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Effect of sugar replacement with stevia on quality of vanilla flavoured cow milk set yoghurt

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Abstract

Stevia extracted from the leaf of *Stevia rebaudiana* Bertoni plant is a natural alternative sweetener that has the potential to be used as a sugar replacer in sweetened dairy products. The current study aimed to investigate the effect of sugar replacement with stevia extract on the quality of vanilla flavoured cow milk set yoghurt. Cow milk was standardized to 3% fat and 12% milk solids non-fat. Commercially available pure stevia extract was used to replace the sugar (maintained at 8% in the control) at the rate of 0 (control), 25% (T1), 50% (T2), 75% (T3) and 100% (T4) in cow milk set yoghurt. Yoghurt mixtures were separately heat treated (85°C/30 mins), cooled to inoculation temperature (42°C), inoculated with commercial yoghurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus* (1:1 ratio) and incubated till the pH reaches nearly 4.6. Set yoghurt samples were tested for chemical composition, organoleptic attributes, physicochemical and physical properties using standard procedures. Total calorie content was calculated per 100 g of yoghurt. Milk fermentation characteristics, water holding capacity and instrumental colour of yoghurt were not affected (p>0.05) by the sugar replacement with stevia. Carbohydrate content showed a decrease while protein and ash showed an increasing trend with an increasing level of sugar replacement in yoghurt. A 100% sugar replacement with stevia resulted in a 16.83% reduction in the calorie content of yoghurt. Control, as well as 100% sugar replaced yoghurt, obtained higher sensory scores while in the other treatments a decreasing trend in the sensory score was observed with the increasing level of sugar replacement suggesting possible interaction of stevia compounds with sucrose. It can be concluded that the replacement of 100% sugar is possible with the commercially available stevia extract used in the current study for the production of vanilla flavoured set yoghurt.

1. Introduction

Yoghurt is considered a nutrient-dense healthy dairy product and therefore, it has a growing consumer demand throughout the world. It has been reported that the global yoghurt market reached a value of nearly US$ 40.6 billion in the year 2019 and is forecasted to reach approximately US$ 51.2 billion by 2024 (Shahbandeh, 2020). Yoghurt is marketed in various physical forms such as set, stirred, drinking, frozen and more, and in different types such as natural, fruit on the bottom, sweetened flavoured and sundae style. Yoghurt is a good source of protein, calcium, phosphorus, iodine vitamin B2, B12 like nutrients (Moore *et al*., 2018) and its consumption is associated with a lower risk of obesity and cardio-metabolic risk in children as well as in adults (Marette and Picard-Deland, 2014; Cormier *et al*., 2016).

Sucrose is the main sweetener used in the production of sweetened yoghurt due mainly to the desirable sweet taste, easy availability and low cost (Giri *et al*., 2014). However, excessive consumption of sugar leads to various health ailments such as type II diabetes, obesity, cardiovascular diseases, tooth decay etc. (Cichosz *et al*., 2011; Kalicka *et al*., 2017). Even a small amount of sugar reduction can significantly improve the diet and the health of an individual (Mahato *et al*., 2020). Therefore, there is a growing interest in the food industry to use low/no-calorie alternatives for sugar. On the other hand, there is a big demand for '*lite*' products among current health-conscious consumers (Narayanan *et al*., 2014). Therefore, low-calorie dairy products with alternative sweeteners have a promising market.

Even though many alternative high-intensity

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synthetic sweeteners such as acesulfame-K, aspartame, neotame, saccharin, sucralose and others are available, their use is restricted since many of these compounds are said to be carcinogenic and associated with some disease conditions (Gonzalez *et al*., 2014). Consequently, the demand for low/zero-calorie natural alternative sweeteners has grown dramatically during the last decade due to health-conscious consumers (Philippe *et al*., 2014). High fructose corn syrup, sugar alcohols (polyols) and quite recently, tagatose, thaumatin and steviol glycosides are among the natural alternative sweeteners currently used in the food industry. In addition to the sweetness, they offer health benefits as well (Saraiva *et al*., 2020). Nevertheless, it is important to note that sucrose provides not only the sweetness for a particular product but also some other functions such as desirable taste and consistency (Nip, 2007). Furthermore, consumers do not like to compromise taste even though they are health conscious (Narayana *et al*., 2020). Therefore, sugar reduction from a product involves multiple technical challenges to maintain the sensory properties and consumer acceptance of that product (Mahato *et al*., 2020).

