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Effect of Germination on the Physical, Chemical, and Sensory Characteristics of Cowpea Products: Flour, Paste, and Akara

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Physicochemical and sensory characteristics of products made from cowpeas that were ungerminated (UN) or germinated at 25 or 30 °C for 24 h (G25 and G30, respectively) were assessed. Nitrogen solubilities of UN, G25, and G30 were similar. Electrophoresis revealed minor changes in proteins due to germination. Pastes of G25 or G30 had higher flow consistency (n) and apparent viscosity and possessed better frying characteristics than paste of UN. Germination increased the hardness, elasticity, gumminess, and chewiness of akara (fried cowpea paste) but did not affect cohesiveness. The derived instrumental color function (ΔH) was higher for G25 and G30 flour and akara than for UN products. Among sensory measurements, germination significantly improved the crust color of akara but slightly reduced the ratings for moistness, tenderness, and flavor. Overall acceptability was not reduced by germination.

Cowpea (*Vigna unguiculata*) flour is the principal ingredient in preparing akara (fried cowpea paste), which is popular in West Africa (Dovlo, 1976). Traditionally, cowpea paste is prepared by a manual process that is time-consuming and labor intensive. Technology for producing a ready-to-use flour, intended to reduce labor and encourage expanded cowpea usage, has been developed and is being implemented (Ngoddy et al., 1986; McWatters et al., 1988). Such flour can be directly hydrated to paste.

Germination, which increases the activity and synthesis of hydrolytic enzymes, has been applied to legume seeds to reduce the levels of flatulence-inducing oligosaccharides (Nnanna and Phillips, 1988). This process may also modify the functionality of protein and starch in food systems, depending upon the duration and temperature of germination (Hsu et al., 1982). It is reasonable to expect that the functional behavior of cowpea flour is controlled by the properties of its polymeric components, protein and starch, and further, that these com-

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ponents may be affected positively or negatively during germination. Proteolysis alters these properties by changing the molecular size, conformation, solubility, and strength of the inter- and intramolecular bonds of the protein molecules (Kinsella, 1976). Previously, germination conditions were optimized to allow maximum reduction of flatulence-inducing oligosaccharides in cowpea while minimizing dry matter loss, microbial contamination, and elaboration of starch- and protein-hydrolyzing enzymes (Nnanna and Phillips, 1988).

Our ultimate goal in this study was to produce a flour from the germinated seed that has low flatulence potential and still possesses the nutritional and functional characteristics necessary for utilization in fried (akara) and steamed (moin-moin) products. To be acceptable in West Africa, cowpea flour and paste made from germinated seeds must perform successfully in preparation of traditional foods. The study reported here was undertaken to evaluate the effect of germination on the physical, chemical, and sensory characteristics of cowpea products: flour, paste, and akara.

MATERIALS AND METHODS

Materials. Cowpeas (*Vigna unguiculata*, cv. California blackeye) were obtained from Grisez Warehouse Co. (Crows Landing, CA) and stored at 4 °C until used. Sodium dodecyl sulfate (SDS), 2-mercaptoethanol (2-ME), acrylamide, *N,N'*-methylenebisacrylamide, ammonium persulfate, tetramethylenediamine (TEMED), tris(hydroxymethyl)aminomethane (Tris), and molecular weight (MW) markers (MW-SDS-200 kit) were obtained from Sigma Chemical Co. Glycine was from Fisher Scientific. All other chemicals were laboratory reagent grade.

Germination and Sample Preparation. Cowpea seeds were soaked in tap water for 9 h at room temperature; 3.6-kg batches of the hydrated seeds were germinated in the dark for 24 h at 25 (G25) or 30 °C (G30). The germinating system was constructed as previously described (Nnanna and Phillips, 1988) but scaled up to accommodate a larger quantity of seeds. The ungerminated (UN) and germinated (G25 and G30) seeds were milled into flour as described by Nnanna and Phillips (1989).

Nitrogen Solubility. The nitrogen pH–solubility profile of a 1% (w/v, protein basis) dispersion of ungerminated (UN) and germinated (G25 and G30) cowpea flour in deionized water was determined according to the method of Hsu et al. (1982). The pH of the sample dispersion was adjusted with either 1 N HCl or 1 N NaOH to values between 2 and 10; the sample was shaken mechanically at room temperature for 1 h and then centrifuged at 10000*g* for 30 min. The supernatant was analyzed for nitrogen by the Kjeldahl method (AOAC, 1980).

