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Effect of micronisation temperature (130 and 170 °C) on functional properties of cowpea flour

Agnes M. Mwangwela [a,c], Ralph D. Waniska [b], Amanda Minnaar [a,*]

[a] Department of Food Science, University of Pretoria, Lynnwood Road, Pretoria 0002, South Africa
[b] Cereal Quality Laboratory, Department of Soil and Crop Sciences, 2474 TAMUS, Texas A&M University, College Station, TX 77843-2474, USA
[c] Department of Home Economics and Human Nutrition, Bunda College of Agriculture, University of Malawi, Lilongwe, Malawi

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Abstract

Functional properties of cowpea flour from seeds micronised at two different surface temperatures (130 and 170 °C) were studied. Micronisation (130 and 170 °C) significantly ($P \leqslant 0.05$) increased the water absorption capacity and least gelation concentration of the flour. The treatment significantly ($P \leqslant 0.05$) reduced the water solubility and swelling indices, gel strength and foaming capacity of the flour. The changes in cowpea flour functional properties, such as the loss of foaming capacity in flours from micronised (130 and 170 °C) seeds, were associated with significant ($P \leqslant 0.05$) increase in the surface hydrophobicity and cross-linking of the cowpea protein. SDS–PAGE of the protein-rich fractions revealed changes in the protein subunit profile which included the formation of disulphide bonds and possibly Maillard cross-links. The flour from M-170 °C seeds was significantly ($P \leqslant 0.05$) darker than was the flour from unmicronised and M-130 °C seeds.

Keywords: Cowpea flour; Micronisation; Protein; Functional properties

1. Introduction

Micronisation is a short time high temperature process that utilises electromagnetic radiation in the infra red region (wavelength of 1.8–3.4 nm) to rapidly heat materials (Zheng, Fasina, Sosulski, & Tyler, 1998). The process has been shown to considerably reduce the cooking time of legumes, such as cowpeas, lentils and split peas (Arntfield et al., 2001; Cenkowski & Sosulski, 1998; Mwangwela, Waniska, & Minnaar, 2006). However, wider use of the micronisation process in cowpeas could be attained by extending the utilisation of micronised legume seeds in the diet beyond the whole seed. Milling of micronised cowpeas into flour could be one such process, since there are existing uses of cowpea flour in food systems. There is a wide variety of products that are made from cowpea flour in different parts of Africa which are dependent on the functionality of cowpea flour (Phillips et al., 2003). Apart from the traditional products, cowpea flour has also been used as a nutritious ingredient in fried (Kerr, Ward, McWatters, & Resurreccion, 2001) and baked (Phillips et al., 2003) products, as well as comminuted meat products, such as chicken nuggets (Prinyawiwatkul, Beuchat, McWatters, & Phillips, 1997b) and meatballs (Serdaroglu, Yildiz-Turp, & Abrodimov, 2005).

The suitability of cowpea flour in such food systems is dependent on its functional properties, such as foaming, water and oil absorption capacities (WAC and OAC), as well as thermally induced gelling (Abu, Muller, Duodu, & Minnaar, 2005; Prinyawiwatkul, Beuchat, McWatters, & Phillips, 1997a). Cowpea protein (24%) is one of the main contributing components to the functionality of the flour (Mwangwela et al., 2006). Cowpea seeds have high protein content which is relatively hydrophilic and water-soluble (Mwasaru, Muhammad, Bakar, & Che Man,

* Corresponding author. Tel.: +27 12 420 3239; fax: +27 12 420 2839.
E-mail address: amanda.minnaar@up.ac.za (A. Minnaar).

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1999). These physicochemical properties of cowpea protein are crucial in retaining the good foaming properties of the flour, necessary for imparting a spongy texture of cowpea flour-based products, such as *akara* (Plahar, Hung, McWatters, Phillips, & Chinnan, 2006). The WAC and OAC and gelling properties of heterogeneous systems, such as flour, are physicochemical characteristics of protein and starch components (Prinyawiwatkul et al., 1997b).

