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Development and Characterization of Reduced-Calorie Gelatin-Based Gummy Jellies Containing Osmo-Dehydrated Santols

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Abstract

Santol flesh are edible and, due to their sourness and astringency, osmo-dehydration using sugar is required in the process of producing confectioneries from santols. The flavor of santols was able to be better preserved using sugar infiltration. However, the sucrose-infiltrated (SI) santol prepared without any preservative can be stored at ambient temperature for only 2–3 weeks due to their high water activities (a_w; 0.91±0.01). Preservative-free gelatin/sucrose/glucose syrup gummy jellies flavored with a paste of SI flesh and arils were developed as a low-water-activity processed santol product. Two-step blanching with hot water was also employed to inhibit the browning in peeled santols. Once cooled, the blanched fruits were peeled. The peeled santols were then soaked in a 3% w/v citric acid solution for 15 min. In the second step, the santol flesh was separated from the inner parts and cut into pieces. The cut santol pieces were kept in water before blanching and then subjected to another blanching process (30-180 s) followed by immediate cooling. There was no significant difference in overall sensory satisfaction among the santol gummy jellies containing the SI santol at 120, 130, 140, 150, or 200 g/100 g glucose syrup. However, the jelly prepared with 120 g of the SI santol exhibited the lowest a_w (0.74±0.01). Formulations containing 120 g SI santol with sucrose substitutions (0, 25, 50, 75, or 100%) using erythritol were then investigated. Crystallization of erythritol occurred in the jellies with 75 and 100% sucrose substitutions and resulted in

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sensorially inferior products. Springiness and cohesiveness of the jellies dramatically decreased whereas their hardness dramatically increased. There was no significant difference in any aspect of sensory attributes among the jellies containing 0, 25, or 50% erythritol. Further reductions in the a_w were observed in the presence of erythritol. The formulation with 50% sucrose substitution exhibited the lowest a_w (0.65±0.00). Therefore, the 50% sucrose/50% erythritol jelly supplemented with 120 g of SI santol was the best reduced-calorie formulation in terms of susceptibility to microbiological spoilage. It provided 332.06 kcal/100 g. Its hardness, springiness, and cohesiveness were 64.23±8.70 N, 4.85±0.05 mm, and 0.74±0.02, respectively. Infiltration with sweeteners can be employed to improve the palatability of fruits or herbs that are sour and/or astringent. The further processing of infiltrated fruits to a product with low water activity, such as gummy jellies, lengthens the shelf life of product.

Introduction

Santol trees (*Sandoricum koetjape* Merr.) are largely distributed in Southeast Asia. The santols are seasonal fruits and are typically available for 4 months from mid-June to mid-October. During the harvest season, the production of santol exceeds the demand, leading to lower prices. Therefore, it is essential to find processing methods that can extend the shelf life of santol and add value to the product. They are recognized as a good source of antioxidants[1-3] and prebiotics.[4] The pericarps, thick spongy tissues enclosing the seeds and arils, of the cultivated varieties in Thailand are thick and account for the majority of the edible fruit parts. In ripe santols, the arils are fluffy, juicy, and delicious with an exquisite flavor whereas the pericarps are sour and astringent. Typically, the pericarps are not consumed directly without seasoning or adjusting their tastes.

One major problem of santol processing is the browning of the pericarps after peeling. The brown color is attributed to the enzymatic oxidation of phenolic compounds[5] and is considered undesirable. Immature fruits exhibit browning at a faster rate than that observed in mature ones.[6] Local people typically keep the peeled santols immersed in a saline solution to prevent the browning. In this way, salt gradually diffuses into the pericarps and affects the taste of the processed santol products.

Blanching with hot water is a simple and practical method and the problem of enzymatic browning could be completely overcome at a short blanching time. Therefore, the study on the extent of browning after hot-water blanching (up to 6 minutes) was examined in detail in the first part of the present study.

About the preservation of the santols, the peeled pericarps are dehydrated by stir-frying with sugar over low-to-moderate heat until the recrystallization of sugar occurs. The dehydrated pericarps are then seasoned with salt and chili powder. Alternatively, the santols are by employing sugar infiltration. In brief, the peeled santols are immersed in syrup, the infiltrated fruits are drained, the liquid is boiled and cooled down, and the infiltrated fruits are stored in the boiled liquid.

Although the santol pericarps account for the majority of the mass of the edible parts, the use of the santol pericarps in food products is limited. This is due to their sour and astringent taste. In this respect, sugar infiltration improves the palatability of the pericarps as the compounds responsible for sourness or astringency diffuse out to the syrup. Moreover, the taste and aroma of santols are better preserved with sugar infiltration compared to the first preservation method. The infiltrated pericarps also retain much of their sponginess and are much softer as a result.

To extend their shelf lives, moisture content and water activity (a_w) are important in formulating products. The moisture content of foods indicates how much water was present in the foods whereas the a_w is a measure of how much of that water is unbound and thus available to support the growth of microorganisms.[7] The a_w of pure water is 1.0 whereas a few microbial spoilages occur in foods exhibiting extremely low a_w (<0.6). Due to the low moisture content, the a_w of santols preserved with the first method is relatively low but it is required that the soft edit parts of the fruits (seed arils and the innermost part of pericarps) are removed. On the other hand, the a_w is found higher in osmotically dehydrated products.[8] As a result, the sugar-infiltrated santol is very prone to microbial spoilage. Typically, the preservative-free sucrose-infiltrated santol can be stored at room temperature for only a few weeks. With refrigeration, the shelf life is longer at the expense of the cost of storage.

The benefit of the sugar infiltration is to adjust the taste of the santol pericarps. However, the high a_w poses a major problem to the product shelf life that needs to be overcome. Combining osmotic dehydration with drying, such as vacuum or air drying, is found expensive.[8] Therefore, the further processing of the sugar-infiltrated santol to a confectionery product with a much lower water activity, such as gummy jelly, is a viable and commercially feasible option.

The gummy jelly or dry jelly is one class of sugar confectioneries based on a hydrocolloid (i.e. a type of candy gel). It derives from a gelling agent such as gelatin[9] mixed with sweeteners including sucrose and glucose syrup.[10] Herb or fruit juice is also added as a natural flavoring agent.[11-15] The gummy jellies containing natural-based bioactive compounds were also reported.[3, 16] Gummy jellies typically exhibit relatively low water activities approaching those of dry foods.

The sweeteners in the santol-free gummy jellies are sugars, i.e. sucrose and glucose syrup, which are also the main ingredients. Sucrose is the most common bulk sweetener but it poses a concern to an individual with diabetes. It was reported that, as part of a balanced diet, the use of a moderate amount of sucrose did not affect the glucose variability or insulin requirements in patients with type I diabetes.[17] However, as part of a light meal that does not provide a balanced diet, consuming gummy jellies could pose a threat to an individual with diabetes. Therefore, it is beneficial to also formulate santol gummy jellies with lower sugar content.

Erythritol is a bulk sweetener that is 60–70% as sweet as sucrose while it is almost completely noncaloric.[18] The glycemic and insulin indices of erythritol are 0 and 2% of those for glucose, respectively.[19] Therefore, erythritol is a promising sweetener used as a sucrose substitute for diabetics. Our study also showed that the partial sucrose substitution with erythritol also led to a reduced-calorie formulation with a substantially lower a_w.

