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Effect of Fermented Rice Dough on the Organoleptic Quality and Shelflife of Rice ''Masa''

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Abstract

The study involved the isolation and identification of Lactic Acid Bacteria (LAB) from fermented rice dough and the use of the fermented rice dough for the production of 'masa'. The study was aimed at determining the effect of the fermented rice dough on the sensory quality and shelf life of 'masa'. The result obtained show that the fermented rice dough had a mean lactic acid bacteria count of $7.9x10^6$cfu/g after five days of fermentation. The mean fungal and aerobic plate counts were $4.2x10^5$cfu/g and $2.9x10^5$cfu/g respectively. Five species of lactic acid bacteria were isolated and identified as *Lactobacillus sanfranciscens, Lactobacillus pontis, Lactobacillus fermentum, Lactobacillus fermentii* and *Lactobacillus brevis*. 'Masa' made with 15% fermented rice dough was most effective in inhibiting fungal spoilage having the longest minimum mould free shelf life (MMFSL) of 8 days, but it was the least accepted in terms of organoleptic quality. The control 'masa' without fermented dough and the commercial 'masa' were the most accepted but have the least MMFSL of 4 days. However, 'masa' made with 5% fermented dough had an appreciable level of acceptance and an average (MMFSL) of 6 days. The findings of this work show that addition of fermented rice dough can improve the shelf life and organoleptic quality of 'masa' produced from rice.

Keywords: Fermented rice dough, Organoleptic quality, Minimum mould free shelflife, ''Masa''

INTRODUCTION

'Masa' is consumed in various forms by all age groups in the Northern States of Nigeria and many other Sahelian African countries (Mali, Burkina Faso, Niger, Chad, and Ghana). It is one of a variety of fermented cereal-based foods and is a good source of income for the women who prepare this traditional product for sale (Ayo *et al.*, 2008).

'Masa' is a fermented puff batter of rice cooked in a pan with individual cuplike depression. 'Masa' also referred to as *waina* is like the Indian *dosa* in taste and different from the Mexican 'Masa' used in tortilla preparation. 'Masa' is a very popular staple food consumed by over 80% of the Northern Nigeria population (Nkama, 1993). 'Masa' is prepared to create variety in cereal for sale; it serves as breakfast and snack item. Though 'masa' is as popular as *akamu* in Nigeria, it has received very little research attraction (Nkama and Malleshi, 1998). 'Masa' is consumed in various forms by all aged groups in the Middle Belt and Northern States of Nigeria. 'Masa' results from frying of the fermented rice dough which is round in shape with brown smooth body and crippling edges. The brown crisp edges and the mild sour taste are considered by many consumers as the quality attribute required of 'masa'. 'Masa' is a good source of income for the women who prepare the traditional product for sale. The addition of cowpea, groundnut or soybeans flour into 'masa' during preparation is reported to improve the organoleptic quality of 'masa' (Nkama and Malleshi, 1998; Ezema and Ihezie, 2006). It serves as a breakfast and snack item.

Some fermented products are known to have a very short shelf-life. Important losses in the fermentation industry are due to microbial attacks that have been reported all over the world Fungal contamination which is known to be the most common source of microbial spoilage is a costly problem in some fermentation industries and the major factor in many cases, governing the shelf-life of fermented products (Gobbetti *et al.,* 2008). Besides the repellent side of visible growth, fungi, may be responsible for off-flavours and may produce mycotoxins and allergenic compounds (Gobbetti *et al.*, 2002; Gobbetti *et al.,* 2008).

Fermented dough (Sourdough) is an important modern fermentation of cereal flours and water based upon an earlier spontaneous process (Chavan and Chavan, 2011). The sourdough microflora is dominated by lactic acid bacteria and, along with yeast; they play a key role in cereals fermentation. The sourdough fermentation has a number of beneficial effects that include prolonged shelf life, accelerated volume gain, delayed staling, improved flavor, and good nutritional value. Sourdough also improves sensory characteristics such as loaf volume, evenness of baking, colour, aroma, taste, and texture of baked products. Sourdough has been reported to contribute to extended shelf life by inhibiting the growth of spoilage bacteria and moulds ((Katina, 2005; Chavan and Chavan, 2011).

