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Biochemical and Proximate Properties of Fermented Milk Products (NONO) in Northern Nigeria

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ABSTRACT

The present study was aimed at analyzing the biochemical, proximate and physiochemical properties of raw milk samples (nono). A total of 60 nono samples were collected from Bauchi, Jigawa and Plateau States in Northern Nigeria at guided random. The proximate composition, biochemical and physiological properties of the nono samples were analyzed using standard methods. Lactic acid producing bacteria were isolated using (MRS) medium. There were significant differences ($p \leq 0.05$) in the proximate composition of the raw and processed cow milk "nono" collected from the three different States. There was a decrease in the moisture content of the processed products across the three states in the raw milk. There was decrease in the ash content of the processed "nono", but the samples from Bauchi were significantly different at ($p \leq 0.05$). Ash content of the samples was 1.83 ± 06 and 0.61 ± 0.03 for raw and processed "nono" respectively. Significant increase was observed in the crude protein, fat, carbohydrate and total solid between the processed samples collected across the three states. Decreased values were obtained in the non- fat solid of samples collected from Bauchi State which were 13.22±0.03 and 11.00±0.20 for raw and processed "nono" respectively.

Keywords: *Biochemical, proximate, fermented milk, Nono, Nothern Nigeria*

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INTRODUCTION

Fermented foods forms an important part of the diet in many cultures, and over time fermentation has been associated with many health benefits. Because of this, the fermentation process and the resulting fermented products have recently attracted scientific interest. In addition, microorganisms contributing to the fermentation process have recently been associated with many health benefits, and so these microorganisms have become another focus of attention. Lactic acid bacteria (LAB) synthesize vitamins and minerals, produce biologically active peptides with enzymes such as proteinase and peptidase, and remove some non-nutrients (Şanlier et al., 2019). The specific composition of the milk microbiota has direct impact on the subsequent development of dairy products. Lactic acid bacteria can bring about fermentation of milk through the production of lactate and have a variety of different impacts on the sensory characteristics, texture, flavour and organoleptic properties of resultant products. Certain microorganisms can also negatively impact on milk quality and shelf life; for example, psychrotolerant bacteria can proliferate during refrigeration and, through the production of

extracellular lipases and proteases, leading to spoilage (Quigly et al., 2013). Fermentation aids the breaking down of large organic molecules into smaller particles through the action of microorganisms (Nkhata et al., 2018). Example, sugars and starches can be converted into alcohol by the activities of yeast enzymes, while proteins are converted to amino acids. The enzyme activities on food ingredients favour food fermentation, resulting to desirable biochemical changes which cause significant modification to the food. Fermentation is a natural method of enhancing vitamins, essential amino acids, anti-nutrients, proteins, food appearance, and enhanced aroma. Fermentation aids significantly in the reduction of the energy required for cooking as well as making a safer product (Nkhata et al., 2018; Xiang et al., 2019).

MATERIALS AND METHODS

Collections of nono samples

Processed and raw milk samples were purchase from three different States; Jigawa, Bauchi and Plateau. A total of sixty (60) nono samples, twenty (20) from each state, ten raw and ten processed were purchased from different markets in the three different States. Each sample consisting of (250 ml) were collected in sterile screw-cap sterile bottles and kept under low temperature using an ice-cooled box to be brought to the Department of Plant Science and Biotechnology laboratory, University of Jos for further experiment.

Physiochemical analysis

Determination of pH

The pH of the fermented milk samples (yoghurt) was measured with a pH meter with a glass electrode every one hour according to Sadler and Murphy (2003). 30 mL of each sample was transferred into a beaker and the probe of the pH meter rinsed with distilled water and dipped into the samples; the probe was rinsed with distilled water after inserting in each sample. The pH of each sample was displayed on the meter and recorded. The pH of all the 60 samples was tested in triplicate. A proximate analysis for all the samples was carried out using AOAC (2005) method to determine the moisture content, proteins, ash content, crude fibre, fat content and non-soluble fat content. Elemental analysis was carried out respectively using AOAC method (2005).

