# Fractionation and characterization of teff proteins

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Running title: Teff proteins

Abbreviations: 2-D, two- dimensional; AL+GL, albumins + globulins; ATP, adenosine triphosphate; DTT, 1,4-dithiothreitol; IEF, isoelectric focusing; IPG, immobilised pH gradient; kDA, kilo Daltons; LMWNC, low molecular weight nitrogen compounds; $M_r$, relative molecular size; MW, molecular weight; NL, nonlinear; NR, Non-reducing Conditions; R, reducing conditions; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; $T_g$, glass transition temperature.

## HIGHLIGHTS

The proportion of aqueous alcohol-soluble teff protein was approx. 40%. Hence, contrary to previous reports, prolamin is the major teff grain storage protein.

Teff prolamins were found to be less cross-linked by disulphide bonding than sorghum prolamins.

By 2-D electrophoresis, teff protein contained more polypeptides than maize or sorghum.

With differential scanning calorimetry, teff prolamin exhibited a single endothermic peak at 69.85°C, while no peak was detected for sorghum prolamin.

The lower polymerisation, hydrophobicity and denaturation temperature of teff prolamins probably make them somewhat functional in bread making, in comparison to the sorghum prolamins.

**Abstract**

The protein fractions in three different teff types were studied in comparison to sorghum to explain teff's superior bread making quality. The proportion of aqueous alcohol-soluble teff protein was approx 40% and it was rich in glutamine and leucine. Hence, contrary to previous reports, prolamin is the major teff grain storage protein. With SDS-PAGE under non-reducing and reducing conditions, teff prolamins showed broad bands at approx. 20.3 and 22.8 kDa. Other bands were at approx. 36.1, 50.2, 66.2 and 90.0 kDa, respectively under non-reducing conditions, but were absent under reducing conditions, indicating that these polypeptides are disulphide bonded. The presence of broad monomeric prolamin bands in teff under non-reducing conditions indicates that teff prolamin is less polymerised than sorghum prolamin. Estimated free energy of hydration of teff prolamins was -161.3 kcal/mol compared to -139.8 kcal/mol for sorghum prolamin. By 2-D electrophoresis, teff protein contained more polypeptides than maize or sorghum. Teff contained a higher proportion of basic polypeptides than maize. With differential scanning calorimetry, teff prolamin exhibited a single endothermic peak at 69.85°C, while no peak was detected for sorghum prolamin. The lower polymerisation, hydrophobicity and denaturation temperature of teff prolamins probably make them somewhat functional in bread making.

**Keywords:** Teff, sorghum, prolamin, electrophoresis

## 1. Introduction

The tropical cereal teff [*Eragrotis tef (*Zucc.) Trotter] is a staple cereal crop in Ethiopia (Ethiopian Central Statistical Authority, 2004, and Eritrea, Djibouti, south-eastern Sudan and northern Kenya (Curtis et al., 2008). The whole grain is ground into flour that can be used as a base ingredient for leavened flatbreads such as injera, added as a thickening agent to soups and sauces, fermented to make beer and ethnic beverages, or made into porridge and puddings (Bultosa and Taylor, 2004).

Recently, the use of teff in food systems is gaining popularity as both a naturally gluten-free alternative to wheat products and a nutrient-rich ingredient in the baby food industry (Hopman et al., 2008; Curtis et al., 2008). However, despite the growing interest in teff, there is limited scientific knowledge on the characteristics of its protein fractions. Teff flour, despite it being gluten-free, has been reported to produce high-quality leavened flatbread that stales much slower than if made from other cereals, in particular sorghum (*Sorghum bicolor* (L.) Moench) (Parker et al., 1989; Yetneberk et al., 2005; Taylor and Emmambux, 2008), which is commonly used to produce gluten-free baked goods and traditional flatbreads (Schober and Bean, 2008).

The reason for teff being the preferred cereal for flatbread has not been scientifically explored in detail. Bekele (1995) reported that teff protein is made up of 3-15% prolamins. However, the amino acid composition reported by this same author showed a low amount of lysine, and high levels of glutamine, alanine, leucine and proline, which according to Taylor and Emmambux (2008) is an indication of a high proportion of prolamins.

This study was therefore conducted to characterize teff protein to understand its superiority to sorghum in the production of baked goods.

## 2. Experimental

### *2.1. Teff, sorghum and maize grains*

South African white (Witkop) and brown (Rooiberg) teff varieties were purchased from Pannar Seeds, Greytown, South Africa. White Ethiopian teff grain was kindly provided by Dr. Senayit Yetneberk (Ethiopian Agricultural Research Institute). Sorghum grain of a mixture of two non-tannin, white tan-plant cultivars PANNAR PEX 606/202 obtained from PANNAR Seeds (Greytown, South Africa and white maize grain (cultivar PAN 6335) obtained from the South African Agricultural Research Council, Potchefstroom, South Africa, were used for comparison.

