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African Journal of Science and Technology (AJST)
Science and Engineering Series Vol. 2, No. 2, pp. 71-80

INFLUENCE OF FERMENTATION AND COWPEA STEAMING ON SOME QUALITY CHARACTERISTICS OF MAIZE-COWPEA BLENDS

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ABSTRACT: *Fermentation and cowpea steaming can be used to improve the protein quality and quantity of fermented maize dough. In the production of maize-cowpea blends, it is important that the quality characteristics are evaluated to determine their functionality in the products. A 5x4x2x2 factorial experiment with cowpea level, fermentation time, cowpea steaming time and fermentation method as the variable was performed. The cowpeas were dehulled, steamed, dried at 65EC for 24 hours and milled into flours. Maize was soaked in water (18 hours), drained and milled into flour. The maize-cowpea blends were made into a 50% moisture dough, fermented for the specified periods, dried at 65EC and milled into flour. Samples were evaluated for pH, titratable acidity, water absorption and sugars. The pH and titratable acidity of the samples were affected by fermentation time, steaming time, and the levels of cowpeas in the blend. Cowpeas was the main source of glucose/galactose. Fermentation caused a reduction in stacchyose and glucose/galactose. The mixing of cowpea flour with fermented maize dough prior to drying (single component fermentation) gave similar effects on sugar concentrations as detected in the co-fermented samples (multi-component fermentation). Fermentation and steamed cowpea fortification can be used to produce high protein fermented cereal foods with reduced anti-nutritional factors.*

Key words : Fermentation, steamed cowpeas, cowpea fortification, chemical composition, functional properties, weaning foods.

INTRODUCTION

Fermentation is widely applied in the processing of cereals for the preparation of a wide variety of dishes in developing countries (Obiri-Danso,1994). In Ghana, over 90% of cereal based traditional foods are prepared from fermented maize dough. The soaking of the grains in excess water allows the selection of desirable micro-organisms, such as lactic acid bacteria, yeasts, and moulds (Sefa-Dedeh *et al*., 1999; Sefa-Dedeh & Cornelius, 2000). The activity of these micro-organisms reduces pH and increases the titratable acidity of the substrate. A number of fatty acids are also produced (Akinrele,1970; Plahar & Leung, 1982). Mensah *et al.* (1990) studying the properties of fermented maize dough observed significant inhibition of pathogenic bacteria. Cooking the dough into porridge reduced the antimicrobial effect but there was still significant inhibition of pathogens. The antimicrobial property could be an important strategy for the reduction of the high levels of faecal bacteria in cereal foods in the developing countries

Most African traditional gruels are made from cereals and the result is gruels that have low nutritional value as they are not adequate sources of micro- and macro-nutrients (Brown,1991). During cooking, the starch binds water, requiring considerable amounts of water to bring the consistency of the porridge to a level suitable for child feeding. This lowers the energy and nutrient density of the porridge considerably. This high volume/high viscosity characteristic referred to as dietary bulk makes it difficult for infants fed on these gruels to satisfy their nutrient requirement, which is considered as a major problem of malnutrition in areas where cereal staples are the main foods (Ljungqvist *et al*., 1981). Recently, more attention has been directed towards the fulfilment of protein energy requirements because of the widespread occurrence of protein energy malnutrition (PEM) and linear growth retardation in developing countries. The techniques commonly employed in traditional weaning food developments, include the formulation of high quality protein mixes (foods) using cereals and legumes such as cowpeas and soybeans.

The application of cowpea-fortification to fermented cereal porridges have been reported to increase the nutritive value. As well, the total nutrient density increases by more than two-folds as a result of reduction in viscosity (Marero *et al*., 1980; Afoakwa,1996; Sefa-Dedeh *et al*, 2000). Cowpea steaming has been developed to control the infestation of cowpea seeds during storage. It involves the exposure of cowpea seeds to steam followed by drying to acceptable storage moisture contents. Sefa-Dedeh and Demuyarko (1994) investigated the effects of steaming and storage on some physico-chemical properties of cowpea seeds and flour. They reported that whilst the steaming resulted in an increase in water absorption capacity of the flour samples, the steamed seeds showed reduced water absorption capacities. They also observed characteristic differences in the viscoamylograph data following steaming, which is an indication of the ease of cooking of the samples. Further studies on some chemical and functional properties of steamed cowpea fortification to weaning foods will provide insight into the compatibility of steamed cowpea as a food ingredient and the success with which steamed cowpeas can be incorporated into various weaning foods.

This study was aimed at investigating the influence of fermentation and steamed cowpea fortification on some chemical and functional properties of fermented maize-cowpea blends.

MATERIAL AND METHODS

Materials

Maize (*Zea mays*) and cowpea (*Vigna unguiculata*) were used in the experiments. An improved variety of maize (Abeleehi) was purchased from Ejura Farms, Accra. Dent corn and Blackeye peas obtained from the Centre for Food Safety and Quality Enhancement (CFSQE), University of Georgia, Atlanta and used for the study. All the samples were stored at a cold room (4°C) during the study period.

