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Functional characteristics of cowpea (*Vigna unguiculata*) flour and starch as affected by soaking, boiling, and fungal fermentation before milling

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Functional characteristics of cowpea flour and starch as affected by soaking, soaking and boiling (S/B), and fermentation of seeds with *Rhizopus microsporus* var. *oligosporus* before milling were investigated. Soaking and fungal fermentation had fewer effects on flour and starch functionality compared to boiling. Boiling drastically reduced foamability and thus increased specific gravity of whipped cowpea paste. The gradual increase in total color differences of pastes prepared from S/B seeds with or without fermentation was due to a gradual decrease in lightness (L*). The more intense yellow color observed in whipped pastes from S/B seeds after fermentation was due to increased b* and decreased L* values. Starch from all treatments had greater swelling volume than flour. Significant reductions in swelling volume of flour and starch were due to the boiling treatment. Pasting characteristics of control flour and starch are concentration dependent. The pasting curve pattern of cowpea flour differed markedly from that of cowpea starch. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Cowpeas (*Vigna unguiculata*), also known as black-eyed peas, Southern peas, and crowder peas, are underutilized in the United States and other industrialized countries. This is due in part to storage-induced textural defects that prolong cooking time and demand higher fuel inputs for food preparation (Aguilera & Stanley, 1985). Another major limitation in expanded cowpea consumption is the presence of certain anti-nutritional factors and non-digestible components. Because of the potential of cowpeas as an economical source of significant amounts of protein, calories, and some water-soluble vitamins, they should be considered a promising food ingredient. Increased utility will depend upon development of appropriate technologies to produce meal or flour with acceptable functional properties and enhanced nutritional quality (Prinyawiwatkul *et al.*, 1996d).

Research has emphasized expanding the utilization of cowpeas in the form of meal and flour (McWatters, 1990) for use as functional ingredients in food products. Dry milling technology, which yields cowpea flour that retains functional and nutritional properties, has been developed (McWatters *et al.*, 1988; Phillips *et al.*, 1988). Attempts to enhance further the quality of cowpea flour have employed a wide range of technologies, e.g. germination, fermentation, γ-irradiation and α-galactosidase treatment (Prinyawiwatkul *et al.*, 1996*a*).

In a search for pulses to be used as substrates for making fermented products resembling tempeh and natto in countries where soybeans are not locally available, cowpeas are a potential alternative. *Rhizopus microsporus* var. *oligosporus* has been used successfully to ferment partially defatted peanuts which subsequently were milled into flour (Prinyawiwatkul *et al.*, 1993). A simplified solid-substrate fermentation and milling process for preparing flour from non-decorticated cowpeas (cv. White Acre) was developed (Prinyawiwatkul *et al.*, 1996*b*). Enhancement of nutritional quality of cowpea flour, including the absence of raffinose and stachyose, increased B-vitamin content, and decreased trypsin inhibitor activity, using a solid-substrate fermentation with *R. microsporus* has been demonstrated (Prinyawiwatkul *et al.*, 1996*a,c*). Scale-up production of cowpea flour, essentially free of flatulence-

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causing oligosaccharides, is feasible and would stimulate prospects for its utilization.

While nutritional quality ultimately is important in considering cowpea flour as an ingredient in food products, successful performance of cowpea flour depends largely on the functional and sensory characteristics required in final products. The great versatility of cowpea flour as a base for many products (McWatters, 1990) points out the need for a better understanding of its functional characteristics. Soaking and cooking of cowpeas to be used as a substrate in solid-substrate fermentation are necessary if an acceptable fermented cowpea flour is to be produced; however, these operations may have a great impact on flour functionality, for example, foaming and pasting characteristics. The objective of this study was to determine selected functional characteristics of cowpea flour and starch as affected by soaking, soaking and boiling (S/B), and solid-substrate fermentation of seeds with *R. microsporus* var. *oligosporus* before milling.

MATERIALS AND METHODS

Preparation of fermented cowpea flour

Mature dry seeds (cv. White Acre, 1993 crop) were obtained from Southern Frozen Foods, Montezuma, GA, USA. Upon receipt, cowpeas were inspected visually and defective seeds were discarded. Cowpeas were stored at 7°C and 60% relative humidity until utilized.

Cowpeas (1.75 kg batch) were used to prepare fermented flour using the procedure described in a previous study (Prinyawiwatkul *et al.*, 1996*b*). Cowpeas were soaked in tap water (cowpeas:water, 1:6, wt/wt) at *ca* 25°C for 24 h, boiled for 45 min, drained, cooled to 25–30°C, and inoculated uniformly with a commercial dried powder *R. microsporus* var. *oligosporus* starter culture (Tempeh Lab, Inc., Summertown, TN, USA) at a ratio of 1:200 (starter:cooked cowpeas, wt/wt). The inoculated seeds (1 kg batch) were placed in perforated Zip-loc® vegetable bags (DowBrands L.P., Indianapolis, IN, USA) and incubated at 30°C for 0 (inoculated but dried immediately), 15, 18, 21 and 24 h. Fermented cowpeas were then oven-dried at 60°C for 13 h, finely ground, and stored at −18°C until used. Two batches of fermented cowpeas were prepared and flour from both batches was mixed thoroughly before subjecting to analyses. Note that 'S/B' stands for 'soaking and boiling', which will be used throughout this report.

Starch extraction

Cowpea flour (100 g) was mixed with 900 ml of 0.2% NaOH (pre-cooled to 4°C) to dissolve most of the protein but not to gelatinize the starch (Schoch & Maywald, 1968). The slurry was stirred for 2 h at room temperature (*ca* 25°C), then filtered through a series of sieves (#50, #80, #140, and a collecting pan, USA Standard Testing sieve, A.S.T.M. E-11 specification, W.S. Tyler Inc., Mentor, OH, USA). The filtrate was collected and allowed to stand undisturbed for 15 h at 4°C to allow prime starch to sediment. The supernatant liquid was decanted carefully and the sediment was resuspended in 500 ml of double de-ionized water, stirred for 5 min in an ice bath, and centrifuged at 15 300*g* for 30 min at 4°C. Washing, stirring and centrifugation were repeated from four to five times until the pH of supernatant was in the range of 6.0–6.5. The prime starch was washed with 500 ml of ethanol (Tolmasquim *et al.*, 1971) to remove fat residues, decanted, dried at 35°C for 48 h and ground with a mortar and pestle.

