wheat and PMSF2-wheat doughs respectively. Bars coloured ¦ represents SH concentrations at dough development time; ¦ represents SH concentrations in dough with L-AA after 1 h resting; while ¦ represents SH concentration of dough without L-AA after 1 h resting. Bars represent ± SD for triplicate analysis.

It is evident from Figures 4.1a - c that sulphydryl (SH) concentration increased with increasing soy flour concentrations in soy-wheat composite doughs. After 1 hour resting of dough, a positive correlation existed between SH concentration and soy flour concentration with r^2 values of 0.85, 0.86, 0.91 without L-AA and 0.91, 0.97, 0.96 with L-AA for RSF-wheat, PMSF1-wheat and PMSF2-wheat dough, respectively. The effect of L-AA on SH concentration was significant from 100 g kg^{-1} of soy flour concentration in PMSF1 and PMSF2-wheat dough; and from 200 g kg^{-1} in RSF-wheat dough. RSF (enzymically active soy-wheat dough) presumably showed its oxidising capacity by suppressing levels of SH at 100 g kg^{-1} soy flour.

Analysis of variance on the effect of L-AA on SH groups during resting of dough in all three soy-wheat doughs showed that there were significant differences between SH groups from initial to I hour resting of dough and between 1 hour resting of dough with and without L-AA for higher concentrations of soy flour (*$P< 0.05$*), but there was no significant difference in SH concentration between initially and after 1 hour without L-AA (*$P >.05$*). SH concentrations without L-AA were significantly lower than SH concentrations with L-AA; this result suggested that L-AA significantly increased SH concentrations in all soy-wheat doughs made from the three soy flour types. It also suggested that SH concentrations in soy-wheat doughs are more affected by L-AA addition than with soy flour concentration or time of dough resting.

Andrews et al. (1995) reported that mechanical or physical mixing of dough could cause stretching of disulphide bonds, causing them to break, resulting in sulphydryl formation. These observations confirm earlier findings (Chapter 3) that stretching of disulphide bonds during dough mixing could overwhelm the effect of oxidising agents. The effect of L-AA on SH groups was evaluated during dough resting, without mechanical stretching of disulphide (SS) bonds. This was done to mimic the practical situations where dough is kneaded for a few minutes and allowed to rest /ferment for an hour before baking.

L-AA works as an oxidising agent in the presence of oxygen, either from the atmosphere or from other oxidants; if oxygen is limiting, the L-AA acts as a reducing agent because dehydroascorbic acid (DHAA) is not formed (Tenido, 2003). Grosch and Wieser (1999) hypothesised that the oxidation of GSH to GSSG by L-threo DHAA is accelerated by glutathione dehydrogenase (GSH-DH) (E.C.1.8.5.1) and that this enzyme was specific to GSH, not CSH (cysteine). As evidenced from the results, soy flour cysteine could not be oxidised with L- AA. The change in free SH in wheat dough was insignificant compared to that in soy-wheat dough.

4.4.1.2. Interaction of soy CSH with gluten proteins

From observations in this experiment, interaction of soy and wheat proteins in soy-wheat doughs was assumed because of the change in SH concentration due to L-AA. The high concentration of cysteine (CSH) in soy-wheat doughs is expected to cause interactions with protein disulphides, thus leading to the formation of GSH and CSH according to Equations 2, 3 and 4. CSH is considered as a strong reducing factor and the presence of L-AA could possibly cause a synergistic effect on the reduction of gluten proteins in the following interchange reactions;

DHAA + 2 GSH $\longrightarrow$ LAA + GSSG	(Equation 1)	(Grosch, 1999)
GSSG + PSH $\longrightarrow$ PSSG + GSH	(Equation 2)	(Grosch, 1986)
P-SS-G + CSH $\longrightarrow$ P-SS-C + GSH	(Equation 3)	(Grosch, 1986)
P-SS-C + GSH $\longrightarrow$ P-SS-G + CSH	(Equation 4)	(Grosch, 1986)

Although the enzymatic reaction (GSH-DH catalysed) in (1) was considered to be faster (Grosch and Wieser, 1999; Sarwin et al., 1993) than the reaction of GSSG with protein thiols (2), the formation of CSH and GSH from interchange reactions between gluten protein and protein cysteine sulphides (2, 3 and 4) was likely to increase free SH content in soy-wheat dough. Because the reaction of GSSG with protein thiols (2) has been observed to be faster in L-*threo*-AA doughs (Grosch and Wieser, 1999) and this coupled with the high amounts of reactive radicals formed in this composite dough, could also disrupt the formation of gluten disulphides (1). The disulphide interchange reaction has been reported to be fast when flour is wetted, with a possibility of free thiols, naturally present in flour (especially low molecular weight), attacking glutenin disulphide bonds (Ewart, 1990).

4.4.1.3. The L-AA consumption pattern in soy-wheat doughs

Results on the consumption of L-AA in soy-wheat composite doughs, after resting the dough for 1 hour, are illustrated in Figure 4.2. From the trend shown, the concentration of L-AA decreased with increases in soy flour concentration for the 3 types of composite doughs. Analysis of variance to compare L-AA consumption between the soy-wheat dough models indicated that there were significant differences between RSF and PMSF1-wheat dough ($P < 0.05$) and between RSF-wheat and PMSF2-wheat dough ($P < 0.05$). There was no significant difference between PMSF1 and PMSF 2 ($P.> 0.05$). This suggested that composite doughs made from physically modified soy flour had similar L-AA consumption patterns and that RSF had a significantly higher consumption pattern. This could be attributed to the higher levels of oxidation products anticipated from the enzymatically active soy flour, as these were likely to participate in L-AA oxidation.

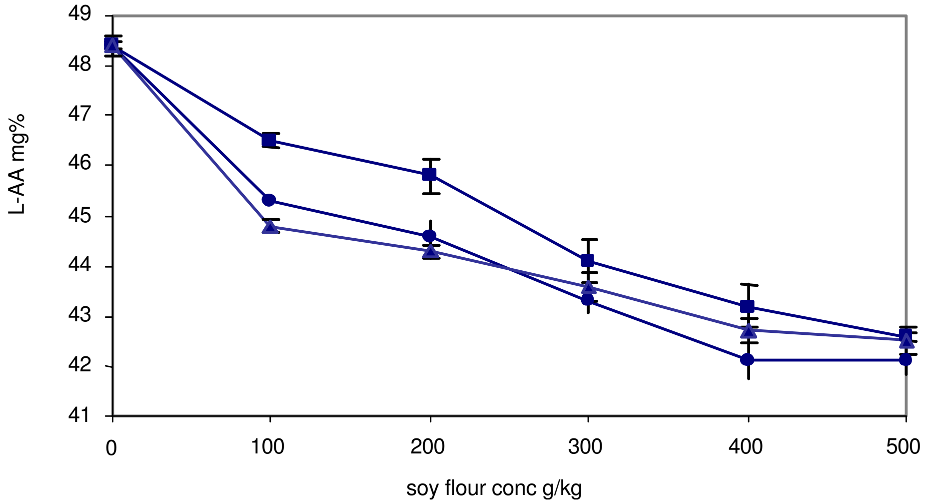


Figure 4.2 Relationships between the amount of L-AA (mg /100g) used and soy flour composition (0 - 500g kg^{-1}) in three soy-wheat composite dough after I hour of resting the dough. Bars represent ± SD. Symbol on lines; ¦ = RSF-W (raw soy flour-wheat) dough; ? = PMSF1-W (physically modified soy flour1- wheat) dough; and ? = PMSF2-W dough

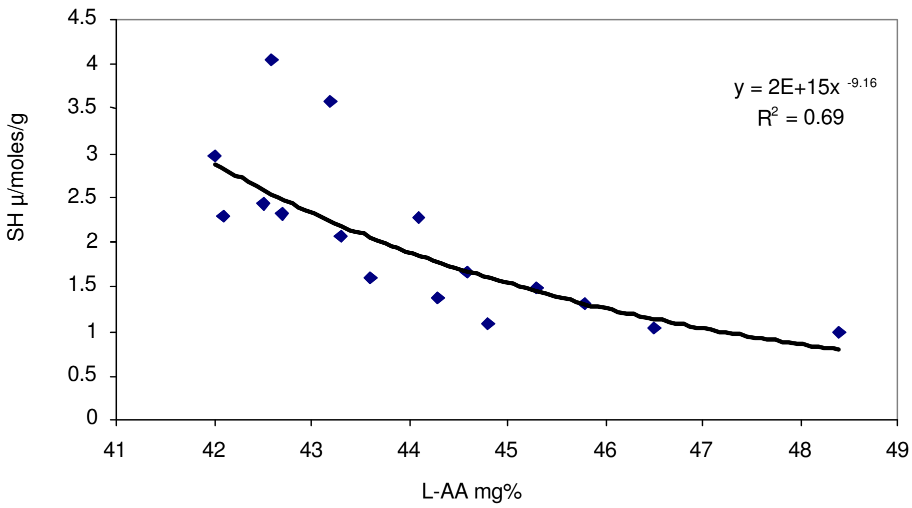


Figure 4.3 Relationship between SH concentration (µmoles /g) and L-AA (mg %) oxidised after 1 hour resting of soy-wheat doughs made from RSF, PMSF1 and PMSF2 in the same ratios as in Figure 4.2.

Figure 4.3 illustrates the relationship between SH concentration and L-AA oxidised after resting the dough for 1 hour. There was a negative correlation between SH concentration and the amount of L-ascorbic acid used in all three soy-wheat dough types, with higher consumption of L-AA in lower soy flour wheat doughs. This could have been due to less interference from soy flour in these doughs.

Table 4.2 Residual L-AA and DHAA left in soy-wheat dough after 1 hour of dough rest L-AA is expressed as mg /100g.

Values are the means of triplicate determinations, ± values are standard deviations.

soy flour (g kg^{-1})			
RSF-W	Total residual L-AA [a]	Residual L-AA [b]	Residual DHAA [c]
0	1.6 ± 0.1	1.3 ± 0.1	0.3 ± 0.0
100	3.5 ± 0.2	2.8 ± 0.2	0.7 ± 0.1
200	4.2 ± 0.1	3.7 ± 0.1	0.5 ± 0.1
300	5.9 ± 0.2	5.4 ± 0.2	0.5 ± 0.0
400	6.8 ± 0.1	6.4 ± 0.1	0.4 ± 0.0
500	7.4 ± 0.2	7.2 ± 0.2	0.2 ± 0.0
PMSF1-W			
0	1.6 ± 0.1	1.3 ± 0.1	0.3 ± 0.0
100	4.7 ± 0.1	3.5 ± 0.1	1.2 ± 0.0
200	5.4 ± 0.1	4.7 ± 0.2	0.7 ± 0.0
300	6.7 ± 0.1	5.9 ± 0.1	0.8 ± 0.1
400	7.9 ± 0.3	7.3 ± 0.2	0.6 ± 0.0
500	7.9 ± 0.2	7.5 ± 0.2	0.4 ± 0.0
PMSF2-W			
0	1.6 ± 0.1	1.3 ± 0.1	0.3 ± 0.0
100	5.2 ± 0.1	3.7 ± 0.1	1.5 ± 0.1
200	5.8 ± 0.1	4.7 ± 0.1	1.1 ± 0.1
300	6.4 ± 0.2	5.8 ± 0.2	0.6 ± 0.0
400	7.3 ± 0.3	7.1 ± 0.3	0.2 ± 0.0
500	7.5 ± 0.3	7.2 ± 0.3	0.3 ± 0.0

[a] represents the total amount of unused L-AA + DHAA in dough sample after 1 hour

[b] represents the amount of L-AA (unused) in sample and [c] represents the difference between a and b and is the DHAA estimated in dough sample after 1 hour.

Results in Table 4.2 show the residual L-AA and DHAA in the dough after 1 hour of resting. Significant differences for residual L-AA in soy-wheat dough were (*$P < 0.05$*); *$P < 0.05$*) and *($P > 0.05$*); for RSF and PMSF1; RSF and PMSF2 and PMSF1 and 2 composite doughs, respectively. These values confirm that L-AA consumptions in PMSF 1 and 2 were not significantly different, and that a greater amount of L-AA had been consumed by the dough, either through redox reactions or more complex reactions within the dough system. Residual DHAA concentrations were unexpectedly low in the dough. From theoretical considerations, oxidation of L-AA in the dough should have resulted in high concentrations of DHAA since the SH concentration indicated no effect from this oxidising agent. The presence of a high concentration of oxidation radicals formed from lipoxygenase activity in soy-wheat dough possibly reacted with DHAA and irreversibly converted it to its 2-3- diketo-L-gulonic acid form with no L-AA activity.

Observations made from this work showed that L-AA had increased the SH concentration in the soy-wheat dough from 100 - 500 (g kg^{-1}) soy flour during resting of the dough. The magnitude of the response in free SH groups from L-AA addition suggested more interaction of soy and wheat proteins in the presence of L-AA. There was no clear evidence to link L-AA oxidation to the free SH groups in the soy-wheat dough, except that L-AA and soy cysteine possibly reduced gluten proteins synergistically. Apart from using oxidising agents as dough strengtheners, physical means of stabilising soy-wheat dough was suggested as another way to improve its baking performance. A more controlled experiment to monitor the interaction of soy cysteine in the presence of an oxidising agent (DHAA) during SS/SH interchange is described in the next section.

4.4.2. Soy flour cysteine in SS/SH interchange

The effect of soy flour cysteine on a model GSSG production system is described in this section.

4.4.2.1. The role of physically modified soy protein in GSSG formation

The interaction of soy flour cysteine (CSH) during the enzymatic oxidation of GSH to GSSG by DHAA in the presence of GSH-DH is described with the following proposed mechanisms for L-AA and GSSG production given in Figure 4.4.

DHAA + 2 GSH + (GSH-DH) ? L AA + GSSG (enzymatic oxidation) (Model 1)

DHAA + 2 GSH + PSH1 (GSH-DH) ? L AA + GSSG (inhibition from PSH) (Model 2)

DHAA + 2 GSH + PSH2 (GSH-DH) ? L AA + GSSG (less inhibition) (Model 3)

DHAA + PSH1 or 2 + (GSH-DH) ? (insignificant reaction) (Model 4)

GSH + DHAA ? LAA + GSSG (non-enzymatic oxidation) (Model 5)

PSH1 soy protein from raw soy flour (RSF)
PSH2 soy protein from thermally treated soy flour (PMSF2)

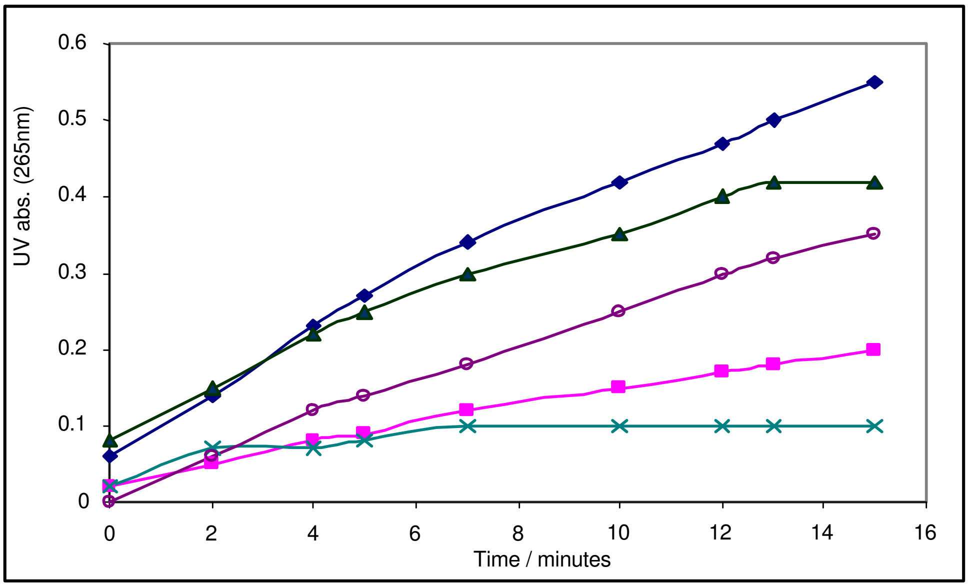


Figure 4.4 Rate of change in UV absorbance of L-AA (265 nm)

? represents model 1; ¦ represents model 2; ? represents model 3; ? represents model 4; and ? represents model 5. Proposed mechanisms for the models are given above. Model systems are shown in Table 4.3.

Results in Figure 4.4 show the rate of L-AA production in the five systems listed in Table 4.3. The oxidation of GSH to GSSG by DHAA in the presence of GSH-DH (Model 1) relates to the hypothesis of Elkassabany and Hoseney (1980), Grosch (1986), Grosch and Wieser (1999) and Every et al. (1999).

The presence of soy PSH {(from raw soy flour) Model 2} in the GSH /GSH-DH model system significantly inhibited GSSG production (from 1.82 to 0.61 µmoles / L / min). However, soy PSH extracted from physically modified soy flour (Model 3) slowed the rate of reaction to a lesser extent compared to raw soy protein (1.21 µmoles / L /minute). This finding may be explained by the fact that PSH (extracted from physically modified soy flour) has been mostly modified to a stable form (PSSP) during thermal treatment of the soy flour preparation; therefore this protein may not interfere to a great extent with the oxidation of GSH to GSSG.

Model 4 system indicated that replacement of GSH with soy PSH would be detrimental to the production of GSSG (0.12 µmoles / L /min). This also indicated that soy PSH is not a substrate for GSH-DH as suggested by other workers on the substrate specificity of GSH-DH (Grosch, 1986; Grosch and Wieser, 1999).

The model system with GSH, DHAA and buffer without GSH-DH (Model 5) produced GSSG at a rate of 1.21 µmoles / L / minute. Although this reaction was slower than the enzymic system (Model 1) at 1.82 µmoles / L/ min, L-AA was still produced from DHAA in the presence of GSH only (non-enzymatic) and this observation confirmed the theory reported by Kaid et al (1997) that significant and non-enzymic reduction of DHAA by GSH could still occur.

Table 4.3 Summary of model systems used for the production of L-AA and the resultant amount of GSSG (µmoles / L) produced per minute.

Exp	*GSH*	*GSH-DH*	*soy-PSH*	*L-DHAA*	*Buffer*	*L-AA abs/min.*	*GSSG /min (µ moles / L)*
1	*	*		*	*	0.03	1.8
2[a]	*	*	*	*	*	0.01	0.61
3[b]	*	*	*	*	*	0.02	1.2
4		*	*	*	*	0.002	0.12
5	*			*	*	0.02	1.2

[a] soy protein thiols (PSH) from raw soy flour (RSF)

[b] soy protein thiols (PSH) from physically modified soy flour (PMSF2)

During the kneading of dough (Grosch and Wieser, 1999), L-AA is rapidly oxidised to L-DAA by atmospheric oxygen and L-DAA becomes an improver in strengthening dough.

L-AA + ½ O_2 ? L-DHAA + H_2O (Grosch and Wieser, 1999)

Experiments to evaluate the oxidation of L-AA in soy-wheat dough during mixing and resting indicated that L-AA consumption correlated negatively with soy flour concentration in wheat dough. These results suggested that more DHAA would be produced in a soy-wheat dough containing less soy flour. L-AA oxidation (from atmospheric oxygen) may be hindered by interferences in soy-wheat dough as free radicals, formed from oxidation products of soy flour dough, are likely to compete for the singlet oxygen molecules in Reaction model 1 and thus formation of DHAA may be inhibited in soy-wheat dough. However for this model experiment, D-HAA was added to a GSH/ GSH-DH system to simulate a wheat dough system. On wetting the wheat dough treated with L-AA, effective mixing of the dough in the presence of oxygen from the air rapidly oxidises L-AA to DHAA. GSSG may rapidly increase as follows:

DHAA + 2 GSH (GSH-DH) ? L AA + GSSG (Grosch and Wieser, 1999)

As dough mixing continues, GSSG decreases, leading to a reaction of GSSG with protein SH groups.

GSSG + PSH **?** PSSG + GSH (Grosch and Wieser, 1999)

Cysteine residues of glutenin also form intermolecular disulphide bonds with GSSG as follows

GSSG + 2CSH ? CSSC + 2GSH (Grosch and Wieser, 1999)

High levels of low molecular weight SH groups that are not substrates of GSH-DH are suggested to have slowed down the production of L-AA and consequently the production of GSSG. As pointed out by Grosch and Wieser (1999) doughs that contain too much of the low molecular weight SH may be negatively affected with L-AA addition. Results from this experiment indicated that native (raw) soy proteins (PSH and CSH) may inhibit S-S/S-H interchange by slowing down the formation of gluten disulphides which are essential for strengthening the dough. On the other hand, soy protein from physically modified soy flour slightly inhibited the rate of reaction (GSH/GSSG) for the formation of gluten disulphides. This observation may indicate that physically modified soy flour has less interference with wheat dough development compared to raw soy flour.

An important consequence of the formation and interchange of disulphide groups between proteins and small molecules is that the molecular-weight distribution of the dough proteins is likely to change, thus altering their contributions to dough quality. An essential next step was thus to determine the size distribution of the dough proteins. These experiments are described in the next section, using SE-HPLC as the analytical method.

4.4.3. Size distribution of dough proteins

This section deals with the evaluation of the relative protein size distribution (UPP%) of the original wheat and soy flours, and composite doughs made from these flours, during mixing and resting, as it also evaluates possible protein interactions at the molecular level using size-exclusion high-performance liquid chromatography (SE-HPLC).

4.4.3.1. SE-HPLC of wheat and soy flours

The elution profiles for extractable protein (EP) from wheat flour (Figure 4.5a) 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 (Figure 4.5b), in accordance with the reports of Gupta et al. (1993) and Carceller and Aussenac (2001). The percentage of unextractable polymeric protein (%UPP) in total wheat-flour proteins [in total Peak-1 dough proteins] 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 (Figure 4.5c and d respectively). The profiles for the physically-modified soy flour (PMSF #2) had higher proportions of large proteins than those for raw soy flour. In fact, the profile for unextractable PMSF protein (Figure 4.5f) 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 #1 were similar to those shown for PMSF #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 render the bulk of the soy protein extractable into solution for analysis by SE-HPLC.

The extractability of soy proteins without sonication was greater for raw soy flour (only 8% UPP) than for physically-modified soy flour (19% UPP and 34% UPP for PMSF #2 and #1 respectively). Unextractable polymeric protein was much greater for the wheat flour (57% UPP) [Appendix 4.3].

Fig.4.5a Wheat flour EP

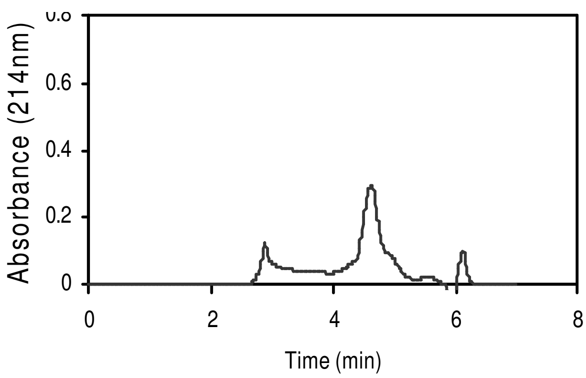


Fig. 4.5b Wheat flour UP

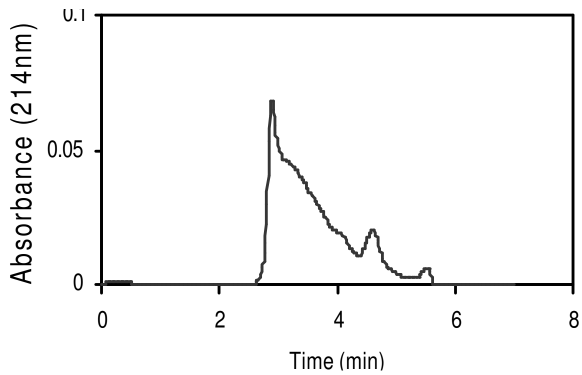


Fig 4.5c RSF EP

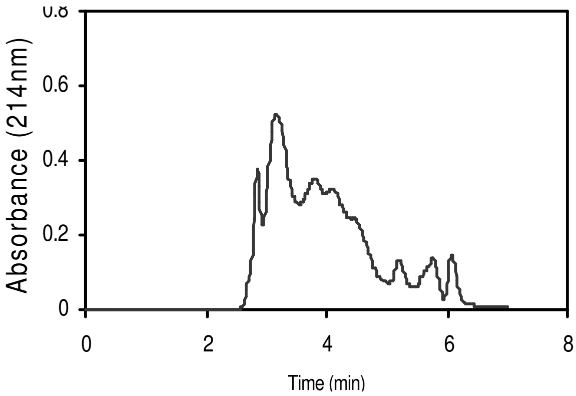


Fig 4.5d RSF UP

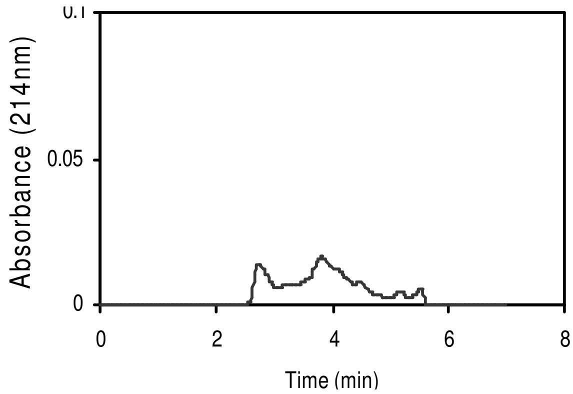


Fig 4.5e PMSF2 EP

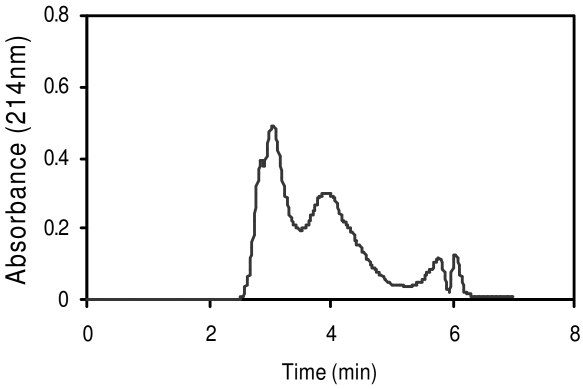


Fig.4.5f PMSF2 UP

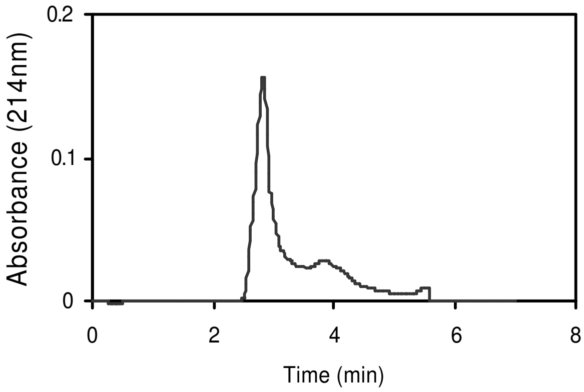


Figure 4.5a -f. SE-HPLC elution profiles for extractable protein (EP) and for unextractable protein (UP) of wheat flour, raw soy flour (RSF) and physically-modified soy flour (PMSF #2). Note the different absorbance scales (y axis)

Peaks at 2.91 and 3.82 minutes (>100,000 Da) in soy flours (EP and UP) corresponded to subunits of 7S and 11S soy proteins, respectively (Fig.4.5c - 4.5f). The two small peaks (<70,000 Da) at 4.5 and 5.25 minutes were regarded as smaller subunits of 11S and 7S soy proteins. Under the same experimental conditions, SE-HPLC profiles for isolated soy protein showed two distinct peaks; the first one at 2.9 minutes was assigned to 7S, while the second one at 3.8 minutes was assigned to11S (results not shown).

Figure 4.6a

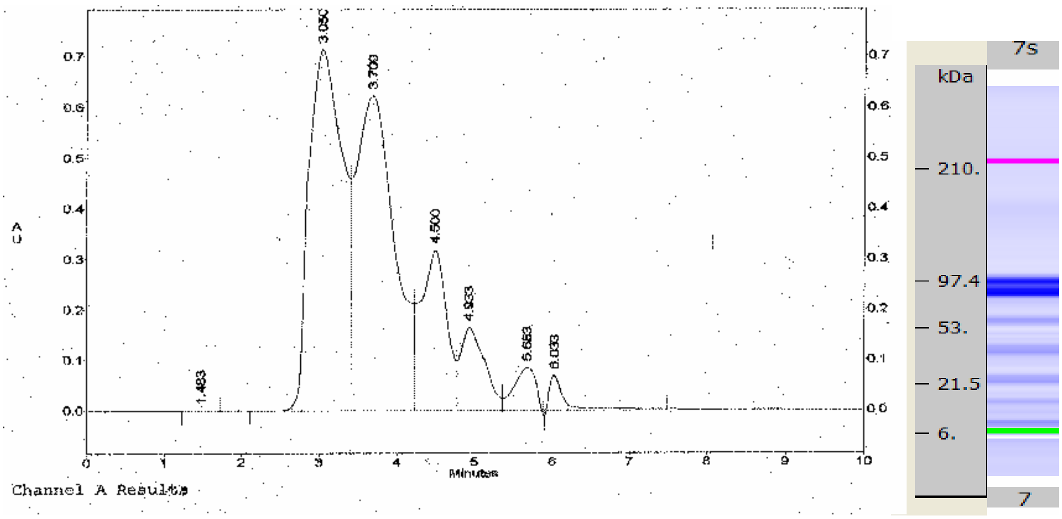
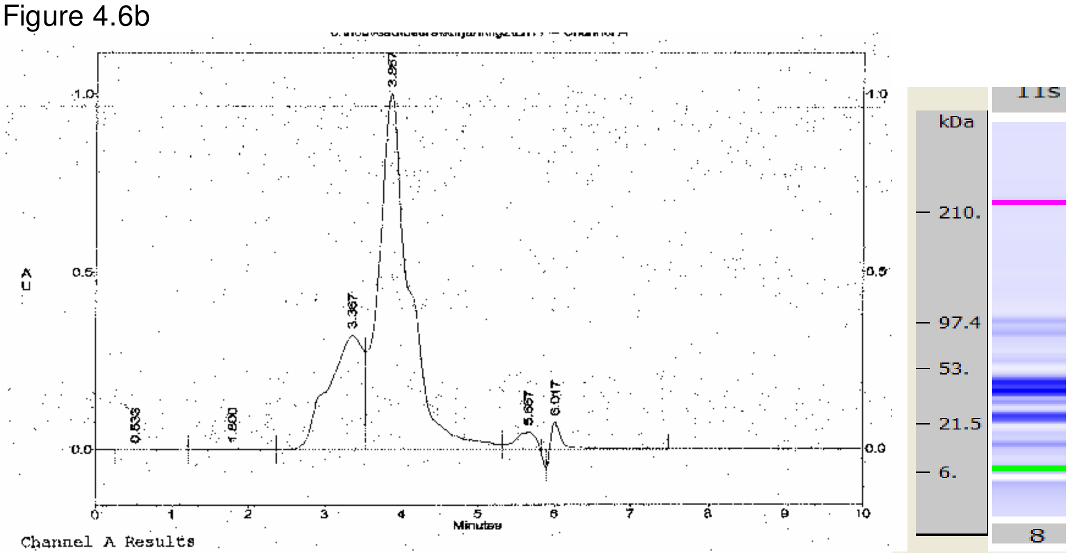


Figure 4.6b



Figures 4.6a and b show SE-HPLC (0.5% w/v SDS in 0.05 M Na phosphate buffer, pH 6.9) for 7S and 11S soy proteins respectively. The corresponding capillary electrophoresis gels (1% SDS) for the same proteins non - reducing conditions appear on the right.

The purified preparation of 7Sand 11S soy proteins were analysed by SE-HPLC and by capillary electrophoresis (Figure 4.6). The 7S major peak [Figure 4.6a, split into two (3 - 3.7 minutes)] corresponded to the distinct band on the 7S electrophoregram (85-90 kDa) confirming that the major peak (3 - 3.7min) is the 7S soy protein. Because of the emergence of this broad peak, it is possible that some 11S soy protein was present in this 7S preparation. The 11S soy protein (Figure 4.6b) showed one distinct peak (3.9min) corresponding to one dense band at approximately 45 kDa on the electrophoregram. Molecular weights (42 - 44 kDa and 58 - 85 kDa) are in accordance with previous work for 11S and 7S subunits respectively (Liu, 1997; Apichartsrangkoon, 2002; Hou and Chang, 2004). From these findings, the major peaks in soy flours (2.9 and 3.8 minutes) were regarded as 7S and 11S soy proteins, respectively.

4.4.3.2. SE-HPLC profiles for wheat and soy-wheat composite doughs

Figure 4.7 (parts a, c and e) show the SE-HPLC profiles for extractable proteins in wheat and in soy-wheat (1:1) doughs. The elution profiles are relatively 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 (Figure 4.7, parts b, 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. It was similar to the corresponding profile for the wheat-only dough. On the other hand, the raw-soy composite dough had a majority of medium-sized proteins, being similar to the corresponding profile for extractable proteins. The protein fraction on Peak 1 (unextractable protein) mostly consists of the large glutenin of wheat (>100,000 Daltons) (Gupta et al., 1993). Studies have confirmed that this polymeric protein fraction governs the technological parameters of wheat flours (Gupta et al., 1993; Carceller and Aussenac, 2001), and that it represents the major criteria for dough strength and baking quality (Fisichella et al., 2003; Grosch and Wieser, 1999).

The subunits of wheat glutenin polymers are linked by inter-chain disulphide bonds and the addition of reducing agents to glutenin results in the 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 essentially intact, thereby preserving the large polymer structure of glutenin. 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 has been shown to 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 the 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 (Wagner and Anon, 1990; Liu, 1997). The process of denaturation involves dissociation and unfolding of proteins, and is often accompanied by the 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 (Wagner and Anon, 1990; Liu, 1997; Puppo et al., 2004). 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 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 of raw (native) soy flour, PMSF2 and PMSF1. Results in Section 4.4.1 showed lower free SH groups in the physically modified soy flours compared to those for raw soy flour, suggesting that the process of physical modification has conformed most of the free SH into disulphide bonds. This development is also supported by the higher values of unextractable polymeric proteins in the physically modified soy flours compared to those of raw soy flour. Soy proteins separated from raw soy flour (SP1) and those made from physically modified soy flour (SP2) gave a polymeric to monomeric ratio of 4 and 20.5 for SP1 and SP2 respectively. This also confirmed the aggregation of proteins into higher molecular weight proteins caused by the physical modification process of 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.

