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Effect of Lactic Acid Bacteria on Microbial, Proximate and Sensory Qualities of Nono (A Nigerian Fermented Milk Product)

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Abstract

The production of Nono using Lactic Acid Bacteria and the effect of *Lactobacillus casei* and *Lactobacillus plantarum* on the Microbial, proximate, and sensory evaluation was investigated. The spontaneous and hawked samples serve as the control. There is a general decrease in the pH value which ranges from 6.40-4.56 during the fermentation period. Sample inoculated with *Lactobacillus plantarum* has the highest TTA of 1.95±0.05 while the purchased Nono sample has the least TTA value of 1.49±0.00. Nono fermented with *L. plantarum* has the highest protein content while Nono with spontaneous fermentation has the lowest protein content. Nono fermented with *L. casei* has the highest fat of 6.02±0.01 and the purchased Nono sample has the least fat of 4.33±0.02. The microbial analysis shows that the inoculated Nono sample has a reduced microbial load compare to the hawked sample of $12\times10^5 \pm 2.5$, $7.0\times10^5 \pm 3.0$; $16.5\times10^5 \pm 3.5$; $18.5\times10^5 \pm 3.5$ cfu/ml for NSLC, NSLP, NSC3 and NSPN Total viable count and there was no growth of Coliform *Staphylococcus* and *Salmonella* count in the inoculated samples respectively. The sensory evaluation shows no significant difference in colour and texture through the four Nono samples but significant difference exist between the taste and flavor at ($P \leq 0.05$) Nono fermented with *L. plantarum* was rated the best with overall acceptability of 8 while the control has the least overall acceptability of 5. The Nono sample stored at refrigerated temperature (4°C) has a shelf life of 8 days. Hence the use of the lactic acid bacteria (*L. plantarum*) has biopreservative should be encourage in Nono production has it improved the nutrient, microbial and sensory quality of the drink.

Keywords

Microbial, Proximate, Sensory, Cow Milk, Latic Acid Bacteria, Nono

1. Introduction

Milk is a nutrient-rich white liquid food produced by the mammary glands of female mammals. It is the first food of young mammals and a primary source of nutrition for infant mammals. It is a mixture of fat and high-quality protein in water and contains some carbohydrate (lactose), vitamins, and minerals. Milk and milk products may be obtained from different species, such as goats, sheep and cow. Milk is said to be the lacteal secretion of the mammary gland. It is practically free from colostrum and is obtained by completely milking one or more healthy cows 15 days before or after parturition [1].

Milk can be referred to animals' complete protein food containing all essential amino acids required by the body. It is an extreme versatile product from which a myriad of commercial products such as cheese, yoghurt, Nunu and Kindirimo are derived. Milk is a food of outstanding interest and has been taken by humans since the earliest pre-historic times and still forms the basis of most nation economics [2]. A number of animals are used to produce milk for consumption by human though cow is considered the most important in commercial terms with white Fulani identified as the principal producer [3]. Fermented dairy products considered an excellent source of calcium, phosphorus and

magnesium, which are highly bioavailable, because of the lower pH of fermented milk compared to that of milk. These minerals in optimum ratio are present in milk and its products and are required for optimum growth and maintenance of bones [4].

Nono is a fermented milk product popularly known as *fura de nono* consisting of a cereal "fura" made from millet and "Nono" a fermented milk product similar to yoghurt. Nono is a general name used for locally fermented cow milk and it is widely sold and consumed in the northern part of Nigeria [5]. It is an opaque white or milky coloured liquid food drink got from fermented raw milk. Nono can be consumed anytime of the day. It is a healthy food drink whose consumption transverses the Saharan tribes of West African sub-region extending to the inhabitants of the Mediterranean region and also the Middle East which is called *"dahi" or "lass"* [6]. Predominantly, Nono is prepared and hawked by the normadic Hausa/Fulani cattle herdsman, who controls over 80% of Nigerians cattle production [7]. Fresh cow milk is unfermented, raw cow milk collected fresh from the cow's udder. Consumers have a strong preference for this traditionally produced and processed milk product due to the satisfying nature and also its high protein content [8]. It could be due to its affordability in comparison with imported, processed milk products whose prices are higher.

Lactic acid bacteria (LAB) are a group of gram-positive bacteria including the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus,* and *Streptococcus.* The general description of the bacteria included in the group is gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. Lactic acid bacteria are nutritionally fastidious, requiring carbohydrates, amino acids, peptides, nucleic acids and vitamins. In discussing the importance of *Lactobacillus* species are important as probiotics as a live microbial feed supplement, which is beneficial to the host animal through improving its intestinal microbial balance [9]. The major Problem of Nono is as a result of low shelf life due to contaminant which can result in food borne outbreak. This can be improved by using a bio-preservative. The objectives of this study is to produce Nono drink from raw cow milk using lactic acid bacteria as bio-preservative, to improve the shelf life of Nono drink, to compare the two starter culture and evaluate the microbial quality, proximate and sensory quality of the Nono drink.

