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Microbiological and physicochemical properties of 'nono' sold at some markets in Enugu north senatorial district

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Abstract

"Nono" is an indigenous fermented milk product. Handlers' poor sanitary cultures and non-scientific practices harm food safety and public health. This study evaluated the microbiological, physicochemical, and sensory properties of "Nono," collected from the marketplaces of Enugu North Senatorial District of Nigeria. The total aerobic bacterial and total coliform counts were higher than Nigeria's standard value whereas, 50% of the overall fungal count was within permissible limits regardless of market samples**.** The bacteria identified were *Staphylococcus aureus* (17.65%), *Salmonella enterica* (17.65%), *Bacillus subtilis* (11.76%), *Klebsiella aerogenes* (11.76%), whereas fungal were *Aspergillus niger* (57.14%) and *Torulopsis dottila* (42.86%). Although significant changes ($P < 0.05$) in proximate and physicochemical parameters were observed, no significant differences in sensory attributes ($P > 0.05$) across these samples were recorded. The means (±SD) for pH (4.47±0.18), protein (8.74±0.44%) etc. were recorded. The presence of pathogenic bacteria in the nono samples could be hazardous to public health.

***Keywords*:** Nono Fermented milk; Microbiological; Physicochemical; Sensory quality; Nigeria

Introduction

In Nigeria, the volume of unregulated or uncontrolled local or indigenous alcoholic and non-alcoholic beverages produced, sold, and consumed has reached alarming levels. Strict regulations and food safety standards must be followed in order to protect the public's health. Okaru *et al.* (2019) stated that in villages and towns, unregulated beverages are sometimes referred to as artisanal, unrecorded, illicit, or illegal food drinks that pose significant health risks due to improper production methods and lack of quality control. As these products gain popularity, it becomes increasingly important for regulatory bodies to implement measures that ensure consumer safety and promote responsible drinking practices. Nigeria, like other countries, has its share of illegal foods and beverages. These types of products' typical features include but are not limited to, the fact that they are produced outside government regulation unchecked, or without regard for standard and safety guidelines; such unregulated beverages are sometimes vulnerable. Products are frequently homemade or alternative drinks that expose consumers to harmful substances; manufacturers occasionally claim that their products with different ingredients offer more nutrients than those found in regulated beverages. Since many sellers offer their goods in discarded bottles and plastic containers, packaging can occasionally be compromised.

Consuming these local beverages had reportedly been connected to a number of health problems, such as gout, obesity, diabetes, hyperuricemia, coronary heart disease, stroke and an elevated risk of death (Collin *et al*. 2019), and as such, their unwholesome consumption may result in unfavorable health outcomes.

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However, food poisoning, intoxication, renal failure, and other chronic ailments have been caused by inadequate protection (Nwaiwu and Itumoh, 2017) from these unregulated products. The fact that these drinks are produced using non-scientific techniques, endangering public health and disregarding food safety, is more worrisome. Gazuwa and Denkok (2017) claim that when scientific methods are not followed, there is often a metabolic shift from precursors to unwanted and hazardous metabolites, which can have lethal cumulative effects on consumers.

A number of these foodstuffs are made via uncontrolled microbial fermentation. These dietary items are significant and connected to many people’s socioeconomic health. Many families sell these drinks on the streets in order to make money. Examples of alcoholic beverages include pito (Florence *et al.* 2016) and burukutu (Okiki *et al.* 2018). Non-alcoholic beverages, such as tiger nut milk (Ukwuru and Ogbodo, 2011), soymilk (Ozoh and Umeaku, 2016), and zobo (Izah *et al.* 2016), are plant-based. Nono (Ani *et al.* 2018), kunu (Adelikan *et al.* 2013), and yoghurts can be produced with plant or animal milk (Ajiboye *et al.* 2014).

‘Nono’ is a locally produced fermented cow milk beverage that is popular throughout Africa and beyond that is similar to yoghurt and other fermented dairy products (Gemechu *et al*. 2015). In Nigeria, Hausa/Fulani herders, who possess over 80% of the cattle population, produce and sell “nono”. It can be consumed raw or prepared into another milk cereal-based dish or cuisine called fura-da-nono. However, the unscientific methods and questionable hygiene standards of "nono" handlers in this unofficial dairy industry are suspicious, posing risks to food safety and public health (Anyanwu, 2019). Nono has a thick, smooth, uniform look and a harsh, sour flavor similar to yoghurt and other fermented dairy products (Egwaikhide *et al.* 2014). Vitamins A, C, E, and the B complex are abundant, just as, calcium, phosphorus, and amino acids (Nebedum and Obiakor, 2007).

