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Biochemical composition of infant weaning food fabricated from fermented blends of cereal and soybean

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Abstract

Infant weaning foods were produced from fermented blends of germinated and ungerminated cereal-soybean flour paste, using *Saccharomyces cerevisiae* and *L. plantarum* ATCC 10776 as starter cultures. A shorter fermentation period of less than 24 h was obtained compared to the traditional 72–96 h. Protein content of the formulated blends increased with fermentation, ranging from 15.5% in F25B to 18.2% in F410B. This could be attributed to the breakdown of nutrients of the substrates especially soybean, by the starter organisms. The relative decrease in the calcium and phosphorus content of the fermented blends could have resulted from leaching during the steeping stage and subsequent loss from the discarded pomace of the grains which contained most of the minerals. Fermentation also provided optimal pH for the degradation of the phytate phosphorus constituents of the fermented blends could be due to the metabolic activities of the fermenting microorganisms. Porridges made from all the formulated cereal-soybean blends were rated above average in terms of overall acceptability, but F15B had the highest preference rating. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In many developing countries, especially in tropical Africa, infant weaning foods as well as foods for adults are based on local staple diet made from cereals, roots and tubers of cassava and potatoes. These foods are usually prepared as thick porridges for adults or as liquid gruels for infants. To be suitable for the feeding of young children, these cereals are prepared in liquid form by dilution with a large quantity of water, thereby resulting in more volume but with a low energy and nutrient density.

However, most cereals are limited in essential amino acids such as threonine, lysine and tryptophan (Horn & Schwartz, 1961), thus making their protein quality poorer compared with animals. Their protein digestibility is also lower than that of animals, partly due to the presence of fibres and tannins which bind to protein thus making it indigestible (Graham, Glover, Lopez De Romana, Morales, & Maclean, 1980). Various methods have been shown to improve the protein quality of cereals such as amino acid fortification, supplementation or complementation with protein concentrates or other food sources and genetic improvement (Jansen, 1974).

Recently, germination and fermentation are adopted as ways of improving cereal protein quality. Fermentation of food is one of the oldest and most economic methods of food processing and it often leads to an improvement in the nutritional value of foods by bioenrichment with microbial proteins, amino acids, lipids and vitamins. The potential of fermented foods for reducing or alleviating food-related precipitating factors of malnutrition, particularly among weaning age children is a viable one, considering the favourable properties inherent in these types of foods (Lorri, 1993).

Since cereal grains will continue to be the major basic diets of infants and adults in developing countries, efforts should be geared towards improving the nutritional status of the food products. This paper therefore reports the biochemical composition of fabricated infant weaning food made from composite blends of cereal and soybean.

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2. Materials and methods

2.1. Preparation of samples

Quantities of maize (*Zea mays*), sorghum (*Sorghum bicolor*) and wheat (*Triticum vulgare*) were obtained from a retail market in southwestern Nigeria. They were cleaned, shelled and thoroughly washed in tap water, during which the floating grains were discarded and the remaining lot air-dried, before dividing them into batches. One batch was left unfermented, while the other batch was soaked in tap water for 18 h at ambient temperature ($30-32^{\circ}$ C). The seeds were later germinated in a wooden tray with a screened bottom measuring 49.55×63.5 cm. The bottom was lined with wet paper towels and germination was carried out in an aircirculating incubator at 30° C for 4 days. Germinated seeds were separated from non-germinated and mouldy dry seeds daily, washed and dried at 50° C for 18-20 h.

The dried, germinated and ungerminated seed batches were separately milled and stored at 5°C. For the soybean seeds, enough quantity was soaked in tap water for 24 h followed by dehulling, washing and drying at 55°C for 36 h before milling. The milled samples were also stored at 5°C.

2.2. Formulation of cereal-soybean blends

About 150 g of different blends of cereal flour were separately weighed into 1 litre fermenters and soybean flour was incorporated into the blends at 5% and 10% (w/w) concentration.

