32

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UPGRADING THE PROTEIN LEVEL OF 'OGI' USING *CANDIDA UTILIS* NN1 ISOLATED FROM 'NONO'

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ABSTRACT

Candida utilis NN4 isolated from nono (fermented cow milk) was used to upgrade the protein content of ogi (fermented cereal gruel prepared from millet). The results revealed that the enriched ogi had pH of 4.50, 45.6% moisture, 0.2% ash, 1.57% protein, 0.1% lipid and 52.37% carbohydrate. When the ogi was enriched with microbial protein (4g of Candida utilis NN4), the protein content increased from 1.57% to 6.56% after 120h of fermentation. Carbohydrate and pH of the enriched ogi decreased while the lipid and moisture contents increased. It was however, observed that the ash content was trace. The results of this study suggest that microbial protein can be used to improve the nutritional quality of ogi, a weaning food in Nigeria.

Keywords: Fermentation, Ogi, Protein, Milk, Candida utilis, 'Nono'

INTRODUCTION

"Ogi" is a fermented cereal gruel made from maize, millet or sorghum. It is enjoyed by adults as a breakfast cereal food and it is a popular weaning food in Nigeria and most West African countries like Ghana. It is easy to prepare. Besides, it is less expensive. These qualities account for its popularity among mothers. Sugar and milk are normally added to ogi porridge to enhance taste. Ogi naturally contains quite a number of microorganisms which are responsible for most of the changes that occur therein. Although non-pathogenic microorganisms constitute no health hazard to consumers, they may make the product unpalatable by their fermentative activities. The microorganisms associated with maize fermentation during ogi production include, *Corynebacterium* sp, *Lactobacillus sp*, *Bacillus* sp, *Penicillium* sp and *Saccharomyces* sp (Ekpo and Ekpo, 2005). Traditional processing methods have been known to adversely affect the nutrient quality of fermented foods. This is a problem with ogi in which about 40% of the protein content is lost during wet-milling of the maize; over 50% of calcium and 75% of phosphorus are lost as reported by Ekpo and Ekpo (2005). These loses are serious. Consequently, ogi lacks the essential amino acids and other nutrients needed for growth. In developing countries, the bulk of diets are mainly carbohydrate foods (cereals, yam, cassava, etc) that are low in protein. High consumption of low protein diets result in high sugar concentration in blood which can cause coronary diseases and even retarded growth in children-Kwashiorkor (Fagbemi and Ijah, 2006). There is therefore, the need to enrich ogi with microbial protein.

Microorganisms (algae, bacteria, fungi,/and 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 (Araujo *et al.*, 2006). However, yeasts are the most widely acceptable group of microorganisms used in carbohydrate food enrichment purposes. For human consumption, yeasts are used in small quantity to supply proteins, especially the essential amino acids, lysine, vitamins and minerals. They have anti-oxidant properties that stabilize food products. Yeasts are incorporated into baby flours, cereals, soup, sauces, as texture and flavor component (Ijah *et al.*, 2004). Generally, microbial protein supplement is less expensive and affordable in some underdeveloped countries than other protein-rich foods such as meat, fish and egg (Ijah *et al.*, 2004). Microorganisms used for food enrichment purposes have been obtained from various sources. For instance, Raimbault *et al.* (1985) enriched cassava using moulds isolated from traditional foods. Similarly, Fagbemi and Ijah (2006) enriched fufu using yeast isolated from burukutu (a local wine brewed from sorghum). The aim of the present study was to improve the protein content of 'ogi' through fermentation with yeasts isolated from nono (fermented cow milk).

MATERIALS AND METHODS

Sample collection

Samples of 'nono' (fermented cow milk) were purchased in sterile one litre capacity plastic bottles from sales points at Mini market, at the Main Campus of the Usman Danfodiyo University Sokoto (UDUS), Nigeria. They were transported to the Microbiology laboratory, UDUS in an ice box for the isolation of yeasts. Millet was purchased in polythene bags from Sokoto Central Market, Sokoto, Nigeria, and transported to the laboratory for the production of ogi.

