

# Effect of Milling and Particle Size on Functionality and Physicochemical Properties of Cowpea Flour

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## ABSTRACT

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Cowpeas (*Vigna unguiculata*) were milled through 0.5-, 1.0-, and 2.0-mm screens, and the flour was subsequently separated into different particle-size ranges. Such procedures caused only minimal changes in moisture, fat, protein, ash, and total carbohydrate. The amount of extractable starch, however, varied from 34.5 to 52%. The effects of both mill screen and sieve mesh size were significant ( $P < 0.05$ ). Differences in milling and separation procedures resulted in significant variations in water absorption (0.41–2.81 g of water/g of flour), solids lost (0.34–1.17 g/g of flour), and protein solubility (21.2–37.4%) ( $P < 0.05$ ). Finely milled flours (91% moisture) had lower initial gelatinization temperatures (70–

73°C), as measured by differential scanning calorimetry (DSC) ( $P < 0.01$ ). Gelatinization peaks in high-moisture flour were similar to that of pure starch. At lower moisture, a second peak was observed indicative of protein. Light-scattering analysis showed that different conditions produced a bimodal particle-size distribution when samples were suspended in water. The small size had relatively constant diameters (19–21  $\mu\text{m}$ ) and was associated with starch granules. The latter had a large size distribution and varying peak size and was associated with aggregated flour particles. These results indicate that changes in processing produces cowpea flours with differing chemical and physical properties.

One important source of nutritious, high protein flour is the cowpea (*Vigna unguiculata*). A typical cultivar contains 11% moisture, 24% protein, 1.3% fat, 56.8% carbohydrate, 3.9% fiber, and 3.6% ash (Deshpande and Damodaran 1990). However, problems in the functionality of cowpea paste have been noted. For example, McWatters (1983) studied the use of commercial Nigerian cowpea flour in the production of *akara*, a fried cowpea paste. The *akara* was dry and dense and had a tough outer surface. Such poor performance was related to particle-size distribution, in particular to the higher fraction of small particles in the commercial flour as compared with noncommercial flour. Similar results were reported by Ngoddy et al (1986). These researchers found that *akara* made from fine cowpea flour had decreased hydration and air incorporation and showed a denser and less spongy texture. They recommended that flours used for *moin-moin* (steamed cowpea paste) or *akara* be milled with 65–75% of the particles in the size range 45–150  $\mu\text{m}$  (350–100 mesh) and seeds dried at  $<60^\circ\text{C}$ .

The functional properties of cowpea flour are critical to the production of associated foods. These properties include water absorption, fat absorption, and protein solubility, which affect the processing, texture, and appearance of the product. Physicochemical changes in starch and protein significantly affect product characteristics. Changes in physical states of starch and protein are particularly important and include starch gelatinization, glass transitions, protein denaturation, and formation of protein networks. This study was undertaken to determine the effect of flour mill screen and particle size on the proximate analysis, functional characteristics, and thermal properties of cowpea flour. In a subsequent study, these properties will be related to the textural properties and appearance attributes of snack foods prepared from cowpea flour.

## MATERIALS AND METHODS

Cowpeas (*V. unguiculata*, California blackeye 5) were decorticated as described by Hung et al (1990) and ground in a Thomas-Wiley laboratory mill (model 4, Arthur H. Thomas Co., Philadelphia, PA) equipped with either a 2.0-, 1.0-, or 0.5-mm screen (Hung et al 1990). The milled flour was separated into particle-size fractions

by retention above 20, 30, 40, 60, 80, 100, 140, 200, and 400 mesh screens (Phillips et al 1988). Fractionation was repeated on three separate samples. Samples were stored at  $-18^\circ\text{C}$  until used.

Proximate analysis of decorticated flour, as well as flour fractions, included measurements of moisture, protein, fat, ash, and carbohydrate. Moisture content was determined using a modified vacuum oven method (Approved Method 44-40, AACC 1995). Crude protein was measured by the Kjeldahl procedure (Approved Method 46-12) using  $\text{N} \times 6.25$  (FAO 1970). Crude fat was determined by extraction with ether in a Goldfish apparatus (Approved Method 30-26). Ash was determined by heating at  $550^\circ\text{C}$ , then weighing the resultant gray ash (Approved Method 08-01). All proximate analyses were done in triplicate and reported on a dry weight basis.

Total carbohydrate was determined by difference after calculation of moisture, protein, fat, and ash. Crude starch content was determined using an industrial analyzer (YSI model 27, Yellow Springs Instrument Co., Yellow Springs, OH). The method of Galvez and Resurreccion (1993) was used in which samples are hydrolyzed in base and  $\alpha$ -amylase, then subjected to amyloglucosidase (Sigma, St. Louis, MO) before reading glucose content. Modifications to the method were that  $\approx 0.43$  g of sample was weighed into a 25-mL volumetric flask, to which 4.5 mL of 4M KOH was added. Starch analysis was done in triplicate. Basic functionality tests of water absorption, fat absorption, and protein solubility were determined on triplicate samples.