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Aliquots of 455 mg of cowpea flour were extracted in centrifuge tubes with 20 mL of 0.2 M Tris-HCl buffer, pH 6.8, containing 2% SDS and 2% S-ME at room temperature for 2 h to give a protein concentration of Ca. 5 mg/mL. The supernatant was collected after centrifugation at 10000*g* for 20 min. SDS-PAGE was performed essentially as described by Laemmli (1970) and Davis (1964). Sample aliquots of 50 and 70 μL were applied on top of the gels. Electrophoresis was carried out on a Bio-Rad protein dual-slab cell (Bio-Rad Laboratories, Richmond, CA) operated initially at 20 mA/gel slab and then at 30 mA/gel slab for 6 h during stacking and separation, respectively. Myosin (subunit molecular mass 205 kDa), β-galactosidase (subunit molecular mass 116 kDa), phosphorylase b (subunit molecular mass 97 kDa), bovine serum albumin (molecular mass 66 kDa), ovalbumin (molecular mass 45 kDa), and carbonic anhydrase (molecular mass 29 kDa) were used as marker proteins. After electrophoresis, gels were fixed in 50% trichloroacetic acid, stained with 0.05% Coomassie Brilliant Blue R in methanol/acetic acid/water (5:1:4 (v/v)), and destained in methanol/acetic acid/water (5:7:88 (v/v)). Gels were scanned with a Bio-Rad Model 1650 scanning densitometer.

Cowpea Paste Preparation and Evaluation. Paste was prepared by adding sufficient water to 200 g of cowpea flour to adjust the moisture content to 58%. The paste was then whipped in an N-50 Hobart mixer at speed 3 for 1.5 min (McWatters, 1983).

Foam volume of the whipped paste was measured with a graduated cylinder. Specific gravity of the foam was determined by the method of Campbell et al. (1979).

Flow characteristics of the whipped paste were determined at 23 ± 1 °C with a digital Brookfield Model HATD viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA). Measurements were made at 0.5, 1, 2.5, 5, 10, and 20 rpm using a cylindrical spindle HA-27. Attempted readings at 50 and 100 rpm were off scale. The rpm values and the viscometer readings were converted to shear rate (s^{-1}) and shear stress by multiplying by 0.34 and 1.7, respectively. These factors were provided by the viscometer manufacturer. Paste apparent viscosity at a given shear rate was calculated as the ratio of shear stress to shear rate. The indices of flow consistency (b) and flow behavior (n) were calculated as described by Chinnan et al. (1985).

Akara Preparation. The formulation, preparation procedure, and frying conditions previously reported by McWatters (1983) were adopted in preparing the akara samples.

Objective Measurements. The textural quality of akara was determined with a Kramer shear compression cell (McWatters, 1983) and with a flat-plate cell (texture profile analysis, Friedman et al., 1963). Each cell was fitted to the Model 1122 Instron universal testing machine (Instron Inc., Canton, MA). A single akara ball was compressed and sheared in the Kramer shear cell at a crosshead speed of 50 mm/min, a chart speed of 200 mm/min, and a full-scale setting of 50 kg. A typical force–deformation curve is described elsewhere (Hung et al., 1987). Force ratio is the ratio of first to second peak force. Shear energy is defined as the area under the force–deformation curve up to a second maximum (Hung et al., 1987).

The texture profile analysis (TPA) parameters (hardness, cohesiveness, elasticity, gumminess, and chewiness), previously defined by Friedman et al. (1963), were derived from force–deformation curves obtained with the flat-plate compression cell. For each measurement, a 1-cm akara cube (crust removed) was compressed twice to 25% of its original height (75% compression) in a reciprocating motion by the Instron instrument (Hung et al., 1987). The flat-plate compression test was operated at crosshead and chart speeds of 50 and 200 mm/min, respectively, and a full-scale setting of 5 kg.

Hunter color values L, a, and b of the ungerminated and germinated akara samples were obtained with a Gardner XL-845 colorimeter standardized against a yellow tile (L = 78.3, a = 1.94, b = 23.5). Surface color values of akara samples (top and bottom sides) were determined by using the reference standard. Four readings per treatment replication were determined. The saturation index (ΔE) and hue difference (ΔH) expressing the color difference between the sample and the standard were derived from the L, a, and b values (McWatters and Chinnan, 1985).

The moisture content of akara was determined by vacuum drying 5-g ground samples (14 mesh) at 70 °C for 24 h. Crude fat content was determined on moisture-free samples extracted for 24 h with Skelly F in a Goldfisch apparatus.