Reports stated that "nono" is the result of spontaneous fermentation caused by the introduction of starter culture - lactic acid bacteria (LAB) on fresh raw unskimmed and unpasteurized milk, and is often kept unrefrigerated (Egwaikhide *et al*. 2014; Dafur *et al*. 2018; Anyanwu, 2019; Fagbemigun *et al*. 2021). The fresh milk is directly obtained from a cow into a properly washed calabash, which is thereafter exposed to the sun for around 2 hours to facilitate separation of the fat layer (Egwaikhide *et al.* 2014). Then after, some overnight fermented milk is added as a source of starter culture, and the inoculated fresh milk is allowed at room temperature overnight to ferment, producing "Kindirmo"—a sour milk. Curdled sour milk is combined-with a significant amount of water and stirred with a T-shaped stick until it achieves a fine consistency. It can be eaten alone or with fura, a millet or maize dumpling. Raw milk that has been fermented locally and its byproducts are believed to be more nutritious than unfermented milk, mainly in the rural settings (Egwaikhide *et al.* 2014). This belief stems from the traditional practices that have been passed down through generations, emphasizing the health benefits associated with fermentation. Moreover, the consumption of fermented products like Kindirmo not only supports local agricultural practices but also promotes food security in these communities.

Some research findings have acclaimed that the fermented milk product is more nutritious and health-promoting than fresh milk (Akabanda and Glover, 2010). However, the inherent microbial load - lactic acid bacteria (Egwaikhide *et al.* 2014; Wakil *et al*. 2014), contaminants from the air, equipment, handlers, etc. (Banik *et al*. 2014), and nutrient-dense nature that promotes microbial growth and proliferation (Pfeuffer and Watzl, 2018) all play a significant role in the acceptability or rejection of milk and milk products (nono). The need for safe and high-quality milk has been growing (Gemechu *et al*. 2015), but it is concerning because the majority of the key players in this value chain in the manufacture of ‘nono’ hardly follow environmental and personal hygiene regulations, thus endangering public health and food safety.

Most locally produced fermented dairy products including nono (Adebayo *et al.* 2013), yoghurt (Omola *et al.* 2019), Kunu zaki (Braide *et al.* 2018), cereal and legume-based drinks, such as pito and burukutu (Eneji *et al.* 2020), soymilk (Akani and Barika, 2019), tiger nut milk (Badua *et al.* 2018; Osho and Shobande, 2019), and plant-based beverages, such as zobo (Ezearigo *et al*. 2014; Ibitoye *et al*. 2017) are produced under poor hygienic conditions. Proper production practices are often abandoned by these handlers. Even more worrisome is the fact that the drinks are made using non-scientific methods. According to Gazuwa and Denkok (2017), this may result in a metabolic shift from a precursor to undesirable and dangerous metabolites, with the overall effect potentially harmful to consumers.

Reports stated that a number of factors influence the safety and quality milk including the chemical makeup of milk (total solid, solids-not-fat, fat, protein, ash, and lactose), physical attributes (temperature, pH, specific gravity, titratable acidity, etc.), sensory attributes (lack of peculiar flavor, color, or odor, etc.), and microbiological qualities. When evaluating the general attributes of milk and milk products, each of these factors is significant (Bhatia *et al.*

2015). Although physicochemical analysis is a valuable method for assessing the quality of milk and its products, thorough analyses of the microbiological properties of 'nono' sold in our communities should not be ignored (Omotosho *et al.* 2013; Teklemichael *et al.* 2015). Therefore, the purpose of this study is to evaluate the physicochemical, sensory, and microbiological characteristics of' "nono", a fermented food milk available in some markets in the Enugu North Senatorial Districts.

Materials and methods

The research area

This research area covered different markets/locations within the Enugu North Senatorial Districts of Enugu State such as; Orie Orba markets (OOM) and Obollo Afor market (OAM) in Udenu L.G.A., Ogige Nsukka market (ONM) and Afor Opi market (AOM) in Nsukka L.G.A., Eke Ekwegbe markets (EEM) and Nkwo Ogbede market (NOM) in Igbo-Etiti L.G.A., Eke Ozzi market (EOM) in Igbo-Eze North and Nkwo Ibagwa market (NIM) in Igbo-Eze South L.G.A. There are semi-urban settlements with a sizable farming population in these areas, as well as rural inhabitants. These markets serve as crucial hubs for commerce, where local farmers and traders converge to exchange goods and services. The vibrant atmosphere not only supports the local economy but also fosters a sense of community, as residents gather to socialize and share in the rich cultural traditions of the region.

Sample collection

The samples collection method of Ogbonna (2011) was adopted in this study with slight modifications. A total of sixteen (16) "nono" samples analyzed were purchased at random from vendors in eight (8) distinct markets located within the Senatorial Districts. These marketplaces were chosen based on patronage, the number of animals, and nono vendors/hawkers. These samples were delivered to the Microbiology Laboratory, Department of Science Laboratory Technology (SLT), Federal Polytechnic, Ohodo, Enugu State, for processing.

