Effect of spontaneous fermentation and amylase rich flour (ARF) on the nutritive value, functional and viscoelastic properties of cowpea-fortified nixtamalized maize

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Abstract
Studies were conducted to evaluate the combined effects of spontaneous fermentation and amylase-rich flours (ARF) on some nutritive value, functional and viscoelastic properties of cowpea-fortified nixtamalized maize. A $2^2 \times 3^2$ factorial design, with fermentation medium, fermentation time and ARF level, was performed. The blends were fermented for the specific times and analysed for their titratable acidity, pH, water absorption capacity, viscoelastic properties, texture, protein and mineral content. Fermentation and ARF addition influenced titratable acidity, pH, water absorption, viscoelastic properties and texture of the cowpea-fortified nixtamalized maize. Addition of ARF decreased the viscoelastic properties, texture and pH of all the blends with a corresponding increase in acidity. Slight increases in protein and ash contents were noted with products fermented in coconut water, but ARF addition had only a marginal effect. Thus, fermentation and ARF addition could be applied to cowpea-fortified nixtamalized maize to enhance the functionalities with reduced viscosity and texture suitable for weaning food formulations.

Keywords: Maize, fermentation, nixtamalization, amylase-rich flour, cowpea fortification, coconut water, functional properties, rheological properties, weaning foods

Introduction
Maize is the principal cereal produced in sub-Saharan Africa with an annual production currently of approximately 1.4 million metric tonnes, and it is used for the preparation of a wide variety of dishes. The main problems associated with maize-based foods are the lack of adequate protein quality or essential amino acids and the viscosity that limits intake, especially in weaned infants. One of several methods for improving the nutritional quality of maize is fermentation, which is also the oldest method of preparing and preserving food. Fermented maize products by tradition constitute an important part of the diet of people in most developing countries (Banigo and Akpapunam 1987; Oluwamukomi et al. 2005; Afoakwa and Aidoo 2006; Lei et al. 2006; Afoakwa et al. 2007). One of the primary objectives for the traditional fermentation of maize is to cause souring of the dough with its associated improvement in taste, flavour and texture (Sefa-Dedeh et al. 2001a, 2003). The main aroma
components in fermented maize doughs have been described to be lactic acid, acetic acid, butyric acid and propionic acid (Plahar and Leung 1982; Akpapunam and Sefa-Dedeh 1995). These fermentations, as carried out traditionally, are spontaneous.

Nixtamalization is the process of alkaline cooking of maize with lime, and traditionally it is the primary processing step during manufacture of several maize chips, maize tortillas and taco shells. The process of nixtamalization is popular in Mexico and Central America, and has been applied to maize for centuries (Bressani et al. 1990; Serna-Saldivar et al. 1990; Martínez-Flores et al. 2002, 2006; Rendón-Villalobos et al. 2009). In addition to the variety that it introduces in the utilization of maize, other important effects of nixtamalization include increased bioavailability of niacin, improved protein quality, increased calcium content and the reduction of aflatoxin concentration of masa products (Serna-Saldivar et al. 1987; Wall and Carpenter 1988; Bressani et al. 1990; Sefa-Dedeh et al. 2004; Ayala-Rodriguez et al. 2009).

The incorporation of amylase-rich flour (ARF) has been reported as an effective means of producing liquefied foods from their solid base, and can be processed using malted cereals such as maize, rice, millet or sorghum to develop amylotic enzymes (Desikachar and Malleshi 1982; Mosha and Svanberg 1983; Chevan and Kandam 1989; Gopaldas et al. 1991; Barragan-Delgado and Serna-Saldivar 2000). Malting is a traditional practice in developing countries primarily used in the production of alcohol and non-alcoholic beverages. During malting of grains, α-amylase and β-amylase activity is developed. These enzymes effectively degrade starch granules, reducing their water-binding capacity. Malting, among the low-cost methods available to reduce the high viscoelastic properties of cereal-based foods, seems to be the most effective (Desikachar and Malleshi 1982). The incorporation of a small amount of germinated grain, ARF, to cereal-based foods has been reported to produce a remarkable reduction in viscosity (Marero et al. 1988; Gopaldas et al. 1991; Akpapunam and Sefa-Dedeh 1995). Also, malting conveys certain safety benefits to foods by the removal of toxic and anti-nutritional substances such as lectin and haemagglutinins (Nnanna and Phillips 1988). Even though cowpea fortification and nixtamalization have been reported to contribute to improved nutrition and sensorial properties of cereal foods, the extent of the combined effects of spontaneous fermentation and ARF addition on the quality characteristics of maize still remains unknown. The rationale of the present study was therefore to investigate the effects of fermentation and ARF addition on some nutritional, functional and viscoelastic properties of cowpea-fortified nixtamalized maize.