Isolation and identification of yeasts
Yeast strains were isolated by plating serially diluted samples of nono on Sabouraud dextrose agar (SDA) and incubated at room temperature (28 ÷ 2^0 C) for 48h. Different isolated colonies were subculture repeatedly on fresh SDA to obtain pure cultures of the isolates. The yeast isolates were characterized based on colony morphology, growth at 37^{0}C, growth on 50% glucose, starch hydrolysis, carbohydrate utilization profiles, and absence or presence of pseudomycelium. The organisms were identified by comparing their characteristics with those of known taxa using the schemes of Barnett *et al.* (1990).

Production of biomass
Based on the results of the screening test, one isolate, *Candida utilis* NN4 was selected for the enrichment study. Cell biomass was produced using sterile nutrient broth. Each flask was inoculated with the yeast isolate. The flasks were plugged with cotton wool, shaken and incubated at room temperature (28±2°C) for 24h. The yeast cells were harvested by centrifugation at 1000rpm for 15min and then filtered using filter paper. The filtered yeasts were dried using hot air oven at 45°C for 8h. The dried yeast cells were stored in sterile universal bottles until required (Fagbemi and Ijah, 2006).

Preparation of 'ogi'
'Ogi' was prepared using the method of Oyeyiola (1991). Two kilograms of millet were soaked in cold tap water for 24h at room temperature (28±2°C). The steep water was drained off and the grains washed with tap water. Water was drained off from the grains. Then the grains were wet-milled and sieved through a fine wire mesh screen (pore size 0.81mm^2) to remove the pomace, which was discarded. The filtered liquid was allowed to settle for 6h to produce sediment (ogi).

Enrichment of 'ogi' with yeast
Four grammes (4g) of a 24h-old yeast strain were added to 100g of ogi contained in 1000ml capacity Erlenmeyer flask, mixed thoroughly and allowed to stand at room temperature (28±2°C) for 120h. A control experiment was set up in which the 'ogi' was not enriched with microbial cells. Samples were withdrawn after 0, 24, 48, 72, 96 and 120h for microbiological and biochemical analyses.

Microbiological analysis
Serially-diluted samples (10^{-1} – 10^{-2}) were plated onto nutrient agar (for viable bacterial counts) and MacConkey agar (for coliforms). The inoculated plates were incubated at 37°C for 24h. Colonies, which developed on the plates were counted and expressed as colony forming units per gramme (cfu/g) of the sample. The counts were determined after 0, 24, 48, 72, 96 and 120 hours.

Biochemical analysis
Determination of pH
The pH of 'ogi' enriched with yeast was determined using the pH meter (Micro Crison Instruments, S.A., Barcelona) after standardization of the pH meter with phosphate buffer solution (Fagbemi and Ijah, 2006).

Determination of moisture
A metallic dish was dried in an oven at 80°C for 20min, cooled in a desiccator and weighed. Five grammes of the sample was placed in the dish and weighed. The dish with the sample was then dried in an oven at 80°C for 24h to achieve a constant weight and was quickly transferred to a desiccator to cool. It was weighed immediately after cooling with minimum exposure to the atmosphere. The loss in weight of the sample during drying was the moisture content. This was calculated using the formula (Fagbemi and Ijah, 2006).

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W_1 . W_0}$$

Where:
w_0 = weight of empty dish, w_1 = weight of sample, w_2 = weight of dry sample

Determination of ash content
The ash content of the enriched 'ogi' was determined by placing a porcelain crucible having 5g of the sample on a burning flame for 15min until smoke ceased. It was later transferred into a muffle furnace, heated at 550^{0}C to burn off all the organic matter. The chars then burnt off as carbon (iv) oxide, leaving a white ash. The weight was again measured and the percentage ash was calculated using the formula (Fagbemi and Ijah, 2006):

$$\% \text{ ash} = \frac{W_2 - W_0}{W_1 - W_0}$$

Where:
W_0= weight of crucible, W_1 = weight of sample, W_2 = weight of ash

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Determination of protein

The crude protein content of 'ogi' enriched with yeast was determined by the micro Kjeldahl method using conversion factor of 6.25 (Fagbemi and Ijah, 2006).

Determination of carbohydrate content

The carbohydrate content of the enriched sample was calculated by subtracting the total protein and' lipid form organic matter Fagbemi and Ijah, 2006).

