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Effect of Yeast and Lactic Acid Bacteria on Nutritional and Sensory Quality of Masa (A Fermented Snack)

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Abstract: Eight microbial yeast and lactobacillus isolates were identified during spontaneous fermentation of mixed cereal gruels (rice, maize and millet) for 78 h at room temperature. The potential of these isolates to act as single starter was further studied in a fermentation set up for Masa (waina) production. The resulting batter from the milled rice used was inoculated with 2.0×10^6 cfu/ml of *Saccharomyces* and *Lactobacillus* inoculum suspension in different set up. The fermented gruel was sized and fried in little oil to produce Masa (Waina). The Masa was analyzed for physical (thickness, volume and spread) and nutritional qualities through an untrained panelist. The dimension of the Masa ranges from 4.50-6.0cm in diameter, 1.5-2.0cm thickness and 50-68g in weight. The result of the proximate analysis showed that for both isolates there was a steady increase of crude fibre ranging from 0.75-1.04%, crude protein for all isolates gave insignificant difference in amount ranging between 7.33 and 8.63% for *Saccharomyces* and *Lactobacillus* spp., respectively. There was reduction in fat between isolates with *Lactobacillus* having the lowest fat value of 12.06%. There was increase in moisture content ranging from 35.5-42.13% for *Saccharomyces* and *Lactobacillus* spp., respectively. There was high production of carbohydrate, whereas there was no significant difference in terms of results obtained between the isolates and baker's yeast used as control in this study. The sensory evaluation showed that all samples of Masa produced were preferred by the panelist.

Key words: Masa, fermentation, proximate analysis, sensory evaluation

INTRODUCTION

Food is one of the basic necessities of life (Okaka and Okaka, 2001) and traditional foods are a recognizable part of these necessities. Nigeria has a variety of people and culture that it is difficult to pick one traditional dish. Each region has its own favourite food and that depends on ethno-cultural and religious inclinations (Abdel *et al.*, 2009 and Adebayo *et al.*, 2010). Fermentation is one of the oldest and most widespread methods of food processing and preservation. More than anything else, man has known the use of microbes for preparation of food products for thousands of years and all over the world a wide range of fermented foods and beverages contribute significantly to the diets of many people (Achi, 2005). Practically all nations have some traditional type of fermented products made by the action of *Lactobacilli* and yeast alone or in combination with other microorganisms. Fermentation has been viewed as a dynamic process during which several catabolic and anabolic reactions proceed simultaneously depending on several conditions, including substrates, microflora and environmental factors. In traditional fermented food preparation microbes are used to prepare and preserve food products, adding to their nutritive value, the flavor and other qualities associated with edibility (Achi, 2005).

Fermentation process play important role in food technology in developing countries.

In Nigeria Masa or waina is one of such traditional foods consumed by all class of ethnic divide. It is a fermented bread-like product which is round in shape with brown smooth body and crippling edges made from millet, maize or rice flour. Masa (or Waina as it is called locally) is like the Indian idle shape and dosa in taste (Ayo *et al.*, 2012). Masa is a very popular staple food consumed by over 80% of the Northern Nigeria population (Ayo *et al.*, 2008). It is also consumed in Niger, Burkina-Faso and Mali (Nkama, 1998). Masa is prepared to create variety in cereal food products for sale. It is one of the major sources of carbohydrate to the natives of the indigenes states in the Northern part of Nigeria. Although, fermented foods traditionally have constituted a significant proportion of our diet, Nigerians have exhibited an ambivalent attitude in terms of consumer's taste and preferences for food.

