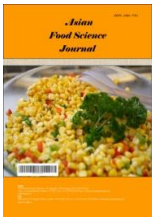

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Assessment of the Effects of Back Sloping on Some Starter Culture Strains and the Organoleptic Qualities of their Yoghurt Products

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Fermented milk is an essential commodity in Africa and beyond. Many techniques have been developed over time for the manufacture of different forms of yoghurt products. One of these Traditional methods includes back slopping). The advantages of this method include faster fermentation rates due to reduction in lag time, and subsequent production of relevant metabolites as well as allowing for a more reliable product formation on a consistent basis. The aim of this study was to better understand, the effects of back sloping on the microbial community as well as on the organoleptic characteristics of the yoghurts produced using the method. The model from this work could be used to study the dynamics of the microbial community associated with back-slopping practices and the understanding of possible associated defects in order to allow better control over the application of the method on commercial levels. We characterized the yoghurt produced from both microbial compositional study using culture-dependent morphological examinations on MRS, M17, Nutrient Agar and Potato dextrose Agar, as well as from organoleptic point of view. The results show that back sloping up to three-fold (batch) gave increasing acceptance but decreased afterwards. Acidification activity which determines proteolysis of casein

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for coagulation to take place also increased up to three folds. Residual lactose, syneresis and moisture content also decreased favourably by three fold order. Therefore, back sloping could be recommended on commercial level especially in the developing countries where facilities for consistent supply of pouched starter culture are limited because, aside the above mentioned advantages, this process also favours the growth of bacteria which release antimicrobial substances thereby ensuring the growth of the same species while reducing the growth of other organisms thereby preserving the products' quality.

Keywords: Organoleptic; fermentation; back sloping; microbial community and yoghurt.

1. INTRODUCTION

A large proportion of the world population now consumes one form of fermented product or the other owing primarily to the health benefits the products contain. Aside the health benefits, fermented milk products like yoghurt are nutritious and are easy to digest because the major carbohydrate it contains which is lactose has been broken down to lactic acid and other easily assimilated end products [1]. Other products that are produced include various forms of flavouring compounds such as acetoin, acetaldehydes, etc which make the product more appealing by improving the organoleptic characteristics, such as flavour, after-taste and textures. Selected microorganisms refer to as starter cultures are involved in the bio-transformation processes [2]. The transformation of these products takes place during their normal activity called fermentation. In the past, the so called spontaneous or natural fermentation were the sources of fermented products. However, the qualities of the end products were grossly unpredictable because undefined number, type and ratio of wild microorganisms are involved in an uncoordinated manner [3]. Even though the process has been practiced for many years, the transformation through such fermentation cannot be fully controlled, automated or correctly predicted because various types of indigenous microflora are involved apart from the lactic acid bacteria (LAB) desired for obvious tastes and outcome [4].Today, groups of efficient microorganisms commercially available are used in dominant and well optimised conditions for the fermentation processes. Consequently, through the application of industrial production protocols, fermented milk products are becoming fashionable, and diverse with one form of innovation to the other modifications with probiotics, use of prebiotics, addition of additive agents to enhance consumer acceptance, addition of fresh fruits, etc [5].

In other to satisfy consumer demands on one hand and to enhance continuity of production despite none availability of shelf-stable starter culture pouched, nevertheless, some artisans apply back-slopping fermentation methods during milk fermentation [6]. In this method, a small amount of fermented products is added into fresh pasteurised milk to initiate fermentation. In the process, a stable dominant microbial community is formed. One of the advantages of back sloping is that the microbial community dominates the product thereby wading off all other microorganisms known as contaminants. This gives room for the production of new products with predictable qualities and at the same time, the products are formed within a shorter fermentation time because adaptation shortens lag period and contamination are reduced to minimal [7].

In this experiment, back-slopping fermentation of pasteurised milk using commercial starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus* were evaluated for the microbial composition including the lactic acid bacteria (LAB) and also the organoleptic characteristics.

2. MATERIALS AND METHODS

2.1 Production of Yoghurt

Two Hundred grams (200g) of standard milk powder was added to each of Two 500ml capacity glass beakers containing distilled water and stirred continuously using a sterile spatula till it dissolves and forms a homogenous solution. The glass beakers were pasteurized with aluminium foil covering. They were cooled to about 40^0C and inoculated with standard LAB starter strains (*L.bulgaricus* and *S.thermophillus*) with 3% culture containing 10^6 cfu/ml. The setup, batch 1 (B1) was incubated for eight hours (at 40^0C) during which all the necessary fermentation parameters like pH were measured every 2hours. This first batch was used to inoculate another set of pasteurised, 200g powdered milk dissolved in 500ml fermentation glass [8].

