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Consumer rejection threshold, acceptability rates, physico-chemical properties, and shelf-life of strawberry-flavored yogurts with reductions of sugar

Running title: Sugar reductions on yogurts…

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ABSTRACT

BACKGROUND: There is an increasing demand for reduced-sugar products due to the worldwide prevalence of obesity, diabetes, and cardiovascular diseases. The aim of this study was to evaluate the effects of sugar (sucrose) reductions on the acceptability, preference, and quality of strawberry-flavored yogurts. A consumer rejection threshold test and an acceptability test (N=53) were conducted using six yogurt samples with decreasing concentrations of sugar (12 to 5 g/100 g). Additional physico-chemical tests [pH, °Brix, water-holding-capacity (WHC), viscosity, and color] were conducted to examine the quality and shelf-life of strawberry-flavored yogurts with reductions of sucrose during 28 days of storage at 4 °C.

RESULTS: Reduction of sucrose affected the acceptability and physico-chemical characteristics of yogurts. The consumer rejection threshold showed that sucrose in strawberry-flavored yogurts could be reduced to 5.25 g/100 g from an initial concentration of 12 g/100 g without affecting the preferences of consumers. The 71%-sucrose (8.50 g/100 g of yogurt) was perceived as the most liked (6.27 using a 9-point hedonic scale) and the most preferred (rank sum=127.50) yogurt sample. For the physico-chemical properties of yogurts, the viscosity (3,263-5,473 cP) decreased, and the color lightness (80.98-85.44) increased during 28 days of storage at 4 °C.

CONCLUSION: Physico-chemical properties and preferences were affected by the reduction of sugar. The consumer rejection threshold analysis showed that sucrose can be reduced to less than half of the initial concentration. These findings are useful to understand consumers' acceptability and shelf-life of yogurts with reduced-sugar formulations in the developing of new products.

Keywords: sugar reduction, yogurt, acceptability, physico-chemical

INTRODUCTION

The demand of healthier food products has been rapidly increasing in recent years [1] due to (1) changes in human lifestyles, (2) a growing worldwide incidence of obesity, diabetes and cardiovascular diseases, and (3) the association between carcinogens and high sugar/calorie contents that are related to traditional snack products [2-4]. Yogurt is among the most popular healthy food choices, which is widely consumed [5-9]. To achieve a broader market, the yogurt industry is continuing to develop novel products that are adapting to current consumer needs. Typical fruit yogurts contain up to 12 g/100 g of sucrose [10]. The World Health Organization (WHO) recommended reducing the daily intake of sugar to 5% of the total energy consumption (approx. 25 g a day) [11]. As healthier food choices are in increasing demand, the development of reduced-sugar yogurts is of great interest.

The formulation of yogurt with reduced-sugar represents a significant challenge for the yogurt industry. When formulating reduced-sugar food products, consumers expect to have the resemblance of the original sensory characteristics from the full-sugar versions [12]. Previous studies reported that consumers rated the acceptability of sugar-reduced yogurts poorly due to their pronounced differences in taste, flavor, and texture compared to their conventional counterparts [13, 14]. These sensorial inconsistencies have been associated with the physical and sensory properties that sugar delivers to yogurt [14]. Sugar (sucrose) is crucial for providing taste and texture to yogurt, and for achieving the desired sensory quality. Moreover, the addition of sucrose during the processing/manufacturing contributes to the sweetness, total solids, texture, body, viscosity and moisture retention of yogurts [4, 15].

Few studies have explored the effects of sugar reductions on the sensory perception of yogurts [15]. Hoppert, Zahn, Jänecke, Mai, Hoffmann and Rohm [16] using a conjoint analysis integrated with a sensory preference test evaluated the reductions of sugar and fat on vanilla yogurts. Processes such as lactose hydrolysis and sugar substitution have been studied as potential alternatives for sugar reduction in yogurts [9]. In contrast, the dose-response relationship of sucrose on other food matrices has been extensively studied [17, 18]. The determination of sugar acceptability thresholds, which can provide the minimum concentration of sugar that is acceptable for consumers, is suggested to be one of the most useful approaches to reduce sugar in food products [19]. In order to minimally alter the sensory quality of the reduced-sugar yogurt, it is essential to identify the optimal sugar concentration for consumers [15]. Therefore, the research objective of the present study was to evaluate the effects of sugar (sucrose) reductions on the acceptability, preference, and quality of strawberry-flavored yogurts. A consumer rejection threshold test was conducted to determine the concentration of sucrose in yogurt at which consumers will reject the samples. In addition, an acceptability test was performed to measure the liking rates of different concentrations of sucrose in the yogurt. Furthermore, physico-chemical tests were conducted to examine the quality and shelf-life of strawberry-flavored yogurts with reductions of sucrose during 28 days of storage period at 4 °C.

MATERIALS AND METHODS

Materials

Full cream fresh milk (Coles Supermarkets Australia Pty Ltd, Hawthorn East, VIC, Australia), full cream milk powder (Devondale Murray Goulburn, Southbank, VIC, Australia), mild yogurt culture including *Lactobacillus delbrueckki ssp. Bulgaricus*, and *Streptococcus thermophilus* (Green Living Australia, Underwood, QLD, Australia), castor sugar (sucrose, Coles Supermarkets Australia Pty Ltd, Hawthorn East, VIC, Australia), and fresh strawberries (Coles Supermarkets Australia Pty Ltd, Hawthorn East, VIC, Australia) were used as the main ingredients in the preparation of the yogurt samples.

Preparation of strawberry-flavored yogurt samples

Strawberry-flavored yogurt samples were formulated at the sensory laboratory of the University of Melbourne, Australia. For the preparation of the strawberry flavoring jam, fresh strawberries were liquefied with a blender (BL480 Auto-IQ One Touch Intelligence, Nutri Ninja, Boston, MA, USA) for 35 s, and the desired sucrose concentration was added for each treatment (Table 1). Pasteurization of each strawberry flavoring treatment was performed for 2 min at 90 °C using a cooking pot in a commercial stove. Strawberry flavorings were hot-filled into sterile glass jars and were stored at 4 °C for 12 h (Figure 1). Mild yogurt was prepared by first mixing 40 g of full cream milk powder with 1 L of full cream milk and pasteurized at 90 ± 2 °C for 5 min (Figure 1). Pasteurized milk was cooled down to 42 ± 2 °C, and the freeze-dried starter culture was inoculated according to the manufacturer recommendation (0.001 g per 1 L of yogurt). The mix was transferred immediately to the yogurt maker (Greek yogurt & cheese maker, Kuvings Australia, Croydon, NSW, Australia) and allowed to incubate for 8-h at 42 °C. A quality evaluation of yogurt was made to ensure that pH reached to 4.6. Yogurt samples were then poured into 1.5 L plastic container with lids (Woolworths Group, Bella Vista, NSW, Australia) and transferred to a cold room at 4 °C for a period of 12 h. Strawberry jam and yogurt were blended the next day and stored at 4 °C prior to consumer and physico-chemical tests (Figure 1). A concentration of 5.5% strawberry jam was used in line with the average strawberry flavoring concentration found in commercial products. Two independent batches of yogurt samples were prepared for the physico-chemical and consumer tests. Additionally, strawberry-flavored yogurt batches used for the physico-chemical analysis were stored in sealed sterile plastic containers at 4 ± 1 °C for 28 days for further shelf-life quality evaluations.

Sensory evaluation

Subjects

All sensory testing protocols were listed as minimal risks with the ethics approval 1543704.2 on February 2017 by the Human Ethics Advisory Group (HEAG) of the Faculty of Veterinary and Agricultural Science at The University of Melbourne, Australia. A total of N = 53 untrained participants (37 females and 16 males; aged 18 to 45 years old) were recruited from a pool of staff and students at The University of Melbourne, Australia via the university noticeboard.

Participants were pre-screened using the following criteria: (1) regular consumers of yogurt (at least once per month) based on self-reported responses, and (2) not having taste/smell disorders. Consumer evaluations took place in individual booths in the Sensory Laboratory at The University of Melbourne under a controlled environment with illuminated modern LED lights (configured with color white; RGB = 255, 255, 255) and a set temperature of 25 °C. Prior to the tasting session, participants were required to sign a consent form approved by the HEAG (The University of Melbourne). All participants were also informed of any allergens (cereals containing gluten, peanuts, milk/dairy products, eggs, fish, crustacean shellfish, nuts, celery, mustard, sesame, sulfites, lupin, mollusks, and soybeans) that may be present in the yogurt samples. Therefore, all participants were healthy individuals who could consume yogurts regularly. Consumers who participated in the sensory evaluation were compensated with a coffee voucher.

Stimuli

The range of sucrose (castor sugar) concentrations evaluated in this experiment (from 12 to 5 g/100 g of yogurt) was determined in previous focus group discussions (n = 6) within the sensory group of The University of Melbourne, in which, overall product attitudes and acceptability, and sweetness and sourness intensities were asked to panelists. Five sucrose concentrations were prepared with equidistant decrements (12, 10.25, 8.5, 6.75, and 5 g/100 g of yogurt) to determine the most liked/preferred sucrose concentration in strawberry-flavored yogurts (Table 1). The 12%-sucrose concentration was selected as it provides a comparable sweetness to commercial strawberry-flavored yogurts found in Australian markets. The 5%-sucrose concentration was selected as the lowest sucrose strawberry-flavored yogurt determined by the previous focus group discussions. The remaining three concentrations were intermediate between 12% and 5% (Table 1).

Sensory procedure

Two separate sensory tests in the same session were conducted: (1) a consumer rejection threshold (CRT) analysis of the sucrose concentration in the strawberry-flavored yogurt, and (2) an acceptability test on different concentrations of sucrose in the strawberry-flavored yogurt samples.