Determination of titratable acidity (T. A.)

This was determined using the alkaline titrimetric method described by AOAC (2005). 20ml of the sample was dispensed into conical flask and 3 drops of phenolphthalein indicator was added. This was titrated against diluent standard alkaline solution (0.01N NAOH solution). Titration was done until a persistent faint pink colouration was obtained. The total titratable acidity was calculated using the formula below.

$$\% \text{ Titratable acidity (T. A)} = \frac{T \times N}{W} \times 100$$

Where, T = Titre value N = Normality of titrant W= Weight of sample used (Igbabul et al., 2014).

Proximate analysis

Proximate analysis of food is the determination of the major components of food. This includes moisture, fats (lipids), ash (mineral), protein carbohydrate and fibre.

Determination of moisture content

This was determined by the method of AOAC (2005) gravimetric method. A measured weight of the sample (5g) was weighed into a previously weighed moisture can. The sample in the can was evaporated to dryness over a steam bath and then dried in the oven 105^{o}C for 3 hours in the first instance. It was cooled in a desiccator and weighed. It was then returned to the oven for further drying. Drying, cooling and weighing were repeated until a constant weight was obtained. By difference, the weight of the moisture lost was obtained and expressed as a percentage of the weight of sample analyzed.

$$\text{Moisture contents (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where, W1 = weight of empty moisture can W2 = weight of can + sample before drying

W3 =weight of can + sample after drying (Igbabul et al., 2014).

Determination of ash content

Ash content was determined by the furnace incineration gravimetric method as described by AOAC (2005). 5g of the sample was weighed into previously weighed crucible. It was evaporated to dryness over a steam bath and then burnt in a muffle furnace at 55^{0} C until it become grey ash. The ash in the crucible was carefully removed and cooled in a desiccator and reweighed. By weighed increased, the weight of ash was obtained and expressed as percentage of the sample analyzed and calculated as

shown below.

Ash content (%) =W2-W1× 100

Where, W1 = weight of empty crucible W2 = weight of crucible + ash.

Determination of protein content

Protein content was determined using kjeldahl method (AOAC, 2005). The total nitrogen was obtained and multiplied with the factor 6.38 (having a milk- based product to obtain the protein). 0.5g of the Nono sample was boiled in 10mls of concentration H_2S0_4 with selenium as catalyst. Boiling (digestion) was done under a fume cupboard until a clear solution was obtained. This digest was transformed quantitatively to a standard flask and diluted to 100ml with distilled water 10ml portion of the digest was mixed with equal volume 45% NaoH solution and distilled in a semi micro-kjeldahl apparatus. The distillate was collected into 10% boric acid solution containing 3 drops of mixed indicator (methyl red and bromocresol green). A total of 50ml distillate was collected and titrated against 0.02N H_2SO_4 solution. Titration was done from green to a deep red end- point. A reagent blank was also treated as described above. The N_2 content and hence protein was calculated as shown below.

$$\% N2 = \frac{100 \times 14 \times N \times VF \times T\text{-}BLK}{W \quad 100 \quad VA}$$

W =weight of sample N =Normality of titrant Vf = Total digest volume Va = Volume of digest analyzed T = Sample titre BLK = Reagent Blank titre.

Determination of carbohydrate

Content Carbohydrate was calculated as nitrogen free extractive using the formula described by James (2013). % CHO = 100-% (Protein + ash +fat +moisture content).

