Effect of Fermentation on Bacteriological and Physicochemical Properties of 'ofada" rice.

Abstract

Rice (*Oryza sativa*) is an important annual crop in Nigeria and one of the major staples, which can provide a nation's population with the required food security of 2,400 calories per person per day. In Nigeria rice is one of the few food items whose consumption has no cultural, religious, ethnic or geographical boundary. Fermented rice is used to produce rice wine, spaghetti and noodles. Work was then carried out on the Nigerian rice VAR. ITA 150 (ofada) to determine the bacteriological and physicochemical activities during fermentation. Standard microbiological and chemical methods were used. Six microorganisms were isolated which include; *Bacillus cereus*, *Micrococcus luteus Lactobacillus plantarum, Staphylococcus aureus, Leuconcostoc mesenteroides*, and *Bacillus licheniformis*. It was observed that the microbial loads increased till the 72nd hour of fermentation except *Staphylococcus aureus* and *Micrococcus luteus* that their loads decreased after the 72nd hour. There was an increase in the moisture, fibre, fat and protein contents, while carbohydrate, ashS and anti-nutrients contents decreased. It was evident that fermentation process contributes to the bacteriological and physicochemical properties of the fermented rice in the production of another consumable product.

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Keywords: Fermented rice, Microorganism, Physicochemical, Bacteriological, Chemical.

Introduction

Oryza sativa (var. ITA 150) commonly called 'Ofada' in Nigeria, is one of the indigenous rice varieties emanated from South-West Nigeria. It is an unpolished medium grain rice. Consumers gave it preference for its unique taste and aroma. However, inefficient processing technology leads to unappealing products because of the presence of stones when it is being eaten (Omotayo, *et al*., 2012).

Rice is fermented to produce rice wine. Rice wine is an alcoholic beverage made from rice. Unlike the European wine, that is made by fermentation of natural sweet grapes and other fruits, rice wine is made from the fermentation of rice starch converted to sugars. The process is akin to that used to produce beer. Beer production employs a mashing process to convert starch to sugars whereas rice wine uses the amylolytic process (Campbell-Platt, 2009). Sake is often referred to in English – speaking countries as rice wine. It is produced by means of a brewing process using a mash, similar to that which is used for beer production. Thus, sake would be more accurately referred to as rice beer rather than rice wine. Rice wine typically has a higher alcohol content, 18% - 25% ABV, than grape wine (9%-16%) which in turn has a higher alcohol content than beer (usually 4%-6%). Rice wine is much used in Chinese cuisine and in other Asian countries. Alcoholic beverages distilled from rice were formerly exclusive in East and Southeast Asia countries. Later, knowledge of the distillation process reached India and Parts of South Asia through trade. Some types of rice wine are: Pangasi – Rice wine from Mindanao in the Phillippines and Raksi - Tibetan and Nepali rice wine (Giwa *et al.,* 2011).

In Nigeria, rice can be used in the production of "kunu-zaki", a beverage usually consumed in the Northern part of Nigeria. Kunu-zaki, known for its moderately high carbohydrate content, sweet taste and low viscosity, is produced mainly from millet (*Pennisetum* species), although sorghum (Sorghum bicolor), maize (*Zea mays*), rice (*Oryza sativa*) and other cereals can be used. It is normally flavored with a combination of spices commonly called "Kayan yaji'' which includes ginger (*Zingiber officinale*), cloves (*Eugenia aromatica*), black pepper (*Piper guinese*) and cinnamon (*Xylopia acthiopica*) (Jay, 1987; Adebayo *et al*., 2013)

During fermentation, each microorganism contributes its quota by producing different enzyme which can act on the microbiological or physicochemical composition of the food either positively or negatively. Therefore, the rationale behind this work was to see the effect of fermentation on microbiological properties of “ofada” rice. The specific objectives of this research work are to:

(a) determine the types and loads of bacteria associated with the fermented Nigerian rice (*Oryza Sativa* (ofada) Var. ITA 150);

(b) investigate the effect of fermentation on the physicochemical properties of the fermented rice.

MATERIALS AND METHODS

Sample collection, Sterilization of materials and media preparation.

Three cups of “ofada” rice were bought in Igbemo Ekiti where the rice is being cultivated in large quantity. The inoculating chamber was sterilized using uv- light, all the glass wares used were sterilized inside the oven at $160^{o}C$ for 1hr. The media used were Nutrient agar (NA) and Nutrient Broth NB) and prepared according to manufacturer’s instruction. The media were then autoclaved at $121^{o}C$ for 15 minutes.

Preparation of Rice Samples for Microbial analysis

One gram of the rice was weighed into 9 mL of sterile water, the whole content was mixed properly. This constituted the stock solution. A syringe was used to dispense 9.0 mL of distilled water into each of four test tubes and the test-tubes autoclaved at $121^{o}C$ for 15 minutes. One mL was transferred from the stock solution of raw rice to a test tube containing 9.0 mL sterile distilled water and it was labeled as 10^{-1}. The serial dilution process was carried out serially until it got to the test tube labeled as 10^{-4}.