Micronisation has been shown to precook legumes, such as cowpeas (Mwangwela et al., 2006), lentils (Arntfield et al., 2001), beans (Bellido, Arntfield, Cenkowski, & Scanlon, 2006) and peas (Cenkowski & Sosulski, 1998). In addition to these products, the flour from micronised cowpeas has potential for utilisation in some food systems, depending on its functionality. Fasina, Tyler, Pickard, Zheng, and Wang (2001) reported increased pasting viscosities of legume (kidney, pinto, and black beans, and green peas) flour following micronisation (<10% moisture, 140 °C). Similarly, Cenkowski and Sosulski (1998) reported significant ($P \leqslant 0.05$) increase in the pasting property of flour from split peas (26% moisture content, 120 °C). Contrary to these findings earlier results from our studies have shown that micronisation of moisture-conditioned (41%) cowpea seeds, especially at 170 °C, adversely reduced the pasting properties of cowpea flour (Mwangwela, Waniska, McDonough, & Minnaar, 2007). Fasina, Tyler, Pickard, and Zheng (1999) reported improved water absorption capacity for hulless and pearled barley following micronisation (26.5% moisture content, 105 and 115 °C). At the same time, a reduction in WAC has been reported for chick pea flour from micronised seeds (Sarantinos & Black, 1996). Micronisation did not have a definite effect on oil absorption capacity of chick pea flour (Sarantinos & Black, 1996). Since information on functional properties of flour from micronised legumes is rather scanty, the objective of this study was to examine the effect of micronising cowpea seeds, at low (130 °C) and high (170 °C) final surface temperatures, on functional properties of the flour.

2. Materials and methods

2.1. Raw materials

Bechuana white (white color) cowpeas supplied by Agricol (Potchefstroom, South Africa) were cleaned to remove chaff and shrivelled and broken seeds. The cleaned seeds were packed in propylene bags and stored at 4 °C until the time of use.

2.2. Hydrothermal process and cowpea flour preparation

The cowpeas were micronised according to the method described by Mwangwela et al. (2006). Cowpea seeds were steeped in deionised water for 6 h, followed by equilibration for 12 h. The moisture-conditioned (41% moisture) seeds were micronised in 160 g batches for 3 and 8 min to final surface temperatures of 130 (M-130 °C) and 170 °C (M-170 °C), respectively, using a tabletop microniser (Technilamp Pty., Johannesburg, South Africa). Surface temperature of the seeds during micronisation was monitored using thermocouples attached to a Grant Squirrel 800 data logging system (Monitoring and Control Lab, Johannesburg, South Africa). Following micronisation, the micronised cowpeas had 25% and 5.0% moisture contents for the M-130 and M-170 °C treatments, respectively. Micronised cowpeas were spread on a tabletop and cooled to room temperature for 1 h before being packed in Zipper bags (Plastilon Packaging, Pretoria, South Africa) and kept at 22 °C. Due to the high residual moisture, the M-130 °C cowpea samples were freeze-dried before storage. Unmicronised and micronised (130 and 170 °C) cowpeas were milled (Falling number mill, 3100) to pass through a 250 μm-aperture sieve. The milled flour samples were vacuum-sealed and stored at 4 °C prior to use.

2.3. Colour values of the cowpea flour

Colours of the unmicronised and micronised cowpea flour were determined using a Chroma Meter CR-400 (Konica Minolta Sensing Inc, Osaka, Japan). The colour of the flours was expressed as L^*, a^*, b^* values, where L^* = lightness, a^* = redness, and b^* = yellowness.

2.4. Moisture content

Moisture contents of the micronised and unmicronised flours were determined using the AOAC method 925.10 (AACC, 2000).

2.5. Crude protein

Crude protein content of the flour from unmicronised and micronised (130 and 170 °C) cowpea seeds was determined using the Dumas method. A factor of 6.25 was used to calculate the crude protein from the nitrogen content determined using a Leco Nitrogen Analyser FP 528 (Leco Africa Pty, Kempton Park, South Africa).

2.6. Nitrogen solubility index

Nitrogen solubility index of the flour was determined according to the AACC method 46-23 (AACC, 2000). One gramme flour samples were dispersed in 50 ml of 0.1 M NaCl solution and stirred continuously for 1 h with the pH maintained at 7. About 20 ml of the suspension was centrifuged (10,000*g*, 15 min, 4 °C) and the supernatant filtered through a Whatman No. 1 filter paper. The nitrogen content of the filtrate was determined using a Leco Nitrogen Analyzer 528 (Leco Africa Pty, Kempton Park, South Africa). Protein content of the filtrate was calculated using 6.25 as the conversion factor. Nitrogen solubility index was expressed as a percentage of the sample protein on a dry basis.

