

# COMPOSITION OF MARAMA BEAN PROTEIN

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## Abstract

The protein composition of marama beans, an indigenous African oilseed legume, was determined in comparison with soya beans. Marama bean protein contained a substantial amount of tyrosine compared with soya bean protein. It was slightly richer in proline than soya. By SDS-PAGE, marama protein contained fewer protein bands compared with soya. The patterns of these bands in marama under non-reducing and reducing conditions were similar, suggesting an absence of disulphide bonds. The vicilin (7S) and acidic 11S subunits seemed to be absent in marama. This is most unusual in legume proteins. Only a major basic legumin (11S) (20 kDa), medium (63 kDa) and high (148 kDa) molecular weight protein bands were separated for marama. Most polypeptides in the marama proteome map are basic compared with soya. Only one polypeptide match was tentatively identified compared with soya. Marama protein composition is very different from that of soya.

**Key words:** marama bean, soya bean, 11S and 7S proteins, tyrosine

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## 1. Introduction

Marama bean is an indigenous southern African leguminous oilseed (Coetzer & Ross, 1976). Its protein (29–38%) and oil (32-42%) contents (Holse, Husted & Hansen, 2010) are very similar to those of soya (Mujoo, Trinh & Ng, 2003) and peanuts (Venkatachalam & Sathe, 2006).

Very limited information is available on the protein composition of marama bean (Maruatona, Duodu & Minnaar, 2010; Holse *et al.*, 2010; Amonsou, Taylor & Minnaar, 2011). Marama flour contains much higher tyrosine content (6.2 g/100 g flour) than soya bean flour (1.8 g/100 g flour) (Maruatona *et al.*, 2010). A study of seed microstructure showed that marama bean protein bodies were similar to those of soya bean in terms of shape and localisation within the parenchyma cells (Amonsou *et al.*, 2010). However, marama protein bodies seemed to contain spherical globoid and druse crystal inclusions, which are absent in soya (Amonsou *et al.*, 2011).

This study compares the chemical characteristics of marama protein in terms of constituent polypeptides and their structure with those in soya. This information is required to facilitate the effective use of marama protein as a functional ingredient in food systems.

## **2. Materials and methods**

### **2.1. Raw materials**

Marama bean specie *Tylosema esculentum* (Burch) A. Schreib was used. This was gathered from Rooidraaitrust, Gauteng Province, South Africa in 2008 (voucher specimen deposited at H.G.W.J. SCHWEICKERDT (PRU), Accession number: 113873). Soya bean (*Glycine max* L. Merr.) was obtained from AGRICOL, Pretoria, South Africa.

### **2.2. Chemicals**

The following chemicals were used: Immobilized pH gradient (IPG) strips pH 3-11 NL and pH 6-11 and carrier ampholytes corresponding to each pH range; acrylamide, bisacrylamide; sodium dodecyl sulfate (SDS); N,N,N,N-tetramethyl-Ethylenediamine (TEMED); ammonium persulfate (APS); thiourea; urea; dithiothreitol (DTT); iodoacetamide and 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) from GE Healthcare (Uppsala, Sweden); methanol, *n*-hexane, 2-mercaptoethanol (2-ME), trichloroacetic acid (TCA), acetone and glycerol from Sigma-Aldrich (St. Louis, MO).

### **2.3. Protein preparations**

Marama beans were dehulled using a cracker (WMC Sheet Metal Works, Tzaneen, South Africa). Soya beans were dehulled with a Tangential Abrasive Dehulling Device (TADD) (Sheperd, 1979). Dehulled marama and soya beans were coarse

milled in a food blender. The bowl (sample holder) and blade of the blender were pre-chilled at -20°C for 10 min to prevent heating during milling.

### ***2.3.1. Defatted flours***

Coarse milled flours were then defatted four times with *n*-hexane, flour: hexane ratio 1:5, for 90 min at room temperature. Defatted flours were placed in a fume hood overnight to remove the remaining hexane. The flours were milled in a laboratory attrition mill (Ika Werke, Staufen, Germany) and sieved through 0.5 mm opening screen. The protein contents of the defatted marama and soya flours were 55.7 and 50.0% (N x 5.71), respectively.

### ***2.3.2. Purified protein extracts***

Proteins were extracted from defatted marama and soya flours with 30 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl, at a flour to solvent ratio of 1:20 (Aluko & Yada, 1995). Protein extraction was done at 40°C for 1 h in a shaking water bath. Crude protein contents (N x 5.71) of bean flours and protein extracts were determined by combustion analysis (968.06) (AOAC, 2000).