Materials and methods

Materials

Maize (*Zea mays*) and cowpea (*Vigna unguiculata*) were obtained from the Crop Research Institute of Ghana and were used for the study. These were stored at cold room temperature (4°C) during the experimental period. Lime (Ca(OH)₂), food grade, was obtained from BOH Chemicals Ltd (Poole, UK).

Experimental design and sample preparation

A 2 × 3 × 3 factorial experimental design with fermentation medium (water, coconut juice), fermentation time (0 h, 24 h, 48 h) and ARF (malt) level (0%, 2%, 5%),
respectively, was used. Nixtamal was prepared by boiling whole maize in 1% lime solution for 30 min and steeping in the cooking liquor for 14 h. The steeped grains were washed thoroughly with water to remove excess lime and milled using a disc attrition mill (Model 10-2A; KE Electrics, New Delhi, India). Part of the meal produced was mixed with water and part was mixed with coconut water to form dough of 55% moisture content, and they were fermented for 0 h, 24 h and 48 h, respectively. After 48 h of fermentation, the dough was dried in an air oven at 50°C for 16 h to obtain moisture contents between 7 and 10%, and the dried samples were milled into fine flour using the hammer mill (Model 2A; Christy and Norris Ltd, Chelmsford, UK) to pass through a 300 μm sieve.

Cowpea was soaked in water for 30 min and dehulled using a disc attrition mill (Model 10-2A; KE Electrics). The dehulled cowpea was dried in an air oven (Compenshet Brit, Pat No. 852942; Haslemere, Surrey, UK) at 50°C for about 12 h. The dry cowpea was then milled using the hammer mill (Model 2A; Christy and Norris Ltd) into fine flour.

The ARF was prepared by cleaning and steeping an amount of maize in water for 24 h at ambient temperature (28°C). After steeping, the seeds were spread on aluminium trays lined with moistened jute sacks, covered in a moistened jute sack and allowed to germinate in the dark for 5 days. The samples were sprinkled with about 700 ml water daily throughout the germination period. The sprouted seedling samples were spread on aluminium trays and then dried using the air oven at a temperature of 50°C for 24 h. The dry malt was then milled using the hammer mill (Model 2A; Christy and Norris Ltd). The flour (ARF) was then sieved using a laboratory sieve with a mesh size of 60 μm. The malt was mixed with the nixtamalized maize flour according to the levels indicated in the experimental design. The flour samples obtained were analysed for the following indices; pH, non-volatile acidity, water absorption capacity (WAC) (27°C and 70°C), viscosity, texture, protein and ash.

**Analytical methods**

**pH and non-volatile acidity (titratable acidity).** Ten grams of dried flour were mixed with 100 ml distilled water. The mixture was allowed to stand for 15 min, shaken at 5-min intervals, and centrifuged at 3,000 rpm for 15 min using a Denley centrifuge (Model BS4402/D; Denley, UK). The supernatant was decanted and its pH was determined using a pH meter. Ten-ml aliquots (triplicate) were titrated against 0.1 M NaOH using 1% phenolphthalein as indicator. Non-volatile acidity was calculated as grams of lactic acid per 100 g sample.

**Water absorption capacity.** Five grams of sample were weighed into a centrifuge tube and 30 ml distilled water at temperatures of 27°C and 70°C was added. The solution was stirred and allowed to stand for 30 min and was centrifuged using a Denley centrifuge (Model BS4402/D; Colchester, Essex, UK), at 3,000 rpm for 15 min. The supernatant was decanted and the increase in weight noted by weighing. The WAC is expressed as a percentage of the initial sample weight. The determination was done in duplicate.

