Nutritional Evaluation of the Starchy Flour Obtained from Cassava Tubers on Fermentation with a Mixed-Culture Inoculum

G. Padmaja,* Mathew George, and S. N. Moorthy

Division of Post Harvest Technology, Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India

Zoe Bainbridge, Vanessa Plumb, J. F. Wood, and C. J. Powell

Natural Resources Institute, Central Avenue, Chatham Maritime, Kent, U.K.

Cassava fermentation improves its textural qualities and reduces the level of toxic cyanogenic glucosides. A mixed-culture inoculum provided fermentation of cassava tubers was found to be self-sustaining and to yield fermented flour having good puffing quality as well as low cyanide content. The nutritional quality of the fermented flour (sour and sweet) studied in comparison to the nonfermented cassava flour indicated that there was a decrease of 32% in the crude protein in sour flour and 69% in sweet flour. The ether extractive fraction increased by 26% in sour flour while there was tremendous reduction in ash content in fermented flours. Although there was a decrease in all of the amino acids in the sour and sweet flours, the decrease in arginine, histidine, and glutamic acid was quite noticeable. Despite the decrease in all amino acids in sweet flour, the protein quality based on the essential amino acid scores in sweet flour appeared to be good. Sucrose, glucose, and fructose were the prominent sugars in nonfermented flour. Fermentation was found to reduce the sucrose content significantly. There was no significant difference in *in vivo* digestibility of fermented and nonfermented flours. The study indicated the need for proper fortification of fermented flour with proteins and minerals to upgrade its food value.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is extensively cultivated throughout the tropics for its starchy tubers which form a primary or secondary staple for about 500 million people of the tropical belt (Cock, 1985). The tubers are consumed after boiling, baking, or frying in most parts of Asia, whereas fermented food products from cassava are popular in Africa, Latin America, Indonesia, and Philippines (Coursey, 1973; Lancaster et al., 1982). Microbial fermentation of cassava is reported to improve the shelf life of the resultant flour due to the organic acids formed through fermentation (Tewe, 1991). In addition, it improves the textural quality of cassava and reduces the levels of the toxic cyanogenic glucosides (Arihantana and Buckle, 1987; Ayernor, 1985; Bokanga et al., 1988; El Tinay et al., 1984; Mahungu et al., 1987; Padmaja et al., 1993). A disadvantageous feature in most cassava fermentations is the obnoxious smell emanating from the fermentation vats (Ohochukwu and Ballantine, 1983; Okafor et al., 1984). Studies conducted earlier by Mathew George et al. (1991) showed that the foul smell during fermentation could be eliminated by providing an inoculum to facilitate lactic fermentation. Using yogurt and palm wine as luxuriant sources for lactic acid bacteria and yeasts, respectively, an initial inoculum was prepared. The inoculum was enriched by successive fermentation of cassava using steep liquor of an earlier batch as the inoculum. Thus a mixed culture inoculum comprising *Lactobacillus* sp., *Streptococcus* sp., *Corynebacterium* sp., and yeasts was formulated. The mixed culture inoculum is stable and self-sustaining. The pH of the steep liquor was found to be lowered within 24 h of fermentation, which helped to prevent extraneous microbial contamination. The biochemical changes during fermentation as well as the modification of functional properties of the starchy flour by the inoculum have been reported earlier (Mathew George et al., 1993; Moorthy et al., 1993). The inoculum source could also enhance cyanide detoxification in cassava, and the resultant flour had only low levels of cyanide (Padmaja et al., 1993). In the present study, we have compared the nutritional qualities of the fermented cassava flour with the nonfermented flour and investigated the effect of fermentation on the *in vivo* digestibility of native and cooked flours.

* Address correspondence to this author at Senior Scientist (Biochemistry), Central Tuber Crops Research Institute, Thiruvananthapuram 695 017, Kerala, India.