The consumer rejection threshold (CRT) was measured by the method described in Prescott, Norris, Kunst and Kim [20]. This approach is based on a standard method for assessing preference (based on selection) similar to the paired preference test within the method of the constant stimuli threshold. A series of concentration steps of the evaluated stimulus are added to the product, so consumers are asked to indicate which of the two samples – one with the added stimulus and one without – they preferred or choose (selection) [20]. Participants were instructed that the study was a test of preference for different strawberry-flavored yogurt samples with various levels of sweetness. Consumers undertook (N = 53) a series of 5-paired comparison tests, one for each sucrose concentration including the control (12%-sucrose concentration). Each pair consisted of a sample of control and a sample of another sucrose concentration (labeled with 3-digit random codes; Figure 2). Sucrose concentrations were presented in descending order (from higher to lower concentrations) [21]. In each test, participants were required to taste both samples and indicate the sample in the pair that they preferred. Participants received a new pair of samples every 2 minutes, with a 5-minute break in between pairs. After each pair, participants were requested to cleanse their palates with water and crackers. The order of consumption of samples within each pair was randomized across all the samples in the series.

Following the CRT test and after a 30 minutes break, participants were asked to complete a consumer acceptance test on all five strawberry-flavored yogurt samples (from 5% to 12%; Table 1). Participants were simultaneously presented with a new set of samples from the same batch used in the CRT. Each sample was poured into a 30 mL plastic cup coded with a 3-digit random number. The presentation order of the samples was randomized within each participant. Yogurt samples were evaluated with an internal temperature of 4 ± 1 °C. Participants were asked to evaluate the overall liking of each sample using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) and rate the relative intensity of sweetness and sourness using a 3-point just about right (JAR) scale (1 = too little, 2 = just about right, 3 = too much). An additional question of purchase intent, where consumers stated whether they would purchase the product if it was commercially available in the marketplace, was determined using a binomial scale (1 = Yes and 2 = No). At the end of the questionnaire, participants were asked to rank all samples according to their preference (1 = most preferred, 5 = least preferred). Sensory evaluations of the yogurt samples were conducted after one day of storage at 4 ± 1 °C.

Physico-chemical analysis

Physico-chemical analyses were performed on the yogurt samples on the 1st, 7th, 14th, 21st and 28th day of storage at 4 ± 1 °C. Total soluble solids content was measured with a digital refractometer for °Brix analysis (HI96801, Hanna instruments, Melbourne, VIC, Australia). The refractometer was calibrated using distilled water. Mean values from six replicate measurements and standard deviations were calculated. The pH of yogurt samples was measured at room temperature using a pH meter (Benchtop pH/mV meter, 860031, Sper scientific direct, Scottsdale, AZ, USA). The pH meter was calibrated with fresh pH 4.00 and 7.00 standard buffers. Furthermore, the viscosity of the yogurt samples was measured using a Brookfield viscometer (model DV-II+, AMETEK Brookfield, Middleborough, MA, USA). For this, 50 g of yogurt sample were retrieved from the storage container and placed into a 50 mL beaker. All samples were placed on chilled ice to maintain the storage temperature (4 ± 1 °C). The viscometer was operated at 20 revolutions per minute (RPM) (spindle number 4) [22]. The viscosity values were expressed as centipoises (cP) and recorded after 40 s of rotation. All samples were allowed to rest for 60 s after each recording to eliminate the effect of immediate time dependence. All determinations were repeated six times on the same batch of the sample, and the average value and standard deviation of six measurements were recorded.

A colorimeter (WR 10, FRU, Shenzhen, China) was used to determine whiteness (L^*), red/greenness (a^*), and yellow/blueness (b^*) values of the strawberry-flavored yogurt samples. A standard white tile was used to standardize the instrument. The color parameters (L^*, a^*, and b^*) were measured three times and averaged on the surface of each yogurt treatment. The water holding capacity (WHC) of yogurt was determined using a refrigerated centrifuge (Allegra X-12R, Beckman Coulter, Indianapolis, IN, USA). Yogurt samples (5 g) were centrifugated at 4500 x g (Relative Centrifugal Force, RCF) for 15 min at 4 °C. After centrifugation, the formed clear supernatant was collected and weighed. Triplicates were measured for each sample and averaged. The extent of the whey separation of yogurt samples was calculated from the weight of the supernatant and that of the yogurt following the Eq. 1

$$WHC(\%) = \left(1 - \frac{Supernatant\ Weight\ (g)}{Yogurt\ Weight\ (g)}\right) \times 100 \qquad \text{Eq. 1}$$

Experimental design and statistical analysis

A Randomized Block Design (RBD) was used to investigate the effect of sugar reductions on the sensory properties of the yogurt samples using the panelists as blocks. A Repeated-Measurements Design (RMD) was used to investigate the effects of sugar reduction on the physico-chemical parameters of yogurt samples during the 28 days of storage at 4 ± 1 °C. For the consumer rejection threshold test, criteria for significant rejection ($\alpha = 0.05$) as a function of sucrose level were based on the binomial distribution for paired comparison tests [20]. A linear regression analysis was used to determine the relation between sucrose concentration and the preference of consumers [23]. Analysis of variance (ANOVA) with a generalized linear model (GLM) and a *post-hoc* Tukey's Honestly Significantly Different (HSD**)** test ($P \leq 0.05$) were used to assess significant differences in the hedonic ratings and instrumental measurements of the strawberry-flavored yogurt samples.

A penalty test on the JAR ratings was performed to determine the effects of the sensory attributes on the hedonic liking of yogurt samples. The total penalty score (TPS) for individual attributes was calculated by multiplying the percentage of "not-JAR" (either "too little" or "too much") by the corresponding mean decrease [the difference between the liking score at "not-JAR" and the liking score at JAR]. Besides, a penalty analysis using the mean drops (decreases in liking due to not achieving the ideal perception of the evaluated attribute) was also conducted using the XLSTAT (Addinsoft, New York, NY, USA) statistical software version 2017 [24]. For the purchase intent data, multiple pairwise comparisons were performed using the Cochran's Q test and the simultaneous confidence intervals testing. The Friedman analysis (non-parametric) was performed for the preference data (ranking scores). Preference and acceptability are two measurements of the affective responses of consumers. The ranking method uses nonparametric statistical techniques for the analysis due to the categorical nature of the data. On the other hand, acceptability employs rating scales using parametric statistics to analyze the liking data, treating the categorical 9-point hedonic scale as a continuous scale. In some cases, the acceptability methodology fails to find significant differences among treatments when true differences exist (Type II error), and this can be attributed to several factors (variability, power, and others);

however, the bias that the scale is introducing to the data can be also affecting the analysis[19]. Therefore, using ranking data with nonparametric statistics can account for the bias of the scale and minimize the Type II error. A Principal Component Analysis (PCA) was applied to interpret relationships between overall liking and the physico-chemical data of the yogurt samples at day 1. A product-attribute biplot was used for the illustration of the PCA. Hierarchical Cluster Analysis (HCA) was performed using the Euclidean distance and the Wards linkage to categorize sample groups that were similar in the sensory and analytical results. Data analyses were performed using the XLSTAT (Addinsoft, New York, NY, USA) statistical software version 2017 [24].

RESULTS

Sensory evaluation of reduced-sucrose strawberry-flavored yogurts

Consumer rejection threshold for sucrose concentrations of strawberry-flavored yogurts

Figure 3 illustrates the proportion of participants selecting the yogurt samples containing reduced sucrose concentrations over the control (100%-sucrose = 12 g/100 g = 0% reduction). Generally, the reduction of sucrose from 0% to 58.3% in strawberry-flavored yogurts decreased the selection of the reduced-sucrose samples over the control from 52% to 35%. The 41.7%-sucrose sample (representing a sucrose reduction of 58.3%) was only selected by 35% of the participants over the control (Figure 3). A regression model was applied to correlate the participants' selections with the sucrose content in the strawberry-flavored yogurt samples. The data was fitted using the linear model [17] with a coefficient of determination $R^2 = 0.86$ (Figure 3). This suggests that there were factors other than sucrose that contributed to the consumers' selections. Based on the regression model, the extrapolated value for the proportion ($P = 0.38$; binomial distribution; $\alpha = 5\%$) of sensory panelists (N = 53) who significantly rejected the reduced-sucrose samples was at 56.3% of sucrose reduction (Figure 3). This analysis proposes that the concentration of sucrose in strawberry-flavored yogurts may be reduced by 56.3% without affecting the consumers selections compared to the full-sucrose samples (12 g/100 g of sucrose). In other words, this means that sucrose can be reduced up to 5.25 g/100 g in strawberry-flavored yogurts from an initial concentration of 12 g/100 g.

Consumers' acceptability, preference and purchase intent of reduced-sucrose strawberry-flavored yogurts

Table 2 shows the overall taste liking scores, purchase intent and preference results of the reduced-sucrose strawberry-flavored yogurt samples. The 70.8%-sucrose sample had the highest overall taste liking score (6.27) compared to other treatments (5.06-5.71). Results of the overall taste liking followed a similar trend compared to that of the critical rejection threshold analysis, in which the 41.7%-sucrose sample (corresponding to a 58.3% of sucrose reduction) obtained the lowest taste liking score and also was the least preferred sample in the CRT analysis (Figure 3). On the other hand, the 85.4%- and 56.3%-sucrose samples (i.e., 14.6% and 43.8% of sucrose reductions) were similarly scored in taste liking (5.71). The 70.8%-sucrose sample received the highest purchase intent (67.3%); however, this percentage was not significantly different ($P \geq 0.05$) to other yogurt treatments (43.3-53.9% of purchase intent; Table 2). For the preference data, the 41.7%-sucrose was the least preferred sample (rank sum = 195.5), which was significantly ($P < 0.05$) different to the 70.8%- and 56.3%-sucrose samples (rank sums = 127.5 and 140.0, respectively). The sucrose (70.8%) sample was the most preferred sample (rank sum = 127.5); however, the preference for this sample was not significantly ($P \geq 0.05$) different to the preferences of the 100%-, 85.4%- and 56.3%-sucrose samples (Table 2).