Sample preparation

Whole cowpeas were soaked in water for 4 minutes to loosen the seed coats. The seeds were drained and dehulled using a disc attrition mill (Agrico Model 2A, New Delhi). The hulls were separated by floatation in water. One portion was dried in an oven temperature of 65°C for a period of 20-24 hours and the remaining portion steamed for 4 minutes. Subsequently, the steamed seeds were oven dried at 65°C and milled into flour using a disc attrition mill (Agrico Model 2A, New Delhi). Whole maize grains were soaked in water (1:3, w/v) at 28°C for 18 hours. The steep water was drained and the grain milled using a disc attrition mill (Agrico Model 2A, New Delhi). Several formulations were prepared by the addition of weighed portions of dehulled and steamed cowpea flour to maize meal before fermentation. The resulting meal was kneaded into a 50% moisture dough, allowed to ferment at room temperature for a period of 0 - 72 hours. Other blends were formulated by the addition of cowpea flours to the maize dough after fermentation. The preparations were dried in an oven at 65°C and milled into flour.

Experimental Design :

A 5 x 4 x 2 x 2 factorial experimental design was used and the principal factors were:

i. Cowpea level : 0, 5, 10, 15 and 20 % ;
ii. Fermentation time : 0, 24, 48 and 72 hours;
iii. Steam treatment : 0 and 4 minutes,
iv. Fermentation method : Single and multiple component.

Samples were evaluated for pH, titratable acidity, water absorption, and mono-, di- and oligosaccharides.

Methods

pH and titratable acidity

Ten grams of dried flour was mixed with 100ml distilled water. The mixture was allowed to stand for 15 minutes, shaken at 5 minutes intervals and centrifuged at 3000 rpm for 15 minutes using a Denley centrifuge (Model BS4402/D, Denley, England). The supernatant was decanted and its pH was determined using a pH meter (Model HM-30S, Tokyo, Japan). Ten (10)ml aliquots (triplicate) were titrated against 0.1M NaOH using 1% phenolphthalein as indicator. Acidity was calculated as grams lactic acid/100g sample.

Water absorption capacity

Five grams of sample was weighed into a centrifuge tube and 30ml of distilled water at temperatures of 25 and 70°C added independently for the analysis at 25 and 70°C respectively. The mixture was stirred and allowed to stand for 30 minutes and centrifuged using a Denley centrifuge (Model BS4402/D, Denley, England), at 3000 rpm for 15 minutes. The supernatant was decanted and the increase in weight noted by weighing. The water absorption capacity was expressed as a percentage of the initial sample weight. The determination was done for duplicate samples.

Determination of mono-, di- and oligosaccharides

Sugars were extracted from maize/cowpea blends using a mixture of chloroform : methanol (1:1, w/v) and water as in Havel, Tweeten, Seib, Wetzel and Liang (1977). Extract was concentrated under vacuum, made to 5 Ml with de-ionized water. The extract was filtered with 0.22m PTFE filters and stored in 5 mL ampoules at a cold room temperature of 0°C. Ten microlitres (10 µm) samples were analyzed by High Performance Liquid Chromatography (HPLC) using a Hitachi system equipped with a Hewlett Packard Integrator and Shimadzu Refractive Index Detector. Separation was done on a 220 x 4.6 mm amino propyl column (amino-spheri-5, Brownlee Labs, Santa Clara) eluted with a 70:30 v/v mixture of acetonitrile : water which contained 0.01% tetraethylene pentamine (TEPA) as recommended by Aitzemuller (1978). Quantification was against authentic external standards of the sugars detected and a lactose internal standard.

Statistical analysis

The data obtained from the chemical and functional determinations were statistically analyzed using Statgraphics (Graphics Software System, STCC, Inc. U.S.A). Comparisons between sample treatments and the indices were done using analysis of variance (ANOVA) with a probability $p < 0.05$.

RESULTS AND DISCUSSION

pH and acidity

The solid-state fermentation of maize dough had a drastic effect on pH. Within the first 24 hours of fermentation, the pH decreased from 6.3-4.0 (Fig. 1). After 72hrs of fermentation, the pH of the dough was 3.87. Fortification with up to 10% of unsteamed cowpea yielded fermented dough with comparable pH as the unfortified maize dough. Samples containing 15-20% unsteamed cowpea however showed a relatively high pH (Fig. 1A).

The proteins in the cowpea may have contributed to the high pH. Steaming of cowpea prior to addition to maize appeared to promote fermentation (Fig.1B). These samples had relatively lower pH than their unsteamed samples. Fortification up to 20% steamed cowpea provided samples with comparable pH as the 100% maize samples. The data suggests that maize can be fortified with up to 20% level and fermented to produce a dough with low pH. Analysis of variance on the data showed that only fermentation time had a significant effect ($p \leq 0.05$) on dough pH. Duncan's multiple comparison tests indicated that the unfermented maize samples were distinctly different from the fermented maize samples. The samples fermented for 24 hours were significantly different from those fermented for 72 hours. The 48 hour-fermented samples however

