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RESEARCH ARTICLE OPEN ACCESS

Development of Low-calorie Functional Yoghurt by Incorporating Mannitol Producing Lactic Acid Bacteria (*Leuconostoc pseudomesenteroides*) in the Standard Yoghurt Culture

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Abstract

As calorie-consciousness becomes a worldwide phenomenon, demand for low-calorie sweeteners is increasing. Compared to other sugars, the reduced calorific value of mannitol (1.6 kilocalories per gram) finds its application as a sweetener in low-calorie foods. The present study was conducted to develop low-calorie yoghurt by adding lactic acid bacteria (LAB) having significant mannitol production potential. *Leuconostoc pseudomesenteroides* IMAU:11666 was incorporated to standard yoghurt culture as adjunct culture. As mannitol is a food-grade sweetener with Food and Drug Administration (FDA) endorsement, the newly identified LAB strain can be used to develop low-calorie dairy products with beneficial effects. Side effects of other artificial sweeteners can also be reduced. Significantly high ($p \leq 0.05$) mannitol content was observed in functional yoghurt samples T1 (12.27 ± 0.18 g/l) and T2 (14.13 ± 0.30 g/l) with *Leuconostoc pseudomesenteroides* when compared to control samples. The calorific value obtained for yoghurt samples viz., C1, C2, T1, and T2 (86, 95, 98, and 92 kcal/100g, respectively) was less than control yoghurt C (99 kcal/100 gm). Microbial and chemical quality parameters of the functional yoghurt were in the safe and acceptable zone. On sensory evaluation of yoghurt samples, significantly higher overall and flavor scores were observed for sample T2 with *Leuconostoc pseudomesenteroides.*

Keywords: Mannitol, *Leuconostoc pseudomesenteroides,* Yoghurt, Calorific value, Microbial quality, Sensory quality

INTRODUCTION

Governments worldwide have been issuing science-based dietary advice for more than a century, regularly changing their recommendations to avoid nutritional shortages, reduce the risk of chronic illness, and enhance human health.[1] Despite these efforts, worldwide malnutrition and non-communicable disease (NCD) trends continue to rise. In addition, several countries lost the ability to achieve their health and sustainability goals due to these fully avoidable diet-related health outcomes.[2] Dairy products are widely consumed in many parts of the world and are a good source of protein and minerals.[3,4] On the other hand, dairy products contain ingredients that may cause health problems, such as cholesterol and saturated fatty acids (SFAs). However, the combination of SFAs found in dairy does not increase cardio-metabolic risk as much as other diets. The interplay of multiple ingredients and processing aspects produces varying outcomes in association studies examining the volume and type of dairy foods with type 2 diabetes risk.[5] This is where the importance of low-calorie dairy food formulation and development comes into play.[3]

D-Mannitol is a sugar alcohol with six-carbon, half sweet as sucrose, and has diverse applications in low-calorie foods and pharmaceuticals.[6] Mannitol imparts a cold, sweet flavour to meals, with sucrose accounting for around half of the sweetness. Mannitol does not dramatically raise the level of blood sugar, resulting in a lower glycemic index, making it beneficial to people with diabetes.[7] Lactic acid bacteria (LAB) are considered food-grade microorganisms with Generally Recognized as Safe (GRAS) status.[8] These microorganisms and their products can be added to foods without any restriction due to their beneficial effect on the gastrointestinal tract. In addition, mannitol produced by such food-grade organisms can act as a natural sweetener in food products and is therefore considered to be the alternative to artificial sweeteners.[9]

A *Leuconostoc citreum* strain was previously isolated from sourdoughs and used as a starter in apple juice. After 48 hours of fermentation, the sucrose and fructose concentrations in the apple juice were dramatically reduced (83%) with a concurrent increase in mannitol concentration. As a result, it had less sugars but retained sweetness, indicating that converting fructose to mannitol might lead to healthier drinks.[10] Moreover, it is a food-grade sweetener with Food and Drug Administration (FDA) endorsement with ADI of 0-50 mg/kg body weight.

The present study was conducted to develop low-calorie yoghurt by adding mannitol-producing lactic acid bacteria *Leuconostoc pseudomesenteroides* to the standard culture. In addition, chemical, microbiological and sensory parameters of yoghurt were also studied.

