

Dosage of starter culture influences gel formation and physico-chemical properties of yoghurt made from zebu milk

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Abstract

In order to determine the optimum dosage of starter culture for yoghurt produced from zebu milk, fresh milk was inoculated with 5, 10, 15, 20, and 25g/L of freeze-dried starter culture. Proximate composition, gel formation, pH, sensory properties and acceptance of yoghurts inoculated with different dosage of starter culture were evaluated after 2, 4, 6, 8 and 10 h of incubation. Total solids, protein and carbohydrate content of yoghurts increased significantly ($p < 0.05$) with the increase of starter culture dosage. Total solids varied from 14.1-15.2 %, protein from 3.54-4.31 %, and carbohydrate from 5.46-6.17 %. Gel formation occurred after 4 h of incubation for 25 g/L dosage and 10 h for 5g/L dosage. The pH of yoghurts decreased with time and with the increasing level of starter culture. Sensory properties of yoghurts improved with higher dosage of starter culture. The order of acceptance was; 25>20>15>10>5 g/L. Increasing dosage of starter culture improved physico-chemical properties of yoghurt and shortened time of gel formation.

Key words: yoghurt, composition, curd formation, sensory properties

Introduction

Yoghurt is a dairy product widely consumed by humans all over the world. The characteristic flavour, nutritional value and perceived health benefits has contributed to its wide acceptance (Hewitt and Bancroft, 1985; Adolfsson et al., 2004). Yoghurt is obtained through the fermentation of milk by action of two lactic acid bacteria; *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Schmidt et al., 2001). However, high cost of pure strains of yoghurt culture has necessitated the search for optimum dosage of culture for yoghurt production in Nigeria.

Yoghurt contains many nutrients essential for growth, development and maintenance of the human body. It is rich in carbohydrate, protein, fat, numerous vitamins and minerals including calcium and phosphorus, and has nutritional benefits that surpass

those of milk from which it is derived (Remeuf et al., 2003). The fermentation process during yoghurt production ensures an increased shelf life and microbial safety due to the conversion of lactose into lactic acid. The partial hydrolysis of milk protein, fat and lactose during fermentation also makes yoghurt more digestible than fresh milk (Kolars et al., 1984; Bystron and Molenda, 2004). Thus, people who cannot tolerate milk, either because of protein allergy or lactose intolerance, can easily digest yoghurt because the present lactose is partially fermented to lactic acid. Consumption of yoghurt has been associated with tremendous health benefits due to improvement of gastrointestinal functions and reduced risk of disease infection (Heyman, 2000). Yoghurt containing live cultures was sometimes used in attempts to prevent antibiotic-associated diarrhoea (Beniwal et al., 2003) while low-fat yoghurt was reported to promote weight loss due to the presence of calcium (Zemel et al. 2005).

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Consumption of yoghurt in Nigeria significantly increased during the last decade, however, poor quality and consistency of locally produced yoghurts remains a serious concern (Dublin-Green and Ibe, 2005). Yoghurt consistency is influenced among other things by characteristics of the starter culture and the total solids content in milk (Omer, 2003). The dosage of starter culture in milk during incubation is also expected to exert some influence on gel formation and organoleptic properties of yoghurt. This study was therefore conducted to determine the effects of starter dosage on time of gel formation and physico-chemical properties of yoghurt made from zebu milk.

Materials and methods

The experiment was conducted at the Dairy Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria.

Milk collection

Raw milk was collected from lactating Sokoto Gudali cows at the Teaching and Research Farm, University of Ibadan and was preserved in the refrigerator at 4 °C until required for processing.

Starter culture

A freeze-dried commercial starter culture (Yogourmet, Lyo-San Inc., Canada) containing a mixture of *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* was used to inoculate the milk before incubation. The inoculum was prepared by dissolving 2 kg of milk powder in 6 L of warm water and inoculating it subsequently with 100 g of freeze-dried culture at 45 °C for 6 h until a gel was formed. The obtained gel was stirred and used as inoculum at the dosages specified below for the preparation of yoghurt.