Fig. 4.7a, Wheat dough EP

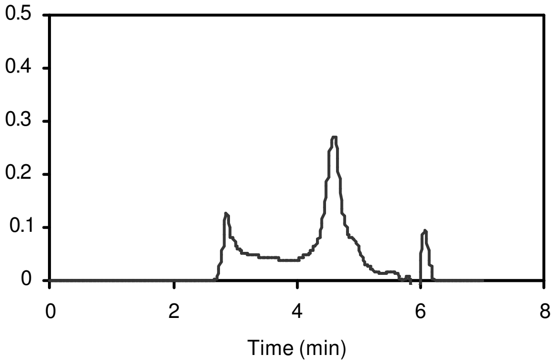


Fig. 4.7b, Wheat dough UP

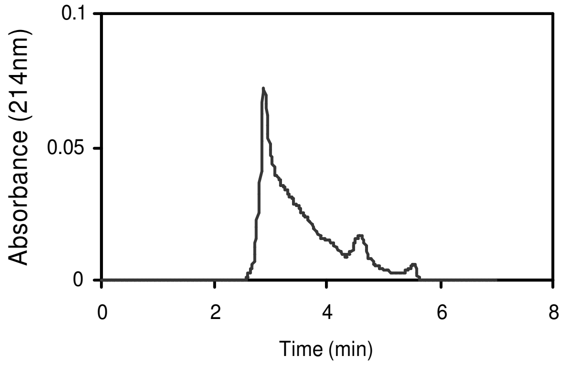


Fig.4.7c, W-RSF dough EP

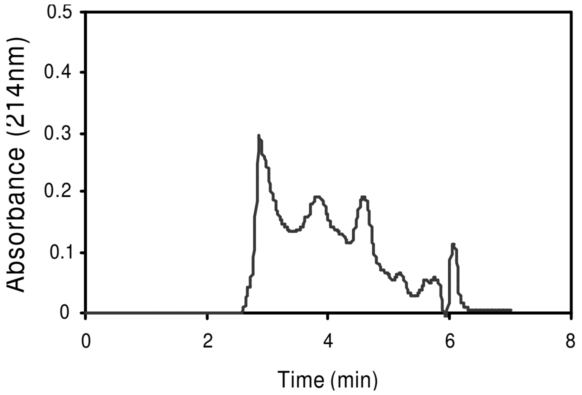


Fig. 4.7d, W-RSF dough UP

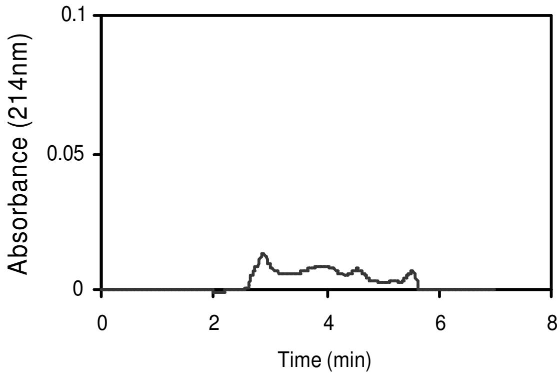


Fig. 4.7e, W-PMSF dough EP

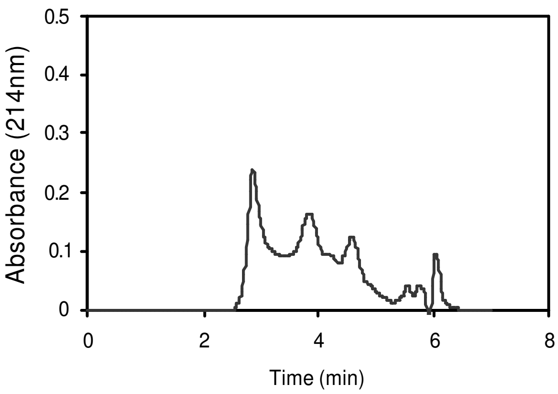


Fig.4.7f, W-PMSF dough UP

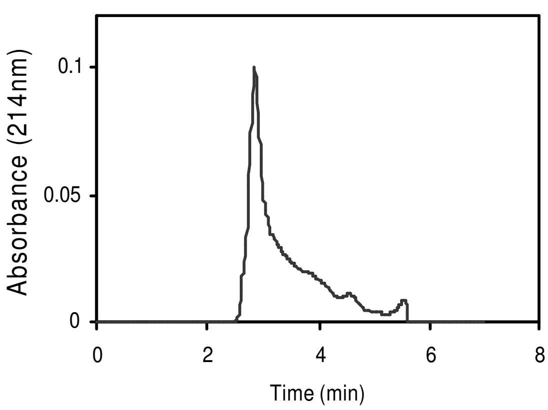


Figure 4.7a –f: SE-HPLC elution profiles for extractable protein (EP) and for unextractable protein (UP) from doughs fermented for 1 hour, made from wheat flour, wheat mixed with raw soy flour 1:1 (W-RSF) and wheat mixed with physically-modified soy flour #2 1:1 (W-PMSF). Note absorbance values (y-axis)

4.4.3.3. Relation between UPP of composite dough and baking quality

Evidence that baking performance is positively related to the disulphide-linked glutenin polymers, and that the largest 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 (Gupta et al., 1995; Grosch and Wieser, 1999; Fisichella et al., 2003). 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 (Surana et al., 1973; D'Appolonia, 1977; Bushuk, 1985). Results from our work now demonstrate that molecular size distribution (shown by %UPP) is reduced by the RSF in soy-wheat doughs during fermentation. The PMSF-wheat dough contributed higher resistance to extension (R_m) and greater mixing tolerance than the RSF-wheat dough in the preceding chapter (App.4.1).

Puppo et al. (2004) observed a big peak at 813 kDa from soy protein isolate at pH 8, which they suggested to be native 7S and 11S subunits with a shoulder at 50 k Da. The same soy protein isolates at pH 3 exhibited two major peaks at elution volumes around 190 and 220 m L and these corresponded to molecular masses lower than 66 kDa. Molina and Anon (2000) also observed one large peak at 917 k Da from non-hydrolyzed soy protein isolates, which they attributed to trimeric and hexameric forms of 7S and 11S protein respectively. Because 7S and 11S proteins have molecular masses between 180-210 and 350 kDa respectively, they attributed this peak to the existence of a high degree of association from these soy proteins.

From the results highlighted in Figure 4.5c –f, soy flours exhibited two distinct peaks (at 2.9 and 3.8 minutes) and the elution profile for these peaks fell into the polymeric protein classification (>100,000 Da) according to Carceller and Aussenac (2001) and Gupta et al. (1993). Subunits of 11S proteins have molecular masses of 35 kDa and 20 kDa, for the acidic polypeptide and the basic polypeptide respectively, while for 7S, the sizes are 57 kDa and 42 kDa for each of α^1 and α

subunits and β subunits, respectively (Grosch and Wieser, 1999). Because these subunits are smaller than 100.000 kDa, the two distinct peaks in these soy flours could theoretically be assigned to aggregates of 7S and 11S of the relative subunits. Results from Molina and Anon (2000); Puppo et al. (2004) (using non-hydrolyzed soy protein isolates; soy protein isolate at pH 8 respectively) showed that 7S and 11S proteins could not be resolved under their given experimental conditions but existed as aggregates. Under acidic conditions (pH 3), soy proteins exhibited two major peaks, which corresponded to molecular masses lower than 66 kDa; high molecular weight polymers remained unextractable (Puppo et al., 2004). However in the current experimental conditions (pH 6.9), the soy protein major peaks indicated that soy protein aggregates (>70,000 kDa) were extractable and that the two major storage soy proteins (7S and11S) were resolved as separate peaks.

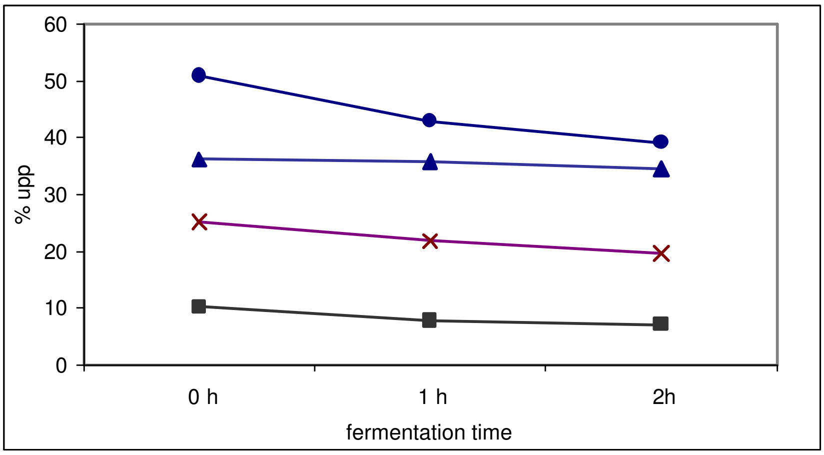


Figure 4.8 shows % UPP of soy-wheat dough versus fermentation time in hours; ? = wheat dough; ? = PMSF 1-wheat dough; x = PMSF 2-wheat dough; ¦ = RSF-wheat dough. Points on the figures represent the mean of duplicate values; error was within ± 3% of the mean.

Unextractable polymeric protein continued to decrease during the fermentation period for all doughs (Figure 4.8), indicating that the polymeric proteins were being gradually broken down during dough fermentation. The rate of decrease in % UPP was greatest during fermentation for the dough made from wheat flour only.

4.4.3.4. Effect of soy flour amount on UPP and pH of soy-wheat dough

Soy-wheat dough made from PMSF2 was used to evaluate the effect of soy flour concentration on UPP during fermentation. SE-HPLC profiles for extractable proteins were the same as for soy-wheat doughs (Figure 4.7c -f) except that the polymeric protein, Peak 1 (>100000 Da), increased proportionally to soy / wheat flour. The unextractable protein HPLC profile for PMSF2-W dough also showed one major peak (2.9 mins) which increased in size with increase of soy protein up to 30% soy flour in the soy-wheat doughs. Above 30% soy flour in the composite dough, this major peak (polymeric protein peak) decreased in size suggesting that interaction of the polymeric proteins in the two flours was optimum up to 30 % soy flour in the composite dough and after that interaction was possibly hindered by the high concentration of non-wheat proteins. Table 4.4 shows the effect of soy flour concentration on UPP% in soy-wheat dough made from PMSF2 during fermentation (up to 1 hour fermentation at 37ºC). Theoretical UPP% values in PMSF-wheat composite flours were calculated and compared with dough made from the flours. Above 30% soy flour in composite dough, UPP% did not change appreciably after 30 minutes of dough fermentation. The decline in UPP from original flour composites to the dough prepared from these flours is possibly due to chemical changes (SS/SH interchanges) which take place when water is mixed with the flour.

Table 4.4: UPP% in flours and in soy-wheat doughs during resting
± Values given are the error of the mean for replicate analyses

% soy flour (PMSF2)	*UPP% in flours*	*UPP% in dough 0 min*	*UPP% in dough 30 min*	*UPP% in dough 1 hr*
0 (wheat flour)	57.0 ± 1.7	49.8 ± 1.0	46.6 ± 0.0	43.1 ± 0.1
10	53.2 ± 1.2	33.3 ± 1.0	34.5 ± 0.2	33.6 ± 0.6
20	49.4 ± 1.0	26.4 ± 0.1	28.9 ± 0.2	27.9 ± 0.6
30	45.6 ± 0.9	24.3 ± 1.7	21.8 ± 0.1	21.3 ± 1.0
40	41.8 ± 1.2	19.1 ± 0.7	19.2 ± 0.3	18.3 ± 0.3
50	38.0 ± 0.8	18.9 ± 1.4	18.5 ± 0.9	17.2 ± 0.2
100 % PMSF2	19.4 ± 0.8			
100 % PMSF1	34.1 ± 0.4			
100 % RSF	7.8 ± 0.2			

A summary of the relationship between UPP, pH and soy flour concentrations in soy-wheat doughs made from PMSF2 is highlighted in Figure 4.9. Information on this figure shows that, while UPP% decreases drastically with soy flour increases, the pH on the other hand increases. More work is needed on the optimisation of the physical modification process for soy flour, soy flour concentration and pH in order to attain higher or optimal levels of UPP using high soy flour concentration in wheat dough.

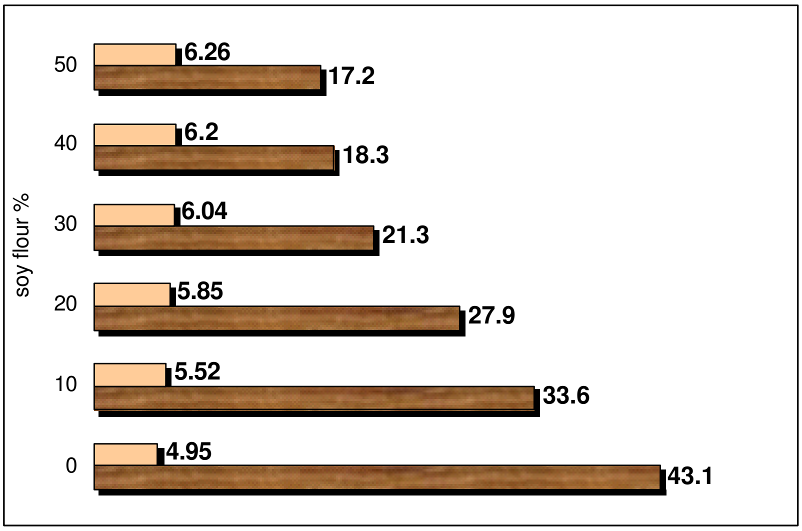


Figure 4.9 show the relationship between pH and UPP % and soy flour concentration in soy-wheat dough made from physically modified soy flour (PMSF 2). Values given are the mean of duplicate analysis.

Symbol ¦ represents pH; while ¦ represents UPP% (1h fermentation) of soy-wheat dough.

4.4.3.4.1 The relationship between protein dispersibility index (PDI) and pH of soy-wheat doughs

The protein dispersibility index (PDI), representing the water dispersible protein in the composite dough, increased with increasing pH. Observations in Figure 4.10 suggest how the use of organic acids (L-AA, citric acid) could lower the pH and PDI in high soy flour doughs and thereby improving UPP (unpublished work). This may stabilise soy proteins and might promote interaction of soy proteins with the hydrophobic wheat proteins. Raw soy flour would not be ideal for this procedure since it would require undesirable amounts of acid to lower its high PDI.

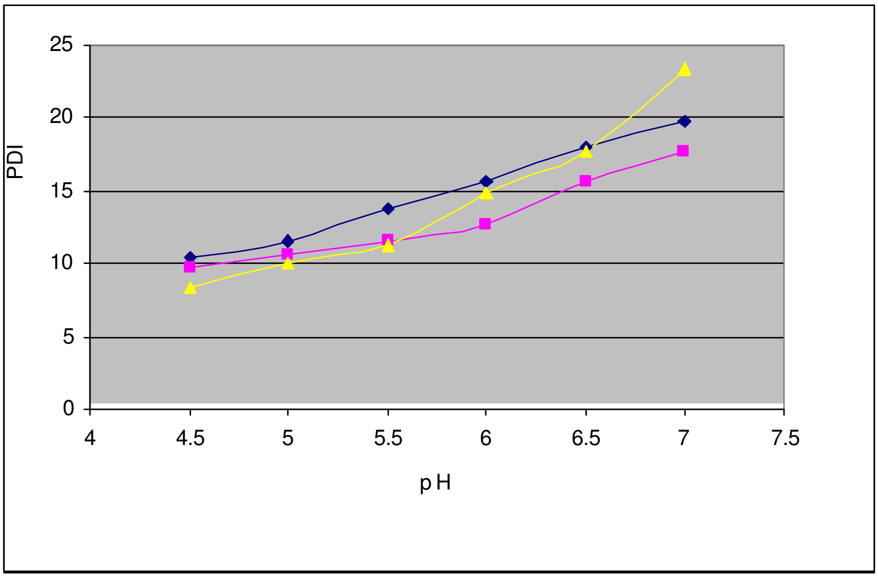


Figure 4.10, pH versus PDI of soy-wheat dough made from PMSF#2 ? is PDI for 20% soy flour dough; ¦ PDI for 30% soy flour dough and ? is the PDI for 50% soy flour dough

WHO guidelines for the production of edible, heat-processed soy grits and flours on PDI values is in the range of 10 – 30, which is indicative of adequate heat treatment for good protein nutritional value. Higher values are associated with under-heating and lower values indicate over-heating (Stauffer, 2002). PDI values for soy flours used on this work were 16, 23 and 68 for PMSF1, PMSF 2 and RSF, respectively. The PDI values for PMSF flours are in accordance with the heat treatment received and also fall within the range of WHO guidelines for adequate nutritional value.

4.4.3.5. Interaction of soy and wheat proteins (partial reduction and oxidation of soy-wheat dough) during Farinograph mixing

SE-HPLC co-elution profiles of large glutenin and soy proteins (>100kDa) for soy-wheat dough (PMSF#2) gave an insight into possible interaction of soy and wheat proteins during the fermentation of doughs. Therefore partial reduction (DTT) and oxidation (KIO_3) of soy-wheat doughs during Farinograph mixing was done in order to evaluate the possible incorporation of soy proteins into a glutenin-soy complex during the oxidation process and contribution of soy proteins to dough chemical properties. Results in Table 4.5 show glutenin peak areas and UPP percentages (SE-HPLC analysis) of treated and non-treated wheat dough and soy-wheat doughs made from raw and physically modified soy flours.

Table 4.5 UPP and glutenin peak areas in soy-wheat (1:1) doughs during mixing; ±values given on UPP% indicate the error of the mean. Peak area (glutenin) values are the mean of duplicate analyses.

	Control [a] dough at Peak time		Treated [b] dough at Peak time	
	[c] UE Glutenin peak area	UPP %	[c] UE Glutenin peak area	UPP %
Wheat dough	2,687307	48.2 ± 0.7	3,290960	52.3 ± 0.7
PMSF-W dough	2,813936	21 ± 2	3,549905	27.1 ± 1.6
RSF-W dough	0,433596	6.4 ± 0.4	0,774760	9.0 ± 0.2
	Control dough at Break time		**Treated dough at Break time**	
	UE Glutenin peak area	UPP %	UE Glutenin peak area	UPP %
Wheat dough	2,117904	26.4 ± 0.5	2,021564	30.4 ± 1.3
PMSF-W dough	2,216336	15.1 ±1.1	3,484143	25.0 ± 1.6
RSF-W dough	0,306200	3.1 ± 0.3	0,446321	6.4 ± 0.3

[b] treated doughs (DTT and KIO_3) for reduction and oxidation during mixing
[a] control doughs with no treatment; [c] Un-extractable

The partial reduction and oxidation procedures provided a means of studying the contribution of glutenin composition to the functional properties of dough (Uthayakumaran et al., 2000). The composite dough used in this study consisted of high levels of foreign proteins (50% soy flour). Because partial reduction opens up polymers through breaking of S-S bonds, the process of oxidation was anticipated to restore the S-S bonds and in the process incorporate added soy protein polymers into the glutenin polymer network. From the results listed in Table 4.5, the glutenin (polymeric) peak area increased from control PMSF-W dough to the partially reduced and oxidised dough (2.8 to 3.5 at peak time and 2.2 to 3.5 at break time). This was presumed to be an indication of soy protein incorporation into the glutenin polymer network of wheat dough. These results also suggested that the simple addition of soy proteins into the glutenin polymer network occurred during mixing (without reduction and oxidation) showing the glutenin peak area of wheat dough to that of PMSF-W dough to be 2.6 to 2.8 and 2.1 to 2.2 at peak time and break time, respectively. This was different for RSF-W dough (2.6 to 0.4 and 2.1 to 0.3). The effect of RSF on wheat dough polymeric proteins was negative (as already observed from previous sections of this Chapter). Appendix 4.2 also shows the major protein peak areas for soy-wheat dough extracts during fermentation.

The performance of this technique was not expected to be the same as in procedures used by previous workers (Bekes et al., 1994; Uthayakumaran et al., 2000), where wheat dough and pure glutenin were used on an optimised oxidation-reduction procedure. In the current experiment, the chemical properties of composite dough and wheat dough (partially reduced and re-oxidised) were not only restored, but improved in terms of larger numbers of polymeric proteins. This could have been attributed to the fact that concentration of potassium iodate used in this experiment was in excess for the reduced disulphides in the dough, resulting in oxidation of free SH groups and forming extra polymeric proteins. SE-HPLC results for samples taken at various levels of oxidation also revealed a shift to higher molecular weight in the molecular weight distribution of proteins (Bekes et al., 1994). In a study to find the contribution of glutenin composition to functional properties of wheat dough, a reduction reaction time of four minutes [using DTT up to 20µg / 2g flour (Bekes et al., 1994); DTT 90µg /2 g flour (Uthayakumaran et al., 2000) as a reductant] was found to be efficient for partial and reversible reduction without destruction of dough structure. This process was followed by five minutes of oxidant reaction time using KIO_3 up to 20µg / 2g flour (Bekes et al., 1994); and 1.25mg /2g flour (Uthayakumaran et al., 2000). The current method used 1.08µmoles (166µg) DTT / g of flour as reductant and1.54µmoles KIO_3 (329.5µg /g) of flour as oxidant in anticipation of the high content of disulphides in soy-wheat dough {(8-9µmoles / g) unpublished results}. Careful optimisation of this procedure using composite flours would provide methods to study the contribution of soy proteins to dough chemical properties.

4.4.4. Lab-on-a-chip-Capillary Electrophoresis

This section evaluates protein interaction between soy and wheat proteins during Farinograph mixing of the composite dough using the lab-on-a-chip capillary electrophoresis technique. Treated (partially reduced and oxidised) and non-treated (controls) doughs were used to evaluate protein interaction and the possible contribution of soy proteins to dough properties.

4.4.4.1. Understanding the mechanism of aggregate formation and the type of interactions between soy and wheat proteins

Results in Figure 4.11a show gel patterns for wheat dough and soy-wheat dough protein extracts after partial reduction-oxidation during Farinograph mixing.

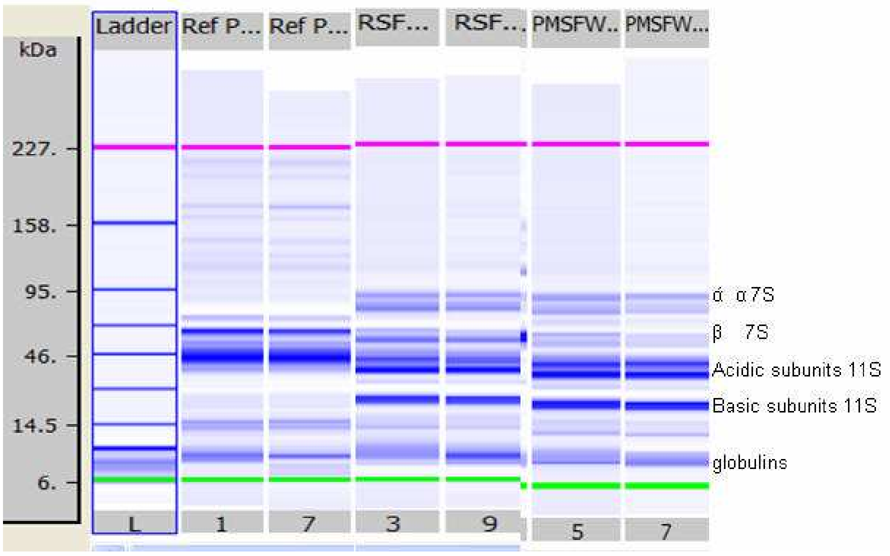


Figure 4.11a Lab-on-a-chip capillary electrophoresis for soy-wheat dough extracts, (reducing conditions in 1% SDS containing 1% DTT), simulated as gel patterns after partial reduction-oxidation (DTT and KIO_3) at peak time during Farinograph analysis. Lane 1 Ref P represents treated, while lane 7 Ref P is non-treated wheat dough; Lane 3 RSF represents treated RSF-W while 9 RSF is non-treated RSF-W dough; Lane 5 PMSF represents treated PMSF-W and 7 PMSF is non-treated PMSF-W dough.

The medium molecular weight (MMW) glutenin subunits (40-60 kDa) highlighted in Figure 4.11a, lane 1 and 7 {ref (wheat proteins)} are not evident in the soy-wheat dough proteins, (RSF-W lane 3 and 9; PMSF-W lane 5 and 7) profiles. It is possible that these MMW wheat proteins have co-eluted with 11S subunits whose bands appear very dense considering that soy flour proteins have been diluted (1:1 ratio) with wheat flour. High molecular weight glutenin subunits were present in all lanes

(Figure 4.11), but these bands were faint at the loadings used, especially for the composite doughs in which the wheat bands are diluted by the presence of soy proteins. Under non-reducing conditions, high molecular weight glutenin subunits, wheat bands would be expected to be more pronounced.

Figure 4.11b

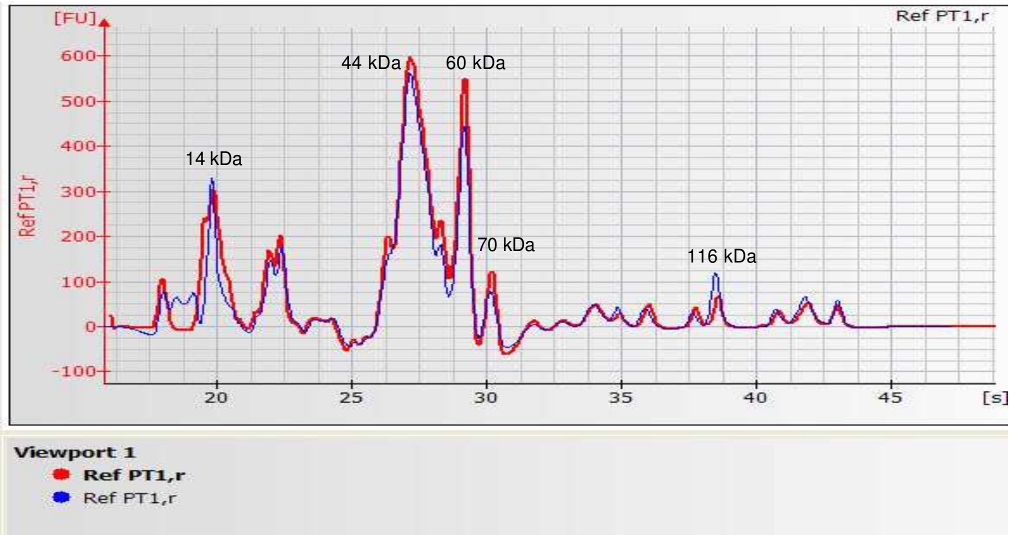


Figure 4.11c

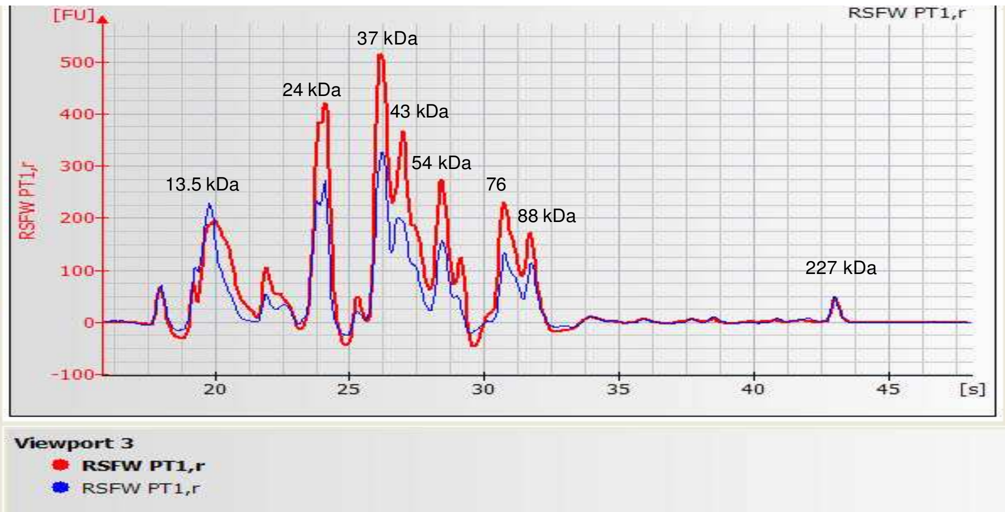


Figure 4.11d

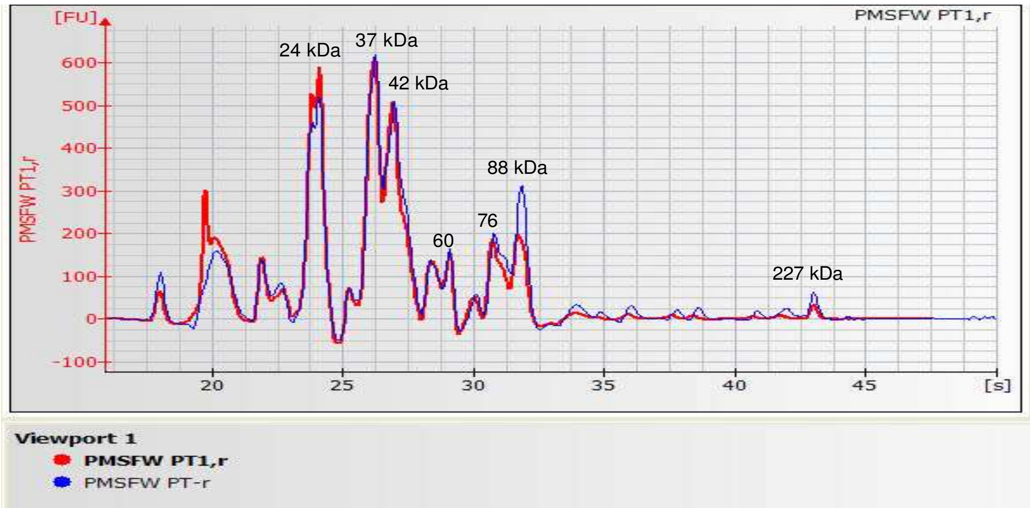


Figure 4.11e

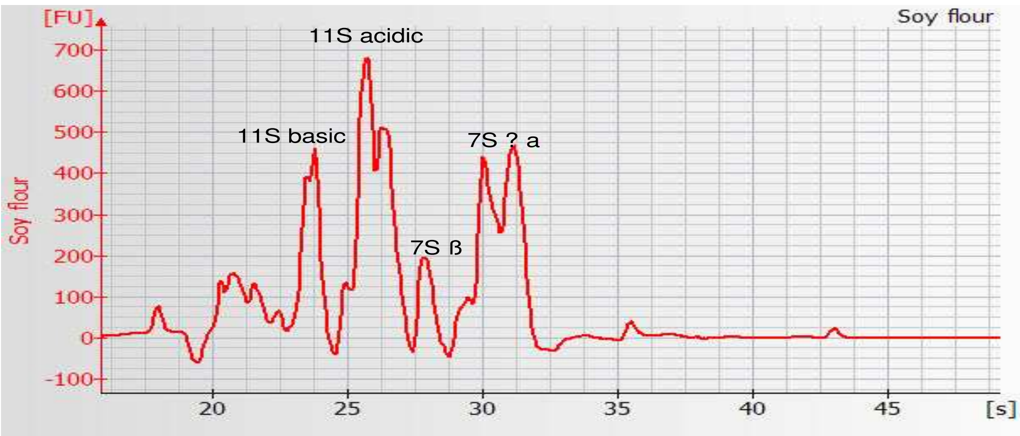


Figure 4.11b –e shown as elution profiles (reducing conditions with 1% SDS containing 1% DTT). Fig. 4.11b shows the elution profile (wheat dough proteins) corresponding to lanes 1 and 7 on Fig 4.11a; Fig 4.11c show elution profiles (RSF-W dough) corresponding to lanes 3 and 9 on Fig 4.11a; while Fig 4.11d shows an elution profile (PMSF-W dough proteins) corresponding to lanes 5 and 7 of Fig 4.11a. The red traces are treated doughs (reduced and oxidised) while the blue traces are non-treated doughs. Fig 4.11e is 100% soy flour protein extract (not treated).

Capillary electrophoregrams on Figure 4.11a –d show molecular distribution for the low molecular weight (LMW) and medium molecular weight (MMW) subunit proteins. LMW subunits of wheat proteins (44kDa in Figure 4.11b) overlap the soy-wheat dough electrophoregrams (Figure 4.11c and d), appearing in the same molecular zone as the 11S acidic soy protein subunits (37-43kDa). There is a possibility that 7S ß soy protein (Fig.4.11e) also co-eluted with the MMW subunits of wheat proteins [60kDa (? 1, 2 gliadins in Figure 4.11b)] as observed from the soy-wheat profiles (Figure 4.11c and d). HMW glutenin subunits (>100kDa) are diluted in Figure 4.11b, in the soy-wheat protein profiles; this effect is more pronounced in the RSF-W dough protein peaks.

Observations from these figures demonstrate that treated doughs (partial reduction - oxidation) and control doughs (without treatment) had the same gel and elution patterns for wheat dough and PMSF-W dough suggesting that partial reduction and oxidation process was effectively reversible in the smaller proteins (LMW and MMW). However, observations on Figure 4.11c showed that areas under peaks for untreated dough in RSF-W dough (blue line) were smaller compared the area under peaks for treated dough (red line), suggesting that there was no interaction between wheat and soy proteins in the untreated dough. The increase in peak area in treated dough suggested that the partial reduction and reoxidation process facilitated the interaction of wheat and soy proteins through oxidation of SH groups, as this also indicated that interactions were covalent. The fact that PMSF-W dough proteins (control and treated) had the same elution profile meant that this composite dough did not require chemical means to facilitate protein interaction and this may confirm that PMSF is superior to RSF. Because the area under the peak for 11S soy protein (acidic subunits) of Figure 4.11e (100% soy flour protein) and soy-wheat dough protein extract in Figure 4.11d (50% soy flour protein) appeared to be the same in size, this may indicate that the 11S peak (Figure 4.11d) consisted of both wheat and soy proteins.

Under non-reducing conditions, large soy proteins (180 and 360 kDa for 7S and 11S) did not show on electrophoregrams, however, electrophoregrams under reducing conditions (Figures 4.11b–d) were considered to be useful in evaluating protein-protein interactions since the profiles clearly highlighted subunits of soy proteins.

4.5. Conclusion

Observations made from this work showed that L-AA addition (0.05% w/w) added to soy-wheat dough (100 - 500 g kg^{-1} soy flour) increased SH concentration during resting, as it also decreased polymeric to monomeric ratios in wheat dough and PMSF-W dough during fermentation of these doughs. This suggested that L-AA acted as a reducing agent in soy-wheat dough.

The SE-HPLC technique demonstrated 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 distribution, 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. UPP% and glutenin peak results (SE-HPLC) from partially reduced and re-oxidised doughs revealed that the chemical properties of composite doughs were not only restored, but improved in terms of higher amounts of polymeric proteins.

Capillary electrophoresis (using Lab-on-a-chip methodology) did not reveal the presence of very large proteins (unreduced), which are presumed to be critical to the improvements provided by the physical modification process. However observations from capillary electrophoregrams revealed that partial reduction and reoxidation of composite doughs facilitated the interaction of wheat (LMW subunits) and soy proteins (11S acidic subunits) through oxidation of S-H groups, and this also indicated that interactions were covalent.

The significant contribution from this chapter is that soy-wheat dough SE-HPLC and capillary electrophoresis results have provided an insight into possible methods to study the contribution of soy proteins to dough chemical properties. The results in general indicated 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.

Chapter 5

Thermally induced component interaction in soy- wheat doughs using differential scanning calorimetry (DSC)

5. Table of contents

Abstract

The effect of soy flour concentration, L-ascorbic acid and water amount on the thermal properties of soy-wheat dough was evaluated using differential scanning calorimetry. Soy-wheat dough was prepared from raw soy flour (RSF) and physically modified soy flour (PMSF#2). The soy flour concentration was varied from 10 to 50% (w/w) substitution for wheat flour. The water addition used for preparation of doughs was 70, 80, 85 and 90% (m L water/100 g flour) and L-ascorbic acid (L-AA) used was 0, 0.1, 0.2 and 0.3% (w/w in total flour). Dough blends were mixed to dough development time at a constant speed of 60 rpm using a timer.

Interactive, main effects plots and regression analysis (ANOVA MINITAB) were used to study interaction of soy flour, water and L-ascorbic acid on the thermal properties of soy-wheat dough. Results revealed that there were significant interactive effects of the three variables on the thermal properties of soy-wheat dough, and that water content was the most significant factor influencing the thermal properties of soy-wheat dough. Linear coefficients and F-statistic values revealed significant interactive effects of water and soy flour on onset temperature (T_o) and enthalpy (? *H*) of soy wheat dough major endotherms (mainly due to water evaporation) (*P <0.05*). Increase of soy flour decreased ? *H* (*P <0.05*), but increased onset and peak temperatures at low water levels (< 85%) in the in soy-wheat dough, while L-AA up to 0.2% increased onset and peak temperatures of soy-wheat dough major DSC curves. Observations from interactive and major effects plots indicated that L-ascorbic acid (L-AA) up to 0.2% significantly increased the onset and peak temperatures of soy-wheat dough major endotherms. Estimated regression coefficients (multiple linear regression analysis) for L-AA and water percentages on soy-wheat dough evaporation enthalpies (? *H* J/g) were used to generate a mathematical model at a fixed soy flour concentration (30% w/w) in wheat dough. This model was used in the next chapter for the formulation of soy-wheat bread.