RESULTS

Nutritional qualities of unriched ogi

'Ogi' used in this study (Tables 1-3) contained 45.6% moisture, trace amount of ash, 1.57% protein, 0.1% lipid, 52.37% carbohydrate and had a pH of 4.53.

Identification and potential of yeast isolates to utilize carbohydrate

A total of ten isolates were obtained from Nono. (Table 4). The organisms were identified as species of *Candida* (constituted 40% of the total isolates) and *Torulopsis* (constituted 60% of the total isolates). All yeast isolates were screened for ability to utilize carbohydrate. One of the isolates grew more luxuriantly in glucose medium than other isolates, based on this quality, the strain *Candida utilis* NN4 was chosen for the protein enrichment study.

Nutritional qualities of ogi enriched with microbial protein

A pH of 4.53 was obtained for ogi enriched with yeast *(Candida utilis* NN4) at zero hour. The pH decreased gradually with increasing fermentation period and reached 3.35 after 120h (Table 2). The protein content of ogi enriched with *Candida utilis* NN4 was 6.56% after 120h of fermentation. This value is higher than the 1.57% crude protein of ogi before enrichment (Table 3). In general, protein content of ogi enriched with *Candida utilis* NN4 increased with increase in fermentation period. The lipid content of the enriched product also increased with increase in fermentation period while the carbohydrate content decreased with increasing fermentation period (Table 3). The results also revealed that ogi enriched with microbial protein had fairly high moisture content but the ash content was trace (Table 4).

Table1: pH of 'ogi' enriched with *Candida utilis* NN4

Fermentation time (Hour)	pH value of 'ogi'	
	Unenriched	Enriched
0	4.53	4.53
24	4.60	4.13
48	4.00	3.82
72	3.77	3.90
96	3.48	3.67
120	2.30	3.35

Table 2: Carbohydrate, protein and lipid contents of 'ogi' enriched with *Candida utilis NN4*

Fermentation time (Hour)	Carbohydrate (%)		Protein (%)		Lipids (%)	
	UEO	ENO	UEO	ENO	UEO	ENO
0	52.37	51.78	1.57	1.92	0.1	0.1
24	52.73	50.75	1.57	3.23	0.1	0.2
48	52.14	49.99	1.66	4.11	0.2	0.3
72	54.45	48.93	1.75	4.37	0.2	0.3
96	51.65	47.38	1.75	5.42	0.2	0.4
120	51.18	45.64	1.92	6.56	0.3	0.4

UEO: Unenriched 'ogi', ENO: Enriched 'ogi'

Table 3: Moisture and ash contents of ogi enriched with *Candida utilis* NN4

Fermentation period (Hour)	Moisture (%)		Ash (%)	
	UEO	ENO	UEO	ENO
0	45.6	46.8	trace	trace
24	45.4	45.8	trace	trace
48	45.8	45.6	trace	trace
72	43.6	46.4	trace	trace
84	46.2	46.8	trace	trace
120	46.6	47.4	trace	trace

UEO: Unenriched 'ogi', ENO: Enriched 'ogi'

Microbiological qualities of protein-enriched 'ogi'

The results (Table5) revealed that the viable bacterial counts increased, from 3.90×10^3 cfu/g to 6.00×10^3 cfu/g of 'ogi' enriched with *Candida utilis* NN4 after 120h fermentation period The coliform counts of the enriched 'ogi' increased from 1.60×10^1 cfu/g to 3.80×10^1 cfu/g after 72h and decreased sharply to 1.90×10^1 cfu/g after 120h fermentation period.