Sample processing

Processing involved a series of physicochemical, proximate and microbiological tests to assess the quality and safety of the nono samples. Each sample was evaluated for microbial contamination levels, including the presence of pathogenic organisms, to ensure compliance with health standards. Samples were processed using the methods described by Fagbemigun *et al.* (2021), with minor modifications. Briefly, lactating cows were milked manually to collect samples. Fresh cow milk samples from several nursing cows' udders were gathered in containers, combined, and fermented spontaneously for 24 hours using a lactic acid fermentation process. These combined samples were then left open in one container in the sun for approximately two hours to allow the fat layer to separate before being scooped out. The samples were then sieved, and some amounts of previously prepared or overnight 'nono' (back slopping culture) were added to enhance lactic acid fermentation. This mixture was then placed in a controlled environment to ferment for an additional 48 hours yielding sour milk known as "Kindirmo". The fat and whey were removed, and a significant amount of water was added to curdle the sour milk, which was then swirled with a T-shaped stick until it reached a fine consistency. The processed 'nono' samples were placed in appropriately labeled sterile corked plastic tubes and stored in ice-filled containers for analysis. The resulting product was evaluated for flavor, taste, aroma, colour, appearance and general acceptability ensuring it met the desired quality standards. All the samples obtained from different locations/markets (OOM, OAM, ONM, AOM, EEM, NOM, EOM, and NIM) within these study areas underwent rigorous physicochemical, proximate and microbiological testing to evaluate their nutritional qualities, identify bacterial counts and assess overall quality. The results would provide valuable insights into the production methods and safety of 'nono' across the various markets in the region.

Microbiological analysis

The International Dairy Federation's (IDF, 2002) recommended protocols for microbiological examination as adopted by the Standard Organization of Nigeria (SON) were followed. Every analysis was carried out in duplicate.

Enumeration, isolation, identification and confirmation of microorganisms from 'nono' samples

Homogenization and serial dilution of the samples

Nono samples were shaken, and 25 ml of the sample was aseptically added to 225 ml of peptone water and homogenized by shaking, followed by further decimal dilutions to 10^{-6} concentrations. Using a sterile automated pipette, 1 ml of the stock 'nono' sample was pipetted into 9 ml of normal saline to dilute the samples serially. This method was repeated, each time using a sterile pipette, until a 10^{-6} dilution was attained. Aliquots (1 ml) of dilution 10^{-3} and 10^{-4} were directly inoculated into petri dishes with the appropriate isolation medium.

Pour plate and spread plate culturing techniques were used to enumerate and isolate microorganisms from various 'nono' samples using appropriate media, including Nutrient agar for

total aerobic bacterial counts (TABC), MacConkey agar total coliform counts (TCC), Potato dextrose agar/Sabourand dextrose agar for total fungal counts (TFC). Other media used include blood agar medium for *Streptococcus* species and Baird-Parker agar (OXOID) for *Staphylococcus aureus*. All these media were incubated at 32 ± 2^0C for 24-48 hours before being counted for bacteria. For fungi, the plates were incubated at 25^0C for 4-5 days before being counted.

The bacterial isolates were identified using cultural, morphological, gram staining, and biochemical techniques described by Cheesebrough (2005) and Mubarack *et al.* (2010). The fungal isolates were identified using macroscopic and microscopic observations, as described by Bala *et al.* (2017). Fungal isolates were also recognized using a color atlas (Frey *et al.* 1979).

Determination of proximate and physicochemical properties of the 'nono' samples.

The AOAC (2005) standard method was used to determine the samples' proximate parameters, which include their crude protein, crude fat, ash and moisture as well as carbohydrate contents which were estimated by differences: % carbohydrate = 100 - % (protein + moisture + fat + fiber + ash). The physicochemical properties, such as the temperature of the samples was measured at the point of collection with a thermometer, while a pH meter was used to measure the potential of hydrogen (pH). Lactometers and viscometers were used to measure the specific gravity and viscosity. The titratable acidity, total solids, and solids-not-fat were all determined according to the method of the AOAC (2005).

Sensory evaluation of the 'nono' samples.

To determine whether these market samples were acceptable, organoleptic tests were conducted. Evaluation based on the Hedonic scale was employed, and a 10-member panel of sensory judges made up of staff of the SLT Department and students participated in the evaluation. Data were classified and analyzed statistically based on taste, aroma, colour, appearance, and overall acceptability.

Results and discussion

Table I shows the average total aerobic bacterial counts (TABC), total coliform count (TCC), and total fungal count (TFC) for 'nono' samples.

The total aerobic bacterial counts (TABC) varied among the different markets/location. The results revealed that the TABC ranged from 2.01×10^5 cfu/ml (NOM) to 3.06×10^6 cfu/ml (AOM), TCC ranged from 6.50×10^3 cfu/ml (ONM) to 3.00×10^4 cfu/ml (OOM), while TFC ranged from 7.00×10^2 cfu/ml (NIM) to 1.40×10^4 cfu/ml (NOM) in all the areas studied.

Table I. Total aerobic bacterial counts (TABC), total coliform counts (TCC) and total fungal counts (TFC) in cfu/ml from 'nono' samples marketed at the different markets/locations

Markets	TABC (cfu/ml)	TCC (cfu/ml)	TFC (cfu/ml)
OOM	2.80×10^6	3.00×10^4	1.15×10^3
OAM	1.18×10^6	1.06×10^4	1.20×10^3
ONM	1.04×10^6	6.50×10^3	9.50×10^2
AOM	3.06×10^6	1.01×10^4	7.10×10^2
EEM	2.01×10^6	2.60×10^4	1.11×10^4
NOM	2.10×10^5	1.25×10^4	1.40×10^4
EOM	1.19×10^6	7.00×10^3	8.50×10^2
NIM	8.30×10^5	1.18×10^4	7.00×10^2
SON Limit	$< 5.00 \times 10^4$	1.00×10^2	1.00×10^2

Legend: OOM = Orie Orba market, OAM = Obollo Afor market, ONM = Ogige Nsukka market, AOM = Afor Opi market, EEM = Eke Ekwegbe market, NOM = Nkwo Ogbede market, EOM = Eke Ozzi market, NIM Nkwo Ibagwa market, SON = Standard Organization of Nigeria, cfu/ml = colony forming unit per milliliters.