Determination of fat content

Determination of fat content was carried out according to the method described by AOAC (2005) Nono sample (5.0g) was mixed with 0.88 ammonia solution and 10mls of 95% ethanol was added to it and mixed well. 25mls of diethyl ether was added to it and shaken vigorously for 1 minute. 25mls of petroleum ether was added and mixed well. The mixture was allowed to separate into phases and after standing for 1 hour. The fat extract (ether phase) was collected and the sample was re-extracted with the same solvent and the extracts pooled together. The extract was then transferred to a weighed flask and the solvent recovered while the fat in the flask was dried in the oven 100 C for 30 minutes cooled in a desiccator and weighed. The weight of fat was determined and the amount of fat determined and expressed as a percentage of the sample analyzed. It was calculated as shown below.

$$\% \text{ fat} = \frac{W2\text{-}W3 \times 100}{1}$$

Where, W1 =weight of flask alone W2 = weight of flask and extract

Determination of mineral element of the NONO samples

Ten (10) ml each of the samples were weighed into sterilized standard flasks containing 10% 250ml HCl and made up to 100ml with deionized water. Potassium (K), Sodium (Na), Sulphur (S), Phosphorus (P), Magnesium (Mg), Nitrogen (N) and Calcium (Ca) contents were determined using Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer).

DISCUSSION

For the proximate composition of nono samples, the parameters considered includes moisture, ash, contents, crude protein, crude fat, carbohydrate total solid and non-solid fat (Table 1). The moisture content across the samples B_1, B_2, Jg_1, Jg_2, Js_1 and Js_2 were 82.07, 41.20, 79.37, 35.01, 80.97 and 37.67 respectively. The results of the proximate analysis reveals that there was a significant difference in the proximate composition of the raw and processed (nono) cow milk collected from the three different states; Plateau State, Jigawa and Bauchi state at $P \leq 0.05$. There was a significant decrease in the moisture of the processed product across the three different states over the raw cow milk; this is in agreement with the work reported by Henry-Unaeze & Ezeugwus (2022). This could be due to microbial cell proliferation; the dried weight of the milk samples that were analyzed. The significance of moisture content in milk is that, high moisture content implies high water activity which supports microbial growth consequently reducing the shelf life of the milk sample. Low moisture contents on the other hand, implies low water activities, low water activities cause reduction in microbial growth and the predominant microbial culture consequently increasing the shelf life of the milk samples as a result of low availability of water for microbial growth.

There was decrease in the ash content of the processed milk, but only the samples in Bauchi was significantly different at $p \leq 0.05$ with ash content of 1.83

Table 1: Proximate Composition of Nono Samples collected from Bauchi, Jigawa and Jos Plateau states of Nigeria

Parameter (%)	B_1	B_2	Jg_1	Jg_2	Js_1	Js_2
Moisture	82.07 ± 0. 15^a	41. 20 ± 0.06^b	79.37 ± 0.21^a	35. 01 ± 0.32^b	80. 97 ± 0.31^a	37.67 ± 0.09^b
Ash	1.83 ± 0.06^a	0.61 ± 0.03^b	1.20 ± 0.01^a	0. 88 ± 0.02^a	1.17 ± 0.06^a	0.59 ± 0.02^a
Crude protein	4.33.± 0.12^b	8.66 0. 18^a	13. 17 ± 0. 04^b	7.70 ±0.06^a	3. 70 ± 0.10^b	5. 63 ± 005^a
Crude fat	9.05 ± 0.04^b	12.58 ± 0.06^a	9.17 ± 0.04^b	14. 37 ± 0.12^a	11. 94 ± 0.03^b	15.23 ± 0.15^a
Carbohydrate	55.63± 0.08^b	59.19 ± 0.05^a	45.75 ± 1.82^b	51. 29 ± 0.07^a	48.28 ± 0.06^b	53.36 ± 0.10^a
Total solid	22. 73 ± 0.038^b	23.57 ± 0.12^a	17. 55 ± 0.08^b	24.30 ± 0.18^a	18.751 ± 0.04^B	24.89 ± 0.29^a
Non- fat solid	13.22 ± 0.03^a	11.00 ± 0.20^b	8.68 ± 0.07^b	10.37 ± 0.06^a	9.67 ± 0.06^a	10.20 ± 0.01^a
L.S.D	0.50					
S.F	****					

B1 = raw milk collected from Bauchi, B2 = Processed nono from Bauchi Jg1 = raw milk from Jigawa, Jg2 = nono from Jigawa, Js1 = raw milk from J, JS2 = nono from Jos. Values are presented as mean ± standard deviation. Values across different column are not significant. Values with different superscript within the same column are significantly different ($p<0.05$); There was a significant difference in the proximate composition of raw and processed milk collected from three states At $P \leq 0.05$.

