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Microbiological and Sensory Evaluations of Fermented Rice Snack (masa) Supplemented with Soybean

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Abstract: The microbiological and chemical quality of rice masa with or without (control) 20% w/w soybean supplement were analyzed during production and ambient (28±2°C) storage. The aroma, visual appearance, firmness of the supplemented (SBS) and unsupplemented (USP) stored samples were also investigated. The Total Viable Counts (TVCs) of SBS decreased from 8.30 to 5.49 $\log_{10}$ cfu g^{-1} while that of USP increased from 5.38 to 8.93 $\log_{10}$ cfu g^{-1} during fermentation. Eleven microbial genera were identified during fermentation with *Lactobacillus, Aspergillus* and *Saccharomyces* spp predominating. Stored samples showed unacceptable sharp population increases after 24 h with maximum load occurring in the SBS. Significant difference ($p = 0.05$) existed in TVCs and pH of SBS and USP during production and storage. The aroma and visual appearance of SBS and USP differed significantly with aroma of SBS being more preferred. No significant difference existed in the firmness of both products.

Key words: Microbiological, aroma, visual appearance, firmness, rice, soybean, supplementation, fermentation

INTRODUCTION

Recently, interest in cereal-based foods especially rice (*Oryzae sativa*) and its products have increased in developing countries due to problem of hunger and malnutrition. Masa a traditional fermented product from rice has become highly popular in Nigeria and other parts of the world where it is known by various names[1,2] and constitute a major dietary component in such countries. However, the relatively low nutritional value of rice based diet has resulted in efforts to improve their nutritional quality by supplementation with more nutritious food such as soybean which had been known to increase protein content of cereal based products[3,4].

The effects of supplementation of other cereal based products such as maize meals (Ugali, Kwoka) with soybeans and their microbiological, sensory and nutritional qualities have been reported[3,5,6]. But that of soybean supplemented rice 'masa' and its acceptability during ambient storage is lacking. The aim of this work was therefore, to assess the influence of natural fermentation characteristics on the type and population of microorganisms associated with the fermentation of soybean supplemented rice masa. In addition, the microbial and sensory attributes of ambient stored soybean supplemented rice 'masa' were also investigated.

MATERIALS AND METHODS

Sources of materials: White polished rice (*Oryzae sativa*), soybean (*Glycin-max*), bakers yeast (Engedura, The Netherlands), granulated sugar and onions were each obtained from Umuahia Main Market. But the earthenware pots consisting of seven shallow 'pots' (kasko masa) used for the baking of masa were obtained from Central Market Bauchi, Nigeria.

Preparation of rice and soybean for fermentation: Sorted rice and dehulled soybean were weighed out in 5:1 ratio (800 g of rice to 160 g of soybean). Of the sorted rice, 600 g of it and the soybean were soaked for 12 h and thereafter drained. The rice and soybean were then added 25 kg^{-1} of onion and blended in 360 mL of tap water (1:2 w/v) using blender (Moulinex, Paris, France) for 2 min to obtain the slurry. The remaining 200 g of the sorted rice was washed, cooked and added to the slurry. These were thoroughly mixed for 60s and 20 g kg^{-1} of Baker's yeast was added and further mixing carried out for 2 min. The slurry was then divided into four portions and allowed to ferment for 12 h as traditionally practiced. Production of the unsupplemented (USP) rice masa (the control) was done without the addition of soybeans. The microbiological and chemical qualities of both soybean

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Table 1: Total viable counts (TVCs), pH and titratable acidity (TA) of fermenting slurries of soybean supplemented and unsupplemented rice masa

		Fermentation time h			
Evaluated parameters		0	4	8	12
TVCs (log_{10} cfu g^{-1})	Supplemented	8.30±0.02a	8.16±0.01a	6.36±0.01b	5.49±0.01b
	Unsupplemented	5.38±0.02b	5.25±0.03b	8.54±0.50a	8.93±0.01a
pH	Supplemented	5.91±0.10a	5.64±0.12a	5.42±0.25a	5.03±0.17a
	Unsupplemented	5.32±0.18b	4.93±0.15b	4.83±0.12b	4.72±0.06b
TA (% lactic acid)	Supplemented	0.34±0.2a	0.35±0.3b	0.48±0.1a	0.47±0.2a
	Unsupplemented	0.38±0.1a	0.44±0.2a	0.46±0.2a	0.49±0.3a