Being a fermented food, Waina (or Masa) is either processed through spontaneous fermentation of the cereal gruel or starter cultures are used to initiate fermentation for better quality control in terms of consistency and microbiological safety of the product. The Baker's yeast is one of the commercial starter

available for waina production. The raw materials and ingredients including millet, rice, salt, sugar, yeast, trona or kanwa, vegetable oil are used. The grain particularly pearl millet, rice or maize is dehulled (Rice and Acha are used directly). Washed, soaked (12 h) dried and milled. The ground rice/maize and millet is sieved to produce flour and grits. The grits are added to boiling water and cooked to gelatinization and allowed to cool before mixing with raw flour in the ratio 1:4. The resulting batter, is inoculated with baker's yeast and its allowed to ferment overnight (14-16 h). Salt and sugar are added. The fairly thick batter is then diluted with trona (Kanwa water) and the batter is stirred (vigourously to incorporate air) and fried in a cup-like depression in which oil has been added to produce masa. The batter is fried for 2 minutes on one side then turned with a small spoon and the other side fried (Nkama and Malleshi, 1998).

One of the major challenge with Masa (waina) production is that it doesn't have a long term storage and handling attribute. One of the economic values of Masa (waina) is that it brings about provision of market opportunities for the middle class natives and availability of food supplement and poverty alleviation (Oyewole and Isah, 2012). In traditional fermentation processes, such as production of Masa, endogenous flora initiates fermentation to yield various attributes of the product. Modern fermentation is initiated by starter cultures consisting of microorganisms that are inoculated directly into food materials to overwhelm the existing flora and bring about desired changes in finished product (Jimoh *et al.*, 2012). Masa is a single cereal food whose protein is very poor and commonly deficient in the essential amino acids such as lysine. Consequently, the use of single starter rather than relying on the indigenous microflora for fermentation as it's commonly practiced in the northern states of Nigeria reflects the poor advancement in upgrading its production process. This work attempted to investigate the potential of single yeast and lactic acid bacteria in enhancing the nutritional and sensory qualities of masa production. Isolation, selection, preservation or collection of starter and making a high efficient microbial strain for use as a starter will make the fermentation process more promising. Lactic Acid bacteria and Yeast are probably the most important groups of microbes in fermented foods such as masa.

MATERIALS AND METHODS

Sample collection: Pearl millet, polished local rice, maize, baker's yeast (*Saccharomyces cerevisiae*), kanwa (sodium bicarbonate) were purchased at kawo market Kaduna State Nigeria. The pearl millet (*Renisetum glaricum*), maize (*Zea maize*), rice (*Oriza sativa*) were cleaned and washed thoroughly in running tap water for 30 minutes. Sterile water was used to rinse the cereals

for 5 minutes. 200g each of the cereals was soaked in sterilized water (500ml) for 18 h. The soaked cereals (millet, rice and maize) was milled in a blender to make a batter. The batter was further allowed to stand for 8hrs to allow for fermentation.

Isolation of yeast and lactic acid bacteria: After eight (8 h) of fermentation of the batter a serial dilution of the fermented gruel was carried out and 0.1ml of the diluent was inoculated unto Yeast Extract Agar and MRS Agar. Ten isolates (5 each from the two growth media) were subcultured and preserved in slants.

Yeasts were isolated with the aid of enrichment techniques using media which while permitting their development, discourages the growth of other organisms like molds and bacteria. Antibiotics were also used to discourage bacterial growth (Beech and Davenport, 1970). The pour plate method of Harrigan and McCance (1976) was adopted for isolation of representative yeast colonies carried out after incubation for 3-5 days at 30°C. A representative number of yeast colonies were isolated at random from plates that showed appreciable growth and further analyzed. The isolates were purified by the conventional streaking method and kept on Malt Extract Agar (MEA) slants. Lactic Acid bacteria were isolated using MRS agar anaerobically and also preserved on MRS slant. All isolates were preserved at 4°C.

The conventional methods described by Kreger-Van Rij (1984) and Van der Walt and Yarrow (1984) which include the study of some morphological features as well as biochemical was employed for yeast characterization. However, Lactic Acid bacteria were characterize microscopically and biochemically.