Among the natural alternative sweeteners, stevia is one of the promising sweeteners obtained from leaves of *Stevia rebaudiana* Bertoni, a shrub-like plant in the *Asteraceae* family native to South America but at present grown in other countries in Asia and Europe as well (Saraiva *et al*., 2020). The plant is having increased attention due to the presence of sweetening compounds, natural origin as well as therapeutic benefits (Gardana *et al*., 2003; Nunes *et al*., 2007; Sehar *et al*., 2008; Rao and Giri., 2009). Glycosides found in the leaves of the *S. rebaudiana* plant are responsible for the sweet taste (Gardana *et al*., 2003; Lisak *et al*., 2012). The most important glycosides found in stevia plant are steviosides, rebaudiosides A to F, steviolbiosides and dulcosides (Gardana *et al*., 2003). Depending on the variety and the growing conditions of the plant, the amount of the above-mentioned compounds present varies between 4 to 20% (Lisak *et al*., 2011). Among a wide array of health benefits provided by stevia apart from providing sweetness, anti-hyperglycemic (Jeppesen *et al*., 2002), anti-hypertensive (Melis, 1996), anti-inflammatory (Zou *et al*., 2020), anti-cancer (Yasukawa *et al*., 2002) effects are remarkable.

Stevia is around 200 to 300 times sweeter than sucrose (Lisak *et al*., 2011). It can be used to make *lite* products without using artificial non-nutritive sweeteners for consumers with dietary restrictions (Narayanan *et al*., 2014). Stevia is available in different commercial formulations and therefore it is important to select the appropriate type and concentration before the product is launched (Narayanan *et al*., 2014). It is stable at high temperatures (up to 200°C) as well as in acidic and alkaline environments. Also, it has good solubility and light stability (Lisak *et al*., 2012). These specific characteristics along with the zero calorific value make stevia suitable for many food applications and processing conditions. Stevia is recommended to be used in food and medicinal applications by over 20 countries in the world including the USA, Japan, Canada, China, Brazil, Argentina, Korea, Mexico, Indonesia and Tanzania (Kolb *et al*., 2001; Giri *et al*., 2014). GRAS (generally recognized as safe) status has been given to steviol glycosides by the US Food and Drug Administration (FDA) in 2008. Furthermore, according to the European Food Safety Authority (EFSA), glycosides found in the stevia are not genotoxic or carcinogenic and therefore it has been allowed to be used as an additive in food and beverages (Kuźma *et al*., 2012). Even though the use of stevia as a natural sweetener in food applications in Sri Lanka is not a common practice, there is an emerging and promising niche market due to the demand of health-conscious consumers. In this context, the current study aimed to investigate the effect of the replacement of sucrose with commercially available pure stevia extract on the properties of vanilla flavoured set-style yoghurt.

2. Materials and methods

2.1 Experimental site

The present study was carried out at the Dairy Technology Laboratory of the Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka.

2.2 Materials

Fresh cow milk was obtained from the Faculty farm. Freeze-dried thermophilic yoghurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbruekii* ssp *bulgaricus* (1:1 ratio) (Chr. Hansens Laboratories, Denmark) was used to ferment the yoghurt. Commercially available 100% pure natural stevia extract (RCC Worldwide, Malaysia; 10 mL equivalent to 3 kg sugar) was used as the alternative sweetener to replace sucrose in the yoghurt. Skim milk powder (Anchor, Fonterra, Sri Lanka), sugar, gelatin, vanilla flavour and egg yellow colour were obtained from the local market.