Approximately 500 g of each grain type was ground with a laboratory hammer mill (Falling Number 3100, Huddinge, Sweden) to pass through a 500 µm opening screen, stopping the mill at every 60 s and allowed to cool for 5 min to prevent sample heating. The milled whole flour samples were packaged in airtight zip-lock type polyethylene sample bags and stored at 4°C until use.

### *2.2. Fractionation of teff proteins*

The flours were used without defatting in the protein fractionation procedure, as described by Taylor et al. (1984). In brief the procedure was as follows. Albumins, globulins

and low molecular weight nitrogenous compounds (LMWNC) were extracted with 1.25 M NaCl (1 part of flour to 5 parts of solvent by weight) three times, and subsequently washed with distilled water, with continuous stirring for at 4°C for successive periods of 1h. The extracts were recovered by centrifugation at 6000 g for 10 min at 4°C. The supernatants were combined and dialyzed against distilled water for 24 h at 4°C (with three changes of water). The dialyzed extract (albumins + globulins fraction) was then freeze dried. The prolamins were then extracted with 60% (v/v) tert-butanol containing 0.05% (w/v) 1,4-dithiothreitol (DTT) at room temp. The extraction was repeated twice for 1 h each and then overnight. The extracts were recovered as described above for the NaCl extracts. The residue after prolamin extraction was resuspended in 100 ml distilled water and the starch hydrolysed with 1000 units of α-amylase (Megazyme International, Bray, Ireland) at 35°C until the suspension was starch free as determined by iodine solution. The suspension was centrifuged at 6000 g for 10 min and washed three times with distilled water to remove the soluble α-amylase and sugars. The resulting pellet (glutelins) was then freeze dried.

In order to analyse the teff prolamins by SDS-PAGE under non-reducing conditions (Fig.1 NR) they were extracted as described above but using 60% (vol./vol.) tert-butanol without DTT.

*2.3. Analyses*

Crude protein (N × 6.25) content of flours and protein fractions were determined by a Dumas combustion method (AACC International, 2000). Amino acid composition was determined

following the PICO.TAG-method of Bidlingmeyer et al. (1984). SDS-PAGE was done according to the procedure of Taylor et al. (2007). Loading was 20 µg protein per well on an X Cell SureLock Mini-Cell electrophoresis unit (Invitrogen Life Technologies, Carlsbad, CA). Gels were stained with Coomassie Brilliant Blue R-250.

Two-dimensional electrophoresis was performed using total protein from whole teff, sorghum, and maize flours. Protein extraction and solubilisation were done as described by Consoli and Damerval (2001). Electrophoresis was performed according to Natarajan et al. (2005). IEF, the first dimension, was performed using 13 cm immobilized pH gradient (IPG) strips [pH 3–10, nonlinear (NL)]. The strips were focused on steps at 500 V for 1 h, 1000 V for 1 h, gradient 1000–8000 V for 8 h, and 8000 V for 0.2 h using an Ettan IPGphor II system (Amersham Biosciences, Uppsala, Sweden). After IEF, the strips were first equilibrated in 0.375 M Tris–HCl buffer at pH 8.8 containing 6.0 M deionized urea, 20% (w/v) glycerol, 2% (w/v) SDS and 2% (w/v) DTT), and then in 0.375 M Tris–HCl buffer at pH 8.8 containing 6.0 M deionized urea, 20% (w/v) glycerol, 2% (w/v) SDS and 2.5% iodoacetamide , each for 15 min. SDS-PAGE was carried out in a Hoefer SE 600 Ruby electrophoresis unit (Amersham Biosciences) at 150 V and 20 mA/gel until the bands migrated from the stacking gel into the separating gel. Then the voltage was set at 600 V and 30 mA/gel. Gels were silver stained using a PlusOne Silver Staining Kit system (Amersham Biosciences). Gel images were acquired using a Versa Doc Documentation system (Bio-Rad, Hercules, Canada). The gel images were analysed using melanie$^{TM}$ 2-D gel analysis software, version 7.05 (Swiss Institute of Bioinformatics, Switzerland). Isoelectric points (pI) and molecular weights (MW) were calculated for all spots using the software. Based on pI values, the numbers of acidic and basic polypeptide spots were counted.

