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Microbiological Quality of Traditionally Fermented Fresh Cow Milk (*Nono*) Retailed in Selected Local Government Areas of Kano State, Nigeria

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Abstract

Nono is an African fermented beverage commonly prepared by the Fulani cattle herdsmen and sold by their maids to both rural and urban people.This study was conducted to assess the microbiologicalquality of traditionally fermented fresh cow milk (*Nono*) retailed in selected local government areas of Kano State, Nigeria using standard protocol.The physico-chemical parameters (pH, titratable acidity and viscosity) were determined according to standard methods. The microbiological analyses carried out were based on the enumeration of aerobic mesophilic bacteria, lactic acid bacteria, *Streptococcus* sp.,fungi, *Shigella*sp. as well as the isolation and identification of *Salmonella* sp., *Staphylococcus aureus* and *Clostridium botulinum* using the method of International Dairy Federation. The results of the analyses showed that the pH ranged from 3.59 - 5.36, titratable acidity (0.73 - 2.17%), viscosity (10.14 – 550 cp). The aerobic mesophilic count ranged between 0.0 – 2.8 x 10^6cfu/ml. Lactic acid bacteria ranged from 4.0 x 10^3 – 6.0 x 10^6 cfu/ml. *Streptococcus* sp. ranged from 0.0 – 4.8 x 10^5cfu/ml.Fungal count ranged from 0.0 – 8.8 x 10^6cfu/mlwhile *Shigella* sp. ranged between 0.0 –9.3 x 10^4 cfu/ml. *Staphylococcus aureus* was not detected in any of the samples analyzed. The incidence of *Salmonella* sp. obtained in this study was 3.5% while *Clostridium botulinum* was 1.75%. The presence of these pathogens in *nono* milk is a source of public health concern.

Keywords: Traditional fermentation, Microbiological Quality, *Nono*.

INTRODUCTION

Nono is traditional fermented fresh cow milk. It is made by a process involving lactic acid fermentation. The fresh milk is directly obtained from a cow into a properly washed calabash and kept wide open in the sun for approximately two hours to facilitate separation of the fat layer (Egwaikhide *et al.*, 2014). Some quantity of overnight fermented milk is added therefore, to serve as source of starter culture and the inoculated fresh milk is left overnight at room temperature for fermentation, to get sour milk known as "*Kindirmo*". The addition of large volume of water to the curdle sour milk, which is then stirred with a T- shaped stick to a liquid of fine consistency gives rise to *Nono*. *Nono* has thick, smooth and uniform appearance with a sharp acid taste like that of yogurt. It can be taken alone or mixed with a dumpling of millet or maize called *Fura*. It is believed, especially in rural areas, that locally fermented raw milk and its by-products have better nutrition than unfermented one (Egwaikhide *et al.*, 2014). It constitutes a primary sour milk product from which other products may be processed (Gonfa *et al.*, 2001). It is a healthful food whose consumption transverses the Saharan tribes of West African Sub- region extending to the inhabitants of the Mediterranean region and also the Middle East. In the Middle East, it is called '*dahi*' or '*lassi*' (Nahar *et al.*, 2007). *Nono* contains good quantities of amino acid, calcium, phosphorous and vitamins A, C, E and the B complex (Nebedum and Obiakor. 2007). Some research findings have acclaimed fermented milk to be more nutritious and health-promoting than fresh milk (Akabanda and Glover, 2010). In Nigeria, *nono* is produced mainly by the nomadic 'Fulani' herdsmen who control over 80% of the cattle population. This study was aimed at assessing the microbiological quality of traditionally fermented fresh cow milk (*nono*) retailed in selected local government areas of Kano State, Nigeria.

MATERIALS AND METHODS

Experimental design

The experiment was carried out using completely randomized design (CRD). Since the experimental material is homogenous with treatment as the only source of variation (Mukhtar, 2013).

Sample collection

Proportionate sampling technique (Mukhtar, 2013) was adopted in the sample collection. Four hundred *nono* samples were purchased from 9 *nono* retailing markets (Table 1) spread across the three senatorial zones of Kano State. The sample collection and analysis were for a period of eighteen months,(October, 2016 to March, 2018). Hawked samples were collected twice weekly in sterile corked bottles and transported in cool box with ice to the microbiology laboratory of the Department of Microbiology, Bayero University, Kano for the analyses.