Sourdough has a natural, additive-free image and lactic acid bacteria which have been used in food for thousands of years and are "generally regarded as safe" (GRAS). The introduction of sourdough would be particularly suitable in cereal foods containing dietary fibre rich plant tissues as both nutritional and technological quality could be considerably enhanced in these products by utilising sourdough (Katina, 2005). Sourdough fermentations as well as baking agents based on sourdough, have retained their importance in contemporary baking technology because of the improved aroma, texture and shelf-life of sourdough products (Ganzle *et al.*, 2003; Corsetti and Settanni, 2007).

Consumer demands for more natural foods have stimulated the research on biological (i.e vegetal and microbial) preservation systems. In this aspect, lactic acid bacteria are organisms of interest for preservation since they have been used for centuries in various fermented foods, either by their natural presence in raw materials (spontaneous fermentation) or their addition as pure starter cultures (Anwaar *et al.*, 2002).

There is a dearth of information on the role of fermented dough (sourdough) on the organoleptic quality and shelf life of *'masa'*. This study was therefore aimed at determining the effect of sourdough addition on the organoleptic quality and shelflife of 'masa'.

Materials and Methods

Sample collection

Rice grain was purchased from terminus market, Jos, Plateau state. The rice was washed, sun-dried and milled to obtain fine rice flour.

Preparation of rice flour dough

The dough samples were prepared by mixing 1000g of the rice flour with 1000ml of sterile water manually under aseptic condition (Teiking, 2005; Saeed, 2009). Three samples were prepared in plastic buckets.

Fermentation of dough to yield fermented dough (Sourdough)

The three dough samples were fermented spontaneously at room temperature for 120 hours (5 days).

Analysis of dough during fermentation

Microbial counts, pH and titratable acidity (TTA) were determined at 0hrs, 24hrs, 48hrs, 72hrs, 96hrs and 120hrs intervals of fermentation.

Determination of pH and TTA

Ten grams of the fermented dough sample was homogenized in 90ml of sterile distilled water and filtered and pH taken using pH meter (Hanna HI 9025). Ten millilitres of the homogenate from each sample was titrated against 0.1N sodium hydroxide (NaOH) for the determination of TTA.

Percentage titratable acidity calculated as lactic acid:

$$TTA = \frac{\text{Titre} \times \text{Normality of base} \times 0.009018 \times 100}{\text{Weight of sample in gram}}$$

(Wakil *et al.*, 2008)

Microbial counts

A serial dilution of each sample was made and the last two dilutions (10^{-5} and 10^{-6}) were plated out in duplicates on appropriate agar for microbial counts as described below:

Lactic acid bacteria count (LABC)

The last two dilutions (10^{-5} and 10^{-6}) were inoculated on De Man Rogosa Sharpe's (MRS) agar plates. The plates were incubated under anaerobic condition at 30^{0}C for 48hours. Colonies were counted and results expressed as CFU/g.

Aerobic plate count (APC)

The last two dilutions (10^{-5} and 10^{-6}) were inoculated on plate count agar (PCA) plates. The plates were incubated at 37^{0}C for 24hours. Colonies were counted and results expressed as CFU/g.

Fungal Count

The last two dilutions (10^{-5} and 10^{-6}) were inoculated on potato dextrose agar (PDA) (containing 0.5ml/l streptomycin sulphate) plates. The plates were incubated at room temperature. Colonies were counted and results expressed as CFU/g.

Identification of LAB isolates

Colonies on the MRS plates were further purified by successive streaking on fresh MRS agar plates. The LAB isolates were identified based on their Gram reaction, catalase test and sugar fermentation tests as described by Mehmood *et al.* (2009), Rubayyi *et al.*, 2010, Sameen *et al.* (2010).

