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Chemical and sensory evaluation of germinated cowpeas (*Vigna unguiculata*) and their products

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Abstract

Cowpeas (*Vigna unguiculata*), germinated for 24, 48, 72, or 96 h or ungerminated, were dehulled and milled. The resultant flour was analysed for oligosaccharide and anti-nutritional factors and subsequently used to prepare three recipes, viz., *akara*, *moin-moin* and cake. The recipes were evaluated by panellists for acceptability based variously on size of product, texture, colour, crust/crumb tenderness and flavour. Trypsin inhibitor was reduced from 12.4 ± 0.15 to 10.2 ± 0.06 TIU/mg; and haemagglutinin from 1:200 to 1:25. Stachyose disappeared from all germinated products and was replaced by sucrose. Apart from the moisture and carbohydrate, which decreased, the proximate composition of the cowpea flour improved with germination. Although recipes prepared from ungerminated and traditionally processed cowpea showed superior organoleptic properties (7.6 acceptability level), the products of the 24-h-germinated cowpea were satisfactory (6.6 acceptability). Therefore, 24-h germination is considered a worthwhile processing because it totally eliminates stachyose, which otherwise would induce flatulence, and reduces anti-nutritional factors to tolerable levels without a significant change in acceptability.

Keywords: Cowpea; Germinated; Recipes; Sensory evaluation

1. Introduction

Legumes are major components of human diet in many developing countries. Cowpea (*Vigna unguiculata*), in particular, contributes about 80% of the total protein intake in certain parts of Nigeria. Dehulled, wet-milled preparations of cowpea can be deep-fried in oil to obtain *akara* or steamed to obtain *moin-moin*. Whole cowpea can be cooked with spices and eaten singly or in combination with cereals such as rice (*Oryza sativa*), sorghum (*Sorghum* spp.) or corn (*Zea mays*). The latter combination recipes are being promoted because of the complementary value of cereal and legume proteins. Recently, recipes have been developed which use cowpea flour for non-traditional foods such as cakes, biscuits, pancakes and even weaning foods (Ngoddy, Enwere & Onuorah, 1986).

In spite of the high protein content (20–30%) of cowpea, it has not been widely applied in geriatric and infant feeding. The constraint has been the presence, in the cowpea, of indigestible oligosaccharide, particularly raffinose and stachyose, which induce flatulence (Flemming, 1981; Sosulski, Elkowiez & Reichert, 1989). In addition to these, anti-nutritional factors, such as trypsin and chemotrypsin inhibitors and haemagglutinins, have been detected (Liener, 1979; Subbulakshmi, Ganeshkumar & Venkataraman, 1976). Any processing methods to reduce these undesirable components would facilitate adoption and acceptance of cowpea-based recipes as weaning foods and in geriatric feeding. A number of processing methods have already been tried and shown to reduce the levels of both anti-nutritional factors and oligosacharides (Batra, 1987; Jaya & Venkataraman, 1981; Silva & Luh, 1979). The purpose of this work was to investigate the effect of germination on the chemical composition of cowpea as well as to evaluate comparatively the effect on the organoleptic properties of cowpea-based traditional and recently developed recipes.

2. Materials and methods

2.1. Processing of cowpea

The cowpeas (*V. unguiculata*) were purchased from the local market. Wholesome seeds of similar sizes were

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selected, soaked in clean tap water for 12 h at room temperature (≅27°C) and then divided into five equal portions (by weight). Four portions were spread each on a separate clean jute bag and left for 24, 48, 72, or 96 h, to germinate. One portion, the control, was not germinated. During germination, the seeds were rinsed at 24 h intervals in running tap water for 10 min, to reduce fungal contamination. The proportion of seeds which germinated or spoiled in the process of germination was noted. All germinated seeds, as well as water-soaked control seeds, were dehulled, dried and hammer-milled in three passes. The resultant flour was sifted through a 40-mesh sieve, packed in polythene bags and stored at room temperature until used. Traditionally wet-milled paste from non-germinated cowpea was also processed and included as an additional control sample.