### ***2.3.3. Total protein preparation***

Total protein extraction was performed by the TCA/acetone precipitation method of Natarajan, Xu, Caperna and Garrett (2005), except that DTT was included. The protein precipitate was suspended in 7 M urea, 2 M thiourea, 2% (w/v) CHAPS, 0.5% (v/v) IPG buffer without DTT. Protein solutions were kept at -20°C until analysed. Protein concentrations were determined by the Bradford method (Bradford, 1976).

## **2.4. Amino acid analysis**

The amino acid contents of bean flour and protein extracts were determined using the PICO.TAG-Method (Bidlemeier, Cohen & Tarvin, 1984). This method is based on

the principle of reverse phase chromatography with pre-column derivatization, following acid digestion.

## **2.5. SDS-PAGE**

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by (Taylor, Bean, Ioerger & Taylor, 2007). Ten microliter of aliquots containing 10, 15 and 20  $\mu$ g protein was loaded onto a gradient gel of 4–12% polyacrylamide. Gels were stained with Coomassie Brilliant Blue R-250.

## **2.6. Two-dimensional gel electrophoresis**

The Isoelectric Focusing (IEF) was performed using 13 cm IPG strips (pH 3–11 Non Linear, NL) and pH 6-11). The strips were focused on a gradient at 150 V for 2 h, 500 V for 1 h, 1000 V for 1 h, and 8000 V for 3 h using the Ettan IPGphor II system (Amersham Biosciences, Uppsala, Sweden). After IEF, the strips were first equilibrated in equilibration buffer with DTT and then in buffer with iodoacetamide each for 15 min.

SDS-PAGE was carried out in a Hoefer SE 600 Ruby electrophoresis unit (Amersham Biosciences) at 30 mA/gels, voltage set at maximum. The 2-DE gels were stained with Coomassie Blue.

Gel images were acquired using a Versa Doc Documentation system (Bio-Rad, Hercules, Canada) and analyzed with PDQuest 2 D Image Software. Master gels were generated for each sample. The 2-D electrophoresis soya protein spots were identified by reference to Natarajan *et al.* (2005) and Zarkadas, Gagnon, Poysa, Khanizadeh, Cober, Chang, and Gleddi (2007) as well as maps from a database

(<http://oilseedproteomics.missouri.edu>). It was attempted to identify the marama protein spots based on these.

### **2.7. Statistical analysis**

The experiment was repeated twice. Chemical analyses were thus done on two separate protein extracts. One way analysis of variance was performed for protein content and amino acid composition and the means compared using Fisher's Least Significant Different Test at  $p \leq 0.05$ .

## **3. Results and discussion**

### **3.1. Protein contents and yields of marama protein extract**

The protein content of the marama protein extract was high (86%) and similar to that of soya (Table 1). The protein yield in the marama extract (63%) was similar to that of soya (65%). The method used for preparing the extracts was a standard procedure for soya (Aluko & Yada, 1995). Therefore for the purpose of this study, the protein yields of the respective extracts were considered to be sufficiently representative.

### **3.2. Amino acid profile**

The glutamic and aspartic acid, which may include glutamine and asparagine, respectively, were the major amino acids in the purified marama protein extract (Table 2) as well as in the flour (Maruatona *et al.*, 2010). These are also the major amino acids in soya (Table 2). The lysine content of marama protein extract (5.7 g/100 g protein) was also similar to that of soya. Based on the FAO/WHO (1989) recommended pattern, marama flour and protein extract are adequate sources of lysine for pre-school children.

The tyrosine contents of marama flour and purified protein extract were very high (11 and 9 g/100 g protein, respectively), almost 3 times those of soya, thus confirming other reports on marama protein composition (Maruatona *et al.*, 2010; Ripperger-Suhler, 1983). In fact, the tyrosine content of marama protein was substantially higher than that in other legumes, including peanuts (Venkatachalam & Sathe, 2006) and lupine (Lqari, Vioque, Pedroche & Millan, 2002). Since tyrosine is involved in polypeptide crosslinking (Takasaki, Kato, Murata, Homma & Kawakishi, 2005), the high tyrosine in marama may contribute to the structural stability of its protein as suggested for gluten (Tilley, Benjamin, Bagorogoza, Okot-Kotber, Prakash & Kwen, 2001). The proline content of marama was also slightly higher than that of soya, which may affect its protein structure (Levit, 1981). Proline can impart a rigid structure to protein by participating in protein folding and unfolding (Levit, 1981).

