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PHYSICO-CHEMICAL, NUTRITIONAL AND FUNCTIONAL PROPERTIES OF DEFATTED MARAMA BEAN FLOUR

BY

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Submitted in partial fulfillment of the requirements for the degree

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DECLARATION

I declare that the dissertation herewith submitted for the degree MSc Food Science at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

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DEDICATION

God is love

To my loving parents Dintle & Galeboe Maruatona,

Lovely sisters Thobo & Botho,

Beautiful daughter Setho,

Relatives and friends.

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- Prof. A. Minnaar and Dr. K.G. Duodu for superb supervision and constructive criticism.
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- My family and friends for offering me support and words of encouragement during the course of this research.
- City of Tshwane for providing me with a fabulous social life.

ABSTRACT

Physico-chemical, nutritional and functional properties of defatted marama bean flours

by

Gaamangwe Nehemiah Maruatona

Supervisor: Prof. A. Minnaar
Co-supervisor: Dr. K.G. Duodu

Marama bean (*Tylosema esculentum* (Burch) A. Schreib) is an underutilised, drought-tolerant legume native to the drier parts of Botswana, Namibia and South Africa. The bean is comparable to soya beans in protein content and quality whereas its oil content is comparable to that of peanuts. By adding value to the marama bean through processing into protein-rich flours, its utilisation may be increased. Therefore, one of the objectives of this study was to adopt suitable low-cost processing technologies used for soya processing to produce protein-rich marama bean flours. The effect of dry heating of whole marama beans on lipoxygenase enzymes of its defatted flour was determined since oxidative rancidity catalysed mainly by lipoxygenase enzymes can reduce the shelf-life of the flour. The presence of trypsin inhibitors can affect the protein digestibility of the marama bean flour adversely. The effect of dry heating of whole marama beans on *in-vitro* protein digestibility and amino acid content of its defatted flour was determined. Lastly, the effect of dry heating of whole marama beans on the protein-related functional properties of the resultant defatted flour was determined.

The presence of lipoxygenase iso-enzymes (L-1 and L-2) activity in marama beans was determined by a visual and spectrophotometeric method using unheated soya beans as reference. Lipoxygenase iso-enzymes (L-1 and L-2) activity was not detected in marama beans. This may possibly suggest that these lipoxygenase iso-enzymes are absent or possibly inhibited in marama beans. In an attempt to optimise dry heating to inactivate trypsin inhibitors in marama beans, whole marama beans were dry heated at 100 ^{o}C, 120 ^{o}C and 150 ^{o}C, respectively for 20 min. Defatted flours prepared from the heated marama beans (HMF's) were analysed for their trypsin inhibitor activity using defatted flours from unheated marama beans (UMF) and soya beans (USF) as control and

reference samples, respectively. Trypsin inhibitor activity in UMF was almost four and half times higher than in USF. Dry heating of whole marama beans at 150 ^{o}C/20 min significantly reduced the trypsin inhibitor activity in its defatted flour to almost zero probably due to inactivation of the trypsin inhibitor.

The effect of dry heating of whole marama beans at 150 ^{o}C/20 min on the physico-chemical, nutritional and protein-related functional properties of defatted marama bean flour was determined. UMF was used as a control while USF and HSF were used as reference samples. HMF had higher protein content but lower fat content than UMF. It is suggested that dry heating disrupted the lipid bodies of the marama beans allowing more oil to be expelled during coarse milling of the flour. Heating significantly reduced the L* values of marama and soya bean flours possibly due to Maillard browning reactions. Heating significantly increased *in-vitro* protein digestibility of marama and soya bean flours probably due to protein denaturation and inactivation of trypsin inhibitors. Heating generally decreased the amino acid contents of marama and soya bean flours possibly due to chemical modification of the amino acids. UMF and HMF can potentially be used to improve protein quality in marama-cereal composite flours, porridges and breads.

Heating significantly decreased the nitrogen solubility index (NSI) and emulsifying capacity (EC) of marama and soya bean flours possibly due to protein denaturation and/or cross-linking. This may make HMF and HSF not suitable for applications in emulsion type meat products such as sausages because emulsion formation is critical during processing of sausages. Heating significantly decreased the foaming capacity of soya flour but did not have an effect on that of marama bean flour probably due to their high residual fat content which may have disrupted protein films during foam formation. UMF has a potential to be used in comminuted meat products because of its relatively high NSI, EC and OAC.

The laboratory process used in this study can be modified and adopted by SME's to produce defatted marama bean flours with potential applications in bakery and meat products and as a protein supplement in composite marama-cereal products.

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1 INTRODUCTION AND PROBLEM STATEMENT

The marama bean (*Tylosema esculentum* (Burch) A. Schreib)) is a long-lived perennial species endemic to the arid areas of Southern Africa, specifically in Botswana (around the Kgalagadi region), Namibia (Hartley, Tshamekang and Thomas, 2002) and smaller populations in the provinces of Limpopo, North-West and Gauteng in South Africa. According to Hartley *et al.* (2002), field observations confirmed that the species is heterostylous (style length differs between same species) and that fruit set and by implication seed set are very low in this species. Thus the low seed set suggests that this may be an adaptation of the species to an environment in which rainfall is scarce. This observation may explain the reason why this wild underutilised crop grows in areas which are characterised by low rainfall and poor soils, for example, the desert areas of Botswana and Namibia. The species, a member of the Caesalpinioideae subfamily of the Fabaceae, produces seeds commonly called *marama* beans which are edible (Hartley *et al.*, 2002).

The high protein and oil contents of marama beans highlight its potential socio-economic value to the indigenous populations of where it grows. Mmonatau (2005) reported that marama beans contain 37% protein. This compares favourably with the protein content of soya beans, which is about 40% (Snyder and Kwon, 1987). Ketshajwang, Holmack and Yeboah (1998) reported an oil content of 48.2% while Mmonatau (2005) reported an oil content of 39% for marama beans. This compares well with oilseeds such as groundnuts (45-55%), sunflower seeds (22-36%), soya beans (21%) and rapeseed (22-49%) (Salunkhe and Kadam, 1989).

However, no research has been done on value-addition through processing and commercialisation of marama bean as a food crop. This is probably because of the ready availability of other food crops from the Southern African region and so the bean remains an underutilised wild crop. Also, since marama beans are not commercially grown, the bean as a raw material at the moment is limited in its potential application. Rural communities, for example, the Basarwa people of the Kalahari and adjoining districts in Botswana roast marama beans and eat it as a snack. Thus, with its high protein and fat

contents, the bean has a potential to provide a good source of protein and energy in the rural areas and improve the food security and diversify livelihoods of the local population.

Mirrored against the soya bean because of the high protein and oil contents, protein-rich flour can be developed from marama beans by adopting and modifying processing methods used for manufacturing soya protein-rich flours. For example, defatted soya flour produced by extrusion-expelling process has a protein content of 65% which can be added to different food products to improve the protein quality and for functional purposes (Lusas and Riaz, 1995). Protein-rich soya flours are often used in food products such as sausages, breads, soups to make use of their functional properties such as emulsification, water absorption and adhesion that impart desirable characteristics to the product (Vaidehi and Kadam, 1989).

Although the extrusion process concept can be applied to marama bean flour, the cost of equipment, in particular the extruder, is expensive and would probably be unaffordable to small-medium enterprises (SME) in less industrialised countries. There is a need to develop appropriate low-cost processes or technologies that can permit production of marama bean flours by SME's in developing countries to promote direct consumption of marama flour as a supplement and/or as a functional ingredient in food systems by the food industry.

The objectives of this research are therefore firstly to adopt suitable low-cost technologies used for soya flour processing to produce protein-rich marama bean flours and secondly to characterise the marama bean flour in terms of physico-chemical and functional properties. The latter objective is necessary because the properties would ultimately determine the potential application(s) of the flour in food systems.

2 LITERATURE REVIEW

2.1 Introduction

The marama bean (*Tylosema esculentum* (Burch) A. Schreib) is a long-lived perennial and nutritious legume species adapted to the arid zones of Southern Africa as shown in Fig. 2.1.1 below, provided by South African National Biodiversity Institute (SANBI).

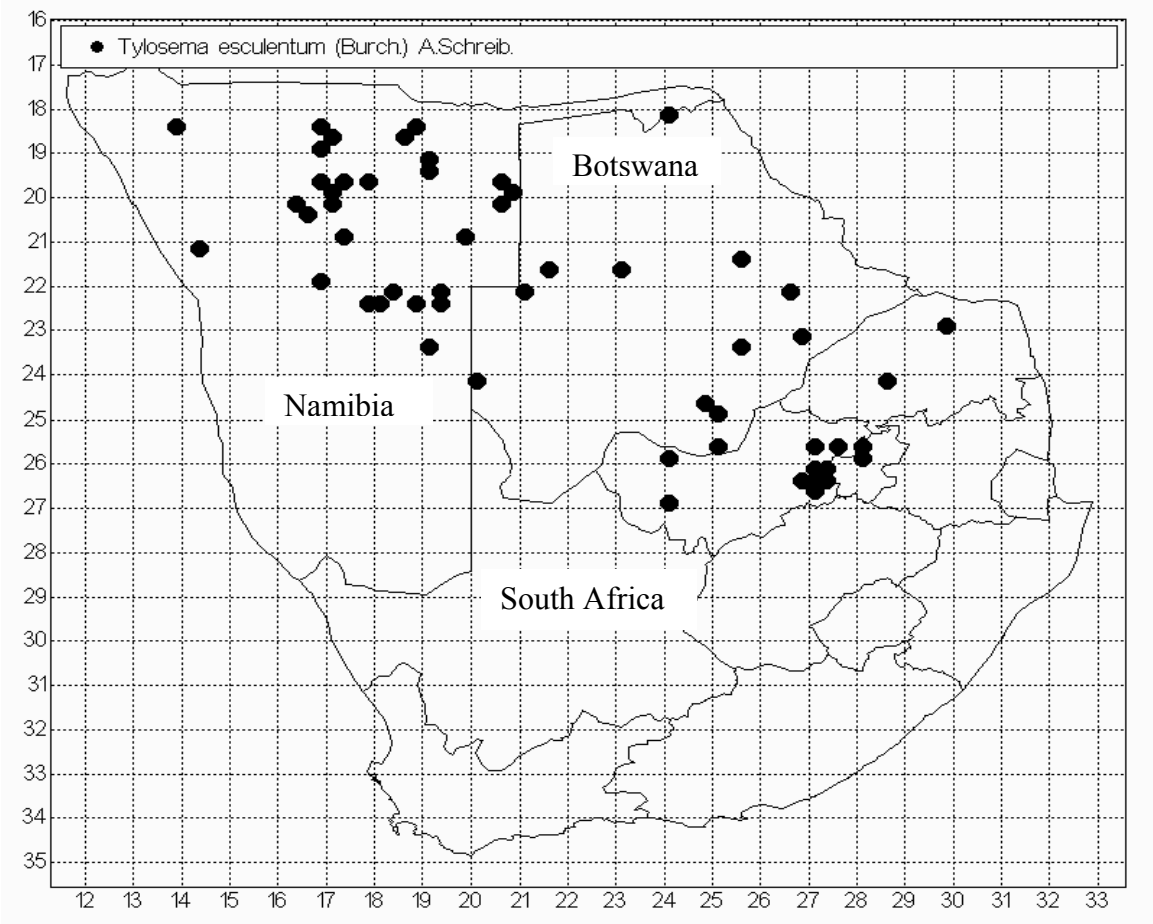


Figure 2.1.1: Geographical distribution of *Tylosema esculentum* (Burch) A.Schreib in Southern Africa (South African National Biodiversity Institute (SANBI), sa).

An updated geographical distribution of marama beans in South Africa (Gauteng Province and its periphery), which is mainly *Tylosema fassoglense* (Kotschy ex Schewinf.) Torre & Hillc is shown in Fig. 2.1.2.

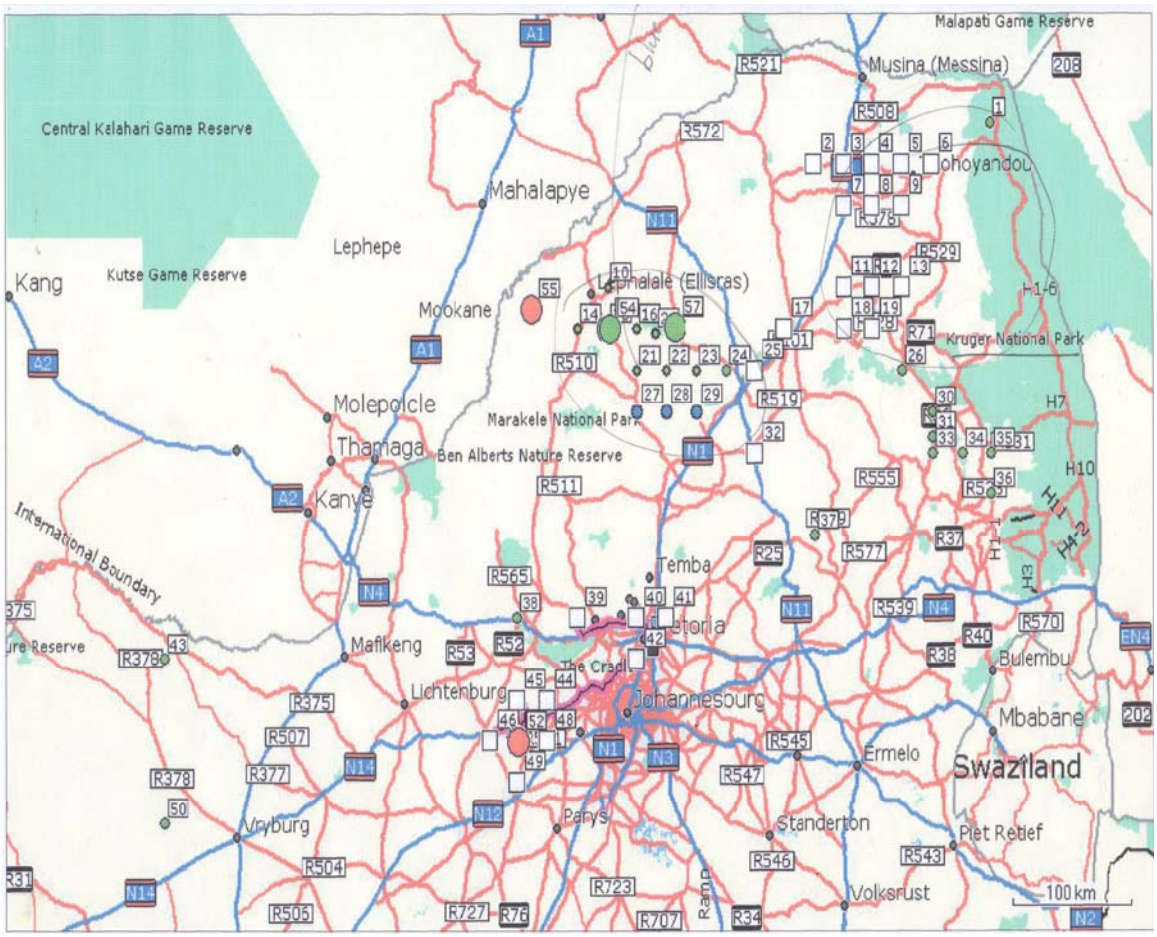


Figure 2.1.2 GPS spots of *Tylosema esculentum* (Burch) A.Schreib and *Tylosema fassoglense* (Kotschy ex Schewinf.) Torre & Hillc in South Africa around the Gauteng Province and its periphery (Personal communication - de Kock, 2008; Senior Lecturer, Department of Food Science, University of Pretoria, SA)

Key:

Red dots – *Tylosema esculentum* (Burch) A.Schreib

Green dots - *Tylosema fassoglense* (Kotschy ex Schewinf.) Torre & Hillc

Blue dots – Points visited but no plants found

Open squares – possible points not yet visited or no access available

The plant is a creeper and its seeds are contained in pods (Fig 2.1.3a) that open up when dry (Fig. 2.1.3b)

Figure 2.1.3a: Picture of a marama bean plant (*T. esculentum* (Burch) A. Schreib)

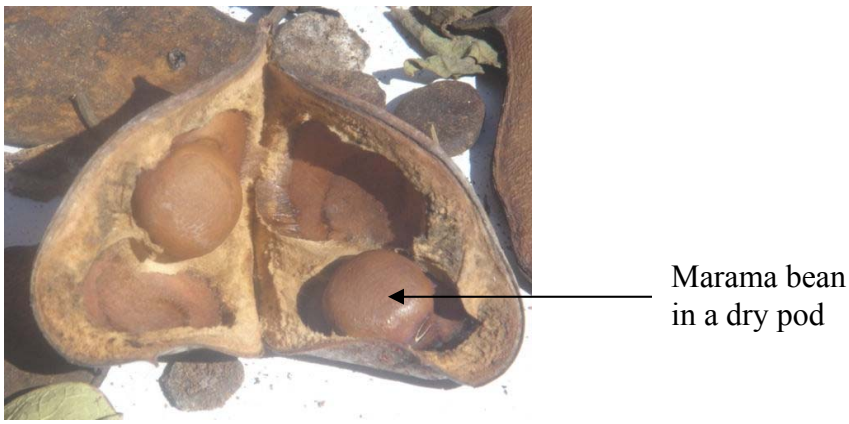


Figure 2.1.3b: Picture of a marama bean pods and dry seeds (*T. fassoglense* (Kotschy ex Schewinf.) Torre & Hillc

The beans, similar in protein and oil contents to that of soya beans and peanuts, respectively (Ketshajwang *et al*., 1998), are roasted and eaten as a snack by the local people where it grows. The harvesting of this wild nutritious crop is usually done by the local people around June/July each year. However, no figures have been recorded on the amount of marama bean that is harvested annually in areas where it grows. Of late, because of its high protein and oil content and other components that are known to have

potential health benefit, research in domestication, utilisation and chemical composition of the marama bean is being undertaken (Marama I and II EU projects).

2.2 Morphology of marama beans

The dry mature marama bean seeds are brown in colour and nearly spherical and disc shaped. However, the seeds vary considerably in size. The *Tylosema fassoglense* (Kotschy ex Schewinf.) Torre & Hillc species mostly found in South Africa has rather flat shaped seeds when compared with *Tylosema esculentum* (Burch) A. Schreib seeds which are spherical. According to Kadam, Deshpande and Jambhale (1989) morphologically, the seeds of leguminous plants are generally similar in structure. Thus, like soya beans, the marama bean consists of three major parts, namely the seed coat (hull), cotyledons and germ. The hard brown seed coat forms the outermost layer of the marama bean. It contains the easily identifiable area known as the hilum (seed scar) which is lighter in colour (Fig. 2.1.4). The marama bean is a dicotyledon, meaning that the seed has two cotyledons. The seed coat holds the two cotyledons together. Upon removal of the seed coat, the two cotyledons separate. The cotyledons are cream-white in colour.

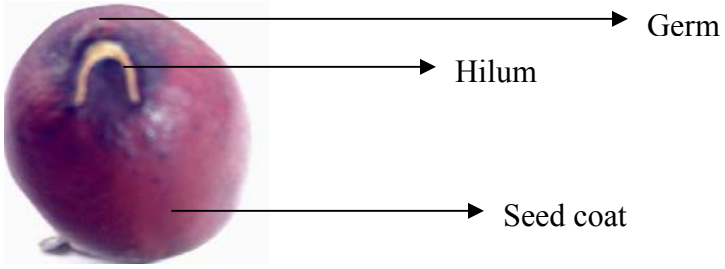


Figure 2.1.4: Picture of a whole marama bean (*T. esculentum* (Burch) A. Schreib)

The microstructure of marama bean cotyledons (Fig. 2.1.5) seems similar to that of peanut (*Arachis hypogaea* L. cv. Florigiant) cotyledons (Young and Schadel, 1991). After staining with Toluidine Blue, the protein bodies appear as large distinct bluish-purple oval bodies whereas the lipid bodies remain white and the cytoplasmic network and cell walls stain bluish-purple (van Zyl, 2007). Raw marama cotyledon stained with

iodine did not develop a blue colour indicating the probable absence of starch in marama beans (van Zyl, 2007a).

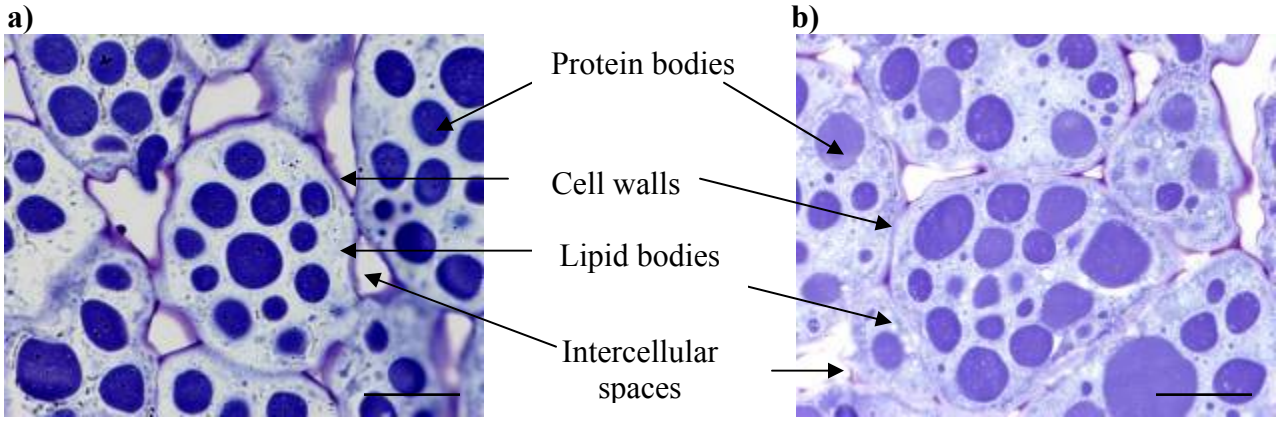


Figure 2.1.5: Light micrograph (LM) of cross section of raw cotyledon of (a) *T. esculentum* (Burch) A. Schreib and (b) *T. fassoglense* (Kotschy ex Schewinf.) Torre & Hillc (Bar = 10 µm) (van Zyl, 2007a)

2.3 Comparison of the chemical composition of the marama bean and soya bean

2.3.1 Protein composition

The protein content of marama beans ranges between 34 and 37% (dry basis) (Amarteifio and Moholo, 1998; Mmonatau, 2005) and is comparable to that of soya beans, which have been reported to be about 43% (Vaidehi and Kadam, 1989) (Table 2.1.1). Values of marama bean protein content reported vary probably due to environmental factors such as soil type and weather. Bower, Hertel, Oh and Storey (1988) found that globulins are the most abundant proteins (53%) followed by albumins (23.3%), prolamins (15.5%), alkali-soluble glutelins (7.7%) and acid-soluble glutelins (0.5%) in marama beans. On the other hand, soya bean proteins contain about 90% globulins and 10% albumins (Gueguen, 1983).

Most legume proteins, including soya bean protein, are particularly valuable because their amino acid composition complements that of cereals. Soya beans are limiting in the sulfur-containing amino acids cysteine and methionine, but contain sufficient lysine to overcome the lysine deficiency of cereals (Vaidehi and Kadam, 1989).

Soya bean protein-rich flour is normally blended with cereals to make high quality composite protein-rich flours (Friedman and Brandon, 2001).

As with soya bean protein, marama bean protein is also relatively high in lysine and limiting in methionine and cysteine (Table 2.1.2) and so has a potential to be used for the same application as soya bean protein-rich flour. In fact, the leucine, phenylalanine, threonine and valine contents of the marama bean protein meet or exceed the level recommended by the Food Agricultural Organisation (FAO) for a protein to be classified as a quality protein (Table 2.1.2).

Table 2.1.1: Comparison of proximate composition of marama beans and soya beans (g/100g) on dry basis

Nutrient	**Marama bean**		**Soya bean**
	Mmonatau (2005)	**Amarteifio & Moholo (1998)**	**Vaidehi & Kadam (1989)**
Crude protein	36.0-37.0	34.1	43.4
Crude fat	37.0-39.0	33.5	24.3
Carbohydrates	19.0	24.1	27.4
Ash	3.0-3.2	3.7	5.0

2.3.2 Fat composition

Amarteifio & Moholo (1998) and Mmonatau (2005) reported marama bean oil content of 33.5 and 39% respectively, which is much higher than that of soya beans (21.0%) but compares favourably with that of peanut (45.0-55.0%) as reported by Salunkhe and Kadam (1989). The cultivation of the bean should therefore be encouraged as it is a potential source of commercial vegetable oil. In terms of the fatty acid composition, the marama bean oil was reported to have palmitic (16:0), stearic (18:0), oleic (18:1n-9) and linoleic (18:2n-6) acids as the principal fatty acids. Oleic acid (47.6%) was the most abundant fatty acid followed by linoleic acid (26.4%) (Ketshajwang *et al*., 1998).