Introduction

Although gelatinisation of starch in aqueous systems is quite well understood and considerable research has been devoted to studies on thermally induced component interactions in wheat dough, there is scanty literature published on the thermal properties of composite doughs, particularly the soy-wheat dough system. This is mainly because the soy-wheat dough system is complex in terms of component interactions that involve wheat starch, wheat gluten, soy proteins and other minor components. Proteins and starches are important food components in increasing viscosity and stabilising dispersions to provide texture and firmness to the final product. These functional attributes are based on the water holding and gelation ability of the polymers during and after thermal treatment (Okechukwu and Rao, 1997). Because soy proteins and wheat starch are thermodynamically different polymers, mutual interactions or phase separation may occur during heating of soy-wheat dough and this could have significant consequences on the texture of the product.

DSC is a long-distance measurement of polymer connectivity and co-operativity and the DSC thermal transition curve (endotherm) reveals the endothermic events at temperatures similar to those at which structural changes are taking place within the polymers (Cooke and Gidley, 1992). This technique was found suitable in revealing possible wheat starch – soy protein interactions during the heating of soy-wheat doughs. Characterisation of biopolymers by thermal analysis has been described as an important approach to understanding the functionality of starch and gluten at structural and molecular levels (Chinachoti, 1994) with the new approach of combining thermal analyses and molecular spectroscopy to better understand the polysaccharides in cereal systems. However, the thermal properties of soy-wheat composite doughs have not been studied and for the current work, it was considered important to understand the thermally induced component interaction of soy-wheat flour components (mainly soy proteins and wheat starch) before attempting to use a combination of techniques for this complex system. DSC, which has been described by White and Lauer (1990) to be a powerful tool in studying starch gelatinisation in dough model systems or baked foods, was found suitable for this study as this technique also provides fundamental information including the gelatinisation temperatures, the total heat absorbed by a sample in millijoules and the amount of heat absorbed per gram of sample.

In classic DSC analysis, pans are mechanically sealed to support vapour pressure brought about by the heated dough sample. If open pans are used (as in real baking), water vapour will be released with consequent release of heat capacity of the sample resulting in a large upward bending of the baseline of the DSC curve (Schiraldi et al., 1996). Because of this explanation, use of aluminium pans is not recommended for DSC analysis of high moisture materials. To minimise this, the tightly sealed dough samples were only heated to 110ºC and nitrogen was used at 20 PSI to remove any volatilised products of heating. Any measurements after 100ºC were regarded as partly and largely contributed to evaporation of water from the dough samples. Several authors have also reported work on moist dough samples using hermetically sealed aluminium pans (White and Lauer, 1990; Addo et al., 2001; Kobylanski et al., 2004; Miyazaki et al., 2004). A method to improve the validity of DSC measurements for volatile samples by using thermal gravimetric analysis (TGA), a simultaneous thermal analyser, and subtracting the evaporation contribution of the volatile components has been developed Artiaga et al. (2005).

Statement of the Problem

The complex interplay between soy proteins and wheat starch in the composite dough matrix and the effect of water (plasticiser) and L-ascorbic acid (bread improver) on the thermal properties of this composite dough were to be studied. Theoretical starch gelatinisation temperatures in soy-wheat dough using L-ascorbic acid, water and soy flour concentration as variables were investigated in an attempt to get fundamental information essential for soy-wheat bread making.

Starch gelatinisation and evaporation of water from the surface cause the formation of the crust layer, which obstructs further expansion (Bloksma, 1985). Experimental observations revealed that soy-wheat bread tends to form crust very rapidly during baking and this is possibly due to the Maillard reactions from lysine and sugars found in soy flour. This understanding would imply that soy-wheat dough might not expand to its full potential because of the early obstruction. In this study, gelatinisation temperatures and soy protein denaturation in soy-wheat composite dough at various ratios will be studied using the differential scanning calorimeter (DSC).

During DSC analysis, the sample is sealed in an aluminium pan and no water is lost during the heating process, this analysis gives fundamental information as to the theoretical gelatinisation temperatures as it follows the gelatinisation process with greater precision. Amounts of soy flour (physically modified), water and L-ascorbic acid were optimised in order to control and predict the heat absorbed by the dough sample [(? *H* (J/g)]. This was done in order to achieve the desirable thermal properties for a specific soy-wheat dough composition as this was also aimed at preventing early dough crusting. This information was then used for the development of a mathematical model and later for the formulation of soy-wheat bread in the next chapter.

Objectives

- To study the endothermic transition properties of soy-wheat doughs and to characterise the molecular contributions of wheat gluten, wheat starch and soy proteins in a soy-wheat dough system using DSC.
- To study the interactive effects of L-AA, water and soy flour on the thermal properties of soy-wheat dough made from physically modified soy flour.
- To develop a model and use it to predict thermal properties (? *H* in J/g) of soy – wheat doughs using DSC.
- To use the predicted enthalpies [(? *H* (J/g)] from soy-wheat dough DSC endotherms and adjust optimum combinations of soy flour, L-Ascorbic acid and water that would result in the desired thermal performance for a specific composition of soy-wheat dough.

Hypothesis

Soy flour concentration, water amount and L-AA concentration can be optimised to control and predict heat absorbed by soy-wheat dough samples during DSC analysis.

5.2. Principles of Methods used

This section describes the principle of differential scanning calorimetry (DSC).

5.2.1. Differential scanning calorimetry for soy and wheat protein

White and Lauer (1990) reported the differential scanning calorimeter (DSC) to simulate the baking process by pasting the starch and heat denaturing the proteins in a sample, while simultaneously measuring the heat flow through the sample as a function of temperature. The differential scanning calorimeter measures the change in heat capacity between the glassy and rubbery states of food and this is indicated by a change in baseline in a DSC thermogram. The model for glass transition, which is responsible for the change in heat capacity, is located at the leading edge of the first DSC peak. Completion of the glass transition permits the crystalline starch to undergo a non-equilibrium melting process giving rise to a second endothermic peak (starch gelatinisation) (Donovan, 1979: Liu et al., 1991; Biliaderis, 1992; Liu et al., 2002). Because water acts as a plasticiser for food systems, the glass transition temperature decreases as the water content increases. For this reason, moisture content should be quoted for thermal transition temperatures (Hoseney et al., 1986).

Of the two major components of soy protein, β-conglycin, also called 7S is less heat stable with a denaturation peak temperature of about 70 °C and glycinin, (11S) fraction with a denaturation peak at about 90 °C (Nagano et al., 1992; Wagner and Anon, 1990). Soy dispersions heated for a second time, do not show DSC endotherms, indicating that heat induced denaturation of the proteins is followed by an irreversible process of aggregation (Wagner and Anon, 1990).

Purified wheat gluten does not exhibit denaturation peaks when heated in a differential scanning calorimeter within the temperature range from 30-130 °C, and this is attributed to the lack of a long range order of gluten giving rise to an amorphous random polymer (Hoseney, 1986). Other workers have attributed this lack of endothermal transition of gluten to endothermic and exothermic events cancelling each other or to the absence of significant co-operativity in the protein structure (Erdogdu et al., 1995; Adoo et al., 2001; Agyare et al., 2004). Because most random polymers exhibit glass transition during heating, gluten (11.3% moisture) has a glass transition (T_g), [change in heat capacity] at 50 °C. When dry, the glass transition for gluten is above 160 °C. With moisture content of above 13%, the glass transition of gluten occurs at room temperature, suggesting that in a dough system with moisture content above 60%, gluten would theoretically not exhibit a glass transition during heating. At higher temperatures or water content, the polymer is rubbery (Hoseney, 1986).

5.3. Methods and Materials

Soy flours (RSF and PMSF2) were prepared as described on Chapter 4.

5.3.1. Preparation of samples for DSC

Doughs were prepared by mixing wheat flour, soy flour and water in various concentrations of soy flour from 0% to 50% (w/w). The composite flours (1g ± 0. 001) were weighed in glass beakers and manually mixed before water addition. Water used for the preparation of the doughs was 70, 80, 85 and 90% (mL of water /100g flour). L-AA used was1000, 2000, and 3000ppm and this was added to the composite flour before water addition. Dough blends were manually mixed in 100 mL glass beakers using a stainless steel hand mixer at an approximate speed of 60 rpm. The speed and time of mixing was monitored using a clock. The optimum time used for dough development was followed from Farinograph results of soy-wheat doughs at various concentrations (Maforimbo et al., 2006). Dough was allowed to rest for 30 minutes at room temperature before the DSC run. Control dough samples (without L-AA) were also run.

5.3.2. Differential Scanning Calorimetry

Samples, from 10-15 mg of the composite dough, were directly weighed into pre-weighed standard aluminium pans. Pans were tightly sealed by crimping the edges of the pan using DSC sample crimper. Samples were equilibrated on the DSC for 10 minutes at 30°C before heating from 30 to 110°C at a rate of 10°C/ minute. To balance the heat capacity of the sample pan, an aluminium pan with distilled water was used. Nitrogen at 20 psi was employed as a purge gas to remove any volatilised products of heating.

The onset temperature (T_o) and peak temperature (T_p) and the enthalpy of transition (?*H*) of dough samples were determined using a Perkin - Elmer Series 7 DSC with a thermal analysis controller (TAC 7) and a PE Model 7700 professional computer. Calculations for T_o, T_p and enthalpy of transition (?*H*) were automatically computed using the PE DSC 7/ Unix Thermal Analysis Software. Area in millijoules (m J) was calculated as the total heat absorbed by sample and the amount of heat absorbed per gram of sample was the calculated as (?*H*) in J /g. Indium [melting point, 156.60°C and standard heat (?*H*), 28.71 J/g] was used to calibrate the DSC prior to analysis.

5.3.3. Statistics

Analysis was done in triplicate until the reproducibility of analyses was ± 5.0ºC for T_o and T_p and ± 3% error of mean for ? *H* values. The major endotherms (due to water evaporation, 11S protein melting) of soy-wheat dough made from physically modified soy flour were regressed against L-AA, water and soy flour concentrations using MINITAB software. The concentrations of variables used was 0, 0.1, 0.2 and 0.3% (w/w) for L-AA; 70, 80, 85 and 90% (mL water/ g flour) for water and 0, 10, 20, 30, 40 and 50% (w/w) for soy flour (Appendix 5.3). Regression coefficients and F-values for each thermal response were estimated by linear regression analysis. F statistical significance was checked by Analysis of Variance to find the effects of moisture content, L-AA, and soy flour concentration on onset temperature (T_o), peak temperature (T_p) and enthalpy change (? *H*) of the soy-wheat dough. In order to evaluate the interactions of the three variables on the thermal properties of soy-wheat doughs, main effects plots and interaction-effects plots were also generated (MINITAB, ANOVA).

5.4. Results and Discussion

Results and discussion for DSC of the major components in soy-wheat dough (soy proteins, wheat starch and gluten) are revealed and discussed with reference to known literature. DSC endotherms for wheat flour, soy flour and soy-wheat dough at various ratios; including effects of L-ascorbic acid addition and water content on the thermal properties of soy-wheat doughs are dealt with in this part.

5.4.1 DSC endotherm curves for soy protein, gluten and wheat starch

Results from the current work revealed that gluten only exhibited a second order glass transition peak (T_g) at 46.2ºC (Appendix 5.1) but did not show any endotherm transition peak. This observation has been explained by the lack of a long range order for gluten protein or by the absence of significant co-operativity in the protein structure (Hoseney et al., 1986; Addo et al., 2001), the lack of a tertiary-ordered structure by which gluten cannot be put into a disordered form by heat (Erdogdu et al., 1995). Because of these explanations, wheat starch and soy proteins would theoretically and dominantly show transition endotherms in soy-wheat doughs during heating in a differential scanning calorimeter.

DSC endotherms in Figure 5.1 revealed that transition ranges for wheat prime starch (60% moisture) were 64 -73ºC and 96-102ºC for first and second transitions respectively; while that for a mixture of soy protein and wheat prime starch exhibited two dominant endotherms. The first endotherm for the mixture of soy protein and wheat starch was deeper and broader and was reasonably assigned to soy protein denaturation and initial melting of wheat starch (78-86ºC); while the second smaller endotherm was considered to be melting of the remaining stable starch (105-112ºC). Soy protein isolate (60% moisture) ranged from 77 - 83ºC and 85 - 98ºC for 7S and 11S proteins respectively (curve not shown).

The gelatinisation temperature range published for wheat starch from different varieties was from 61 to 76 °C (Blanshard, 1986; White and Lauer, 1990; Kobylanski et al., 2004), and these observations were moisture dependant. Regarding two endotherms for starch gelatinisation on Figure 5.1, two endotherms have also been observed in limited water starch-water systems (Donovan, 1979; Billiaderis et al.,1986) and in excess water starch-water systems (Fukuoka et al., 2002). Two models were proposed for the two DSC endotherms for water/starch mixtures (Donovan, 1979; Billiaderis et al., 1986): swelling of the amorphous parts of the starch granules and coupling with crystallites. These processes lead to melting of the crystallites in the presence of excess water (first peak). When the water starts to be limiting for the process to be completed, the remaining crystallites melt at a higher temperature (second peak). Billiaderis (1992) and Agyare et al. (2004) also offered an alternative hypothesis for the two stage process of starch gelatinisation (<61% moisture) namely, the rapid melting of the less stable crystallites followed by second melting stage of the more stable starch crystallites. Erdogdu et al. (1995) reported two endotherm peaks for gelatinisation of prime and tailing starch;

gelatinisation at 64ºC and melting of the amylose-lipid complex at 103ºC. Therefore values observed on this experiment are in accordance with published values for the thermal transition for wheat starch.

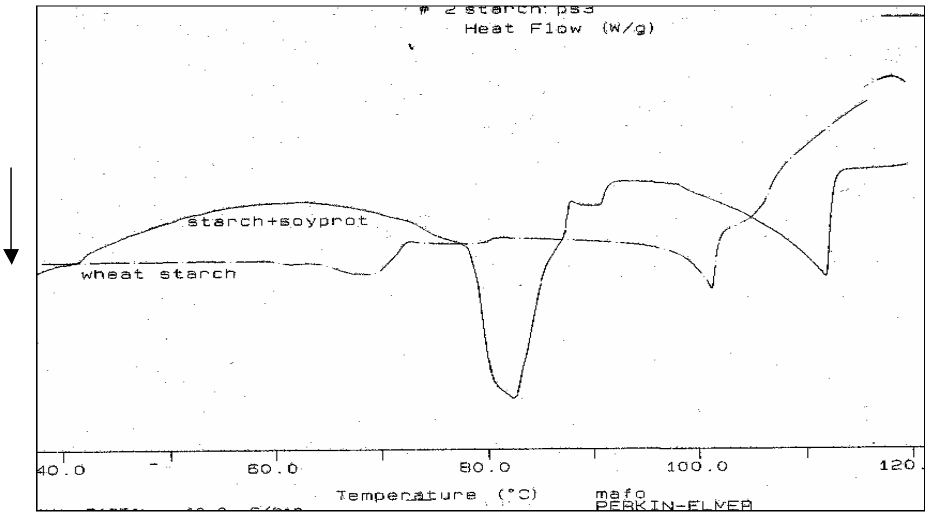


Figure 5.1, DSC endotherms for prime wheat starch, soy protein and wheat starch mixture (1:1). The endothermic transition (W/g), appear on the y axis versus temperature (ºC) on the x axis; the arrow denotes endothermic flow direction. For prime wheat starch, prime wheat starch / soy protein mixture (1:1) moisture 60%.

5.4.2. DSC endotherms for wheat and soy flours

Figure 5.2 shows DSC curves for wheat flour, raw soy flour (RSF) and physically modified soy flour (PMSF); at 80% moisture. Wheat flour had two DSC endotherms, the first one ranged from 73ºC to 77ºC and the second one from 79-88ºC, with peaks, at 74 and 88ºC. Agyare et al. (2004) reported the starch gelatinisation ranges for wheat flour dough to be 64-70°C and 80-94°C for the two transitions respectively, while White and Lauer (1990) reported the starch gelatinisation temperature for wheat flour in sucrose and water system to be 97.7 °C. The observed starch gelatinisation temperatures for wheat flour are consistent with the published values (Agyare et al., 2004) although the moisture used in the current experiment was above 61% (mL/100g). Explanation for this could be the different experimental conditions and instrumentation used and more so because gelatinisation of starch occurred in two stages (Figure. 5.1). The presence of non-starch polysaccharides in the flour (Kobylanski et al., 2004) and of more stable starch-lipid complexes (Erdogdu et al., 1995) is capable of elevating starch gelatinisation temperatures. In wheat flour dough, starch gelatinisation exists within the gluten gel matrix (Agyare and others, 2004); therefore the delay in starch gelatinisation during the heating of wheat flour dough could be explained by this phenomenon.

Fig 5.2

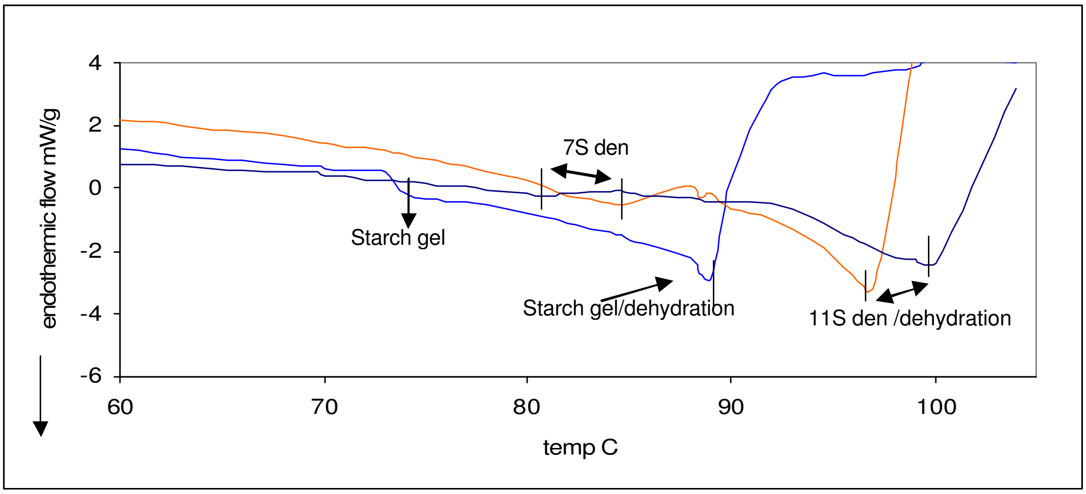


Figure 5.2, DSC transition curves for wheat flour, raw soy flour (RSF) and physically modified soy flour (PMSF). The endothermic transition (W/g) on the y axis versus temperature (ºC) on the x axis for wheat flour - ; RSF - ; and PMSF - ; (moisture 80%) Heating rate 10ºC /minute, all data files were normalized to a constant sample weight of 10mg.

RSF (raw soy flour) exhibited two endothermic transitions, from 74.8 - 84°C and 93-102°C with peaks at 81 and 100°C respectively. The first and second peaks corresponded to 7S and11S soy protein denaturation respectively. PMSF (physically modified soy flour) had two endotherms ranging from 79.6 - 87.6°C and 89 – 98°C with peaks at 85°C and 96°C respectively and peaks were similarly assigned to 7S and 11S soy protein denaturation respectively. Results for soy protein denaturation in soy flours are close to those from published literature, 74 and 90°C (Wagner and Anon, 1990); 74 and 87°C (Puppo et al., 2004); 65-75 and 85-95 for 7S and 11S respectively (Liu et al., 2004). The slight delay in 7S protein denaturation could have been due to the existence of soy proteins in flour whereby other flour components were possibly interacting.

The results in Table 5.1 revealed that the enthalpy of transition for 7S protein in soy flour dough was -19.5 and -7.6 J/g for PMSF and RSF, respectively, whilst that for the 11S protein in soy flour dough was -74 and -80 J/g for PMSF and RSF, respectively. The transition enthalpy for starch gelatinisation in wheat flour was 5.7 J/g and that for water evaporation from wheat dough (and possible melting of more stable wheat starch) was 189 J/g. Because the second transition enthalpy in wheat flour (at 88°C) was large, it was largely attributed to the evaporation of water from the wheat dough.

Table 5.1 DSC enthalpies (J/g) for soy proteins, as isolated and in soy flour

Values are the mean of triplicate values; ± are the error of the mean

Soy protein form	*7S (J/g)*	*11S (J/g)*
Isolated proteins (current work)	-1.56 ± 0.12	- 8.2 ± 1.5
Pure soy protein (published)*	-1.74	- 6.57
In raw soy flour (RSF)	-7.6 ± 1.0	- 80.2 ± 3.3
In physically modified soy flour (PMSF)	-19.5 ± 3.1	- 73.6 ± 4.3

* *Cai et al.(2002)*

From previous work, the enthalpy of starch gelatinisation for different varieties of pure wheat starch ranged between 9.6 and 11.4 J/g (Wickramasinghe et al., 2005); 4 and15 J/g (Fukuoka et al., 2002) at various moisture contents, whilst that for starch gelatinisation in wheat flour was 2.07J/g (Addo et al., 2001) and 3.177 J/g (White and Lauer, 1990). Results from Cai et al. (2002) revealed that pure soy protein denaturation enthalpy was 1.74 and 6.57 J/g for 7S and 11S respectively. From current work, the denaturation enthalpy for partially purified 7S and 11S soy proteins was 1.56 and 8.2 J/g respectively, whilst soy protein denaturation in soy flour was -19.5 and -74 J/g for 7S and 11S in physically modified soy flour and; -7.6 and -80 J/g for 7S and 11S respectively in raw soy flour (Table 5.1). Results for transition enthalpies of wheat starch and soy proteins are in accordance with reported findings from literature with slight variations due to experimental conditions. On the other hand, results for transition enthalpies of soy proteins in soy flour are obviously higher as this may have been caused by the existence of other components in the soy flour. In particular, the enthalpy for 11S was extraordinarily high (-80 and -74 J/g for RSF and PMSF, respectively); for this reason, this endotherm was attributed to water evaporation from the soy flour dough. Therefore 11S denaturation enthalpy possibly became invisible within this large water evaporation enthalpy.

5.4.3. Effect of physical modification of soy flour on thermal properties of soy- wheat dough

Figure 5.3a shows the thermal transition of soy-wheat dough (10-50% w/w) made from raw soy flour (RSF). All DSC curves exhibited their second order transition [glass transition (T_g)] at approximately 48ºC. Endothermal transitions (first order) in soy-wheat doughs were generally biphasic, with peaks at 76 and 91ºC; 70 and 83ºC; 68 and 84ºC; 70 and 82ºC for 20, 30, 40 and 50% soy flour dough, respectively. Figure 5.3b highlights DSC profiles for the PMSF composite dough at 80% moisture. A broad transition endotherm was almost revealed by 20% soy flour dough, suggesting that all components melted on one broad phase, although basically 20, 30 and 40% soy flour dough revealed two distinct endothermic peaks at 80 and 99ºC; 83 and 99ºC; 82 and 98ºC respectively. The first peaks in soy-wheat dough endotherms corresponded to 7S denaturation and wheat starch gelatinisation while the second larger peaks were generally proportional to soy / wheat flour ratios and these were mainly assigned to water evaporation of soy-wheat dough and melting of the more stable 11S soy protein.

Thermal transition in soy-wheat dough made from PMSF shifted to higher temperatures compared to that made from RSF. As reported by Bloksma (1986) and Therdthai et al. (2004), water released from denatured proteins is used for starch gelatinization during baking in wheat bread; this could explain the delay in starch gelatinisation by soy protein denaturation in PMSF-wheat dough. The shift of wheat starch gelatinisation temperatures to higher values in the presence of cowpea protein has been reported by Okechukwu and Rao (1997).

Results in Table 5.2 show comparison of major enthalpies in RSF and PMSF wheat dough at various concentrations, including major enthalpies of the flours.

Table 5.2 DSC enthalpies for soy-wheat dough at various concentrations

Values given are the mean of three replicates: ± SD

Percentage of soy flour in wheat flour dough	*Enthalpy of RSF- wheat dough (J/g)*	*Enthalpy of PMSF-wheat dough (J/g)*
10%	-167 ± 7	-187 ± 8
20%	-156 ± 8	-216 ± 6
30%	-102 ± 5	-160 ± 5
40%	-124 ± 4	-150 ± 10
100% PMSF dough		- 74 ± 4
100% RSF dough	- 80 ± 4	
100% Wheat dough	-189 ± 7	-189 ± 7

DSC transition enthalpies for the second major peaks for RSF – wheat dough were generally smaller (167, 156, 102 and 124 J/g); compared to those for PMSF-wheat dough (187, 216, 160 and 150 J/g for 10, 20, 30, and 40% soy flour dough respectively). Because these transition endotherms were larger compared to those of 11S soy protein denaturation in soy flour, and smaller compared to those of wheat dough's major endotherm, it was theoretically sound to assign them to 11S (more stable) protein denaturation and evaporation of water from soy-wheat dough, although the 11S denaturation peak became undetectable due to the existence of the large enthalpies. A paired t-test indicated that the major transition enthalpies for PMSF-wheat dough were significantly larger compared to those of RSF – wheat dough *(P<0.05),* meaning that soy-wheat dough made from PMSF had greater heat capacity compared to that made from RSF. The delay of thermal transition endotherms in PMSF-wheat dough could be an advantage in allowing more time for bread oven spring before starch gelatinisation and melting of other dough components as this would increase loaf volumes. The larger evaporation endotherms in PMSF-W doughs compared to RSF-W dough suggested that composite dough made from physically modified soy flour required more heat energy to break hydrogen bonds in water for the release of steam (evaporation) than that from raw soy flour. Contrastingly, physically modified soy flour should theoretically have smaller enthalpies compared to raw soy flour due to the fact that soy proteins have already undergone partial denaturation during the thermal preparation process. The possible explanation for this could be that the PMSF-wheat dough has greater water absorption capacity compared to the RSF-wheat dough (highlighted in Chapter 3) and therefore would possibly release more water during evaporation. The other reason could have been that PMSF (previous chapter) was suggested to consist of a larger polymeric protein network in its structure (due to a physical modification process) and therefore hydrogen bonds may theoretically be more difficult to break than in raw soy dough (containing smaller protein polymers).

Water evaporation enthalpies decreased proportionally to soy flour concentrations in the composite doughs and the possible explanation to this phenomenon would be greater potential for soy proteins to hold on to water (hydrophilic) compared to wheat proteins during heating. This means that the less the soy flour proteins in the dough, the more water is given off during evaporation resulting in larger enthalpies; while the higher the soy flour proteins in the composite dough, the less water is given off and thus smaller enthalpies.

Fig 5.3a RSF-W dough

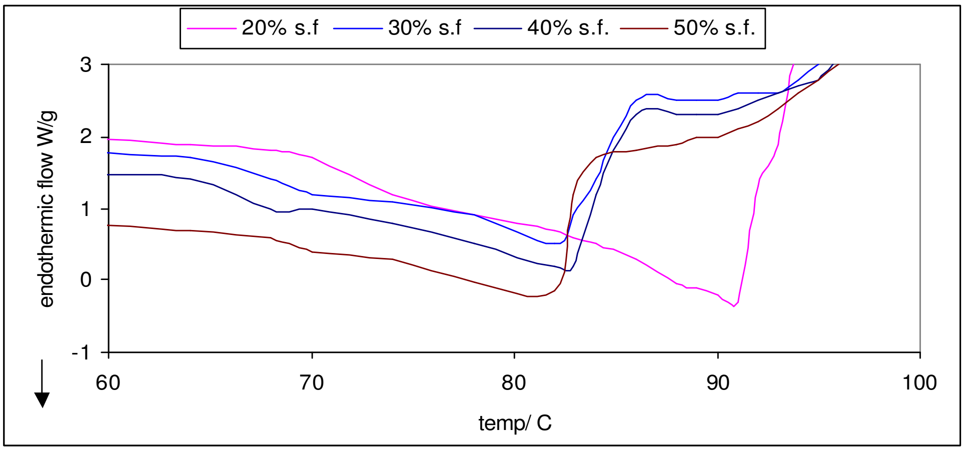


Fig 5.3b PMSF-W dough

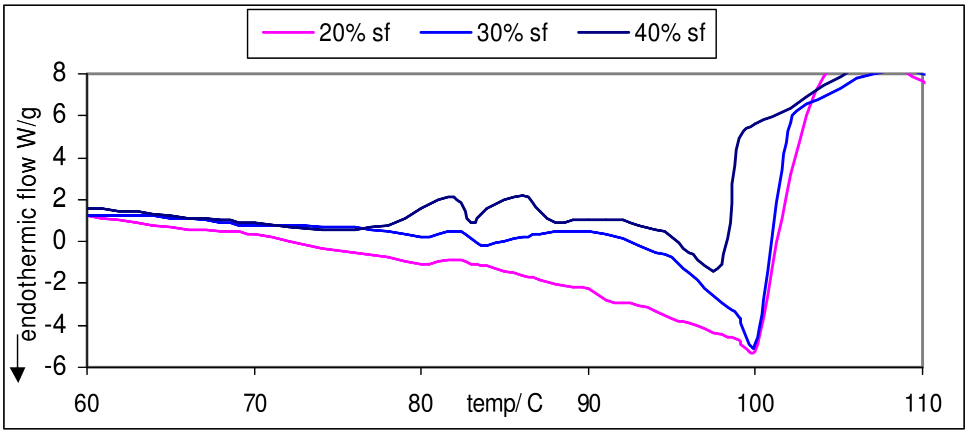


Figure 5.3 Parts a and b show temperature (ºC) versus endothermic flow (W/g) for soy wheat dough from 20% -50% RSF and PMSF; 20sf = 20% soy flour; 30sf = 30% soy flour; 40sf = 40% soy flour. Points on figure represent the mean of triplicate analysis; reproducibility was ± 3.0ºC. Heating rate, 10ºC /min. All data files were normalised to a constant sample weight of 10mg

Figures 5.3a and 5.3b show DSC thermal transitions for soy-wheat doughs made from RSF and PMSF at 80% moisture. The shift in starch gelatinisation and 7S protein denaturation (Figure. 5.3b) is shown as well as the existence of large endotherms for water evaporation including 11S protein denaturation between 90 - 100ºC. There was no shift in starch gelatinisation and 7S protein denaturation in RSF-W dough (Figure. 5.3a) with the larger endotherms mainly around 80ºC.

5.4.4 Effect of Moisture Content on DSC Endotherms of Soy-Wheat Dough

The effects of moisture content on DSC transition curves for soy-wheat dough are shown on Figures 5.4a, 5.4b and 5.4c for 70, 80 and 85% water in soy-wheat doughs made from physically modified soy flour (PMSF). Soy-wheat dough endotherms showed multiple transitions at 70% moisture but DSC endotherms for soy–wheat dough were generally biphasic. The first and smaller endotherms were assigned to denaturation of 7S soy protein and wheat starch gelatinisation, while second and large endotherms were assigned to 11S soy protein denaturation and water evaporation from dough. As expected, low moisture in the composite dough (70%) had lower evaporation enthalpies compared to high moisture doughs (80-85%). At higher moisture content, the dominant soy wheat dough endotherms were larger and tended to decrease proportionally with soy flour concentration in the dough for reasons explained earlier that more water in dough would give rise to large evaporation enthalpies. This observation also highlights the plasticising effect of water in food systems (Hoseney et al., 1986; Kalichevisky and Blanshard, 1992; Biliaderis, 1992; Kobylanski et al., 2004).

5.4.5. Effect of L-AA on DSC endotherms of soy-wheat doughs

Figures 5.5a, 5.5b and 5.5c show the effect L-ascorbic acid (0.1% w/w) on the thermal transition curves of soy-wheat dough at various ratios. The effect of L-ascorbic acid was characterised by smoother DSC endotherms in 70, 80 and 85% soy-wheat dough indicating co-operative melting of starch and soy protein denaturation as these events overlapped the starch gelatinisation and soy protein denaturation temperatures. These events were also included in the water evaporation broad peak. This observation suggested that L-AA increased component interaction in soy-wheat dough. Soy-wheat bread to which 0.05% (w/w) L-AA was added has shown to increase in loaf volumes with smooth rounded tops as compared to the loaves without L-AA (unpublished data).This phenomenon coupled with the L-AA effect on thermal transition curves may suggest that DSC curves directly influence bread quality. Broad endotherms as a result of increased moisture or L-AA addition might support the theory that food blends behave as homogeneous systems in DSC thermograms (Kobylanski et al., 2004).The delay and broadening of transition peaks caused by L-AA addition to low water soy-wheat dough (70% water, Figure 5.5a) may allow for more time for bread oven spring and increase loaf volumes.