To the best of our knowledge, there has been no prior research or documented attempts at processing santol into gummy jelly, making this study a novel exploration in the field of food product development. Therefore, gummy jelly is one of the most apparent options for the production of a santol-flavored confectionery possessing a low a_w. Our research aimed to develop a preservative-free santol gummy jelly with a lower sugar content and an extended shelf life. The taste of the santol pericarps was adjusted employing sucrose infiltration and erythritol was used to substitute sucrose. Gelatin-based formulations supplemented with the infiltrated santols and reduced-calorie santol gummy jellies were examined in the second and third parts, respectively.

Material and Methods

Materials

Santols (cv. *Nimnual* and cv. *Puifai*) were obtained from an orchard in Lopburi Province, Thailand. Bovine skin gelatin (250 bloom), glucose syrup (dextrose equivalent (DE) 42), and citric acid were purchased from T.S. Inter Lab Part., Ltd., Thailand. Sucrose and erythritol were purchased from Mitr Phol Sugar Co., Ltd., Thailand, and Tinnakorn Chemical & Supply Co., Ltd., Thailand, respectively.

Preparation of the Sucrose-Infiltrated Santol

Santol fruit can be separated into 3 parts: (1) pericarp, (2) seed arils, and (3) seeds (Figure 1). It typically contains 5–6 seeds of which up to 4 seeds are undeveloped. The developed seeds are inedible (Figure 1(a)). The outer skin of the pericarp is edible but generally discarded after peeling as it is fibrous and rubbery. The santol pericarp left after peeling consists of soft spongy tissues. The innermost layer of the pericarp that is in direct contact with the seed aril is fluffy (Figure 1(b) and Figure 1(c)). The seed aril is juicy but, unlike that of a rambutan, longan, or durian, it is very hard to separate from the seed. Since the santol variety cultivated in Thailand has a thick pericarp and relatively large seeds, the pericarp constitutes more than half of the edible fruit parts. Therefore, the peeled pericarp was called "flesh" rather than peel. To avoid ambiguity, the santol seed aril was called "pulp." The word "peeling" referred to the removal of the outer fruit skin.

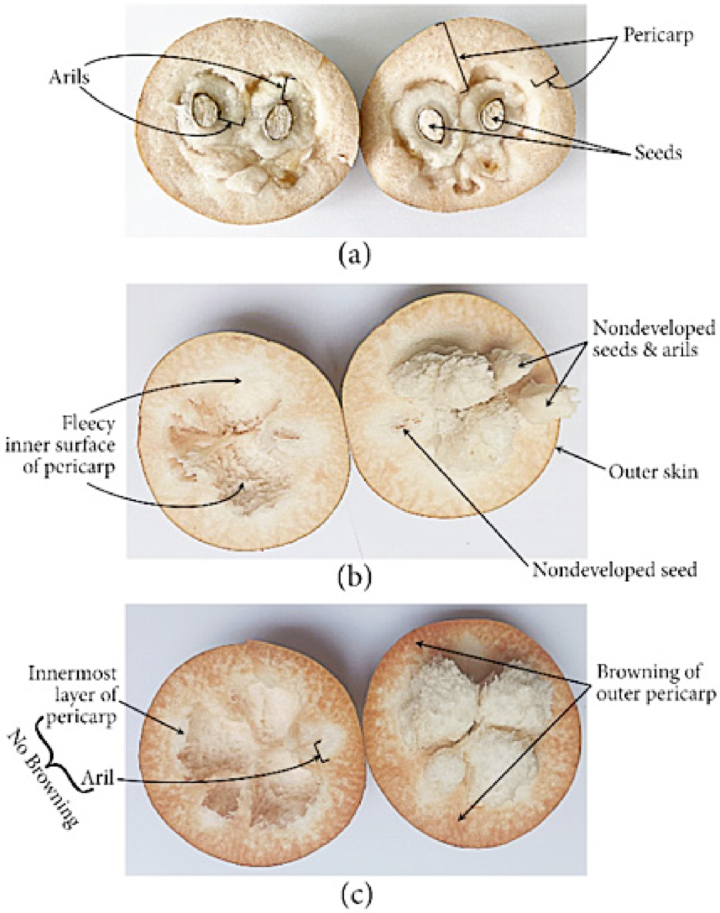


Fig. 1: Photographs of split santols, (a) a santol with 2 developed seeds, (b) a santol with 3 withered (non-developed) seeds, and (c) a santol with 2 withered seeds showing an intense brown color of the outer pericarp. The photograph in (a) was taken immediately after the fruit was split whereas the ones in (b) and (c) were taken 1 and 5 minutes after the fruits were cut

Blanching Treatments

In one-step treatments, peeled and unpeeled santols were blanched with hot water (95–98°C) for 30–180 seconds and immediately cooled down using a large amount of tap water for at least 5 minutes. The peeled fruits were kept immersed in water before being blanched.

In two-step treatments, the hot-water blanching of unpeeled santols was carried out as described above. After cooling down, the blanched fruits were peeled. The peeled santols were then directly immersed in 3% w/v citric acid solution for 15 minutes. An alternative treatment in the second step was to separate the santol flesh, cut into pieces, and blanched (30–180 seconds and immediately cooled down) again. The cut pieces of the flesh were kept immersed in water before being blanched.

Sucrose Infiltration

Thin sucrose syrup (30% w/v) was boiled for 5 minutes and allowed to cool down to room temperature. Sucrose infiltrations of double-blanched flesh and seed with aril were carried out separately in closely sealed containers for 5 days at room temperature.

Preparation of the Santol Blend

As summarized in Figure 2, the sucrose-infiltrated (SI) flesh and seeds aril were processed separately. The sucrose-infiltrated santol (SIS) blend was prepared by mixing the flesh paste and aril and used in the preparation of gelatin-based santol gummy jellies.