Differential scanning calorimetry (DSC) was performed on the extracted teff and sorghum prolamins using a Metler Toledo (Schwerzenback, Switzerland) HPDSC-827 DSC. The procedure was according to Ju et al. (2001). Approximately 5 mg sample was weighed directly into an aluminium pan and 10 µl 0.01 M phosphate buffer, pH 7.5 was added. The pan was sealed; the mixture allowed to equilibrate for 3 h, and then scanned over the range 25 to120°C at 10°C/min.

*2.3. Statistical analysis*

The extraction experiments were repeated three times. Crude protein, amino acid composition, SDS-PAGE, 2-D electrophoresis and DSC were done in duplicate. Protein extraction data were subjected to one way analysis of variance and the means compared using Fisher's Least Significant Different Test at $p \leq 0.05$.

## 3. Results and Discussion

*3.1. Fractionation of teff and sorghum proteins*

The protein contents of the Witkop, Rooiberg and white Ethiopian teff (10.2-11.6%) were higher than that of the sorghum (Table 1) but similar to values reported by previous authors (Jansen et al., 1962; BOSTID, 1996). When compared to other millets the teff protein content was lower than values reported for proso millet (Kalinova and Moudry, 2006), common millet and foxtail millet but slightly higher than values reported for finger millet (Ravindran, 1991).

However, such comparisons must be treated with caution as cereal grain protein content is strongly affected by cultivar and cultivation conditions.

The distribution of the protein fractions in different teff varieties was similar, although the proportion of LMWNC varied (Table 1). Teff protein contained a higher proportion of albumins + globulins than sorghum, 11% of total protein compared to about 6%. The proportion of glutelins was rather lower, approx. 22% compared to about 30% in sorghum. It is difficult to assign a particular reasons for this, as the glutelin fraction in sorghum, for example, comprises very heterogenous proteins and occurs in both the endosperm and pericarp tissues (Taylor and Schüssler, 1986). However, the higher proportion of albumins and globulins in teff protein may have an influence on its functionality. Chakraborty and Khan (1988) reported that compositional differences in protein fractions such as albumins and globulins can result in differences in wheat flour functional properties, such as baking performance and dough rheology. Dreese and Hoseney (1990) found that when water soluble proteins were removed, gluten dough became more elastic and less viscous.

Prolamins accounted for approximately 40% of the total teff protein (Table 1). Bekele (1995) reported a much lower prolamin content of teff protein, 3-15%. The difference can be attributed to differences in the method of extraction. In this study, extraction of prolamins was done with 60% tert-butanol containing 0.05% DTT as opposed to extraction with 60% ethanol only by Bekele (1995). Tert-butanol is a more hydrophobic solvent than ethanol and is used to extract the prolamins from tropical cereals such as sorghum (Belton et al., 2006), which are more hydrophobic than those of wheat (Duodu et al., 2003). Also, the presence of DTT should have led to extraction of more prolamins compared to only aqueous ethanol (Taylor et al., 2005; Moroni, et al., 2010). Several authors have reported different values for protein fractions in

cereals as a result of differences in the extraction conditions especially solvent used in the extraction (Taylor et al., 1984; Chandna and Matta, 1990; Taylor et al., 2005; Moroni, et al., 2010). A large proportion of the storage proteins in cereals is disulphide bonded into large polymeric networks, so a reducing agent is necessary to efficiently extract these proteins (Bean and Lookhart, 2000; Taylor et al., 2005).

*3.2. Amino acid composition*

The teff albumins + globulins fraction contained higher proportions of arginine, aspartic acid/asparagine and lysine compared to the prolamins and glutelins fractions. The teff prolamins fraction was very much richer in glutamic acid/glutamine and richer in leucine compared to other fractions. Interestingly, however, teff prolamin contained much lower amounts of leucine and proline, and higher content of glutamic acid/glutamine compared to sorghum prolamin. Not withstanding this, it is clear from its amino acid composition that the teff prolamin fraction extracted with aqueous tert-butanol plus DTT is prolamin. Thus, based on its amino acid composition and proportion of total protein, it is evident that prolamin is in fact the major storage protein in teff as in other tropical cereals such as sorghum (Taylor et al., 1984), pearl millet (Chandna and Matta, 1990), finger millet (Ramachandra et al., 1978) and maize (Chandna and Matta, 1990).

