The Pharma Innovation Journal 2022; 11(11): 2063-2066

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(11): 2063-2066

www.thepharmajournal.com
Received: 20-09-2022
Accepted: 25-10-2022

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Herbal (*Stevia rebaudiana*) sugar substitute for the preparation of low-calorie yoghurt

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Abstract
Sucrose plays a significant role in many dietary health problems such as diabetes mellitus, coronary heart disease and obesity. *Stevia rebaudiana* (sugar leaf) is one of the most non-caloric natural sweeteners known to humankind, and due to its beneficial attributes on human health; there is a greater possibility of its use in dairy industry. Therefore, the aim of this study was to investigate the effect of replacing cane sugar/sucrose with *Stevia rebaudiana* leaf extract powder (at 25%, 50%, 75% and 100% levels) on the physiochemical, textural, microbiological and organoleptic qualities of the developed low-calorie yoghurt. There were no significant differences in curd setting time, pH, fat and total solids content of the yoghurt supplemented with cane sugar, stevia and a mixture of cane sugar and stevia. A panel of six judges, using the nine-point hedonic scale, carried out sensory profiling of the control and treatment yoghurt samples. The supplementation of stevia's leaf extract powder in yoghurt increased the flavour and taste, body and texture, and overall acceptance scores, but did not affect the appearance and colour. The textural properties like firmness, consistency, cohesiveness and viscosity index of the control and treatments exhibited significant difference among them. As the cane sugar replacement level increased, the textural parameters of the treatments were also increased. The viscosity index was lower in cane sugar yoghurts when compared to the yoghurts made with stevia or stevia + cane sugar, which was also reflected in the sensory scores. The coliform and yeast and mould count of control and treatments exhibited no significant difference among them and were within the FSSAI (2012) prescribed limits. From the above results, it is clear that the use of *Stevia rebaudiana* in the production of yoghurt not only has a positive effect on the energy composition of the product but also improves the organoleptic and textural characteristics. Hence, it can be concluded that low-calorie yoghurt can be prepared by replacing cane sugar with *Stevia rebaudiana* leaf extract powder up to 100 per cent levels without affecting the physiochemical, microbiological, textural and sensory properties.

Keywords: Low-calorie yoghurt, *Stevia rebaudiana*, sensory and textural properties

1. Introduction
According to FDA (1991) [1], "Yoghurt is produced by culturing cream, milk, partially skimmed milk or milk either alone or in combination with lactic acid-producing bacteria *viz*., *Lactobacillus bulgaricus and Streptococcus thermophilus*". The regulations specify that yoghurt before the addition of bulky flavours contains not less than 3.25 per cent milk fat, 8.25 per cent milk solids-not-fat and titratable acidity of 0.9 per cent. Yoghurt is rich in nutrients like potassium, calcium, protein and vitamin B and has beneficial effects on human health by supplying prebiotic and probiotic bacteria. It helps to strengthen the immune system and improves lactose digestion and gastrointestinal conditions including lactose intolerance, constipation, diarrhoea, colon cancer, inflammatory bowel disease and allergies (Fitzgerald *et al*., 2004) [2].
Sucrose plays a significant role in many dietary health problems such as diabetes mellitus, coronary heart disease, and obesity. Raw cane sugar (or brown sugar) normally contains 94 to 98.5 per cent sucrose and 1.5 to 6 percent non-sucrose components (Koltuniewicz, 2010) [3]. Probably, the cane sugar aggravates blood sugar in people with diabetes mellitus and has no nutritional value. *Stevia rebaudiana* (sugar leaf) is one of the most non-caloric natural sweeteners known to humankind, and due to its beneficial attributes on human health; there is a greater possibility of its use in the dairy industry. Hence, instead of cane sugar, *Stevia rebaudiana* leaf extract powder was used to enhance the sugar content in the yoghurt.
Stevia rebaudiana owes its sweet taste to the steviol glycosides contained in its leaves. More than eight glycosides have been identified, of which stevioside and rebaudioside A are the most important glycosides. Stevioside is present in the leaves in the largest amounts (4–13% DM) and it is 150–300 times sweeter than sucrose.

Although, rebaudioside A is present only in smaller amounts than stevioside (2–4% DM), it is the sweetest of all steviol glycosides (200–400 times sweeter than sucrose) and has the best taste. It dissolves in water better than stevioside and it is resistant to various pH environments (Carakostas *et al.* 2008; Lemus-Mondaca *et al.* 2012) [4, 5]. Therefore, by considering the valuable aspects of *Stevia rebaudiana*, the present study was envisaged to prepare a low-calorie yoghurt by using *Stevia rebaudiana* leaf extract powder and to evaluate the physiochemical, textural, microbiological and sensory properties of the developed product.

