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Quality assessment of 'oti-oka' like beverage produced from pearl millet

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ABSTRACT

Objective: The effects of single and co-cultures of predominant lactic acid bacteria and yeast as starters on the nutritional, anti-nutritive component and physical properties of "oti-oka" like beverage using pearl millet as substrate was investigated.

Methodology and Results: Lactic acid bacteria and yeast isolated from spontaneous fermented pearl millet were screened for the production of various enzymes, antagonistic activity against selected pathogenic and spoilage microorganisms, production of lactic acid and diacetyl. Single and co-cultures of predominant organisms were selected based on the parameters stated above and used to produce "oti-oka" like beverage. The nutritional, anti-nutritive component, physical and sensory properties of "oti-oka" like beverage produced was investigated. Lactobacillus fermentum 01 recorded the highest occurrence of 20.60% while Saccharomyces cereviseae had occurrence of 14.71%. Most Lactic acid bacteria (LAB) species could breakdown pectin while few were able to produce amylase, protease, invertase and lipase. However Saccharomyces cereviseae exhibited the best enzymatic profile amongst tested isolates. The quantity of lactic acid produced by LAB species ranged between 0.6 to 2.6g/l. Lactobacillus brevis produced the highest (8.6g/l) diacetyl. "Oti-oka" like beverage produced using combined starter cultures of L. fermentum and S. cereviseae had protein content of 7.50% and calcium content of 8.76mg/100ml. Phytate content reduced from 1.13±0.15mg/100ml to 0.60±0.10mg/100ml, Polyphenol content reduced from 1.33±0.64mg/100ml to 0.50±0.10mg/100ml while Tannin content reduced from 1.83±0.17mg/100ml to 1.00±0.11mg/100ml. 'Oti-oka' like beverage produced from pearl millet using combined starter culture of L. fermentum and S. cereviseae was rated best in terms of taste and aroma when compared with control sample.

Conclusion and application: Pearl millet could be substituted successfully for sorghum to produce improved "oti-oka" like beverage that is more acceptable to the 'Western palate' in terms of nutritional, anti-nutritive contents and sensory properties using combine starter culture of *S. cereviseae* and *L. fermentum*. **Key words:** Pearl millet, "Otika like" beverage, lactic acid bacteria, *Saccharomyces cereviseae*.

INTRODUCTION

There exists at least nine species of millet around the world with the total production of 28.38 million tons, out of which 11.36 million tons (40%) produced in Africa (FAO, 1995). Pearl millet (*Pennisetum glaucum*) remains the only species grown in Africa (Fasasi, 2009) and which forms major sources of energy and protein for about 130 million people in Sub-Saharan Africa (SSA). Despite its importance however, pearl millet is considered a "lost crop" because its untapped potential is still very vast (Iren, 2004). The making of beverages from pearl millet is not common in Africa though it has been reported to give little alcohol (Gregory, 1984). 'Oti-oka' is an indigenous alcoholic beverage produced originally from sorghum. It is a fermented cereal gruel whose production is carried out using indigenous fermentation technology in the South-Western Region of Nigeria (Ovo, Oke-ogun, and Sagamu). It has a good flavor; it is sweet, slightly sour with a brownish-opaque colour and has a pleasant taste during the third day of fermentation. Intensified efforts are going on in the developed world to isolate and characterize microorganisms responsible for the production of indigenous fermented foods but microorganisms associated

MATERIALS AND METHODS

Sample collection: Pearl millet (*Pennisetum glaucum*) was purchased at Bodija Central Market at Ibadan , Oyo State Nigeria and was brought into the laboratory in clean polyethylene nylons for immediate use. The seeds were carefully freed from foreign materials as well as broken and shrunken seeds.

Traditional preparation of "Oti-oka" like beverage from pearl millet: The cleaned grains were steeped in water for 18 hours after which they were rinsed thrice and the grains drained. The hydrolyzed drained grains were spread evenly on a clean travs lined with cloth and kept wet by frequent spraying of water for 36 hours at 25°C after which the germinated grains were sundried for 48 hours and ground to pass a 0.4 mm sieve. One hundred and Fifty (150)g of ground germinated pearl millet was weighed and 50g each was distributed into 3 portions. Each portion was mixed with distilled water (1:5), sieved with muslin cloth and allowed to boil for 3hours. The boiled mixture was allowed to cool to 30°C and fermented at 30°C for 72 hours.

