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Protein enrichment of cassava with yeasts for garri production

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ABSTRACT

Saccharomyces cerevisiae strains PWO2A, PWO3C and PWO3D isolated from palm wine were used to enrich cassava for garri production. During the fermentation process, there were changes in microbiological and biochemical characteristics of the cassava mash. The total viable bacterial counts increased from 2.7×10^2 cfu/g to 4.2×10^2cfu/g for the enriched cassava mash after 48 hours while that of the unenriched increased from 2.7×10^1cfu/g to 3.0×10^1 after 24 hours and then decreased to 2.5×10^1cfu/g after 48 hours. The fungi counts of the enriched cassava ranged from 3.5×10^2cfu/g to 6.8×10^2cfu/g while the fungi counts of the unenriched sample ranged from 2.5×10^1cfu/g to 2.9×10^1cfu/g. Various bacterial species (*Bacillus, Staphylococcus, Klebsiella, Corynebacterium, Escherichia*), moulds (*Aspergillus, Fusarium, Mucor, Rhizopus*) and yeasts (*Saccharomyces cerevisiae, Candida sp*) were found to be associated with the fermentation process and probably contributed to the reduction of cyanide content of the cassava. The enriched garri had a protein content of 7.6% while the unenriched had a protein content of 6.3%. It was observed that commercially prepared garri had a protein content of 4.0%. Generally, good acceptability and organoleptic qualities (taste, aroma, colour, texture) of the protein enriched garri were best achieved within 48hours of enrichment. The results suggest that garri can be made more nutritious with yeasts particularly *Saccharomyces cerevisiae* strains PW02A, PW03C and PW03D.

KEYWORDS

Protein;
Enrichment;
Cassava;
Yeast;
Palm wine.

INTRODUCTION

There is much concern for the development of food to meet the increasing population of the world, especially in developing countries like Nigeria, where there are cases of malnutrition arising from low protein intake. The quality of a diet is determined to a large extent by the amount of protein it contains. Traditional foods of animal origin are often relied upon to meet the protein needs. However, conventional protein sources such as fish, meat and eggs are expensive and unaffordable with low income of a higher population of the developing countries. The bulk of diets in the developing countries are mainly carbohydrate foods (cereals, yam, cassava, etc.) that are low in protein. High consumption of foods low in

protein results in high sugar concentration in the blood giving rise to diabetes and a reduction in the mental and physical activity as well as a disease in children called kwashiorkor.

Cassava (*Manihot esculenta*) is a major crop and staple food in developing countries. It has 0.4% protein[9] and 86% carbohydrate. It is easy to cultivate, resist drought and tolerate poor soil. Cassava is an essential diet of more than half a billion people; it can be eaten after boiling or roasting. It can be processed into many products such as lafun (a wet dry fermented product), fufu (a creamy white fermented paste). At present most of the cassava is consumed in the form of garri, a preparation which involves an anaerobic fermentation step which gives the final product a typical sour taste. Although garri is widely consumed in West Africa, scanty information exists on the microbiological and physicochemical qualities of garri. In addition there is little or no information on protein enrichment of cassava for garri production.

Microorganisms (algae, fungi, bacteria, yeasts) can serve as a substitute for protein supplement because they contain high amount of protein, have rapid growth rate and have the possibility of being cultured on diverse substrates[1]. The growth of these microorganisms which is more rapid than that of higher plants makes them very attractive as higher protein crops. While only one or two grain crop is grown per year, a crop of yeast or mold may be harvested weekly and bacteria may be harvested daily.

However, yeast has been the most widely accepted and used microbe for enriching the food and feeds of man and animals respectively. It has the greatest advantage in protein composition, in that it has a broad amino acid composition and is the richest source of lysine[6]. Microorganisms used for food enrichment purposes have been obtained from various sources. For instance[16], enriched cassava using molds isolated from traditional foods. Similarly[5,6], enriched cassava for fufu production with yeasts isolated from burukutu (a local wine brewed from sorghum). The aim of this study is to improve the protein content of cassava through fermentation with yeasts isolated from palm wine. It is usually consumed with the yeasts it contains. The yeasts are not pathogenic.

