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NUTRITIVE VALUE AND SENSORY QUALITY OF FERMENTED CASSAVA SOY SUPPLEMENTED FLOUR

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ABSTRACT
In this study unfermented cassava mash was prepared and developed into four different samples A, B ,C and D as an approach to add and improve nutritive value. To obtain flour sample (A) containing only 100% unfermented cassava, the mash was dried and milled. Part of the unfermented cassava mash was supplemented with to prepare flour sample (B) in the ratio of 80:20 respectively. Flour sample (D), 100% was obtained by fermenting cassava mash for 48 hours followed by drying and milling. Flour sample (C) was obtained from fermented cassava mash supplemented with soy mash of ratio of 80:20 respectively. All the cassava flour samples were each subjected to proximate composition, hydrocyanic acid, sensory quality and nutritive value analyses. The results showed that protein, fat, ash, crude fiber increased with fermentation. The protein content of fermented cassava-soy flour was 12.14±0.28 and was found to be greater than the unfermented cassava-soy flour (9.84±0.04). Hydrocyanic acid deceased with soy supplementation and fermentation. The results of sensory quality analysis showed that there was no significant difference (p>0.05 in the overall acceptability of the various cassava flours at. However, the results revealed significant differences in colour, aroma, texture and taste of the four cassava flours. The fermented soy supplemented cassava flour was higher in nutritive value than the rest of the flour samples in all aspects including; WG (4.01), NU (0.02), FND (52.56),PER (0.033), and FCE (54.76). These were closely related to the control (casein).

Keywords: Supplementation, fermentation, cassava, Sensory, Nutritive(c)

INTRODUCTION

The increasing demand for high quality cassava composite flour in Nigeria occasioned by a deliberate Federal Government policy of 40% cassava inclusion in bread has thrown up a series of new challenges to food scientist and technologist in Nigeria. This is because of the need to develop flours that possess qualities close to those of wheat, which has for many years been the primary source of flour used by bakers. Apart from the above, there is also the challenge of developing nutritionally rich flours that can serve as food vehicle for transporting vital nutrients to local populations. The use of composite flours for this reason has however, been known for a long time now. Seibel (2007) defined composite flour as a mixture of flours from tubers rich in starch (e.g. cassava, yam, sweet potato) and/or protein-rich flours (e.g. Soy, peanut) and/or cereals (e.g. maize, rice, millet, buckwheat), with or without wheat flour.

Soy supplemented cassava flour as the name implies is composite flour made out of soybeans and cassava. The compounding ratios may vary, but the most commonly known ratio is 80% cassava: 20% soybean. Soybean is a legume and is rich in protein content. This legume can therefore, compliment the low protein and fatty acid content of cassava. Soybean is a known rich source of dietary fiber, magnesium, manganese, phosphorus, copper, iron and vitamin K It is also low in cholesterol and sodium (Liu and Pan, 2011) and most of these mentioned nutrients are absent in cassava. (Hahn and Keyser ,1985). Optimum utilization of cassava is often prevented in developed countries because of its poor nutritive value and presence of cyanogenic glucosides (Cooke and Coursey, 1981) not properly processed and can cause serious health hazards. Cassava products are also known to be deficient in vital vitamins and minerals. Supplementation of cassava with soybeans therefore enhances its nutritive value as vitamins, essential amino acids and proteins get formed through biosynthesis. Furthermore, fermentation of the cassava roots used in the production of fermented cassava flour can also enhance micronutrient bioavailability and ensure removal of the toxic cyanogenic glucosides according to previous studies (Hahn and Keyser, 1985). Information on the quality of fermented

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cassava flours supplemented with soybean for product development is lacking.

The objectives of this study were therefore, to provide information on the proximate composition, hydrocyanic acid content, sensory quality and nutritive value of fermented cassava flours supplemented with soybean for the production of baked goods.

MATERIALS AND METHODS:

Sources of raw materials

Both cassava tubers (TMS 30572 variety) and soy-beans (variety) used for the study were obtained from the University of Agriculture, Makurdi farms. Pure strains of *Saccharomyces cerevisae* was obtained from Benue Brewery Ltd, Makurdi and transported under controlled conditions to the Food Microbiology Laboratory of Department of Food Science and Technology, University of Agriculture Makurdi.