**Water absorption capacity and solids lost.** Water absorption was determined according to the method of Phillips et al (1988). Flour samples (2 g) were suspended in 20 mL of distilled water and gently agitated on a mechanical shaker for 1 hr. The suspension was then centrifuged at  $48,000 \times g$  for 1 hr. The wet pellet was collected and drained on Whatman #1 filter paper, then freeze-dried. Water absorption was reported as:

$$\text{g of water absorbed/g of insoluble matter} = (\text{g of wet pellet} - \text{g of dry residue}) / \text{g of dry residue} \quad (1)$$

**Oil absorption capacity.** Oil absorption capacity was determined by suspending 1 g of cowpea flour in 20 mL of peanut oil (Sessions Co., Enterprise, AL) and agitating for 1 hr. The slurry was then centrifuged for 1 hr at 20,000 rpm ( $48,000 \times g$ ). The solids were collected on Whatman #1 filter paper and drained for 3 hr. Oil absorption was determined as:

$$\text{g of oil absorbed/g of flour} = (\text{g of pellet} - \text{g of flour sample}) / \text{g of flour sample} \quad (2)$$

Mean values for oil absorption were calculated from five measurements.

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**Protein solubility.** The supernatant from the water absorption capacity assay was used to determine the protein solubility of the cowpea flours. The ratio of protein in the supernatant to that in the untreated cowpea flour was determined using Kjeldahl analysis (Approved Method 46-12). All analyses were made in triplicate.

### Starch Gelatinization

Transition temperatures and enthalpies associated with the gelatinization process were determined using differential scanning calorimetry (Perkin Elmer DSC 7, Norwalk, CT). Cowpea flour samples with known moisture content were mixed with distilled water to give total moisture contents of 10, 42, 64, 78, and 91%. Samples of  $14 \pm 3$  mg were hermetically sealed in aluminum pans and then scanned from 20 to 150°C at 10°C/min. Thermal transitions for gelatinization were characterized by  $T_o$  (onset temperature),  $T_p$  (peak temperature),  $T_c$  (conclusion temperature), and  $\Delta H$  (enthalpy of gelatinization). Enthalpy of gelatinization was expressed on a dry weight basis as:

$$\Delta H_{\text{gel}} (\text{J/g}) = \text{peak area (J)} / [\text{sample wt (g)} \times \% \text{ flour in sample}] \quad (3)$$

Samples of cowpea starch were also analyzed with DSC for comparison with the flour samples. Starch was extracted from the sample using the method of Schoch and Maywald (1968).

### Particle Size of Suspended Flours

To assess the affects of milling and particle size on the degree of association of flour particles, the particle-size distribution of samples suspended in water was measured. Samples were prepared as for water absorption and protein solubility assays: 2 g of dry flour were suspended in 20 mL of water at 25°C and gently stirred for 1 hr. Particle-size distribution of the suspension was determined using a light-scattering instrument (Mastersizer, Malvern

Instruments Ltd., Worcestershire, UK). Particle size refers to hydrated and suspended particles after mixing with water, and not to particle size of the dry ingredients as collected on mesh screens.

### Statistical Analysis

A  $3 \times 4$  complete factorial experimental design was used with three mill screen sizes and four particle sizes after fractionation. Unless otherwise noted, samples were processed and analyzed in triplicate. Analysis of variance (ANOVA) was used to test the hypothesis that means were not different for the measured proximate and functional properties and to test for main effects and interactions between mill screen and particle size. Duncan's multiple range test was used to test for differences between groups ( $\alpha = 0.05$ ). Calculations were performed using SAS version 5 (SAS Institute, Cary, NC) for the Windows 95 operating system. Correlation analysis between two sets of data were performed using the CORREL analysis tool of Microsoft Excel, which calculates the covariance of the data sets divided by the product of their standard deviations.

## RESULTS AND DISCUSSION

### Flour Particle-Size Distribution

Particle-size distributions for the three mill screen sizes investigated are shown in Table I. As expected, seeds milled through a

**TABLE III**  
Significant Correlations Between Attributes of Proximate Composition, Functional Properties, and Thermal Properties of Cowpea Flour

Attribute	Mill Screen Size	Mesh Sieve Size	Mill $\times$ Mesh
Proximate composition			
Moisture	** <sup>a</sup>	ns	ns
Fat	*	*	**
Protein	ns	**	ns
Total carbohydrate	ns	*	ns
Ash	ns	**	ns
Starch	*	*	ns
Functional properties			
Water absorption	*	*	ns
Solids lost	*	*	ns
Oil absorption	*	*	ns
Protein solubility	*	*	ns
Thermal properties <sup>b</sup>			
Onset ( $T_o$ )	ns	**	ns
Peak ( $T_p$ )	ns	*	ns
Conclusion ( $T_c$ )	ns	*	ns
Peak width ( $T_c - T_o$ )	ns	*	ns
Enthalpy ( $\Delta H$ )	ns	ns	ns

<sup>a</sup> \*\*, \* = Significant at  $P < 0.01$  and  $P < 0.05$ , respectively; ns = not significant.