2.3 Manufacturing of yoghurt

Raw cow milk (3.67±0.58% fat and 8.74±1.31% SNF) was preheated to 40°C and cream was separated using a cream separator. Milk was standardized to 3% fat and 12% SNF by adding the required amount of cream and SMP. The calculated amount of commercially available pure stevia extract (based on the relative degree

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of sweetness to sucrose) was used to replace the sugar (maintained at 8% in the control) at the rate of 0 (control), 25% (T1), 50% (T2), 75% (T3) and 100% (T4) in the yoghurt mix. Gelatin was added as the stabilizer at the rate of 0.5% and the resultant yoghurt mixes were separately pasteurized at 85°C for 30 mins. Then the yoghurt mixes were cooled to 42°C in a water bath. Direct vat set (DVS) yoghurt starter culture containing *S. thermophilus* and *L. delbruekii* ssp. *bulgaricus* (1:1) was added at the rate of 2% of the yoghurt mix and mixed well for the proper distribution of the culture microorganisms. The vanilla flavour was added as the flavouring agent, mixed well and the yoghurt mix was poured into 100 mL polystyrene cups. They were kept in an incubator maintained at 42±1°C temperature until the desired pH of nearly 4.6 was attained. The resultant yoghurts were immediately kept in a refrigerator maintained at 4±1°C until further analysis. The study was repeated 3 times.

2.4 Physicochemical properties

2.4.1 pH and titratable acidity

The pH of raw milk and yoghurt mix during incubation and in the finished yoghurt were measured using a benchtop digital pH meter (Agilent, 3200P), after calibration with fresh pH 4.0 and 7.0 standard buffers at 25°C. Titratable acidity of raw milk and yoghurt mix during incubation and in the finished yoghurt was determined by titrating with 0.1 N NaOH using a 0.5% phenolphthalein indicator.

2.5 Proximate composition

Total solids (TS) and solid-not-fat (SNF) contents of milk were determined by lactometer method. Gerber method was used to determine the fat content of raw milk and the finished yoghurt. The gravimetric method was used to determine the TS content of yoghurt. The protein content of the finished yoghurt was determined by the Kjeldahl method. Ash content of yoghurt was determined by the ignition method (AOAC, 2000). The Carbohydrate (CHO) content of the yoghurt samples was calculated by deducting protein, fat and ash percentages from the percentage of the total solids.

2.6 Physical properties

2.6.1 Viscosity

The viscosity of the finished yoghurt samples was measured using a Brookfield RV DV-1 viscometer at 10˚C. The spindle number 07 at 100 rpm was used for all the samples. Readings were taken after allowing the viscometer spindle to rotate 60 secs in the middle of the sample.

2.6.2 Spontaneous whey syneresis

Spontaneous whey syneresis was determined in undisturbed set yoghurt samples according to the method proposed by Amatayakul *et al*. (2006). A cup of yoghurt (80 mL) was tilted at an angle of 45° to collect the surface whey, immediately after removing it from the refrigerator. Collected whey was siphoned out by using a needle fitted to a graduated syringe. Siphoning was completed within 10 s to avoid forced leakage of whey from the yoghurt. Percent SWS was calculated as follows.

$$\text{SWS} = \frac{\text{Volume of siphoned whey}}{\text{Volume of initial yoghurt sample}} \times 100$$

2.6.3 Water holding capacity

The WHC of yoghurt was measured by a centrifugation method proposed by Narayana and Gupta (2013) with slight modifications. Ten grams of yoghurt sample was centrifuged at 1,500×*g* for 10 mins at 10±1°C within 12 hrs of the production of set yoghurt. The supernatant was removed within less than 10 mins and the wet weight of the pellet was recorded. The WHC was calculated by the following equation.

$$\text{WHC (\%)} = \frac{\text{NY} - \text{WE}}{\text{NY}} \times 100$$

Where NY is the weight of native yoghurt and WE is the weight of expelled whey

2.6.4 Instrumental colour

Instrumental colour analysis of the yoghurt samples was performed by using PCE-CSM 2 colourimeter calibrated with the white calibration plate provided and as specified by the manufacturer. CIELab colour scale was used to get L^*, a^* and b^* values. The L^* value ranges from 0 to 100, indicating the brightness from black to white; the a^* value indicates the variation from red ($+a^*$) to green ($-a^*$); the b^* value indicates the variation from yellow ($+b^*$) to blue ($-b^*$) in the CIELab colour scale.

