RESEARCH ARTICLE

Pilot study on chilli stalks as a source of non-dairy lactic acid bacteria in yogurt making

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ABSTRACT

Chilli stalks were investigated as a source of non-dairy lactic acid bacteria (LAB) in the fermentation of milk for yogurt production. Milk samples used for this study were obtained from four different milk types which are evaporated whole milk, powdered whole milk, groundnut whole milk and coconut whole milk. The milk samples were prepared, pasteurized at 82°C for 30 minutes, cooled to 45°C and inoculated with chilli stalks. Two reference samples were similarly treated and inoculated with starter 'A' and starter 'B', (LAB from store bought yogurts Tm). The pH and titratable acidity reported as percentage lactic acid were recorded as fermentation indicators. The results were significantly different ($p \leq 0.05$) and showed that chilli stalks cultured evaporated milk yogurt (CEY), chilli stalks cultured powered milk yogurt (CPY), chilli stalks cultured groundnut milk yogurt (CGY) and chilli stalks cultured coconut milk yogurt (CCY) have the pH values of 4.1, 5.4, 4.4 and 4.0, respectively. The pH values for starter 'A' cultured yogurts are starter 'A' cultured evaporated milk yogurt (AEY) 5.0, starter 'A' cultured powered milk yogurt (APY) 5.4, starter 'A' cultured groundnut milk yogurt (AGY) 3.4 and starter 'A' cultured coconut milk yogurt (ACY) 4.5 while those of starter 'B" cultured yogurts are starter 'B' cultured evaporated milk yogurt (BEY) 4.2, starter 'B' cultured powered milk yogurt (BPY) 5.3, starter 'B' cultured groundnut milk yogurt (BGY) 4.6 and starter 'B' cultured coconut milk yogurt (BCY) 4.6. The titratable acidity (% lactic acid) are (CEY) 1.15, (CGY) 1.16 and (CCY) 1.32. In chilli stalks inoculated powdered milk (CPY), cheese was produced after the 18 hours incubation period. The titrable acidity (% lactic acid) of reference samples are AEY 1.06%, APY 1.32%, AGY 0.95%, ACY 1.19%, BEY 1.09% and BCY 1.61%. From the results obtained in this study, chilli stalks contain microorganism that resulted in increased acidity in yogurt products.

Key Words: *Chilli stalks; Non-dairy lactic acid bacteria; Yogurt; pH; % Lactic acid*

Lactic acid is one of the most important organic acids which is being extensively used around the globe in a range of industrial and biotechnological applications [1]. Yogurt is a fermentation dairy product obtained from lactic acid fermentation of milk. It is made by adding certain bacterial called probiotics to milk. These bacteria produce lactic acid, which decreases milk pH and coagulate milk proteins to give yogurt its texture and characteristic tang [2]. Probiotics are defined as live microorganisms that when administered in adequate amount confer a health benefit on the host [3]. Yogurt helps in maintaining a healthy digestive system due to the presence of the probiotics [4]. Milk fermentation is one of the oldest methods by human beings to preserve milk and extended shelf life [5]. The decrease in pH inhibits the growth of pathogenic bacteria. The lactic acid produced is also responsible for the characteristic flavor and aroma of yogurt and helps to maintain the quality of yogurt during storage and packaging [6].

Traditionally, yogurt is produced from dairy milk, but in recent years due to lactose intolerance in some individual, milk allergy, vegans, scarce and expensive dairy milk in developing countries and sub Saharan Africa, various plant milk substitutes such as coconut milk, groundnut milk, tigernut milk, soyamilk have been used in the production of acceptable yogurt-like products [7]. Historically, many probiotic microorganism are associated with milk or dairy products and a large group of known probiotics used in human food belong to the general lactobacillus and bifidobacterium [8,9]. In recent years, there has been increasing interest in lactic acid bacteria isolated from non-dairy products due to their diverse metabolic profile, unique flavor – forming activities and potential for use in starters or starter adjuncts for the dairy industry [10].

There have been claims that the calyx of capsicums fruits such as chilli, are often rich in various lactobacilli. These natural bacteria create a starter for lacto-fermentation of milk. In addition capsaisin from the chilli appears to increase the metabolic rate of the lactobacilli [11,12].

The stalks (calyx) of chilli (*Capsicum* spp.) are general removed as waste during food processing. The use of this considered waste in the curding of milk can however change its status from being a waste product to a product of high economic value therefore turning waste to wealth. Also it will provide an easy access to healthy starter culture in yogurt making; as the pure freeze-dried starter culture are scarce in the market, which makes people resort to store bought yogurt as starter culture in yogurt making. The effectiveness of these store bought yogurt are not always guaranteed due to certain hidden additives from the manufacturers and the fact that the active culture tends to decrease over time [13].

Despite several claims on the use of chilli stalks (calyx) in yogurt making, there have been paucity of scientific documentation of its effectiveness in terms of pH change and the percent lactic acid content of the yogurt produced with it. This work is however undertaking to investigate the effectiveness of chilli stalks in yogurt making by monitoring the fermentation of yogurt produced with chilli stalks as a source of lactic acid bacteria (LAB) on both dairy and non-dairy milk by measuring the pH and the % lactic acid of the yogurt as fermentation indicators.