Figure 5.4a. 70% water no L-AA

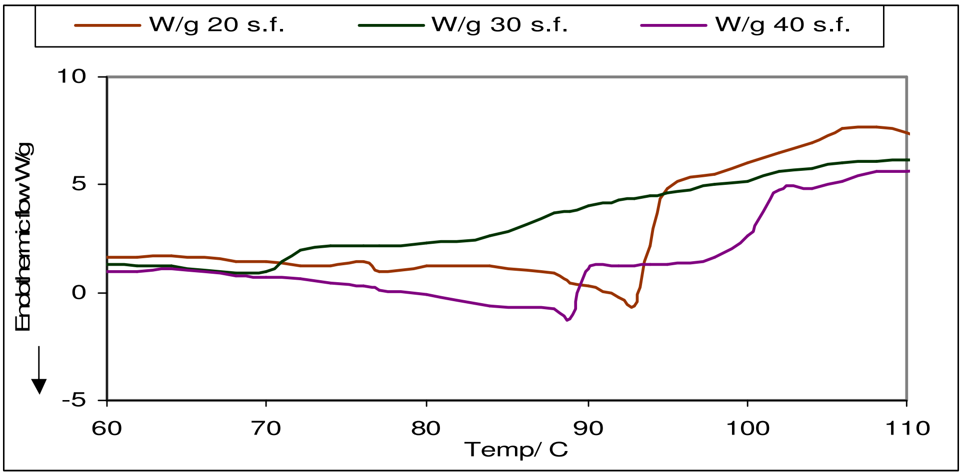


Figure 5.5a. 70 % water L-AA (0.1% w/w)

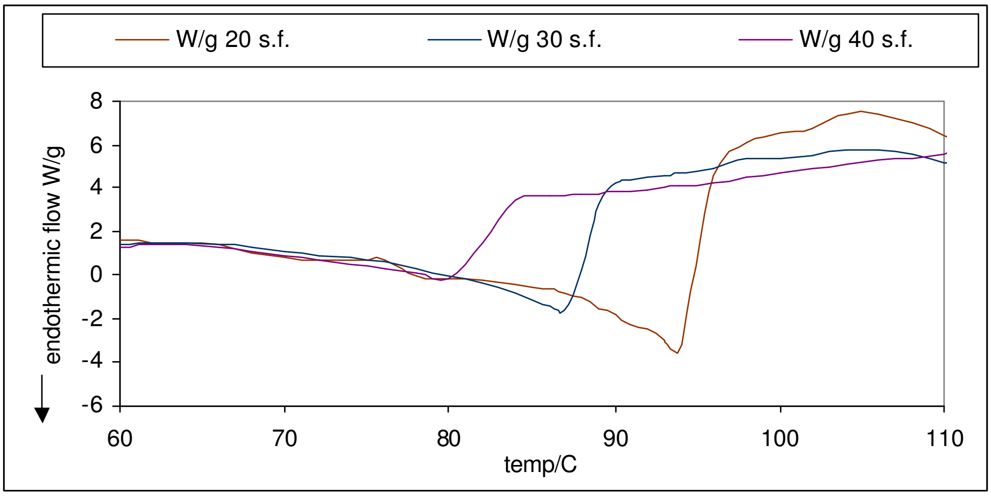


Figure 5.4b. 80% water, no L-AA

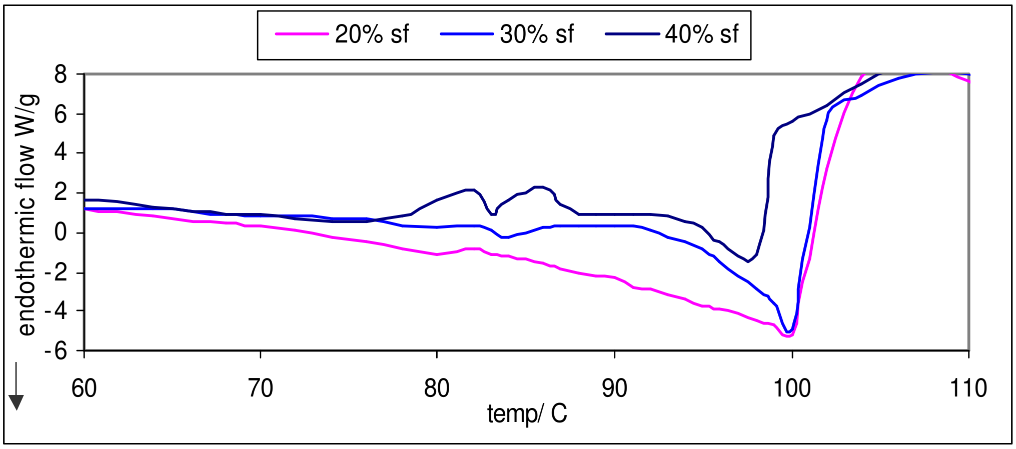


Figure 5.5b. 80% water, L-AA (0.1% w/w)

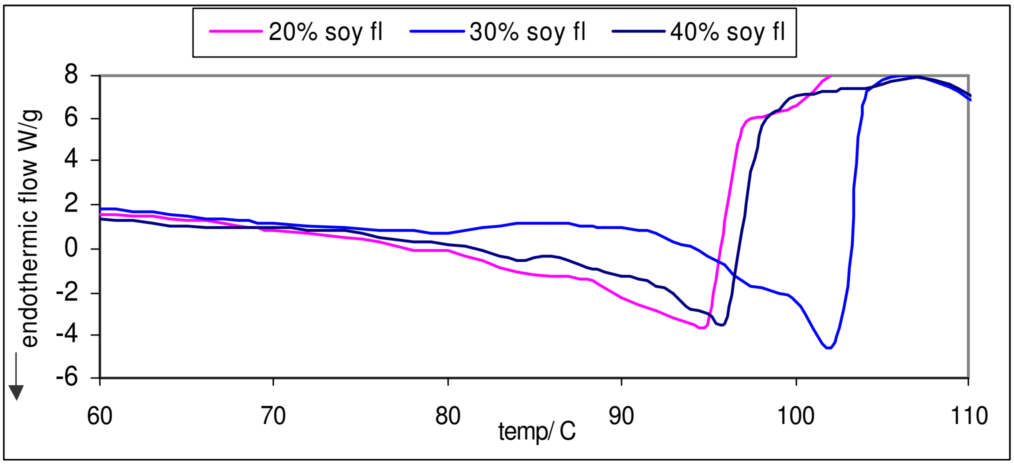


Figure 5.4c. 85% water, no L-AA

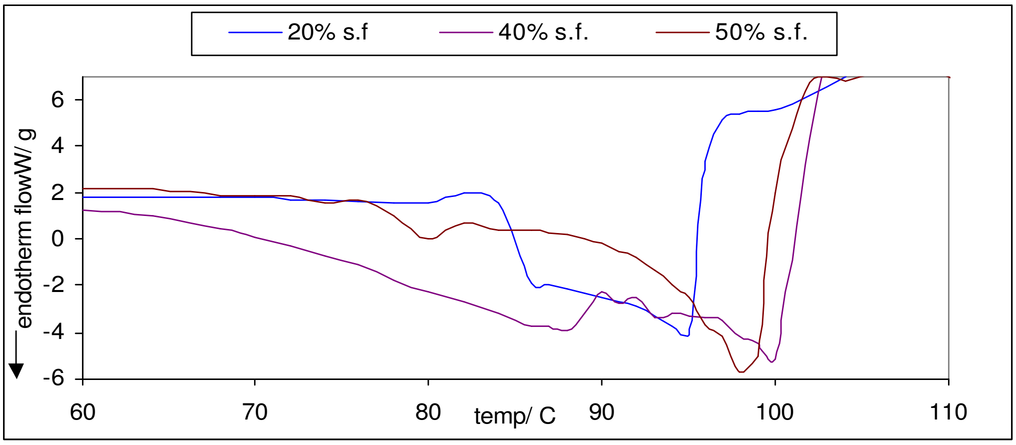


Figure 5.5c. 85% water, L-AA (0.1% w/w)

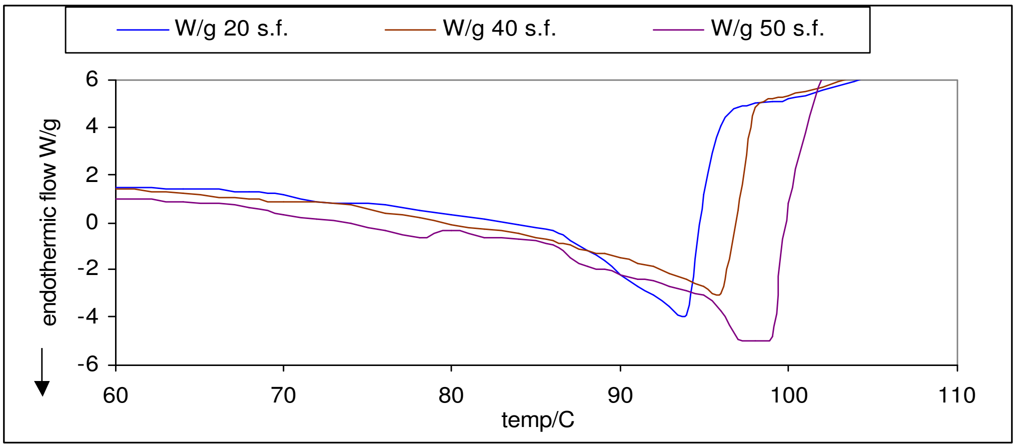


Figure 5.4a, b and c. Endothermic transition (W/g) on y axis versus temperature (ºC) on x axis in soy wheat dough at 70%, 80% and 85% moisture respectively; Fig 5.5a, b and c show the same with the addition of L-AA (0.1% w/w) in the composite doughs. s.f. = soy flour (PMSF) in composite dough. Heating rate was 10ºC /min. All data files were normalised to a constant sample weight of 10mg. The arrows (down) show endothermic flow (W/g).

Figures 5.6a and b show typical DSC endotherms for soy-wheat doughs at 70% and 85% moisture respectively, and the effect of L-ascorbic acid (0.2% w/w) on the doughs. In the low moisture dough (70%, Figure 5.6a) endotherms were almost bimodial, with the first transition attributed to cooperative melting of starch and 7S protein denaturation, while the second transition (11S protein denaturation and water evaporation) occurred in one endotherm, being proportional to soy flour concentration. By way of contrast, endotherms on Figure 5.6b show three transitions suggesting that starch gelatinisation and 7S protein denaturation occurred separately. The effect of L-AA on smoothening endotherms (cooperative melting of components) appeared to be more effective at lower water levels in the composite doughs.

During sample running, there was generally a large upward bending of the baseline of the DSC curves after the major endotherms (water evaporation >100ºC) and this occurrence was possibly due to loss of heat capacity from the samples (Schiraldi et al., 1996).

Figure 5.6a

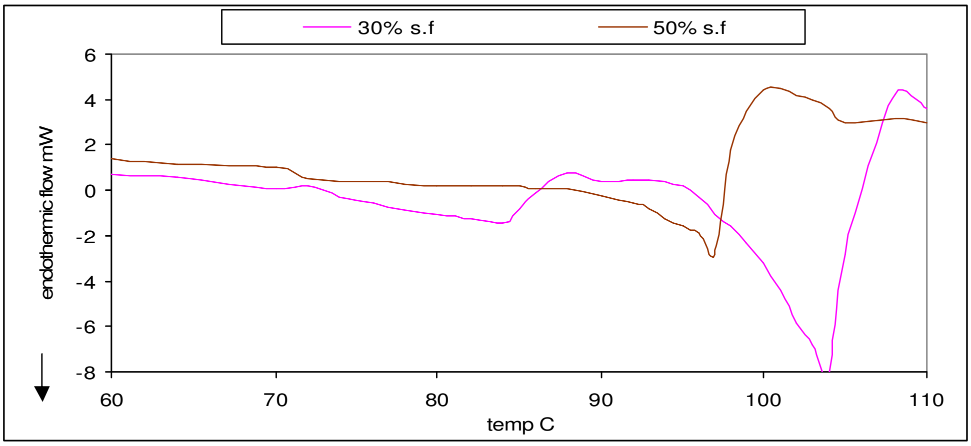


Figure 5.6b

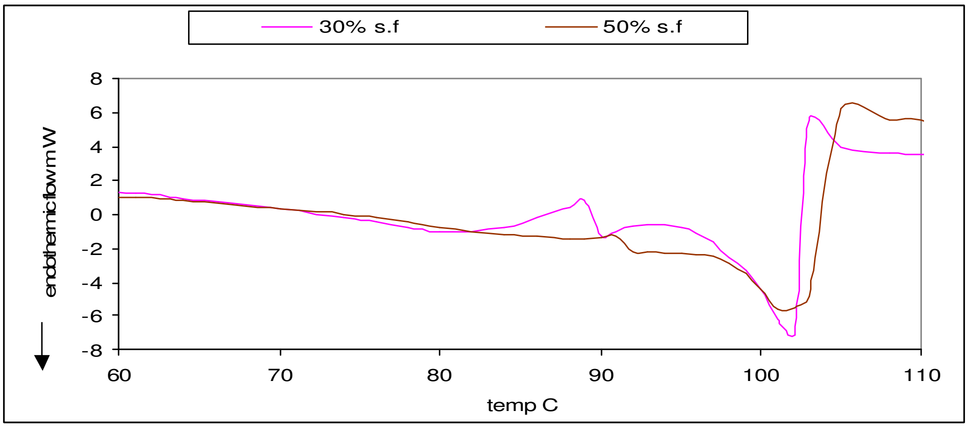


Figure 5.6a (top) and 5.6b (bottom) show typical soy-wheat dough DSC curves (30 and 50% soy flour). Endothermic transition (W/g) on y axis versus temperature (ºC) on x axis for typical soy-wheat dough DSC endotherms at 70% and 85% moisture (fig. 5.6a and b respectively) and the effect of L-ascorbic acid (0.2% w/w) in the dough.

Results showed that soy flour proteins caused a delay in starch gelatinisation being the resultant effect dependent on water and L-AA available in the dough. Addo et al. (2001) reported that rheological properties of dough change greatly during cooking where starch granules and gluten proteins undergo structural changes to form three-dimensional matrices via protein-protein and protein-starch interactions. DSC endotherms in soy-wheat dough were dominantly biphasic and the occurrence of two dominant phases in this complex dough system suggested that there were significant interactions between wheat starch, soy proteins and other components in the dough as all these components were mainly undergoing thermal transition in two phases. The complex behaviour of soy flour components in wheat dough including competition of water between soy proteins and wheat starch, phase separation between incompatible components is still not understood. Bloksma (1986) and

Therdthai et al. (2004) reported that during the heating of wheat dough, the process of protein denaturation releases water from the gluten proteins and this water is used for starch gelatinisation. The hydrophilic soy proteins could theoretically continue to hold water during heating of the composite dough and this would disrupt/delay starch gelatinisation. For this reason, the major endotherms in soy-wheat dough could possibly and partly be attributed to melting of the remaining starch and other components in the complex dough since most water will still be held by soy proteins until after 90ºC when 11S soy proteins denature and release the rest of water. However, the major (mainly water evaporation) endotherms have shed some light into the role of soy protein in modulating the thermal performance of soy-wheat dough, with an insight into more research on product and process control using these peaks as indicators for product quality.

From the outcome of these results, interactions of water, soy flour and L-ascorbic acid on enthalpies of dehydration, onset and peak temperatures of soy-wheat dough major endotherms (dehydration) will be studied in an attempt to predict thermal performance and bread making properties of soy-wheat doughs.

5.4.6. Interactive effects of soy flour, L-ascorbic acid and water content on T_o, T_P and water evaporation enthalpy (? H) using plots, reduced regression analysis and Multivariate Analysis.

Results from the first part of this experiment indicated that L-AA, water and soy flour concentrations all influenced the thermal properties of soy-wheat doughs and that these influences seemed to be interactive. That meant that the effect of each variable on thermal transition properties depended on the concentration of other variables in the soy-wheat doughs. The next part of the experiment was therefore aimed at pursuing further this interactive behaviour and using the information for optimisation and formulation of soy-wheat bread. DSC water evaporation endotherm values for soy-wheat doughs made from physically modified soy flour were regressed against L-AA, water and soy flour concentrations using MINITAB software. The concentrations of variables used were 0, 0.1, 0.2 and 0.3% (w/w) for L-AA; 70, 80, 85 and 90% (mL water/ g flour) for water and 0, 10, 20, 30, 40 and 50% (w/w) for soy flour. Regression coefficients and F-statistic values for each transition temperature were estimated by linear regression analysis and results are highlighted in Table 5.3. F statistical significance was also checked using Multivariate analysis (Table 5.4) to evaluate significant effects of moisture, L-AA and soy flour concentration on onset temperature (T_O), peak temperature (T_p) and enthalpy (delta *H*) of the soy-wheat dough dehydration endotherms. Results in Table 5.3 highlight coefficients and F-statistic values estimated by linear regression analysis. Thermal transition values used for this analysis were taken from the mean values (water evaporation endotherms) of soy-wheat dough DSC curves.

Table 5.3, Coefficients and F-statistic values of soy flour, water and L-AA on thermal properties of soy-wheat dough

Factor	T_o	T_p	***Delta H***
Soy flour	0.09*	0.05	-0.04**
Water	0.46**	0.44**	0.07**
L-ascorbic acid	8.66	5.57	0.54
F-value (ANOVA)	9.26**	10.31**	38.43**

The symbol (*) represents significance (P <0.05); ** represents significance (P <0.005)

Table 5.4 F-statistics from multivariate analysis (General Linear Model)

	T_o	T_p	? H
Soy flour	5.8**	1.26	174.98**
Water	28.3**	38.9**	64.14**
L-AA	84.26**	71.03**	12.33**
Soy flour * water	11.26**	5.76**	8.92**
Soy flour * L-AA	9.08**	7.31**	7.97**
Water * L-AA	10.56**	10.87**	18.87**

* symbol represents interaction

** symbol represents significant effect (P<0.005) on thermal properties of dough

Multivariate analysis in Table 5.4 (General linear model) revealed highly significant F-statistic values *(P<0.005)* for main and interactive effects of the three independent variables on T_O, T_p and ? *H* of the soy-wheat dough dehydration endotherms. The overall model was statistically significant (F = 13.58; 13.45; 20.79; *P 0.000*) and R squared values for the corrected model were 0.87, 0.869 and 0.911 for T_O, T_p and ? *H* respectively.

5.4.6.1. Main-effects plots of water, soy flour and L-ascorbic acid on enthalpy (? *H*), T_o and T_p of soy-wheat dough major endotherms.

Data means (ANOVA) used on these plots were generated from the DSC dominant endotherms (water evaporation). The plot for the main effects of soy flour on delta H (? H_{vap}) is highlighted on Figure 5.7a. A linear negative correlation of soy flour concentration with delta H as also attested by the F-statistic (Table 5.3 and 5.4) was shown. These results are consistent with observations made from figures on the first part of this Chapter (Section 5.4) and also emphasise the explanation given earlier that increases of hydrophilic soy flour proteins decreased enthalpies proportionally due to less water being available for evaporation. Water increase in the composite dough (up to 85%) increased delta H of vaporisation (? H_{vap}) as this was also attested by the positive water coefficient (Table 5.3). Although the coefficient (0.54) indicated that L-AA effects on enthalpy were not statistically significant *(P <0.05),* observations in Figure 5.7a revealed that L-AA additions (up to 0.1% w/w) to soy-wheat doughs increased dehydration enthalpy considerably after which there was a decrease of enthalpy up to 0.3% of L–AA.

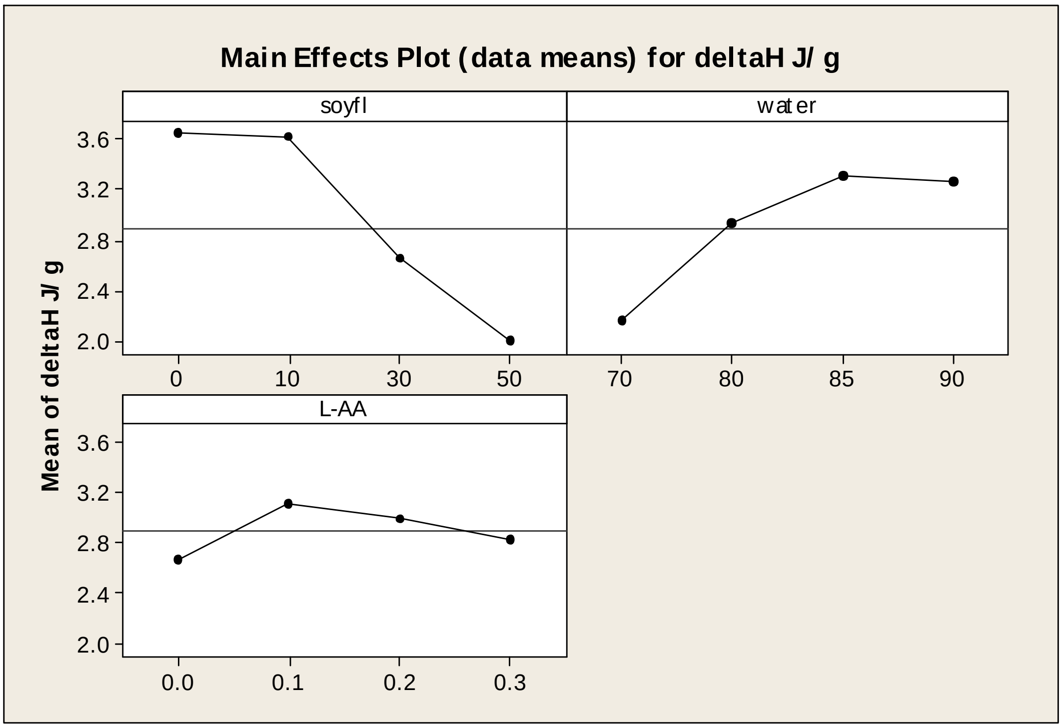


Figure 5.7a The main-effects plot of soy flour, L-AA and water on ? H (J/g) of soy-wheat dough dehydration endotherms. The soy flour figure shows percentage soy flour in dough (x axis) versus ? H means (J/g x10^2). The water figure shows percentages of water (x axis) versus ? H means in J/g (x 10^2), while the L -AA figure shows the percentage L -AA (x axis) versus ? H means {(J/g x 10^2) y axis}.

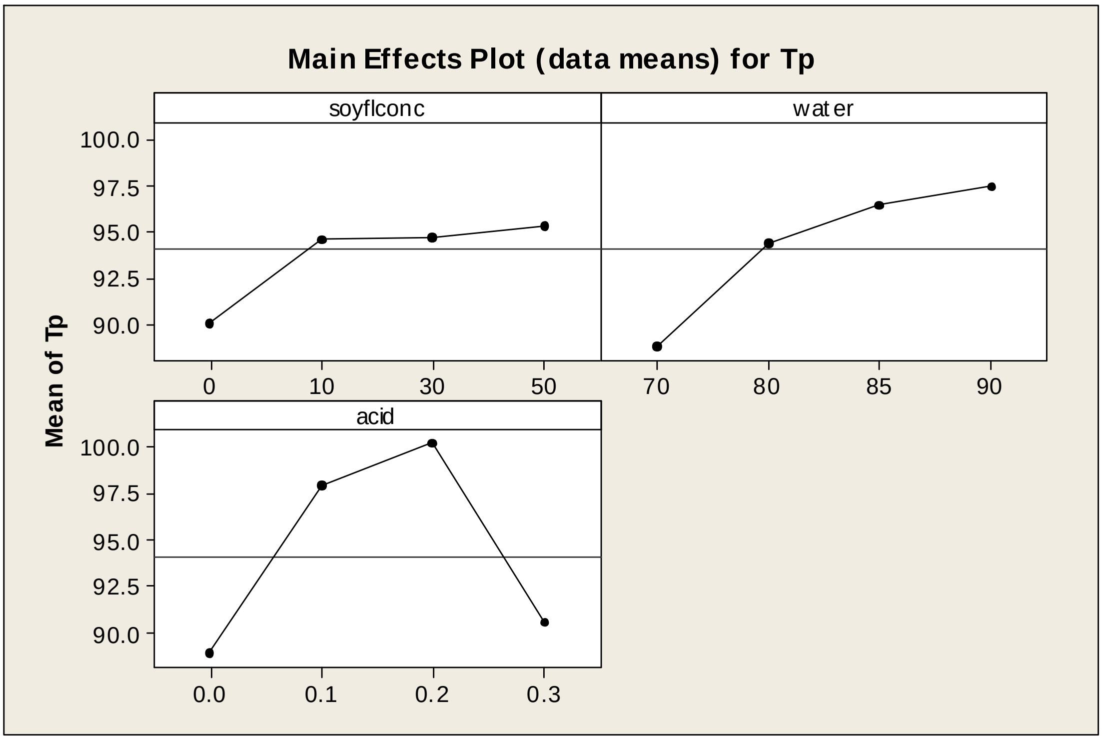


Figure 5.7b The main-effects plot of soy flour, L -AA and water on peak temperature (T_p) of soy-wheat dough dehydration endotherm. The soy flour figure shows soy flour % (w/w) in dough (x axis) versus peak temperature (T_p) in ºC(y axis). The water figure shows the water % (v/w) in dough (x axis) versus (T_p) in ºC (y axis); while the acid figure shows L-AA % (w/w) in dough (x axis) versus (T_p) in ºC (y axis).

The main effects of soy flour on T_p (Figure 5.7b) showed that soy flour up to 50%, water amounts up to 90% and L-AA up to 0.2% (w/w) all increased peak temperatures (T_p) of soy-wheat dough dehydration endotherms.

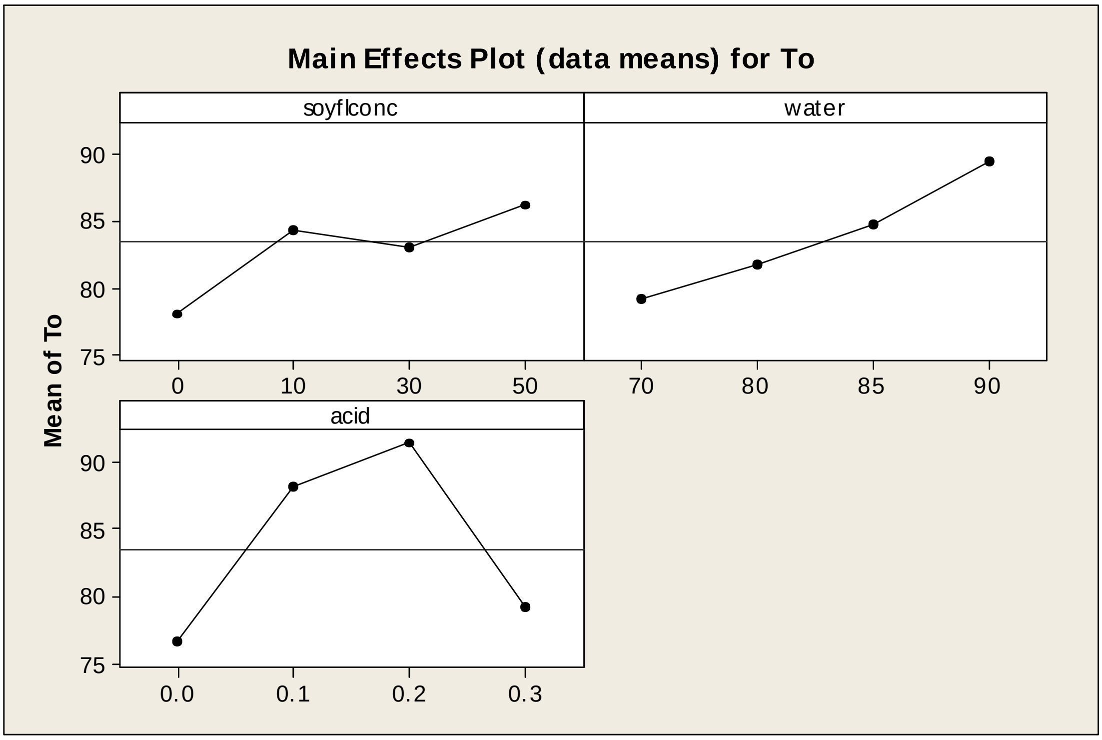


Figure 5.7c The main-effects plot for soy flour, L -AA and water amount on onset temperature (T_o) of soy-wheat dough dehydration endotherm. The soy flour figure shows soy flour % in dough (x axis) versus peak temperature (T_o) in ºC(y axis). The water figure shows the water % in dough (x axis) versus (T_o) in ºC (y axis). The acid figure shows L-AA % in dough (x axis) versus (T_o) in ºC (y axis).

Plots on Figure 5.7c show the similar trend of the effect of soy flour on onset temperatures of the major DSC curves showing a saddle at 30% soy flour, as also observed on T_p response to soy flour on Figure 5.7b. Apart from the saddle, onset temperatures for dehydration endotherms have generally increased with soy flour increase up to 50% (w/w). Similarly to peak temperatures, water increase has also resulted in increased onset temperatures for the water evaporation endotherms. Addition of more water (>85%) to the composite dough increased the onset of dehydration enthalpies (T_o) appreciably. Although these onset and peak temperatures increased significantly with more water, delta H did not increase appreciably after 85% water was used in the dough (Figure. 5.7a). This phenomenon could indicate that higher water volumes in the soy wheat dough may cause components to interact with water more than they interact with each other during heating. Component interaction during heating may promote co-operative melting of components allowing uniformity and larger transition enthalpies. L-AA influence on T_o was similar to that on T_p, increased onset temperatures for dehydration with a maximum (0.2%) of L –AA in the dough. This observation suggested an optimum for L-AA influence on controlling the thermal transition of soy-wheat dough. These results indicated that increase in water, soy flour and L-AA all delayed onset and peak temperatures of soy-wheat dough dehydration endotherms, the resultant of which may allow for more time for bread oven spring before crust formation. Therefore optimisation of these variables would provide useful information for the soy-wheat bread baker.

5.4.6.2 Interaction effects, water, soy flour and L-ascorbic acid on thermal properties of soy-wheat dough

Interaction plots for soy flour, water and L-AA on delta H (? *H*) of soy-wheat dough DSC dehydration endotherms are shown in Figure 5.8a.

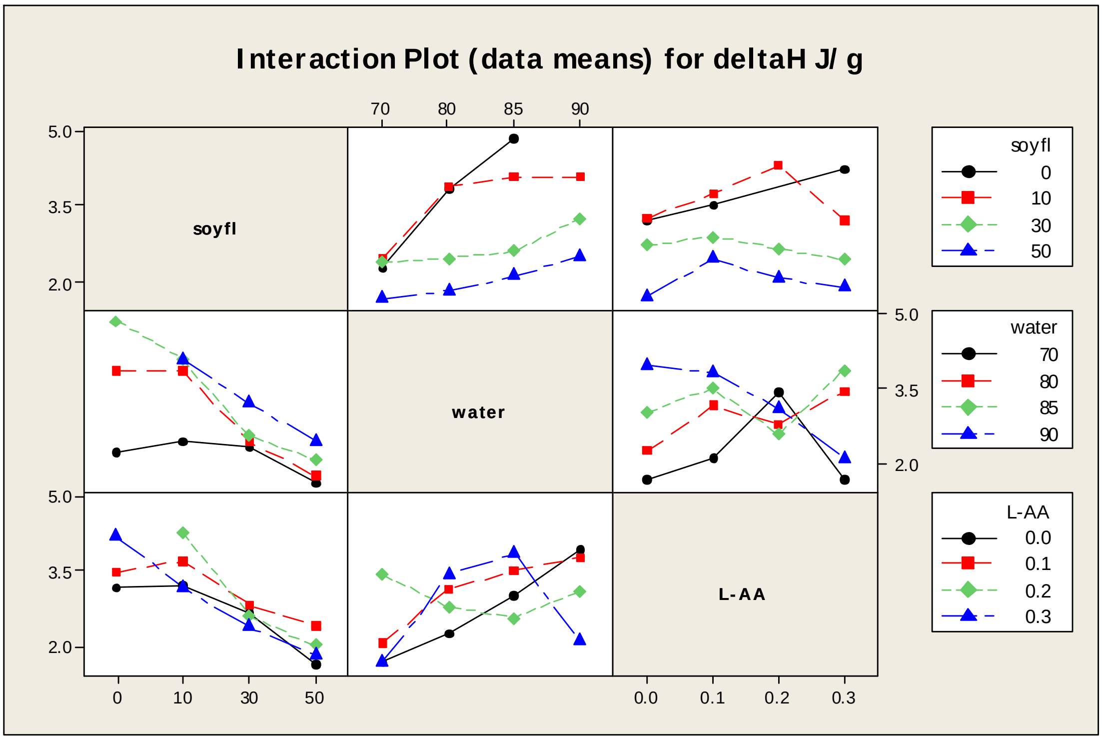


Figure 5.8a The interaction of soy flour (% w/w in total flour), water amount {% (m L of water /100g flour)} and L-AA amount (% w/w) on delta H (J/g) of soy-wheat dough; all three variables are given as x-axis while delta H is given as y axis. Values for delta H = x 10^2.

Results in Table 5.3 indicate that F-value was highly significant for the linear effects of soy flour and water on delta H *(P <0.005*) of soy-wheat evaporation endotherm. Increases of soy flour significantly decreased delta H, while increases in water significantly increased delta H *(P <0.05).* The negative (linear) coefficient of soy flour on enthalpy was attested from the interaction plots (Figure.5.8a). The positive influence of water on delta H was also attested by the positive (linear) coefficient for water (Table 5.3). The linear coefficient of L-AA on delta H was not significant (*P<0.05*). However, observations from the interactive plots, showed that the presence of L-AA up to 0.2% (w/w) in soy-wheat dough did not alter the influence of water on delta H, but high levels of L-AA (0.3% w/w) and high water levels (>85%), decreased the enthalpy of water evaporation. This could also be explained by the fact that high levels of L-AA may interact with water molecules and therefore limiting water for evaporation.

Soy flour and L-AA

Interactive plots of L-AA and soy flour on delta H showed that delta H increased up to 0.3% (w/w) L-AA in the control dough (wheat); increased up to 0.2% of L-AA in the 10% soy flour dough while this increase was only up to 0.1% of L-AA in the 30-50% soy flour dough. L-AA was more influential in increasing the evaporation enthalpies of low soy flour doughs. This observation suggested that the influence of L-AA on delta H was dependent on soy flour amount and that high soy flour doughs did not respond to the effect of L-AA on enthalpies possibly due to the high levels of soy proteins in controlling the amount of water for evaporation.

Water and L–AA

Similarly water and L-AA interactions influenced the enthalpy of dehydration in soy-wheat dough. At 70% moisture in the dough, enthalpy of water evaporation was highest at 0.2% L-AA, while this increase continued up to 0.3% L-AA at higher moisture dough (80 - 85 %) and delta H decreased with increase of L-AA (0.3%) in the 90% moisture dough. This observation indicated that the effect of water on delta H may have been influenced by the L-AA amount and vice versa, although enthalpy of evaporation was theoretically more dependent on water available than L-AA in soy-wheat dough as also attested by the non-significant coefficient of L -AA on ? *H*.

Soy flour and water

Generally, lower soy flour doughs (<30% soy flour) needed less water to absorb more heat energy (larger enthalpies), while high soy flour doughs (>30% soy flour) had very low enthalpy values at low water levels (<80%) and these improved with the addition of more water (>80%). The same explanation highlighted earlier on the hydrophilic nature of soy proteins and their capacity to hydrate more than the wheat proteins is emphasised as this phenomenon might restrict the mobility of water with consequent decrease of enthalpies of evaporation. Increase of water levels in these high soy flour doughs would obviously improve the water evaporation enthalpies. Results from this experiment indicated that more water (>80%) was needed for high soy flour composite dough (>30% soy flour) in order to attain higher enthalpies of dehydration.

Results for the interaction of soy flour, water and L-AA on peak temperatures (T_p) / ºC of soy-wheat dough dehydration endotherms are highlighted on Figure 5.8b.

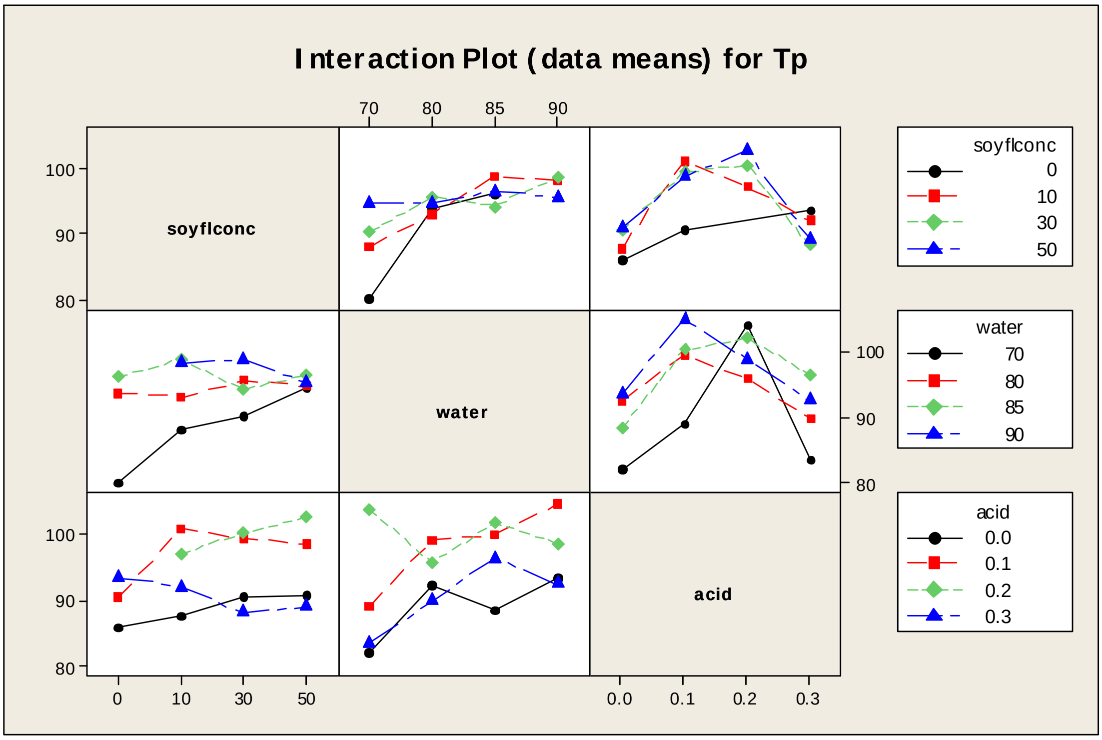


Figure 5.8b Plots for the interaction of soy flour (% w/w in total flour), water amount {% (m L of water /100g flour)} and L -AA amount (% w/w) on peak temperatures (T_p) of soy-wheat dough. All three variables are given as x-axis while T_p is given as y axis

Soy flour, water and L-AA on T_p

The linear regression coefficient for water on peak temperature (T_p) of dehydration was positive, meaning that the increase of water increased T_p *(P <0.05).* Water influence on T_p was optimum at 30% soy flour, and this increase was effective up to 0.2% of L -AA in the soy-wheat dough. Soy flour and L-AA had non significant linear coefficients, which meant that these two factors had non linear effects on T_p (*P <0.05*). However, F-statistics in Table 5.4 revealed highly significant interactive effects between soy flour, water and L-AA on T_p (*P <0.005*). Observations from the main and interactive plots generally revealed that L-AA (up to 0.2% w/w) increased peak temperatures of soy-wheat dough dehydration endotherms, and that the further addition of L-AA decreased T_p. Increases in soy flour increased peak temperatures at low water levels (<80%) in the composite dough. This confirms that the soy flour influence on onset of dehydration depended on the amount of water available.