JAR responses and total penalty scores of reduced-sucrose strawberry-flavored yogurts

The percentages of just about right scores illustrate the proportion of consumers who perceive the relative intensity ("too little", "just about right" (JAR), and "too much") of a product attribute [25]. Figure 4 depicts the frequency distribution of participants' responses over the JAR scores of sweetness and sourness. In general, the JAR frequencies of sweetness ranged from 33% to 56%. On the other hand, the JAR frequencies of sourness ranged from 33% to 71% (Figure 4). For sweetness, the frequency distribution of the participants selections showed a shift towards "too much" as the concentration of sucrose increased in the yogurt samples (from 6% in the 41.7%-sucrose sample to 62% in the 100%-sucrose sample). The opposite occurred for the "too little" selection, in which the frequencies decreased from 56% in the 41.7%-sucrose sample to 5% in the 100%-sucrose sample. The 70.8%-sucrose samples obtained the highest JAR frequency of selection for sweetness (56%). Interestingly, selections of "too little" and JAR were the same

(44%) for the 56.3%-sucrose sample. The overall trend of the JAR data was that as the concentration of sugar increased, the frequency of participants that rated "too little" in sweetness decreased. Variations in the JAR and hedonic responses have been attributed to differences in the taste perception of participants [26]. It is usual to have marked differences in the taste sensitivity of the participants in the sampling population. This potentially can have an effect on the perception of the sample at the time of tasting. For sourness, the selection of being "too much" decreased as the sucrose concentration in samples increased [from 44% in Sucrose (41.7%) to 3% in Sucrose (100%)]. The selection frequencies of JAR and "too little" varied across yogurt samples for the sourness attribute. Yogurt containing 70.8%-sucrose obtained the highest percentage of JAR selection for sourness (71%). In comparison, yogurts sweetened with 41.7%- and 100%-sucrose were respectively rated "too much" and "too little" in sourness by 44% and 60% of the participants, respectively (Figure 4).

Total penalty scores in the overall liking of strawberry-flavored yogurts according to the JAR deviations of sweetness and sourness are illustrated in Figure 4. According to Walker [27], attributes with penalty scores higher than 0.5 can potentially affect consumer acceptability. Yogurts sweetened with 41.7% and 56.3% of sucrose were significantly penalized as being "too little" in sweetness (TPS = 1.08 and 0.63, respectively). On the other hand, participants penalized the 85.4%- and 100%-sucrose samples for being "too much" in sweetness (TPS = 0.86 and 0.92, correspondingly, Figure 4). The sourness in the Sucrose (41.7%) treatment was strongly penalized for being "too much" in that attribute (TPS = 1.00). This contrasts with treatments sweetened with 85.4% and 100% of sucrose, which were penalized for their low sourness intensity (TPS = 0.6 and 0.7, respectively, Figure 4).

Physico-chemical properties of reduced-sucrose strawberry-flavored yogurts

Table 3 shows the effects of sugar reductions on pH, °Brix, WHC, and viscosity of the yogurt samples during 28 days of storage at 4 ± 1 °C. Sucrose reductions did not significantly ($P \geq 0.05$) affect the pH values (4.28) of the strawberry-flavored yogurt samples at day 1. Moreover, pH values of the samples 41.7% to 70.8% of sucrose did not significantly ($P \geq 0.05$) change during the 28 days of storage period (4.26-4.28). However, the pH value of the 85.4%-sucrose sample at 28 days of storage was significantly lower ($P < 0.05$) compared to that of 1 day of storage (from 4.28 at day 1 to 4.26 at day 28; Table 3). The °Brix values decreased significantly ($P < 0.05$) as the sucrose concentration decreased in the yogurt samples [from 22.52 (100%-sucrose) to 14.89 (41.7%-sucrose) at day 1]. Storage time did not significantly ($P \geq 0.05$) affect the °Brix values of the yogurt samples except for the 100%-sucrose sample that showed a significant ($P < 0.05$) decrease after 28 days of storage (from 22.52 at day 1 to 21.82 at day 28; Table 3). At day 1, sucrose reductions significantly ($P < 0.05$) decreased the WHC of the yogurt samples (from 97% of the 100%-sucrose to 58% of the 41.7%-sucrose). Throughout the 28 days of storage, the 100%-sucrose samples had significantly higher ($P < 0.05$) WHC values compared to those of the reduced-sucrose treatments (Table 3). The WHC values of the 100%-sucrose sample decreased significantly ($P < 0.05$) during the storage time (from 97% at day 1 to 81% at day 28). For the reduced-sucrose treatments (85.4-41.7%), storage time had a marginal effect on the WHC values. The sucrose reductions in the yogurt samples marginally affected the viscosity, although the viscosities of the 41.7%-sucrose samples were generally higher than the viscosities of the 100%-sucrose samples (3,668-5,308 vs. 3,263-4,805 cP) throughout the storage period. Generally, all yogurt samples showed significant ($P < 0.05$) reductions in their viscosity values after 28 days of storage (from 5,473-4,805 at day 1 to 3,263-3,932 at day 28; Table 3).

Regarding the color, lightness (L^*), red/greenness (a^*), and yellow/blueness (b^*) values of the yogurts are shown in Table 4. In general, sucrose reductions significantly ($P < 0.05$) increased the L^* values of yogurt samples (from 81.12 of the 100%-sucrose to 84.32 of the 41.7%-sucrose at day 1). The storage time showed no significant effects ($P \geq 0.05$) on the L^* value of all treatments (from 81.12-84.32 at day 1 to 83.05-85.44 at day 28). However, the 41.7%-sucrose samples showed the highest L^* values (83.79-85.44) among all treatments throughout the 28 days of storage (Table 4). For the a^* values of the yogurt samples, sucrose reductions showed marginal changes at day 1 (a^* values of 1.75-1.91). During the 28 days of storage time, the a^* values of the yogurt treatments were significantly ($P < 0.05$) reduced (from 1.75-1.91 at day 1 to 0.47-0.62 at day 28). On the other hand, the b^* values of the yogurt treatments did not show notable changes from sucrose reductions at day 1 (b^* values of 5.66-6.33). However, the 41.7%-sucrose sample had a significantly ($P < 0.05$) higher b^* value compared to that of the 100%-sucrose sample (6.33 vs. 5.66). The storage period showed no significant effects ($P \geq 0.05$) on the b^* values of all yogurt treatments (Table 4).

Multivariate data analysis of reduced-sucrose strawberry-flavored yogurts

For all yogurt samples at day 1 of storage, the Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) results are shown in Figure 5. The PC biplot explained 91.1% (PC1 = 70.4% and PC2 = 20.7%) of the total data variability considering all the physico-chemical properties (°Brix, WHC, viscosity, and color) and overall taste liking altogether. The °Brix and WHC vectors (factor loadings = 0.97-0.99; Table S1) contributed largely to the discrimination of the yogurt samples in the PC1. On the other hand, the overall taste liking vector (factor loading = 0.96; Table S1) contributed largely to the discrimination of samples in the PC2. The °Brix vector was positively associated with WHC and the *a** color value, and negatively associated with *b** and *L** color values. The 100%-sucrose sample was associated with higher °Brix, WHC, and *a** color value, and lower *b** and *L** color values. On the other hand, the 70.8%-sucrose sample was related to higher overall taste liking, while the 41.7%-sucrose sample was associated with lower liking. Figure 5 shows the HCA of the five yogurt samples considering all physico-chemical and sensory variables. Three main cluster groups were formed: (1) 100%- and 85.4%-sucrose, (2) 70.8%-sucrose, and (3) 56.3%- and 41.7%-sucrose samples. In terms of the significant correlations of variables in this study, °Brix was positively correlated with WHC (r = 0.94; Table S2), and negatively correlated with *L** and *b** values (r = -0.98 and r = -0.91, respectively; Table S2). On the other hand, the *b** value was negatively correlated with WHC (r = -0.92; Table S2).

DISCUSSION

Sensory evaluation of reduced-sucrose strawberry-flavored yogurts

Consumer rejection threshold for sucrose concentrations of strawberry yogurts

The critical rejection threshold (CRT) analysis allows the determination of a critical point at which the preference of a product can be adversely affected [20]. Previous studies showed that there is a positive linear relationship between sugar concentrations and consumer acceptability of yogurt products [23]. In the present study, the preference for yogurt samples decreased with reductions of sucrose in the formulations (Figure 3). A consumer study conducted in Switzerland reported that the optimum sugar reduction in strawberry- and coffee-flavored yogurts was 7%. Further reductions of sugar to 5% in yogurts were shown to reduce the consumer acceptance due to the low intensity of sweetness and aroma [15].

The CRT appeared to be associated with the overall taste liking results from this study. The least preferred sample (41.7%-sucrose), which had the lowest concentration of sucrose in the CRT analysis, was found to obtain the lowest overall taste liking score (5.06; Figure 3; Table 2). However, the 70.8%-sucrose treatment obtained the highest overall taste liking score (6.27) compared to that of the 100%-sucrose sample (treatment with the highest concentration of sucrose), which only had a score of 5.50 (Table 2). These results suggest that the preference of strawberry-flavored yogurts was not solely based on the sucrose concentration but other multiple sensory factors including flavor, aroma, texture, and aftertaste [28]. In the present study, sourness also had a sensory effect on the acceptability and preference of yogurt samples. Based on the JAR results (Figure 4), the selection of being "too much" in sourness increased with reductions of sucrose, and a TPS = 1 was found for the 41.7%-sucrose sample, which indicated that this yogurt was "too much" sour for consumers. Future studies should examine the different characteristics of sensory attributes and emotional responses for better inferences of consumers' preferences towards yogurt products.

In regards to the CRT determination, the methodology of Prescott, Norris, Kunst and Kim [20] was designed to determine the level of additive that caused consumers to prefer/choose the base (unadulterated sample). However, the method of Prescott, Norris, Kunst and Kim [20] had to be adapted and modified to achieve the research objectives of the present study. The rationale behind this is that in Prescott, Norris, Kunst and Kim [20] the objective was to determine the CRT value of increasing concentrations of 2,4,6-trichloroanisole (TCA) in white wine. The TCA (cork taint) is associated with negative quality and perception in wines, and the increases of this compound generate the rejection of consumers. The opposite happens with sugar in yogurts (and/or other dairy products), as this ingredient is associated with positive quality and liking. Therefore, reducing sugar in yogurt (to a specific limit) can generate the rejection of consumers. Besides, a similar methodology of reducing sugar concentrations to obtain the CRT has been implemented by de Oliveira Pineli, de Aguiar, Fiusa, de Assuncao Botelho, Zandonadi and Melo [21] for orange nectars.