Isolation of bacteria

From 10^{-4} of the serially diluted sample above, 0.5 mL was transferred to the center of a sterile Petri dish. About 15 mL of each molten NA cooled to $45^{o}C$ was then added to each plate and rocked gently to facilitate mixing the agar with the sample. The NA plates were placed at $37^{o}C$ for 24 hours.

Determination of Microbial Population and Cultural Morphology

The distinct bacterial colonies that grew on the NA were counted using colony counter and examined physically for their characteristics features: colour, shape, size, elevation, surface and edges.

Purification of Bacterial isolates

The bacteria isolated were purified by streaking each type of the colonies onto fresh nutrient agar using inoculating loop and flame. Incubation was carried out for 24 hr at $37^{o}C$. Gram staining was carried out and observed under the microscope for purity. The purified isolates were then placed on agar slants incubated at $37^{o}C$ for 24 hours and kept in the fridge until required for further tests.

Biochemical Characterization of Bacterial Isolates.

Gram’s staining, Motility test, Catalase test, Spore test, Fermentation of sugars and coagulase test were done according to standard microbiological methods.

Preparation of the Microbial Inocula for Fermentation of Rice

Three loopfuls of microbial cells were taken with an inoculating loop from each stock of the purified isolates in to the already autoclaved, cooled 10mL nutrient broth. It was incubated at $37^{o}C$ for 24 hours. Afterwards, 10 ml of each grown microorganism was centrifuged at 1,500 rpm for 15 minutes and the supernatant was discarded. The cells were washed with 10 mL sterile distilled water and re-suspended in another 10 mL sterile distilled water. This was used as bacterial inoculum preparation. 5 ml was withdrawn and 15mls of water was added to 10g of the raw rice and mixed together. The inoculation was done by using only one microbial type for fermentation. Fermentation was allowed to take place for 3 days (72 hrs) at room temperature of 28 ± 2ºC. The Control was allowed to ferment naturally without inoculation. Sampling was done at 24 hrs interval to determine the microbial loads.

Physicochemical properties of the fermented rice

Physicochemical properties like moisture, fat, ash, carbohydrate, and fibre content of the fermented rice was determined according to the methods of AOAC, 2000.

RESULTS AND DISCUSSION.

A total of 6 different bacterial types were observed in the fermented rice. The microbial loads varied. After assessing the microbiological parameters like color, surface, shape and elevation on nutrient agar and their Gram staining reactions with biochemical tests, *Bacillus cereus*, *Bacillus licheniformis*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Micrococcus luteus* and *Staphyloccus aureus* were the inherent organisms on the ofada rice. The highest colony forming unit bacteria observed was *Staphylococcus aureus* and least was *Bacillus cereus* as seen in Fig 1.

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The population *of Bacillus cereus*, *Bacillus llicheniformis*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* increased from 24 hrs till 72 hrs of fermentation. However, *Staphylococcus aureus* and *Micrococcus luteus* showed a reduction in population after 72 hours of fermentation(Fig 2). The isolated bacteria were similar to the findings of (Adekoyemi *et al.,* 2012) who reported the isolation of these bacteria during the fermentation of a Nigerian rice var"ofada". Odunfa, 1983 first reported that the predominant fermentation microorganism during maize fermentation in the production of 'ogi' was a *Bacillus subtilis* and *Lactobacillus plantarum*. The reduction in *Staphycoccus aureus* colony count after the 72 hours of fermentation could be due to decrease in pH , (Fleming, 1982; Bernardeau, 2006) reported that pH reduced gradually as the fermentation progressed, which only allows acidophiles (acid microorganisms) to grow, hence reduction in Staphylococcal count and increase in *Lactobacillus* sp count. More so, *Staphylococcus aureus* needs salt before it can grow well. This can also be due to reduction in the amount of available nutrients needed for their growth and also some microorganisms might have released toxic substances into the fermenting substrate which could have adverse effect on other microorganism, as supported by (Gaggia *et al.*, 2011) who worked on the fermentation of *Hura crepitans* seeds. A significant differences were observed in each of the fermented samples at $p \leq 0.05$ % confidence interval. There was an increase in the moisture, protein and fibre contents of the fermented rice sample within the 72 hours of fermentation. However contents of Ash, Fat and carbohydrates decreased as shown in figure 3 below. This was similar to the report given by Eka, (1998) who reported decrease also in fat content of locust beans after fermentation. The reduction in the crude fat content of the fermented grains may be due to the activities of microorganisms, which led to the breakdown of some amino acids with liberation of ammonia (Onifade *et al*., 2001) or leach out of the fat and other metabolic activities that occurred during the fermentation.

After the third day (72 hour) the protein content increased. This may be due to the fact that some microorganisms were dead and released their constituents into the rice samples which could have increased the protein concentration as suggested by Abu *et al.,* (1998). The biomass could equally have contributed to the increased protein content (Achinewhu and Isichei, 1990; Ross *et al,* 2009).