Determination of pH, titratable acidity and viscosity of *nono*

These parameters were determined according to the method of the Association of Official Analytic Chemists (AOAC, 2005). For pH, the glass electrode was pushed into the sample to 3/4of the sample, then swirled for 5 seconds and allowed to become steady before taking reading on the Jenway- U.K pH meter.

Titratable acidity was determined by mixing the content of *nono* and indicator by gentle shaking and 1 ml of 0.1N sodium hydroxide was added within 15 seconds from the burette. This was followed by drop mix addition of the 0.1N NaOH till a faint pink color which persisted appeared. The acidity of the sample was calculated using the following equation: Titratable acidity (%) = 0.009 x Vol. of NaOH used x 100 / Weight of the sample.

Apparent viscosity was determined at 20^{o}C with a viscometer (Model DV-E Brookfield) equipped with a rotor. Samples were equilibrated for 20 minutes at the desired temperature of 20^{o}C. After making sure the marker was on zero, it was then set on for 3 minutes until a stable mark was reached. The value was then read and expressed in Centipoise.

Microbiological Analysis

Microbiological analysis was carried out based on procedures recommended by the International Dairy Federation (IDF, 2002).

Nono samples were shaken and 25ml of the sample was aseptically introduced into 225ml of peptone water and homogenized by shaking followed by further decimal dilutions up to 10^{-6} concentrations. From appropriate dilutions, 1ml each was placed in duplicate petri dishes using the pour plate technique.

Media employed for the isolation and enumeration of the organisms included: nutrient agar (Zayo-Sigma Germany) for aerobic mesophilic bacteria, de Man Rogosa and Sharpe medium (OXOID) for lactic acid bacteria, Blood agar medium for *Streptococcus* species,Potato dextrose agar (Rapid Labs – UK) for fungi, Baird parker agar (OXOID) for *Staphylococcus aureus*, SSA agar (Labs - UK) for *Shigella*, Deoxycholate Citrate Agar (Hi-Media) for *Salmonella* and cooked meat medium (OXOID) for *Clostridium botulinum*. The nutrient agar plates were incubated at 30^{o}C for 24hrs while SSA agar, and DCA plates were incubated at 37°C for 24 hrs, The Baird parker agar plates were inverted and incubated at 37^{0}C for 24 hrs. Blood agar plates were incubated at 37^{0}C for 48 hrs, MRS plates were incubated anaerobically at 30^{0}C for 48 hrs, Cooked meat medium tubes were incubated at 30^{0}C for 5 days while potato dextrose agar plates were incubated at ambient temperature.

Statistical analyses

The microbiological data obtained from the research was subjected to Analysis of Variance (ANOVA), using one – way classification, Least significant difference (LSD) test was carried out at $p < 0.05$ to determine whether there was significant difference between the means (Mukhtar, 2013)

RESULTS AND DISCUSSION

The results of the physico chemical characteristics of the *nono* samples (Table 2) showed that the mean pH fell between 4.22 – 4.70. The market range of this study 3.59 - 5.36 supports that obtained by Okonkwo (2011) in Maiduguri, El Bakri and El Zubeir (2009) in Khartoum State, Sudan but differ from 5.51-6.29 reported by Adesokan *et al*. (2011) in Ibadan and 5.7 by Obi and Ikenebomeh (2007) in Benin. The low pH prevents the growth of most spoilage and pathogenic organisms (Varga, 2007). The mean titratable acidity fell between 1.34 – 1.50% while the range is from 0.73-2.17, which is higher than 0.08-0.13 obtained by Egwaikhide *et al*. (2014). There is no difference ($p>0.05$) between the means of pH, titratable acidity and viscosity. The high acidity explains why *nono* has a sour taste and may be due to the variation in duration of fermentation period and method of production. The viscosity of the *nono* samples ranged between 10.14-550 centipoise while the mean fell between 133.5 - 168.7 cP. These values are lower than means of 213 and 360 obtained by Okeke *et al*. (2016). Generally, viscosity of milk is important in determining the rate of creaming, heat transfer and the flow conditions in dairy processes.

The result of the microbiological quality shows comparison of the mean microbial count of *nono* samples (Table 3). Dawakin tofa has the least mean aerobic mesophilic bacteria count of 4.10 x 10^{4} cfu/ml while Gaya has the highest mean mesophilic aerobic bacteria count of 7.50 x 10^{4}cfu/ml.

