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Effect of Lactic Acid Bacteria and Yeast Starter Cultures on the Soaking Time and Quality of "Ofada" Rice

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ABSTRACT

Freshly harvested paddy rice was randomly obtained from three different farms in "Ofada" town, Ogun State, Nigeria and processed according to the traditional parboiling method. The rice was inoculated singly with cultures of *Lactobacillus amylophilus*, *Leuconostoc mesenteroides*, *Saccharomyces uvarum* and *Saccharomyces cerevisiae* which were isolated from the soak-water. In order to assess the effect of starter cultures on the soaking time of the rice, the pH and titratable acidity were determined at 12 h interval. The chemical composition and the sensory quality of the rice were also determined at the end of the soaking period while uninoculated rice served as control. The chemical composition of the rice as well as the pH and TTA of the soak-water were significantly ($p < 0.05$) different from the control while the processing time was shortened to 48 h. The rice inoculated with *Saccharomyces uvarum* and *Saccharomyces cerevisae* had the highest protein content and best sensory attributes.

Keywords: "Ofada" Rice; Starter Cultures; Soak-Water; Fermentation Time; Quality

1. Introduction

Rice is one of the most important cereals of the world. It is widely consumed and cultivated in most countries. It is the staple food for over three billion people, constituting over half of the world's population [1,2]. There are two important varieties namely, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). "Ofada" rice, a peculiar Oryza sativa species [3] was first grown in Ofada town, Ogun-State, South Western Nigeria and has since become very popular in the Western part of the country. It plays very important role in the diet of many Nigerians and is utilised mostly in households where it is consumed in many forms such as boiled, jollof, fried or "tuwo shinkafi" (local rice paste). Industrially, it is important in the production of local beverages such as "kunu", "pito" and cereal gruels. "Ofada" rice is processed traditionally by parboiling method that involves three stages of moisture treatment (soaking, steaming and drying). This rice is specially relished because of its characteristics flavour that develops during soaking as a result of the fermentative activities of some microorganisms. In addition, Otegbayo [4] reported that soaking contributes mainly to organoleptic, physical and nutritional changes in rice.

Fermentation is a metabolic process in which carbohydrates and related compounds are oxidised with the release of energy in the absence of any external electron acceptor; bacteria, yeast and moulds being mainly responsible. Lactic acid bacteria and yeasts have been implicated in the fermentation of some African indigenous foods and have also been reported to be responsible for the production of flavour compounds, aroma, microbial stability and food safety in "*ogi*" [5,6]. Lactic acid bacteria and yeasts have also been used as starter cultures in various food products because of their desirable effects in such foods [7].

A starter culture is a culture containing specifically selected known microorganisms which is used for initiating and controlling fermentation. It aims at increasing consistency and speed and also improving the nutritional quality, sensory attributes, degrading antinutritional factors such as phytate and reducing biogenic amines [8,9]. The use of starter cultures such as *Saccharomyces* sp, *Lactobacillus* sp, and *Pediococcus* spp. have also been employed in the dairy, brewery, baking and wine industries for ages.

Rice is nutritionally poor and subsistence on rice is responsible for *beriberi* in children and this has been reduced by parboiling or enrichment of rice [4]. Rice was also genetically modified with Beta carotene to combat

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vitamin A deficiency and blindness in children of developing countries [2]. To improve the quality of rice and also shorten the processing time, various modifications to parboiling such as soaking in hot water for a short period of time (3 - 4 h) as practiced in India and steaming above atmospheric pressure in Nigeria [4,10] have been employed. However the use of starter cultures isolated from the soak-water of the rice to improve the quality of "ofada" rice has not been investigated considering the numerous advantages of starter cultures. Also, literature is deplete of information on the role of lactic acid bacteria and yeast cultures in improving the quality of "ofada" rice.

2. Materials and Methods

2.1. Sample Preparation

"Ofada" rice was purchased from Ofada town in sterile polythene bags. Fifty kilograms of the freshly harvested rice was randomly obtained from three different farms in the town and mixed together to form a composite sample of 150 kg. Sixty kilograms of the rice was further obtained from the sample by a process of quartering and transported to the laboratory for analyses.

2.2. Processing of "Ofada" Rice

The rice was processed using the local method as described by Adeniran [11]. The paddy rice was soaked in water for 72 h as done in the traditional processing of "ofada" rice and parboiled under steam. The parboiled rice was sun dried for 24 h and then milled to obtain the rice grains. Samples from the soak-water were aseptically collected into sterile bottles and subjected to chemical and microbiological analyses. All experiments were conducted in triplicates while uninoculated rice served as control.