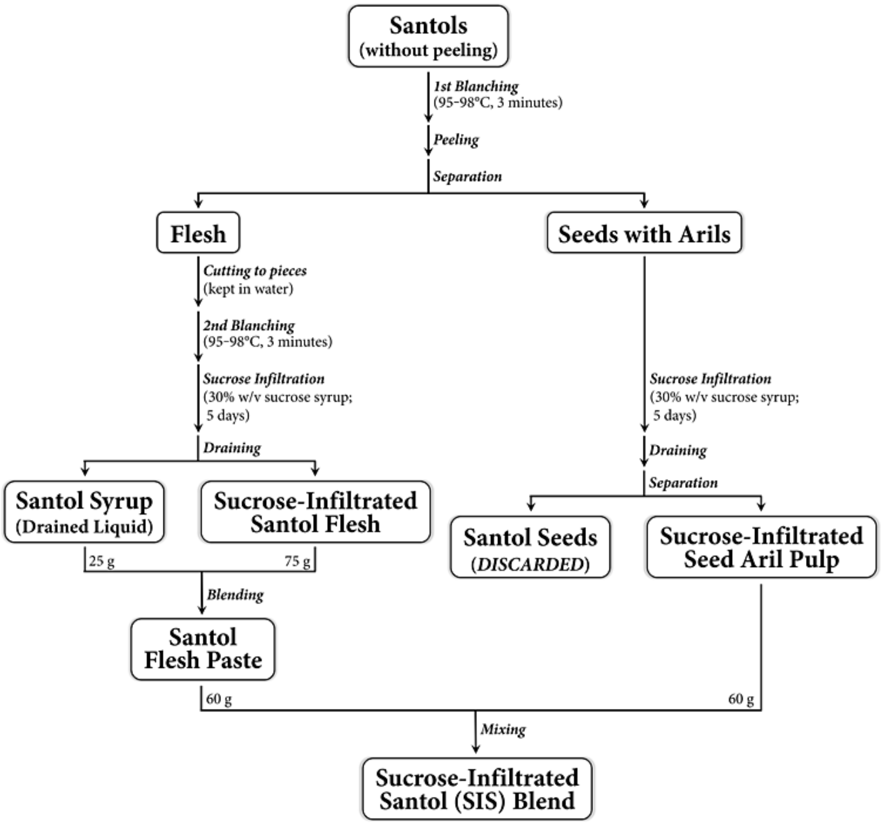


Fig. 2: Double blanching treatment of santols and preparation of the SIS blend

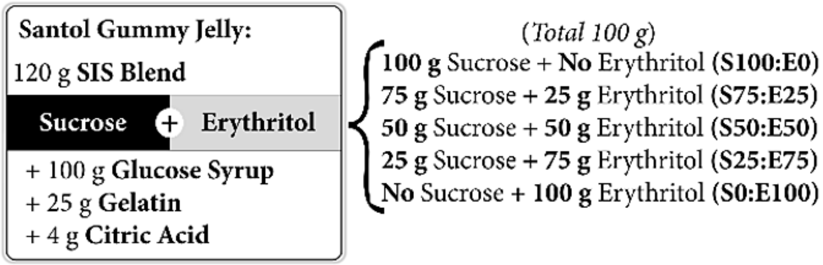


Fig. 3: Ingredients of the reduced-calorie santol gummy jellies

Preparations of the Santol Gummy Jellies

The gummy jellies contained gelatin, sucrose, glucose syrup, and citric acid (25, 100, 100, and 4 g, respectively). Gelatin was hydrated with 50 mL of water for 10 minutes. Dissolve sucrose in the preheated SIS blend and boil the mixture for 4 minutes. Hydrated gelatin, preheated glucose syrup, and citric acid were then added separately in the order. Mix the ingredients well for 1 minute after each addition. The thick mixture was poured into a silicone mold (with 1.5×1.5×1-cm^3 holes), cooled down to room temperature, and placed in a refrigerator (<10°C) for 5 hours. Santol jellies with 120, 130, 140, 150, and 200 g of the SIS blend were prepared and investigated.

To prepare the reduced-calorie santol jellies, the sucrose/erythritol mixture was added to the preheated SIS blend. The amounts of the SIS blend were fixed at 120 g per 100 g of sucrose/erythritol mixture. The reduced-calorie santol jellies were prepared with sucrose/erythritol mixture at weight ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 (Figure 3).

Peroxidase Test

The peroxidase activities of the treated santols were assessed using the method described in Kachhadiya *et al.*[20] with modifications. The santol flesh (10 g, peeled) was taken and ground, and 20 mL of distilled water was added. After the content was thoroughly mixed by vortexing for 1 minute, 1 mL of 0.5% w/v guaiacol in 95% ethanol and 1 mL of 0.08% hydrogen peroxide (H_2O_2) were added and thoroughly mixed. The colors due to peroxidase activities were allowed to develop for 5 minutes. The peroxidase activities were determined semi-quantitatively as positive (++, reddish brown solution), light positive (+, light brown solution), or negative (-, colorless solution).

pH Measurement

Ten g of gummy jelly was cut into small pieces, mixed with 10 g of water, and heated to 90°C. When the mixture turned to liquid, the sol was thoroughly mixed and allowed to cool down to room temperature. The liquid samples were taken to determine the pH using a pH meter (FE20, FiveEasy Plus, Switzerland).

Determination of Total Soluble Solid

The total soluble solid (TSS) was determined using the liquid samples prepared in the same way as that used for the pH measurement. It was determined using a hand refractometer (RHB-32ATC N3, JEDTO Instrument, Thailand).

Determination of Water Activity

Samples (5 g) were cut into small pieces and the water activities were determined using a water activity meter (AquaLab CX 3 TE, Decagon Devices, Inc., USA).

Textural Properties

The texture profile analysis (TPA) was carried out at 27°C by applying two cycles of deformation to samples (i.e. employing the double compression test). The force-time graphs were generated using a TA-XT Plus texture analyzer (Stable Micro Systems, UK) equipped with a 50-kg load cell. The compression probe was 25.4 mm in diameter (TA11/1000). A single piece of gummy jellies (1.5×1.5×1 cm^3) was used without trimming and the sample was placed on the platen so that the sample's height was 1 cm (i.e. with a 1.5×1.5-cm^2 base). The TPA parameters were as follows: test speed at 5 mm/s, target distance at 5 mm, trigger box at 30 g, data acquisition rate at 200 points/s, and delay between the compression cycles at 5 s.

Colors Measurement

The colors of santol gummy jellies were measured using a colorimeter (Minolta CR-10, Japan) in CIE L*, a*, and b* coordinates in which L* ranges from 0 to 100 whereas both a* and b* are unbound. Chroma (C*) and hue angle (h*) were converted from the a* and b* values.

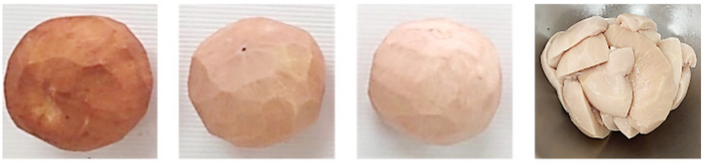
Sensory Evaluations. The 30 trained panelists consisted of 6 males and 22 females aged between 19 and 25 years old. Samples were placed separately on white polystyrene plates labeled with three-digit random numbers and presented to the panelists in a randomized order. Between each sensory test, the panelists were given drinking water to rinse their tongues and palates and to reduce sensory fatigue. The sensory evaluations were conducted using a unipolar 7-point hedonic scale in which 1 represented extreme dislike and 7 represented the opposite extreme. Five sensory attributes (appearance, color, aroma, taste, and texture) were evaluated along with the overall satisfaction. The study employed a randomized complete block design (RCBD). Data were analyzed using analysis of variance (ANOVA), and mean differences were compared using Duncan's New Multiple Range Test (DNMRT). The nutritional content of the chosen reduced-calorie santol jelly was determined according to methods described in AOAC 21 by Central Laboratory (Thailand) Co., Ltd.

Statistical Analyses

All statistical analyses, except those concerning sensory scores, were carried out employing a one-way ANOVA test. A general linear model/Univariate was employed to analyze the data related to sensory evaluations. When applicable, Duncan's Multiple Range Test (DMRT) was employed for multiple comparisons. Treatments with no DMRT post hoc letter (a, b, c, etc.) in common were significantly different. All statistical interpretations were carried out at the confidence interval of 95% (α = 0.05).

