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EFFECT OF PARTIAL REPLACEMENT OF SUCROSE WITH STEVIA AND SUCRALOSE ON THE PHYSICOCHEMICAL AND STRUCTURAL-MECHANICAL PROPERTIES OF APPLE MARMALADE

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ABSTRACT

In this study, low-sugar apple marmalade formulations were developed by partial replacement of sucrose with stevia and sucralose. Their rheological, textural, physicochemical properties and microstructures were evaluated. The concentration of sweeteners was found to have a significant effect on the physicochemical and rheological properties of the formulations. The hardness of marmalades decreased with addition of sweeteners. Herschel–Bulkley model was found to be the best model describing rheological behavior. The consistency index decreased with increasing sweeteners substitution, whereas the flow behavior index showed an increasing trend with the increase of the sweeteners content. Additionally, the microstructure of marmalades with sweetener substitution exhibited a porous structure in the gel network. The increase in sucralose concentration resulted in more surface deformation resulting in weaker gel formation than stevia. Marmalade prepared with 50% stevia substitution was found the best combination and resulted in good sensory properties like marmalade samples containing 500 g sugar.

Keywords: Marmalade, texture, rheology, sweetener, microstructure

SÜKROZUN STEVİA VE SÜKRALOZ İLE KISMEN DEĞİŞTİRİLMESİNİN ELMA MARMELATININ FİZİKOKİMYASAL VE YAPISAL-MEKANİK ÖZELLİKLERİ ÜZERİNDEKİ ETKİSİ

ÖZ

Bu çalışmada, sükrozun kısmen stevia ve sükraloz ile değiştirilmesiyle düşük şekerli elma marmelatı formülasyonları, geliştirilmiştir. Formülasyonların reolojik, dokusal, fizikokimyasal özellikleri ve mikro yapıları değerlendirilmiştir. Tatlandırıcıların konsantrasyonunun formülasyonların fizikokimyasal ve reolojik özellikleri üzerinde önemli bir etkiye sahip olduğu bulunmuştur. Tatlandırıcı ilavesiyle marmelatların sertliği azalmıştır. Herschel-Bulkley modelinin reolojik davranışı açıklayan en iyi model olduğu bulunmuştur. Tatlandırıcı ikamesinin artmasıyla kıvam indisi azalırken,

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tatlandırıcı içeriğinin artmasıyla akış davranış indisi artan bir eğilim göstermiştir. Ayrıca tatlandırıcı ilaveli marmelatların mikro yapısı, jel ağında gözenekli bir yapı sergilemiştir. Sükraloz konsantrasyonundaki artış, steviadan daha zayıf jel oluşumuyla sonuçlanan daha fazla yüzey deformasyonuna neden olmuştur. %50 stevia ikamesi ile hazırlanan marmelat en iyi kombinasyon olarak bulmuş ve 500 g şeker içeren marmelat örnekleri gibi iyi duyusal özellikler sağlamıştır.
Anahtar kelimeler: Marmelat, tekstür, reoloji, tatlandırıcı, mikro yapı

INTRODUCTION

Marmalade is a mixture brought to a suitable gelled consistency by adding sugar and water to the pulp, purée, juice, and juicy extracts and/or edible parts of one or more fruits by CXS 296-2009, adopted in 2009, amended in 2017 food standard (International Food Standard - Codex Alimentarius, 2020). For hundreds of years, food preservation techniques have been applied to fresh and perishable fruits to extend their shelf life and increase their availability out of season. Production of jams, jellies, marmalades, and fruit preserves is among those techniques. Marmalade, a common type of fruit-derived product, is known as a traditional delicacy. It is a semisolid food obtained by boiling fruit pulp with sugar, acid, pectin, and other ingredients like preservatives, coloring, and flavoring items until reaching the suitable consistency (Lal et al., 1960). Due to high sucrose content with its sweetening effect and caloric value, marmalade is also a great source of energy and carbohydrate. However, a high sucrose diet has been associated with some health problems including diabetes, cancer, metabolic and cardiovascular diseases (Rippe and Angelopoulos, 2016). Because of the negative connotations related to sugar consumption, low-calorie products are made by fully or partially replacing sugar with sweeteners depending on the properties required in the product.

It is technologically possible to reformulate marmalades to be a healthy alternative to traditional ones. Carbohydrate or non-carbohydrate artificial sweeteners, especially sorbitol, maltitol, xylitol, acesulfame-K, saccharin, cyclamate, stevia, sucralose, or combinations of these, can be used to maintain or improve the properties of marmalades. However, the consumption of sweeteners is restricted. For example, the daily amounts of stevia and sucralose limited to 4 mg/kg and 5 mg/kg body weight, respectively (Chattopadhyay et al., 2014; Fry, 2012).