Enumeration and Isolation of Microorganisms: Serial dilutions were made from the fermented samples using sterile pipette. This was done by mixing 10 ml of the fermented samples thoroughly with 90 ml of sterile distilled water to give 1:10 dilution. The dilution was made up to 10⁻⁶. Using sterile pipette of 1ml, appropriate dilutions was plated out on different culture media. De Man Rogosa and Sharpe (MRS) agar plates were incubated in a carbon-dioxide enriched jar at 37°C with 'oti-oka' production are yet to be identified (Abdelgadi *et al.*, 2001; Mathara *et al.*, 2004; Adnan & Tan, 2007).

The major problem of 'oti-oka' production in Africa is that there is lack of consistency in quality (Ogundiwin & Tehinse, 1981) because the substrate from which it is produced (Red variety of sorghum) contains a high level of polyphenol, tannin (Hounhouigan *et al.*, 2006) and dhurrine a cyanogenic glycoside which on hydrolysis yields hydrogen cyanide (HCN), a cyanhydric acid (Osuntogun *et al.*, 1989; Hounhouighan *et al.*, 2006). This study investigated the use of pearl millet as a substitute for sorghum using different starter cultures for production of "oti-oka" like beverage.

for 48 h. Other bacterial plates were incubated aerobically at 30°C for 24 h while yeast extract agar plates were incubated aerobically at 30°C for 24-48 h. At the end of incubation period, representative colonies were selected at random and sub- cultured repeatedly to obtain pure cultures. The cultures were examined for colony and cell morphology, biochemical tests (Harrigan & McCance, 1976), the use of API 50 CH strips in conjunction with API 50 CHL medium and API 20C AUX diagnostic kits (Bio Meriux) for Lactic acid bacteria and yeast isolates respectively.

Antimicrobial activity of LAB metabolites: Well diffusion assay method was employed (Shillinger & Lucke, 1989). Pre-poured indicator agar plates (4mm, containing the indicator microorganisms) was overlaid with a 10ml soft agar (0.7%) lawn of indicator culture to generate a potential mat of the indicator bacteria. The indicator lawn was prepared by adding 0.1ml indicator organism to 10ml soft agar. Wells of 5mm in diameter were cut into these agar plates using a sterile cork borer and 10µl of the cell-free metabolites of the test isolates were placed in each well. The plates were incubated overnight aerobically at 37°C and examined for zones of inhibition.

Enzyme Screening of LAB and Yeast isolates: The LAB and yeast isolates were screened for amylase, lipase, protease and pectinase production using the plate method described by Harrigan and McCance (1990) while invertase production was carried out using the method of Fiedurek and Gromada (2000).

Quantitative determination of lactic acid and diacetyl produced by LAB: The test organisms were grown in MRS broth for 72 h. Each sample was centrifuged at 3000×g for 15minutes (Ogunbanwo *et al.,* 2003) while the supernatant from each sample was used for quantitative estimation of lactic acid and diacetyl content (A.O.A.C, 1980).

Selection of Starter culture: The starter cultures used in production of "oti-oka" like beverage were selected based on the following parameters; enzyme screening profile, quantity of lactic acid, diacetyl, predominant microorganism, antagonistic activity and growth at different acidic pH and temperature.

Laboratory production of "oti-oka" like beverage: 400g of cleaned seeds were washed three times with clean water and soaked with tap water for 12 h (1:3 w/v) at room temperature (25 ± 2 °C). The grains were

drained and spread evenly on wet cotton cloth at room temperature (25 \pm 2 °C) for 48 h. Another cotton cloth was used in covering the spread grains and wetting was carried out every 12 hours. Removal of mouldy, sprouting seeds and devegetation was carried out before the grains were washed with tap water. The washed germinated seeds were thereafter steeped in 1% sodium metabisulphate for 15 minutes and rinsed thrice in clean water before drying in the oven at 80°C for 12h. The dried, germinated grains was milled and diluted in water (1:5w/v), sieved and divided into 4 portions after which they were boiled at 100°C for 3h. Three portions of the cooled (30°C) slurry were inoculated with starter culture(s) while the last portion was allowed to ferment naturally at 25 ± 2 °C for 72h. The fermented products were refrigerated at 4°C after they were dispensed into screw capped bottles.