Consumers acceptability and preference of reduced-sucrose strawberry-flavored yogurts

Participants perceived the sugar-reduced strawberry-flavored yogurts as being less sweet and much sour. This is expected as sugar has also the role of offsetting the acidic taste of the yogurt base [23, 29]. In the present study, reducing sucrose concentrations in the yogurt samples increased the selection of "too little" in sweetness, and increased the selection of "too much" in sourness (Figure 4). These findings coincide with results from previous studies of strawberry-flavored yogurts [15, 30]. The optimum sucrose level in terms of product pleasantness not only depends on the perceived sweetness but also on the overall food taste experience [31]. In the present study, the 70.8%-sucrose was the most liked (6.27) and most preferred (rank sum = 125.50) sample among all yogurts (Table 2). This liking score was directly associated with the perceived ideal sweetness and sourness intensities from the JAR results (JAR = 56% for sweetness, and JAR=71% for sourness; Figure 4). On the other hand, the 41.7%-sucrose was the least liked (5.06) and least preferred (rank sum 195.50) yogurt sample (Table 2). From the TPS results, the 100%- and 85.4%-sucrose samples were penalized for being too sweet and too little in sourness. In contrast, the 41.7%- and 56.3%-sucrose samples were penalized for being less sweet. The least liked sample (41.7%-sucrose) was also penalized for being too sour by 44% of the participants (Figure 4).

Although significant differences among the yogurt samples were found for the overall taste liking, the purchase intent (%) showed marginal differences. Results showed that consumers would not mind purchasing reduced-sugar strawberry-flavored yogurts compared to the full-sugar versions that are sold in the marketplace. In fact, for the present study, the 100%-sucrose sample, which corresponded to the sweetness level of commercial strawberry-flavored yogurts in Australia, obtained a medium-low purchase intent percentage (42.3%). This appeared to be associated with the intensity of sweetness, in which 61.5% of the participants penalized the overall taste of the 100%-sucrose for being too sweet. These results are in line with the current trends in consumer diets, which are systematically shifting towards healthier products with lower sugar concentrations. Other techniques can be used to measure the effect of non-sensory factors on the acceptability of yogurt samples in future studies. Projective techniques are used for measuring the feeling and beliefs of participants using the conscious and unconscious parts of their minds. These techniques can help to overcome communication barriers throughout the subconscious perceptions of participants [32, 33].

Physico-chemical properties of reduced-sucrose strawberry-flavored yogurts

Sucrose reductions showed no effect on the pH values of the strawberry-flavored yogurts. An average pH range of 4.26 to 4.28 was observed for all the samples. The values of this range were higher than the pH value (4.11) reported by Cinbas and Yazici [22], in which blueberry yogurts sweetened with 6% of sugar were evaluated. The slightly higher pH values of the yogurt samples obtained in the present study may be partially attributed to a lower ratio of *Streptococcus thermophilus* over *Lactobacillus bulgaricus*, which has been proven to reduce the pH lowering effect in the incubation of yogurt mixes [34]. Previous studies reported a significant pH reduction in strawberry- and blueberry-flavored yogurts during 14 and 20 days of refrigerated storage [22, 35].

Contrary to the results of the present study, a 28 days of storage period did not show any significant change in the pH of the yogurt samples. Although the same strains of starter culture (*S. thermophilus* and *L. bulgaricus*) were used, inconsistencies in the results between studies may be attributed to the metabolic activity (production of organic acids, flavors/aromas compounds, and fatty acids) of the bacterial strains [36]. The metabolic activity can be affected by the conditions of the medium in which bacteria grow and perform the fermentation; therefore, having different sugar concentrations could be a factor for the differences in quality. An inhibitory effect has been reported in the growth of lactic acid bacteria when the content of sucrose in milk increased (10-12%) [37]. Besides, small variations in the production of yogurt batches can also affect the metabolic activity of bacteria. After 28 days of storage, the pH values of the 100%- and 85.4%-sucrose samples were marginally reduced compared to that of other treatments (Table 3). This can partially be explained by the higher sugar concentrations in these samples (100%- and 85.4%-sucrose) since sugar has been proven to optimize the growth of lactic acid bacteria and promote the formation of lactic acid during fermentation [38]. Further microbiological studies are needed to understand the effects of sugar reduction on the performance of lactic acid bacteria in yogurts.

Sucrose reductions significantly decreased the °Brix values of yogurt samples (Table 3). This result was expected due to the reduction of the sugar mass fraction that decreased the total solid content of yogurts [39]. The results in the present research are in agreement with the study of Cinbas and Yazici [22], in which a significant difference in the total solid content of blueberry yogurts was observed between the 0%- and 6%-sugar samples. Strawberries can add sweetness to the yogurt samples beyond the added sugar of the formulations. In the present study, the total °Brix values of the unsweetened jams were measured for the batches used for sensory analysis (°Brix = 12.5±1.0) and physico-chemical measurements (°Brix = 11.0±1.0). A previous study by Samykanno, Pang and Marriott [40] reported that Australian strawberries have °Brix values of 10.6-10.8. Strawberries with these °Brix values were perceived as slightly sweet by a descriptive panel [41]. The °Brix values reported in the present study (jam) are similar to the values reported by Samykanno, Pang and Marriott [40] from the fruit puree. This confirms that there is a baseline sweetness in the yogurt products imparted by the °Brix values of the strawberries. As mentioned in the materials and methods of the present study, the strawberry jam was prepared by liquefying the fresh strawberries with a blender, and then the desired sucrose concentration was added for each treatment. However, variations between batches exist since although the process of making the strawberry jams was the same for each batch, the fruits have an inherent variation in terms of composition. However, the results of the present study indicated that the preparations of the strawberry jams were made correctly since increases in sugar concentrations generated increases in both sensory responses and °Brix values as it can be observed in Figure 4 and Table 3.

The WHC is defined as the cohesiveness of the yogurt gel microstructure [42]. It measures the whey separation and stability of coagulation in yogurt products [43]. In the present study, the WHC of the strawberry-flavored yogurt samples decreased with reductions in the sucrose concentration (Table 3). These results may be associated with the lower total solid content and the water binding capacity from sugar reductions [43]. The water holding capacity of yogurt has been associated with the ability of milk proteins to retain water [44]. The significant decrease of the WHC in the 41.7%- and 56.3%-sucrose samples during storage (Table 3) may be explained by the degradation of proteins that is commonly catalyzed by bacterial proteases that exist in yogurt [45].

The apparent viscosity is a quality parameter that determines the flow behavior; thereby, it measures the consistency of yogurts [46]. In the present study, the influence of sucrose reductions on yogurt consistency was marginal, with no significant differences found among the viscosity values (Table 3). These results are comparable to previous studies, in which it is shown that viscosity of yogurts was stable with different sugar reductions in the formulations [22, 46]. The present study showed that the extended storage time of 28 days at 4 °C significantly reduced the viscosity of strawberry-flavored yogurts, which is consistent with the findings of Celik, Bakırcı and Şat [47]. According to Jooyandeh, Mortazavi, Farhang and Samavati [42], viscosity is an attribute that is influenced by many factors, including formulation composition, types of starter culture, and processing methods. The decline of viscosity over time may be related to the pH reduction which could have weakened the covalent cross-link of protein polymer in the yogurt [48]. According to Sodini, Remeuf, Haddad and Corrieu [43], yogurt exhibits certain non-Newtonian behavior such as shear-thinning, yield stress, viscoelasticity, and time dependency. Future studies are needed to understand the textural properties of yogurt and evaluate how sucrose reductions affect its rheological behavior.

Regarding color, Cinbas and Yazici [22] demonstrated that unsweetened blueberry yogurts were significantly higher in lightness (*L**) but lower in yellow/blueness (*b**), and not different in red/greenness (*a**) values compared to yogurts sweetened with 6%-sucrose. In contrast to these results, sucrose reductions increased the lightness (*L**) values in yogurts from the present study (Table 4). A previous study demonstrated that the addition of red color improved the sweetness sensation of strawberry-flavored drinks [49]. Color is the first perceived attribute that can determine product acceptance and preference [50]. The 28 days of storage period significantly increased the lightness (*L**) and decreased the redness (*a**) of yogurt samples (Table 4). The loss of color pigments during food storage has been associated with numerous factors, including pH, acidity, oxidation, storage humidity and temperature [51]. Other studies showed that the pigments of strawberry (anthocyanins) are unstable under changes in pH, light, oxygen, enzymes, ascorbic acid and thermal treatments [52]. In the present study, the degradation of the red color, which increased the lightness of yogurt samples during the 28 days of storage, may be associated with a combination of the aforementioned factors. Further studies are needed to evaluate the combined effects of sugar reduction and storage time on the color and pigment changes of strawberry-flavored yogurts.

CONCLUSIONS

A critical rejection threshold and acceptability tests were conducted to determine the most liked/preferred concentration of sugar (sucrose) in strawberry-flavored yogurts based on consumer's responses. From the results, sucrose can be reduced to 5.25 g/100 g in strawberry-flavored yogurts from an initial concentration of 12 g/100 g without affecting the preferences of consumers. However, the 70.8%-sucrose was perceived as the most liked and preferred yogurt sample. In general, reduction of sucrose affected the acceptability and physico-chemical characteristics of yogurts. Lighter colors (higher L^* value), lower total soluble contents (°Brix), and lower water holding capacities were perceived acceptable by consumers. Future microbiological studies should be conducted to explore the effects of sugar reductions on the lactic acid bacteria growth in yogurts. These findings are useful for understanding consumers' acceptability and preferences of yogurts with reduced-sugar formulations.

