Original Research Article

COMPARATIVE EFFECTIVENESS OF WILD AND COMMERCIAL STRAINS OF LACTIC ACID BACTERIA STARTER CULTURE DURING BACKSLOPING FOR YOGHURT PRODUCTION

Abstract

Yogurt is one of the traditionally fermented dairy products which are usually prepared with lactic acid bacteria cultures containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* and has gained widespread consumer acceptance as a healthy diet. The roles of exotic starter cultures for dairy milk processing in Nigeria food supply chain cannot be over stated. However, there are concerns over growing food and food-related import dependency in the sub-African countries and Nigeria in particular which have increased during the last few years. The selection of indigenous strains with capacity for backsloping in yoghurt production might reduce importation and improve economy in a way. Yoghurt fermentation was carried out by backsloping method using three commercial (Exotic) formulated cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophillus*; *ES07, ES32* and *ES14* respectively as well as wild (isolated) formulated culture strains; *WS11, WS65* and *WS13* respectively and were used to produce yoghurt using backsloping method. Their pH, coagulation, microbial profile and sensory attributes were evaluated after three consecutive batch of backsloping. The overall sensory attributes of yoghurts produced using exotic compared with indigenous strains showed no significant ($p > 0.05$) difference. There was also no significant ($p < 0.05$) variation for all set of yoghurts in term of acidification and coagulation ability. The pH of fermented products using commercial cultures ranged from 4.53-5.32, total plate count 1.1×10^6- 4.0×10^7 cfu/ml during the 10 hours fermentation. While pH of yoghurts produced using isolated cultures ranged from 4.35 – 4.72, total plate count 2.7×10^7- 2.3×10^8 cfu/ml during the 10 hour fermentation times. The overall acceptability were in the decreasing order; *WS65>WS11>ES07>WS13>ES14>ES32*. Starter culture formulation using indigenous strains could be further developed genetically for commercial applications because they compete favourably with their exotic counterparts.

Keywords: ***Acidification, Backsloping, Cultures, Importation and Indigenous***

Introduction

There is high demand for yoghurt and fermented milk owing to their numerous health benefits. In recent years, more people are getting aware of the health protections that could be derived from the consumption of controlled-fermented products such as yoghurt. (Bristone *et al.*, 2015). For centuries, the health benefits of the consumption of fermented milk such as yoghurt with its lactic acid bacteria contents reducing the incidence of intestinal toxin, virgina infections, blood pressure and osteoporosis have been reported (Shahnawaz *et al*., 2013). Fermented products like yoghurt have been known to contain elements with some therapeutic and health maintenance properties (Igbadul *et al*., 2014). Aside the above mentioned benefits, the presence of *S. thermophilus* and *L. bulgaricus* as the culture organisms in the fermented

products like yogurt means the availability of functional probiotic properties (Aryana *et al*., 2017). The use of Lactic Acid Bacteria strains in dairy starter cultures is highly recommended because of their safety considerations (GRAS status) (Rychen *et al.,* 2018).

Some studies have shown that another beneficial property of LAB is their possession of biomolecules with autophagy triggering capacity which are said to be important in cell recycling for antimicrobial protection and maintenance of epithelial competence (Dinić *et al*., 2017). Although LAB is wide spread in nature, they are commonly associated with dairy materials where they mostly play the role of acid fermentation. These functions have been harnessed for the commercial production of many dairy products with good sensory characteristics for the benefit of mankind (Bluma *et al*., 2017). Indigenous Lactic acid bacteria strain isolated from the spontaneous fermented milk refered to as the 'wild' have specific properties that can be harnessed for controlled fermentation of milk (Bluma *et al*., 2017).

Considering the limited availability of exotic commercial starter cultures in Nigeria, there has been a trend of selection and development of new starter cultures from the autochthonous habitat for development into viable commercial status with potent traits for backsloping applicationa and stable shelf life. The effort at using such newly-isolated and well-characterized cultures for use in place of the exotic strains will lead to the production of standard products with predictable characteristics as their exotic peers in terms of both nutrition enhancement and health promoting features.

There is currently limited documentation of the technological consequences of the different traditional techniques employing backsloping for producing controlled fermented products such as yoghurt.