Preparation of whipped cowpea paste

Whipped cowpea paste was prepared by adding sufficient water (231–253 ml) to 200 g portions of cowpea flour (ranging from 4.8 to 9.4% moisture) to give a final moisture content of 72%. The paste was stirred gently for 2 min with a rubber spatula and whipped in a mixer (Model N-50, Hobart Corp., Troy, OH, USA) at high speed (#3) for 1.5 min.

Foam capacity and specific gravity

Foam capacity of cowpea pastes, expressed as the percent increase in foam volume (ml) after whipping, was measured in a pharmaceutical cylinder. Specific gravity of pastes before and after whipping was determined according to the method of Campbell *et al.* (1979). Triplicate measurements were made for each flour.

Color

Colorimetric measurements of hydrated cowpea flour, i.e. paste before and after whipping, were determined in triplicate (*ca* 150 g each) as previously described (Prinyawiwatkul *et al.*, 1994). Measurements were recorded using a Gardner XL-800 tristimulus colorimeter (Pacific Scientific, Bethesda, MD, USA) equipped with an XL-845 circumferential sensor. The instrument was calibrated with a yellow standard tile ($L^* = 83.59$, $a^* = -3.11$, $b^* = 28.52$). Surface color differences were minimized by reporting an average of eight readings per paste sample. Psychometric color terms involving hue angle $[\tan^{-1}(b^*/a^*)]$, chroma $[(a^{*2} + b^{*2})^{1/2}]$, and total color difference, ΔE, $[(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2]^{1/2}$, where L^*_0, a^*_0, and b^*_0 represent the respective readings of control samples, were computed.

Swelling volume

Cowpea flour (1 or 2 g) and/or starch (1 g) was weighed into 125×20 mm screw-capped test tubes and mixed

thoroughly with excess water to hydrate flour and/or starch. The tubes were heated in a water bath at 92 ± 1°C for 30 min with intermittent stirring, and immediately cooled in tap water for 30 sec and in ice water for 5 min to accelerate gel formation. After centrifuging at 1800*g* for 5 min at *ca* 25°C, all tubes were placed in ice water for another 10 min and the supernatant liquid was removed gently. Water was then added to the sediment to bring the volume to 20 ml. Swelling volume (ml) was calculated as [20 ml − (ml of water required to adjust the volume to 20 ml)]. Three replicates were conducted for each flour and starch sample.

Pasting characteristics

Pasting characteristics of cowpea starch and flour were determined in triplicate using a Brabender Visco-Amylograph (Type PT-100/VA-VE, C. W. Brabender Instruments, Inc., NJ, USA) equipped with a 700 cm.gf cartridge. A suspension of 16% cowpea flour in de-ionized water (dry wt/v) or 7% starch (dry wt/v), unless otherwise stated, was heated from 50 to 95°C at a uniform rate of 1.5°C/min with constant stirring at 75 rpm. The sample was held at 95°C for 30 min (break-down), then cooled to 50°C at a rate of 1.5°C/min (set-back), and held for 30 min. Pasting temperature was defined as the temperature at which an increase in viscosity was first observed. Hot-paste stability was defined as the ratio of viscosity at break-down to viscosity at 95°C. Retrogradation tendency was defined as the ratio of viscosity at set-back to viscosity at break-down. Cool-paste stability was defined as the ratio of viscosity at 50°C held for 30 min to viscosity at set-back.

Statistical analysis

Analysis of variance (ANOVA) was performed to determine differences in properties of flour and/or starch samples. Tukey's Studentized range test was performed for *post hoc* multiple comparisons. Group differences, expressed in terms of differences in mean vectors of all flour and starch properties, were determined using multivariate analysis of variance (MANOVA). Descriptive discriminant analysis (Huberty, 1994) [PROC CANDISC, SAS version 6.03 (SAS, 1988)] was performed subsequently to identify functional characteristics of flour and starch underlying group differences among cowpea flours prepared using various treatments.

RESULTS AND DISCUSSION

Foam volume and specific gravity

Foam volume and specific gravity are indices of texture lightness of food products (Prinyawiwatkul *et al.*, 1994). Theoretically, cowpea foam consists of air droplets encapsulated by a liquid film containing soluble surfactant protein. Uniform distribution of fine air bubbles usually imparts body, smoothness and lightness to foods (Cheftel *et al.*, 1985) and facilitates flavor dispersion (Kinsella, 1981). After whipping, the foam volume of hydrated control cowpea flour (paste) increased (*ca* 31%) from about 430 to 565 ml, and the specific gravity decreased from 1.058 to 0.760 (Table 1). Soaking (25°C, 24 h) slightly increased foam volume (34.5%) and decreased specific gravity (from 0.760 to 0.741) of hydrated flour after whipping, compared to control flour. Pastes prepared from pre-soaked cowpeas produced greater increases in foam volume after whipping than did pastes from non-soaked cowpeas (McWatters & Brantley, 1982). The soaking treatment provided an environment in which protein and starch components could imbibe more water (McWatters & Brantley, 1982) which may have facilitated foam formation. Henshaw and Lawal (1993), however, used various methods of cowpea flour preparation and observed that processes involving soaking lowered foam capacity, although no explanation was given.

Boiling (45 min) eliminated foamability and concomitantly increased specific gravity of cowpea paste

Table 1. Foam volume (% increase) and specific gravity of hydrated cowpea flour (paste) before and after whipping as affected by soaking, boiling, and fungal fermentation*

Treatment	Foam volume		Specific gravity			
	(% increase after whipping)		Before whipping		After whipping	
Control	31.4	$(1.3)^b$	1.058	$(0.002)^c$	0.760	$(0.004)^c$
Soaking	34.5	$(0.0)^a$	0.892	$(0.003)^d$	0.741	$(0.002)^d$
Soaking and boiling	0.0	$(0.0)^c$	1.117	$(0.002)^a$	1.087	$(0.003)^a$
0 h Fermentation	0.0	$(0.0)^c$	1.117	$(0.001)^a$	1.086	$(0.004)^a$
15 h Fermentation	0.0	$(0.0)^c$	1.104	$(0.005)^b$	1.088	$(0.001)^a$
18 h Fermentation	0.0	$(0.0)^c$	1.100	$(0.002)^b$	1.081	$(0.003)^{ab}$
21 h Fermentation	0.0	$(0.0)^c$	1.105	$(0.001)^b$	1.080	$(0.001)^{ab}$
24 h Fermentation	0.0	$(0.0)^c$	1.104	$(0.001)^b$	1.075	$(0.002)^b$

*Numbers in parentheses refer to standard deviation of three measurements. Mean values in a column not followed by the same letter (as superscript) are significantly different ($p \leq 0.05$).