2. Materials and Method

2.1. Sample Collection

Fresh milk samples were collected from a local dairy farmer along Ilaro-Owode road, Ilaro, Ogun State, Nigeria. The milk samples were collected in a sterile tightly sealed container, placed in cooler containing ice packs. The milk sample was transported to the laboratory for immediate analysis.

2.2. Bacteria Strains and Culture

The Lactic Acid Bacteria strains were gotten from the Federal Institute of Industrial Research Oshodi (FIIRO). Strains of *Lactobacillus Casei and Lactobacillus plantarum* were inoculated into the milk.

2.3. Nono Sample Preparation

The fresh milk sample was pasteurized at 72°C for 20 secs and allowed to cool down and divided into three portions. Two of the three portions of the milk sample were inoculated with *L. Plantarum* and *L. Casei* cultures (1%) singly. The third portion was left un-inoculated. The three portions were allowed to ferment at 30°C for 24 hrs. The third portion was allowed to ferment spontaneously. At the end of the fermentation period, the milk butter was removed by churning and the remaining sour milk. Figure 1 depicts flow diagram of Nono processing steps.

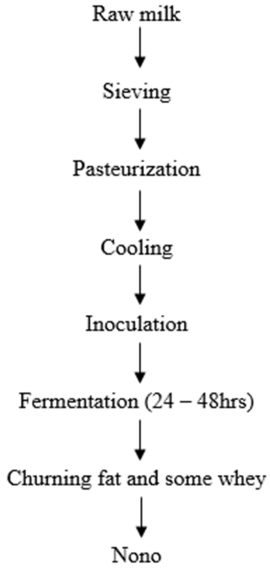

Figure 1. *Flow diagram of Nono processing.*

2.4. Microbial Analysis

The freshly prepared Nono and Hawked samples were brought into the laboratory for analysis. The culture medium; MacConkey Agar for Coliform count, Potato Dextrose Agar for Mold and Yeast count, Bismuth Sulphite Agar for *Salmonella* count, Baird Parker Agar for *Staphylococcus* count and Nutrient Agar for total viable count were prepared according to the manufacturer's specification.

Isolation of microbes - In each isolation, Nono sample was shaken while 10 ml of the sample was aseptically introduced into 90 ml of sterile normal saline solution and homogenized by shaking followed by further decimal dilutions to up to 10^{-6} concentrations. A 0.1 ml quantity of appropriately diluted sample was used to inoculate freshly prepared media and surface-plated. All plates were incubated for total viable count 30°C for 24 hrs, *Staphylococcus, Salmonella* and Coliforms were incubated 35°C for 48 hrs while yeast and mold was incubated for 28±2°C for 3 to 5 days. The resulting

colonies following inoculation and incubation were counted using digital colony counter [10].

Proximate Analysis - The proximate analysis (Moisture, content, crude fibre protein, ash, fat) was determine by AOAC [11] while Carbohydrates was calculated by differences.

Sensory Evaluation - Samples of Nono were subjected to sensory evaluation by nine (9) panelists familiar with the drink. Each of the panelist was asked to rate the samples on the basis of flavour, taste, colour, texture and overall acceptability using a nine point hedonic scale.

3. Results

Table 1. *Microbial Analysis of the Laboratory Prepared and Hawked Nono Samples.*

	Total Viable Count (cfu/ml)	Coliform Count (cfu/ml)	Salmonella Count (cfu/ml)	Staphylococcus Count (cfu/ml)	Yeast & Mould Count (cfu/ml)
NSLC	$12\times10^5 \pm 2.5$	ND	ND	ND	$6\times10^5 \pm 1.00$
NSLP	$7.0\times10^5 \pm 3.0$	ND	ND	ND	$3\times10^5 \pm 1.5$
NSC3	$16.5\times10^5 \pm 3.5$	$17.5\times10^5 \pm 2.5$	$1.5\times10^5 \pm 0.5$	$17\times10^5 \pm 1.0$	$10\times10^5 \pm 2.0$
NSPN	$18.5\times10^5 \pm 3.5$	$32.5\times10^5 \pm 2.5$	$11\times10^5 \pm 1.0$	$17.5\times10^5 \pm 2.5$	$20\times10^5 \pm 2.0$

NSLC=Nono sample inoculated with *L. casei,* NSLP=Nono sample inoculated with *L. plantarum*, NSC3=Nono sample without any inoculums (Control), NSPN=Purchased Nono Sample. Values are Mean of duplicate sample ± standard deviation. ND= Not Detected