<sup>b</sup> 91% moisture.

**TABLE I**  
Mean Weight Percentage of Cowpea Flour Particles Collected on Sieves of Stated Size After Milling Through Three Screens ( $n = 3$ )

Mesh Sieve Size	Opening (mm)	Mill Screen (% particles)		
		0.5 mm	1.0 mm	2.0 mm
400	0.037	53.13	6.9	6.22
200	0.074	18.64	6.38	3.69
140	0.105	4.89	16.9	5.22
100	0.149	2.24	2.8	1.29
80	0.177	2.33	9.14	1.18
60	0.25	7.06	2.36	4.12
50	0.297	9.77	9.74	5.58
40	0.42	1.51	20.1	10.06
30	0.595	0.22	22.56	20.1
20	0.841	0.21	3.13	42.54

**TABLE II**  
Mean Proximate Composition (%) of Cowpea Flours Milled Through Three Screens and Separated by Particle Size<sup>a</sup>

Sample	Moisture	Fat	Protein	Ash	Total Carbohydrate	Starch
2.0-mm mill screen						
Unsieved flour	10.50bc <sup>b</sup>	0.75a	23.66c	3.72a	61.37a	35.58a
0.297-mm sieve	10.63bc	0.52a	23.28c	3.76ab	61.81a	36.46ac
0.149-mm sieve	9.60a-c	1.12cd	25.49c	3.97bc	59.83ab	34.49ab
Collecting pan	10.27bc	1.01b-d	22.42bc	3.61a	62.70a	44.71d
1.0-mm mill screen						
Unsieved flour	9.10ab	0.81b	23.63c	3.75ab	62.71a	40.66c
0.297-mm sieve	9.33a-c	0.82bc	24.40c	3.92bc	61.51a	35.99a
0.149-mm sieve	8.93ab	1.10cd	26.29a	4.10bc	59.58b	36.37a
Collecting pan	8.66a	1.07cd	22.66c	3.63a	63.97a	48.37ef
0.5-mm mill screen						
Unsieved flour	9.30ab	1.16cd	23.40c	3.76ab	62.38a	51.99f
0.297-mm sieve	9.13ab	0.96b-d	25.36c	4.00bc	60.55a	49.07ef
0.149-mm sieve	8.50a	1.18cd	25.08c	4.05bc	61.20a	39.15c
Collecting pan	8.70a	1.22cd	23.02c	3.67ab	63.38a	47.29e

<sup>a</sup> Above 0.297- and 0.149-mm mesh sieves or in a collection pan below a 0.037-mm sieve;  $n = 3$ .

<sup>b</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

fine (0.5-mm) screen had a greater percentage of particles collected on sieves with smaller openings. Those milled through coarser (1.0- and 2.0-mm) screens showed more particles in the sieves with larger openings. For example, seeds milled into flour with the 0.5-mm screen had 72% of particles retained on the 400 mesh (0.037-mm) and 200 mesh (0.074-mm) screens. The medium (1.0-mm) mill screen produced a broader distribution of particle sizes, with ≈43% of the particles collected above the 40 mesh (0.420-mm) and 30 mesh (0.595-mm) sieves. The coarse (2.0-mm) mill screen produced more particles of larger size; ≈73% of the particles were retained in the 40 mesh (0.420-mm), 30 mesh (0.595-mm), and 20 mesh (0.841-mm) sieves.

### Proximate Composition

The data in Table II show the mean proximate composition for cowpea flour milled through 0.5-, 1.0-, and 2.0-mm screens and collected on 50 mesh (0.297-mm), 100 mesh (0.149-mm), or after the final 400 mesh (0.037-mm) sieves. In most cases, the effects of mill screen or sieve size on proximate composition of collected samples were not great. The ranges of composition (wt%) were moisture (8.50–10.63%), fat (0.52–1.22%), protein (22.42–26.29%), ash (3.61–4.10%), and total carbohydrate (59.58–63.97). Some differences were statistically significant, however, and are shown in Table II. The main effects of mill screen size, mesh sieve size, and interactions (mill × mesh) are summarized in Table III. Mill

screen size affected moisture, fat, and starch levels. Particle size (or mesh sieve opening size) was important to every attribute except moisture content. Two-way interactions (mill × mesh) were only significant for fat levels. In general, moisture content decreased with decreasing mill size ( $P < 0.05$ ), while crude fat and starch levels increased ( $P < 0.01$ ). The 0.149 mesh screen had the highest levels of fat, protein, and ash. Protein and ash content were the lowest in the collection pan fraction.