MATERIALS AND METHODS

Collection of samples

Samples of palm wine were collected in sterile bottles from sales point at Tradoc Military Cantonment, Minna, Nigeria and transported in ice box to the laboratory for the isolation of yeasts. Fresh tubers of cassava (*Manihot esculenta crantz)* were obtained from Gwari market, Minna, Nigeria in polythene bags and transported to the laboratory. The fresh cassava mash had a moisture content of 58%, 1% protein, 10% fat, 1% ash, 141.51mg/kg cyanide and pH 7.0.

Isolation and identification of yeasts

Yeast strains were isolated by plating serially diluted samples of palm wine on Sabouraud dextrose agar (SDA) and incubated at room temperature (28± 2^0C) for 48 h. Different isolated colonies were subcultured repeatedly on fresh media to obtain pure cultures of the isolates. The isolated yeasts were characterized using colonial morphology, cellular characteristics, ascospore formation, vegetative reproduction and utilization of sugars. The organisms were identified by comparing their characteristics with those of known taxa using the scheme of[2,8]

Screening of yeasts isolates for potential to grow in cassava medium

The ability of the yeast isolates to grow on cassava was investigated by incubating the organisms in mineral salt broth (MSB) containing cassava filtrate (carbon source) and incubating the culture under stationary condition at room temperature (28±2^0C) for 72h. At the end of the incubation, the medium was observed visually for turbidity as index utilization of the incorporated carbon source.

Production of biomass

Based on the result of the screening test, three strains of *Saccharomyces cerevisiae* (PW02A, PW03C, PW03D) were selected for the enrichment experiment. Cell biomass was produced using potato dextrose broth in which 250mg of chloramphenicol was incorporated to inhibit bacterial growth. The medium was sterilized by autoclaving at 121^{0}C for 15 min. Each flask was inoculated with each of the yeast strain and plugged

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with cotton wool, and incubated at 28^{0}C for 72hrs using a flask shaker. The yeast cells were harvested by centrifugation at 400rpm. The harvested yeast cells were washed using sterile distilled water and dried in an oven at 45^{0}C. The dried yeast cells were stored in sterile McCartney bottle until required.

Biochemical analysis of fresh, enriched and unenriched cassava mash

Estimation of protein content

The protein content of the sample was determined using the micro kjeldal method using a protein conversion factor of 6.25.

Determination of lipid (fats)

The fat content of the samples was determined by direct soxhlet extraction using petroleum ether (bp 40-60^{0}C) as solvent. 0.5g of the sample contained in a filter paper was placed in an extractor and the set up was placed on a heating mantle. The heat source was adjusted so that the solvent boiled gently and the refluxed several times for 6h until the ether had siphoned over and boiled off until the extractor was empty. On removal, the filter paper was placed in an oven at 50^{0}C and dried to constant weight. The percentage lipid was then calculated.

Analysis of ash content

The ash content of the samples was determined by placing a porcelain crucible having 30g of the dry sample in a murfle furnace and heated to 500^{0}C to burn off all the organic matter, leaving a white ash. The crucible was then removed and immediately covered and placed in a desiccator to cool. The weight was again measured and the percentage ash was calculated.

Determination of carbohydrate

The carbohydrate content of the samples was determined by subtracting the total protein and lipid from the organic matter.

Determination of moisture

A Petri dish was dried in an oven at 105^{0}C for 20min, cooled in a desiccator and weighed. 30g of the sample was placed in the dish and weighed again. The dish and sample was then dried in an oven at 105^{0}C for 24h to achieve a constant weight and was quickly transferred to a desiccator to cool. It was weighed again after cooling with minimum exposure to the atmosphere. The loss in weight of the sample during drying is the moisture content.

pH measurement

The pH of the samples was determined using a pH meter after standardizing it with phosphate buffer solution.

Determination of cyanide

The cyanide content of the samples was determined using the method of[11]. In this method, 20g of the crushed cassava roots was homogenized in distilled water for 10min. The homogenate was incubated for 18h at room temperature (28±2^{0}C) after which 100ml of 5% $NaHCO_3$ was added to it before distillation. The filtrate was collected and titrated against 0.2% iodine solution using 1% starch as indicator. Cyanide content was calculated using the values.