2.3. Fermentation studies

2.3.1. Test organisms

Stock cultures of *Saccharomyces cerevisiae* was obtained from Dr. T. Ekunsanmi of the Department of Botany and Microbiology, University of Ibadan, while *Lactobacillus plantarum* ATCC 10776 was from the Culture Collection of Laboratory of Food Hygiene, Department of Food Technology, Chemical Centre, Sweden. *L. plantarum* was propagated in MRS broth (pH 5.5), while *S. cerevisiae* was grown in Malt Extract broth.

2.3.2. Inoculation

Approximately 150 g of each formulated blend (F15-F410) were weighed into the fermenters and sterilized at 121°C for 10 min. Soybean flour was separately sterilized and added aseptically to each blend. The flour mixture was stirred with a glass rod followed by addition of 500 ml sterile distilled water. Thorough mixing was achieved using a magnetic stirrer. The resulting mixture was inoculated with 1 ml each of *S. cerevisiae* and *L. plantarum* (3×10^6 CFU ml⁻¹). Fermentation was carried out at 30° C for 24 h for duplicate batches of each formulation.

2.4. Analysis

For the analyses below, appropriate weights of fermented and unfermented samples were used:

- 1. Estimation of crude protein, fibre, ash and lipid contents was by the methods of AOAC (1980).
- 2. Determination of calcium was by the Atomic Absorption spectrophotometric method while phosphorus was measured on a Technicon Autoanalyser (EEL 201) (AOAC. 1980).
- Measurement of vitamins: riboflavin, ascorbic acid and niacin were determined employing the procedures of AOAC (1980), while thiamine was extracted by using a combination of Pearson's (Gyorgy & Pearson, 1967) acid and enzymatic extraction procedures. Measurement was by the chemical assay method involving alkaline oxidation of thiamine to thiochrome.
- 4. Amino acid analysis: samples were analysed using the modified method of Ketiku (1973). Seven millilitres of 6 N-HCl were added to 2 ml defatted sample in a glass ampoule. Nitrogen gas was bubbled in to prevent oxidation during hydrolysis. The samples were then hydrolysed at $100 \pm 5^{\circ}$ C for 22 h. After cooling, the hydrolysate was filtered and 4 ml of the filtrate were then vacuum-dried. Citrate buffer (pH 2.2) was used in reconstituting the samples. 1 ml of each sample solution was used in loading the Technicon TSM 50 Amino acid autoanalyser.
- 5. Estimation of phytic acid: a modified method of McCance and Widdowson (1935) was used. About 10 g of the sample was shaken with 100 ml of 0.5 N HCl in a mechanical shaker for 2 h and filtered. A 20 ml portion was neutralised with 2% NaOH using phenolphthalein indicator and made up to 50 ml. Duplicate 20 ml portions were treated with 4 ml of 0.05% FeCl₃, heated in a water bath for 15 min, cooled and centrifuged. The precipitate was washed with 5 ml of 6 N-HCl, stirred with 2 ml distilled water and boiled. Two millilitres of 2% NaOH was added and allowed to stand for 15 min before making up to 100 ml. Suitable aliquots were used for the determination of phosphate by the vanadomolybdate method of Kitson and Mellon (1944).

3. Sensory evaluation of the fermented samples

Sensory evaluation of the porridges prepared from each blend was carried out to determine their organoleptic characteristics. A nine member panel of judges consisting of students and laboratory workers of the University familiar with porridge made from fermented maize or sorghum (ogi), was constituted. The panel members were asked to rate these samples for colour, texture, taste, consistency and aroma. The ratings were presented on a 9-point Hedonic scale ranging from 9 = like extremely to 1 = dislike extremely.

4. Results

Table 1 shows the chemical contents of the unfermented and fermented cereal-soybean blends. There was an increase in the crude protein content at the end of fermentation, while a decrease was recorded for the crude fibre, total ash and total lipid of the fermented blends. In the fermented samples, the calcium and total phosphorus contents were 54 mg 100 g⁻¹ in F15B to 66 mg 100 g⁻¹ in F410B and 161 mg 100 g⁻¹ in F15B to 217 mg 100 g⁻¹ in F410B, respectively. The phytate phosphorus content was also reduced from 117 mg 100 g⁻¹ in F15A to 87 mg 100 g⁻¹ in F15B (Table 2).

