

HIGHLY VISCOUS DOUGH FORMING PROPERTIES OF MARAMA PROTEIN

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Abstract

Marama bean is an indigenous Southern African oilseed legume with an unusual protein composition. Hence, its rheological properties were studied. Marama protein formed a highly viscous and extensible dough when compared to soya and gluten. With a dough of 38% moisture, marama protein extensibility was very high (304%), twice that of gluten and soya, and this increased considerably (> 3 fold) when the moisture content was increased to 45%. With added peroxidase, the storage modulus (G') of marama protein dough increased with time, suggesting the formation of new and strong protein networks. Dityrosine crosslinks were detected in the doughs. Marama protein showed a single transition with a denaturation temperature higher than soya glycinin. Marama protein appeared more hydrophobic and contained more β -sheet structure than soya. Thus, the highly viscous and extensible rheological behaviour of marama protein is probably related to its high β -sheet conformation, hydrophobic interactions and tyrosine crosslinks.

Key words: Marama, soya, gluten, protein rheology

1. Introduction

Marama bean, *Tylosema* species, is an underutilised indigenous Southern African oilseed legume (Coetzer & Ross, 1976). In comparison to commercial oilseed legumes such as soya and groundnuts, the marama plant is highly drought-tolerant and therefore potentially well-adapted to other semi-arid and arid regions around the world (Travalos & Karamanos, 2006).

The protein content of marama bean (approx. 35%) is very similar to that of soya bean (Amonsou, Taylor, & Minnaar, 2011). However, the composition of marama protein is very different from that of soya (Ripperger-Suhler, 1983; Maruatona, Duodu, & Minnaar, 2010, Amonsou, Taylor, Beukes, & Minnaar, 2012). Marama protein contains a high level of tyrosine (9-12 g/100 g protein), 3 to 5 times of that in soya (Ripperger-Suhler, 1983; Amonsou et al., 2012). Since tyrosine is involved in polypeptide crosslinking (Tilley, Benjamin, Bagorogoza, Okot-Kotber, Prakash, & Kwen, 2001; Takasaki, Kato, Murata, Homma, & Kawakishi, 2005), this could influence the rheological behaviour of marama protein as is proposed for wheat gluten (Tilley et al., 2001). Further, the globulin storage protein composition of marama is unusual in that it consists mainly of basic (11S) legumin and no acidic (11S) subunits (Amonsou et al., 2012), unlike soya. The vicilin (7S) also seems to be absent in marama protein. The absence of vicilin (7S) and the presence of more 11S basic proteins may also affect its functionality. Hence, the rheological properties of marama protein were studied in comparison with soya protein and gluten.

2. Materials and methods

2.1. Materials

Marama beans *T. esculentum* (Burch) A. Schreib was used. Detailed descriptions of marama sample acquisition and voucher specimen deposition are given in Amonsou et al. (2011). Soya bean (*Glycine max* L. Merr.) and vital gluten were obtained from commercial sources.

2.2. Protein extraction

Marama protein was extracted from defatted flour with 30 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl, at a flour to solvent ratio of 1:20 (Amonsou et al., 2012). Two separate extractions were made. Soya protein was extracted in the same way. Marama and soya protein extracts were freeze dried and stored at 4°C until analysed. Crude protein contents (N x 5.7) of the protein extracts and gluten were determined by combustion analysis (AOAC method no. 968.06) (AOAC, 2000). Protein contents of marama, soya and gluten were 85.2, 84.7, 74.4 g/100 g dry basis, respectively. Ash contents: 1.1, 1.5 and 0.6 g/100 g, and dry matter contents: 95.5, 95.4, 96.3 g/ 100 g were obtained for marama, soya and gluten respectively.

2.3. Analyses

2.3.1. Rheology

Protein doughs at three levels of moisture contents (38, 45 and 52%, dry powder basis) were prepared by mixing marama protein, soya protein and gluten, each with water using a spatula and kneaded by hand until a homogenous dough was obtained as described in Schober, Bean, Boyle, & Park (2008).

The extensibilities of the protein doughs were measured using a TA-XT2 Texture Analyser (Stable Micro Systems Ltd, Godalming, England). Dough (3 g) was left to rest for 15 min and then cut into strips (60 mm x 5 mm). A hook to lift the strip of dough was attached to the Texture Analyser. Extensibility measurements of the dough strips of initial length 23 mm, placed just above the hook were carried out within 1 min to prevent moisture loss. Each treatment was measured in triplicate. The resistance force to extension and the distance of dough extension were measured. Extensibility parameters (e.g. actual force acting on the dough strip, % elongation at break/maximum extension) and rheological properties (e.g. Henchy strain, ϵ_H , strain rate, $\dot{\epsilon}$ and stress, δ) were determined as described in Zaidel, Chin, & Yusof (2010). Extensional viscosity (η_E) was estimated as ratio of stress over the strain rate. Both the cross-sectional area and volume of the dough strip were assumed to be constant.