Production of garri

Fresh cassava roots were peeled, washed, grated and stored in a jute bag for dewatering. Fermentation of the fresh cassava roots was allowed to take place for 48h. After 48h, the dewatering process was completed using a dewatering machine to drain off the remaining water from the cassava mash. The cake formed was broken into smaller lumps, sieved and fried to a temperature of 80^{O}C until the moisture content reduced to 10%. During fermentation, the microbiological analysis of the fresh cassava mash was carried out after 0, 24h and 48h. Similarly, biochemical analysis (as described above) of the fermenting cassava was carried out.

Microbiological analysis of garri

A serially diluted sample of the fresh cassava mash was plated on Nutrient agar (for total viable bacterial counts), MacConkey agar (for coliform counts), mannitol salt agar (for Staphylococci), and sabouraud dextrose agar (for fungi). The inoculated plates were incubated at 37^{0}C for 24h for bacteria and at room temperature (28± 2^{0}C) for 3-5 days for fungi. Colonies which developed on the plates were counted and expressed as colony forming units per gramme (cfu/g) of sample. The counts were determined after 0h, 24h, and 48h. The isolates were purified by repeated sub culturing on fresh media and characterized based on gram staining, colony morphology, biochemical tests includ-

ing production of catalase, coagulase and carbohydrate fermentation. The bacterial isolates were identified by comparing their characteristics with those of known taxa using the scheme of[3]. Fungi were identified using their morphological and biochemical characteristics[15]

Enrichment of garri with the yeasts

1.5g of 72h old yeast strain were inoculated into 200g of the fresh cassava mash, mixed thoroughly and allowed to stand for 48h for fermentation as the dewatering process proceeded. A control experiment was set up in which the cassava mash was not enriched with yeast cells. Samples were withdrawn at interval of 0h, 24h and 48h for the determination of protein, pH, ash, moisture, lipid, and cyanide contents. Microbiological analysis was also carried out during these intervals for bacterial, fungi, coliform and staphylococci counts.

Sensory evaluation of protein enriched garri

The method of[17] was used. 150g of the enriched garri was prepared with boiling water, allowed for five minutes and then stirred. The garri (eba) was compared with commercially prepared garri (eba) for the following parameters: taste, colour, texture, aroma and general acceptability by a panel of ten judges (using questionnaire) of regular eba consumers using the Hedonic scale product. The sensory scores were analyzed statistically.

RESULTS

Yeasts in palm wine

Of the nine yeasts isolated from the palm wine, three isolates, identified as *Saccharomyces cerevisiae* (PW02A), Saccharomyces sp (PW03C), and Saccharomyces cerevisiae (PW03D) exhibited high ability to grow and utilize cassava as a carbon source. While other isolates *Saccharomyces cerevisiae* (PW01B), *Saccharomyces cerevisiae* (PW02D), *Saccharomyces* sp (PW03B), *Saccharomyces* sp (PW03A), *Candida* sp (PW02B) and *Candida* sp (PW01A) would need more than 72h to grow maximally in the cassava medium. *S. cerevisiae* PW02A, PW03C and PW03D were able to grow maximally within 72h (TABLE 1). These three strains were selected for protein enrichment experiments.

Microbial counts and identification

The results revealed that the total viable bacterial counts for the enriched cassava mash increased from 2.7 $\times 10^2$cfu/g to 4.2 $\times 10^2$cfu/g after 48h. The coliform counts increased at the early stage (2.6 $\times 10^2$cfu/g) and decreased sharply after 24h to 1.0 $\times 10^2$cfu/g and then increased slightly again after 48h, 1.4 x 10^2cfu/g. The counts of staphylococci also decreased from 2.0 $\times 10^2$cfu/g to 1.3 $\times 10^2$cfu/g after 48h fermentation. Fungi counts increased from 3.5 $\times 10^2$cfu/g to 6.8 $\times 10^2$cfu/g over the same period (TABLE 2). It was observed that coliforms dominated at the initial stage of fermentation, the aerobic heterotrophic bacteria dominated after 24h while yeasts dominated at the end of the fermentation period.