**Viscoelastic properties.** The Brookfield Viscometer (Model RVTB) equipped with disc spindles was used to measure the viscoelastic properties of the samples. Paste viscosities were measured for 500 ml slurries contained in a 600-ml beaker. Preliminary studies
were performed to determine the best slurry concentration and the spindle number, and a concentration of 8% (dry matter basis) and a number 3 disc spindle were found to be appropriate at rotational speed of 10 and 20 rpm, respectively, for the cooked and hot paste viscosities. The slurries were heated on a hot plate with a magnetic stirrer, until the temperature was 95°C. The viscosity was measured at that temperature as the hot paste viscosity for the sample. The sample was then cooled at ambient temperature to 50°C and the cooled paste viscosity was determined.

Texture. Twelve per cent slurries of the dry sample flours were cooked into a porridge, which was allowed to set to room temperature (25°C) within a period of 40 min. The texture of the set slurries were determined using a TA-XT2 texture analyser, equipped with a back extrusion rig equipped with a compression disc of 45 ml diameter. The work done in back extruding about 90 ml set sample slurry was determined. The test was replicated three times at a crosshead speed of 5 mm/sec and a distance of 35 mm. The force deformation curve was plotted using the XT.RA Dimension, version 3.78 computer software (Stable Micro System, Haslemere, Surrey, England).

Protein. The protein content of the dry flours was determined by the Kjeldahl method using AOAC approved method 923.03 (AOAC 1990). The factor of conversion of nitrogen to protein was 6.25.

Ash. The ash content was determined using the standard AOAC approved method 920.87 (AOAC 1990). Approximately 2 g sample were weighed into a known weight crucible (pre-heated in the furnace and cooled.). The samples were then put in a pre-heated furnace (GallenKamp Muffler Furnace) of 550–600°C overnight. After this the crucibles were removed, cooled and weighed to determine the weight of the ash samples. The ash content was calculated on a grams per 100 g samples basis. Determinations were done in duplicate.

Statistical analysis

The data obtained from the analyses were statistically analysed using Statgraphics (Graphics Software System; STCC, Inc, Rockville, MD, USA). Comparison between sample treatments and the indices were done using analysis of variance and multiple range tests, with probability $P \leq 0.05$.

Results and discussion

pH profile

The pH of nixtamalized maize is an important quality parameter that influences the flavour and shelf-life of products made from alkaline maize (Serna-Salvador et al. 1990). The pH of all of the cowpea-fortified nixtamalized maize sample increased with increasing ARF level for both fermentation media (Figure 1). The results showed a slight consistent pH increase from 4.62 at 0% ARF concentration to 4.66 at 5% ARF level for samples fermented in water and from 4.52 at 0% ARF concentration to 4.54 at 5% ARF level for samples fermented in coconut water, respectively, indicating that the pH values of samples fermented in coconut water were slightly lower than those
fermented in water—an indication that samples steeped in coconut juice generated slightly less acid than those steeped in water.

On the contrary, the pH of the products steeped in both water and coconut juice decreased consistently with increasing fermentation time (Figure 1). Previous reports by Afoakwa et al. (2004, 2007) showed that the pH of lime-treated maize decreased with fermentation time, irrespective of the fermenting media. Sefa-Dedeh (1991) showed that a final pH of 4.13 was obtained after fermenting lime-treated maize for 72 h. The decreases in pH observed during fermentation suggest the presence and activity of lactic acid bacteria during the spontaneous fermentation of the blends. These lactic acid bacteria are largely responsible for the production of lactic acids during fermentation of maize-based foods (Afoakwa et al. 2004; Sefa-Dedeh et al. 2007).

![Figure 1. Effect of fermentation and ARF on the pH of cowpea-fortified nixtamalized maize.](image-url)
The low pH obtained in the cowpea-fortified nixtamalized maize blends during fermentation is important since bacteria, including pathogenic organisms, do not survive in such low pH environments. This imparts microbial safety as well as increased shelf-life of the final products.

Analysis of variance on the data showed that the fermentation time significantly affected (\(P \leq 0.05\)) the pH, but the fermentation medium and ARF level had no significant effect (\(P > 0.05\)) on the pH. Multiple range analysis on the effect of fermentation time revealed that samples fermented for 24 and 48 h were distinctly different (\(P \leq 0.05\)) from the unfermented blends, suggesting that fermentation is vital for acid generation in maize-based foods.

**Non-volatile acidity (titratable acidity)**

Titratable acidity is a measure of the total non-volatile acid (dissociated and undisassociated) produced in the fermentation product. Sefa-Dedeh (1991) reported that operations such as soaking, size reduction and fermentation contribute to the development of flavour, colour, texture and other product quality attributes in cereal products.