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CONFLIC OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Rouhi M, Mohammadi R, Mortazavian A and Sarlak Z, Combined effects of replacement of sucrose with D-tagatose and addition of different probiotic strains on quality characteristics of chocolate milk. *Dairy Sci Technol* **95**:115-133 (2015).
2. Scott SK, Rabito FA, Price PD, Butler NN, Schwartzbaum JA, Jackson BM, Love RL and Harris RE, Comorbidity among the morbidly obese: a comparative study of 2002 US hospital patient discharges. *Surg Obes Relat Dis* **2**:105-111 (2006).
3. Aidoo RP, Depypere F, Afoakwa EO and Dewettinck K, Industrial manufacture of sugar-free chocolates–Applicability of alternative sweeteners and carbohydrate polymers as raw materials in product development. *Trends Food Sci Tech* **32**:84-96 (2013).
4. Chattopadhyay S, Raychaudhuri U and Chakraborty R, Artificial sweeteners–A review. *J Food Sci Technol* **51**:611-621 (2014).
5. ABS, *Australian health survey: Nutrition first results – Food and nutrients, 2011-12*. http://www.abs.gov.au/ [August 18 2018].
6. Yildiz-Akgül F, Enhancement of torba yoghurt with whey protein isolates. *Int J Dairy Technol* **71**:898-905 (2018).
7. Heydari S, Amiri-Rigi A, Ehsani MR, Mohammadifar MA, Khorshidian N, Koushki MR and Mortazavian AM, Rheological behaviour, sensory properties and syneresis of probiotic yoghurt supplemented with various prebiotics. *Int J Dairy Technol* **71**:175-184 (2018).
8. Chetachukwu AS, Thongraung C and Yupanqui CT, Development of reduced-fat coconut yoghurt: physicochemical, rheological, microstructural and sensory properties. *Int J Dairy Technol* **72**:524-535 (2019).
9. McCain H, Kaliappan S and Drake M, Invited review: Sugar reduction in dairy products. *J Dairy Sci* **101**:8619-8640 (2018).
10. Slocum S, Jasinski E, Anantheswaran RC and Kilara A, Effect of sucrose on proteolysis in yogurt during incubation and storage. *J Dairy Sci* **71**:589-595 (1988).
11. WHO, *WHO calls on countries to reduce sugars intake among adults and children*. http://www.who.int/mediacentre/news/releases/2015/sugar-guideline/en [August 28 2018].
12. Johansen SB, Næs T, Øyaas J and Hersleth M, Acceptance of calorie-reduced yoghurt: Effects of sensory characteristics and product information. *Food Qual Prefer* **21**:13-21 (2010).

13. Reis RC, Minim VP, BOLINI HM, Dias BR, Minim LA and Ceresino EB, Sweetness equivalence of different sweeteners in strawberry-flavored yogurt. *J Food Qual* **34**:163-170 (2011).
14. Nikooie A, Ghandehari Yazdi AP and Shamsaei M, Effect of sucrose replacement by Stevia as a non-nutritive sweetener and bulking compounds on physiochemical properties of foodstuffs. *J Herb Drugs (An International Journal on Medicinal Herbs)* **6**:121-128 (2015).
15. Chollet M, Gille D, Schmid A, Walther B and Piccinali P, Acceptance of sugar reduction in flavored yogurt. *J Dairy Sci* **96**:5501-5511 (2013).
16. Hoppert K, Zahn S, Jänecke L, Mai R, Hoffmann S and Rohm H, Consumer acceptance of regular and reduced-sugar yogurt enriched with different types of dietary fiber. *Int Dairy J* **28**:1-7 (2013).
17. Li X, Lopetcharat K, Qiu Y and Drake M, Sugar reduction of skim chocolate milk and viability of alternative sweetening through lactose hydrolysis. *J Dairy Science* **98**:1455-1466 (2015).
18. Oliveira D, Antúnez L, Giménez A, Castura JC, Deliza R and Ares G, Sugar reduction in probiotic chocolate-flavored milk: Impact on dynamic sensory profile and liking. *Food Res Int* **75**:148-156 (2015).
19. Lawless HT and Heymann H, *Sensory evaluation of food: principles and practices*. Springer Science & Business Media (2010).
20. Prescott J, Norris L, Kunst M and Kim S, Estimating a "consumer rejection threshold" for cork taint in white wine. *Food Qual Prefer* **16**:345-349 (2005).
21. de Oliveira Pineli LdL, de Aguiar LA, Fiusa A, de Assuncao Botelho RB, Zandonadi RP and Melo L, Sensory impact of lowering sugar content in orange nectars to design healthier, low-sugar industrialized beverages. *Appetite* **96**:239-244 (2016).
22. Cinbas A and Yazici F, Effect of the addition of blueberries on selected physicochemical and sensory properties of yoghurts. *Food Technol Biotech* **46**:434-441 (2008).
23. Barnes DL, Harper SJ, Bodyfelt FW and McDaniel MR, Prediction of consumer acceptability of yogurt by sensory and analytical measures of sweetness and sourness. *J Dairy Sci* **74**:3746-3754 (1991).
24. XLSTAT, *XLSTAT (Addinsoft)*. http://www.xlstat.com/en/ [November 15 2018].
25. Pohjanheimo T and Sandell M, Explaining the liking for drinking yoghurt: The role of sensory quality, food choice motives, health concern and product information. *Int Dairy J* **19**:459-466 (2009).
26. Masi C, Dinnella C, Monteleone E and Prescott J, The impact of individual variations in taste sensitivity on coffee perceptions and preferences. *Physiol Behav* **138**:219-226 (2015).
27. Walker, *Using penalty analysis as an aid in product development. White paper – development resources*. http://www.fona.com [November 15 2018].
28. Routray W and Mishra HN, Scientific and technical aspects of yogurt aroma and taste: a review. *Compr Rev Food Sci Food Saf* **10**:208-220 (2011).

29. Vickers Z, Holton E and Wang J, Effect of ideal–relative sweetness on yogurt consumption. *Food Qual Prefer* **12**:521-526 (2001).
30. Kälviäinen N, Roininen K and Tuorila H, The relative importance of texture, taste and aroma on a yogurt-type snack food preference in the young and the elderly. *Food Qual Prefer* **14**:177-186 (2003).
31. Moskowitz HR, Kluter RA, Westerling J and Jacobs HL, Sugar sweetness and pleasantness: Evidence for different psychological laws. *Science* **184**:583-585 (1974).
32. Judacewski P, Los PR, Lima LS, Alberti A, Zielinski AAF and Nogueira A, Perceptions of Brazilian consumers regarding white mould surface-ripened cheese using free word association. *Int J Dairy Technol* **72**:585-590 (2019).
33. Pinto LdPF, Silva HL, Kuriya SP, Maçaira PM, Cyrino Oliveira FL, Cruz AG, Esmerino EA and Freitas MQ, Understanding perceptions and beliefs about different types of fermented milks through the application of projective techniques: A case study using Haire's shopping list and free word association. *J Sens Stud* **33**:e12326 (2018).
34. Purwandari U, Shah N and Vasiljevic T, Effects of exopolysaccharide-producing strains of Streptococcus thermophilus on technological and rheological properties of set-type yoghurt. *Int Dairy J* **17**:1344-1352 (2007).
35. Vahedi N, Tehrani MM and Shahidi F, Optimizing of fruit yoghurt formulation and evaluating its quality during storage. *Am-Eurasian J Agric Environ Sci* **3**:922-927 (2008).
36. Batista AL, Silva R, Cappato LP, Almada CN, Garcia RK, Silva MC, Raices RS, Arellano DB, Sant'Ana AS and Junior CAC, Quality parameters of probiotic yogurt added to glucose oxidase compared to commercial products through microbiological, physical–chemical and metabolic activity analyses. *Food Res Int* **77**:627-635 (2015).
37. Zourari A, Accolas J and Desmazeaud M, Metabolism and biochemical characteristics of yogurt bacteria. A review. *Le lait* **72**:1-34 (1992).
38. Hartati AI, Lactose and reduction sugar concentrations, pH and the sourness of date flavored yogurt drink as probiotic beverage. *Jurnal Aplikasi Teknologi Pangan* **1** (2012).
39. Popa D and Ustunol Z, Sensory attributes of low-fat strawberry yoghurt as influenced by honey from different floral sources, sucrose and high-fructose corn sweetener. *Int J Dairy Technol* **64**:451-454 (2011).
40. Samykanno K, Pang E and Marriott PJ, Chemical characterisation of two Australian-grown strawberry varieties by using comprehensive two-dimensional gas chromatography–mass spectrometry. *Food Chem* **141**:1997-2005 (2013).
41. Wozniak W, Radajewska B, Reszelska-Sieciechowicz A and Dejwor I, Sugars and acid content influence organoleptic evaluation of fruits of six strawberry cultivars from controlled cultivation, in *III International Strawberry Symposium 439*, Ed, pp 333-336 (1996).
42. Jooyandeh H, Mortazavi SA, Farhang P and Samavati V, Physicochemical properties of set-style yoghurt as effected by microbial transglutaminase and milk solids contents. *J Appl Env Biol Sci* **4**:59-67 (2015).

43. Sodini I, Remeuf F, Haddad S and Corrieu G, The relative effect of milk base, starter, and process on yogurt texture: a review. *Crit Rev Food Sci Nutr* **44**:113-137 (2004).
44. Wu H, Hulbert GJ and Mount JR, Effects of ultrasound on milk homogenization and fermentation with yogurt starter. *Innov Food Sci Emerg Technol* **1**:211-218 (2000).
45. Tamime A and Robinson R, Yogurt Science and Technology, Woodhead Publishing. *Cambridge, England* (1999).
46. Lisak K, Jeličić I, Tratnik L and Božanić R, Influence of sweetener stevia on the quality of strawberry flavoured fresh yoghurt. *Mljekarstvo* **61**:220 (2011).
47. Celik S, Bakırcı I and Şat I, Physicochemical and organoleptic properties of yogurt with cornelian cherry paste. *Int J Food Prop* **9**:401-408 (2006).
48. Lorenzen PC, Neve H, Mautner A and Schlimme E, Effect of enzymatic cross-linking of milk proteins on functional properties of set-style yoghurt. *Int J Dairy Technol* **55**:152-157 (2002).
49. Calvo C, Salvador A and Fiszman SM, Influence of colour intensity on the perception of colour and sweetness in various fruit-flavoured yoghurts. *Eur Food Res Technol* **213**:99-103 (2001).
50. Ares G and Deliza R, Studying the influence of package shape and colour on consumer expectations of milk desserts using word association and conjoint analysis. *Food Qual Prefer* **21**:930-937 (2010).
51. Ahmed J, Shivhare U and Sandhu K, Thermal degradation kinetics of carotenoids and visual color of papaya puree. *J Food Sci* **67**:2692-2695 (2002).
52. Francis FJ and Markakis PC, Food colorants: anthocyanins. *Crit Rev Food Sci Nutr* **28**:273-314 (1989).
53. Torrico DD, Tam J, Fuentes S, Gonzalez Viejo C and Dunshea FR, D-Tagatose as a sucrose substitute and its effect on the physico-chemical properties and acceptability of strawberry-flavored yogurt. *Foods* **8**:256 (2019).