2.2. Chemical analysis of samples

2.2.1. Protein, fat, fibre, carbohydrate, moisture and ash

Each sample, including the controls, was analysed for crude protein, crude fat, fibre, carbohydrate, moisture and ash, according to AOAC (1980) methods.

The crude protein content was determined using the improved Kjedahl method for nitrate-free samples (AOAC, No. 2.057). Approximately 2.0 g of sample was digested with 25 ml concentrated H_2SO_4 in a digestion tank in the presence of 0.7 g HgO, and anhydrous Na_2SO_4. A small amount of paraffin was added to reduce frothing. Samples were boiled until the solution became clear and $\geqslant$30 min beyond. To the digest, cooled to $<$25°C, was added 200 ml of water before 25 ml of sodium thiosulphate was added to precipitate Hg. Sodium hydroxide solution was added until the reaction mixture became strongly alkaline. Then the contents were distilled and the NH_3 distillate received in a flask with the tip of the condenser immersed in standard HCl. Excess HCl in the distillate was titrated with standard NaOH solution. The percent nitrogen was calculated according to AOAC 2.057 (1980) and total protein calculated by amplifying result with a factor of 6.25 (AOAC 14.068, 1980).

To determine crude fat content, 2 g of sample on Whatman No. 1 filter paper in a funnel was first extracted with five 20-ml portions of water prior to drying; and ether-extracted and analysed for fat according to AOAC 7.056 (1980).

Moisture content was determined by drying 2 g of each sample contained in a weighed porcelain dish with lid in a hot air oven at 100°C until constant weight was obtained. The covered porcelain dish used had an opening for ventilation.

Ash was determined by the AOAC (1980) method, 14.006. Approximately 4.0 g of sample in a weighed shallow, wide ashing dish was ignited to a dull red heat at 550°C in a furnace until light grey ash resulted. The dish and contents were re-weighed soon after cooling to room temperature in a desiccator containing re-ignited CaO as drying agent.

Carbohydrate content was calculated as the difference in weight between dry sample (after all moisture has been evaporated to constant weight) and the ash.

Crude fibre was determined after extracting 2 g of sample with petroleum ether by the AOAC (1980) method, 7.065, in the presence of asbestos. Crude fibre was taken as the loss in weight on ignition expressed as a percentage of the weight of the initial sample.

2.2.2. Trypsin inhibitor and haemagglutination

Trypsin inhibitor activity was determined by the method of Kakade, Rackis, McGhee, and Puski (1974). Extraction of the flour sample (5 g) for 2 h at room temperature was in 0.5 M Tris-HCl buffer (pH 8.2) supplemented with 0.5 M NaCl. Bovine pancreatic trypsin (BDL) was used at initial concentration of 2% (w/v) in 0.001 M HCl. The substrate was 40 g of *N*-benzoyl-DL-arginine-*p*-nitroaniline, BAPNA (Sigma), dissolved in 1 ml of dimethyl sulfoxide, DMSO (Sigma) and diluted to 100 ml with Tris-buffer at 37°C in a water bath.

Haemagglutinin activity was determined using sheep red blood cells (srbc). Approximately 1 g of sample was soaked in 25 ml of phosphate-buffered saline (PBS) for 6 h and then filtered through Whatman no. 1 paper. The filtrate was diluted in 2-fold series in a 96-well microtitre plate (Sterilin), after which an equivalent volume of 1% srbe was added. Filtered PBS, without any sample, was included as a negative control. The experimental sample was incubated at 37°C for 1 h and the last dilution showing agglutination was taken as the haemagglutination (HA) titre.