Table 2.1.2: Essential amino acid composition of marama beans and soya beans (g/100g protein)

Amino acid	Marama bean	Soya bean	FAO Reference Pattern
	Mmonatau (2005)	Vaidehi & Kadam (1989)	Snyder & Kwon (1987)
Isoleucine	3.43	4.6	6.4
Leucine	5.46	7.8	4.8
Lysine	4.06	6.4	4.2
Methionine	0.69	1.1	2.2
Cysteine	0.42	1.4	4.2
Phenylalanine	3.32	5.0	2.8
Threonine	3.39	3.9	2.8
Tryptophan	ND	1.4	1.4
Valine	4.30	4.6	4.2

ND No data reported

Interestingly, the oleic acid content (23.4%) and linoleic acid content (53.2%) of soya bean oil as reported by Gunstone (2002) are almost a half and twice that of marama bean oil respectively. This implies that marama bean oil and/or marama cake meal would probably be less susceptible to oxidation than soya bean oil and/or soya bean cake meal because it has less 1,4 penta-diene structure in linoleates. This structure has been reported by Nawar (1996) to be more susceptible to oxidation by a factor of about 20 than the propene of oleate because it has two double bonds in the cis form as opposed to the propene structure in oleate which has one double bond.

However, both oils contain appreciable amounts of mono and diunsaturated fatty acids and thus are susceptible to oxidation by lipoxygenases and autooxidation which may lead to oxidative rancidity and formation of undesirable off-flavours (Nawar, 1996). This may reduce the shelf-life of the soya bean or marama bean flours if defatting of the flour was not effective.

2.3.3 Anti-nutritional factors

Legumes are known to contain anti-nutritional factors such as protease inhibitors that limit the digestibility and reduce the nutritional quality of legume proteins (Chavan and Kadam, 1989). The major anti-nutritional factors in soya beans that have been studied include trypsin and chymotrypsin inhibitors. They are discussed below because their degree of inactivation to low levels is an indicator of effective heat treatment of soya beans. The presence of trypsin inhibitors in marama bean has been reported by Bower *et al.* (1988).

2.3.3.1 Trypsin inhibitors

The soya bean protease inhibitor is composed of two major fractions: those that have a molecular weight of 20,000 to 25,000 with relatively few disulphide bonds and specific toward trypsin (Kunitz inhibitor) and those that have a molecular weight of 6,000 to 10,000 with high proportion of disulphide bonds and are specific to both trypsin and chymotrypsin (Bowman-Kirk inhibitor) (Liener and Kakade, 1980). The inhibition of trypsin and chymotrypsin by soya bean trypsin inhibitor have been reported to depress growth in rats since they reduce digestibility of amino acids and increase the sulphur-containing amino acid requirement (Vaidehi and Kadam, 1989). This observation has since been extended to a variety of experimental monogastric animals. The mechanism of trypsin secretion by the pancreas (Fig. 2.1.6) has been postulated to be controlled by feedback inhibition which depends upon the level of trypsin and chymotrypsin present at any given time in the small intestine (Liener, 1981). If the trypsin is complexed with the trypsin inhibitor or dietary protein and the level of trypsin falls below a certain critical threshold value, the hormone cholecystokinin (CCK) induces the pancreas to produce more trypsin. This is believed to be the mechanism of the cause of pancreatic hypertrophy in rats due to trypsin inhibitor.

Fortunately, the soya protein inhibitors are inactivated by heat and thereby losing their ability to inhibit the proteolytic activity of trypsin (De Valle, 1981). Vaidehi and Kadam (1989) reported that the inhibitors were inactivated by blanching in boiling water for 5 min whole soya beans that were soaked overnight. As a food legume, marama beans

contain protease inhibitors. Bower *et al*. (1988) reported that the total marama bean protein contains about 20% trypsin inhibitor and dry heating the defatted meal at 140 °C for 30 min decreased it by 70%. Ripperger-Suhler (1983) reported that no trypsin inhibitor activity was observed in roasted marama beans. Both researchers reported high levels of trypsin inhibitor in marama beans compared to soya beans. However, in studies to compare the effectiveness of inhibition of trypsin by soya and marama bean trypsin inhibitor, the soya bean trypsin inhibitor exhibited the anticipated 1:1 (Inhibitor: Trypsin) molar ratio for complete inhibition. The marama bean inhibitor was less effective, exhibiting a 2:1 ratio of inhibitor to enzyme for complete inhibition (Elfant, Bryant and Starcher, 1985).

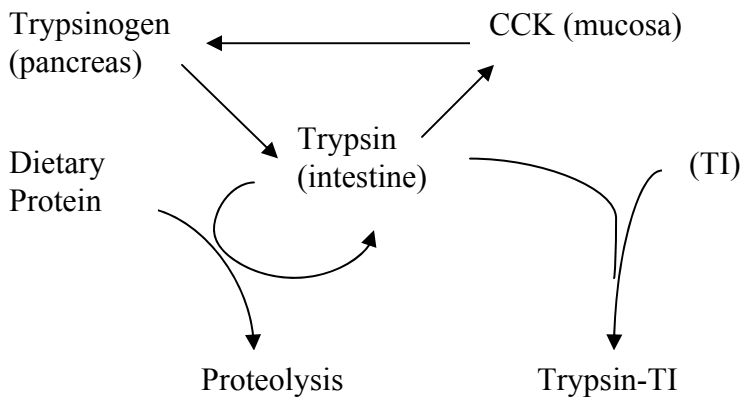


Figure 2.1.6 Regulation of the secretion of trypsin by the pancreas. CCK, cholecystokinin; TI, trypsin inhibitor (Liener, 1981)

2.4 Production of defatted protein-rich flours from leguminous oilseeds

This review concentrates on the production of defatted soya bean flour as the process used can be adopted and modified to produce defatted marama flour. Most of the literature that deals with processing of soya beans into flours is old because the technology was developed many years ago. The following processing flow diagram (Fig. 2.1.7) describes the process for manufacturing defatted soya flour. The process can be divided into four major steps: dehulling, oil extraction, heat treatment and milling.

2.4.1 Dehulling

The purpose of this operation is to separate the hulls of the beans from the cotyledons and to break the soya beans into smaller particles to prepare them for flaking. In soya bean processing, beans are cracked to coarse particles by cracking machines which consist of counter-rotating, corrugated rolls. This operation loosens the hulls and permits their separation by aspiration (Snyder and Kwon, 1987). After dehulling, the smaller bean particles are conditioned to the required moisture content by heating to 65 °C with indirect steam or direct steam injection to increase the plasticity of the bean particles in preparation for flaking (Snyder and Kwon, 1987). Flaking facilitates oil release in the screw press by decreasing the distance that the oil has to travel to reach the flake particle surface whereas in the solvent extraction process it facilitates the solvent penetration into the lipid bodies (Snyder and Kwon, 1987).

2.4.2 Oil extraction

In the soya bean industry, the most commonly used oil extraction processes are the screw-press (expeller) process and extraction by solvents (Berk, 1992). The purpose of this operation is to reduce the oil content of the soya beans so that the flour can have a higher protein content and longer shelf-life because it would be less susceptible to rancidity. The extraction of oil from oilseeds is usually preceded by a heat treatment step to enhance coalescence of the oil droplets and thus increase the oil yield. It has been found that extrusion cooking of coarsely ground whole soya beans at 10 to 14% moisture at 130 to 135 °C for 30 s produces press cake with about 6% oil in a single pass through the press (Nelson, Wijeratne, Yeh and Wei, 1987).

In the screw-press process, the material is usually maintained at about 60 °C, which is usually the set temperature of the screw press to facilitate oil extraction. The material to be pressed is fed between the screw and the barrel and propelled by the rotating screw in a direction parallel to the axis. As the pressure gradually increases, the oil is released and flows out of the press through the slots provided on the periphery of the barrel while the press cake continues to move in the direction of the shaft to be discharged at the other end of the machine (Berk, 1992).

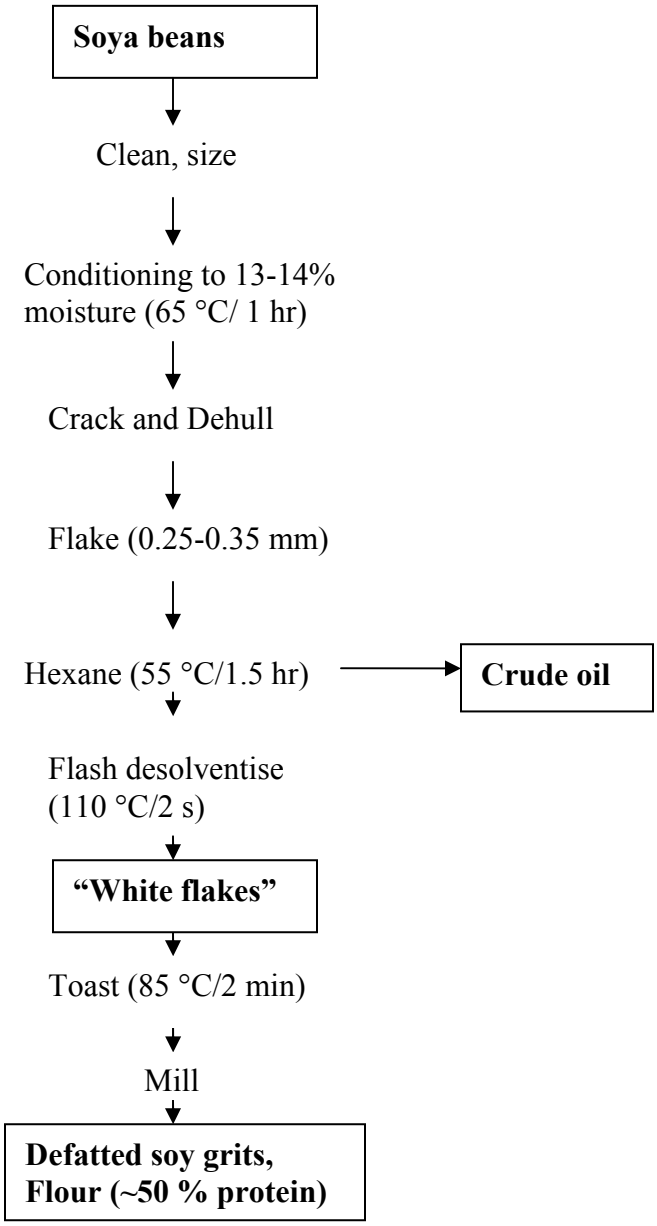


Figure 2.1.7: An example of scheme for industrial production of defatted soya flour ingredients using solvent extraction for defatting (Lusas and Riaz, 1995 - modified)

Although this process is simple and not expensive, the oil yield is generally low resulting in a press cake with a high oil content which may have a reduced shelf-life due to development of off-flavours. However, oil yield can be increased by recovering the residual oil using solvent extraction although this extra processing step might not be economical (Berk, 1992).

On the other hand, the solvent extraction process involves the extraction of oil from oilseeds, for example, soya beans by use of non-polar solvents like petroleum ether or hexane. The transfer of oil from the solid to the surrounding oil-solvent solution (miscella) may be divided into three steps, namely the diffusion of the solvent into the solid, dissolution of the oil droplets in the solvent and diffusion of the oil from the solid particle to the surrounding liquid (Snyder and Kwon, 1987). The defatted soya bean flakes are then fluidised in a stream of superheated solvent vapours for a short time (110 °C /2 s) (flash desolventising) to evaporate the solvent from the flakes and then rapidly cooled to minimise protein denaturation (Milligan, 1981; Snyder and Kwon, 1987).

2.4.3 Heat treatment

The purpose of this operation is to bring about several changes in physical, biochemical and nutritional qualities of the soya bean. Heat treatment of soya beans, which can be accomplished by either air drying the beans in an oven at 150 °C – 200 °C for a short period of time, toasting (cooking with steam at 85 °C/2 min) or by extrusion cooking (200 °C/5 s) deactivates protease inhibitors such as trypsin and chymotrypsin inhibitors which have a negative effect on digestion of proteins by humans (Liener, 1986). Quin, ter Elst, Bosch and van der Poel (1996) reported that the level of trypsin inhibitor activity (TIA; mg/g) in raw soya beans toasted at 120 °C for 10 min and 134 °C for 2 min was reduced from 23.4 mg/g to 1.70 mg/g and 1.83 mg/g respectively. At these temperature-time combinations, the level of lectin was also decreased to almost zero.

It appears that a certain amount of energy is required to achieve the inactivation of anti-nutritional factors, either by heat treatment at a high temperature for a short time or for a longer time at a lower temperature. This process, if properly controlled, also improves important functional properties such as water hydration capacity, foaming and emulsion capacity of protein-rich flours (Morr, 1990).

Heating also deactivates the lipoxygenase enzyme that catalyses the first step in the pathway leading to the formation of a number of off-flavour compounds in soya bean products that reduce the shelf-life of the products. Stephens, Watkins and Nielsen (1997)

found that defatted soya bean meal that was pre-heated at 85 °C did not develop a peroxide value (PV) above 10 when stored at 23 °C for 8 weeks. Buranasompob, Tang, Powers, Reyes, Clark and Swanson (2006) reported that heating walnut kernels at 60 °C for 10 min inactivated 81% of the initial lipoxygenase enzyme activity. These findings indicate that short time heat treatments are effective in inactivating lipoxygenase enzymes and thus extend the shelf-life of these oilseed products.

Heat treatment of marama beans, if properly controlled, also leads to development of acceptable flavour and colour which make them more palatable. Mmonatau (2005) conducted a descriptive sensory analysis of marama bean flour prepared from beans roasted at 120 °C/40 min, 150 °C/30 min, 150 °C/25 min and 150 °C/20 min using a trained panel of 10 people. The flour prepared from beans roasted at 150 °C/20 min was found to have a good sensory profile in terms of the descriptors used when compared to the other samples.

2.4.4 Milling

The purpose of this operation is to mill the press cake to meet the criteria of being classified as a flour. According to Berk (1992), soya flour is classified as flour if at least 97% of the product can pass through a U.S. 100-mesh standard screen (0.149 mm). Milling can be accomplished by using a conventional hammer mill or pin mill. However, to process flours with finer particle size, impact turbo mills or high-speed pin mills are used (Snyder and Kwon, 1987). The milling equipment used influences paste functionality and end product quality because milling affects particle-size distribution of the flour. A flour with coarser particles has less exposed surface area and moisture absorption is retarded hence a viscous paste is produced (Singh, Hung, Phillips, Chinnan and McWatters, 2004). In products such as akara and moin-moin, cowpea flours with a larger average particle size (0.84 mm), are required to make a quality paste as they are able to hold more water and have good foaming properties (Singh, Hung, Corredig, Phillips, Chinnan and McWatters, 2005). Soya grits are identical in composition to soya flours, the only difference is larger particle size of the grits (Johnson, 1970). As such soya grits are used in coarsely ground meats, cookies, crackers and specialty bread to enhance their nutritional and textural quality. In bread making, soya flour with fine particles is

preferred because it absorbs water at a much faster rate and thus increases the shelf-life of the bread by retarding moisture loss (Johnson, 1970).

2.5 Effects of processing on selected functional and physico-chemical properties of defatted protein-rich flours

Food processing of a raw material can modify proteins and other components which can change the functionality and nutritional quality of the final product. The term "functionality" as applied to food ingredients, is defined as "any property, aside from nutritional attributes, that influences an ingredient's usefulness in food" (Fennema, 1996). The ultimate success of using protein-rich flours in food formulations depends largely upon their functional attributes after their processing and how they interact with other ingredients in the final product.

According to Wolf (1970) the functional properties of soya flours are generally due to their proteins. However, flours contain other components such as water-soluble carbohydrates, fiber and lipids that may also contribute to the overall effect observed. Table 2.1.3 shows the functional properties of soya flour in food systems. It is suggested that marama bean protein-rich flours have the potential to be used in some of these food systems.

2.5.1 Protein-related functional and physico-chemical properties

According to Moure, Seneiro, Dominguez and Parajo (2006), the functional properties of food proteins can be classified into three main groups: (i) properties related to protein-water interactions (e.g. protein solubility, water hydration, viscosity, gelation, texturisation) (ii) properties related to protein-protein interactions (e.g. gelation and precipitation) and (iii) surface properties (e.g. emulsifying, foaming activities and surface tension). Selected functional properties due to protein-water interactions, protein-protein interactions and surface properties will be discussed below.

2.5.1.1 Protein solubility

Nitrogen solubility index (NSI) is one of the terms that can be used to describe protein solubility. It is usually the first property measured at each stage of preparation and processing of a protein ingredient due to its significant influence on the other functional properties of proteins. The NSI value is the percentage of total nitrogen in the sample that is soluble (Milligan, 1981).

Table 2.1.3: **Functional properties of soya flour in food systems (Wolf, 1970; Endres, 2001)**

Functional property	Food system
Emulsification	
Formation	Frankfurters, bologna, sausages
Stabilisation	Frankfurters, bologna, sausages, soups
Fat absorption	
Promotion	Frankfurters, bologna, sausages, meat patties
Prevention	Doughnuts, pancakes
Water absorption	
Uptake	Breads, cakes, macaroni, confections
Retention	Breads, cakes
Texture	
Viscosity	Soups, gravies

The NSI value indicates the extent of protein denaturation and hence the intensity of heat treatment which has been applied to the starting material. Thus the NSI method can be

used to measure the solubility of the proteins and thus determine its uses in food systems. The NSI measurement has been found to correlate with protein functionality; a decrease in NSI is generally accompanied by a decrease in functionality (Kinsella, 1979). Table 2.1.4 below shows the uses of soya flours of different NSI values.

Table 2.1.4: **Uses of soya flours of different nitrogen solubility index (NSI) values (Johnson, 1970; Endres, 2001)**

NSI (%)	Uses
>85	Enzyme bleaching of bread
50-60	Breads, cakes, sweet doughs, macaroni, doughnuts
25-35	Beverages, pancakes, waffles, gravies, soups, sausages
10-20	Crackers, cookies, infant foods

Soya flours with minimum heat treatments (NSI > 85%) show high lipoxygenase activity and are used at 0.5% to bleach wheat flour, improve mixing tolerance and to impart flavour to bread (Endres, 2001). According to Johnson (1970), soya flours with NSI between 50 and 60% are mostly used in breads, cakes, cookies, macaroni and doughnuts because they have milder soya flavour than soya flours with an NSI > 85% and improved water absorption capacity. Soya flours with NSI of 25-35 % are mostly used in beverages and soups because they have the least soya flavour since they received high heat treatments while those with an NSI of 10-20 % are used in crackers, cookies and natural grain breads to add colour and a nutty flavour (Johnson, 1970).

Protein solubility is an important attribute of proteins as the degree of their solubility influences their other functional properties such as water absorption, gelation, emulsification and foaming (Kinsella, 1979). Solubility of proteins is required if these functional properties are to be achieved in product formulations. Several factors are known to influence protein solubility, for example, pH, temperature, processing conditions and ionic strength. From the literature, marama bean protein appears to be comparable to soya bean protein in amino acid profile, suggesting possible similarity in solubility of these proteins. However, this may not be strictly true because the marama

and soya bean protein have different content of protein fractions which could influence their protein-related functional properties. Wolf (1970) found the maximum nitrogen solubility of defatted soya bean meal proteins to be at pH of about 6.5. NSI values also increased substantially at both ends of the pH scale whereas minimum solubility was found at pH 4 to 5, the isoelectric point (pI). Similar results were reported for peanut protein by Yu, Ahmedna and Goktepe (2007) as they observed minimum protein solubility at pH 4.5 and maximum solubility at pH 10. At the pI, protein-protein interaction increases because the electrostatic forces of the molecules are at a minimum and less water interacts with the protein molecules; the protein molecules aggregate and possibly precipitate (Shen, 1981). However, the situation is different at pH values above and below pI as a protein has a net negative or positive charge and thus more water interacts with the protein charges.

Hydrophobic interactions between proteins, which depend on the amino composition, also influence protein solubility. A low number of hydrophobic residues coupled with an elevated charge and the electrostatic repulsion and ionic hydration occurring at pH above or below the isoelectric pH increase the solubility of proteins (Moure *et al*., 2006).

Food processing operations such as heat treatment also influence solubility of proteins. Heating above 50 °C denatures proteins by breaking the non-covalent bonds, for example, hydrogen, hydrophobic and electrostatic bonds involved in stabilisation of secondary and tertiary structure. This leads to unfolding of the proteins and the exposed hydrophobic groups interact and reduce water binding as well as protein solubility.
Yu *et al* (2007) found that roasting of peanuts significantly decreased protein solubility in peanut flour from 32% to 12% at pH 7.0 when compared to raw peanut flour.

2.5.1.2 Water hydration capacity (WHC)

Water hydration describes the ability of a matrix of molecules, usually macromolecules, to entrap large amounts of water in a manner such that exudation is prevented (Fennema, 1996). It is a broad term that encompasses food properties such as water absorption, swelling, wettability, water holding capacity, cohesion, adhesion, dispersability and

viscosity all of which are related to progressive hydration of proteins. As most foods are hydrated solid systems (Cheftel, Cuq and Lorient, 1996), their sensory properties, for example, texture, viscosity and mouth-feel are influenced by the interaction of proteins and other constituents of the food with water.

Kuntz and Kauzmann (1974) stated that the amount of water associated with proteins is closely related with its amino acid profile and increases with the number of charged residues. Since hydrogen bonding of water can occur with polar groups such as hydroxyl, amino, carbonyl and sulphydryl groups, which are usually part of most proteins, water absorption of proteins varies with the number and type of polar groups present (Hutton and Campbell, 1981). The conformation, temperature, protein concentration, hydrophobicity, pH and ionic strength also affect water binding capacity of proteins. Conformational changes in the protein molecules can affect the nature and availability of the hydration sites; transition from globular to random coil conformation may expose previously buried amino acid side chains, thereby making them available to interact with water (Hutton and Campbell, 1981). It must be noted in products such as flours, that carbohydrates also have an effect on water absorption because of their hydrophilic nature (Hutton and Campbell, 1981).

The results reported by Johnson (1970) of a study to correlate NSI of soya flour and its water absorption capacity (WAC) revealed that as the NSI decreased from 85% to 70%, WAC increased from 270% to 385%. However, as the NSI further decreased to 55% and 15%, the WAC now decreased from 370% to 290%, respectively. Since NSI is temperature dependent, it was concluded that WAC of soya flour generally increases to a certain maximum point as the temperature is increased and then decreases as the temperature continues increasing. This trend in WAC is probably due to the fact that excessive heating denatures proteins, thereby exposing more hydrophobic sites leading to aggregation of proteins. Therefore, this may reduce the protein surface area and availability of polar amino acids for water binding; the net effect of which is a weakened hydrogen bonding (Fennema, 1996). Obatolu, Fasoyiro, Ogunsunmi (2007) reported a significant increase in the water absorption capacity from 131% to 167% of roasted yam beans flour when compared with raw flour. They attributed the increase in water

absorption capacity to the increase in the availability of the polar amino acids of the denatured proteins as well as gelatinization of starch. Other workers (Onimawo and Akpojovwo, 2006) reported increases from 4.5 g/g to 4.9 g/g in the water binding capacity of pigeon pea flour when toasted at 100 °C for 1 h.

Kinsella (1979) reported that the amount of bound water (unfreezable water) increased with protein concentrations of soya protein-rich flours, with soya isolate having the highest water binding capacity. Soya flour, soya concentrate and soya isolate preparations used had a water binding capacity of 0.24, 0.28 and 0.37 g/g solids, respectively. The increased water binding by the soya isolate compared to the soya flour and concentrate was attributed to the greater ease with which the isolate proteins swell, dissociate and unfold to expose additional binding sites. On the other hand, the carbohydrates (cellulose and hemicellulose) and other components of the flour and concentrate may have impaired an increase in water absorption because they poorly absorb water.

Changes in salt concentration can also affect the water absorption capacity of proteins as it leads to the unfolding of the compact structure of some proteins thus exposing and increasing the number of potential water binding sites. However, higher salt concentration led to dehydration of *Canavalia cathartica* and *Canavalia maritima* (wild legumes of India) flours and a decrease in water absorption capacity (Seena and Sridhar, 2005). This is possibly due to the competition of proteins and salt ions in binding water, which is usually also accompained by a decrease in solubility of proteins, a phenomenon known as the “salting-out” effect (Vojdani, 1996).

Other processing conditions such as fermentation and enzymatic hydrolysis have been reported to increase the water hydration capacity of protein flours. Yu *et al*., (2007) reported an increase in water hydration capacity (ml/g) of fermented defatted flour prepared from raw peanuts. This was attributed to the proteolytic activity of fungal enzymes which produced soluble oligo-peptides that absorbed more water. High water hydration capacity of proteins is a desirable functional property in food such as sausages, custards and dough because these products should imbibe water without dissolution of proteins to attain a viscous and thick body.

2.5.1.3 Oil absorption capacity (OAC)

The interaction of oil with proteins, which is usually by physical entrapment, is very important in food formulations because of its effect on the flavour, mouth-feel and texture of foods (Kinsella, 1976). According to Carvalho, Garcia and Amaya-Farfan (2006), the ability of a particular protein to interact with both water and oil indicates that they possess well balanced proportions of externally oriented hydrophilic and hydrophobic groups, and could thus be used as thickeners, viscosity and adherence enhancers in addition to flavour retention. Intrinsic factors affecting oil binding capacity of proteins include amino acid composition, protein conformation and surface polarity/hydrophobicity. Seena and Sridhar (2005) found that the oil absorption capacity of the Indian legume *Canavalia maritime* flour (1.53 ml/g) was higher than that of *Canavalia cathartica* flour (1.43 ml/g) and attributed the difference in OAC to the variation in the presence of non-polar side chains, which interact with the non-polar hydrocarbon portions of the oil.