Table II. Morphological, cultural and biochemical characteristics of bacterial isolates from the 'nono' samples

GR	CA	OX	CT	UR	IN	MR	VP	M	HAE	SH	COA	GL	FR	LA	SU	RH	ORGANISM
-R	+	-	+	-	-	-	+	+				+	+	+	+	+	*Klebsiella aerogenes*
+R	+	-	+	-	-	-	+	+	+	-		+	+	-	+	-	*Bacillus subtilis*
+R	-	+	-	-	+	-	+				+	-	-	-	-	-	*Salmonella entericas*
-R	+	-	-	-	+	+	-	+	-	-	-	+	+	+	+	+	*E. coli*
-C	+	-	+	+	-	+	+	-	+	-	+	+	+	+	+	-	*Staphylococcus aureus*
+R										+		+	+	+	+	+	*Lactobacillus sp*
-C		-		-			+	-	+			+	+	+	+	+	*Streptococcus sp*

Legend: + = Positive, - = Negative, GR= Gram reaction, CA= Catalase test, OX= Oxidase test, CT= Citrate test, IN= Indole, MR= Methyl red, VP= Voges Proskauer, Motility= Motility, HAE= Hemolysis test, SH= Starch hydrolysis, COA= Coagulase test, GL= Glucose fermentation, FR- Fructose test, LA= Lactose, SU= Sucrose, RH= Rhamnose

Table III. Frequency of occurrence of microbial isolates from 'nono' samples at different markets

Isolates	OOM	OAM	ONM	AOM	EEM	NOM	EOM	NIM	Total	%age
Bacterial organisms										
B. snbtilis	0	1	0	0	0	0	0	1	2	11.76
S. aureus	1	0	1	0	0	0	1	0	3	17.65
K. aerogenes	0	1	0	0	1	0	0	0	2	11.76
S. enterica	1	0	0	1	0	0	0	1	3	17.65
E. coli	0	0	1	0	1	0	1	0	3	17.65
Lactobac.sp	0	1	0	1	0	0	0	0	2	11.76
Strept. sp	0	0	1	0	0	1	0	0	2	11.76
Total	2	3	3	2	2	1	2	2	17	99.99
Fungal organisms										
A. niger	1	0	0	1	1	0	0	1	4	57.14
T. dottila	0	0	1	1	0	1	0	0	3	42.86
Total	1	0	1	2	1	1	0	1	7	100

Key: 1 = Isolated, 0 = Not isolated

The organisms identified after the morphological, cultural and biochemical tests are shown in Table II. A total of seven bacterial organisms were isolated from all the samples.

Table III depicts the frequency of occurrence of these microbial isolates. The microbial isolates depicted a total of seventeen (17) bacterial and seven (7) fungal organisms. The bacterial organisms such as *Staphylococcus aureus, Salmonella enterica,* and *E. coli attained* the highest frequency of occurrence of 3 (17.65 %), while *Bacillus subtilis*, *Klebsiella aerogenes*, *Lactobacillus species,* and *Streptococcus species* had a frequency of 2 (11.76 %). However, the fungal isolates recorded 4 (57.14 %) occurrences for *As,pergillus niger* and 3 (42.86 %) for *Torulopsis dottila*.

The mean (±SD) proximate parameters and physicochemical properties of 'nono' samples evaluated are presented in Table IV. The proximate analysis results showed that the mean (±

Table IV. Mean (±SD) for proximate parameters and physicochemical properties of 'nono' obtained from different markets/locations