MATERIALS AND METHODS

Organisms and Fermentation Procedures. Preparation of the inoculum and fermentation procedures were reported earlier (Mathew George et al., 1991). A high-yielding medium cyanide cassava variety H-1687 released from CTCRI was used in the study. Tubers of uniform maturity were collected from plants grown at the Institute Farm. Prismoid tuber pieces of 3- × 2-cm base without the outer hard cortex were dispensed in double the quantity of water (1:2 w/v) and inoculated with 20 mL of mother liquor/kg of cassava and left for fermentation for 72 h under ambient conditions (temperature 30 ± 2 °C). The medium was found to be self-sterilizing against adventitious infestation by undesirable microorganisms as reported by Akinrele (1964). The mother liquor contained per milliliter of Lactobacilli 7.5×10^6, Streptococci 3.5×10^5, bacterial count in nutrient agar comprising of Corynebacteria at 6.5×10^6 and yeast 0.7×10^5. Duplicate beakers were maintained for each fermentation experiment, and this was repeated three times. After a 72-h fermentation, the tuber pieces were taken out, sun-dried, powdered, and sieved through a 30-mesh-size sieve to obtain fermented sour flour (FSO). To obtain fermented sweet flour (FSW), the fermented pieces were disintegrated in water to remove the organic acids and centrifuged, and the residue was sun-dried, powdered, and sieved. Nonfermented cassava flour

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Table 1. Composition of Experimental Diets[a]

ingredient	group I: nonfermented cassava flour (NFC), %	group II: fermented sour flour (FSO), %	group III: fermented sweet flour (FSW), %
cassava flour	70.5	70.5	70.5
cellulose	3.0	3.0	3.0
groundnut oil	10.0	10.0	10.0
casein	11.5	11.5	11.5
vitamin–mineral premix	5.0	5.0	5.0

[a] Groups IV, V, and VI were fed the respective cooked diets of I, II, and III.

was prepared from sun-dried cassava chips of the variety H-1687 by powdering in a hammer mill and sieving through the same mesh size sieve.

Proximate Analysis of Fermented and Nonfermented Cassava Flours. Protein, crude fiber, ether extractives, ash, and carbohydrates were determined via the methods recommended by the Association of Official Analytical Chemists (AOAC, 1980) and suitably modified at NRI, U.K.

Amino Acid Analysis. Amino acid profiles were studied using a Biotronik LC 5000 amino acid analyzer at Natural Resources Institute, Chatham, U.K. For the amino acids except methionine and cystine, the dried and defatted samples of fermented (sour and sweet) and nonfermented cassava flour were hydrolyzed with 6 M HCl (Spackman et al., 1958). The hydrolysate was concentrated under vacuum, dissolved in citrate dilution buffer, and fed to the amino acid analyzer.

For the analysis of cystine and methionine, performic acid oxidation was carried out (Spindler et al., 1984) and the hydrolysate was processed as before. Tryptophan was not determined.

Sugar Analysis. Sugar profiles of fermented (sour and sweet) and nonfermented cassava flours were qualitatively characterized using a SP-8800 series HPLC. A 7.8- × 300-mm Aminex HPX-87 P cation-exchange column connected to a Carbo P microguard column was used to achieve chromatographic separations.

The standard sugar solution contained the individual sugars, D-glucose, sucrose, maltose, and fructose, at a concentration of 0.5 mg/mL. Samples for HPLC sugar analysis were prepared by homogenizing 2 g of dried and defatted cassava flour (fermented and nonfermented) in 20 mL of HPLC-grade water at high speed, for 2 min in an ultra-Turrex homogenizer. The supernatant obtained through centrifugation was cleaned as follows:

Five milliliters of homogenate was passed through a mixed bed resin TMD-8 (cation–anion-exchange resin) to decolorize it. The eluate was then passed through a Waters Sep-Pak C_{18} cartridge to ensure that all non-carbohydrate material was removed. The eluate was filtered through a 0.46-μm filter and fed to the HPLC. The flow rate was 0.5 mL/min and the column temperature was maintained at 70 °C. Attenuation of the RI detector was set at 5 for the nonfermented cassava flour and at 2 for the fermented flour. Sugars were identified by comparison of the retention time with standard sugars.

***In Vivo* Digestibility Studies.** *In vivo* digestibility of fermented and nonfermented cassava flour (native vs cooked) was assessed in albino rats. Native flour diets were prepared as per the composition in Table 1. For preparing the cooked flour diets, 70.5 g each of fermented as well as nonfermented flour were steam-cooked for 15 min. This was then mixed with the other ingredients and fed to rats. After an acclimatization period of 1 week, observation was taken on the starch content of the feed and excreta collected over a period of 21 days. The reducing sugars and other reducing groups likely to appear in the excreta were removed using 80% ethanol. Residue was hydrolyzed with 2 N HCl to convert starch to reducing sugars, and the sugar content was determined using potassium ferricyanide. Starch content was computed from this using a Morris factor of 0.9. Percentage digestibility of starch was calculated from

$$\frac{(\text{starch fed} - \text{starch excreted})}{\text{starch fed}} \times 100$$