MATERIALS AND METHODS

The materials used in this study which included evaporated whole milk (peak brand), powdered whole milk (peak brand) groundnut, coconut, store bought yogurt samples (Hollandia and Supershake brands) and chilli pepper were purchased from stores and local markets in Esa-Oke and Ilesa in Osun State. The chemicals and reagents used are of analytical grades purchased from chemical stores in Ile-Ife, Osun State. The model Hanna H196107 pH meter made in Italy was used for pH measurement. All the experimental procedures were carried out in the Chemistry Laboratory of Science Laboratory Technology Department, Osun-State College of Technology, Esa-Oke, Osun- State, Nigeria.

Experimental design

Four milk types were used as milk base materials, which are two dairy milk (evaporated whole milk and powdered whole milk) and two non-dairy milk (groundnut milk and coconut milk). Two reference starter cultures were used in similar way as the materials under test (chilli stalks). The two reference starter cultures labelled as 'A' and 'B' are store bought yogurts that contained active live cultures of lactic acid bacteria (LAB). The pH and titratable acidity of the milk base and final products were determined using pH meter and titration with 0.1N NaOH and phenolphthalein.

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Samples preparation: The evaporated whole milk and powdered whole milk were each reconstituted by mixing with potable warm water at ratio 1:2 of milk to water and allowed to dissolve as recommended by their manufacturer.

Extraction of groundnut milk: About 100g of sorted groundnut sample was cleaned and soaked in water for 18 hours. The water was changed at regular interval of 3 hours to prevent fermentation. The soaked groundnut was drained and blanched in hot water for 5 minutes. It was then blended and sieved using muslin cloth with 750ml of potable water to produce fresh groundnut milk [14,15].

Extraction of coconut milk: The Coconut shell was opened and the flesh removed. 200g of the flesh was shredded, blended with 500ml of potable water and sieved using muslin cloth to produce fresh coconut milk [16].

Chilli stalks (calyx): Chilli with the stalk attached was washed with clean water and stalks were detached whenever needed for the inoculation (Figures 1 and 2).

Chemical Analyses

Determination of pH values: The electrode of the pH meter was standardized and used to measure the pH of the sample [17,18].

Titratable acidity: 10ml of sample was titrated against 0.1N NaOH using phenolphthalein indicator. The titre value was used to calculate the titratable acidity as percent lactic acid [18].

$$Total\ Acidity = \frac{Volume\ of\ NaOH}{Volume\ of\ sample\ taken}$$

RESULT AND DISCUSSION

The production of lactic acid from lactose by lactic acid bacteria (LAB) results in lowering of pH and thus increased acidity. There are two fundamentally different methods of expressing acidity, the titratable acidity expressed as % lactic acid and hydrogen ion concentration expressed as pH. Titratable acidity measures the total acidity while pH indicates the strength of the acid [19].

pH of milk base and yogurt

The pH of the fresh milk sample 6.0-6.3 are within the rage of 6.0-7.0 reported for fresh milk [20,21]. After the 18hours incubation period, a general trend of decreased pH was observed in all the yogurt samples produced (Figure 3). The pH of the fresh milk base and yogurts are as presented in Figure 4. The pH values obtained for the sample Chilli stalks cultured Evaporated milk yogurt (CEY) 4.1, Chilli stalks cultured powdered milk yogurt (CPY) 5.4, Chilli stalks cultured Groundnut milk yogurt (CGY) 4.4, and Chilli stalks cultured coconut milk yogurt (CCY) 4.0. The pH values of yogurts produced with starter 'A' and 'B' are as follows Started 'A' cultured Evaporated milk yogurt (AEY) 5.0, Starter 'A' culture powered milk yogurt (APY) 5.4, starter 'A' cultured Groundnut milk yogurt (AGY) 3.4, starter 'A' cultured coconut milk yogurt (ACY) 4.5, started 'B' cultured powdered milk yogurt (BEY) 5.3, started 'B' cultured Groundnut milk yogurt (BGY) 4.6 and starter 'B' cultured Coconut milk yogurt (BCY) 4.3. A chronicle of all the pH values obtained showed that it was within the range of 3.5-5.0 reported for yogurt [22-27]. However for the powdered milk yogurt samples the pH of 5.3-5.4

Figure 1) *Chilli stalks*

Figure 2) *Chilli with stalks attached*

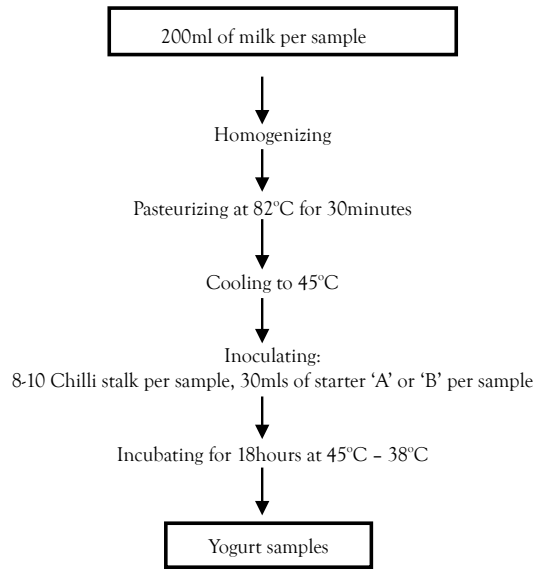

Figure 3) *Yogurt preparation*

The flow chart of yogurt preparation described by Farinde (17) was adapted with slight modification.