The newly formulated product with sweeteners should meet the consumer's demands in terms of its textural, structural and flavor characteristics when compared with traditional products (Renard et al., 2006). Currently, low-sugar or sugar-free confections are also continuing to gain in immense popularity. Due to a steady increase in interest in a balanced diet and a healthy lifestyle, reduced sugar or sugar-free products have a place in the dietary choices of humans. At the same time, fruits also provide essential nutrients in a healthy diet. They have a vital role for the health and maintenance of the body because of their concentrations of vitamins and minerals, and especially being good sources of dietary fiber and antioxidants. Gorinstein et al. (2001) studied the contents of dietary fiber in the whole apple, along with its pulp and its peel. They found that the peel of the apple is unusually a well-balanced and the richest source (0.91% fresh weight) in terms of total fiber, and insoluble fiber (0.46% fresh weight) and soluble fiber (0.43% fresh weight) proportions. Vetter et al. (2001) also emphasized that the phytochemicals and nutrients of apple pomace as well as having its functional characteristics like water holding, gelling, thickening and stabilizing abilities. It was demonstrated that apple with nutritional properties have a good potential in a variety of food formulations, as well.

This study aims to formulate the best quality reduced sugar apple marmalade (RSAM) by optimizing the composition of ingredients using artificial and natural sweeteners without adding commercial pectin, determining its rheological, textural, and physicochemical properties, and examining its microstructure.

MATERIALS AND METHODS

Raw Materials

Apples (*Malus domestica 'Gala'*) were purchased from marketplace in Izmir, Turkey. Stevia (Pure Stevia Extract 95 % Rebaudioside-A) and Sucralose (Vitasweet® Sucralose) were kindly provided by Egepak A.Ş., Izmir, Turkey. Sucrose and lemons were purchased from a market in Izmir, Turkey.

Sample Preparation

Apples were sorted and cleaned. They were washed then stalks and cores were removed. After peeling, they were put into the boiling water to soften the tissue and they were filtered through a sieve to obtain the pulp. Prior to cooking, sucrose and sweetener were added to the mixture just before the end of cooking, 15 mL of lemon juice was added to the mixture to prevent sugar from crystallization and to regulate acidity. While the marmalade was still hot, it was filled to the sterilized and hot jars and immediately closed. Formulation of the samples was given in Table 1. While determining the amount of sweetener, the equal sweetness was considered.

Table 1. Apple marmalade formulations with sweeteners (basis: 1 kg apple)

Formulation No	Sucrose Basis, g	Sucrose Replaced, %	Sucrose Amount, g	Stevia Amount, mg	Sucralose Amount, mg
1	500	0	500	0	0
2	500	25	375	416.67	0
3	500	50	250	833.33	0
4	500	25	375	0	208
5	500	50	250	0	416
6	600	0	600	0	0
7	600	25	450	500	0
8	600	50	300	1000	0
9	600	25	450	0	250
10	600	50	300	0	500

Physicochemical Properties

The water activity (a_w) of the RSAM samples were measured by a benchtop water activity analyzer (HygroLab 3, Rotronic, Bassersdorf, Switzerland) at room temperature. Total soluble solid (TSS) content was measured with a refractometer (PAL-3, ATAGO Co. LTD., Tokyo, Japan). The ash content of the formulations was measured gravimetrically by burning them in an oven at 550 °C. Moisture content was determined by vacuum oven at 70 °C for 16 hours. pH of the samples was measured by a bench top pH meter (InoLab 7310, WTW, GmbH, Germany). Titratable acidity (TA) was measured against 0.1 N NaOH solution, and it was reported as a percentage of citric acid. TSS, ash content, moisture content and TA analyzes were conducted using methods by Cemeroglu (2013). Color of the formulations was measured by chromometer (CR400, Konica Minolta, Osaka, Japan) using D65 illuminant. Color values were presented in the form of CIE L*, a* and b*.