2.7. Water solubility index (WSI), water and oil absorption capacities (WAC, OAC)

Water and oil absorption capacities (WAC, OAC) of the flour from unmicronised and micronised (130 and 170 °C) cowpeas were determined according to the AACC method 56-20 (AACC, 2000) with slight modifications. A 2 g (M0) flour sample was dispersed in 40 ml of deionised water or refined sunflower oil and vortexed for 10 min. The samples were centrifuged (1000*g*, 15 min at 20 °C) and the supernatant decanted. The centrifuge tubes were then inverted for 5 min on a paper towel, followed by weighing of the residue (M2). The residue from the water absorption samples was dried in a hot air oven for 24 h at 50 °C and weighed (M1). WAC, OAC and WSI were calculated as follows:

$$\text{WAC or OAC} = \{(M2 - M0)/M0\} \times 100; \quad \text{and}$$

$$\text{WSI} = \{(M0 - M1)/MO\} \times 100$$

where M0 is the sample weight (db); M1 is the weight of dried residue; and M2 is the weight of wet or oily residue.

2.8. Swelling index

Samples of flour from unmicronised and micronised (130 and 170 °C) cowpeas were dispersed in deionised water (1:20, w/v) and vortexed for 1 min, followed by heating in a water bath at 90 °C for 30 min with intermittent mixing. The heated samples were cooled for 30 s under running water and for 10 min in an ice bath to accelerate gel formation. The tubes containing the gels were centrifuged (4500*g*, 20 °C) for 10 min, after which the samples were allowed to stand for 5 min at 24 °C. The supernatant was decanted and the residue weighed. Swelling index was calculated as the ratio of the weight of the final residue to the initial sample weight (Abu et al., 2005).

2.9. Concentration on gelation

Cowpea flour dispersions in deionised water with concentration from 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20% (w/v) were prepared in test tubes. The dispersions were heated in a water bath at 80 °C for 1 h, followed by rapid cooling under running cold water. The test tubes were left to set at 4 °C for 2 h. Least gelation concentration was determined as the concentration when sample from the inverted tube did not fall or slip (Adebowale, Olu-Owolabi, Olawumi, & Lawal, 2005).

2.10. Gel strength

The strength of gels formed by the flour from unmicronised and micronised (130 and 170 °C) cowpeas was determined, following the method reported by Abu et al. (2005). Eighteen percent (w/v) flour dispersions were heated in a water bath (90 °C) for 50 min. The hot pastes were cooled in running water and allowed to set for 15 min in an ice bath, following which the gels with a height of 20 mm were kept at 4 °C for 16 h. Gel strength was measured using a TA-XT2 Texture analyser (Stable micro systems, Goldalming, Surrey, UK). A 20 mm diameter probe (P20) with a punch area of 314.16 mm^2 was used. The probe operated at a pre test speed of 1 mm/s and penetrated the gel at the speed of 0.5 mm/s. Force required for the probe to penetrate the gel to 8 mm was recorded as a measure of gel strength.

2.11. Foaming capacity

The foaming capacity of the flour from unmicronised and micronised (130 and 170 °C) cowpea was determined according to the method reported by Akubor, Isolokwu, Ugbane, and Onimawo, 2000. A 5% (w/v) dispersion of the flour in deionised water was whipped using a Power Five mixer (Kenwood Ltd., Hants, England) for 8 min at 24 °C. The foam was quantitatively transferred into a graduated cylinder. Foam volume was expressed as a percentage of the volume occupied by the sample prior to whipping.

2.12. Protein extraction

Cowpea protein was isolated from unmicronised and micronised (130 and 170 °C) seeds according to a modified method of Mwasaru et al. (1999) and Horax et al. (2004). The flour was defatted with hexane (flour:hexane, 1:6, w/v) at 25 °C for 4 h and air-dried in a fume hood overnight. Ten percent of the defatted flour in 0.1 M NaOH at pH 8.5 was homogenised using a Ultra Turrax T25 tissue macerator (Janke and Kunkel GmbH & Co., K.G., Stauffen, Germany) at 24,000 rpm for 30 min (4 °C), followed by centrifugation at 10,000*g* (4 °C) for 1 h. The residues were extracted twice in 0.1 M NaOH at pH 8.5. The supernatants were pooled and precipitated by adjusting the pH to 4.5 using 0.1 M HCl. The precipitated protein was recovered by centrifuging, followed by three washings with deionised water (pH 4.5). The protein isolate was solubilised by adjusting the pH to 7.0, followed by 24 h dialysis (12–14 kDa, Labretoria, Pretoria, South Africa) at 4 °C. The dialysed material was freeze-dried and termed the protein-rich fraction (PRF). The nitrogen content of the PRF was determined using a Leco Nitrogen Analyzer 528 (Leco Africa Pty, Kempton Park, South Africa). Protein content of the PRF was calculated using 6.25 as the conversion factor.