Giami, Adindu, Akusu and Emelike (2000) as well as Onimawo and Akpojovwo (2006) reported improved fat absorption capacities for African breadfruit (*Treculia africana*) flour roasted at 160 °C /10 min and pigeon pea (*Cajanus cajan*) flour roasted at 100 °C/1 h when compared to the raw flours. They attributed this trend to heat dissociation of proteins and protein denaturation which occurred during roasting thus unmasking the non-polar residues from the interior of the protein molecules. A similar trend was observed for WHC. However, Yu *et al*., (2006) found that peanut flour roasted at 175 °C reduced both its water hydration capacity and oil absorption capacity. This is possibly because high temperatures exposed more hydrophobic sites causing aggregation of proteins thus decreased WHC and also irreversibly denatured the peanut protein and hence destroying both hydrophilic and hydrophobic groups the result of which is a reduced OAC. Based on the observations above, it appears that mild heating is positively correlated to WHC and OAC as opposed to severe heating.

2.5.1.4 Foaming properties

Food foams are dispersions of air cells in a continuous liquid (for example beer foam) or semi-solid phase (for example bread dough) that contains a soluble surfactant. There is a similarity between formation of foams and emulsions as both have a continuous and discontinuous phase and their stability is affected by protein surface activity (Moure *et al*. 2006).

According to Moure *et al*. (2006) good foam-forming proteins must: (i) rapidly adsorb during whipping and bubbling (ii) must unfold, concentrate and spread quickly to lower interfacial tension and (iii) form a continuous viscoelastic air permeable film around each gas bubble. These properties allow the particular protein to facilitate formation of stable oil-water and air-water interactions. Proteins are ideally more suited than small molecular weight surfactants (phospholipids, mono and diglycerides) to act as macromolecular surfactants in foam-type products because they function as a double barrel by lowering the interfacial tension and forming a continuous and highly viscous film at interfaces via complex intermolecular interactions (Cheftel *et al*., 1996). Foams are generally unstable because of very large interfacial areas and this is explained by the following three destabilising mechanisms: (i) drainage – the liquid lamella drains down due to gravity, pressure differences and/or evaporation leading to coalescence of the gas bubbles (ii) gas diffusion – small bubbles diffuse into the large bubbles leading to a disproportionation phenomena and very large unstable bubbles (iii) Rupture of liquid lamellae separating gas bubbles (Cheftel *et al*., 1996). Foaming is affected by temperature, protein concentration, pH, structural flexibility, surface hydrophobicity, solubility, lipids and processing conditions (Wilde and Clark, 1996; Adsule and Kadam, 1989; Kinsella, 1979).

Kinsella (1979) and Morr (1990) reported that heating of soya protein dispersions to 75 - 80 °C was optimum to obtain maximum foam expansion and foam stability. Mild heating possibly increases the tendency of the proteins to rearrange at the air/water interface and interact with each other by formation of electrostatic, hydrophobic, hydrogen and covalent bonds, thereby forming a thick, visco-elastic film that reduces air leakage and stabilises the foam (Wilde and Clark, 1996).

On the other hand, Obatolu *et al*., (2007) reported a foaming capacity value of 40.2% for raw yam bean flour which decreased to 4.9% after the flour was prepared from beans roasted at 120 °C. Similar decreases in foaming capacity were reported for toasted pigeon pea (*Cajanus cajan*) flour (Onimawo and Akpojovwo, 2006) and this could be attributed to protein cross-linking caused by the more severe heat treatment which decreased the solubility and flexibility of the proteins (Nakai, 1983).

Kinsella (1979) reported that foam expansion and stability of soya isolate was improved with concentrations up to 3 % (w/v). At this concentration, air leakage (% in 2 h) was reduced to almost zero possibly because the viscous colloidal solution favours foam formation. However, this was influenced by pH, with the maximum foam expansion and stability occurring at pH 2 and pH 9 while the minimum occurred between pH 4 and pH 6, the isoelectric point where soya protein has the least solubility. It appears that solubility of soya proteins is closely correlated with foaming while foam stability was related to denaturation (Cherry and McWatters, 1981). A precipitated protein is unable to diffuse through a solution, and thus it cannot be transported to the air/water interface. Conversely, a protein that is able to diffuse to the interface, penetrates, unfolds, re-organises and forms a thick visco-elastic film around the air bubble will promote foam stability.

The structural flexibility of the protein also affects the formation of protein-stabilised foams, that is, protein flexibility correlates positively with foamability (Wilde and Clark, 1996). Graham and Phillips (1979) conducted a study on the adsorption properties of three proteins with different conformations, namely bovine serum albumin (maleable globular), lysozyme (compact globular) and β-casein (random coil). They found that β-casein, a structurally disordered, flexible protein, adsorbed at the air-water interface and gave good foamability. On the contrary, because of their rigidity, the globular proteins (bovine serum albumin and lysozyme) were slow to unfold and expose hydrophobic regions at the air-water interface and as a result had poor foamability. However, foams formed with solutions containing globular proteins are more stable than those of flexible β-casein because intermolecular interactions of globulins are more rigid. Based on the above observation, soya flour is likely to have stable foams because 90% of its proteins

are globulins. It is suggested that the marama proteins would exhibit less stable foams compared to soya proteins because they contain a lower percentage of globular proteins (53 %).

In studies on the effect of soya protein preparations on foam formation and stability, soya isolate was found to exhibit superior foaming properties when compared to concentrates or flours. Soya isolate had a foam volume increase of 65% and 165% compared to soya concentrate and soya flour, respectively (Kinsella, 1979). The lower volume increase in soya concentrate and flour was attributed to the presence of lipids in these flours which destabilise the protein films and cause the foam to collapse.

2.5.1.5 Emulsifying properties

An emulsion is a system containing two immiscible liquid phases, one of which is dispersed in the other as tiny droplets. The phase in the form of droplets is called the internal or dispersed or discontinuous phase and the matrix in which the droplets are dispersed is called the external or continuous phase (Cheftel *et al*., 1996). Food emulsions can be of the oil-in-water or water-in-oil types. Emulsion of fats and water in the absence of an emulsifier are thermodynamically unstable due to the formation of small dispersed droplets which lead to an increase in interfacial area between the two liquids and thus the larger the surface area, the larger the surface tension at the interface (Adsule and Kadam, 1989). Droplets will tend to coalesce so that the surface area and therefore the surface tension can be reduced; this ultimately causes the emulsion to break.

Emulsifiers, such as proteins, help with the formation and stabilisation of emulsions by reducing the surface tension between the oil and aqueous phase. The proteins stabilise the emulsion by diffusing to the interface, where they form an interfacial membrane around the oil droplets, with the hydrophobic residues interacting with oil and hydrophilic residues with water, leading to a decrease in interfacial energy barrier and thus preventing coalescence of the oil droplets (Adsule and Kadam, 1989). However, the ability of proteins to unfold at interfaces and act as emulsifiers varies with the molecular properties of proteins such as their flexibility, solubility, concentration, conformational stability,

distribution of hydrophilic and hydrophobic residues in the primary structure as well as external factors (pH, ionic strength and temperature) (Cheftel *et al*., 1996).

According to Kinsella (1979), protein flexibility is one of the most important factors that determine the effectiveness of proteins as emulsifiers hence proteins with high molecular flexibility show high surface activity because they easily unfold to expose hydrophobic regions which enhance interfacial film formation. As proteins need to be in solution to act as emulsifiers, the solubility of proteins is important for emulsion properties because the higher the solubility of the proteins, the faster they are able to migrate to the interface and align to reduce the interfacial tension between water and oil (Hill, 1996). In the food industry, the hydrophilic: lipophilic balance (HLB) scale is used to select emulsifiers for emulsification of oil-in-water and water-in-oil emulsions. This is based on the ratio of polar and non-polar portions in each protein molecule. Emulsifiers with HLB values in the range 3-6 promote water-in-oil emulsions, whereas oil-in-water emulsions are formed with emulsifiers having HLB values between 8 and 18 (Lewis, 1990). Since soya proteins are mostly hydrophilic (Kinsella, 1979), they are likely to act as good emulsifiers in oil-in-water emulsions.

Although no values were provided, it has been reported that soya proteins progressively reduce interfacial tension as protein concentration is increased (Kinsella, 1979). This is because the higher the protein concentration, the more coverage the proteins provide at the interface. However, if the protein concentration is low, flocculation may occur possibly because the droplets will share protein molecules and reduce coverage at the interface (Hill, 1996).

Heating of proteins affects both their solubility and hydrophobicity and this may lead to changes in emulsifying properties (Nakai and Li-Chan, 1989). Mild denaturation of proteins by heating could improve their emulsifying properties due to increased hydrophobic surface, flexibility and solubility (Kilara and Sharkasi, 1986). However, if heating leads to a decrease in solubility of proteins, the emulsifying capacity of proteins can be reduced. Onimawo and Akpojovwo (2006) found that dry heating decreased the emulsion activity of pigeon pea flour from 80% to 50% after roasting at 100 °C in an

oven for 1 h. This may probably be explained by heat-induced aggregation of proteins which resulted in a decrease in the exposure of hydrophobic sites and a subsequent loss in solubility of the proteins (Nakai and Li-Chan, 1989). Varying pH and sodium chloride concentration has been reported to have an effect on emulsion capacity of proteins. Ramanatham, Ran and Urs (1978) observed that shifting the pH to levels above or below the iso-electric point improved emulsion capacity of peanut protein isolate in 0.1M or 0.2M NaCl because of improved protein solubility.

2.5.1.6 Colour

Colour is usually the major criterion used by consumers to evaluate quality. Colour has been described by Bloiun, Zarins and Cherry (1981) as a sensory property dependent on both physical and psychological factors related to the object, the conditions of observation, and the individual making the observation. Colour pigments (chlorophyll, carotenoids, flavonoids and other phenols) readily degrade during processing and storage of plant foodstuffs. This can have a huge impact on colour quality and may also affect nutritional properties (Fennema, 1996). Plant phenols have been singled out as the most important contributor to colour problems in products containing oilseed protein (Bloiun *et al*., 1981) because they are substrates for enzymatic browning reactions. Since marama bean contains phenols (van Zyl, 2007b), this problem is likely to occur in marama bean flour. Non-enzymatic browning reactions also occur upon thermal treatment and storage of foods containing reducing sugars and proteins, and depending on the conditions used, may produce brown colours that are desirable or undesirable in some foods (Bemiller and Whistler, 1996).

2.5.1.7 Protein quality

The nutritional quality of a legume protein is mostly determined by three different factors, namely: amino acid composition, amino acid digestibility or availability and the presence or absence of antinutritional factors (Liener, 1981). It is well known that of the 20 amino acids found in nature, 8 of them, namely isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine are termed ‘essential’ amino acids because they cannot be synthesised by the human body (De Valle, 1981).

Therefore, these amino acids must be obtained from the diet. It has been reported that the ratio or pattern of each amino acid requirement to another is more important than the absolute content of each essential amino acid in determining protein quality (De Valle, 1981). However, amino acid patterns alone are not sufficient to predict protein quality because soya beans and other legumes (such as marama beans) contain antinutritional factors such as protease inhibitors that lower their nutritional value (Liener, 1981; Friedman and Brandon, 2001).

The Food and Agricultural Organisation/World Health Organisation (FAO/WHO) has developed suggested human amino acid requirements for 1 year old, 2-5 years old, 10-12 years old and adults (Table 2.1.5) using beef as a reference pattern. This table can be used to determine the quality of a given protein by comparing the levels of amino acids of the test protein with corresponding levels in the reference pattern. The ratio of the level of the most limiting amino acid in the test protein to that of the corresponding amino acid in the reference pattern is multiplied by 100 to determine the "chemical score" of the protein (Del Valle, 1981). The "chemical score" of a protein is dependent on the amino acid composition and not on amino acid availability (digestibility) and thus may give false information about the quality of the protein (Hsu, Vavak, Satterlee and Miller, 1977; Del Valle, 1981).

The digestibility of a legume protein can be affected by heat treatment and protease inhibitors (De Valle, 1981; Friedman and Brandon, 2001). As widely reported in literature, heating unfolds proteins by destroying the helical regions of the protein and weakening the covalent bonds, thereby making it easier for digestive enzymes to hydrolyse the protein and thus improving the nutritional quality of the protein (Friedman and Brandon, 2001). It has been reported that mild heat treatment also inactivates trypsin inhibitor, and this is paralleled by the improvement in nutritive value of the protein. In experiments to determine the effect of heat treatment on trypsin inhibitory activity and protein efficiency ratio (PER) of soya bean meal fed to rats, it was found that the PER of soya bean meal heated at 100 °C increased from 1.40 to 2.63 when compared with the raw soya bean meal (Liener, 1981). This improvement in PER was attributed to the inactivation of the trypsin inhibitor.

Table 2.1.5: Essential amino acids reference patterns and patterns for beef and soya bean protein (Friedman and Brandon, 2001)

	FAO/WHO suggested amino acid requirements (mg/g protein)				Amino acid (mg/g protein)	
Amino acid	1 y old	2-5 y old	10-12 y old	Adult	Beef	Soya protein
Threonine	43	34	28	9	42.1	38.4
Cys + Met	42	25	22	17	32.7	68.1
Valine	55	35	25	13	45.4	49.1
Isoleucine	46	28	28	13	41.8	47.1
Leucine	93	66	44	19	77.5	85.1
Tyr + Phe	72	63	22	19	70.2	96.6
Histidine	26	19	19	16	32.0	25.4
Lysine	66	58	44	16	79.4	63.4
Tryptophan	17	11	9	5	9.9	11.4

Cys + Met: Cysteine + Methionine

Tyr + Phe: Tryptophan + Phenylalanine

However, it was observed that when purified trypsin inhibitor was added to the heated soya bean meal, the PER was reduced to 1.95, a value higher than the PER of raw soya bean meal. Thus, it was evident that heat treatment not only inactivates the trypsin inhibitor but also unfolds the proteins for easier access by digestive enzymes. The reduced PER of the heated soya bean meal with the added purified trypsin inhibitor was possibly due to the loss of endogenous sulphur-containing amino acids, which are abundant in pancreatic enzymes such as trypsin and chymotrypsin (Liener, 1981). These enzymes are secreted by the pancreas in response to lower levels of these enzymes in the rat body as they bind to the trypsin inhibitor, thereby draining the body of the essential amino acids. This scenario, coupled with the fact that soya bean protein is deficient in sulphur-containing amino acids, possibly led to the observed growth depression in rats.

Excessive heat treatment has been reported to have detrimental effect on the nutritive value of soya bean proteins (Westfall and Hauge, 1948; De Valle, 1981; Liener, 1981; Friedman and Brandon, 2001). The Maillard browning reactions of the ε-NH_2 group of lysine with carbonyl groups of reducing sugars produce fructosyl-lysine and, at high pH, cross-linking of lysine to form lysinoalanine and conversion of L- to D-lysine. All this reactions can lead to losses of lysine (Del Valle, 1981; Friedman and Brandon, 2001) and thus lower protein quality. This is possibly due to the fact the modified lysine is not hydrolysed by the pancreatic enzymes, thus the digestibility of proteins is reduced. Certain amino acids such as arginine, trytophan, histidine and serine have been found to be destroyed as result of excessive heating in the presence of carbohydrates (Bressani and Elias, 1974).

2.6 Gaps in knowledge

No research has been done on optimising post-harvest methods for dehulling and processing of marama bean into value-added products such as protein-rich defatted marama bean flours that can be used as functional ingredients in food systems. Since soya beans are comparable in chemical composition to marama beans, especially with regard to protein content and amino acid composition, processing methods that are used to manufacture defatted soya flour can be modified and adopted to develop low cost appropriate technologies that can be used by SME's to produce defatted marama bean flour. The various processing steps and parameters used from the time the bean is received until it is processed into flour can affect its quality. In soya bean processing, time, temperature and moisture content are critical factors in controlling heat denaturation of soya proteins (Milligan, 1981).

Denaturation of marama proteins due to heat treatment may potentially lead to modification of the functional properties of protein-rich flour as well as its protein quality. Denatured proteins have low solubility, and this can negatively affect other protein-related functional properties such as foaming, emulsification and water hydration capacity. However, heat treatment of proteins can also result in improved protein-related functional properties and protein quality due to unfolding of proteins, inactivation of antinutritional factors (trypsin inhibitors) and enzymes (lipoxygenase iso-enzymes). Little

is known regarding the effect of dry heating of whole marama beans on functional properties and protein quality of its protein-rich defatted marama flour. There is need for studies to develop low-cost processing technologies that can be adopted by SME's for processing of marama beans into value-added products such as protein-rich marama flours. Furthermore, the effect of critical steps in processing of these flours, in particular time/temperature combinations, need to be studied because they will determine their potential applications as protein supplements and functional ingredients in food systems. Research on value addition to the marama bean would stimulate the domestication and eventually, commercial cultivation of marama beans and enhance its utilisation.

2.7 Hypotheses

- Dry heating of whole marama beans will inactivate lipoxygenase enzymes and prevent oxidation of polyunsaturated fatty acids and consequently the production of conjugated unsaturated fatty acid hydroperoxides that cause development of off-flavours (Robinson, Zecai, Claire and Rod, 1995).

- Dry heating of whole marama beans will improve the protein digestibility of its defatted flour because it will inactivate anti-nutritional factors such as trypsin inhibitors. Furthermore, it will unfold proteins therefore making them more accessible to proteolytic enzymes (Liener, 1981). This may improve the digestibility of the proteins (Nielsen, 1991) provided that excessive cross-linking of proteins did not occur.

- Dry heating of whole marama beans will affect protein-related functionality of its defatted flour because of the partial denaturation of protein. This will possibly decrease the solubility of the protein and thus may subsequently decrease emulsifying capacity, foaming capacity and increase the water hydration capacity and oil absorption capacity of the defatted marama bean flour. The proposed decrease in solubility can be explained by the effect of heating which will increase surface hydrophobicity of protein due to unfolding of molecules upon

2.8 Objectives

- To determine the effect of dry heating of whole marama beans on lipoxygenase enzymes and trypsin inhibitor activity of its defatted flour.

- To determine the effect of dry heating of whole marama beans on *in-vitro* protein digestibility and amino acid content of its defatted flour.

- To determine the effect of dry heating of whole marama beans on the protein-related functional properties such as solubility, water/oil absorption capacity, emulsifying capacity and foaming capacity of its defatted flour.

3 RESEARCH

This research chapter is divided into two sections:

3.1 Optimisation of dry heating to inactivate lipoxygenase enzymes and trypsin inhibitors in marama beans (*Tylosema esculentum* (Burch) A. Schreib).

3.2 Effect of dry heating of whole marama beans (*Tylosema esculentum* (Burch) A. Schreib) on the physico-chemical, nutritional and functional properties of its defatted marama bean flour.

3.1 Optimisation of dry heating to inactivate lipoxygenase enzymes and trypsin inhibitors in marama beans (*Tylosema esculentum* (Burch) A. Schreib)

ABSTRACT

Marama bean is a wild underutilised legume whose potential usage as a protein-rich flour may be affected by the presence of lipoxygenase enzymes that can reduce the shelf-life of marama flour by catalysing oxidative rancidity resulting in the production of off-flavours. Also, marama bean as a legume contains trypsin inhibitors that can reduce the protein digestibility of defatted marama bean flour and hence its nutritive quality. Lipoxygenase iso-enzymes activity (L-1 and L-2) was not detected in marama beans because of the possible presence of inhibitor(s) or simply because it was absent in marama beans. However, there is possible presence of L-3 in marama beans since the test for it was inconclusive. Whole marama beans were dry heated at 100 °C, 120 °C and 150 °C, respectively for 20 min and analysed for their trypsin inhibitor activity using unheated soya beans as a reference. Defatted flour prepared from unheated marama beans was found to be four and half times higher in trypsin inhibitor activity than defatted flour prepared from unheated soya beans. This may probably be due to the fact that beans may develop a defence mechanism by increasing trypsin inhibitor activity to avoid being eaten by wild animals, insects and fungi. Dry heating significantly ($p \leq 0.05$) reduced the trypsin inhibitor activity in defatted marama bean flour from 250.8 TUI/mg flour in unheated samples to 3.3 TUI/mg flour in 150 °C dry heated samples probably due to irreversible denaturing of the trypsin inhibitor. Therefore, this temperature/time combination can be used for dry heating whole marama beans when processing marama protein-rich flour provided it does not negatively impact on the protein-related functional properties of the flour.

3.1.1 Introduction

Marama [*Tylosema esculentum* (Burch) A. Schreib] is a wild, perennial, underutilised legume indigenous to the Kalahari Desert region of Southern Africa (Monaghan and Halloran, 1996). The marama bean is high in protein (37%) and oil (39%) (Mmonatau, 2005), and is a source of protein and energy for the locals in areas where it grows. However, the utilisation of the marama bean is limited due to fact that it is not domesticated and commercialised and also little research has been done on the processing of marama bean into value-added products such as protein-rich flours.

The marama oil is composed largely of mono and di-unsaturated fatty acids (Ketshajwang *et al*., 1998) that are susceptible to oxidation by lipoxygenase enzymes and auto-oxidation which may lead to oxidative rancidity and loss of desirable flavours (Fennema, 1996). This may reduce the shelf-life of marama bean flour and/or lower nutritive value of marama beans or food products in which they are incorporated. Fortunately, the lipoxygenase enzymes are easily inactivated by heat. Buranasompob *et al*., (2006) reported that walnuts lost 81% of lipoxygenase enzyme activity after being dry heated at 60 °C.

Marama beans, like other legumes, contain trypsin inhibitors (Elfant, Bryant, Starcher, 1986), which are known to lower digestibility of protein by inhibiting the proteolytic activity of trypsin and thus reducing the nutritional quality of legume proteins (Leiner, 1986). However, heating of legume proteins has been reported to improve the nutritional quality of the proteins (DiPietro and Leiner, 1989). This improved performance is partly attributed to inactivation of anti-nutritional factors such as trypsin inhibitors (Liener, 1981). Dry heating (roasting), can be used as one of the methods to inactivate trypsin inhibitors. This process involves the application of dry heat to legume seeds using a hot pan or roaster at a temperature of 150 to 200 °C for a short time (Iyer, Kadam and Salunkhe, 1989).

Various factors may affect the effectiveness of the dry heating in terms of reducing the trypsin inhibitor activity of legumes. One of the most important factors that is considered

during processing of legumes to reduce trypsin inhibitor activity is the time and temperature combinations used during the heat treatment step. Bower *et al*., (1988) reported that dry heating of defatted marama meal at 140 °C for 30 min decreased the trypsin inhibitor activity of the meal by 70%.

The objective of this research was therefore to optimise the dry heating process to inactivate lipoxygenase enzymes and trypsin inhibitors in whole marama beans during the preparation of defatted marama bean flour.

3.1.2 Materials and methods

Marama beans (*Tylosema esculentum* (Burch) A. Schreib) that were harvested in June 2006 from the Kalahari Desert, Gantsi in Botswana were obtained from National Food Technology Research Centre, Botswana. The beans were sorted by removing chaff and shrivelled beans and then washed with water before being dried with a dry cloth. The cleaned beans were then packed in polypropylene bags and stored at 5 °C until the time of use.

The experimental design (Fig. 3.1.1) below was used for optimisation of dry heating processing to inactivate lipoxygenase enzymes and trypsin inhibitors in whole marama beans.