Table 2. *Biochemical test of Microbial Isolates of Nono samples.*

Colony Characteristics	Cellular Characteristics	Gram's Reaction	Catalase Test	Oxidase	Coagulase	Motility	Spore	Sugar Dermentation					Suspected Organism
								L	M	G	F	S	
Cream, round, flat	Rod	-	+	-	-	-	-	-	-	-	-	-	*Alcaligenes spp*
Yellow, entire, flat	Cocci	+	+	-	-	-	-		-	+	+	+	*Micrococcus luteus*
Cream, soft, smooth	Cocci	+	+	-	+	-	-	+	+	+	+	-	*Staphylococcus aureus*
Cream, glossy, flat	Rod	+	+	-	-	+	+	v	+	+	-	+	*Bacillus cereus*
Cream, raised, entire	Rod	+	-	-	-	-	-	+	+	+	+	+	*Lactobacillus casei*
Cream, soft, round	Cocci in chain	+	+	-	-	-	-	-	-	+	+	-	*Streptococcus spp*
Cream, circular, entire	Rod	+	-	-	-	-	-	+	+	+	+	+	*Lactobacillus plantarum*
Pink	Rod	-	+	-		+	-	+	+	+	+	+	*Entorobacter aerogenes*

+ve means positive reaction, -ve means negative reaction, L = lactose, M = maltose, G = glucose, F = fructose, S = sucrose

Table 3. *Proximate Composition of Laboratory Prepared and Hawked Nono Samples.*

	NSLC	NSLP	NSC3	NSPN
Moisture (%w/w)	79.81±0.05	74.47±0.04	80.67±0.03	78.24±0.04
Protein (%w/w)	8.08±0.00	8.46±0.04	6.97±0.01	7.82±0.04
Fat (%w/w)	6.02±0.01	5.58±0.02	6.00±0.02	4.33±0.02
Carbohydrate (%w/w)	10.15±0.02	10.20±0.01	5.18±0.01	8.46±0.02
Ash (%w/w)	1.08±0.03	1.29±0.03	1.17±0.05	1.15±0.02
TTA (% lactic acid)	1.71±0.09	1.95±0.05	1.51±0.02	1.49±0.00
Total Solids (%w/w)	20.19±0.05	25.53±0.04	19.13±0.03	21.76±0.04

NSLC=Nono sample inoculated with *L. casei*, NSLP= Nono sample inoculated with *L. plantarum,* NSC3=Nono sample without any inoculums (Control), NSPN=Purchased Nono Sample. Values are means of duplicate sample ± standard deviation.

Table 4. *pH of the milk sample before and after fermentation at room temperature.*

	NSLC	NSLP	NSC3	NSPN
Before	6.32±0.05	6.46±0.09	6.40±0.02	**
After	4.62±0.05	4.56±0.05	4.92±0.05	4.60±0.05

NSLC= Nono sample inoculated with *L. casei*, NSLP=Nono sample inoculated with *L. plantarum*, NSC3=Nono sample without any inoculums (Control), NSPN=Purchased Nono Sample ** = Not Determined

Table 5. *Sensory Evaluation of Laboratory Prepared and Hawked Nono Samples.*

	Taste	Flavour	Colour	Texture	Overall Acceptability
NSLC	7.2^{bc}	6.3^{b}	6.9^{a}	6.4^{a}	7.1^{bc}
NSLP	8.0^{a}	7.1^{a}	7.4^{a}	7.2^{a}	8.1^{a}
NSC3	6.9^{bc}	7.0^{a}	7.4^{a}	6.4^{a}	6.9^{bc}
NSPN	7.6^{ab}	7.1^{a}	7.1^{a}	6.9^{a}	7.3^{b}

NSLC=Nono sample inoculated with *L. casei*, NSLP=Nono sample inoculated with *L. plantarum*, NSC3=Nono sample without any inoculums (Control), NSPN=Purchased Nono Sample. Each sensory property is significantly different at ($p \leq 0.05$)