(Table 1). Differences in protein content of flours markedly influence foamability. For instance, Lazos (1992) reported pumpkin seed flour (61.4% protein, 0.2% fat) to have a 211% increase in foam volume, whereas a 48–84% increase for cowpea flour (24.6–25.4% protein, 0.3–2.4% fat) and a 5.1–31.8% increase for taro flour (2.0–3.8% protein, 0.8% fat) were reported by Abbey & Ibeh (1988) and Tagodoe & Nip (1994), respectively. In our study, boiling did not affect the protein content (27.1%) of flour prepared from S/B seeds, compared to that (26.2%) of the control flour (Prinyawiwatkul *et al.*, 1996*b*). This indicates that protein quality, rather than quantity, governs the foamability of cowpea flour under the conditions used in this study.

The negative effect of severe heat treatment on foamability of cowpea flour has been demonstrated (Enwere & Ngoddy, 1986; Ngoddy *et al.*, 1986; Padmashree *et al.*, 1987; Abbey & Ibeh, 1988; Hung *et al.*, 1988; Giami, 1993). Yasumatsu *et al.* (1972) suggested that protein denaturation decreased protein solubility, which in turn decreased foam capacity. Hung *et al.* (1988) and McWatters *et al.* (1988), however, reported that foam volume and specific gravity of whipped cowpea paste were enhanced by mild heat treatment. These discrepancies can be rationalized. During heating, protein–protein interaction could occur and the consequences of heat-induced interaction would be unfolding, aggregation and coagulation. Mild heat treatment, which induces partial unfolding of globular, flexible protein without causing coagulation, improves foam formation (Kinsella, 1981) by favoring protein absorption at the air–liquid interface with the foam lamella. The improvement may, however, be observed within a narrow range of conditions (Megha & Grant, 1986). Severe heat treatment may have prevented protein from rapidly migrating and orienting to form an interfacial film around nascent air bubbles, as was the case observed in this study.

Improvement of foam capacity of denatured soy protein by fungal protease at 50°C was reported by Bernardi Don *et al.* (1991). In the present study, fermentation of cowpeas with *R. microsporus* var. *oligosporus* up to 24 h did not restore the foamability of flour that was impaired by heat treatment (Table 1). Reports on the improvement of foam capacity of flour as a result of natural or lactic acid bacterial fermentation indicate a lack of general agreement (Canella *et al.*, 1984; Schaffner & Beuchat, 1986; Giami & Bekebain, 1992). One explanation for these conflicting observations is that foam capacity generally is improved if severe heat treatment is not involved in the fermentation process.

Color

After whipping, all pastes were lighter (higher L*) and less yellow (lower b*), except for the paste from S/B seeds, which was slightly more yellow (Table 2). Without exception, whipped and unwhipped pastes prepared from soaked seeds were lighter and less yellow than all other paste samples. During soaking, seed color pigment leached out into the soak water. Acidic (pH 5.54) soak water after 24 h at 25°C may be a contributing factor to lightness (L*) of paste (Prinyawiwatkul *et al.*, 1996*b*). All whipped and unwhipped pastes prepared from S/B seeds before and after fermentation had lower L* and higher a* (more toward redness) values than pastes prepared from control seeds.

Boiling caused a significant reduction in b* values of unwhipped paste compared to that of the control sample. Whipping significantly lowered the b* of paste from control seeds but did not affect the b* of paste from S/B seeds before and after fermentation. Color lightness (L*) of pastes gradually decreased as the fermentation time of seeds increased. The gradual increase in ΔE of whipped and unwhipped pastes prepared from fermented seeds was attributed to the gradual decrease in lightness (L*). Production of dark pigments and breakdown of carbohydrates that facilitate Maillard browning reactions during drying of freshly fermented cowpeas (Prinyawiwatkul *et al.*, 1993) occurred during fungal growth.

Hue angle values of all pastes were slightly greater than 90°. Pastes with hue angles between 90 and 180° are more toward greenish yellow. Although whipped paste prepared from soaked seeds had the highest hue angle value (98.40°), its color as observed visually was greenish white rather than greenish yellow. This was attributed to its highest lightness (L* = 84.20), lowest yellowness (b* = 18.89) and lowest color saturation (chroma = 19.09) compared to all other whipped pastes. The more intense blackish yellow color (increased chroma) observed in whipped pastes from fermented S/B seeds was due to increased b* and decreased L* values.

Swelling volume

Swelling of starch granules is the first stage in the initiation of changes in hydration-related properties. Legume starch usually exhibits a restricted-swelling pattern (Reddy *et al.*, 1984), depending on starch content as well as the presence of impurities (e.g. proteins and lipids) and pre-treatment or processing history. The swelling behavior of cowpea flour and starch (Table 3) is influenced strongly by processing treatments. On an equal weight basis, starch had a greater swelling volume than did flour. The swelling volume of control starch (1 g) was 20.7 ml compared to 16.1 and 5.2 ml observed for control flour (2 and 1 g, respectively, Table 3).

For flour prepared from control and soaked seeds, swelling volume increased about three times as the amount of flour increased from 1 to 2 g, indicating a non-linear relationship. Swelling volumes of flour and starch from soaked seeds were slightly greater than those of control flour and starch. Particle size of flour

has been shown to influence strongly the swelling of rice flour, with finer flours having greater swelling capacity (Sandhya Rani & Bhattacharya, 1989). Limited moisture diffusion has not been reported in a starch suspension apparently due to its small particle size (Okechukwu *et al.*, 1991). One explanation for the greater swelling volume of flour from soaked seeds compared to control seeds is the finer flour particle size (Prinyawiwatkul *et al.*, 1996*b*).

Significant reductions in swelling volume of starch at 1 g (from 21.1 to 6.6 ml) and flour at 2 g (from 17.9 to 8.1 ml) were observed as a result of boiling for 45 min (Table 3). In contrast, the boiling effect on swelling volume of flour at 1 g (from 5.9 to 5.0 ml) was not obvious. Enwere and Ngoddy (1986) reported decreased swelling capacity of cowpea flour with increased temperature used for drying cowpeas destined for flour production. According to Uchendu (1982), proteins in cowpea flour also contribute to swelling, and the ability of starch and protein to swell is adversely affected by both thermal and mechanical damage occurring during flour preparation.