The overall mean was 4.95 x 10^4cfu/ml. This value is higher than that obtained by Okonkwo (2011) in Maiduguri but lower than that reported by Tankoano *et al*. (2016) in Ouagadougou. The range values for aerobic mesophilic bacteria fell between not detected to 2.80 x 10^6cfu/ml (Table 4). These values are lower than 3.00 x 10^3 - TNTC reported by Egwaikhide *et al*. (2014) but higher than that reported by Shittu *et al*. (2016). The result is also in agreement with that reported by Omotosho *et al*.(2013) and Laba and Udonsek (2013). Aerobic counts are used to estimate viable bacteria populations in milk and reflect the hygienic practices used in the production and handling of the milk (Houghtby *et al*., 1994). Madobi has the least mean lactic acid bacteria (LAB) count of 2.80 x 10^4cfu/ml while Wudil had the highest mean LAB count of 3.10 x 10^5cfu/ml. The mean concentration of LAB (8. 93 x 10^4 cfu/ml) was lower than that reported by Tankoano *et al*. (2016). The mean of lactic acid bacteria is higher than that of other treatment means. Range values for LAB fell between 4.00 x 10^3 – 6.00 x 10^6 cfu/ml (Table 4). These values are within the same range with those obtained by Savadogo *et al*. (2004) but differ from the value obtained by Beukes *et al*. (2001) in South Africa. The production of lactic acid gives the fermented product a sour taste and also results in the formation of a smooth gel. Wudil has the least mean count for *Streptococcus* sp.(1.10 x 10^4 cfu/ml) while Kabo has the highest mean count of 4.90 x 10^4 cfu/ml with an overall average of 2.41 x 10^4cfu/ml. The difference between the means of *Streptococcus*sp., aerobic bacteria, fungi and LAB do not differ ($p>0.05$) significantly. The range values for *Streptococcus* spp. fell between ND – 4.80 x 10^5cfu/ml. These results are higher than that reported by Olatunji *et al*. (2012) in Abuja, Nigeria. These are the principal lactic acid producing bacteria in milk and are responsible for fermentation of carbohydrate to lactic acid. Thus these organisms are responsible for normal souring of milk (O'Connor and Tripathi, 1992). Madobi has the least mean fungal count of 2.00 x 10^4cfu/ml while Makoda has the highest mean fungal count of 2.70 x 10^5 with an overall mean value of 9.60 x 10^4cfu/ml. This value is lower than that reported by Idise *et al*. (2009) for *nono* in Zaria but higher than that of Okonkwo (2011) in Maiduguri. The range values for fungi in this study fell between ND - 8.80 x 10^6cfu/ml (Table 4). The results are in agreement with the findings of Akabanda *et al*. (2013) in Ghana and Adebesin *et al*. (2001) but higher than that reported by Omotosho *et al*. (2013). The incidence of fungi (yeasts) in all these samples, however, may suggest that yeasts are a common flora of the milking parlours, containers and fermentation vessels (Gadaga *et al*. 2000). *Staphylococcus aureus* was not detected in any of the market samples analyzed. Wudil has the least mean count for *Shigella* (4.70 x 10^3 cfu/ml) while Dawakin tofa has the highest mean count of 6.30 x 10^3cfu/ml. The overall mean for *shigella* species in this research (5.3 x 10^3cfu/ml) was higher than that reported by Okonkwo (2011). The range values of *Shigella* spp. fell between not detected to 9.30 x 10^4 cfu/ml which is similar to that obtained by Omotosho *et al*. (2013).The presence of this organism in food is a major public health problem (WHO, 2005).

Table 1: Lists of Markets that were Surveyed

Senatorial Zone	Local Government Area	Markets
Kano North	Dawakin tofa	Dawakin tofa
		Kwa
		Tatarawa
	Kabo	Kabo
		Garo
		Katsalle
	Makoda	Makoda
		Kore
		Ganji
Kano Central	Warawa	Makole
		Gano
		Warawa Dai
	Gwale	Tal udu
		Gadon kaya
		Aisami
	Madobi	Madobi
		Kwankwaso
		Gora
Kano South	Sumaila	Sumaila
		Sitti
		Angwa manzo
	Wudil	Wudil
		Dukawa
		Gorubobi
	Gaya	Gaya
		Utai
		Ganaiki