Dynamic small oscillatory analyses were performed using a Physica MCR 301 Rheometer (Anton Paar, Ostfildern, Germany) equipped with two parallel plates ($d=25$ mm). The gap between the plates was set at 2 mm for all experiments. Dough (3 g) was loaded on the temperature controlled (25°C) lower plate and the upper plate was lowered onto the dough. Excess dough was trimmed off using a razor blade and the periphery of the sample was covered with a thin layer of paraffin oil to prevent moisture loss (Oom, Pettersson, Taylor, & Stading, 2008). The dough was allowed to equilibrate for 15 min before the start of each test. To determine the linear viscoelastic range, a series of amplitude sweep tests were conducted over a strain amplitude range of 0.01 to 100% at a constant angular frequency of 6.28 rad/s.

The mechanical spectra of protein doughs were determined by performing oscillatory shear tests over a frequency range of 0.01 to 100 rad/s, at constant strain amplitude of 0.5%, which was within the linear viscoelastic region. Storage modulus G' , loss modulus G'' and loss tangent $\tan \delta$ were continuously monitored during the test. All tests were repeated at least twice.

The effect of peroxidase on dough rheological properties was monitored by performing a time sweep test. Horseradish peroxidase (POX), 85 U (236 units/mg, Sigma, P8250) and 100 μ l 30% hydrogen peroxide were added to 3 g dough at 45% water content (dry basis). Dough was incubated in-situ in the rheometer with the plates set at 37°C. The dough was allowed to equilibrate for 15 min and the time scan was conducted over a period of 10800 s at a constant angular frequency of 6.28 rad/s and 0.5% strain amplitude (within the linear viscoelastic range). The control dough sample was incubated in the same manner but without POX and hydrogen peroxide. G' , G'' and $\tan \delta$ were recorded every 60 s. At the end of the time scan, the dough was recovered, flash frozen in liquid nitrogen. Frozen dough samples were freeze dried.

2.3.2. Hydrophobicity

The free energy of hydration (FEH) for marama protein was calculated based on known FEH of individual amino acids (Shewry, Popineau, Lafiandra, Halford, Tatham, & Belton, 2003) and amino acid composition of marama protein. The values of the free energy of hydration were then used to estimate the hydrophobicity of marama protein as described by Duodu, Taylor, Belton and Hamaker (2003). Marama protein amino acid composition was determined by the PICO.TAG-Method (Bidlingmeyer, Cohen, & Tarvin, 1984). Protein samples were hydrolysed in 6 M HCl

containing 0.5% phenol at 116°C under vacuum for 24 h prior to chromatographic analysis. For tryptophan determination, hydrolysis was done under alkaline conditions. To determine the cysteine and methionine contents, marama protein was first oxidized with performic acid.

2.3.3. FTIR

The secondary structure of marama protein was determined using Vertex 70/70v ATR-FTIR spectrometer (Bruker Optik, Ettingen, Germany). Marama and soya protein powders were placed on the ATR diamond crystal. These were scanned at a 4 cm⁻¹ resolution from 4000 to 400 cm⁻¹.

2.3.4. DSC

This was performed using a Mettler DSC 1 (Toledo, Columbus, OH). Protein powders (10% w/v) were dispersed in 0.05 M sodium phosphate buffer (pH 7.0). Well mixed dispersions (15 mg) were weighed into 40 µl aluminium pans and hermetically sealed. These were allowed to equilibrate for 2 h and scanning was performed from 20-120°C at 10°C/min. A sealed empty pan was used as reference. Measurements were done in triplicate.

2.3.5. HPLC/MS

Protein doughs (treated and untreated with POX) and powders were hydrolysed with 6 M HCl (0.05 µg protein/µl) containing 0.1% phenol under vacuum for 24 h at 110°C (Tilley et al., 2001). The amino acids were separated on a reverse phase C₁₈ column (250 x 4.6 mm I.D., 5 µm, 12 nm, YMC, Kyoto, Japan), at a flow rate 1 ml/ min with 20 µl loading.

The amino acid peaks separated by HPLC were further analysed using Linear Ion Trap Triple Quadrupole Mass Spectrometry (4000 Q Trap AB, Applied Biosystems, Carlsbad, CA). The positive mode was used for ionisation. The mass scan was performed in the following ranges: 180-185 m/z for tyrosine and 350-370 m/z for dityrosine (Takasaki et al., 2005).

2.4. Statistical analysis

One way Analysis of Variance was performed on the data where appropriate. Fisher's Least Significant Difference test was used for mean separation at a 5% level of significance.