Production of experimental 'masa'

The rice sourdough was added to the rice dough for 'masa' production at 5%, 10% and 15% concentration. For the 'masa' production, the rice grains were soaked in water for 2 days after which the water is decanted and washed with fresh clean water followed by wet milling with little water. The run–off water from the milling mash is collected in a separate bowl and allowed to settle. The slurry is boiled for 20 min and then allowed to cool. The mash is then mixed with the rice slurry, covered and allowed to ferment for 24 hours. After the fermentation, the fermented dough was rolled by hand into balls and fried in vegetable oil for 5-10 minutes using earthen ware pot (Ogunbanwo and Fowoyo, 2010).

Analysis of experimental 'masa'

The pH and TTA of the experimental 'masa' samples were determined as earlier described.

Sensory evaluation of 'masa' samples

The 'masa' samples were subjected to sensory evaluation using a panel of 25 enlightened judges to evaluate the following physical parameters; taste, texture, appearance, and aroma. Five point grade was used for the analysis; Excellent-5, Very good-4, Good-3, Satisfactory-2, and Poor-1 (Boboye *et al*., 2008). The judges were instructed to rinse their mouth with water before and after taking each sample.

Proximate analysis of the 'massa' samples and rice flour

The 'masa' samples as well as the rice flour used for the production of the 'masa' were analysed for moisture, crude protein, fat, crude fibre, total ash and nitrogen free extract (NFE) content according to their respective standard methods as described in AOAC, (2006).

Evaluation of minimum mould-free shelflife of experimental 'masa' and conventional 'masa'

The experimental 'masa' and conventional 'masa' purchased from local producers were stored at room temperature and their shelf-life evaluated by determining the minimum mould-free shelf-life (MMFSL) as described by Pattison *et al*. (2004).

Statistical Analysis

The data obtained from the sensory evaluation were subjected to statistical analysis using one way analysis of variance (ANOVA).

RESULTS

The microbial counts during the sourdough development are as shown in Table 1. Lactic acid bacteria mean count was the highest after 120 hours of fermentation (4.7 x 10^6CFU/g), followed by mean fungal count of 4.2.x10^5 CFU/g and mean aerobic plate count of 2.9x10^5 CFU/g. In all cases there is a significant difference in the mean counts ($P \leq 0.001$)

Five (5) species of lactic acid bacteria of the genus *Lactobacillus* were isolated from the fermented rice dough and identified as *Lactobacillus sanfranciscens, Lactobacillus pontis, Lactobacillus fermentum, Lactobacillus fermentii* and *Lactobacillus brevis* (Table 2).

The pH of the fermented rice dough (sourdough) decreased progressively from 6.5 at the beginning of the fermentation to 3.8 at day five while the TTA increased progressively from 0.16 – 0.73 (Table 3).

Table 4 shows the pH and TTA of the experimental 'masa' samples, the 'masa' supplemented with 5%, 10% and 15% sourdough have lower pH values and higher TTA values than the control and commercial 'masa' without sourdough.

The result of the sensory evaluation of the various 'masa' samples is as shown in Table 5. The commercially prepared (conventional) 'masa' has the highest mean overall acceptance of 4.57±0.55, followed by the 5% sourdough 'masa' with overall acceptance of 3.47±1.07, 10% sourdough 'masa' with overall acceptance of 3.22±0.95, 15% sourdough 'masa' with overall acceptance of 2.90±0.99 and the least being the control 'masa' with overall acceptance of 2.87±0.64. There is significant difference in the mean overall acceptability between the commercial 'masa' and the other 'masa' samples ($P \leq 0.05$).

Table 6 shows the proximate composition of the rice flour and those of experimental masa. The experimental 'masa' samples have higher moisture content, fat content and crude fibre content than that of the rice flour but lower crude protein, ash content and nitrogen free extract than the rice flour.