The results revealed general decreases in titratable acidity with increasing ARF concentrations. Titratable acidity decreased from 0.059 g lactic acid/100 g sample to 0.05 g lactic acid/100 g sample at 0% and 2% malt level, respectively, for samples fermented in water (Figure 2). Similar decreasing trends in titratable acidity with increasing ARF concentration were observed for samples fermented in coconut juice. These findings are in agreement with an earlier report (Afoakwa and Aidoo 2006) that titratable acidity decreases with increasing ARF concentrations at all levels of lime concentrations in maize. The observed decrease could be due to the fact that malt may have an acidity-suppressing effect on the products due to relatively higher pH levels reported for malted cereals. Cereal \(\alpha\)-amylase, which is the main enzyme responsible for the breakdown of starch, has an optimum pH in the range 5.7–6.3 (Mosha and Svanberg 1983).

On the contrary, fermentation caused consistent increases in titratable acidity in all of the cowpea-fortified nixtamalized maize samples (Figure 2). This observation might be due to the generation of acid by lactic acid bacteria produced during the fermentation period. Increases in acidity of cereal-based foods during fermentation have been widely reported (Sefa-Dedeh et al. 2001b; Afoakwa et al. 2004, 2007; Afoakwa and Aidoo 2006). Souring of maize dough has been linked to lactic acid fermentation during which lactic acid and other organic acids are produced (Plahar and Leung 1982). Nout et al. (1995) reported that lactic acid fermentation exhibits antimicrobial effects on pathogenic microorganisms due to the presence of the acids. This suggests that the combined effects of fermentation and ARF addition could be applied to cowpea-fortified nixtamalized maize to produce safe, acceptable foods that possess all of the nutritional improvements that nixtamalization imparts.

Analysis of variance showed that the fermentation time and fermentation medium significantly affected (\(P \leq 0.05\)) titratable acidity but the malt level had no significant (\(P > 0.05\)) effect on the titratable acidity. Further analysis using the multiple range tests revealed that a significant (\(P \leq 0.05\)) difference existed in the effect of water and coconut media on titratable acidity. It also revealed that each of the fermentation times
(0 h, 24 h and 48 h) produced a distinctly different effect with respect to acidity of the blends.

**Water absorption capacity**

The WAC refers to the weight of water bound per gram of dry sample and has been reported to be dependent on the availability of hydrophilic groups that bind water molecules and on the gel-forming capacity of macromolecules (Gomez and Aguitera 1983). The results showed variations in trends in the WAC of the cowpea-fortified nixtamalized maize samples as fermentation proceeds with both incubation temperatures (27°C and 70°C) used. These trends suggest possible relationship in WAC with fermentation processes and temperature (Figures 3 and 4).

Generally, trends in the WAC of the samples incubated at 70°C showed initial lower values than those at 27°C (Figures 3 and 4). The initial decrease at 70°C in the WAC of the fortified nixtamalized maize dough could be due to the fact at 70°C there were reductions in the availability and action of the hydrophilic groups, which bind...
water. Oosten (1982) suggested that divalent cations bind tightly with starch molecules, actually causing water holding capacity to decrease. This trend, however, was not observed in samples fermented in coconut water. The result obtained therefore suggest a saturation and excess Ca\(^{2+}\) and Ca(OH)\(^+\) at the surface of the starch granules during fermentation. Sefa-Dedeh et al. (2003) reported an increase in WAC on addition of cowpea to nixtamalized maize.

The WAC decreased with increasing ARF concentration at both 27\(^\circ\)C and 70\(^\circ\)C for all treatments. The results showed decreases in WAC from 66.0\% at 0\% ARF level to 56.9\% at 5\% ARF for samples fermented in water when the temperature was 27 \(^\circ\)C, and from 65.0\% at 0\% ARF concentration to 58.3 \% at 5\% ARF for samples fermented in coconut water (Figure 3). Similar trends were observed at 70\(^\circ\)C temperature (Figure 4). The decrease in water absorption with increasing ARF concentration is due to the breakdown of the starch by amylolytic enzymes (\(\alpha\)-amylase and \(\beta\)-amylase) present in the ARF, which were suspected to be produced in the aleurone layer during the malting process.

