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Production of Nigerian Nono Using Lactic Starter Cultures

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Abstract: The effect of Lactic Acid Bacteria (LAB) on nutritional quality, acceptability and shelf life of nono was investigated. The lactic starter cultures were selected based on their ability to produce diacetyl. *Lactobacillus casei*N18 produced the highest quantity (1.65 g/ml) of diacetyl while *Lactobacillus brevis*N15 produced the lowest amount (0.9 g/ml). During 24 h fermentation a general decrease in pH was observed with a corresponding increase in Titratable Acidity (TA). The pH ranged between 5.51 and 6.29; while the TA ranged between 19 and 21%. The mixed starter culture comprises of *L. casei*N18 and *L. plantarum*N07 produced the highest quantity of diacetyl (2.40 g/ml) while the lowest quantity of diacetyl (2.00 g/ml) was recorded for the sample produced with spontaneous fermentation. Nono fermented with mixed culture of *L. casei*N18 and *L. plantarum*N07 had the highest protein content (69.98%) while nono produced with spontaneous fermentation (control) had the lowest (59.20%). Nono fermented with *L. casei*N18 and *L. plantarum*N07 was rated best with overall acceptability of 7 while the control had overall acceptability of 4. The Nono sample stored in refrigerator had a shelf-life of 6 days while the sample stored at room temperature (28°C±2°C) had a shelf life of 3 days.

Key words: Lactic acid bacteria, nono, fermented foods, protein content

INTRODUCTION

Nono is Nigerian locally fermented milk product commonly prepared by Hausa/ Fulani cattle rearers. It is mostly available in the northern part of Nigeria (Yahuza, 2001; Bankole, 1990). Nono is rich in protein, essential amino acids, phosphorous and vitamins (Nebedum and Obiakor, 2007). Lactic acid bacteria are mostly associated with the production of fermented milk products. They play key role in producing desirable flavour, aroma and good physical appearance in fermented milk products (Tannock, 2004). Although, lactic acid is the principal product of the fermentation but lesser amount of flavouring substance diacetyl is also being produced (Reyee *et al*., 2003).

Diacetyl is a major flavour compound in many cultured diary products. This compound is synthesized primarily by *Leuconostocs, Lactobacilli* and some *Lactococci* that can metabolize citrate (Boumerdassi *et al*., 1997; Cogan *et al*., 1981). Diacetyl is an essential property that contributes to the organoleptic quality of foods. It is also an attribute of diary starter culture which confers desirable flavour characteristics in certain products (Marshall, 1987). Jay and River (1986) documented the antimicrobial property of diacetyl.

In this study the quantity of diacetyl produced by LAB isolated from purchased nono samples was determined and selected LAB with highest quantity of diacetyl was used as starter culture for the production of nono. Nutritional quality and the shelf life of nono produced were determined.

MATERIALS AND METHODS

Sample collection: Fresh milk samples were collected from a local dairy market at Bodija, Ibadan, Nigeria and Nono samples were bought from local producers at Mokola, Ibadan and transported to the laboratory for immediate analysis.

Bacteria strains and cultures: Lactic acid bacteria strains were isolated from the nono samples and characterized using API50CH strips and API50CHLmedium (API System, Biomerieux Sa, France).

Determination of diacetyl formation by the LAB isolates: This was carried out by transferring 25 ml of the separate fluid of the test organisms into conical flasks and 7.5 ml of hydroxylamine solution was used for the residual titration. These flasks were titrated with 0.1 M HCl to a greenish yellow end point using bromophenol blue as indicator. The equivalence factor of HCl to diacetyl is 21.5 mg (AOAC, 1990). The LAB isolates with highest quantity of diacetyl were selected as the starter cultures for nono production.

Nono preparation: Fresh milk sample was pasteurized at 72°C for 20 sec and was allowed to cool down and was divided into four portions. The milk samples were inoculated with selected lactic starter cultures (1%) singly or in combination. The uninoculated sample served as control and the milk samples were fermented at 30°C for 24 h.