Table 2: Chemical composition of Nono samples collected from three States in Nigeria

Parameter (mg/l)	B1	B2	Jg1	Jg2	Js1	Js2
Nitrogen	4.33 ± 0. 12^b	4. 68 ± 0.03^a	3. 17 ± 0.04^b	3. 44 ± 0.02^a	3.70 ± 0.01^b	4.01 ± 0.13^a
Calcium	0.63 ± 0.02^b	1.93 ± 0.01^a	0.65 ± 0.02^b	1. 89 ± 0.02^a	0.59 ± 0.01^b	1.42 ± 0.05^a
Magnesium	0.33.± 0.01^a	0.33 ± 0. 01^a	0.28 ± 0. 01^a	0.31 ±0.01^a	0. 31± 0.01^a	0. 31 ± 02^a
Phophurus	2.43 ± 0.01^a	1.54 ± 0.02^a	1.94 ± 0.02^a	0. 75 ± 0.02^b	1. 83 ± 0.02^a	0.85 ± 0.02^b
Potassium	1.21 ± 0.02^a	1.05 ± 0.11^b	1.77 ± 0.08^a	1. 71 ± 0.04^a	0.76 ± 0.01^a	0.68 ± 0.03^a
Sodium	0. 54 ± 0.00^a	0. 48 ± 0.01^a	0. 42 ± 0.01^a	0. 41 ± 0.01^a	0. 51 ± 0.03^a	0.54 ± 0.03^a
Sulphur	0. 24 ± 0.01^a	0.28 ± 0.01^a	0.42 ± 0.01^a	0.40 ± 0.01^a	0.33 ± 0.01^a	0.32 ± 0.01^a
L.S.D	0.06					
S.F	****					

Values with different superscript within the same column are significantly different ($p<0.05$). There was a significant difference at ($P \leq 0.05$) in the elemental composition of the raw and processed samples collected from three different states.

Table 3: Physiochemical properties of raw and processed cow milk collected from the three States in Nigeria

Parameter (%)	B_1	B_2	Jg_1	Jg_2	Js_1	Js_2
Viscosity	180.53 ± 0.47^b	213.38 ± 0.33^a	151.00 ± 1.32^b	365.00 ± 3.61^a	135.67 ± 0. 25^b	432. 50 ± 0.44^a
Ph	6.51 ± 0.02^a	3. 72 ± 0.02^b	5.47 ± 0.01^a	3. 21 ± 0.01^a	6. 85 ± 0.03^a	4. 35 ± 0.02^b
Titratable acidity	0.121 ± 0.001^a	0.903 ± 0.002^a	0.183 ± 0.002^a	0.977 ± 0.002^a	0.095 ± 0.002^a	0.45 ± 005^a
L.S.D	1.50					
S.F	****					

Values with different superscript within the same column are significantly different ($p<0.05$). There was a significant difference (at $P \leq 0.05$) in physiochemical properties of the raw and processed cow milk collected from the three different states.

± 06 and 0.61 ± 0.03 for raw and processed cow milk respectively (Table 2). The decrease in ash content is usually change in certain chemical composition such as carbohydrates, moisture content and fat as reported by Obadina et al., (2013). There was a significant increase in the crude protein showing the lowest level in Js_1 3.70 ± 0.10 and the highest content in Jg_1 13.17 ± 0.04. This supports the work of Obadina et al., (2013).