Sample preparation

Fresh cassava roots were peeled, washed and cut into equal sizes of 8 – 12 cm long before grating. The grated cassava mash was dewatered and made into solid grated cassava mash. The mash was sun dried to attain moisture content of about 20% and subjected to solid media fermentation by mixing 0.5 g yeast with the solid cassava mash on a tray using a sterilized glass rod. Afterwards 100 g of the mash was spread on the aluminum tray of diameter of 50 cm and 2 cm average layer thickness. The *S. cerevisae* (yeast) mixed solid cassava mash was then covered with foil paper and allowed to ferment for 48 hours. The first treatment was unfermented, the second and third were fermented and supplemented with soybean flour and the fourth was only fermented cassava flour. Soybean seeds were sorted and blanched at $90^0C \pm 2^0C$ for 30 minutes, then dehulled, dried, milled and sieved into flour.

Proximate composition

The nutritional parameters included the determination of moisture, crude fat, protein, ash content and crude fibre by AOAC (1995). Carbohydrate was determined by simple difference as described by Ihekoronye and Ngoddy (1985).

Determination of hydrocyanic acid content

To determine the effectiveness of the fermentation and reduction of the cyanogenic glucosides, hydrocyanic content of the sample was determined using the alkaline titration method of AOAC (1995).

Sensory evaluation

The flour was presented into four clean transparent beakers for evaluation of colour, aroma, texture and overall acceptability by ten semi-trained panelist by using a 9-point hedonic scale (Ihekoronye and Nggoddy, 1985) . The flour samples were further made into stiff porridge by reconstituting in water and heating over fire while stirring until a thick stiff porridge was formed. The porridge was evaluated for taste.

Procedure for feeding trial

Experimental diets of known chemical composition were prepared and evaluated for their nutritive values using wistar strains albino rats of 21 days old and 29 g average weight. (Ossai and Malomo 1985). The albino rats were purchased from the College of Veterinary Medicine, University of Agriculture, Makurdi Nigeria. Five rats were randomly allotted to each of the five experimental diets. The rats were housed in individual metabolic cages placed on cardboard to permit collection of faeces Ten grammes of each experimental diet including casein were given daily and water ad-libitum. Milk casein served as the control. Data from daily feed intake, and weight gain were obtained by weighing the remained fed diet and all the rats individually. Protein consumption was calculated from the feed intake. Day 10 marked the beginning of the feaces collection which lasted for 16 days. The feaces were stored in small screw bottles tightly capped and stored in a refrigerator until used. At the end of the collection period, the bulk feaces for each rat were dried, weighed and milled into fine powder. The nitrogen content of the faeces was determined by the standard Kjeldah method (AOAC, 1995).

Statistical evaluation:

The statistical significance of the observed differences among the means of triplicate readings of experimental results were evaluated for mean and standard deviation while means were separated using Duncan's Range Test. (Duncan,1995).

RESULTS AND DISCUSSIONS:

Proximate composition of cassava flour samples (dry weight basis):