Sensory Evaluation. A 10-member panel, previously familiarized with the organoleptic characteristics of akara and the scoring guidelines, participated in the test. Four-point rating scales were developed (McWatters et al., 1988) for the attributes of shape, exterior color uniformity, sponginess, moistness, tenderness, and flavor. The test was done in triplicate. One replicate comprised of a control (UN), G25, and G30 was evaluated per day. Samples were prepared in the morning and evaluated at midafternoon. Samples were arranged in random order on oven-proof white plates, heated at 204 °C for 4 min in a conventional oven, and evaluated while warm in individual booths equipped with incandescent lighting.

Statistical Analyses. Linear regression analysis was used in estimating the flow consistency and flow behavior indices of whipped cowpea paste; other data were analyzed for analysis of variance (ANOVA) and Duncan's multiple range test. All analyses were according to the procedures of Statistical Analysis System (SAS, 1985).

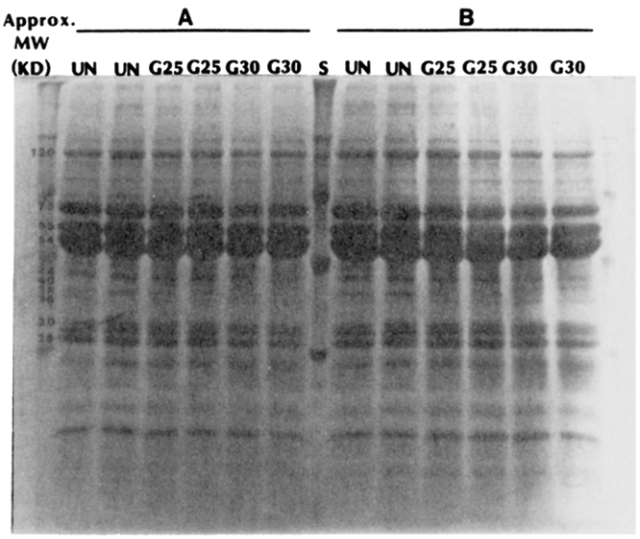

Figure 1. SDS-PAGE patterns of ungerminated (UN) and germinated (G25 and G30) cowpea flour proteins: sample duplicate loading of 50 (A) and 79 μL (B). Gels of 12% polyacrylamide run in the presence of 2-ME. S = standard marker proteins.

RESULTS AND DISCUSSION

Nitrogen Solubility. One important measure of the functional utility of a protein is its solubility in an aqueous environment. Generally, a pH–solubility profile is determined as the proportion of nitrogen that is soluble at pH values from 2 to 10. Such solubility profile measurement provides a good index by which several other functional characteristics may be predicted. The solubility profile of UN was not significantly different ($P \leq 0.05$) from that of G25 or G30. As in most legumes, the pH of minimum solubility occurred between pH 4 and 5, and nitrogen solubility increased toward the acidic and basic sides. Mean solubilities of 81.9, 84.2, and 88.1% corresponding to UN, G25, and G30 were observed at their natural pH values, 6.6, 6.7, and 6.7, respectively. The pH–solubility values reported here for ungerminated cowpea protein are in close agreement with values reported by Sosulski et al. (1987) but somewhat higher at high pH values than those of Schaffner and Beuchat (1986). These variations exist because protein solubility values are dependent on the measurement conditions, sample preparation, and sample history.

G25 and G30 showed somewhat higher solubility (92.2–99.1%) in the alkaline region compared to UN (82.4%). The improved solubility in the alkaline region may have resulted from changes in protein molecular species during germination. A similar increase in solubility above pH 6 has been reported for germinated faba bean (Hsu et al., 1982).

SDS-PAGE. Khan et al. (1980) extensively characterized the major cowpea seed proteins by a two-dimensional SDS-PAGE with nonreducing conditions in the first dimension and reducing conditions in the second dimension. These authors showed that the major albumin protein was a monomer of molecular mass 105 kDa and that the major globulin protein consisted of two components, one composed of three subunits of molecular masses 52, 58, and 63 kDa and the other containing 52 and 58 kDa molecular mass subunits.

Electrophoretic patterns of cowpea proteins with subunit molecular masses ranging from 14 to 193 kDa are presented in Figure 1. PAGE revealed only minor differences between protein subunits from UN and G25/G30.