2.7 Total calorie content

The calorific value of CHO, fat and protein are assumed as 3.87, 8.79 and 4.27, respectively (Arbuckle, 1986) and the total calorie content of set yoghurt samples was calculated by using the following equation (Giri *et al*., 2014).

Total Calorie content = {(% CHO × 3.87) + (% Fat × 8.79) + (% Protein × 4.27)}

2.8 Sensory evaluation

Sensory evaluation was carried out by using an untrained panel of thirty judges, following the method

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proposed by Mason and Nottingham (2002) for the finished yoghurt samples for flavour, body and texture, colour, sweetness, after taste and overall acceptability after one day of storage. Non-smoking, regular yoghurt consuming healthy adults who were willing to participate in the test were selected from the Faculty of Agriculture University of Ruhuna, Sri Lanka. Individual sensory booths were prepared with the yoghurt samples, drinking water to wash off the mouth and a sensory evaluation sheet. The place was well ventilated and ample lighting was provided to eliminate disturbances from nuisance odours and insufficient light condition, respectively. Each yoghurt sample was labelled with random three-digits. Panellists were advised to evaluate the samples in a quick manner but not in a hurry using a 5-point hedonic scale (1 = dislike extremely, 2 = dislike slightly, 3 = neither like nor a dislike, 4 = like slightly and 5 = like extremely).

2.9 Statistical analysis

All the experiments were conducted in triplicates. Data were tabulated and analyzed using SPSS (ver. 20) statistical software package. Repeated measure ANOVA in the General Linear Model procedure was used to analyze pH and TA data during incubation. Differences in the mean values of the parameters tested among the treatments were examined using the one way ANOVA procedure followed by Tukey's test. Sensory data were analyzed using Friedman's non-parametric test.

3. Results and discussion

3.1 pH and titratable acidity profile of yoghurt during incubation

Figure 1 shows the reduction in pH of the yoghurt mixes prepared by replacing sugar with pure stevia extract during incubation of 4 hrs in ½ hr intervals. It has been observed that in all the treatments pH was decreased from an initial value ranging from 6.50-6.63 to a final value ranging from 4.37-4.53, without showing any significant ($p>0.05$) differences between treatments.

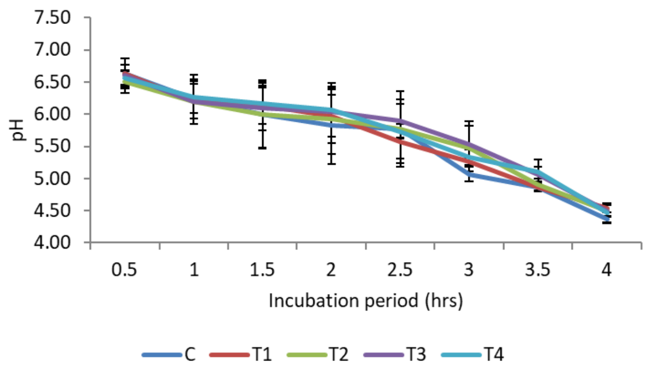

Figure 1. Reduction of pH of yoghurt during incubation at 42±1°C. Values are mean±SD, n = 3. C: control (100% sugar), T1, T2, T3 and T4: 25, 50, 75 and 100% sugar replaced with stevia, respectively.

This implies that the addition of pure stevia extract used in the current study did not have any significant effect on the metabolic activity of starter culture microorganisms. This is in line with the observations made by Lisak *et al*. (2011), who examined the influence of the stevia sweetener on the quality of strawberry-flavoured yoghurt. They reported that the addition of sweeteners namely sucrose, stevia or a combination of sucrose and stevia did not have an effect on the metabolic activity of the starter culture microorganism used.

Similarly as shown in Figure 2, the increment of titratable acidity in the yoghurt mixes during the incubation ranging from an initial value of 0.24-0.26 % LA to a final value of 0.72-0.81% LA was not affected by the addition of stevia extract.

As well established, gradual pH reduction of the yoghurt mixes during the incubation is due to the increment of the lactic acid produced by the action of starter culture microorganisms in milk sugar lactose.