MATERIALS AND METHODS

Preparation of functional yoghurt

Yoghurt was prepared according to Tamime and Robinson.[11] The cultures used in different treatment yoghurt samples are mentioned in Table 1.

Estimation of calorific value

The calorific values were estimated at Confederation for Ayurvedic Renaissance Keralam Ltd (CARE, Keralam) laboratory, Thrissur, Kerala, using formula method.

The equation used was: kcal 100g-1 = (% protein × 4) + (% lactose × 4) + (% fat × 9).

Chemical analysis

Titratable acidity

Ten grams of yoghurt sample was weighed in a suitable dish or basin. To this, 30 ml of warm water and 1 ml of phenolphthalein indicator were also added. Sample along with reagents mixed well and titrated against 0.1N NaOH solution. 10 g of material diluted with 30 ml of water taken in another dish served as a control for comparison of colour.

pH

pH was measured using an electronic digital type pH meter Hanna.[12]

Syneresis

The yoghurt (10 g) was spread across the Whatman No. 1 filter paper as a thin layer to cover the surface. The yoghurt was filtered for a period of 10 min. The quantity of liquid that passed through the filter paper was estimated and recorded. The percentage of syneresis was calculated by dividing the weight of the liquid passed with the weight of the initial sample multiplied by 100.[13]

Microbiological analysis

LAB count

Enumeration of LAB in the yoghurt sample was carried out (IDF, 117: 2003) in selective media for *Lactobacillus delbrueckii* sub-sp. *bulgaricus* (MRS media) and for *Streptococcus thermophillus* (M-17 media). Serial dilutions of the samples were prepared using peptone diluents. Yoghurt samples were plated at 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-7} dilution and incubated at 37°C for 48h.

Coliform count

Direct samples were decimally diluted at 10^{-1} in sterile peptone water and plated 1 ml sample with 20 ml of VRBA plated into Petri dishes for enumeration of coliform bacteria as coliform colony forming units per millilitre by pour plate method. Plates were incubated at 37°C for 24 hours.[14]

Yeast and mould count

Potato dextrose agar plates were prepared with 10% tartaric acid. Spread plating technique was adopted by spreading 0.1 ml of direct and 10^{-1} diluted sample over sterile plates. Plates were incubated at 25°C for five days. The development of colonies was observed.

Sensory evaluation

Sensory evaluation of yoghurt samples was carried out using a nine-point hedonic scale.[15] Scorecard was also prepared. Different sensory parameters such as appearance and colour, body and texture, flavour, and overall scores were analyzed.

Statistical analysis

Six replications were carried out, and the data obtained was subjected to statistical analysis using SPSS version 24.0. Non-parametric tests and one-way ANOVA were used to evaluate the sensory parameters of yoghurt.

Table 1. Different treatments of yoghurt prepared for analysis

Treatments	Details of starter lactic acid bacteria added
Control 1 (C)	Standard yoghurt cultures (*Streptococcus thermophilus, Lactobacillus bulgaricus*)
Control 2 (C1)	Standard yoghurt cultures + *Leuconostoc mesenteroides*
Control 3 (C2)	Standard yoghurt cultures + *Leuconostoc mesenteroides*+ optimum sugar
Treatment (T1)	Standard yoghurt cultures + *Leuconostoc pseudomesenteroides*
Treatment (T2)	Standard yoghurt cultures + *Leuconostoc pseudomesenteroides* + optimum sugar

RESULTS

Estimation of mannitol content and calorific value in functional yoghurt

Estimation of mannitol content in functional yoghurt samples, colorimetric assay with slight modifications was performed as per the method suggested by Sanchez.[16] Yoghurt samples prepared were given in Table 1. Comparison of mannitol content in Control yoghurt samples (C1 & C2) and treatments (T1 & T2) were carried out. Overall mean mannitol content was estimated as 7.67 ± 0.31, 6.13 ± 0.18, 12.27 ± 0.18 and 14.13 ± 0.30 g/l respectively for C1, C2, T1 and T2. A significant increase ($p \leq 0.05$) in mannitol content was observed in treatment groups T1 and T2 than C1 and C2, which indicates that the functional yoghurt with *Leuconostoc pseudomesenteroides* shows higher mannitol production.