3. Results and discussion

With a dough of 38% moisture content, the extensibility of marama protein was very high (304% of its original length), about twice that of gluten and soya (Table 1). Furthermore, the extensibility of marama protein increased considerably (more than 3 fold) when the moisture content was increased from 38 to 45%. This is probably due to the plasticising effect of water. In comparison with gluten dough, marama dough at 45% moisture content did not break at the texture analyser set distance of 120 mm (Fig. 1). Thus, the extensional viscosity of marama protein was very high, about twice that of gluten at 38% moisture content. This property was also found to increase with increasing moisture content from 38 to 45% for marama, similar to gluten. At approximately 52% moisture content, marama protein flowed and it was not possible to measure its extensibility. This is unlike the situation with gluten where it was still possible to form dough with well defined shape at all the moisture contents studied. In

comparison with gluten, marama protein showed less resistance force to extension. The resistance to extension of gluten has been reported to be related to its high molecular weight glutenin subunits (Tronsmo, Magnus, Baardseth, Schofield, Aamodt, & Færgestad, 2003), which are stabilised mainly by disulphide bonds (Kinsella, 1979; Song & Zheng, 2007). In terms of its viscous flow behaviour and extensibility, marama protein appears similar to wheat gliadin (Song & Zheng, 2007; Zaidel et al., 2010).

Dynamic rheological measurements were conducted on marama protein at 45 and 52% moisture contents in comparison with gluten. Soya protein was not included due to the difficulties in forming a dough and the lack of the repeatability of results at these moisture contents. The linear viscoelastic range (LVR) of marama protein dough was low (within 5% strain) compared to that of gluten (15%) (data not shown). Thus, it appeared that LVR was not influenced by the increase in moisture content from 45% to 52% for both marama protein and gluten. LVR of the gluten was within the range reported by Letang, Piau, & Verdier (1999).

The storage G' (representing the elastic component) and loss modulus G'' (representing the viscous component) increased with increasing frequency (1–100 Hz) when small oscillatory deformations were performed within the linear range for marama protein (Table 2), as with gluten. However, G' and G'' appeared to decrease with increasing moisture contents within the above specified frequency range for both marama protein and gluten. This was because water behaves like a plasticiser, reducing both the viscous and elastic behaviour of proteins (Masi, Cavella, & Sepe, 1998). Similar reductions in G' and G'' with increasing water content have been

reported for wheat flour dough (Letang et al., 1999). The loss tangent ($\tan \delta$) values were low for marama protein (0.2-0.5), similar to those of gluten (Table 2). $\tan \delta$, defined as the ratio of G'' over G' , reflects the balance between the viscous and the elastic character of a viscoelastic material (Mezger, 2006). As small dynamic deformation analysis is non-destructive, it can provide some information on the types of molecular bonding that may be responsible for the structural stability of proteins (Mezger, 2006). A low $\tan \delta$ value is often associated with a high degree of crosslinking (Tsiami, Bot, Agterof, & Groot, 1997; Mezger, 2006) and therefore a strong protein network. Marama protein, thus, appeared to have some structural stability, but only when small deformations were applied. The complex viscosity (η^*) decreased at increasing frequency for marama protein, which was similar to gluten (Table 2). According to a review by Tunick (2011), breaking and reformation of molecular bonds in proteins during frequency sweeps may lead to structural changes that affect rheological properties. At low experimental time scale (that is high frequency), there is not enough time for broken inter and intra molecular bonds to reform. Possibly, this phenomenon leads to permanent molecular alignment or disentanglement of long chain polymers and consequence decrease in complex viscosity.

To understand the behaviour of marama protein in terms of its dough forming ability and high extensibility, composition and structural analyses were performed. Marama protein contains a high proportion of aromatic amino acid, tyrosine (Table 3), in comparison with soya (Ripperger-Suhler, 1983; Maruatona et al., 2010; Amonsou et al., 2012). Aromatic and non polar aliphatic amino acids can impart a hydrophobic character (Nakai, Li-chang, & Hayakawa, 1986). The constituents' polypeptides of

marama protein, which were found to be mainly basic (11S) legumin (Amonsou et al., 2012) can also impart hydrophobicity to marama protein. This is because similar basic (11S) proteins in soya have been found to be more hydrophobic than the acidic proteins (11S) and total glycinin (Yuan, Yang, Tang, Zheng, Ahmad, & Yin, 2009). Therefore, the substantial level of tyrosine and the abundance of basic (11S) protein in marama could reduce the solubility of its protein in aqueous solution and favour dough formation. The free energy of hydration, calculated from amino acid composition also suggests that marama protein is more hydrophobic than soya, having a less negative value of free energy of hydration (-185.25) compared to soya protein (200.81)(Table 3).

FTIR was used to study the structure of marama protein. In the amide I region, a single major peak at 1636.36 cm^{-1} , which corresponds to β -sheet structure (Meng & Ma, 2002; Withana-Gamage, Wanasundara, Pietrasika, & Shanda, 2010) was identified in marama proteins, as with soya protein (Fig. 2). No peak (corresponding to α -helical conformation) was observed in the amide I region in either marama or soya proteins. It is well reported that soya protein contains a very low proportion (approx 15%) of α -helical structure (Damodaran, 1989; Yuan et al., 2007). Thus, marama protein appears to have a very low proportion of alpha helical structure, similar to soya protein. This is in apparent contrast with Hulse, Larsen, Hansen, & Engelsen (2011) who reported almost equal amount of α -helical and β -sheet structures when analysing marama bean flour. In this study, purified marama protein was used, which may account for the difference. Some apparent differences in secondary structures between marama and soya proteins were evident in the amide II region, a peak at 1533.02 cm^{-1} corresponding to α -helix and a second peak at 1518.47 cm^{-1} ,

possibly corresponding to β -turns / stretching of aromatic amino acids (Holse et al., 2011). The second peak (at 1518.47 cm^{-1}) was absent in soya protein. Marama protein thus appeared to contain more β -sheet structure than soya.