### **3.3. Protein subunit composition**

SDS-PAGE revealed differences in protein compositions between marama and soya (Fig. 1 & 2). The protein profiles from defatted marama and soya flours (Fig. 1) were similar to those of their respective protein extracts (Fig. 2). This indicates that these protein extracts were representative of the storage proteins of each of the respective seeds. By SDS-PAGE, marama protein contained fewer major protein bands (three) compared with soya (approx. 8) (Mujoo *et al.*, 2003).

The patterns of these bands in marama under non-reducing and reducing conditions were similar, suggesting an absence of disulphide bonds. This was unlike the situation in soya where 11S storage proteins were separated under reducing conditions (lane 2), due to the fact that the 11S acidic and basic units are linked by disulphide bonds

(Mujoo *et al.*, 2003; Kinsella, 1979). Further in comparison with soya bean, the acidic 11S legumin subunits were absent in marama. Only a major broad band (around 20 kDa), was present in marama, corresponding to a basic 11S legumin was present.

Two major higher molecular weight protein bands were also observed in marama. There was a medium intensity protein band of approx. 63 kDa. Under non-reducing conditions, this appeared to match a major band in soya. However, while this band remained in marama under reducing conditions, it disappeared in soya probably forming the 11S sub-units when separated under reducing conditions. The other protein band was a very high molecular weight band (148 kDa), which did not match any bands in soya. This protein band was also not reduced by mercapthoethanol, suggesting that its constituent polypeptides are not linked together by disulphide bonds. Since marama protein contains such a high level of tyrosine compared with soya (Maruatona *et al.*, 2010) and tyrosine can participate in polypeptide crosslinking (Tilley *et al.*, 2001; Takasaki *et al.*, 2005), it is suggested that dityrosine type of crosslinks may be responsible for the stability of this high molecular weight protein in marama.

Marama protein was further analysed using proteomic type 2-D electrophoresis to attempt to identify its constituent polypeptides by comparison with soya.

### **3.4. Two-dimensional gel electrophoresis**

Marama appeared to contain a similar number of polypeptides to soya, as indicated by protein spots (Fig. 3, Table 3). However, the total protein of marama and its purified



protein extract contained more basic polypeptides than those of soya (Fig. 4 & Table 3).

As indicated by the proteome map, the vicilin (7S) subunits were apparently absent in marama, unlike soya (Fig. 3). The absence of 7S subunits in legumes is most unusual, if not unique. Variations in the major storage protein in terms of vicilin (7S) and legumin (11S) contents (Kinsella, 1979; Horax *et al.*, 2004) have been reported in the literature for some legume species (Kinsella, 1979; Horax, Hettiarachchy & Chen, 2004; Chel-Guerrero, Scilingo, Tintoré, Dávila & Añón, 2007). For instance both 7S and 11S have been found to be major storage protein fractions of lupine (*Lupinus albus*) (Duranti *et al.*, 1981) and soya bean (Kinsella, 1979) whereas the 7S has been found to be the major protein fraction of dry bean legumes such as mucuna bean (Adebowale, Adeyemi, Oshodi & Niranjana, 2007) and cowpeas (Horax *et al.*, 2004).

The protein band of 63 kDa from SDS-PAGE (Fig. 1 & 2) most likely corresponds to polypeptides with pI in the range of 6.4-7.5 and molecular weight of about 64 kDa on the marama proteome map (Fig. 3).

To provide a better separation of basic protein of marama, proteomic analysis was performed on 13 cm strips, pH 6-11 (Fig. 3 A, B). The proteome map of marama that was obtained with IPG strip pH 6-11 (Fig. 4 A-B) further confirmed differences in proteome patterns between marama and soya with respect to the high number of basic polypeptides in marama. Compared with other legumes, the protein profile of marama seemed to be different from those of peanuts (Kottapalli, Payton, Rakwal, Agrawal, Shibato, Murow & Puppala, 2008) and protein-rich oilseeds like canola (Uruakpa &

Arntfield, 2006) and sunflower (Sammour, El-Shourbagy, Abo-Shady & Abasary, 1995). Based on amino acid composition data of purified protein from this study and those published by Maruatona *et al.* (2010), marama protein contains less acidic amino acids content than soya. The proteome pattern of marama thus seems to be related to its amino composition.

