International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 11 (2014) pp. 649-656**
http://www.ijcmas.com

Original Research Article

Chemical and microbiological characteristics of *Soy-Kununzaki* (a non-alcoholic beverage) produced from millet (*Pennisetum typhodeum*) and soybean (*Glycine max*)

N.Danbaba[1*], S.B.Oyeleke[2], A.T.Maji[1], I.N.Kolo[1], H.Hauwawu[1] and I.F.Kolo[3]

[1]National Cereals Research Institute (NCRI), Badeggi, PMB 8, Bida, Niger State, Nigeria
[2]Department of Microbiology, Federal University of Technology, PMB 65, Minna, Niger State, Nigeria
[3]Department of Food Science and Technology, Federal University of Technology, PMB 65, Minna, Niger State, Nigeria
**Corresponding author*

Keywords

Soy-*Kununzaki*, Chemical, Microbiological properties, Safe food

A B S T R A C T

Improved technique for the production of the local cereal-based beverage *Kununzaki* with the addition of soybean has been developed. Acceptance and consumption are on the increase. This research was conducted to evaluate changes in both chemical and microbiological qualities of this beverage during its production. The study was carried out at the Processing and Utilization Laboratory of NCRI and Microbiology Laboratory of the Federal University of Technology Minna. Raw materials were sourced from the Bida modern market and soybeans were obtained from the seed store of NCRI. Soy-*kununzaki* was prepared using the improved technique. pH, total titratable acidity (TTA) and total soluble solids (TSS), total viable bacteria, coliform and mold/yeast counts were observed and microbes were identified on raw materials and product during fermentation. The result showed that pH decreased significantly (p□0.05) from 6.02 to 3.16, TTA increased from 0.053 to 0.099, while TSS, decreased from average of 8.05 to 6.68. *Listeria*, *Staphylococcus*, *Bacillus*, *Micrococcus*, *Lactobacillus*, *Aspergillus*, *Penicillium* and *Trichophyton* were among the genus of microorganisms isolated. The changes in chemical attributes and organisms isolated have public health implications. The chemical and microbiological qualities of Soy-*kununzaki* are critical and therefore require monitoring during its production for the production of safe food.

Introduction

Kununzaki is a popular non-alcoholic beverage produced from cereals. It is usually spiced with ginger, red and black pepper, cinnamon, and cloves. Its production is traditionally carried out on small scale batches, being made widely in the northern part of Nigeria. The complementation of cereal products with legume has been

advocated and the usefulness of legume in the production of high protein food to meet the need of the vulnerable group of the population is now well recognized, and several high protein foods have been developed industrially. But, the cost of these products makes them out of reach for the low and average income owners (Nkama, 1993). Alternative for the production of high protein food to meet daily protein, vitamins and mineral requirement of vulnerable group of the population expert suggest is the improvement of traditional food product techniques through fortification with protein and mineral rich raw materials and process optimization through careful evaluation of each process stage to identify critical control points. An improved traditional technique for the production of *kununzaki* with the addition of soybeans has been developed at NCRI, and similar work has been carried out the Federal Institute of Industrial Research (FIIRO), Oshodi (Anounye, 1997; Bankole and Olatunji, 2001). The new technology involves the addition to cereal base soybean for the production of *kununzaki* to improve its nutritional composition. The acceptance and consumption of this product is on the increase, the need to evaluate food safety parameters therefore becomes critical. This study therefore was designed with aimed to evaluate the chemical changes (pH, titratible acidity, total soluble solids) and microbiological qualities of soy-*kununzaki* and the raw materials used in its preparation as risk management tool. The results we believe will improve the process technology and enhance adoption for up-scaling.

Materials and Methods

Samples of soybeans (*Glycine max*) were obtained from the Soybean Research Program, National Cereals Research Institute (NCRI) Badeggi, Nigeria, while millet (*Pennisetum typhodeum*) ginger (*Zingiber officinale*), Black pepper (*Pipper spp*), Clove (*Eugenia caryopyllata*) and Cinnamon (*Xylopia aethiopica*) were purchased from Bida local market, Niger State, Nigeria. The samples were taken in sterile sample bottles plugged with cotton wool and covered with aluminum foil. Samples were prepared at Central Services Laboratory of NCRI, Badeggi and Microbiology Laboratory, Federal University of Technology, Minna. The samples were analyzed for their chemical and microbial contamination at each stage of the production process and the raw materials (Table 5 and 7).