Therefore, the aim of this work was to determine and document the comparative differences between backsloping using commercial strains and those of the isolated cultures during yoghurt production practice. Such indigenous knowledge in traditional fermentation practices utilising backsloping method may enhance further development, up-scaling and utilization of local resources to the level of other renowned fermented milk products.

Materials and Methods

Preparation of Glass Wares and Media

Glass wares were oven sterilized by dry heat (Medified Equipment and Scientific DHG-9023A, England) at 160°C for 60 minutes as described by Fawole (1988) and Cheesbrough (2000). Each microbial medium was prepared by dissolving the appropriate powder in 1000 ml (1 litre) of distilled water as described by the manufacturers. Each was autoclaved at 121°C for 15 minutes (Fawole, 1988; Jideani and Jideani, 2006).

Sample collection

Commercial starter cultures for this research were obtained from standard supermarkets within Abuja metropolis and maintained at 40C until needed. The locally fermented milk product (Nunu) for the isolation of indigenous lactic acid bacteria strains were collected from the households processing them from around the FCT. The samples were collected in sterile bottles and kept cool until they arrived at the laboratory, where they were kept at 4°C for further use.

Isolation of Lactic Acid Bacteria.

One (1ml) ml aliquot was taken from the collected samples aseptically after thorough homogenization. Serial dilution was then made using sterile peptone water by adding 1ml into 9ml. A 0.1ml aliqut o 10^{-2} and 10^{-4} of the serially diluted samples were aseptically added to M17 and MRS agar for the isolation of LAB (Badis *et al*., 2004a). In order to prevent the growth of yeast, the agar media were supplemented with $100mg^{-1}$ of cycloheximide and were incubated for 5 days at 40^0C anaerobically using the Gas Pack system (Merck Anaerocult type A) (Kalavrouzioti *et al*., 2005). After incubation, discrete colonies were randomly selected and purified on sterile media. The resulting pure strains were kept in two different conditions including at 4°C for MRS and M17 plates and at -20°C for M17 and MRS broths supplemented with 20% glycerol for further use (Mathara *et al*., 2004).

Identification of the Bacterial Strains

All the purified bacteria strains were tested for gram reaction, catalase production and spore formation (Harrigan and McCance, 1976). Colonies were characterized on MRS and M 17 agar. Only strains with gram positive and catalase negative reactions were finally used for further subjected to further identification (Sharpe, 1979). Growth at different temperatures (10, 15, 37, 40 and 45°C) for 5 days, resistance to 63°C for 30 min (Sherman test), growth in the presence of 2, 3, 4 and 6.5% NaCl and different pHs (4.5 and 6.5) were used for further identification of the strains. Ability to hydrolyse arginine and asculin, utilization of citrate, production of acetone, gas formation from glucose and dextran production from sucrose were also tested according to the method of Samelis *et al*., 1994. The strains were also tested for fermentation of L-arabinose, D-xylose, galactose, D-fructose, sorbitol, lactose, melibiose, saccharose, D-raffinose, melezitose, mannose and glucose (Tserovska *et al*., 2002). The growth of bacterial strains at 10, 15, 37, 40 and 45°C was visually confirmed by the changes in turbidity of MRS or M17 broth after 24, 48 and 72 h of incubation. The tolerance of microorganisms to the different levels of salt, pH and heat (60°C) was also visually evaluated. Arginine dihydrolase agar and asculin azid agar (Merck, Germany) were employed to perform the hydrolysis tests. For evaluation of citrate utilization and acetone production, citrate and MR-VP agars (Merck, Germany) were used. MRS or M17 broths containing inverted Durham tubes were used for evaluation of gas production and the production of dextran from sucrose was done in MRS agar (Mayeux *et al*., 1962).

Preservation of LAB Strains

In order to prevent loss of properties, the identified strains were stored in skimmed with 30% (v/v) glycerol at 4^0C till further use (Badis *et al*., 2004). Cultures were also kept on MRS agar or M17 agar slant at 4ºC and streaked forth-nightly.

Technological Characterization

Acidifying activity

The acid-production potential of the strains was measured according to the International Dairy Federation (IDF) standard 306 (Kihal *et al*., 1996). Fermentation was carried out at 40^0C by preparing 10% milk and introducing 24 hour old cultures at 1% rate. The pH was

measured during the 12 hour fermentation period by inserting the cleaned probe of the pH meter in the fermenting medium and determining the values..