The calorific value of functional yoghurt was estimated in CARE Keralam laboratory, Thrissur, and compared with control, and the result is depicted in Table 2. The value observed for plain yoghurt prepared with standard cultures *S. thermophillus* and *L. bulgaricus* was 99 kcal in 100 gm. The calorific value obtained for C1, C2, T1, and T2 were 86, 95, 98, and 92 kcal, respectively, in 100 gm using a bomb calorimeter. It was clear that the functional yoghurt with *Leuconostoc pseudomesenteroides* and optimum sugar produce low caloric yoghurt than normal standard yoghurt.

Table 2. Calorific value of yoghurts

Yoghurt samples	Calorific Value (kcal/100gm)
C (*S. thermophillus* and *L. bulgaricus*)	99
C1 (*S. thermophillus, L. bulgaricus* and *L. mesenteroides*)	86
C2 (C1 and sugar 4 per cent)	95
T1 (*S. thermophillus, L. bulgaricus* and *L. pseudomesenteroides*)	98
T2 (T1 and sugar 4 per cent)	92

Chemical analysis

The result of titratable acidity, pH, and syneresis were given in Table 3 for samples C, C1, C2, T1, and T2. A significant difference ($p \leq 0.05$) in acidity and pH was observed among the samples. In addition, freshly prepared yoghurt samples were examined for syneresis. Statistical analysis using one-way ANOVA (p-value ≤0.05) showed a significant difference between the treatment samples.

Microbiological analysis

Lactic count obtained (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*), coliform count, and yeast and mould count obtained were given in Table 4. The mean Streptococcus count observed in C, C1, C2, T1, and T2 were 7.62±0.01, 7.81±0.01, 7.8±0.01, 7.74±0.01, and 7.87±0.01 at log cfu/g, respectively. Concerning L. bulgaricus count, the mean count observed in C, C1, C2, T1, and T2 were 7.62±0.02, 5.71±0.03, 5.36±0.03, 3.28±0.02, and 2.36±0.03 log cfu/g. The entire control and treatment yoghurt samples showed the absence of coliform in direct plating. No significant difference was noted in any of the samples used for yeast and mould count.

Sensory evaluation

Overall score obtained for C, C1, C2, T1 and T2 were 14.63 ± 0.40, 15.49 ± 0.51, 16.97 ± 0.56, 15.63 ± 0.45 and 18.35 ± 0.54 respectively. The score obtained shows that the sample T2 had a higher value and was comparatively more acceptable by the panelist with its appearance, colour, body, texture, and flavour. On non-parametric test analysis, the p-value was ≤0.05, indicating a significant difference between different yoghurt samples with respect to overall

Table 3. Chemical quality of yoghurt samples

Chemical parameters	Control			Treatment	
	C	C1	C2	T1	T2
Titratable acidity (Per cent of lactic acid)	0.72±0.01[c]	0.75±0.00[b]	0.73±0.01[cb]	0.74±0.00[cb]	0.79±0.00[a]
pH	4.59±0.03[ab]	4.51±0.01[b]	4.60±0.03[a]	4.58±0.03[ab]	4.52±0.01[b]
Syneresis (%)	27.17±0.88[a]	25.92±0.50[a]	20.45±1.05[c]	23.35±0.88[b]	14.08±0.81[d]

Each value is a mean of six observations with SE
Means with different superscript in same rows differ significantly ($p \leq 0.05$).

Table 4. Microbial quality of yoghurt samples

Lactic acid bacteria cultures	Microbial counts				
	Control (log cfu/g)			Treatment (log cfu/g)	
	C	C1	C2	T1	T2
S. thermophilus counts	7.62±0.01[d]	7.81±0.01[b]	7.8±0.01[b]	7.74±0.01[c]	7.87±0.01[a]
L. bulgaricus counts	7.62±0.02[a]	5.71±0.03[b]	5.36±0.03[b]	3.28±0.02[c]	2.36±0.03[d]
Coliform count	NIL	NIL	NIL	NIL	NIL
Yeast and mould counts	0.49±0.04[ns]	0.48±0.10[ns]	0.51±0.06[ns]	0.48±0.07[ns]	0.48±0.05[ns]

Each value is a mean of six observations with SE; Means with different superscript in same row differ significantly (p≤0.05); ns – non significant (p>0.05).