2.13. Surface hydrophobicity

Surface hydrophobicity of the PRF samples was determined according to the method of Hayakawa and Nakai (1985). Ten millilitre solutions of protein were made in 0.01 M phosphate buffer (pH 7) with concentrations ranging from 0.0001% to 0.0008% (w/v). The probe for aromatic hydrophobicity, 1-anilino-8-naphthalene sulfonate

(ANS) (25 μl, 8 mM in 0.01 M phosphate buffer (pH 7.0)), was added to each protein solution, and fluorescence intensities of these solutions were measured at 390 nm excitation and 470 nm emission using LS 30 Luminescence spectrometer (PerkinElmer Inc, Boston, MA). The surface hydrophobicities, expressed as a slope of fluorescence intensity (arbitrary units) against protein concentration, were calculated by linear regression (Statistica 6.0).

2.14. Gradient SDS–PAGE

The effect of micronisation temperature on the molecular size and distribution of cowpea proteins was studied using gradient SDS-Gel electrophoresis according to the method reported by Byaruhanga, Erasmus, and Taylor (2005). The gels (12.5 × 16 cm, 1.5 mm thick) had a concentration gradient from 4% to 18% and were polymerised with 0.1% (w/v) ammonium persulphate (APS) and tetramethylenediamine (TEMED). The protein was dispersed in sample buffer (0.125% (w/v) Tris/HCl, 20% (v/v) glycerol, 2% (w/v) SDS and 0.005% (w/v) bromophenol blue). For the electrophoresis under reducing conditions, 0.1% 2-mercaptoethanol was added to the sample buffer. The gels were loaded to a constant protein content of 49.5 μg. Molecular weight marker solution, low range (Roche Diagnostics Corporation, Indianapolis, USA) was diluted by 1–10 with reducing sample buffer. The mixture consisted of phosphorylase B (M_r 97.4 × 10^3), bovine serum albumin (M_r 66.2 × 10^3), aldolase (M_r 39.2 × 10^3), triose phosphate isomerase (M_r 26.6 × 10^3), trypsin inhibitor (M_r 21.5 × 10^3) and lysozyme (M_r 14.4 × 10^3). The diluted molecular weight marker was boiled for 5 min and 20 μl (96 μg protein molecular weight marker) were loaded onto the gels. Electrophoresis was carried out at a constant voltage of 25 mA per gel and 150 V for 14 h at 8 °C, using a Protean II xi vertical cell with a 1000 Powerpac (Bio-Rad Laboratories, Hercules, CA). Proteins were stained with 0.03% (w/v) Coomassie Brilliant Blue R250 in 7% (v/v) acetic acid and 20% (v/v) methanol and 3.2% trichloroacetic acid (TCA). After staining, the gels were de-stained with 4% (v/v) acetic acid and 29% (v/v) methanol and 3% TCA. The de-stained gels were scanned on a flat bed scanner.

2.15. Statistical analysis

Mean values for the functional properties, colour and surface hydrophobicity of the cowpea flours were obtained from three repetitions. One-way analysis of variance (ANOVA) of the data and correlations of variables were performed using Statistica version 6 (StatSoft, Inc., Tulsa, OK) statistical software. The least significance difference test at $P \leqslant 0.05$ was used to separate the means.

3. Results and discussion

Cowpea flour from unmicronised seeds contained 23.9% crude protein, which is within the range reported for cowpeas (Chan & Phillips, 1994). Although there were no prominent changes in crude protein content with micronisation (Table 1), the rate of protein extraction, as well as the purity of the PRF, declined with increasing micronisation temperature (Fig. 1). The protein extraction rate attained in this work is higher than what some workers have obtained for cowpea, although the protein content of the PRF is within the range reported for cowpea protein isolates (Mwasaru et al., 1999).

Micronisation significantly ($P \leqslant 0.05$) reduced the lightness of cowpea flour with increasing temperature (Table 1). Cowpea flour from the M-170 °C seeds was the darkest of the samples and it had higher yellowness and redness values. The browning of the cowpea flour with micronisation was possibly due to Maillard reactions, since cowpeas do contain reducing sugars (Longe, 1980; Phillips et al., 2003) and have high protein contents.