The free energies of hydration of the teff protein fractions were calculated from their amino acid content according to Shewry et al. (2003) and compared to those of sorghum (Table 3). The teff prolamins free energy of hydration (-161.31 kcal/mol) was similar to that of the teff glutelins (-160.80 kcal/mol) but less negative, i.e. more hydrophobic than the teff albumins + globulins fraction. Compared to sorghum prolamins (-139.800, teff prolamins was more

negative. This indicates that teff prolamins are more hydrophilic than sorghum prolamins. In fact, the free energy of hydration of teff prolamins is much closer to values reported for wheat gliadins (-159.794 kcal/mol) and glutenins (-165.817 kcal/mol) by Shewry et al. (2003), as well as -140.36 and -113.63 kcal/mol reported for α- and γ-kafirins, respectively by Duodu et al. (2003).

*3.3 SDS-PAGE*

The SDS–PAGE patterns of teff prolamins for the three teff varieties were similar but somewhat different from that of those of sorghum (Fig. 1). Under non-reducing conditions, teff prolamins (extracted with 60% tert-butanol without DTT) showed broad protein bands at approx $M_r$ 20.3 and 22.8 kDa (Fig. 1 NR lanes 1-3), assumed to be the teff prolamin monomers. Tatham et al. (1996) reported two major prolamin bands with $M_r$ approx. 22.5 and 25.0 kDa in teff under reducing conditions. The sorghum prolamin monomers were of somewhat higher apparent molecular weight and the bands were much fainter (Fig.1 NR lane 4). Other teff bands were of $M_r$ approx. 36.1, 50.2, 66.2 and 90.0 and kDa, respectively. These bands were absent under reducing conditions (R lanes 1-3), indicating that they were polypeptides linked by disulphide bonding and may be considered as prolamin oligomers (dimers, trimers and tetramers), similar to sorghum (El Nour et al., 1998; Emmambux and Taylor, 2009).

Under reducing conditions, the two major prolamin monomer bands of $M_r$ approx. 20.3 and 22.8 kDa were present in teff (Fig. 1 R lanes 1-3) at similar intensity as under non-reducing conditions. In contrast, the sorghum prolamin monomers (Fig 1 R lane 4) were present at much higher intensity than under non-reducing conditions. This indicates that the teff prolamins are less polymerised than sorghum prolamins. The ability of teff flour to produce good quality baked goods

may be related to this. Emmambux and Taylor (2009) reported that sorghum kafirin (prolamin) contained a higher proportion of cross-linked polypeptides compared to maize zein, suggesting a higher propensity towards intermolecular disulphide crosslinking among kafirins than occurring in zeins. According to Hamaker and Bugusu (2003) this crosslinking of kafirins encapsulates alpha-kafirin, the major kafirin subclass, within the sorghum protein bodies, thus preventing the kafirin from being functional in dough systems.

*3.4 2-D electrophoresis*

The protein spot patterns on the 2-D gels for teff, maize and sorghum total proteins were different (Fig. 2). The teff and maize protein spots were more clearly resolved than those of sorghum. This might be due to the relative insolubility of sorghum prolamins, as 2-D electrophoresis resolution is affected by the solubilisation buffer (Görg et al., 2004; Natarajan et al., 2005). Notwithstanding these issues of resolution and prolamin solubility, teff seemed to contain more different proteins than maize and sorghum, as indicated by the number of spots counted in the 2-D electrophorograms, some 646, 552 and 294, respectively. This could be related to differences in chromosome number. Maize (Kynast et al., 2001) and sorghum (Kim et al., 2005) are diploid with a chromosome number of $2n = 2x = 20$, while teff is tetraploid with a chromosome number of $2n = 4x = 40$ (Yu et al., 2004). The number of protein spots obtained from maize 2-D gel in this study was considerably higher than values (113) reported by Albo et al. (2007) for genetically modified maize protein. The differences might be due to the fact that this present gels were stained with silver stain, while Albo et al. (2007) used Coomassie dye. Silver staining is generally more sensitive in detecting polypeptide spots than Coomassie staining (Rabilloud et al., 1994). The number of acidic polypeptide spots (pI 3.0-6.5) was higher than

basic polypeptide spots (pI 7.5-10.0) with all three cereal grains. However, teff protein contained a higher proportion of basic polypeptides (47%) than maize (34%) or sorghum (43%). This may be of significance with regard to protein functionality in "sour dough type" fermented products such as injera (Yetneberk et al., 2005), as during the bread making process lactic acid production would result in a substantial proportion of teff proteins being charged.Since there are no data on total proteins of teff and sorghum grains in proteome databases, the polypeptide spots on teff 2-D gel were tentatively identified by comparing with published pIs and molecular weights for proteins from maize endosperm (Mechin et al., 2004) and flour (Albo et al., 2007). About 80 protein spots, representing 12% of the total protein spots in the teff 2-D gel were found to match with maize. For sorghum, about 24 spots (7%) of the protein spots matched. This suggests that there are more qualitative similarities between teff and maize proteome maps compared to sorghum. The results of the tentatively identified polypeptide spots are presented in supplementary Tables 1, 2 and 3. Spots tentatively identified in teff included proteins involved in metabolism, development, adenosine triphosphate (ATP) synthesis, protein transcription, cell rescue, defence, death and ageing, as well as heat shock protein precursors.