The result of the vitamin analysis showed an increase in the values obtained for the fermented samples relative to the unfermented (Table 3). Generally, the amino acid content of the blends increased at the end of fermentation, but decreases were observed for aspartic acid, alanine, proline, asparagine and glutamic acid in all the samples (Table 4). For the organoleptic evaluation (Table 5), all the formulated blends were rated above average in terms of overall acceptability but F15B was the most preferred.

Table 1 Chemical contents of cereal-soybean blends

Samples		Dry matter (%)							
	-	Crude protein	Crude fibre	Total ash	Total lipid				
F ₁₅	Α	14.5 ± 0.09	0.08 ± 0.00	2.44 ± 0.07	6.33 ± 0.35				
	В	16.8 ± 0.09	0.04 ± 0.00	2.03 ± 0.01	2.12 ± 0.05				
F ₂₅	А	14.7 ± 0.01	0.06 ± 0.00	2.44 ± 0.07	6.46 ± 0.02				
	В	15.5 ± 0.66	0.03 ± 0.00	2.03 ± 0.01	2.05 ± 0.01				
F ₃₁₀	А	14.2 ± 0.45	2.85 ± 0.04	2.85 ± 0.04	6.82 ± 0.02				
	В	18.1 ± 0.09	0.06 ± 0.00	2.16 ± 0.08	2.15 ± 0.04				
F ₄₁₀	А	15.4 ± 0.46	2.85 ± 0.01	2.86 ± 0.01	6.69 ± 0.09				
	В	18.2 ± 0.04	0.50 ± 0.00	2.12 ± 0.04	2.08 ± 0.01				

A=unfermented sample; B=fermented sample. F_{15} =Germinated sorghum, ungerminated wheat, germinated maize, 5% soybean. F_{25} =Ungerminated sorghum, germinated wheat, ungerminated maize, 5% soybean. F_{310} =Ungerminated sorghum, germinated sorghum, ungerminated maize, germinated maize and 10% soybean. F_{410} =Ungerminated wheat, germinated wheat, germinated maize and 10% soybean.

5. Discussion

Porridge meals were produced from fermented cerealsoybean flour paste using starter cultures. The relatively shorter fermentation period (<24 h) compared to the traditional spontaneous fermentation process (72–96 h), was in agreement with the earlier reports of Lorri (1993) and Sanni, Lonner, Marklinder, Johansson, and Molin (1994).

The increase in the protein content of the fermented blends could be attributed to the breakdown of nutrients of the substrates, especially soybean, by the starter organisms. Soybean is known to be a protein-rich seed. The decrease in the ash content could have resulted from the fermentation activity of the starter-cultures and the processing method. The important dietary constituents of the ash are calcium and phosphorus. The values obtained for the crude fibre at the end of fermentation meet the requirement recommended for children (USDA, 1975).

The decrease in the lipid content at the end of fermentation may have resulted from oxidation due to