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Physico-chemical analysis: The pH of the fermenting milk samples was taken at four hours intervals during the 24 h fermentation using a pH meter (Crison MicropH). The Titratable Acidity (TTA) was determined at 4 h intervals during 24 h fermentation by titrating 20 ml of decanted homogenate against 0.1 M NaOH to a pH of 6.8 using phenolphthalein as an indicator. The titre volume of each homogenate was multiplied by 0.09 to give % TTA as lactic acid. Diacetyl production of the fermenting milk samples was determined at 4 h interval during 24 h of fermentation as described above.

Sensory evaluation: Samples of nono produced were assessed for organoleptic qualities by five regular consumers of nono for colour, odour, taste, viscosity and overall acceptability using a 9 point Hedonic scale where 9 = like extremely and 1 = dislike extremely.

Proximate analysis: Determination of fibre, ash, crude protein, dry matter, moisture content and crude fat were carried out on the nono samples using standard procedures as described by AOAC (1990).

Determination of shelf-life: Some of the nono samples were stored at room temperature (28±2°C) while others were stored at refrigerated temperature (4°C). Samples were taken every 24 h for microbiological analysis to determine the microbial load of the nono samples stored at both room and refrigerated temperature.

RESULTS

A total of twenty LAB were isolated from traditionally fermented nono purchased from local producers. They were identified to be *Lactobacillus brevis, Lactobacillus casei, L. plantarum, L. bulgaricus* and *L. fermentum*. The quantity of diacetyl produced by the LAB isolates was investigated. *L. casei*N18 had the highest quantity of 1.65 g/ml after 72 h of fermentation. This was followed by *L. plantarum*N07 that had 1.45 g/ml while *L. brevis*N15 had the least value of 0.9 g/ml (Table 1).

Changes in the pH during the fermentation of milk for nono production is shown in Table 2. The pH of the samples decreased throughout the fermentation period. At the end of fermentation, milk fermented with mixed starter culture of *L. casei*N18 and *L. plantarumN07* had the lowest value of pH 5.51 while the highest value (pH 6.29) was observed for milk that was fermented spontaneously (control). However, increase in Titratable Acidity (TTA) was noted throughout the fermentation period. The TTA value ranged between 21% and 19% at the end of 24 h fermentation (Table 3).

Production of diacetyl when milk was fermented with lactic starters for nono production was determined (Table 4). As fermentation progressed, the quantity of diacetyl increased. The sample produced with the mixed lactic starters contained the highest amount of diacetyl (2.40 g/ml) at the end of 24 h fermentation while the sample produced with spontaneous fermentation had the lowest amount (2.00 mg/ml) within the same period of time.

Table 5 showed the proximate analysis of nono samples produced with different starter cultures. The control sample had the highest moisture content (87.11%) while milk fermented with *L. plantarum*N07 had the least moisture content (80.78%). Nono fermented with mixed culture of *L. casei*N18 and

Table 1: Quantities of diacetyl produced by the LAB isolated from nono-a Nigerian fermented milk product