Protein solubility in an aqueous environment is an essential property that affects functionality of cowpea flour in terms of foaming, emulsification and gelation (Nnanna, Phillips, McWatters, & Hung, 1990). Nitrogen solubility index of unmicronised flour was comparable to values reported in the literature (Abu et al., 2005) (Table 1). Micronisation (130 and 170 °C) reduced ($P \leqslant 0.05$) the NSI of the cowpea flour by 32% and 45%, for the M-130 °C and M-170 °C flours, respectively. Micronisation (130 and 170 °C) caused denaturation of the cowpea protein, hence reducing its solubility. Similar reductions in protein solubility have been reported in micronised peas (Cenkowski

Table 1
Effects of high (170 °C) and low (130 °C) final micronisation temperature for cowpea seeds on physicochemical properties of cowpea flour

Physicochemical property	Micronisation		
	Unmicronised	130 °C	170 °C
Moisture (%)	9.2 a (0.7)	5.0 b (0.9)	4.8 b (0.5)
Protein (%)	24.2 c (0.2)	24.7 b (0.3)	25.9 a (0.6)
L^*	84.36 a (0.42)	81.34 b (0.55)	70.06 c (0.33)
a^*	5.00 c (0.04)	5.23 b (0.06)	8.45 a (0.26)
b^*	6.01 b (0.22)	6.28 b (0.36)	13.33 a (0.28)
Protein solubility (%)	87.4 a (13.7)	59.7 b (13.04)	47.7 c (5.7)
PRF surface hydrophobicity (slope ratio)	228 c (45)	369 b (34)	624 a (23)

Means followed by the same letter within a row are not significantly different at level $P \leqslant 0.05$. Standard deviations of the means are in parentheses. L^*, lightness; a^*, redness; b^*, yellowness; PRF, protein-rich fraction; unmicronised, raw.

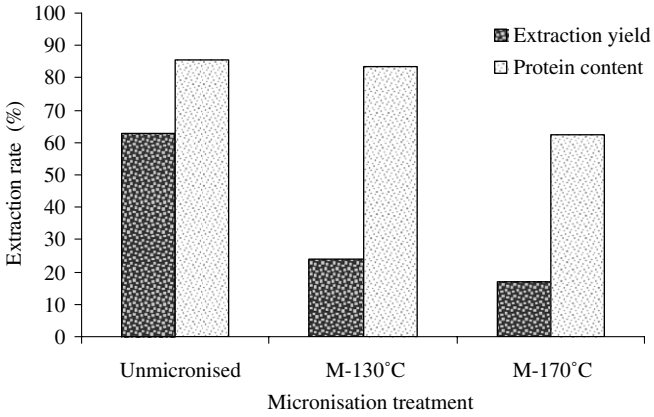

Fig. 1. Effect of micronisation temperature on the extractability of the protein rich fraction from cowpea seeds and its protein content.

& Sosulski, 1998), cowpeas (Mwangwela et al., 2006), beans (Bellido et al., 2006), and lentils (Arntfield et al., 1997; Arntfield et al., 2001). Zheng et al. (1998) attributed the reduction in protein solubility of micronised legumes to hydrophobic interactions, which render the protein less soluble in water. Cowpea protein is generally hydrophilic (Horax, Hettiarachchy, Chen, & Jalaluddin, 2004), indicating that the non-polar/hydrophobic side chains are buried inside the protein. PRF from unmicronised cowpeas had lower surface hydrophobicity values than had those reported for cowpea protein isolate (Table 1) (Horax et al., 2004). The difference in surface hydrophobicity of the cowpea proteins could be due to difference in variety (Horax et al., 2004) and protein isolation methods. However, micronisation (130 and 170 °C) significantly ($P \leqslant 0.05$) increased the surface hydrophobicity of the PRF, possibly by changing the cowpea protein conformation to expose more hydrophobic sites. The PRF from the M-170 °C had significantly ($P \leqslant 0.05$) higher surface hydrophobicity than had the PRF from M-130 °C cowpeas (Table 1). This may imply that more hydrophobic sites were exposed with increasing micronisation temperature.