2. Materials and Methods

The experiments were carried out by utilizing the facilities available at the Department of Livestock product technology (Dairy Science), Veterinary College and Research Institute, Namakkal. Fresh cow milk was purchased from Livestock Farm Complex, Veterinary College and Research Institute, Namakkal. Spray-dried skim milk powder (Aavin Dairy, Erode) was used to adjust the solids-not-fat content in yoghurt. Ampoules of freeze-dried culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were obtained from National Dairy Research Institute, Karnal, Haryana. Cane sugar, purchased from the local market, was used in the experiment. *Stevia rebaudiana* leaf extract powder was purchased Arboreal Bioinnovations Pvt. Ltd.

2.1 Experimental design

Different treatments of low-calorie yoghurt were designed as follows:

Treatments	Details
Control	Plain yoghurt without replacement of cane sugar
T_1	Yoghurt with 25% replacement of cane sugar
T_2	Yoghurt with 50% replacement of cane sugar
T_3	Yoghurt with 75% replacement of cane sugar
T_4	Yoghurt with 100% replacement of cane sugar

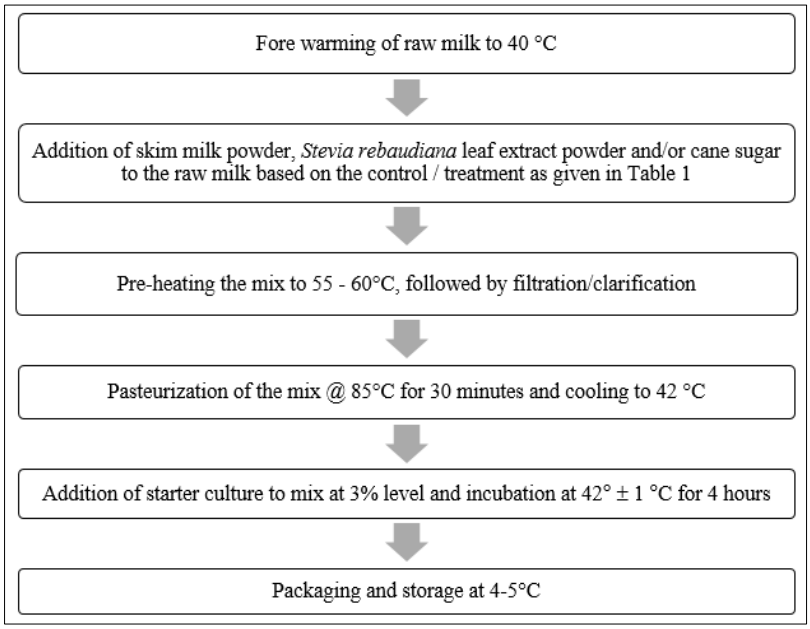
2.2 Preparation of low-calorie yoghurt using *Stevia rebaudiana* leaf extract powder

Plain yoghurt was prepared as per De (2004) [6]. Yoghurt mixes were prepared by incorporating cow milk, skim milk powder and cane sugar, and the *Stevia rebaudiana* leaf extract powder was used to replace the cane sugar at 25, 50, 75 and 100 per cent levels. The yoghurt mixes, after filtration/clarification at 55-60 °C, was pasteurized at 85°C for 30 minutes and then it was cooled to 42°C. Yoghurt starter culture containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* was added at the rate of 3 per cent, and incubated at 42 ± 1 °C for 4 hours until a firm coagulum of yoghurt was formed, which is then packed and stored at 4-5 °C.

Table 1: Ingredients for 100 grams of low-calorie yoghurt mixes

Ingredients (in grams)	Control	T_1 (25%)	T_2 (50%)	T_3 (75%)	T_4 (100%)
Cow milk	87.5	87.5	87.5	87.5	87.5
Skim milk powder	6.5	6.5	6.5	6.5	6.5
Sugar	6	4.5	3	1.5	0
Stevia rebaudiana leaf extract powder	0	1.5	3	4.5	6
Total	100.00	100.00	100.00	100.00	100.00

2.3 Process flow chart for the preparation of low-calorie yoghurt



2.4 Physicochemical analysis of yoghurt

pH was estimated using a digital pH meter. The total solids content was determined according to AOAC (1990), 15th edition. Fat was estimated as per the procedure described in IS:SP:18 (Part XI) – 1981.