Rheological Measurements

Rheological measurements of the formulations were performed at 30 °C by using an AR 2000-ex rheometer (TA Instrument, New Castle, DE) equipped with an Environmental Temperature Controller Unit. The temperature controller unit has no capability of cooling, that is why measurements were conducted at 30 °C. Parallel plate geometry with 25 mm diameter and 1 mm gap height was used. By this configuration, oscillatory time sweep, oscillatory stress sweep, and stepped flow tests were conducted. For oscillatory time sweep test, shear rate was kept at 0.05 s^{-1} and for 15 min, measurement was carried out. For oscillatory stress sweep, torque scanning range was set to 0.1 – 10000 μN.m. At the frequency of 1 Hz, the test was carried out and storage modulus (G') and loss modulus (G'') were measured. For the stepped flow test, controlled stress (CS) mode was used with the torque range of 250 – 2500 μN.m. Rheological models were fitted to Power Law and Herschel-Bulkley (HB) equations (see Eqn. 1 and 2, respectively) by using MATLAB software (MathWorks, 2021b, Natick, Massachusetts).

$\tau = K\dot{\gamma}^{n}$ (Eqn. 1) (Sahin and Sumnu, 2006)

$\tau = \tau_0 + K_{HB}\dot{\gamma}^{n_{HB}}$ (Eqn. 2) (Sahin and Sumnu, 2006)

Where; τ is shear stress (Pa), K is consistency index for Power Law (Pa.s^{n}), $\dot{\gamma}$ is shear rate (1/s), n is flow behavior index for Power Law, τ_0 is yield stress (Pa), K_{HB} is consistency index for HB model (Pa.s^{nHB}) and n_{HB} is flow behavior index for HB model.

Texture Profile

The textural properties of marmalade samples were measured using a texture analyzer (TA-XT Plus Texture Analyzer, Stable Micro System, UK) with a load cell of 5 kg at 25 ℃. Texture profile analysis (TPA) curve consists of compression cycles. Trigger force, pre-test speed, compression speed and post-test speed were set to 0.05 N, 2 mm/s, 2 mm/s and 5 mm/s, respectively. Sample was put to container of the instrument with the fill height of 3 cm and cylindrical probe (25.4 mm diameter) was compressed the depth of 20 mm. Hardness (N), adhesiveness (J), cohesiveness, springiness (m), gumminess (N) and chewiness (J) were calculated from TPA curves.

Microstructure of Marmalades

Scanning electron microscopy (SEM) was used to provide information about the food microstructure using images at high resolution. It was carried out on freeze-dried samples. Before the analysis, freeze-dried samples were fastened onto conducting sticky carbon tape and then coated with gold to impart electrical conductivity to the sample by Sputter Coater (Emitech K550X). RSAM samples were covered at 15 milliamps flux and under 6×10^{-2} mbar vacuum for 1.5 min. All samples were assayed and photographed with Philips XL 30S FEG scanning electron microscope operating at an accelerated voltage of 5 kV and magnification in the range of ×250-2500. SEM images were collected from different places of the RSAM samples.

Sensory Evaluation

The appearance, taste, color, texture, and overall acceptability of different marmalade formulations were determined by a 9-point hedonic scale (Lawless and Heymann, 2010). Water was used for cleansing palates. The sensory analysis was performed with the panel consisting of 21 semi-trained people familiar with the marmalade taste. Average values were determined for each evaluated attribute. Test was conducted after 3 days of production time. The samples were kept at room temperature for 3 hours prior to test.

Statistical Analysis

Analysis of variance (ANOVA) was used to find out the differences by using MINITAB software (Version 19, Minitab Inc., Coventry, UK). Tukey's comparison test at 95% confidence interval was used for pairwise comparisons. All measurements were conducted at least three replicates.

RESULTS AND DISCUSSION

Physicochemical Properties of Low Sugar Apple Marmalade

The results of physicochemical properties of RSAM samples are given in Table 2.

The water activity of apple marmalades increased with increasing sweetener concentration due to lower interaction of water with the matrix. All samples prepared in this study showed similar a_w values to the lemon marmalades that are prepared by replacing sucrose with tagatose and isomaltulose (Rubio-Arraez et al., 2017). Abid et al. (2018) reported that bacteria growth in pomegranate jam was observed at the a_w values higher than 0.86. Vilela et al. (2015) pointed out that the a_w value must be at least 0.80 for mold growth in strawberry, raspberry, and cherry jams made by replacing sucrose with sweeteners. In this study, all formulations have relatively high a_w value. The main reason of this could be hot filling consecutively condensate formation in the headspace. The preservative function of these formulations belongs to lowered pH values instead of a_w. Rubio-Arraez et al. (2017) stated that sweet orange marmalades having sweeteners (tagatose and oligofructose) showed proper microbiological stability at pH values below 3.8.