Yoghurt preparation

Raw milk was clarified manually using a muslin cloth. The milk was then heated to 60 °C and homogenized using a high speed mixer (10000-13000 rpm; Qlink[®], Shangai, China). The milk was subjected to modified low temperature-long time pasteurization by heating in a water bath to a temperature of 75 °C for 20 minutes under continuous stirring. This temperature-time combination was used because it was amenable to the manual pasteurization method

used in this study. Hereafter, the milk was cooled down to 45 °C inside a cold water bath, divided into five plastic containers (5 L each) and inoculated with the starter culture at dosages of 5, 10, 15, 20, and 25 g/L which corresponds to the treatments. Each treatment was further divided into five plastic cups (500 mL each, with lids) and placed inside an incubator set at 43 °C. Gel formation and pH were assessed for each treatment at 2, 4, 6, 8 and 10 hours of incubation. After incubation, yoghurt samples were cooled and refrigerated at 4 °C for further analysis.

pH of yoghurt samples

pH of fresh milk and yoghurt samples was determined at 25 °C using an electronic pH meter (PHS-3C, TBT, Jiangsu, China). The pH meter was calibrated with buffer standards of pH 4 and 10 prior to use and samples were assessed at 2, 4, 6, 8 and 10 hours of incubation.

Chemical analysis

Total solids in raw milk and yoghurt was determined by drying in an oven at 105 °C to constant weight, protein was determined by Kjeldahl method ($N \times 6.38$), ash was determined using a muffled furnace and fat by a modified Rose Gottlieb method following the general procedures of AOAC (1995). The carbohydrate fraction of the samples was determined as the difference between the total solids and other milk solids (protein, fat and ash).

Sensory evaluation

All the samples were evaluated for sensory characteristics and overall acceptability by a 10-man semi-trained panel drawn from the Department of Animal Science, University of Ibadan, Nigeria. Yoghurt samples were identified by three-digit random numbers and presented to the panel in a random manner. A nine-point hedonic scale ranging from 9 (highest score) to 1 (lowest score) was used (Iwe, 2002). Sensory characteristics evaluated include: aroma, colour, taste and mouth feel. Overall acceptability of yoghurt was determined as the average score for sensory characteristics.

Statistical analysis

The experimental design adopted for the study was the Completely Randomized Design. All data obtained were subjected to Analysis of Variance using

procedures of SAS (1995). Means were separated using Duncan’s Multiple Range test.

Results and discussion

The composition of raw milk and yoghurt inoculated with different dosage of starter culture are presented in Table 1.

The total solids, protein and fat in the raw milk were within the range reported for zebu cattle in Nigeria (Ndubueze et al., 2006; Adesina, 2012; Olorunnisomo, 2013).

Total solids, protein, and carbohydrate fraction of yoghurts were significantly ($p < 0.05$) influenced by dosage of starter culture while fat and ash fractions were not significantly affected ($p > 0.05$). Total solids increased with higher dosage of starter culture in the milk. The inoculum used in this study was prepared from a 3:1 mixture of water and milk powder with higher solids content than fresh milk. Hence, the higher total solids observed in yoghurts with higher dosage of starter culture may be correlated to the higher

solids content in the inoculum. Higher syneresis and concentration of solids associated with higher dosage of starter culture in yoghurts (Lee and Lucey, 2010) also may have influenced the solids content of yoghurts. Total solids of yoghurts in this study varied from 14.1-15.2 %. This is similar to solids content of 14-15 % reported for most commercial yoghurts (Tamime and Robinson, 1999). Protein content of yoghurt varied from 3.54-4.31 % and increased with higher dosage of starter culture. Such findings may be attributed to the higher milk and microbial proteins in the inoculum and the concentration of solids in the set yoghurt at higher dosage of starter culture. Carbohydrate fraction of the yoghurts followed similar trend with total solids and protein fractions.

Time required for gel formation in set yoghurt inoculated with different dosage of starter culture is presented in Table 2.