Note: Every market is made up of 3 sub markets

Table 2: Physicochemical Characteristics of *Nono* Samples

Markets	pH	Titratable acidity (%)	Viscosity (cP)
DTF	4.24 ± 0.04	1.49 ±0.03	161.9 ±12.9
KBK	4.22 ± 0.05	1.50 ±0.05	148.3 ±11.9
MKK	4.36 ± 0.07	1.44 ±0.05	168.7 ±20.8
WRW	4.42 ± 0.06	1.40 ±0.04	135.9 ±9.20
GWL	4.35 ± 0.05	1.40 ±0.04	145.7 ±9.70
MDB	4.36 ± 0.05	1.47 ±0.04	146.8 ±13.50
SML	4.70 ±0.05	1.44 ±0.04	139.8 ±14.40
WDL	4.35 ± 0.05	1.49 ±0.04	146.8 ±12.10
GYA	4.33 ±0.06	1.34 ±0.04	133.5 ±11.80
Statistics	NSD	NSD	NSD

Values are the mean of different markets. No of samples: DTF (40), KBK (39), MKK (39) WRW (46), GWL (45), MDB (45), SML (49), WDL (49), GYA (48). ± = S.E, NSD = No significant difference.

Table 3: Comparison of the Mean Microbial Count (Cfu/ml)of *Nono* Samples

Organisms	Markets								
	DTF n = 40	KBK n = 39	MKK n = 39	WRW n = 46	GWL n = 45	MDB n = 45	SML n = 49	WDL n = 49	GYA n = 48
Aerobic mesophilic bacteria	$4.1x\ 10^4$	$1.3x\ 10^4$	$3.1x\ 10^4$	$2.4x\ 10^4$	$5.8x\ 10^4$	$3.4x\ 10^4$	$1.2x\ 10^5$	$2.0x\ 10^4$	$7.5x\ 10^4$
Lactic acid bacteria	$3.1x\ 10^4$	$4.2x\ 10^4$	$1.4x\ 10^5$	$5.6x\ 10^4$	$8.6x\ 10^4$	$2.8x\ 10^4$	$2.9x\ 10^4$	$3.1x\ 10^5$	$8.2x\ 10^4$
Streptococcus sp.	$2.6x\ 10^4$	$4.9x\ 10^4$	$4.8x\ 10^4$	$1.6x\ 10^4$	$1.3x\ 10^4$	$1.4x\ 10^4$	$1.6x\ 10^4$	$1.1x\ 10^4$	$2.4x\ 10^4$
Fungi	$2.1x\ 10^5$	$2.2x\ 10^5$	$2.7x\ 10^5$	$3.8x\ 10^4$	$2.2x\ 10^4$	$2.0x\ 10^4$	$3.1x\ 10^4$	$3.4x\ 10^4$	$2.3x\ 10^4$
S. aureus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Shigella sp.	$6.3x\ 10^3$	$5.4x\ 10^3$	$5.0x\ 10^3$	$5.0x\ 10^3$	$5.9x\ 10^3$	$5.1x\ 10^3$	$5.0x\ 10^3$	$4.7x\ 10^3$	$5.7x\ 10^3$

Table 4: Range of Counts (Cfu/ml) of Organisms Isolated from *Nono* Samples

Organisms	Markets								
	DTF n = 40	KBK n = 39	MKK n = 39	WRW n = 46	GWL n = 45	MDB n = 45	SML n = 49	WDL n = 49	GYA n = 48
Aerobic mesophilic bacteria	0.0 -7.9x 10^5	0.0 - 8.5 x 10^4	0.0 -4.2 x 10^5	0.0 - 4.2 x 10^5	0.0 -8.3 x 10^5	0.0 -4.0 x 10^5	0.0 -2.8x 10^6	0.0 -2.2 x 10^5	0.0 -1.5 x 10^6
Lactic acid bacteria	8.6 x 10^3 - 9.7x 10^4	8.0 x 10^3 - 2.2 x 10^5	4.0 x 10^3 - 4.2x 10^6	8.4 x 10^3 - 8.0 x 10^5	9.7 x 10^3 - 2.2 x 10^6	3.89 x 10^3 - 7.9 x 10^4	7.9 x 10^3 - 1.7 x 10^5	1.0 x 10^4 - 6.0 x 10^6	6.9 x 10^3 - 4.5x 10^5
Streptococcus sp.	0.0 -3.6x 10^5	0.0 -4.3 x 10^5	0.0 -4.3 x 10^5	0.0 -7.3 x 10^4	0.0 - 5.1 x 10^4	0.0 -3.8 x 10^4	0.0 -1.5x 10^5	0.0 -5.3 x 10^4	0.0 -4.8 x 10^5
Fungi	0.0 -2.7 x 10^6	0.0 -2.3x 10^6	0.0 -8.8 x 10^6	0.0 - 2.6 x 10^5	0.0 -9.1 x 10^4	0.0 -8.2 x 10^4	0.0 - 3.4x 10^5	0,0 -4.7 x 10^5	0.0 -2.1 x 10^5
S. aureus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Shigella sp.	0.0 - 5.3x 10^4	0.0 - 5.6x 10^4	0.0 - 6.1 x 10^4	0.0 - 9.3 x 10^4	0.0 -5.1 x 10^4	0.0-8.1 x 10^4	0.0-8.2 x 10^4	0.0 - 6.2 x 10^4	0.0 - 6.9 x 10^4