Greatest differences in compositional values were for starch levels at 34.49–51.99%. For example, starch levels increased in the order 35.58, 40.66, and 51.99% for unsieved flours milled through 2.0-, 1.0-, and 0.5-mm screens, respectively. All differences were significant at  $P < 0.01$ . Both mill screen and particle size affected starch levels ( $P < 0.01$ ). Similar changes were not seen in total carbohydrate, however, where only one treatment was significantly different from the rest. This suggests that while milling and sieving do not change total carbohydrate, the starch may become more extractable in some cases. One possible cause is that greater degradation of starch occurs at smaller mill screen sizes (and presumably higher shear conditions) that facilitate starch extraction. In addition, the flour milled at 0.5 mm had a distribution skewed toward smaller particle sizes (Table I). For samples milled at 2.0 and 1.0 mm, the collection pan fines had higher starch content than other fractions. Clouett et al (1986) showed that the amount of starch entering the fines (during air particle-size classification) depends

**TABLE IV**  
Mean Values for Functional Properties of Cowpea Flours Milled Through Three Screens and Separated by Particle Size<sup>a</sup>

Sample	Water Absorption (g of water/g of flour)	Solids Lost (g of solids/g of flour)	Oil Absorption (g of oil/g of flour)	Protein Solubility (%)
2.0-mm mill screen				
Unsieved flour	1.69b <sup>b</sup>	0.66d	0.41a	21.65ac
0.297-mm sieve	1.62b	0.34ab	0.46ab	18.82a
0.149-mm sieve	2.41b–d	0.69d	0.66b–d	33.71bd
Collecting pan	0.79a	0.57bc	0.59bc	37.38d
1.0-mm mill screen				
Unsieved flour	1.56b	0.75d	0.52ab	28.30bc
0.297-mm sieve	2.12bc	0.77de	0.48ab	21.17a
0.149-mm sieve	2.68b–d	1.08f	0.75b–d	29.37b
Collecting pan	0.41a	0.23a	0.56bc	33.00bd
0.5-mm mill screen				
Unsieved flour	0.73a	0.44b	0.53ab	32.97bd
0.297-mm sieve	2.40b–d	0.83e	0.51ab	30.57bd
0.149-mm sieve	2.81b–d	1.17f	0.55a–c	31.56bd
Collecting pan	0.70a	0.46b	0.54a–c	32.42bd

<sup>a</sup> Above 0.297- and 0.149-mm mesh sieves or in a collection pan below a 0.037-mm sieve;  $n = 3$ .

<sup>b</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

**TABLE V**  
Mean Onset ( $T_o$ ), Peak ( $T_p$ ), and Conclusion ( $T_c$ ) Temperatures, Peak Width ( $T_c - T_o$ ), and Enthalpy ( $\Delta H$ ) for Cowpea Flour Separated by Particle Size at Three Moisture Levels

Sample	91% Moisture				64% Moisture				42% Moisture			
	$T_o$ (°C)	$T_c - T_o$ (°C)	$T_p$ (°C)	$\Delta H$ (J/g)	$T_o$ (°C)	$T_c - T_o$ (°C)	$T_p$ (°C)	$\Delta H$ (J/g)	$T_o$ (°C)	$T_c - T_o$ (°C)	$T_p$ (°C)	$\Delta H$ (J/g)
2.0-mm mill screen												
Unsieved flour	78a <sup>a</sup>	15c	86a	8.7ab	81a	18a	88a	6.8a	80a	20ab	88a	3.3a
0.297-mm sieve	75b	12ab	83a	8.4b	77b	16ab	85b	6.5b	76b	21a	86b	3.3a
0.149-mm sieve	74b	14bc	84a	9.0a	76b	18a	84b	6.0c	79ab	18bc	86b	3.4a
Collecting pan	74b	11a	80b	8.2bc	77b	15b	84b	6.2bc	80a	16bc	86b	3.0bc
1.0-mm mill screen												
Unsieved flour	76ab	16c	86a	8.4b	78b	16ab	85b	6.9a	79ab	22a	89a	3.4a
0.297-mm sieve	75b	13b	83a	8.7ab	78b	16ab	86ab	6.3b	81a	20a	90a	2.9c
0.149-mm sieve	76ab	15c	84a	9.0a	77b	20c	86ab	6.3b	78ab	14c	86b	3.1ab
Collecting pan	72c	12a	79b	8.0c	76b	15b	84b	6.6ab	78ab	20a	87ab	2.8c
0.5-mm mill screen												
Unsieved flour	73bc	14bc	82a	7.8c	76b	20c	85b	6.3b	80a	22a	90a	3.2ab
0.297-mm sieve	72c	11a	78b	8.0c	75bc	18a	83b	6.6ab	76b	18bc	84bc	3.0bc
0.149-mm sieve	73bc	12a	80b	7.6d	77b	15b	85b	6.2bc	78ab	21a	88a	2.9c
Collecting pan	70d	11a	77b	7.8cd	74c	18a	84b	6.0c	79ab	20a	89a	3.1ab

