http://dx.doi.org/10.22161/ijeab/3.1.34 *ISSN: 2456-1878*

Effect of Temperature on the Shelf life of *Nono* (Locally Fermented Milk) and Yoghurt

Samson Kyakma. S., Attah Sunday

Department of Animal Production, University of Agriculture Makurdi, PMB, 2373, Makurdi, Benue State, Nigeria

Abstract**—** *Effect of temperature on the shelf life ofnono(locally fermented milk) and yoghurt was carried out for 7 days and 3 months respectively. Freshly made nonowas kept under room and refrigerated temperature for 7 days. Chemical parameters such as protein, fats, carbohydrate, moisture and ash were analyzed within one hour of collection and on the 7th day. Some physical parameters such as texture and flavour were measured using visual appraisal just before the preservation and then on daily basis. Freshly made yoghurt was treated alike and kept for the period of 3 months (which is the claimed shelf life of yoghurt by most manufacturers). The physical, chemical parameters and microbial load were also measured at weekly intervals. The result of the physical and chemical parameters explains deterioration before the end of the experiment in both samples. It was also concluded that freshly made yoghurt kept at room temperature be consumed only on the first day of production and fermented milk is advised to be pasteurized before consumption due to the high microbial load.*

Keywords*— *Nono, Locally Fermented Milk, Yoghurt.

I. INTRODUCTION

Yoghurt is made by the control thermoduric fermentation of pasteurized non-fat or low-fat milk, carried around 45°C (Prescott *et al., 2005*).It is probably the most popular fermented milk product in the United states and in Nigeria too. (Prescott *et al.,2005*). It is produced both commercially and by individuals, using yoghurt-making kits. Apart from the microbiological quality of raw milk been of great importance in regard to product and food safety, raw milk should be unadulterated and free from taints, antibiotics, blood and visible segments. Since*nono*and fresh cow milk are produced by illiterate fulani's in village with poor knowledge of shelf life, product safety, sanitation and aseptic milking techniques, handlers of this product may unknowingly introduce pathogenic microorganisms into the product. Since these products do not undergo further processing before being sold for consumption, this food may become potential source of illness to their consumers (Uzeh*et al., 2006*).

Nono is a general name used for locally fermented cow milk and it is widely consumed in many African countries including Nigeria (Uzeh*et al., 2006*). It is an opaque white to milky colour liquid food gotten from fermented raw milk. It is a healthy food which consumption transverses the Sahara tribes of West African sub-region extended to the inhabitant of the Mediterranean region and also the Middle East. In the Middle East it is called "Dahi" or "Lassi" (Nahar*et al.*, 2007). Predominantly, *nono* is prepared and hawked by the nomadic Hausa Fulani cattle herders who control over 80% of Nigeria's cattle production.

Dairy product is essential part of the Nigerian food; therefore attention ought to be paid in to hygienic aspect of handling and distributing of such foods. This study was under taken to ascertain the shelf life of *nono* and yoghurt under room and refrigeratedtemperatures.

II. MATERIALS AND METHODS

The study was carried out at the Microbiology Laboratory of University of Agriculture Veterinary Teaching Hospital Makurdi, Benue state of Nigeria located in the Southern Guinea Savanna zone (Latitude 7°43′N and longitude 8°3′E). The area is warm with a minimum temperature of 24.20ºC and maximum temperature of 36.33ºC. The rain fall is between 508 and 1016mm and the relative humidity is between 39.50±2.20 and 64.00±4.8% (TAC,2009)

Sample Collection/Processing

Fresh milk collected in the evening was poured in to a calabash containing the starter culture and left to ferment over night.The leftover of *nono* made on the previous day served as the starter culture. The locally fermented milk popularly known as *nono*was then stored at room and refrigerated temperatures (4°C) for analyses.