The structure of marama protein was further characterised by DSC. Marama protein showed a single endothermic peak at around 96°C , in contrast with soya, which had two peaks at around 76°C and 91°C (Fig. 3). These peaks in soya correspond to thermal denaturation temperatures of β -conglycinin and glycinin, respectively (Shand, Ya, Pietrasik, & Wanasundara, 2007). The single transition observed in marama occurred at a higher temperature than did soya glycinin (11S) and was very broad. This is an indication of high thermal stability and resistance to denaturation of marama protein compared to soya glycinin (11S). High thermal denaturation temperature of soya glycinin (11S) has been related to its high proportions of β -sheet structure (Damodaran, 1988). DSC data thus also suggest that marama protein contains a high content of β -sheet than helical structures.

Since marama protein is rich in tyrosine (Maruatona et al., 2010; Amonsou et al., 2012) and this amino acid can be involved in protein crosslinking (Takasaki et al., 2005), POX was added to marama protein to crosslink the tyrosine residues. With added POX, the storage modulus (G') of marama protein dough increased to a greater extent than the untreated control (Fig. 4). In contrast, only a slight increase of G' with time was observed for POX treated gluten, while the untreated gluten dough remained almost unchanged. The increase in G' for POX treated marama protein dough indicates that new protein networks were formed, possibly through dityrosine crosslinks as was observed previously with gluten (Takasaki et al., 2005)

To further elucidate the types of crosslinks responsible for the structural stability of marama protein, marama protein and soya protein and POX treated marama protein and gluten dough samples were analysed by HPLC. Tyrosine eluted at about 12 min in marama protein (Fig. 5) and soya protein (data not shown). In addition to the tyrosine peak, POX treated marama protein dough had about 10 smaller eluting peaks as also did gluten (gluten data not shown). The concentrations of these peaks were lower in the untreated proteins compared to those treated with POX. Among these peaks, those eluting at around 16.5, 17.5, 18, and 22.3 min were tentatively identified as tyrosine crosslinks by reference to Michon, Wang, Ferrasson, & Gueguen (1999) and Tilley et al. (2001).

By Quadrupole MS, a compound with m/z of 182.2, corresponding to tyrosine (Tilley et al., 2001; Peña et al., 2006) was detected for the HPLC peak recovered at about 12 min (Fig. 6). Among the smaller peaks, only those recovered at 16.5 and 17.5 min revealed compound with m/z 360.5 or 361, which corresponds to dityrosine crosslinks (Tilley et al., 2001; Takasaki et al., 2005).

4. Conclusions

Marama protein is characterised by a highly viscous and extensible dough when compared to other plant storage proteins like soya and gluten. The dough forming ability and rheological behaviour of marama protein is most likely related to its high β -sheet conformation with some contribution from hydrophobic interactions. Tyrosine crosslinks appear to be important in the rheology of marama protein. The highly

viscous and extensible nature of marama protein indicates that it has potential as stabiliser in food systems.

5. References

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Table 1Tensile properties of marama protein, soya protein and gluten¹

38% moisture content (dry basis)					
Proteins	Peak stress (kPa)	Strain at break (%)	ϵ_H	Extensional Viscosity (η_E , kPa.s)	Area under curve (N.mm)
Marama	31.1 ^c ± 1.9	304 ^c ± 10	1.1 ^c ± 0.1	329.7 ^c ± 36.5	108.8 ^d ± 4.5
Soya	13.6 ^a ± 1.0	174 ^b ± 7	0.6 ^b ± 0.0	82.6 ^a ± 17.8	25.0 ^a ± 4.3
Gluten	35.9 ^d ± 1.7	165 ^a ± 6	0.5 ^a ± 0.0	184.1 ^b ± 34.2	55.5 ^b ± 3.0
45% moisture content (dry basis)					
Marama ²	15.9 ^b ± 1.5	>1043 ^e ± 125	>2.4 ^e ± 0.1	>579.0 ^d ± 48.5	>93.7 ^c ± 5.2
Soya ³	nd	nd	nd	nd	nd
Gluten	40.8 ^e ± 2.0	486 ^d ± 60	1.6 ^d ± 0.1	690.5 ^e ± 44.5	160.1 ^e ± 10.5

¹Mean ± SD (n=4). Mean values with different superscript letters in column are significantly different(p < 0.05), ϵ_H : Hencky strain / true strain²Marama dough at 45% moisture content did not break at the maximum extension (120 mm)³Soya protein at 45% moisture content flowed, nd : not determined