Results

Suppression of Enzymatic Browning in the Santol Flesh. On exposure to air, enzymatic browning begins to occur on the surfaces of the peeled santols. Without any pretreatment, the prolonged exposure of the peeled santols to oxygen is problematic as it results in a brown color that is typically undesirable for processed santol products. The browning occurred readily when the santol flesh was injured (peeled or cut) and the intensity of the brown color of the outer layer of the flesh rapidly increased (Figure 1(c) and Figure 4(a)). Blanching with hot water is a simple and practical method to suppress the browning process. The one-step treatments via blanching were carried out with peeled and unpeeled santols (the PB and UB series of treatments, respectively). We limited the continuous blanching time to a maximum of 3 minutes because longer continuous blanching caused the mushiness of the outermost layer of the flesh just under the rubbery peel. The results of the peroxidase tests are given in Table 1. The peroxidase activities of peeled santol were assessed semi-quantitatively as positive (++; Figure 4(b)), light positive (+; Figure 4(c)), and negative (–; Figure 4 (d))



(a) (b) (c) (d)

Fig. 4: Photographs showing the colors of (a) a peeled santol without any pretreatment, and treated and peeled santols exhibiting (b) positive, (c) light positive, and (d) negative peroxidase activities, respectively. The photographs were taken 30 minutes after peeling or pretreatments

Table 1: Peroxidase activities in peeled santols after the application of 4 methods to suppress enzymatic browning

First Blanching Time (s)	Peroxidase Test*			
	PB	UB	UB+CA	UB+B
30	+	++	++	++
60	+	++	++	+
90	+	++	++	-
120	+	++	+	-
150	+	+	+	-
180	+	+	+	-

* '++' = Positive; '+' = Light Positive; '-' = Negative.

PB: Blanching of 'peeled' fruits;

UB: Blanching of 'unpeeling' fruits followed by peeling;

UB+CA: UB treatment followed by 3% w/v citric acid treatment for 15 minutes;

UB+B: UB treatment followed by blanching of flesh only for 180 seconds.

The blanching of peeled santols (PB) was able to partially suppress the browning process even at 30 seconds. However, the total suppression of enzymatic browning was not possible even after 3 minutes of blanching. The blanching of unpeeled fruit (UB) was less effective. The degrees of browning suppression were only comparable to those of the PB treatments when the fruits were blanched for at least 2.5 minutes.

Browning of santols mostly occurred on the flesh, and arils. It progresses rapidly in the flesh. The reaction is catalyzed by an enzyme named 1,2-benzenediol: oxygen oxidoreductase (polyphenol oxidase, PPO) released in plant tissues.[6,22] Oxygen and phenolic compounds are the stimulator to activate the reaction. However, it is reported that all phenolic compounds present are not the substrates of PPO; certain phenolic compounds in fruits and vegetables linearly correlate with antioxidant properties, thus considered to be beneficial to inhibit the discolouration rate.[23]

Benjawan and Chutichidet [5] studied the effects of ascorbic acid or citric acid on the slowing down of browning in santols. Unpeeled santols were immersed in an anti-browning solution (at 5% or 10%) and stored at 28°C and 90% relative humidity. Santols treated with 5% citric acid exhibited the highest L* value and the lowest PPO activity in the flesh tissue.

Since the application of either of the one-step treatments for 3 minutes was unable to completely suppress the enzymatic browning in the flesh, we further examined 2 variants of double (2-step) treatments. Unpeeled santols were blanched followed by the immersion of the peeled fruits in 3% citric acid for 15 minutes (UB+CA series; Table 1). The degrees of browning suppression were slightly better than that of the UB treatments alone but total browning suppression was not achieved within 3 minutes of blanching. This suggests that the concentration of citric acid employed in the present study may be too low for peeled santols. It is also very likely that, for browning prevention with a chelating agent to be effective, the treatments need to be applied before wounding occurs in the pericarps (i.e. before peeling).

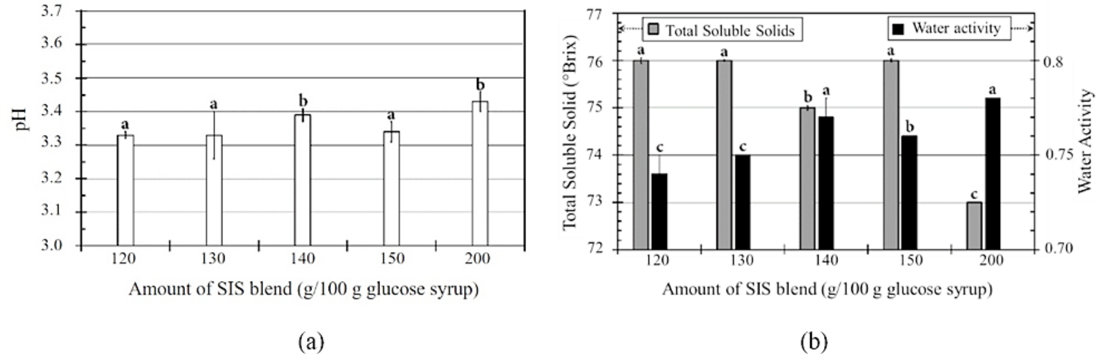


Fig. 5: (a) pHs, and (b) total soluble solids (TSS) and water activities of the santol gummy jellies with varying amounts of the SIS blend. Post hoc homogeneous subsets are marked with letters a, b, c, etc

In the UB+B series of treatments, unpeeled santols were first blanched, peeled, and separated from the seeds/arils. The obtained flesh was cut into pieces that were then subjected to the second blanching step for a fixed 3 minutes. With the double blanching treatment, the total suppression of browning was achieved when unpeeled santols were blanched for at least 90 seconds in the first blanching step. Therefore, we employed the double blanching treatment (3-minute blanching in both steps) in the subsequent processing of santols.

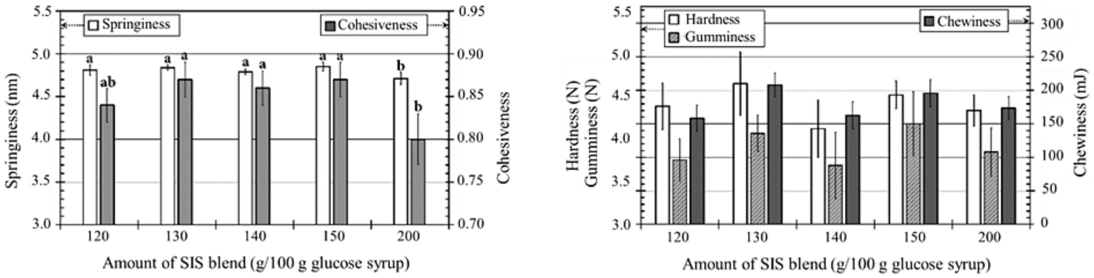
The SIS blend was the main flavoring and coloring agent in santol gummy jelly. Sucrose infiltration of the santol flesh is required to dilute the concentrations of certain organic compounds responsible for the sour and astringent taste of the flesh. In effect, the paste of the SI flesh provided a combination of sweetness and sourness more appropriate for a gummy jelly. Five formulations of santol gummy jellies containing varying amounts of the SIS blend (120, 130, 140, 150, or 200 g/100g of glucose syrup) were investigated.

The pH of the santol gummy jellies without erythritol is given in Figure 5(a). They were significantly different and ranged from 3.33 to 3.43.

The total soluble solids (TSS) and water activities are given in Figure 5(b). The TSS of the santol gummy jellies ranged from 73.00 to 76.00 °Brix. The TSS of the formulation with 200 g of SIS blend (SIS200) was significantly lower than those of the other formulations (p≤0.05).

The water activities (a_w) ranged from 0.74–0.78. An upward trend was observed in the case of the a_w (Figure 5(b)). Formulating the gummy jellies with higher amounts of the SIS blend resulted in higher a_w since the SIS blend was prepared using thin sucrose syrup. For reference, the a_w of the SI santol flesh was 0.91±0.01. The lowest a_w (0.74±0.01) was observed in the SIS120 formulation. The a_w of the santol gummy jellies without erythritol are in the same range as those reported for jam, marmalade, marzipan, molasses, and dried figs.