Proteolytic Activity

The proteolytic activity of the isolates were determined by fermenting 10% skimmed milk for 12 hours at 40^0C and recording the coagulation which is either positive (+) or negative (-).

Biomass production

The bacteria strains were sub-cultured into MRS broth (100ml) of the medium with 10% of the active culture. The respective growth was monitored by measuring the Optical Density at 600nm (OD600) using a spectrophotometer. The biomass was determined by centrifuging aliqut samples. The dry weight was determined as needed by drying the pellet of samples at 55ºC for 24h (Ayad *et al*., 2004).

Experimental Design for Yoghurt Production

In order to fully attain the goal of this research, two sets of milk fermentation experiments were set-up in parallel; one used commercial starter culture strains, viz; co-culture samples of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (*ES07, ES32* and *ES14*) respectively while the other one used the isolated and purified LAB strains, (*WS11, WS65* and *WS13*) respectively.

The freeze dried samples were prepared from the commercial strains by weigh 0.5g of the lyophilized cultures and dissolved in 5ml of the pasteurized milk. The culture was incubated at 40ºC for 18h. Three sets of reconstituted skimmed milk (10%) were homogenised and pasteurized at 65ºC for 30 minutes and cooled to 42ºC. Each set was inoculated with about 5ml (containing10^6 CFU/mL) commercial, prepared starter cultures (*ES07, ES32* and *ES14*). Five (5ml) of isolated cultures (*WS11, WS65* and *WS13)* containing 10^6 CFU/ml which were determined by the initial viable counts. All the set-up was incubated at 40^0C until the pH reached 4.5 then kept in the refrigerators set at 4ºC. Sample from the fermented samples were used to initiate consecutive batch (2.5%) to get a three-fold sample. During fermentation, the pH, coagulation, aerobic total plate count, flavour and organoleptic properties were measured for each set of the sample. The third-fold fermented sample were compared.

Organoleptic Analysis

This analysis was carried out by ten taste panellists who were trained to identify the characteristics of yoghurt without sugar added. They were served with the chilled samples in coded cups and the respective records were made after tasting. The panellists were asked to rank the coded samples on the basis of their quality attributes made up of; consistency, flavour, taste, colour, texture and overall acceptance using nine-point hedonic scale, expressing degree of liking or disliking as in the questionnaire (Igbadul *et al*., 2014).

Results

Table 1: pH, Proteolytic ability and Total Plate Count of the third-fold fermented Yoghurt using Commercial and Isolated LAB strains

Parameter	Commercial Cultures			Isolated Cultures		
	ES07	ES32	ES14	WS11	WS65	WS13
pH (EPV)	4.53	5.32	5.21	4.42	4.35	4.72
Proteolysis	+	+	+	++	++	+
Total Plate Count	$4.0x10^7$	$3.4x10^6$	$1.1x10^6$	$2.3x10^8$	$1.2x10^8$	$2.7x10^7$

EPV = End point value; += Coagulation

Table 1 shows the pH, Proteolytic ability and total plate count of both the commercial and isolated LAB strains recorded at the end of a third-fold milk fermentation (Backsloping). The lowest pH (4.35) was recorded in the yoghurt culture fermented with isolated lactic acid bacteria culture (WS65) while the highest pH (5.32) was recorded in yoghurt fermented with bacteria culture (5.32). Proteolytic activity (Coagulation rate) occurred fastest in yoghurt products produced with cultures WS65 and WS11. Total aerobic plate count of yoghurt culture were recorded overall higher in yoghurt cultures fermented with isolated lactic bacteria strains than in the commercial strains. The highest being $2.3x10^8$ CFU/mL (WS11) while the lowest was $1.1x10^6$ CFU/mL (ES14).