Variables	OOM	OAM	ONM	AOM	EEM	NOM	EOM	NIM	Overall mean
Protein (%)	9.05± 0.21	7.74± 0.37	7.80±0 .40	7.45±0 .07	8.22± 0.03	9.75±0. 35	10.10± 0.99	9.82± 1.10	8.74±0.4 4
Fat (%)	2.70± 0.14	3.70±0. 14	3.78±0 .31	4.11±0 .13	3.13±0. 04	3.39±0. 16	3.99± 0.04	3.94±0.2 5	3.59±0.1 5
Ash (%)	0.76± 0.06	0.80±0. 09	0.90±0 .08	0.98± 0.02	0.84± 0.08	0.90±0. 04	0.84±0.1 4	0.90±0.0 9	0.87±0.0 8
Moisture (%)	59.10± 1.27	63.75±1 .06	60.05± 1.63	66.55± 0.64	64.80±0 .42	54.65±0 .92	57.60±0. 57	54.69±0. 58	60.15±0. 89
Carbohydrate (%)	28.39±1 .68	24.0±1. 38	27.47± 2.42	20.90± 0.82	23.02±0 .57	31.32±0 .69	27.47±0. 33	30.65±0. 18	26.66±1. 01
Temp (°C)	21.7± 0.35	21.55±2 .19	23.80± 0.28	20.54± 0.65	22.50±0 .71	21.90±0 .00	21.50±0. 71	23.00±1. 41	22.07±0. 79
Ph	4.46± 0.51	5.11±0. 01	4.39±0 .01	4.02± 0.06	4.18±0. 25	5.09±0. 16	3.88± 0.11	4.65±0.3 1	4.47±0.1 8
Sp. G (g/ml)	1.031± 0.00	1.029± 0.00	1.032± 0.00	1.031± 0.00	1.028±0 .00	1.030±0 .00	1.031±0. 00	1.032±0. 00	1.031±0. 00
TTA (%)	0.190± 0.00	0.190±0 .01	0.202± 0.00	0.187± 0.00	0.214±0 .00	0.199± 0.01	0.208±0. 01	0.199±0. 00	0.197±0. 00
Viscosity (cp)	131.0±1 .41	146.10± 2.76	139.6± 0.57	128.6± 0.57	143.6± 0.57	142.7± 0.42	137.7±1. 91	143.0±0. 10	139.03± 1.04
TS (%)	11.50±0 .42	12.51±0 .69	12.92± 0.14	11.09± 0.16	10.75±0 .07	9.94± 0.37	9.90±0.2 8	11.04±0. 34	11.21±0. 31
SNF (%)	8.80± 0.28	8.81± 0.83	9.14±0 .17	6.99± 0.02	7.63±0. 04	6.55± 0.21	5.91±0.3 3	7.10±0.5 9	7.62±0.3 1

Results are presented as mean ± SD of two replicates
Legend: pH = potential of hydrogen, Sp. G = Specific gravity, TTA = Titratable acidity, TS = Total solids, SNF = Solids -not-fat.
Results are presented as means ± SD of two replicates.

SD) protein values ranged from 7.45±0.07 to 10.10±0.99 % with an overall mean of 8.74±0.44. Other parameters such as fat ranged from 2.70±0.14 to 4.11±0.13%, ash ranged from 0.76±0.06 to 0.98±0.02%, moisture ranged from 54.69±0.58 to 66.55±0.64%, and carbohydrate ranged from 20.90±0.82 to 31.32±0.69%, with their overall means of 3.59±0.15, 0.87±0.08, 60.15±0.89 and 26.66±1.01, respectively.

The physicochemical analysis revealed that temperature ranged from 20.54±0.65 to 23.80±0.28, pH values ranged from 3.88±0.11 to 5.11±0.01, titratable acidity ranged from 0.187±0.00 to 0.214±0.00%, viscosity ranged from 131.00±1.41 to 146.10±2.76cp, total solids ranged from 9.90±0.28 to 12.51±0.69%), and solids-not-fat ranged from 5.91±0.33 to 9.14±0.17, with their corresponding overall means of 22.07±0.79, 4.47± 0.18, 0.197± 0.00, 139.03±1.04, 11.21±0.31, and 7.62±0.31, respectively.

The sensory evaluation of these products revealed no statistically significant difference ($P > 0.05$) among the 'nono' samples obtained from different markets (Table V).

The results of the microbiological analysis of the "nono samples showed that the products were contaminated by microorganisms of public health concern. The records revealed that total aerobic bacterial counts (TABC) ranged from 2.01×10^5 cfu/ml (NOM) to 3.06×10^6 cfu/ml (AOM), total coliform counts (TCC) ranged from 6.50×10^3 cfu/ml (ONM) to 3.00×10^4 cfu/ml (OOM), while the total fungal counts (TFC) ranged from 7.00×10^2 cfu/ml (NIM) to 1.40×

Table V. Mean (±SD) sensory evaluation of 'nono' samples obtained from different markets

Variables	OOM	OAM	ONM	AOM	EEM	NOM	EOM	NIM	Overall Mean
Taste	4.37± 0.31	5.72± 0.32	6.07± 0.23	6.33± 0.91	7.00± 0.20	5.57± 0.20	5.97± 0.45	5.07± 0.16	5.76± 0.36
Aroma	6.24± 0.24	5.53± 0.42	5.88± 0.44	5.37± 0.32	5.30± 0.17	4.76± 0.25	5.43± 0.06	5.33± 0.06	5.48± 0.23
Colour	4.57± 0.40	5.92± 0.60	5.77± 0.60	6.93± 1.41	5.83± 0.15	7.00± 0.10	6.30± 1.12	5.57± 0.32	5.99± 0.43
Appearance	5.59± 0.36	5.93± 0.21	6.40± 0.53	6.77± 0.38	6.17± 0.21	6.33± 0.29	5.17± 0.45	5.90± 0.36	6.03± 0.35
Gen. Acceptability	5.97± 0.25	6.67± 0.15	6.53± 0.25	5.48± 0.11	6.23± 0.21	6.40± 1.13	6.40± 0.20	5.73± 0.42	6.18± 0.34

Results are presented as mean ± SD of two replicates

10^4 cfu/ml (NOM) in all the areas studied. The TABC was higher in OOM (2.80×10^6) than in the OAM (1.18×10^6) even though both markets are in the same local government area but in different locations. The high counts recorded could be due to the handlers' poor sanitary conditions, the quality of water used in processing the 'nono,' tools used in the production, the use of previous fermented samples, and storage before distribution to consumers. The ONM and AOM, though they share the same local government, have high counts of 3.06×10^6 (AOM) and lower counts in ONM (1.04×10^6), which could be due to their settings. The awareness of sanitary issues and improved protocols for processing and handling may have been indoctrinated into 'nono' vendors in this urban setting – ONM the through mass media and public health awareness/education. The application of such processing protocols is better appreciated by the ONM (an urban setting) than its counterpart – the rural setting (AOM) - which may have contributed to the lower counts in this area.