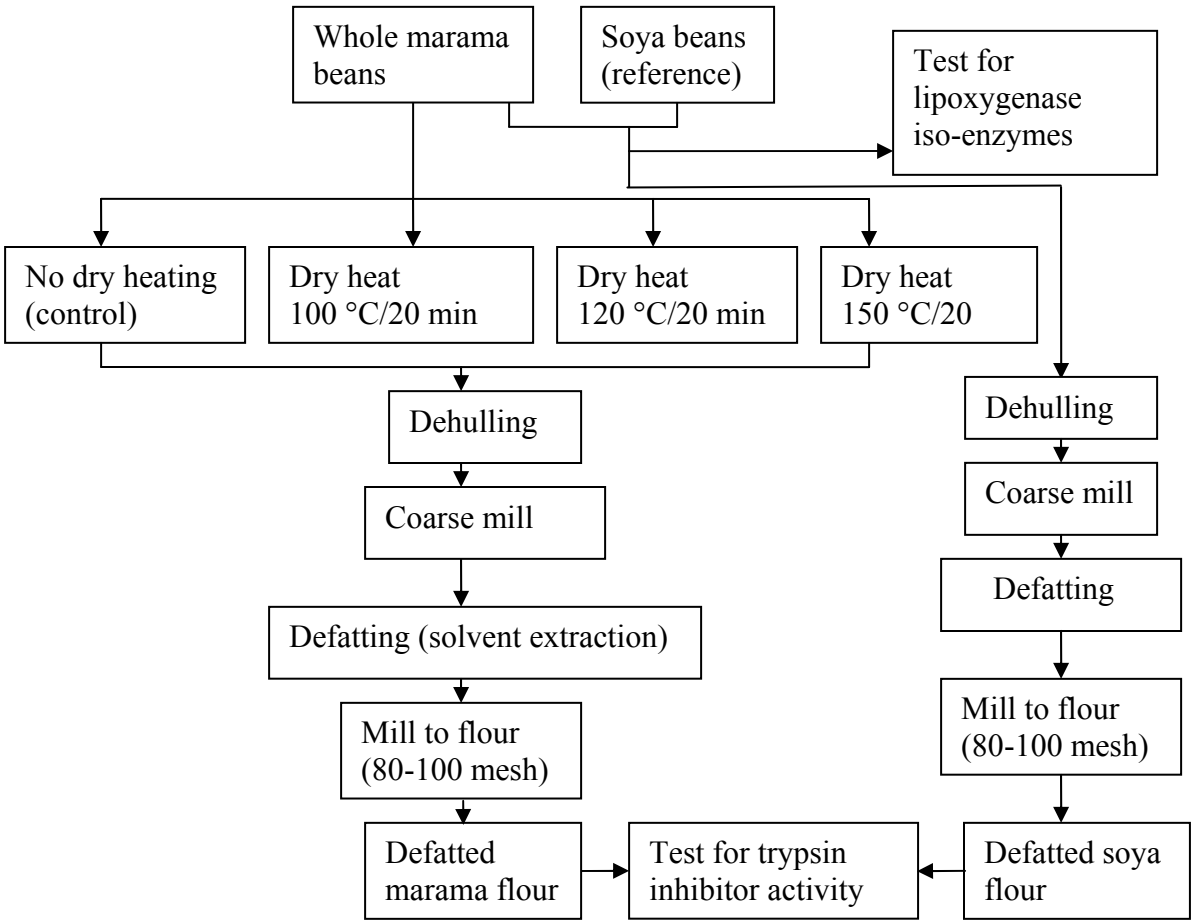


Figure 3.1.1: Experimental design for optimisation of dry heating processing to inactivate lipoxygenase enzymes and trypsin inhibitors in whole marama beans

3.1.2.1 Preparation of flour samples and extracts for detection of lipoxygenase iso-enzymes.

Clean whole marama beans were dehulled using the DF sample cracker (WMC Sheet Metal Works, Tzaneen, South Africa) (Fig. 3.1.2). The cracker has two discs carrying blades, in which one of the discs rotates and the other remains stationary. A groove machined into the stationary disc channels the beans to a seat carrying the only blade on this disc. The rotating disc, on the other hand, has four knives, all made of High Speed Steel. As this disc rotates, the blades cut through the bean for as long as it still remains on the stationary disc (Personal communication - Tjiparuro, 2007; Principal Engineer, RIIC, Kanye, Botswana). The dehulled beans were then ground with a chilled mortar and pestle

to fine flour (80-100 mesh). Soya bean flour was prepared in a similar way but the beans were dehulled manually. Flour (0.5 g) of each sample was homogenised with 49 ml of ice-cooled deionised water at 9500 rpm with an Ultra-turrax T25 homogeniser (Ika-Labtechnik, Germany) and allowed to stand for 1 h at 4 °C. The homogenates were centrifuged in 15 ml centrifuge tubes (1000 rpm, 10 min, 4 °C) and the supernatant obtained was used as the extract sample for detection of lipoxygenase iso-enzymes by the spectrophotometric method.

Figure 3.1.2: DF sample cracker used for dehulling marama beans

3.1.2.2 Preparation of substrate for the detection of lipoxygenase iso-enzymes

Sodium linoeate substrate was prepared from linoleic acid (99%) (Sigma Chemical Co., St. Louis, USA) as described by Axelrod, Cheesbrough and Laasko (1981). Tween 20 (70 mg) was added to 70 mg linoleic acid and the mixture was mixed in 4 ml deionised water by drawing back and forth in a pasteur pipet, avoiding formation of air bubbles. To obtain a clear solution, 0.55 ml 0.5 M NaOH was added and the solution was made up to 25 ml with deionised water. This solution was prepared daily before conducting the test.

3.1.2.3 Preparation of β-Carotene at 50% saturation in acetone for the detection of lipoxygenase iso-enzymes

β-Carotene (10 mg) obtained from Sigma Chemical Co., St. Louis, USA was dissolved in 10 ml acetone. The mixture was mixed vigorously using a vortex mixer and centrifuged at 1000 rpm for 5 min. The orange-coloured supernatant was diluted with the same volume of acetone and stored in a brown vial at 4 °C. This solution was prepared daily before conducting the test.

3.1.2.4 Detection of lipoxygenase iso-enzymes – Visual method

The presence of lipoxygenase iso-enzymes in unheated marama beans was determined by the method described by Suda, Hajika, Nishiba, Furuta and Igita (1995) using unheated soya beans as the standard sample. The principle of the method is based on the ability of the individual lipoxygenase iso-enzymes to bleach methylene blue and β-carotene. The method consists of three tests designated Tests I-III which test for lipoxygenase iso-enzymes 1 (L-1), 2 (L-2) and 3 (L-3) respectively. Test I was conducted by mixing 2.5 mg soya bean or marama bean flour with 0.5 ml distilled water in a test tube and allowing the mixture to stand for 3-10 min. Then 2 ml of the dye-substrate (25 ml 0.2 M sodium borate (pH 9.0), 5 ml of 100 µM methylene blue, 5 ml 10 mM sodium linoleate prepared as described in 3.1.2.2 above and 5 ml distilled water) were added to the test tube and after 3 min, the colour of the solution was checked visually.

Test II involved the same procedure as Test I, except for the following modifications: (i) 5.0 mg flour was used; (ii) 154.25 mg dithiothreitol was weighed into a 100 ml glass-stoppered bottle, and then 25 ml 0.2 M sodium phosphate buffer (pH 6.0), 5 ml 100 µM methylene blue, 5 ml acetone, and 5 ml 10 mM sodium linoleate substrate were added, and the mixture was then swirled in the test tube. The colour was checked after 5 min.

Test III involved the same procedure as Test I, except for the following modifications: (i) 25 ml 0.2 M sodium phosphate buffer (pH 6.6), 5 ml 10 mM sodium linoleate substrate, and 5 ml distilled water were mixed and added to a 100 ml glass-stoppered bottle

containing 5 ml β-carotene at 50% saturation in acetone (prepared as described in 3.1.2.3 above) and the mixture was shaken vigorously. The colour was checked after 5 min.

The presence or absence of lipoxygenase iso-enzymes was visualized within 5-10 min through the bleaching of methylene blue (L1 and L2) and β-carotene (L3).

3.1.2.5 Detection of lipoxygenase iso-enzymes – Spectrophotometeric method

In screening for L-1 activity of unheated soya beans (standard) and unheated marama beans, 1.0 ml 0.2 M sodium borate buffer (pH 9.0), 0.2 ml 100 µM methylene blue, 0.2 ml of 10 mM sodium linoleate substrate, 0.2 ml distilled water and 0.6 ml soya bean or marama bean flour extracts were mixed and the absorbance at 660 nm was measured with a Lambda EZ150 UV/Vis Spectrophotometer (Perkin-Elmer Corporation, USA) at intervals of 10 s for 3 min at 23 °C as described by Suda *et al.* (1995). For screening for L-2, the reaction mixture contained 0.8 ml 0.2 M sodium phosphate buffer (pH 6.0), 0.2 ml 100 µM methylene blue, 0.2 ml 0.2 M dithiothreitol in 0.2 M sodium phosphate buffer (pH 6.0), 0.2 ml acetone, 0.2 ml 10 mM sodium linoleate substrate and 0.4 ml soya bean or marama bean extracts. The absorbance at 660 nm was measured with a Lambda EZ150 UV/Vis Spectrophotometer (Perkin-Elmer Corporation, USA) at intervals of 1 min for 10 min at 23 °C.

3.1.2.6 Measurement of trypsin inhibitor activity of marama beans

Trypsin inhibitor activity of unheated and dry heated whole marama beans was assayed using the AACC method 22-40 (1991). This method has been used to determine total and residual trypsin inhibitors in soya products, including raw and toasted soya bean meals and flours, soya protein concentrates and isolates and maize-soya mixtures. This method uses N-benzoyl-$_{DL}$-arginine *p*-nitroanilide (BAPA) as a substrate for porcine trypsin and the ability of aliquots of meal extract to inhibit the activity of trypsin towards this substrate is utilized to estimate the amount of trypsin inhibitor in a soya meal sample.

Marama and soya beans were grounded to a fine powder using chilled pestle and mortar to avoid generating heat. The flours were defatted using n-hexane at room temperature in

the ratio 1:3 (flour: hexane) for 1 h using a magnetic stirrer at low setting. The mixture was allowed to settle for 30 minutes and then the hexane decanted. The procedure was repeated three times with fresh hexane each time. The defatted samples were placed in a fume cupboard overnight to evaporate the n-hexane. The samples (not exceeding 1g) were extracted with 50 ml 0.01N NaOH/g sample for 3 h, with magnetic stirrer at low setting. The sample extracts were filtered through Whatman filter paper no. 2 or no. 3. This filtering step is a modification of the method as it is less time consuming. Stauffer (1990) reported that filtering the soya extract before running the assay does not change the concentration of trypsin inhibitor.

The sample extracts were diluted to the point where 1 ml produces trypsin inhibition of 40-60%. This reduces the standard deviations. Trial dilutions were conducted to establish this inhibition value. In the case of defatted marama flour prepared from unheated marama beans, 1 ml of the extract was diluted to 50 ml with Tris buffer (0.05 M, pH 8.2). For defatted soya flour prepared from unheated soya beans, a ratio of 1:20 v/v was used. Portions (0, 0.6, 1.0, 1.4, and 1.8 ml) of diluted suspension were pipetted into duplicate sets of test tubes and adjusted to 2.0 ml with water. Then 2 ml of trypsin solution was added to each test tube and placed in a water bath at 37 ^{o}C. The mixture was mixed using a vortex mixer.

Then 5 ml substrate solution previously warmed to 37 ^{o}C was added to the mixture and exactly 10 min later, the reaction was stop by adding 1 ml acetic acid solution. The mixture was mixed using a vortex mixer. For blank preparation, 5 ml of BAPA was added to 2 ml sample extract, incubated at 37 ^{o}C for 10 min, and then 1 ml acetic acid solution was added followed by addition of 2 ml trypsin solution. The absorbance at 410 nm was measured with a Lambda EZ150 UV/Vis Spectrophotometer (Perkin-Elmer Corporation, USA) at 23 °C. One Trypsin unit is arbitrarily defined as an increase of 0.01 absorbance unit at 410 nm per 10 ml of reaction mixture in terms of Trypsin inhibitor units (TIU). Trypsin inhibitor activity was expressed as trypsin units inhibited (TUI)/ml of extract.

3.1.2.7 Statistical analyses

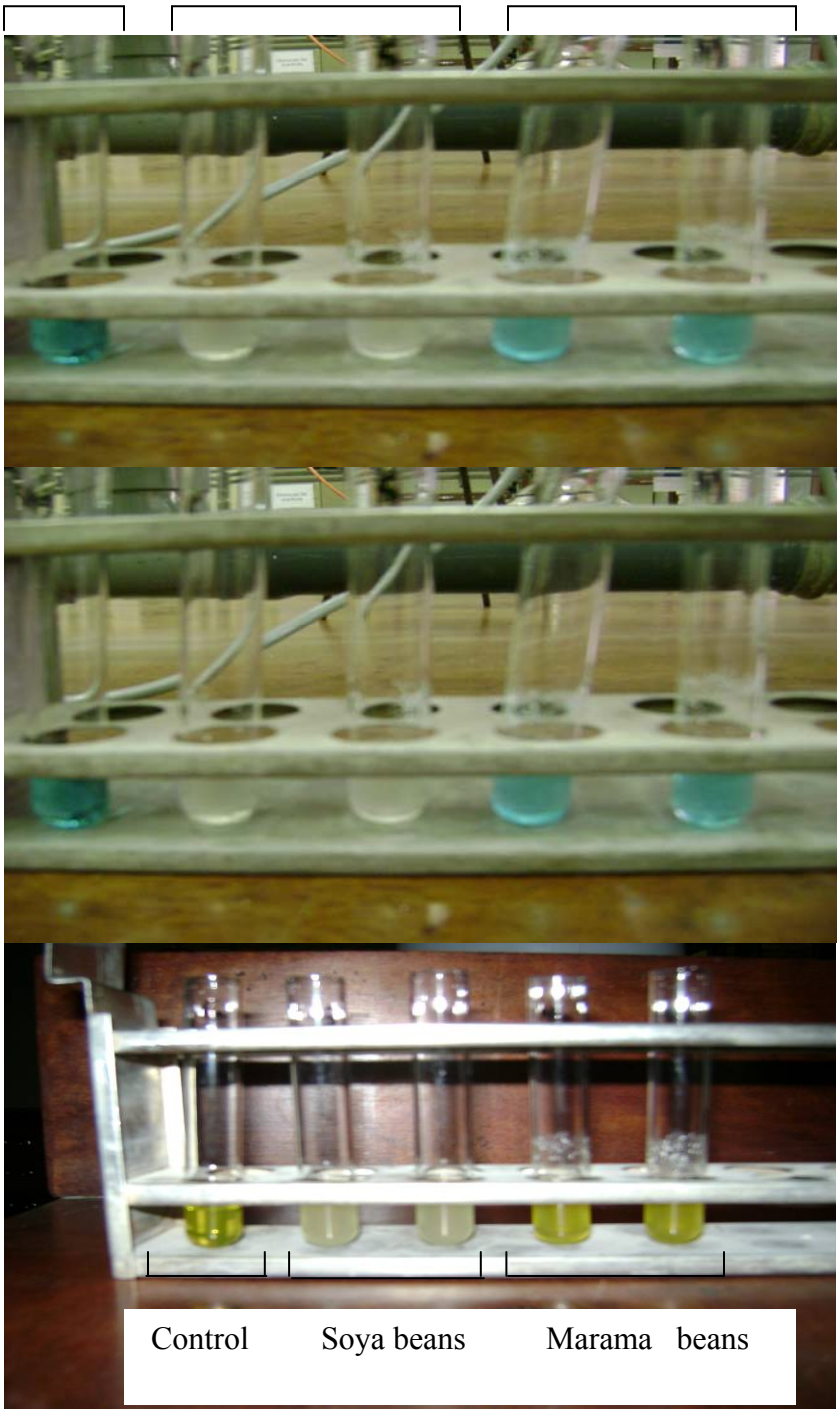
The experiment was repeated twice and all analyses were performed in duplicate (n=2) per repeat. Data obtained was analysed by one-way ANOVA. Mean differences were evaluated at the 95% significance level ($p \leq 0.05$) using the least significant test. The analyses were performed using Statistica Version 6.0 (Statsoft, Tulsa, USA).

3.1.3 Results and Discussion

3.1.3.1 Detection of lipoxygenase iso-enzymes in unheated marama beans

The marama beans extract did not bleach either methylene blue or β-carotene whereas soya beans extract had a strong bleaching activity towards methylene blue and β-carotene, thus indicating the presence of L-1, L-2 and L-3 iso-enzymes in soya beans (Fig 3.1.3). This suggests that the lipoxygenase iso-enzymes may be absent or be naturally inhibited in some way in marama beans. The investigation was conducted on marama beans harvested in June 2006 to determine if the activity might have disappeared during storage. The investigation was conducted on freshly harvested marama beans (June 2008) and similar results were obtained for L-1 and L-2 only. These results suggest that the age of the marama beans harvested in 2006 was not a factor in the observed absence of its lipoxygenase (L-1 and L-2) activity.

Although no amount was given, St. Angelo, Kuck and Ory (1979) reported that peanut tannins (catechol-like) gave 67% inhibition of soya bean lipoxygenase at pH 8.4 possibly through cross-linking with the enzyme. Since marama bean cotyledons contain a total phenolic content of 2.8 mg catechin equivalent CE/100 mg on a dry basis (van Zyl, 2007b), these compounds may possibly be responsible for inhibiting lipoxygenase in marama beans. Gallic acid polymers have been reported to be effective in retarding lipoxygenase oxidation of linoleate (Nawar, 1996). The cotyledons of marama beans contained about 23.4 mg of gallic acid per 100 g (dry basis) of sample (van Zyl (2007b). However, it is not clear whether the gallic acid in marama beans is in monomeric or polymeric form.



Test 1 for lipoxygenase (L-1) 1 at pH 9.0

Test II for lipoxygenase 2 (L-2) at pH 6.0

Test III for lipoxygenase 3 (L-3) at pH 6.6

Figure 3.1.3: Bleaching of methylene blue and β-carotene by soya bean and marama bean flours (test I-III) in visual judging method for detecting the presence of L-1, L-2 and L-3 iso-enzymes

Skrzypczak-Jankun, Kangjing and Jankun (2003) also demonstrated using X-ray analysis at 21 Å that the flavonoid quercetin, that is also found in marama beans (van Zyl, 2007b) complexed with soya bean lipoxygenase thereby inhibiting it. Another phenolic compound found in marama beans, caffeic acid (331.8 mg/100 g) (van Zyl, 2007b) inhibited rat leukocyte 5-lipoxygenase at 200 µm (Puerta, Gutierrez and Hoult, 1999). It appears that phenolic compounds in general have some form of inhibitory activity towards lipoxygenase enzymes. The mechanism of inhibition of lipoxygenase by phenolic compounds (catechol-like) has not yet been determined. However, it has been proposed that tannins may inhibit the enzyme by hydrogen, covalent, ionic and hydrophobic bonding with the enzyme (St. Angelo *et al*., 1979).

Marama bean flour has been reported by Mmonatau (2005) to contain 0.42g/100g of erucic acid, an unsaturated fatty acid with a 22-carbon chain length. This fatty acid was also identified by St. Angelo *et al.* (1979) and St. Angelo and Ory (1984) as a lipoxygenase inhibitor as it completely inhibited soya bean and peanut lipoxygenase at a minimum concentration of 7.3 µmol/100g. Of the oilseeds that were studied (soya beans, rapeseed and peanuts), lipoxygenase activity was not detected in rapeseed only and this was attributed to the presence of erucic acid and tannins in rapeseed (St. Angelo *et al.* 1984).

The absence or presence of lipoxygenase 1 and 2 iso-enzymes (L-1 and L-2) in marama bean extracts was also determined by the spectrophotometric method. Marama bean extracts did not bleach methylene blue at pH 9.0 as the absorbance of the methylene blue did not decrease with time, suggesting the absence of L-1 iso-enzyme in marama beans (Fig. 3.1.4). Similar results were obtained for the L-2 iso-enzyme at pH 6.0 (Fig. 3.1.5), also suggesting the absence of L-2 iso-enzyme in marama beans.

However, soya bean extracts exhibited bleaching activity toward methylene blue as indicated by the decrease in absorbance of methylene blue with time (Figs. 3.1.4 and 3.1.5), confirming the presence of L-1 and L-2 iso-enzyme in soya beans. According to Toyosaki (1992), methylene blue decolourising reactions involve hydroperoxide, especially 13C-OOH isomers (i.e. hydroperoxides with 13 carbons), that are formed

during lipid peroxidation. The mechanism of methylene blue (MB) bleaching by lipoxygenase involves the specific abstraction of hydrogen from the hydroperoxide 13C-OOH isomer by methylene blue, which is then reduced to MB-H, which is colourless.

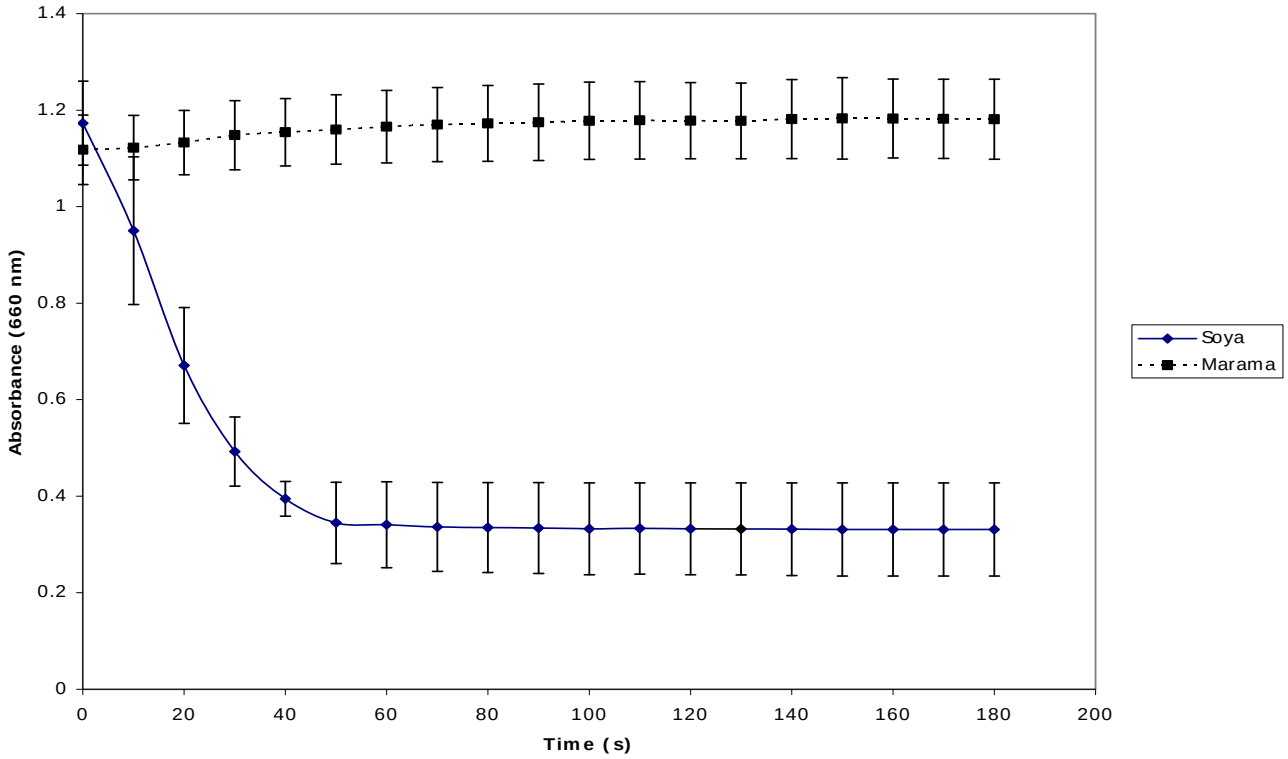


Fig 3.1.4. Methylene blue bleaching by soya bean and marama bean extracts in spectrophotometric method for detecting the presence of L-1 isozyme (pH 9.0) at 660 nm

3.1.3.2 Trypsin inhibitor activity of unheated and dry heated marama beans compared with unheated soya beans

The trypsin inhibitor activity of defatted flour from unheated soya beans (USF), defatted flour from unheated marama beans (UMF) and defatted flour from dry heated marama beans (HMF) are shown in Table 3.1.1. The trypsin inhibitor activity of USF falls within the values (43-84 TIU/mg sample) for different soya bean cultivars reported by Guillamon, Pedrosa, Burbano, Cuadrado, de Cortes Sanchez and Muzquiz (2008). The trypsin inhibitor activity of UMF was almost four and half times higher than that found in USF. A much higher trypsin inhibitor activity of marama beans was reported by Bower *et al.* (1988), who found the marama beans trypsin inhibitor activity to be six times more

than that found in soya beans. The difference observed in the trypsin inhibitor activity of the marama beans may be due to the variations in chemical composition of the beans that were used.

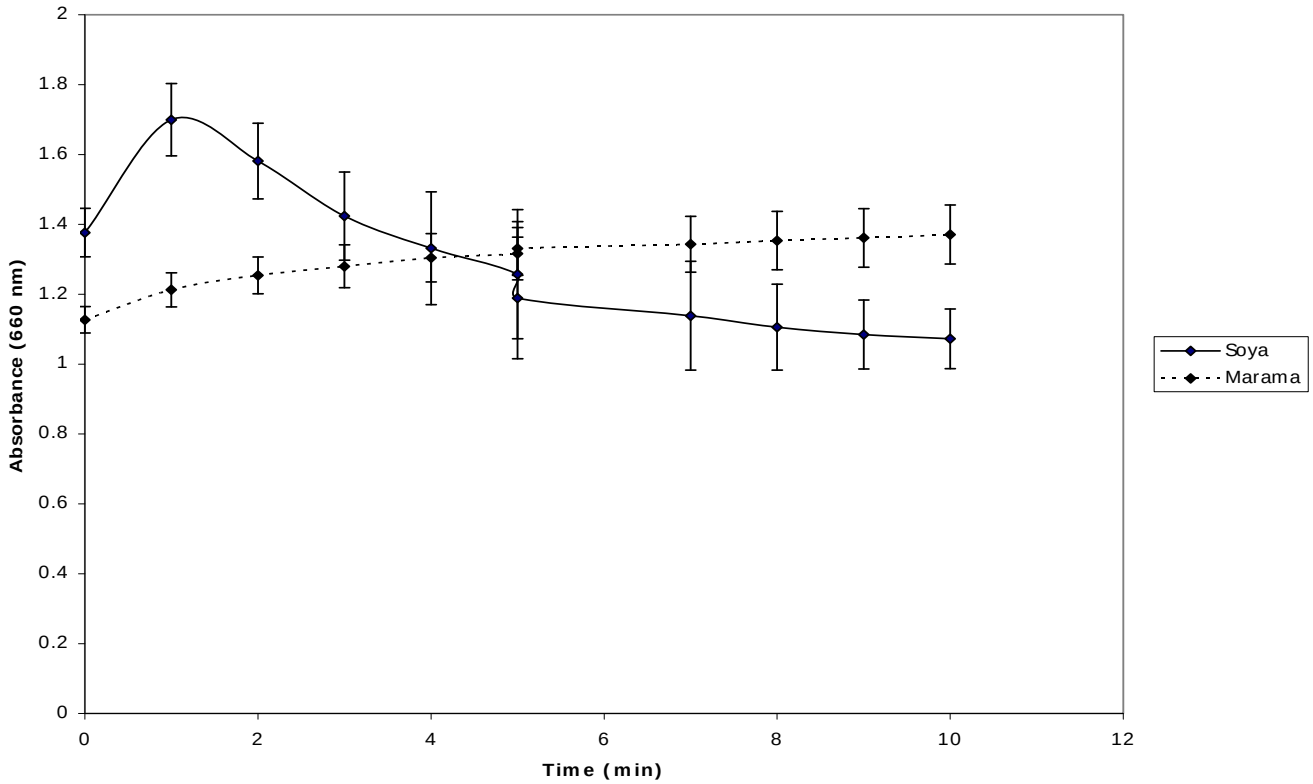


Fig. 3.1.5 Methylene blue bleaching by soya bean and marama bean extracts in spectrophotometric method for detecting the presence of L-2 isozyme (pH 6.0) at 660 nm

It was observed that dry heating of whole marama beans at 100 °C and 120 °C for 20 min decreased the trypsin inhibitor activity by 12.6% and 25.7% respectively. On the other hand, dry heating at 150°C for 20 min practically decreased the trypsin inhibitor activity by almost 100%. This suggests that heating whole marama beans at this temperature for 20 min was effective in altering the conformation of the inhibitors and thus permanently inactivated them (DiPietro and Liener, 1989). Although information on the temperature/time combinations used for heating marama beans were not provided, Ripperger-Suhler (1983) observed no trypsin inhibitor activity in roasted marama beans and very low levels in autoclaved marama beans.