In this study, conditions used to measure the swelling volume of flour and starch were selected with the intention of mimicking those of the Brabender Visco-Amylograph. Crosbie (1991) observed a high correlation ($r=0.81$) between starch swelling volume and starch paste peak viscosity for wheat flour. It was

Table 2. Color of hydrated cowpea flour (paste) before and after whipping as affected by soaking, boiling, and fungal fermentation†

Color‡	Control	Soaking	Soaking and boiling	Fermentation time (h)				
				0	15	18	21	24
L* — bf	77.59	79.76	75.29	74.30	73.23	71.38	69.90	69.31
	(0.00)^{b}	(0.01)^{a}	(0.03)^{c}	(0.07)^{d}	(0.02)^{e}	(0.06)^{f}	(0.00)^{g}	(0.00)^{h}
L* — af	83.41	84.20	76.24	75.31	74.36	72.22	70.87	70.44
	(0.09)^{b}	(0.08)^{a}	(0.00)^{c}	(0.00)^{d}	(0.01)^{e}	(0.03)^{f}	(0.04)^{g}	(0.01)^{h}
a* — bf	−2.16	−2.01	−0.53	−1.51	−2.01	−1.87	−1.62	−1.60
	(0.00)^{d}	(0.01)^{d}	(0.10)^{a}	(0.05)^{b}	(0.15)^{d}	(0.11)^{cd}	(0.17)^{bc}	(0.19)^{bc}
b* — af	−2.22	−2.79	−1.35	−1.58	−1.81	−1.73	−1.63	−1.97
	(0.18)^{c}	(0.10)^{d}	(0.30)^{a}	(0.01)^{ab}	(0.15)^{bc}	(0.16)^{ab}	(0.03)^{ab}	(0.08)^{bc}
b* — bf	28.08	23.27	24.13	23.76	23.75	23.86	23.66	24.33
	(0.02)^{a}	(0.03)^{d}	(0.06)^{bc}	(0.11)^{bcd}	(0.04)^{bcd}	(0.04)^{bcd}	(0.51)^{cd}	(0.31)^{b}
b* — af	20.65	18.89	24.29	22.54	22.62	23.62	23.04	23.95
	(0.15)^{e}	(0.54)^{f}	(0.02)^{a}	(0.16)^{d}	(0.09)^{d}	(0.09)^{bc}	(0.07)^{cd}	(0.02)^{ab}
Hue angle — bf	94.40	94.94	91.25	93.65	94.83	94.49	93.91	93.76
	(0.00)^{abc}	(0.03)^{a}	(0.24)^{d}	(0.14)^{c}	(0.37)^{a}	(0.25)^{ab}	(0.35)^{bc}	(0.47)^{bc}
Hue angle — af	96.14	98.40	93.19	94.01	94.57	94.52	94.05	94.70
	(0.46)^{b}	(0.07)^{a}	(0.72)^{d}	(0.24)^{cd}	(0.38)^{c}	(0.51)^{c}	(0.07)^{cd}	(0.19)^{c}
Chroma — bf	28.16	23.36	24.14	23.80	23.84	23.93	23.71	24.38
	(0.02)^{a}	(0.02)^{d}	(0.06)^{bc}	(0.11)^{bcd}	(0.02)^{bcd}	(0.05)^{bcd}	(0.51)^{cd}	(0.30)^{b}
Chroma — af	20.77	19.09	24.33	22.60	22.71	23.68	23.10	24.03
	(0.17)^{e}	(0.55)^{f}	(0.03)^{a}	(0.16)^{d}	(0.08)^{d}	(0.10)^{bc}	(0.07)^{cd}	(0.03)^{ab}
ΔE — bf	0.00	5.27	4.85	5.47	6.14	7.52	8.89	9.11
	(0.00)^{f}	(0.02)^{d}	(0.09)^{e}	(0.04)^{d}	(0.04)^{c}	(0.07)^{b}	(0.26)^{a}	(0.12)^{a}
ΔE — af	0.00	2.03	8.09	8.34	9.27	11.59	12.78	13.38
	(0.00)^{g}	(0.45)^{f}	(0.03)^{e}	(0.03)^{e}	(0.02)^{d}	(0.02)^{c}	(0.05)^{b}	(0.01)^{a}

†Numbers in parentheses refer to standard deviation of three measurements. Mean values in a row not followed by the same letter (as superscript) are significantly different ($p\leq0.05$).
‡bf = before whipping, af = after whipping.

Table 3. Swelling volume (ml) of cowpea flour and starch as affected by soaking, boiling, and fungal fermentation*

Treatment	Flour (2 g)		Flour (1 g)		Starch (1 g)	
Control	16.11	(0.47)^{b}	5.20	(0.10)^{b}	20.70	(0.30)^{a}
Soaking	17.86	(0.34)^{a}	5.86	(0.07)^{a}	21.12	(0.49)^{a}
Soaking and boiling	8.12	(0.04)^{cd}	5.04	(0.14)^{bc}	6.61	(0.08)^{b}
0 h Fermentation	8.09	(0.15)^{cd}	5.11	(0.06)^{bc}	6.73	(0.07)^{b}
15 h Fermentation	8.24	(0.09)^{c}	4.76	(0.14)^{c}	7.02	(0.04)^{b}
18 h Fermentation	8.08	(0.17)^{cd}	5.16	(0.09)^{bc}	6.82	(0.08)^{b}
21 h Fermentation	7.59	(0.06)^{d}	4.86	(0.27)^{bc}	6.60	(0.07)^{b}
24 h Fermentation	7.73	(0.05)^{cd}	4.98	(0.14)^{bc}	6.70	(0.04)^{b}

*Dry weight basis. Numbers in parentheses refer to standard deviation of three measurements. Mean values in a column not followed by the same letter (as superscript) are significantly different ($p\leq0.05$).

suggested that starch swelling volume may be a useful alternative index to starch paste peak viscosity in characterizing the performance of starch in starch-based food products. We observed a much lower and, in some cases, negative correlation between flour or starch swelling volume and flour pasting characteristics (Table 6). Therefore, the concept proposed by Crosbie (1991) may not be applicable to cowpea flour in which the preparation involves severe heat treatment or to products in which the quality depends on cool-paste viscosity.