Table 2. Physicochemical properties of reduced sugar apple marmalade (RSAM) samples

Formulation No	a_w	TSS, °Brix	Dry Matter, %	pH	TA, %
1	0.80±0.00^{d}	65.78±0.58^{b}	73.55±0.77^{b}	3.62±0.01^{a}	0.24±0.00^{f}
2	0.87±0.01^{b}	61.63±0.29^{d}	64.91±0.69^{d}	3.54±0.00^{bc}	0.32±0.00^{bc}
3	0.91±0.01^{a}	52.49±0.00^{h}	54.55±0.55^{f}	3.58±0.00^{ab}	0.32±0.00^{bc}
4	0.87±0.01^{b}	60.16±0.58^{e}	60.00±0.41^{e}	3.51±0.01^{cd}	0.33±0.01^{abc}
5	0.91±0.01^{a}	50.29±0.58^{i}	52.39±0.75^{g}	3.54±0.00^{bc}	0.34±0.00^{ab}
6	0.77±0.01^{e}	74.79±0.29^{a}	77.36±0.02^{a}	3.63±0.01^{a}	0.23±0.00^{f}
7	0.84±0.01^{c}	62.31± 0.29^{cd}	69.29±0.17^{c}	3.55±0.00^{bc}	0.29±0.01^{e}
8	0.88±0.01^{b}	53.79±0.29^{g}	59.35±0.60^{e}	3.56±0.00^{bc}	0.30±0.01^{de}
9	0.83±0.01^{c}	63.44±0.50^{c}	69.71±0.17^{c}	3.47±0.04^{d}	0.23±0.00^{f}
10	0.87±0.01^{b}	55.63±0.29^{f}	58.65±0.54^{e}	3.51±0.04^{cd}	0.35±0.01^{a}

The total soluble solid content was found to be highest in the formulation having only sucrose. Council Directive 2001/113/EC of 20 December 2001 relating to fruit jams, jellies, marmalades, and sweetened chestnut puree intended for human consumption allows the soluble solids content to be lower than 60 °Brix with the sweetener use in the formulation. The sweetener added formulations met the criterion of the Council Directive. The amount of dry matter is associated with the extended shelf life due to higher interaction of solids with water (Abid et al., 2018).

The pH values of the marmalade products ranged between 3.47 and 3.63. Highest pH value belongs to formulation 6 whose sucrose content is highest. Gajar and Badrie (2002) found that the pH value to be 3.62 for the low-calorie christophene jam. They also reported that the pH value was in the recommended range of 3.00 and 4.00. Similarly, the pH values of jams prepared with peach, plum, strawberry, and apricot (Carbonell et al., 1991; García-Martínez et al., 2002) were in the same range. Titratable acidity is about the total acid content of the product. It comes from the organic acids presenting in fruits or added acidulants to the formulations (Kanwal et al., 2017). Also, presence of acids provides texture by contributing to the gelation mechanism of pectin and enhances the natural fruit flavor (Onoğur, 2001).

Color analysis results are given in Table 3. Among formulations, formulation 6 has the darkest color value due to highest sucrose content. Igual et al. (2010) indicated that high heat treatments could result in sucrose caramelization consequently a darker color could occur in the jam product. A significant increase was observed in the L* value by the increased stevia substitution (*P < 0.05*). In general, the marmalade formulations made by using Sucralose sweeteners appeared in a lighter color than those made with stevia. This may be due to the response of different sweeteners to the heating process. Sucralose is known to be highly stable at elevated temperatures that are often used in food, beverage, and drug manufacturing processes so that product sweetness levels can be maintained following cooking, baking, and pasteurization (Frazier, 2007).

It was found that the a* value of formulation 6 was the highest. The reason of this can be explained by elevated pH value so that the enhanced Maillard reaction occurrence in the medium. Abid et al. (2018) stated that increasing proportions of pomegranate fruits in jam results in a decrease of a* value. The jams (with higher amount of fruit) were less reddish which could be due to decomposition of the anthocyanins during thermal treatment. The color parameter b* ranged between 6.08 and 9.15. Abolila et al. (2015) did not find a significant difference in color scores between orange jam formulations prepared with fructose, stevioside and Sucralose. Basu et al. (2013) explained the color change in low calorie mango jam as the fact that the acid degradation reaction yields with smaller sugars having reducing end so contributing to Maillard reaction. In addition, in the study of Peinado et al. (2012), the color change in isomaltulose and sucrose containing strawberry products because of sugar degradation by citric acid was examined. They found the effect of citric acid on the color change is significant.