Each value is the mean±SD of 4 determinations. Means within the same columns for respective evaluated parameter followed by different letters are significantly different at p = 0.05

supplemented (SBS) and unsupplemented (USP) slurries were determined at intervals of 0, 4, 6 and 12 h of fermentation. Aseptic procedures were followed for samples for microbiological analyses.

Baking and storage of soybean supplemented and unsupplemented rice 'masa': Into each of the fermented portion was added 60g kg^{-1} granulated sugar to improve the taste as traditionally practiced. Heating 'kasko masa' containing little oil as traditionally practiced and allowed to cool baked a 30 g quantity of the fermented slurry. The samples (SBS and USP) were wrapped respectively in aluminum foil and analyzed at intervals of 0, 1 and 2 days during tropical ambient (28±2°C) storage.

Microbiological analyses of slurry and stored rice 'masa': Total Viable Counts (TVCs) of samples (slurry or masa) were determined following serial dilutions prepared using a Stomacher model 400 (Colworth Seward Medical, London, UK). Pour-plating of the serially diluted pooled sample (20 g in 180 mL of 1 g L^{-1} peptone water) was carried out in duplicate on Tryptone Soy Agar (TSA); Biotec Laboratory Ltd. Suffolk, UK) and incubated at 35-37°C for 24-48 h to obtain 25-250 colonies per plate[7]. Lactic acid bacteria were isolated using deMan Rogosa Sharpe (MRS) agar following pour-plate and anaerobic incubation (Gas Pak, BBL, Becton Dickinson and Co USA) at 30°C for 5 days. Coliforms were determined using MacConkey agar incubated at 35-37°C for 24 h[8]. Other microorganisms isolated involved the use of Baird-Parker agar for *Staphylococcus aureus*, Bacillus cereus selective agar for *Bacillus cereus* and modified Malt extract agar (pH 3.5) for fungi.

Procedures used in identifying the microorganisms: Typical colonies picked at random from countable plates were purified (by streaking), kept in slants under refrigeration and subjected to different morphological (gram stain and spore tests) and biochemical tests. The confirmation of the presumptive isolates was carried out as detailed[9,10]. Similarly, fungal isolates were identified after morphological (lactophenol staining of moulds) and biochemical tests including fermentation of maltose, sucrose, mannitol, glucose, arabinose and lactose used for yeast[8,11].

Chemical analysis of the samples: The pH of the samples was measured in duplicate with a pH meter (model CD 640, WPA, Linton, Cambridge, UK) after homogenization (1:2 sample to deionized water). Titratable acidity was determined by titration against 0.I N Na0H to a phenolphthalein end point (pink) and expressed as percentage lactic acid[7].

Sensory evaluation: A trained 10-member panel consisting of graduate students and staff evaluated the stored samples for aroma, visual appearance and firmness at intervals of 0, 1 and 2 days. The objective evaluation of firmness was determined by using a Universal Automatic penetrometer (Stanhope-seta, Surrey, UK). A nine point hedonic scale was used[12].

Statistical analysis: The data obtained was subjected to analysis of variance (ANOVA)[13] for mean comparison of different products at the probability level of p = 0.05.