Determination of chemical properties

pH

The pH values were measured using a referenced pH meter (Model 291 mk2 PYE UNICAM, England) after standardization with pH 4 and pH 7 buffers. Values were recorded as the meter becomes steady. The meter electrode is first dipped into a buffer solution before dipping into sample for analysis; the pH is read on the meter automatically.

Determination of Total Titratable Acidity (TTA)

Titratable acidity was determined using the method described by Pearson (1991). Ten ml of soy-*kunuzaki*was put into a volumetric flask and three drops of phenolphthalein indicator were added and the flask thoroughly shaken. This was titrated against 0.1N NaOH to a pink color end-point. Total titratable acidity was expressed as lactic acid;

Titratable acidity = titer value (ml) x 0.009 lactic acid

Determination of Total Soluble Solids (TSS)

Total soluble solids were determined in duplicate using Abbe 60 Refractometer and results expressed as degree of brix (^{o}Brix). A drop of the sample is placed on graduated face of the meter and closed; the reading was taken by viewing through the refractometer and records the high on the graduated slide. Three readings were taken and mean recorded.

Isolation of microorganisms

The culture media used were nutrient agar (NA) Sabourauds dextrose agar (SDA) and Eosin methylene blue agar (EMB). The NA was used for the isolation of bacteria, SDA for fungi, while EMB for coliforms. One percent streptomycin was added to SDA after sterilization to inhibit the growth of bacteria (Fawole and Oso, 1995). One gram or ml of each sample were taken and placed or poured into a test tube containing 9ml sterile distilled water. The test tubes were shaken to dislodge the associating microorganisms, or homogenize the liquid samples. The suspensions were serially diluted to 10^6 in accordance with Mackie and McCartney (1988). 1ml of each dilution was inoculated on the NA, EMB and SDA in Petri dishes (Fawole and Oso, 1995). The inoculated media were incubated at 37^0 for 3–5 days; pure colonies of both bacteria and fungi were obtained through sub-culturing, form the initial mixed cultures and stored on NA slant.

Identification of bacteria

Microscope examination, gram staining, motility and biochemical test were carried out in accordance with the procedures of Collins and Lyne, (1984) (Table 8).

Identification of fungi

The isolates were observed physically to determine color and type of colony formed. Morphological characteristics such as type and arrangement of spores produced as well as the mycelia type was also used. The isolates were assigned to probable identify using taxonomic description in Sasom and RenenHockstra (1988) (Table 6).

Total aerobic microbial count

The total viable microorganisms were counted and recorded as colony forming unit per gram or ml (cfu/g or ml) of the sample. Duplicated plates of the inoculated samples were divided with lines into four parts, and one part was counted. The number of distinct colonies recorded was then multiply by four to get the appropriate total. Average of the duplicate plates was multiplied with the dilution factor (10^6cfu/g or ml).

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA). This was used to evaluate variation in terms of changes in microbial count and chemical parameters at each stage (Table 4). Probability level was maintained at 0.05 (confidence limit) (Gomez and Gomez, 1984). While Least Significant Difference Test (LSD) was used to test significance within the means of the treatments (Ignatius, 1986).

Results and Discussion

Change in chemical attributes of Soy-*kununzaki* during production process

There was a significant ($P \leq 0.05$) change in the pH from weak acidic reaction at the beginning of the process to relatively strong acidic reaction at end of the process (Table 1).