Figures captions

Figure 1. Flow chart of steps involved in the preparation of strawberry-flavored yogurt samples.

Figure 2. Consumer rejection threshold (CRT) methodology for the yogurt samples*.
*****In each test, participants are required to taste both samples and indicate the sample in the pair that they prefer. The order of the pair presentation (control and reduced-sucrose sample) was randomized within each test.

Figure 3. Proportions of participant selecting the reduced-sucrose yogurt sample over the control (full-sucrose sample)*.
*The dotted grey line at y = 0.5 represents the baseline preference at random chance while the solid grey line illustrates the 5% significance criterion (1 - 0.62 = 0.38) of sucrose reduction rejection based on the binomial distribution for the paired comparison test (N = 53). Consumer rejection threshold (CRT) for sucrose reduction on strawberry-flavored yogurt samples was reached at 56.26%, which was calculated based on predicted linear model equation.

Figure 4. Selection frequencies (%) of Just-About-Right (JAR) results and the total penalty scores in overall taste liking for sweetness and sourness of strawberry-flavored yogurt samples (Treatment labels are shown in Table 1).

Figure 5. (a) Principal component analysis (PCA) bi-plot and (b) Cluster analysis visualizing treatments* (strawberry-flavored yogurt samples), taste liking, and physico-chemical properties at day 1 of storage time.
*Treatment labels are shown in Table 1. WHC = Walter Holding Capacity.

Table 1. Formulations* of the different sucrose concentrations for the strawberry-flavored yogurt samples

Treatment label	Concentration of Sucrose	Sucrose reduction
Sucrose (100%)	12.00 g/100 g yogurt	0.00%
Sucrose (85.42%)	10.25 g/100 g yogurt	14.58%
Sucrose (70.83%)	8.50 g/100 g yogurt	29.17%
Sucrose (56.25%)	6.75 g/100 g yogurt	43.75%
Sucrose (41.67%)	5.00 g/100 g yogurt	58.33%

*The yogurt sample with the sucrose concentration of 12.00 g/100 g represented the full-sucrose sample (100 %). The subsequent sucrose concentrations represent reductions from the initial sucrose concentration. The sucrose was mixed with the pasteurized strawberry flavoring (5.5%) that was incorporated into the pre-made mild yogurt.

Table 2. Overall taste liking, purchase intent values and ranking sums of the strawberry-flavored yogurt samples

Treatment*	Overall taste liking**	Purchase intent (%)***	Preference (rank sums)****
Sucrose (100%)	5.50 ± 1.51[ab]	42.31%[a]	163.50[ab]
Sucrose (85.42%)	5.71 ± 1.56[ab]	53.85%[a]	153.50[ab]
Sucrose (70.83%)	6.27 ± 1.42[a]	67.31%[a]	127.50[b]
Sucrose (56.25%)	5.71 ± 1.39[ab]	42.31%[a]	140.00[b]
Sucrose (41.67%)	5.06 ± 1.76[b]	42.31%[a]	195.50[a]

*Treatment labels are shown in Table 1.

**Overall taste liking data are represented as mean and standard deviation values (N=53). The liking scores were based on a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely).

[a–b] For overall taste liking, mean values that share the same letter within the same column are not significantly different ($P \geq 0.05$).

***For the purchase intent results, percentage values which share the same superscripts within the same column are not significantly different [$P \geq 0.05$; Cochran Q test and simultaneous confidence interval test].

****For the ranking results, rank sum values with the same superscripts within the same column are not significantly different [$P \geq 0.05$; Friedman test].

Table 3. Effects of sucrose reductions on pH, °Brix, water holding capacity (WHC), and viscosity of strawberry-flavored yogurt samples during 28 days of storage at 4 °C

Parameter	Treatment*	Time of storage (days)				
		1	7	14	21	28
pH	Sucrose (100%)	4.28 ± 0.01^{abc}	4.26 ± 0.01^{cd}	4.27 ± 0.01^{abcd}	4.26 ± 0.01^{d}	4.26 ± 0.01^{cd}
	Sucrose (85.42%)	4.28 ± 0.01^{abc}	4.27 ± 0.01^{abcd}	4.26 ± 0.01^{bcd}	4.26 ± 0.01^{d}	4.26 ± 0.01^{d}
	Sucrose (70.83%)	4.28 ± 0.01^{ab}	4.27 ± 0.01^{abcd}	4.26 ± 0.01^{bcd}	4.26 ± 0.01^{cd}	4.27 ± 0.01^{abcd}
	Sucrose (56.25%)	4.28 ± 0.01^{a}	4.27 ± 0.01^{abcd}	4.27 ± 0.01^{abcd}	4.27 ± 0.01^{abcd}	4.27 ± 0.01^{abcd}
	Sucrose (41.67%)	4.28 ± 0.01^{a}	4.28 ± 0.01^{ab}	4.28 ± 0.01^{abc}	4.28 ± 0.01^{abc}	4.28 ± 0.01^{abc}
°Brix	Sucrose (100%)	22.52 ± 0.52^{a}	21.93 ± 0.22^{ab}	22.09 ± 0.31^{ab}	22.21 ± 0.18^{ab}	21.82 ± 0.43^{b}
	Sucrose (85.42%)	20.13 ± 0.59^{c}	20.52 ± 0.12^{c}	20.60 ± 0.17^{c}	20.63 ± 0.39^{c}	20.74 ± 0.28^{c}
	Sucrose (70.83%)	19.12 ± 0.86^{de}	19.14 ± 0.84^{d}	18.33 ± 0.12^{f}	18.47 ± 0.11^{ef}	18.74 ± 0.30^{def}
	Sucrose (56.25%)	16.18 ± 0.44^{g}	16.81 ± 0.08^{g}	16.21 ± 0.28^{g}	16.40 ± 0.28^{g}	16.37 ± 0.30^{g}
	Sucrose (41.67%)	14.89 ± 0.31^{h}	15.17 ± 0.32^{h}	15.17 ± 0.15^{h}	15.19 ± 0.39^{h}	15.03 ± 0.22^{h}
WHC (%)	Sucrose (100%)	97.00 ± 0.20^{a}	81.41 ± 0.70^{b}	81.57 ± 1.01^{b}	81.61 ± 2.10^{b}	80.89 ± 1.69^{b}
	Sucrose (85.42%)	75.13 ± 0.81^{c}	75.13 ± 0.13^{c}	75.84 ± 1.66^{c}	72.81 ± 1.19^{cd}	74.59 ± 0.60^{c}
	Sucrose (70.83%)	69.47 ± 0.50^{def}	71.01 ± 0.03^{de}	72.56 ± 1.05^{cd}	68.99 ± 0.30^{ef}	71.04 ± 0.15^{de}
	Sucrose (56.25%)	62.87 ± 0.42^{i}	66.98 ± 1.13^{fgh}	68.37 ± 1.12^{efg}	64.98 ± 0.80^{ghi}	69.75 ± 1.14^{def}
	Sucrose (41.67%)	58.00 ± 1.00^{j}	67.84 ± 1.14^{efgh}	67.51 ± 1.11^{fgh}	64.87 ± 0.29^{hi}	67.04 ± 0.57^{fgh}
Viscosity (cP)	Sucrose (100%)	4,805 ± 587^{abcd}	4,413 ± 578^{abcdef}	4,112 ± 485^{bcdef}	3,920 ± 408^{cdef}	3,263 ± 268^{f}
	Sucrose (85.42%)	4,962 ± 563^{abc}	4,962 ± 536^{abc}	4,365 ± 571^{abcdef}	4,282 ± 435^{abcdef}	3,522 ± 412^{ef}
	Sucrose (70.83%)	5,473 ± 661^{a}	5,150 ± 712^{abc}	4,403 ± 594^{abcdef}	4,240 ± 663^{abcdef}	3,607 ± 429^{def}
	Sucrose (56.25%)	5,147 ± 733^{abc}	4,552 ± 634^{abcde}	4,375 ± 639^{abcdef}	4,432 ± 600^{abcdef}	3,932 ± 477^{cdef}
	Sucrose (41.67%)	5,287 ± 631^{ab}	5,308 ± 798^{ab}	4,540 ± 686^{abcde}	4,193 ± 630^{bcdef}	3,668 ± 684^{def}

Values represent means and standard deviations (SD) of six replicates from two independent batches of yogurt.

$^{a\text{-}j}$ Mean values that share the same letter within the same parameter are not significantly different ($P \geq 0.05$).

*Treatment labels are indicated in Table 1.