Table 2 Calcium total and phytate phosphorus of cereal-soybean blends

Calcium, total and phytate phosphorus of cereal-soybean blends	Calcium,	total	and p	phytate	phosp	horus of	f cerea	l-soybean	blends
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Samples		$mg \ 100 \ g^{-1}$				
	-	Ca	P (total)	Phytate (P)		
F ₁₅	A B	$54.7 \pm 2.61 \\ 54.0 \pm 2.82$	$\begin{array}{c} 181\pm1.41\\ 161\pm1.06 \end{array}$	$\begin{array}{c} 117 \pm 2.12 \\ 87.5 \pm 2.12 \end{array}$		
F ₂₅	A B	$\begin{array}{c} 60.3 \pm 0.35 \\ 52.1 \pm 0.14 \end{array}$	$\begin{array}{c} 201 \pm 1.41 \\ 172 \pm 0.56 \end{array}$	$\begin{array}{c} 127 \pm 1.41 \\ 92.0 \pm 0.00 \end{array}$		
F ₃₁₀	A B	$\begin{array}{c} 70.5 \pm 0.00 \\ 61.9 \pm 0.14 \end{array}$	$267 \pm 1.76 \\ 216 \pm 0.14$	$\begin{array}{c} 151 \pm 1.41 \\ 147 \pm 1.41 \end{array}$		
F ₄₁₀	A B	$\begin{array}{c} 72.3 \pm 0.35 \\ 66.1 \pm 0.42 \end{array}$	$\begin{array}{c} 239\pm1.41\\ 217\pm1.69 \end{array}$	$\begin{array}{c} 154 \pm 0.70 \\ 150.0 \pm 0.00 \end{array}$		

Interpretation of sample symbols is as stated in Table 1.

 Table 3

 Vitamin content of cereal-soybean blends

Samples		Vitamins (mg 100 g ⁻¹)						
	_	Riboflavin	Ascorbic acid	Niacin	Thiamine			
F ₁₅	А	0.12	0.004	4.02	0.12			
	В	0.34	0.013	5.32	0.35			
F ₂₅	А	0.12	0.004	4.10	0.12			
	В	0.32	0.012	5.36	0.34			
F ₃₁₀	А	0.21	0.005	4.58	0.15			
510	В	0.46	0.013	6.04	0.39			
F ₄₁₀	А	0.24	0.008	4.50	0.21			
- 410	В	0.52	0.024	6.20	0.56			

Interpretation of symbols is as stated in Table 1.

Table 4 Amino acid content of cereal-soybean blends (g 16 g^{-1} N)

Amino acid	Samples								
	F ₁₅ A	$F_{15}B$	F ₂₅ A	$F_{25}B$	F ₃₁₀ A	F ₃₁₀ B	F410A	F ₄₁₀ B	
Lysine	4.32	6.96	4.01	8.8	4.72	8.86	4.38	6.94	
Valine	5.66	6.18	5.75	6.14	5.59	6.34	5.60	6.3	
Threonine	4.85	7.90	5.02	8.02	5.02	8.92	4.75	9.26	
Methionine	3.65	4.80	3.76	4.66	3.82	5.02	3.64	4.81	
Tryptophan	1.76	1.92	1.96	2.01	1.96	2.36	1.98	2.06	
Aspartic acid	5.02	3.18	5.18	3.26	5.05	3.45	5.02	3.62	
Leucine	10.0	12.6	10.6	12.8	10.7	12.9	10.9	12.8	
Phenylalanine	5.75	6.45	5.58	6.54	5.68	7.93	5.62	6.92	
Isoleucine	4.84	5.10	5.02	5.18	5.00	6.02	5.02	5.86	
Cystine	1.92	0.18	1.90	0.16	1.98	n.d.	2.00	n.d.	
Tyrosine	4.62	5.94	4.22	6.04	4.54	6.22	4.52	6.20	
Serine	4.96	5.52	4.90	5.20	5.08	5.85	5.10	5.92	
Arginine	5.20	5.46	5.16	5.40	5.20	5.84	5.20	5.68	
Glycine	5.24	4.78	5.26	4.72	5.10	4.84	5.60	4.92	
Alanine	7.98	4.15	7.44	4.18	7.78	4.25	7.36	4.14	
Proline	7.26	4.30	7.30	3.24	7.62	3.12	8.02	4.18	
Histidine	2.12	2.58	2.10	2.50	2.18	2.84	2.26	2.97	
Glutamic acid	14.7	11.2	14.0	11.8	13.2	5.22	13.7	9.05	
Asparagine	0.93	0.65	0.90	0.68	1.06	0.78	1.07	0.85	

Interpretation of symbols is as stated in Table 1. n.d. = not detected.

pre-fermentation treatment of the substrates, especially soybean. The microorganisms could also oxidize the lipid to obtain energy for their metabolic activities. Lipid yields a considerable amount of energy for microorganisms when oxidized. Similar results were obtained by Ejiofor, Oti, & Okator (1987), and Sanni & Ogbonna (1992). However, this low level of fat is desirable to enhance the storage stability and keeping qualities of the fermented blends.