The textural properties of the santol gummy jellies without erythritol are given in Figure 6. Both the springiness and cohesiveness of the SIS200 gummy jelly were significantly lower than those of the other formulations (Figure 6(a)). The ranges of springiness and cohesiveness were 4.71–4.85 mm, and 0.84–0.87, respectively. There was no significant difference in hardness, gumminess, and chewiness (Figure 6(b)). Higher amounts of the SIS blend had no significant effect on the hardness, gumminess, and chewiness of the gummy jellies. The ranges of hardness, gumminess, and chewiness were 39.27–45.99 N, 33.77–38.55 N, and 158.21–207.97 mJ, respectively.



(a) (b)

Fig. 6: (a) Springiness and cohesiveness, and (b) hardness, gumminess, and chewiness of the santol gummy jellies with varying amounts of the SIS blend. Post hoc DMRT homogeneous subsets are marked with letters a, b, c, etc. whereas ns indicates no significant difference

Table 2: Color coordinates of the santol gummy jellies with varying amounts of the SIS blend

	Amounts of SIS Blend (g/100 g glucose syrup)				
	120	**130**	**140**	**150**	**200**
L*	44.84^{bc} ± 0.56	44.46^{c} ± 0.63	46.32^{a} ± 0.32	45.22^{b} ± 0.70	44.77^{bc} ± 0.81
a*	3.05^{b} ± 0.16	36.01^{b} ± 0.20	2.89^{b} ± 0.10	3.06^{b} ± 0.29	3.89^{a} ± 0.26
b*	15.94^{b} ± 0.38	16.76^{b} ± 0.69	15.91^{b} ± 0.56	15.13^{c} ± 0.78	14.64^{c} ± 0.72
c*	16.23^{b} ± 0.39	17.03^{a} ± 0.70	16.17^{b} ± 0.55	15.44^{c} ± 0.8	15.15^{c} ± 0.72
h*(°)	79.17^{bc} ± 0.38	79.82^{a} ± 0.51	79.70^{ab} ± 0.55	78.58^{c} ± 0.79	75.11^{d} ± 0.99

Post hoc DMRT homogeneous subsets are marked with letters a, b, c, etc.

The color coordinates of santol gummy jellies were given in Table 2. The L* a* b* values were in the range of 44.46–46.32, 2.89–3.89, and 14.64–16.76, respectively. Significant differences were observed among the color coordinates (p≤0.05). At higher amounts of the SIS blend, slightly lower chroma (C*) values were observed whereas the hue (h*) angles were shifted toward brown (low-saturation orange). Higher amounts of the SIS blend led to less saturated colors with more intense brown hue. However, this is not unexpected because higher amounts of the SIS blend resulted in higher concentrations of coloring agents provided by the SIS blend.

The mean scores of the sensory evaluation are given in Figure 7. All of the sensory mean scores were more than 5. There was no significant difference in the mean scores regarding the color, taste, and overall satisfaction (p>0.05) in which the mean scores ranged from 5.45 to 6.00. Significant differences in appearance, aroma, and texture were observed (p≤0.05). Higher amounts of the SIS blend resulted in the significantly higher texture and aroma scores. However, a clear trend of the appearance scores with respect to the amounts of the SIS blend was not observed.

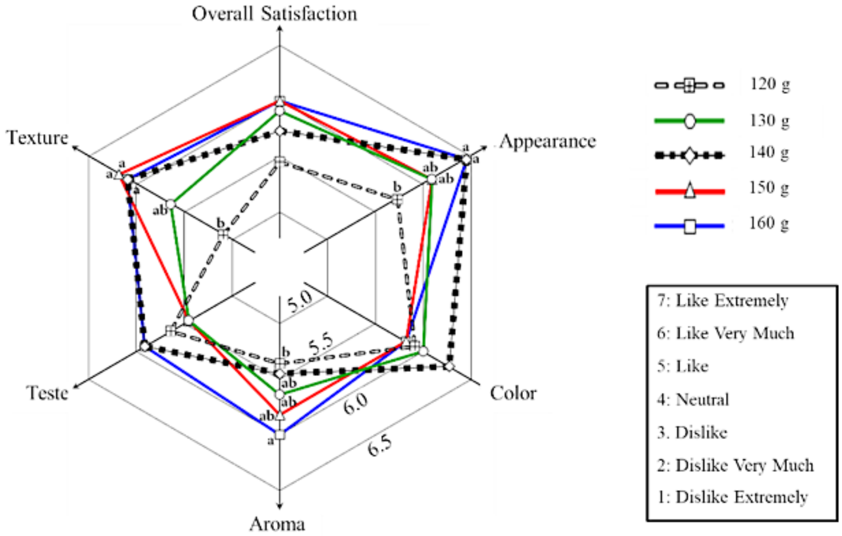


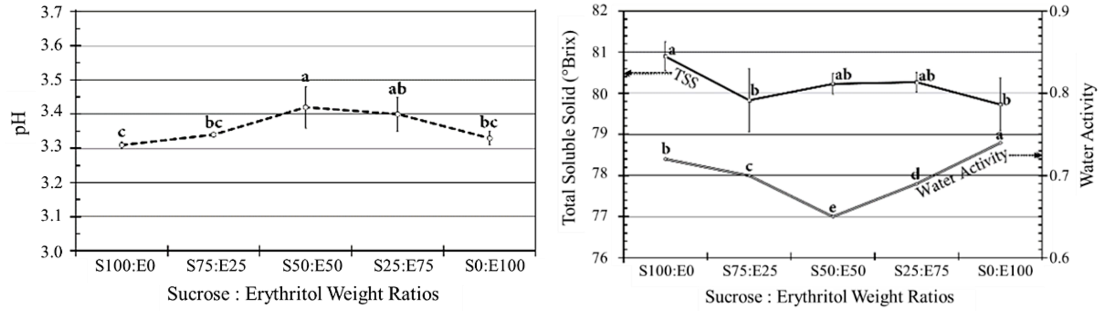
Fig. 7: (Sensory evaluation mean scores of the santol gummy jellies with varying amounts of the SIS blend. *Post hoc* DMRT homogeneous subsets are marked with letters a, b, c, etc. whereas ns indicates no significant difference

Concerning the base formulation for the further study with sucrose substitution, there was no clear winner. Since there was no significant difference among the overall satisfaction scores, the selection was made using chemical and textural criteria. As already mentioned, the a_w increased with increasing amounts of the SIS blend. The springiness and cohesiveness were not significantly different among formulations containing ≤150 g of the SIS blend. Therefore, the santol gummy jelly formulated with 120 g of the SIS blend (SIS120) was used in subsequent experiments because its a_w was the lowest (0.74±0.01). Even though the SIS120 gummy jelly a_w was much lower compared to that of the SI flesh (0.91±0.01), a santol gummy jelly with lower is preferable and beneficially affected its shelf life.

Reduce-Calorie Gummy Jellies. Based on the SIS120 formulation containing 100 g of sucrose, the santol gummy jellies with lower sugar were prepared by using erythritol as the sucrose substitute (Figure 8). Erythritol is zero-calorie and the reduced-calorie formulations were the S75:E25, S50:E50, S25:E75, and S0:E100. In these reduced-calorie santol gummy jellies, the degrees of sucrose substitution were 25, 50, 75 and 100%, respectively. Note that, for the sake of simplicity, the sucrose contributed by the SIS blend was not taken into consideration when we mentioned about degrees of sucrose substitution. The S0:E100 jellies still contained an appreciable amount of sucrose.