2.5 Texture analysis of yoghurt

The firmness and consistency of low-calorie yoghurt were characterized using the Instron Texture Analyser (Model: TA.XT Plus, Stable Microsystems) and Texture Expert Software. A Back Extrusion Cell (A/BE) with a 35mm disc

and extension bar using a 5kg load cell was used to measure the firmness and consistency of the developed low-calorie yoghurt. Six measurements for each sample were recorded using a 5 mm diameter and 150 mm long stainless steel probe adapter attached to a 5kg load cell. The penetration depth at the geometrical centre of the samples contained in a standard size back extrusion container (50mm diameter) was 30 mm and the penetration speed was set at 1.0 mm/s. The firmness of the samples was determined as the peak compression force during penetration. The maximum negative force is taken as the indication of consistency/resistance to flow off the disc during back extrusion. All determinations were carried out at 15 °C.

2.6 Organoleptic evaluation

Yoghurt samples were evaluated for appearance, flavour, body and texture and total sensory by a panel using a 9-point Hedonic scale (Dubey *et al*., 2011) [7]. All the samples were appropriately coded before being subjected to sensory evaluation.

2.7 Statistical analysis

The data obtained in all the experiments were analysed statistically by applying two-way ANOVA by approved statistical methods of SPSS (version 28.0.1.1).

3. Results and Discussion

3.1 Physicochemical analysis of yoghurt

Table 2 shows the Mean ± SE values of physicochemical properties of control and treatment yoghurt samples. Statistical analysis revealed no significant differences in the curd setting time, pH, fat and total solids content of the yoghurt supplemented with cane sugar, stevia and a mixture of cane sugar and stevia. The results are in accordance with the findings of Vijay *et al*. (2022a) [8], who analysed the dietetic yoghurt optimized with palm jaggery and reported that no variation in the physicochemical properties was found between plain yoghurts and palm jaggery yoghurts.

The curding setting time of the control and treatments ranged from 4.09 – 4.20. The pH value of control and treatments yoghurts ranged from 4.55 – 4.60. The fat and total solids content of the control and treatments were within the FSSAI (2012) [9] prescribed limits of 3.0 and 13.5 per cent respectively. Therefore, substitution of *Stevia rebaudiana* leaf extract powder in the place of cane sugar did not affected the physicochemical properties of yoghurt. The results are also in agreement with the values reported by Malarkannan and Geevarghese (1996) [9] and Ghadge *et al.* (2008) [10].

Table 2: Physicochemical properties of yoghurt samples (Mean ± SE)

Parameters	Control	T1	T2	T3	T4
Setting time (hours)	4.09 ± 0.02	4.13 ± 0.01	4.14 ± 0.02	4.18 ± 0.03	4.20 ± 0.02
pH	4.55 ± 0.01	4.57 ± 0.01	4.58 ± 0.00	4.59 ± 0.01	4.60 ± 0.00
Fat (%)	3.02 ± 0.14	3.02 ± 0.16	3.07 ± 0.11	3.03 ± 0.13	3.06± 0.12
Total solids (%)	23.79 ± 0.13	23.78 ± 0.16	23.77 ± 0.21	23.80 ± 0.23	23.78 ± 0.20

Different mean values in a row do not differ significantly (P ≥ 0.05)

3.2 Textural and microbial analysis of yoghurt

The results pertaining to the textural and microbiological properties of control and treatment yoghurt samples are presented in Table 3. Statistical analysis of control and treatment yoghurts revealed a significant difference in the textural parameters such as firmness, consistency, cohesiveness and viscosity index. The textural parameters exhibited an increasing trend towards the 100 per cent replacement of cane sugar with *Stevia rebaudiana* leaf extract powder. This supported by the findings of Vijay *et al*. (2022b) [11], who analysed the palm sugar substituted yoghurt and reported similar results with respect to textural properties. Therefore, as the cane sugar replacement level increased, the textural properties of the treatments also tend to be increased. The results are also in accordance with the findings of Yilmaz-Ersan and Topcuoglu (2021) [12].