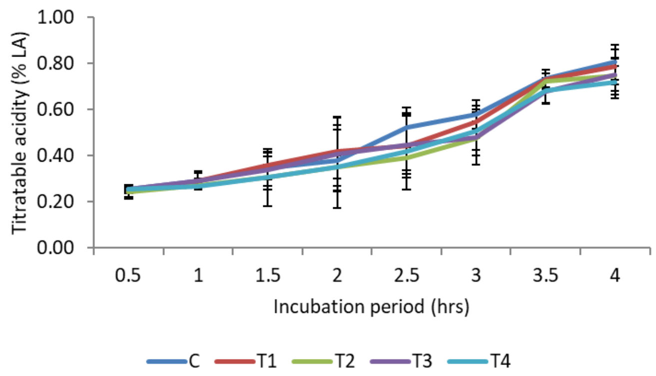

Figure 2. Changes in titratable acidity of yoghurt during incubation at 42±1°C. Values are mean±SD, n = 3. C: control (100% sugar), T1, T2, T3 and T4: 25, 50, 75 and 100% sugar replaced with stevia, respectively.

3.2 Proximate composition of yoghurt

Table 1 shows the effect of sugar replacement with stevia on the proximate composition of vanilla-flavoured set yoghurt. It was observed that the total solids and CHO percentages decreased dramatically with the increase of stevia level in the yoghurt mix with significant ($p<0.05$) variations in between some treatments. Sucrose is the major contributor to the CHO present in the sweetened yoghurt. This will be the reason for the above observation of a dramatic reduction in TS and CHO in the yoghurt with decreasing sugar levels. Giri *et al*. (2014) also made similar observations where they produced *kulfi*, an Indian dairy product, by partially replacing the sugar with pure stevia extract powder. They have reported a significant ($p<0.05$) reduction of

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Table 1. Effect of sugar replacement with stevia on proximate composition of vanilla flavoured set yoghurt

Treatment	Level of sugar	Level of stevia	Parameter				
			TS (%)	Ash (%)	Protein (%)	Fat (%)	CHO (%)
Control	100	0	22.33±0.93[c]	0.67±0.02[a]	2.49±0.00[a]	3.26±0.15	15.93±0.87[b]
T1	75	25	21.24±1.14[bc]	0.81±0.07[a]	2.75±0.04[a]	3.40±0.17	14.34±1.09[b]
T2	50	50	20.40±0.87[bc]	0.85±0.04[a]	2.56±0.06[a]	3.40±0.10	13.59±0.78[b]
T3	25	75	18.85±1.26[ab]	1.23±0.11[b]	3.36±0.05[b]	3.43±0.05	10.93±1.13[a]
T4	0	100	17.69±0.45[a]	1.25±0.13[b]	3.41±0.32[b]	3.40±0.20	10.22±0.53[a]

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different (p<0.05).

CHO percentage in *kulfi* with the increased level of sugar replacement.

Due to lower levels of sugar, there was a reduction in the total weight of *kulfi* and hence, the fat, protein, ash and moisture percentages increased proportionately in these samples (Giri *et al*., 2014). In the current study also, it was observed that the ash and protein percentages increased with the increase of sugar replacement with stevia extract. Nevertheless, changes in the fat percentage were not significant (p>0.05) in between the treatments, even though there were mild variations. Further Hernández-Rodríguez *et al*. (2017) conducted a study to find out the effect of protein-*Lactobacillus plantarum*-polysaccharide coacervate and stevia in the substitution of milk fat and sucrose, respectively on microstructure and rheology of yoghurt. They have reported that the moisture content of yoghurt variants containing stevia was significantly higher due to the presence of lower TS with decreasing sugar content.