The pH of the santol gummy jellies with different degrees of sucrose substitution were significantly different ($p \leq 0.05$; Figure 9(a)). However, the S50:E50 formulation was the least acidic (3.42±0.06) whereas the santol gummy jelly without erythritol (S100:E0, SIS120) was the most acidic (3.31±0.01). The pHs of the S75:E25 and S0:E100 formulations were comparable.

Fig. 8: Photographs of normal (SIS120; leftmost) and the other 4 reduced-calorie santol gummy jellies



(a) (b)

Fig. 9: (a) pHs, and (b) total soluble solids and water activities of the reduced-calorie santol gummy jellies. Post hoc homogeneous subsets are marked with letters a, b, c, etc

The total soluble solid and a_w of the reduced-calorie gummy jellies are given in Figure 9(b). They ranged from 79.73 to 80.27 °Brix. The TSS of the S25:E75 and S0:S100 formulations were significantly lower than that of the S100:E0 formulation ($p \leq 0.05$). There was no significant difference in terms of the TSS among the gummy jellies containing erythritol.

The a_w Water activities were significantly different ($p \leq 0.05$; (Figure 9(b)). The smallest a_w (0.65±0.00) was observed in the S50:E50 formulation whereas the a_w of the 0:100 formulation was the highest (0.74±0.01). When the a_w of the S100:E0 (SIS120) formulation from 2 separated preparations (0.74±0.01 and 0.72±0.00) were pooled together, the a_w of the S100:E0 and S0:E100 formulations were not significantly different. Tekeungwongtakul 24 also reported that the application of sugar substitute in strawberry gummy jellies led to a reduction in the a_w. This result indicated that more water molecules were bound in the presence of erythritol and the water activity was lowered as a result. Gelatin, as a gelling agent, is responsible for the formation of a three-dimensional network via its triple helical structures acting as junction zones [25] (discussed in more detail below).

Both sucrose and sugar alcohols (sorbitol and xylitol) were reported to reduce the water activity of gelatin gels.[26] The lowering of the a_w occurred owing to the increased total concentration of sweeteners. However, at 75 and 100% erythritol, the a_w

dramatically increased. This is arguably because of the crystallization of erythritol. The solubility in water of erythritol (~61 g/dL; ~499.5 mmol/dL) is much lower than that of sucrose (~200 g/dL; ~584.3 mmol/dL) or glucose (~90.9 g/dL; ~504.6 mmol/dL). The photographs in Figure 8 show that the entire surfaces of the S100:E0 gummy jellies were covered in white power. Compared to the formulations with lower amounts of erythritol, much more sugar crystals were present on the surfaces of the S25:E75 jellies. The crystallizations of erythritol at high concentrations are discussed in more detail below.

The color coordinates of the reduced-calorie gummy jellies are given in Figure 10. Significant differences were observed among the L* a* b* coordinates ($p \leq 0.05$) of which values ranged from 45.87–77.23, 1.13–4.13, and 4.47–13.60, respectively. Except the 50%-erythritol (S50:E50) formulation, higher degrees of sucrose substitution with erythritol led to higher L* values (lighter color) and lower a* and b* values. The white powder on the surfaces of the S0:E100 gummy jelly indicated that crystallization of erythritol took place. The L* values of the jellies containing 75 and 100% erythritol markedly increased due to the presence of erythritol crystals. There was no significant difference among the C* values of the S100:E0, S75:E25, and S50:50 formulations ($p > 0.05$). The crystallizations of erythritol in the S25:E75 and S0:E100 gummy jellies also resulted in a marked decrease in the C* values.

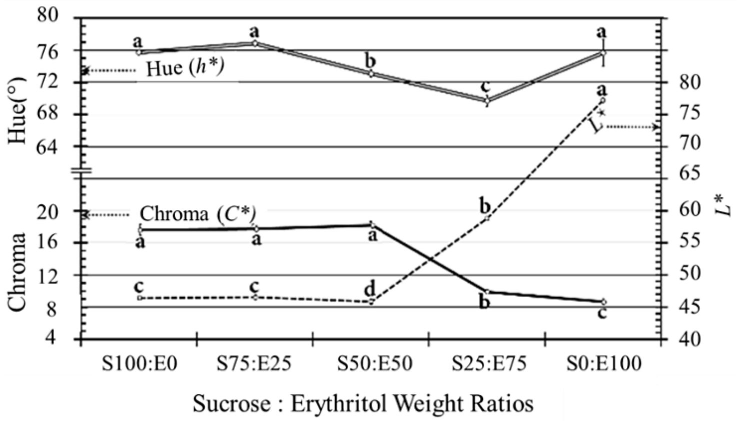
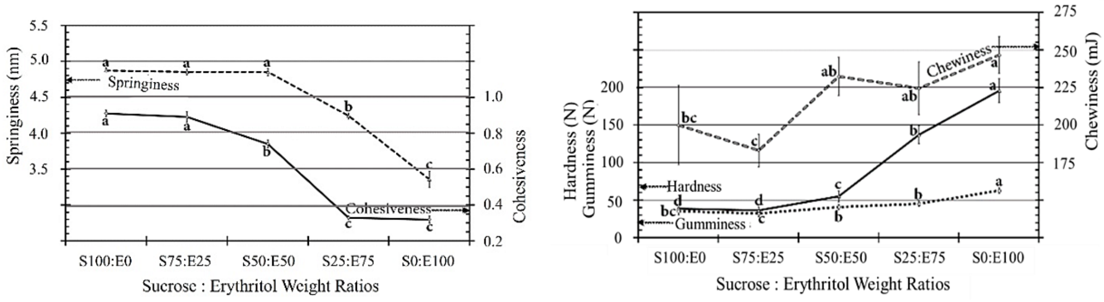


Fig. 10: Color coordinates of the reduced-calorie santol gummy jellies.
***Post hoc* homogeneous subsets are marked with letters a, b, c, etc**

The color hues of the reduced-calorie gummy jellies significantly shifted toward brown at 50 and 75% sucrose substitutions ($p \leq 0.05$). Interestingly, the hue angle of the S0:E100 formulation was comparable with those of the S100:E0 and S75:E25 formulations. The densely present erythritol crystals likely masked the true color of the S0:E100 gummy jelly.

Textural Profiles all aspects of the textural properties of the reduced-calorie formulations were significantly different. The cohesiveness exhibited a downward trend with the highest (0.91±0.02) and lowest (0.32±0.02) values observed in the S75:E25 and S0:E100, respectively (Figure 11(a)). The springiness values ranged from 3.36 to 4.87 mm. The springiness of the S100:E0, S75:E25, and S50:E50 formulations were not significantly different and were among the highest. Marked decreases in the springiness were observed at 75 and 100% sucrose substitutions.

Figure 11(b) revealed the upward trend of both the hardness and gumminess in which the S0:E100 formulation exhibited the highest values. The hardness, gumminess, and chewiness were in the ranges of 42.33–227.79 N, 36.67–72.89 N, and 183.00–246.31 mJ, respectively. A sharp increase in the hardness was observed between the S50:E50 and S25:E75 formulations. The gumminess and chewiness of the S0:E100 formulation were the highest even though its springiness and cohesiveness were the smallest. This is because its hardness was ~355% of that of the S50:E50 gummy jellies. Too high hardness and too low springiness and cohesiveness were detrimental to the texture of the gummy jellies. Therefore, the sucrose/erythritol at a 1:1 weight ratio provided the best compromise between these counteracting textural parameters.