Table 3.1.1: Trypsin inhibitor activity of extracts of defatted flour from unheated soya beans, defatted flour from unheated marama beans and defatted flour prepared from whole marama beans dry heated at various temperatures for 20 min using benzoyl-D L-arginine-*p*-nitroanilide (BAPA) as substrate

	Trypsin inhibitor activity	
Flour sample	TUI[f]/ ml extract	TUI/mg flour
USF	57.6[b] (4.1)	57.6[b] (4.1)
UMF	100.3[e1] (5.7)[2]	250.8[e] (14.3)
HMF (100 °C/20min)	87.7[d] (4.3)	219.3[d] (10.8)
HMF (120 °C/20 min)	74.5[c] (1.3)	186.3[c] (3.3)
HMF (150 °C/20 min)	1.3[a] (1.3)	3.3[a] (3.3)

[1] Means within a column with different letters are significantly different ($p \leq 0.05$)
[2] Standard deviations are given in parentheses
[f] Average of trypsin units inhibited determined at five different levels of crude extract
Results are expressed on a fresh weight basis
USF – Defatted flour from unheated soya beans
UMF – Defatted flour from unheated marama beans
HMF – Defatted flour from dry heated whole marama beans

Marama bean protease inhibitors have been isolated by Elfant *et al*., (1985) and have been reported to represent about 10.5% of the total protein. Two major inhibitors, namely the Kunitz and the Bowman-Birk inhibitors have been purified and studied in soya beans (Vaidehi and Kadam, 1981). These protease inhibitors are known to form complexes with porcine and bovine trypsin in the intestinal tract of animals thus reducing the digestibility of proteins and consequently inhibiting growth (Woodworth, Tokach, Goodband, Nelssen, O'Quinn, Knabe and Said, 2001). The high trypsin inhibitor content of raw

marama beans underlines the need for a controlled dry heat treatment during processing to inactivate most of the trypsin inhibitors. However, a balance must be struck to optimise the heat treatment to avoid decreasing protein quality and functionality.

3.1.4 Conclusions

Lipoxygenase iso-enzymes (L-1 and L-2) were not detected in marama beans suggesting that they are either absent or naturally inhibited in some way in marama beans. This means that off-flavour in marama bean flour development in as a result of lipoxygenase iso-enzymes does not seem likely. However, the potential presence of L-3 and other enzymes such as lipase which can be involved in rancidity of defatted marama flour has not been confirmed. Dry heating of whole marama beans at 150 °C/20 min is effective in inactivating almost all the trypsin inhibitors in marama beans. This temperature/time combination can be used for dry heating of whole marama beans during production of marama bean flour. However, the effect of this temperature/time combination on protein-related functional properties and nutritional quality of defatted marama bean flour is important and will be reported in the next section.

3.2 Effect of dry heating of whole marama beans (*Tylosema esculentum* (Burch) A. Schreib) on the physico-chemical and functional properties of the resultant defatted marama bean flour

ABSTRACT

Defatted flour from unheated and dry heated whole marama beans (150 °C /20 min) were analysed for their proximate composition, colour, in-vitro protein digestibility, amino acid composition and selected protein functional properties. Commercial defatted flour from unheated and heated soya beans (USF and HSF) were used as reference samples. Defatted flour from dry heated whole marama beans (HMF) had higher protein content but lower fat content than defatted flour from unheated whole marama beans (UMF). This is probably due to the fact that oil in the flour from dry heated marama beans was more readily dissolved in the hexane during the defatting process. HMF and HSF had lower L* values but higher a* and b* values compared to UMF and USF, respectively, probably due to Maillard browning reactions. Heating slightly increased in-vitro protein digestibility of HMF and HSF compared to UMF and USF, respectively, possibly because of protein unfolding and denaturation and inactivation of trypsin inhibitors. Heating generally decreased the amino acid composition of HMF and HSF compared to UMF and USF respectively. Protein solubility and emulsifying capacity of HMF and HSF were significantly lower than that of UMF and USF possibly due to protein denaturation and/or cross-linking. It was observed that there was a significant positive correlation between protein solubility and emulsifying capacity of the flours. Heating significantly increased the water absorption capacity of HMF compared to UMF but the increase for HSF compared to USF was not of practical significance. Although heating decreased the oil absorption capacity of HMF and HSF compared to UMF and USF respectively, the decrease was not of practical significance. It was observed that foaming capacity of UMF, although lower than that of USF, was not affected by heating probably due to the rigidity of marama proteins and the high fat content of the marama bean flours. UMF exhibited good protein-related functional properties compared to HMF. However, both flours have potential for use as ingredients in selected food systems. UMF was

superior to USF in terms of protein-related functional properties except for water absorption and foaming capacity.

3.2.1 Introduction

The marama bean ((*Tylosema esculentum* (Burch) A. Schreib) is an underutilized legume crop native to the Kalahari Desert and sandy regions of Botswana and Namibia and South Africa. Its protein content (37%) (Mmonatau, 2005) is comparable to that of soya beans (43.4%) (Vaidehi and Kadam, 1989). As a result, value-added protein-based ingredients can be potentially developed from marama beans similar to those commercially available from soya beans. These include protein-rich flours, concentrates and isolate. Minimum protein contents of these products are 50%, 70% and 90%, respectively, depending on the efficiency of fat extraction (Wolf, 1970, Lusas and Riaz, 1995). Protein-based ingredients prepared from marama beans have a potential to be used to improve the protein quality of cereal-based foods through compositing and to act as functional ingredients in foods. The term functional in this context refers to a property of an ingredient, aside from nutritional attributes, that influences an ingredient's usefulness in food (Fennema, 1996).

There are a number of functional characteristics desired in protein-containing ingredients. The importance of any one characteristic is dependent upon the particular use of the ingredient (Kinsella, 1979). The following are some of the protein-related functional properties desired in functional ingredients: protein solubility, water and oil binding capacity, emulsification, foaming, viscosity and gelation. The importance of each of these properties varies with the different uses, for example, emulsification in comminuted meats, water absorption in bakery products and viscosity in soups. Functional properties of proteins are affected by intrinsic factors such as molecular structure and size of proteins, as well as extrinsic factors including the method of protein extraction, pH, ionic strength and the components in the food system (Moure, Sineiro, Dominguez and Parajo, 2006).

Heat treatment is one the most common methods used in the processing of soya flours to denature proteins and inactivate protease inhibitors (Wright, 1981; De Valle, 1981) and improve the nutritional value of the soya flours (Liener, 1981; Torun, Viteri and Young,

1981). On the other hand, heating of soya beans may decrease total and available lysine in the resultant soya flours depending on the time and temperature that used during the heat treatment (Faldet, Satter and Broderick, 1992). Lysine is vulnerable to heat damage because of its reactive epsilon-amino group that reacts with reducing sugars in Mallaird type reactions during heat processing (Damodaran, 1996). As such the availability of this essential amino acid may be reduced in the soya flours. Heat denaturation of proteins may affect functional properties of the soya flours such as protein solubility, emulsification, foaming and water absorption capacity (Kinsella, 1979; Morr, 1990). Heating has been reported to decrease the protein solubility, emulsification and foaming capacity of low fat soya flour (Heywood *et al*., 2002). Similar results were reported by Yu *et al*. (2007) for peanut flour and yam bean flour (Obatolu *et al*., 2007).

Marana bean flour, which can be prepared by adopting procedures used for soya flour processing, seems to be a promising product which can be used as a protein supplement and functional ingredient. However, studies on the physico-chemical and functional properties of marama bean flours are non-existing. The objective of this research was to determine the effect of dry heating of whole marama beans on the chemical composition, *in-vitro* protein digestibility and protein-related functional properties of its defatted flour. This study could provide some basic information, which would help determine potential applications for defatted marama bean flours in food products.

3.2.2 Materials and methods

3.2.2.1 Preparation of defatted flour from dry heated whole marama beans

Marama beans described previously in the chapter 1 (3.1.2) were dry heated with a forced convection continuous tumble roaster (Roastech (www.roastech.co.za), South Africa) (Fig. 3.2.1). Marama beans used to prepare unheated defatted marama flour were not dry heated (control sample). The continuous tumble roaster has four main components, namely the control system, seed hopper, drum (perforated cylinder and screw conveyer) and a holding unit. A speed set of 290 was selected because it resulted in a heating time of 20 min (first bean) to 23 min (last bean) at 150 °C which was found to inactivate most of the trypsin inhibitors as shown previously. The beans were then fed to a hopper and

transferred to the container by gravity where they were heated with hot air blown by a fan attached to the container. The drum consists of a rotating perforated cylinder which mixes the beans and a screw conveyer which also mixes and propels the beans forward at the set speed. This ensures continuous uniform distribution of heat to the beans and is probably more effective in inactivating trypsin inhibitors than a stationary roaster (Personal communication – Teseling, 2007; Director, Roastech, South Africa).

All bean samples were then cracked using a DF sample cracker (WMC Metal Sheet Works, Tzaneen, South Africa) described previously (Fig. 3.1.2) and the seed coat separated from the cotyledons manually and by use of sieves of different sizes.

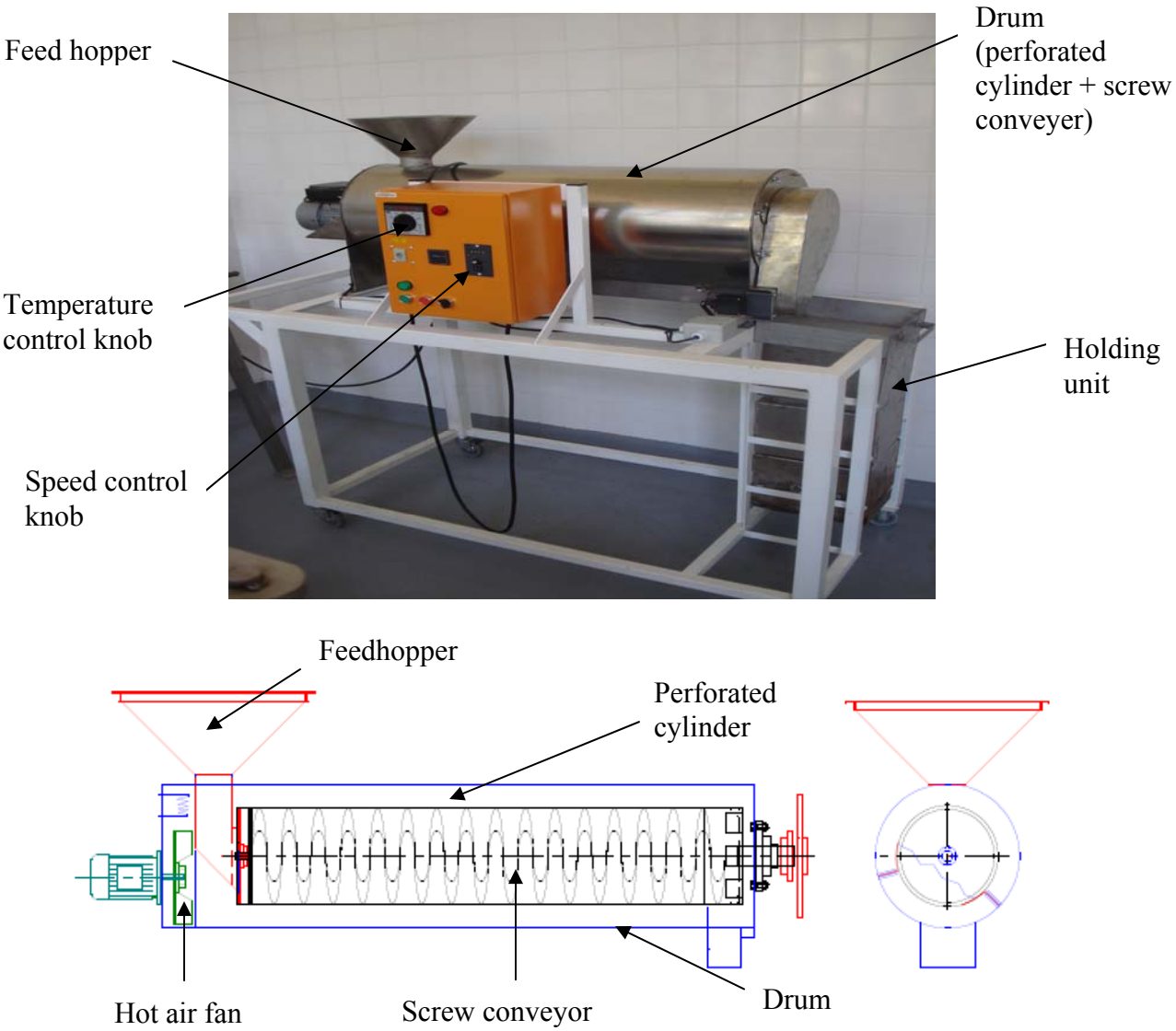


Figure 3.2.1: Picture and schematic diagram of the forced convection continuous tumble roaster used for drying whole marama beans (Personal communication - Teseling, 2007; Director, Roastech, South Africa)

The beans were coarsely ground using a Waring blender into a meal and defatted using *n*-hexane in the ratio of 1 part meal: 3 parts hexane by stirring the meal-hexane mixture with a magnetic stirrer for 1 h. The mixture was allowed to stand for 30 min before decanting the *n*-hexane supernatant. The process was repeated 3 times to effectively defat the sample. The defatted samples were then left in a fume cupboard overnight to evaporate the remaining solvent in the samples. The processing trials were repeated twice because of limited quantities of available raw material. The defatted meal was milled to flour particle size to pass through a 0.8 mm screen using a laboratory hammer mill 3100 (Falling number, Sweden), packaged in zip lock polyethylene bags and stored at 5 °C.

The experimental design (Fig. 3.2.2) was used for determining the effect of heating of whole marama beans on the physico-chemical and functional properties of its defatted marama bean flour. Commercial unheated and heated defatted soya flours obtained from Nedan Oil Mills (Pty) Ltd (South Africa) were used as reference samples in this study for comparison. Table 3.2.1 shows the information provided about the soya flours by the supplier.

The independent variable in the experiment was the dry heating process. The dependent variables were as follows: % moisture, % protein, % fat, % ash, % carbohydrate (by difference), amino acid composition, protein digestibility, colour measurements, nitrogen solubility index, water and oil absorption capacities, foaming capacity, emulsion capacity.

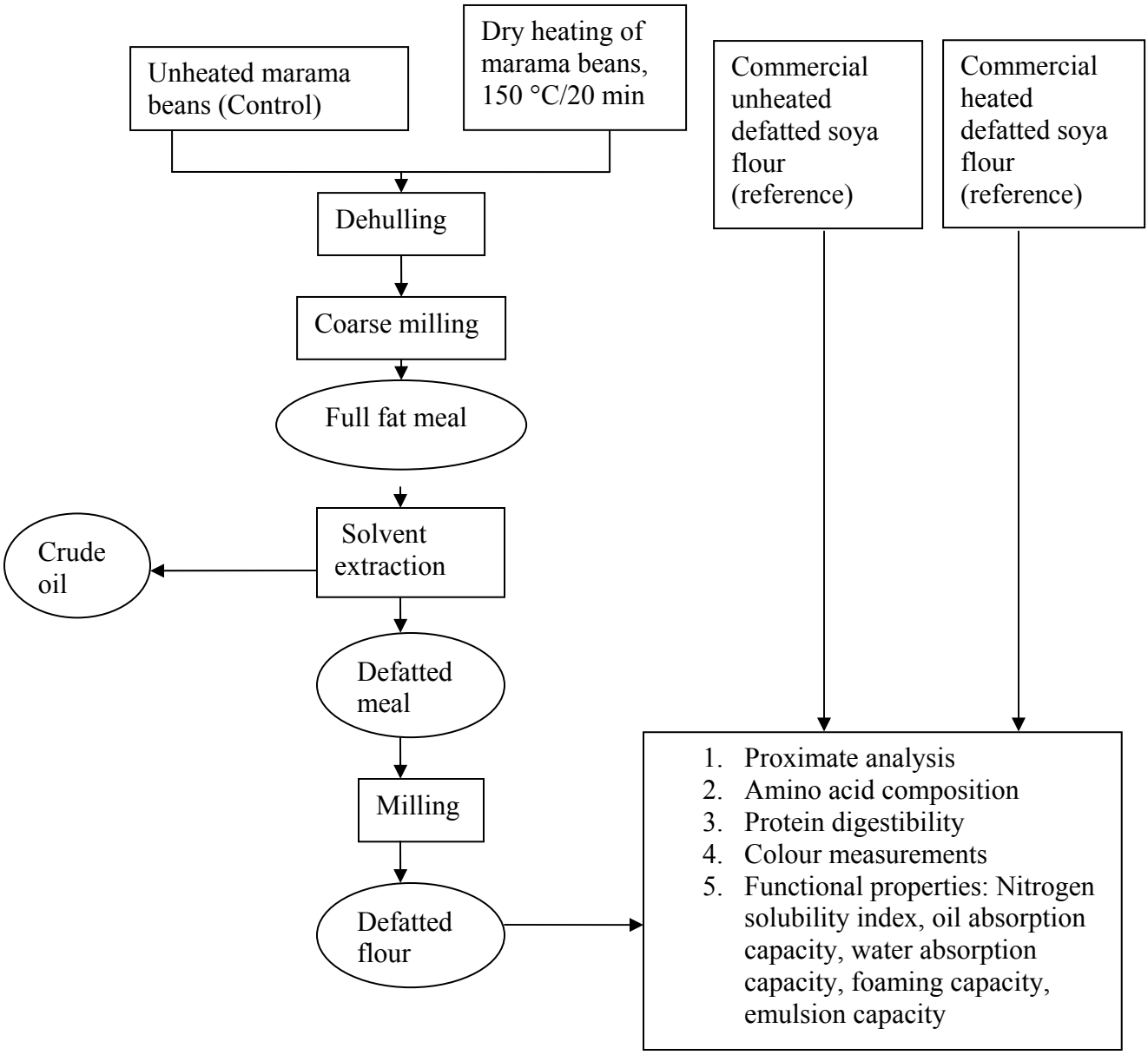


Figure 3.2.2: **Experimental design for determining the effect of dry heating of whole marama beans on physico-chemical and functional properties of defatted marama bean flour using commercial defatted soya flours as reference samples**

Table 3.2.1: Description and specifications of the commercial defatted soya flours used in the study as reference samples

Commercial soya flour	Description	Moisture	Protein	Colour	PDI (%)	Processing parameters
Unheated soya flour (USF)	Untoasted Flour	8% max	48 % max	Light cream	60-70	Conditioning (60 °C /1 h) FDS* (100-110 °C/2 s)
Heated soya flour (HSF)	Toasted flour	8% max	48% max	Light brown	30-40	As for USF Preconditioning (90 °C/2 min) Extrusion (140 °C/15 s)

- Flash desolventising system (FDS)

3.2.2.2 Proximate analysis of defatted flour from unheated and dry heated whole marama beans

Moisture

Moisture was assayed in duplicate using the AACC Method 44 – 15A (AACC, 1999). Moisture tins were dried in a forced draught oven at 103 °C for 1 h. The tins were then cooled in a dessicator for about 20 min. The tins were weighed using an analytical balance and 2 g of flour sample weighed into the tins. The samples were covered with aluminium foil and dried in a forced draught oven for 4 h at 103 °C. The samples were then cooled for 10 min and weighed using an analytical balance.
The moisture content (%) was calculated as follows:

$$\% \text{ moisture} = \frac{((\text{mass food} + \text{tin}) - (\text{mass tin})) - ((\text{mass dry food} + \text{tin}) - (\text{mass tin}))}{(\text{mass food} + \text{tin}) - \text{mass tin}} \times 100$$

Crude protein

Crude protein was determined using the Leco FP – 528 Protein/Nitrogen Analyser (Leco Corporation, USA). This procedure is a three-phase analysis where the nitrogen in the protein is released through chemical decomposition by heat. The phases are as follows:

- *Sample drop purge phase*: The encapsulated samples are placed in the loading head, sealed and purged of any atmospheric gases that have entered during sample loading. The ballast volume and gas lines are also purged.
- *Burn phase*: The sample is combusted at 850 °C in a stream of oxygen.
- *Analyse phase*: Nitrogen containing compounds are converted to nitrogen which is oxidized to oxides of nitrogen; water produced is condensed and removed. Oxides of nitrogen are carried by helium gas to a thermal conductivity detector and reduced to nitrogen for estimation. The carbon dioxide and sulphur dioxide formed are removed by selective absorption.

The final result was presented as weight percentage of nitrogen. The nitrogen amount was converted to percent protein with the protein conversion factor (N x 5.71).

Crude fat

Fat was extracted from the sample using semi-continuous extraction: Soxhlet apparatus, AACC Method 30-25 (AACC, 1983), with a few modifications. The samples (weights of between 2 – 5 g) were placed in the Soxtest apparatus and extracted with petroleum ether for 4 h. The ether was then removed from the collection flask at low temperature volatilisation before oven drying. The residue fat was dried in an oven at 100 °C for 30 min. Percent fat was calculated as follows:

$$\% \text{ Fat} = \frac{(\text{mass of beaker} + \text{fat}) - (\text{mass of empty beaker})}{\text{Mass of sample}} \times 100$$

Ash

AACC Method 08 – 01 (AACC, 1999) was used to determine the ash content. Ash is the material remaining after oxidative combustion of all the organic matter in food; in proximate analysis of foods, "ash" is therefore a measure of the food's mineral content. Silica crucibles were dried for 5 h in a muffle oven, allowed to cool in the muffle oven, then transferred to a dessicator using metal tongs. The crucibles were weighed to the nearest 0.1 mg using an analytical balance. Approximately 2 g of the finely flour sample was transferred into the crucible and spread as a thin layer. The crucibles containing the sample were then re-weighed. The crucibles were placed on a tripod and gauze and heated until the samples were charred. The samples were then placed in the muffle oven and heated at 550 °C for 5 h. After this, the samples were visually examined. They should be a uniform light grey. If not, a few drops of distilled water was added to spread out the sample and the drying process repeated until uniform light grey ash was obtained or to constant weight. The ash was cooled in a dessicator and weighed soon after room temperature was attained. Percentage ash was calculated as follows:

$$\% \text{ Ash} = \frac{(\text{mass ash + crucible}) - (\text{mass crucible})}{(\text{mass food + crucible}) - (\text{mass crucible})} \times 100$$

Carbohydrate content by difference

Total carbohydrate was obtained by calculation after estimating all other components by proximate analysis.

% Carbohydrate = 100 – (% Moisture + % Ash + % Crude fat + % Crude protein)

3.2.2.3 Colour

Colour was measured using a Chroma Meter CR-400 (Konica Minolta Sensing, Inc. Japan) and expressed in terms of lightness (L*), red-green characteristics (a*-value), blue-yellow characteristics (b*), Hue angle (h°) and chroma (C*); $h° = \tan^{-1}(b^*/a^*)$ and $C^* = [(a^{*2} + b^{*2})^{1/2}]$ (McGuire, 1992).

3.2.2.4 Determination of amino acid composition of defatted marama bean flours and commercial soya flours

The amino acid composition was determined using a modification of the method described by Bidlingmeyer, Cohen and Tarvin (1984). Defatted flour samples were centrifuged at 15 000 rpm for 5 min and then dried under vacuum for 1.5 to 2 h. The pH of the samples was adjusted by adding 20 µl solution of ethanol:water:triethylane in the ratio of 2:2:1 and then dried for a further 1.5 to 2 h. The resulting sample was derivatised by adding 20 µl derivatising solution of ethanol:water:triethylamine:phenylisothiocyanate in the ratio of 7:1:1:1. The mixture was allowed to react at room temperature for 10 min prior to drying under vacuum (minimum of 3 h). The columns used were application-specified Pico-Tag columns. The sample was resuspended in 200 µl of Picotag sample diluent and an 8 µl sub-sample was then injected for separation by HPLC (2X Model 510 pumps, Model 440 Absorbance detector, 717 plus Autosampler (Waters Corporation, MA, USA)). A gradient which runs for the separation consisted of 10% B traversing to 51% B in 10 min using a convex curve (number 5). The amino acid composition was expressed as g/100 g flour on a dry basis.