2.2.3. Oligosaccharides

Oligosaccharide constituents were qualitatively detected by the thin-layer chromatographic (TLC) method described by Akpapunam and Markakis (1979). Sucrose, raffinose and stachyose (East Anglia Chemicals, Suffolk) were included as standards. The chromatographic solvent consisted of a mixture of ethyl acetate (65 ml), butanol (15 ml), water (15 ml) and acetic acid (20 ml). The plate was stained by spraying with a solution of aniline (6.938 g) and phenolphthalein (1.66 g) in 100 ml of water-saturated *n*-butanol.

2.3. Preparation of akara, moin-moin and cake

The recipes for *akara* and *moin-moin* are shown in Table 1, and for cake in Table 2. The procedures for preparation of the three are outlined in Figs. 1 and 2, respectively.

The control *akara*, *moin-moin* and cup cakes were prepared with standard recipes and procedures.

Table 1
Recipe for *akara* and *moin-moin*

Ingredients	Food	
	Akara	*Moin-moin*
Cowpea flour	50.0 g	50.0 g
Red pepper	5.0 g	5.0 g
Onions	5.0 g	5.0 g
Maggi seasoning	–	0.5 g
Water	75 ml	150 ml
Oil	1 l[a]	18 ml

[a] required for deep oil frying.

Table 2
Recipe for cake

Ingredients	Amount
Fine cowpea flour	192 g
Fine corn flour	200 g
Margarine	200 g
Sugar	200 g
Baking powder	18 g
Salt	0.5 g
Eggs	50 g
Lemon	2 g
Water	100 ml

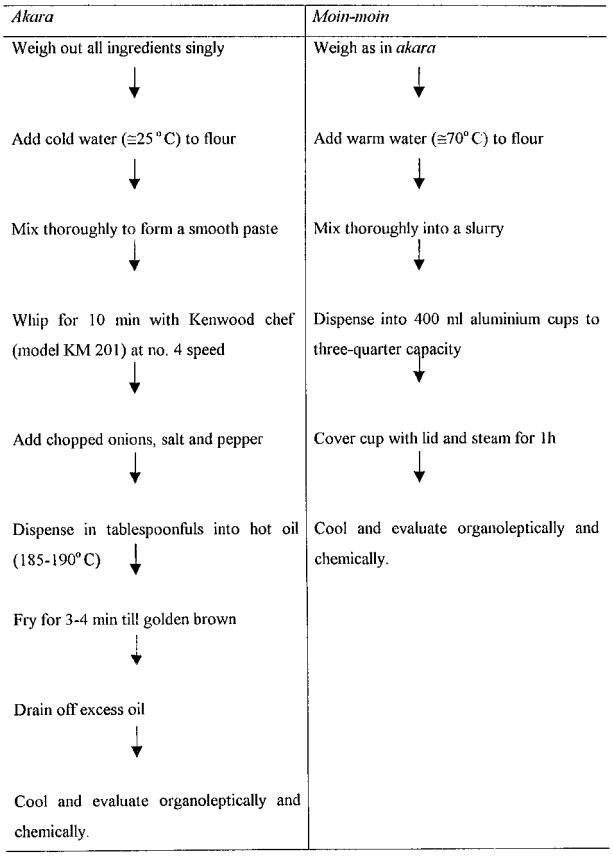

Fig. 1. Flow diagram for preparation of *akara* and *moin-moin*.

3. Sensory evaluation

The *akara*, *moin-moin* and cake were evaluated organoleptically by 50 untrained panellists using a nine-point hedonic scale. Scores were awarded for colour, texture, flavour and overall acceptability of the preparations. In addition, shape, crust and/or crumb tenderness were scored for *akara* and cake, and size and distribution of air cells for *akara*.

4. Statistical analysis

Analysis of Variance (ANOVA) was applied in comparing the levels of acceptability of products from ungerminated cowpeas and cowpeas germinated for varying lengths of time. Duncans New Multiple Range Test (Steele & Torrie, 1960) was used to identify means with significant differences.