The first fermented sample (B1) was used to inoculated another set of pasteurized milk solution and fermented for 6 hours to produce another batch cycle tagged Back slope sample 1 (BS1). The cycle (batch fermentations) was repeated until a fourth cycle (BS4) was attained). A batch was produced from the original starter culture and used as culture control (CC).

2.2 Determination of pH

The pH of the fermented milk samples (yoghurt) in all the batched including the control was measured with a handheld, glass electrode pH meter every one hour for 8 hours [9].

2.3 Determination of Moisture Content

The moisture content was determined by the AOAC [10] gravimetric method. In the method, a measured quantity (10g) was weighed into a previously weighed foil and evaporated to dryness in an oven at 105^0Cfor 3 hours. The process was repeated until a constant weight was attained after cooling.The weight of the moisture lost was calculated and expressed as a percentage of the weight of sample [11].

% moisture (% MC) = W2 –W1

Where,
W1 = weight of empty foil
W2 = weight of foil + sample before drying
W3 =weight of foil + sample after drying

2.4 Determination of Lactose Content (Total carbohydrates analysis)

The lactose content of each yoghurt sample was examined using the phenol-sulphuric acid method [12]. In the procedure, one gram of the sample (in boiling tube) was hydrolyzed by keeping it in a boiling water bath for 3 h with 5 ml of 2.5 HCl, it was then cooled at room temperature, neutralized with solid sodium carbonate until effervescence ceases, made up to 100 ml and centrifuged. After sample preparation, the working standard was pipetted out into a series of test tubes with 0.2, 0.4, 0.6, 0.8, and 1 ml as well as 0.1 and 0.2 from the sample in another two test tubes, then 1 ml of phenol and 5 ml of 96% the acid were added, the composition was shaken for 10 min and was placed in a water bath at 25–30 °C for 20 min, finally, the color that developed was read at 490 nm using a colorimeter (Ultrospec 2100 pro Biochrom Ltd., Cambridge C B 4 0FJ England). The total carbohydrate present in the solution (Lactose) was calculated using the predetermined standard curve and the formulaAbsorbance of0.1ml of the test=x mg of glucose in100ml of the sample solution contains=x/0.1×100mg of glucose=%of total carbohydrate present was used for the calculation [13].

2.5 Forced Syneresis Assay

The tendency for whey separation in the yoghurt samples were done by using a standard method [14]. In the procedure, 25ml of set yoghurt at 5^0C was slowly transferred to 50 ml capacity centrifuge tubes without disturbing the coagulum and centrifuged at 3394 RPM in a Remi centrifuge (Make-Remi, India) for 20 min. The amount of whey separated at the top of the coagulum inside centrifuge tubes was recorded as millilitres. The weight fraction of the supernatant liquid was used as index of whey syneresis (ml/100 g yoghurt).The higher the volume of whey separated, the higher was the whey separation and vice versa.

2.6 Microbiological Analysis of Fresh Yoghurt

The microbial composition of the yoghurt samples were determined using the method of Kumar *et al*.,2015 n the procedure, one millilitre of each Yoghurt sample was homogenized in 9.0 ml of sterile distilled water and was used as stock solution. Then dilutions were made up to 10^{-6} from the stock solution. Aliquot 0.1ml was of 10^{-4} was spread on appropriate media, MRS, M17, Nutrient agar (Modified with 20ml/l benomyl), Malt extract agar (containing 0.1g/l chloramphenical) and PDA (containing 0.1 g/l chloramphenicol and 0.5mg/ml cycloheximide) for the microbial analysis of yoghurt (Table 5) of *L.bulgaricus, S.thermophilus*, other bacteria, yeast and mold respectively [15,16].

2.7 Sensory Evaluation

All the yoghurt samples (BS1-BS4) and the control(CC) were evaluated for organoleptic characteristics and overall acceptability by 10 panelists that comprised individuals that already had experience with yoghurt characteristics and a five point hedonic scale ranging from very good (score = 5) to very poor (score = 0) as extremes [17].

3. RESULTS

The study revealed that LAB strains recorded increased activities from back sloping cycle 1 to cycle 3 but reduced in the 4th cycle (That is, when used the 4th time) during fermentation.

The result on Table 6 shows the overall acceptance score percentage in decreasing order, BS3 (24%)>BS2 (21%)> CC (20.4%)> BS4 (19.6%)> BS1 (15%). Table 1 shows the acidification activities of the respective culture at each batch. The pH decreased from CC (4.48), BS1 (4.43), BS2 (4.20) to BS3 (4.19).

Table 2 shows the moisture contents of the yoghurt samples that were produced. It decreased from BS1 (85.4%)>BS2 (83.7%)>BS3 (82.8%) to the lowest which is the control, CC 80.6%).