Preparation of sample for masa (waina) production:

200g of polished local rice was washed thoroughly in running tap water for 20 minutes. Final washing and rinsing was done with sterile boiled water severally to make the medium free of microbes. The rice was soaked in sterilized water (500ml) for 12 h and milled. Two fermentation protocol was set up for each of the isolates and one control using a baker's yeast. The resulting batter was inoculated with the identified yeast and LAB isolates of 2.5×10^6 cfu/ml of each inoculum and allowed to ferment for 12 h. After fermentation 50g of boiled rice was added to the batter to enhance its gelatinization properties. This was stirred vigorously (using mortar and pestle) to incorporate air. It was then fried in a local clay pot with individual cuplike depression in which 10cm³ of oil has been added. The batter was fried for 3-4 minutes on one side, then turned with a frying spatula spoon and the other side fried to produce Masa. The thickness and width of the Masa ball was measured using a digital weigh-in balance and ruler respectively.

Proximate analysis: The AOAC (1990) methods were used in the determination of the hydrogen ion concentration and ash content. Moisture, fat, protein and carbohydrate were also determined.

Sensory quality: The sensory qualities were evaluated by 15 untrained panelists comprising staff and students of the Kaduna State University Nigeria. Attributes assessed include flavor, taste, colour, texture, appearance and the overall acceptability of Masa using seven Hedonic Scale (1 and 7 for extremely dislike and extremely like respectively) According to Ogunjobi *et al.* (2005). Standard deviation and Standard error mean were calculated respectively.

RESULTS AND DISCUSSION

The Proximate quality results obtained on the fermentation of rice by isolated *Saccharomyces* and *Lactobacillus* spp for waina production is as summarized in Table 1.

The moisture content showed an increasing margin among all masa produced. There was an appreciable increase from 35.15-39.89 and 39.06-42.13% for masa fermented with *Saccharomyces* spp and *Lactobacillus* spp., respectively. However, Baker's yeast used as a standard control had a higher moisture content of 44.12%. This is not unusual as cereal grains are relatively high in moisture content especially during soaking, similarly the boiled rice added to the soaked rice varied between the masa produced with Baker's yeast and the *Saccharomyces* and *Lactobacillus* spp. The high moisture content makes shelf-life to be shorter and may be liable to spoilage during storage (Okaka, 2005). Moisture content creates a favourable condition for microbial proliferation as well as enhances enzymatic deterioration as also reported by Oduro *et al.* (2009). In this finding crude fibre content was 1.08% for masa fermented with Baker's yeast, much higher than that produced by *Saccharomyces* spp. and *Lactobacillus* spp. fermented masa with 0.75-0.71 and 0.79-1.04%, respectively. Fibre generally has numerous health benefits; fibre is reported to have beneficial effects on preventing cancer (Appiah *et al.*, 2011; Shankar and Lanza, 1991), however, Ayo *et al.* (2008) observed that Low fibre of flour from rice cereal could improve baking quality. Protein content of masa fermented with *Saccharomyces* spp., *Lactobacillus* spp. and Baker's

yeast was 8.63, 7.7 and 7.80%, respectively. Protein is found in all tissues of cereal grains but the concentration varies from grain to grain due to their chemical composition (Kent, 1984). Rice grain has protein content of 6.8-8.0% while Maize and Millet have protein content of 9-10% (Ihekoronye and Ngoddy, 1985). However, it has been noted in other research that protein content could increasingly be higher when cereals are enriched with legumes before masa production. Ayo *et al.* (2012) reported an increasing effect of groundnut paste on protein content of masa from (9.56-13.59 and 9.48-13.23%) which has been proofed to contain high quantity of protein (36-40%) (Ayers and Davenport, 1997). The average ash (mineral) and fat content of masa produced from rice fermented with *Saccharomyces*, *Lactobacillus* and baker's yeast were 1.07-0.85, 0.72-0.83 and 1.12%, respectively. While fat content were 15.80-12.80, 13.60-12.06 and 13.40%, respectively. The relatively low ash content in the respective masa could be due to the low quantity of trona and salt added during production, as higher quantity of trona and salt have been shown to increase the % ash (Ayo *et al.*, 2008). The high fat content despite the low fat/oil level of the raw material (rice) could be due to the oil used in toasting with its tendency of being absorbed by the batter. This could endanger the keeping quality of the product (Nkama, 1993, 1998).