	Diacetyl production (g/ml)					
LAB isolates	12	24	36	48	60	72
Lactobacillus brevis N01	0.60±0.01*	0.70±0.02	0.80±0.02	0.81±0.01	0.90±0.00	0.93±0.00
Lactobacillus brevis N02	0.50±0.00	0.60±0.01	0.70±0.01	0.79±0.01	0.85±0.04	0.90±0.03
*Lactobacillus bulgaricus*N03	0.70±0.02	0.80±0.03	0.91±0.00	0.49±0.02	1.10±0.03	1.30±0.05
*Lactobacillus brevis*N04	0.60±0.01	0.63±0.02	0.67±0.03	0.70±0.00	0.80±0.02	0.91±0.05
*Lactobacillus brevis*N05	0.50±0.02	0.60±0.04	0.70±0.02	0.80±0.01	0.90±0.02	0.95±0.04
*Lactobacillus plantarum*N06	0.95±0.01	1.00±0.03	1.10±0.01	1.20±0.03	1.30±0.03	1.40±0.00
*Lactobacillus plantarum*N07	1.00±0.00	1.10±0.01	1.20±0.04	1.30±0.02	1.37±0.00	1.45±0.03
*Lactobacillus brevis*N08	0.55±0.03	0.62±0.02	0.67±0.01	0.70±0.04	0.80±0.00	0.91±0.02
*Lactobacillus brevis*N09	0.63±0.02	0.67±0.03	0.70±0.00	0.80±0.04	0.90±0.02	0.95±0.03
*Lactobacillus brevis*N10	0.60±0.01	0.64±0.02	0.69±0.04	0.76±0.01	0.80±0.01	0.90±0.03
*Lactobacillus brevis*N11	0.60±0.02	0.67±0.02	0.70±0.01	0.75±0.01	0.80±0.05	0.93±0.04
*Lactobacillus brevis*N12	0.50±0.03	0.60±0.03	0.65±0.02	0.69±0.02	0.78±0.04	0.90±0.02
*Lactobacillus brevis*N13	0.60±0.02	0.69±0.00	0.74±0.02	0.79±0.00	0.84±0.02	0.93±0.02
*Lactobacillus brevis*N14	0.55±0.01	0.60±0.01	0.69±0.02	0.83±0.04	0.87±0.00	0.91±0.04
*Lactobacillus brevis*N15	0.50±0.01	0.60±0.03	0.71±0.00	0.82±0.04	0.85±0.03	0.90±0.02
*Lactobacillus planetarium*N16	0.90±0.03	1.01±0.01	1.11±0.01	1.22±0.04	1.34±0.05	1.41±0.00
*Lactobacillus planetarium*N17	0.90±0.02	1.00±0.00	1.11±0.02	1.21±0.02	1.35±0.02	1.40±0.02
*Lactobacillus casei*N18	1.52±0.01	1.60±0.02	1.61±0.02	1.62±0.04	1.64±0.03	1.65±0.04
*Lactobacillus fermentum*N19	0.80±0.02	0.91±0.00	0.95±0.03	0.97±0.02	0.98±0.03	1.00±0.01
*Lactobacillus brevis*N20	0.60±0.01	0.70±0.02	0.80±0.02	0.89±0.02	0.92±0.00	0.95±0.02

*Values are means (n = 3) ±standard variation

Table 2: Changes in pH during fermentation of milk for nono production in Nigeria

	Fermentation period (Hours)						
Samples	0	4	8	12	16	20	24
A*	7.93±0.00^{aa**}	7.91±0.02^{ab}	7.85±0.03^{ac}	7.48±0.02^{ad}	6.43±0.02^{ae}	6.40±0.01^{af}	6.29±0.03^{ag}
B	7.02±0.01^{ba}	6.30±0.01^{bb}	6.22±0.04^{bc}	6.17±0.01^{bd}	5.89±0.02^{be}	5.66±0.00^{bf}	5.58±0.01^{bg}
C	7.40±0.00^{ca}	7.37±0.00^{cb}	6.23±0.04^{cc}	6.18±0.03^{cd}	6.00±0.01^{ce}	5.76±0.01^{cf}	5.57±0.03^{cg}
D	7.79±0.01^{da}	7.35±0.02^{db}	7.24±0.03^{dc}	6.22±0.00^{dd}	6.06±0.02^{de}	5.75±0.04^{df}	5.51±0.02^{dg}

*Sample codes: A = Nono produced with spontaneous fermentation; B = Nono produced with *Lactobacillus plantarum* N07; C = Nono produced with *Lactobacillus casei* N18; D = Nono produced with *L. plantarum* N07 and *L. casei*N18. **Values are means (n = 3) ±Standard Variation. Means followed by different superscripts are significantly different ($p \leq 0.05$) along rows and columns according to Duncan multiple range test

Table 3: Total titratable acidity during fermentation of milk for nono production in Nigeria

	Fermentation period (Hours)						
Samples	0	4	8	12	16	20	24
A*	0.40±0.03^{aa**}	10.00±0.01^{ab}	10.00±0.03^{ac}	16.00±0.01^{ad}	17.00±0.02^{ae}	18.00±0.04^{af}	19.00±0.03^{ag}
B	10.00±0.02^{ba}	10.00±0.00^{bb}	15.00±0.05^{bc}	15.59±0.04^{bd}	17.00±0.03^{be}	19.90±0.02^{bf}	20.00±0.02^{bg}
C	06.00±0.01^{ca}	15.00±0.02^{cb}	16.00±0.00^{cc}	16.00±0.02^{cd}	17.00±0.02^{ce}	19.00±0.03^{cf}	20.00±0.03^{cg}
D	15.00±0.03^{da}	16.00±0.02^{db}	17.00±0.03^{dc}	17.60±0.04^{dd}	18.00±0.03^{de}	19.10±0.00^{df}	21.00±0.04^{dg}