Table 2Rheological properties of marama protein and gluten¹

45% moisture content (dry basis)					
Proteins	ω (1/s)	G' [kPa]	G''[kPa]	Tan δ	η^* [kPa.s]
Marama	1	319.3 ^h \pm 38.9	86.7 ^h \pm 10.6	0.265 ^b \pm 0.011	330.5 ^k \pm 40.4
	10	487.8 ⁱ \pm 57.9	119.6 ⁱ \pm 14.6	0.255 ^b \pm 0.023	50.2 ⁱ \pm 6.1
	63	648.1 ^j \pm 79.4	157.5 ^j \pm 18.7	0.279 ^a \pm 0.015	10.6 ^g \pm 1.3
	100	681.0 ^j \pm 83.2	167.5 ^k \pm 19.5	0.315 ^b \pm 0.026	7.0 ^f \pm 0.8
Gluten	1	6.4 ^a \pm 1.8	2.3 ^a \pm 0.7	0.356 ^c \pm 0.007	6.8 ^f \pm 1.9
	10	10.8 ^b \pm 3.0	4.9 ^b \pm 1.2	0.455 ^c \pm 0.005	1.2 ^c \pm 0.3
	63	17.9 ^{cd} \pm 5.1	10.0 ^{cd} \pm 2.8	0.558 ^d \pm 0.004	3.3 ^d \pm 0.1
	100	20.5 ^d \pm 5.8	12.1 ^d \pm 3.4	0.586 ^d \pm 0.003	2.4 ^e \pm 0.1
52% moisture content (dry basis)					
Marama	1	90.8 ^e \pm 2.4	24.3 ^e \pm 6.2	0.266 ^b \pm 0.003	93.6 ^l \pm 2.5
	10	138 ^f \pm 31.8	31.4 ^f \pm 6.1	0.236 ^a \pm 0.008	14.1 ^h \pm 3.2
	63	178.5 ^g \pm 34.8	39.4 ^{fg} \pm 6.2	0.228 ^a \pm 0.007	2.9 ^c \pm 0.6
	100	187.0 ^g \pm 31.1	42.2 ^g \pm 5.7	0.231 ^a \pm 0.009	1.9 ^d \pm 0.3
Gluten	1	7.2 ^a \pm 0.1	2.5 ^a \pm 0.0	0.339 ^b \pm 0.007	7.6 ^f \pm 0.1
	10	11.4 ^b \pm 0.3	4.9 ^b \pm 0.2	0.424 ^c \pm 0.005	1.2 ^c \pm 0.0
	63	16.8 ^c \pm 0.2	8.7 ^c \pm 0.9	0.524 ^d \pm 0.006	0.3 ^b \pm 0.0
	100	18.8 ^d \pm 0.2	10.2 ^d \pm 0.1	0.550 ^d \pm 0.004	0.2 ^a \pm 0.0

¹Mean \pm SD (n = 4). Mean values with different superscript letters in columns are significantly different (p < 0.05). G': storage modulus, G'': loss modulus, Tan δ : phase angle, η^* : complex viscosity

Table 3

Free Energy of Hydration (FEH) of amino acids in marama protein compared to soya protein¹

Amino acid (AA)	FEH (kcal/mol) (Shewry et al., 2003)	Marama		Soya	
		mol% AA	FEH	mol% AA (Zakardas et al., 2007)	FEH
Lysine	-3.77	5.23	-19.72	6.46	-24.35
Isoleucine	0.07	4.03	0.28	4.99	0.35
Leucine	0.07	5.5	0.39	7.95	0.56
Methionine	-0.10	0.68	-0.07	2.12	-0.21
Cysteine	-0.27	0.79	-0.21	2.21	-0.60
Phenylalanine	-0.28	3.62	-1.01	5.17	-1.45
Tyrosine	-2.82	7.62	-21.49	3.88	-10.94
Tryptophan	-0.88	0.26	-0.23	1.32	-1.16
Valine	0.04	4.89	0.20	5.17	0.21
Threonine	-1.69	3.44	-5.81	5.04	-8.52
Histidine	-2.18	2.42	-5.28	2.86	-6.23
Asparagine	-3.11	10.56	-32.84	10.22	-31.78
Glutamine	-3.15	13.94	-43.91	18.51	-58.31
Serine	-2.36	7.31	-17.25	5.47	-12.91
Glycine	-0.23	10.78	-2.48	3.64	-0.84
Arginine	-6.85	5.03	-34.46	6.29	-43.09
Alanine	-0.66	5.12	-3.38	3.97	-2.62
Proline	0.23	8.82	2.03	4.72	1.09
Total			-185.25		-200.81

¹Aspartic acid and glutamic acid were computed as asparagine and glutamine, respectively