2.3. Isolation of Pure Cultures

Microorganisms from soak-water were cultured on plate count, MRS and PDA agar respectively and then subcultured repeatedly to obtain pure cultures. The isolates obtained were then characterised and identified using standard schemes [12].

2.4. Screening of Isolates from Soak-Water as Starter Cultures

The criteria for the choice of inoculum as starter cultures were principally frequency of occurrence of isolates and appearance of isolates at the end of soaking period which is a function of the stability and adaptation of isolates to the substrate (soak-water) [13]. The selected lactic acid bacteria isolates were maintained on MRS agar slants and the yeasts on PDA agar slants and stored at 4˚C.

2.5. Inoculation of the Selected Isolates into "Ofada" Rice

The selected starter cultures were harvested by aseptically adding 10 ml of sterile water into the agar slants in order to make pure culture suspensions. The suspension was adjusted with sterile water to give a concentration of 10^6 cfu/ml with the improved Neubauer hemocytometer. "Ofada" rice was sorted to remove dirt's and stones and the rice washed with three changes of sterile water to reduce contaminants. The rice was further soaked in cold water (1:5) after which 5 ml of 10^6 cfu/ml of the starter cultures were aseptically inoculated into the soaked rice and the rice allowed to ferment for 48 h. The rice was parboiled until the seed coat opened, dried to moisture content of 14% and milled with locally fabricated rice milling machine. Traditionally soaked paddy rice (without starter culture) served as control. The milled rice was subjected to chemical and sensory analyses while the soak-water was used to assay the soaking time of "ofada" rice.

2.6. Effect of Starter Cultures on Soaking Time of "Ofada" Rice

In order to assess the effect of starter cultures on the soaking time of "ofada" rice, the pH and titratable acidity content of the soak-water was measured at 12 h intervals for 72 h.

The pH value of the soak-water was measured using a digital pH meter (Jenway, 3015, UK) according to AOAC [14] method while the titratable acidity content of the fermenting soak-water was also determined using the method described by Edema [12]. This involved diluting ten millilitres of the soak-water in 90 ml of sterile distilled water out of which 25 ml of it was titrated against 1 N NaOH with 10% phenolphthalein (0.5% in 5% ethanol) as indicator.

The titratable acidity content was calculated from the formula:

$$\text{Lactic acid}\,(\text{mg/ml}) = \frac{\text{Average titre value} \times 90.08}{\text{Volume of sample}}$$

where 90.08 is the lactic acid equivalent factor.

2.7. Effect of Starter Cultures on the Chemical Composition of "Ofada" Rice

The content, protein, fat, ash, carbohydrate, crude fibre, mineral (atomic absorption Spectrophotometer, Buck, 210, USA), amylase (Spectrophotometer, Unispec, 23D, England) and vitamin (titrimetric method) contents of the samples were determined by the AOAC method [13].

2.8. Sensory Evaluation

The sensory evaluation of the cooked rice was carried out

with 23 panelists who were asked to indicate their preference in terms of attributes such as aroma, taste, appearance and overall acceptability using 9 point hedonic scale where I and 9 represents dislike extremely and like extremely respectively.

2.9. Statistical Analysis

All data were reported as means of replicates. Means were separated by Duncan's multiple range tests to establish significant differences at $p < 0.05$ between samples respectively, using SPSS version 10.0.

3. Results and Discussion

3.1. Selection of Isolates from Soak-Water

Table 1 shows the organisms isolated from the soak-water. *Leuconostoc mesenteroides*, *lactobacillus amylophillus*, *Saccharomyces cerevisiae*, and *Saccharomyces uvarum* were selected because cultures for food fermentation are primarily selected on the basis of dominance during fermentation and appearance at the end of fermentation [12]. They are also selected by their ability to produce the desired products or changes efficiently [14, 15].

3.2. Effect of Starter Cultures on the Soaking Time of "Ofada" Rice

The pH and titratable acidity content of the starter cultures is shown in **Table 2**. It was observed that while the pH reduced, the TTA increased significantly ($p < 0.05$) during the soaking period. The pH of the lactic acid bacteria and yeasts were significantly different ($p < 0.05$) from each other as well as from the uninoculated rice and fermentation time was also shortened to 48 h. The production of acids which are the main metabolites of lactic acid bacteria provides enabling environment for yeasts in mixed fermentations [16]. In single cultures there is no competition for nutrients and growth factors hence there is increased production of metabolites in a short time (**Table 2**).