Only one polypeptide in marama was tentatively identified by comparison with soya. This was spot 5 (Fig. 3 & 4), which appear to correspond with Glycinin G2: *A2B1*, a subunit of the 11S basic soya proteins. This single match with the 11S soya proteins and the apparent absence of 7S proteins indicates the storage proteins of marama are very different from those of soya.

Storage protein compositions have been found to significantly influence the functionality of protein properties (German, Damodaran & Kinsella, 1982; Yuan, Yang, Tang, Zheng, Ahmad & Yin, 2009). The absence of vicilin (7S) and the presence of more 11S basic protein in marama may increase the stability of protein to heat and extreme pH's as suggested for 11S basic soya glycinin (Yuan *et al.*, 2009). The stability of basic 11S of soya protein was associated with its high hydrophobicity compared with the acidic subunits and the total glycinin (11S) (German *et al.*, 1982; Yuan *et al.*, 2009). According to Creighton (1993), hydrophobic interactions are endothermic and therefore more stable at increasing temperature compared with hydrogen bonding and electrostatic interactions. A close look at the amino acid composition of marama revealed that its protein contains more aromatic amino acids. These together with the aliphatic amino acids may increase the hydrophobicity and stability of marama protein in comparison with soya. Further, the high tyrosine in

marama may also contribute to the stability of its protein structure as described previously.

#### **4. Conclusions**

The protein composition of marama bean is very different from that of soya bean. The vicilin (7S) and the acidic 11S subunits seem to be absent in marama protein. The only major proteins in marama are basic legumin (11S) and two additional proteins of relatively higher molecular weight. Also, marama protein seems to lack disulphide bonds. These findings suggest that marama protein may have increased heat stability compared with soya due to high hydrophobic interactions that are associated with the basic 11S. Since tyrosine is involved in polypeptide crosslinking, the tyrosine in marama may also contribute to the structural stability of its protein.

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**Table 1.**

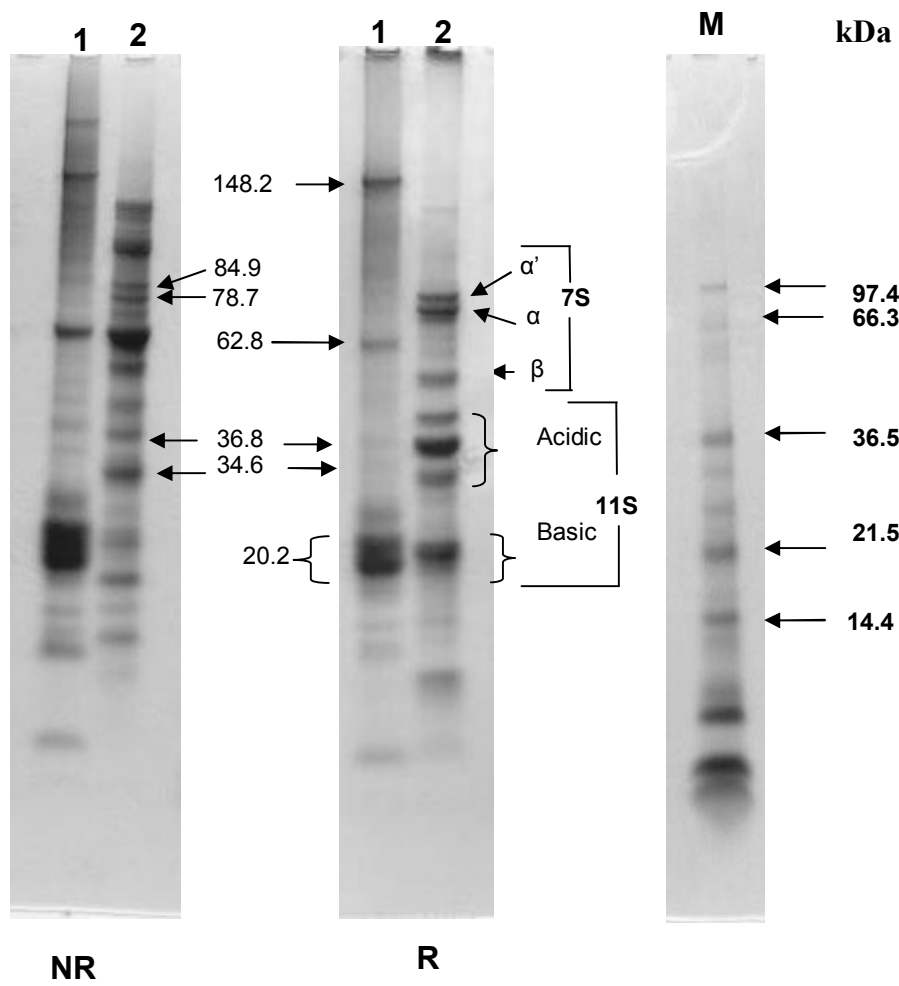
Protein yield and protein content (dry basis) of marama and soya protein extracts<sup>1</sup>