Pasting characteristics

Among the most important practical functional properties of starch or starchy materials is their pasting characteristics. The swelling of starch granules leads to significant changes in viscosity and other rheological properties of the paste that are characteristic of the particular type of starch. Pasting characteristics of non-treated cowpea starch [Table 4 and Fig. 1(a)] and non-treated cowpea flour [Table 5 and Fig. 1(c)] are concentration dependent. According to the classification by Schoch and Maywald (1968), cowpea starch exhibits A-type (high swelling) and B-type (moderate swelling) as reported, respectively, by El Faki *et al.* (1983) and Tolmasquim *et al.* (1971). In our study, control cowpea starch exhibited B-type swelling at higher concentration (7–8%) and C-type (restricted swelling) at lower concentration (4–6%) [Fig. 1(a)]. Differences in pasting curve patterns were due mainly to the starch concentration and varietal differences.

The pasting temperature of control cowpea starch ranged from 72.5 to 75.5°C, depending on the concentration (Table 4). As the concentration increased from 4 to 8%, the pasting temperature gradually decreased, the viscosity at 95°C progressively increased, and the pasting curve shifted slightly toward lower temperature [Fig. 1(a)]. The initial flat portion of the pasting curve represents the period in which any swelling is insufficient to register an increase in viscosity with the equipment. On the basis of viscosity changes, comparison of the pasting temperature with that of the pasting peak before reaching 95°C (data not shown) indicates an approximate gelatinization range of 72.5–89.0°C [Fig. 1(a)]. However, based on birefringence properties, El Faki *et al.* (1983) and Tolmasquim *et al.* (1971) reported lower gelatinization ranges of 65–73°C and 64–78°C, respectively. The lower gelatinization ranges (Tolmasquim *et al.*, 1971; El Faki *et al.*, 1983) were probably due to the loss of birefringence which usually occurs before appreciable swelling and increased viscosity take place (Fujimura *et al.*, 1995*b*). At all control starch concentrations, the differences between peak viscosity before and at 95°C were relatively small [Fig. 1(a)], indicating the ease of cooking of cowpea starch. At higher concentrations, besides the broad pasting peak, cowpea starch showed low shear-thinning (break down) behavior or good hot-paste stability

Table 4. Pasting characteristics of cowpea starch as affected by soaking, boiling, and fungal fermentation*

Treatment	Pasting temp. (°C)	BU at 95°C	BU at 95°C-hold	BU at 50°C	BU at 50°C-hold	Hot-paste stability	Retrogradation tendency	Cool-paste stability
Control — 4%	75.5	80.0	83.3	90.0	90.0	1.04	1.10	1.00
	(0.0)	(10.0)	(11.6)	(0.0)	(0.0)	(0.07)	(0.16)	(0.00)
Control — 5%	75.5	170.0	150.0	200.0	213.3	0.88	1.33	1.07
	(0.0)	(0.0)	(0.0)	(0.0)	(5.8)	(0.00)	(0.00)	(0.03)
Control — 6%	74.0	320.0	270.0	416.7	456.7	0.84	1.54	1.10
	(0.0)	(0.0)	(0.0)	(5.8)	(5.8)	(0.00)	(0.02)	(0.00)
Control — 7%	74.0	610.0	523.3	910.0	986.7	0.86	1.74	1.08
	(0.0)^{b}	(0.0)^{a}	(5.8)^{a}	(10.0)^{a}	(11.6)^{a}	(0.01)	(0.01)	(0.01)
Control — 8%	72.5	816.7	746.7	1353.3	1393.3	0.91	1.81	1.03
	(0.0)	(5.8)	(5.8)	(11.6)	(5.8)	(0.01)	(0.00)	(0.01)
Soaking	73.0	546.7	506.7	843.3	966.7	0.93	1.66	1.15
	(0.9)^{b}	(5.8)^{b}	(5.8)^{b}	(5.8)^{b}	(5.8)^{b}	(0.02)	(0.02)	(0.01)
Soaking and boiling	72.5	30.0	30.0	46.7	46.7	—†	—	—
	(0.0)^{b}	(0.0)^{e}	(0.0)^{e}	(5.8)^{d}	(5.8)^{cd}			
0 h Fermentation	70.5	56.7	50.0	60.0	60.0	—	—	—
	(0.9)^{c}	(5.8)^{c}	(0.0)^{c}	(0.0)^{c}	(0.0)^{c}			
15 h Fermentation	68.0	30.0	43.3	40.0	40.0	—	—	—
	(0.0)^{d}	(0.0)^{e}	(5.8)^{cd}	(0.0)^{de}	(0.0)^{de}			
18 h Fermentation	68.5	40.0	40.0	50.0	50.0	—	—	—
	(0.9)^{d}	(0.0)^{d}	(0.0)^{d}	(0.0)^{cd}	(0.0)^{cd}			
21 h Fermentation	67.5	36.7	30.0	30.0	30.0	—	—	—
	(0.9)^{d}	(5.8)^{de}	(0.0)^{e}	(0.0)^{e}	(0.0)^{e}			
24 h Fermentation	—†	10.0	10.0	10.0	10.0	—	—	—
		(0.0)^{f}	(0.0)^{f}	(0.0)^{f}	(0.0)^{f}			

*Seven percent starch (dry wt/v), unless otherwise indicated, especially for the control sample; BU refers to Brabender unit for viscosity. Numbers in parentheses refer to standard deviation of three measurements. Mean values in a column not followed by the same letter (as superscript) are significantly different ($p \leq 0.05$).

†Not determined, because there was insignificant gelatinization and retrogradation.

(Table 4) indicating the great resistance of swollen starch granules to mechanical disintegration upon holding for 30 min at 95°C. As starch concentration increased, an increase in retrogradation tendency was observed as indicated by drastic increases in viscosity upon cooling to 50°C; however, the cool-paste stability was fairly constant [Table 4 and Fig. 1(a)].