Table 4. Effects of sucrose reductions on color (*L**, *a**, and *b**) values of strawberry-flavored yogurt samples during 28 days of storage at 4 °C

Parameter	Treatment*	Time of storage (days)				
		1	**7**	**14**	**21**	**28**
*L** value	Sucrose (100%)	81.12 ± 0.88^{n}	81.54 ± 0.24^{mn}	82.12 ± 0.38^{klm}	80.98 ± 0.63^{n}	83.05 ± 0.13^{hi}
	Sucrose (85.42%)	81.65 ± 0.36^{lmn}	82.69 ± 0.25^{ijk}	82.75 ± 0.38^{ijk}	83.35 ± 0.20^{ghi}	83.80 ± 0.36^{efg}
	Sucrose (70.83%)	82.08 ± 0.64^{klm}	82.32 ± 0.14^{jkl}	82.96 ± 0.41^{hij}	83.95 ± 0.47^{defg}	84.55 ± 0.28^{bcd}
	Sucrose (56.25%)	83.87 ± 0.73^{defg}	83.54 ± 0.35^{fgh}	83.98 ± 0.44^{defg}	84.56 ± 0.42^{bcd}	84.91 ± 0.14^{abc}
	Sucrose (41.67%)	84.32 ± 0.47^{cde}	83.79 ± 0.29^{efg}	84.24 ± 0.20^{cdef}	85.20 ± 0.15^{ab}	85.44 ± 0.15^{a}
*a** value	Sucrose (100%)	1.91 ± 0.16^{a}	1.47 ± 0.09^{c}	1.12 ± 0.11^{fgh}	0.81 ± 0.12^{k}	0.47 ± 0.05^{mn}
	Sucrose (85.42%)	1.89 ± 0.07^{a}	1.36 ± 0.08^{cd}	1.05 ± 0.09^{ghi}	0.88 ± 0.07^{jk}	0.62 ± 0.05^{l}
	Sucrose (70.83%)	1.83 ± 0.09^{ab}	1.26 ± 0.02^{de}	0.95 ± 0.07^{ij}	0.78 ± 0.10^{k}	0.61 ± 0.04^{lm}
	Sucrose (56.25%)	1.75 ± 0.08^{b}	1.23 ± 0.05^{ef}	0.86 ± 0.05^{jk}	0.80 ± 0.06^{k}	$0.47 \pm 0.\ 03^{n}$
	Sucrose (41.67%)	1.82 ± 0.04^{ab}	1.15 ± 0.07^{efg}	0.99 ± 0.10^{hij}	0.78 ± 0.03^{k}	0.57 ± 0.10^{lmn}
*b** value	Sucrose (100%)	5.66 ± 0.12^{g}	6.18 ± 0.11^{bcdef}	6.28 ± 0.10^{abcde}	6.25 ± 0.11^{abcdef}	5.84 ± 0.11^{fg}
	Sucrose (85.42%)	5.99 ± 0.10^{defg}	6.28 ± 0.09^{abcde}	6.42 ± 0.10^{abc}	6.23 ± 0.04^{bcdef}	6.20 ± 0.12^{bcdef}
	Sucrose (70.83%)	5.96 ± 0.15^{efg}	6.37 ± 0.05^{abcde}	6.40 ± 0.19^{abcd}	6.27 ± 0.16^{abcde}	6.25 ± 0.07^{abcdef}
	Sucrose (56.25%)	6.03 ± 0.30^{cdefg}	6.25 ± 0.07^{abcdef}	6.53 ± 0.20^{ab}	6.66 ± 0.19^{a}	6.32 ± 0.05^{abcde}
	Sucrose (41.67%)	6.33 ± 0.09^{abcde}	6.64 ± 0.23^{a}	6.51 ± 0.12^{ab}	6.34 ± 0.06^{abcde}	6.48 ± 0.12^{ab}

Values represent means and standard deviations (SD) of six replicates from two independent batches of yogurt.

$^{a\text{-}n}$ Mean values that share the same letter within the same parameter are not significantly different ($P \geq 0.05$).

*Treatment labels are indicated in Table 1.

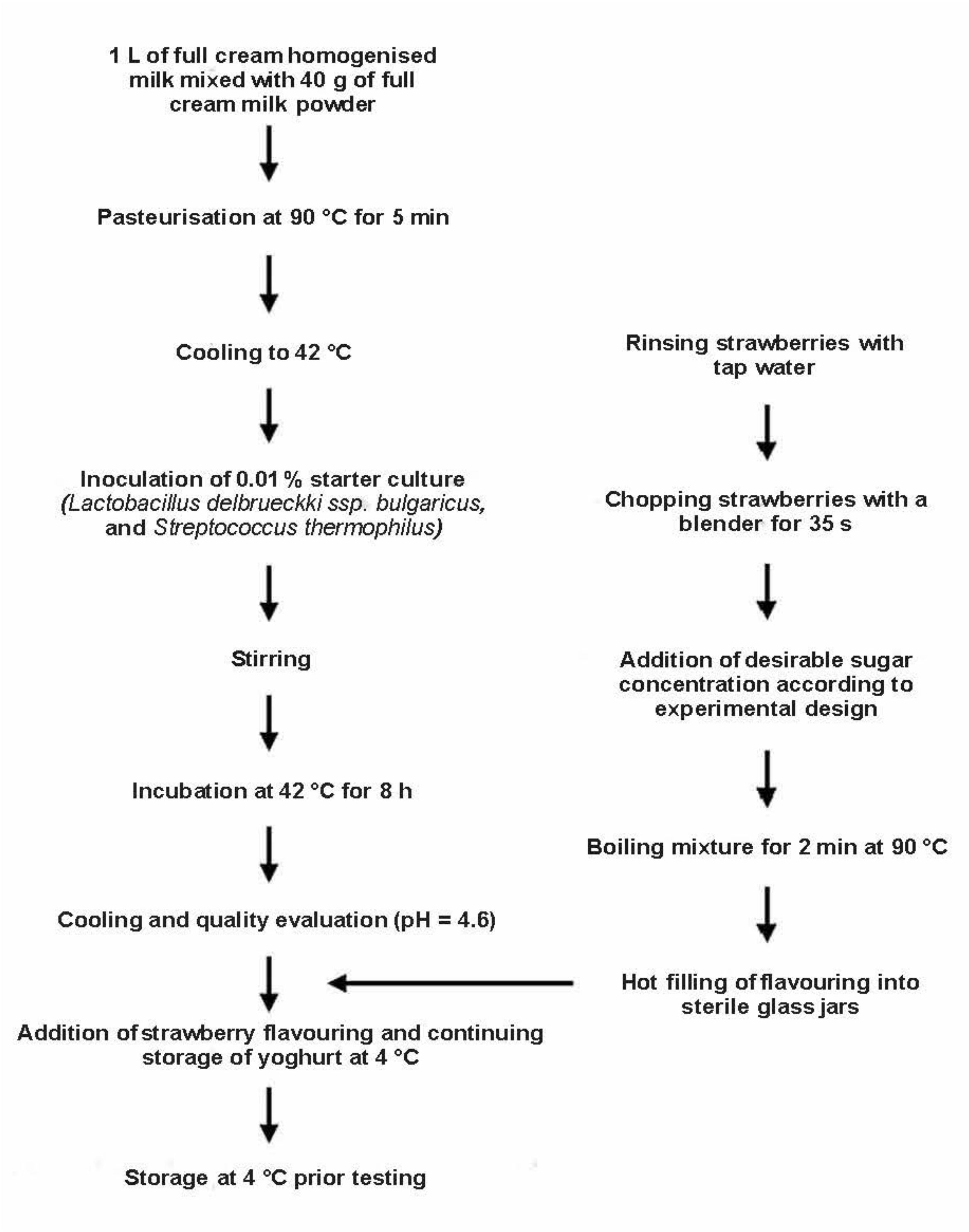
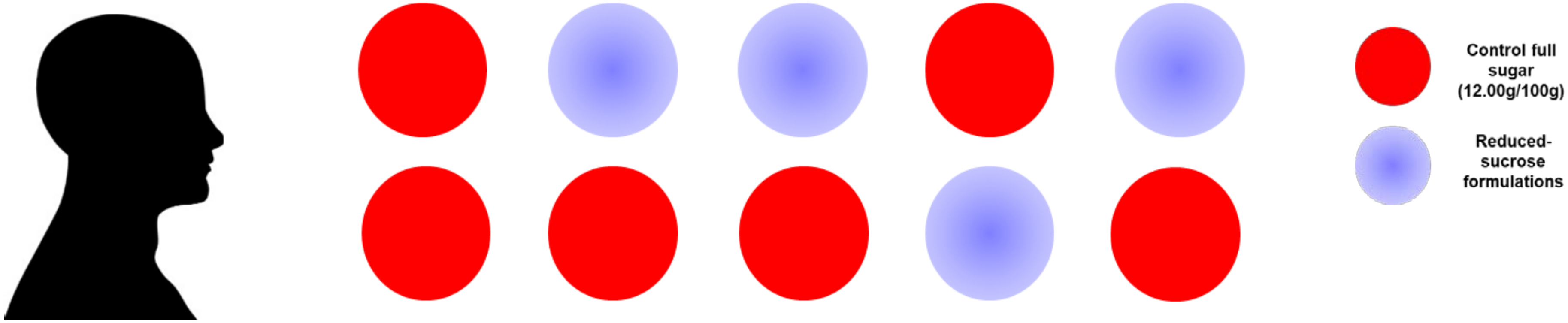


Figure 1. Flow chart of steps involved in the preparation of strawberry-flavored yogurt samples. Extracted from Torrico et al (2019)[53].



Reductions of sucrose	0%	14.58%	29.17%	43.75%	58.33%
Sucrose concentration	12.00g/100g	10.25g/100g	8.50g/100g	6.75g/100g	5.00g/100g
Sucrose concentration (%)	100%	85.42%	70.83%	56.25%	41.67%

Figure 2. Consumer rejection threshold (CRT) methodology for the yogurt samples*.

*In each test, participants are required to taste both samples and indicate the sample in the pair that they prefer. The order of the pair presentation (control and reduced-sucrose sample) was randomized within each test.