3.3. Effect of Starter Cultures on the Chemical Composition of "Ofada" Rice

3.3.1. Proximate Composition

Changes in the proximate composition of "ofada" rice inoculated with lactic acid and yeast cultures are presented in **Table 3**. The proximate composition of rice treated with lactic acid bacteria and yeast starter cultures were significantly different ($p < 0.05$) from the control and the changes in composition more pronounced in the crude protein. This agrees with the work of Edema [12] who reported an increase in the crude protein content of

Table 1. Percentage frequency of occurrence of lactic acid bacteria and yeasts isolated from soak-water of "ofada" rice.

Isolate	Frequency of Occurrence (%)
Bacteria	
Lactobacillus farciminis	50
Leuconostoc mesenteroides	75
Lactobacillus maltarominicus	50
Lactobacillus kefir	50
Lactobacillus amylophillus	100
Lactobacillus alimentarius	25
Pediococcus halophillus	50
Yeasts	
Saccharomycodes sinensis	50
Saccharomyces uvarum	75
Pichia angusta	50
Candida mesenterica	50
Torulospora delbrueckii	50
Saccharomycodes cerevisiae	100

Table 2. Effect of starter cultures on the pH and total titratable acidity (mg/ml) of fermenting soak-water of "ofada" rice.

Inoculum	Time (h)											
	0		12		24		36		48		72	
	pH	TTA	pH	TTA	pH	TTA	pH	TTA	pH	TTA	pH	TTA
Uninoculated	7.57^{e}	0.02^{a}	7.50^{e}	0.04^{a}	7.35^{e}	0.09^{a}	6.92^{d}	0.16^{a}	6.78^{e}	0.20^{a}	6.33	0.28
L_1	7.22^{b}	0.12^{c}	6.84^{b}	0.19^{d}	6.40^{b}	0.27^{c}	6.10^{b}	0.34^{c}	5.45^{b}	0.44^{c}	ND	ND
L_2	7.01^{a}	0.10^{c}	6.62^{a}	0.22^{e}	6.21^{a}	0.30^{d}	6.03^{a}	0.37^{d}	5.23^{a}	0.48^{d}	ND	ND
Y_1	7.36^{d}	0.07^{b}	7.02^{c}	0.13^{c}	6.71^{d}	0.23^{b}	6.43^{c}	0.26^{b}	6.01^{d}	0.36^{b}	ND	ND
Y_2	7.31^{c}	0.07^{b}	7.00^{c}	0.10^{b}	6.50^{c}	0.24^{b}	6.02^{a}	0.37^{d}	5.80^{c}	0.42^{c}	ND	ND

Mean values with different superscripts along the columns differ significantly by Duncan's multiple range tests at 5% level of significance. Key: L_1 *Leuconostoc mesenteroides*; L_2 *Lactobacillus amylopophillus*; Y_1 *Saccharomyces uvarum*; Y_2 *Saccharomyces cerevisiae*; ND Not determined.

sour bread made with lactic acid bacteria and yeasts. This increase could be due to the biosynthesis of protein by starter cultures [12]. Mackay and Baldwin [17] also reported a significant improvement in the protein quality of some fermented cereal products as a result of fermentation. The carbohydrate content of rice inoculated with starter cultures was also less than the uninoculated rice probably due to the breakdown of some starch and sugar components of "ofada" rice as a result the amylolytic and fermentative action of enzymes and fermenting organisms [18].

3.3.2. Mineral Content

The changes in the mineral content of rice inoculated with starter cultures are shown in **Table 4**. There was no significant difference ($p < 0.05$) in the ash content of *Saccharomyces uvarum* and *S. cerevisiae* and also between *Lactobacillus amylophillus* and uninoculated rice while the ash content of rice inoculated with *Leuconostoc mesenteroides* was significantly different from the other samples.

3.3.3. Vitamin Content

The riboflavin and thiamin contents of the rice samples inoculated with *Saccharomyces cerevisae* and *Saccharomyces uvarum* were significantly different from each other and the remaining isolates (**Table 4**).

This result is in agreement with the work of Batharcaray [19] and Otegbayo [4] who reported an increase in the vitamin content of rice especially riboflavin and thiamin as a consequence of the migration and redistribution of the water soluble vitamins [4]. The possibility of the synthesis of some of these vitamins by the starter cultures during their metabolic activities could be another factor.