Yoghurt was produced from powdered milk as described by Food Science and Technology Laboratory, University of Agriculture Makurdi using the Food Safety Standards. 1000ml of freshly prepared reconstituted milk was pre-heated to about 45°C and filtered. 15g of sugar was added, mixed and pasteurized at 95°C and held for 30 minutes. It was cooled to an inoculation temperature of 45°C in a closed container placed in a bath of iced water. 2.5g of starter culture *(Lactobacillus bulgarius and Lactobacillus acidophilus)* and 2ml of food flavor was added, mixed and incubated at 45°C for 3hours.

http://dx.doi.org/10.22161/ijeab/3.1.34 *ISSN: 2456-1878*

Experimental Treatment/ Procedures

Table.1: Effect of Storage Time and Temperature on The Microbial load of Nono

TNC= Too numerous to count, Tem=Temperature, REF =Refrigerated, a,b,c means with different subscript on the same row are significantly different (p<0.05),

Days	Room temp.	Ref temp(4°C)
At collection	3.8± 0.46	3.8± 0.46C
Day 1	6.0± 051bc	3.0± 046
Day2	8.8± 0.51a	3.0± 0.46
Day3	TNC	3.1± 046
Day4	TNC	3.0± 0.46
Day5	TNC	3.0± 0.46
Day6	TNC	3.9± 0.46
Day7	8.3± 0.54b	5.2± 0.46a

Nono sample was collected and divided in to two. The first part was stored at room temperature and the 2nd part was kept under refrigerated condition (4°C). The microbial load and the pH at both temperatures were studied for the period of seven (7) days.

Freshly made Yoghurt was equally treated alike, but the parameters were studied on a weekly basis for a period of 3 months.

Microbial load

The determination of microbial load was done using 1ml yoghurt and*nono* samples serially diluted (10^1 to 10^5) in sterile water and 200µl of samples were plated unto nutrient agar plates and incubated at 37°C for 24hrs. The numbers of colonies were counted afterwards using a Standard Counter. Sampling was carried out for daily for *nono* and weekly for yoghurt.

Chemical Parameters

In all the experiments**,** the samples were analyzed for protein, fat, carbohydrate, moisture and ash. The fat was estimated by the Roese-Gottlieb Method following the procedures of Supplee and Bellis (2014), milk protein (N x 6.38) was determined using the semi-micro Kjeldahl and Markhams Distillation Apparatus and the ash content was obtained by drying and ashing a weighed milk sample (10ml) to a constant weight as 550 °C for 48 hours, while the moisture content was determined based on the principle of drying to constant weight has described by Osborne and Voogt (1978). The pH was determined by the use of a pH meter (WPA CD6). Determinations were done at the onset of Treatments and at the end of 30 hours, 7days and 3 month for Experiment 1, 2 and 3, respectively.

Physical Parameters

Sensory qualities of milk were evaluated by a jury of 5 panelists to determine the texture and flavour at 6 hourly intervals following the procedure of Meilgaard*et al.* (1999). The panelists tasted the samples and were asked to keep the milk products in their mouth for 12 seconds before scoring. The milk product samples were presented in random order. Water was used for rinsing mouth between samples (International Dairy Federation, 2002).

Statistical Analysis

Microsoft Excel spread sheet (2006) was employed for raw data entry. Transformation of microbial count was done using average dilution x 1/dilution factor x $^1/_{0.1}$ before the analysis, the data obtained were subjected to statistical analysis. Means that were significantly different were separated using least significant difference (LSD) as contained in SPSS (2010) for Windows (version 16). For all analysis, 95 % CF (confident factor) and P (probability)-value<0.05 was set for statistical significance of an estimate.

Table.2: Effect of Storage Time and Temperature on The pH readings of Nono

Days	Room temp.	Ref. temp.(4°C)
At collection	5.0± 0.46	3.8± 0.46C
Day 1	4.13± 051bc	3.0± 046
Day2	3.80± 0.51a	3.0± 0.46
Day3	3.73	3.1± 046
Day4	3.03	3.0± 0.46
Day5	2.43	3.9± 0.46
Day6	1.73	5.2± 0.46
Day7	1.13	

a,b,c means with different subscript on the same row are significantly different (p<0.05), TEMP= Temperature, Ref=Refrigerated

~~TABLE 3: Effect of Storage Time and Temperature on The Proximate Composition Of Nono~~