3.3 Physicochemical and physical properties

3.3.1 pH and titratable acidity after gel stabilization

Table 2 shows the physicochemical and physical properties of the sugar replaced vanilla flavoured set yoghurt after one day of refrigerated (4±1°C) storage. It was observed that the pH and the titratable acidity of the yoghurt were not significantly (p>0.05) different between the treatments. However, it is clear that the sugar replaced yoghurt had a slightly lower pH and higher titratable acidity (Table 2) than the control sample. Increment of the titratable acidity after the incubation period is due to the post-acidification which is caused by the starter culture microorganisms added. The progressive transformation of lactose to lactic acid is the reason for the post-acidification (Ramírez-Santiagoa *et al*., 2010). Cooling of the yoghurt after the production limits starter culture activity but does not inhibit it completely (Kalicka *et al*., 2017). It was observed in the current study that the post-acidification is comparatively higher in the yoghurts manufactured by replacing sugar with stevia compared to the control (Table 2). Kalicka *et al*. (2017) have also observed that the reduction of pH and increment of TA of yoghurt during refrigerated storage was more rapid in yoghurt manufactured by replacing sugar with stevia.

3.3.2 Spontaneous whey syneresis and water holding capacity

One of the most important properties of set style yoghurt is the strength of the coagulum and its ability to immobilize water. In this respect, water holding capacity (WHC) which is an important physical property of a yoghurt gel plays a major role. The WHC of yoghurt is defined as its ability to hold all or part of its water (Bierzuńska *et al*., 2019). It reflects the stability of the coagulum and its ability to hold water in the yoghurt matrix. This, in turn, leads to a reduction in spontaneous whey syneresis. It is an important index when evaluating yoghurt quality and is considered a major defect, especially in set style yoghurt that may lead to consumer rejection (Narayana and Gupta, 2013). Higher TS, principally proteins cause the yoghurt gel structure to be denser (Sodini *et al*., 2004) and thereby helping to keep more water in the matrix. Further, Torres *et al*. (2013) reported that sucrose contributes to moisture retention in yoghurt gels. In the current study, sucrose was replaced

Table 2. Effect of sugar replacement with stevia on physicochemical and physical properties of vanilla flavoured set yoghurt

Treatment	Level of sugar	Level of stevia	Parameter			
			pH	TA (% LA)	WHC (%)	Viscosity (cP)
Control	100	0	4.53±0.15	0.89±0.05	73.07±2.86	6421±275.74[a]
T1	75	25	4.40±0.10	o.95±0.04	71.35±7.96	8125±925.00[b]
T2	50	50	4.50±0.10	0.97±0.02	73.90±4.16	7000±0.00[ab]
T3	25	75	4.37±0.23	0.93±0.06	70.30±1.53	6090±90.00[a]
T4	0	100	4.43±0.15	0.96±0.13	71.35±7.96	8125±925.00[b]

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different (p<0.05).

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by pure stevia extract up to 100%. Nevertheless, none of the yoghurt samples exhibited spontaneous whey syneresis. Furthermore, it was observed that the WHC was not significantly (p>0.05) affected by the addition of stevia, even though the TS level was decreased maximum of up to 8% due to the reduction of sugar content in the yoghurt. Haque and Aryana (2002) mentioned that the type of sweetener affects the state of association of casein micelles in yoghurt. Furthermore, according to Wan *et al.* (2014), stevioside in stevia can form a complex with soy protein isolate through hydrophobic interactions. Hernández-Rodríguez *et al.* (2017) hypothesized that stevia has the ability to interact with milk proteins. Strengthening their idea, the results of the current study also assumes that stevia has the ability to interact with milk proteins and thereby making the yoghurt gel matrix stronger due to the non-affected WHC and absence of SWS even at 100% sugar replacement level.

3.3.3 Viscosity

Even though viscosity was observed to be significantly (p<0.05) different between treatments, a clear trend was not observed with an increased level of sugar replacement with stevia (Table 2). Nevertheless, viscosity in stevia added yoghurt samples was observed to be comparatively higher than that of the control sample except at 25:75 sugar: stevia treatment. Lisak *et al*. (2012) also reported in their study that the apparent viscosity of yoghurt made with stevia was higher than that of the yoghurts made with only sucrose and the combination of sucrose and stevia. This might be due to the ability of stevia to interact with milk proteins and thereby strengthening the protein structure as previously mentioned elsewhere in this report. Kalicka *et al*. (2017) mentioned in their study that the addition of stevia does not influence the consistency but improves the hardness of yoghurt.