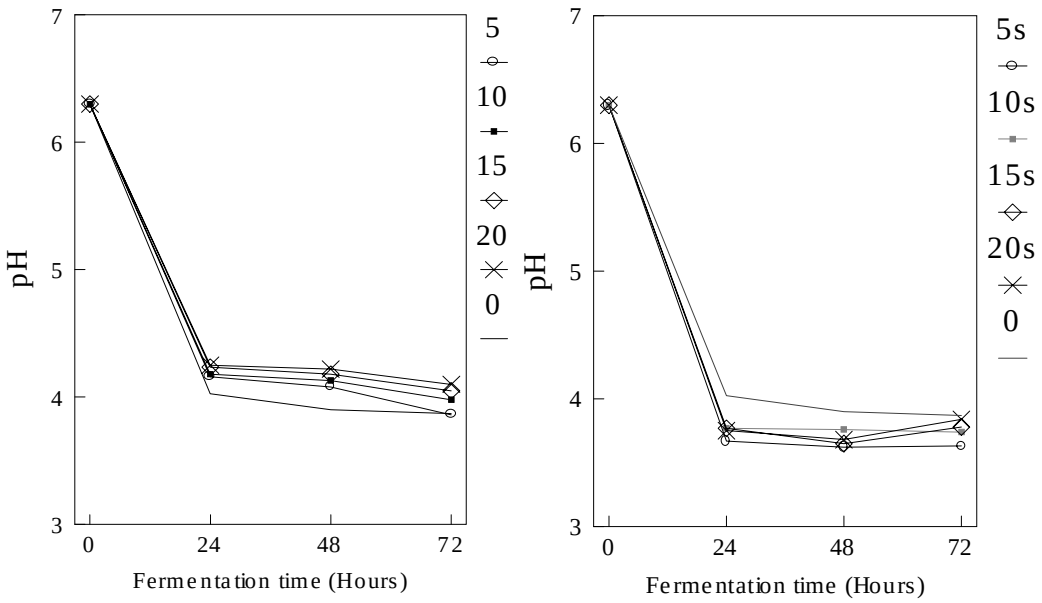

Figure 1. Effect of steam treatment and cowpea concentration on the pH of unsteamed (A) and steamed (B) cowpea-fortification at concentrations of 0 - 20% of fermented maize-cowpea blends

compared favourable with the other two samples (Table 1).

Table 1. F-ratios of process variables of the functional properties

Process variable	pH	Acidity	Water absorption
Fermentation time	65.175*	187.35*	14.783*
Cowpea level	0.2815	1.917	6.786*
Steam treatment	0.343	0.387	0.009

* Significant F-ratios at $p \leq 0.05$

The major carboxylic acids produced during the fermentation of maize dough have been identified as lactic, butyric, acetic and propionic acids (Plahar & Leung, 1982). The acidity of maize dough increased with fermentation. The addition of unsteamed cowpea lowered the acidity of the maize dough (Fig. 2A).

Steaming the cowpea prior to the addition to maize led to increases in acid production during fermentation for upto 48 hours. However, acidity decreased slightly during the final 24 hours of the fermentation period monitored (between 48 and 72 hours). Analysis of variance on the data showed that fermentation time had a significant (p#0.05) effect on acidity. In addition the acidity level associated with each fermentation time was dependent on whether the cowpea has been steamed (Table 1) the ability of the cowpea-fortified maize dough system to ferment and produce acids comparable to the traditional maize dough system is beneficial. Mensah et al. (1990) reported that the antimicrobial properties of fermented maize dough due to the acids produced during fermentation. This was reported to reduce incidence of diarrhoea in infants consuming fermented maize porridge. The cowpea-fortified maize dough will have two important attributes, such as antimicrobial properties and high protein content. This will make it useful in the formulation of weaning foods.

Sugars and Oligosaccharides

Cowpea

Xylose, fructose, glucose, galactose, sucrose, maltose, raffinose and stachyose were determined in different concentrations in whole cowpea (Table 2). Dehulling led to an increase in the concentration of all sugars except for maltose, glucose and galactose. This suggest that maltose, glucose/galactose are concentrated in almost all the sugars with the exception of sucrose, glucose and galactose. The "-galactosides are known to constitute the major portion of sugars in legumes seeds (Fleming, 1980). Stacchyose was observed to be the major oligosaccharide, followed by raffinose in the cowpea samples as reported by other workers (Akpapunam & Markakis, 1979; Abdel-Gawad,

