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Development of starter culture with driedMalabar tamarind (*Garcinia gummi-gutta*) fruits for buffalo milk curd

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***Abstract*-** Nowadays typical starter cultures are very expensive and because of this reason people tend to use a small amount of curd from a previous batch as the starter culture. But doing this continuously causes quality reduction of curds. When consecutive curds are used as starter cultures, wild cultures can be prominent because of dilution of Lactic Acid Bacterialconcentration. Therefore, major objective of this study was to prepare a freeze dried starter culture using dried Malabar tamarind (*Garcinia gummi-gutta*) fruits to avoid such situations. In addition, there was a necessity to check whether there is a difference between curds prepared with commercial culture and newly developed culture regarding consumer preference for sensory attributes. And also it was important to know the microbial composition of the end product. From the first sensory evaluation, the best inoculation rate of Malabar tamarind pulp was chosen and the curd made from it was compared with curd made from commercial starter culture in the second sensory evaluation. For chemical analysis, pH and titratable acidity were determined for three successive days. Microbial identification procedures were used in addition to the determinationof totalplate count (TPC), yeasts & molds counts and coliformcounts. Data were appropriately analyzed statistically. Consumer preference was higher for the curd made from highest concentration of Malabar tamarindpulp;therefore it was compared with the curd made from thecommercialstarter culture. Three species of *Leuconostoc* and four species of *Candida*were identified in the end product made from 5% of Malabar tamarind pulp inoculated starter culture. Therefore, results suggested that the Malabar tamarind can be used to prepare a starter culture successfully which can be freeze dried for further usage.

***Index Terms*-** curd, sensory evaluation, Malabar tamarind, *Leuconostoc*

I. INTRODUCTION

A starter culture can be defined as amicrobial preparation of a large number of cells of at least one microorganism that canbe added in to a raw material to produce a fermented food by accelerating and steering its fermentation process.Starter cultures have a multifunctional role in dairy fermentations. The production of lactic acid by fermenting lactose is the major role. Lactose is responsible todevelop thecharacteristic texture and overall flavor of the fermentedmilk products, and enhances preservation [1].Curd is a popular dairy productwhich possesses a great history that is prepared by inoculating a starter culture for milk fermentation as many other fermented dairy products.

Mainly in South Asian countries like India, Sri Lanka, Pakistan and Nepal curd is an ordinarily consumed food usually as a dessert. India remains at the top in buffalo curd production followed by Pakistan. On the other hand, milk obtained fromdairy animals such as goat, sheep and mare is used for curd preparation in Russia. Customarily Westerners do not prefer consumption of curd apart from Italians.

It is considered as a nutritious food which contains adequate amounts of proteins, carbohydrates, vitamins and minerals plus ample amount of fat for the wellbeing of life. Additionally, curd itself contains less cholesterol and high amount of calories. Nutritional composition,specially the amounts of fat can vary slightly with the buffalo breed that is used to obtain the milk. Curd is reflected to be more nutritious than milk as it is highly digestible while calcium and phosphorous become more bio-available in addition to the enhanced probiotic effect of Lactic Acid Bacteria (LAB).

Lactose fermentation is the technique of curd preparation which is performed by LAB. In other words, this is known as sour milk preparation or milk acidification which has become easy to be prepared by adding a small amount of inoculum. An alternative approachto attain milk acidification is the addition of acidogens, such as citric acid, hydrochloric acid, propionic anhydride or lactide[2].Lactose fermentation of food is the widely accepted method of preservation, which may also impart desirable sensory & nutritional properties to the fermented milk products[3].Although there are various fermentation methods, very few studies have carried out for buffalo milk curd as it is not a popular international dairy product rather a mouth feeling dessert consumed in parish level. Milk can be fermented solely by acid provision or adding acid together with Lactic Acid Bacterial strains.Basically,*Streptococcus lactis*,*Streptococcus diacetylactis*, *Streptococcus cremoris*,*Lactobacillus bulgaricus* and *Streptococcus thermophillus* are the strains using in commercial starter cultures for fermented dairy products according to the standards established by Sri Lanka Standard Institution (SLSI) [4]. Mostlyquality curds are prepared by rural community as they maintain a traditional mother culture.