Table 7 shows the minimum mould free shelflife of the of the 'masa' samples. The masa supplemented with 15% sourdough has the longest MMFSL of eight (8) days, followed by the 10% sourdough 'masa' with a MMFSL of seven (7) days, the 5% sourdough 'masa' has a MMFSL of six (6) days while both control 'masa' without sourdough and the commercial 'masa' without sourdough have MMFSL of four (4) days.

Table 1: Microbial counts during fermentation of rice dough to sourdough

Fermentation time	TLABC	FC	APC
0 hour	$1.3x10^4$	$1.6x10^4$	$3.2x10^7$
24 hours	$9.6x10^5$	$3.1x10^5$	$2.0x10^6$
48 hours	$1.4x10^6$	$5.9x10^5$	$5.1x10^5$
72 hours	$4.0x10^6$	$3.6x10^6$	$3.0x10^5$
96 hours	$8.9x10^6$	$4.8x10^6$	$2.1x10^4$
120 hours	$3.2x10^7$	$6.2x10^6$	$1.7x10^3$
Mean Count	$\mathbf{4.7x10^6{}_a}$	$\mathbf{4.2.x10^5{}_b}$	$\mathbf{2.9x10^5{}_c}$
P-Value	**<0.001**		

Key: TLABC= Total lactic acid bacteria count, **APC**= Aerobic plate count, **FC**= Fungal count

Table 2: Morphological and Biochemical Characteristics of the isolates

Tests	**Result**					
Catalase		-	-	-	-	-
Oxidase		-	-	-	-	-
Gram reaction		+ rods	+ rods	+ rods	+ rods	+ rods
Sugar Fermentation Test						
Glucose	+	+	+	+	+	
Sucrose	+	+	+	+	-	
Sorbitol	+	-	-	+	-	
Xylose		-	-	+	+	-
Starch		+	+	-	-	-
Lactose	+	+	+	+	+	
Arabinose		+	+	+	+	+
Fructose		+	+	+	+	+
Maltose	+	+	-	-	-	
Mannitol		-	-	-	-	-
Galactose		+	+	+	+	+
Isolates	A	B	C	D	E	

Key: A: *L. fermentum*, B: *L. brevis*, C: *L. pontis*, D: *L. fermentii*, E: *L. sanfranciscens*

Table 3: Effect of fermentation time on pH and TTA of fermented dough samples

Fermentation time	pH	TTA
0 hour	6.5	0.16
24 hours	4.6	0.45
48 hours	4.3	0.37
72 hours	4.0	0.59
96 hours	3.9	0.72
120 hours	3.8	0.73

Table 4: pH and TTA values of the experimental 'masa' samples

Sample	pH	TTA
A	4.1	0.39
B	3.8	0.41
C	3.7	0.48
D	5.2	0.50
E	5.0	0.54

Key: A= 'Masa' produced with 5% sourdough; **B**= 'Masa' produced with 10% sourdough; **C**= 'Masa' produced with 15% sourdough; **D**= Control 'masa' produced without sourdough; **E**= Commercial control 'masa'.

Table 5: Sensory evaluation of the ''masa'' samples

	Taste	Appearance	Aroma	Texture	Overall acceptability
A	3.40 ± 1.12^b	3.12 ± 1.01^b	3.92 ± 1.01^b	3.44 ± 1.12^b	3.47 ± 1.07^b
B	3.12 ± 0.97^b	3.08 ± 0.99^b	3.36 ± 0.95^b	3.32 ± 0.90^b	3.22 ± 0.95^b
C	2.76 ± 1.05^a	2.80 ± 0.91^a	2.88 ± 1.05^a	3.16 ± 0.94^b	2.90 ± 0.99^a
D	2.80 ± 0.58^b	2.88 ± 0.67^a	2.92 ± 0.64^a	2.88 ± 0.67^a	2.87 ± 0.64^a
E	4.68 ± 0.48^c	4.76 ± 0.53^c	4.40 ± 0.65^c	4.64 ± 0.64^c	4.57 ± 0.58^c
P- value	<0.003	<0.003	<0.043	0.045	0.031

P ≤ 0.05. Means with different superscript differ significantly.