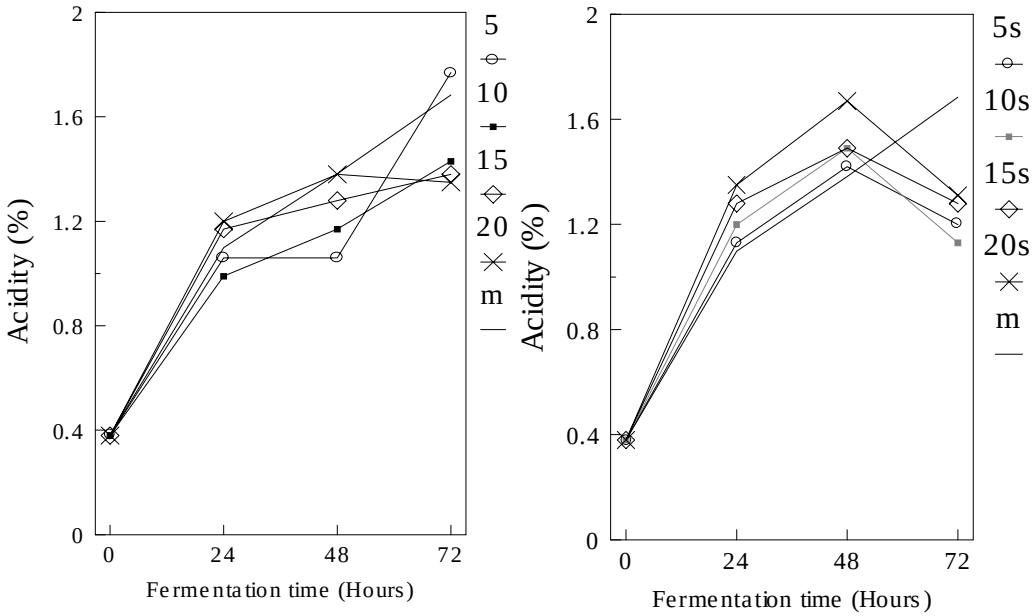

Figure 2. Effect of steam treatment and cowpea concentration on acid production of unsteamed (A) and steamed (B) cowpea-fortification at concentrations of 0 - 20% of fermented maize-cowpea blends

1992; Sosulski *et al.*, 1982). The levels of stacchyose and raffinose increased with dehulling. This observation was contrary to initial studies reported by Akinyele and Akinlosotu (1991) who detected a decrease in oligosaccharide levels after dehulling cowpea seeds. The longer pre-soaking period of 4 hours allowed in their experiment might have facilitated leaching or initiated the process of fermentation in the seed resulting in increased "-galactosidase activity on the oligosaccharides.

Table 2. Levels of mono-, di- and oligosaccharides determined in cowpea (*Vignia unguiculata*) samples (mg/100g dry matter)

Cowpea sample	Xylose	Fructose	Glucose + Galactose	Sucrose	Maltose	Raffinose	Stachyose
Whole	0.38	0.012	0.337	1.652	0.118	0.552	2.545
Dehulled	0.612	0.436	0	1.828	0.051	0.63	3.661
Dehulled & Steamed	0.332	0.068	0.028	2.034	0.039	0.234	3.34

Maize

Maize was observed to have high concentrations of glucose and galactose. Other sugars detected in the maize were maltose, xylose and fructose (Table 3). The effect of fermentation on the concentration of the sugars varied. During the first 24 hours of fermentation, the concentration of fructose, glucose and galactose decreased. Xylose and maltose however showed a slight increase in concentration. Further fermentation for 24 hours showed an increase in all the sugars except xylose. Fermentation for 72 hours showed a drastic reduction in the concentration of glucose/ galactose and maltose (Table 3). The levels of maltose, glucose and galactose appeared to increase during the first 48 hours of fermentation and drop drastically after 72 hours. Sucrose, raffinose and stacchyose were not detected in the maize samples.

Table 3. Levels of mono-, and disaccharides as determined in maize (*Zea mays*) samples (mg/100g dry matter)

Fermentation Time (Hours)	Xylose	Fructose	Glucose + Galactose	Maltose
0	0.705	1.093	9.636	0.21
2 4	0.788	0.678	9.566	0.277
4 8	0.481	0.69	10.213	0.438
7 2	0.473	0.597	1.056	0.14

Co-fermentation

Fermented maize and cowpea blends showed varied effects of process variables on sugar and oligosaccharide concentrations. In the system in which cowpea was co-fermented with maize (Figs. 3 & 4) the effects of process