Table 3: Textural and microbiological properties of yoghurt samples (Mean ± SE)

Parameters	Control	T1	T2	T3	T4
Firmness (g)	$50.33^{c} \pm 4.67$	$55.28^{c} \pm 6.55$	$88.54^{b} \pm 6.51$	$94.68^{b} \pm 5.93$	$126.71^{a} \pm 5.42$
Consistency (g-sec)	$1013.69^{e} \pm 70.43$	$1324.82^{d} \pm 94.35$	$1689.61^{c} \pm 96.41$	$2087.68^{b} \pm 79.96$	$2434.68^{a} \pm 83.47$
Cohesiveness (g)	$29.24^{e} \pm 2.39$	$47.02^{d} \pm 4.82$	$67.23^{c} \pm 5.11$	$88.70^{b} \pm 5.05$	$103.92^{a} \pm 4.93$
Viscosity index (g-sec)	$25.65^{e} \pm 3.88$	$35.56^{d} \pm 4.01$	$68.62^{c} \pm 4.94$	$79.39^{b} \pm 4.89$	$97.16^{a} \pm 4.22$
Yeast and mould count (log_{10} cfu/ml)	1.64 ± 0.14	1.39 ± 0.18	1.38 ± 0.39	1.14 ± 0.30	1.02 ± 0.52
Coliform count (log_{10} cfu/ml)	Nil	Nil	Nil	Nil	Nil

Means bearing differ superscripts in a row differ significantly ($p < 0.05$)

The yeast and mould count (cfu/ml) of control and treatments exhibited no significant difference among them and were within the FSSAI (2012) [13] prescribed limits of 100 per gram (maximum) respectively. Also, the results revealed that the coliforms were absent in both control and treatment yoghurt samples, which confirms that the product has been produced under the hygienic standards.

3.3 Sensory evaluation of yoghurt

The samples of yoghurt were subjected to sensory evaluation by a panel of six judges using the scorecard adopted by Pearce and Heap (1974) [14] and the results are presented in Table 3.

Table 4: Sensory properties of yoghurt samples (Mean ± SE)

Parameters	Control	T1	T2	T3	T4
Appearance and Colour	8.44 ± 0.02	8.43 ± 0.03	8.41 ± 0.03	8.42 ± 0.02	8.41 ± 0.03
Body and Texture	8.58^{c} ± 0.02	8.61^{bc} ± 0.02	8.62^{b} ± 0.01	8.64^{b} ± 0.01	8.67^{a} ± 0.01
Flavour	8.86^{b} ± 0.01	8.87^{b} ± 0.01	8.87^{b} ± 0.01	8.89^{a} ± 0.01	8.89^{a} ± 0.01
Overall acceptability	8.58^{c} ± 0.02	8.60^{c} ± 0.02	8.60^{c} ± 0.02	8.63^{b} ± 0.01	8.67^{a} ± 0.01

Means bearing different superscripts in a row differ significantly ($p < 0.05$)

The appearance and colour scores of control and treatments exhibited no significant difference among them. But, the body and texture and flavour scores of control and treatments exhibited a significant difference ($p < 0.05$) among them. Similarly, the overall acceptability scores of control and treatments exhibited a significant difference ($p < 0.05$) among them. The sensory properties, except appearance and colour, was found to be improved in treatment yoghurt samples when compared to the control yoghurt samples. In addition, the treatment with the 100 per cent substitution of cane sugar by *Stevia rebaudiana* leaf extract powder scored the maximum, when compared with the lesser percent substituted treatment yoghurts. Therefore, substitution of cane sugar by *Stevia rebaudiana* leaf extract powder in yoghurt was found to improve the sensory qualities of the resultant low-calorie yoghurt.

The results are in accordance with Vijay *et al*. (2022a) [11], who reported that the replacement of cane sugar with palm jaggery along with turmeric solution as a colouring agent did not affect the overall acceptability. The appearance, body and texture, flavour and total scores exhibited in the present study are in accordance with the reports of Malarkannan and Geevarghese (1998) [9] for yoghurt prepared with condensed coconut water and also in resemblance with Ghadge *et al.* (2008) [10] for apple pulp incorporated yoghurt.

4. Conclusion

From the results, it can be concluded that the low-calorie yoghurt samples can be prepared by replacing cane sugar with *Stevia rebaudiana* leaf extract powder up to 100 per cent without affecting the sensory properties. Moreover, the use of *Stevia rebaudiana* in the production of yoghurt not only has a positive effect on the energy composition of the product but also improves the organoleptic and textural characteristics. In addition, no marked variation has been noticed in the physicochemical and microbiological properties. Therefore, the consumers can harvest the benefits of consuming *Stevia rebaudiana* and catch up on a new yoghurt variety.

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