The effects of soaking, S/B, and fermentation on pasting characteristics of isolated starches (7% dry wt/v) are shown in Table 4 and Fig. 1(b). The pasting curve pattern of starches extracted from control and soaked seeds was similar, having a moderate-swelling pattern, but was distinctively different from that of starches extracted from S/B and fermented seeds [Fig. 1(b)]. Paste viscosity of control starch was slightly greater than that of starch from soaked seeds. Starch extracted from seeds that had been boiled for 45 min showed very low viscosity throughout the heating and cooling cycles indicating that insignificant gelatinization and retrogradation occurred. Similar observations were also reported for drum-dried cowpea powder (Okaka & Potter, 1979) and parboiled cassava flour (Raja & Ramakrishna, 1990). During boiling, cowpea starch apparently approached complete gelatinization and hence only minute swelling occurred during the Brabender heating cycle. This phenomenon, to a large

Table 5. Pasting characteristics of cowpea flour as affected by soaking, boiling, and fungal fermentation*

Treatment	Pasting temp.(°C)	BU at 95°C	BU at 95°C-hold	BU at 50°C	BU at 50°C-hold	Hot-paste stability	Retrogradation tendency	Cool-paste stability
Control — 10%	75.5	140.0	110.0	133.3	120.0	0.76	1.25	0.90
	(0.0)	(0.0)	(0.0)	(5.8)	(0.0)	(0.04)	(0.06)	(0.04)
Control — 13%	75.0	306.7	186.7	230.0	213.3	0.61	1.23	0.93
	(0.9)	(5.8)	(5.8)	(0.0)	(5.8)	(0.03)	(0.04)	(0.03)
Control — 16%	75.0	430.0	393.3	513.3	440.0	0.91	1.31	0.86
	(0.9)^{a}	(0.0)^{c}	(5.8)^{e}	(5.8)^{e}	(0.0)^{f}	(0.01)^{e}	(0.00)^{e}	(0.01)^{b}
Control — 16%†	75.0	443.3	316.7	370.0	343.3	0.71	1.17	0.93
	(0.9)	(5.8)	(15.3)	(17.3)	(5.8)	(0.03)	(0.04)	(0.03)
Soaking	75.3	686.7	450.0	700.0	663.3	0.66	1.56	0.95
	(0.4)^{a}	(23.1)^{a}	(10.0)^{e}	(17.3)^{c}	(25.2)^{d}	(0.01)^{e}	(0.02)^{ab}	(0.02)^{a}
Soaking†	74.5	713.3	376.7	526.7	493.3	0.53	1.40	0.94
	(0.9)	(11.5)	(5.8)	(11.5)	(5.8)	(0.00)	(0.03)	(0.01)
Soaking and boiling	65.5	340.0	593.3	973.3	883.3	1.75	1.64	0.91
	(0.5)^{bc}	(20.0)^{d}	(23.1)^{c}	(41.6)^{b}	(32.2)^{b}	(0.06)^{c}	(0.02)^{a}	(0.01)^{a}
0 h Fermentation	65.8	273.3	796.7	1140.0	1040.0	2.93	1.43	0.91
	(0.8)^{b}	(20.8)^{e}	(5.8)^{a}	(45.8)^{a}	(17.3)^{a}	(0.21)^{a}	(0.05)^{cd}	(0.03)^{a}
15 h Fermentation	58.5	493.3	666.7	980.0	770.0	1.35	1.47	0.79
	(1.7)^{d}	(28.9)^{b}	(41.6)^{b}	(20.0)^{b}	(30.0)^{c}	(0.02)^{d}	(0.07)^{bc}	(0.01)^{c}
18 h Fermentation	60.5	453.3	716.7	963.3	806.7	1.58	1.34	0.84
	(0.0)^{d}	(23.1)^{bc}	(15.3)^{b}	(5.8)^{b}	(11.6)^{c}	(0.11)^{cd}	(0.07)^{de}	(0.01)^{b}
21 h Fermentation	63.5	243.3	513.3	726.7	603.3	2.11	1.42	0.83
	(0.0)^{c}	(5.8)^{e}	(32.2)^{d}	(30.6)^{c}	(23.1)^{d}	(0.16)^{b}	(0.03)^{cd}	(0.01)^{b}
24 h Fermentation	63.5	273.3	436.7	600.0	516.7	1.60	1.37	0.86
	(0.0)^{c}	(5.8)^{e}	(15.3)^{e}	(10.0)^{d}	(11.6)^{e}	(0.09)^{cd}	(0.03)^{cde}	(0.01)^{b}

*Sixteen percent flour (dry wt/v), unless otherwise indicated, especially for the control sample; BU refers to Brabender unit for viscosity. Numbers in parentheses refer to standard deviation of three measurements. Mean values in a column not followed by the same letter (as superscript) are significantly different ($p \leq 0.05$).
†Sixteen percent flour (dry wt/v) in soak water.

Table 6. Correlation between swelling volume and pasting characteristics of cowpea flour and starch*

Variable	Swelling volume of		
	Starch	Flour (1 g)	Flour (2 g)
Flour			
BU at 95°C	0.65	0.66	0.73
BU at 95°C-hold	−0.62	−0.31	−0.58
BU at 50°C	−0.61	−0.24	−0.55
BU at 50°C-hold	−0.51	−0.08	−0.44
Starch			
BU at 95°C	0.99	0.68	0.98
BU at 95°C-hold	0.99	0.70	0.99
BU at 50°C	0.99	0.69	0.99
BU at 50°C-hold	0.99	0.71	0.99

*BU refers to Brabender unit for paste viscosity.

extent, can be explained by the swelling behavior of starch (Table 3). The correlation between swelling volume and starch pasting characteristics was highly positive (Table 6).

Protein content of starches extracted from control and soaked seeds ranged from 0.5–0.7% (dry wt basis); starches extracted from soaked/boiled and fermented seeds contained 3.3–6.3% protein. The protein residues in starch from S/B and fermented seeds did not influence paste viscosity, although the presence of protein has been reported to increase viscosity of starch paste (Anker & Geddes, 1944). The effect of fermentation on starch paste viscosity was insignificant compared to boiling. The lower paste viscosity of starch from fermented (24 h) flour compared to that of starch from non-fermented (0 h) flour was due to starch degradation caused by *R. microsporus* amylase activity.