Regardless of the dosage treatments, there was no gelation in milk after 2 h of incubation. However, at 4 h of incubation there was little gel formation in milk inoculated with 25 g/L of starter while lower dosages showed no gelation. At 6 h of incubation,

Table 1. Chemical composition of raw zebu milk and yoghurt with different dosage of starter culture

Parameter	Dosage of yoghurt culture						
	Raw zebu milk	5 g/L	10 g/L	15 g/L	20 g/L	25 g/L	SEM
Total solids	12.85	14.1 ^b	14.2 ^b	14.4 ^b	15.2 ^a	15.2 ^a	0.62
Protein	3.40	3.54 ^b	3.96 ^{ab}	3.98 ^{ab}	4.28 ^a	4.31 ^a	0.28
Fat	4.30	4.18	4.18	4.13	4.12	4.09	0.30
Ash	0.65	0.66	0.60	0.64	0.63	0.64	0.07
Carbohydrate	4.50	5.72 ^{ab}	5.46 ^b	5.65 ^{ab}	6.17 ^a	6.16 ^a	0.37

a,b: means with different superscripts along the row differ significantly ($p < 0.05$)

Table 2. Gel formation in yoghurt inoculated with different dosage of starter culture

Hours	Dosage of yoghurt culture				
	5 g/L	10 g/L	15 g/L	20 g/L	25 g/L
2	No gel	No gel	No gel	No gel	No gel
4	No gel	No gel	No gel	No gel	Little gel
6	No gel	No gel	No gel	Gel formed	Gel formed
8	No gel	Little gel	Gel formed	Fully formed	Fully formed
10	Little gel	Gel formed	Fully formed	Fully formed	Fully formed

Table 3. pH of yoghurt inoculated with different dosage of starter culture during incubation

Hours	Dosage of starter culture					SEM
	5 g/L	10 g/L	15 g/L	20 g/L	25 g/L	
2	6.60 ^{b,w}	6.35 ^{ab,x}	6.30 ^{ab,w}	6.10 ^{a,x}	6.05 ^{a,x}	0.33
4	5.86 ^{a,x}	5.70 ^{ab,y}	5.35 ^{b,x}	4.90 ^{bc,y}	4.65 ^{c,y}	0.28
6	5.10 ^{a,y}	4.90 ^{a,z}	4.80 ^{a,y}	4.40 ^{ab,z}	4.30 ^{b,yz}	0.26
8	4.90 ^{a,yz}	4.68 ^{ab,z}	4.60 ^{ab,yz}	4.30 ^{b,z}	4.20 ^{b,yz}	0.22
10	4.65 ^{a,z}	4.55 ^{a,z}	4.30 ^{ab,z}	4.25 ^{ab,z}	4.05 ^{b,z}	0.22
SEM	0.25	0.26	0.22	0.24	0.26	

a,b,c: means with different superscripts along the row differ significantly ($p < 0.05$)

w,x,y,z: means with different superscripts along the column differ significantly ($p < 0.05$)

substantial gel was formed in milk inoculated with 20 and 25 g/L starter while lower dosages showed no gel formation. By 8 h, substantial gel was formed in milk inoculated with 15, 20 and 25 g/L of starter while little gel was formed in 10g/L and none in 5 g/L. At 10 h of incubation, gel was formed in all dosage treatments although quality of gel appeared better at higher dosages. Such results indicate that curd formation in yoghurt is time and starter dose-dependent at constant temperature.

The pH of yoghurt inoculated with different dosage of starter culture is presented in Table 3. Results showed a significant ($p < 0.05$) reduction in pH as level of starter culture and time of incubation increased.

Casein in milk normally precipitates at the iso-electric pH of 4.6 to form a curd or gel through the action of lactic acid bacteria or direct addition of acids (Lee and Lucey, 2010; O'Mahony, 1988). At 2 h of incubation, none of the treatments reached the iso-electric point; hence no gel was formed (Tables 2 and 3). At 4 h of incubation, 25 g/L dosage had a pH of 4.65 and formed a little gel. Lee and Lucey

(2010) explained that gelation occurs at pH 5.2 to 5.4 for milk given a high heat treatment. Lack of gelation at pH of 4.8 to 5.1 in this study may be attributed to the low heat treatment of milk before fermentation. At incubation period of 6 h, milk inoculated with 20 and 25g/L of starter had pH below 4.6 and formed a soft curd while at 8 h of incubation all but 5 g/L dosage formed some curd (pH 4.2-4.68). At 10 h of incubation, all dosage levels, except 5 g/L, had pH below 4.6 and formed a substantial gel. At this incubation period, milk inoculated with 5 g/L of starter (pH of 4.65) formed negligible amounts of gel. However, such observations indicate that given more time, also low dosage of starter will form a gel.