*3.5 DSC*

DSC can be used to study the thermal denaturation properties of proteins, as thermal denaturation will cause an endothermic peak. Teff prolamin showed a single endothermic peak at around 69.85°C (Fig. 3). No peak was detected for sorghum prolamin up to 120$^{o}$C, the maximum temperature applied. This indicates that teff prolamin is less thermally stable than kafirin. Lawton (1992) using DSC found that the glass transition temperature ($T_g$) (endothermic

peak temperature) of zein, maize prolamin, ranged between >140$^{o}$C and <30$^{o}$C, over a moisture content of <5% to >25%, respectively. He further observed that zein dough exhibited good visco-elastic properties above its $T_{g.}$ The relatively low thermal stability of teff prolamin compared to kafirin may be related to the good bread making functionality of teff flour.

## 4. Conclusions

As in most other cereals, prolamins are the major protein group in teff grain. There are several significant differences between teff and sorghum prolamins. Teff prolamins are more hydrophilic, less polymerised and have lower thermal stability. These differences probably make them more functional in bread making compared to sorghum prolamins.

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LEGENDS TO FIGURES

Fig. 1. SDS-PAGE profiles of teff and sorghum prolamins under non-reducing (NR) and reducing (R) conditions

Prolamins subjected to SDS-PAGE under non-reducing conditions were extracted with 60% tert-butanol without DTT.

Lane 1. Witkop teff, Lane 2. Rooiberg teff, Lane 3. Ethiopian white teff, Lane 4. white sorghum, M. Molecular weight standards (kDa).

Fig. 2. Two-dimensional electrophoresis map of protein from whole Witkop teff, maize and sorghum flours using immobilized pH gradient (IPG) strips (3-10), silver stained. a. teff, b. maize, c. sorghum.

Fig. 3. DSC thermograms for Witkop teff and white sorghum prolamins extracted with 60% tert-butanol plus DTT

Table 1

Distribution of teff and sorghum protein fractions

| mple | Protein content of flour (g/100 g)(db) | LMWNC[1] (g/100 g protein) | AL+GL[2] (g/100 g protein) | Prolamins[3] (g/100 g protein) | Glutelins (g/100 g protein) | Protein recovery (%) |
|---|---|---|---|---|---|---|
| tkop teff | $10.8^{c}$±0.1 | $14.0^{a}$±1.8 | $12.2^{b}$±1.6 | $42.5^{c}$±1.2 | $21.0^{a}$±0.7 | $92.5^{a}$±1.0 |
| oiberg teff | $11.6^{d}$±0.1 | $20.2^{b}$±3.2 | $11.3^{b}$±4.1 | $41.2^{bc}$±2.2 | $20.6^{a}$±0.6 | $93.2^{a}$±2.9 |
| hite hiopian teff | $10.2^{b}$±0.9 | $24.1^{c}$±2.1 | $10.1^{b}$±1.0 | $38.4^{a}$±1.0 | $24.9^{b}$±1.3 | $97.4^{b}$±1.5 |
| hite rghum | $8.8^{a}$±0.1 | $13.5^{a}$±1.0 | $6.7^{a}$±0.6 | $40.9^{b}$±0.7 | $30.3^{c}$±2.0 | $91.5^{a}$±2.0 |

[1] Low molecular weight nitrogenous compounds

[2]Albumins+Globulins

[3]Extracted with 60% tert-butanol plus DTT

± Mean and standard deviation of three separate extractions

Mean values with different superscript within the same column are significantly different ($P<0.05$)

Table 2
Amino acid composition (g/100 g protein) of Witkop teff and white sorghum protein fractions[1]