Table I. Apparent Viscosity[a] and Corresponding Shear Rates of Whipped Paste Made from Ungerminated (UN) and Germinated (G25 and G30) Cowpeas

	app viscosity, Pa·s		
shear rate, s^{-1}	UN	G25	G30
0.17	51.1 (3.29)[b]	78.8 (5.73)	73.2 (6.95)
0.34	33.9 (1.37)	51.9 (3.60)	46.8 (3.16)
0.85	23.5 (1.00)	34.0 (1.86)	30.6 (1.37)
1.70	19.2 (0.70)	27.2 (1.25)	24.9 (1.17)
3.40	14.3 (0.30)	20.9 (0.87)	20.0 (0.76)
6.80	9.8 (0.23)	14.4 (0.68)	14.4 (0.69)

[a] Calculated from the equation $\eta_a = b\gamma^{n-1}$, where η_a = apparent viscosity, b = flow consistency index, γ = shear rate, and n = flow behavior index. [b] Numbers in parentheses refer to standard error of means.

The major bands at molecular masses 120, 75, 65, and 54 kDa correspond to the major albumin (~100 kDa) and globulin subunits (63, 56, and 52.5 kDa) identified by Murray et al. (1983) and Khan et al. (1980). Bands at 44 and 38 kDa diminished upon germination and were replaced by bands at 40 and 36 kDa. This change was more pronounced for G30 than for G25. Murray et al. (1983) have identified the bands in this region as albumin components. Additionally, the two largest of three intermediate-intensity subunits (30–26 kDa) became more diffuse and merged together in gels of seeds germinated at 30 °C. This region has been shown to be composed of both albumin and globulin subunits by Murray et al. (1983).

Cowpea Paste Evaluation. McWatters and Chinnan (1985) found that the level of water used to hydrate cowpea flour had a greater effect on physical characteristics of paste and akara than hydration time. A water level of 58% was adopted in this study. Foam volume and specific gravity are good indicators of the dispensing and frying characteristics of the whipped paste in the preparation of akara (Cherry and McWatters, 1981). Here, we found that the foam volume of the UN paste (733 mL) differed somewhat from those of G25 (690 mL) and G30 (630 mL). The difference in foaming behavior between the ungerminated and germinated cowpea paste may be attributed to the small changes in native albumins (Figure 1) discussed earlier since these are the components most likely to be involved in functional expression in foams (Cherry and McWatters, 1981).

Differences were also observed in the pastes' flow consistency and apparent viscosity (Table I), which are parameters that also govern the quality characteristics of akara (Phillips et al., 1988). The flow consistency index (b) of the UN paste (22.86) differed from those of the germinated samples (G25 = 33.88; G30 = 31.41), whereas the flow behaviors (n) were relatively similar (0.57, 0.56, and 0.58, respectively). It was also observed that the germinated treatment produced pastes of better dispensing/frying characteristics than the ungerminated treatment. This implies that b is a better index than n in predicting paste characteristics, a view consistent with an earlier observation by McWatters and Brantley (1982) that proper paste consistency was essential for ease of dispensing, shape retention during frying, and acceptable end product quality. Apparent viscosity (η) and corresponding shear rates of whipped paste are given in Table I. Paste from the ungerminated sample exhibited a lower apparent viscosity profile at each shear rate than germinated counterparts. These differences further suggest a modification of cowpea protein during germination even though the electrophoretic pattern revealed only minor

Table II. Objective Textural Characteristics of Akara Prepared from Ungerminated (UN) and Germinated (G25 and G30) Cowpea Paste[a]

	textural characteristics							
	Kramer shear cell			flat-plate compression test[b]				
treatment	akara wt, g	force ratio	energy/wt, 10^{-3} J/g	hardness	elasticity	cohesiveness	gumminess	chewiness
UN	10.0	1.09	101.3 b	19.1 b	17.9 b	0.5	8.7 b	155.9 b
G25	9.6	1.10	111.5 a	26.6 a	22.2 a	0.5	13.7 a	303.2 a
G30	10.2	1.14	117.2 a	29.7 a	22.1 a	0.5	13.9 a	308.1 a

[a] Means calculated from 12 replicated measurements. Values in a column with different letters differ significantly ($P \leq 0.05$). [b] Textural parameters were obtained by using the texture profile analysis method.