Samples	Protein content (N x 5.71) (g/100 g)	Protein yield (%)
Marama extract	86.1 <sup>a</sup> ± 1.5	62.8 <sup>a</sup> ± 1.4
Soya extract	85.6 <sup>a</sup> ± 1.3	65.3 <sup>b</sup> ± 1.6

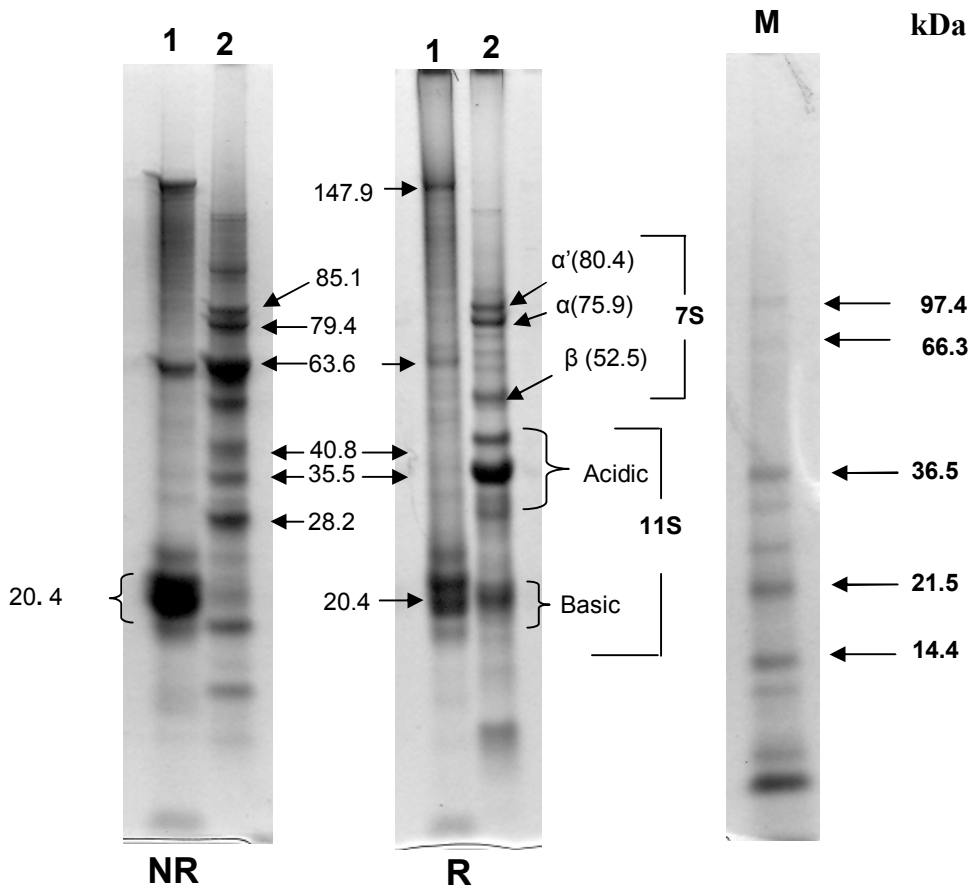
<sup>1</sup>Mean ± SD (n = 4) is reported;

Mean values with different superscript letters in columns are significantly different (p<0.05)