Several bacterial genera were identified during the fermentation period: *Bacillus, Klebsiella, Escherichia, Corynebacterium* and *Staphylococcus*. Fungi isolated included *Aspergillus, Fusarium, Mucor* and *Rhizopus*. It was observed that *Aspergillus* had the highest occurrence. Yeasts *Saccharomyces cerevisiae* and *Candida sp* were also identified.

The pH of the enriched cassava mash decreased from 6.98 to 6.96 after 48h while that of unenriched cassava mash increased from 7.0 to 7.02. The cyanide content of the fermenting enriched cassava mash decreased after 24h from 141.75mg/kg to 97.88mg/kg and further decreased to 52.13mg/kg after 48h. The cyanide content of the unenriched fermenting cassava mash decreased from 128.25mg/kg to 101.25mg/kg after 48h (TABLE 3).

TABLE 1 : Screening test for growth of yeasts in cassava medium

Coded yeast strain	Growth in cassava medium after 72 hours
Saccharomyces cerevisiae PW03D	+++
Saccharomyces cerevisiae PW02A	+++
Saccharomyces sp PWO3C	++
Saccharomyces cerevisiae PW01B	+
Saccharomyces cerevisiae PW02D	+
Saccharomyces sp PW03B	+
Saccharomyces sp PW03A	+
Candida sp PW02B	+
Candida sp PW01A	+

+++: maximum growth; ++: moderate growth; +: minimal growth

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TABLE 2 : Microbial population during cassava fermentation for garri production

Fermentation period (hrs)	Enriched cassava mash				Unenriched cassava mash			
	TVB ($\times10^2$ cfu/g)	CC ($\times10^2$ cfu/g)	ST ($\times10^2$ cfu/g)	FUN ($\times10^2$ cfu/g)	TVB ($\times10^1$ cfu/g)	CC ($\times10^1$ cfu/g)	ST ($\times10^2$ cfu/g)	FUN ($\times10^2$ cfu/g)
0	2.7	2.6	2.0	3.5	2.7	4.6	2.0	2.5
24	4.0	1.0	1.7	2.0	3.0	3.0	1.5	4.0
48	4.2	1.4	1.3	6.8	2.5	5.0	1.3	2.9

TVB: Total viable bacteria; CC: Coliforms; ST: Staphylococci; FUN: Fungi; cfu/g: Colony forming units per gramme.

TABLE 3 : Biochemical qualities of enriched and unenriched cassava mash

Time (hours)	Sample	Crude protein (%)	Moisture (%)	Lipid (%)	Ash (%)	Cyanide (mg/kg)	pH
0	E	2.38	59.03	10	2.99	141.75	6.98
	UNE	2.10	58.22	10	1.25	128.25	7.0
24	E	3.24	47.2	14	2.25	97.88	7.01
	UNE	2.75	44.04	9	1.75	121.50	6.94
48	E	4.96	45.01	18	1.50	52.13	6.96
	UNE	4.22	2.53	7	1.25	101.25	7.02

E: Enriched, UNE: Unenriched sample

Sensory evaluation of the protein enriched garri

The results of sensory evaluation of enriched garri using Hedonic scale are presented in TABLE 4. Statistical analysis of the data showed that there were significant differences ($P<0.05$) among the enriched, unenriched and commercially prepared garri. However, the texture and taste of the enriched garri was preferred to that of the commercially prepared garri after 48h of fermentation. Thus, good acceptability of the sensory parameters (colour, taste, aroma, texture) of the enriched garri was best achieved after 48 hours fermentation.