| | Albumins+Globulins | | Prolamins[2] | | Glutelins | |
|---|---|---|---|---|---|---|
| Amino acid | Teff | Sorghum | Teff | Sorghum | Teff | Sorghum |
| Histidine | 2.9$^{d}$ | 2.5$^{d}$ | 1.7$^{b}$ | 1.2$^{a}$ | 2.2$^{c}$ | 3.5$^{e}$ |
| Threonine | 3.8$^{e}$ | 3.9$^{e}$ | 3.6$^{d}$ | 2.5$^{a}$ | 3.8$^{e}$ | 3.5$^{d}$ |
| Lysine | 5.6$^{g}$ | 4.8$^{f}$ | 0.2$^{a}$ | 0.1$^{a}$ | 3.9$^{e}$ | 3.6$^{d}$ |
| Tyrosine | 3.2$^{a}$ | 3.2$^{a}$ | 5.4$^{e}$ | 4.9$^{b}$ | 3.8$^{c}$ | 3.9$^{c}$ |
| Methionine | 1.8$^{c}$ | 0.0 | 4.7$^{e}$ | 1.3$^{ab}$ | 5.2$^{f}$ | 1.5$^{bc}$ |
| Valine | 5.0$^{bc}$ | 5.2$^{c}$ | 4.8$^{b}$ | 4.4$^{a}$ | 4.4$^{a}$ | 5.0$^{bc}$ |
| Isoleucine | 3.3$^{a}$ | 3.3$^{a}$ | 4.4$^{c}$ | 4.4$^{c}$ | 3.6$^{b}$ | 3.6$^{b}$ |
| Leucine | 6.1$^{a}$ | 6.1$^{a}$ | 9.0$^{b}$ | 16.1$^{d}$ | 6.7$^{a}$ | 8.4$^{b}$ |
| Phenylalanine | 3.4$^{a}$ | 3.8$^{b}$ | 6.0$^{e}$ | 6.2$^{e}$ | 4.5$^{d}$ | 4.2$^{c}$ |
| *Subtotal essential amino acids* | 35.1 | 32.8 | 39.8 | 41.1 | 38.1 | 37.2 |
| Serine | 4.0$^{bc}$ | 4.5$^{d}$ | 4.2$^{c}$ | 4.2$^{c}$ | 4.5$^{d}$ | 3.7$^{a}$ |
| Arginine | 7.7$^{f}$ | 8.1$^{g}$ | 1.2$^{a}$ | 1.6$^{b}$ | 4.8$^{e}$ | 4.4$^{d}$ |
| Glycine | 6.1$^{g}$ | 5.2$^{e}$ | 1.3$^{a}$ | 1.5$^{a}$ | 5.5$^{f}$ | 4.4$^{d}$ |
| Aspartic acid/ Asparagine | 7.9$^{f}$ | 7.9$^{f}$ | 3.2$^{a}$ | 5.4$^{bc}$ | 6.3$^{d}$ | 6.8$^{e}$ |
| Glutamic acid/ Glutamine | 18.3$^{c}$ | 22.5$^{d}$ | 33.9$^{f}$ | 28.3$^{e}$ | 16.6$^{b}$ | 13.6$^{a}$ |
| Alanine | 5.9$^{b}$ | 5.9$^{b}$ | 5.1$^{a}$ | 7.1$^{c}$ | 5.0$^{a}$ | 5.8$^{b}$ |
| Proline | 3.7$^{a}$ | 4.0$^{ab}$ | 5.7$^{d}$ | 8.9$^{f}$ | 5.0$^{c}$ | 6.6$^{e}$ |
| *Subtotal non-essential amino acids* | 53.6 | 58.1 | 54.6 | 57.0 | 47.7 | 45.3 |
| % Recovery | 88.7 | 90.9 | 94.4 | 98.1 | 85.8 | 82.5 |

[1]Mean (n = 2) values with different superscripts within the same row are significantly different (P<0.05)

[2]Extracted with 60% tert-butanol plus DTT

Table 3

Free energy of hydration of amino acids of witkop teff and white sorghum protein fractions