*Sample code is as stated in Table 2. **Values are means (n = 3) ±Standard Variation. Means followed by different superscripts are significantly different ($p \leq 0.05$) along rows and columns according to Duncan multiple range test

Table 4: Diacetyl production (g/ml) during fermentation of milk for nono production

	Fermentation period (Hours)						
Sample	0	4	8	12	16	20	24
A*	1.00±0.00^{aa**}	1.20±0.02^{ab}	1.40±0.00^{ac}	1.60±0.01^{ad}	1.70±0.03^{ae}	1.90±0.01^{af}	2.00±0.01^{ag}
B	1.30±0.01^{ba}	1.65±0.00^{bb}	1.68±0.03^{bc}	1.80±0.00^{bd}	1.85±0.04^{be}	1.90±0.00^{bf}	2.30±0.02^{bg}
C	1.15±0.02^{ca}	1.50±0.03^{cb}	1.60±0.01^{cc}	1.80±0.01^{cd}	1.90±0.01^{ce}	2.00±0.04^{cf}	2.20±0.03^{cg}
D	1.05±0.04^{da}	1.50±0.00^{db}	1.60±0.01^{dc}	1.70±0.00^{dd}	1.80±0.03^{de}	2.20±0.03^{df}	2.40±0.00^{dg}

*Sample code is as stated in Table 2. **Values are means (n = 3) ±Standard Variation. Means followed by different superscripts are significantly different ($p \leq 0.05$) along rows and columns according to Duncan multiple range test

Table 5: Proximate analysis of nono samples produced with different LAB

Sample	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)
A*	87.11±0.04^{aa**}	59.20±0.01^{ab}	11.53±0.01^{ac}	8.26±0.02^{ad}	Nill
B	80.78±0.03^{ba}	61.60±0.03^{bb}	15.26±0.03^{bc}	7.00±0.02^{bd}	Nill
C	83.74±0.02^{ca}	60.09±0.02^{cb}	14.50±0.02^{cc}	6.01±0.03^{cd}	Nill
D	85.92±0.03^{da}	69.98±0.04^{db}	16.54±0.01^{dc}	7.96±0.01^{dd}	Nill

*Sample code is as stated in Table 2. **Values are means (n = 3) ±Standard Variation. Means followed by different superscripts are significantly different ($p \leq 0.05$) along rows and columns according to Duncan multiple range test

Table 6: Sensory evaluation of nono samples produced with different lactic acid bacteria

Sample	Appearance	Taste	Texture	Colour	Aroma	Overall acceptability
A*	4.21±0.01^{aa**}	3.32±0.00^{ab}	4.22±0.04^{ac}	5.05±0.01^{ad}	5.01±0.02^{ae}	4.04±0.02^{af}
B	7.25±0.04^{ba}	5.13±0.01^{bb}	5.20±0.02^{bc}	8.41±0.05^{bd}	6.02±0.01^{be}	5.22±0.04^{bf}
C	6.30±0.02^{ca}	4.15±0.02^{cb}	5.22±0.04^{cc}	7.22±0.00^{cd}	5.03±0.02^{ce}	6.02±0.01^{cf}
D	8.14±0.03^{da}	6.23±0.02^{db}	6.41±0.01^{dc}	9.12±0.01^{dd}	7.11±0.00^{de}	7.01±0.02^{df}

*Sample code is as stated in Table 2. **Values are means (n = 3) ±Standard Variation. Means followed by different superscripts are significantly different ($p \leq 0.05$) along rows and columns according to Duncan multiple range test

*L. plantarum*N07 had the highest protein content (69.98%); the least protein content was detected in the control sample (59.20%). Nono produced with mixed starter culture had the highest fat content (16.54%) whereas the control sample had the lowest fat content (11.53%). The sensory evaluation of nono samples produced is shown in Table 6. Nono fermented with *L. casei*N18 and *L. plantarum*N07 was rated best with overall acceptability of 7 while the control sample had the overall acceptability of 4. The shelf life of nono samples produced indicated that nono stored at refrigerated temperature (4°C) had a longer shelf life of 6 days compared to nono stored at room temperature which had a shelf life of 3 day.