Table III. Hunter Color Values, Moisture, and Oil Composition of Akara Prepared from Ungerminated (UN) and Germinated (G25 and G30) Cowpeas[a]

	Hunter color values[b]						
treatment	L	a	b	ΔE	ΔH	moisture, %	crude fat, dry basis
UN	38.9	3.8 b	16.3 b	40.4	13.1 b	47.8	25.9
G25	37.3	5.4 a	16.5 a, b	42.3	14.3 a	46.2	26.5
G30	37.8	5.7 a	16.6 a	42.5	14.7 a	47.0	26.0

[a] Values in a column with different letters differ significantly ($P \leq 0.05$). [b] Saturation index (ΔE) and hue difference (ΔH) were calculated from the L, a, and b values as follows: $E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$; $\Delta H = [(\Delta E)^2 - (\Delta L)^2 - (\Delta C)^2]^{1/2}$, where c refers to chroma and the prefix Δ refers to the difference between sample and reference standard.

changes (Figure 1). Viscosity is a useful index of structural changes in proteins (Kinsella, 1976).

There is no doubt that the differences in foaming properties, flow consistency, and apparent viscosity are largely due to protein modification during germination since data (Nnanna and Phillips, 1988) from amylase activity suggest negligible action on starch during the first 24 h of germination. Furthermore, the embryo utilizes oligosaccharide instead of starch as a source of energy during the developing stage. Therefore, starch fraction contributed very little, if any, to the differences in paste characteristics.

Objective Measurements. Data for objective texture characteristics are presented in Table II. All of the textural characteristics (hardness, elasticity, gumminess, and chewiness) obtained with the flat-plate compression cell except cohesiveness were increased significantly ($P \leq 0.05$) by germination (Table II). For all the samples, shear energy and gumminess correlated well, $r = 0.99$ ($P \leq 0.05$), with sensory score for tenderness.

The exterior color of akara samples prepared from the various flours had similar ΔL and ΔE values (Table III). The degree of redness and ΔH were the same for G25 and G30 but were greater ($P \leq 0.05$) than for UN.

Akara from UN did not differ significantly ($P \leq 0.05$) from that from G25 or G30 in moisture and crude fat contents (Table III). Since none of the treatments produced akara samples having thick crusts, one would expect the rate of moisture loss from the interior during frying to be the same. A linear relationship between moisture content of paste and oil uptake of akla (akara) during frying has been reported by Osei-Yaw and Powers (1986). Therefore, the similarity in crude fat content of UN, G25, and G30 may be attributed to the equal water levels and perhaps the specific gravities (0.57, 0.59, and 0.59, respectively) of their pastes.

Sensory Evaluation. Sensory attributes of akara are given in Table IV. Among sensory measurements, germination significantly ($P \leq 0.05$) improved the crust color of akara but reduced slightly the ratings for moistness, tenderness, and flavor. Panelists found the surface browning of akara prepared from germinated samples to be significantly ($P \leq 0.05$) more appealing than the pale brown color of akara from the ungerminated sample. Some panelists perceived the moistness and tenderness attributes of germinated samples as being drier and heavier than those of UN. This is not surprising considering the higher apparent viscosity of the germinated samples. The flavor was described by some as slightly feedlike, savory sweet, or slightly stale but generally not objectionable. There were no significant differences in scores for shape and sponginess. Overall, paste from UN, G35, and G30 produces akara samples that were acceptable organoleptically, rounded in shape with small air cells.

Table IV. Comparison of Sensory Ratings[a] of Akara Prepared from Ungerminated (UN) and Germinated (G25 and G30) Cowpeas

treatment	shape	exterior color	sponginess	moistness	tenderness	flavor
UN	3.3	2.7 b	3.0	2.9 a	3.6 a	3.5 a
G25	3.2	3.4 a	2.7	2.3 b	3.2 b	2.6 b
G30	3.1	3.2 a	2.7	2.3 b	3.2 b	2.9 b

[a] Values in a column with different letters differ significantly ($P \leq 0.05$). The following scoring scales were used. Shape: 4 = uniform, 3 = reasonably uniform, 2 = slightly distorted, 1 = very distorted. Exterior color: 4 = uniform, 3 = reasonably uniform, 2 = slightly uneven, 1 = uneven browning. Sponginess: 4 = very spongy, 3 = spongy, 2 = moderately spongy, 1 = slightly spongy. Moistness: 4 = very moist, 3 = moist, 2 = moderately moist, 1 = slightly moist. Tenderness: 4 = tender, 3 = moderately tender, 2 = moderately tough, 1 = tough. Flavor: 4 = good, 3 = reasonably typical of akara, 2 = slightly off, 1 = severely off.

This work has demonstrated that cowpea seeds, germinated under conditions that significantly reduced the oligosaccharide that contributes to flatulence potential (Nnanna and Phillips, 1988, 1989), retained the essential functional characteristics necessary for making akara. Therefore, a technology that integrates germination with a milling process would provide cowpeas not only with improved nutritional appeal but also good functional properties that may be exploited in other food formulations.

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