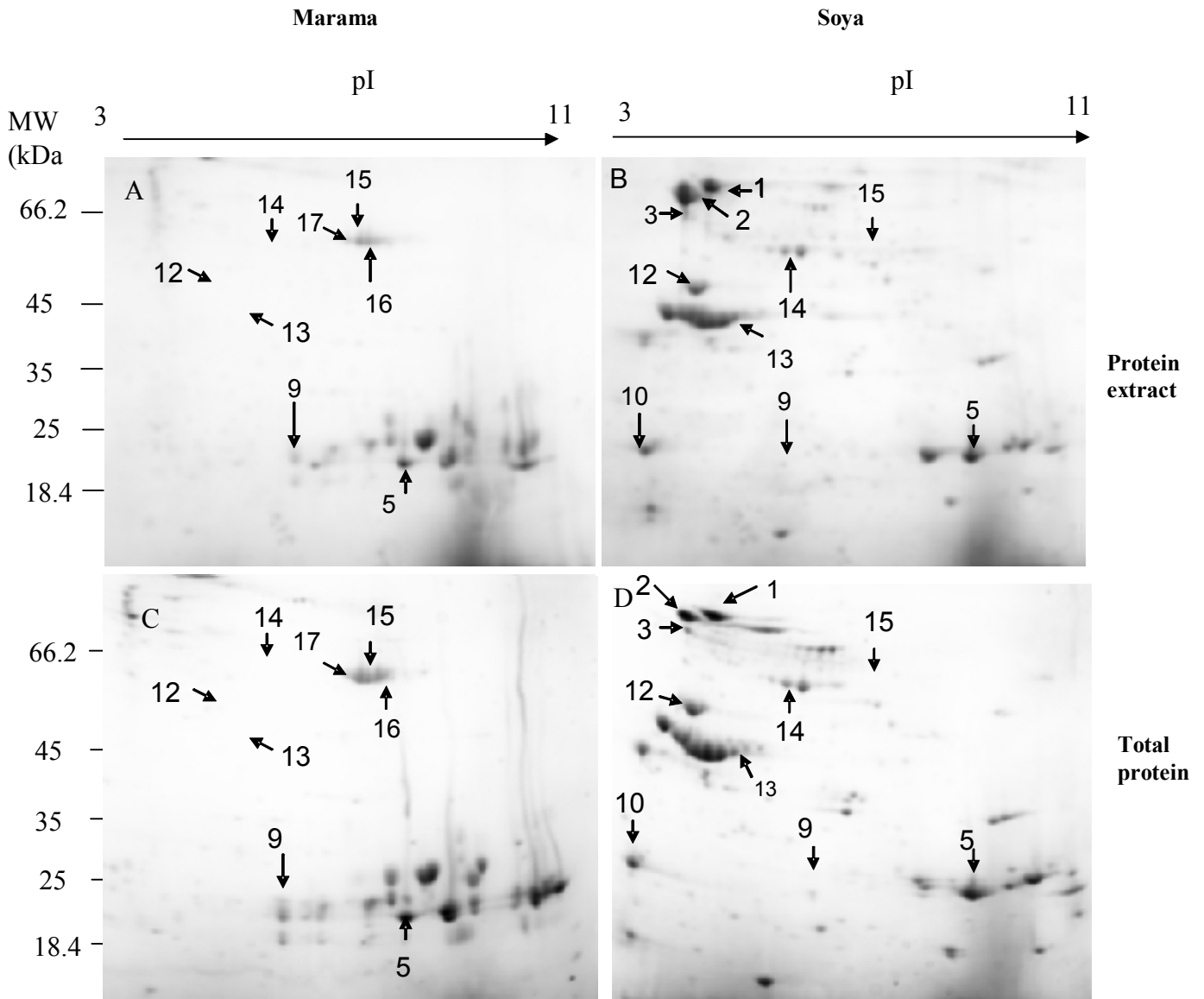




**Fig. 1.** SDS-PAGE profile of defatted marama and soya bean flours under non-reducing (NR) and reducing (R) conditions. Lane 1: Marama, Lane 2: Soya, Lane M: Molecular weight standards