RESULTS AND DISCUSSION

There was a significant difference (p = 0.05) in the Total Viable Counts (TVCs) of SBS and USP Table 1, which may be due to the raw materials used in their production[7]. Furthermore, combining rice, soybean and other minor ingredients may have led to the initial higher microbial population at the fermentation stage of SBS production. The mean TVCs of SBS decreased from 8.30 to 5.49 log_{10} cfu g^{-1} while there was initial 4 h decrease in TVCs of USP followed by subsequent population increase. The decrease in TVCs of SBS with time may be attributed to the decrease in pH Table 1, which invariably inhibited the growth of some of the microorganisms in the slurry. But the initial 4 h decrease in TVCs of the USP may be probably due to various microorganisms adapting to the slurry environment. Thus, it was likely that those favoured by the fermenting medium were also probably

Table 2: Microorganisms isolated at different time intervals during fermentation of soyabean supplemented and unsupplemented rice masa production

	Supplemented				Unsupplemented			
	Fermented time (h)				Fermented time (h)			
Isolates	0	4	8	12	0	4	8	12
Bacteria								
Bacillus cereus	+	+	+	-	+	+	-	-
Lactobacillus plantarium	+	+	+	+	+	+	+	+
Acetobacter sp.	+	+	+	-	+	+	-	-
Enterobacter aerogenes	+	+	-	-	-	-	+	+
Escherichia coli	+	+	-	-	-	-	-	-
Micrococcus luteus	+	+	+	+	-	-	+	+
Mycoflora								
Aspergillus flavus	+	+	+	-	+	+	+	-
Aspergillus niger	+	+	+	-	+	+	-	-
Penicillium sp.	+	+	-	-	+	+	-	-
Fusarium sp.	-	-	-	-	+	-	-	-
Rhizopus sp.	+	+	-	-	-	+	+	+
Saccharomyces cerevisiae	+	+	+	+	+	+	-	-

+ = Isolated; - = not isolated

Table 3: Total Viable Count (TVCs), pH and titratable acidity of soybean supplemented and unsupplemented rice masa stored at ambient temperature

		Storage time (days)		
Evaluated parameters		0	1	2
TVCs (log_{10} cfu g^{-1})	Supplemented	3.88a	4.71a	6.93a
	Unsupplemented	3.48b	4.68b	5.84b
pH	Supplemented	8.42a	5.83a	4.03a
	Unsupplemented	4.34b	3.84b	3.44b
TA (% lactic acid)	Supplemented	0.38b	0.43a	0.45a
	Unsupplemented	0.45a	0.46a	0.47a

Each value is the mean of 4 determinations. Means within the same columns for respective evaluated parameter followed by different letters are significantly different at p = 0.05

inhibited initially until they were able to establish themselves. Similar trends have been observed during puto (a Phillipine rice product) production during the first 2 h of its second stage fermentation[14]. However, the increase in TVCs thereafter may be attributed to ready availability of adequate nutrients and favourable pH. Thus, the appox. 4 log increase Table 1 in TVCs of USP may not be unexpected.

The pH and Titratable Acidity (TA) of SBS ranged from 5.91 to 5.03 and 0.34 to 0.47, respectively while that of USP ranged from 5.32 to 4.72 and 0.38 to 0.49, respectively with significant difference (p = 0.05) existing between the pH of SBS and USP Table 1. The lower but significantly different pH of USP may be due to the carbohydrate composition of the rice which was probably degraded to organic acids such as lactic, acetic acids while the more protein content of SBS may have contributed to the corresponding increase in pH of the medium. Furthermore, the result strongly suggest the lactic type fermentation of the products since Murdock and Field[15] and Ekweani[16] had earlier stated that cereal fermentation is of the lactic type where pH of the fermenting mass decreases with TA.

The microbial flora of the fermenting slurries varied widely with that of the SBS being most heterogeneous and containing eleven microbial genera Table 2. The bacterial genera included *Bacillus, Lactobacillus*, *Acetobacter*, *Enterobacter*, *Escherichia* and *Micrococcus* while that of fungi include *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus* and *Saccharomyces* with *Lactobacillus*, *Aspergillus* and *Saccharomyces* predominating. Soybean supplementation is known to enhance the diversity of microbial population[6]. Thus, the heterogeneous microflora exhibited in the SBS slurry collobarates this assertion. However, as fermentation progressed beyond 8 h less desirable microflora such as *Enterobacter*, *Escherichia coli* and *Aspergillus* sp. disappeared with more desirable flora such as Lactic Acid Bacteria (LAB), *Micrococcus* and *Saccharomyces* sp. predominating. The presence of *Fusarium* sp at the initial stage of USP slurry fermentation may not be unconnected with diverse microbial flora associates with cereal grains at harvest[17].