The high TABC counts in all the samples, although exceeding the Standard Organization of Nigeria's (SON) limit of 5.00×10^4 cfu/ml or 4.6 log 10 cfu/ml, were all lower than the Codex Alimentarius Standard of 10^7 cfu/ml, and so, are all indications of contaminations, which may not be unconnected with the unhygienic practices such as poor sanitary conditions of handlers, non-potable water, and uncleaned containers during production. The samples from EEM (2.01×10^6) and NOM (2.10×10^5), all in Igbo-Etiti, as well as EOM (1.19×10^6) and NIM (8.30×10^5), both in Igbo-Eze North, presented their various levels of TABC. The length of time these fresh samples were stored before being utilized in production, water quality, and the handlers' sanitary conditions may have contributed to their varying levels of these contaminants. Other factors are unhygienic practices such as poor sanitary conditions of handlers, non-potable water, and uncleaned containers during production. The TABC results are not within the limit reported by Abdulrahman and Sanmi (2021), who recorded $1.29\times 10^5 - 7.63 \times 10^5$ cfu/ml. They reported that the high counts might be due to the handlers' poor sanitary conditions, low level of cleanliness observed and tools used in production and storage. We recorded much higher TABC values that ranged from 2.10×10^5 (NOM) to 3.06×10^6 (AOM) than earlier reported by Abdulrahman and Sanmi (2021). These values may not be unconnected with the quality of water used in processing, the length of time of storing milk samples, and the poor sanitary conditions of production staff. In our quest to know why there were high TABC in most areas, we discovered that some of these vendors patronize non-potable water (well water and stream), which they share in common their herds. Also, lack of refrigerators to keep the samples immediately after milking and sanitary conditions of these vendors have all contributed to the increase in high TABC counts.

The total coliform counts (TCC) in this study ranged from 6.50×10^3 cfu/ml (ONM) to 3.00×10^4 cfu/ml (OOM). The

highest TCC recorded in OOM (Table I) is lower than 5.12 $\times 10^{10}$ cfu/ml reported by Maikai and Madaki (2018), which, notwithstanding, is an indication of contamination. All the TCC results recorded were higher than SON's acceptable limits (1.00×10^2), however, the high rates of coliforms among different vendors may not be unconnected with the local technology applied during processing, the sanitary conditions of the handlers, and the hygienic status of the udder of the animal. Elsewhere, it has been reported that coliforms are indicator organisms of post handling contagion in manufacture (Rojas *et al*. 2020). The occurrence of coliforms bacteria in all the market samples, suggest that the milks were tainted with fecal material from the unhygienic udder and teats of the lactating cows, the milking vessels and environments, as well as cows with clinical mastitis and as such, harmful to consumers' health. Since these vendors share their drinking water (mostly well water and streams) in common with their herds, the possibility of fecal contamination from humans and herds who defecate indiscriminately can hardly be ruled out. These findings corroborate the works of Owusu-Kwarteng *et al*. (2020) and also the report of Frew and Abebe (2020), who reported that the presence of coliforms in great amount in dairy products is a sign that the products are possibly harmful to the consumers' health.

The presence of fungi, even in minute quantities in milk, and its product is unwanted because of the unpleasant changes that affect the quality of the products. Reports have shown that fungi constitute the major indicator of general essential quality (Huera-Lucero *et al*., 2020). These undesirable changes caused by the presence of fungi may have come from air, uncleaned apparatuses, and poor personal hygiene. The range of the total fungal counts recorded in this study (7.00×10^2 to 1.40×10^4 cfu/ml) is beyond the standard limit for milk and its products (FAO/WHO, 2018). This value is, however, slightly lower than the report from Anyanwu (2019), who recorded mean fungal counts that ranged from 2.27×10^5 to 5.3×10^5 cfu/ml. The TFC is higher in OAM (1.20×10^3) than in OOM (1.15×10^2) even though both markets are in the same locality and are patronized by the same vendors. The perceived disparities that cut across all other markets could be due to prolonged exposure to certain environmental conditions favoring proliferation of fungal organisms, the surrounding air, uncleaned apparatuses, and poor personal hygiene. More so, the distance between the point of collection and transportation from farms to processing contributed in the level of TFC recorded in this study. All the TFC values recorded in this study were higher than the limit by the SON, which is 1.00×10^2 cfu/ml. These values may have helped to ensure that the fermented milk product did not have excessive bacterial contamination, which can lead to spoilage or the presence of harmful mycotoxins.