From the results in Table 3, it was observed that protein values increased with the addition of 20% soy flour to 80% cassava flour. However, fermentation seemed to increase the protein value further from 9.84±0.04 in sample B (80% unfermented cassava flour '80% UCF' : 20% soy flour '20 % SF') to 12.14±0.28 in sample C (80% fermented cassava flour '80% FCF' : 20% soy flour '20% SF'). A significant increase was also observed in the fat (5.73±0.07), ash (3.84±0.64) and crude fibre (6.94±0.02) content values of sample C (80% FCF : 20% SF) compared to those sample A, B and D. These results show the viability of using fermented soy-cassava in enhancing the nutritive value of cassava flours. The increased protein value of sample C (80% FCF : 20% SF) compared to the other samples can be attributed to two factors. First is fermentation of the cassava aided by *S. cerevisiae* which has been previously reported by Antai and Mbongo (1994); Boonnop *et al*., (2009) and Akintomide and Antai 2012) to increase the growth of single cell proteins as well as increase enzyme activity. The authors reported a 20% increase protein content of cassava after solid state fermentation and the work of Balagopalan and Padmaja (1988) who developed a solid state fermentation process for the protein enrichment of cassava flour and cassava starch factory wastes using the fungus *Trichoderma pseudokoningii* Rifai. Similarly, Oboh and Elusiyan (2007) reported increases in proximate composition of cassava fermented with pure strain of *S. cerevisae* for 3 days. The second factor is the presence of 20% soybeans in the cassava flour since soybeans is a known rich source of protein (Berk, 1992; Liu and Pan, 2011). The case of increased fat content of sample C (80% FCF : 20% SF) compared to the other samples can also be discussed from two perspectives.First, it has been reported in Akindumila and Glatz (1998) and Boonnop *et al*., 2009) that some group of fungi produce microbial oils during fermentation. On the other hand, the addition of 20% soybeans in the cassava flour can also be said to be responsible for the increase in fat content since soybeans is rich in fats (Liu and Pan 2011; Sato, 2011). The later can be seen from Table 3, where the fat content of sample B and C both of which contained 20% soy beans were relatively higher than the other samples even sample B was unfermented. Fermentation cannot therefore be said to be the principal reason for the increase in fat content although it also played a marginal role as can be seen when comparing fat content values for sample A and D in Table 3.

Table 1: Blend Formation of components per 100g

Sample	Cassava Flour	Soy Flour	Nature of product	Fermentation time
A	100	0	unfermented	0 hours
B	80	20	unfermented	0 hours
C	80	20	fermented	48 hours
D	100	0	fermented	48 hours

Key: A – 100% Unfermented cassava flour (100% UCF): 0% soy flour
B – 80% Unfermented cassava flour (80% UCF) : 20% soy flour (20% SF)
C – 80% Fermented cassava flour (80% FCF) : 20% soy flour (20% SF)
D – 100% Fermented cassava flour (100% FCF) : 0% soy flour

Table 2: Composition of experimental diet (g/100g diet)

Components(g/100g) of A.M Experimental Diets Samples	A	B	C	D	M
Cassava flour	10	10	10	10	10
Casein (Milk Protein)	0	0	0	0	10
Corn Starch	70	70	70	70	70
Non-Nutritive Fibre	1	1	1	1	1
Sugar	5	5	5	5	5
Groundnut oil	8	8	8	8	8
Vitalyte (Salt/Vitamin)	6	6	6	6	6
Total Mass	100	100	100	100	100

Key: A – 100% Unfermented cassava flour (100% UCF): 0% soy flour
B – 80% Unfermented cassava flour (80% UCF) : 20% soy flour (20% SF)
C – 80% Fermented cassava flour (80% FCF) : 20% soy flour (20% SF)
D – 100% Fermented cassava flour (100% FCF) : 0% soy flour

Hydrocyanic acid content:

Table 4 shows the results of hydrocyanic acid content analyzed. From the Table, it was observed that fermentation had a significant effect ($P \leq 0.05$) on the hydrocyanic acid Content of the cassava flour reducing it from 16.42±0.615 in sample A (100% UCF) to 9.85±0.177 in sample D (100% FCF). Supplementation with soybeans also played a significant ($P \leq 0.05$) effect on the cyanide acid content of the flour as the 80% unfermented cassava flour (sample B) supplemented with 20% soy flour had a lower HCN value (9.32±0.115) than sample A (100% UCF) value of 16.42±0.615. Sample C (80 %FCF:20% SF) and D (100% FCF) also behaved in a similar way as the HCN value reduced from 9.85±0.177 in Sample D to 9.25±0.577 in Sample C due to supplementation. The values recorded for Hydrocyanic acid content for all the samples except sample A fall below the recommended maximum safe level of 10 mg/kg for ready to eat (RTE) cassava chips set by the Food Standards Australia New Zealand (FSANZ 2008a and FSANZ 2008b); Codex Alimentarius Commission (1989). The observed reduction in HCN content with soy flour supplementation can be explained from the view point of the reaction pathway for cyanogenic glucosides in the human system. This can be seen if we decide to compare reducing it from 9.42±0.615 to 16.42±0.615. The values obtained for samples B, C and D fall below the codex. The result in Table 4 showed that the hydrocyanic acid content unfermented cassava flour was higher (16.42 mg) than the 10 mg recommended safe level. This hydrocyanic acid content was reduced to 9.57 mg after 48 hours fermentation and was safe below the recommended level. This was in agreement with the report of Achinewhu *et al.*, (1998) who reported that solid media fermentation aids in degrading anti-nutritional factors and toxic components such as cyanide.