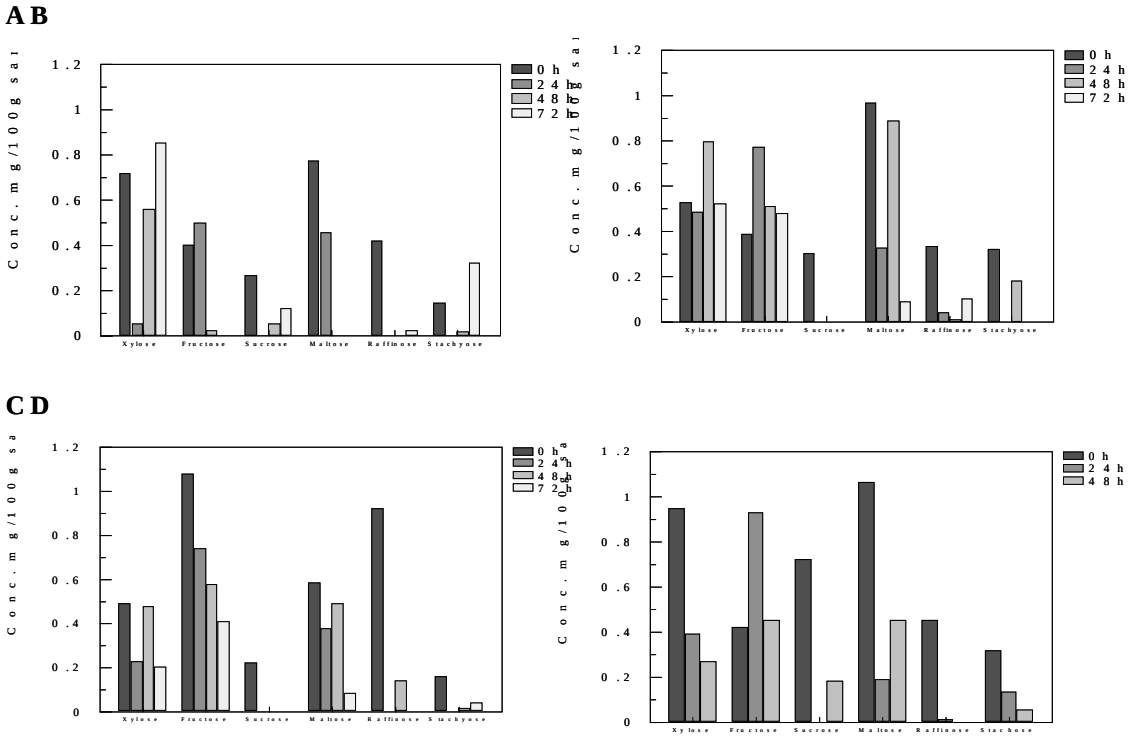

Figure 3. Effect of fermentation on the sugar levels in 5% (A), 10% (B), 15% (C) and 20% (D) unsteamed cowpea on co-fermented maize-cowpea blends

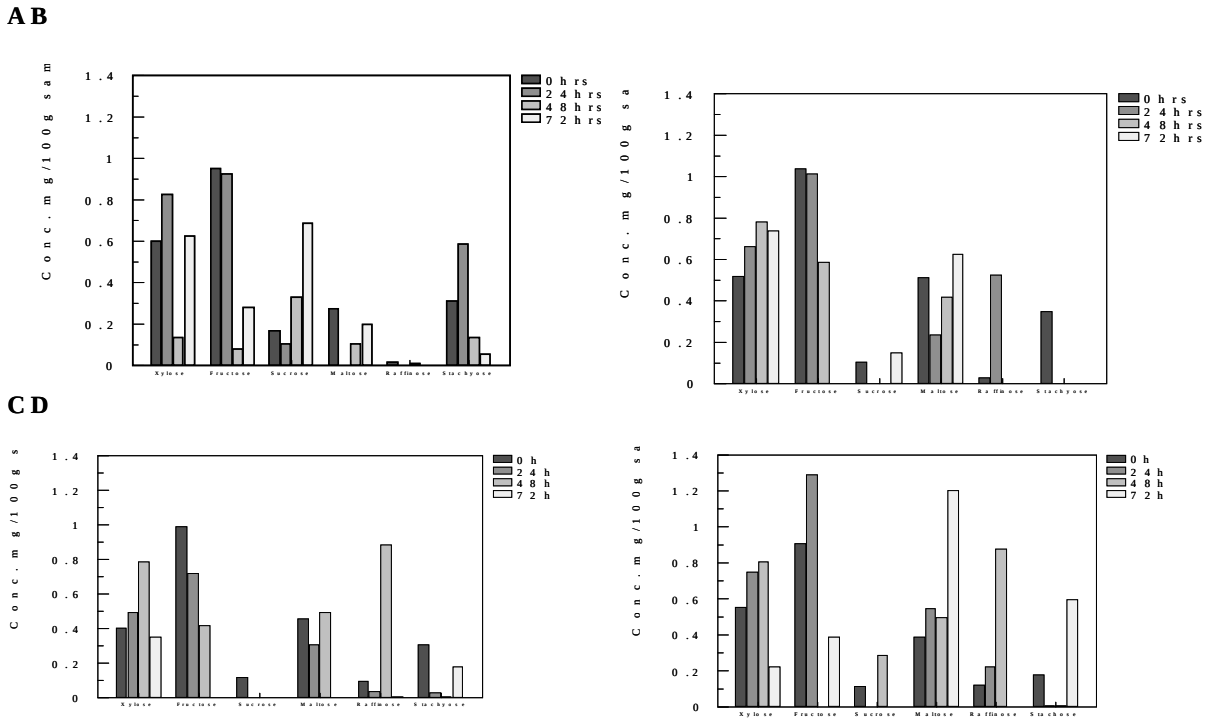

Figure 4. Effect of fermentation on the sugar levels in 5% (A), 10% (B), 15% (C) and 20% (D) steamed cowpea on co-fermented maize-cowpea blends