Table 4: Colony morphology and biochemical characteristics of yeast isolates from 'nono'

Isolate code	Colony Morphology	shape	Ppseudomycelium	Starch hydrolysis	growth at 37°C	Growth on 50% glucose	carbohydrate utilization					Organism
							Gal	Glu	Suc	Mal	Lac	
NN1	Smooth colonies Cream colored	oval	-	+	+	+	-	+	-	-	-	*Torulppsis apicola*
NN2	Smooth colonies, White colored	oval	-	+	+	+	+	-	+	-		*Torulopsis candida*
NN3	Smooth colonies Cream colored	oval	+	+	+	+	-	+	+	-	-	*Candida tropicalis*
NN4	Smooth colonies, elongated Cream colored		+	+	+	+	+	+	-	+	-	*Candida utilis*
NN5	Smooth colonies Cream colored	oval	-	-	+	+	+	+	-	+	-	*Torulopsis candida*
NN 6	Smooth colonies White colored	oval	-	-	+	+	+	+	-	+	-	*Toralopsis candida*
NN 7	Smooth colonies, Cream colored	elongated	-	+	+	+ +	+ +	+	-	-	-	*Candida lipolytical*
NN 8	Smooth colonies Cream colored	elongated	-	+	+	+ +	+ +	+	-	-		*Candida lipolytica*
NN 9	Smooth colonies Cream colored	oval	-	+	+	+ -	+ +	-	-	-		*Torulopsis apicola*
NN 10	Smooth colonies, Cream colored	oval	-	+	+	+ -	- -	-	-	-		*Torulopsis lipolytica*

+: Positive:- Negative, Gal: galactose, Glu: glucose, Suc: sucrose, Mal: maltose, Lac: lactose

Table 5: Changes in microbial flora during fermentation of 'ogi' enriched with yeast (*Candida utilis* NN4)

Fermentation time (Hour)	Viable bacteria ($x10^3$cfu/g)		Coliforms ($x10^1$cfu/g)	
	UEO	ENO	UEO	ENO
0	4.7	3.9	1.7	1.6
24	5.1	3.5	1.9	2.5
48	5.9	4.2	2.3	3.2
72.	6.4	4.8	2.8	3.8
96	6.8	5.6	3.0	2.6
120	8.0	6.0	2.2	1.9

UEO: Unenriched ogi, ENO: Enriched ogi

DISCUSSION

It has been revealed in the present study that ogi prepared from millet contained protein, carbohydrate, lipid and moisture which are necessary for healthy growth. However, the initial crude protein of enriched ogi was 1.57%, which is very low, but when enriched with *Candida utilis* NN4, the protein content was raised to 5.56% after 120h of fermentation. This means that the organism has a good enrichment capability in terms of percentage crude protein value (Fagbemi and Ijah, 2006). The initial content of lipid, moisture and carbohydrate of ogi before enrichment were 0.1%, 15.6%, and 52.3% respectively. When the product was enriched with microbial protein, the levels of lipid and moisture increased as fermentation period increased, while the carbohydrate content decreased. This shows that the organism had competent degradative enzyme system for the utilization of the carbohydrate. This agrees with the report of Wainwright (1992), that carbohydrate can promote the growth of yeasts for single-cell protein (SCP) production. The present study has revealed that ogi from millet enriched with microbial protein had trace amount of ash probably due to the protein content that has increased. The pH of ogi enriched with microbial protein decreased from 4.53 to 3.35. The progressive fall in pH that occurred in the protein enriched product is characteristic of fermenting cereal grains (Akinrele, 1970; AU and Fiselds, 1981; Umoh and Fields, 1998). During the process of fermentation of ogi enriched with *Candida utilis* NN4, there was increase in bacterial counts probably due to favourable conditions, which allowed the multiplication of the organisms. The proliferation of coliforms, especially in the early and intermediate stages of fermentation is characteristic of mixed acid fermentation (Davis *et al.*, 1980). The microorganisms probably originated from the water used for fermentation of millet, surrounding air or container used for the steeping of the millet. Their subsequent decrease in number could be attributed to increased acidity of millet mash. Adegoke and Babalola (1988) had made similar observation. The involvement of microorganisms such as bacteria and fungi in the fermentation of ogi has been reported by Adegoke and Babalola (1988), Oyeyiola (1989) as well as Ekpo and Ekpo (2005).

CONCLUSION

The use of yeast isolated from 'nono' *(Candida utilis* NN4) has increased the protein content of ogi prepared from millet. This is of great importance especially as ogi serves as a weaning food for infants. More detailed studies should be carried out on the yeast isolate as it can be used for single-cell-protein (SCP) production and enrichment of other carbohydrate foods.

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