(a) (b)

Fig. 11: (a) Springiness and cohesiveness, and (b) hardness, gumminess, and chewiness of the reduced-calorie santol gummy jellies. *Post hoc* DMRT homogeneous subsets are marked with letters a, b, c, etc

Discussion

Effects of Erythritol on the Gelatin Matrix. An important characteristic of gelatin is the thermo-reversible sol-gel transition. In the course of gel setting after the jelly melt gradually cools down, the gelatin colloidal solution (sol) gradually transforms from the dispersion state into an infinite network by the formation of cross-linkages that retain its solvent (gel).[27] Parts of the gelatin chains consisting of a specific sequence of amino acid residues begin to change their conformation from random coils to α-helices. The assembly of 3 α-helices leads to the formation of a triple helix junction zone. The remaining parts of the gelatin chains are in a non-helical single chain state. These single chains entangle with nearby chains and are collectively called random coils. The junction zones act as the cross-linking sites between the gelatin chains and are responsible for the formation of a 3-dimensional network that perceives macroscopically as a gel.

The presence and the relative amounts of sugars and polyols (sugar alcohols) can promote or prevent the formation of the junction zones, both in terms of number and length. Arguably, higher proportions of the junction zones (and hence lower proportions of the random coils) lead to gels with higher hardness and lower springiness and vice versa. Erythritol was reported to promote the formation of the gelatin junction zones. Gekko *et al*[28] showed that, compared to water, the helix formation of gelatin molecules was enhanced in aqueous solutions of polyols whereas alcohols depressed the helix formation. The helix-forming ability of erythritol was the highest compared to those of longer linear polyols. On the other hand, glucose and/or sucrose at very high concentrations were reported to help stabilize the gelatin chains in the random coils state 29. In other words, the formations of α-helices were prevented to a great extent at high concentrations of glucose and/or sucrose. A smaller proportion of gelatin chains forms α-helices leading to smaller numbers and shorter junction zones.

All formulations (S100:E0, S25:E75, S50:E50, S25:E75, and S0:E100) contained the same amounts of gelatins (~7.16 wt%), glucose syrup, and the SIS blend. Therefore, the types and relative concentrations of the sweeteners were responsible for the differences in texture profiles. Figure 12 shows the conceptual structures of the gelatin matrices in santol gummy jellies without, with 50%, or with 100% erythritol. To facilitate the comparisons of the structures of the gelatin networks, the diagrams in the top row of Figure 12 show only the gelatin network and sugar crystals (if present). The numbers and length of the junction zones were exaggerated to emphasize the effects of erythritol and glucose/sucrose on gelation. In the absence of erythritol, the majority of gelatin chains adopted the random coil conformation with a short and small number of junction zones since the total concentration of sweeteners is very high in santol gummy jellies (Figure 12(a)).

In the S50:E50 formulations, the total number of sucrose (12 carbons, molecular weight: 342.30 g/mol) and glucose (6 carbons, molecular weight: 180.16 g/mol) were outnumbered by erythritol (4 carbons, molecular weight: 122.12 g/mol) since the glucose syrup used this study had a moderate dextrose equivalent. More junction zones formed in the presence of erythritol. Erythritol also promoted the formations of longer junction zones whereas the random coils were shorter and less entangled (Figure 12(b)). As a result, the hardness of the S50:E50 gummy jelly increased while its springiness decreased. The numbers of unbound water molecules decreased as the total concentrations of the sweeteners increased. Higher numbers of the hydroxyl (—OH) groups contributed by erythritol increase the ability of the gelatin matrix to hold and tightly bind water.

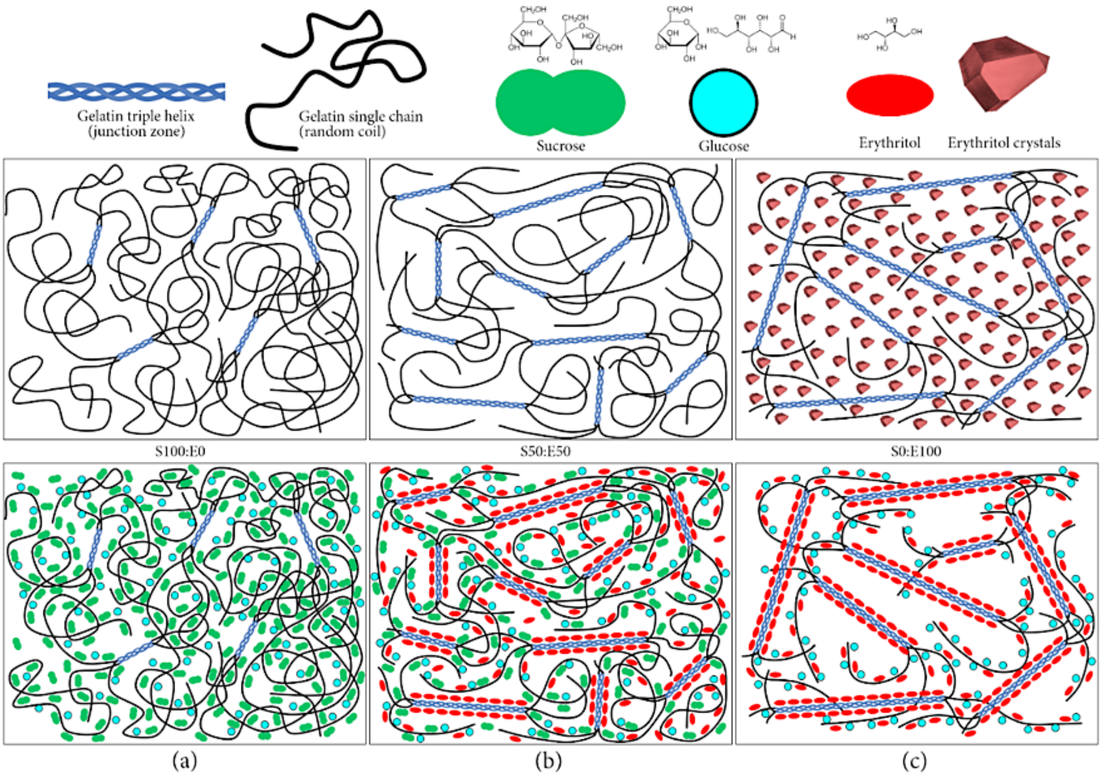


Fig. 12: Conceptual structures of the (a) S100:E0, (b)S50:E50, and S0:E100 santol gummy jellies. In the top row, only the gelatin network and sugar crystals (if present) are shown whereas soluble sugars in the gelatin network are shown in the bottom row

In the S0:E100 gummy jelly, the erythritol crystals were present (Figure 12(c), top diagram) due to the lower solubility of erythritol as mentioned above. At this concentration, the number of soluble erythritol could be comparable to or even higher than that of the soluble glucose. The number of junction zones may be comparable to that present in the S50:E50 gummy jelly but the total length of all junction zones combined was much longer. A much smaller proportion of gelatin chains was in the random coil state. The erythritol crystals also acted as reinforcing grains. The high number and longer junction zones combined with reinforcement by the erythritol crystals resulted in the extremely high hardness of the S0:E100 gummy jelly.

The increase in water activity of the S25:E75 gummy jelly suggests that the crystallization of erythritol also occurred at 75% sucrose substitution. The crystallization of erythritol prevented a large proportion of the hydroxyl groups of erythritol from forming hydrogen bonds with water molecules. A higher proportion of water was unbound and water activities increased as a result.