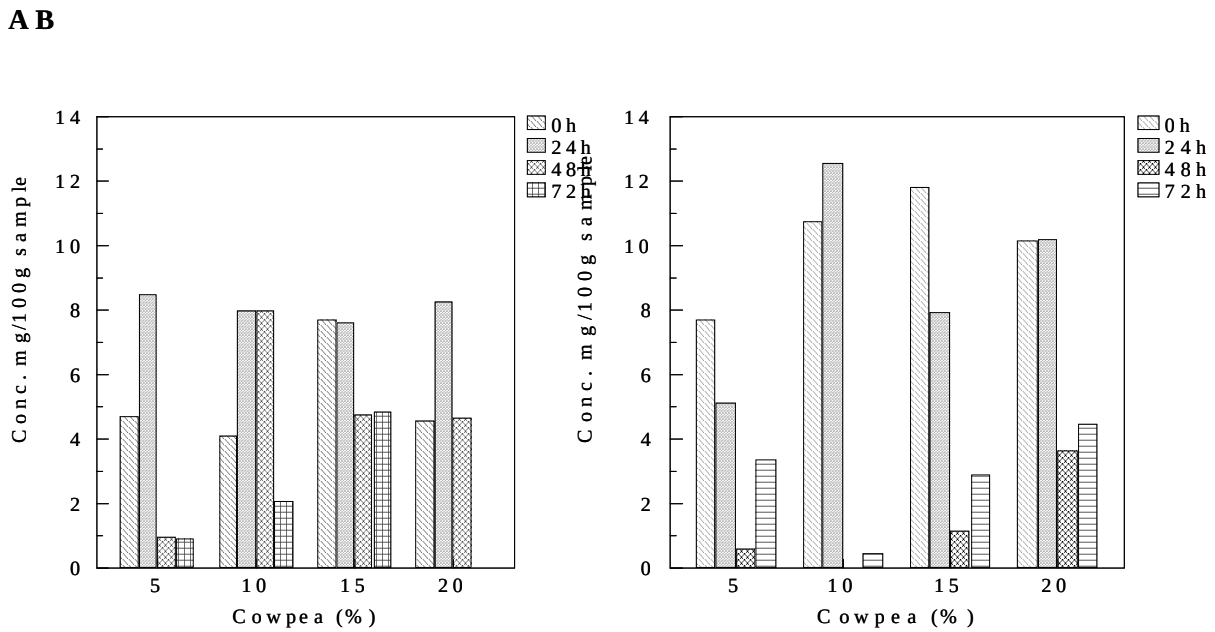

Figure 5. Effect of fermentation on glucose concentration in co- fermented maize-(A) unsteamed and (B) steamed cowpea blends

variables on xylose concentration was not conclusive. Xylose was derived from both the maize and cowpeas. The co-fermentation of maize with steamed cowpeas (10, 15 and 20%) appeared to increase the xylose concentration (Figs. 4B, C & D) up to 48 h of fermentation after which a decrease was observed. The system containing unsteamed cowpeas however showed a consistent decrease in xylose concentration with the 20% cowpea-fortified system (Fig. 3D). The sugars which are derived solely from cowpeas ; sucrose, raffinose and stacchyose seemed to be affected by fermentation. A general decrease with fermentation and complete removal were observed. The sugars predominantly from maize, being glucose/galactose (Fig. 5), maltose and fructose (Fig. 3 & 4) showed a general reduction with fermentation. Steaming of cowpeas appeared to assist this reduction.

Blend after maize fermentation

This system showed an interesting trend. Sugars such as sucrose, raffinose and stacchyose derived solely from cowpeas showed great reduction in concentration with fermentation time (Figs. 6 & 7). This reduction was more pronounced in the 5, 10, and 15% cowpea-fortified fermented maize system. Apart from xylose, all other sugars being glucose/galactose (Fig. 8), fructose and maltose showed a general reduction with process variables. The data suggest that the fermented maize dough with its 50-55% moisture is an important media for the reduction of sugars and oligosaccharides derived from cowpeas. It is proposed that the reactions involving the sugars and oligosaccharides from cowpeas may have occurred from the time of mixing with the fermented maize dough to the early part of drying at 65° C.

The data from the two fermented maize-cowpea systems was subjected to analysis of variance. Table 4 is a summary of the significant F-values from the analysis. Xylose was affected by any of the process variables. Fermentation time had a significant effect ($p \leq 0.05$) on fructose, glucose/ galactose, maltose, raffinose and stachyose concentrations (Table 4). The level of cowpeas in the blend affected the glucose/galactose and stachyose concentrations. Steam treatment affected only the maltose concentration. Significant interactions were found between fermentation time and all other process variables.

Table 4. Table of significant F-ratios of mono-, di- and oligosaccharides fermented weaning foods

Process variables	Fructose	Glucose + Galactose	Maltose	Raffinose	Stacchyose
Fermentation time	43.079	48.972	3.501	5.142	20.372
Fortification level	-	4.074	-	-	10.453
Steam treatment	-	-	5.400	-	-

Water absorption capacity

Process treatments of raw material are known to affect their hydration properties (Philips *et al.*, 1988). Water absorption capacity was measured at room temperature (26°C) to determine the behaviour of the cereal-legume flours in cold water. This is an important index which can give valuable information on the behaviour of the blend during processing. Addition of cowpea improved the water absorption potential of fermented maize dough (Table 6). This was probably due to the influence of added protein in the blends. Proteins are mainly responsible for the bulk of water uptake and to a lesser extent the starch and cellulose at room temperature. Sefa-Dedeh and Osei (1994) made similar observations on a cowpea fortified fermented maize dough system.

Steam treatment of cowpeas was observed to increase its water absorption capacity from 166.8349% - 221.9613%. For the unfermented and 24-hour fermented maize-cowpea blends, steam treatment of cowpea seemed to cause a decrease in water absorption potential.