Our findings have shown that the fungal growth observed could be as a result of exposure of the milk in the course of transportation, from the air, handlers, or even from the proliferation of these organisms in the skin and teat of the cow when the condition (acidity) became favourable. Since the handlers are not privy to pasteurization techniques, which can reduce or eliminate these organisms, there is a possibility that human health is being threatened. These findings corroborate those of Cisse *et al*. (2018), who, in addition, reported an emergence of contaminants at the various stages (from the udder, after mixing milk from several cows, sieving, fermentation, and after churning) of 'nono' samples collected.

Research has shown that fungi in fermented milk thrive by using some of the acids (sour taste), thus supporting the proliferation of putrefying bacteria, and other microbes, e.g *Staphylococcus aureus* and *Listeria monocytogenes* (Cisse *et al*., 2018; Banwo *et al*. 2020). We isolated and characterized similar organisms, including gram positive and negative bacteria as well as fungi. Bacteria such as *Bacillus subtilis, S. aureus, Klebsiella aerogenes, Salmonella enterica, Escherichia coli, Lactobacillus species,* and *Streptococcus species* (Table II), as well as two fungi such as *A*spergillus *niger* and *Torulopsis dottila* were all isolated. The proliferation of bacterial organisms was due to the growth of *A. niger* and *T. dottila* (Table III).

Milk and its products are likely sources of bacterial pathogens. The organisms recorded in this study, corroborate the report of Bazata *et al*. (2020), who reported specifically *Lactobacillus plantarum* in addition to the organisms we obtained in this study. Maikai and Madaki (2018) and Tamba *et al*. (2021), also isolated in addition, *Enterobacter, Proteus* and *Citrobacter species*. The incidences of both *E. coli* and *S. aureus* 3 (17.65 %) as well as *K. aerogenes* 2 (11.76 %) suggest contamination of the product from water and utensils. The sample quality is improved due to the presence of lactic acid bacteria that produce antibacterial material, including organic acids (Mortera *et al*. 2018). The incidences of *Salmonella* and *Shigella* had been implicated as causative agents for typhoid fever and shigellosis - health debilitating illnesses. The presence of *Salmonella enterica* 3 (17.65 %) agrees with the work of Esonu *et al*. (2021), and this could be due to poor hygiene level, lack of potable water used in the

production processes and use of water from streams that are polluted (defecated) by humans and animals (Table III). The incidence of *Bacillus subtilis* at 2 (11.76%) is pathogenic, and this organism is highly resistant to stress due to spore production. The occurrence of *Bacillus species* had been reported as the causative agents for emetic syndrome (nausea and vomiting) and food-borne intoxication (Anyanwu, 2019; Noah and Salam, 2020). The incidence of *K. aerogenes* at 2 (11.76 %) could be a suggestion of a likely contact with discharge and excretory products of either human handlers or cows. Wiri *et al*. (2020), in addition to, isolated *Pseudomonas aeruginosa*, confirmed that the food product may have been in contact with the personnel's body wounds or discharge. The occurrence of *Aspergillus niger* 4 (57.14%) recorded in this study had been reported by Anyanwu (2019), who maintained that molds are the major contaminants of fermented milk in Nigeria. Aspergillus organisms have been implicated in most food intoxication due to the mycotoxins they produce.

The total frequency of occurrence of the bacterial isolates were highest at OAM and ONM with three (3) organisms, while NOM was the least, recording one (1) organism. All other markets, however, recorded two (2) organisms. On the other hands, the fungal isolates achieved two (2) organisms at AOM, zero or no organisms were seen from samples collected at OAM and EOM, while the remaining markets such as; OOM, ONM, EEM, NOM and NIM recorded one (1) organism each. The occurrences of these isolates may not be unconnected with pre- and post-handling operations such as; equipment used, poor hygiene levels of the producers, unscientific procedure used, lack of potable water etc. It had been reported that fungi in fermented milk thrive by using some of the acids (sour taste), thus supporting the proliferation of putrefying bacteria, and other microbes, for example, *Staphylococcus aureus* and *Listeria monocytogenes* (Cisse *et al*. 2018; Banwo *et al*. 2020). Most of the bacterial organisms recorded were not part of the contaminants abinitio, rather they may have colonized the samples arising from the presence of fungal that provided the conditions supporting the proliferation of spoilage and pathogenic organisms.

There were variations in the mean (±SD) of the proximate and physicochemical properties of the different 'nono' samples (Table IV). However, the overall mean (±SD) for the proximate parameters, namely protein, fat, ash, moisture, and carbohydrates, were 8.74±0.44, 3.59±0.15, 0.87±0.08, 60.15±0.89 and 26.66±1.01, respectively. The overall mean (±SD) physicochemical values also were 22.07±0.79, 4.47±0.18, 1.031±0.00, 0.197±0.00, 139.03±1.04, 11.21±0.31, 7.62±0.31 for temperature, pH, specific gravity, titratable acidity, viscosity, total solids and solids-not-fat, respectively.