Sensory quality of cassava flours: Table 5 represent the sensory quality mean scores for cassava flours. The results showed that the panelist preferred all the experimental cassava flours evidence from the sensory quality attributes except aroma of sample A. There was however, no significant difference ($P > 0.05$) in the texture and color of samples B, C and D. It should also be noted that sample B had similar constituents as sample C (both contain 20% soy flour). This means that supplementation did not impact much on the sensory properties of the flour.

For the stiff porridge, sample C was observed to be more acceptable to the panelist in terms of colour, aroma, taste and general acceptability (GA). GA was however not significantly different ($P > 0.05$) for sample A, B and C. This shows that the supplemented flour was more acceptable when made into stiff porridge.

Table 3: Proximate Composition of Cassava Flour Tables-*Garamond*, 10 points:

Sample	Moisture	Ash	Protein	Fat	Crude Fibre	Carbohydrate	Energy
A	11.25±0.35[a]	1.6±0.07[a]	2.36±0.07[a]	1.10±0.1[a]	3.89±0.49[a]	91.11±0.78[a]	384.97[a]
B	11.25±0.35[a]	3.66±0.35[b]	9.84±0.04[b]	5.20±0.04[b]	4.80±0.01[b]	76.77±0.34[b]	393.24[b]
C	10.25±0.35[a]	3.84±0.64[b]	12.14±0.28[c]	5.73±0.07[c]	6.94±0.02[b]	71.40±0.78[b]	385.75[b]
D	11.95±0.07[a]	2.55±0.35[c]	3.85±0.16[d]	1.42±0.35[d]	5.79±0.14[b]	86.38±0.93[d]	373.70[a]

Means with different superscripts on the same column are significantly different ($p \leq 0.05$). Values represent means of duplicates.

Key:A – 100% Unfermented cassava flour (100% UCF): 0% soy flour
B – 80% Unfermented cassava flour (80% UCF) : 20% soy flour (20% SF)
C – 80% Fermented cassava flour (80% FCF) : 20% soy flour (20% SF)
D – 100% Fermented cassava flour (100% FCF) : 0% soy flour

Table 4: Hydrocyanic Acid Content of Experimental Composite Flour:

Sample	HCN Content (m**g)**
A	16.42±0.615[a]
B	9.32±0.115[c]
C	9.25±0.577[d]
D	9.85±0.177[b]

Means with different superscripts on the same column are significantly different ($p \leq 0.05$). Values represent means of duplicates.

Key: A – 100% Unfermented cassava flour (UCF)
B – 80% Unfermented cassava flour (UCF) + 20% soy flour (SF)
C – 80% Fermented cassava flour (FCF) + 20% soy flour (SF)
D – 100% Fermented cassava flour (FCF)

Nutrition quality of protein enriched fermented and unfermented cassava flour:

Table 6 showed that the rats fed with diet sample A (100% UCF) had a negative values for weight gain (– 10.85g), feed conversion efficiency 'FCE' (– 19.69), protein efficiency ratio 'PER' (– 2.49), nitrogen utilization 'NU' (–2.25), feed nitrogen digestibility 'FND' (–15.02), and corrected protein efficiency ratio 'C.PER' (–3.13). Rats fed with diet sample B (80% UCF:20% SF) had positive values for all the parameters except FND (-64.50) which had a negative value while those fed with diet sample C (80% FCF:20% SF), D (100% FCF) and M(control) all had positive values for all the parameters tested.