3.3.4 Instrumental colour

The colour is a vital factor affecting consumer acceptability, quality, flavour expectation, freshness and commercial value of yoghurt (Yilmaz-Ersan and Topcuoglu, 2021). Table 3 depicts the effect of sugar replacement with stevia on the instrumental colour of vanilla flavoured set yoghurt.

It was observed that none of the values obtained for L^*, a^* and b^* were having significant differences between the treatments. In fact, the L^* value ranged from 75.19±29.29 in 100% sugar replaced yoghurt to 92.37±1.10 in 75% sugar replaced yoghurt with no significant (p>0.05) differences between the samples. The commercial preparation of stevia used in this study was in pure form and colourless. This is the reason why there was no effect on the colour parameters of the yoghurt by replacing sugar with stevia. Kalicka *et al*. (2017) also reported that the addition of stevia did not cause any changes to the colour of the yoghurt. According to Dufossé and Galaup (2009) due to the presence of milk fat, protein and natural pigments, yoghurt should have a bright white colour. However, in the production of sugar replaced yoghurt in the current study, market available egg yellow colour was used as an additive leading to the reduction of lightness of yoghurts. Colour was added especially since the consumer acceptance for the set yoghurt is higher when it is light yellow among the consumers in Sri Lanka. Yilmaz-Ersan and Topcuoglu (2021) also highlighted that the colour features and sensorial perceptions differ with the geographical differences and ethical concerns of the consumers. All the yoghurt samples having minus a^* value confirms the dominating green tone over the red. Moreover, b^* values with all the measurements above zero, confirm that the yellow colouration dominates over the blue in all the samples.

3.4 Sensory attributes

Consumer's choice determines the market acceptance of a novel food product and hence, its organoleptic attributes become critically important. After 24 hrs of refrigerated storage at 4±1°C and gel stabilization, yoghurt samples were subjected to sensorial evaluation. Mean values of the subjective sensory attributes such as flavour, body and texture, colour, sweetness, aftertaste and overall acceptability of yoghurt are shown in Table 4. From the statistical point of view, a non-trained panel of 30 judges found significant (p<0.05) differences between the yoghurt

Table 3. Effect of sugar replacement with stevia on instrumental colour of vanilla flavoured set yoghurt

Treatment	Level of sugar	Level of stevia	Colour		
			L^*	a^*	b^*
Control	100	0	82.32±13.61	-0.46±0.56	18.65±3.69
T1	75	25	81.98±17.39	-0.26±0.73	17.42±5.05
T2	50	50	84.14±14.42	-0.47±0.71	18.98±2.86
T3	25	75	92.37±1.10	-0.68±0.24	19.73±0.88
T4	0	100	75.19±29.29	-0.23±0.27	17.25±6.85

L^* = Lightness; a^* = redness (+) and greenness (-); b^* = yellowness (+) and blueness (-)

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Table 4. Effect of sugar replacement with stevia on sensory attributes of vanilla flavoured set yoghurt

Treatment	Level of sugar	Level of stevia	Sensory score*					
			Flavour	Body and	Colour	Sweetness	After taste	Overall
Control	100	0	4.42±0.50^{bc}	4.18±0.68^{bc}	4.15±0.71^{b}	4.06±0.86^{b}	4.09±0.81^{bc}	4.18±0.64^{bc}
T1	75	25	3.70±0.68^{b}	3.85±0.80^{b}	3.97±0.81^{ab}	3.42±0.97^{a}	3.67±0.78^{b}	3.73±0.63^{b}
T2	50	50	3.21±1.05^{ab}	3.52±0.97^{ab}	3.97±0.81^{ab}	3.33±1.02^{a}	3.33±0.99^{ab}	3.30±0.95^{ab}
T3	25	75	2.91±0.72^{a}	3.09±0.95^{a}	3.67±0.82^{a}	2.97±0.98^{a}	3.06±1.00^{a}	3.00±0.83^{a}
T4	0	100	4.45±0.79^{bc}	3.76±0.94^{b}	4.24±0.79^{b}	4.21±0.96^{b}	4.21±0.86^{bc}	4.33±1.11^{bc}