Interaction plots of soy flour, water and L-AA on onset temperature (T_o)/ ºC of soy-wheat dough DSC dehydration endotherm are highlighted on Figure 5.8c.

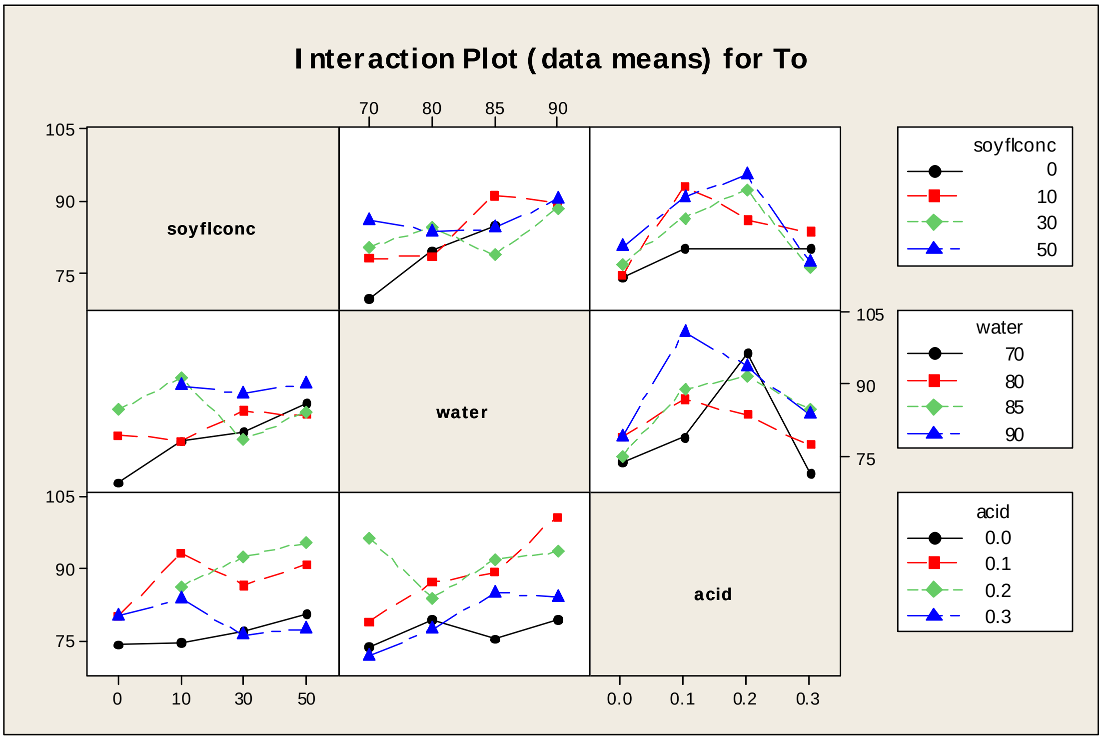


Figure 5.8c Plots for the interaction of soy flour (% w/w in total flour), water amount {% (m L of water /100g flour)} and L -AA amount (% w/w) on onset temperature (T_o) of soy-wheat dough. All three variables are given as the x-axis, while T_o is given as the y axis

Soy flour, water and L-AA on T_o

Estimated linear coefficients of soy flour, L-AA and water on the onset temperature (T_o) of dehydration were positive and this indicated that increase of all three variables increased onset temperature for the soy-wheat dough major endotherms. However, this effect was significant for water and soy flour while L-AA effect was non-significant (*P <0.05*) meaning that increases of water or soy flour in the dough significantly increased onset temperatures for soy-wheat dough evaporation endotherms (*P <0.05*). Observations on Figure 5.8c revealed that onset temperatures for dehydration increased gradually with increase of soy flour at low water levels in the dough (70 - 80%) and that this increase was insignificant at higher water levels, meaning that the onset of dehydration was delayed in limited water doughs. This could have been due to limitation of water in high soy flour doughs causing a delay in the melting of the rest of dough components including dehydration. Interaction between soy flour and L-AA revealed that onset temperatures for dehydration increased in all soy flour concentrations with L-AA up to 0.2% (w/w) in the dough. Interaction between water and L-AA highlighted that increases of L-AA up to 0.2% increased onset temperatures of dehydration and this occurred at all water levels. The significant shift of onset and peak temperatures of soy-wheat dough dehydration endotherms to higher temperatures caused by the increase in soy flour could have been caused by the presence of non-starch polysaccharides (from soy flour) which would cause starch to resist gelatinisation (Kobylanski et al., 2004) which effect was also dependant on water available.

High soy flour doughs required high water levels to attain higher enthalpies of dehydration, and this tended to decrease onset temperatures. White and Lauer (1990) reported that early starch gelatinisation caused early setting of bread during baking and this ultimately decreased the loaf volumes. These results highlighted the importance of optimising water, L-AA and soy flour in controlling starch gelatinisation and soy-wheat dough dehydration during baking of soy - wheat bread in order to achieve desirable loaf volumes. Despite the negative effects of soy flour on thermal properties of soy-wheat dough, soy flour has been reported to have a positive role in modulating bread staling (Vittadini and Vodovotz, 2003) (Appendix 5.2).

5.5 Development of a mathematical model

The important and useful parameter for optimisation of water, L-AA and soy flour was considered to be on water evaporation enthalpy (? H_{vap}) of the composite dough. This was because DSC evaporation enthalpies of soy-wheat dough were proportional to soy flour increases in the dough and therefore these enthalpies were theoretically assumed to relate to loaf volumes. This phenomenon was found useful in developing a mathematical model which would be used to predict and control enthalpy of vaporisation in soy-wheat doughs. Predicted enthalpies would allow a choice of optimum combinations of water, L-AA and soy flour that would result in desirable thermal performances and thus bread volumes. No work has been reported on the use of DSC water evaporation endotherms for optimisation, in particular with the use of composite dough. Because of this explanation, subtraction of the evaporation contribution of the volatile dough samples, as suggested by Artiaga et al. (2005) to improve the validity of DSC measurements was not considered.

5.5.1. Experimental matrix used to fit second order regression model

Methods for developing the model were followed from White and Lauer (1990), Horton et al. (1990), Toufeili et al. (1994) and Kobylanski et al. (2004), with modifications. The experimental matrix for the interactive effects of L-AA, water and soy flour amounts on enthalpy (J/g) yielded 50 results, of which results were the means of three replicate analyses. In order to develop a model with high soy flour in bread, a mathematical model was generated from known ratios of L--AA and water percentages at a fixed ratio of soy flour in the composite dough (30% soy flour) and the corresponding DSC major enthalpies (evaporation endotherms). This meant that enthalpy results for 30% soy flour dough only were selected from the experimental matrix so as to end up with 16 values. Table 5.5 shows the experimental matrix which was used to develop the model. A full quadratic model containing 6 coefficients was used to describe the responses observed and to fit the following equation.

$Y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_1^2 + b_4 x_2^2 + b_5 x_1 x_2$;

Where y = ? *H*; b_0 b_1, b_2, b_3, b_4, b_5 are coefficients, x_1 is water percentage and x_2 is the L-AA percentage

Estimated regression coefficients for delta H (? *H*) were obtained from responses by multiple linear regression analysis. The results were used to fit the following second order regression model;

[? *H* (J/g) = 3.2342 + (- 0.0868 x W) + (28.3846 x A) + (0.00093 x W^2) + (- 15.125 x A^2) + (- 0.29166 x W A)];

Where W = water % and A = L-AA %.

Table 5.5 Experimental matrix used to fit the model

? H	*Water*	*Acid*				*? H*
Actual y	x1	x2	x1*x1	x2*x2	x1*x2	Predicted y
1.90	70	0.0	4900	0.00	0.0	1.715
1.90	80	0.0	6400	0.00	0.0	2.242
2.38	85	0.0	7225	0.00	0.0	2.575
3.23	90	0.0	8100	0.00	0.0	2.954
2.23	70	0.1	4900	0.01	7.0	2.361
2.26	80	0.1	6400	0.01	8.0	2.596
3.20	85	0.1	7225	0.01	8.5	2.783
3.32	90	0.1	8100	0.01	9.0	3.016
3.37	70	0.2	4900	0.04	14.0	2.704
2.63	80	0.2	6400	0.04	16.0	2.647
2.08	85	0.2	7225	0.04	17.0	2.688
2.48	90	0.2	8100	0.04	18.0	2.776
1.98	70	0.3	4900	0.09	21.0	2.744
3.36	80	0.3	6400	0.09	24.0	2.396
2.33	85	0.3	7225	0.09	25.5	2.291
2.08	90	0.3	8100	0.09	27.0	2.233

* b_0	b_1	b_2	b_3	b_4	b_5
3.2342	-0.0868	28.3846	0.00093	-15.125	-0.29166

* b_0 is a constant; b_1 is linear water coefficient; b_2 is linear L -AA coefficient; b_3 is the quadratic water coefficient; b_4 is the quadratic L-AA coefficient and b_5 is the interactive coefficient for water and L–AA. (all coefficients on ? H).

The regression coefficient (R^2) for delta H on linear, quadratic and interactive effects for L-AA and water amounts was high (0.8), meaning that the model was able to explain 80% of the variations in the responses. Therefore this model was found adequate and useful for the formulation of soy-wheat bread.

As a key part of statistical modelling, model residuals were examined to ensure that assumptions made were reasonable and the choice of the model was appropriate. Figure 5.9.shows the normal probability plot for this model.

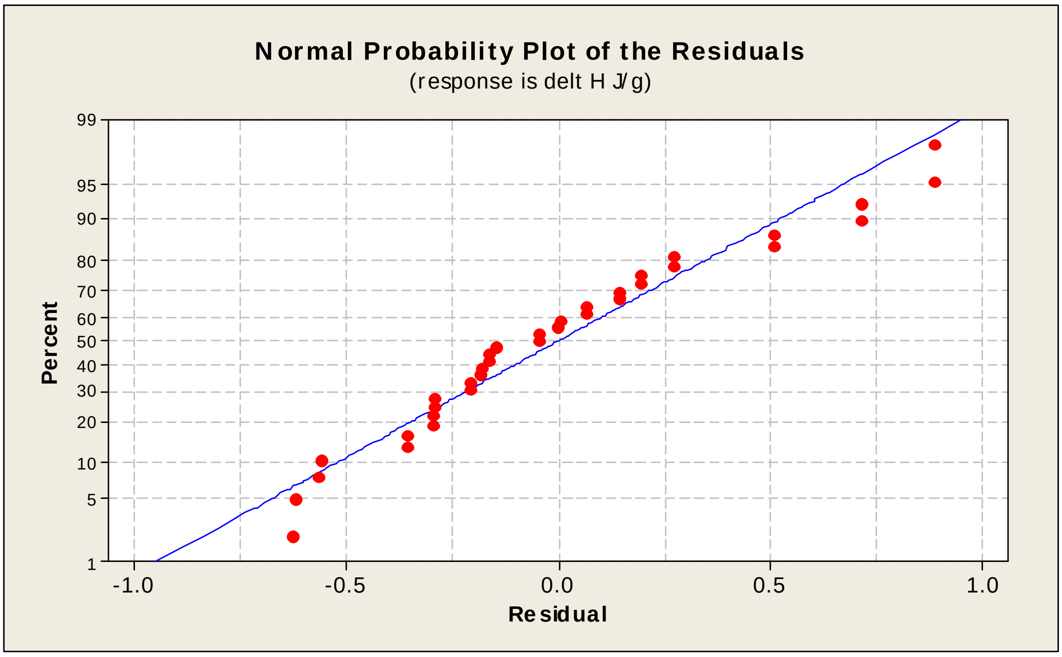


Figure 5.9 The normal probability plot for this model highlighting residuals (x-axis) against % frequencies (y-axis).

Residuals (*estimates of experimental error)* were obtained by subtracting the observed responses (delta H J/g) from the predicted responses. A normal probability plot was generated for these residuals from the fitted model. The purpose of the dot plot was to provide an indication of the distribution of the residuals. The overall pattern of residuals should be similar to the bell-shaped pattern observed when plotting a histogram of normally distributed data. If residuals from a fitted model are not normally distributed, then one of the major assumptions of the model has been violated (Chambers et al., 1983). The points on this plot form a nearly linear pattern, which indicates that the normal distribution is a good model for this data set.

5.5.2 Interactive effects of moisture and L-AA on enthalpy, using the reduced model equation

Because of the need to use high levels of soy flour in bread, interactive effects were also studied at a fixed soy flour level (30%) using the second order regression model. Plots generated from the model equation are highlighted on Figure 5.10a (surface) and Figure 5.10b (contour lines). The plots are presented in two forms to emphasise and follow interactions between water and L-AA percentages in the model chosen. The plots also highlight the ability of the model to predict water evaporation enthalpy of soy-wheat dough. A surface response chart for interaction between water and L-AA % on soy-wheat dough enthalpy of vaporization (? H $_{vap}$) using model equation is shown on Figure 5.10a.

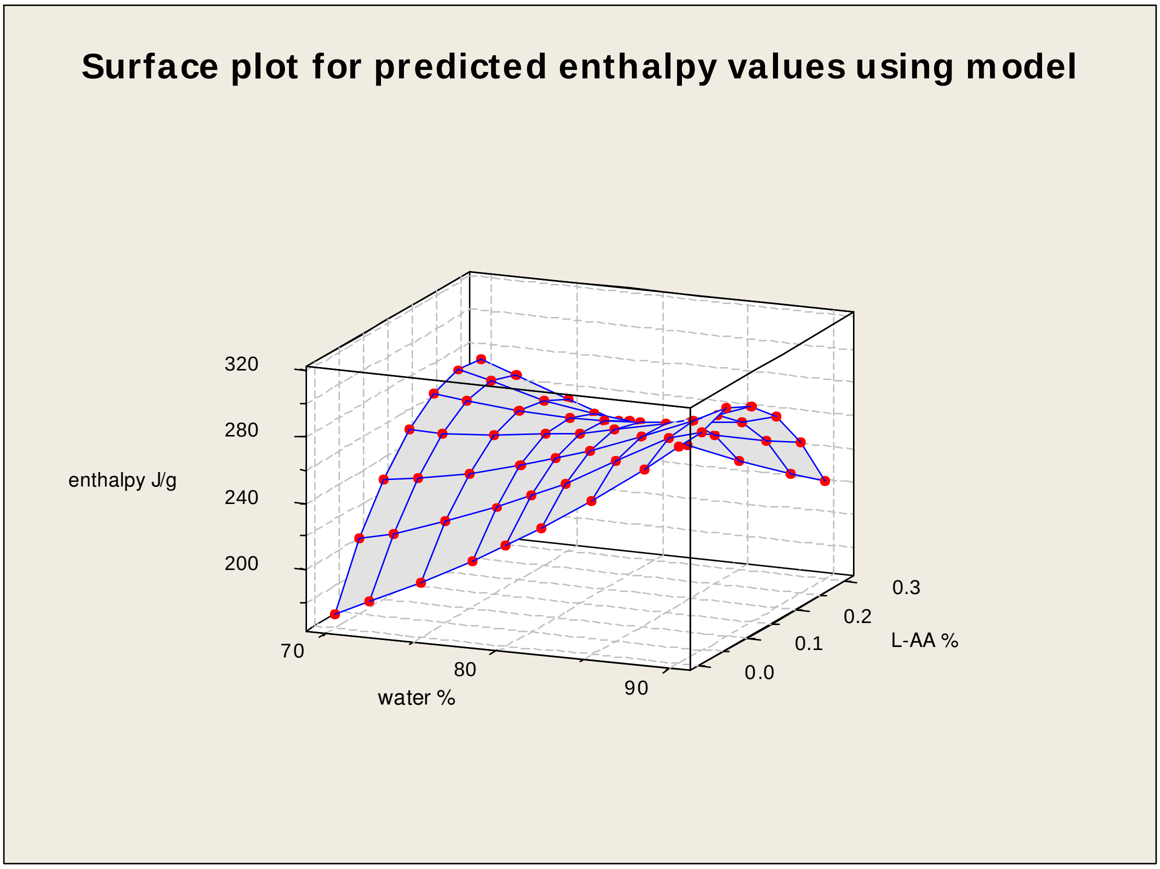


Figure 5.10a. A surface chart showing the interaction between water (x-axis) and L-AA % (y-axis) on soy-wheat dough enthalpy of vaporisation [(? H $_{vap}$) z-axis]

At low water levels (<80%), ? *H* continued to increase with increases in L-AA, whilst at high water levels in the dough (> 80%), increases of L-AA increased ? *H* to a maximum after which ? *H* started to decrease. The increase of water up to 90% in the soy-wheat dough continued to increase ? *H* at low levels of L-AA, whilst high levels of L-AA did not favour high amounts of water as ? *H* started to decrease. In summary, observations from the surface chart indicate that soy-wheat dough absorbed greatest amount of heat at high concentrations of L-AA in the low water doughs (<80%); at lower levels of L-AA (< 0.2%) and high water (>80%) composite doughs. The effect of L-AA on improving ? *H* in intermediate water level (80%) composite dough was less significant compared to that in low moisture dough.

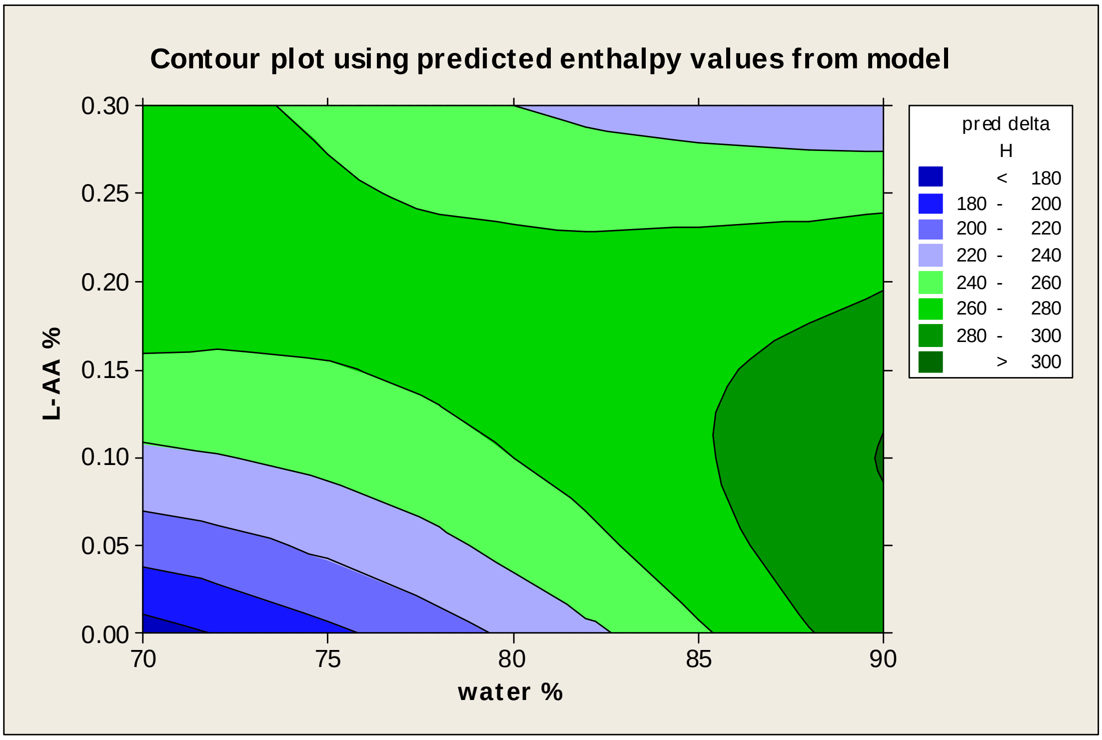


Figure 5.10b.The interaction of water and L-AA on soy-wheat dough DSC evaporation enthalpies (? H_{vap}), shown on contour plots.

Contour lines obtained from reduced regression model were also plotted as a function of water amount and L-AA concentration in the soy-wheat dough at a fixed soy flour concentration. The contour lines on Figure 5.10b also revealed similar observations made from the surface chart (Figure.5.10a). Soy-wheat doughs at low water levels (70-77%) and low L-AA (<0.07% w/w), had the least heat capacity (<220 J/g), and the composite dough had its greatest heat capacity at high levels of water (>86 -90%) with low L-AA (<0.2% w/w). Desirable heat capacities (260 -280 J/g) can be obtained from a wide range of water and L-AA variables ranging from 70 to 88 % water and 0.16 -0.3% of L-AA. The contour lines emphasise the influence of water on ? *H* being the resultant effect dependent on the concentration of L-AA in the dough. The model generated contour lines can be easily used to find optimum combinations of water and L-AA for a desirable thermal performance and in turn improve bread volumes. Enthalpies for any formulation can be calculated using this model. If bread volumes have been correlated with enthalpies of transition for the doughs, this means that bread volumes can be predicted. This also means that percentages of L-AA and water can be calculated and adjusted from the model whilst minor ingredients, soy flour and wheat flour remain constant and the desired bread volume is attained. A model for all three variables (water, soy flour and L-AA) would have been developed but it would have resulted in the choice of low amounts of soy flour in the bread (<30%) for optimum enthalpies. The model used on this experiment (with fixed 30% soy flour) was designed to maximise the use of soy flour for the targeted group of people who cannot afford to buy wheat flour.

5.6. Conclusion

DSC transition endotherms for soy-wheat dough were generally bimodal from 10 to 50% soy flour. The first and smaller transition peaks were characteristic of 7S soy protein denaturation and wheat starch gelatinisation while the second major peaks were attributed to 11S protein denaturation and soy-wheat dough water evaporation. Because the second major peaks were unexpectedly large, it was also possible to attribute these peaks to the melting of the more stable starch/polysaccharide - lipid complexes in the composite dough. Soy flour proteins delayed starch gelatinisation being the resultant effect dependent on water and L-AA available in the dough.

F-statistic values (Multivariate analysis) for soy flour, water and L-AA on thermal properties of soy-wheat dough revealed highly significant interactive effects between L-AA, water and soy flour on onset temperatures, peak temperatures and enthalpy of transition (? *H*) of soy wheat dough evaporation endotherms ($P < 0.05$). Increases in soy flour shifted the onset temperature (T_o) for starch gelatinisation and for the major DSC endotherm (water evaporation of soy-wheat dough) to higher temperatures while soy flour increases significantly decreased ? *H* ($P<0.05$). Water had highly significant and positive linear effects on T_p T_o and ? *H* ($P < 0.005$) indicating that water increased onset (T_o), peak temperatures (T_p) and ? *H* of soy-wheat dough evaporation endotherms. Although statistic coefficients for L-AA effects on thermal properties were insignificant ($P<0.05$), L-AA up to 0.2% in the dough increased onset and peak temperatures (main effects plots) and improved cooperative melting of components as observed on DSC smoothened curves.

Optimisation of soy-wheat flour composition, L-AA concentration and water was considered to be an important step in improving component interaction during baking as this would enhance thermal properties and baking performance of soy-wheat doughs. The DSC method used revealed large soy-wheat dough enthalpies, which were attributed to water evaporation. Because these enthalpies were proportional to soy flour increase in the dough, they were used to develop a mathematical model for the formulation of soy-wheat bread.

Chapter 6

Evaluation of the model in the formulation of soy-wheat bread

6. Table of contents

Abstract

This section of study determined the optimum combinations (enthalpy adjusted) of L-AA and water using fixed soy flour and other ingredients in concentrations which would result in improved bread-making qualities through objective and subjective analysis. The model developed in the preceding chapter was tested for its ability to predict loaf volumes in soy-wheat doughs. Results revealed that predicted enthalpies positively correlated with loaf volumes (R^2 =0.9). Enthalpy-adjusted values for water and L-AA (from the model) were successfully used for formulating soy-wheat bread with comparable loaf volumes to that of wheat bread.

This part also dealt with formulation of soy-wheat bread in an attempt to raise protein and amino-acid content in this formulation to at least 50% of the estimated safe intakes recommended by FAO/WHO (Food and Agricultural Organization / World Health Organization). Theoretical and published data values for the recommended protein and amino-acid intakes {required dietary allowances (RDA)} were compared to the estimated values in the formula for soy-wheat bread.

Results showed that the optimum soy-wheat bread formulation (highest enthalpy, 303 J/g) had the largest loaf volume (820 cm^3) with a light dense crumb texture (0.45g /cm^3). This bread was however perceived as less acceptable on overall acceptability (descriptive sensory analysis) to other formulations with less water, smaller loaf volumes (750 cm^3 and 760 cm^3) and denser crumb texture (0.47g /cm^3).

Results also showed that a daily serving of 100 grams of the formula soy-wheat bread would provide approximately 25% of the estimated safe intakes of daily protein requirements for adults and 60-100% of daily protein requirements for children (FAO/WHO recommendations), while the same amount of this bread would provide at least 50% of the daily lysine requirements for children and 70 -100% for adults (assuming 100 g /day per capita consumption of bread).

6.1. Introduction

The use of composite flours in bakery products has been a focus for research and development for the last two decades in both developed and developing countries. This is because composite bakery products offer beneficial opportunities, including nutrition, function and economy. The importance of supplementation of wheat flour with inexpensive cereals and legumes has been emphasised by many workers (Dhingra and Jood, 2001, Yousseff et al., 1976, Fleming et al., 1978; Shogren et al., 2003; Hugo et al., 2005; Nishita et al., 1976; Sharma et al., 1999; Tsen and Tang, 1971; D'Appolonia, 1977, Pomaranz et al., 1969). Wheat (*Triticum aestivum*) is the world's most important cereal crop in terms of production and consumption, but its production is costly for developing countries (Sanginga, 1999; Dhingra and Jood, 2001; Othira et al., 2004; Mojisola, et al., 2005). On the other hand, grain legumes are less costly to produce and they contribute significantly towards the protein, mineral and B-complex vitamin needs for people in developing countries (Neilson et al., 1979; Bressani et al., 1982).

The realisation of the health benefits from whole grain foods has become more possible with the improved acceptability of more dense bread over the past few years. The development of such functional foods in bread not only improves nutritional status, but also helps those suffering from degenerative diseases associated with today's life styles and environment (Sanchez et al., 2002; Gujral et al., 2002; Kobylanski et al., 2004). On the other hand, the use of high levels of soy flour in bread as a vehicle for soy proteins is of prime importance in developing countries where malnutrition levels among children and women have increased. Wheat importation in developing countries represents adverse economic effects as well as displacing indigenous crops with resultant detrimental effects on agricultural and technological development (Mojisola et al., 2005). As such, the soybean is one of the indigenous crops being popularised in developing countries in order to address food security and nutrition. Furthermore, the promotion of indigenous flours in the formulation of soy-cereal products as staple foods is becoming widely accepted by under-privileged communities. In order to utilise the full potential of soy protein and to meet the recommended protein intakes for these populations, soy flour has to be substituted for wheat flour in large proportions, since legumes form a major part of their dietary protein. Bread is a universally accepted food item and is a good medium for protein supplementation. Full-fat soy flour was used for bread making on this current work since it is claimed to have better functional properties than the defatted flour (Hoover, 1979). This flour also has more practical significance in developing countries where solvent or mechanical extraction of oil to prepare defatted soy flour is a costly procedure.

Observations from the preceding chapters indicate that L-AA and water directly influenced soy-wheat bread quality. The model equation proposed in the preceding chapter successfully predicted DSC major enthalpies of soy-wheat dough using these two variables (L-AA and water). Therefore it was found useful to optimise combinations of L-ascorbic acid, water and soy flour for the formulation of soy-wheat bread.

Statement of the problem

It is well known that high levels of soy flour in wheat bread results in adverse organoleptic properties (D'Appolonia, 1977; Grosch, 1985; Schofield, 1986; Dhingra and Jood, 2001). There is therefore a need for the formulation of a soy-wheat bread that is acceptable in terms of organoleptic properties. Although composite breads made from soy flour are popular in developing countries, the quality has mostly been inferior, due to beany flavours which are imparted to the bread. As pointed out by many workers (D'Appolonia, 1977; Ryan et al., 2002; Shogren et al., 2003; Coultate, 1996), one of the continuing impediments to the acceptance of soybean products is their beany flavour which is brought about by the lipoxygenase-catalysed oxidation of soybean oil to volatile compounds; this process leads to the accumulation of oxidation products in a series of biochemical reactions forming (volatile) aldehydes and ketones. It is because of these volatile compounds that the seed meal and oil flavour are spoiled (Coultate, 1996). Heat treatment of soybeans prior to flour milling is recommended to avoid formation of these volatiles since they will be difficult to remove in the later stages of processing.

Because of the dilution of the gluten matrix in composite breads, it has been found that treatments, such as the addition of oxidants like bromates, L-ascorbic acid, surfactants and extra water, improved loaf volumes (Shogren et al., 2003). Response surface methodology (RSM) has been successfully used in objective and subjective optimisation of bread formulations, with combinations and optimisation of water, egg white and gums as gluten replacements in rice and corn-flour breads (Ylimaki et al., 1988; Toufeili et al., 1994; Kobylanski et al., 2004). On the other hand, less work on the development and optimisation of soy-cereal products has been reported.

Surfactants and oxidising agents improve the dough strength of soy-wheat bread, but they do not minimise the bean odour imparted into the final product. Shogren et al. (2003) were the first to demonstrate that soy-wheat bread (up to 40% soy flour) could be made with only low levels of bean flavour, but he attributed this to high-quality yeast masking the bean flavour. L-ascorbic acid will be used in this work to strengthen the composite bread as well as to promote yeast growth and yeast flavour (Shogren and others) and thereby help in masking the bean flavours in soy breads. This study will employ the use of physically modified full-fat soy flour made from optimally heated soybeans (PMSF2) (lipoxygenase theoretically destroyed before flour preparation). The process of flour preparation was optimised in order to retain the nutritional properties of soy flour and thus to improve soy-product acceptance. This flour was found suitable for village use, and for small-scale farmers who do not have ingredients to mask bean flavours in soy breads.

Because the diets for most people in developing countries mainly comprise cereals, essential amino-acids (in particular lysine) need to be protected during processing of soy products. Lysine is a limiting amino acid in staple cereals like wheat and maize, it therefore serves to complement the amino-acid profile requirements in these cereal diets. It is therefore essential to monitor and develop optimum processing methods in order to retain available lysine in soy products (Bressani et al., 1982; Neilson et al., 1979; Liu, 1997). Available lysine in the formula breads will be estimated in order to determine the extent of losses during flour processing and baking.

Objectives

- To test the ability of the model to predict loaf volumes in soy-wheat dough
- To use enthalpy-adjusted L-AA and water values for formulating soy-wheat bread with loaf volumes comparable to those of wheat bread.
- To determine if the optimum combinations of L-AA and water (enthalpy-adjusted formulation) at fixed levels of soy flour and other ingredients result in improved bread qualities through objective and subjective analysis.
- To compare the nutritional value of formula bread with that of estimated safe intakes or required daily allowances (RDA) for protein and lysine.

Hypothesis

Because the dominant DSC enthalpies of soy-wheat dough correlated with soy flour concentration, the model predicted enthalpies should theoretically correlate with soy-wheat bread loaf volumes.

6.2. Methods

6.2.1. Evaluation of the ability of the model to predict loaf volumes in soy-wheat doughs

As observed from the preceding chapter, soy flours added in increased levels in soy-wheat doughs proportionally decreased DSC enthalpies of water evaporation (second and major endotherms) from the respective doughs. The ideal/optimum formulations would require ratios of L-AA and water (using a 30% soy-flour dough) that would raise the enthalpy of evaporation (? *H* in J/g) to match or become closer to that of a wheat-flour dough. Larger enthalpies of evaporation indicate large endothermic heat capacities and therefore more heat needed to evaporate water from the dough. From a practical point of view, high soy-flour doughs had smaller heat capacities, presumably due to the hydrophilic nature of soy proteins in holding onto the water. In order to facilitate optimum cooking and mostly to allow for optimum rising of the soy-wheat bread, high soy-flour doughs would theoretically need to have larger endothermic heat capacities. The model equation from the preceding chapter was successfully used to predict evaporation enthalpy using various levels of L-AA and water (30% soy flour). This part of the study deals with enthalpy-adjusted formulations (15 randomly peaked, predicted enthalpies), which were tested through baking to find if they can produce loaf volumes that correlate with enthalpies.

6.2.2. Bread formulation for affective sensory analysis

The next step was to use the model equation to determine optimum combinations of L-AA and water in soy-wheat dough that would give large loaf volumes. The selected combinations of L-AA and water would then be used (optimum) for soy-wheat bread formulation at fixed concentrations of soy flour (30%) and all other ingredients (except where indicated). Optimum formulations (enthalpy adjusted) were then used for the preparation of soy-wheat bread for affective sensory evaluation.

A separate model for determining values of L-AA and water required in 50% soy-flour dough was obtained (not shown) although this formulation was included in the initial sensory evaluation (affective). A surface response chart generated from the model (Appendix 6.4) was used to obtain formulations for the preparation of 50% soy-flour breads. Although a lot of work has been published on the effects of increased levels of soy-flour in wheat bread previously, this 50% soy-flour model was included on the first sensory analysis to evaluate the score margins between 30 and 50% soy-flour breads using the pre-treated (PMSF2) soy flour and L-ascorbic acid. This exercise was planned with the aim of providing an insight into the feasibility of using higher amounts (>30%) of soy flour for future formulations, and thereby improving the protein and amino-acid levels in the bread.

Samples listed in Table 6.1 include Sf30a, Sf30b and Sf50a, Sf50b for formulations of 30% and 50% soy flour, respectively, where sf30a and sf30b were optimum formulations at 30% soy flour, while Sf50a and Sf50b were optimum formulations at 50% soy flour.

Table 6.1 Bread formulation for affective sensory analysis

Breads	*Soy flour g)*	*Wheat flour (g)*	*Sugar (g)*	*Salt (g)*	*Compressed Yeast (g)*	*L-AA (g)*	*Water (m L)*	*?H (J/g)*
Sf30a	60	140	10	3	1.8	0.19	180	301
Sf30b	60	140	10	3	1.8	0.5	140	276
Sf50a	100	100	10	3	1.8	0.2	180	281
Sf50b	100	100	10	3	1.8	0.3	170	263

6.2.3. Preparation for soy-wheat breads

Doughs were prepared using the formulations given in Table 6.1. Wheat flour was replaced by soy flour to make total flour weights of 200 grams. The dough was mixed using the straight-dough procedure following the methods of BRI Australia (2002) with slight adjustments to cater for the composite dough.