Sensory attributes of yoghurt dosed with different levels of starter cultures are presented in Table 4. attributes such as aroma, taste, mouth feel and overall acceptability of yoghurt were affected ($p < 0.05$) by dosage of starter culture while color was not significantly ($p > 0.05$) affected.

The effect of yoghurt culture on aroma, taste and mouth feel was not significant at lower dosage

Table 4. Sensory scores* of yoghurt inoculated with different dosage of starter culture

Parameters	Dosage of starter culture					SEM
	5 g/L	10 g/L	15 g/L	20 g/L	25 g/L	
Aroma	6.14 ^b	6.54 ^b	6.43 ^b	7.29 ^a	7.50 ^a	0.34
Colour	6.41	6.50	6.53	6.51	6.55	0.35
Taste	6.79 ^b	6.90 ^b	7.42 ^a	7.71 ^a	7.57 ^a	0.36
Mouth feel	6.86 ^b	7.00 ^b	7.35 ^a	7.42 ^a	7.50 ^a	0.36
Overall acceptability	6.55 ^b	6.74 ^b	6.93 ^{ab}	7.23 ^a	7.28 ^a	0.37

a,b,c: means with different superscripts along the row differ significantly ($p < 0.05$)

*Scores were based on a 1-9 hedonic scale, where 9 is the highest score and 1 the lowest

however, at higher dosage, aroma, taste and mouth feel of yoghurts were significantly enhanced. Overall acceptability score of yoghurt also increased with higher dosage of starter culture. This indicates that higher dosage of starter culture has positive influence on physical qualities of yoghurt and consumer acceptance. Lee and Lucey (2010) reported that physical and sensory properties of yogurt gels were greatly influenced by the total solids content of yoghurt milk. Hence, the increased total solids content of yoghurt with higher dosage of starters may have contributed to improved sensory qualities.

Conclusions

Total solids, protein and carbohydrate content of yoghurts increased with higher dosage of starter culture. There were significant reductions in pH of yoghurt with the increase in level of starter culture and the time of incubation. Sensory qualities and acceptance of yoghurt improved with higher inclusion of starter culture. Although all dosage treatments formed a gel at 10 h of incubation, gelation occurred earlier in yoghurt with higher dosage of starter culture. Such results indicate that gel formation in yoghurt is time and starter dose-dependent. Decisions on dosage to adopt should be based on cost of pure strains of starter culture and consumer preferences.

Utjecaj količine starter kulture na oblikovanje gela i fizikalno-kemijska svojstva jogurta proizvedenog od zebu mlijeka

Sažetak

U svrhu određivanja optimalne doze starter kulture za proizvodnju jogurta od zebu mlijeka, svježe mlijeko je inokulirano dodatkom 5, 10, 15, 20, i 25 g/L priprava liofilizirane starter kulture. Pri tome je praćena neposredna priprema kulture i tvorba gela, a jogurtima proizvedenim inokulacijom mlijeka različitim dozama starter kulture određivani su pH vrijednost, senzorska svojstva i prihvatljivost nakon 2, 4, 6, 8 i 10 sati inkubacije. U proizvedenim uzorcima jogurta uočen je značajan ($p < 0,05$) porast udjela suhe tvari, proteina i ugljikohidrata s povećanjem doze starter kulture. Ukupan udjel suhe tvari

kretao se između 14,1 i 15,2 %, proteina između 3,54 i 4,31 %, a ugljikohidrata između 5,46 i 6,17 %. Gel se oblikovao nakon 4 sata inkubacije kod dodatka starter kulture u količini od 25 g/L, odnosno nakon 10 h za količinu starter kulture od 5 g/L. pH vrijednost proizvedenih uzoraka jogurta snižavala se s vremenom i s povećanjem količine dodane starter kulture. Dodatak većih količina starter kulture utjecao je na poboljšanje senzorskih svojstava jogurta, a redosljed prihvatljivosti bio je $25 > 20 > 15 > 10 > 5$ g/L. Povećanje doze starter kultura također je poboljšalo fizikalno-kemijska svojstva jogurta te rezultiralo skraćivanjem vremena potrebnog za oblikovanje gela.

Ključne riječi: jogurt, sastav, oblikovanje gela, senzorska svojstva

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