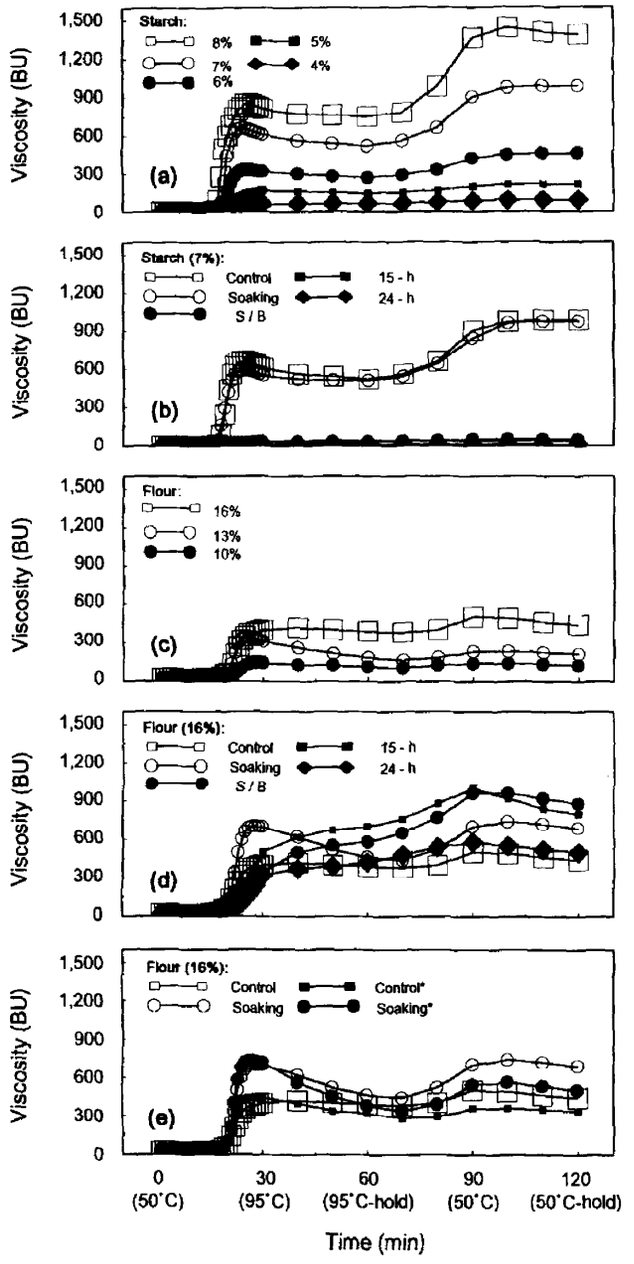

Fig. 1. Brabender Visco-Amylograms of starch and flour suspensions in de-ionized water. (a) Starch from untreated cowpea flour (control) as affected by concentration; (b) starch (7% dry wt/v) as affected by soaking, soaking and boiling (S/B), and fermentation up to 24 h; (c) control flour as affected by concentration; and (d) flour (16% dry wt/v) as affected by soaking, soaking and boiling, and fermentation. Brabender Visco-Amylogram of (e) flour (16% dry wt/v) from control and soaked seeds as affected by soak water (*).

Pasting characteristics of cowpea flour as affected by soaking, S/B, and fermentation are shown in Table 5 and Fig. 1(d). The pasting curve pattern of cowpea flours differed markedly from that of cowpea starches [Fig. 1(d) vs Fig. 1(b)]. Although it is believed generally that paste viscosity patterns are governed by two simultaneous phenomena, i.e. the extent of swelling of starch granules and the extent of disintegration of swollen starch granules (Schoch & Maywald, 1968), the pasting characteristics of flours as observed in this study cannot be explained solely by the swelling characteristics of flour and starch (Table 3). In fact, a positive correlation between starch or flour swelling volume and flour pasting characteristics is virtually non-existent (Table 6).

Without exception, the pasting temperature (58.5–65.8°C) of flours prepared from S/B seeds before and after fermentation was lower than that (*ca* 75°C) of flours from control or soaked seeds. Unlike starch, flour prepared from control seeds exhibited a pasting curve pattern different from that of flour from soaked seeds [Fig. 1(b) vs Fig. 1(d)]. Soaking seeds significantly increased the hot paste viscosity (686 BU) of flour at 95°C compared to that (430 BU) of the control flour (Table 5). During soaking, hydration and softening of seed occur, thus facilitating leaching of water-soluble components (e.g. organic acids, simple sugars and oligosaccharides) into soak water (Mulyowidarso, 1988; Prinyawiwatkul *et al.*, 1996*a*,*c*). The chemical reactions between water-soluble components and starch in cowpea seeds were thought to suppress the gelatinization property of the control flour. One observation supporting this hypothesis is the increased temperature of endotherm recorded by the differential scanning calorimeter as a water extract of adzuki beans (*V. angularis*) was added to the isolated starches and/or cotyledon cells (Fujimura & Kugimiya, 1995*a*). However, results [Table 5 and Fig. 1(e)] from our study reveal that soak water extract of cowpeas did not adversely affect paste viscosity below and at 95°C, but suppressed paste viscosity thereafter. Therefore, the suppression of gelatinization of cowpea flour made from whole intact unsoaked seeds may not be accounted for solely by the presence of water-soluble components. The suppression of starch gelatinization in intact cells was reported for lima beans (Hahn *et al.*, 1977), adzuki beans (Fujimura & Kugimiya, 1993, 1995*a*), faba beans (Fujimura *et al.*, 1995*b*) and kidney beans (Fujimura & Kugimiya, 1994). Fujimura and Kugimiya (1995*a*) proposed that, in addition to the presence of water-soluble substances,

suppression of starch gelatinization in the intact cells may be caused by the physical strength of cell walls and limited available water.

Priestley and Avumatsodo (1977) reported that water penetration is a major factor influencing gelatinization of cowpea starch. Before soaking, cowpea cotyledon cells are densely packed and contain starch granules which appear rough and tightly embedded in a protein matrix (Liu *et al.*, 1992). During soaking, water first penetrates the seed coat and cotyledon, then diffuses through the cell wall and finally through proteinaceous materials on the surface of or between starch granules (Priestley & Avumatsodo, 1977). After soaking, starch granules become smoother and are loosely embedded in the protein matrix (Liu *et al.*, 1993). The increase in paste viscosity of flour from soaked cowpea seeds observed in our study may have been due to the availability of sufficient water and space required for starch granules to quickly hydrate and swell during gelatinization.

The pasting curve patterns of flours prepared from S/B seeds before and after fermentation were similar but distinctively different from those of flour from control and soaked seeds [Fig. 1(d)]. Unlike starch paste viscosity, paste viscosity of flours prepared from S/B and fermented seeds progressively increased during heating and cooling but decreased slightly upon holding for 30 min at 50°C [Fig. 1(b) vs Fig. 1(d)]. Because of the high protein content (26–28%), pasting characteristics of cowpea flour are influenced by a physical competition for water between protein coagulation/gelation into a continuous network and starch swelling during cooking.