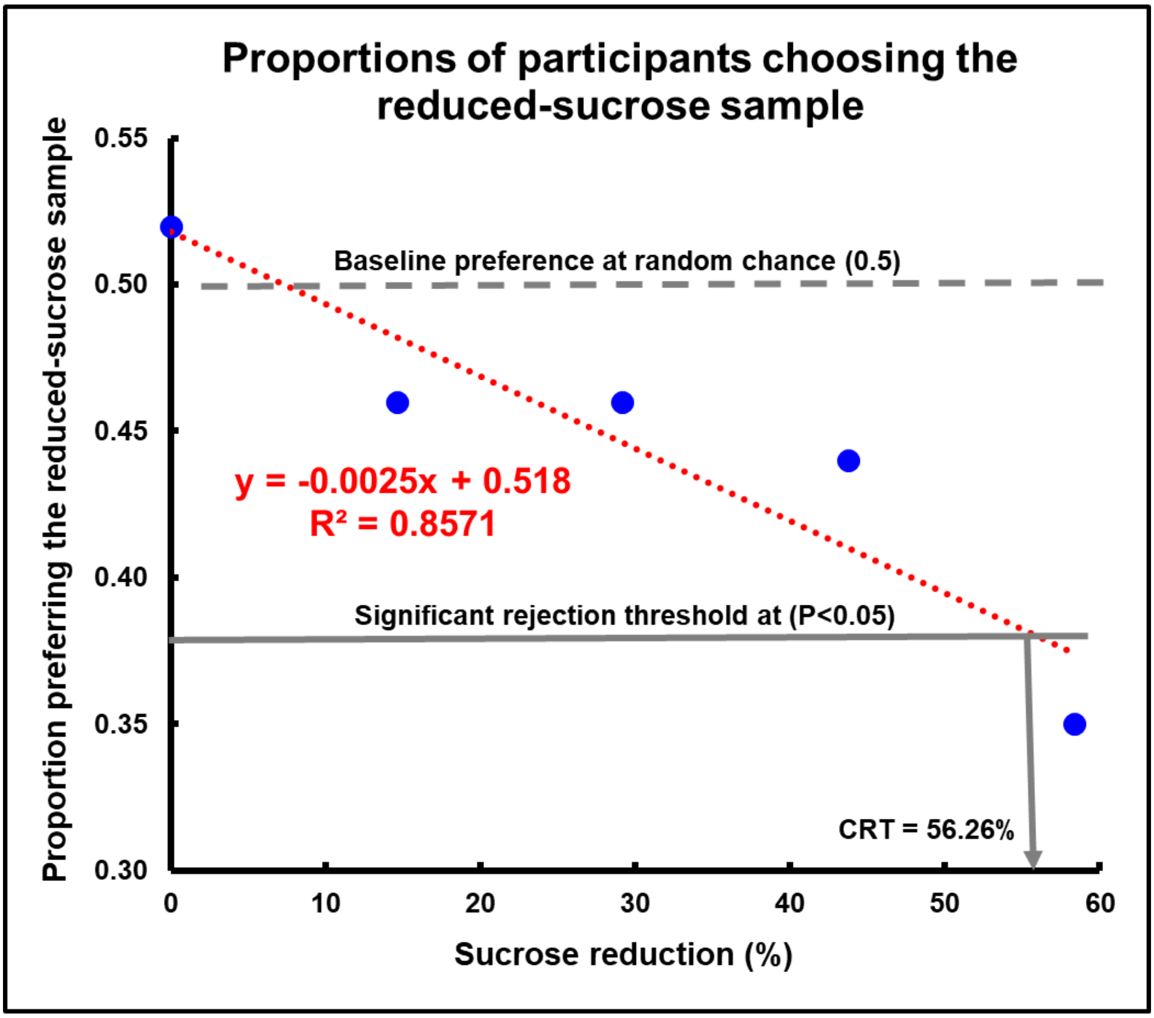


Figure 3. Proportions of participant selecting the reduced-sucrose yogurt sample over the control (full-sucrose sample)*.

*The dotted grey line at y = 0.5 represents the baseline preference at random chance while the solid grey line illustrates the 5% significance criterion (1 - 0.62 = 0.38) of sucrose reduction rejection based on the binomial distribution for the paired comparison test (N = 53). Consumer rejection threshold (CRT) for sucrose reduction on strawberry-flavored yogurt samples was reached at 56.26%, which was calculated based on predicted linear model equation.

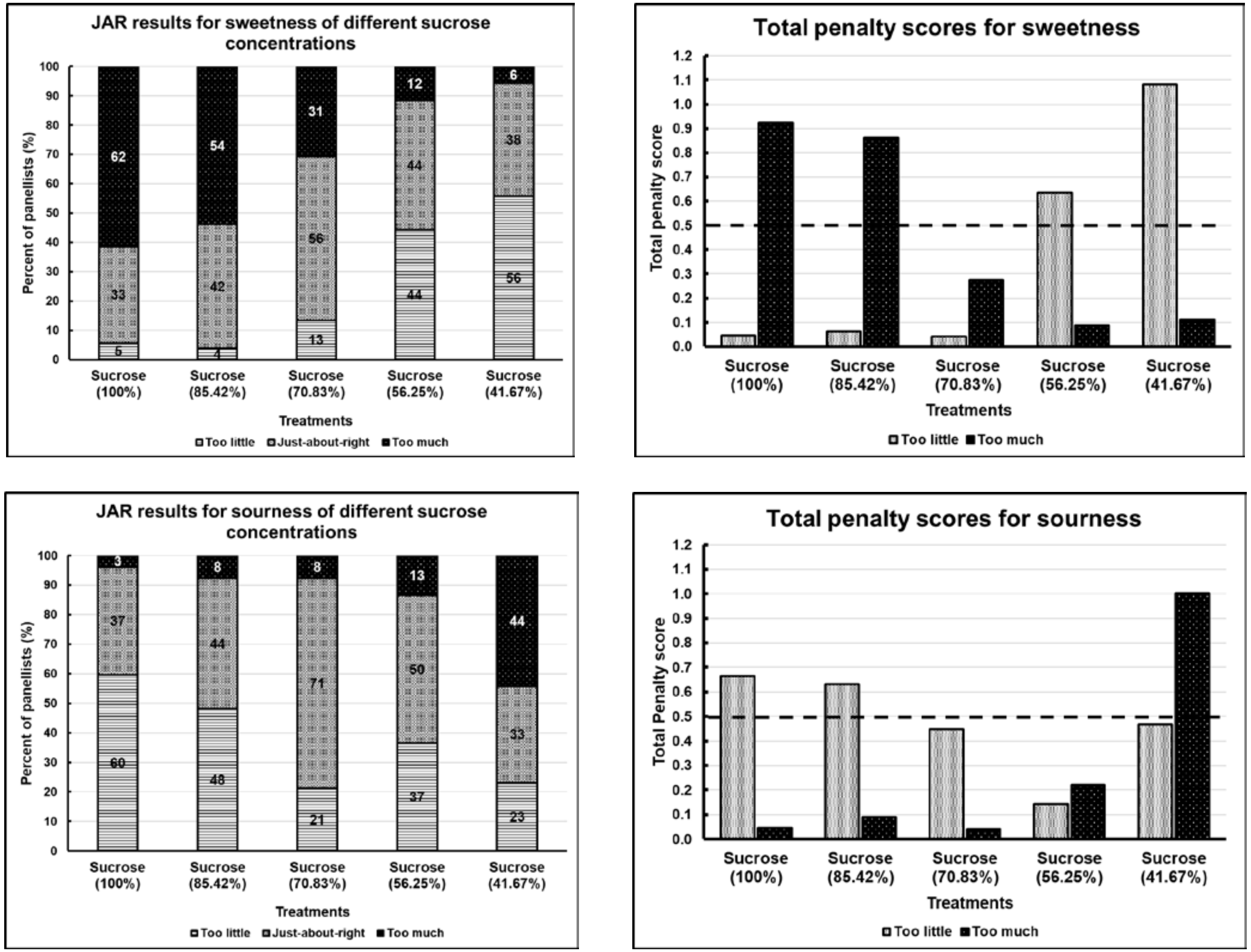


Figure 4. Selection frequencies (%) of Just-About-Right (JAR) results and the total penalty scores in overall taste liking for sweetness and sourness of strawberry-flavored yogurt samples (Treatment labels are shown in Table 1).

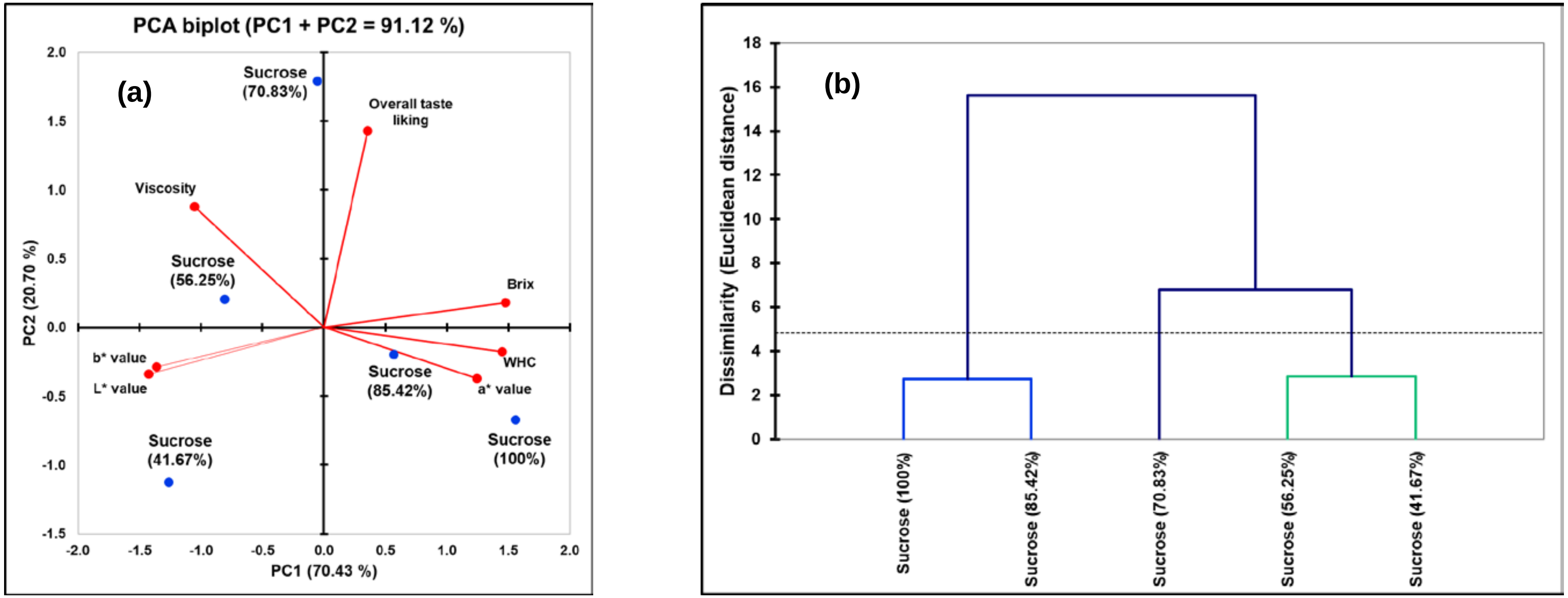


Figure 5. (a) Principal component analysis (PCA) bi-plot and (b) Cluster analysis visualizing treatments* (strawberry-flavored yogurt samples), taste liking, and physico-chemical properties at day 1 of storage time.

*Treatment labels are shown in Table 1. WHC = Walter Holding Capacity.