All ingredients were mixed with the simultaneous addition of water at medium speed 3 using a Kenwood Chef Dough mixer until cohesive dough was obtained (3 minutes). The water temperature used (13.5 -14ºC) was calculated using the Major Factor method (BRI Australia, 2002). The finished dough temperature ranged from 26 to 27ºC for all doughs. The resultant dough was allowed to rest for 30 minutes at room temperature. The dough was then moulded and placed in greased and floured baking tins (21 cm x 10 cm x 5.5 cm). These were allowed to ferment/proof in a warm, moist proofer (WTC binder78532 Tuttlingen Germany) at a relative humidity of 80-85%, maximum temperature of 40ºC until the size of the dough had risen (approximately double its original size). The process of proofing took 1 hour. The dough was baked in an oven (CPC Rational GmbH, D86899) for 10 minutes at 180ºC. Desired crust colour was obtained from the given optimums.

Baking tests were replicated at least twice. Weights and volumes were determined 2 hours after the bread was taken from the oven and allowed to cool on stainless steel racks (Appendix 6.3). Loaf volumes of breads were determined by the rapeseed displacement method (Fleming and Sosulki, 1978; Ylimaki et al., 1988; Gujral et al., 2002) using rice instead of rapeseed. Specific loaf volumes (cm^3/ g) and density (g /cm^3) were determined from the weights and volumes of the breads.

6.2.4. Evaluation of crumb texture

Methods for evaluating crumb texture were followed from Gujral et al. (2002).
Crumb texture was measured using a texture analyser. For replicate samples, three equal pieces of bread-crumb samples (8.3 x 5 x 1.5cm) were subjected to texture profile analysis using a Texture Analyser (Expert Exceed, stable Micro Systems). The bread sample was placed on the platform of the testing machine. A circular plate of 4 cm diameter, attached to a 25 kg load cell, was used to compress the bread crumb to a 10mm distance at a speed of 1mm per second. The force in grams per unit time in seconds (g/sec) was plotted. Texture analysis was done 4 hours after baking.

6.2.5. Procedure for affective sensory analysis

The first part of sensory evaluation was used as part of a product-screening effort to remove products which did not warrant further testing. The products preferred would then be used for more informative tests (descriptive).

Acceptability of the soy-wheat bread samples was determined by taste panels composed of 40 judges including university lecturers, food-science students and other technical staff from the University of Western Sydney, Hawkesbury Campus. The panels were asked to rate the unidentified breads using the nine-point Hedonic Rating Scale ranging from "like extremely" (9) to "dislike extremely" (1) for each organoleptic characteristic (Dhingra and Jood, 2001). The breads from the oven were allowed to cool for at least 3 hours, after which they were sliced (stainless-steel knife) into samples, approximately 6 x 3 x 1 cm in size. After slicing, bread samples were placed in plastic disposable plates and wrapped in plastic materials to prevent drying. The plastic plates were coded with 3-digit codes (generated from Excel software) and served to panelists. Panelists were asked to rinse their mouth with water after the evaluation of each product.

6.2.6. Bread formulation for descriptive sensory analysis

Soy-wheat bread formulations sf30a and sf30b from the affective sensory evaluation were regarded as superior over the four formulations tested using the analysis of variance. Average scores of sensory attributes for these two formulations (30sfa and 30sfb) were not significantly different *(P 0.05).* Therefore these two formulations (including 30sfc which was in between 30sfa and 30sfb for water and L-AA concentration) were selected for a detailed descriptive sensory analysis. Descriptive analysis was aimed at giving more insight into the effects of each formulation on the organoleptic properties of the soy-wheat bread.

Theoretically, water increases continued to increase enthalpies of vaporisation in soy-wheat doughs during DSC but L-AA appeared to reach an optimum. Therefore, in order to determine the optimum amount of L-AA (enthalpy adjusted), a derivative equation was drawn from the model to find the optimum concentration of L-AA (at constant water amount) that would give the highest enthalpy. The following procedure was used for the derivative;

$?H = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_1^2 + b_4 x_2^2 + b_5 x_1 x_2$; (model equation)

Derivative: $d\,?H / dx_2 \quad = \quad b_2 + 2b_4 x_2 + b_5 x_1$

$$2b_4 x_2 = - b_2 - b_5 x_1$$

$$x_2 = - (b_2 + b_5 x_1) / 2b_4$$

Where x_1 = water %; x_2 = L-AA %; b_0, b_1, b_3, b_4 and b_5 are all model coefficients. Data for x_2 (L-AA) values were calculated from the derived equation at fixed water values and enthalpy (?*H*) values obtained were plotted on 3D scatter plot on Figure 6.1.

Figure 6.1

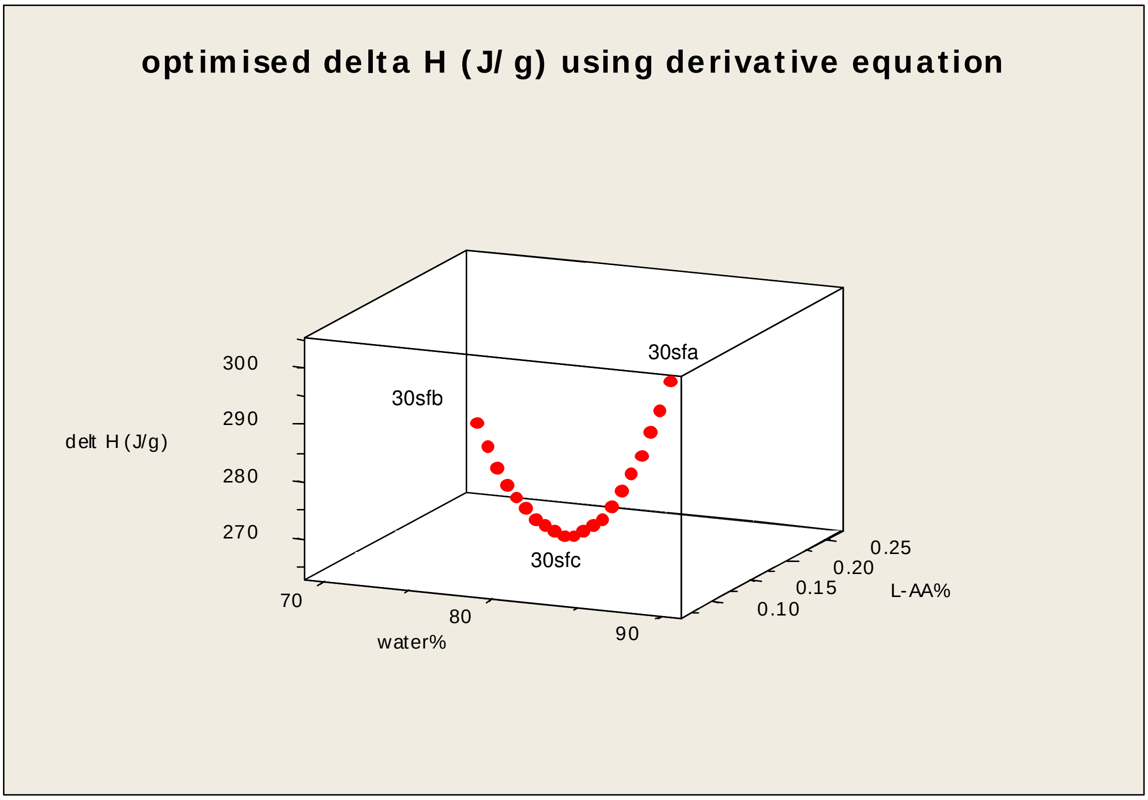


Figure 6.1 3D scatter plots for calculated values of enthalpy using the derivative equation. Delta H values (model derived) versus water and L-AA percentages highlighting the three points selected for soy-wheat bread formulations used for descriptive analysis.

Three points were selected for this formulation, the optimum ?*H* at 70% water and 0.275% L-AA; optimum (?*H*) at 90% water and 0.075% L-AA; minimum (?*H*) at 80% water and 0.17% L-AA and these formulations correspond to 30sfb, 30sfa and 30sfc, respectively (Figure 6.1 and Table 6.2).

Table 6.2 Bread formulations for descriptive sensory evaluation

Breads	*Soy flour g)*	*Wheat flour (g)*	*Sugar (g)*	*Salt (g)*	*Compress yeast (g)*	*Fat (g)*	*L-AA (g)*	*Water (m L)*	*Pred ?H (J/g)*
Sf30a	60	140	10	3	1.8	4	0.15	180	303
Sf30b	60	140	10	3	1.8	4	0.52	140	276
Sf30c	60	140	10	3	1.8	4	0.34	160	266

6.2.7 Procedure for descriptive sensory analysis

Unstructured scaling, consisting of a horizontal line 15 cm long with anchor points 1.5 cm from each end (with appropriate descriptors), was used for descriptive analysis of bean flavour and texture attributes of soy-wheat bread samples (optimum formulations). The panel consisted of 33 trained panelists who were selected on the basis of their ability to discriminate and scale the key sensory attributes of soy-wheat bread. Panel orientation included familiarisation with bean flavour using commercial soy milk. Orientation on texture attributes (crumb denseness and moistness) and overall acceptability was also given to the panellists. Reference and attribute scores were based on a 15-point scale according to Shogren et al. (2003) with 0.5 point increments (1 = threshold; 1.5 to 5 = slight; 5.5 to 10 = moderate; 10.5 to 15 = extreme). Bread samples were prepared as in Section 5.3.5 (affective analysis). The panelists were asked to make a vertical line on the horizontal line to indicate their rating for each sensory attribute. The descriptor ranges for the key flavour attribute were; bean flavour (not bean – extremely bean), and those for key texture attributes were; crumb denseness (not dense – extremely dense), crumb moistness (not moist – extremely moist), while overall acceptability (dislike extremely – like extremely) was asked lastly.

6.2.8. Nutritional value of breads

Because of the liability of lysine to heat destruction during processing, lysine availability and proximate analysis were evaluated in the processed soy-wheat breads.

6.2.8.1 Lysine availability

Lysine availability was evaluated following the methods of Tejada (1985), Linden (1996) and Fernandez-Artigas et al. (1999) with modifications. The available lysine is the quantity of lysine in which the amine situated on the ε-carbon has not reacted with an aldehyde (sugar or other) during processing. Because the bond which is formed between lysine and the aldehyde cannot be hydrolysed by the digestive enzymes, the measurement of the intact lysine fraction is important in all processed products. These free amino groups are reacted with FDNB (flouro-2-4-dinitrobenzene) and can be quantified. The sample is reacted with FDNB in ethanol for 2 hours to form a DNP (dinitrophenol) - protein derivative. DNP- protein is in turn subjected to hydrolysis in 6N HCl for 24 hours to form DNP–amino acids. The amount of sample analysed should contain at least 12 mg of available lysine (Tejada, 1985).

The bread sample (2g), 10 mL $NaHCO_3$ (8%) solution, 15 mL FDNB solution (in 3% ethanol) and 3 glass beads were added to 50 mL Pyrex round-bottomed flask (fitted with a Quickfit stopper). The mixture was mechanically shaken for 2 hours at room temperature (ensuring that no sample sticks to the sides) after which ethanol was evaporated over a steam bath (95ºC). The hydrolysis of DNP proteins was achieved by adding 30 mL of 6N HCl. This was gently refluxed for 16 hours after removing CO_2 by stirring. After hydrolysis, the hot solution was filtered into a 250 mL volumetric flask and topped up to the mark with distilled water when it was cold. To separate and remove DNP-amino acids from DNP-lysine, 2 mL of filtrate was put in two test tubes (A and B); ether extraction from the two tubes was done following steps on Appendix 6.6. The ether-extraction process was also repeated for standard DNP-lysine. The absorbance of solutions in both tubes was read at 435 nm (UV/VIS Spectrophotometer Shimadzu mini 1240 Pharmacia Biochrom 4060). The absorbance of tube A, minus that of tube B (blank) was the DNP-lysine absorbance. A stock solution (100mg mL^{-1} of *N*- ?-2, 4-DNP-L lysine HCl dissolved in concentrated HCl) was diluted 10 times with distilled water (10mg mL^{-1}) to prepare a working standard solution (containing 0.1mg / 2mL). Concentration of lysine (g/100g protein) was calculated following Tejada (1985).

6.2.8.2 Proximate composition

Proximate composition of the breads for crude protein (*N x 6.25*), moisture, ash and fat was determined following methods from the Association of Official and Analytical Chemists (AOAC, 1995).

6.2.9. Statistical analysis

The data for objective and subjective analysis were subjected to analysis of variance (ANOVA) from Excel software. Significant differences to separate means were determined using least-squares significant difference (LSD). MINITAB ANOVA (general linear model and main-effects plots) were used to study effects of L-AA and water on organoleptic properties of soy-wheat bread. A model equation (developed in the preceding chapter) was used to optimise L-AA and water amounts in the formulation of soy-wheat bread.

6.3. Results and discussion

6.3.1. Ability of the model to predict soy-wheat dough loaf volumes

Loaf volumes were successfully predicted as a result of the model-adjusted enthalpies. Results for predicted enthalpies and resultant loaf volumes are shown in Table 6.3.

Table 6.3. Predicted enthalpies (?H (J/g) and the resultant loaf volumes, crumb texture and density from several formulations. Values given are the mean of duplicate analysis. (±values given in the table are the error of the mean)

Sample [a]	*Predicted ?H (J/g)*	*loaf vol. (cm^3)*	*Crumb texture. ($g{\cdot}s^{-1}{\cdot}m^{-1}$)*	*Density g/cm^3*
30 % soy flour wheat bread	303	820 ±15	14.0 ± 0.5	0.45 ± 0.01
	295	800 ±20	14.6 ± 0.8	0.46 ± 0.03
	295	810 ±5	14.2 ± 0.6	0.45 ± 0.02
	293	810 ±25	14.9 ± 0.5	0.46 ± 0.02
	274	760 ±15	17.9 ± 0.8	0.46 ± 0.03
	278	800 ±15	17.5 ± 0.9	0.47 ± 0.01
	277	800 ±20	17.9 ± 0.3	0.46 ± 0.02
	278	790 ±20	15.3 ± 0.7	0.46 ± 0.02
	276	760 ±10	16.3 ± 0.6	0.47 ± 0.02
	270	740 ±15	14.9 ± 0.8	0.47 ± 0.01
	269	780 ±20	14.9 ± 0.7	0.47 ± 0.02
	266	750 ±15	14.7± 0.8	0.47 ± 0.03
	266	740 ±10	16.5 ± 0.8	0.48 ± 0.02
	265	720 ±10	17.6 ± 0.9	0.49 ± 0.03
	260	700 ±20	16.9 ± 0.2	0.51 ± 0.01
ref/wheat	**289**	**780** ±15	**18.0** ± 0.8	**0.44** ± 0.01

[a] symbol means; all dough samples were fixed at 30% soy flour

The results in Table 6.3 show the trend for predicted enthalpies and the resultant bread qualities. It is clear from this observation that the addition of soy flour to the soy-wheat bread has influenced crumb texture as crumb texture in wheat bread was hardest (18 g $s^{-1}{\cdot}mm^{-1}$). Several workers have already pointed out that soy-flour proteins improve crumb texture in wheat bread as they have attributed this to the excellent water-binding capability of soy proteins (Kilara and Sharkasi, 1986; Liu, 1997; Utsumi and Kinsella, 1985; Hoover, 1979; Marsili, 1993). Loaf volumes were generally higher in the higher values of enthalpy, while the lower enthalpies gave smaller loaf volumes. Bread-crumb texture and density seemed to be influenced by the amount of water and L-AA in the formulation and therefore crumb texture and density did not seem to correlate with enthalpy areas. But from general observation, bigger loaf volumes were less dense than the smaller ones.

The results on Figure 6.2 show the polynomial relationship between loaf volumes and predicted enthalpies, and thereby highlighting the ability of this model to predict loaf volumes (from adjusted enthalpies) in soy-wheat dough. The correlation coefficient (R^2) for the loaf volumes and predicted enthalpies was 0.90, meaning that the equation on Figure 6.2 was able to explain at least 90% of the data. The high correlation coefficient showed that evaporation enthalpies of dough samples were reliable predictors of loaf volumes. Loaf volumes were determined as a measure of adjusted formulation from predicted enthalpies as this confirmed that the desired soy-wheat bread loaf volumes can be achieved using the model (from Chapter 5).

Figure 6.2

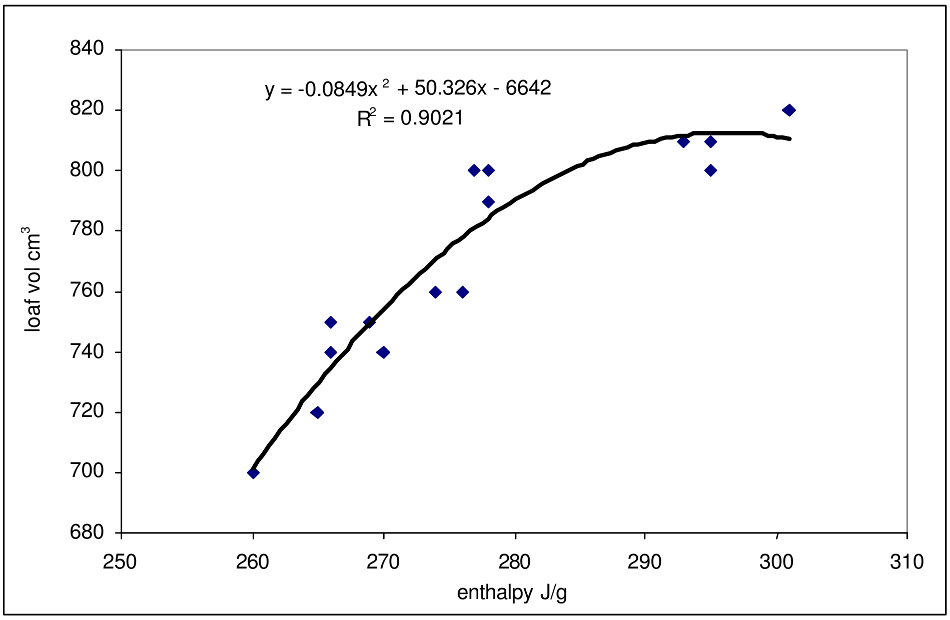


Figure 6.2 Polynomial relationship between loaf volumes (cm^3) and enthalpy (J/g) for the soy-wheat bread using model-predicted enthalpies.

6.3.2. Affective sensory analysis

The results in Table 6.4 show the average scores for affective sensory evaluation.

Table 6.4 Sensory evaluation characteristics (average scores), maximum score for extreme liking was 9 and objective analysis for soy-wheat bread using different formulations (±values given for objective analyses are the error of mean values)

Breads	Crumb colour	Crumb Texture	Flavour	Taste	Loaf vol (cm^3)	Spec loaf vol (cm^3/g)	Crumb text. ($g\ s^{-1}\ mm^{-1}$)
Sf30a	7.13^{a}	5.85	6.38^{ac}	6.35^{a}	820 ±15	2.19 ±0.05	14.0 ± 0.5
Sf30b	6.85^{ab}	6.18	6.50^{a}	6.50^{a}	760 ±10	2.16 ±0.08	16.3 ± 0.6
Sf50a	6.40^{b}	5.75	5.78^{bc}	5.68^{b}	780 ±20	2.06 ±0.05	17.9 ± 0.8
Sf50b	6.53^{b}	5.70	5.82^{bc}	6.00^{ab}	760 ±15	2.07 ±0.07	20.1 ± 1.0

Superscripts indicate significant difference. Values followed by the same letter (down each sensory attribute) were not significantly different ($P < 0.05$).

Results observed in Table 6.4 indicate that crumb colour score (subjective analysis) and specific loaf volume (objective analysis) were highest in the Sf30a formulation (optimum) and that crumb texture was softest in this formulation.

Significant differences among the four bread samples for each organoleptic attribute were determined by the F statistic value following Analysis of variance from Excel software (Poste et al., 1991). Significant studentised range values ($P = 0.05$) from statistical tables were used. Least significant differences (LSD) among the means were also determined for each sensory attribute.

From the F-statistic value, there was significant difference in taste between the four bread samples ($P < 0.01$). Using LSD (least significance difference) to compare the means for the four bread samples, Sf30a and Sf30b tasted the same while Sf30a and Sf30b tasted differently from Sf50a ($P < 0.05$). However, Sf30a and sf30b were not significantly different from Sf50b in taste. The less water (85%) or higher amount of L-AA (0.15%) could have influenced the better taste in Sf50b compared to its counterpart Sf50a. Using letters to indicate differences, average values for taste scores were 6.35^{a}; 6.5^{a}; 5.68^{b}; 6.00^{ab} for Sf30a, Sf30b, Sf50a and Sf50b, respectively, where values followed by the same letter were not significantly different (*P <0.05*). Since Sf30a and Sf30b were not significantly different, these observations would suggest that the formulation using the 30% soy flour was more accepted than the 50% soy flour formulation.

For flavour perception, again there was significant difference in flavour among the four samples according to the F-statistic value (*P <0.05*). Using LSD, Sf30a and Sf30b had the same flavour; Sf50a and Sf50b were not different either (*P <0.05)*. Average values for flavour were 6.38^{ac}; 6.5^{a}; 5.78^{bc}; 5.82^{bc} for Sf30a, Sf30b, Sf50a and Sf50b, respectively. The results on flavour preference suggested that 30% soy flour bread was more accepted than the 50% soy flour bread.

Colour differences were also perceived as significantly different using the f-statistical value (*P <0.05*). Comparing the means, (LSD) Sf30a and Sf30b were not significantly different in colour, whilst Sf30a was significantly different from Sf50a and Sf50b and; Sf30b was not significantly different in colour from Sf50a and Sf50b (*P <0.05*). The average values for the colour scores were 7.13^{a}; 6.85^{ab}; 6.4^{b}; 6.52^{b} for the same order as flavour. Sf30a had the highest score in colour, followed by Sf30b. These results also suggested that 30% soy flour formulation had a more appealing colour than the 50% soy flour breads. No significant difference was observed in texture among all soy-wheat bread formulations (*P <0.05*).

In order to accurately assess the significant differences among the four samples and four sensory attributes, the scores for each sensory attribute were converted into ranks and the rank sums were tested for significance using the Friedman test for ranked data. Rank sum data was in the following order 7^{a}; 5^{a}; 15^{b}; 17^{b} for Sf30a Sf30b, Sf50a and Sf50b, respectively (*P <0.05*), where values followed by the same letter were not significantly different. The results revealed that 30sfa 30sfb were the same in appeal and that Sf50a and Sf50b were also the same in appeal. Therefore, the first two formulations (Sf30a and Sf30b) were considered superior and selected for a detailed descriptive sensory analysis.

6.3.3 Descriptive sensory analysis

Table 6.5 Effect of formulation on organoleptic and objective characteristics of soy-wheat dough formulations [average scores for sensory analysis and mean values for objective analysis (maximum score was 15), ± values given as error of mean values]

Sample	Bean flavour	Dense-ness	Moist-ness	Bean flavour liking	Overall acceptance	Crumb text. (g s^{-1} mm^{-1})	Density g/cm^3
Sf30a	4.8	7.6	9.5	8.2	8.2	14.0 ± 0.5	0.45 ± 0.01
Sf30b	5.1	8.5	8.2	9.1	8.9	16.3 ± 0.6	0.47 ± 0.02
Sf30c	5.5	8.0	8.5	7.0	8.9	14.7 ± 0.8	0.47 ± 0.03

Average scores shown in Table 6.5 indicate that bean flavour in soy-wheat bread was least perceived in Sf30a bread (optimum formula). The soy-wheat bread (Sf30b) which contained the least amount of water in the formulation was perceived slight-moderate in bean flavour and most dense, while bread (Sf30a) with the highest amount of water in its formulation was perceived as being the least in bean flavour, least in density and highest in moistness. Sample Sf30c, which contained in-between levels of L-AA and water (0.175% and 80% respectively) also scored moderate in bean flavour, and fell between Sf30a and Sf30b in denseness and moistness. From the average scores on overall liking, Sf30a was the least accepted although it was the optimum formula with the highest loaf volume. This was possibly due to the high moisture in the bread.

The data for descriptive sensory analysis was subjected to analysis of variance (Excel software) with the F statistic value used to determine significant differences among the bread samples for each organoleptic attribute. There were no significant differences in bean flavour, crumb moistness, crumb denseness and overall liking between the 3 bread samples (Sf30a, Sf30b and Sf30c), ($P>0.05$). Because there was no statistical difference between the samples, the point classification system (Shogren et al., 2003) was used to separate the bread samples. Frequency distribution histograms (using the point system) were plotted to evaluate the number of responses against soy-wheat bread samples for each attribute tested, where 1= threshold; 1.5 to 5 = slight; 5.5 to 10 = moderate; 10.5 to 15 = extreme. Histogram charts were also used to characterize location and variability of data sets, Appendix 6.1).

Figure 6.3

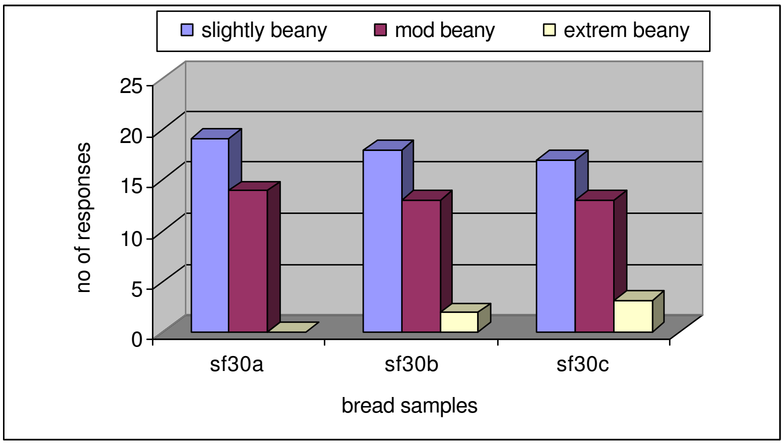


Figure 6.3 Sensory evaluation on bean flavour, the number of responses (y-axis) against the soy-wheat bread samples (x-axis). The bars represent the extent of bean flavour classified as slightly bean = ; moderately bean ; and extremely bean .

Observations in Figure 6.3 showed that the majority of responses tasted the bean flavour as slight in all three soy-wheat breads. The bean flavour in sample Sf30a was mostly muted, with the highest number of responses tasting it to be slightly beany. The order of bean flavour in decreasing order from observations on histogram was Sf30c, Sf30b and Sf30a. The reasons for bean flavour perception to be generally slight in these soy breads could have been attributed to the active dry yeast, which was suggested to be an important component in reducing soy bean flavours by Shogren et al. (2003); they also added that sugar and ascorbic acid in soy bread may promote yeast growth as well as acting as an anti-oxidant (ascorbic acid) in retarding oxidation of unsaturated lipids and reducing the formation of oxidation products which are responsible for the soy bean flavour.

Figure 6.4

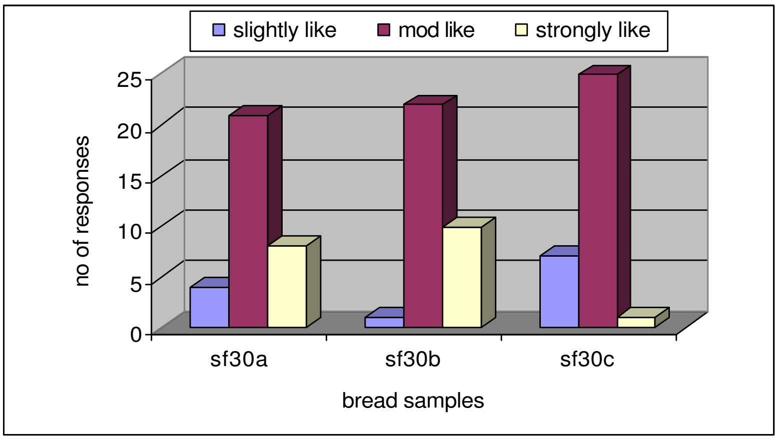


Figure 6.4 Sensory evaluation on bean flavour liking highlighting the number of responses (y-axis) against soy-wheat bread samples (x-axis). The bars represent bean flavour liking classified as slightly = ¦ moderately ¦ and strongly liked ¦ .

From observations in Figure 6.4, bean flavour was strongly liked in sample Sf30b, followed by sample Sf30a and this flavour was mostly and moderately appreciated in sample Sf30c. Generally, bean flavour was moderately appreciated in all 3 samples suggesting that panelists had central tendency behaviour in their preferences.

Figure 6.5

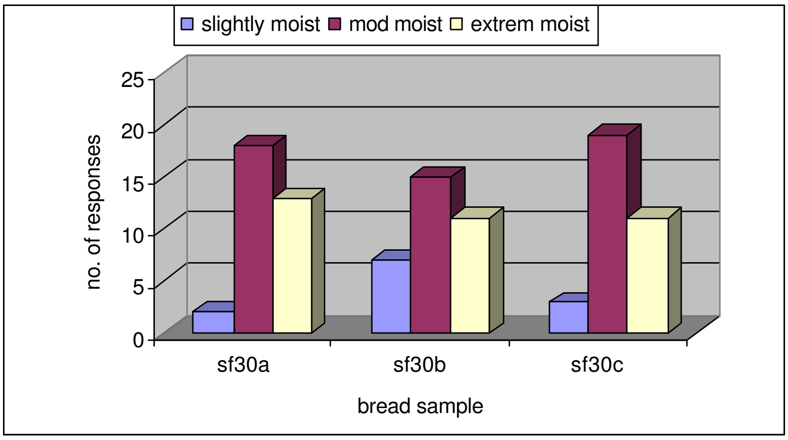


Figure 6.5 Sensory evaluation on crumb moistness, the number of responses (y-axis) against the soy-wheat bread samples (x-axis). The bars represent the extent of moistness classified as slightly moist = ¦ moderately ¦ and extremely moist ?.

Although the F-statistic test revealed no significant differences in moisture between the three bread samples, observations from Figure 6.5 showed that the Sf30a sample was rated extremely moist by more respondents compared to the Sf30b and Sf30c bread samples. This would be expected from the amount of water used in formulation for Sf30a (90%). Similarly to the liking for bean flavour, the majority of respondents tasted all the breads to be moderately moist.

Figure 6.6

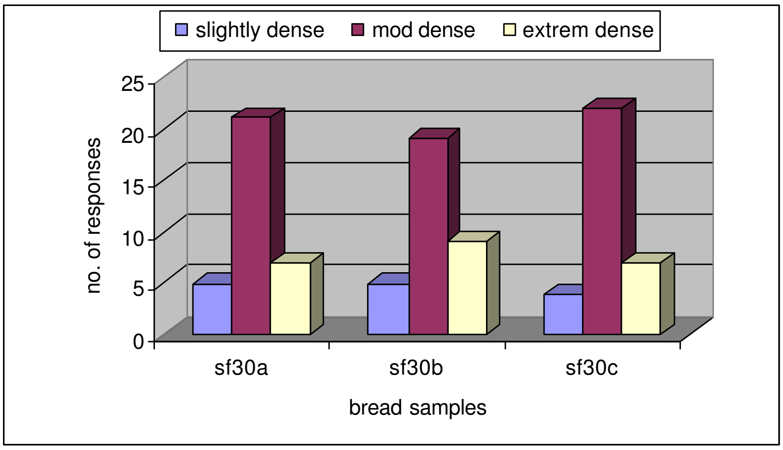


Figure 6.6 Sensory evaluation crumb denseness, the number of responses (y-axis) against the soy-wheat bread samples (x-axis).The bars represent denseness classified as slightly dense = ¦ moderately dense ¦ and extremely dense ?.

The greater number of respondents felt that the three breads were moderately dense (Figure 6.6) and again, this highlighted the central tendency behaviour showed by the panelists. For the smaller number of respondents who tasted the soy-wheat breads to be extremely dense, they rated sample Sf30b (70% water) to be the most dense bread, followed by Sf30c (80% water) while Sf30a (90% water) was tasted as the least dense. This indicated that water had a role to play on the density of the soy-wheat bread as these results also correlated with bread crumb textures. Samples in decreasing order of denseness were Sf30b, Sf30c and Sf30a; while the decreasing order for crumb texture was 16.3 grams, 14.7 grams and14.0 grams for Sf30b, Sf30c and Sf30a, respectively.

Figure 6.7

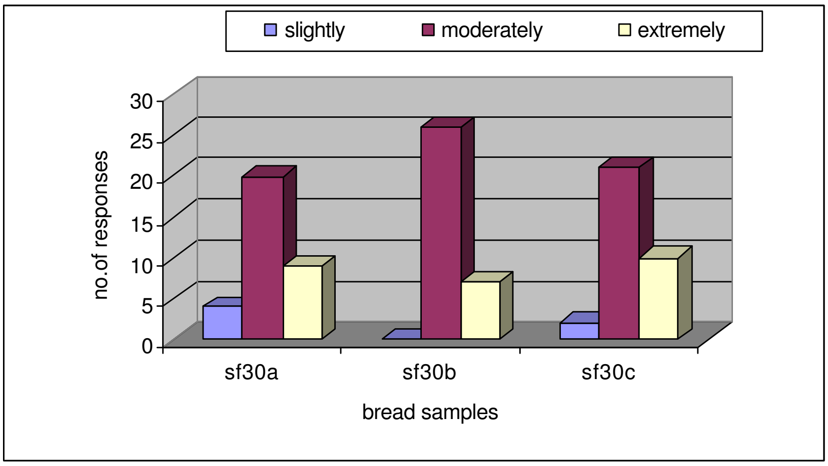


Figure 6.7 The overall liking of soy-wheat bread samples; number of responses (y-axis) against the soy-wheat bread samples on x-axis. The bars represent overall liking classified as slightly ¦ moderately ¦ and extremely liked ?.

Generally the three soy-wheat breads were moderately liked, with Sf30b scoring highest on moderate acceptance (Figure 6.7). A smaller number of respondents liked this bread very much and extreme liking was in the order of Sf30c, Sf30a and Sf30b. Because the F-statistical values indicated no significant differences in the overall liking of the three breads, this coupled with the fact that many respondents liked all three breads moderately (from histogram observations) may indicate that all the three formulations could be used acceptably. The fact that these breads were found acceptable in a developed country would mean that communities in developing countries would find this bread more acceptable since they do not have much choice in terms of bread preferences. Communities in developing countries mainly consume dense bread made from various cereals and therefore they are assumed to appreciate these breads. Dense bread is however more appreciated than the traditional white bread today due to health awareness among people.

The inclusion of overall liking on this evaluation was to find out if other sensory attributes could be altered to improve the acceptability of these breads (Stone and Sidel, 1993; Popper et al., 2004). The results on this study revealed that bean flavour, moistness and denseness did not correlate with overall liking of all three breads, except for Sf30b formula, where bean flavour slightly and negatively correlated with overall acceptability {-0.51 *(P <0.05)*} meaning that increases of bean flavour slightly decreased acceptability. This could have been due to the denseness in this bread, influencing the perception for bean flavour and therefore slightly affecting the bread's overall acceptability. This would suggest that increasing moisture in the formula would improve acceptability. Appendix 6.2 shows crumb porous structure for the breads.

6.3.4 Effects of L- AA and water on key organoleptic properties and density of soy-wheat breads

Because there was no significant difference in the overall acceptability (sensory evaluation) of the three variations in bread formulation, the effects of variables (L-AA and water) on organoleptic properties of soy-wheat bread were also deemed to be insignificant. More so, these results did not clearly reveal the interactive behaviour of the two variables on organoleptic properties. In order to verify these, MINITAB ANOVA (Appendix 6.5) was used to estimate the effects of the two variables (water and L-AA) on the key organoleptic properties of soy-wheat bread formulation. Figures 6.8, 6.9 and 6.10 highlight the main effects of these two variables on the average bean score, average taste score and on the density of formula breads.