~~PARAMETERS~~	~~DAYS~~	
	~~1~~	~~7~~
~~Crude protein~~	~~2.82~~	~~2.64~~
~~Moisture~~	~~80.79~~	~~79.14~~
~~Carbohydrate~~	~~3.12~~	~~1.17~~
~~Fat~~	~~0.80~~	~~0.70~~
~~Ash~~	~~0.31~~	~~0.03~~

http://dx.doi.org/10.22161/ijeab/3.1.34 *ISSN: 2456-1878*

~~TABLE 4: Effect of Storage Time and Temperature on Flavour Quality and Score Of *Nono*~~

~~Days of Storage~~	~~FLAVOUR QUALITY~~		~~FLAVOUR SCORE~~	
	~~Room .TEMP~~	~~REF. TEMP~~	~~Room .TEMP~~	~~REF .TEMP~~
~~1~~	~~PL~~	~~PL~~	~~100.00~~	~~100.0~~
~~2~~	~~SR~~	~~PL~~	~~68.0~~	~~100.0~~
~~3~~	~~SR~~	~~PL~~	~~52.0~~	~~100.0~~
~~4~~	~~OF~~	~~PL~~	~~28.0~~	~~100.0~~
~~5~~	~~OF~~	~~PL~~	~~20.0~~	~~100.0~~
~~6~~	~~OF~~	~~SR~~	~~20.0~~	~~64.0~~
~~7~~	~~OF~~	~~SR~~	~~20.0~~	~~60.0~~
~~Mean SD~~	~~-~~	~~-~~	~~24.0 ±73.3~~	~~18.5 ± 93~~

PL=Pleasing, SR= Sour, OF=Off flavour, TEMP= Temperature, REF=Refrigerated

Table.5: Effect of Storage Time and Temperature on The Proximate Composition of Yoghurt on the 1st and 90 Days (3months)

Parameters	1ST day	3 months(Refrigerated4°C)
Crude Protein	3.49	1.21
Moisture	90.49	84.61
Carbohydrate	3.45	2.80
Fat	2.31	1.86
Ash	0.45	0.10

Table.6: Effect of Storage Time and Temperature on The pH Readings of Yoghurt

Week	Room temp.	Ref. temp.(4°C)
At collection	4.6± 0.05	4.6± 0.05
Week 1	3.1± 0.01[a]	4.5± 0.05[a]
Week 2	2.96± 0.05[b]	4.53± 0.05[a]
Week 3	2.70±0.17[b]	4.13± 4.11[a]
Week 4	2.43±0.01	4.13± 4.11[a]
Week 5	2.20±0.17[b]	3.96± 0.05[a]
Week 6	1.96±0.11[c]	3.83± 0.11[a]
Week 7	1.50±0.06	3.43±0.11[a]
Week8	1.20±0.00[b]	3.20±0.00[a]
Week9	1.40±0.17[b]	3.06±0.01[a]
Week10	1.23±0.25[b]	2.83±0.05[a]
Week11	1.00±0.00[b]	2.70±0.17
Week12	1.00±0.00	2.56±0.11[a]

Table.7: Effect of Storage Time and Temperature on The Microbial load of Yoghurt (103 Cfu/ml)

Week	**Room temperature**	**Refrigerated temperature(4°C)**	**Week**
At collection	TS	TS	At collection
Week 1	3.7 ±0.006a	1.8 ± 0.007c	Week 1
Week 2	TNC	1.8 ±0.001 c	Week 2

Week 3	TNC	1.8 ± 0.002c	Week 3
Week 4	TNC	2.7 ± 0.004bc	Week 4
Week 5	TNC	2.3 ± 0.005 b	Week 5
Week 6	TNC	2.3 ± 0.001 b	Week 6
Week 7	3.7 ± 0.006a	2.3 ± 0.001 b	Week 7
Week 8	2.9 ± 0.004b	2.3 ± 0.001 b	Week 8
Week 9	1.7 ± 0.005b	2.3 ± 0.003 b	Week 9
Week 10	TS	3.0 ± 0.003 b	Week 10
Week 11	No growth	3.0 ± 0.003b	Week 11
Week 12	No growth	3.6 ± 0.003a	Week 12