Table 7: Microbial load (Log10 cfu/g) of nono samples stored at room and refrigerated temperatures

	Days															
	1		2		3		4		5		6		7		8	
Temp.	4°C	28°C	4°C	28°C	4°C	28°C	4°C	28°C	4°C	28°C	4°C	28°C	4°C	28°C	4°C	28°C
A*	ND	ND	ND	ND	ND	7.48±0.01^{aa}	ND	#	ND	#	7.42±0.03^{ab}	#	7.53±0.04^{ac}	#	7.43±0.01^{ad}	#
B	ND	ND	ND	ND	ND	7.31±0.02^{ba}	ND	#	ND	#	7.08±0.02^{bb}	#	7.18±0.02^{bc}	#	7.32±0.02^{bd}	#
C	ND	ND	ND	ND	ND	7.32±0.02^{ca}	ND	#	ND	#	6.92±0.00^{cb}	#	7.05±0.01^{cc}	#	7.21±0.00^{cd}	#
D	ND	ND	ND	ND	ND	7.16±0.00^{da}	ND	#	ND	#	6.89±0.02^{db}	#	7.00±0.02^{dc}	#	7.42±0.02^{dd}	#

*Sample code is as stated in Table 2. # = Not determined, ND = Not detected. **Values are means (n = 3) ±Standard Variation. Means followed by different superscripts are significantly different ($p \leq 0.05$) along rows and columns according to Duncan multiple range test. Temp. = Temperature

DISCUSSION

In this study LAB were isolated from traditionally fermented nono samples purchased from local producers. They were identified as belonging to the genus *Lactobacillus*. These include *Lactobacillus brevis L. casei, L. fermentum* and *L plantarum*. Members of *Lactobacillus* species can be detected in a variety of habitat including fermented foods and diary products (Tannock, 2004; Savadogo *et al*., 2004; Ogunbanwo *et al*., 2004; Nebedum and Obiakor, 2007; Adesokan *et al*., 2009).

The isolated LAB produced diacetyl which increase from 0-72 h of fermentation. *L. casei* produced the highest quantity of diacetyl followed by *L. plantarum*N07. Diacetyl production is a common property among the *Lactococci, Lactobacilli* and *Leuconostoc* (Cogan, 1980; Marshall 1987). Boumerdassi *et al*. (1997) reported that citrate is the principal precursor of diacetyl in fermented dairy products. Jay and River (1986) reported that diacetyl has antimicrobial properties. Diacetyl could act in synergy with other antimicrobial agents and contribute to combined preservation systems in fermented foods (Earnshaw *et al*., 1988). It also brings about desirable flavour in dairy products.

The result also further shows that the pH of all the nono samples decreased as the fermentation progressed while the titratable acidity increased. The pH dropped from 7.93-5.51 and this agreed with the work of Oyewole (1990) who stated that the acidity of fermenting milk is normally only noticeable when the pH falls to about 5.5. This showed that the LAB inoculated into the pasteurized milk were able to carry out acid fermentation which resulted in the production of lactic acid. Achi and Akobor (2000) and Nout (1991) also reported the decrease in pH and increase in TTA during fermentation process of traditional fermented food products. Due to the addition of the starter culture, the protein and fat content of the samples increased. It was observed that milk fermented with mixed starter culture had the highest protein and fat contents. This result is in accordance with the findings of Ibeawuchi and Dalyop (1995) who stated that pasteurized milk fermented with starter culture had their crude protein and fat contents increased.

Sensory evaluation of quality and acceptability of samples showed that nono produced with mixed starter culture of *L. casei*N18 and *L. plantarum*N07 was rated the best. This is in accordance with the earlier report of Fenwema (1985) which showed that pasteurization and fermentation with starter culture improve the flavour and taste of milk and milk products. The microbial analysis of all the samples stored at room temperature (28±2°C) and at refrigerated temperature (4°C) was carried out. It was discovered that nono stored at refrigerated temperature had the least microbial load and longer shelf life compared to that of the room temperature. Oyawoye *et al*. (1997) documented that fermented foods inoculated with starter culture extents the shelf life of such product when stored at 4°C. The result from this study indicated that nono produced with starter culture that possesses antimicrobial properties and stored at refrigerated temperature will not only improve the flavour and nutritional value but it will also extend its shelflife.

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