Cowpea flour from unmicronised seeds absorbed 1137 g kg^{-1} oil and micronisation (130 and 170 °C) did not change this attribute (Table 2). Prinyawiwatkul et al. (1997a) reported that oil absorption capacity of cowpeas flour did not change with most processing treatments, such as boiling, milling and fermentation. The presence of non-polar side chains, which bind the hydrocarbon side chain of oil, would promote oil binding capacity of flours; however, in this study, the significantly ($P \leqslant 0.05$) higher surface hydrophobicity (Table 1) of the protein-rich fraction isolated from cowpeas micronised to 170 °C did not enhance the OAC of the flour. Oil absorption capacity is an important property of cowpea flour that could be used as an extender in comminuted meat formulations, such as meat balls and sausages, where flavour retention and palatable mouth feel is desired. Since micronisation did not change the OAC of the flour, flour from micronised seeds could be used to extend meat products without adverse effect on texture and mouth feel (Prinyawiwatkul et al., 1997b; Serdaroglu et al., 2005).

Micronisation of cowpea seeds (especially 170 °C) significantly ($P \leqslant 0.05$) increased the WAC of the cowpea flour (Table 2). The flour from M-170 °C cowpeas absorbed more ($P \leqslant 0.05$) water than did M-130 °C samples. Fasina et al. (1999) reported that micronisation (26% moisture and 115 °C) improved the water-holding capacity of hulless and pearled barley flour. Prinyawiwatkul et al. (1997a) also reported improved water retention capacity for cowpea flour from soaked and boiled seeds. Starch and protein are important constituents that determine water absorption properties of heterogeneous systems such as flour. Fasina et al. (2001) demonstrated that micronisation (<10% moisture, 140 °C) of legumes (pinto and black beans, lentils and green peas) resulted in increased WAC, which was mainly attributed to protein denaturation, since the amount of starch gelatinised under the micronisation conditions was minimal.

Despite the increase in water absorption capacity with micronisation, swelling index of the flour had a significant ($P \leqslant 0.05$) negative correlation ($r = -0.83$) with WAC. Micronisation significantly ($P \leqslant 0.05$) reduced the swelling index of the flours by 17.8% and 18.2% for the M-130 °C and M-170 °C, respectively (Table 2). Swelling index is indicative of starch granule swelling during gelatinisation, as well as water retention due to protein gelation. It has been reported that the starch in micronised (moisture conditioned) legumes is gelatinised and protein solubility is reduced (Arntfield et al., 1997; Bellido et al., 2006; Mwangwela et al., 2006). Hence the flour from micronised

Table 2
Effects of high (170 °C) and low (130 °C) final micronisation temperatures for cowpea seeds on functional properties of cowpea flour

Functional property	Micronisation		
	Unmicronised	130 °C	170 °C
Oil absorption capacity (g kg^{-1})	1137 (174)	1208 (177)	1177 (147)
Water absorption capacity (g kg^{-1})	1384 c (89)	2509 b (75)	2871 a (101)
Water solubility index	38.6 a (1.6)	22.6 b (1.51)	17.4 c (1.6)
Swelling index	7.24 a (0.43)	5.95 b (0.44)	5.92 b (0.39)
Gel strength (N mm^2)	115.7 a (8.9)	72.3 b (4.6)	28.8 c (2.5)
Least gelation concentration (w/v)	8 c (0.8)	11 b (1.0)	13 a (1.0)
Foam capacity (%)	291 a (11.5)	112 b (2.1)	102 c (2.1)

Means followed by the same letter within a row are not significantly different at level $P \leqslant 0.05$. Standard deviations of the means are in parentheses. Unmicronised, raw.

(130 and 170 °C) cowpeas had lower swelling indices than the unmicronised cowpea flour. Similar reduction in swelling index has been reported in irradiated cowpea flour (Abu et al., 2005).

Cowpea flour from unmicronised seeds had a water solubility index (WSI) of 38.67 which was significantly ($P \leqslant 0.05$) reduced by 42% and 55% by micronisation at 130 and 170 °C, respectively (Table 2). Water solubility index is an indication of the water soluble fractions in the flour, such as protein and sugars. WSI had a significant ($P \leqslant 0.05$) positive correlation with NSI of the flour. Hence the reduced WSI could partly be due to the reduced protein solubility.