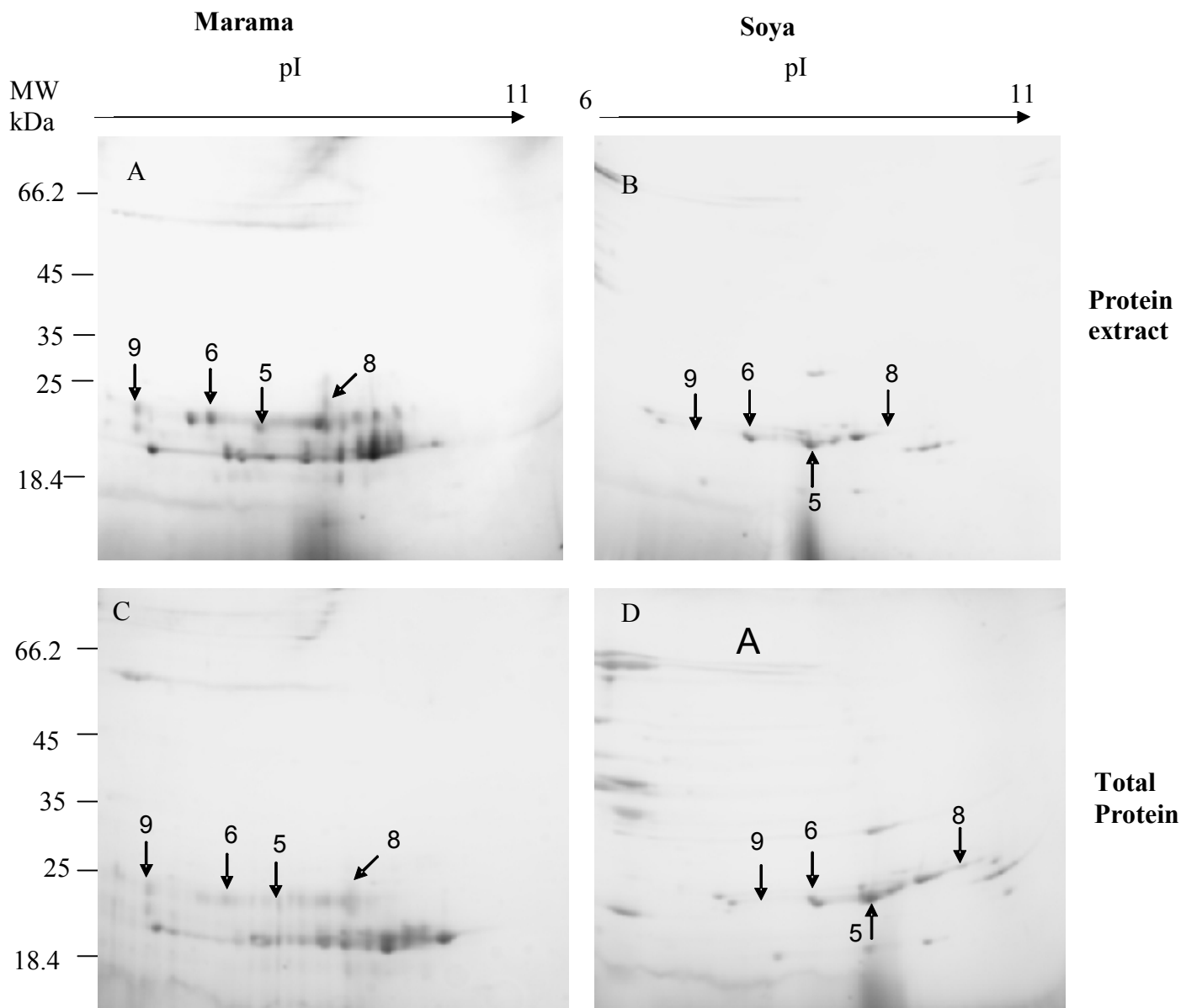
**Fig. 2.** SDS-PAGE profile of extracted marama and soya bean proteins under non-reducing (NR) and reducing (R) conditions. Lane 1: Marama, Lane 2: Soya, Lane M: Molecular weight standards



**Fig. 3.** Two-dimensional maps of marama (A & C) and soya (B& D)

proteins using Immobilized pH gradient (IPG) strips (3-11 Non Linear, NL).

A: marama protein extract, C: total marama protein, B: soya protein extract, D: total soya protein. 1:  $\alpha'$ -subunit of  $\beta$  conglycinin, 2 & 3:  $\alpha$ -subunit of  $\beta$  conglycinin, 5: glycinin G2 A2B1, 10: soybean trypsin inhibitor, 12: Glycinin A3b4 subunit homohexamers, 13: Soybean proglycinin A1ab1b homotrimer, 14:  $\beta$ -Conglycinin  $\beta$ -homotrimer (Natarajan *et al.* (2005); Zarkadas *et al.* (2007); <http://oilseedproteomic.missouri.edu>). The same numbers on maps indicate spot matches. **Spots** 9, 15, 16, 17 not identified.



**Fig. 4.** Two-dimensional proteome maps of marama (A & C) and soya (B& D) proteins using Immobilized pH gradient (IPG) strips (6- 11).