<sup>a</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

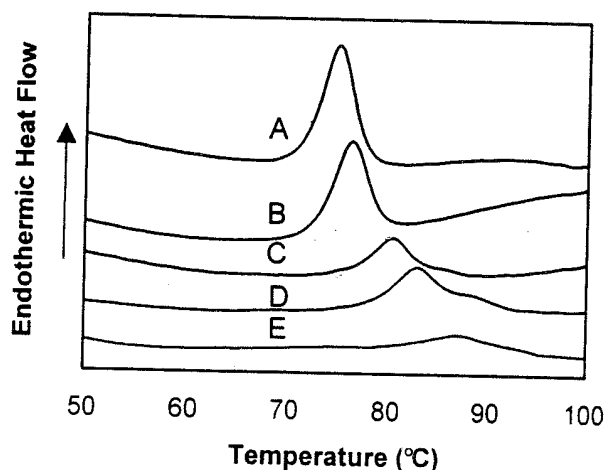
on the size of the starch granules of a specific species or cultivar of legume. They showed that cowpea flour, with small starch granules (<3.5–18.0 μm), gave fines with greater starch content than that of other legumes with larger granules such as pigeon pea or faba bean.

### Functional Properties

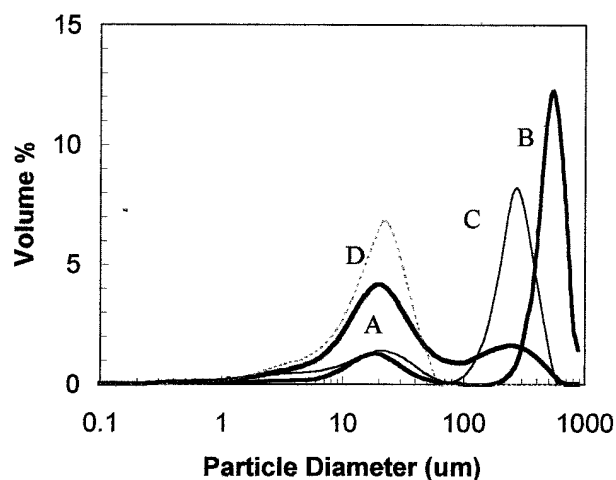
**Water absorption capacity and solids lost.** ANOVA showed that both mill screen and mesh screen size were significant factors on water absorption and solids lost. For unsieved flour, water absorption capacity decreased with mill screen size (Table IV) in the order: 1.69 g of water/g of flour (2.0-mm screen), 1.56 g of water/g of flour (1.0-mm screen), and 0.73 g of water/g of flour (0.5-mm screen) ( $P < 0.05$ ). The same was not true for sieved fractions of a given size that had been milled through different mill screens. For example, the 0.297-mm fractions showed increasing water absorption with decreased milling size: values of 1.62, 2.12, and 2.40 g of water/g of flour ( $P < 0.05$ ) for 2.0-, 1.0-, and 0.5-mm mill screens, respectively. Within a given mill screen group, water absorption capacity increased from the 0.297- to the 0.149-mm fraction ( $P < 0.05$ ). However, water absorption was significantly less in fine fractions caught in the collecting pan ( $P < 0.05$ ). These results agree with McWatters (1983), who observed poor water absorption

in finely milled flours. Solids lost increased with decreasing particle size down to the 0.149-mm fraction. Solids lost in the collection pan fines, however, were much less than in the 0.149-mm fraction ( $P < 0.05$ ).

In general, water absorption correlated with solids lost ( $r = 0.86$ ) and amount of starch extracted ( $r = -0.63$ ). The ability of water to be absorbed depends on limitations to mass transport as well as chemical composition of the sample. As seen in Table I, milling through smaller size openings (e.g., through the 0.5-mm screen) tends to produce distributions skewed toward smaller particle sizes. Thus, the ability of water to penetrate the sample and interact with particle macromolecules may be hindered. The observation that fractions lowest in solids lost also displayed lowest water absorption supports this observation. That is, solids that were lost to the supernatant are hydratable substances that break free from the matrix. If water permeation was thorough and these components remained in the flour matrix, then one would expect higher water absorption capacity. McWatters (1983) found that cold paste viscosity decreased with decreasing flour particle size and also suggested this occurred because water was not fully absorbed into the fine flours.



**Fig. 1.** Thermal curves derived from differential scanning calorimetry for cowpea flour samples milled through a 1.0-mm screen and collected on a 0.149-mm sieve. A, Extracted starch (91% moisture content); B, cowpea flour (91% moisture); C, cowpea flour (64% moisture); D, cowpea flour (42% moisture); E, cowpea flour (10% moisture).