Table 3 is the mean forced syneresis (%) result of yoghurt samples while BS1 had the highest of 3.8, BS3 recorded the lowest of 3.2.

Table 4 shows the lactose reduction profile of the cultures subjected to back sloping treatment.The culture control (CC)recorded 4.17% which is the highest, while BS3 had the lowest (4.04%).

Table 5 shows the microbial population dynamics on MRSA and M17 respectively which increased for the two starter cultures used up to the 3rd batch

Table 6 is the sensory analysis results and it showed increased product acceptance (BS1-BS3) but decreased afterwards (BS4).

Fig. 1. shows the pie-chart presenting the the yoghurt preference judgement by the panellist. BS3 had the highest fraction while BS1 had the least.

Fig. 2. shows the bar-chart of the comparative preference of the yoghurt samples which increased from BS1 upto BS3.

4. DISCUSSIONS

Five samples of fermented yoghurt were produced using standard commercial strains of LAB, (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). Four sets were back sloped (BS1-BS4) while one (CC) was not back sloped as control. Back sloping could be beneficial but to an extent as we could see from the result of this work where there were improvement in technological and functional properties of both the microorganisms responsible for the fermentation as well as the functional characteristics of the producing microorganisms.

Table 1. Mean pH Values (Acidification Activity) of Yoghurt Produced Using Back SlopingMethod

Time (Hour) Sample 0	24	68			
BS1	6.50	5.85	4.57	4.20	4.19
BS2	6.50	5.72	4.46	4.23	4.04
BS3	6.50	5.60	4.30	4.19	3.95
BS4	6.50	5.81	5.36	4.68	4.58
CC	6.50	5.76	5.51	4.50	4.47

BS= Back Slope- CC= Commercial Culture

Table 2. The Moisture Contents of Yoghurt Samples

Yoghurt Sample	Initial Wt (g)	Final Wt (g)	Mean Wt (g)	Moisture Content (%)
BS1	30	4.38	25.62	85.4
BS2	30	4.89	25.11	83.7
BS3	30	5.16	24.84	82.8
BS4	30	3.72	26.28	87.6
CC	30	5.82	24.1880.6	

BS= Back Slope- CC= Commercial Culture

Table 3. Mean of Forced Syneresis (%) of Yoghurt Samples

Sample BS1	BS2	BS3	BS4	CC
Syn. (%)3.8	3.4	3.2	3.9	3.7

BS= Back Slope- CC= Commercial Culture- Syn. = Syneresis

Table 4. Mean Residual Sugar (Lactose) Content of Yoghurt Samples (%)

	Milk Fermentation Time (Hour)				
Isolate	**0**	**2**	**4**	**6**	**8**
BS1	4.56	4.49	4.22	4.12	3.48
BS2	4.58	4.46	4.20	4.09	3.31
BS3	4.57	4.42	4.10	4.04	3.67
BS4	4.55	4.51	4.49	4.46	4.17
CC	4.56	4.47	4.28	4.17	3.74

BS=Back Sloping

Table 5. Microbial Enumeration of Yoghurt

Sample	NA	MRSA	M17	Yeast/Mold
BS1	$1.1x10^3$	$1.6x10^6$	$6.5x10^5$	$1.0x10^2$
BS2	$1.4x10^1$	$1.0x10^4$	$2.1x10^4$	$1.6x10^2$
BS3	$2.9x10^3$	$7.1x10^5$	$6.2x10^4$	3.7x102
BS4	$1.7x10^1$	$2.1x10^3$	$1.2x10^4$	$6.4x10^4$
CC	$3.8x10^1$	$7.0x10^5$	$5.2x10^6$	$1.3x10^1$

BS=Back Sloping; CC=Commercial Culture; NA=Nutrient Agar

Table 6. The Panelists' results for sensory evaluation

Panelist	Yoghurt Samples/Scores				
	BS1	**BS2**	**BS3**	**BS4**	**CC**
1	12	14	17	14	13
2	11	14	18	16	15
3	10	15	13	10	14
4	14	17	18	13	16
5	10	16	17	14	12
6	07	14	18	16	13
7	14	14	18	15	16
8	12	13	14	09	14
9	08	16	18	15	12
10	15	11	13	12	15
Total Score	103 (15%)	144 (21%)	164 (24%)	134 ((19.6%)	140 (20.4%)