5. Results

Maximum germination was achieved at 96 h, 96% of the seeds being already germinated at 24 h. Spoilage set in within 48 h and increased to 16.9% by 96 h (Table 3). Germination resulted in decreased anti-nutritional factors — trypsin inhibitor from 12.4 ± 0.15 to 10.2 ± 0.06 and haemagglutinin from 1:200 to 1:25 — in 96 h (Table 4). The proximate composition of flours prepared from cowpeas following 0–96 h germination generally increased in comparison with ungerminated seeds, except for moisture and carbohydrate, the latter rather decreasing with germination time (Table 5). Fat, protein and crude fibre apparently increased with germination time. Sucrose and stachyose were the two oligosaccharides detected. Sucrose was not present in the ungerminated control and CG-0 which was soaked for 12 h but was detected in germinated samples. Conversely, stachyose was detected in CG-0 but not in the germinated samples (Table 6).

Germination variously affected the organoleptic properties of *akara*, *moin-moin* and cake prepared from cowpea samples. For *akara* (Table 7), the colour of the ungerminated wet-milled sample (CAM) was most accepted. The differences in mean scores for the organoleptic evaluation of CG-0, and CG-24 were similar ($P > 0.05$). The colour of the CG-96 sample was the least acceptable. Germination did not adversely affect the shape of the *akara*, but for sponginess, all germinated samples, except CG-72, scored better (though not significantly, $P > 0.05$) than CWM. While CWM had finer and more evenly distributed air cells than other samples,

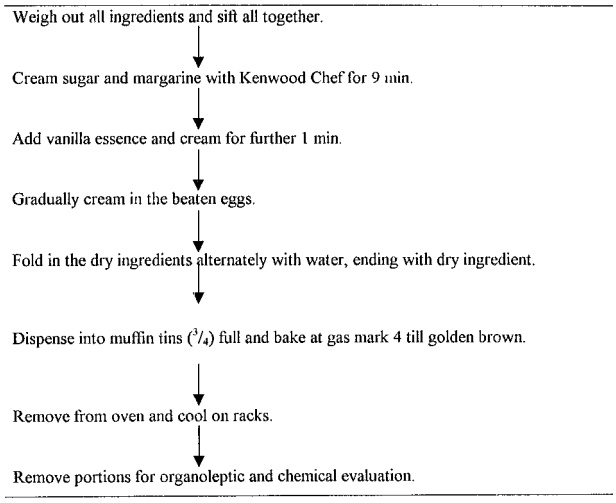

Fig. 2. Flow diagram for cake making.

Table 3
Percentage germination and spoilage of cowpea seeds at various time intervals

Germination time (h)	Percent germination	Percent spoilage
	$(\overline{X} \pm \sigma^{-1})$	$(\overline{X} \pm \sigma^{-1})$
0	0.00	0.00
24	96.3 ± 0.12	0.00
48	98.7 ± 0.15	3.9 ± 0.30
72	99.3 ± 0.15	7.7 ± 0.70
96	99.6 ± 0.53	16.9 ± 0.17

Table 4
Changes in trypsin inhibitor and haemagglutinin activity during germination

Samples[a]	Trypsin inhibitor activity (TIU[a]/mg)	Haemagglutinin activity (titre)
CG-0	12.4 ± 0.15	1:200
CG-24	11.2 ± 0.21	1:25
CG-48	10.5 ± 0.10	1:25
CG-72	10.3 ± 0.17	1:25
CG-96	10.2 ± 0.06	1:25

[a] Ungenninated cowpea (CG-0), cowpea genninated for 24 h (CG-24), 48 h (CG-48), 72 h (CG-72) and 96 h (CG-96).

CG-96 had more tender crust than all others including CWM ($P > 0.05$). For flavour, CWM was judged superior. CG-0 and CG-24 were comparable and the latter two significantly better ($P < 0.05$) than CG-48, CG-72 and CG-96. The overall acceptability decreased with germination time.