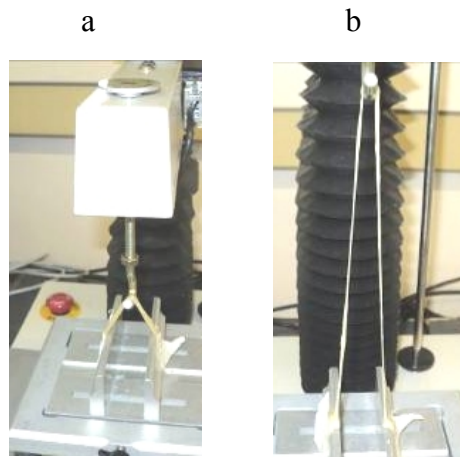


Fig. 1. Extensibility of marama protein at 45% moisture content (dry basis) measured with a TA-XT2 Texture Analyser. a: Just after the start of the extension, b: Extension at maximum set distance (120 mm)

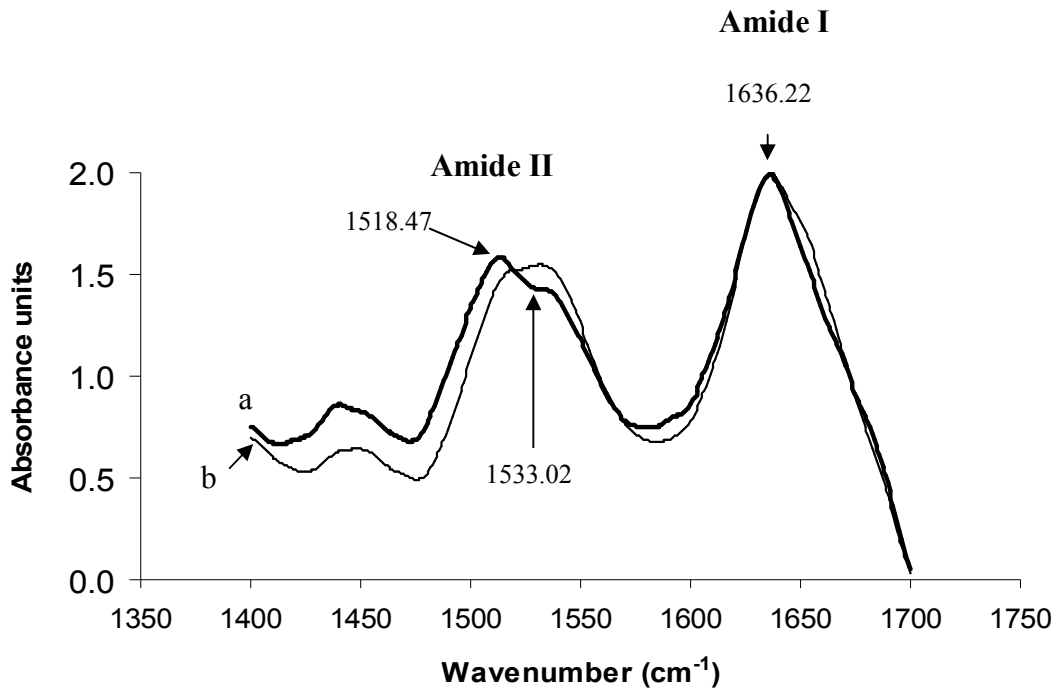


Fig. 2. Typical FTIR spectra of a: marama protein and b: soya protein