Significant differences (p = 0.05) existed between SBS and USP in TVCs and pH during ambient storage of the products. However, there were increases in TVCs and TA with storage time as decreases in pH were observed. The initial decrease in TVCs (3 log cfu g^{-1}) on day O Table 3 caused by the baking of the fermented SBS and USP is suggestive of the safety of the products after production. However, the remarkable increase in TVCs after 24 h of ambient storage may be partly attributed to heat tolerance and recovery of the microorganisms especially *Bacillus cereus* and *Aspergillus* sp Table 4 thereby indicating questionable safety of the products[18] after 24 h of production. There have been similar sharp increases in the microbial counts of 'tortilla' (a maize product) or soybean supplemented kwoko or fura after 24 h of production[6,19,20].

Table 4: Variations in the occurrence of the microbial genera during ambient storage of soybean supplemented and unsupplemented rice masa

Products	Storage time (days)	Bacteria					Moulds		
		LAC	MIC	STR	BAC	STA	MUC	PEN	ASP
Supplemented	0	+	+	+	+	+	-	-	-
Unsupplemented	+	-	-	+	-		-	-	-
Supplemented	1	+	+	+	+	-	-	-	+
Unsupplemented	+	+	-	+	+		-	-	+
Supplemented	2	+	+	+	+	-	+	+	+
Unsupplemented		+	+	-	+	+	+	+	+

+ = isolated; - = not isolated LAC = *Lactobacillus*; MIC = *Micrococcus*; STR = *Streptococcus*; BAC = *Bacillus*; STA = *Staphylococcus;* MUC = *Mucor;* PEN = *Penicillium*; ASP = *Aspergillus*

Table 5: Sensory attributes of soybean supplemented and unsupplemented rice masa stored at ambient temperature

Products	Storage time (days)	Sensory evaluation (mean values)		
		Aroma	Visual appearance	Firmness
Supplemented	0	8.2a	5.0b	6.0a
Unsupplemented		7.0b	6.4a	6.8a
Supplemented	1	6.4a	4.7b	6.2a
Unsupplemented		5.3b	5.6a	6.6a
Supplemented	2	4.2a	4.0b	4.9a
Unsupplemented		3.6b	4.4a	5.2a

Values are means of 4 determinations. Values in column (for each storage time) with different letters are significantly different at p = 0.05

The *Lactobacillus* and *Bacillus* sp constituted the major microorganisms in both SBS and USP stored samples Table 4. However, it is likely that *Lactobacillus* sp may have created sub-optimal acidic conditions for many microorganisms such as *Staphylococcus*, *Micrococcus, Bacillus* sp and the moulds to thrive in the products.

The aroma and visual appearance of both SBS and USP stored samples were significantly different (p = 0.05) with the aroma of SBS being more preferred. However, the firmness of USP was better but not significantly different from SBS samples Table 5. The preferred aroma may likely be as a result of *Saccharomyces* and *Lactobacillus* sp which dominated the final stages of fermentation producing ethanol and organic acids respectively which then combined to produce esters for desirable flavour and aroma[21]. The light-brown creamy colour of USP samples may have resulted in its visual appearance (6.4) being more preferred since the SBS had darker colour and rough texture and significantly different (p = 0.05) than the SBS, which had darker colour and rough texture.

CONCLUSION

Soybean supplementation (20% w/w) of rice masa resulted in existence of diverse genera of microorganisms with population decrease during fermentation as *Lactobacillus*, *Aspergillus* and *Saccharomyces* spp predominated. Ambient stored samples of SBS showed higher unacceptable population increase after 24 h with less diverse microbial genera in the USP samples. However, significant difference (p = 0.05) existed in microbial population and pH of both SBS and USP stored samples. In addition, aroma of SBS samples was better preferred with no significant difference in the firmness of the products.

While enhancing the nutritive value of rice masa through soybean supplementation, greater safety margin of the product should also be enhanced through the application of hazard analysis critical control point system.

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