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($p<0.05$).
5-point hedonic scale was used where 1 = strongly disagree and 5 = strongly agree

samples manufactured by replacing sugar with pure stevia extract. With the increase of sugar replacement level with stevia extract in the vanilla flavoured set yoghurt, a decrease in all the sensory scores were observed. However, it is interesting to note that at 100% sugar replacement level where only pure stevia extract was used to sweeten the product, the sensory scores were significantly ($p<0.05$) higher and similar to that of the control sample where 100% sugar was used to sweeten the yoghurt. Therefore, there is a suspicion that a sugar-stevia interaction might be present leading to the deterioration of organoleptic attributes in sugar replaced yoghurt in the presence of sucrose. On the contrary, Lisak *et al*. (2012) reported that the scores for odour and colour attributes were similar in all the samples where they used 100% sugar, 100% stevia and 50:50 sugar: stevia mixture for the production of yoghurts. Furthermore, stevia yoghurt was awarded lower sensory scores due to the after-mouth taste. However, they reported that the samples sweetened with a mixture of stevia and sugar (50:50) had no after-mouth taste. In the current study even though the instrumental colour was not significantly different between the yoghurt samples, the colour score was also observed to be higher in the T4 sample and comparable to that of the control. This might be due to the emotional influence of the consumers.

On the other hand, it has been reported that different commercially available stevia formulations have different effects on the organoleptic quality of the products as well as other qualities and hence, careful selection of the stevia sweetener with different products is needed to get the desired outcome to satisfy the consumers (Narayanan *et al*., 2014). Therefore, further studies are suggested to find out the reasons for the observations made in this study.

3.5 Total calorie content

Figure 3 shows the changes in the calorific value (cal/100 g) of the yoghurt samples due to the replacement of sugar with stevia. It was observed that the calorific value of all the treatments where stevia was used to replace the sugar was lower. Furthermore, at 75 and 100% levels of sugar replacement with stevia, a significant ($p<0.05$) reduction of calorific value was observed compared to the control. The main reason behind this is the decrease in the sugar level which is a major contributor to the calorie content in the yoghurt samples. Giri *et al*. (2014) observed that 70% sugar replacement with pure stevia extract powder reduced the calorific value of dietetic *Kulfi* by 33%. In the current study, 100% sugar replacement with pure stevia extract resulted in 16.83% of the calorific value of yoghurt.

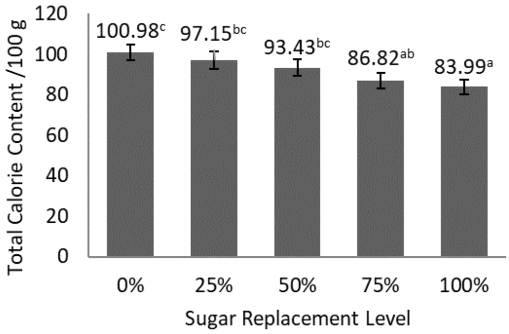

Figure 3. Effect of sugar replacement with stevia on the total calorie content of yoghurt. Values with different superscripts are significantly different ($p<0.05$).

4. Conclusion

Milk fermentation characteristics, water holding capacity and instrumental colour of yoghurt were not affected ($p>0.05$) by the sugar replacement with stevia. Carbohydrate content showed a decrease while protein and ash showed an increasing trend with an increasing level of sugar replacement in yoghurt. A hundred per cent sugar replacement with stevia resulted in a 16.83% reduction in the calorie content of yoghurt. Control, as well as 100% sugar replaced yoghurt, obtained higher sensory scores for all the sensory attributes while in the other treatments a decreasing trend in the sensory score was observed with the increasing level of sugar replacement suggesting possible interaction of stevia compounds with sucrose. Finally, it is concluded that the replacement of 100% sugar is possible with the commercially available stevia extract used in the current experiment for the production of vanilla flavoured set

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yoghurt without any significant alterations in the product quality. Further studies are needed with different stevia brands and forms to find out the possible sugar replacement percentage.

Conflict of interest

The authors declare no conflict of interest.

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