| | | Free energy of hydration (kcal/mol) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Albumins+Globulins | | Prolamins | | Glutelins | |
| Amino acids | Free energy of hydration | Teff | Sorghum | Teff | Sorghum | Teff | Sorghum |
| Histidine | -2.18 | -5.68 | -4.89 | -3.38 | -2.30 | -4.66 | -7.43 |
| Serine | -2.36 | -12.71 | -14.15 | -13.23 | -12.41 | -15.01 | -12.76 |
| Arginine | -6.85 | -42.45 | -44.80 | -6.79 | -8.29 | -27.75 | -26.54 |
| Glycine | -0.23 | -2.64 | -2.24 | -0.55 | -0.61 | -2.49 | -2.05 |
| Aspartic acid | -3.11 | -26.02 | -25.92 | -10.69 | -16.58 | -21.86 | -24.47 |
| Glutamine | -3.15 | -57.68 | -68.09 | -102.52 | -79.86 | -52.63 | -44.47 |
| Threonine | -1.69 | -7.59 | -7.73 | -7.18 | -4.71 | -8.01 | -7.65 |
| Alanine | -0.66 | -6.12 | -6.16 | -5.37 | -6.89 | -5.48 | -6.60 |
| Proline | 0.23 | 1.03 | 1.14 | 1.61 | 2.35 | 1.48 | 2.02 |
| Lysine | -3.77 | -20.44 | -17.43 | -0.62 | -0.48 | -14.99 | -14.28 |
| Tyrosine | -2.82 | -7.07 | -6.98 | -11.95 | -10.10 | -8.78 | -9.39 |
| Methionine | -0.10 | -0.17 | 0.00 | -0.44 | -0.12 | -0.51 | -0.15 |
| Valine | 0.04 | 0.24 | 0.25 | 0.23 | 0.20 | 0.22 | 0.26 |
| Isoleucine | 0.07 | 0.25 | 0.24 | 0.33 | 0.31 | 0.28 | 0.30 |
| Leucine | 0.07 | 0.46 | 0.46 | 0.67 | 1.13 | 0.53 | 0.69 |
| Phenylalanine | -0.28 | -0.81 | -0.91 | -1.44 | -1.38 | -1.14 | -1.09 |
| Total | | -187.40 | -197.21 | -161.31 | -139.75 | -160.80 | -153.61 |

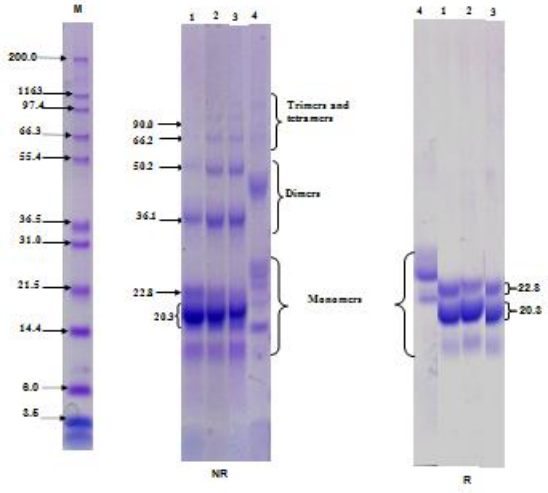
M
200.0
116.3
97.4
66.3
55.4
36.5
31.0
21.5
14.4
8.0
3.6
1 2 3 4
90.0
66.2
50.2
36.1
22.8
20.3
Trimers and tetramers
Dimers
Monomers
NR
4 1 2 3
22.8
20.3
R