![Figure 3. Effect of fermentation and ARF on the water absorption capacity of cowpea-fortified nixtamalized maize at 27\(^\circ\)C.](Image)
The WAC also showed consistent decreases with increasing fermentation time at 27°C for both fermentation media, but increased with slight increases in fermentation time at 70°C (Figure 3). The effect of malt level on the water absorption capacity was more significant at 70°C than at 27°C, and this could be due to the increased activity of the enzymes (especially α-amylase) at 70°C than at 27°C. Cereal malt α-amylase is reported to have optimum temperature for maximum activity at 60–70°C (Mosha and Svanberg 1983).

Analysis of variance showed that the malt level and fermentation time significantly affected ($P \leq 0.05$) the WAC at 27°C but the fermentation medium had no significant effect on the WAC at 27°C. Multiple range tests conducted to determine the effect of fermentation time on the WAC showed that 24 and 48 h of fermentation had the same effect on the WAC at 27°C, but these were significantly ($P \leq 0.05$) different from the WAC at 27°C of the unfermented (0 h) blends. On the other hand, multiple range tests conducted to determine the effect of the ARF concentration on the WAC revealed that the 2% malt level was not significantly ($P \leq 0.05$) different from the 0% and 5% malt levels; however, there was a significant difference between the effect of 0% and 5% ARF levels at 27°C. Analysis of variance showed that the fermentation medium, ARF
concentration and fermentation time all had no significant ($P > 0.05$) effect on the water absorption capacity at 70°C.

**Viscoelastic properties**

The viscoelastic properties of the samples were evaluated using Brookfield Viscometer (Model RVDV1+; Stoughton, MA, USA). This was to assess the apparent viscosity of porridges prepared from the cowpea-fortified nixtamalized foods in order to establish the changes in viscosity with the treatments during processing and consumption of the samples. Apparent viscosity of fluids is the measure of fluid resistance to flow at a given shear rate. The Brookfield viscometer measures the spindle speed, spindle number and torque, from which the viscosity is measured. For a material of a given viscosity, the resistance is greater as the spindle size and/or rotational speed increases. The minimum viscosity range is obtained using the largest spindle at the highest speed, and the maximum range is obtained using the smallest spindle at the slowest speed (Afoakwa and Sefa-Dedeh 2002). The apparent hot (95°C) and cooked (50°C) paste viscosity of 8% slurries made from different flour samples of cowpea-fortified nixtamalized maize with ARF addition at different concentrations (0%, 2% and 5%) were measured to establish their viscosity levels during cooking and consumption, respectively.

At both 95°C and 50°C, similar trends were observed in all of the samples fermented in water and coconut water. The results of the apparent viscosity of the different cooked samples measured using the Brookfield viscometer indicated that the viscosity levels of most of the samples were determined using spindle number 2 at 50 and 100 rpm and spindle number 3 at 2.5 rpm. This means that the viscosities of the various samples were comparable without any difficulty based on their respective rpm and spindle numbers. The results showed that at 95°C, the samples fermented in water with 0% ARF had the highest viscosity with 377.4 cP, 446.3 cP and 486.4 cP at 0 h, 24 h and 48 h fermentation times, respectively, an indication that viscosity increases with increasing fermentation time (Table I). The viscosity also decreased with increasing malt level, with samples containing 5% ARF being the least viscous. The decrease in the viscosity with ARF level is due to the amylase enzymes present in the malted maize, which tends to break down the starch into smaller molecular weight dextrin and sugars (Serna-Saldivar et al. 1990).

At 50°C, comparatively higher viscosity levels were observed for all the samples than at 95°C. It was observed that samples fermented in water exhibited higher viscosity than samples fermented in coconut water (Table II). This could be attributed to higher

<table>
<thead>
<tr>
<th>Fermentation medium</th>
<th>ARF level (%)</th>
<th>0 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0</td>
<td>377.4</td>
<td>446.3</td>
<td>486.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.357</td>
<td>13.96</td>
<td>113.8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.112</td>
<td>2.404</td>
<td>0.190</td>
</tr>
<tr>
<td>Coconut water</td>
<td>0</td>
<td>226.2</td>
<td>264.9</td>
<td>365.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.347</td>
<td>17.41</td>
<td>15.14</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.098</td>
<td>0.12</td>
<td>0.111</td>
</tr>
</tbody>
</table>