Table 5. Water absorption capacities of fermented maize-unsteamed cowpea blends (% dry matter basis)

Fermentation time (Hours)	Cowpea level (%)	Water absorption of co-fermented blends	Water absorption of fermented blends
0	0	114.6731	114.6731
	5	142.6048	136.6179
	10	143.3218	140.0624
	15	144.9571	140.5073
	20	137.3242	146.1079
24	0	118.6975	118.6975
	5	124.1301	132.2226
	10	124.2137	139.5657
	15	121.8473	146.8565
	20	134.0411	133.8545
48	0	120.7402	120.7402
	5	127.1835	133.3353
	10	130.1206	145.6074
	15	134.2309	128.7172
	20	137.0246	143.9276
72	0	114.2845	114.2845
	5	122.7185	143.0052
	10	136.0395	131.9409
	15	138.8273	132.8310
	20	139.9509	144.3295

However, 48 hours and 72 hours of fermentation the steamed cowpea fortified blends had higher water absorption capacities. Sefa-Dedeh and Demuyarko (1994) reported that

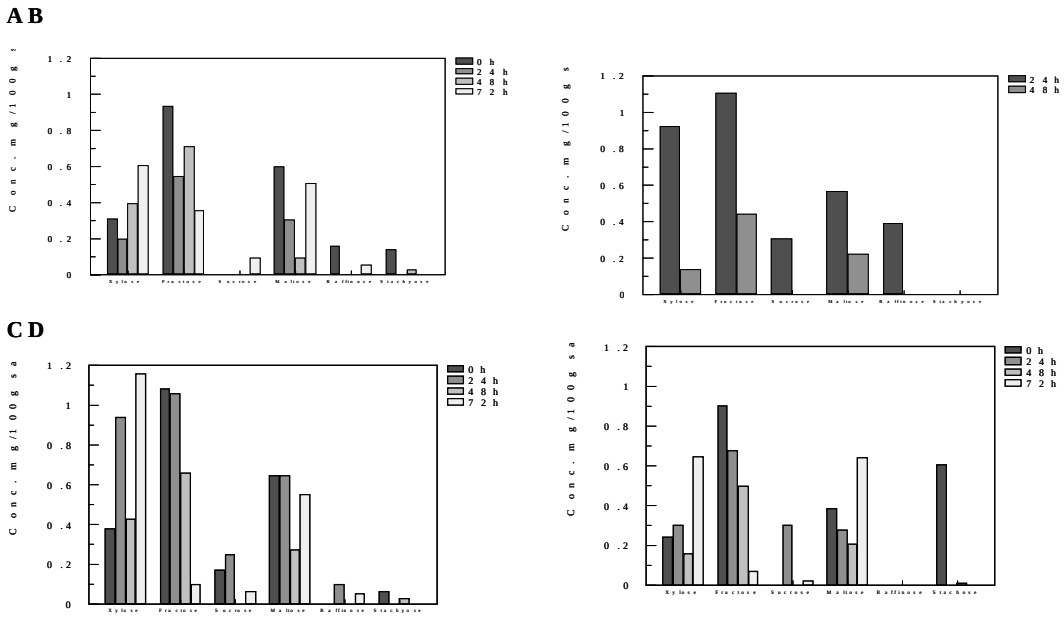

Figure 6. Effect of fermentation on the sugar levels in5% (A), 10% (B), 15% (C) and 20% (D) unsteamed cowpea on fermented maize- cowpea blends