**Fig. 2.** Light-scattering analysis of particle size in suspensions of 2 g of cowpea flour in 20 mL of water. Samples were milled through a 0.5-mm screen, then collected on sieve screens. A, Whole flour; B, 0.297-mm sieve; C, 0.149-mm sieve; D, collection pan fines.

**TABLE VI**  
Mean Parameters Derived from Light-Scattering Detection of Particle Size for Cowpea Flours Milled Through Three Screens and Separated by Particle Size<sup>a</sup>

Sample	First Peak			Second Peak		
	Range (μm)	Peak (μm)	Volume (%)	Range (μm)	Peak (μm)	Volume (%)
2.0-mm mill screen						
Unsieved flour	1–93	19	72ae <sup>b</sup>	93–594	249	28
0.297-mm sieve	1–124	19	71a	124–879	443	29
0.149-mm sieve	0.9–76	17	41b	84–594	270	59
Collecting pan	0–60	21	100c	...	...	...
1.0-mm mill screen						
Unsieved flour	0.7–93	19	85d	93–879	539	15
0.297-mm sieve	1.3–93	18	30e	184–879	489	70
0.149-mm sieve	0.9–76	18	67a	76–594	272	33
Collecting pan	0.2–62	21	98c	112–272	167	2
0.5-mm mill screen						
Unsieved flour	0.5–93	19	77ed	93–594	247	23
0.297-mm sieve	1.5–93	18	21f	203–879	539	79
0.149-mm sieve	0.9–76	21	30e	93–594	272	70
Collecting pan	0.6–63	21	100c	...	...	...

<sup>a</sup> Above 0.297- and 0.149-mm mesh sieves or in a collection pan below a 0.037-mm sieve;  $n = 3$ .

<sup>b</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

Although starch and other macromolecules contribute to water absorption, protein is the chief water-imbibing component of seeds before starch gelatinization (Mayer and Poljakoff-Mayber 1975). Kinsella (1979) found that as water absorption of food proteins increased, viscosity also increased. Protein content (Table II) was somewhat lower in the finer fractions of the cowpea flour, and this may also contribute to decreased water absorption in these fractions.

**Oil absorption.** Differences in oil absorption among the various treatments were small. The range of oil absorption was 0.41 g of oil/g of flour (2.0-mm mill screen, whole flour) to 0.75 g of oil/g of flour (1.0-mm mill screen, 0.149-mm sieve). Within the various groups, oil absorption was highest for the 0.149-mm fraction. These results were significantly different from the other particle-size fractions ( $P < 0.05$ ), except in the 0.5-mm milled group. Both mill screen and mesh screen were significant factors (Table III).

**Protein solubility.** Protein solubility increased with decreasing mill screen size for the unsieved flours as well as the 0.297-mm sieved fractions. No clear trends were observed among various fractions within a given mill screen size group. For the 2.0- and 1.0-mm groups, the 0.297-mm fraction displayed the least protein solubility, while the collection pan fraction had the highest. No significant differences were found among the fractions in the 0.5-mm mill screen group. Flour that had been milled through a 0.5-mm screen showed increased protein solubility. This may indicate that more rigorous milling helps disrupt cell components and allows macromolecules to be more readily extracted.

Han and Kahn (1990) studied navy beans, pinto beans, and chick-peas and found that starch-rich fractions (coarse particle size) had the highest water-holding capacity, nitrogen solubility, and cold paste viscosity, whereas protein-rich fractions (fine particle size) were superior in emulsification and foamability. Horvath et al (1989) found similar results with air-classified yellow pea fractions, where fine fractions were high in protein and exhibited greater oil absorption capacity, emulsifying activity, and stability. They also determined that coarse fractions ( $>16 \mu\text{m}$ ) were characterized by high water absorption capacities. We found that fines were rich in measurable starch with low water absorption, quantity of solids lost, and high in protein solubility. This indicates that apparent starch content, per se, does not dictate water absorption capacity. We also found that middlings (0.149-mm screen) were high in protein with high water and oil absorption capacities and quantity of solids lost. Protein may act as both a water-binding agent and surfactant. One might expect protein-rich fractions to exhibit greater oil absorption as well as enhanced emulsification and foamability. This agrees with the results of Ngoddy et al (1986), who recommended that cowpea flour milled for akara has 65–75% of the particles between 45–150  $\mu\text{m}$ .