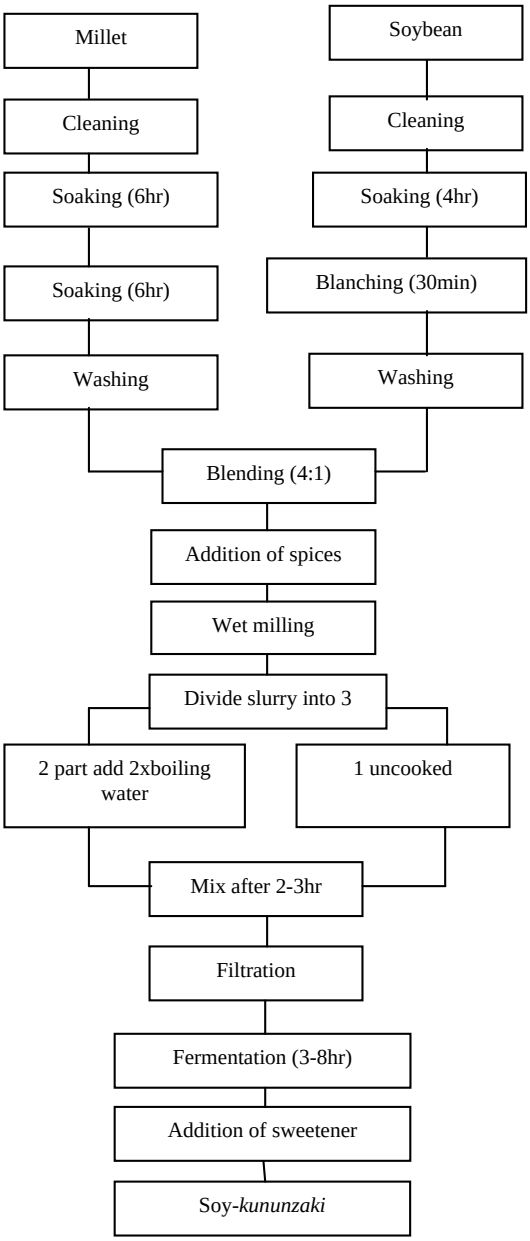

Figure.1 Improved method of *kununzaki* processing with addition of soybeans

Table.1 Changes in chemical properties of soy-*kununzaki* during its production

Fermentation time (hr)	pH	Titritable acidity	Total soluble solids
0	6.314	0.180	14.140
2	6.122	0.274	13.388
4	5.538	0.382	12.592
6	4.230	0.482	11.820
8	4.038	0.604	9.646
10	3.806	0.706	8.814
12	3.634	0.820	8.221
Mean	4.811	0.493	11.231
LSD (5%)	0.0195	0.0167	0.0655
CV (%)	0.20	1.90	0.30

Values are mean of three determinations. Means in the same column having difference greater than or equal to LSD are significantly different at 5% level of probability.

Table.2 Pearson correlation coefficients between fermentation time and changes in chemical properties

	FT	pH	TTA	TSS
FT	1			
pH	-0.96	1		
TTA	0.99	-0.95	1	
TSS	-0.98	0.94	-0.98	1

FT = Fermentation time (hr), TTA = Titratable acidity, TSS = Total soluble solids

Table.3 Number of microorganisms isolated on the raw materials used in the production of Soy-*kununzaki*

Raw Material	TVC (CFU/ml)	CFC (CFU/ml)	MYC (CFU/ml)
Soybeans	$1.26x10^3$	$1.58x10^4$	$<1.00x10^1$
Millet	$1.68x10^3$	$2.98x10^3$	$6.20x10^3$
Ginger	$4.18x10^4$	$<1.00x10^1$	$2.21x10^3$
Black pepper	$1.43x10^3$	$2.72x10^4$	$1.10x10^3$
Cinnamon	$1.01x10^3$	$4.42x10^3$	$<1.00x10^1$
Clove	$<1.00x10^1$	$5.36x10^4$	$<1.00x10^1$

Values of three counts recorded as mean, **TVC** = Total Viable Count, **CFC** = Coliform Count. **MYC** = Mold and Yeast Count.

Table.4 Change in the number of microorganisms counted at different stages of Soy-*kununzaki* production

Production Stages	TVC^+ (CFU/ml)	CFC^+ (CFU/ml)	MYC^+ (CFU/ml)
Steeping (SB)	$4.26x10^4$	$1.85x10^3$	$3.27x10^4$
Steeping (ML)	$4.89x10^4$	$3.18x10^3$	$3.11x10^3$
Paste	$6.48x10^4$	$4.02x10^3$	$5.12x10^3$
Cooked +uncooked	$2.26x10^2$	$2.89x10^3$	$5.00x10^3$
Filtrate	$3.86x10^3$	$2.41x10^2$	$2.13x10^3$
Filtrate	$4.92x10^4$	$1.10x10^2$	$<1.00x10^1$
Filtrate	$5.64x10^4$	$1.18x10^2$	$<1.00x10^1$
Filtrate (final product)	$6.13x10^5$	$<1.00x10^1$	$<1.00x10^1$

Value of three counts recorded as mean, **TVC**= Total Viable Count, **CFC** = Coliform count, **MYC** = Mold and Yeast Counts.