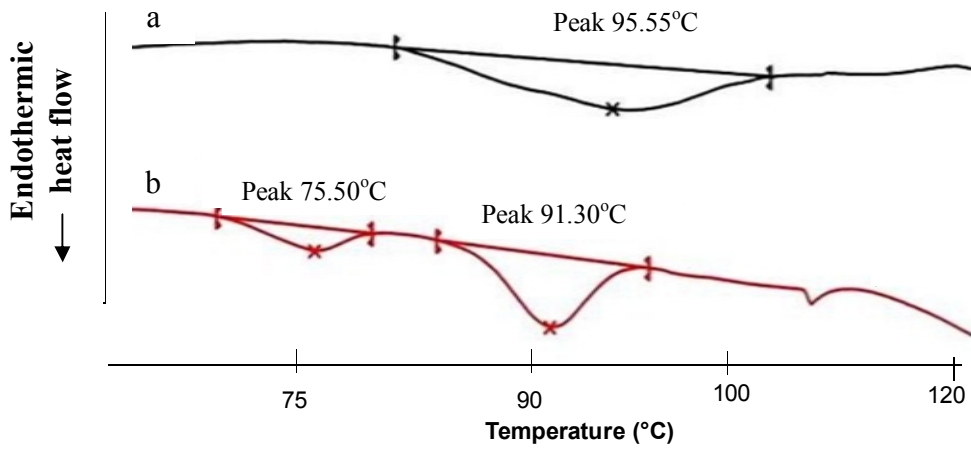


Fig. 3. Typical DSC thermograms of a: marama and b: soya protein

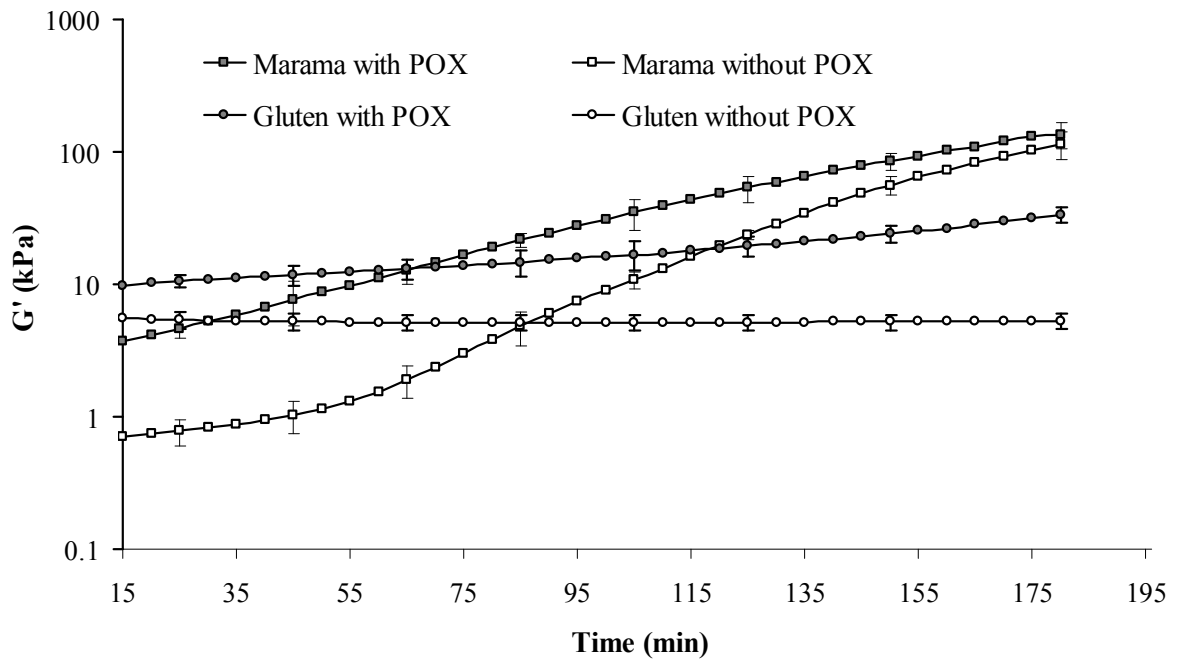


Fig. 4. Storage moduli (G') of marama protein and gluten treated with peroxidase (POX) as a function of time. Error bars indicate standard deviations of means ($n = 4$)