The carbohydrate contents decreased, the decrease was obviously due to the fact that the carbohydrates were used up as sources of energy by the metabolizing microbes during fermentation. Uzomah and Ogunsanya (2011) worked on *Mucuna sloanei*, *Detarium microcarpum* and *Brachystegia euccycoma* and found out that the carbohydrate contents of each of them decreased till the 3rd day of fermentation. The fermentation process involves the conversion of materials to the peculiar substances needed by the microorganisms for various activities like build up cell wall. As the microorganisms were not separated from the biomass.

Reduction in the ash content of all the samples may be due to boiling loss and leaching of the soluble inorganic salt during fermentation. There was a general noticeable reduction in all antinutrients quantified for, at every hour of the rice fermentation. This could be attributed to the activity of the microbes used in the fermentation process, which could have initiated the activities of the enzymes that degraded these antinutrients (Mubarak, 2005) who worked on the nutritional composition and antinutritional factor of mung beans *(Phaseolus aureus)*

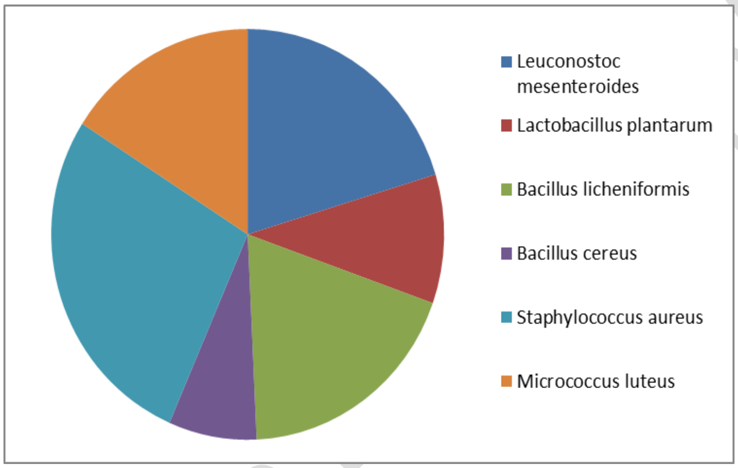

Fig 1. Occurrence of bacteria population before fermentation

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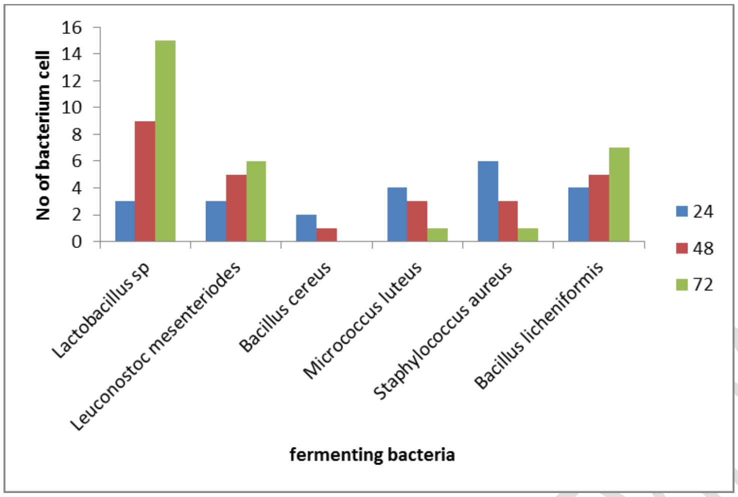

Fig 2: Microbial loads of the fermenting rice

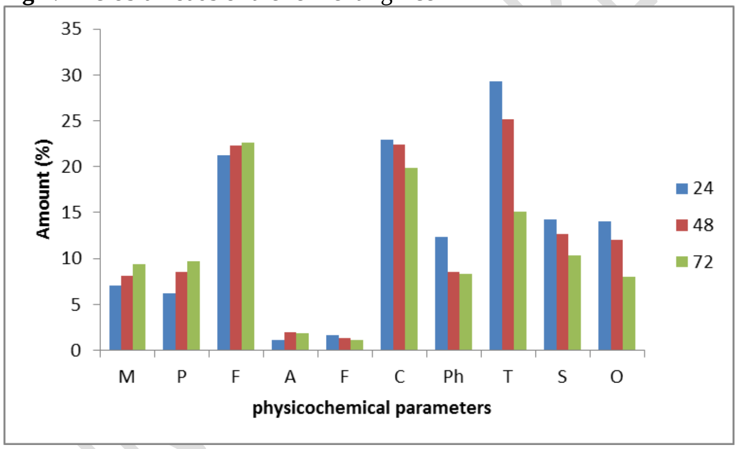

Fig 3: Percentage physicochemical parameters during fermentation
Key: M: moisture content, P: Protein content, F: Fibre content, A; Ash content, F: Fat content, C: Carbohydrate content, Ph: Phytate content, T: Tannin content, s: Saponin content, O: Oxalate content.

CONCLUSION AND RECOMMENDATION

It can be concluded that every food product has its own resident microflora and each microbe contributes to the fermentation process. From the results of this study, fermentation process produced significant and noticeable effect on the microbial load of the fermenting and fermented Nigerian 'ofada' rice samples. Fermentation of this variety of rice can be acceptable in order to produce a fermentable food product, since, its originality can still be retained because, it has not gone through any pre-treatment before fermentation.

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