Table 5. Sensory scores of functional yoghurts

Yoghurt samples	Sensory scores		
	Appearance and colour	Body and texture	Flavour
C	4.00 ± 0.10[ns]	3.75 ± 0.20[ns]	6.86± 0.22[bc]
C1	4.04 ± 0.17[ns]	3.91± 0.18[ns]	7.53± 0.20[d]
C2	4.03 ± 0.16[ns]	4.05± 0.16[ns]	8.89± 0.26[c]
T1	4.25 ± 0.14[ns]	4.17 ± 0.17[ns]	7.19± 0.26[a]
T2	4.47 ± 0.18[ns]	4.46± 0.16[ns]	9.39± 0.25[ab]

Each value is a mean of six observations with SE; Means with different superscript in same column differ significantly (p≤0.05); ns – non significant (p>0.05).

mean scores. No significant differences were noticed in scores of different samples related to appearance and colour and body and texture (Table 5). However, a significant increase in flavour score was noted in sample T2.

DISCUSSION

Estimation of mannitol content and calorific value in functional yoghurt

Otgonbayar et al.[17] evaluated the mannitol production of different Leuconostoc strains isolated from kimchi. They discovered that the strain *L. citreum* KACC 91348P grew faster (high growth rate) and generated more mannitol (high production rate) than the other strains. The mannitol production was reported to be maximum (14.83 g/L/h) when it was grown in a batch fermenter (30°C and pH 6.5). Jung et al.[18] made baechu kimchi, fermented traditional Korean vegetable cuisine with a mannitol-producing *L. mesenteroides* strain (10^7 cells/g kimchi) as the starter. The mannitol level of starter kimchi was greater than that of non-starter kimchi. So far, there haven't been any reports of kimchi fermenting with fructose added on purpose. The addition of fructose to kimchi is expected to increase the formation of mannitol. Rice et al.[19] described a novel use of a Leuconostoc strain to create a healthful beverage. Helanto et al.[20] used chemical treatment to create a *Leuconostoc pseudomesenteroides* mutant with a fructokinase activity of only 10% of the wild-type. In the presence of glucose and fructose, the mutant generated more mannitol than the wild type. This is because of the blocking of fructose 6-phosphate production, and more fructose was transformed into mannitol.

Dwivedi[21] observed that mannitol intake would be slightly laxative if it exceeded 20g per day. Mannitol is a food-grade sweetener with Food and Drug Administration (FDA) endorsement with ADI of 0-50 mg/kg body weight. The mannitol content observed in the current research meets the above-mentioned recommendation. Previous research used different techniques in the detection of mannitol from organic materials. Yun and Kim[22] detected mannitol content with the help of HPLC (High-Performance Liquid Chromatogram) using a cation exchange resin column at 85°C. Sanchez[16] and Grobben et al.[23] used the colorimetric assay method to detect mannitol from complex biological material. Chandan et al.[24] estimated the amount of sugar alcohol content in the yoghurt sample and found it negligible in quantity compared with other mono and disaccharide sugars.

Benedict and Fox[25] determined energy values of various foods and excreta by heat combustion using a bomb calorimeter. They estimated the calorific value of dried skimmed milk as 4.89 calories per litre of oxygen. The study conducted by Kroger and Weaver[26] mentioned that the average calorific value of 44 yoghurt samples examined from central Pennsylvania ranged 62.3 - 127.0 kcal/100g with an average of 103.2 kcal/100 g. The average calorific value of yoghurt lies between ice cream (200 kcal/100g) and whole milk (60 kcal/100g). The values obtained in the present study are in accordance with the reported values. AOAC[27] detailed the method of estimating calorific value in feed and food samples using a bomb calorimeter. Smit et al.[28] determined the calorific value of whole milk, milk with two percent fat, milk powder (skimmed), gouda cheese, cottage cheese, cheddar cheese, and cottage cheese using a bomb calorimeter. In the study conducted by Salman et al.,[29] the calorific values of buffalo milk ($p<0.05$) were found to be higher than cow milk. Dairy Council of California reported the calorific content of low-fat plain yoghurt as 154 kcal in 245g of samples. The calorific value of plain whole milk yoghurt per 100g is 61 kcal.[30]