## Thermal Properties

**Effects of moisture content.** Typical DSC thermal curves for cowpea flour are shown in Fig. 1. This example depicts DSC scans for 1.0-mm mill screen/collection pan fractions. Thermal curves were made at 10, 42, 64, and 91% total moisture. Also shown is the thermal curve for a pure starch extract (Fig. 1A). Cowpea starch at 91% moisture displayed an endothermic peak typical of starch gelatinization and was characterized by  $T_o = 71^\circ\text{C}$ ,  $T_p = 74^\circ\text{C}$ , and  $T_c = 79^\circ\text{C}$ . As calculated from the area bounded by the peak and a continuation of the baseline before and after the peak,  $\Delta H = 9.0 \text{ J/g}$  of dry starch. Based on microscopy studies, El Faki et al (1983) indicated gelatinization temperatures for cowpea starch of  $T_o = 65^\circ\text{C}$  and  $T_c = 75^\circ\text{C}$ . Using DSC, Okechukwu and Rao (1997) show average values of  $T_o = 66^\circ\text{C}$ ,  $T_c = 78^\circ\text{C}$ , and  $\Delta H = 8.5 \text{ J/g}$  for 15% cowpea starch; these values were generally increased by the addition of cowpea protein.

When cowpea flour was heated at 91% moisture content, a single endothermic transition was observed (Fig. 1B) and was similar to the peak for pure starch. Hosney (1984) showed there

is little difference in endotherms of starch and flour in excess water systems. At lower moisture content ( $\leq 64\%$ ), the peaks became progressively smaller, shifted to higher temperatures, and began to show a small secondary peak at  $\approx 83\text{--}85^\circ\text{C}$ . This secondary peak was not seen in pure starch at lower moisture content (Fig. 1A). It is well known (Hosney 1984) that starch needs sufficient moisture ( $>80\%$  water) to fully gelatinize, that peak areas for gelatinization diminish at successively lower moisture contents, and that at very low moisture content gelatinization will not occur. The complexity of the intermediate moisture DSC curves indicates that additional factors are at work. Unlike pure starch, cowpea flour contains 35–53% starch and  $\approx 24\%$  protein. Cowpea protein denatures at  $\approx 72\text{--}86^\circ\text{C}$  (Okechukwu and Rao 1997), which coincides with the secondary peak seen in Fig. 1. For cowpea starch and proteins, Wright and Boulter (1980) found  $T_p = 83^\circ\text{C}$  and  $90\text{--}94^\circ\text{C}$ , respectively. At high moisture content, starch can fully gelatinize; the fact that there is more starch than protein and that the heat of gelatinization is greater than the heat of denaturation ( $\approx 9 \text{ J/g}$  as compared with  $5 \text{ J/g}$ ) results in an endotherm that is dominated by gelatinization. At lower moisture contents, gelatinization is partial at best, while protein denaturation can still occur. Thus, a secondary peak becomes more obvious. Okechukwu and Rao (1997) showed related results for cornstarch and cowpea protein. As the ratio of cowpea protein to cornstarch was increased, the formation of a second peak related to protein denaturation was observed. However, in mixtures of cowpea protein and cowpea starch, increased protein-to-starch ratio caused broadening of the peaks to higher temperature but without formation of a distinct second peak. Sosulski et al (1985) also were unable to observe clear separation of starch and protein peaks in cowpea flour.

The effects of moisture content, mill screen size, and collected particle size on  $T_o$ ,  $T_c - T_o$ ,  $T_p$ , and  $\Delta H$  are summarized in Table V. Data from samples containing 91% moisture are shown because such conditions ensure maximum gelatinization. Moisture contents of 64 and 42% are also shown because they correspond to those found in dough used for snack chip preparation (Ward et al 1995) and fried cowpea paste (McWatters and Brantley 1982), respectively. In general as moisture content was increased, the  $T_o$  and the width of the peak ( $T_c - T_o$ ) decreased while the  $\Delta H$  increased. These findings agree with those of Biliaderis et al (1980), who studied starch from Adzuki bean, smooth pea, and lentil. They determined that with increasing volume fraction of water  $T_c$  decreased,  $\Delta H$  increased, and ( $T_c - T_o$ ) decreased. For wheat starch, Hosney (1984) found that  $T_o$  did not change at higher moisture levels but that the  $T_p$  decreased. Ghiasi et al (1982) found that wheat starch at lower moisture had a higher  $T_c$ , as well as a higher temperature at which birefringence was lost.

**Effect of mill screen.** The effect of mill screen size on the thermal properties of cowpea flour is shown in Tables III and V. The mill screen size was a major effect on  $T_o$  ( $P < 0.01$ ),  $T_c - T_o$ , and  $\Delta H$  ( $P < 0.05$ ). The unsieved flour (2.0-mm mill screen) had the highest  $T_o$  at all moisture contents and generally had the highest  $T_c - T_o$ . However, only the  $T_o$  of unsieved flour at 0.5-mm mill screen and 91% moisture was significantly different from the others ( $P < 0.05$ ). A similar trend was seen for the collection pan fractions at all moisture levels. For example,  $T_o$  values at 91% moisture decreased in the order:  $74^\circ\text{C}$  (2.0-mm screen),  $72^\circ\text{C}$  (1.0-mm screen), and  $70^\circ\text{C}$  (0.5-mm screen) ( $P < 0.05$ ). Gelatinization enthalpies for the 91% moisture samples were significantly lower in all the 0.5-mm mill screen fractions at similar particle size. Presumably, more extensive milling causes greater particle degradation, thus less thermal energy is needed to allow hydration and unfolding of starch molecules.