The considerable decrease in the calcium and phosphorus contents of the fermented blends would have been due to losses through the discarded pomace of the substrates which contained most of the minerals. Loss can also occur by leaching during steeping. However, the amount retained after fermentation falls within the recommended levels for weaning infants and young children (Passmore, Nicol, Rao, Beaton, & Demaeyer, 1974).

The reduction in the phytate phosphorus was due to hydrolysis during fermentation. Phytases, which hydrolyse phytate into inositol phosphate, are inactivated during germination and the fermentation process. Hydrolysis of phytate has been reported in lactic fermented maize (Lopez, Gordon, & Fields, 1983), pearl millet (Mahajan & Chauhan, 1987) and in germinated finger millet (Udayasekhara-Rao & Deosthale, 1988). This inhibitor has also been found to be greatly reduced during the fermentation processing of maize and sorghum into Nigerian ogi (Oke, 1967; Adewusi, Orisadara, & Oke, 1991). The fermentation process provides optimal pH conditions for the degradation of phytate.

 Table 5

 Quality attributes of porridges from cereal-soybean blends

Samples	Organoleptic characteristics							
	Colour	Consistency	Texture	Taste	Aroma			
F ₁₅ B	7.6 ^{ab}	7.1 ^{ab}	7.8 ^{ab}	7.6 ^{ab}	7.3 ^{ab}			
$F_{25}B$	7.0 ^a	6.2 ^a	7.2 ^a	6.4 ^a	5.9 ^a			
$F_{310}B$	5.5 ^a	5.6 ^a	6.2 ^a	5.1ª	5.1 ^a			
$F_{410}B$	7.7 ^a	7.1 ^a	7.1 ^a	6.8 ^a	6.8 ^a			

Scores followed by the same letters are not significantly different by Duncan's Multiple Range test at 5% level of significance. However, higher values indicate greater preference.

There was an increase in the vitamin content of the formulated blends at the end of the fermentation period. Several lactic acid bacteria are capable of synthesizing vitamins (Gurr, 1987). *S. cerevisiae* can also synthesize vitamin B_{12} during growth. The result obtained agreed with that of Cameron and Hofvander (1971) that fermentation of whole maize grain in West African foods resulted in an increase in riboflavin and niacin contents. The authors also reported that fermenting a mixture of rice and black grain mungo resulted in increase in riboflavin, niacin and vitamin B_{12} .

Fermentation of the flour blends resulted in an increase in most of the individual amino acids, while decrease was also obtained in some. The decrease may be attributed to the *L. plantarum* utilizing these amino acids for growth. On the other hand, increase may be due to the ability of the starter-cultures to synthesize some amino acids. Odunfa (1985) reported an increase in the amino acid contents of fermented legumes and

cereals. Methionine, the limiting amino acid in legumes, has been reported to increase during tempeh kedele production. Lysine, the limiting amino acid in maize, also increased during germination and fermentation (Wang, 1977).

The consumer acceptability of the porridges produced from the different blends showed that all the samples were rated above average but F15B had the highest preference rating. Based on other characteristics such as viscosity, gross energy density, bulk density, reconstitution index and water-holding capacity (Sanni, Onilude, & Ibidapo, in press), F15B will be recommended as a potential infant weaning food. However, information on protein digestibility, iron availability, tannin content and shelf-life of the flour would need to be provided.

In conclusion, infant and adult foods have been fabricated from composite blends of cereals and soybean, and their nutritive and organoleptic properties have been improved upon by germination and fermentation. All the blends meet the requirements for an infant weaning food in terms of the protein, minerals, vitamins and amino acid contents (FAO, 1970; USDA, 1975).

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