The result of Table 5 shows frequency of occurrence (%) of bacteria isolated from *nono* samples. The incidence of *Salmonella* spp. in this study was three and half percent which differ from Ekici *et al.*(2004) who reported not detected but agrees with Lingathurai and Vellathurai (2010), Okonkwo (2011) and Omotosho *et al.* (2013). All *salmonellae* are of public health concern having the ability to produce infection ranging from a mild self - limiting form of gastroenteritis to septicemia and life threatening typhoid fever (WHO, 2004).

Dairy products have low rates of sporadic contamination and likewise few botulism outbreaks have been associated with these types of products. In this research, percentage detection of *Clostridium botulinum* was 1.75%. This value is lower than 4% obtained by Chukwu *et al*. (2016) from food sold in Lagos and that of 30% of mascarpone cheese samples associated with an outbreak of *C. botulinum* spores (Glass and Marshal, 2013). Similarly, a survey conducted in France identified 7.8% of fish and Shellfish used as ingredients for refrigerated foods as being positive for *C. botulinum* (Glass and Marshal, 2013). *C. botulinum* is the cause of a life threatening food-borne illness called botulism due to the neurotoxin production that grows in food. The source of contamination could be from soil, water, vegetation and silage used to feed the cattle.

The incidence of *Streptococcus* sp. in this study was 8.5% while that of *Shigella* sp. was 4%. Also, in this research, fungi isolated includes *Aspergillus* sp,, *Mucor* sp., *Cladosporium* sp., *Curvularia* sp., and *Rhizoctonia* species.

Table 5: Occurrence (%) of Bacteria Isolated from *Nono* Samples.

Isolated organisms	Markets								
	DTF n=40	KBK n=39	MkK n=39	WRW n=46	GWL n=45	MDB n=45	SML n=49	WDL n=49	GYA n=48
*Salmonella*sp	2 (5)	0.0	2 (5.1)	2 (4.3)	0.0	4 (8.9)	0.0	0.0	4 (8.3)
C. botulinum	1(2.5)	0.0	0.0	1(2.17)	0.0	1(2.22)	1(2.04)	2(4.08)	1(2.08)
Streptococcus sp	5(12.5)	3(7.69)	3(7.69)	4(8.69)	3(6.66)	4(8.8)	4(8.16)	4(8.16)	4(8.33)
Shigella species	2(5.0)	1(2.56)	2(5.12)	2(4.34)	2(4.44)	2(4.44)	2(4.08)	2(4.08)	1(2.08)
Fungi	1(Asp)		1(Muc)	1(Cur)	1(Asp)	1(Cla)	1(Asp)	1(Asp)	1(Rhi)

Key: Values in parenthesis () are percentages. Asp = *Aspergillus* sp, Muc =*Mucor* sp., Cla = *Cladosporium* sp., Cur = *Curvularia sp.*, Rhi = Rhizoctonia sp

CONCLUSION

The results of the present study indicated that the pH ranged between 3.59-5.36, titratable acidity 0.73 - 2.17% while aerobic mesophilic bacteria ranged between 0.0 - 2.80 x 10^6 cfu/mland LAB ranged between 4.00 x 10^3 – 6.00 x 10^6cfu/ml.

Bacteria of public health significance were detected. The presence of these potential pathogenic organisms in *nono* milk which is highly cherished and consumed is a source of public health concern.

Recommendations

Based on the findings of this research, it can be recommended that:

1. Processed *nono*be stored in cool environment probably inside fridge to control the pH and acidity.
2. *Nono*retailing environments should be kept clean to avoid contamination of the product.
3. The use of old portion of previously fermented *nono* as starter should be discouraged as they could be the possible source of contaminating organisms.

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