TABLE 4 : Sensory evaluation scores for protein enriched garri

Sensory attributes	Commercial garri	Unenriched garri	Enriched garri
Colour	5.9	8.1	9.0
Taste	4.5	6.8	8.7
Aroma	5.4	7.3	8.2
Texture	6.1	7.6	8.9
General acceptability	5.9	8.1	8.9

DISCUSSION

The present study revealed that palm wine contains yeasts particularly *Saccharomyces cerevisiae* and *Candida* spp. This could be as a result of high sugar content of palm wine which encourages the proliferation of the yeasts in the drink. Oke[13] reported that *S. cerevisiae* constituted 89-92% of total microbial isolates present in burukutu, a locally brewed fermented wine like palm wine. The screening of the yeast isolates for potential to utilize cassava as a carbon source showed that *S. cerevisiae* (PW02D, PW02Aand PW03C) exhibited maximum growth in cassava medium within 72h. This means that these organisms have competent degradative enzyme system for the utilization of the carbon source. The quality of the fresh cassava roots used has a protein content of 2.1%, which does not agree with[7] that cassava has a protein content of less than 1%. The protein content of a cassava root may depend on the type of cassava.

During the fermentation process, the total viable bacterial counts of the enriched cassava roots increased from 2.7×10^2cfu/g to 4.2×10^2cfu/g (48h). The increase in counts may be due to favorable conditions, which allowed the proliferation of the organisms. The unenriched cassava increased from 2.7×10^1cfu/g (at 0h) to 3.0×10^1 cfu/g (at 24h) and then decreased to 2.5×10^{-1}cfu/g (at 48h). The coliform counts increased

TABLE 5 : Comparison of the nutritional quality of the protein enriched garri with unenriched and commercially prepared garri

Sample	Moisture (%)	Ash (%)	Lipid (%)	Protein (%)	Crude fibre (%)
Enriched garri	6.0	1.4	2.0	7.6	19
Unenriched garri	7.1	1.4	2.0	6.3	21
Commercially prepared garri	5.3	3.0	2.4	4.0	26

at the early stage of the cassava fermentation for both the enriched and unenriched cassava and then decreased at the intermediate and the final stages. This may be due to the quality of the water used in washing the peeled cassava roots, or from the milling where it was grated, or bowls used in washing the cassava roots. The staphylococci counts were high at the early period of fermentation. The organisms probably originated from the water used in washing the cassava roots. The bacteria isolated during fermentation were *Staphylococcus, Bacillus, E. coli, Klebsiella* and *Corynebacterium*. This agrees with the report of Nwachukwu and Okafor[10,12] that these organisms are associated with cassava fermentation. *Bacillus* sp was the most frequently isolated; this may be due to the widespread of the organism in nature, and the ability to form spores.

Aspergillus, Fusarium, Mucor and *Rhizopus* were also isolated in the present study[14] in their study on the microbiology and technology of cassava starch fermentation isolated moulds of these genera. *Aspergillus* occurred more frequently which may be due to the abundance of the organism in nature. Yeasts of the genera *Saccharomyces* were also isolated. Several investigators[10,12,14,18] had also reported the involvement of these organisms in cassava fermentation. Okafor[12] reported that these organisms are indigenous microflora of cassava tubers.

At present, little has been documented on the biochemical processes involved in the different stages of cassava fermentation for garri production. The cyanide content of the cassava mash was high at the initial stage and then decreased after 72h. This may be due to the degrading ability of the fermenting organisms. Esser[4] reported that the bacteria *Bacillus* have linamarase enzyme that is capable of reducing hydrogen cyanide level within 72h. This helps in the detoxifying process.

The initial protein content of the enriched and unenriched cassava mash was 2.38% and 2.10% respectively (TABLE 3). This high protein content may be due to the type of cassava used. After 72h of fermentation the cassava mash was fried and the protein content rose to 7.6% (for enriched cassava mash) and 6.3% (for unenriched cassava mash) respectively (TABLE 4). The considerable great increase in the protein content of the enriched garri may be due to the mixed culture used for the enrichment process and the aseptic nature in which the production was handled. The low protein content of the commercially prepared garri may be due to the handling process, the other microorganisms present that may interfere with the fermentation process by competing successfully with the yeast cells leading to low protein content.

CONCLUSION

Carbohydrate based food are poor in protein but basically rich in energy. Therefore increasing the protein content of cassava for garri production using inexpensive source, microbial cells is important. Prospect for the transformations of cassava based food into protein rich foodstuff are promising since less will be expended for the cultivation to replace imported fish meal. Protein enrichment of carbohydrate based food present an improved food economic that cannot be met by agriculture alone.

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