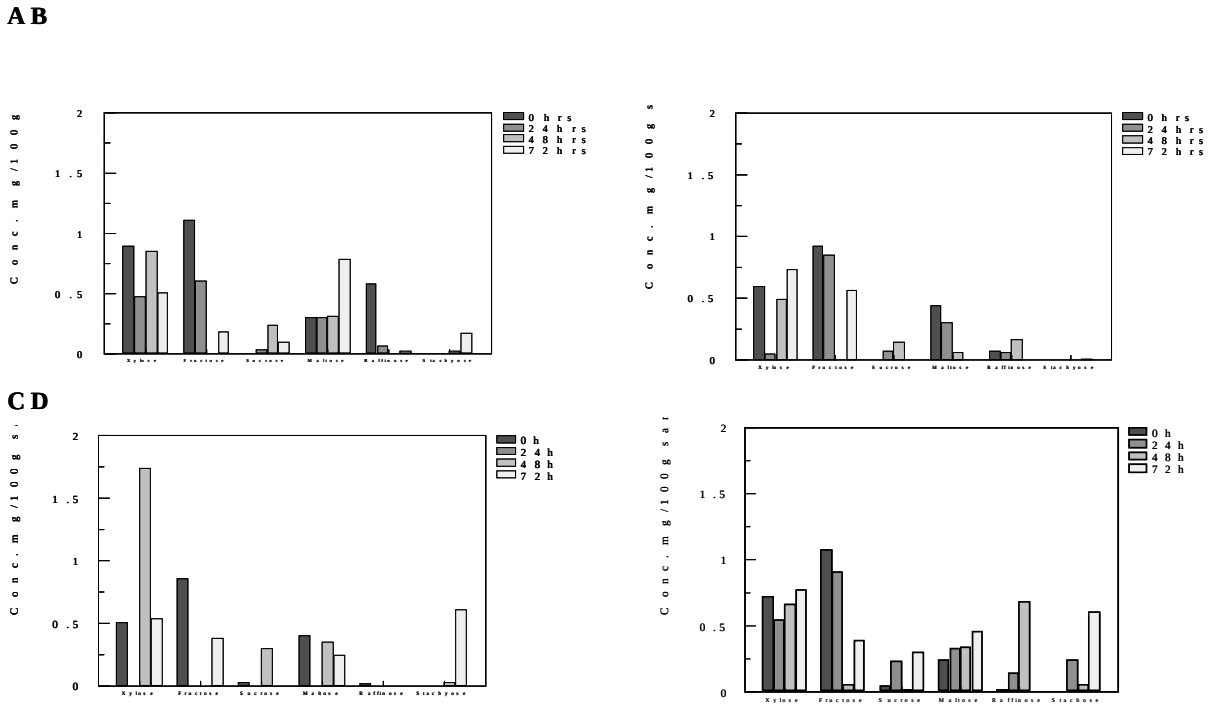

Figure 7. Effect of fermentation on the sugar levels in5% (A), 10% (B), 15% (C) and 20% (D) unsteamed cowpea on fermented maize- cowpea blends

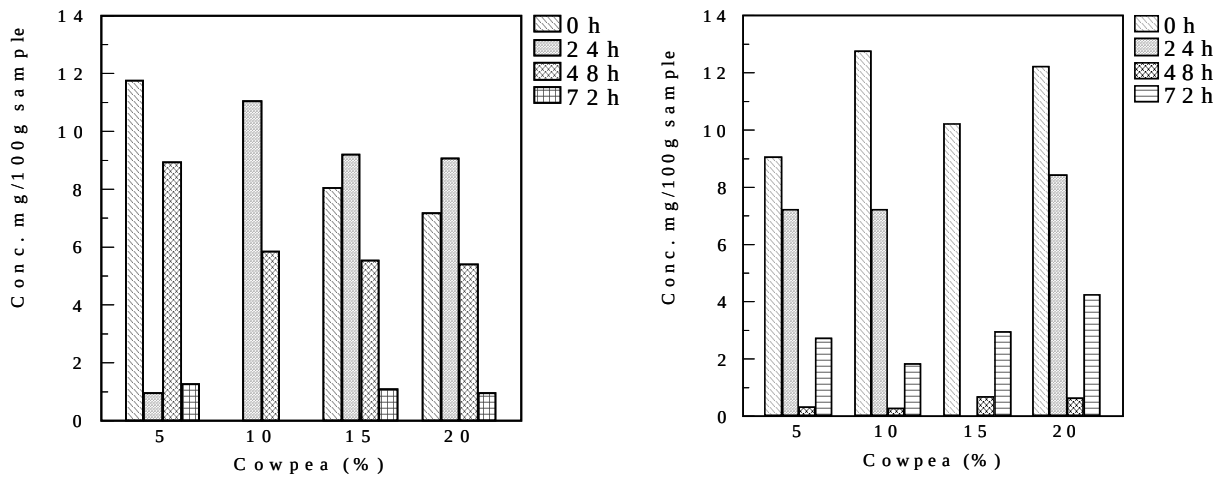

Figure 8. Effect of fermentation on glucose concentration in fermented maize- (A) unsteamed and (B) steamed cowpea blends

steamed cowpea flour possessed better water absorption than raw cowpea flour.

Table 6. Water absorption capacities of fermented maize-steamed cowpea blends (% dry matter basis)

Fermentation time (Hours)	Cowpea level (%)	Water absorption of co-fermented blends	Water absorption of fermented blends
0	0	114.6731	114.6731
	5	121.1686	129.8615
	10	121.7534	135.8566
	15	121.8169	133.2053
	20	135.1617	140.4852
24	0	118.6975	118.6975
	5	115.4199	125.4236
	10	130.6627	122.1084
	15	113.3782	115.4199
	20	136.0908	133.6514
48	0	120.7402	120.7402
	5	136.2266	137.4582
	10	142.3521	146.7009
	15	147.8524	148.1080
	20	144.1065	152.9293
72	0	114.2845	114.2845
	5	148.5680	138.0563
	10	129.3342	149.2652
	15	148.3491	148.0684
	20	134.0860	153.3773

The gelatinization of starch and the denaturation of protein that is the result of the application of heat treatment to cowpeas has been suggested to improve the water imbibing capacity of cowpea and mung bean proteins (Abbey & Ibeh,1988; del Rosario & Flores, 1981). Analysis of variance indicated that cowpea fortification level and the water absorption capacity of the blends. The effects of steaming the cowpeas prior to its incorporation into the maize dough was not statistically significant (Table 1).

CONCLUSION

Fermentation and steam cowpea fortification can be used to produce high protein weaning foods with reduced anti-nutritional factors without significant changes in product quality profiles of the fermented weaning foods.

ACKNOWLEDGEMENT

This study was funded through the Bean-Cowpea Collaborative Research Support Program by the United States Agency for International Development - Grant No. DAN-1310-G-SS-6008-00.

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