3.3.4. Amylose Content

The amylose content of the samples were also significantly different ($p < 0.05$) from each other with the uninoculated rice having the highest value of 17.61% (**Table 4**). The amylose content of parboiled rice reduces during parboiling as a result of starch solubulisation and leaching of the amylose molecules into the surrounding water during soaking and subsequent steaming during parboiling [4].

3.3.5. Sensory Quality of Cooked Rice Inoculated with Starter Cultures

The effect of starter cultures on the sensory quality of cooked "ofada" rice is presented in **Table 5**. There was a significant difference ($p < 0.05$) in the sensory quality of the rice with the rice that was treated with *Saccharomyces cerevisiae* rated best in aroma, taste, appearance and over all acceptability. Furthermore, the rice inoculated with *Saccharomyces cerevisiae* had the highest score of 7.5 and was the most acceptable rice while the rice inoculated with *Leuconostoc mesenteroides* had the lowest score of 5.3. Earlier workers had postulated that starter cultures enhanced the flavour of foods [7,14,6] and the production of volatile compounds may be responsible for the aroma perceived in such fermented foods. Heterofer mentative *lactobacilli* usually produce sharp and not too

Table 3. Proximate composition (%) of inoculated "ofada" rice.

	Moisture	Dry Matter	Fat	Ash	Crude Fibre	Crude Protein	Carbohydrate
Uninoculated	14.22^{b}	85.77^{d}	1.36^{c}	1.43^{c}	0.92^{d}	11.37^{a}	71.00^{e}
Lactobacillus amylophillus	14.94^{e}	85.02^{a}	0.33^{b}	1.0^{b}	0.84^{c}	16.72^{e}	66.10^{c}
Saccharomyces uvarum	14.65^{d}	85.35^{b}	0.26^{a}	0.96^{a}	0.78^{b}	17.48^{d}	65.87^{b}
Leuconostoc mesenteroides	14.29^{c}	85.71^{c}	2.04^{e}	1.43^{c}	1.02^{e}	17.07^{c}	64.15^{a}
Saccharomyces cerevisiae	13.79^{a}	86.21^{e}	1.72^{d}	0.93^{a}	0.76^{a}	12.27^{b}	70.53^{d}

Mean values with different superscripts along the columns differ significantly by Duncan's multiple range tests at 5% level of significance.

Table 4. Mineral, vitamins (mg/100g) and amylose (g/100g) content of inoculated "ofada" rice.

	Sodium	Potttassium	Thiamin	Riboflavin	Amylose
Uninoculated	0.31^{b}	1.20^{b}	4.34^{c}	1.54^{b}	17.61^{e}
Lactobacillus amylophillus	0.34^{c}	1.20^{a}	4.32^{c}	1.62^{d}	14.06^{a}
Saccharomyces uvarum	0.31^{b}	1.20^{a}	4.10^{b}	1.57^{c}	15.32^{c}
Leuconostoc mesenteroides	0.35^{c}	1.50^{b}	4.32^{c}	1.62^{d}	14.49^{b}
Saccharomyces cerevisiae	0.28^{a}	1.30^{ab}	4.01^{a}	1.42^{a}	16.3^{d}

Mean values with different superscripts along the columns differ significantly by Duncan's multiple range tests at 5% level of significance.

Table 5. Mean sensory scores of cooked "ofada" rice.

	Taste	Aroma	Appearance	Overall Acceptability
Uninoculated	5.70^{ab}	5.00^{a}	5.60^{b}	5.30^{ab}
Lactobacillus amylophillus	5.70^{ab}	5.40^{b}	4.70^{a}	5.20^{a}
Saccharomyces uvarum	5.90^{ab}	6.40^{c}	6.20^{abc}	6.20^{bc}
Leuconostoc mesenteroides	6.30^{abc}	6.50^{c}	6.70^{cd}	6.60^{c}
Saccharomyces cerevisiae	7.00^{c}	7.20^{d}	7.50^{d}	7.50^{d}

Values with different superscripts along the column differ significantly by Duncan's multiple range tests at 5% level of significance.

pleasant odours during fermentation and this may be responsible for the low sensory score observed in the rice inoculated with *Leuconostoc mesenteroides*.

4. Conclusion

The findings showed that the starter cultures reduced the soaking time of "ofada" rice to 48 h, enhanced the nutrient composition and improved the sensory quality of the rice. There is need for further work on the effect of mixed cultures on the quality and soaking time of "ofada" rice.

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