The difference in the protein content may be due to polyer build-up or microbial cell proliferation. The lowest fat content was recorded in sample B_1 (9.05 ± 0.04) and the highest fat content was recorded in Js_2 (15.23 ± 0.15). Increase in the amount of fat may be attributed to increased activities of lipolytic enzymes during fermentation Increase in carbohydrate content is determined by the actions of fermenting microorganisms which they use in to convert them into energy needed for growth and other metabolic activities as explained by Osundahunsi et al (2007). A decrease in the non- fat solid was observed in samples collected from Bauchi state with values of 13.2 2 ± 0.03 and 11.00 ± 0.20 for raw and processed milk respectively. Protein, lactose and minerals are referred to solid not fat content; hence addition of fat makes it total solid content. The differences in total milk solids may be due to variation in relative synthesis and secretion of milk components from the mammary gland. Sometimes this may be due to differences among species between individuals as well as conditions affecting individuals (Kapadiya et al., 2016). The results of the elemental composition reveal that there was a significant increase $P \leq 0.05$ in the Nitrogen, and calcium between the raw and processed cow milk across all the samples (Table 3). There was no any significant difference in the magnesium, sodium and sulphur

Table 4: The Biochemical identification of Lactic Acid producing Bacteria

Test	**E9Js2**	**E10Js3**	**E4Js1**	**E4Jg1**	**E7Bau3**	**E9Js1**	**E1Bau3**
Cell morphology	Cocci/ovoid	Cocci/round	Rods	Rods	Rods	Rods	Cocci/round
Colony appearance	Creamy	Creamy	Creamy white	Creamy white	Creamy white	Creamy white	Creamy
Gram test	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-	-
CO_2 from glucose	+	-	+	+	+	+	-
Fermentation of carbohydrate							
Glucose	+	-	+	+	+	+	-
Lactose	+	+	+	+	+	+	+
Xylose	±	+	±	±	±	±	+
Sucrose	±	+	+	+	+	+	+
Melibiose	±	-	±	±	±	±	-
Raffinose	+	±	-	-	-	-	±
Sorbitol	-	±	+	+	+	+	±
Growth at (T^o)							
10 ^oC	-	-	-	-	-	-	-
15 ^oC	+	+	+	+	+	+	+
45 ^oC	-	±	±	±	±	±	±
Growth at (%NaCl)							
2%	+	+	-	-	-	-	+
4%	-	+	±	±	±	±	+
6%	-	-	-	-	-	-	-
Probable organisms	Leuconostocs	Pediococcus	Lactobacillus	Lactobacillus	Lactobacillus	Lactobacillus	Pediococcus

+= Positive,-= negative, ±= varies between isolates

Table 5: Identification and carbohydrate fermentation by LAB isolates (API 50 CHL).

Samples	**E9Js2**	**E10Js3**	**E4Js1**	**E4Jg1**	**E7Bau3**	**E1Bau3**	**E9Js1**
Control (No sugar)	-	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-	-
D – arabinose	-	-	-	-	-	-	-
L – arabinose	-	-	-	-	-	+	-
D – ribose	-	-	-	+	-	+	-
D – xylose	-	-	-	-	-	+	-
L – xylose	-	-	-	-	-	-	-
D – adonitol	-	-	-	-	-	-	-
Xyloside	-	-	-	-	-	-	-
D – galactose	+	+	-	+	-	+	-
D – glucose	+	+	+	+	+	+	+
D – fructose	-	+	+	+	+	+	+
D – mannose	-	+	+	-	-	-	+
L – sorbose	-	-	-	-	-	-	-
L – rhamnose	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-
Mannitol	-	-	-	+	-	-	-
Sorbitol	-	-	-	+	-	-	-
D – mannoside	-	-	-	-	-	-	-
D – glucoside	-	-	-	-	-	-	-
Glucosamine	+	+	-	+	+	-	-
Amygdalin	-	-	-	+	-	-	-
Arbutin	-	-	-	+	-	-	-
Esculin	-	+	-	+	-	-	-
Salicin	-	-	-	+	-	-	-

composition between all the samples collected. It was also observed that there was a decreased in the phosphorus composition between the samples and potassium decreased in sample collected. Viscosity, pH and titratable acidity were at variance. There was a significant difference ($P \leq 0.05$) in the viscosity of raw and processed cow milk (Tables 3 and 4). The processed products had the highest viscosity of 213. 38, 365.00 and 432.50 as against the raw milk 180.53, 151.00 and 135.67 for sample collected from Bauchi, Jigawa and Jos respectively. The results of the pH also revealed that there was a significant decreased in the pH of the