The colour, flavour and overall acceptability of *moin-moin* also declined with germination time (Table 8). The texture remained similar in CWM, CG-0 and the germinated samples. In the case of the cakes (Table 9), WF, CUG and CG-0 were similar in colour and ranked higher than cakes from germinated samples. Cakes from WF, CUG, CG-0 and CG-24 were more tender and more spongy than others. The flavours of WF, CUG, CG-0, CG-24 and CG-48 showed no significant differences in acceptance ($P > 0.05$) but were significantly better than CG-72 and CG-96 ($P < 0.05$). Overall WF, CUG, CG-0 and CG-24 were significantly more acceptable than preparations from samples germinated for over 24 h.

6. Discussion

Changes in the proximate composition of the germinated seeds are expected physiological effects of germination (Jaya & Vankataranam, 1981; Ologhogbo & Fetuga, 1986). By some of these changes, the factors limiting the application of cowpea in geriatric and infant feeding were apparently reduced even within 24 h of germination.

Amino acids are needed for growth by the sprouting shoots of the cowpea seed and would be obtained by hydrolysis of native proteins including trypsin inhibitors and haemagglutinin. The reduction of the latter two components which occurred in this work had been observed by Gupta and Wagle (1980) and Sathe, Dashpande, Reddy, Gell and Salunkhe (1983). The apparent increase in the total protein with increase in germination time, which was not observed by Obizoba and Egbuna (1992), is not easily understood but confers nutritional advantage on the germinated product. The decrease in trypsin inhibitor and haemagglutinin and the disappearance of the oligosaccharides invariably improve the digestibility and assimilation of cowpea.

Table 5
Proximate composition (%) of cowpea flours

Samples[a]	Moisture	Fat	Protein	Crude fibre	Ash	Carbohydrate
CG-0	15.6 ± 0.11	3.5 ± 0.1	24.2 ± 0.36	1.3 ± 2.0	1.3 ± 0.17	50.3 ± 0.36
CG-24	17.6 ± 0.63	4.0 ± 0.21	25.0 ± 0.1	1.5 ± 0.10	8.0 ± 0.17	44.2 ± 0.15
CG-48	19.50 ± 0.20	6.5 ± 0.30	27.1 ± 0.1	1.8 ± 0.1	6.8 ± 0.31	28.1 ± 0.17
CG-72	20.30 ± 0.30	7.6 ± 0.18	31.5 ± 0.18	1.8 ± 0.40	5.5 ± 0.26	33.1 ± 0.25
CG-96	18.2 ± 0.10	5.5 ± 0.20	32.4 ± 0.17	6.5 ± 0.26	7.0 ± 0.06	30.0 ± 1.05

[a] Ungerminated cowpea (CG-0), cowpea germinated for 24 h (CG-24), 48 h (CG-48), 72 h (CG-72) and 96 h (CG-96).

Table 6
Changes in the oligosaccharide content of cowpea during germination[a]

Oligosaccharide	RF	CG-0	CG-24	CG-48	CG-72	CG-96
Sucrose	2.4/13	−	+	+	+	+
Stachyose	1.4/13	+	−	−	−	−

[a] Present, −not present.

Table 7
Mean scores for organoleptic evaluation of *akara* prepared from the different flour samples and CWM[a]

Qualitative characteristics	CWM	CG-0	CG-24	CG-48	CG-72	CG-96
Colour	8.0a[b]	7.3a	6.8a	4.7b	4.0b	3.0c
Shape	7.3a	7.2a	7.0a	6.0b	6.5b	7.0a
Sponginess	6.2ab	6.4a	6.4a	6.3ab	6.4a	6.6a
Air cell	6.9a	5.3b	4.7b	5.1b	5.5b	4.4b
Crust						
Tenderness	5.6b	6.2b	6.3b	6.2b	6.2b	7.4a
Flavour	7.9a	7.1b	6.8b	3.9c	3.1d	2.5d
Overall						
Acceptability	7.6a	6.9ab	6.6b	3.8c	2.9d	2.6d

[a] CWM = wet-milled (non-germinated) cowpea.
[b] a–d = mean values in the same row with similar letter(s) are not significantly different at 5% level ($P > 0.05$).