Table.5 Bacteria isolated from raw materials used in the preparation of soy-*kununzaki*

Raw materials	Microorganisms
Soybean	*Staphylococcus spp, Bacillus spp*
Millet	*Shigella spp, Lactobacillus spp*
Ginger	*Klebsiella spp, Bacillus spp, Staphylococcus spp*
Black pepper	*Bacillus spp*
Cinnamon	*Klebsiella spp, Micrococcus spp*
Cloves	*Staphylococcus spp*

Table.6 Fungi isolated from raw materials used in the production of Soy-*kununzaki*

Raw materials	Organisms
Millet	*Aspergillus niger, Mucor spp*
Soybean	*Aspergillus niger*
Clove	*Trichophyton spp*
Ginger	*Penicillium spp*
Cinnamon	*Rhizopus spp*

Table.7 Yeast isolated from soy-*kununzaki* during production

Isolates code	organism
Sk-12	*Trichosporon spp*
Sk-13	*Torulopsis spp*
Sk-22	*Saccharomyces cerevisiae*
Sk-32	*Candida spp*
Sk-11	*Candida spp*
Mm-1	*Cryptococcus neoformance*
Mm-31	*Candida spp*
Fl-43	*Candida spp*
Fl-21	*Torulopsis spp*

Table.8 Bacteria isolated from soy-kununzaki during production

Isolate code	Organism
Bt 00	*Bacillus spp*
Bt 43	*Bacillus spp*
Bt 54	*Lactobacillus spp*
Bt 12	*Klebsiella spp*
Tr 32	*Corynebacterium spp*
Re 21	*Staphylococcus spp*
We 33	*C. haemolyticum*
Wq 51	*Listeria monocytogenes*
Rf 34	*Micrococcus*

The pH ranged between 6.314 to 3.634 which were almost the same with that reported by Adeyemi and Umar (1994). TTA increase (Fig. 2) even as pH decrease from an average of 0.053 to 0.099. The percentage soluble solid measured in ^{0}Brix gradually decrease from an average of 8.08 at the slurry level to 6.68 at the finished product level (Fig. 3). This result agrees with works of Efiuveweuwere and Akoma (1995) and Akoma *et al.,* (2002). The decrease in TSS solids was likely as a result of action of fermentative microbes on the carbohydrate of the filtrate as the solid has been partially gelatinized.

Change in Microbial counts during Soy-*kununzaki* Preparation

There was a relatively high viable bacteria, coliform and mold/yeast counts at the start of the process, this may likely be as a

result of long steeping time which allow seepage of solid into the soaking water and therefore favored the growth of microorganisms introduced from the raw materials and water used for soaking. The counts steadily increase to the paste level, and then drastically reduced during the mixing of cooked and uncooked paste (Table 1).

Microorganisms of public interest isolated on the raw materials include *Listerian* spp, *Staphylococcus* spp, and *Bacillus* (Table 1). *Listeria monocytogenes* has since been recognized as an important food borne pathogen. According to Lovett and Twedt (1998), septicemia is the most common Listeric manifestation in adults. The isolation of this organism in the raw materials agrees with the findings of Anounye (1997). Form of listeriosis involving the central nervous system includes meningitis, encephalitis and abscesses (Lovett and Twedt, 1998). *Staphylococcus* food intoxication is one of the most common foods borne illness giving rise to nausea, vomiting, abdominal cramping, prostration and diarrhea (FAO, 2004). Some species of *Staphylococcus* are also considered lipolytic (Gilbert *et al.*, 2000), therefore, capable of causing hydrolytic and oxidative deterioration of the beverage since soybean is an oil seed. During the production operations, *Aspergillus niger* and *Aspergillus flavus* were the most isolated mold (Table 2), and *Saccharomyces cerevisiae* is the dominant yeast isolated (Table 3). *Bacillus* species are involved in fermentation (Jideani and Osume, 2001). The increased acidity may prove some kind of assurance of microbiological safety of the product. Isolates such as genus *Lactobacillus, Candida,* and *Aspergillus,* apart from being contaminants, are reported to be involved in most fermentation process. *Torulopsis* species are associated with cereal based beverages being common contaminant in breweries. L*actobacillus* and yeast species are not pathogen (Bryan *et al.*, 1992), but they may cause significant quality and economic losses, making their control necessary. FDA (2005) reported that many of these organisms are pathogenic, and occur naturally in the environment where foods are grown and processed.