Apart from these cultures,Malabar tamarindhas been using in southern Sri Lanka as a milk fermenter since ancient years while tamarind (*Tamarindus indica*) is used in Mexico as North American countries do not have specific starter cultures for curd as it is not a widespread dairy product in western republics. *Garcinia gummi-gutta*trees which belong to Family Guttiferae, grown in Southeast Asia, bear fruits which contains 30% acid.Objective of this study was to develop a starter culture for curd using *Garcinia gummi-gutta* in a cost effective way and to determine the microbial composition of the end product made using the newly developed starter culture.

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II. MATERIALS & METHODS

Preparation of curd

Curd preparation is an easy process that has been used since ancient times which has passed forward generation by generation. It can be implemented in house hold level very easily alike commercial and large scale manufacturing.A quality curd can be prepared by involvement of few steps that a majority of people learnt through their experience or as they were taught by cohorts.Ordinarily full fat milk is used for curd making which adds natural quality to its texture being a high fat food. The production steps are illustrated in the Figure 01as stated in Dassana (2014) [5].

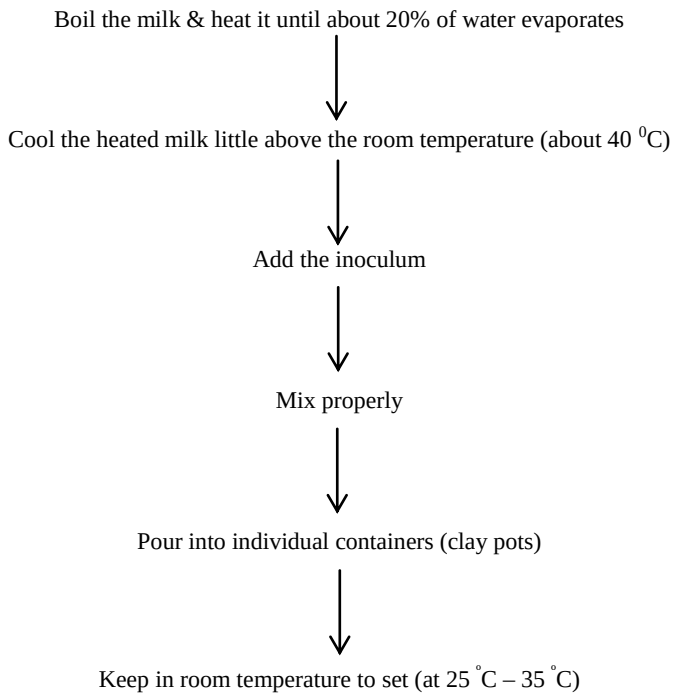

Figure 01: Production process of curd.

Development of starter culture with Malabar tamarind

A pulp of Malabar tamarindwas prepared by grinding 100 g of dried fruits with 200 mL of distilled water. The dried fruits were soaked in water for several days prior to the pulp preparation. A small quantity of Malabar tamarindpulp was added into boiled milk to form mother culture which causes milk curdlingwith higher amount of syneresis. A small quantity of that was used as the starter culture for the next curd series. Likewise, four consecutive curd series were preparedas intermediate cultures until to a point where the syneresis stops. Therefore, the 4th stage was identified as the starter culture which was freeze driedand the curds in 5th stage were used for sensory evaluations and other testing purposes, assuming that if it is in a better condition that provides evidence for goodness of its starter culture.

Sensory evaluations for the product

Two sensory evaluations were carried out appropriately for thirty panelists. They were instructed to rinse the palate with water and to wait at least thirty seconds between samples.The first sensory evaluation was done to find out the best curd made using the starter cultures which were prepared fromthree concentrations of Malabar tamarindpulp as 0.5%, 2.5% and 5%. The pulp was inoculated into boiled milk &the cultures were prepared as statedabove.Odour, texture, sourness, after-taste and overall acceptability were ranked according to the preference.Best curd, out of three products was selected from first sensory and that was compared with the commercial product in the second sensory evaluation. The sensory attributes of curd were evaluated by consuming curd without sugar and later three spoonful of sugar was provided to check whether overall acceptability will be changed.