Table 2. Proximate Composition of Fermented and Nonfermented Cassava Flour

nutrient	composition, g/100 g of DM		
	NFC	FSO	FSW
crude protein (N × 6.25)	2.36 ± 0.005	1.61 ± 0.0008 (−31.8)[a]	0.732 ± 0.002 (−69.0)
crude fiber	2.53 ± 0.04	2.39 ± 0.072 (−5.5)	2.13 ± 0.008 (−15.8)
ether extractives	0.312 ± 0.08	0.392 ± 0.0002 (+25.6)	0.285 ± 0.0008 (−8.3)
ash	2.48 ± 0.01	0.61 ± 0.01 (−75.4)	0.652 ± 0.02 (−73.7)
nitrogen-free extract (by difference)	92.36 ± 0.12	95.48 ± 2.0 (+3.4)	96.20 ± 0.008 (+4.2)

[a] Values in parentheses indicate percent deviation from nonfermented cassava flour.

RESULTS AND DISCUSSION

Changes in Proximate Composition. Proximate components in fermented cassava (sour and sweet) have been compared with those in nonfermented cassava (NFC) in Table 2. There was a decrease of ca. 32% in the crude protein in fermented sour flour (FSO) and 69% in the fermented sweet flour (FSW). Bokanga et al. (1988) obtained a 33% decrease in protein in the fermented cassava obtained using a starter culture isolated from naturally fermenting cassava. It has been well-documented that textural changes occur in cassava tuber during fermentation (Ayernor, 1985; Mathew George et al., 1991; Okafor et al., 1984). The mixed culture used in our study was found to elaborate polysaccharide and pectin degrading enzymes which help to lyse the cell membranes and alter their integrity (Mathew George et al., 1991). These changes are likely to leach out the soluble nutrients from cassava, and the decrease in protein might have resulted partly from the leaching into steep water. Ezeala (1984) also obtained a 45% decrease in protein in cassava which was naturally fermented. The mother liquor used in our study contained *Lactobacilli*, *Streptococcus*, *Corynebacteria*, and yeast, and no external source of nitrogen was provided for their growth and multiplication. It was quite likely that the microorganisms proliferated by utilizing the nitrogen present in cassava tubers (Ezeala, 1984).

There was a ca. 26% increase in ether extractive fraction in the FSO which might have resulted from the organic acids like lactic acid which are formed as a result of fermentation. Akinrele (1964) reported that organic acids were enriched in fermented cassava. We also have found that there is a substantial increase in lactic acid content of the sour flour. The washing step that followed in the preparation of FSW leaches out the soluble lactic acid. Accordingly, FSW has a sweet taste, and a decrease of 8.3% was observed in the ether extractive fraction. An increase in ether extractives was reported in fermented cassava by Ezeala (1984) while Bokanga et al. (1988) observed a decrease of 38% in fat.

A tremendous decrease (73–75%) was observed in ash content of fermented cassava flour. Our results corroborate with those of Bokanga et al. (1988) who have reported a 59% decrease in total ash content in fermented cassava. Most of the soluble minerals would have been leached out during fermentation while some essential minerals might have been assimilated by the organisms. Similar decreases in the proximate components were reported during the preparation of placali, a traditionally fermented food product from cassava (Firmin, 1992). These results

Table 3. Amino Acid Composition of Fermented and Nonfermented Cassava Flour

amino acid	composition, mg/100 g of flour		
	NFC	FSO	FSW
aspartic acid	181.48	107.71	45.97
threonine	83.31	49.91	25.33
serine	100.30	51.36	28.40
glutamic acid	470.11	162.29	73.64
glycine	87.79	64.09	37.48
alanine	155.76	97.08	36.97
valine	106.91	67.46	39.09
isoleucine	73.40	52.65	37.26
leucine	122.96	76.80	42.90
tyrosine	73.16	47.66	33.38
phenylalanine	69.86	38.48	41.65
histidine	396.95	156.17	31.70
arginine	380.43	14.65	35.94
proline	37.76	25.76	11.71
total lysine	120.83	88.55	45.82
cystine	39.18	25.12	15.66
methionine	29.03	24.79	19.54

indicate the need for adequate supplementation of fermented cassava with proteins and minerals to upgrade its food value.