The flow chart for laboratory production of "oti-oka" is showed thus:



Cooled at 4°C in refrigerator and dispense into screw capped bottles.

Figure 1: Flow diagram for the production of "oti-oka" like beverage using starter culture.

Physico-chemical Analysis: The pH of "oti-oka" like beverage produced was determined in triplicate using a pH meter after standardization with pH 4 and 8 buffers (Sigma) and the titratable acidity of "oti-oka" like beverage was determined in triplicates by titrating 10ml of the sample with 0.1N NaoH using phenolphthalein as indicator until a pink color appeared. The titratable acidity was expressed as % lactic acid (A.O.A.C, 1980). **Proximate composition of "Oti-oka" like beverage:** This was carried out on all the produced samples on wet matter basis according to conventional methods of A.O.A.C. (1980). Carbohydrate, moisture content, crude fibre, protein, ether extract, ash as well as

RESULT

The result of the research shows that a total of eight species of Lactic acid bacteria and seven species of yeast were isolated from fermented pearl millet. *Lactobacillus fermentum* 01 *recorded* the highest occurrence of 20.60% followed by *Lactobacillus brevis*

calcium, phosphorous, sodium, iron, zinc, alcohol, and anti-nutritive content were determined.

Sensory Analysis: Sensory evaluation of "oti-oka" like beverage samples was carried out by 15 member trained panel of judges. The parameters used were colour, aroma, sourness, taste and general acceptability. A 7 point hedonic scale ranging from 7= Excellent to 1=dislike extremely was used for the rating. **Analysis of Data:** Results obtained in this study were subjected to analysis of variance using ANOVA and separation of means was carried out by Duncan's Multiple Range Test (Duncan, 1955).

(11.77%) while the least occurrence were *Pediococcus pentosaceus* and *Streptococcus salivarus* with percentage occurrence of 2.94% amongst the lactic acid bacteria isolates (Table1).

 Table 1: Frequency of occurrence of Lactic acid bacteria and Yeast isolated from traditionally fermented pearl millet.

Isolate	Number of Occurrence(n)	Percentage Occurrence (%)
L. brevis	4	11.77
L. plantarum	3	8.82
L. fermentum	7	20.60
L. lactis	2	5.88
S. faecium	2	5.88
S. salivarus	1	2.94
P. pentosaceus	1	2.94
L. mesenteroides	2	5.88
Saccharomyces cereviseae	5	14.71
Saccharomyces chevelaria	2	5.88
Pichia ohmeri	1	2.94
Geotrichum candidum	1	2.94
Debaryomyces hansenii	1	2.94
Rhorotorula graminis	1	2.94
Torulospora delbrekii	1	2.94
Total	34	100

Amongst the yeast isolates, *Saccharomyces cereviseae* had occurrence of 14.71% followed by *Saccharomyces chevelaria* (5.88%). Enzyme screening profile of the isolates reveals that *Lactobacillus fermentum* 01 produce three enzymes namely amylase, protease and invertase while in addition to these Saccharomyces *cereviseae* produced lipase and esterase and exhibited the best enzymatic profile amongst tested isolates (Table2).

Table 2: Enzyme screening profile of LAB and Yeast strains isolated from traditionally fermented pearl millet Enzymes / zone of hydrolysis (mm)

	Amylase	Protease	Lipase	Invertase
Isolate			·	
L. lactis	-	15	-	
L. plantarum	20		-	
L. fermentum	35	15	-	25
L. brevis	-	12	-	-
S. cereviseae	10	2	11	8
S. chevelaria	3	-	-	-

Lactobacillus fermentum 01 was able to inhibit the growth of most of the spoilage and pathogenic indicator microorganisms tested, followed closely by

Lactobacillus plantarum 01 and Lactobacillus brevis recorded the least (Table 3).