Chemical parameters

Analysis of titratable acidity

The titratable acidity of the products was determined according to the guidelines provided in the SLSI 824: part 2: 1988 [4].

pH analysis

The pH of the products was determined according to the guidelines provided in the SLSI 824: part 2: 1988 [4]by 510 cyber scan digital pH meter.

Identification of microorganisms

Identification of microorganisms could be done upto the Genera level. Microorganisms present in the product were determined by inoculating samples onto different media.Loop full of curd samples were inoculated onto Sabouraud agar (Oxoid Ltd., Hampshire, UK)to isolate fungi which wereincubated at 37 $^\circ$C.The samples spread on MRS agar(Oxoid Ltd., Hampshire, UK)incubated anaerobically for 48 h at 35°C in order to isolate mesophilicLactobacilliand *Leuconostoc* which is a method previously discussed by Aly Savadogo, (2004) [6].Samples spread on Rogosa agar (Oxoid Ltd., Hampshire, UK) were incubated anaerobically for 48 h at 35°C for the isolation of lactobacilli as described by De Man J. C., (1960) [7]. Firstly, LAB was grown in MRS broth(Oxoid Ltd., Hampshire, UK)and small amount of it was inoculated onto MRS agar medium aseptically. Colonies which grew in different mediawere

identified by different colony morphologies;whereasGram's staining was done to observe the cell morphology.Isolated colonies were sub cultured by streaking on agar plates in order to obtain pure cultures. Pure cultures were initially Gram's stained. For Gram-positive cocci, catalase test was performed to differentiate *Staphylococcus* from *Streptococcus*. The presence of *Staphylococcus* is usually determined by addition of a drop of 3% H_2O_2 to a heavy bacterial suspension and observation of effervescence due to the release of Oxygen. *Streptococcus*and *Leuconostoc* were differentiated by gas production in MRS broth with inverted Durham tubes. Growth in 6.5% NaCl was observed, hemolysis test was performed for the isolated colonies of *Leuconostoc*for further confirmation [8]. For *Candida* species, blood serum test was performed to check the germ tube to confirm the presence of*Candida albicans* which is known as a causative agent for opportunistic human infection.

Bacteriological parameters

Total yeast & mold count

Plate counts for yeasts and molds were taken separately by performing spread plate method on acidified potato dextrose agar. Countswere taken by STUART SC6PLUS colony counter(Bibby Scientific Limited, Staffordshire, ST15 OSA, UK)according to the method previously described by Food and Drug Administration,(2001) [9].

Total Plate Count (TPC)

Total plate counts were taken by inoculating 1 mL of diluted samples on solid total plate count agar by spread plate method as described by Munsch-Alatossava, (2007) [10]. Colonies were counted by STUART SC6PLUS colony counter(Bibby Scientific Limited, Staffordshire, ST15 OSA, UK).

Number of coliforms

Three-tube method was performed with MacConkey agar as discussed in Harrigen, W. F., (1998) [11].

Statistical analysis

Statistical analysis was done basically by Minitab 15.0 statistical software at 95% confidence level & MS Excel 2010.Data which were obtained from the first sensory evaluation were analyzed by Friedman non parametric test for ranking. Data obtained from the second sensory evaluation were analyzed by Mann-Whitney Test and chi – square goodness of fit test. Data from the Chemical Analysis were analyzed by Tukey's Comparison Test for mean comparison.

III. RESULTS & DISCUSSION

The ranks given by the panelists for the products are summarized inthe Figure 02.