Protein and starch interact due to attraction of their opposite charges to form a complex during gelatinization. In the presence of excess water and sufficiently high temperature, however, the internal starch–protein bonds can be disrupted, allowing starch granules to expand and expose their hydroxyl groups to freely form hydrogen bonding with water (Watson & Johnson, 1965). In our study, it is likely that boiling cowpeas (45 min) induced denaturation of the protein network around starch granules which otherwise functions as a physical or water-restricting barrier (Liu *et al.*, 1993) and thus hinders water absorption and suppresses gelatinization of starch granules. In our study, almost complete starch gelatinization occurred during boiling (45 min) as indicated by the loss of birefringence of starch granules (Fig. 2) and the flat pasting curve of starches extracted from S/B seeds before and after fermentation [Fig. 1(b)]. Consequently, the progressive increase in paste viscosity of flour from S/B seeds [Fig. 1(d)] was probably not due to starch gelatinization, but rather to protein swelling and gelation, although the possible role of other constituents (e.g. fiber) of flour should not be overlooked. As fermentation time increased, the paste viscosity of flour tended to decrease [Table 5 and Fig. 1(d)] due to protein and starch degradation, respectively, caused by *R. microsporus* protease and amylase activities.

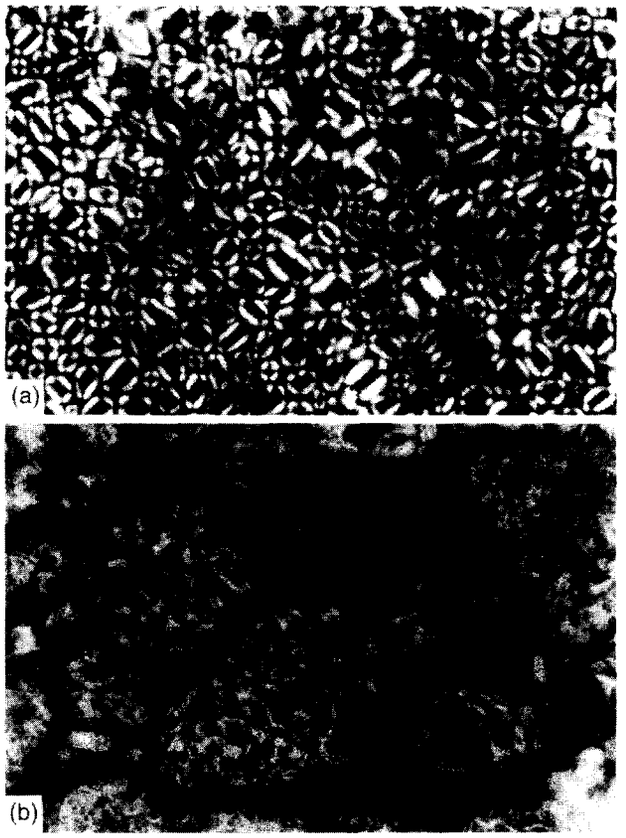

Fig. 2. Micrograph of starch granules from control cowpea flour shows typical birefringence (a). Micrograph of starch granules from flour prepared from soaked and boiled cowpeas shows loss of birefringence (b) as observed under a light microscope (400× magnification) with polarizing film.

Group differences

In addition to ANOVA, multivariate analysis of variance (MANOVA) was used to determine if there was a difference among the eight cowpea flours, considering simultaneously the effects of all flour and starch properties evaluated in this study. The p value of MANOVA's statistics are Wilks' Lambda ($p=0.0001$), Pillai's Trace ($p=0.0001$), Hotelling–Lawley Trace ($p=0.0001$), and Roy's Greatest Root ($p=0.0001$) indicating the existence of differences among flours. Results from descriptive discriminant analysis, DDA, (Table 7) identify variables that largely account for the group differences. Analysis of dimensionality (data not shown) indicates that only two dimensions can explain 99.78% of the variance. The first dimension (CAN1), which accounts for 99.06% of the variance, consists of two major properties, i.e. flour foamability and starch pasting characteristics (swelling volume, BU at 95°C, BU at 95°C-hold, BU at 50°C, and BU at 50°C-hold). As clearly shown in Tables 1 and 3 and Fig. 1(b), flours prepared from control and soaked seeds differed from flours prepared from S/B and fermented seeds. Implications for food systems based on the DDA results must be made with caution because the purpose of DDA used

Table 7. Canonical structure *r*'s describing group differences among cowpea flours based on selected variables

Variable	CAN1	CAN2
Flour		
Foam volume	0.037608*	0.027782
Swelling volume (2 g flour)	0.002001	0.010880
Pasting temperature	0.008574	0.044712*
BU at 95°C	0.005993	−0.029602
BU at 95°C-hold	−0.004808	−0.018367
BU at 50°C	−0.005989	−0.004114
BU at 50°C-hold	−0.005696	0.020733
Hot-paste stability	−0.005521	0.022584
Retrogradation tendency	−0.000441	0.024176
Cool-paste stability	0.001479	0.037931*
Starch		
Swelling volume	0.035449*	0.008383
Pasting temperature	0.000321	0.034375*
BU at 95°C	0.083927*	0.026459
BU at 95°C-hold	0.073978*	0.019727
BU at 50°C	0.099802*	0.037025
BU at 50°C-hold	0.101770*	0.050147
Variance explained (%)	99.06	0.72

*Indicates properties which most account for the group differences; BU refers to Brabender unit for paste viscosity; CAN1 and CAN2 refer to canonical correlation for the first and second dimension, respectively.

here was to identify the properties which underlie group (cowpea flour) differences, rather than the properties which indicate the potential uses of flour in food products.

CONCLUSION AND IMPLICATIONS

Previous studies have shown that scale-up production of flour essentially free of flatulence-causing oligosaccharides is feasible and would stimulate increased utilization of cowpeas (Prinyawiwatkul *et al.*, 1996*a*). Because of its high protein (28%) and low fat (2%) content, absence of oligosaccharides, and enhanced B-vitamin content (Prinyawiwatkul *et al.*, 1996*a,b,c*), fermented cowpea flour would be a promising functional ingredient in various food products. However, the potential uses of this flour depend on the quality required in final products.

The present study has shown that functional characteristics of cowpea flours are affected greatly by various processing treatments during flour preparation. Soaking and fungal fermentation had less impact on flour functionality compared to boiling. Boiling eliminated foamability of flour; thus, the use of flour should be targeted to products in which quality is not dependent on foam properties. Boiling, however, resulted in unique flour pasting characteristics. Flour prepared from S/B and/or fermented (15 h) seeds showed high water absorption properties upon heating and cooling. The broad paste peak exhibited along with high cool-paste viscosity and stability of flour are desirable in certain food systems where gel formation or gelation must occur after the cooking process. This is especially important in the canning industry and in the preparation of extended ground beef, pork, and poultry products, sausages, wieners and luncheon meats. Potential applications of cowpea flour in other products such as bakery and snack foods are also numerous and need further investigation.

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