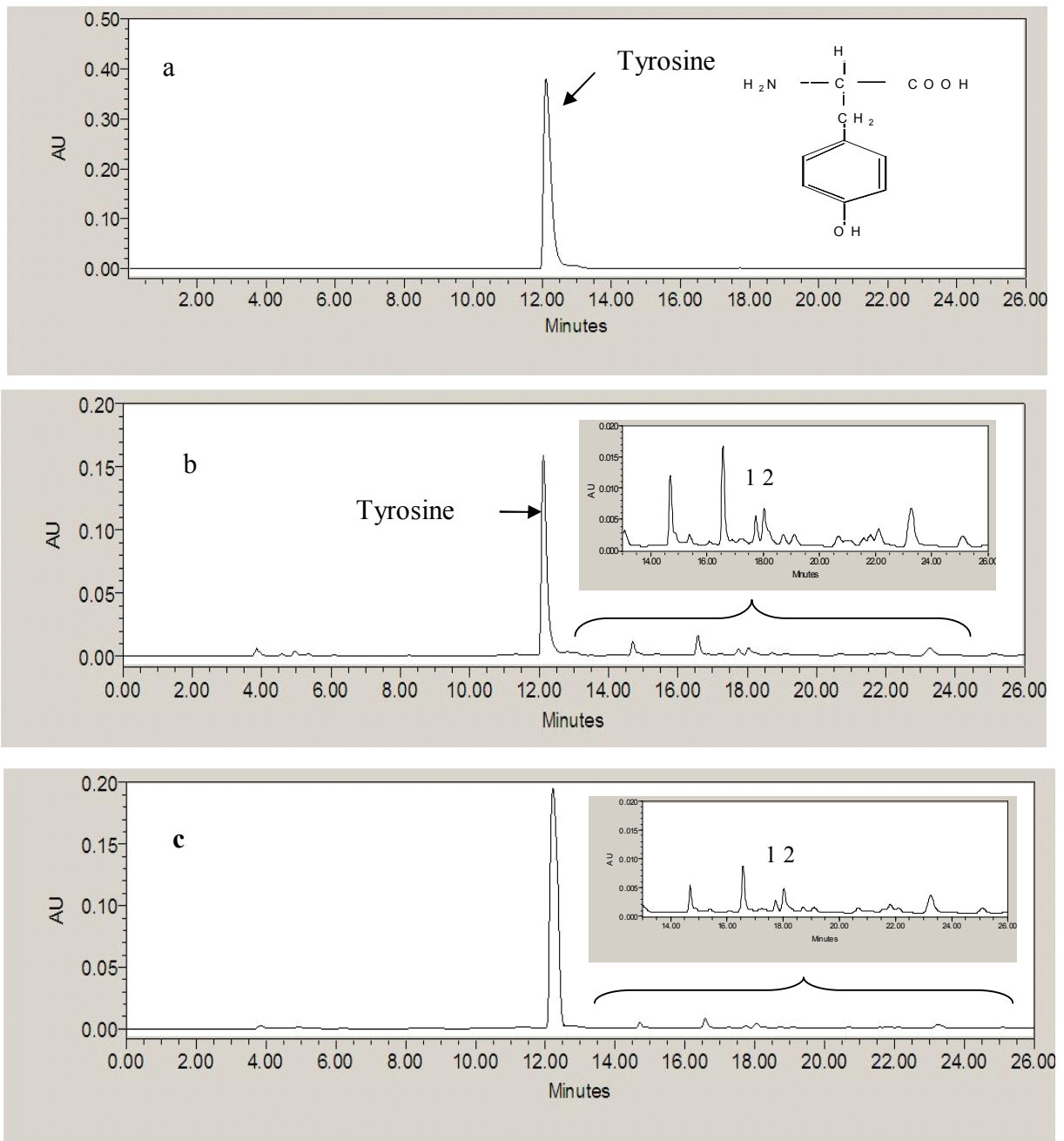


Fig. 5. Reverse-phase HPLC of hydrolysed marama protein dough treated with peroxidase (POX). a: tyrosine Standard, b: marama with POX, c: marama without POX, 1, 2, 3 crosslinked tyrosine peaks (Michon et al., 1999; Tilley et al., 2001) and MS analysis (Fig. 6)

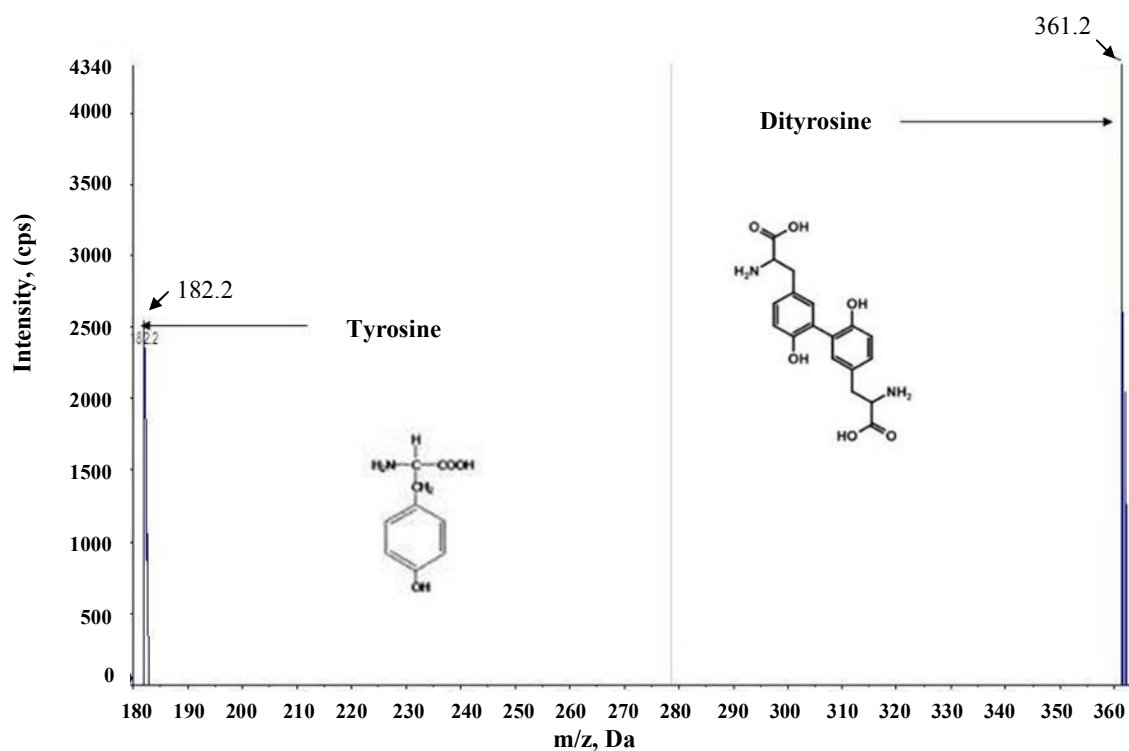


Fig. 6. Typical tyrosine and dityrosine MS peaks of amino acid separated at 17.5 min (Fig 5, peak 2) for marama protein incubated with peroxidase (POX), Positive mode gwas used for ionisation