Mean Daily Feed Intake (MDFI)

It can be observed from Table 6 that MDFI was relatively higher for diet samples A (100% UCF) and D (100% FCF). Such a high feed intake by the test subjects (rats) can be related to the poor protein content of the administered diets. Diet samples A and D contained only cassava with no protein supplementation and as such the rats had to consume the diets more (without getting satisfied) to compensate for the low protein lost during feeding on the diets. The opposite can be said for diets B (80% UCF : 20% SF), C (80% FCF : 20% SF) and M (Milk control). This is because B and C were both supplemented with protein rich soy flour, while the control M contained protein rich milk casein.

The presence of essential amino acids in these diets (B, C and M) explains the significant weight gain observed in rats fed with diets M (5.41), C (4.01) and B (2.75) and weight loss observed in rats fed with diet samples A (–10.85). The low feed intake of rat fed with B, C and M could also be because of the higher protein contents which indicate the availability of essential amino-acids that supported growth. In diet B (7.87), the mean daily feed intake was higher than mean daily feed intake of (7.83) by rats, this indicated the protein superiority of C over B although, both rich in protein.

Table 5: Sensory Attributes of Cassava Flour products

Sample	Sensory attributes of cake					Sensory Attributes of porridge			
	Color	Aroma	Texture	Acceptatability		Colour	Aroma	Texture	Taste
A	8.0[a]	6.0[a]	7.5[a]	7.4[a]	7.2[a]	5.8[a]	7.0[a]	6.0[a]	7.3[a]
B	6.4[b]	6.7[b]	7.0[b]	7.2[a]	6.5[a]	6.5[b]	7.2[b]	7.2[a]	7.0[a]
C	6.0[b]	6.2[b]	7.0[b]	6.4[a]	7.0[c]	7.5[c]	6.3[b]	6.3[b]	7.3[a]
D	6.3[b]	5.8[c]	6.7[b]	6.4[a]	6.2[b]	6.1[b]	5.8[c]	5.8[c]	5.3[b]

Means with different superscripts on the same column are significantly different ($p \leq 0.05$). Values represent means of duplicates.

Key: A – 100% Unfermented cassava flour (100% UCF): 0% soy flour
B – 80% Unfermented cassava flour (80% UCF) : 20% soy flour (20% SF)
C – 80% Fermented cassava flour (80% FCF) : 20% soy flour (20% SF)
D – 100% Fermented cassava flour (100% FCF) : 0% soy flour

Table 6: Nutritive Values of Cassava Flours

Diet	Initial Weight (g)	Final Weight gain (g)	Weight gain (g)	Daily Weight gain (g)	Mean daily feed intake	FCE	PER	NU	FND	C.PER
A	65.35[a]	45.50[d]	-10.85[d]	-0.98[d]	8.09[a]	-19.69[e]	-2.49[d]	-2.25[c]	-15.02[d]	-3.13[e]
B	29.50[e]	32.25[e]	2.75[b]	0.098[b]	7.87[b]	80.20[b]	0.029[c]	0.029[b]	-64.50[e]	0.75[d]
C	43.00[d]	47.01[c]	4.01[a]	0.143[a]	7.83[b]	54.76[c]	0.033[b]	0.029[b]	52.56[b]	0.91[b]
D	52.05[b]	53.23[a]	1.18[c]	0.042[c]	8.06[a]	191.9[a]	0.031[b]	0.028[b]	36.70[c]	0.84[c]
M	44.60[c]	50.01[b]	5.41[a]	0.193[a]	7.05[c]	41.19[d]	0.094[a]	0.084[a]	78.98[a]	2.50[a]

Means with different superscripts on the same column are significantly different ($p \leq 0.05$).

Key: A – 100% Unfermented cassava flour (100% UCF): 0% soy flour
B – 80% Unfermented cassava flour (80% UCF) : 20% soy flour (20% SF)
C – 80% Fermented cassava flour (80% FCF) : 20% soy flour (20% SF)
D – 100% Fermented cassava flour (100% FCF) : 0% soy flour
M – 100% Casein protein (Control)
FCE – Feed conversion efficiency
PER – Protein Efficiency Ratio
NU – Nitrogen Utilization
FND – Feed Nitrogen Digestibility
C.PER – Corrected Protein Efficiency Ratio

Mean Daily Weight Gain (MDWG)