BS=Back Sloping; CC=Commercial Culture

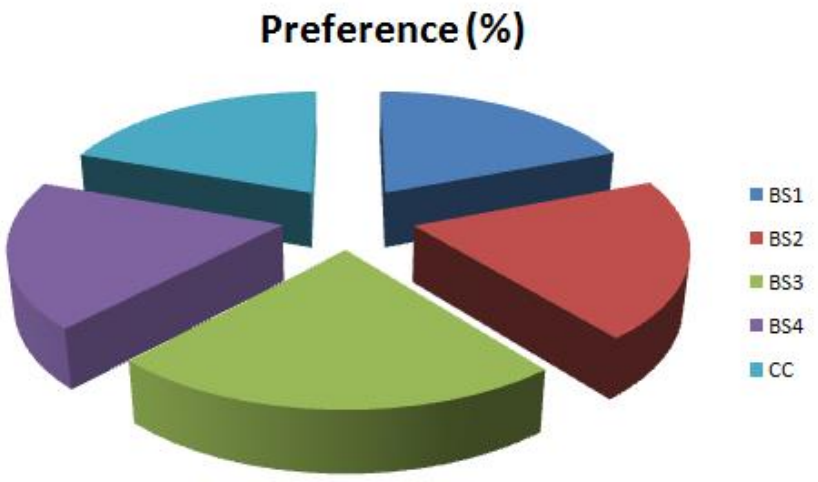

Fig. 1. Comparative Product Preference
BS=Back Sloping; CC=Commercial Culture

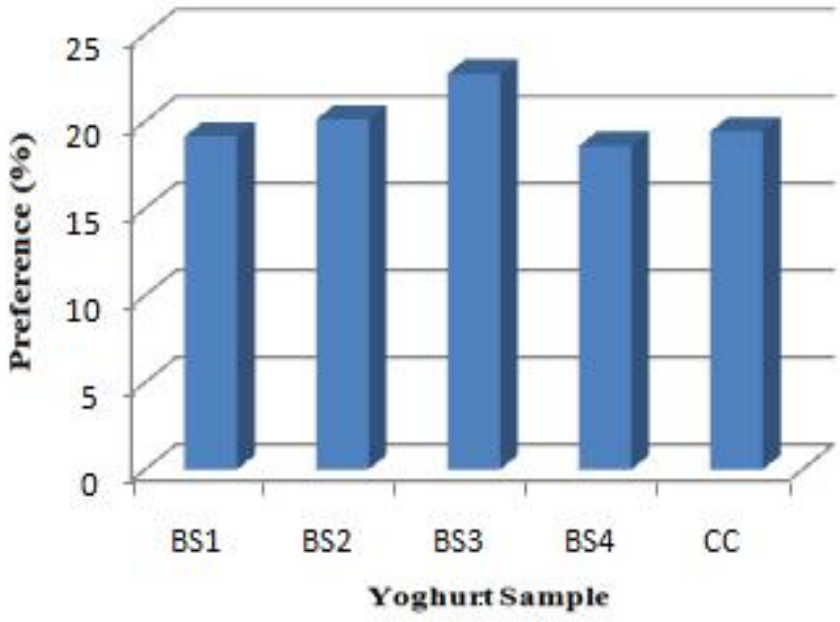

Fig. 2. Comparative Product Preference and Qualities
BS=Back Sloping; CC=Commercial Culture

The results of the study as shown inTable 1 showed that the pH of the product decreased favourably unto three cycles in yoghurt fermented with back-slopping method which is better than that produced without back sloping. This is similar to the results obtained by Kumar, 2015 [18]. The bacteria loads also increased and this was possibly an effect of back-slopping method application due to microbial dominance advantage gained because of shorter lag phase [19,20,]. This advantage also reflected in the organisms able to break down more lactose in the medium resulting in lower residual lactose which is beneficial to the lactose intolerant consumers. In Summary, the small amount of previously fermented products that wereadded to initiate milk fermentation not only caused this increase but also accelerated the fermentation time.

5. CONCLUSIONS

In the past, fermentation was traditionally used for the preservation of food materials and as such has been used for centuries. Today, however, one of the main reasons for food fermentation is not only to preserve but also to enhance the nutritional values as well as for producing wide products with customer's acceptance in terms of tastes, texture, flavour and the likes. In the developing countries such as Nigeria, not many dairy producing companies can run standard starter cultures production facility on a sustainable bases because of the overall cost and technical skills that they required [21]. Therefore, research and application of back sloping method could be a good way of sustaining such huge investments. Aside cost and technical skills involved such as strain selection and processing in maintaining starter culture, a lot of time is often saved when back sloping method is used [22]. This is however not mean that back sloping does not have its own challenges which should be researched into.

For this purpose, therefore, new studies need to be carried out to explore more options of media and modifications of back sloping that have not yet been industrially tested.

This research showed that back-slopping fermentation offers greater opportunities for small scale dairy industries as well as those affected by Covid-19 to hold strong their fermentation standards in the face of limited starter culture supplies.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely

no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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