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Iable 3. Antagonistic activity	of Lactic acid hac	toria icolatos anainst	some indicator mil	croordanieme
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		Indicator microorganisms / Zone of Inhibition (mm)									
LAB	S.	В.	Е	S.	Р.	Р.	L	Klebsiella	Enterobacteria	А.	А
isolates	typhi	subtilis	.coli	aureus	aeruginosa	florescence	monocytogens	Spp.	Spp.	flavus	.niger
L. lactis	11	11	-	14	18	16	10	-	11	10	15
L.	17	18	7	12	20	20	8	-	12	-	16
plantarum											
L.	20	13	10	17	20	20	10	20	-	22	15
fermentum											
L. brevis	15	10	-	-	21	12	-	-	-	-	-

Lactic acid bacteria isolates were tested for their ability to produce lactic acid and diacetyl, *Lactobacillus brevis* produce the highest quantity of lactic acid and diacetyl with values of 2.6g/l and 8.61g/l respectively and closely follow by *Lactobacillus fermentum* with values of 2.4g/l and 7.53g/l for lactic acid and diacetyl respectively (Table 4).

Table 4: Quantity of Lactic acid and Diacetyl produced by LAB isolates

Isolate	Lactic acid production(g/l)			Diacetyl production (g/l)		
	24h	48h	72h	24h	48h	72h
L. lactis	0.6	0.8	1.0	1.076	5.38	5.59
L. plantarum	1.8	1.4	2.2	0.215	2.152	7.232
L. fermentum	1.4	2.0	2.4	0.215	2.352	7.532
L. brevis	1.8	2.4	2.6	1.076	5.38	8.608

Based on the above results and growth at various pH and temperatures (data not show) *L. fermentum* and *S. cereviseae* were selected and used in single and in combination for laboratory production of "oti-oka" like beverage using pearl millet as substrate. The result of

the proximate composition of "oti-oka" like beverage produced with and without starter cultures and the unfermented pearl millet used in the production of "otioka" like beverage on wet weight basis showed significant increase (p<0.05) in the protein level (Table 5), from 4.90% in control (unfermented sample) to 8.17%, 7.30% and 7.50% in beverages produced with starter culture(s) of *L. fermentum, S. cereviseae* and

combination of *L. fermentum* and *S. cereviseae* respectively.

Table 5: Proximate A	Analysis of "Oti-oka"	like beverage produced	d using different starter cultures
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Proximate Analysis	Unfermented		Starter cult	ture(s)
(%)	Pearl millet	L. fermentum	S. cereviseae	L. fermentum & S. cereviseae
Carbohydrate	6.96±0.10	7.02±0.05	7.06±0.05	7.26±0.05
Crude fibre	0.3±0.00	0.15±0.00	0.20±0.05	0.10±0.00
Ash	0.25±0.05	0.20±0.11	0.07±0.05	0.26±0.05
Protein	4.9±0.50	8.17±0.11	7.30±0.32	7.50±1.4
Ether extract	4.0±0.23	3.4±0.2	4.0±0.20	3.50±1.3
Moisture content	83.6±0.2	81.7±0.3	81.28±0.49	83.49±0.2
Alcohol	0.00	0.00±0.17	1.56±0.20	0.33±0.00
Total reducing sugar	2.4±0.1	1.10±0.10	1.26±0.64	1.93±0.11
Total soluble solid	3.46±0.12	3.50±0.36	3.76±0.30	3.16±0.15
Starch	4.6±0.26	3.50±0.36	3.56±0.11	4.56±0.05

The lowest protein content was however observed in the "oti-oka" like beverage produced spontaneously from pearl millet. Alcohol content increases from 0.00% in the control to 1.56% and 0.33% in "oti-oka" like beverage produced using S. cereviseae and combination of L. fermentum and S. cereviseae respectively as starters. Ether extract content showed no significant increase (p<0.05) in "oti-oka" like beverage produced using starter cultures. The ether extract levels were found to decrease with the lowest level (3.40%) found in beverage produced with L. fermentum and the highest (4.0%) in beverage produced with S. cereviseae. Carbohydrate content increased from 6.96% in the control to 7.02%, 7.26% and 7.06% in beverages fermented with starter culture(s) of *L. fermentum*, *S. cereviseae* and combined starter cultures of L. fermentum and S. cereviseae. A reduction in carbohydrate content was however obtained in "oti-oka" like beverage produced spontaneously from pearl millet.