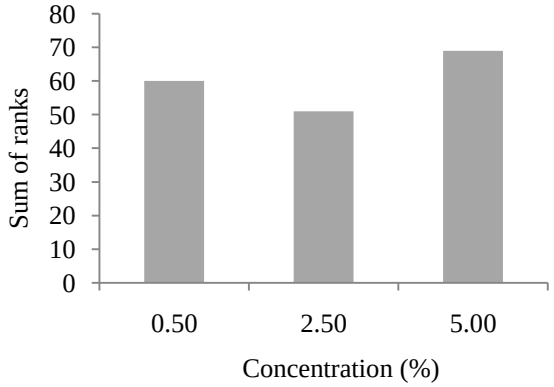

Figure 02: Sum of ranks of overall acceptability in the first sensory evaluation

There were no difference ($p>0.05$) observed for texture, sourness, after-taste and overall acceptability among the products made of three inoculation levels of Malabar tamarind (0.5%, 2.5% and 5.0%). However, the odour was varied significantly among the products ($p<0.05$). Highest sum of ranks wereobtained for the curd made from the highestconcentration of Malabar tamarind pulp (5%) inoculated starter culture. However, the values were not significant ($p>0.05$).

Based on the verbal communicationsdone with the panelists, it was found that when consume with sugar, the curd made from highest concentration of Malabar tamarind pulp inoculated starter culture, gave a better taste. Therefore,it was compared with a curd made from commercial starter culture in the second sensory evaluation. Mean scores for the sensory attributes tested in newly developed product using 5% Malabar tamarind pulp inoculated starter culture and curd made using commercial starter culture were summarized in the Table 01.

Table 01: Mean score for tasting panelists for sensory properties of curd.

Criteria	Curd made from commercial starter culture	Curd made from newly developed starter culture
Odor	3.43	3.90
Texture	4.00	3.67
Sourness	2.97	3.23
After taste	3.70	3.70
Overall acceptability	3.73	3.83

Values are presented as mean values, n=30, 1-5 hedonic scale (1=Dislike very much, 2=Dislike, 3=Neither like nor dislike, 4=Like, 5=Like very much)

The preference of the panelists for the curds made with the commercialstarter culture and 5% Malabar tamarind pulpinoculated starter culture is displayed in the Figure 03.

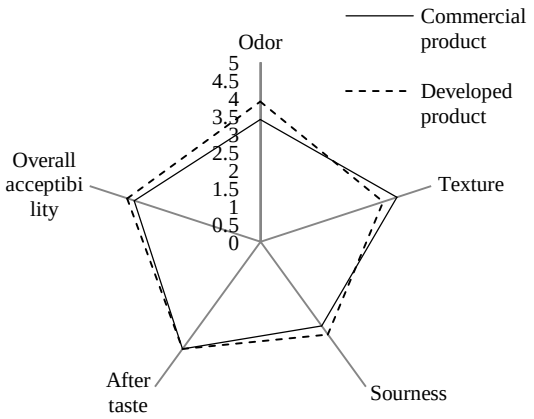

Figure 03: Mean scores for sensory properties of curds

Consumer preference ($p>0.05$) was not different for two curds made from newly developed starter cultureandcurd made from commercial starter culture when they were consumed with sugar. Furthermore, 96.67% people consider cost when buying curds. Only 3.45% people do not like to buy curds made from newly developed starter culture even its price is 33% less than the commercial product.

pH and titratable acidity were measured in triplicates in 3 consecutive days (Table 02).

Table 02: pH and titratable acidity of curds.

	pH			Titratable Acidity		
	1st day	2nd day	3rd day	1st day	2nd day	3rd day
CSCFC	4.47± 0.01	4.07± 0.010	3.72±0 .015	1.19±0 .035	1.30±0 .0485	1.65± 0.041
MTFC	3.98± 0.0057	4.33± 0.017 3	4.26±0 .036	1.47±0 .1124	1.16±0 .1003	1.07± 0.0271

Note: CSCFC = Commercial Starter Culture Fermented Curd; MTFC = Malabar Tamarind Fermented Curd

pH was quite higher in the curd made from commercial starter culture rather than the curd made from newly developed starter culture; however, But not significant ($p>0.05$).pHfulfilled the SLSI standards which should be<4.5 in curd. There are no standards established for titratable acidity of curd. When time passes, pH of the curds made from newly developed starter culture was gradually decreased and again increased,probably due to the treatment applied, but still within the limits established by SLSI [4]. pH of curds made from commercial starter cultures was drastically decreased.