Table I. Effect of fermentation and ARF on the viscoelastic properties of cowpea-fortified nixtamalized maize at 95°C.
sugar levels in coconut water (Duke 2001), which might have reduced the fermentative influence on the maize, resulting in reduced viscosity values in the samples fermented in coconut water. The viscosity at 50°C decreased with increase in ARF concentration, an indication that ARF addition influences the viscosity at 50°C, and reflects the retrogradation tendency of the cooked paste. Increased retrogradation property of the paste can be attributed to the high degree of association of the starch molecules caused by strong tendency of hydrogen bond formation between hydroxyl groups on adjacent molecules. Retrogradation has been associated with undesirable properties such as staling in bread, skin formation, paste gelling and loss of clarity in prepared starch paste (Afoakwa and Sefa-Dedeh 2002). The decrease in viscosity of cowpea-fortified nixtamalized maize on the addition of ARF and the increase in protein content on addition of cowpea to nixtamalized maize (Afoakwa and Aidoo 2006) both suggest that fermentation and ARF addition could be applied to cowpea-fortified nixtamalized maize as a means of increasing the protein content as well as reducing the high viscosity associated with traditional maize-based weaning foods, which have been identified as major causes of malnutrition in sub-Saharan Africa.

Analysis of variance showed that the ARF level significantly affected \((P \leq 0.05)\) the viscosity at 50°C but the fermentation medium and fermentation time had no significant \((P > 0.05)\) effect on the viscosity at 50°C; however, the fermentation medium significantly affected viscosity at 95°C. Further analysis using multiple range tests revealed that a significant decrease in viscosity at 50°C and 95°C can be achieved at all levels of malt (0%, 2% and 5%). It also revealed that a significant difference existed in the effect of fermentation medium on the viscosity at 95°C.

Table II. Effect of fermentation and ARF on the viscoelastic properties of cowpea-fortified nixtamalized maize at 50°C.

<table>
<thead>
<tr>
<th>Fermentation medium</th>
<th>ARF level (%)</th>
<th>Fermentation time (viscosity value (\times 10^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>24 h</td>
</tr>
<tr>
<td>Water</td>
<td>0</td>
<td>345.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.960</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.147</td>
</tr>
<tr>
<td>Coconut water</td>
<td>0</td>
<td>44.08</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.886</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.833</td>
</tr>
</tbody>
</table>

Texture

The texture of nixtamalized maize is one of the most important quality parameters used in the production of quality of alkaline cooked foods. The texture of alkaline cooked products, among other quality characteristics, has significant influence on their acceptability. A number of objective tests using various equipment, such as the mechanical stickiness device described by Ramirez-Wong (1989), have been used for the evaluation of the texture (stickiness) of nixtamalized maize. In this experiment, texture (stiffness) of the blends of cowpea-fortified nixtamalized maize was measured as work (g) required to back extrude a specific amount of cooked slurry of the blends that had been allowed to cool and set to ambient temperature (25°C).
Generally, the effect of ARF addition on the texture of cowpea-fortified nixtamalized maize samples showed consistent decreases at all fermentation times, with samples containing 5% ARF showing the lowest texture values and those of 0% ARF having the highest values (Table III). The texture of the samples generally increased with increasing fermentation time for samples fermented in water as well as those in coconut water. However, the texture of the set samples fermented in water had slightly higher values than their corresponding samples fermented in coconut water at all ARF concentrations and fermentation times. The decrease in texture by the presence of the ARF could be attributed to the breakdown of starch by amylolytic enzymes, which are produced during germination in the aleurone layer of the cereal. This suggest that ARF could be effectively incorporated into cowpea-fortified nixtamalized maize to produce porridges with reduced hardness for use as weaning food for infants. Increasing fermentation time also caused significant increases in texture of the products at all levels of ARF and fermentation media. Previous findings reported similar increases in hardness in nixtamalized maize foods with increasing fermentation time (Sefa-Dedeh et al. 2003; Afoakwa and Aidoo 2005).

Analysis of variance showed that ARF level and fermentation time significantly affected ($P \leq 0.05$) the texture, but the fermentation medium had no significant ($P > 0.05$) effect on the texture. Multiple range analysis on the effect of fermentation time revealed that samples with 2% and 5% malt added were distinctly different ($P \leq 0.05$) from the samples without ARF.