Figure 1

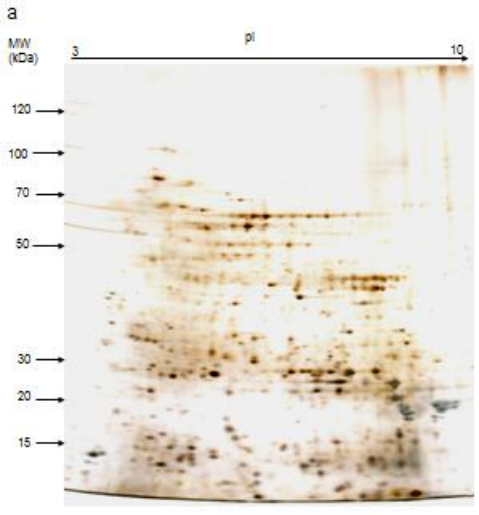


Figure 2a

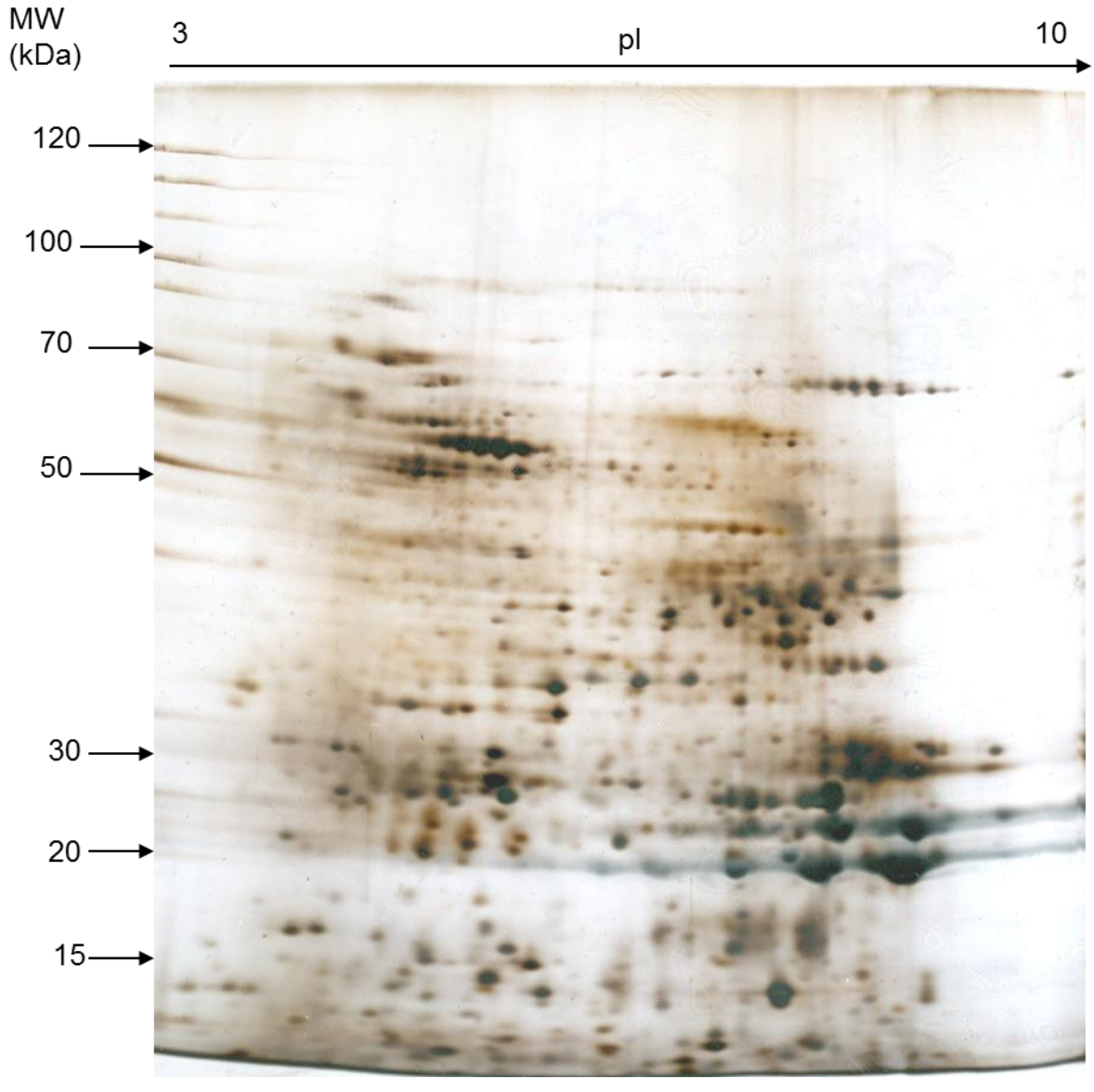


Figure 2b

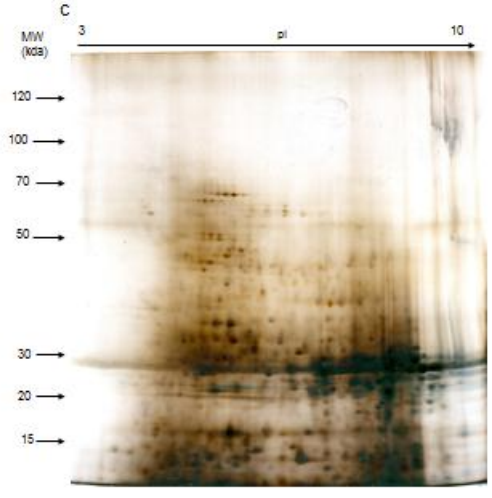


Figure 2c

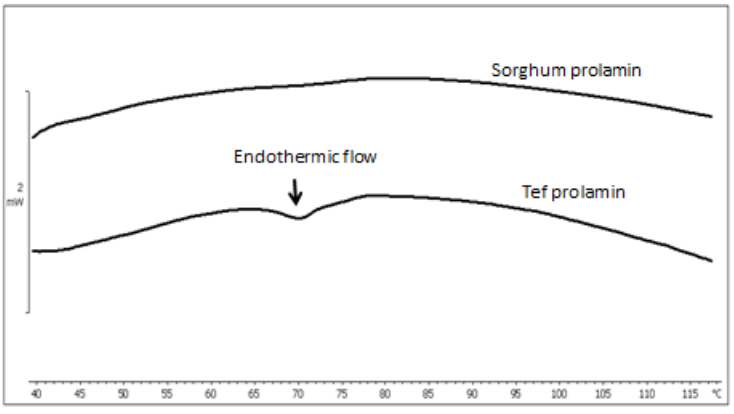
Sorghum prolamin
Endothermic flow
Tef prolamin
2
mW
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
°C

Figure 3