The protein contents ranged from 7.45±0.07 (AOM) to 10.10±0.99 (EOM), with an overall mean of 8.74±0.44. These values recorded here may be a reflection of the time of milking and diet of the cows. Noah and Salam (2020) got similar values; however, our present study disagrees with the 1.50 to 1.61% reported by Abdulrahman and Sanmi (2021). The high amount of protein recorded in EOM could be the reflection of high conversion rate of feed and the time of milk collection. The ideal time for milk collection should be when the cow is relaxed. It should be noted that EOM recorded the highest protein among others owing to the fact that these prevailing factors were addressed. The fat content could be attributed to the time of milking, changes in the temperature of the environment, age and diet of the cow. The fat content of milk decreases with high temperature and increases at low and cold temperatures as well. The overall mean fat content (3.59±0.15) obtained is within the standard of 3.5% for fermented milk (FAO, 2004; Omola *et al*. 2020), thus suggesting the level of water consumed by cow and the quantity added to 'nono' samples during the production process. The low-fat content recorded in OOM (2.70±0.14) could be that the sample was collected at high temperature and from an aged cow, while 4.11±0.13 recorded at AOM may likely be due to the fact that the milk was collected at low and cold temperatures, a diet of high-fat content of the cow as well as milking at the prime age of the cow.

The ash content of 0.87±0.08 is lower the than 1.25±0.8 reported by Trachoo and Mistry (2011), and this is an indication of mineral content which is necessary for bone and teeth development and other body functions. In general, the low ash contents that ranged from 0.76±0.06 (OOM) to 0.98±0.02 (AOM) suggest supplementation of 'nono' with minerals. The moisture content that ranged from 54.65±0.92% (NOM) to 66.55±0.64% (AOM) with an overall 60.15±0.89%, is lower than Uzeh *et al*. (2006), who reported a higher value of 86.03% in similar work in Lagos, Nigeria, while Noah and Salam (2020), equally reported 79.81%. This lower value we recorded may be due to inadequate regular water supply to the cattle from these farms, leading to dehydration. "Nono" with low moisture content may likely harbor less microbial load than the very high content samples.

The overall mean temperature of 22.07±0.79 was recorded in this study. The industrial standard recommended for

fermented milk products is a holding temperature not higher than 8°C (Omola *et al*. 2020). In this study, the lack of a cooling system and refrigerator for nono storage by these vendors might have contributed to high temperatures with a concomitant increase in the microbial contaminants. The pH ranged from 3.88±0.11 to 5.11±0.01 with an overall mean of 4.47±0.18 recorded in this study, agrees with the values reported by Noah and Salam (2020). These values were within the international standard limit for fermented milk. This low pH prevented the growth of spoilage and pathogenic organisms. The persistence of pathogenic organisms such as Klebsiella even after fermentation is an indication that the 'nono' should be pasteurized, since it was able to survive at that pH. The titratable acidity (TTA) has been used to measure the quality (freshness) of milk and its products. The TTA range of 0.190±0.00% (OOM) to 0.214±0.00% (EEM) with the mean value 0.197±0.00%, is lower than 0.90±0.10 obtained by Okeke *et al*. (2016), and also, it did not fall within the value of 0.70 to 1.20% as reported by Choi *et al*. (2016). This showed that the fermentation proceeded normally. The variation in pH and TTA is a function of the fermentative activities by lactic acid bacteria (LAB) present in the product, the method of production, and the duration of the fermentation. These observations agree with a report by Orji *et al.* (2010), who noted that changes in TTA of dairy beverages depend on the rate of growth of bacterial organisms, especially LAB, which contribute to the sour taste of milk products.

The overall mean apparent viscosity value of 139.03±1.04 recorded is within the range of 133.50 to 168.70 cp obtained by Omola *et al*. (2019). Janahar *et al*. (2021) reported that the viscosity is essential in determining the rate of creaminess, heat transfer, the conditions in dairy processes and also varies with changes in temperature. The more the viscosity of the 'nono' sample, the higher the rate of acceptability by consumers. Less dense 'nono' samples available in some markets may have stemmed from the quantity of water added during production, thereby compromising the quality, which may likely affect the market demand.

The range of total solids from 9.94±0.34 (NOM) to 12.92±0.14 (ONM), with a corresponding mean total solid of 11.21±0.31, is higher than the 12.87% reported by Gemechu *et al*. (2015), and that of the established standard of not being less than 12.50% (FAO/WHO, 2007). These variations could be due to differences in breed, feeding and management practices that finally affect the milk composition and quality as was reported by Teklemichael *et al*. (2015). The range of solids-not-fat (SNF) from 5.91±0.33 (EOM) to 9.14±0.17 (ONM) with an overall mean of SNF of 7.62±0.31 was lower than 8.55 and 8.59% reported by Dafur *et al*. (2018) and Gemechu *et al*. (2015), respectively. Our findings corroborate the report of Gemechu *et al*. (2015), who pointed out that these variations could be due to differences in the feeding practices, season, milking method, and lactation period of the cows.

Remarks from sensory tasters and the statistical analysis of the organoleptic test results did not show significant differences ($P \geq 0.05$) in all the parameters, such as taste, aroma, colour, appearance, and general acceptability, among all the 'nono' samples evaluated.

Conclusion

Based on the analyses carried out, some of their proximate and physicochemical properties fell within the acceptable limits. However, 'nono' samples hawked by vendors in these areas were not safe for public consumption. The presence of these pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Bacillus subtilis*, *Aspergillus niger,* etc.) was suggestive of organisms of public health concern.

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