Amino Acid Profile. Amino acid composition of fermented and nonfermented cassava as given in Table 3 indicates that glutamic acid, histidine, arginine, and aspartic acid were the predominant amino acids in the nonfermented cassava flour from variety H-1687. Several workers have reported that glutamic acid and aspartic acid dominated the amino acid profile of fresh cassava (Bokanga et al., 1988; Ezeala, 1984; Muindi and Hanssen, 1981). Gomez et al. (1984) and Ekpenyong (1984) observed that glutamic acid and arginine were the predominant amino acids in dried cassava chips. The contradictory reports indicate the possibility of varietal variations influencing the amino acid profiles.

There was a decrease in all the amino acids in FSO and FSW compared to the NFC (Table 3). Decreases in arginine, histidine, and glutamic acid were quite evident in FSO and FSW. A considerable decrease was also noticed in aspartic acid, alanine, leucine, and total lysine in both fermented flours. The decrease in amino acids observed in FSO and FSW was mainly due to the low protein content of these flours compared to the NFC. The free amino acids in the fermented flours may be low due to leaching of them into the steep liquor. A considerable amount of bound amino acids (of protein) is also leached out/utilized by the microflora. As a consequence, the net amino acid levels in fermented flours (FSO and FSW) were found to be lower than the corresponding values in NFC (Table 3).

The chemical scores of essential amino acids calculated with comparison to the FAO/WHO (1973) reference pattern indicated that most of the amino acids were limiting in NFC and FSO (Table 4). Leucine followed by isoleucine were the first two limiting amino acids in NFC, while leucine and threonine were the two most limiting amino acids for FSO. In the fermented sweet flour, only leucine and threonine were found to be limiting while the others exceeded the FAO/WHO reference scores. Varietal variations in amino acid profile are likely to influence the chemical scores. Bokanga et al. (1988) observed that lysine was the most limiting amino acid in fresh and fermented cassava, while Ezeala (1964) reported that (Cys + Met) were the most limiting amino acids. We found that the sulfur-containing amino acids (Cys + Met) exceeded the FAO/WHO reference pattern by 37% in FSW. Nevertheless, when the total amount of (Cys + Met) present in NFC and FSW are considered on a weight basis (mg/100 g of flour), there is a ca. 50% reduction in the content of these sulfur-containing amino acids in FSW. This points to the fact that although there is reduction in the quantity of amino acids in FSO and FSW, the protein quality of FSW is superior to NFC and FSO. The protein fraction retained in FSW appears to be of high quality, although the explanation for such a phenomenon is difficult.

Sugar Profile. Sucrose, glucose, and fructose were the prominent sugars in nonfermented cassava (variety H-1687) flour, and among these sucrose was present in large amounts (Figure 1a). These findings are in agreement with those of Ketiku and Oyenuga (1970). Maltose was present in only insignificant amounts in nonfermented cassava flour, and the peak was difficult to separate from that of sucrose.

It was observed that the sucrose peak reduced tremendously with fermentation while the fructose peak increased. This suggests possible hydrolysis of sucrose to glucose and fructose by the organisms during fermentation and the preferential utilization of the glucose so formed. Three specific peaks representing some unidentified water-soluble oligosaccharides appeared in the fermented sour flour (Figure 1b). These might have been formed as intermediary hydrolytic products of starch breakdown.

There was appreciable leaching out of soluble oligosaccharides and sugars during the preparation of FSW (Figure 1c). There are no reports on the sugar profiles of fermented cassava. The emergence of new oligosaccharide peaks in FSO suggests that some starch is utilized for the growth of the microorganisms.

***In Vivo* Digestibility.** The percent digestibility of starch in fermented and nonfermented cassava flour (native vs cooked) is given in Table 5. The nonfermented flour in the native state was digestible to the extent of ca. 81%. Cooking was found not to influence the digestibility of cassava flour. In the case of cassava starch, cooking was found to raise the digestibility from 76% for native starch to 90% (Moorthy and Padmaja, 1991). It is likely that the fiber and other components in cassava flour may influence the intestinal retention time of the diet, thereby affecting its digestibility. Bjorck et al. (1988) found that the addition of fiber to native potato starch diet decreased its *in vivo* digestibility. Fermentation did not significantly alter the digestibility of starch in cassava flour, and only a 2–6% increase was observed in digestibility due to fermentation. Cooking also did not increase the digestibility of fermented or nonfermented cassava flour (Table 5).