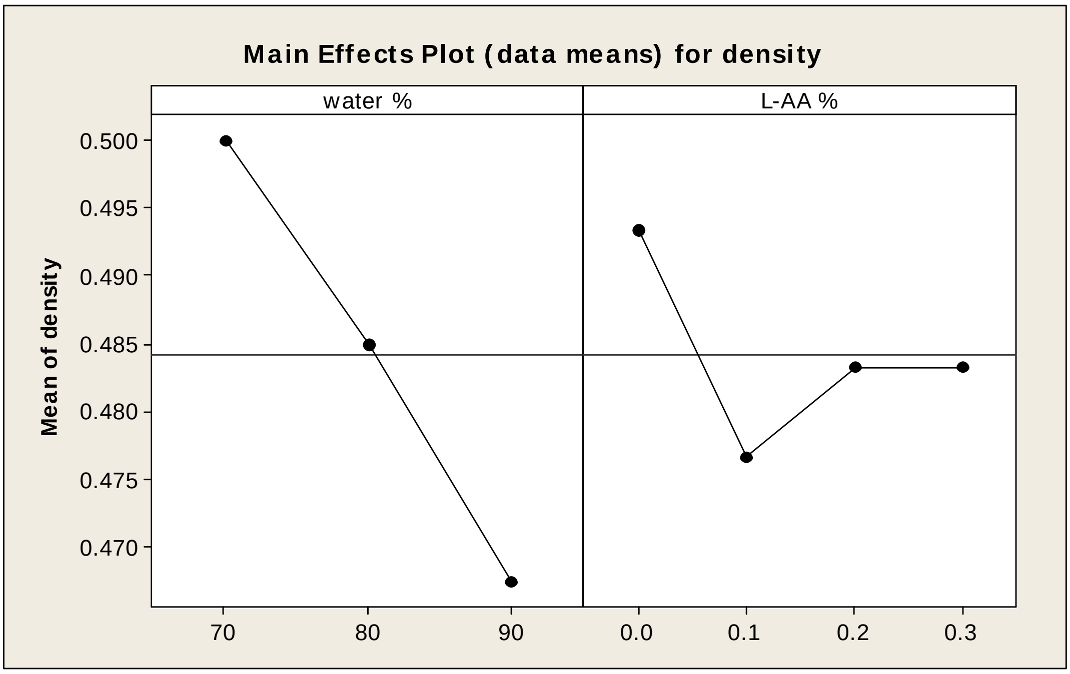


Figure 6.8 The main effects plots for water and L-AA on average density (g/cm^3)

Results from ANOVA F-statistic revealed that water significantly influenced the density of this soy-wheat bread *(P=0.05)* (r^2 = 0.65) as this is also highlighted on Figure 6.8 where increases of water markedly reduced the density of the bread.

F –statistic for the L-AA effect on density was insignificant *(P<0.05)* although observations from the main effects plot suggest that L-AA at 0.1% (w/w) was influential in improving the density of this bread and above 0.1% this effect was non-significant.

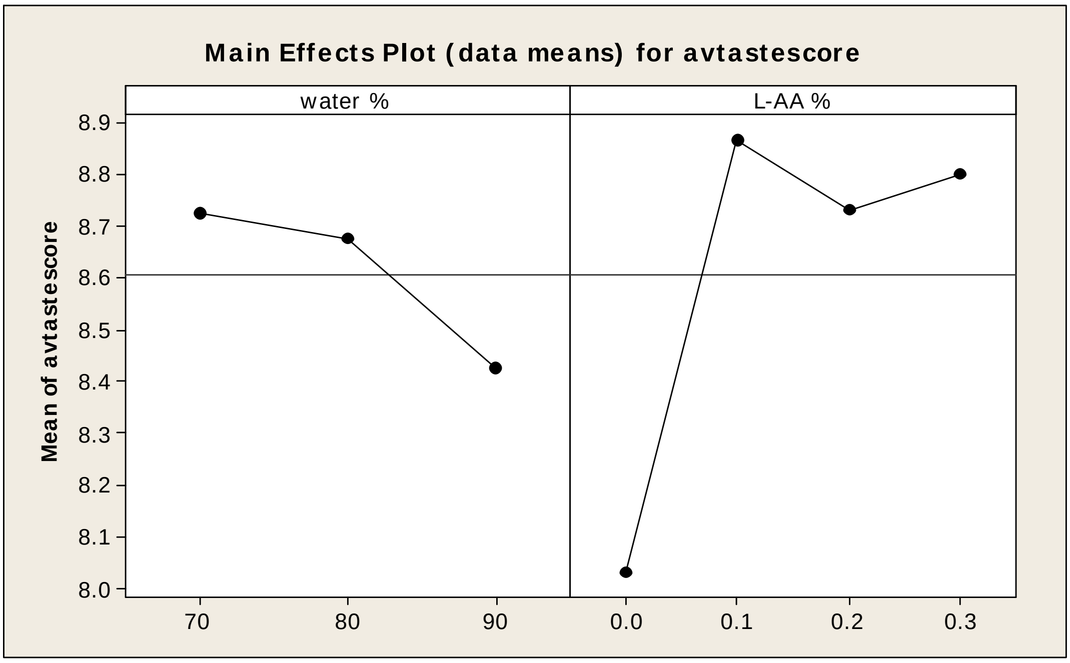


Figure 6.9 The main effects plots for water and L-AA on average taste scores

The F-statistic values for the effects of water and L-AA on taste scores were both insignificant *(P <0.05)*, but the mean taste scores are generally shown to decrease with increases of water while average taste scores increased with increases of L-AA to an optimum of 0.1% (Figure 6.9). This observation confirms the earlier finding that a soy-wheat bread formulation with 90% water had the least acceptability score compared to 70 and 80% water formulas.

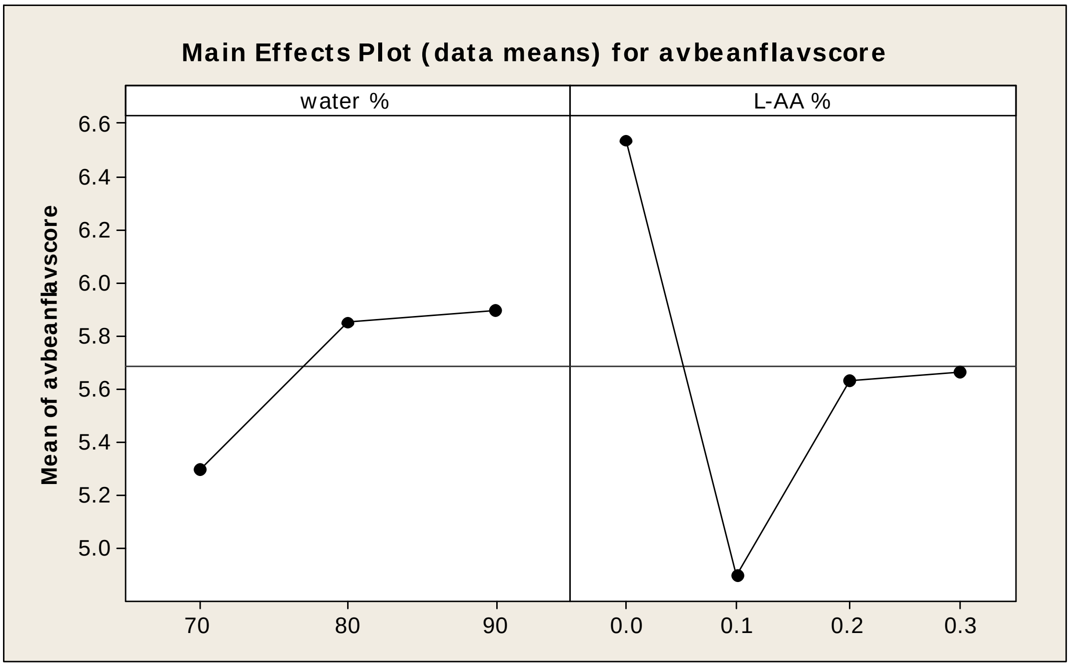


Figure 6.10 The main effects plots for water and L-AA on average bean flavour scores

The F-statistic value for the effect of water on average bean scores was non-significant although increases of water (Figure 6.10) slightly increased bean flavour scores (5.3 to 5.9) from 70 to 90 % of water respectively. On the other hand, the L-AA effect on average bean score was significant *($P<0.05$)* with r^2 of 0.83, as this is also highlighted on Figure 6.10. As explained before, the positive influence of L-AA on taste and the masking of bean flavour in soy-wheat bread has been reported (Shogren et al., 2003).

Correlation between bean flavour and taste average scores revealed a negative r^2 value of -0.564 *($P = 0.05$)*, suggesting that respondents' perception of taste was slightly influenced by the bean flavour as this correlation was only small. Correlation between density and average taste scores was insignificant (r^2 = - 0.035 $P => 0.05$) as this suggested that perception of taste was not dependant on the actual density of the breads.

6.3.5. Nutritional value of formula breads

This section describes the nutritional significance of the formula breads in terms of available lysine and protein content. The values of protein content and available lysine found in formula breads are compared to the Recommended Dietary Allowances (RDA) by FAO/WHO. The nutritional significance to the targeted groups is also described.

Table 6.6 Proximate composition and lysine available in soy-wheat bread made from PMSF2 and wheat flour. Values given are the mean of duplicate analyses, ± values are errors of the mean

Soy flour %	*Protein %*	*Available Lysine %*	*Fat %*	*Moisture %*	*Minerals/ash %*
30	13.6± 0.6	0.68 ± 0.08	9.5± 0.9	38.6± 1.1	2.0± 0.2
50	14.9± 0.5	0.88 ± 0.08	10.2± 0.7	44.8± 1.8	2.1± 0.1

The values in Table 6.6 for protein and lysine and moisture % in soy-wheat bread (30% soy flour) are close to values published by Shogren et al. (2003) who reported the (calculated) nutritional profile of soy-wheat bread (30% defatted soy flour blended with whole wheat and white wheat) to be; protein16.2%, fat 4%, moisture 37%, total dietary fibre 6%, lysine 0.83% and cysteine 0.52%.

Estimated safe intakes or Recommended Dietary Allowances (RDA) for protein are 0.8g/kg body weight and 1g/kg body weight (FAO/WHO, 1985) in adults and children (2-6 years) respectively. Amino acid requirements (mg/kg body weight /day) are 12 and 60 (FAO/WHO/UNO, 1985); 16 and 58 (Liu, 1997) for adults and children respectively. The current formulated soy-wheat bread was estimated to contain 13.6% (w/w) of protein (*N x 6.25*), meaning that there was 13.6 grams of protein in 100 grams of bread. Table 6.7 summarises the estimated amounts of protein and lysine in the formula bread, including RDA values in adults and children.

Table 6.7 Estimated amounts of protein and available lysine, comparison of formula soy-wheat bread and RDA values in adults and children

	RDA [a] (FAO/WHO)			*Soy-wheat bread formula (per 100g serving)*		
	2-5 yrs	10-12 yrs	adults	2-5 yrs	10-12yrs	Adults
Lysine (mg /kg body wt)	58 mg	44 mg	16 mg	680mg	680mg	680mg
protein (g/kg body wt)	1g	1g	0.8g	13.6g	13.6g	13.6g

[a] RDA is the required daily allowances (FAO/WHO)

Table 6.8 Estimates of daily protein and lysine intakes compared to RDA values for three age groups, calculations performed based on estimated daily consumption

		Daily Intake		RDA [a]	
Age	Bread consumption/day	Protein in grams	Lysine in mg	Protein in grams	Lysine in mg
2 -5 yrs	3 slices (91g)	12.4	619	10 - 20	580 - 880
10 – 12 yrs	5 slices (150g)	20.4	1020	20 - 30	880 -1320
Adults	6 slices (183g)	24.5	1224	48	960

[a] RDA is the required daily allowances (FAO/WHO)

Table 6.8 shows the estimates of protein and lysine available in the formulated soy-wheat bread. It is clear that this bread would provide the requirements needed considering that people are likely to consume more than 100 grams of soy bread. The average mass of 1 slice of this composite bread is 30.5 grams. Therefore bread consumption of this bread would range from 3 to 6 slices (91 to 183 g) for children and adults respectively. From this consumption pattern, protein and lysine intakes would vary from those given in Table 6.7 (increase in adults and slightly decrease in children).

The results in Table 6.8 suggest that from the amount of bread consumed, 60-100% of safe intakes of protein and lysine can be met for all ages. Although protein intake for adults falls short (24.5g compared to 48g required), lysine requirements are met. Other sources of protein from legume/cereal meals during the day also complement the need for added protein.

6.4 Conclusions

The model developed in the preceding chapter was able to predict loaf volumes of soy-wheat dough. Loaf volumes correlated well with predicted enthalpies ($r^2 = 0.9$). Enthalpy adjusted values for water and L-AA were successfully used for the formulating soy-wheat bread with comparable loaf volumes to those of wheat bread.

Results (ANOVA) from affective sensory analysis revealed that the soy-wheat bread formulation using 30% soy flour (Sf30) was significantly more appealing in taste, flavour and colour than that from bread with 50% soy flour (Sf50) *($P<0.05$)*. However, there was no significant difference in texture between 30% and 50% soy flour breads *($P<0.05$)*. Results from descriptive sensory analysis revealed that soy-wheat breads from three selected formulations (Sf30a, Sf30b and Sf30c) were perceived as slightly beany in flavour, moderately moist and dense and generally perceived as acceptable. These results showed that soy-wheat bread, using at least 30% physically modified soy flour (PMSF#2) could be prepared with acceptable organoleptic properties (9/15 average acceptability score) , improved loaf volume (750 -760 cm^3) and crumb texture (14.7 to 16.3g /sec·/mm). Although loaf volume for the indicative optimal formulation (Sf30a) was large (820 cm^3), formulations (Sf30b and Sf30c) with smaller loaf volumes (750 cm^3 and 760 cm^3 respectively) were preferred on overall acceptance by panelists. Reasons for their preference to Sf30a could have been the lower moisture content and higher L-AA concentrations.

F-statistics (ANOVA) revealed that water significantly influenced the density of this soy-wheat bread *($P=0.05$, $r^2 = 0.65$)* while L-AA had non-significant effects on density. The effects of water and L-AA on average taste scores were both insignificant *($P <0.05$)* although observations from ANOVA plots revealed a slight decrease of average taste scores (8.7 to 8.4) from 70 to 90% water used for bread making, respectively, and an increase in average taste scores from 8 to 8.8 with L-AA up to

0.1% in the bread. There was a significant and positive influence of L-AA on masking the bean flavour with r^2 of 0.83 *($P<0.05$)*.

The current formula for soy-wheat bread was estimated to contain 13.6% (w/w) of protein (*N x 6.25*) and 0.68 % available lysine; (13.6 grams of protein and 680 mg of available lysine in 100 grams of this bread). A daily serving of 100 grams of this bread was estimated to provide approximately 25% of the estimated safe intakes (FAO/WHO) of protein requirements for adults and 60-100% of protein requirements for children; while the same amount of bread serving would provide at least 50% of the lysine requirements for children and 70 -100% for adults. However, considering the normal consumption pattern (100 to 200 grams) and the density of this bread, calculations revealed that children and adults would get between 60- 100% of lysine and protein requirements (FAO/WHO) from this bread depending on the amount of bread consumed and the weight of the individual.

Chapter 7

Feasibility studies and Recommendations

7. Table of contents

Page

7.1 Introduction

Socio-cultural feasibility was described by Sanginga et al. (1999) as one of the most important areas to be examined during social impact assessment, explaining that this assessment should be based on accurate understanding of the social and cultural organisation of productive activities. This process involves finding out how the intended beneficiaries have access to, make use of and control the natural resources available in their area. This section seeks to address the feasibility of the current work and findings in relation to the needs described in the first chapters and how this project could be implemented to satisfy the target groups described earlier.

As emphasised by Bressani (1985), Sanginga (1999) and Mojisola et al. (2005), as also recognised by the Food and Agricultural Organization (FAO), the need for inexpensive local resources in the production of popular foods, such as bread, is of prime importance in technology development for third-world countries. The composite flour programs whose objectives are to seek ways of substituting expensive wheat flour with starches and proteins from indigenous crops (cassava, sorghum, millet, soybean and other legumes) are already in place in Zimbabwe. These programs need technical and financial support in order to establish full sustainability.

7.2 Ingredients availability

The ingredients used in this formulation are all available locally except for wheat, which is imported. Composite breads made in these communities still require substantial amounts of wheat flour (>70% in most cases) to attain reasonable loaf volumes and hence implementation of wheatless bread still has limitations. Gluten substitutes, active surface emulsifiers, margarine are usually expensive for an ordinary village household. Because many nations develop their bread specialties based on their available agricultural sources (Mojisola et al., 2005), the formulation proposed on this project would be easily adaptable as it would involve special soy-wheat bread consisting of ingredients that are available from agricultural outputs and in local outlets. Soybeans are locally grown and most wheat flour is imported, although some communities produce wheat for use at the household level. Because of wheat importation costs, wheat is very costly for the ordinary people raising the need to substitute it at higher levels with indigenous crops in the preparation of soy-cereal foods.

The other ingredient which would have to be purchased from pharmacies is L-ascorbic acid. Research into the development of L-ascorbic acid from natural resources would be important for this work. However, villagers are used to the idea of purchasing ingredients for baking and cooking, with bicarbonate of soda, yeast, sugar and salt being some of the common ingredients usually afforded by villagers. L-ascorbic acid (vitamin C) required for this formulation is very little, only a pinch (< 0.25g / 100 grams of flour) and therefore will not be costly. This vitamin is available in most pharmacy outlets including rural outlets. The vitamin has been chosen as a bread improver because of availability, it is permitted as a food ingredient and mostly that it does not leave toxic substances in the product after cooking.

7.3 Preparation of soy flours

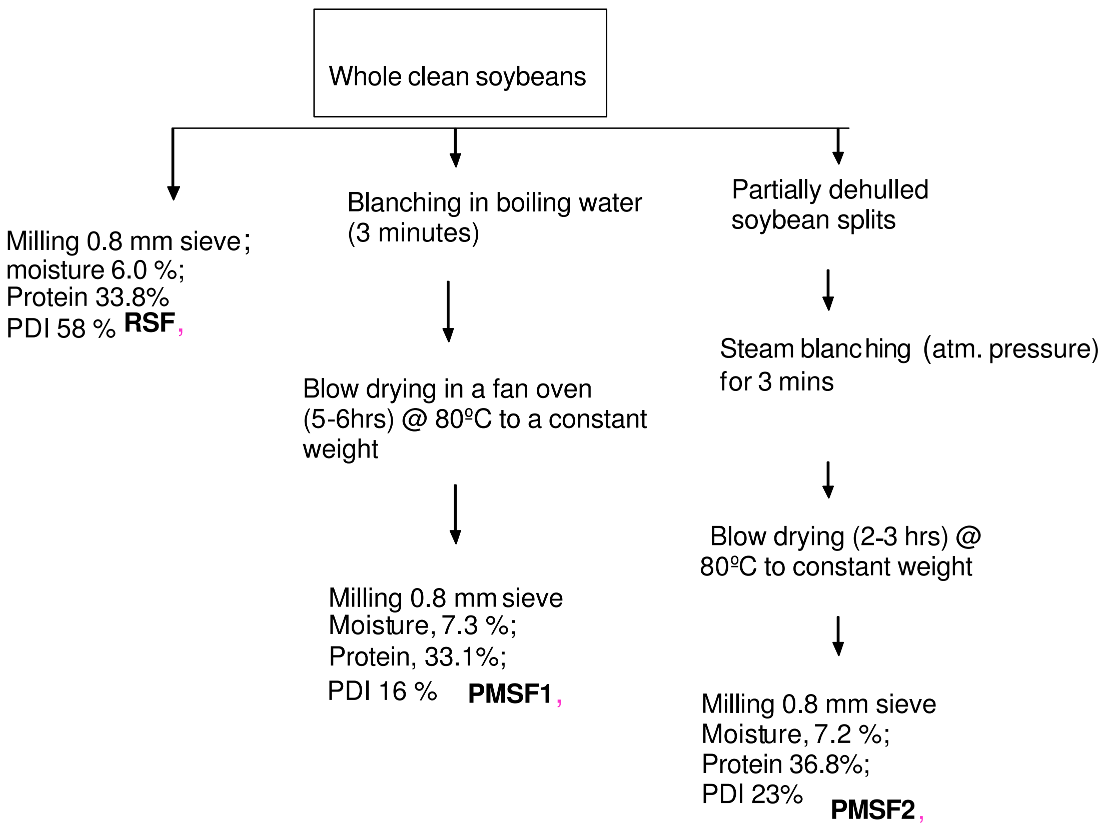
The flow chart for the preparation of soy flours at the village level appears in Figure 7.1. This process has been made simple to facilitate its use at a village and household level. It is also feasible for community groups who may want to start bakery projects at a small-scale. Preparation steps for raw soy flour and physically modified soy flour 1 is common procedure for most villagers as they use "boiled soy flour" to prepare some soy foods. The main difference between the traditional boiled soy flour and the one shown in Figure 7.1 is that in the latter method, optimum processing conditions of temperature and time are used and this is done to achieve lipoxygenase destruction whilst retaining nutrient and functional properties of the soy proteins. Drums for the provision of boiling water or steam blanching for soybeans for preparation of PMSF1 and 2 respectively are available to villagers.

Direct sun drying temperatures are usually between 80 to 90ºC and these conditions have been traditionally employed for drying all forms of vegetables and cereals (Kordylas, 1991). Because sun drying is usually successful if temperatures are high and humidity is low (Hillers, 2001), such conditions are similar to those found in most parts of Southern Africa. Solar dryers are also available to some people and these serve to speed the drying process since drying temperatures are higher.

Milling of flours can be done at local growth points where electric and diesel mechanical millers and dehullers are available. Different sizes of sieves are also available at these mills and their use determines the coarseness/ fineness of flour required. Because staple cereals are usually served as porridge or thick paste, grinding mills are available at growth points in both rural and urban communities.

Standardised procedures for boiling or blanching of beans, drying and baking without the use of thermometers, would have to be developed through calibration trials in order to develop appropriate guidelines for preparation of this flour including the baking procedure at village level.

Figure 7.1



Flow diagram for the preparation of soy flours; RSF is raw soy flour; PMSF1 and 2 is physically modified soy flours 1 and 2 respectively.

7.4 Baking equipment

Because of the wood fuel demand, clay stoves (usually made from clay and cement), (Figure 7.2 and 7.3) have been described as an efficient way to cook with biomass fuels including dung, crop residues, wood and charcoal. This has also been described as an ideal way to address a wide range of socio-economic and environmental goals in developing countries like Latin America, Asia and Africa (Kammen, 1995). This cooking and baking technology has been adapted widely by Zimbabwean rural communities, as well as the use of heavy clay pots or cast iron pots on firewood (Figure.7.4) which can also be used for successful baking. The adoption of these stoves has impacted on social, cultural, economic and environmental aspects including reduction of wood spent and time spent on wood collection, energy conservation, economic opportunities for rural and semi-urban communities, capacity building for women, reducing household smoke exposure and global atmospheric pollution (Kammen, 1995).

Other initiatives have also been made to improve on the cooking environment from interventions by the Biomass Energy Conservation (BEC) in Southern Africa. These include: "*chingwa stove*" meaning bread stove (Figure 7.3); "*tsotso stove"* (stove using small wood pieces). These are high mass brick and mud stoves that allow for multiple cooking. The stoves feature two to three potholes and one hot plate that keeps food warm (*chingwa stove*). They are fitted with a chimney and an oven for baking. The dissemination of these stoves gained momentum in the 1990s when NGOs (non-governmental organisations), Church organisations, Plan International and the Zimbabwe Women's Bureau took the stove project up in some of their activities in rural communities. The *chingwa* (bread) stove uses small firewood chips while the *tsotso* stove uses small pieces of firewood that would otherwise be left unused. Communities produce designs applicable in their own settings for their cooking practices. Apart from the widespread use of clay and mud stoves, some rural communities (very few) in Zimbabwe make use of electric stoves, thanks to the most recent rural electrification programs.

Bread quality could be improved if flour processing and baking was done in commercial bakeries or small-scale enterprises where equipment and processing conditions could be optimised and monitored efficiently. Since these processes are mainly carried out at the household and village levels, guidelines for optimum processing conditions would have to be developed using the available apparatus and equipment at the household level. Critical processes for guideline development will include blanching of beans and sun drying, as these determine the shelf life of the soy flour together with the baking procedure which all determines product quality. Training the villagers in the effective use of simple and available equipment including implementation of simple guidelines to the whole process of flour making and baking is feasible.

Figure 7.2

Figure 7.3

Figure 7.4

Figures 7.2 to 7.4. The baking equipment used in Southern African countries

Figure 7.2. The clay stove, Figure 7.3. High mass brick and mud stove, Figure 7.4. Cast-iron pots commonly used for baking in Southern Africa.

Pictures retrieved from Biomass Energy Conservation projects in Southern Africa.

7.5 Nutritional advantages of the bread

As described earlier, diets for most people in developing countries comprise mainly cereals and therefore soybean meal complements the amino acid deficient in common cereal grains (Bressani et al., 1982; Liu, 1997; Mojisola et al., 2005). These cereals need to be blended with soybean to give a combined protein that meets the FAO standards. Most importantly, soy-cereal combinations make better products in terms of protein quality as well as flavour and functionality, attributes that also enhance acceptability (Fleming and Sosulski, 1978; Yousseff et al., 1976; Sharma et al., 1999; Dhingra and Jood, 2002).

Estimated safe intakes or required daily allowances (FAO/WHO, 1985) for protein and amino-acids have been given in chapter 6, section 6.3.5. Estimates of protein and amino-acid intakes from 100-gram servings of the current soy-wheat bread formula have also been described in the concluding section. As stated earlier, the normal consumption of bread is much higher (100 to 200 grams) and because this bread is dense, it is possible that children and adults would get between 60- 100% of lysine and protein requirements (FAO/WHO) from this bread depending on the consumption and weight of the individual. These protein intakes are considered safe since other forms of protein in the diet are assumed to supplement protein needs as required (RDA).

In order to improve the protein intake, soy flour would need to be increased up to 50% in the bread although this would have negative effects on organoleptic properties. Because 30% soy-wheat bread was found acceptable during sensory evaluation tests, it is reasonable at this stage to advocate this formulation and enable people to utilise this than to encourage higher levels of soy flour that would discourage utilisation of soy flour in bread. Higher level soy flour in wheat bread formulations can always be implemented at later stages when the current formulation has been successfully adapted.

7.6 Product acceptance

Like in many countries, soybean-food acceptance and popularity in Zimbabwe has increased over the past few years due to the health benefits of soy foods. Because of the high production costs for wheat and maize and the prevailing economic and food crisis in Zimbabwe, production and processing of indigenous crops including soybean at the household level have taken a leading role in the lives of rural populations. Although bean flavour from soybean products has been of less importance compared to the food crisis in Zimbabwe, special recipes for various soybean products including soy-cereal parties, soy-vegetable dishes and soy-wheat/maize breads have been formulated to suit household needs at the village level. In most of these soy product recipes, soy bean flavours have usually been masked with the addition of other strong flavour-imparting ingredients like onions, chillies and spices. Therefore consumer perception for the soy-wheat bread formula is expected to be good.

In an experiment carried out in two rural communities in Zimbabwe during a pilot-processing project in 2001, soy-wheat bread (1:1 ratio) was made from soy flours milled from raw soybeans, blanched soybeans, fermented soybeans and toasted soybeans. The breads were subjected to sensory evaluation by 40 judges who were selected from 4 rural communities on the basis of their ability to scale different sensory attributes. Subject opinion was used to evaluate taste, texture and colour superiority of the breads, and products were ranked from 1- 4 in order of superiority for each sensory attribute (1 being best). The rank sum was used to evaluate superiority of products (Kramer, 1963). The results (Figure 7.5) revealed that blanched soy meal gave the most superior product, with the lowest rank sum (*P <0.05).* Bread made from fermented soy (highest rank sum) was considered an inferior product (*P <0.05*). These results showed the acceptance of blanched soybean meal as compared to other forms of soy meals.

Figure 7.5

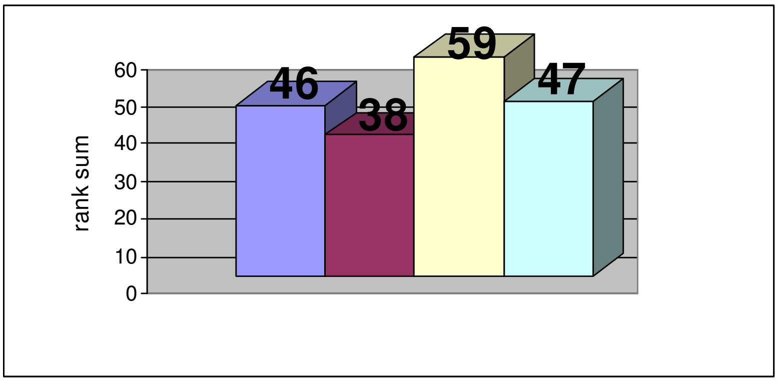


Figure 7.5 Sensory evaluation of soy-wheat bread during baseline surveys in a Zimbabwean village. Rank sum scores for soy–wheat breads (ratio 1:1) made from raw soy meal (46); blanched meal (38); fermented meal (59) and roast meal (47).

7.7. Project implementation and limitations

As reported by Sanginga et al. (1999), soybean adoption has had a positive impact on household income generation, material welfare, gender relations and social equity in Nigeria. A similar trend is taking place in Zimbabwean rural communities who have taken up soybean production and utilisation to a new level despite economic constraints in crop production. A summary of the constraints to the adoption of agro-processing technologies in Southern Africa include increased input costs for crop production, lack of production skills to produce adequate soybean for utilisation, lack of small scale processing equipment (Fellows and Hampton, 1992) and inadequate research infrastructure for product development. Several church organisations and NGOs (non-governmental organisations) funded by international organizations operate in Southern Africa on various projects aiming to generate agricultural technologies and improve productivity, small-scale farmer's welfare and household nutritional status in their continued efforts to alleviate economic stress on rural populations. Therefore, the establishment of pilot processing centres for this work could be achieved through financial and technical support from such organisations.

7.8. Recommendations

The demand for processed soy foods is huge, particularly in the communal and rural sectors of the country. The constraints outlined above are mostly the limitations to the utilisation of soybean. There are no cultural and social strings attached to this technology, it is widely accepted in the Southern African region. There is the need for government policy intervention, to lobby for adequate supplies of production inputs for small-scale farmers. External organisations and non-governmental organisations (NGOs) need more supportive policies from the government to work towards the achievement of this goal. Scientists, in particular food technologists, food and crop nutritionists, need to extend outreach programs for the promotion and production of quality food products which meet both sensory and nutritive demands.

There is the need for large-scale mechanisation for agricultural technologies and agro-processing, with local manufacturers in developing countries challenged to develop and test agro-processing technologies through demonstrations and the use of simple hand-operated tools, which are suitable for use at the village level, (rural ovens, mechanical grinders, oil and milk extractors for seeds).

Efficient mechanisms should be put in place to monitor and ensure that food-processing equipment complies with Food Safety regulations. This is important to avoid migration of components from the processing equipment to the processed foods. Several food-processing machines which are manufactured locally or imported in the developing countries are often made of potentially harmful metals which are capable of reacting with foods and forming toxic substances which may be imparted to the food product.

Comprehensive training workshops for use at farmer-training facilities should be developed to cater for agro-processing training in all crops including tubers, grain legumes and cereals and vegetables. This training should be extended to the improvement of nutrition knowledge for villagers and farmers. “Train-the-trainer” programs are also useful to encourage capacity building. Lastly, adoption and impact assessment surveys during project implementation should always be carried out to assess the impact of technologies adopted on the lives of small-scale farmers.

7.9 Significant contribution of this thesis

The significant contribution from this work is that the process of physical modification of soy flour was successful in modulating the physical and chemical properties of soy-wheat dough. The SE-HPLC technique demonstrated that the improved contribution to dough properties of physical modification of soy proteins was due to changes in the molecular size distribution of the soy proteins, which results indicated that a soy-wheat composite dough made from PMSF#2, forms a stronger dough with potentially better baking qualities. This process of physical modification of soy flour is considered to be of practical significance to developing countries.

Thermal properties (DSC water evaporation endotherms) of soy-wheat dough made from the modified soy flour were used to develop a mathematical model for the formulation of soy-wheat bread. Soy-wheat bread formulations using at least 30% physically modified soy flour (PMSF#2), with acceptable organoleptic properties and improved nutritional value were developed. These resultant breads offer an attractive and sustainable food that is nutritionally ideal.

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Appendices

Appendix 2.1 Structured questionnaire results (Baseline survey).

DIET	*MREWA*	*MHONDORO*
BREAKFAST MAIZE PORRIDGE	68% at least daily	62 % at least daily
WHEAT BREAD &TEA	45 % regularly	78 % regularly
SOYA BREAD & TEA	35 % average of 1 -2 times /week	1.7 % average 1—2 times/week
MAIZE BREAD & TEA	39.1 % regularly	19 % regularly
SOYA-MAIZE PORRIDGE	5 % occasionally	2 % occasionally
SWEET POTATO & PUMPKIN	43.5% seasonally	58.5% seasonally
BREAKFAST (LEGUMES)	15 %	9.4 %
MAIZE *SADZA**	Almost 100 %	Almost 100%
VEGETABLES	Almost 100 %	Almost 100 %
LUNCH MEAT; of which, CHICKEN BEEF FISH	56 % 38 % 15 % 3 %	68 % 37 % 25 % 6 %
LUNCH (LEGUMES)	44 %	51 %
LUNCH (*MAHEU*) (fermented millet brew)	53 %	47 %
LUNCH SEASONAL PRODUCE Corn, pumpkin, groundnuts etc.	44 %	41 %
LEGUME CONSUMPTION Relish form	74 %	72 %
REQUENCY FOR LEGUME CONSUMPTION –relish form	Once/wk 26 .0 % Twice/wk 22.8 % Thrice/wk 6.5 % Seldom 15.0 %	37.7 % 19.0 % 6.0 % 6.0 %
SUPPER MEAT	62 % occasionally	85 % occasionally
FRUIT CONSUMPTION	85 % ; seasonally	35 % ; seasonally

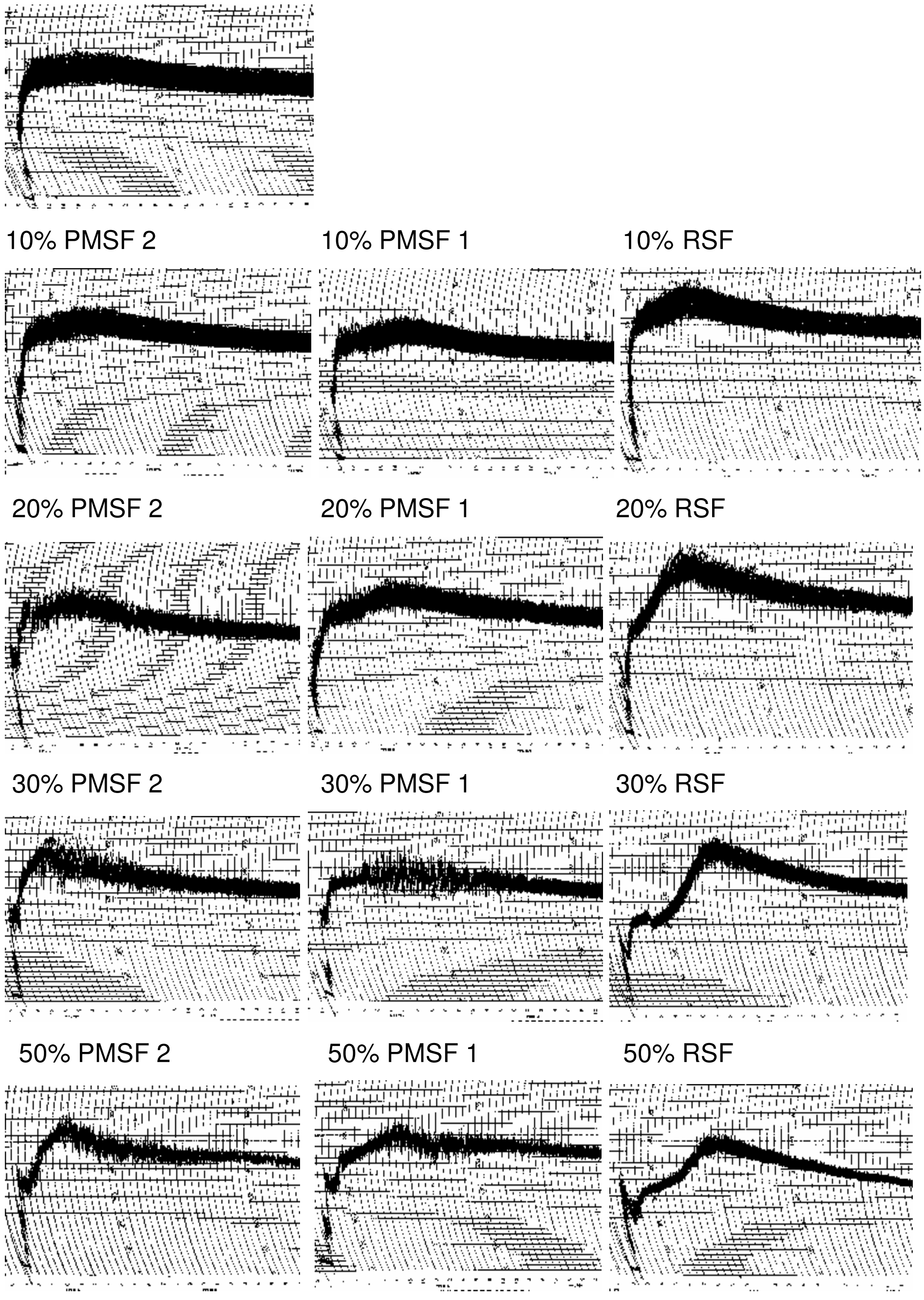
Results in Appendix 2.1 highlight the dietary pattern for respondents in 2 districts of Zimbabwe (2000). N (number of respondents) = 92 for Mrewa and 89 for Mhondoro communal lands.