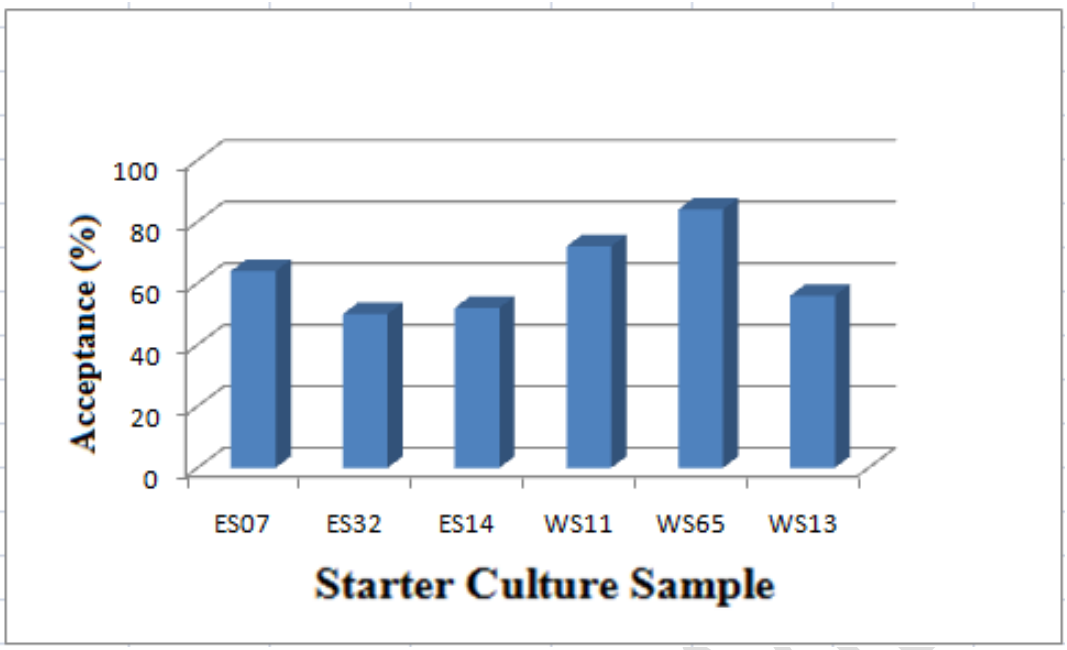

Fig.1: The Percentages Acceptance of the Yoghurt Samples Produced by the Respective Cultures

Figure 1 shows the mean percentage acceptance of the yoghurts produced by the various lactic acid bacteria cultures. The highest being WS65 (84%) while the lowest was ES32 (50%).

A. Soft, smooth, pudding-like, firm textured Product- Consistent Throughout Backsloping Batches

B. Smooth-and-creamy Product **C. Panel of Taste Analysts**

Fig.2: COMPARATIVE BACKSLOPING ABILITY OF SOME LACTIC ACID BACTERIA STRAINS

Discussions

The average acidification activity of the isolated lactic acid bacteria culture was better than their commercial counterparts. As a result, there was a faster coagulation of pasteurised milk protein thereby producing yoghurt with better quality as elucidated by the panelists' results. This is in agreement with the reported work on other product but same principle by Holzapfel (2002) that various carriers such as porous material of gourd have been used to achieve backslopping. That 'inoculation belt' was used for the initiation of *pito* beer fermentation in work carried out in Ghana. And that In Egypt *khisk* is prepared using cow/camel milk and wheat where one-third (w/w) of dried *khisk* is used as a starter inoculum. These practices in the preparation, preservation and distribution of inoculums according to the research could be used as a basis for the development of starters for other fermented foods.

Also, the high acid production means that the product could be better preserved thereby giving it longer shelf life because acid content of food matrix reduce the population spoilage microorganisms. It is said that within a spoiling food, there is often a succession of different populations that rise and fall as different nutrients become available or are exhausted. Notably, some microbes, such as lactic acid bacteria, secrete compounds such as acid that inhibit competitors (Gram, *et al*., 2002). The aerobic total plate count recorded higher values in products fermented with indigenous strains. The implication is that such strains could be better as probiotic cultures since they would be delivered into the gastro-intestinal tract in larger concentrations resulting in better and more colonization for enhanced activity. According to the Food and Agriculture Organisation of the United Nations (FAO)

and the World Health Organisation (WHO), (2014), probiotics are defined as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host.

Conclusions

From the data and observations recorded from our study, it can be concluded that backsloping method could be used for large-scale production of yoghurt because there was no significant difference in the performance of the backsloped lactic acid bacteria cultures as compared with the commercial strains. In fact, the yoghurts produced with indigenous strains showed better overall acceptance over the ones produced with commercial strains.

Recommendations

This old traditional method of fermentation should be employed/upscaled for large scale yoghurt production without delay and with ulmost assurance of predictable success.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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