TNC= Too numerous to count, TS= Too scanty, Tem=Temperature, REF =Refrigerated, a,b,c means with different subscript on the same row are significantly different ($p<0.05$),

Table.8: Effect of Storage Time and Temperature on Flavour Quality and Score control of Yoghurt

	Flavour Quality		*Flavour Score (%)*	
week 0	PL	PL	100.00	100.00
week 1	SS	PL	100.00	100.00
week 2	BT	PL	60.00	100.00
week 3	OF	PL	44.00	100.00
week 4	OF	PL	20.00	100.00
week 5	OF	PL	20.00	92.00
week 6	OF	PL	20.00	92.00
week 7	OF	PL	20.00	92.00
week 8	OF	PL	20.00	92.00
week 9	OF	PL	20.00	92.00
week 10	OF	PL	20.00	80.00
week 11	OF	PL	20.00	80.00
week 12	OF	PL	20.00	76.00

III. RESULTS AND DISCUSSION

The microbial load of fermented milk (*nono*) ranged between $3.8x10^3$ - $5.2x10^3$. The microbial load of *nono* on the first day of production was 3.8 $x10^3$and did not agree with the findings obtained by Savadogo*et al*.,2004. The reason for the contradiction could be linked to the different fermentation process practiced by many local producer of same product as documented by EL-Bakri and EL-Zubeir (2009). The microbial load multiplied rapidly at room temperature compared to refrigerated temperature (4°C). It stands to reason that storage of products at such temperature could increase its shelf life. The microbial load of yoghurt kept at room temperature multiplied rapidly to its peak and started declining until there was no visible colonies seen in the culture. This explains the four stages of bacterial growth. The lag phase which is delay in growth following inoculation of bacteria in to new medium during which time bacteria adapt to its medium. The log phase which is when they adapt to its medium and they reproduced rapidly, it is said here that the cells are in its highest activity during this phase. It is in this phase that the bacteria dominate the growth medium, deplete available nutrients and toxic waste accumulation slowing the state of production. The third phase is the stationary phase were the state of equilibrium is reached between the death of old cells and formation of new cells resulting in non-change in cell number. Afterwards the formation of new cells cease and the existing cells gradually die off. This is called the death phase.The microbial load in yoghurt kept under refrigeration (4°C) maintained the same number of microbial count from week 1 to week 3 as 1.8 x 10^3CFU/ml but increased in the 4^{th} week to 2.7 x 10^3. This might be due to the failure in power supply which leads to the rise in temperature giving room to microorganism to multiply. It is a well-known fact that microorganism multiply rapidly under normal temperature than at lower temperatures. There was a slight decrease to 2.3 x 10^3 on the 6^{th} and 7^{th} week this might possibly be as a result of the growth of psychotropic bacteria which grows in lower temperatures even below 5°C.

The pH of *nono* was 5.0 on the first day of production falls within the range of pH reported by EL-Bakri and EL-Zubeir (2009) but contradicts the 5.51- 6.29 (Adesokan*et al., (*2011), and 5.7 (Obi and Ikenebomeh, 2007). The

difference in pH could be as a result of some factors including the length of fermentation and the starter culture used. If the keeping time of *nono* is increased prior to the consumption, the acidity determines the number and kind of contaminating organism. It is assumed that at lower pH pathogenic organisms should be destroyed making *nono* safe for consumption. Jawetz et al.(1995) noted the presence of *S. cerevisiae* which is a pathogenic organism present in *nono* even at the pH below 5.47 and suggested that *nono* should be pasteurized before consumption.

Interestingly, the slight acidic nature of *nono* is of good medicinal value to human health as the implantation of the lactic acid in the intestines reportedly replaces the putrefying micro-flora there in whose metabolites have been considered to be responsible for various ailments may lead to premature death.

The difference in pH could be as a result of some factors including the length of fermentation and the starter culture used.