When it comes to microbial composition, microorganisms cultured in MRS broth werestained.Gram+ bacilli wereclearly shown under light microscope. Catalase test, Endospore staining and motility test which are the confirmation tests for lactobacilli couldnot perform because the culture was not pure. Therefore,loop full of sample and broth were inoculated into separate solid MRS agar media and Rugosa agar media; however,Gram + bacilli werenot grown. When little amount of diluted sample was spread on solid MRS agar and incubated for 24 hours at 37 ^{0}C three colonies were identified with different morphologies. On the spread plate of Sabouraud agar, four different colonies were there. Those were streaked to obtain pure cultures on the same media and incubate under same conditions as previous.Those were then Gram stained and observed under light microscope. Two Gram + cocci and yeast spp. could be isolated on MRS agar and one Gram + cocci, three yeasts spp. were isolated on Sabouraud agar plates. Gram + cocci could be *Staphylococci*, *Streptocooci* or *Leuconostoc* spp. which appear in same morphology in Gram staining.To identify whether *Staphylococcus* was present catalase test was performed which possibly give positive results for *Staphylococcus*. All three species gave negative results for the catalase test.To differentiate between *Streptococcus* and *Leuconostoc*genera, gas production in MRS broth was observed. One species showed gas production in 24 hours of incubation, the other after 48 hours of incubation and the next when the lid was opened in the culture tubes after 72 hours of incubation, all at 37 ^{0}C. Then as confirmation tests growth in 6.5% NaCl broth was observed. As it was *Leuconostoc* no growth could be seen.When streaked on blood agar,*Leuconostoc*commonly shows α hemolysis or no hemolysis. Two species showed α-hemolysis and the other didn't show any hemolysis. Yeasts grown on both plates possess whitish, round colonies which were more or less same to colony morphology of *Candida*. None of the species did not show growth of germ tube in blood serum which proved that the *Candida albicans* were absent. TPC, yeast and mold counts and Coliform counts obtained for two products are summarized in the Table 03.

Table 03: Total plate counts, yeast and mold counts and Coliform counts for two curds.

Factor	Curd made from commercial starter culture	Curd made from newly developed starter culture
(TPC) 10^5 × CFU/g	1.95 ± 0.16	4.92 ± 0.21
Yeasts & molds	NC	NC
Coliforms	Nil	Nil

Note: NC – Not considerable, Values are presented as mean ± SD.

TPC for curd made from newly developed starter culture was considerably higher than the curd made from commercial starter culture. Normally only the plates that possess colonies around 30 – 300 were counted. Yeasts colonies & molds were less than 30 in all plates, therefore not counted. Furthermore, culture tubes which contained MacConkey agar didn't show any positive results therefore the Brilliant Green Bile broth test wasnot performed.Molds should be < 1 per gram, yeasts should be

<1000 per gram and Coliforms should be absent in a 1 gram portion [4].

IV. CONCLUSION

The results of this study reveal that the *Garcinia gummi-gutta* can beused to make a curd starter culture successfully for milk fermentation as an alternative to expensive commercial starter cultures. Moreover, results showed that the sensory attributes of both curds: curds made using newly developed starter cultureand commercial starter culture, are more or less the same in sensory properties. Therefore, it can be concluded that the Malabar tamarind pulp inoculated starter culturecan be used to produce a curd with the same sensory qualities comparatively tothat of the commercial curd. Furthermore, the consumer preferences observed for both curds were not different to each other with or without sugar. In addition, it is concluded that the microbial composition of the curd made withMalabar tamarind pulp inoculated starter culture contains sufficient amounts of LAB such as *Leuconostoc* for lactic acid fermentation.

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