A: marama protein extract, C: total marama protein, B: soya protein extract, D : total soya protein 5:

Glycinin G2 : A2B1 (Natarajan *et al.*, (2005); Zarkadas *et al.*, (2007);

<http://oilseedproteomic.missouri.edu>). The same numbers on maps are used to indicate polypeptide matches. Spots 6, 8, 9 not identified.

**Table 2.**

Amino acid composition (g/100 g protein) of marama and soya flours and their respective protein extracts<sup>1</sup>

Amino acid (AA)	Flours		Protein extracts		FAO/WHO <sup>2</sup> recommended pattern	
	Marama	Soya bean	Marama	Soya bean	Pre-school children (2-5 years)	Adult
Lysine	5.7 <sup>a</sup>	6.3 <sup>b</sup>	6.6 <sup>c</sup>	6.9 <sup>c</sup>	5.8	1.6
Isoleucine	4.3 <sup>a</sup>	4.7 <sup>b</sup>	5.0 <sup>c</sup>	4.9 <sup>c</sup>	2.8	1.3
Leucine	7.9 <sup>a</sup>	9.8 <sup>c</sup>	7.8 <sup>a</sup>	9.4 <sup>b</sup>	6.6	1.9
Methionine	1.0 <sup>a</sup>	1.5 <sup>b</sup>	1.2 <sup>a</sup>	1.8 <sup>c</sup>	2.5	1.7
Cysteine	0.1 <sup>a</sup>	0.2 <sup>b</sup>	0.2 <sup>b</sup>	0.5 <sup>c</sup>		
Phenylalanine	3.7 <sup>a</sup>	4.0 <sup>b</sup>	4.1 <sup>b</sup>	4.9 <sup>c</sup>	6.3	1.9
Tyrosine	11.4 <sup>c</sup>	3.9 <sup>a</sup>	9.1 <sup>b</sup>	4.0 <sup>a</sup>		
Valine	4.8 <sup>a</sup>	5.0 <sup>b</sup>	5.2 <sup>b</sup>	4.9 <sup>a</sup>	3.5	1.3
Threonine	3.2 <sup>a</sup>	4.0 <sup>b</sup>	3.2 <sup>a</sup>	3.9 <sup>b</sup>	3.4	0.9
Histidine	2.7 <sup>a</sup>	3.1 <sup>b</sup>	3.0 <sup>b</sup>	2.7 <sup>a</sup>	1.9	1.6
<i>Subtotal</i>	<i>44.8</i>	<i>42.5</i>	<i>45.4</i>	<i>43.9</i>		
<i>Essential AA</i>						
Aspartic acid/ Asparagine	9.4 <sup>a</sup>	10.2 <sup>b</sup>	9.5 <sup>a</sup>	9.9 <sup>b</sup>		
Glutamic acid/ Glutamine	15.2 <sup>b</sup>	18.9 <sup>c</sup>	13.2 <sup>a</sup>	19.0 <sup>c</sup>		
Serine	5.5 <sup>a</sup>	5.6 <sup>a</sup>	5.9	5.2		
Glycine	5.9 <sup>b</sup>	4.3 <sup>a</sup>	6.7 <sup>c</sup>	4.4 <sup>a</sup>		
Arginine	8.0 <sup>c</sup>	7.8 <sup>b</sup>	7.1 <sup>a</sup>	7.6 <sup>b</sup>		
Alanine	3.5 <sup>a</sup>	4.5 <sup>b</sup>	3.6 <sup>a</sup>	4.4 <sup>b</sup>		
Proline	7.2 <sup>b</sup>	5.5 <sup>a</sup>	8.3 <sup>c</sup>	5.2 <sup>a</sup>		
<i>Subtotal Non- essential AA</i>	<i>54.7</i>	<i>56.8</i>	<i>54.3</i>	<i>55.7</i>		
<i>Recovery</i>	<i>99.5</i>	<i>99.3</i>	<i>99.7</i>	<i>99.6</i>		

<sup>1</sup>Mean (n = 2) values with different superscript letters in rows are significantly different (p<0.05)

<sup>2</sup>FAO/WHO (1989) recommended pattern (Pre-school children age 2-5 years; Adults)

**Table 3.** Polypeptide distribution and matches between marama and soya protein<sup>a</sup>

Samples	pH range			
	pH 3-11		pH 6-11	
	Marama	Soya	Marama	Soya
Protein spots	276 ± 13	286 ± 21	153 ± 6	96 ± 5
Tentative spot matches	52 ± 5		26 ± 7	

<sup>a</sup>Mean ± SD number of protein spots in gels from two separate protein extracts.