Proximate composition of *nono* at 7 days maintained under refrigeration showed a reduction in all the compositions as compared with the composition on the first day of production this could mean that microorganisms were present and active below 4°C. There was much decrease in all the nutrients of yoghurt samples stored under refrigeration (4°C).Most yoghurt producers claim 3 months as the shelf life of Yoghurt stored at less than 5°C, this seems not to be true from this study because of the changes in the nutritional values at this temperature when stored for this long.

Theflavour quality control as shown below showed *nono* to be pleasing on the first day of production and sour subsequent days if stored under room temperature. This explains the activity of microorganism (lactic acid bacteria) which turns it sour. *Nono*preserved under refrigeration was pleasing for 5 days and got sour on the 6th and 7th day. This may probably be the activities of psychrophiles that grows below 5°C or a fluctuation in the temperature due to failure in power supply that encourage the growth of microorganism which leads to souring of the product.

The flavor score maintained 100% up to 5 days under refrigeration and decreased due to deterioration. The sample stored under room temperature showed a rapid decrease in the score from day 1 due to the effect of microorganism that probably caused the deterioration.

The microbial load of yoghurt kept at room temperature multiplied rapidly to its peak and started declining until there was no visible colonies seen in the culture. This explains the four stages of bacterial growth. The lag phase which is delay in growth following inoculation of bacteria in to new medium during which time bacteria adapt to its medium. The log phase which is when they adapt to its medium and they reproduced rapidly, it is said here that the cells are in its highest activity during this phase. It is in this phase that the bacteria dominate the growth medium, deplete available nutrients and toxic waste accumulation slowing the state of production. The third phase is the stationary phase were the state of equilibrium is reached between the death of old cells and formation of new cells resulting in non-change in cell number. Afterwards the formation of new cells cease and the existing cells gradually die off. This is called the death phase.

The microbial load in yoghurt kept under refrigeration (4°C) maintained the same number of microbial count from week 1 to week 3 as 1.8 x 103CFU/ml but increased in the 4th week to 2.7 x 103. This might be due to the failure in power supply which leads to the rise in temperature giving room to microorganism to multiply. It is a well-known fact that microorganism multiply rapidly under normal temperature than at lower temperatures. There was a slight decrease to 2.3 x 103 on the 6th and 7th week this might possibly be as a result of the growth of psychotropic bacteria which grows in lower temperatures even below 5°C. Rodríguez-Alcalá*et al*., (2009) suggested that longer refrigeration time allows increased growth of psychotropic microorganisms and concomitant production of heat-stable enzymes, especially proteinases and lipases. The result of this present study contradicts the findings of Rodríguez-Alcalá*et al*., 2009 which states that Cooling to a temperature of 4ºC makes the bacteria inactive and prevents them to grow and produce the lactic acid.

Li and Li (1998) suggested that the tolerable limit of microbial load of yoghurt should be equal or less than $1.0x10^5$cfu/ml comparing this to the present studies, it is advisable to take refrigerated yoghurt from the very first day of production to the seventh day of production while it is suggested to take yoghurt kept under room temperature only on the first day of production. Taking it later than this may be detrimental to human health.

The pH of the yoghurt kept under room temperature dropped quickly compared to that kept under refrigeration. The pH of the yoghurt kept under refrigeration dropped and this agrees with the findings of Stamer (1976) who stated that Lactic bacteria are Mesophilic and can grow below 5^0C and some as high as 45^0C, with respect to growth pH some can grow as low as 3.2 some as high as 9.6 and most grow in the pH range 4.0-4.5. However, Henning(1999) also stated that the Coccus of produce 0.05% lactic acid and the rod about 0.6-0.8% (pH of 4.2-4.8). If incubation extend, the pH can decrease to about 3.5 with lactic acid increasing to about 2%.

The pH range 1.0-4.7 in the current study was lower than those reported by Omola*et al*, (2014). The minimum acceptable standard for pH is 4.4 in yoghurt (FAO, 1979)

therefore, the pH of the yoghurt kept under refrigeration and room temperature met the requirement of FAO,(1997) at 2 weeks and less than one week respectively. The pH of yoghurt decreased during the manufacturing process from the time it was inoculated with bacterial culture to the time it was manufactured from 6.7 – 4.7 this is because the lactic strains have the ability to ferment lactose to lactic acid which increases acidity and decrease pH. This result is comparable to the findings of Sokolinska et al, 2004.