Cowpea flour from unmicronised cowpeas had the lowest ($P \leqslant 0.05$) gelation concentration of 8% and formed a significantly ($P \leqslant 0.05$) stronger gel at 18% concentration than did the flour samples from seeds micronised at 130 and 170 °C (Table 2). The least gelation concentration found in this work is within the values reported for cowpea flour (Olaofe, Umar, & Adediran, 1993; Prinyawiwatkul et al., 1997a). Micronisation (130 and 170 °C) increased the lowest gelation concentration to 10% and 12% for the flour from cowpeas micronised at 130 and 170 °C, respectively. The strength of the gels formed from the 18% (w/v) dispersion was significantly ($P \leqslant 0.05$) reduced by 39% and 75% for the M-130 °C and M-170 °C flours, (Table 2). Prinyawiwatkul et al. (1997a) reported a similar increase in the least gelation concentration for cowpea flour following soaking and boiling. In a heterogeneous system, where both the protein and starch are in their native form, both protein and starch contribute toward the gelation properties of the flour. Mwangwela et al. (2007) reported that micronisation at 170 °C reduced the pasting viscosities more severely than did the mild 130 °C micronisation treatment. Consequently the significant ($P \leqslant 0.05$) increase in the least gelation concentration and the weak gel formed by the cowpea flour from seeds micronised at 170 °C was possibly due more to protein gelation than to starch gelation. In addition, Prinyawiwatkul et al. (1997a) reported that high protein (denatured) flour with pregelatinised starch required greater flour concentration for thermal gel formation to occur.

Foaming reflects the capacity of protein to form stable layers surrounding gas droplets in a liquid phase. In order for this to be possible, the proteins need to be soluble in the aqueous phase and to be in a position to diffuse and concentrate at the air/water interface, partially unfolding to form a cohesive layer around the gas bubbles, as well as possess sufficient viscosity and mechanical strength to prevent rupture and coalescence of droplets (Damodaran, 1996). Cowpea flour from unmicronised seeds had a foaming capacity of 291% as compared to the 112% and 102%, respectively, observed in M-130 °C and M-170 °C flours (Table 2). Similar reduction in foaming capacity has been reported in irradiated cowpea flour (Abu et al., 2005). The significant ($P \leqslant 0.05$) reduction in foaming capacity of micronised (130 and 170 °C) samples was possibly due to extensive protein denaturation. Foaming capacity had a significant ($P \leqslant 0.05$) positive correlation ($r = 0.79$) with protein solubility index of the flour. Aluko and Yada (1993) reported that protein solubility had a positive correlation with foaming capacity/expansion while aliphatic hydrophobicity had a negative impact. In addition, Townsend and Nakai (1983) reported that protein flexibility was crucial for foaming properties in protein-stabilised foams. Reduction in foaming properties of partially purified cowpea globulin treated with microbial calcium-independent transglutaminase has shown that protein cross-linking may lead to gradual loss of flexibility and the proteins' ability to unfold at the water air interface (Aluko & Yada, 1999).

SDS–PAGE of the extracted PRF from the unmicronised cowpeas showed two main (56 and 50 kDa) and two minor (63 and 61 kDa) bands in the region between 39.2 and 66 kDa and five minor bands in the 20–39 kDa region (Fig. 2a). These bands correspond to the four major polypeptides (65, 60, 56 and 50 kDa) reported by Chan and Phillips (1994) in the globulin fraction. The cowpea albumins have bands in the 90–100 kDa region and minor bands were observed in this region (Fig. 2a). Rangel, Domont, Pedrosa, and Ferreira (2003) observed two major bands, 50 and 52 kDa, following SDS–PAGE of cowpea protein isolate. These are in the typical molecular mass range for 7S storage proteins (Horax et al., 2004). Although disulphide links have been reported in the α-vignin component of purified cowpea globulins, involving a 60 and a 20 subunit, there was no distinct change in the subunit profile of the PRF from unmicronised cowpea seeds under reducing conditions (Fig. 2b). The lack of a substantial difference in the polypeptide pattern between '*a*' and '*b*' gels may be due to insufficient mercaptoethanol (MCE) in the electrophoresis reagent to reduce the disulphide bonds.

There was a relative reduction in the size of the two major bands in the protein isolated from cowpeas micronised at 170 °C (Fig. 2a). The reduction in size could possibly be due to associations involving the 52 and 55 kDa polypeptides in the formation of higher molecular weight polymers that could not pass through the gradient gels. Micronisation (130 and 170 °C) also resulted in the formation of two minor bands, at 69 and 73 kDa (Fig. 2a-i and 2a-ii), and the 69 kDa (Fig. 2a-ii) band was prominent in the fraction extracted from cowpeas micronised at 170 °C. The 73 kDa band (Fig. 2a-i) was possibly a result of disulphide cross-links involving the 21 and 61 kDa monomers, since it was reduced by MCE (Fig. 2b). The 61 (Fig. 2a and 2b-iii) and 21 kDa monomers (Fig. 2a and b-vi) disappeared in micronised samples under non-reducing conditions and reappeared under reducing conditions (Fig. 2a and b). Freitas, Teixeira, and Ferreira (2004) reported that the γ-vignin (22 kDa) fraction of cowpea globulins had an intra-polypeptide disulphide bond. It is possible that micronisation, especially at 170 °C, resulted in the reformation of these bonds, leading to the formation of inter-polypeptide disulphide cross-linking.