Appendix 2.2

Pictures above show soy –wheat bakeries, soy-cereal patties and soybean-curd products taken during field days in Zimbabwe rural areas (2001)

Appendix 3.1 Typical farinographs of soy-wheat doughs (10-50% soy flour)

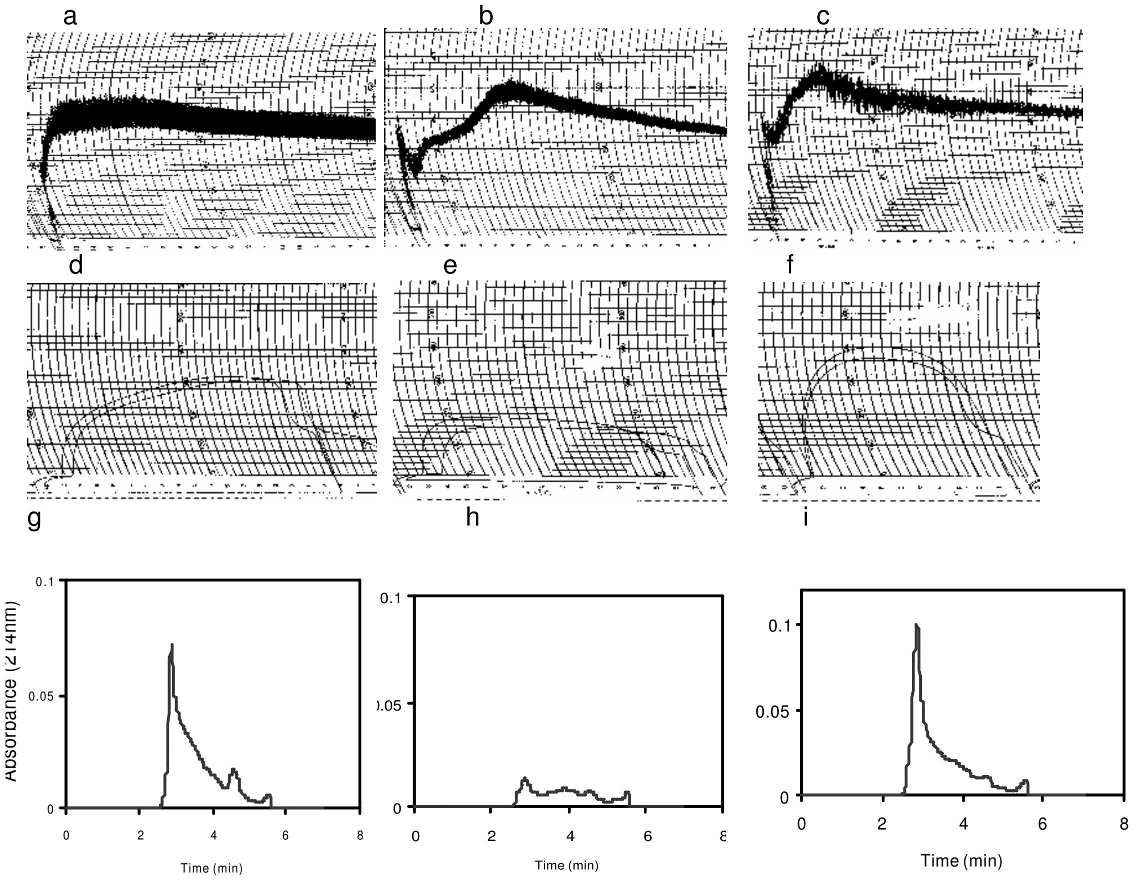
Reference (wheat dough)



Appendix 3.1. Typical Farinograph of soy-wheat dough from 10 to 50 % soy flour

First column (extreme left) shows PMSF2-W dough, 10 -50% soy flour; middle column shows PMSF1-W dough 10 to 50% soy flour; third column (extreme right) shows RSF-W dough from 10 to 50 % soy flour

Appendix 4.1



Appendix 4.1 Parts a-c show Farinograms. Parts d-f show Extensograms. Parts g-i show SE-HPLC profiles. Parts a, d, and g are wheat doughs. Parts b, e and h are wheat-RSF doughs. Parts c, f and i are wheat-PMSF#2 doughs.

The wheat-PMSF dough had better stability and resistance to extension (R_m) compared to RSF-W dough, although its extensibility (*E)* was still well below that of the wheat dough. SE-HPLC showed that the raw-soy-wheat dough had much of its protein of intermediate size distribution, 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 similar to that of the wheat dough. Because the polymeric protein fraction represents the major criteria for dough strength and baking quality (Fisichella et al, 2003; Grosch and Wieser, 1999), these observations are postulated to explain the improved dough-making properties of PMSF compared to RSF.

Appendix 4.2

Comparison of the major protein peak areas in soy - wheat dough during fermentation
Values given on this table are the means of duplicate analysis, standard error of the mean was within ± 3%.

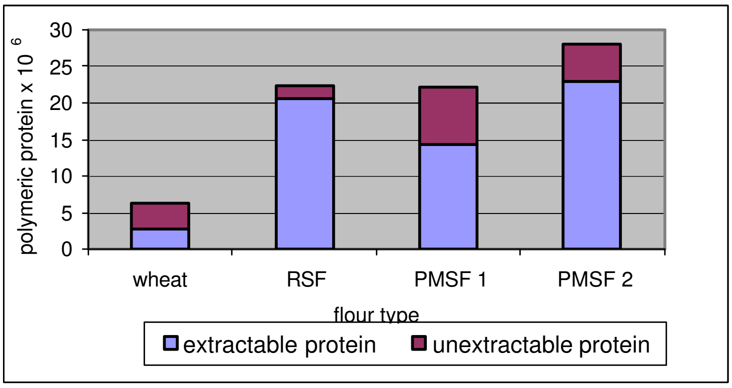
	Peak1 area; 2.90 min Polymeric protein		*Peak 2 area; 3.82min polymeric protein*		*Peak 3 area; 4.6min Monomeric protein*		*Peak 3.23min polymeric protein.*	
	EPP*	UPP*	EPP*	UPP*	EMP*	UMP*	EPP*	UPP*
wheat dough	3517789[a]	3512892[a]	Nil		6363461[a]	583336[a]	Nil	nil
	3693276[b]	2792745[b]			6373241[b]	610303[b]	Nil	nil
	3716986[c]	2382492[c]			6179579[c]	443674[c]	Nil	nil
wheat - RSF	3121836[a]	932969[a]	7768559[a]	944727[a]	5168270[a]	431492[a]	1982790 [a]	nil
	5074298[b]	373595[b]	8634938[b]	1006864[b]	5640483[b]	505015[b]	nil	nil
	4869622[c]	365246[c]	8757595	796771[c]	5643594[c]	407752[c]	nil	nil
wheat - PMSF1	4080811[a]	5074274[a]	5727631[a]	1022943[a]	4392596[a]	658876[a]	Nil	nil
	2491934[b]	4419489[b]	6132726[b]	1157884[b]	4431086[b]	704152[b]	1418369[b]	nil
	2239851[c]	5584636[c]	6587526[c]	nil	4516012[c]	525789[c]	1583922[c]	nil
wheat - PMSF2	5966073[a]	3356878[a]	6831309[a]	1124027[a]	4474502[a]	519387[a]	Nil	nil
	6148849[b]	2817593[b]	8055239[b]	1014415[b]	4732042 [b]	502485[b]	Nil	nil
	5713308 [c]	2859334[c]	8364951[c]	740993 [c]	4667754 [c]	376069 [c]	Nil	nil

[a] represent proteins at 0 minute dough fermentation; [b] represent proteins at 1 hour of dough fermentation; [c] represent protein at 2 h of dough fermentation.

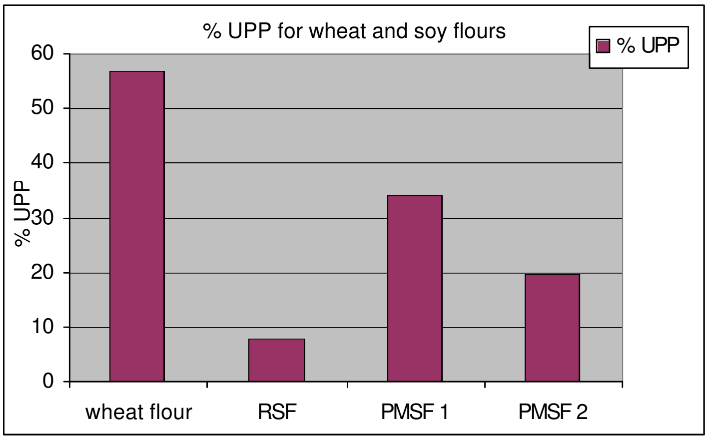
[*] EPP = Extractable polymeric protein; UPP = unextractable polymeric protein;
EMP = extractable monomeric protein; UMP = unextractable monomeric protein

Appendix 4.3

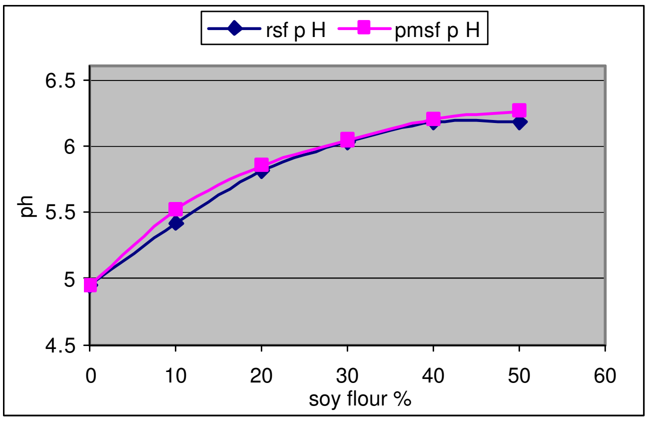
Comparison of extractable (EPP) and unextractable polymeric proteins (UPP) in soy and wheat flour



Percentage of unextractable polymeric protein (UPP) in soy and wheat flours

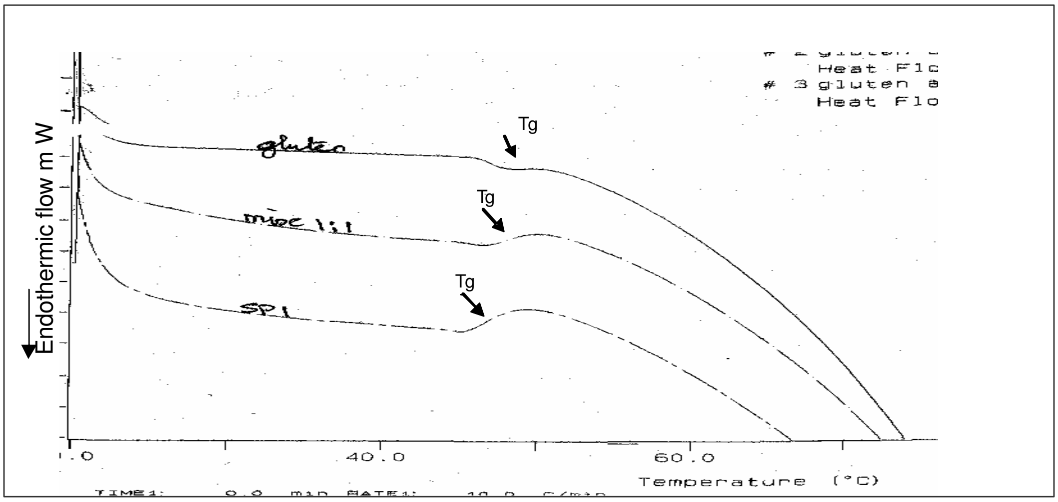


Effect of soy flour concentration on pH of soy-wheat dough



Appendix 5.1 Interaction of soy proteins and gluten using DSC

Glass transition (T_g) curves for gluten, soy protein isolate (spi) and a mixture of soy protein and gluten (1:1 ratio).



Glass transition (T_g) for a mixture of soy protein isolate [80% protein (N $\times$ 6.3) 8.1% moisture and 1.3% lipid] and commercial wheat gluten (approximately 80% protein, 7% fat and 6% moisture); mixed to 1:1 ratio with water to final moisture of 80% (mL water /100g protein).

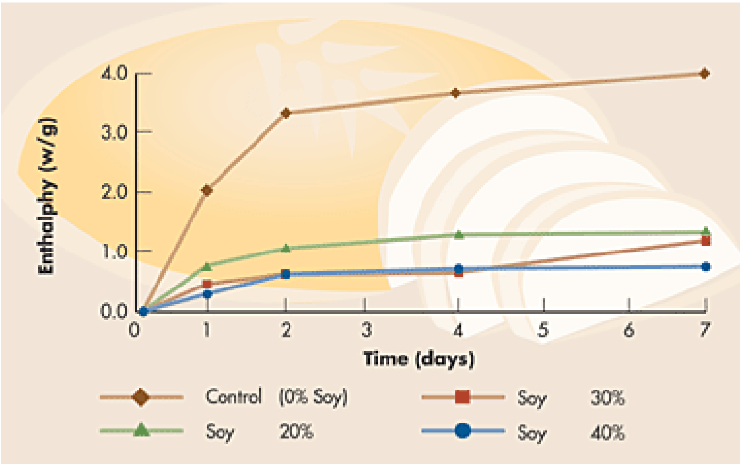
	T_o	T_c	T_g	delta C_p
gluten	46.3	46.1	46.2	0.02
spi	47.6	47	47.3	0.04
*glt + spi	48.8	49.6	49.1	0.04

*gluten and soy protein isolate mixture

Because biopolymer mixtures tend to be incompatible at low water content, 80% water was found suitable for this study to enhance compatibility of the two proteins (soy and gluten). Miscible polymer structures have a single T_g between the T_gs of the two components and broadening of the transition is observed as the components become more immiscible. An incompatible system will have two T_g s corresponding to the two components (Kalichevsky and Blanshard 1992). The results above showed that gluten and soy protein isolate appeared to be compatible showing one T_g at 49.1ºC, although this T_g did not fall between 46.2 and 47.3ºC. The transition did not show broadening as delta C_p (heat capacity) for the mixture was the same as that for soy protein [spi (0.04)].

Appendix 5.2

Rate of staling (enthalpy J/g) versus time in days for soy-wheat bread and control (wheat bread)



Source: Vittadini and Vodovotz, 2003

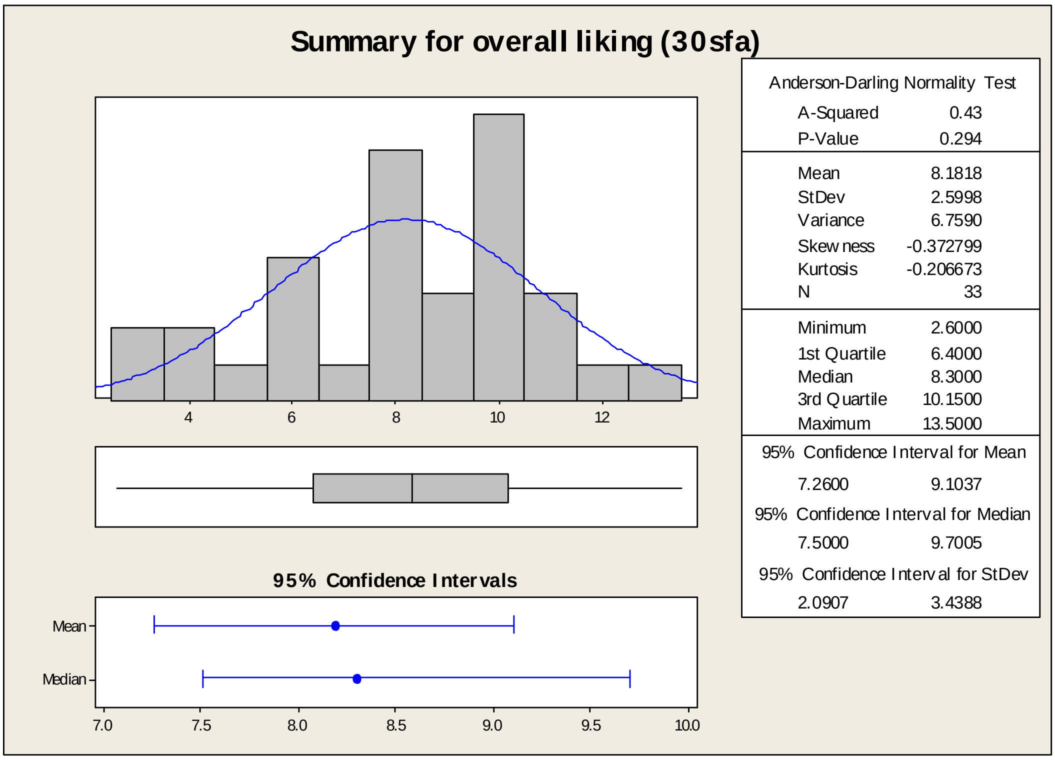
The rate of staling in wheat bread was quantified by the amount of starch molecule (amylopectin) recrystallisation, measured by DSC (differential scanning calorimetry). Soy flour decreases recrystallisation and retards staling in soy-wheat bread. Control bread without soy shows the highest rate of staling.

This figure serves to highlight that although soy flour decreases enthalpy on starch gelatinisation as well as shifting onset of gelatinisation during baking, soy flour has a positive role in modulating bread staling as shown from the figure above.

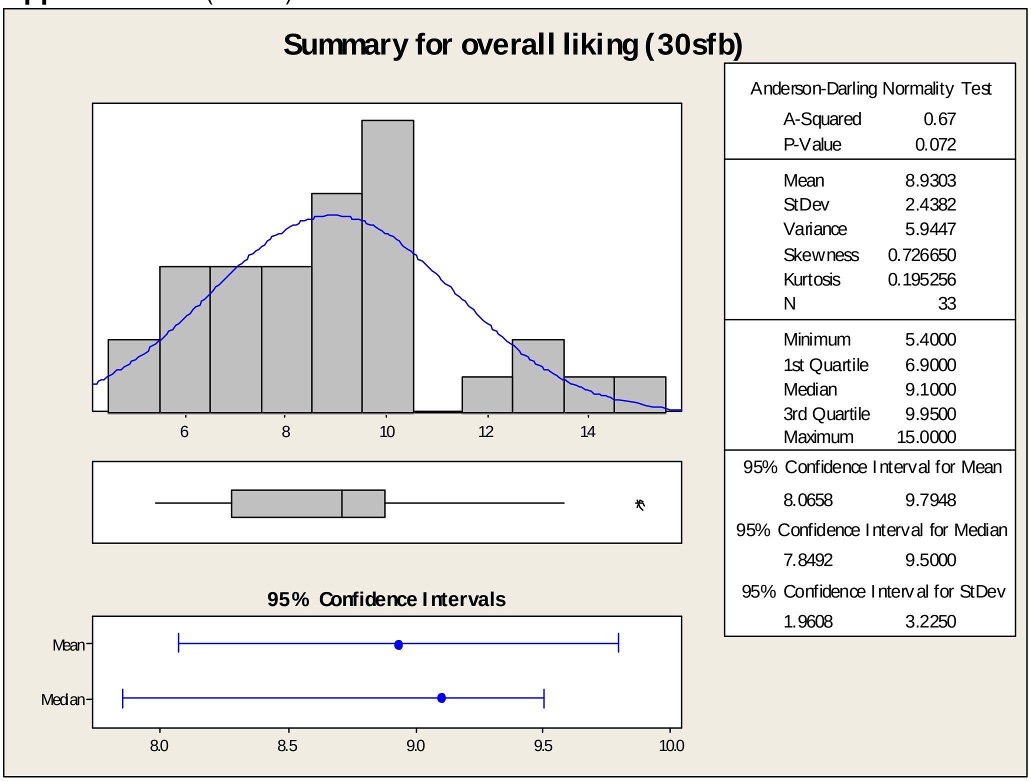
Appendix 5.3. Thermal properties of soy-wheat dough at various levels of water, soy flour and L- ascorbic acid Two thermal values given on same column show the first (smaller endotherm) and second (major endotherm) respectively. (Values are the mean of triplicate values; error was ± 3% for all values given)

		L-AA 0%			*L-AA 0.1%*			*L-AA 0.2 %*			*L-AA 0.3 %*		
Water	Soy flour%	T_o (ºC)	T_p (ºC)	? H (J/g)	T_o (ºC)	T_p (ºC)	? H (J/g)	T_o (ºC)	T_p (ºC)	? H (J/g)	T_o (ºC)	T_p (ºC)	? H (J/g)
70 %	0*	72; 77	76; 84	195	63	74	257	N/A	N/A	N/A	69	82	230
	10	64; 72	69; 84	8.6; 183	78; 91	84; 102	9.8 ;222	75; 86	82; 99	3.8 ;437	67; 76	74; 79	8; 143
	30	69; 74	74; 84	5 ; 180	70; 75	74; 87	1.0; 221	73;105	84;110	1.5;295	76	88	198
	50	72; 90	74; 97	3.6 ;110	74; 97	80; 102	8.8 ;147	72; 92	73; 97	1.7;274	67	80	122
80 %	0*	73: 78	80: 84	2.2: 289	87:96	87: 100	1.2: 409	N/A	N/A	N/A	69: 73	72: 81	3; 460
	10	74: 78	86: 85	3.1: 265	86;100	91;104	8 ; 384	76: 81	78: 95	2.8 :467	73: 77	76: 88	8; 446
	30	76; 82	80; 93	2.6 ;160	80: 90	90: 97	3.2: 226	68;91	74;99	2.4;263	75: 83	78: 91	6; 336
	50	76; 85	78; 99	3 ;201	76;89	80;98	2.0 ;260	80; 91	86;101	2.2;116	66; 75	68; 89	2.2;142
85 %	0*	75: 85	84: 87	2.2: 483	74;91	80;101	3 ; 417	N/A	N/A	N/A	90	104	545
	10	78: 84	81: 90	2.8: 351	78; 97	82;102	2.2 ;388	76; 98	84;104	3.8;421	76; 98	89;104	12;486
	30	70; 76	82; 92	3.8 ;238	76; 93	86;104	1.1;320	71; 90	80;102	11 ;208	76	83	233
	50	74: 80	82: 93	1.8: 150	74; 89	78; 98	4.1;263	85; 91	92;101	9 ; 165	76; 81	80; 98	6.4;275
90 %	10	67; 78	76; 94	2.4 ; 505	82; 99	86;104	10 ;480	69; 93	70; 97	3 ; 420	89	98	198
	30	79; 81	84;99	2.4 ; 323	85;100	98;103	9.5 ;332	74; 97	83; 102	2.1;248	75; 86	76; 91	4; 208
	50	52; 75	57;82	1; 214	56;100	57;104	9 ; 296	73; 101	79; 104	2.9; 260	84	86	225

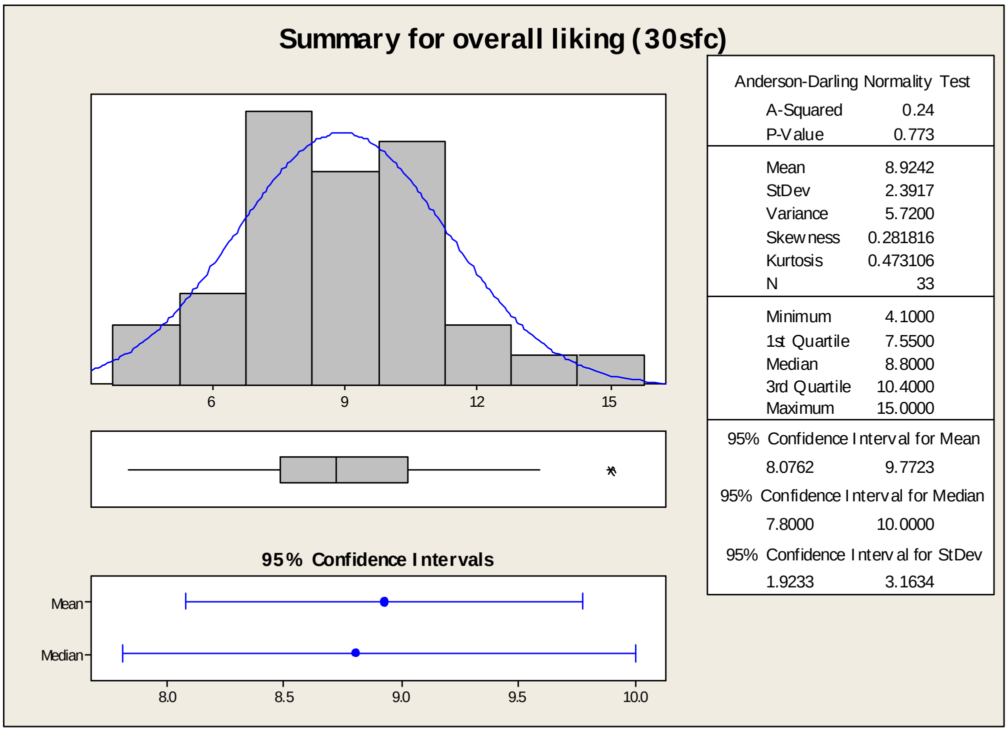
Appendix 6.1a Histogram charts, overall liking responses of soy-wheat bread (30sfa)



Appendix 6.1b (30sfb)



Appendix 6.1c Contd. (30sfc)



In order to characterise the location and variability of data sets, histograms were used. The histograms above (Appendix 6.1a, 6.1b and 6.1c) highlight frequency distribution charts for overall liking scores for Sf30a Sf30b and Sf30c soy-wheat bread formulations respectively. The histogram for Sf30c bread verifies symmetry as most scores fell under the normal distribution curve. This curve has a distinct peak near the mean. Data from this formulation are more likely to represent the truth from panelists. The histogram for Sf30a has a low and negative kurtosis (flat top near the mean), the negative kurtosis referring to the flat distribution of scores. While the histogram for Sf30b indicated that scores were more to the right side of the curve (right tail was heavier in skewness).

Appendix 6.2 Porous dough structure of soy-wheat bread

Soy-wheat bread (30% soy flour, 70% water, 0.2% L-AA used in dough)

Soy-wheat bread (30% soy flour, 80% water, 0.2% L-AA used in dough)

Soy-wheat bread (30% soy flour, 90% water 0.2% L-AA, used in dough)

Appendix 6.2 contd.

Optimum formula soy-wheat bread (30sfa) 30% soy flour, 90% water, 0.075% L-AA used

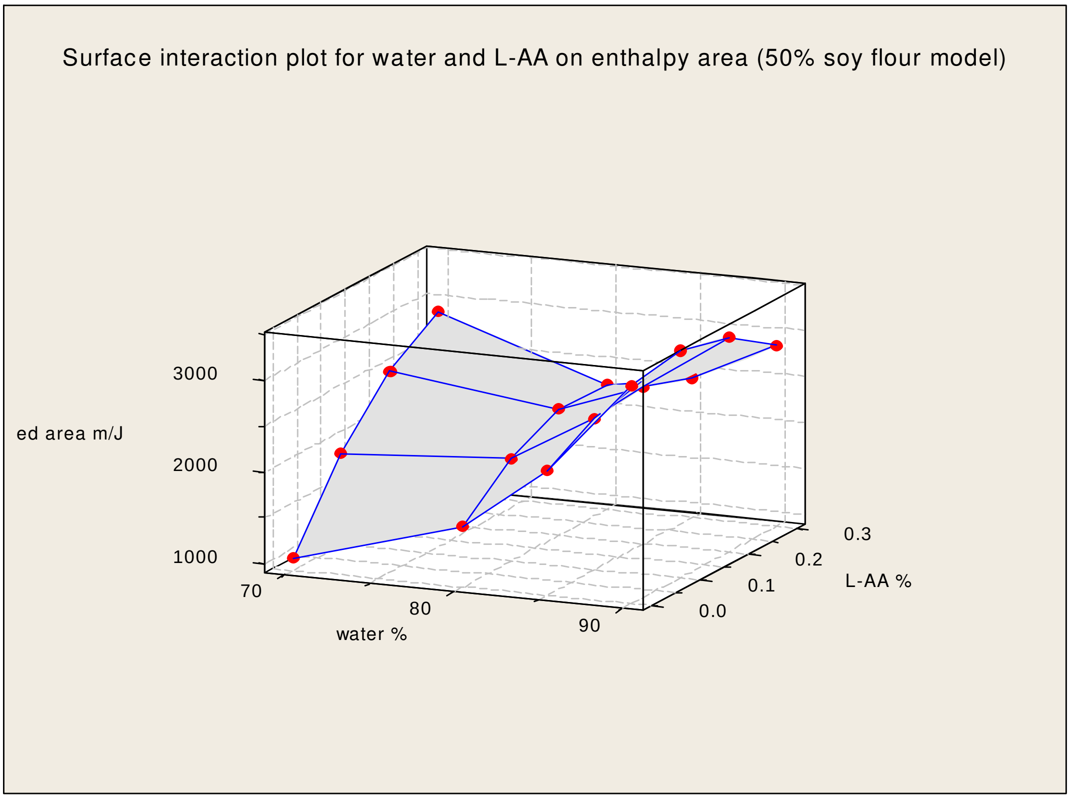
Ref; wheat bread, 68% water used in dough

The mode of release of water was reported to be critical to crumb porous structure (Kumar, 2002) adding that during baking, the more evenly the water is released to the starch, the finer and more even the porosity and the crumb texture of the bread would be. Porous structure of the soy-wheat breads was uneven suggesting that water was unevenly distributed and released to the starch during baking.

Appendix 6.3: Soy-wheat bread on cooling racks before objective analysis

Appendix 6.4

Surface plot for the interaction of L-AA and water on predicted evaporation enthalpy area of soy-wheat dough using a 50% soy flour model equation



Predicted enthalpy area (m J) versus L-AA and water percentages in a 50% soy flour model

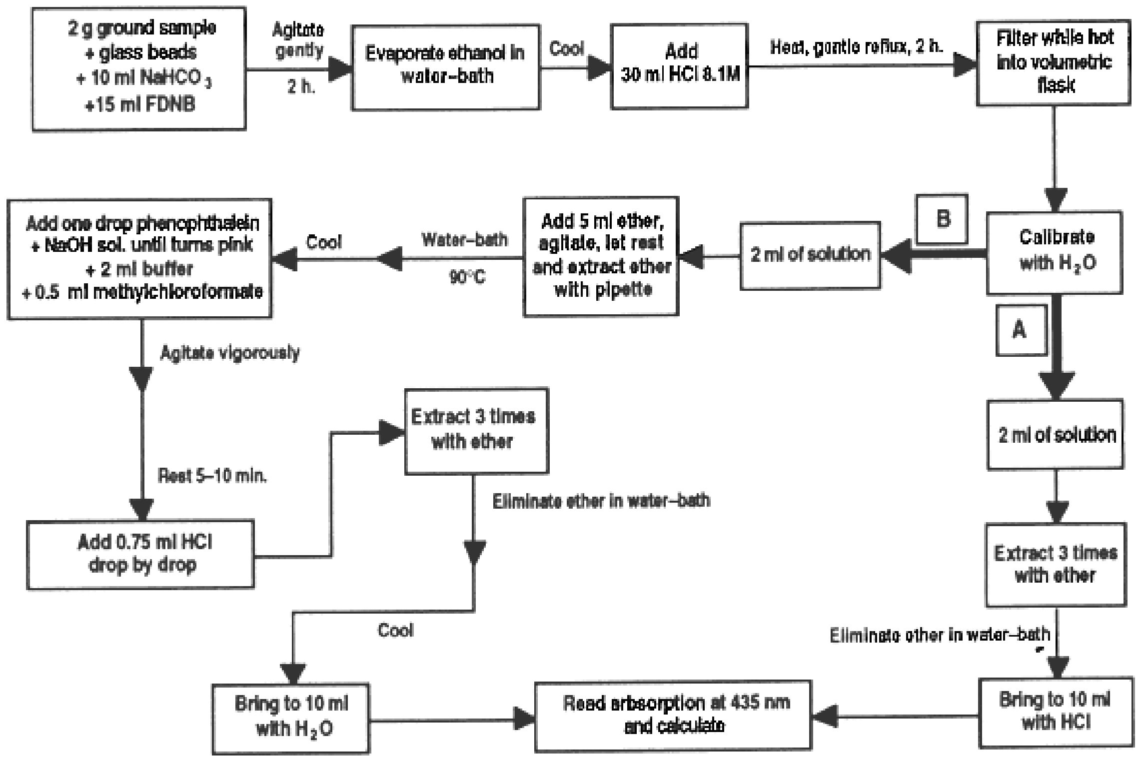
Response surface methodology (RSM) was used to determine levels of L-AA and water needed to attain highest enthalpies of evaporation in soy-wheat dough, 1:1.

These values were then used for the formulation of 50% soy flour bread used for Affective sensory analysis on this part of study.

Appendix 6.5 Table of results used to study (ANOVA) effects of L-AA and water on key organoleptic properties of soy-wheat bread (30% soy flour in bread for all samples). (Values for density are the mean of duplicate analyses and error was ± 3% of the mean; while bean and taste scores are average scores from 30 panelists).

Bread sample	Water %	L-AA %	Density g/cm^3	Average bean score	Average taste score
30sfa	90	.075	0.45	4.8	8.2
30sfb	70	0.27	0.48	5.1	8.9
30sfc	80	0.17	0.47	5.5	8.9
30sfd	70	0.1	0.50	4.5	9.0
30sfe	80	0.1	0.48	5.4	9.4
30sff	80	0.2	0.47	6.1	8.9
30sfg	80	0.3	0.50	5.9	10.0
30sfh	70	0	0.52	6.6	8.2
30sfi	70	0.2	0.50	5.0	8.8
30sfj	90	0.2	0.49	6.4	8.5
30sfk	90	0.3	0.47	5.9	9.1
30sfl	80	0.3	0.50	6.0	8.4
30sfm	80	0	0.47	6.5	7.9

Appendix 6.6 Method followed for the determination of available lysine in soy-wheat bread.



Carpenter's method for determination of available lysine (Tejada, 1985)