The results of the sensory evaluation of the reduced-calorie santol gummy jellies are given in Figure 13. Significant differences were observed in every aspect of the sensory evaluation (p≤0.05). The lowest mean scores were observed in the S0:E100 formulation, i.e. 3.73±0.79, 4.09±0.94, 3.73±0.65, 3.55±0.93, 3.73±0.79, and 3.36±0.67 for the appearance, color, aroma, taste, texture, and overall satisfaction, respectively. The highest appearance (5.82±0.75), aroma (5.64±0.81), taste (5.91±0.94), and overall satisfaction (5.91±0.83) scores were observed in the S50:E50 formulation. The highest color (5.73±0.90), and texture (6.00±0.63) scores were observed in the S75:E25 formulation whereas the S50:E50 formulation ranked second with a score of 5.55±0.93, and 5.91±0.94, respectively.

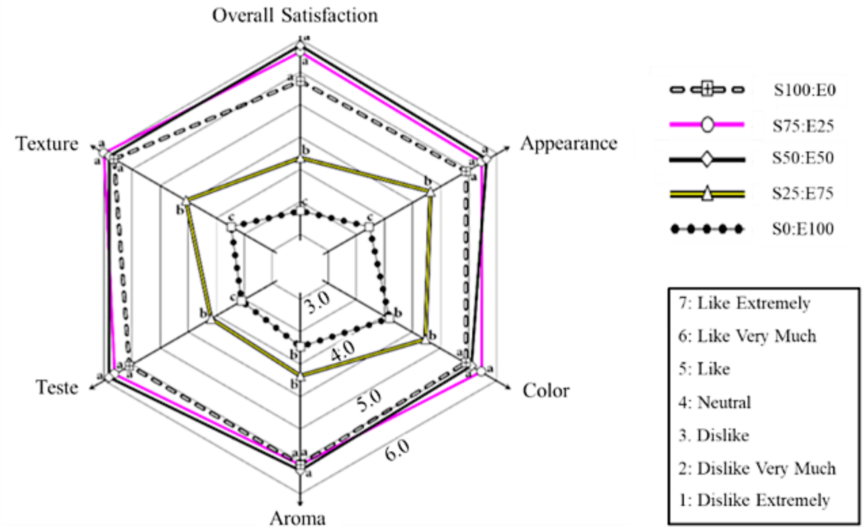


Fig. 13: Sensory evaluation mean scores of the reduced-calorie santol gummy jellies. *Post hoc* DMRT homogeneous subsets are marked with letters a, b, c, etc

The mean scores of the respective sensory attributes of the S100:E0, S75:E25, and S50:E50 formulations were not significantly different (p>0.05). At 75% erythritol (S25:E75) and 100% erythritol (S0:E100), the effects of erythritol on the sensory attributes of the jellies were dramatic. The mean scores of all sensory attributes of the S25:E75 and S0:E100 formulations significantly decreased to below 5 and 4.1m respectively. The detrimental effects of too-high erythritol were more pronounced in the aroma and taste attributes.

In terms of the sensory, the S50:E50 formulation was the best reduced-calorie formulation containing 120 g of the SIS blend. Furthermore, it also exhibited the lowest water activity (0.65±0.00) which is close to the upper limit of those of dry foods (<0.6).

The proximate composition of the S50:E50 santol gummy jelly consisted of 17.33% moisture, 0.34% total fat, 7.74% protein, 74.51% total carbohydrate, and 0.08% ash. Approx. 0.99% and 48.45% of the total carbohydrates were dietary fiber and sugar, respectively. No saturated fat and cholesterol were detectable. Vitamin C was present at 189 mg/100 g whereas no vitamins A, B1, or B2 were not detected.

The S50:E50 santol gummy jelly consisted of relatively small moisture content and also exhibited water activity of 0.65±0.00 which is highly beneficial to the product shelf life. Microbiological assessment showed that, when stored in a tightly closed vial without any desiccant, *Escherichia coli* and *Salmonella* spp. were not present for up to 90 days of storage (data not shown).

The total energy provided by the S50:E50 santol gummy jelly was 332.06 kcal/100 g. Energy from fat and protein accounted for 0.92% and 9.32%, respectively.

The santol plant is used in traditional medicine and it contains various anti-inflammatory triterpenes including sentulic acid and koetjapic acid.[30] The latter exhibits marked anti-inflammatory and anticancer properties. However, there was no report concerning bioactive compounds other than anti-oxidants in the pericarps or seed arils. Approx. 13 and 14% of the dry matter of the santol fruits were soluble and insoluble dietary fibers, respectively. Approx. 25% of the dry matter was crude polysaccharide extractable with hot water. The crude polysaccharide extract was reported to exhibit a distinct effect on the support of selective probiotic growth of *Lactobacillus acidophilus* against *Bifidobacterium lactis*.[4] Inulin, a fructan, while promoting the growth of *L. acidophilus* to 350% of that of the crude polysaccharide extract from santol fruit, it was not selective against B. lactis.

The majority (80–90%) of erythritol was absorbed in the small intestine. As a result, erythritol does not normally cause laxative effects, and gas or bloating when it is consumed in small amounts. Symptoms of mild gastrointestinal disturbance often occur after the consumption of other sugar alcohols (such as maltitol, sorbitol, xylitol, and lactitol).[31] Large doses (>0.66 g/kg male body weight or 0.8 g/kg female body weight) may cause laxation [32] and diarrhea is possible with very large doses. Erythritol also induces the secretion of gastrointestinal hormones that modulate satiet.[33]

Conclusion

Santol gummy jellies were prepared using sucrose-infiltrated (SI) peeled santols. The reduced-calorie santol gummy jellies were prepared to partially compensate for the increase in sugar content due to the addition of the SI santol. Mixtures of sucrose and erythritol were used instead of 100 g sucrose. With 75 and 100% erythritol, the sensorial satisfaction dramatically dropped due to the crystallization of erythritol. Despite the significant decrease in its cohesiveness and the significant increase in its hardness compared to those of the erythritol-free and 25%-erythritol jellies, no significant reduction in the sensory scores of the jelly prepared with 50 g sucrose and 50 g erythritol (S50:E50) was observed. Moreover, the S50:E50 formulation also exhibited the lowest water activity at 0.65±0.00. At 50% sucrose substitution, erythritol reduced both the calorie and water activity of the jellies and, at the same time, was able to maintain the flavorfulness of the product.

Concerning the commercialization of the santol gummy jellies, a comprehensive microbiological risk assessment is required to determine to maximum storage life of the product. Furthermore, formulation adjustments to further decrease the water activity of the gummy jellies are highly beneficial for a preservative-free product. The osmo-dehydrating santol before gummy jelly production enhances its flavour, resulting in an improved product taste. This may be possible with a mixture of erythritol and other sugar substitutes. Alternatively, herbs exhibiting antifungal and/or antibacterial activities may be added.

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Conflict of Interest

All authors disclosed no relevant relationships.

Data Availability Statement

All the experiments were conducted as original research by the research team and were not copied from any previous studies.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

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Author Contribution

- **Suchada Maisont:** Preparation of solution, preparation and characterization of all samples, research planning, sensory evaluation, gamma checking and manuscript preparation.
- **Wantana Leebonoi:** Conceptualization, methodology, writing – original draft.
- **Sutatip Thonglem:** Testing of chemical and physical properties of all samples, statistical analysis.
- **Jaroenporn Chokboribal:** Testing of chemical and physical properties of all sample, statistic analysis, gamma checking and manuscript preparation.

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