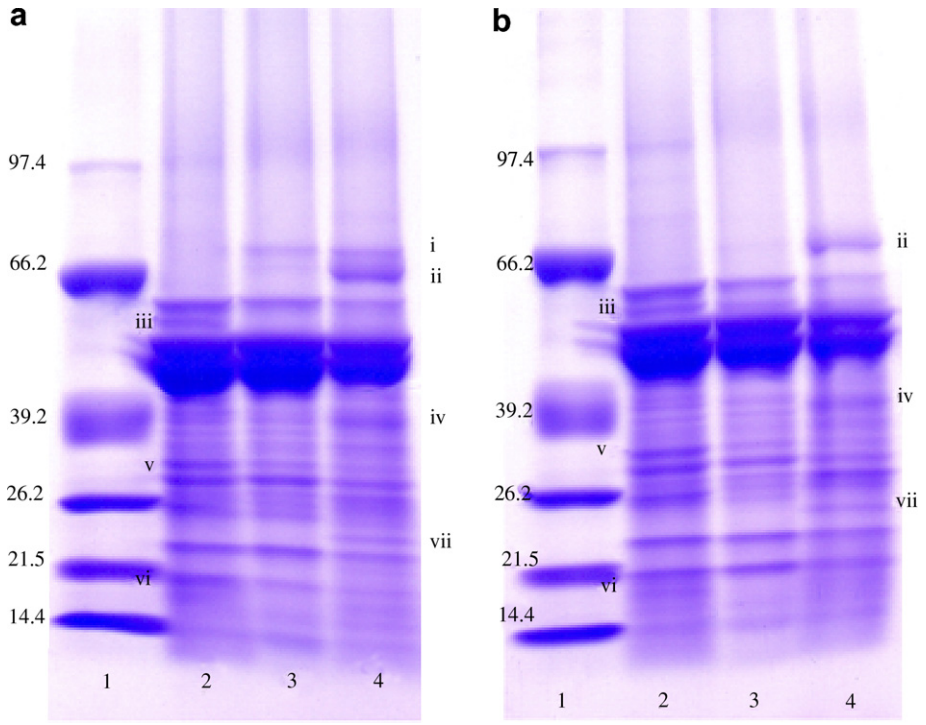

Fig. 2. SDS-gradient gel electrophoresis profiles of cowpea protein extracts from unmicronised and micronised seeds under non-reducing (a) and reducing conditions (b). Lane 1, molecular markers; lane 2, unmicronised; lane 3, micronised at 130 °C and lane 4 micronised at 170 °C. Changes in protein band profile are denoted with (i) to (vi). Unmicronised, raw.

In addition to disulphide cross-linking, it is evident that other forms of cross-linking may have taken place during micronisation. Other bands that were present (Fig. 2a, b-ii, b-iv, b-vii) in the PRF from cowpeas micronised at 170 °C were not reduced by MCE and could possibly be due to other forms of cross-linking that involved the 2 prominent bands (Fig. 2b-v), since these bands decreased with micronisation temperatures both under reducing and non-reducing conditions (Fig. 2a and b). Maillard reactions might have led to the formation of some cross-links in the cowpeas protein (Gerrard, 2002). The decrease in L^* values with increasing micronisation temperatures indicates that Maillard-type browning reactions possibly occurred in micronised (especially at 170 °C) samples. The higher micronisation temperature (170 °C) resulted in darker flour than did the lower micronisation temperature (130 °C) (Table 1). Cowpeas contain both reducing and non-reducing sugars (Longe, 1980; Phillips et al., 2003) which could result in both Maillard and caramelisation reactions. In addition, dityrosyl cross-links would also be another possible form of cross linking (Gerrard, 2002) due to the severe heat treatment, especially for the flour from the cowpeas micronised at 170 °C.

4. Conclusions

Micronisation of cowpeas severely affected the functionality of cowpea protein, resulting in the loss of foaming capacity. Despite this shortfall, mild micronisation temperatures could still be used to process flour with modified functionality. However, flours from cowpeas micronised at high temperatures have limited application in food systems, due to the decline in most of the functional properties measured in this study.

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