Within the experimental period of 28 days rats fed diet A lost weight by -0.83 on daily basis. This amounted to -10.85 weight loss of rat at the end of the experimental period. Conversely, diets B, C, D and M gained weight. Although all gained weight as follows: B (2.7 g), C (4.0 g), D (1.18 g) and M (5.41), there was significant difference in weight gain.. Rats fed with casein (M) gained more weight and rats fed with C had significantly similar weight gain with that of rats fed with casein. The mean daily weight gain also was in that order as seen in Table 6. The difference in daily weight gain were attributed to the protein quality and amount of feed intake Obizoba and Obiano, 1987; Ossai and Malomo, 1985). They reported that protein quality and amount of feed intake have a combined effect on weight gain of tested laboratory animals. The casein fed group had the highest weight gain compared with that of group A and D whose protein was of low quality. The study also revealed that cassava flour protein qualities increased with fermentation. This was proven by the fact that diet B (soy unfermented cassava flour) supplied less nutrients for growth compared with C (soy fermented cassava flour). Similarly, rats fed with fermented cassava flour showed an increase (1.18 g) in weight while the group fed with fresh cassava flour showed a decrease by -10.85 of weight. Thus fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essentials amino acids and proteins by improving proteins quality and fibre digestibility, it also enhances micronutrient bioavailability and aids degradation of anti-nutrient factors (Achinwhu *et al.*, 1998).

Feed Coversion Efficiency (FCE)

The feed conversion efficiency (FCE) of group of rats fed with diet A (fresh cassava flour) was evaluated to be negative since there was weight loss. However, the variable values of feed conversion efficiency of groups of rat fed with diet D (fermented cassava flour) showed the greatest value (191.9), followed by B (soy-fermented cassava flour) (80.20). The FCE of rats fed with diet C (soy-fermented cassava flour), (54.76). This could be that poorer diet showed higher and negative FCE.

These results are in agreement with the result of Ossai and Malomo (1985), who reported that the diet which was poor for protein tended to show higher and negative FCE.

Protein Efficiency Ratio (PER)

The PER of rats fed with diet A showed -2.49 and was least, followed by B, C and D which were not significantly different, indicated weight gain by the respective groups of rats. However, the PER of rats fed with casein as control was higher indicating presence of lysine, the limiting essential amino acid (Ossai and Malomo, 1985).

The groups fed with diet B, C and D was not significantly different from each other but close to group fed with casein indicating also presence of limiting essential amino acids.

The corrected-protein efficiency was directly proportional to the PER and its increase in respective groups was also explained by the report of Ossai and Malomo (1985).

Nitrogen Utilization

The Nitrogen utilization in group of rats fed with diet A (fresh cassava flour) was evaluated negative which implied non-nitrogen utilization. However nitrogen utilization evaluated in group fed with casein (0.084) was greater followed by C (0.029), D (0.0277) and B (0.026)

Thus, increase in nutrient values was attributed to improved protein quality and weight gain as reported by Hughes (1997). The amino acid was responsible for the increasing values as reported by Ossai and Malamo (1985). Faecal Nitrogen collected showed higher nitrogen percentage than nitrogen obtained in the diet. Though the faecal collection of group fed with casein was small followed by group C, B, D and much in group A. Feed Nutrient Digestibility. (FND) of five experimental diets ranged from -15.02 as least value by diet A group fed rats to 78.98 for diet M group. Other values for B, C and D were 64.5, 52.56 and 36.7 respectively. According to Ossai and Malamo (1989), fibre content of a diet affect digestibility, From the result, group fed with casein (M) had highest digestibility followed by 64.5 (B), 52.56 (C) and group D (36.7). The diet with high nutrient digestibility indicated low content of dietary fibre irrespective of the cellulose added which affected digestibility in groups the same way.

CONCLUSION

The study showed that supplementing some full-fat soybean flour with fermented cassava flour will result in considerable improvement in the flour's protein content and gross energy for growth. The adoption and use of this composite flour by populations in which cassava flour is a staple food will likely reduce prevalence of protein malnutrition. This study revealed that fermented cassava flour supplemented with soybean in the ratio of 80:20, respectively, seemed to be the best and therefore, highly recommended for development of baked goods.

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