4. Discussion

According to Table 1, it can be seen that sample NSPN has the highest Total viable count with $18.5\times10^5\pm3.5$ cfu/ml while sample NSLP has the least TVC of $7.0\times10^5\pm3.0$ cfu/ml. For the Coliform count, there was no coliform detected in the inoculated samples. NSPN has the highest coliform count of $32.5\times10^5\pm2.5$ cfu/ml while sample NSC3 has the lowest count of $17.5\times10^5\pm3.0$ cfu/ml. the presence of *Coliform* in the sample may arise from the water used for processing the Nono sample. No *Salmonella* was detected in both sample NSLC and NSLP, but a count of $1.5\times10^5\pm2.5$ cfu/ml and $11.0\times10^5\pm1.0$ cfu/ml was recorded for sample NSC3 and NSPN respectively. There was no *Staphylococcus* count for sample NSLC, NSLP, but was detected in NSC3 and NSPN samples recorded as $17.0\times10^5\pm1.0$ and $17.5\times10^5\pm2.5$ cfu/ml respectively. This was in agreement with the study of Ogbonna [7] who sample hawked Nono and the laboratory sample recorded no pathogens. This may be due to the bio-preservative characteristic of lactic acid bacteria. The yeast and mold count was recorded as $6.0\times10^5\pm1.0$cfu/ml, $3.0\times10^5\pm1.5$cfu/ml, $10.0\times10^5\pm2.0$cfu/ml $20.0\times10^5\pm2.0$cfu/ml, for sample d NSLC, NSLP, NSC3 and NSPN respectively. Microbes isolated were subjected to biochemical test and the isolates as shown in Table 2 are of the genera *Lactobacillus, Micrococcus, Alcaligenes, Staphylococcus, Bacillus, Streptococcus, Enterobacter aerogenes, Salmonella, Sacharomyces, Candida, and Rhizopus.*

The presence of some of the microbes associated with the Nono sample are not surprising as most of them are known to thrive in medium rich in fermentable substrate such as sugars which often led to production of acid after fermentation. Odunfa and Oyewole [12] reported that *Lactobacillus plantarum* was the predominant organism in the fermentation responsible for the production of lactic acid while *Streptococcus* and *Micrococcus acidophilus* are known to be involved in the fermentation of agricultural produce. *Streptococcus and Staphylococcus species* were possible contaminant from the milk handlers, utensils used for milking, air from the environment, though some *Pseudomonas* are spoilage organism at refrigerated temperature. The presence of *Enterobacter sp* is an indication of faecal contamination of the product. Some of the bacteria isolates are normal flora of milk products and animal skin, while others are spoilage and pathogenic species. *Pseudomonas* spp., *Lactobacillus* spp. and *Bacillus* spp. have all been implicated in the spoilage of milk and milk products. *Bacillus* spp is known to be pathogenic and resistant to environmental stress due to its sporing nature, and can cause an emetic syndrome and food-borne intoxication that leads to diarrhea.

This research shows that the pH of the Nono sample increased in acidity as it ferments for 24 hours period. The pH increase across the fermentation period agrees with the report of Oyewole and Odunfa [13], who stated that "the acidity in the fermenting milk is normally noticeable when the pH falls to about 5.5 and below". Milk fermented with starter culture of *L. plantarum* has the highest TTA of 1.95% and milk fermented spontaneously has a least TTA of 1.51% amongst the laboratory prepared Nono sample. The presence of high TTA in sample NSLP may be as a result of the action of the *L. plantarum* culture yielding high acidity content in the fermented milk.

The proximate composition of the inoculated starter culture with LAB and hawked samples were presented in Table 3. The highest moisture content was recorded in sample without inoculation control (NSC3) sample while the least was recorded in NSLP Inoculated with *L. plantarum*. The protein and the fat content of the sample varied. It was observed that the milk fermented with *L. casei* has less protein and more fat content of 8.08±0.00 and 6.02±0.01 respectively compared to milk fermented with *L. plantarum* that has more protein but less fat of 8.46±0.04 and 5.58±0.02 respectively. This does not agree with Adesokan *et, al* [5], where milk fermented with *L. plantarum* has more protein and fat compared to that of milk fermented with *L. casei* which has less protein and fat. This may be as a result of the amount of culture inoculated in the milk. Both sample NSLC and NSLP has more carbohydrate content than sample NSC3 and NSPN.

The sensory evaluation is presented in Table 5, the Nono Inoculated with *L. plantarum* was rated the best in taste, texture and overall acceptability and this is in accordance with the report of Adesokan *et, al* [5]. There is less significant difference between sample NSLC and NSC3. There is no significant difference in colour and texture through the four Nono samples.

From the shelf life study, it was found that sample NSLP lasted for 8 days at refrigerated temperature while that of sample NSLC only last for 6 days. The control sample lasts for 3 days at refrigerated temperature. While the presence of off-flavour and change in colour of the Nono sample shows deterioration in the sample.

5. Conclusion and Recommendation

5.1. Conclusion

From this research work, the samples inoculated with *L. casei and L. plantarum* has shown a lower microbial load compared to the control sample of spontaneous fermentation and the purchased Nono sample. From the sensory evaluation carried out, the milk sample inoculated with *Lactobacillus plantarum* is the most accepted of all. The shelf life of the drink is found to be 6 days and 8 days for milk inoculated with *lactobacillus casei and Lactobacillus plantarum* respectively, while that which ferments spontaneously is 3 days.

5.2. Recommendation

The health of the cow that produces the milk is very important to be monitored. The cows should be vaccinated often to keep them healthy. The milk handler and the milking utensils should be kept hygienically. Other probiotic

organisms may be inoculated in the raw milk to compare the shelf life stability.

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