Table 8
Mean scores for organoleptic evaluation of *moin-moin* samples

Qualitative characteristics	CWM[a]	CG-0	CG-24	CG-48	CG-72	CG-96
Colour	7.9a[b]	7.1b	6.8b	3.9c	3.9c	2.5d
Texture	6.2a	6.2a	6.1a	6.2a	5.6a	5.6a
Flavour	8.0a	7.3a	6.8a	4.7b	4.0b	3.0c
Overall						
Acceptability	7.9a	6.9b	6.8b	4.5c	3.7cd	2.9d

[a] CWM = wet-milled (non-germinated) cowpea.
[b] a–d = Mean values in the same row with similar letter(s) are not significantly different at 5% level ($P > 0.05$).

Table 9
Mean score of organoleptic evaluation of cake samples[a]

Qualitative characteristics	WF	CUG	CG-0	CG-24	CG-48	CG-72	CG-96
Colour	8.2a[b]	8.1a	7.5a	7.1a	5.9b	5.1c	4.5c
Shape	8.0a	7.8a	7.4a	7.8a	7.5a	7.5a	7.6a
Crust tenderness	7.7a	7.3a	7.1ab	7.2b	6.2c	5.8c	5.5c
Crumb							
Tenderness	7.5a	7.3a	7.2ab	7.1b	6.3c	6.0c	6.1c
Flavour	8.2a	8.3a	6.9ab	7.4a	6.9ab	6.1b	5.7b
Overall							
Acceptability	8.1a	7.1ab	6.9b	7.3a	6.1cd	5.4d	5.3d

[a] WF = Wheat flour; CUG = Undehulled germinated cowpea.
[b] a–d = Mean values in the same row with similar letter(s) are not significantly different.

In spite of these desirable changes, germination tended to decrease flavour and overall acceptability of the cowpea products. Germination would generally lead to hydrolysis of the protein components with resultant appearance of amino acids as well as increased sugar content from hydrolysis of starch and all these would give a more intense Maillard reaction, which invariably produces a deterioration in colour.

Akara of desirable quality should be soft, spongy, moist and non-greasy. Sponginess and crust and crumb tenderness are influenced by solubility of proteins in the products as well as reactions among ingredients in the food. These qualities were favourably affected by germination in *akara* which is a highly spongy food but not in cake and *moin-moin*. Thus, while sponginess may elicit higher scores in *akara* it would lower the acceptability of cake and *moin-moin*. Changes in macromolecules during germination may also affect their reaction with other ingredients in the recipe (Lowe, 1966).

Fermentation during germination could account, at least in part, for the deterioration in flavour and overall acceptability. There are products in which fermented flavour are desirable but *akara*, cake and *moin-moin* are not included. Moreover, as the cowpeas approach spoilage stage, deteriorative changes may affect the flavour. However, it should be observed that although their flavour and overall acceptability scores were lower than those of products derived from CWM, WF, CUG and CG-0, they were significantly high (Table 6) to be considered satisfactory. The advantages conferred on products by germination for 24 h are sufficiently high to recommend them for infant and geriatric feeding. This time was not long enough to allow appreciable spoilage to occur or progress to an organoleptically noticeable degree. Jodd, Mehta and Singh (1986) had earlier observed that a 24 h germination was sufficient to eliminate flatulence-producing factors without causing loss of total available carbohydrate.

Although CG-0 was not germinated, differences which were sometimes significant were observed between it and CWM. These may be due to loss of solids which invariably occurs with soaking and better hydration of the macromolecules in soaked and dehulled cowpea cotyledons.

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