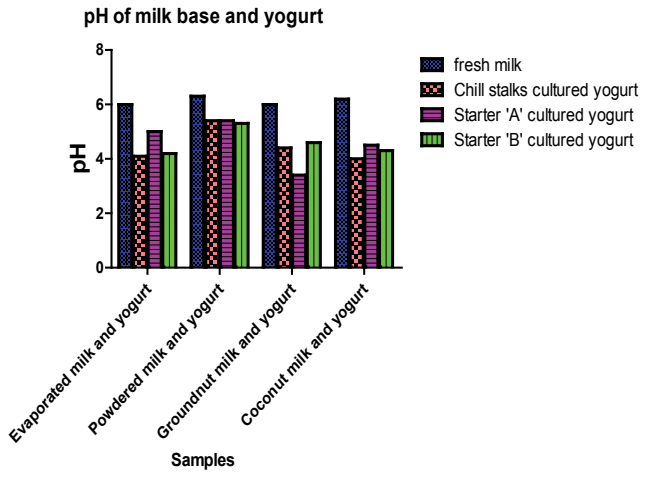

Figure 4) *pH of Milk Base and Yogurt*

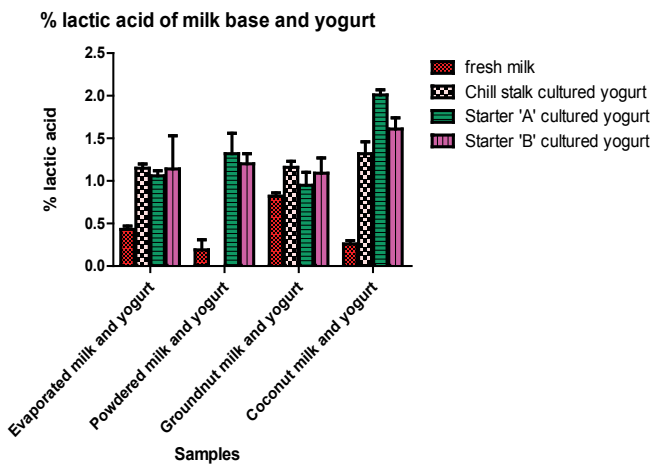

Figure 5) *% lactic acid of milk base and yogurt*

obtained for both the test sample and reference samples are a bit higher that the highest limit of 5.0 reported for yogurt. Nevertheless these yogurt samples also showed a decrease in pH from the starting milk base of pH 6.3.

Percent lactic acid of milk base and yogurt

The percent lactic acid of milk base and yogurt is depicted in Figure 5. The natural acidity of milk is due to citrates and phosphate present in the milk and dissolved CO_2 during the process of milking and thereafter. The natural acidity of milk as reported by DRINC [19] and Marshall [28] are 0.16-0.18% and 0.10-0.26%, respectively. The percent titratable acidity of fresh reconstituted powdered milk and fresh coconut milk were 0.19% and 0.26% accordingly and these values are within the range of acidity reported for milk. The high value of 0.82% for fresh groundnut milk and 0.43% for fresh reconstituted evaporated milk samples may be attributed to the pre-treatment given to the groundnut before blending and also the processing of the evaporated milk.

The presence of lactic acid in milk is due to the fermentation of lactose caused mainly by lactic acid bacteria and an increased in the natural acidity of the milk [29,30]. The % lactic acid of the test samples CEY, CGY, and CCY are 1.15, 1.16, and 1.32 respectively. For CPY, solid cheese was produced with very high attendant of gas production which was evidenced in the bulging out of the polyvinylchloride (PVC) bottle used for the incubation and gaseous release when the bottle was opened. The % lactic acid of reference samples are AEY 1.06, APY 1.32, AGY 0.95, ACY 2.01, BEY 1.14, BPY 1.20, BGY 1.09, BCY 1.16. The % lactic acid obtained for most of the yogurt samples are within the range of 1.3-1.8% reported for yogurt [23,31].

CONCLUSION

The result obtained in this work showed that chilli stalks contain microorganisms that is responsible for decreased pH and increased titratable acidity in yogurt. Therefore probiotic-like microorganism responsible for lactose fermentation could be isolated from chilli stalks. This will serve as a cheap and easily available source of non-dairy yogurt starter and a very good store of probiotics which is a desirable product, considering its immense health benefits. Isolation and characterization of probiotics microorganisms from chilli stalks is hereby recommended.

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