Carbohydrate content was appreciably higher and results showed no difference between values obtained for all products. Masa fermented with *Saccharomyces* and *Lactobacillus* spp had 64.85-60.11 and 60.94-57.87%, respectively. The relatively high carbohydrate content could make the product of significant source of energy to the consumers.

Sensory quality of masa fermented with *Saccharomyces* spp., *Lactobacillus* spp. and baker's yeast: The sensory quality of the fermented masa products is summarized in Table 2, the average means score for texture of masa produced from fermented *Saccharomyces* spp., *Lactobacillus* spp. and baker's yeast were 4.60-5.20, 5.10-4.90 and 5.40, respectively. The average mean score for appearance were 6.55-6.30, 6.20-5.95 and 6.05 for *Saccharomyces* spp., *Lactobacillus* spp. and baker's yeast, respectively. The high scores for appearance could be due to the reason that masa generally has an organoleptic appeal when it is fried. Flavor was scored good with values ranging from 5.45-4.70, 5.60-5.25 and 5.70 for *Saccharomyces* spp., *Lactobacillus* spp. and baker's yeast respectively. Taste was scored 5.00-4.35, 5.10-5.55 and 5.20, respectively. The average score for taste could be due to the reason that in most masa production other spices like onions, pepper and sugar are added to enhance its tasty quality and acceptance. However, in this research masa produced was not fortified with any spice

Table 1: Proximate analysis of processed Masa

Parameters (%)	MLS1	MLS 2	MYS 3	MYS 4	BY
Moisture	35.15	39.89	39.06	42.13	44.12
Crude fibre	0.75	0.71	0.79	1.04	1.08
Protein	7.33	8.63	7.56	7.71	7.80
Ash	1.07	0.85	0.72	0.83	1.12
Fat	15.80	12.80	13.60	12.06	13.40
Carbohydrate	64.85	60.11	60.94	57.87	66.50

OMLS= Massa fermented with *Lactobacillus* spp., MYS= Masa fermented with *Saccharomyces* spp., BY= Masa fermented with Baker's yeast

Table 2: Sensory evaluation of processed Massa

Procedure	Texture	Appearance ^a	Flavour ^b	Taste	Chewiness ^c	General acceptability
MLS 1	4.60±0.14	6.55±0.21	5.45±0.21	5.00±0.14	5.60±0.28	4.55±0.78
MLS 2	5.20±0.14	6.30±0.28	4.70±0.28	4.35±0.21	5.10±0.00	5.05±0.07
MYS 3	5.10±0.00	6.20±0.14	5.60±0.00	5.10±0.14	5.20±0.00	5.10±0.00
MYS 4	4.90±0.14	5.95±0.07	5.25±0.07	5.55±0.07	5.20±0.00	5.15±0.21
BY	5.40±0.00	6.05±0.07	5.70±0.14	5.20±0.00	5.80±0.00	5.80±0.00

Results are mean±SD of values. Include appeal; b- includes aroma; c- includes ease of fragmentation. Scoring system: 1=very poor; 2= Poor; 3= Fair; 4= Average; 5= Good; 6= Very good; 7= Excellent. MLS= Massa fermented with *Lactobacillus* spp., MYS= Masa fermented with *Saccharomyces* spp. BY= Masa fermented with Baker's yeast

ingredients. Chewiness was assessed based on the ability for easy fragmentation of the masa product which also showed mean score to be good for all the fermented masa. The average mean scores in terms of general acceptability were 4.55-5.05, 5.10-5.15 and 5.80 which showed that none was poorly accepted.

Conclusion: Despite that Rice, maize and millet can be used to produced masa, rice is the most commonly used cereal. The use of baker's yeast in the fermentation of these cereals has been a routine activity especially in the urban areas. There is the need to encourage the use of mixed cultures especially *Lactobacillus* spp. As observed in the findings of this research it can be safely encouraged that spontaneous fermentation by microflora from the gruel of these cereals be used in masa production; as results showed close competitiveness in the nutrient and sensory quality with that of baker's yeast.

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