The present study correlated pH directly with the number of colony forming unit which is in agreement with the findings of Micheal *et al.*(2013) who also observed a direct correlation of pH with number of bacterial cell.

Theproximate composition of yoghurt on the 1st day of production showed 3.49 , 90.49 , 3.45 , 2.31, and 0.45 for crude protein, moisture carbohydrate, fat, and ash respectively. At refrigerated temperature of 4°C , the values were 1.21, 84.61, 2.80, 1.86, and 0.10 of crude protein, moisture, carbohydrate, fat, and ash respectively was recorded. Microorganisms use the carbon present in the carbohydrate to produce energy for their survival. Lactic acid bacteria has the capacity to break down complex carbohydrate for their own use, this explains the decrease in the carbohydrate noted in this study at both temperatures. There was much decrease in all the nutrients of samples stored under refrigeration (4°C). Most yoghurt producers claim 3 months as the shelf life of Yoghurt stored at less than 5°C, this seems not to be true from this study because of the changes in the nutritional values at this temperature when stored for this long.

IV. CONCLUSION AND RECOMENDATIONS

From this study, it was concluded that *nono* prepared locally might not be safe for consumption due to the high microbial load however, it is advice that *nono* be pasteurized before consumption to reduce the microbial load.

The result obtained from this study suggests that refrigerated yoghurt is safe for consumption within seven days after production in contrast to one day only for yoghurt kept under room temperature. Taking it later than this may be detrimental to human health.

pH is also used for measuring spoilage in milk because of the correlation observed between the pH and the number of coliform forming unit(CFU).

pH should be considered for commercial and individual use for spoilage detection because of its low cost and ease of use.

REFERENCES

[1] Adesokan, I.A., B.B. Odetoyinbo, Y.A. Ekanola, R.E. Avanrenren and S. Fakorede, (2011). Production of Nigerian nono using lactic starter cultures. Pak. J. Nutr., 10:203-207

[2] El Bakri, J.m and I.E.M. Elzubeir, (2009). Microbiological evaluation of plain and Fruit and Fruit Yoghurt in Khartum State, Sudan. Int.J. Dairy Sci; 4: 1-7

[3] Henning,D.R Personal communication. Tetra Pak.(1999). Dairy Processing handbook..

[4] International Dairy Federation (IDF,2002). Fermented milk: Proceedings of the IDF Isomers content of cow, ewe and goat milks processed by high pressure homogenization. Innov.

[5] Li, G and Lu.J.A. (1998). Streptococcus uberis: a permanent barrier to the control of bovine mastitis.*Vetrinary Journal 157:225-238*

[6] Nahar, A., M. Al-Amin, S.M.K. Alam, A. Wadud and M.N. Islam, (2007). A comparative study on the quality of dahi (Yoghurt) prepared from cow, goat and buffalo milk. Int.J.Dairy.Sci.,2:260-267.CrossRef | DirectLink |

[7] PrescottM.L.,P.J. Harley and A.K. Donald, (2005). Microbiology, Mc graw hill, Newyork, ,6,938-952

a. *rambelliis*p. nov. Syst. Appl. Microbiol., 28(2005), 442–453.

[8] *Rodzegues-Alcala L.M,. Harte, H. and Fortecha, J, L(2009)Fatty acid profile and CLA plasma lipid profile in young healthy women. Anals of Nutrition metabolism 50(4): 387 -393*

[9] Sokolinska, D.C Michalski, M.M, and Pikul,J (2004) Role of the proportion of youghurt bacteria strains in milk souring and the formation of curd qualitative characteristics. Bulletin of vetinary institute pulawy 48;437-441

[10] Stamer, J.R (1976) Lactic acid Bacteria in food microbiology; public health and spoilage aspect Journal of Diary Science Res., 64 (1997), 261-270

[11] Uzeh E.R, E.R. Ohenhen and K.A. Rojugbokan, (2006). Nature and science, ,4(3),37-40