Key:

A= 'Masa' produced with 5% sourdough.
B= 'Masa' produced with 10% sourdough.
C= 'Masa' produced with 15% sourdough.
D= Control 'masa' without sourdough.
E= Commercial 'masa'.

Table 6: Proximate composition of rice flour and 'masa' samples

Sample	Moisture Content(%)	Ash content(%)	Crude protein(%)	Fat content(%)	Crude fibre(%)	Nitrogen Free Extract(%)
Rice Flour	12.75	9.10	12.75	0.41	0.60	64.40
A	51.22	1.20	6.31	4.37	1.92	34.98
B	48.33	0.30	6.57	2.79	1.83	40.18
C	46.54	0.15	7.58	5.03	1.76	38.97
D	44.80	0.30	8.15	5.58	1.70	24.17
E	44.10	0.24	5.54	4.64	1.52	20.21

Key: A= 'Masa' made with 5% sourdough, **B**= 'Masa' made with 10% sourdough,
C= 'Masa' made with 15% sourdough, **D**= Control 'masa' made without sourdough,
E= Commercial 'masa'.

Table 7: Minimum mould free shelf life (MMFSL) of experimental 'masa' and commercial 'masa' samples

'Masa' sample	Minimum Mould Free Shelf Life (MMFSL)
5% fermented dough 'masa'	6
10% fermented dough 'masa'	7
15% fermented dough 'masa'	8
Control 'masa'	4
Commercial (conventional) 'masa'	4

DISCUSSION

The lactic acid bacteria were the most dominant group during sourdough fermentation, with counts ranging between 1.3x10^{3}CFU/g to 3.2x10^{7}CFU/g, this agrees with the findings of other workers; Saeed (2009) also reported LAB as the dominant bacteria in sourdough with counts in the range of 6.24 x 10^4 CFU/g to 6.92 x 10^7 CFU/g. In mature sourdoughs LAB counts range from 1x10^9 to 3x10^9 cfu per gram and yeasts from 1x10^6 to 5x10^7 cfu per gram sourdough (Hammes *et al.*, 2005). According to Katina (2005), the dominant microbes in spontaneously fermented doughs are homofermentative lactobacilli and pediococci, which are found both in wheat and rye sourdoughs at the level of 3 x10^8 to.3 x10^9 CFU-g. Luangsakul *et al.*, 2009 reported LAB counts of 1.8 × 10^4 to 10^8 colonies/g sample, and low fungal counts of 10 to 2.3 × 10^2 colonies/g sample. The high LAB counts compared to yeasts and APC counts is because sourdough fermentation begins with aerobic growth immediately upon mixing flour and water. Once oxygen is depleted, anaerobic fermentation begins with the growth of LAB which produce acids (lactic and acetic) which enhance their rapid growth when the pH value has dropped too low for other microorganisms to grow (Savic *et al.*, 2006). So, the LAB become the most abundant microorganisms in the sourdough and they are therefore responsible for the final stages of the sourdough development (Savic *et al.*, 2006).

The lactic acid bacteria isolates obtained in this work *Lactobacillus sanfranciscens*, *Lactobacillus* pontis, *Lactobacillus fermentum*, *Lactobacillus frumentii*, *Lactobacillus brevis* were in agreement with the findings of Korakli *et al.,* (2004); Rattanachaikunsopon and Phumkhachorn (2010) and Minervini *et al.* (2013), who also reported the presence of similar isolates in cereal sourdough. *Lactobacillus* has been reported as the only genera of LAB isolated from sourdough made from cereals (Korakli *et al*., 2004; Luangsakul *et al*., 2009).