3.2.2.5 In-vitro protein digestibility of defatted marama bean flours from unheated and dry heated marama beans and commercial soya flours

The method described by Hsu, Vavak, Satterlee and Miller (1977) was used to determine the *in-vitro* protein digestibility of defatted flour prepared from unheated and dry heated marama beans. This method uses a multi-enzyme system consisting of trypsin, chymotrypsin and peptidase to hydrolyse the protein and has good correlation (0.90) with the *in-vivo* protein digestibility method (Hsu *et al*., 1977). The samples used for the *in-vitro* protein digestion study were ground to a fine powder to pass through an 80 mesh screen. The pH of 50 ml of the aqueous flour suspension (6.25 mg flour/ml) in distilled water was adjusted to pH 8.0 with 0.1N HCl and/or NaOH and transferred to a 37 °C shaking water bath. The multi-enzyme solution (1.6 mg porcine pancreatic trypsin (IX), 3.1 bovine pancreatic chymotrypsin (II) and 1.3 mg porcine intestinal peptidase/ml) prepared from enzymes obtained from Sigma Chemical Co., (St. Louis, USA) was maintained in an ice bath and the pH adjusted to pH 8.0 with 0.1N HCl and/or NaOH.

Then 5 ml of the multi-enzyme solution was added to the flour suspension which was being shaken at 37 °C. The pH of the enzyme-flour mixture was recorded after 10 min and the digestibility of the protein calculated using the following formula: Y = 210.46 – 18.10X where Y is the % protein digestibility and X is the pH of the enzyme flour mixture after 10 min (Hsu *et al*., 1977).

3.2.2.6 Protein functional properties of defatted marama bean flours prepared from unheated and heated marama beans and commercial soya flours

Functional properties evaluated were nitrogen solubility index, water and oil absorption capacity, foaming capacity, emulsion capacity.

Nitrogen solubility index

Nitrogen solubility index was determined by the AACC Method 46-23 (AACC, 1999) with few modifications. One gram of sample was weighed into 100 ml beaker and mixed with 50 ml of 0.1 M NaCl solution. The pH of the mixture was adjusted to pH 7.0 with 0.1N HCl and/or NaOH and then the mixture was shaken at speed 4 (1024 shaking water bath, Tecator, Sweden) for 1 h at 30 °C. The mixture was left to stand for a few minutes and 20 ml was decanted into 50 ml centrifuge tubes. The sample was centrifuged at 10 000 g, 15 min, 4 °C (Super Minor Centrifuge, MSE, UK) followed by filtering the clear supernatant obtained through a Whatman No.1 filter paper. The nitrogen content of the filtrate was determined using a Leco nitrogen analyser (Model FP-2000; LECO Corp., St. Joseph, Michigan, USA). The protein was obtained by multiplying nitrogen contents by the 5.71 conversion factor.

NSI was calculated using the following:

$$\text{NSI (\%)} = \frac{\text{supernatant protein concentration (mg/ml) x 50}}{\text{sample weight (mg) x (sample protein content/100)}} \times 100$$

Water absorption capacity (WAC)

Water absorption capacity (WAC) of the flours was determined according to the AACC method 56-20 (AACC, 2000) with slight modifications. Two grams of flour (M0) sample was weighed into a pre-weighed 50 ml (M1) centrifuge tube and mixed thoroughly with 40 ml of deionised water for 10 min using a vortex mixer. The samples were centrifuged at 1000 g, 15 min, 20 °C (Rotanta 460 R Centrifuge, Heltich Zentrifugen, Germany) and the supernatant decanted. The centrifuge tubes were then inverted for 5 min on a paper towel, followed by weighing of the residue (M2).

WAC (g water/g flour) was calculated as follows:

$$\text{WAC (g/g)} = \frac{M2 - (M1+M0)}{M0}$$

Triplicate samples were analysed for each replicate.

Oil absorption capacity (OAC)

Oil absorption capacity (OAC) was determined using the method of Chakraborty (1986). One gram of the flour (W_0) was weighed into pre-weighed 15 ml centrifuge tubes and thoroughly mixed with 10 ml of vegetable oil (V_1) using a Vortex mixer. Samples were allowed to stand for 30 min. The flour-oil mixture was centrifuged at 3000 g for 20 min (Rotanta 460 R Centrifuge, Heltich Zentrifugen, Germany). Immediately after centrifugation, the supernatant was carefully poured into a 10 ml graduated cylinder and the volume was recorded (V_2).

OAC (g water/g flour) was calculated as:

$$\text{OAC (g/g)} = \frac{(V_1 - V_2)}{W_0}$$

Triplicate samples were analysed for each replicate.

Foam capacity (FC)

Foaming capacity (FC) was determined in triplicate using the method described by Makri, Papalamprou and Doxastakis (2005). Concentrations of 1% flour (w/v) were prepared in de-ionised water and adjusted to pH 7.4 with 1.0 M NaOH and 1.0 M HCl. A volume of 100 ml of the flour suspension (V_I) was blended for 3 min using a commercial Waring blender (Laboratory & Scientific equipment, USA) at high speed, then transfered into a 250 ml graduated cylinder, and the volume of foam (V_F) was immediately recorded.

FC was calculated using the following equation:

$$FC\ (\%) = \frac{V_F}{V_I}$$

Triplicate samples were analysed for each replicate.

Emulsion capacity (EC)

Emulsifying capacity was determined in duplicate according to the method described by Yasumatsu, Sawad, Moritaka, Mikasi, Wada and Ishi (1972). One gram of the flour was suspended in 50 ml distilled water, then 50 ml of refined canola oil was added. The mixture was emulsified with an Ultra-turrax T25 homogeniser (Ika-Labtechnik, Germany) at 9 500 rpm for 1 min. The emulsion obtained was divided evenly into two 50 ml centrifuge tubes and centrifuged at 4 100 g, 5 min, 20 °C (Super Minor Centrifuge, MSE, UK).

EC was calculated as:

$$EC\ (\%) = \frac{\text{Height of emulsified layer}}{\text{Height of whole layer}} \times 100$$

3.2.2.7 Statistical analyses

The experiment (Fig. 3.2.1) was repeated twice and all analyses, except for emulsion capacity and amino acid composition, were replicated three times (n=3) per repeat. All the data was analysed by one-way ANOVA. Mean differences were evaluated at the 5% significance level ($p \leq 0.05$) using the least significant difference test. Correlation coefficients (r) of functional properties were also performed. The analyses were performed using Statistica Version 6.0 (Statsoft, Tulsa, USA).

3.2.3 Results and Discussion

3.2.3.1 Proximate composition

Defatted flour from dry heated whole marama beans (HMF) had a significantly higher protein content and a significantly lower fat content than defatted flour from unheated whole marama beans (UMF) (Table 3.2.2). The lower protein content of UMF was possibly due to its higher fat content. The lower fat content of HMF was possibly due to the disruption of lipid bodies of the marama beans during dry heating at 150 °C/20 min. Dry heating of marama beans consequently allowed the oil to be more readily expelled from the lipid bodies during coarse milling. A similar difference in fat content of defatted flour from dry heated peanuts (12.5%) compared with defatted flour from unheated peanuts (17.0%) was reported by Yu *et al*. (2006). Although the carbohydrates and ash contents of the two flours were statistically different from each other, the differences are not of practical significance. Both HMF and UMF had significantly higher protein contents compared to USF and HSF despite the low fat content of the defatted soya flours. This is probably due to the inherent high protein content of marama beans which is reported to be 37% on a dry basis (Mmonatau, 2005).

On the other hand, USF and HSF had significantly higher carbohydrate contents than UMF and HMF. Similar to defatted soya flours, the high protein contents of UMF and HMF highlight the potential of the flours to be used as protein supplements in composite flours with cereals to improve protein quality.

Table 3.2.2: Effect of dry heating of whole marama beans at 150 °C /20 min on the proximate composition (g/100g, dry basis) of its defatted flour compared with commercial defatted soya flours

Component (%)	UMF	HMF	USF	HSF
Crude protein	52.7^{c}[1](0.3)[2]	56.0^{d} (0.3)	47.4^{b} (0.1)	43.2^{a} (0.1)
Crude fat	7.0^{c} (0.2)	1.9^{b} (0.2)	0.8^{a} (0.01)	0.8^{a} (0.01)
Carbohydrates*	35.2^{a} (0.4)	36.6^{b} (0.3)	44.8^{c} (0.2)	48.9^{d} (0.1)
Ash	5.1^{a} (0.1)	5.5^{b} (0.2)	7.0^{c} (0.3)	7.1^{c} (0.1)

[1] Means within a row with different letters are significantly different ($p \leq 0.05$)
[2] Standard deviations are given in parentheses
* Calculated by difference
NB: Moisture content (g/100 g flour): UMF= 6.7, HMF = 5.4, USF = 4.7 & HSF = 5.6

UMF – Defatted flour from unheated marama beans
HMF – Defatted flour from dry heated marama beans
USF – Commercial defatted unheated soya flour
HSF – Commercial defatted heated soya flour

3.2.3.2 Colour

UMF was significantly lighter (CIELAB L*) than HMF as indicated by the higher L* value of the UMF (Table 3.2.3). Similarly, USF was significantly lighter than HSF (Table 3.2.3). The darker colour of the HMF and HSF was probably due to browning from Maillard-type reaction products, since both flours contain carbohydrates (including reducing sugars) and have high protein contents. However, HMF was significantly lighter than USF possibly because marama bean cotyledons are less pigmented than soya bean cotyledons. In terms of the a* value, HSF was significantly redder, as indicated by the higher a* value, than HMF, USF and HSF respectively (Table 3.2.3).

Table 3.2.3: Effect of dry heating of whole marama beans at 150 °C /20 min on colour (L, a*, b*, C*, h° values) of its defatted flour compared with commercial defatted soya flours

Flour type	L*	a*	b*	h°	C*
USF	89.1[b] (0.5)	5.4[b] (0.1)	10.1[c] (0.4)	62.0[c] (1.1)	11.4[b] (0.4)
HSF	72.2[a] (0.7)	9.2[d] (0.2)	17.5[a] (0.7)	62.2[c] (0.6)	19.8[d] (0.6)
UMF	96.5[d] (0.3)	4.8[a] (0.1)	2.3[b](0.1)	25.5[a] (0.8)	5.3[a] (0.1)
HMF	92.2[c] (0.5)	6.3[c] (0.2)	10.4[c] (0.7)	58.6[b] (1.0)	12.1[c] (0.7)

L* = Lightness (0=black, 100=white), +a* = red-purple, -a* = bluish-green,
+b* = yellow, -b* = blue, h° = Hue angle, C* = Chroma.
[1]Means within a column with different letters are significantly different ($p \leq 0.05$)
[2]Standard deviations are given in parentheses
USF – Commercial unheated defatted soya flour
HSF – Commercial heated defatted soya flour
UMF – Defatted flour from unheated marama beans
HMF – Defatted flour from dry heated marama beans

A similar pattern was observed for the b* value, indicating that HSF was significantly more yellow (CIELAB b*). However, no significant difference was observed between yellowness of HMF and USF (Table 3.2.3). This does not mean that the two flours (HMF and USF) were of the same colour since samples with identical +a* values may exhibit colours ranging from yellow-orange, yellow-orange-reddish and orange-red-purplish red (Voss, 1992). In this case, this is confirmed by the difference in h° and C* values which denotes the colour differences and colour intensity or saturation of the two flours respectively.

The h° for UMF was significantly lower than that of HMF, USF and HSF respectively, indicating the colour was more orange than yellow. The latter is based on the hue sequence and hue-angle orientation on a CIELAB diagram (with ISCC-NBS colour names) (Voss, 1992). The h° for USF and HSF was not significantly different from each other (Table 3.2.3), indicating similar yellowness. However, the yellowness was more intense in the HSF as exhibited by a higher C* value. It must be noted that the colour indices derived from CIELAB measurements do not give an accurate definition of colour

but only measures the colour differences and colour changes during processing and storage (Wrolstad, Durst and Lee, 2005).

3.2.3.3 In-vitro protein digestibility

The *in-vitro* protein digestibility of HMF was 2.7% higher than that of UMF (Table 3.2.4). Similarly, the *in-vitro* protein digestibility of HSF was higher than USF (Table 3.2.4). The improvement in the protein digestibility of flours prepared from heated beans was probably due to the unfolding of the proteins during heating, thus making them more accessible and easier to be hydrolysed by proteases (Hsu *et al*. 1977). Also, heating possibly inactivated trypsin inhibitors, thereby increasing protein digestibility. Adeyeye (1997) reported that heat treatment (boiling) improved the *in-vitro* protein digestibility of African yam bean (dehulled seeds) by 5.43% when compared with raw samples. Hsu *et al*. (1977) made a similar observation with soya flour where *in-vitro* protein digestibility of a 20 protein dispersibility index (PDI) soya flour (82.11% digestibility) increased by 5.01% when compared to a 70 PDI soya flour (77.10% digestibility). The decrease in PDI of the flour from 70 to 20 was due to the high temperature used. Both authors attributed the improved digestibility of the flours to protein denaturation and destruction of protease inhibitors by heat, enabling easier hydrolysis by proteases.

However, the *in-vitro* protein digestibility of defatted marama bean flours was lower compared to that of defatted soya flours possibly due to slower degradation of marama proteins by the multi-enzyme system used. This may suggest that the marama proteins are more folded (stable) than soya proteins and thus were not readily accessible to the enzymes.

Table 3.2.4: Effect of dry heating of whole marama beans at 150 °C/20 min on *in-vitro* protein digestibility of its defatted flour compared with commercial defatted soya flours as determined by the multi-enzyme assay

Flour type	*In-vitro* protein digestibility (%)
USF	85.7[c1] (0.2)[2]
HSF	88.9[d] (0.7)
UMF	76.5[a] (1.3)
HMF	79.2[b] (0.6)

[1] Means within a column with different letters are significantly different ($p \leq 0.05$)
[2] Standard deviations are given in parentheses
USF – Commercial defatted unheated soya flour
HSF – Commercial defatted heated soya flour
UMF – Defatted flour from unheated marama beans
HMF – Defatted flour from dry heated marama beans

The lower protein digestibility of marama flour when compared to soya flour may also be attributed in part to the higher phenolic content of marama bean cotyledon (2.8%) (van Zyl, 2007b) when compared to the phenolic content of whole soya bean (0.4%) (Seunghyn, Hongkeun, Joungkuk, Jungtae, Joonsang & Illmin, 2004). Although there has not been any conclusive evidence, phenolic acids have been reported to form complexes with digestive enzymes and/or dietary proteins, thus lowering their nutritional value (Shahidi & Naczk, 1992). The mechanism involved in the formation of phenolic acid-protein complexes is possibly through hydrogen bonding between the phenolic hydroxyl group and the carbonyl of the peptides of proteins (reversible reaction) or irreversibly by oxidation to quinones, which combine with reactive groups of protein molecules (Kumar & Singh, 1984; Reddy & Pierson, 1985).

3.2.3.4 Amino acid composition

Table 3.2.5 shows the total amino acid content (with the notable exception of tryptophan) of defatted marama bean flour from whole unheated and heated marama beans (UMF and HMF) compared with that of USF and HSF. The dry heating process significantly reduced lysine, threonine, tyrosine, alanine, proline, phenylalanine, valine, glycine,

leucine in HMF. Reductions in leucine (14.29%) and lysine (11.9%) were the highest. Mmonatau (2005) reported similar losses in lysine in flour made from roasted marama beans.

HSF had significantly lower amino acids content than USF with decreases in threonine (14.62%) and lysine (13.27%) being the highest. The lysine contents of USF, HSF and UMF are comparable to the value recommended by FAO/WHO (1991). Lysine has been reported to be the most vulnerable to heat damage due to Maillard browning reactions of the ε-NH_2 of lysine with reducing sugars. This decreases the lysine content and makes the modified lysine to be nutritionally unavailable (Faldet, Satter and Broderick, 1992). The Maillard browning reactions may explain why there are high reductions in lysine content of flours prepared from heated beans. Since lysine is one of the essential amino acids because it is not synthesised by the human body, damage caused by heat treatment could be considered a quality control parameter in heat processing of marama beans to produce flour.

Not surprisingly, the levels of methionine plus cysteine in both UMF and HMF were limiting when compared to the FAO/WHO reference pattern for these sulphur-containing amino acids (FAO/WHO, 1991). Methionine plus cysteine were also found to be limiting in the USF and HSF. It has been reported by Duranti and Gius (1997) that sulphur-containing amino acids (methionine + cysteine) are limiting in legume seeds. Both the UMF and HMF appear to be comparable to USF and HSF respectively in terms of their amino acid profile with the major difference being the high contents of tyrosine and proline in marama bean flours. Similar amino acid profiles have been reported for roasted marama bean flour (Ripperger-Suhler, 1983; Mmonatau, 2005) and soya flour (Friedman and Brandon, 2001). The higher contents of tyrosine and proline in marama flours compared with the soya flours may possibly be due to the presence of glutelin and prolamine protein fractions as identified by Bower *et al.* (1988) in marama beans. These are not found in soya beans. It is widely known that the gluten protein of wheat contains tyrosine and proline amino acids residues (Wieser, 2007).

Table 3.2.5: Effect of dry heating of whole marama beans at 150 °C/20 min on the amino acids content (g/100 g flour, dry basis) of its defatted flour compared with commercial defatted soya flours

Amino acid	UMF[3]	HMF	USF	HSF
Acidic side chains:				
Aspartic	5.12[c1] (0.22)[2]	4.60[b] (0.02)	4.51[b](0.07)	3.99[a] (0.14)
Glumatic	8.58[a] (0.33)	8.22[a] (0.01)	8.74[a](0.05)	8.01[a] (0.16)
Sub-total	13.70	12.82	13.25	12.00
Basic side chains:				
Histidine	1.57[c] (0.08)	1.44[b] (0.01)	1.42[b](0.02)	1.27[a](0.01)
Arginine	4.02[c] (0.13)	3.93[bc] (0.00)	3.72[b](0.01)	3.25[a](0.11)
Lysine	3.17[b] (0.06)	2.79[a] (0.05)	3.09[b](0.02)	2.68[a](0.05)
Sub-total	8.76	8.16	8.23	7.21
Polar side chains:				
Serine	3.11[d] (0.13)	2.90[c] (0.01)	2.52[b](0.01)	2.23[a](0.06)
Threonine	1.70[b] (0.02)	1.52[a] (0.01)	1.71[b](0.01)	1.46[a](0.06)
Tyrosine	6.20[d] (0.01)	6.00[c] (0.06)	1.79[b](0.01)	1.66[a](0.00)
Cysteine	0.03[a] (0.03)	0.05[a] (0.00)	0.15[b](0.00)	0.12[b](0.01)
Sub-total	11.04	10.47	6.17	5.47
Non-polar side chains:				
Alanine	2.00[b] (0.05)	1.79[a] (0.07)	2.16[c](0.01)	1.97[b](0.01)
Proline	4.29[d] (0.04)	4.07[c] (0.01)	2.53[c](0.03)	2.25[b](0.10)
Valine	2.72[d] (0.06)	2.48[c] (0.01)	2.34[b](0.01)	2.14[a](0.04)
Methionine	0.46[a] (0.02)	0.41[a] (0.06)	0.71[b](0.00)	0.68[b](0.01)
Isoleucine	2.46[c] (0.06)	2.37[c] (0.02)	2.17[b](0.04)	2.00[a](0.06)
Phenylalanine	2.65[c] (0.04)	2.55[b] (0.01)	2.67[c] (0.04)	2.36[a](0.01)
Glycine	3.47[d] (0.11)	3.30[c] (0.00)	2.16[b](0.00)	1.94[a](0.01)
Leucine	3.64[b] (0.06)	3.12[a] (0.02)	3.86[c](0.03)	3.53[b](0.08)
Sub-total	21.69	20.09	18.6	16.91
Total	**55.19**	**51.54**	**46.25**	**44.29**

[1] Means within a row with different letters are significantly different ($p \leq 0.05$) of two determinations (n=2)
[2] Standard deviations are given in parentheses
[3] g amino acid/100 g flour

UMF – Defatted flour from unheated marama beans
HMF – Defatted flour from dry heated marama beans
USF – Commercial defatted unheated soya flour
HSF – Commercial defatted heated soya flour

Based on the values reported in Table 3.2.5, marama flours had much higher levels of amino acids with polar side chains compared to soya flours. However, all the flours (UMF, USF, HSF, HMF) had comparable total values of amino acids with hydrophilic side chains, which were much higher than the values of amino acids with hydrophobic side chains. This is an indication that marama bean and soya bean proteins are predominantly hydrophilic. This ratio between the hydrophilic and hydrophobic amino acids groups can partly affect the emulsifying and foaming properties of the flours because they depend on a proper balance between hydrophilic and hydrophobic amino acid groups (Morr, 1990).

3.2.3.5 Nitrogen Solubility Index (NSI)

The NSI at pH 7.0 of the four flours studied are shown in Table 3.2.6. Dry heating of whole marama beans reduced the NSI of its defatted flour by 28.6%. A similar trend in reduction of NSI was observed when USF was compared to HSF. The decrease in NSI is most probably due to protein denaturation followed by a subsequent increase in surface hydrophobicity and aggregation of proteins through hydrophobic, electrostatic and disulphide interactions (Kinsella, 1979; Morr, 1990). The reduction in NSI due to heat processing has been reported in the case of peanut flour produced by roasting (Yu *et al*., 2007), cowpea flour produced by micronisation (Mwangwela, Waniska and Minnaar, 2007) and low fat soya flour produced by extrusion-expelling system (Heywood, Myers, Bailey and Johnson, 2002).

The lower NSI observed for USF compared UMF was probably due to the fact that USF, although described as an “untoasted” flour by the supplier, was slightly toasted during conditioning and after solvent extraction to inactivate the lipoxygenase enzymes and evaporate hexane, a standard procedure normally practiced in the soya industry (Milligan, 1981; Wright, 1981; Personal communication - Diederiks, 2008; Manager, Nedan Oil Mills (Pty) Ltd, South Africa). Also, since toasting involves moist heating by steam, this has a significant effect in reducing the solubility of soya proteins when compared to dry heating (Kinsella, 1979; Milligan, 1981). It is difficult to compare the NSI of USF and HSF to those values found in literature because researchers use a variety of methods; conditions used in laboratories are different and samples used were processed differently.

Proteins with low solubility values have limited functional properties (Kinsella, 1979) because protein solubility affects other protein-functional properties such as emulsification and foaming (Morr, 1990).

Table 3.2.6: Effect of dry heating of whole marama beans at 150 °C/20 min on protein-related functional properties of its defatted flour compared with commercial defatted soya flours

Flours	NSI (%, db, pH 7.0)	WAC (g/g flour, db)	OAC (g/g flour, db)	FC (%, db, pH 7.4)	EC (%, db)
USF	63.5^{b1} (3.8)	4.3^{c} (0.0)^{2}	1.8^{b} (0.1)	54.9^{c} (1.5)	53.2^{c} (0.8)
HSF	45.5^{a} (3.9)	4.0^{d} (0.1)	1.5^{a} (0.1)	9.5^{a} (1.1)	15.9^{b} (0.7)
UMF	74.8^{c} (6.6)	1.5^{a} (0.0)	2.7^{d} (0.1)	31.1^{b} (1.1)	59.9^{d} (0.7)
HMF	46.4^{a} (6.4)	2.4^{b} (0.1)	2.4^{c} (0.1)	30.7^{b} (1.1)	4.2^{a} (0.4)

[1] Means within a column with different letters are significantly different ($p \leq 0.05$)
[2] Standard deviations are given in parentheses
db – dry basis
USF – Commercial defatted unheated soya flour
HSF – Commercial defatted heated soya flour
UMF – Defatted flour from unheated marama beans
HMF – Defatted flour from dry heated marama beans

3.2.3.6 Water absorption capacity (WAC)

Dry heating of marama beans increased the WAC of marama bean flour significantly (Table 3.2.6). Although not of practical significance, an increase in WAC was observed when HSF was compared with USF. This was probably due to unfolding of the protein molecules upon heating, which exposed previously buried hydration sites, thereby making them available to interact with water (Hutton and Campbell, 1981), resulting in increased WAC. Similar improvement in WAC of heat-treated cowpea flour compared to unheated cowpea flour has been reported by Giami (1993). The WAC of USF compares reasonably well with reported values for raw soya flour (Giami, 1993). However, no values of WAC for marama bean proteins have been reported in literature.

However, WAC values of USF and HSF were both double that of HMF (Table 3.2.6). This may be explained in part by the fact that approximately 90% of the soya proteins are globulins (Kinsella, 1979) as opposed to 53 % globulins in marama proteins (Bower *et al.*, 1988). These storage proteins (globulins), which consist mainly of the 7S (conglycinin) and 11S (glycinin) have more polar-charged amino acids oriented toward the surface and this facilitates hydration (Kinsella, 1979; Duranti and Gius, 1997). Since they are more concentrated in soya proteins than in marama proteins, they are possibly largely responsible for the high WAC of HSF and USF. Although UMF had a higher value of amino acids with hydrophilic chains than USF and HSF (Table 3.2.4), it still had a lower WAC. Therefore, predictions made on the basis of the amino acid classification in Table 3.2.5 alone may not always provide a true reflection of the protein functional properties. On the other hand, the higher content of carbohydrates in the USF and HSF compared to HMF (Table 3.2.2) could have also contributed to the high WAC observed. Carbohydrates are hydrophilic and therefore absorb and retain water (Fennema, 1996). It must be noted that UMF and HMF had a higher fat content than USF and HSF (Table 3.2.2). The presence of this additional fat (a hydrophobic material) could result in less available hydrophilic binding sites available for water holding by the protein.