References

Adeyemi, I.A., Umar, S. 1994. Effect of method of manufacture on quality characteristics of *kununzaki* a millet based beverage. *Nigerian Food J.*, 12: 34–41

Akoma, O., Omuala, S.A., Ajibola, M.O., Akoma, A.O., Alawaki, A.M. 2002. The nutritive and sensory quality characteristics of *kununzaki* produced with the addition of hydrolytic enzymes from malted rice (*Oryza Sativa). J. Food Technol. Afr.*, 7(1): 24–26.

Anounye, J.C. 1997. Development and evaluation of soybeans-millet blend for Soy-*kununzaki* preparation. M.Sc. Thesis, Department of Food Science and Technology, University of Agriculture, Makurdi.

Bankole, A.O., Olatunji, O. 2001. Development of unfermented malted cereal – Soy beverage. *Nigerian Food J.*, 19: 106–114.

Bryan, F.L. 1992. Hazard analysis critical control point evaluatios: A guide to identifying hazards and assessing risks associated with food preparation and storage. World Health Organization, Geneva. Available at http://www.who.doc.org (Accessed 15th March, 2005).

Collins, H., Lyne, P.M. 1984.

Microbiological Method, 5th edn., Butterworts and Co. Publisher Ltd., London. Pp. 40 – 45.

Efiuvweuwere, B.J.O., Akoma, A 1995. The microbiology of *kununzaki* a cereal based beverge from northern Nigeria, during the fermentation (production) process *World J. Microbiol. Biotechnol.,* 11: 491–493.

Efiuvweuwere, B.J.O., Akoma, O., 1997. The effects of chemical preservatives and pasteurization on the microbial spoilage and shelf-life of *kununzaki. J. Food Safety,* 17: 203–213.

Fawole, M.O., Oso, B.A. 1995. Laboratory Manual of Microbiology. Spectrum Books Limited, Ibadan, Pp. 14–56.

Federal Department of Agriculture (FDA), 2005. Notice to manufacturers. http://www.cfsan.fda.gov/-led/ allerg7.html.

Food and Agricultural Organization (FAO), 2004. Food safety management. Scientific certification system.http//:www.fooddag-haccp.html (accessed February 23rd, 2005).

Gilbert, R.J., de-Louvois, J., Donovan, T., Little, C., Nye, K., Ribeiro, C.D., Richard, J., Robert, D., Bolton, F.J. 2000. Guideline for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Commun. Dis. Public Health,* 3: 163–167.

Gomez, K.A., Gomez, A.A. 1984. Statistical procedure for agricultural research. Wiley/Interscience, New York, 680 Pp.

Jideani, I.A., Osume, B.U. 2001. Comparative studies on the Microbiology of three Nigerian fermented beverages – *Burukutu, Pito* and *Nbal. Nigerian Food J.,* 19: 25–33.

Lovett, J., Twedt, R.M. 1998. *Listeria:* A scientific status summary by expert panel on Food Safety and Nutrition of the Institute of Food Technologists (IFT), Chicago, IL, Pp. 8–11.

Nkama, I. 1993. Traditional methods of production of high protein energy foods from grain legumes in the north-eastern state of Nigeria. *Ann. Borno* 10: 138–148. University of Maiduguri Press, Maiduguri, Nigeria.

Pearson, 1991. Composition and analysis of foods, 9th edn. John Willey & Sons Inc., New York, USA.