**Effect of particle size.** Mesh size was also a significant factor for  $T_p$  and  $T_c - T_o$  (Table III). At 91% moisture, there was a general increase in  $T_o$  and  $T_c - T_o$  with increasing particle size (within a given mill screen group) (Table V). A similar trend was observed at 64% moisture but was less significant. Obviously,

thermal properties of flours have a complex dependence on milling conditions and final particle size. Marshall (1992) determined that starch gelatinization can be altered by changing particle size in milled brown rice; decreases in particle size caused a large decrease in  $T_p$ ,  $T_c$ , and  $\Delta H$ , but only small decreases in  $T_o$ . Marshall concluded that gelatinization is affected by the degree of exposure of the starch granules to water; the more accessible granules are to water, the lower the gelatinization temperature. However, for particles <250  $\mu\text{m}$ , increased damage to starch granules may cause lower gelatinization enthalpies.

### Light Scattering and Particle Size

Figure 2 shows plots of volume percent of particles suspended in water over a range of particle diameter as determined by light-scattering analysis. To allow visual clarity, only curves from the 0.5-mm mill screen samples are shown. Critical parameters describing all curves are shown in Table VI, and include particle-size range (sizes with volume percent >0.1), particle size at maximum volume percent, and total volume percent under a given peak. Most samples showed a bimodal distribution of particle size, except for the collection pan fractions, which were unimodal. The lower size distribution was spread within the 1–100  $\mu\text{m}$  particle-size range for each sample, with a peak somewhere between 17 and 21  $\mu\text{m}$ . The larger size distribution occurred in the 100–1,000  $\mu\text{m}$  range, with the actual range and peak varying somewhat with the sample. Most likely, the smaller size particles are suspended starch granules, while the larger particles correspond to undissociated or partially dissociated flour particles. The variability in the distribution and maximum of the second peak support this notion. Larger particles produced curves that had size distributions and maximum values at larger sizes. In addition, the peak maximum occurred at values above the nominal screen size.

The volume of particles associated with each distribution was determined by integration of the peaks. Table VI shows the size range over which peak integration was accomplished. The collection pan fines had first peak (lower size range) volume percents of 100, 98, and 100%, for the 2.0-, 1.0-, and 0.5-mm milled samples, respectively. Whole flours had 72, 85, and 77% of the volume within the lower size peak. In general, lesser amounts were found in the lower peak for the 0.297- and 0.149-mm collected fractions. The 0.297-mm fractions had 71, 30, and 21% volume of particles within the lower peak (for 2.0-, 1.0-, and 0.5-mm milled samples, respectively); the 0.149-mm fractions had 41, 67, and 30% volume of particles within the first peak.

Water absorption was greatest in those particles that were least dispersed. Water absorption is defined in terms of the weight of water absorbed per weight of insoluble material. Two factors are at work. Certainly, water must be able to penetrate in order to be absorbed by flour macromolecules. However, if the particle is disrupted in the process, hydrated solutes may be lost to the supernatant.

### CONCLUSIONS

Milling at different screen sizes, and subsequent separation of flours into different particle-size ranges, had various effects on chemical and physical properties of cowpea flour. With the exception of starch, such procedures caused only small changes in proximate composition. As starch was determined by a procedure in which starch granules were extracted in water, it may be that prior mechanical and different particle sizes allow water to extract starch to different extents. In contrast, milling and separation procedures had a greater effect on the functional properties of cowpea flours. Water absorption, solids lost, and protein solubility were particularly affected, presumably influenced by the ability of water to penetrate flour particles and to either absorb or carry away soluble components. Thermal properties were also affected by different milling conditions. In general, more finely milled flours

had lower initial gelatinization temperatures; this may be an indication that greater shear helps break apart starch granules. Light-scattering analysis showed that different conditions produced different particle-size distributions when samples were suspended in water.

Differences in chemical and functional properties in differently milled flours are expected to influence how the flour performs in food products. For example, akara (fried cowpea paste) may be dry and dense when prepared with flour containing a high percentage of fine particles. Moin-moin (steamed cowpea paste) may be too dense and lack sponginess when prepared from fine flour. The results from this study suggest that milling conditions and particle size influence key properties such as water binding, as well as the type and amount of materials extracted by water. For example, water-binding capacity was lower in finely milled flour, while starch extraction was greater. Such differences may contribute to products that are denser and less able to incorporate air into a spongy structure.

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