3.2.3.7 Oil absorption capacity (OAC)

Although not of practical significance, dry heating of marama beans decreased the OAC of marama bean flour. A similar decrease in OAC was observed when USF was compared with USF (Table 3.2.6). It has been reported that the greater the amount of heat treatment that is given to a protein, the more hydrophobic the protein becomes as a result of a greater number of hydrophobic groups being exposed through unfolding of the protein molecules (Nakai, 1983). The results obtained from this study show a trend that deviates from this accepted theory. Similar observations were also reported for autoclaved and oven-dried cowpea flour (Giami, 1993), micronised cowpea flour (Mwangwela *et al.,* 2007), roasted peanut flour (Yu *et al*., 2006) and low fat soya flour (Heywood *et al*., 2002). The decrease in OAC was attributed to irreversible protein denaturation caused by heating which might have destroyed both hydrophilic and hydrophobic groups of the proteins.

The higher OAC of UMF may partly be related to the fact that UMF contains more amino acids with non-polar side chains, thereby contributing to increased oil absorption. Also, the higher fat content of UMF (7.0%) compared to that of HMF, USF and HSF could have contributed significantly to the high OAC of UMF due to increased lipid-lipid interactions. The higher OAC exhibited by UMF and HMF was possibly partly due to the fact that these flours had higher protein content than USF and HSF. It has been reported (Kinsella, 1979; Hutton and Campbell, 1981) that the percent of fat absorption of soya protein preparations increased as protein concentration was increased; fat absorption (%) of soya flour was 49% lower than that of soya concentrate. A similar trend was observed by Carvalho *et al*. (2006) when comparing OAC of defatted cupuassu (*Theobroma grandiflorum* Schum) seed flour to its protein concentrate. The mechanism of oil absorption by proteins has not yet been explained although oil absorption is attributed to the physical entrapment of the oil by the protein (Zayas, 1997).

3.2.3.8 Foaming capacity (FC)

Dry heating of marama beans did not have a significant effect on the foaming capacity of marama bean flour. UMF and HMF had similar low FC at pH 7.4 (Table 3.2.6) despite their higher NSI compared to USF and HSF respectively. The residual oil in the defatted marama bean flours could have negatively affected their FC by destabilising the protein films surrounding the air droplets (Kinsella, 1976) and causing the foam to collapse. A similar trend in reduction of FC due to lipids has been reported in case of beer foam and it was attributed to the damage of the protein stabilised interface and reduction in surface elasticity of the protein films by the lipids (Clark, Wilde and Marion, 1994). A poor positive correlation ($r = 0.31$) between foaming properties and nitrogen solubilities of marama bean flours was observed (Table 3.2.8). This observation deviates from the accepted theory that protein solubility is positively correlated with foaming (Kinsella, 1979, Nakai, 1983). This may partly suggest that the marama proteins are more rigid or folded than the soya proteins; hence they exhibited a lower foaming capacity. Since foam formation relies on the ability of the proteins to quickly unfold and adsorb at the interfacial region (Cherry and McWatters, 1981), the partial unfolding and rearrangement of marama proteins in the interface was possibly slower compared to soya proteins. This

difference in adsorption behaviour may also probably be due to a difference in molecular functional properties such as for example the exposed hydrophobicity.

The foaming capacity of USF at pH 7.4 was significantly higher than that of HSF (Table 3.2.6). The low FC observed for HSF may possibly be due to the fact that heating denatured the soya proteins, thus promoting the formation of protein aggregation through hydrophobic and disulphide interchange bonding mechanisms (Morr, 1990). It is well known that for a protein to have good foaming properties, it has to be very soluble because foam capacity requires rapid adsorption of protein at air-water interface during whipping, penetration into the surface layer and re-organisation at the interface (Were, Hiettiarachchy and Kalapathy, 1997). HSF had lower NSI at pH 7.0 than USF and hence a lower FC. A similar trend in reduction of FC due to heating and/or low protein solubility has been reported for defatted roasted peanut flour (Yu *et al*., 2007). Significant positive correlations (r = 0.96) between foaming properties and nitrogen solubility in soya flours (Table 3.2.7) have been reported for soya flours (Kinsella, 1979) and cowpea flour (Abu, 2005).

3.2.3.9 Emulsifying capacity (EC)

Dry heating of marama beans significantly decreased the EC of marama bean flour (Table 3.2.6). A similar trend was observed when USF was compared with HSF. The low EC observed for the HMF and HSF was possibly partly due to the lower NSI of HMF and HSF compared to UMF and USF, respectively. It has been reported in literature that emulsion capacity generally depends directly on protein solubility (Carvalho *et al*., 2006). This is because emulsion formation depends on the rapid adsorption, unfolding and reorientation of the proteins at the oil-water interface; thus proteins with low solubility have a decreased capacity to act as surface-active agents and adsorb at the oil/water interface (Morr, 1990). Significant positive correlations were observed between EC and NSI in both soya flours and marama bean flours (Table 3.2.7 and Table 3.2.8).

Table 3.2.7: Correlation coefficients (r) for protein-functional properties of commercial defatted soya flours (USF and HSF)

Functional property	NSI	WAC	OAC	FC	EC
NSI	1.00	0.81*	0.80*	0.96*	0.94*
WAC	0.81*	1.00	0.79*	0.90*	0.93*
OAC	0.80*	0.79*	1.00	0.88*	0.91*
FC	0.96*	0.90*	0.88*	1.00	0.99*
EC	0.94*	0.93*	0.91*	0.99*	1.00

Values with asterisks (*) are significantly correlated ($p \leq 0.05$)
USF – Commercial defatted unheated soya flour
HSF – Commercial defatted heated soya flour

Table 3.2.8: Correlation coefficients (r) for protein-related functional properties of defatted marama bean flours (UMF and HMF)

Functional property	NSI	WAC	OAC	FC	EC
NSI	1.00	-0.90*	0.72*	0.31	0.86*
WAC	-0.90*	1.00	-0.86*	-0.28	-0.99*
OAC	0.72*	-0.86*	1.00	0.01	0.87*
FC	0.31	-0.28	0.01	1.00	0.27
EC	0.86*	-0.99*	0.87*	0.27	1.00

Values with asterisks (*) are significantly correlated ($p \leq 0.05$)
UMF – Defatted flour from unheated marama beans
HMF – Defatted flour from dry heated marama beans

The high residual oil of UMF may also account for its higher EC when compared with USF possibly because as the oil content increases, the hydrophobicity of the flour increases and it allows a greater amount of oil to be emulsified (Heywood *et al*., 2002). Protein surface hydrophobicity has been reported to be positively correlated with emulsifying capacity (Nakai, 1983).

3.2.4 Conclusions

Dry heating of whole marama beans (150 °C/20 min) affects the proximate composition, colour, in-vitro protein digestibility, amino acid composition and protein-related functional properties of defatted marama bean flour in a statistically significant way. The protein content of defatted marama flour from dry heated whole marama beans is higher than that of defatted marama flour from unheated beans because oil is readily expelled from heated beans during coarse milling. The defatted marama bean flours have higher protein content but lower fat content than commercial defatted soya flours. The L* value of defatted marama flour from dry heated whole marama beans (HMF) and commercial defatted flour from heated soya beans (HSF) are significantly lower than that of UMF and USF, respectively, partly due to the Maillard browning reactions. On the other hand, heating significantly increases the in-vitro protein digestibility of defatted marama bean and commercial defatted soya bean flours. However, heating generally reduces the amino acids content of defatted marama bean and commercial defatted soya bean flours. Heating significantly decreases protein-related functional properties of defatted marama bean and commercial defatted soya bean flours such as protein solubility, emulsifying capacity and oil absorption because of protein denaturation and/or protein cross-linking. However, other flour components such as fat influence functional properties such as foaming capacity by destabilising the protein films surrounding the air droplets. Due to its high protein content, HMF can be mainly used to improve the nutritional value of cereal-based foods such as sorghum/maize flours and bakery products. The UMF can enhance nutritional quality of food products, since it provides high protein content and good functional properties (NSI, EC and OAC).

4 DISCUSSION

This chapter is divided in three sections. The first section discusses the principles of the major methods used in this study, namely lipoxygenase detection, trypsin inhibitor activity, *in-vitro* protein digestibility and selected protein-related functional properties of defatted marama bean flour. The problems encountered in the experimental work together with the strengths and weaknesses of the methods will also be discussed. The second section discusses the lab-scale procedure for manufacturing defatted protein-rich flour from whole dry heated marama beans and its potential adoption by Small and Medium Enterprises (SME's). The last section discusses the effect of dry heating on physico-chemical and protein-related functional properties of defatted marama flour and its potential application in food systems.

4.1 Critical evaluation of experimental design and methodologies used

In this study, marama beans harvested in June 2006 in Ghanzi area, Botswana were used because the quantity of beans harvested in June 2007 was not enough. This was due to abundant rainfall which possibly facilitated growth of moulds on the seeds. Since the marama beans are collected by locals from the wild during the harvesting period, it appears this request was not well communicated to the collectors in time. This might also have contributed to the low quantity of beans harvested in 2007. The main limitation encountered with sourcing of the marama beans was that the beans were not easily available in sufficient quantities. This is probably because they are seasonal and grow in the wild. Thus due to the limited beans available, all the tests conducted on the flours were repeated only twice.

Initially, the extrusion-expelling system was considered for the processing trials of defatted marama bean flour because it relies on the mechanical extraction of the oil without use of any chemicals in the extraction process (Heywood *et al*., 2002). However, this technology requires a lot of raw material for processing trials and could not be used. Instead, dry heating of marama beans using a continuous forced convection roaster and defatting by solvent extraction was used in this study.

Commercial unheated and heated defatted soya flours obtained from Nedan Oil Mills (Pty) Ltd (South Africa) were used as reference samples in the study of physico-chemical and protein-related functional properties because soya flour is the most common legume that is used in food systems as a functional ingredient (Wolf, 1970; Dubois and Hoover, 1981) and for improving protein quality (De Valle, 1981). Defatted soya flours are used in a wide variety of food applications such as bakery, meat and beverage products to impart desirable functional properties and enhance consumer acceptability. Defatted marama bean flour has a potential to be used in some of these food systems if it exhibits similar protein-related functional properties. Furthermore, the basic process of manufacturing defatted soya flour which involves heat treatment, dehulling, oil extraction, and milling can be modified and adopted for manufacturing of defatted marama bean flour.

Since marama bean oil contains a high percentage of unsaturated fatty acids (Ketshajwang *et al.*, 1988), marama bean flour would be susceptible to oxidative rancidity and this may present a storage problem. This could reduce the shelf-life of the flour. Lipid oxidation reactions can be catalyzed by enzymes, particularly lipoxygenase (Yoon and Klein, 1979; Nawar, 1996). This enzyme is present in the seeds of most leguminous plants such as soya beans (Axelrod *et al*., 1981; Hildebrand, Versluys and Collins, 1991), peanuts (St. Angelo *et al.,* 1979) and winged beans (Gordon and Mtebe, 1987).

In this study, marama and soya flours prepared from unheated beans were tested for three lipoxygenase iso-enzymes, designated L-1, L-2 and L-3, using a visual judging method (Suda *et al.,* 1995). These three lipoxygenase iso-enzymes have been isolated from soya beans and have pH optima at 9.0, 6.0 and 6.6 respectively when using sodium linoleate as substrate (Suda *et al.,* 1995). This method is based on the principle that L-1 and L-2 are able to bleach methylene blue (Toyosaki, 1996) while L-3 plus L-2 is able to bleach β-carotene (Hildebrand and Hymowitz, 1982) in the presence of linoleic acid as a substrate. The chemical basis of the bleaching is possibly through the abstraction of hydrogen from the hydroperoxide isomer formed during lipid oxidation by methylene and β-carotene,

thereby leading to the reduction (decolourising) of these two compounds (Hildebrand and Hymowitz, 1982; Toyosaki, 1996).

This method is simple, rapid, selective and can be effectively used as a routine test for lipoxygenases in oilseeds. Unheated beans were used because lipoxygenase is easily inactivated by roasting at 100 °C /15 min (St. Angelo *et al.,* 1979). The mortars and pestles used to mill the flours were chilled to avoid inactivating the lipoxygenase iso-enzymes by heat generated during grinding as the iso-enzymes are heat sensitive.

The L-3 test had a limitation in that the method recommended the addition of a soya bean extract containing L-2 iso-enzyme alone (which was not available) to the reaction mixture for a more sensitive test for detecting the L-3 iso-enzyme (Suda *et al*., 1995). Unfortunately this was not available at the time, and therefore the test for L-3 was incomplete. The soya flour samples were able to bleach methylene blue and β-carotene, confirming the presence of lipoxygenase iso-enzymes in soya beans as reported by Axelrod *et al.* (1981) and Suda *et al.* (1995). However, marama bean flour did not bleach methylene blue and β-carotene, indicating the absence of lipoxygenase iso-enzymes in marama beans. In order to determine if the age of the marama beans could have affected the activity of the lipoxygenase iso-enzymes, the test was conducted on fresh marama beans harvested in June 2008. The results obtained indicated the absence of L-1 and L-2 in marama beans while the results for L-3 were unclear because the test was incomplete. This may imply that marama bean flour would not be susceptible to oxidative rancidity catalysed by L-1 and L-2 iso-enzymes in particular.

The L-1 and L-2 iso-enzyme results were further confirmed by measuring the absorbance of the reaction mixture at 660 nm over a period of time. Another method that can be used to determine lipoxygenase activity involves the use of an oxygen electrode to measure the rate of oxygen consumption (Buranasompob *et al*., 2006). However, this method could not be used because of the unavailability of the equipment and also it is not able to distinguish between the different lipoxygenase iso-enzymes. However, it is recommended in future to test for the presence of other enzymes such as lipase and L-3. If present in

marama beans, lipase could possibly convert triglycerides found in oils to free fatty acids which can increase the rate of lipid oxidation (Nawar, 1996).

A spectrophotometric method (AACC method 22-40) with a few modifications was used to determine the trypsin inhibitor activity of defatted marama bean flours prepared from whole marama beans dry heated at three different temperatures for 20 min. Defatted marama flour prepared from unheated marama beans was used as control while defatted soya flour prepared from unheated soya beans was used as a reference sample. Soya flour prepared from unheated soya beans was used a reference sample so that results obtained for this sample could be easily compared with those found in literature and also because it was the first time this method was used on a marama bean product. This method uses N-benzoyl-$_{DL}$-arginine *p*-nitroanilide as a substrate for porcine trypsin, and the ability of aliquots of flour extract to inhibit the activity of trypsin towards this substrate is used to estimate the amount of trypsin inhibitor in a flour sample (Kakade, Simons & Liener, 1969; AACC Method 22-40, 1991).

Since the method recommends that the aliquots used should be diluted to the point where 1 ml of the dilute extract produces trypsin inhibitor activity of 40-60% to reduce relative standard deviation, trial dilutions of 1:20 and 1:50 (v/v) were tested on the samples. It was found that the 1:20 and 1:50 dilutions were acceptable for soya and marama bean flours, respectively because they produced a trypsin inhibitor activity of 40-60%. A 1:20 dilution initially used for marama sample was too concentrated and no reading was recorded by the spectrophotometer at 410 nm for the five different levels of the marama bean flour extract used. This indicated that marama beans are rich in trypsin inhibitors. This method was modified by filtering the sample extract before beginning the assay to make it less time consuming. It has been reported by Stauffer (1990) that filtering the soya extract before running the assay does not change the concentration of trypsin inhibitor. Although this method is used widely, it is very difficult to obtain reproducible results possibly because of the inconsistent extraction of trypsin inhibitor. Furthermore, each assay requires trial dilutions, attempting to arrive at a dilution such that the standard aliquot of 2 ml would give 40-60 % inhibition of trypsin.

Another method that can be used to determine the trypsin inhibitor activity in legume flours involves the addition of trypsin to a substrate-inhibitor mixture in a pH stat at pH 9.0. The amount of base added per unit time to keep the pH at 9.0 is used as a measure of tryptic hydrolysis (Hill *et al*., 1982). However, this pH stat method is not widely used and thus it is difficult to compare results.

In-vitro methods for estimating protein digestibility include dialysis cell method, 'filtration' method, pH-drop and pH-stat methods. The dialysis cell method involves digestion of proteins with pepsin with continuous removal of low-molecular-weight products by dialysis but the procedure is complicated and time consuming (Boisen & Eggum, 1991). The 'filtration' methods involve the use of either a single-enzyme (pepsin), two-enzyme (pepsin and trypsin) or multi-enzyme (pepsin, pancreatin and rumen fluid) systems. However, the single-enzyme method has been found to underestimate protein digestibility in dry beans because legume proteins are more complex (Rombo, 2002) while the other two procedures are not widely used. The pH-drop and pH-stat methods are reliable, rapid, and have good correlations with *in-vivo* values for proteins of plant origin determined in rats (Hsu *et a*l., 1977; Pedersen and Eggum, 1983). These methods involve the use of three enzymes, namely trypsin, chymotrypsin and an intestinal peptidase (Pedersen and Eggum, 1983). The methods are based on the principle that during proteolysis, protons are released from the peptides, resulting in a decrease in pH in a protein suspension (Boisen and Eggum, 1991). The pH-stat method requires the use of an automatic titration apparatus to keep the protein suspension at pH 8.0 during the incubation period to avoid variations. This apparatus was not available and the pH-drop method was used in this study. The pH-drop method is simple and was sensitive enough to detect the possible effects of trypsin inhibitors and heat treatment on protein digestibility of legume proteins (Hsu *et al*., 1977). The main problem that was encountered with the pH-drop method was that it is not possible to keep the pH of the sample suspension uniform at pH 8.0 by using a bench top microprocessor pH meter because it is difficult to manually dispense appropriate quantities of 0.1 M NaOH or HCL. It is possible that enzyme catalysis would not take place at the same pH which may cause variation in the results because the activity of enzymes is pH specific

(Fennema, 1996). However, in this study, the standard deviation was within acceptable limits.

For nitrogen solubility index (NSI), the slower-stirring technique, described in AACC Method 46-23 (AACC, 1999), was used in this study because of the availability of apparatus and the fact that it uses a small sample size (1 g). However, this method was modified by dissolving the flour samples using 0.1 M NaCl instead of distilled water because globulins are the predominant proteins in marama and soya beans (Bower *et al*., 1988; Gueguen, 1983) and are soluble in dilute salt solutions (Fennema, 1996). The use of a shaker at speed 4 (1024 shaking water bath, Tecator, Sweden) for 1 h at 30 °C with the pH maintained at 7.0 yielded reproducible results as opposed to using a magnetic stirrer because the speed and temperature test conditions were easily controlled. However, there were slight variations in pH of the protein suspensions because it was difficult to manually dispense appropriate quantities of 0.1 M NaOH or HCL to keep the pH of the samples uniform. This may affect protein solubility because of the pH-solubility dependence of most plant proteins (Vojdani, 1996; Moure *et al*., 2006). Higher standard deviations for NSI values were observed in this study compared to Abu (2005). Abu (2005) used an automated titrating unit with interchangeable unit operated by a Tinet 2 software programme to keep the pH of samples uniform. This prevented variations in NSI values of same samples and hence reduced the standard deviation. Abu (2005) and Mwangwela *et al*. (2007) used this modified NSI method to determine the protein solubility of irradiated and micronised cowpea flours at pH 7.0 respectively. The determination of the protein solubility of the flours over a wider pH range of 2.0-12.0 could have provided a better explanation about the effect of pH on the solubility of their proteins.

Another method that can be used for determining protein solubility of protein-containing samples is the protein dispersibility index (PDI). The method involves stirring a protein sample in distilled water in a Waring blender at 8500 rpm at 25 °C (Vojdani, 1996) followed by centrifugation and determination of the amount of protein in the supernatant and slurry. Then PDI % is calculated by dividing the % water dispersible protein by % total protein multiplied by 100. Since the marama bean flours were limited, this method

could not be used because it requires a relatively large sample size (20 g) and also the speed of the Waring blender used could not be adjusted to 8500 rpm. Since the Waring blender can generate heat during fast-stirring, this may possibly reduce protein solubility of the samples by partly denaturing the proteins. It must be noted that it is difficult to compare results of one researcher to the other because of the different conditions (pH, stirring speed and duration) and samples used in tests.

Major absorption/rehydration techniques for determining the water absorption of a protein powder ingredient include the excess water, water saturation and swelling methods. The excess water method is the most frequently used for measuring water uptake by protein powder ingredients such as whey protein, casein, soya bean and wheat flours (Hutton and Campbell, 1981) and it was used in this study. In this method, the sample is mixed with excess water and centrifuged to separate bound water from free water. The amount of water retained, usually determined as the weight gain, is reported as water absorption expressed as a percentage of the dry sample weight (AACC method 56-20, 2000). Challenges that were encountered in this method were that low density components of the flours floated on the supernatant surface and this may have lowered the weight gain of sample. Also, protein and carbohydrates that solubilised during mixing were discarded with the supernatant and are no longer available to be hydrated thus possibly affecting measurement. Notwithstanding the limitations discussed above, the excess water method was able to provide very valuable information regarding the effect of dry heating on the water absorption capacity of the flours. In the water saturation method, just enough water is added to saturate the sample but not to cause a liquid phase (AACC method 56-30, 2000) and this eliminates the problem of solubilised protein and carbohydrates. However, this method uses a large sample size (60 g per replicate) and could not be used because of the limited availability of the test sample. The swelling method involves dusting a small amount of sample on a wet filter paper and following the uptake of water in a stationary capillary but it is difficult to reproduce the test conditions (Hutton and Campbell, 1981).

The method of Chakraborty (1986) was used to determine the fat absorption capacity of the marama bean and soya bean flours. The method involves the addition of excess

vegetable oil to the flours, mixing and holding, centrifuging and determining the amount of absorbed oil (total oil – supernatant oil). This technique is similar to the one previously described for water absorption. However, its main limitation is that because of the viscous nature of the oil, not all of it is totally transferred to the measuring cylinder. The oil stuck to walls of centrifuge tubes, and therefore fat absorption capacity may be overestimated. This limitation was reduced by tipping the centrifuge tubes several times to dispense the oil stuck to the walls of the tube.

In this study, the method described by Yasumatsu *et al.,* (1972) was used to determine the emulsifying capacity of marama bean and soya bean flours. The method involves vigorously stirring equal volumes of aqueous solution of protein samples and vegetable oil, centrifuging and measuring the height of the emulsified layer relative to the whole layer. Initially, a centrifuge with fixed angle rotor was used but it was observed that the emulsified layer was slanted. It was therefore difficult to accurately measure the height of the emulsified layer. This problem was solved by using a centrifuge with a swinging bucket rotor which produced an emulsion layer that was easy to measure. The centrifuge with swinging bucket rotor allows the tubes to hang on hinges so that tubes reorient to the horizontal as the rotor initially accelerates while the fixed angle rotor holds tubes in cavities screwed at a predetermined angle hence the slanted emulsion. Another popular method that can be used to determine emulsifying capacity involves the continuous addition of oil to a protein dispersion of known concentration while vigorously mixing until the emulsion breaks down (Cherry and McWatters, 1981; Hill, 1996). The volume of oil required to reach this "break-point", which is revealed by an abrupt change in viscosity, is expressed as the emulsion capacity of the protein sample. However, this method is very sensitive because small variations in technique, equipment, blender speeds, temperature and rate of oil addition can affect the emulsifying properties of proteins (Cherry and McWatters, 1981). Since a standardised test does not exist for determining emulsifying properties of protein samples, this makes it difficult to compare results from different studies.

Whipping is the most commonly used method for foam generation by proteins. This method involves whipping protein suspensions of known concentrations and pH in a

Waring blender for a specified period of time. After blending, the whipped protein suspension is transferred to a graduated cylinder and the increase in volume is expressed as foam capacity. Whipping produces stable foams and if carefully controlled, the results obtained are highly reproducible (Wilde and Clark, 1996). One limitation of this method is that some of the foam is left in the blender when transferring the foam to the measuring cylinder and this could result in the underestimation of foam capacity. The foam left in the blender was carefully transferred to the measuring cylinder using a long spatula. Another limitation of this method is that the foams are stiff and tend not to form a flat top surface in the measuring cylinder, so it is not easy to record the actual volume of the foam. Many workers have employed this method in the study of foam capacity of legume flours (Giami, 1993; Giami and Isichei, 1999; Obatolu and Cole, 1999; Onimawo and Akpojovwo, 2006; Seena and Sridhar 2005 and Obatolu *et al*., 2007). However, the researchers used different conditions (e.g. protein concentration, blender speed, time of blending, pH and temperature) and these influence the foaming characteristics of proteins. Thus because of the variety of conditions used, it is difficult to compare data from different sources.

In a preliminary experiment, the rapid visco analyser (RVA) was used to determine the pasting properties of defatted marama bean flour. The RVA did not give any viscosity measurements when a flour suspension of 10% (m/v) concentration in water was used because the defatted marama bean flour settled at the bottom of the metal container to form dough-like strands which coiled around the spindle paddle during mixing with water. Although a low viscosity reading was observed when a flour suspension of 20% (m/v) was used, this still did not give a better understanding of the possible pasting properties of the defatted marama bean flour. The RVA did not give any viscosity measurements at low viscosity possibly because of the absence of starch in marama beans (van Zyl, 2007a). Since viscosity measurements were not feasible on the flour, rheological properties could rather be conducted when the flour is used in a food system, e.g. composite porridges of sorghum/maize and marama bean flours.