The pH of the fermented dough obtained in this study agrees with the findings of Saeed, (2009) who reported a pH range of 3.53 to 5.74 and Korakli *et al.* (2004) who reported pH of between 4·0 and 4·2. Savic *et al.* (2007) also reported a decrease in pH from 6.5 to 5.5 during sourdough fermentation. The decrease in pH and incease in TTA is due to lactic and acetic acid production by the LAB isolates (Savic *et al*., 2007).

The findings of the present study also tallies with the findings of Saeed, (2009) who reported that pH of sourdough bread follows the same pattern as has been observed in the corresponding sourdoughs. The homofermentative LAB are able to convert hexoses almost completely into lactic acid whereas heterofermentative LAB degrade hexoses into lactic acid, acetic acid/ethanol and CO_2. These acids are responsible for the drop in pH of the sourdoughs and their products (Saeed, 2009).

The result of the sensory evaluation with respect to the overall acceptability show that the commercial 'masa' is the most preferred by the panelist, followed by the control 'masa' without sourdough, most of the panelist complain of the sour nature of the 'sourdough masa'.

The lower crude protein content of the sourdough 'masa' samples compared to that of the rice flour and the sourdough-free 'masa' samples can be explained by the fact that lactic acid bacteria contribute to overall proteolysis during the fermentation of sourdough by creating optimum conditions for activity of cereal proteinases (Gobbetti *et al*., 2005; Saeed, 2009). Generally, sourdough fermentation with lactic acid bacteria resulted in an increase of amino acid concentrations during fermentation, whereas the concentration of free amino acids reduced when the dough was fermented with yeast alone (Thiele *et al.,* 2002). The higher crude fiber of the sourdough 'masa' samples is an added advantage in the nutritional content of 'masa' since dietary fiber is known to promote health and aid the digestive system of humans (Jideani and Onwubali, 2009; Elleuch *et al*., 2011). It is well-documented that dietary fibre plays a significant role in the prevention of several diseases such as; cardiovascular diseases, diverticulosis, constipation, irritable colon, cancer and diabetes (Slavin, 2005; Elleuch *et al*., 2011, Ndife *et al*., 2011).

All the sourdough 'masa' have longer minimum mould-free shelflife than the two sourdough-free 'masa'. The ability of sourdough to improve the shelflife of baked products is well-documented. Zannini *et al.* (2013) reported that the use of 20% sourdough in bread making increased the mold-free shelf life by 2 to 3 days or from 2 to more than 6 days. Gerez *et al.* (2010) also reported that sourdough LAB such as *Lactobacillus brevis* CRL 772, *L. brevis* CRL 796, *L. plantarum* CRL 778, and *L. reuteri* are able to improve the shelflife of bread to five days compared to 2 days for bread produced by baker's yeast alone. The anti-fungal activity of *L. plantarum* 21B was also reported in sourdough bread, compared to breads started with *Saccharomyces cerevisiae* 141 alone, the sourdough bread produced with the association of *S. cerevisiae* 141 and *Lb. plantarum* 21B delayed fungal contamination upto 7 days of storage at room temperature (Gobbetti *et al.,* 2005). The antifungal activity of sourdough is due to a number of metabolites produced by LAB during sourdough development; this include lactic, acetic, caproic, formic, butyric, n-valeric, phenyllactic, 4-hydroxy-phenyllactic and mono-hydroxy octadecenoic acids (Gobetti *et al.,* 2000; Gobetti *et al.,* 2005; Gerez *et al.,* 2010; Zannini *et al.,* 2013).

Although the positive effect of sourdough on the organoleptic quality and shelflife of bread is well researched and documented, there is no published information on the effect of sourdough addition on the organoleptic quality and shelflife of 'masa'. This research work has therefore help bridged this information gap.

Conclusion

From the findings of this work, it can be concluded that addition of rice sourdough during rice 'masa' production can improve the shelf life of rice 'masa'. Five percent (5%) sourdough addition showed promise in improving the organoleptic quality and general acceptability of the 'masa'. The addition of the sourdough at concentrations of 6% - 9% may lead to improvement in both the organoleptic quality and shelflife of the 'masa'.

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