Chemical analysis

Younus et al.[31] determined titratable acidity of three different brands of plain yoghurt, with AOAC method No. 947.05 (1990) and observed an acidity of 0.89, 0.87 and 1.13 percent lactic acid with standard deviation 0.02, 0.04, and 0.05 respectively. In a study conducted by Joseph et al.,[32] a pH range of 4.08-3.7 was observed for plain yoghurt and 4.11 - 4.10 for fruit yoghurt. The study conducted by Nazni and Komathi[33] on papaya and banana pulp incorporated fruit yoghurt observed that pH was higher (6.3 and 6.8) when compared with control cow milk yoghurt and commercial yoghurt (4.5 and 4.9). According to Foda et al.,[34] prolonged yoghurt storage at low temperature 4°C decreased syneresis to half of its initial value compared with fresh yoghurt samples. Roy et al.[35] conducted a study on yoghurt supplemented with fruit pulp and reported that syneresis decreased during the initial five days of storage and increased with an increase in period of storage in yoghurt. In this study, fresh control cow milk yoghurt had a syneresis percent of 48.5.

Microbiological analysis

In the case of *Lactobacillus bulgaricus*, the count gets reduced in the present study. A significant difference was observed between control and treatment in both LAB counts. Adjunct culture might have influenced the growth of yoghurt cultures. This was in accordance with the research done by Ranasinghe and Perera,[36] where Lactobacillus count was less than Streptococcus count in fresh yoghurt. Arnott et al.[37] used the bacteriological media violet red bile agar (Difco) to detect coliforms. Plates were incubated at 37°C for 24 h, and the result ranged from < 1 to 110 cfu/g. Mohammed and El Zubeir[38] determined the yeast and mould count of powdered milk and fresh milk yoghurt as 5.78 ± 1.56 log10 cfu/ml and 5.65 ± 1.86 log10 cfu/ml, respectively. As per FSSA standards, the yeast and mould count in fermented products should not exceed more than 100 cfu/ml. Less than 10 log cfu/g in the current study indicate good sanitary condition.

Sensory evaluation

McGregor and White[39] judged fruit flavoured yoghurt with various sweeteners and observed that yoghurt with no added sweetener had a significantly ($P<0.001$) lower flavour score than the sweetened. All sweetened yoghurts scored good to excellent. In the present study, a significantly higher flavour score was observed for sweetened yoghurts than others. In a study on whether sucrose esters improved the quality of non-fat low-calorie yoghurt by Farooq and Haque,[40] fat less low-calorie yoghurt sweetened with aspartame had a calorific value of 101.4 kcal per 226.8g than regular yoghurt. Yogurts with aspartame had better body, texture, mouthfeel, and acceptance. Skriver et al.[41] observed that the sensory texture of stirred yoghurt varied with different fermentation time-temperature combinations, dry matter content, heat treatment of milk, and composition of starter organisms.

Guven and Karaca[42] studied the effect of different sugar levels 18, 20, and 22 percent and fruit content 15, 20, and 25 percent on the sensory parameters of frozen yoghurt. Sensory evaluation was done on colour, appearance, structure, taste, smell, and consistency at a 20-point scale by five expert panel members. Frozen yoghurt with 22 percent sugar and 25 percent fruit concentrations was most preferred by the panel members. According to Mani-Lopez et al.,[43] yoghurt with L. casei had pH 4.11 was better perceived by the sensory panels because of its less acidic nature than control yoghurt of pH 3.96.

CONCLUSION

Microbial fermentation, chemical synthesis, and enzymatic conversion are the different techniques used to make mannitol. Although chemical methods are the most common for producing mannitol, microbial fermentation has several advantages enabling it to be produced on a large scale. Heterolactic fermenters, Leuconostoc species are the most significant among them. As a result, Leuconostoc species like *Leuconostoc pseudomesenteroides* appear to be appropriate hosts for mannitol production, according to the present study. The higher mannitol synthesis allows the manufacturing of low-calorie fermented dairy products, which improve its application in healthy diets. In addition, microbial and chemical quality parameters of the functional yoghurt prepared here were in the safe and acceptable zone. The findings from this study indicate that *Leuconostoc pseudomesenteroides* can be added to the standard culture to develop low-calorie yoghurt with acceptable sensory, chemical, and microbial quality.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not Applicable.

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