Table 4. Essential Amino Acid Levels and Their Chemical Scores in Fermented and Nonfermented Cassava Flour

amino acid	essential amino acid levels g/16 g of N				chemical scores, %		
	FAO/WHO (1973) ref pattern	NFC	FSO	FSW	NFC	FSO	FSW
isoleucine	4.0	3.11	3.27	5.09	78	82	127
leucine	7.0	5.21	4.77	5.86	74	68	84
lysine	5.5	5.12	5.50	6.26	93	100	114
methionine + cystine	3.5	2.89	3.10	4.81	83	89	137
phenylalanine + tyrosine	6.0	6.06	5.35	10.25	101	89	171
threonine	4.0	3.53	3.10	3.46	88	78	87
valine	5.0	4.53	4.19	5.34	91	84	107

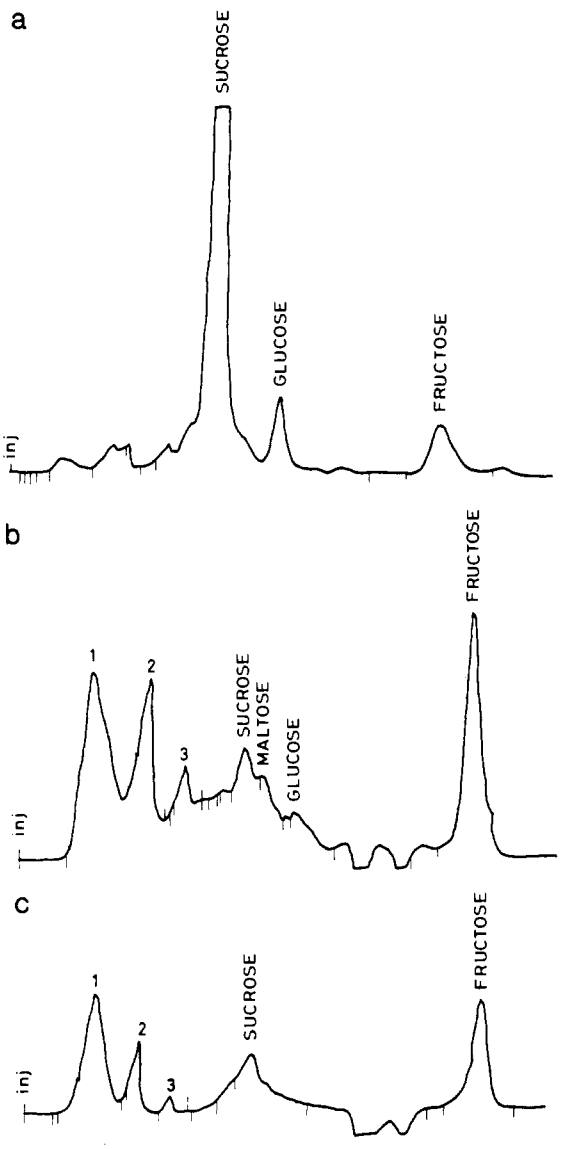

Figure 1. (a) Sugar profile in nonfermented cassava; inj = injection. (b) Sugar profile in fermented sour cassava; 1, 2, and 3 = water-soluble oligosaccharides. (c) Sugar profile in fermented sweet cassava; other legends as in b.

Table 5. Percent Digestibility of Starch in Fermented and Nonfermented Cassava Flour

type of flour diet	% digestibility of starch mean ± SEM	% increase in digestibility of fermented cassava[a]
nonfermented cassava flour (native)	80.85 ± 1.05	
fermented sour flour (native)	82.80 ● 1.32	2.36
fermented sweet flour (native)	85.67 ± 0.26	5.63
nonfermented cassava flour (cooked)	81.83 ± 0.20 (+1.20)[b]	
fermented sour flour (cooked)	85.71 ± 0.20 (+3.4)	4.53
fermented sweet flour (cooked)	85.93 ± 0.81 (±0.30)	4.77

[a] Compared with the nonfermented native and cooked flour. [b] Values in parentheses indicate percent increase in digestibility of cooked flour over native flour.

Our earlier studies showed that fermentation of cassava with the mixed-culture inoculum detoxifies much of the cyanide in fresh cassava, and it is possible to obtain a product with 10.50 mg/kg of cyanide (fresh weight; Padmaja et al., 1993) and a dried product with only small quantities of cyanide (25 mg/kg of DM). However, during the course of fermentation, the soluble proteins as well as free amino acids are leached out/utilized by the organisms which slightly deteriorates the nutritional quality of fermented cassava flour. Nevertheless, amino acid profiles indicate that the protein retained in fermented cassava is of superior quality. Our study suggests the need for suitable protein and mineral fortification of fermented cassava flour to make it a balanced food, especially in countries where it is used as a primary staple.

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