### Protein content

The crude protein measures the total amount of protein from the nixtamalized maize dough, cowpea and malt added. Most researchers report a small increase in nitrogen content during nixtamalization, which has been attributed to a concentration effect. The protein content decreased slightly with the addition of malt to both samples fermented in water and coconut water, respectively. At 20% cowpea level the protein content decreased slightly from 10.89 g/100 g dry sample at 0% ARF level to 10.78 g/100 g dry sample at 5% ARF level, and from 10.98 g/100 g dry sample at 0% ARF level to 10.88 g/100 g dry sample at 5% ARF level for samples fermented in water and coconut water, respectively (Figure 5). Protein contents for both media, however, were comparable. Preliminary studies (unpublished) showed no effect of fermentation time on the protein content of cowpea-fortified nixtamalized maize and thus were not included in the present study. Several workers have reported marked improvements in the protein quality and quantity of cereals with cowpea fortification and nixtamalization.

Table III. Effect of fermentation and ARF on the texture (hardness) of cowpea-fortified nixtamalized maize.

<table>
<thead>
<tr>
<th>Fermentation medium</th>
<th>ARF level (%)</th>
<th>0 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0</td>
<td>32.9</td>
<td>60.0</td>
<td>65.95</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15.05</td>
<td>20.85</td>
<td>22.85</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12.15</td>
<td>17.05</td>
<td>17.40</td>
</tr>
<tr>
<td>Coconut water</td>
<td>0</td>
<td>26.1</td>
<td>55.65</td>
<td>85.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14.05</td>
<td>23.75</td>
<td>19.75</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>11.7</td>
<td>14.75</td>
<td>12.8</td>
</tr>
</tbody>
</table>
(Akpapunam and Sefa-Dedeh 1995; Sefa-Dedeh et al. 2001b, 2003; Afoakwa and Aidoo 2006). Alkaline cooking, cowpea fortification, ARF addition and fermentation can therefore be employed in the improvement of the nutritional quality of nixtamalized foods made from maize. These have been the basis of advocating cereal–legume complementation as a means of improving the protein content of cereal-based foods.

Analysis of variance showed that the fermentation medium, malt level and fermentation time all had no significant \((P > 0.05)\) effect on the protein content. The insignificant effect observed for the malt level could be due to the small decreases that occurred with increasing ARF concentration.

**Ash content**

The ash content of the cowpea-fortified nixtamalized maize samples showed no observable trend with ARF addition in the different fermentation media. The ash contents of 4.64%, 4.72% and 4.68% were noted for the samples fermented in water, while ash levels of 4.86%, 4.78% and 4.72% were noted for samples fermented in coconut water, respectively, with 0%, 2% and 5% ARF concentrations (Figure 6). The observed trend suggest that ash contents were slightly higher in the samples fermented in coconut water than in those fermented in water. This might be due to the ash content of 0.46% reportedly in coconut water (Duke 2001), which might have been added to the ash content of the product thereby increasing their levels slightly higher than those fermented in water with no predetermined ash content. Preliminary studies

![Figure 5. Effect of fermentation and ARF on the protein content of cowpea-fortified nixtamalized maize.](image-url)
(unpublished) showed no effect of fermentation time on the ash content of cowpea-fortified nixtamalized maize, and thus were not included in this study. Similar higher levels of ash in nixtamalized maize when fermented with coconut water as compared with water have been reported (Afoakwa and Aidoo 2006). Analysis of variance showed that fermentation medium and ARF level all had no significant ($P > 0.05$) effect on the ash content.

**Conclusions**

Variations in fermentation medium had no significant effect on the studied indices, with the exception of protein and ash content where samples fermented in coconut water had slightly higher levels that their corresponding samples fermented in water. This suggests that coconut juice can be used as an effective fermentation medium for maize fermentation in situations of water shortages. Fermentation of the cowpea-fortified nixtamalized maize dough resulted in decreased pH, WAC and texture and increased levels of non-volatile organic acids. The addition of ARF to the product effectively reduced the viscosity of fermented cowpea-fortified nixtamalized maize at 2% and 5% concentrations at both the processing temperature (95°C) and the consumption temperature (50°C). ARF addition also significantly influenced the titratable acidity, WAC and texture of the fermented cowpea-fortified nixtamalized maize. ARF addition in combination with fermentation can therefore be employed to further improve the nutritive value and functionality of energy-dense nixtamalized

![Figure 6. Effect of fermentation and ARF on the ash content of cowpea-fortified nixtamalized maize.](image-url)
maize products for the production of liquefied porridges suitable for use as weaning foods for infant feeding.

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**References**