Samples	E9Js2	E10Js3	E4Js1	E4Jg1	E7Bau3	E1Bau3	E9Js1
Cellobiose		-	-	+	-	-	-
Maltose	-	-	-	+	-	+	+
Lactose	+	-	+	+	+	+	+
Melibiose	-	-	-	+	-	+	-
Sucrose	-	-	-	+	+	+	-
Trehalose	-	+	-	+	+	-	+
Inulin	-	-	-	-	-	-	-
L – xylose	-	-	-	-	-	-	-
D – adonitol	-	-	-	-	-	-	-
Xyloside	-	-	-	-	-	-	-
Melizitose	-	-	-	+	-	-	-
D - raffinose	-	-	-	-	-	+	-
Starch	-	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-	-
L – rhamnose	-	-	-	-	-	-	-
Gentiobiose	-	-	-	+	-	-	-
D-Turanose	-	-	-	-	-	-	-
L - Lyxosis	-	-	-	-	-	-	-
D -Tagatosis	-	-	-	-	-	-	-
D - Fucose	-	-	-	-	-	-	-
L - Fucose	-	-	-	-	-	-	-
D - Arabitol	-	-	-	-	-	-	-
L - Arabitol Gluconate	-	-	-	-	-	-	-
	-	-	-	+	-	+	-
2 – Gluconate	-	-	-	-	-	-	-
5 – Gluconate	-	-	-	-	-	-	-

+: Positive reaction: Negative reaction; E9Js2 *Leuconostoc mesenteroides* subsp. *Cremoris*, E10Js3 *Pediococcus damnosus*, E4Jg1 *Lactobacillus delbrueckii* subsp. *Bulgaricus,* E4Jg1 *Lactobacillus plantarum*, E7Bau3 *Lactobacillus delbrueckii* subsp. *Lactis*, E1Bau3 *Lactobacillus brevis*, E9Js1 *Lactobacillus delbrueckii* subsp. *delbrueckii*.

processed product. This could be due to the activities of the lactic acid producing bacteria. There was no significant in the titrable acidity of the entire product. This could explain why all milk products are sour. Results of carbohydrate fermentation test using the API 50 CHL kit (Biomereux) comparing 49 unique biochemical tests appropriate for bacilli. Bacteria isolated from the nono samples from the three states were identified as lactobacilli by observing their colony morphology, physiological and biochemical characteristics using API kits (Tables 4 and 5). Phenotypic data including morphological, physiological and biochemical characteristics and the data were collected by probabilistic identification using API web database (Yelnetty et al., 2014). The probable organisms identified were: E9Js2 *Leuconostoc mesenteroides* subsp. *Cremoris*, E10Js3 *Pediococcus damnosus*, E4Jg1 *Lactobacillus delbrueckii* subsp. *Bulgaricus,* E4Jg1 *Lactobacillus plantarum*, E7Bau3 *Lactobacillus delbrueckii* subsp. *Lactis*, E1Bau3 *Lactobacillus brevis*, E9Js1 *Lactobacillus delbrueckii* subsp. *delbrueckii*.

Conclusion

Based on observations, it was determined that lactic acid producing bacteria demonstrate strong potential for the fermentation of milk products. As a result, these bacteria are highly recommended for the production of desirable milk products. Their ability to produce lactic acid makes them particularly well-suited for this purpose, and their inclusion in the fermentation process can significantly enhance the quality and characteristics of the final products. This recommendation is based on their proven effectiveness and positive impact on the overall quality of fermented milk products.

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