4.2 Critical review of the process used for preparing defatted marama bean flour for use by SME's

It is a well-known fact that the inactivation of the trypsin inhibitor by heating improves the nutritional value of soya beans (Liener, 1981). Since marama beans contain trypsin inhibitors, it was important to optimise the dry heating process to inactivate trypsin inhibitors in whole marama beans. The 150 °C/20 min treatment was effective in inactivating most of the trypsin inhibitor in whole marama beans. This temperature/time combination was used for dry heating whole marama beans using a forced convection continuous tumble roaster (Roastech (www.roastech.co.za), South Africa). The control system of the roaster (Fig. 4.2.1) is used to set the temperature and speed of the screw conveyer. The speed of the screw conveyer determines the holding time the material would be subjected to at the set temperature and this makes the equipment versatile because different types of oilseeds can be dry heated using it. That is, it allows the operator to determine the heating time of different raw materials through trial production runs based on their size and weight by adjusting the speed of the screw conveyor.

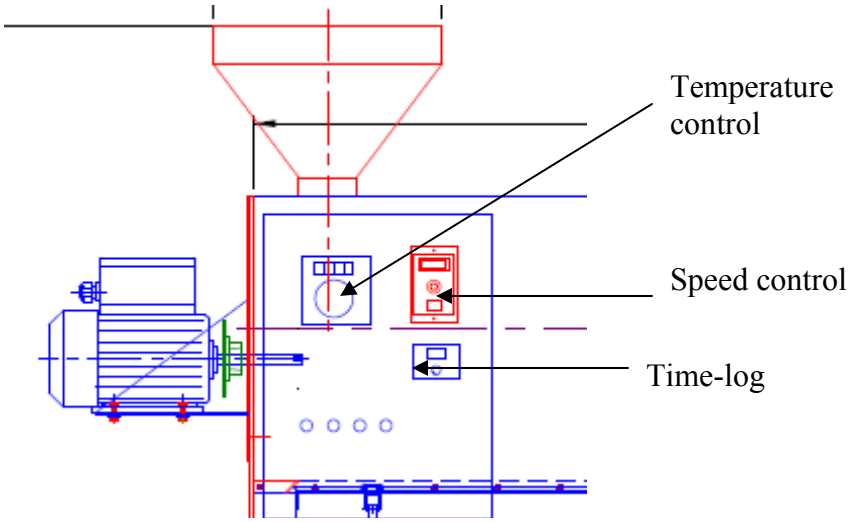


Figure 4.2.1: Drawing of the forced convection continuous roaster control system used for drying heating whole marama beans (Personal communication - Teseling, 2007; Director, Roastech (Ltd) Pty, South Africa)

The continuous roaster is suitable for SME's because it is affordable [R39 330.00 ($ 4 013.27) (21/05/2008)], effective, low maintenance, requires minimal attendance and can be used for dry roasting other oilseeds such as peanuts, sunflower and macadamia (Personal communication – Teseling, 2007; Director, Roastech (Ltd) Pty, South Africa). Since this continuous roaster can process a maximum of 20 kg of material per hour, it is suitable for SME's because of the low material quantity it can process.

As it was time consuming and laborious to manually crack marama beans because their seed coat is very hard, a macadamia cracker developed and built by WMC Metal Sheet Works, Tzaneen, South Africa) was modified to crack marama beans. At a cost of R7 500.00 ($ 765.31) (10/12/2007), the equipment is relatively inexpensive and can be afforded by SME's. The major limitation with this equipment was that it could not fully crack all the marama beans. The cracking process had to be repeated up to three times to completely crack the beans. Furthermore, it was difficult to sort very small pieces of cotyledons and seed coats. As such these were discarded as waste, thereby decreasing the yield. The cracker may be modified by installing an aspirator unit which can effectively separate cotyledons from the seed coats by air separation. In an SME set up, the seed coats and the small pieces of cotyledons can be milled into a fine powder by a grain grinder and supplied to nutraceutical companies because they are rich in phenolic compounds (van Zyl, 2007). These compounds are known to exhibit antioxidant activity (Fennema, 1996) and can be of health benefit. Alternatively, the seed coat powder can be introduced into existing foods to improve nutritional quality provided that its components are not toxic.

During the lab-scale operations, cotyledons were coarsely ground using a Waring blender and defatted by solvent extraction to obtain a defatted meal. Losses in the flour were experienced here because some of the flour was decanted with the hexane. Solvent extraction is not suitable for SME's because of high capital and operation costs, the risk of fire and explosion from solvents and the complexity of the operation. Also, it requires large amounts of raw material, usually a minimum of 1 ton, for processing per day and waste management of solvents such as hexane is a problem (Berk, 1992). SME's can use the screw press process (the expeller) for the extraction of oil from marama beans

because the process is simple and not capital intensive. Also, screw presses of small capacities (100-120 kg/h) are easily available in the market at an affordable cost of R32 000.00 ($ 3 265.31) (08/12/2008) (Personal Communication – Grobler, 2008; Manager, ABC Hansen (Ltd) Pty, Pretoria, South Africa). The oil produced by screw pressing can be filtered and sold to local shops and restaurants or alternatively added back to the flour to make full fat flour. The laboratory hammer mill 3100 (Falling number, Sweden) used to mill the defatted meal to flour particle size that pass through a 0.8 mm screen is not suitable for SME's because of its limited capacity to mill larger quantities (50-100 kg) of the defatted meal. SME's can use a small scale hammer mill M1 developed by RIPCO Botswana (www.ripco.co.bw) which has the capacity to mill meals at 50 kg/h. This equipment is used to mill sorghum into flour through the milling process which is effected by the hammering action of the metal hammers attached to the shaft that rotates in the milling chamber.

The process used in this study above can be modified and adopted by SME's (Fig 4.2.2) to manufacture marama bean flour with potential applications in bakery and meat products and as a protein supplement in composite flours.

Among the three countries, namely South Africa, Namibia and Botswana, where marama beans were harvested, a marama processing plant may attain a certain degree of viability in Botswana and Namibia compared to South Africa. This is due to the organised collection and abundance of marama beans in the veld during the harvesting period (May-July). In South Africa, most of the areas where marama bean plants were previously found are developed mainly because of commercial game farming. Thus since these farms are private properties, it is difficult for locals to have access to the marama beans. However, since marama beans are seasonal and not grown on a commercial scale, it is advisable for SME's to diversify their production line to include other oilseeds such as peanuts, sunflower, macadamia, rape-seed and soya beans to make the enterprise viable. These oilseeds are abundant and easily available and can be processed during the marama bean off season using the same equipment used for processing defatted marama bean flour.

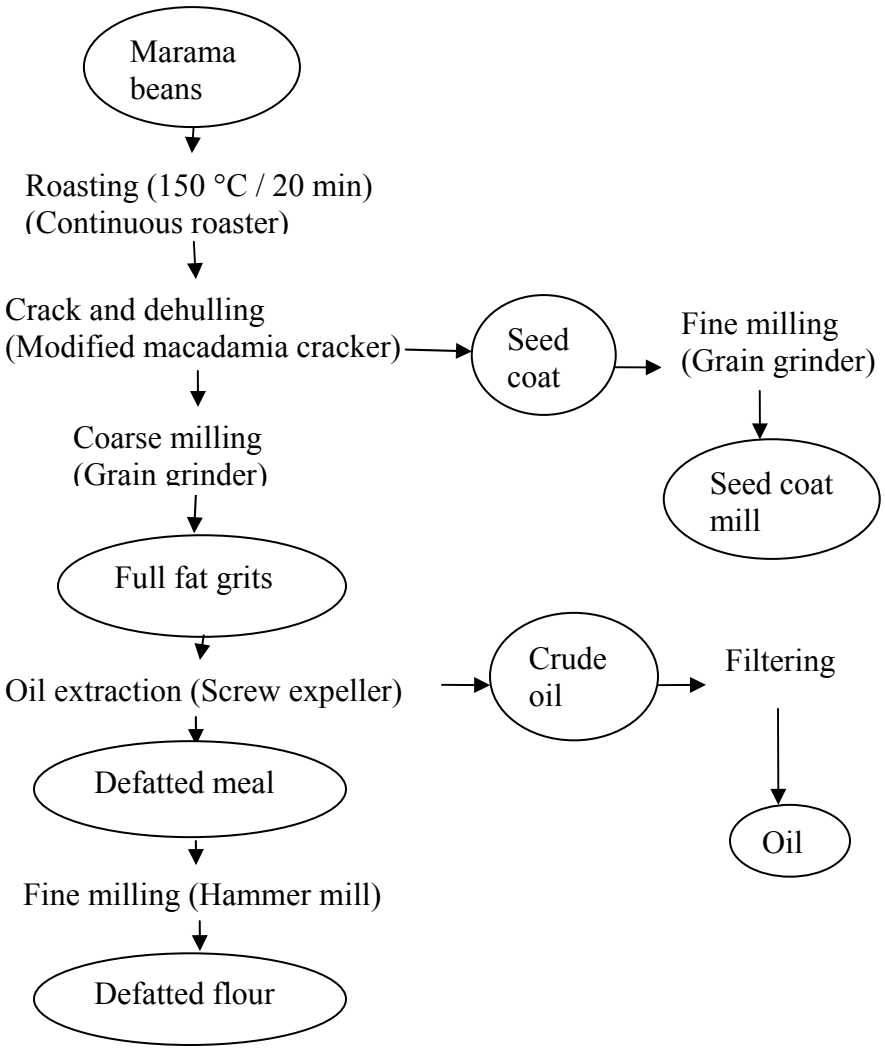


Figure 4.2.2: Recommended marama bean flour manufacturing process for small medium enterprises (SME's)

4.3 Effect of processing on physico-chemical and protein-related functional properties of defatted marama bean flour and its potential applications in food systems

The proposed changes in physico-chemical and protein-related functional properties of defatted marama bean flour following dry heating of whole marama beans at 150 °C/20 min and its potential applications in food systems are summarised in Fig. 4.2.3. It is proposed that dry heating induces marama bean protein denaturation by destabilising hydrogen bonding and electrostatic interactions leading to unfolding of polypeptide chains and a probable exposure of previously buried hydrophobic amino acids groups. Since trypsin inhibitors are proteins, their native structure is also destabilised (denatured) by dry heating and therefore are inactivated (del Valle, 1981; Liener, 1981; Nielsen, Deshpande, Hermodson and Scott, 1988; Friedman and Brandon, 2001). Denatured unfolded protein is more accessible to enzymes than native folded protein (Nielsen *et al*., 1988). This in-part possibly led to the observed increases in the *in-vitro* protein digestibilities of flours prepared from heated beans compared with flours from unheated beans. Other workers have also reported improved *in-vitro* protein digestibility for legume heat-treated flours compared to their native flours (Hsu *et al*., 1977; Adeyeye, 1997; Carbonaro, Cappelloni, Nicoli, Lucarini & Carnovale, 1997).

In this study, defatted commercial soya flour from unheated soya beans had a higher *in-vitro* protein digestibility than defatted marama bean flour from heated marama beans. This observation was rather interesting because most of the trypsin inhibitors had been inactivated in the defatted marama flour prepared from heated marama beans. These results suggest that there may be some structural constraints (compact/twisted structure) that exist in the marama bean protein that were not completely overcome by heating thus limiting the accessibility of the proteolytic enzymes. Furthermore, it is possible that marama bean, as a legume, contain other antinutritional factors such as phytates that inhibit the proteolytic activity of enzymes (Chavan and Kadam, 1981).

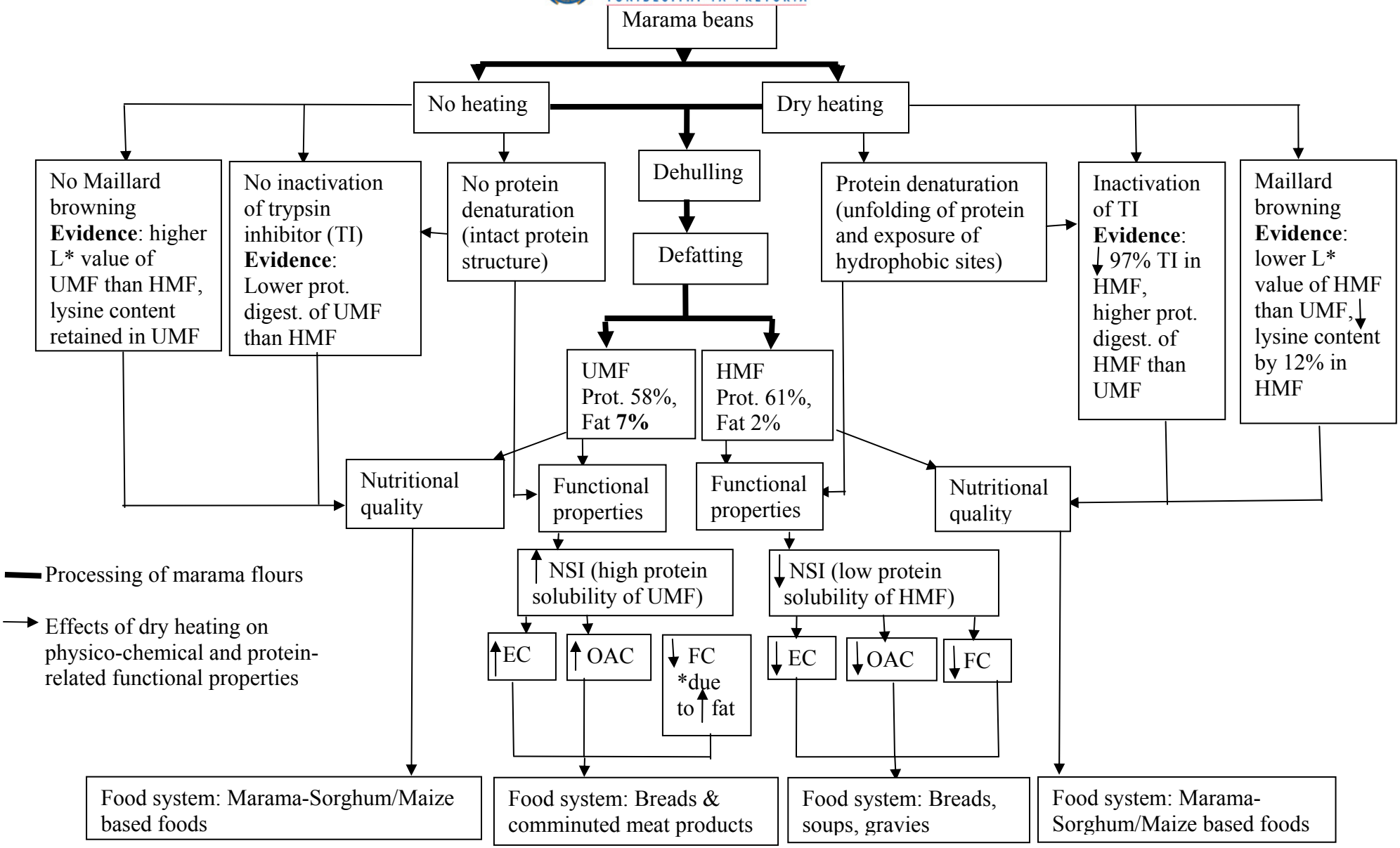


Figure 4.2.3: Proposed changes in defatted marama bean flour physico-chemical and protein-related functional properties following dry heating at 150 °C/20 min and its potential applications in food systems

The improvement in *in-vitro* protein digestibility of the flours due to dry heating was coupled with a decrease in lysine content and L* value. This finding supports the generally held view that heat can lead to the destruction of essential amino acids such as lysine and isoleucine (del Valle, 1981) and initiation of Maillard browning reactions thus affecting protein nutritional quality (Friedman and Brandon, 2001) and decreasing the L* colour value respectively. It has been reported that the formation of Mallard browning reaction products are positively correlated with a decrease in lysine availability (Friedman, 1996). A decrease in available lysine and L* colour value of irradiated cowpea flour was reported by Abu (2005). Excessive heat can induce cross-link formation of isopeptides through displacement of the amide group of aspartate or glutamine by the ε-NH_2 group of lysine which can cause significant losses in available lysine (Thompson and Erdman, 1980). Lysine is reported to be the first essential amino acid to be rendered unavailable during thermal processing (Friedman, 1996). In this study, lysine availability was not determined but significant decreases in lysine content were observed in flours prepared from heated beans compared to flours prepared from unheated beans. Therefore measurement of nutritionally available lysine would be a valuable indicator of protein digestibility of the marama bean flours.

Lower values in NSI of defatted marama bean and soya flours prepared from heated beans compared to flours prepared from unheated beans were observed in this study (Fig. 4.2.3). Other workers have also reported lower NSI values for legume heat-treated flours compared to their native flours (Giami *et al*., 2000; Abu, 2005; Mwangwela *et al*., 2007). The increased exposure of hydrophobic sites due to protein denaturation upon dry heating probably increased hydrophobic interactions between the polypeptide chains leading to the observed decreases in NSI (Nakai, 1983; Nakai and Li-Chan, 1989). The decreases in NSI with dry heating may in part account for most of the changes in protein-related functional properties such as emulsification and oil absorption capacities. In agreement with this, the NSI values of defatted marama bean and soya flours used in this study correlated positively with emulsification capacity. However, the higher residual oil of UMF compared to USF may have partly enhanced the emulsification capacity of UMF. Notwithstanding the slight decreases in oil absorption capacity of flours prepared from heated beans compared to flours prepared from unheated beans, heating does not

seem to affect oil absorption capacity. Therefore, the suggested theory that the mechanism of fat absorption is mainly attributed to physical entrapment of oil as opposed to protein-lipid interactions may be true.

Other reports have also shown protein-related functional properties to be directly dependent on protein solubility (Odoemelam, 2003; Carvalho *et al*., 2006; Onimawo and Akpojovwo, 2006). However, contrary to the accepted theory that NSI positively correlates to foaming capacity (Nakai, 1983; Morr, 1990), this was not true for defatted marama bean flours. This may partly be due to the compact nature of the marama protein structure which may affect the flexibility of marama proteins, hence a lower foam capacity. It is suggested that the higher content of proline in marama proteins compared to soya proteins may be a good indicator that the marama proteins are more folded than the soya proteins and thus are less flexible. This amino acid is mostly found at the bends of folded proteins because of its cyclic side chain which "kinks" the helix structure of proteins (Stryer, 1988; Woolfson and Williams, 1990). In a study to investigate the relationship between the surface properties and the flexibility of proteins by the protease digestion method, a positive correlation was observed between the foaming capacity and digestion velocity of proteins (Kato, Komatsu, Fiyimoto and Kobayashi, 1983). The results suggested that flexibility of protein structure detected by protease digestion may be an important structural factor governing foam formation. Although the digestion method in this study was used to determine the *in-vitro* protein digestibilities of the defatted marama and soya bean flours, it was observed that the defatted marama bean flours had significantly lower protein digestibilities compared with defatted soya flours. This may also partly suggest that the marama proteins are less flexible than the soya proteins, hence they exhibited lower foaming capacities.

Although not of practical significance, slight increases in water absorption capacity of flours prepared from heated marama beans compared to flours prepared from unheated marama beans were observed in this study. However, the water absorption of the soya flours was significantly higher than that of marama flours. Water absorption characteristics represent the ability of a product to associate with water under conditions where water is limiting (Singh, 2001). This variation in water absorption capacity could

possibly in part be due to the conformational behaviour and hydrophilic/lipophilic balance of the proteins in soya and marama bean flours. The higher residual oil in the marama flours compared with the soya flours could have also contributed to their low water absorption capacity by hindering interactions of water with the hydrophilic sites of the marama proteins. Similar results were reported by Jitngarmkusol, Hongsuwankul and Tananuwong (2008) when comparing the water absorption capacity of partially and totally defatted macadamia flours. Presently there is no detailed information on the structural-functional relationship of marama proteins.

Defatting of the marama bean flours led to a significant increase in the protein content of the flours (Fig. 4.2.3). The high protein and lysine content coupled with the low sulphur-containing amino acid content of UMF and HMF implies that they have a potential to be used in composite flours with sorghum or maize flours to improve protein quality (Fig 4.2.3). Cereal grains are known to be low in lysine but contain sufficient amounts of sulphur-containing amino acids (Friedman, 2001). Therefore, with respect to lysine and sulphur amino acid contents, cereal and marama based foods are nutritionally complementary. On the other hand, since marama beans have a high fat content, it may be desirable to use full fat marama bean flours in composite flours to produce a product that has high protein-energy nutrition. Although the protein content of the full fat marama bean flours would be much lower than that of the defatted marama bean flours, it is still higher than most of the full fat flours prepared from legumes such as cowpeas and soya beans and therefore can be used to improve protein quality of cereal based foods.

Just like defatted soya flour, defatted marama bean flour has a potential to be incorporated into foods to impart desirable functional properties. Its potential application in food systems would be based on the functional property results obtained. However, it must be noted that the only realistic way to determine how the defatted marama bean flour will function in a food system is to incorporate it into a food formulation and assess the finished product quality relative to the ‘traditional product’.

The high protein solubility, emulsification capacity and oil absorption capacity of defatted marama bean flour prepared from unheated beans suggests that it has a potential

to be used in emulsified meats (meat sausages) and coarse ground meats (beef patties) (Fig. 4.2.3). Defatted soya flour has been used successfully to partially replace animal proteins in traditional meat products without changing the quality of the product. It has been reported that soya proteins are used in the range of 1-4% to aid in the formation of emulsions and also stabilise the emulsions during processing, as in the production of emulsified meat products (Waggle, Decker and Kolar, 1981). Also, since soya protein is much cheaper than animal protein, the unit cost of the meat product is reduced. However, since marama beans are not commercially available, this may not be the case with marama bean flours.

However, the effect that the defatted marama bean flour could have on the sensory properties, for example, flavour, texture and colour of cereal-based flour should be considered. In this study, it was observed that defatted marama bean flour prepared from unheated beans had a high L* value. Therefore, if the flour is incorporated into cereal-based flour, it would possibly not have a significant effect in changing the colour of the product. Furthermore, the L* value of the defatted marama bean flour prepared from heated beans was high, thus the flour is unlikely to increase possibility of unacceptable browning when it is added to a food system.

HMF has a potential to be applied in breads to improve crust colour through Maillard browning reactions (from sugars and proteins) and nutty roasted flavours. Defatted soya flours have been used successfully in bread and buns to improve crumb body, crust colour and toasting characteristics (Dubois and Hoover, 1981). The low foaming capacity of the defatted marama bean flours implies that it cannot be used in dairy products such as ice creams where foaming is required.

5 CONCLUSIONS AND RECOMMENDATIONS

The lipoxygenase iso-enzymes (L1 and L2) are probably either absent or inhibited in some way in marama beans. This means that off-flavour development due to lipid oxidation catalysed by L-1 and L-2 iso-enzymes in marama bean flour does not seem likely. The presence of L-3 iso-enzyme should be investigated because the test for this iso-enzyme was inconclusive. Furthermore, the potential presence of other enzymes such as lipase should be investigated because they can be involved in catalysing rancidity of marama bean flour and reduce its shelf-life.

The 150 °C/20 min temperature-time combination used in this study for dry heating is effective enough to inactive most of the trypsin inhibitors in marama beans. Trypsin inhibitor inactivation due to dry heating of whole marama beans is coupled with an improvement in the *in-vitro* protein digestibility of the defatted marama flour. Heating probably opens up the structure of proteins and makes them more accessible for cleavage by proteolytic enzymes. However, dry heating of whole marama beans generally decreases the content of most amino acids in defatted marama bean flour. The 150 °C/20 min temperature/time combination is recommended for dry heating of whole marama beans to produce defatted marama bean flour.

HMF has higher protein content but lower fat content than UMF possibly due to the disruption of lipid bodies in marama beans during dry heating and this allows more oil to be expelled from the lipid bodies during coarse milling.

Dry heating of marama beans at 150 °C/20 min modifies most of the protein-related functional properties of the resultant defatted flour. The modification in protein-related functional properties of marama bean flour are partly due to protein denaturation and exposure of more hydrophobic protein sites leading to aggregation of protein molecules through hydrophobic interactions. Dry heating of marama beans may be a potential technique of improving the water absorption capacity of defatted marama flour as evidenced by the increase in WAC of flour with dry heating of the bean. UMF exhibits

better protein-related functional properties than HMF and have potential applications in meat products where protein solubility, emulsion formation and stability is required.

The lab scale process developed and used in this study for producing defatted marama bean flour is relatively successful. However, for the process to be adopted by SME's, it can be modified by using a screw expeller (100-120 kg/hr) instead of solvent extraction for defatting the marama beans because the process would be more affordable to SME's. Furthermore, the lab scale process can be up-scaled to SME's level by using a bigger hammer mill (50-100 kg/h) instead of the lab hammer mill. It may be desirable for SME's to produce full-fat flour, especially for compositing with cereal based flours as this will increase the energy value of the marama-cereal composite flour.

It is necessary to determine the performance of marama bean flour as an ingredient in widely consumed food systems, for example, bread, meat sausage or marama-cereal porridge. This is because the prediction of protein-related functional properties based on simple model systems, though useful, do not provide the conditions, for example, pH, ionic strength, temperature treatments, processing, mixing, other food components, chemical and physical interactions that may occur in the actual food system. Studies on the applications of marama bean flours may potentially trigger the commercialisation of marama beans and therefore increase its utilisation.

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