Production of "oti-oka" like beverage from pearl millet using selected starter culture(s) significantly increased (p<0.05) the mineral content of the product with an exceptional increase in the calcium content from 7.36mg/100ml in the control to 8.73mg/100ml, 8.53mg/100ml and 8.76mg/100ml in the "oti-oka" like beverage produced with starter culture of *L. fermentum*, *S. cereviseae* and combined starter cultures of *L. fermentum* and *S. cereviseae* respectively. The least calcium content was observed in the spontaneously fermented "Oti-oka" like beverage. Significant increase (p<0.05) was also observed in iron, zinc, and sodium composition in all the beverages produced using starter culture(s) (Table 6).

Table 6: Mineral content of	"Oti-oka" like	beverage	produced	using	different	starter	cultures
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Mineral	Unfermented		Starter culture(s)				
Composition (Ma/100ml)	Pearl millet	L. fermentum	S. cereviseae	L. fermentum & S. cereviseae			
Calcium	7.36±0.05	8.73±0.11	8.53±0.15	8.76±0.05			
Iron	0.46±0.05	0.73±0.05	0.83±0.05	1.06±0.11			
Zinc	0.02±0.00	0.05±0.00	0.06±0.01	0.08±0.15			
Sodium	0.66±0.05	0.75±0.10	0.76±0.05	0.56±0.11			
Phosphorous	1.00±0.00	1.10±0.00	1.0±0.20	1.30±0.10			

Values shown indicate mean of three independent readings \pm SD.

Table 7 shows the Anti-nutritive component of "oti-oka" like beverage produced from pearl millet. Significant reduction was observed in the polyphenol content of the beverage. The least reduction effect was observed in beverage produced with combined starter culture of *L. fermentum* and *S. cereviseae*. Phytate content reduced from 1.13 ± 0.15 mg/100ml as observed in the control to 0.60 ± 0.10 mg/100ml in "oti-oka" like beverage

produced with combined starter culture of *L. fermentum* and *S. cereviseae*. Tannin content also reduced in value from 1.83 ± 0.17 mg/100ml to 1.00 ± 0.11 mg/100ml and polyphenol from 1.33 ± 0.64 mg/100ml to 0.50 ± 0.17 mg/100ml in the beverage produced using combined starter cultures of *L. fermentum* and *S. cereviseae*.

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Table 7: Anti-nutritive com	ponent of "Oti-oka" lik	e beverage produce	ed using different	starter cultures

Antinutritive	Unfermented		Starter culture(s)				
content	Pearl millet	L. fermentum	S. cereviseae	L. fermentum & S. cereviseae			
(Mg/100ml)							
Polyphenol	1.33±0.64	0.52±0.11	0.51±0.10	0.50±0.17			
Phytate	1.13±0.15	0.70±0.10	0.71±0.10	0.60±0.10			
Tannin	1.83±0.17	1.01±0.00	1.02±0.15	1.00±0.11			

Values shown indicate mean of three independent readings ± SD.

"Oti-oka" like beverage produced with combined starter culture of *L. fermentum* and *S. cereviseae* using pearl millet as substrate was rated best in term of taste, aroma and overall acceptability and compared well with "Oti-oka" beverage from spontaneous fermented

sorghum (Table 8). Results of our study suggest that the use of combined starter cultures of *L. fermentum* and *S. cereviseae* to ferment pearl millet as substrate for Oti-oka" like beverage production positively affects its sensory attributes.

Table 8: Sensor	v qualitv attrib	outes of "Oti-oka'	' like beverage	produced using	different starter	cultures
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Starter culture(s)	Taste	Sourness	Aroma	Appearance	General
					Acceptability
S. cereviseae	5.20±0.30	3.50±0.26	4.50±0.45	5.40±0.38	3.20±0.15
L .fermentum	5.50±0.30	3.40±0.40	4.70±0.45	5.40±0.38	2.58±0.15
L. fermentum & S. cereviseae	5.53±0.32	3.30±0.28	4.80±0.30	5.40±0.36	4.62±0.30
Otika from spontaneous fermented	5.52±0.32	3.60±0.56	4.40±0.42	5.20±0.38	4.60±0.13
sorghum					
Otika from spontaneous fermented	4.00±0.42	3.70±.26	3.30±0.51	3.80±0.91	3.20±0.35
pearl millet					

Each value is the mean \pm standard error of 15 member panelist.

DISCUSSION

Lactic acid bacteria (LAB) and yeast species were isolated from traditionally fermented pearl millet during "otika" like beverage production. The predominant fermenting species isolated were *L. fermentum* and *S. cereviseae* confirming the report of Oyewole (1997) and Ogunbanwo *et al.* (2003). Their previous studies indicated these organisms as dominant fermenting microorganisms in fermented foods. The cultural and biochemical properties of LAB and yeast isolates were similar to those described by Bergey's Manual of Systemic Bacteriology (Sneath, 1986), Deak and Beuchat (1994) and Sanni *et al.*, (1994). All LAB isolates evaluated in this study produced metabolites

that showed antagonistic activity to both pathogenic and spoilage bacteria and fungi. Lactic acid and diacetyl were the metabolites tested and produced by the LAB in this study and this has been previously documented by Yasmine (2002) and Agarry *et al.* (2010). This conforms to the report of De Martins *et al.* (2001) and Ogunbanwo (2003) who reported the ability of antimicrobial compounds produced by LAB to exert strong antagonistic activity against food contaminating microorganisms. Production of acids and antimicrobials create a competitive and unfriendly environment for growth of pathogens and spoilage microorganisms during fermentation thereby may prolong the shelf-life of the product.

None of the LAB isolates showed lipase activity while majority exhibited pectinase activity and few showed amylase, protease and invertase activity, Lund (1972) reported LAB and other anaerobic microorganisms as pectinase producers while Juven et al. (1985) confirmed the production of pectinase by Leuconostoc mesenteroides. The ability of these microorganisms to produce enzymes is generally rare and has been detected only on few cases (Giraud et al., 1994; Anderson, 1988). S. cereviseae was the only yeast species that showed high enzyme activity amongst other tested yeast strains. This is similar to the report of Gainvors et al. (1994) who stated that though S. cereviseae produce variety of enzymes. These enzymes may hydrolyze the polysaccharides, proteins and lipids to give the taste, aroma and mouth feel that is pleasant and attractive to food consumers (Glover et al., 2005). Based on parameters such as predominance of microorganisms, optimum growth pH and temperature, production of diacetyl, lactic acid and enzymes and antagonistic activity to both pathogenic and spoilage microorganisms, L. fermentum and S. cereviseae were selected as starters and used in singly and in combination for fermentation of pearl millet to produced "Otika" like beverage. The use of starter culture is in line with the research of Sanni (1993) and Kimaryo et al. (2000) as an appropriate approach for control and optimization of the fermentation process in order to alleviate problems of variations in organoleptic quality and microbiological stability observed in African indigenous fermented foods. Yeast and LAB usually coexist in the microbial ecosystem of fermenting beverages (Ogunbanwo et al., 2008) and thus, the use of combined starter culture of LAB and yeast improved the nutritional and microbiological value of the fermented foods. "Oti-oka" produced using starter cultures increased the protein content, reduced ether extract content and improved alcohol production and mineral content of the product compared to the naturally produced "oti-oka" like beverage. According to Obizoba and Atii (1994) and Fasasi (2009), mineral compositions of cereals increase significantly during fermentation due to the activity of the fermenting microorganisms. The reduction of anti-nutritive components present in pearl millet by fermentation agrees with the report of Fasasi (2009) and Oladele et al. (2009). Microorganisms have the ability to reduce these factors in cereals and reduce their levels even further (Fagbemi et al., 2005). The result of the sensory evaluation from this study has shown that "Oti-oka" like beverage produced using combined starter cultures of L. fermentum and S. cereviseae had higher acceptability in term of taste, aroma and overall acceptability and compared favorably with "Oti-oka" beverage from spontaneous fermented sorghum. This was in accordance with the report of Kristek et al. (2004) who stated that the use of starter culture during fermentation gave better organoletic properties than those obtained from spontaneous fermentation

It can be concluded that the use of *L. fermentum* in coculture with *S. cereviseae* enhances the nutritional, sensorial attributes and brought about a significant decrease in the anti-nutritive content of the "oti-oka" like beverage produced from pearl millet compared to the spontaneously produced "oti-oka" beverage form fermented sorghum.

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