Table 3. Color Data of reduced sugar apple marmalade (RSAM) formulations

Formulation No	Lightness/Darkness, L*	Redness/Greenness, a*	Yellowness/Blueness, b*
1	23.81±0.15^{e}	-0.36±0.13^{b}	6.08±0.26^{f}
2	24.93±0.04^{d}	-0.73±0.07^{c}	6.72±0.20^{e}
3	28.16±0.18^{b}	-1.36±0.08^{e}	8.63±0.03^{ab}
4	26.51±0.30^{c}	-1.27±0.08^{e}	7.96±0.18^{c}
5	29.55±0.06^{a}	-1.96±0.01^{f}	9.15±0.18^{a}
6	22.70±0.07^{f}	0.42±0.10^{a}	6.17±0.03^{ef}
7	24.77±0.05^{d}	-0.37±0.06^{b}	7.91±0.09^{cd}
8	26.68±0.27^{c}	-1.14±0.02^{d}	6.59±0.06^{ef}
9	24.81±0.09^{d}	-0.32±0.09^{b}	7.34±0.49^{d}
10	27.74±0.08^{b}	-1.27±0.00^{de}	8.19±0.18^{bc}

Results were reported as mean±standard deviation of 3 replicates. Means±standard deviation within a column followed by different letters is significantly different ($P < 0.05$).

Rheological Properties

From the result of oscillation time sweep test, it was found that there is no significant change within the range of 0 – 900 s ($P < 0.05$). As the consequence of the time sweep test, the equilibration time for the stress sweep test was chosen 600 s. From an industrial point of view, it is desirable to have a short time to reach steady state gel structure (Torres et al., 2013). The oscillation stress sweep test was performed with the equilibration time from time sweep test for each marmalade samples. Dynamic rheological viscoelastic properties of the low sugar apple marmalade formulations were measured within the linear viscoelastic region (LVR) ranging from 0.41 to 50 Pa for G', and 0.41 to 200 Pa for G''. The results of both dynamic moduli showed similar behaviors largely independent of stress values. The values of both moduli are given in Table 4.

Table 4. Viscoelastic properties of reduced sugar apple marmalade (RSAM) formulations

Formulation No	G', kPa	G'', kPa
1	13.024±0.299^{ab}	3.586±0.050^{ab}
2	12.287±0.314^{ab}	3.140±0.132^{ab}
3	11.404±0.672^{b}	2.555±0.154^{b}
4	12.733±0.324^{a}	3.709±0.078^{a}
5	12.965±0.517^{ab}	3.044±0.186^{ab}
6	13.756±0.298^{ab}	3.647±0.095^{ab}
7	11.763±0.432^{ab}	3.891±0.119^{a}
8	12.588±0.477^{ab}	3.276±0.103^{ab}
9	15.188±0.371^{ab}	3.292±0.036^{ab}
10	15.509±0.478^{ab}	3.850±0.056^{a}

Results were reported as mean±standard deviation of 3 replicates. Means±standard deviation within a column followed by different letters is significantly different ($P < 0.05$).

Storage or elastic modulus (G’) is related to the elastic quality, whereas loss modulus (G”) is also associated with the viscous quality of the products. For all samples, the elastic modulus (G’) was extremely higher than the loss modulus throughout the stress range, indicating a predominant contribution of the value G’ to the viscoelastic properties of the marmalade samples. In other words, the marmalade samples exhibited a dominant elastic/solid-like character. The firmness/consistency of the structure of the product was evaluated by the elastic modulus, which was obtained by the strength of gel (Garrido et al., 2015). The formulation 4 significantly contributed to the highest degree of both elastic modulus and loss modulus in all formulations. On the other hand, the lowest values of the modulus were significantly observed in formulation 3, as seen in Table 4. This may be explained as a reduction of the sucrose content, which resulted in the increase of the liquid-like character of the formulation. In the jam gelation process, the pectin molecule chains are aligned and stretched in sucrose and fruit pulp mix and consequently, the intermolecular formation of hydrogen bonding occurs in more available sites. To form a three-dimensional network, the pectin molecules are surrounded by hydrogen bonds. Nevertheless, it is provided to hold the sucrose within the structures of pectin network. Thus, an increased sucrose concentration and therefore an increase in TSS leads to the development of strong elasticity in the jam product (Basu et al., 2011). Similarly, the formulation 7 having a higher TSS degree led to higher values of elastic modulus, compared to the formulation 8. Table 4 showed that there were marked differences in all formulations prepared with sucralose. At the same time, the formulations containing sucralose sweeteners yielded higher values of G’ and G”, compared to the formulations containing stevia sweeteners. This could be due to the different structure of the nature of bond in the sucralose, compared to the stevia. On the other hand, there were no significant differences between the formulations 1 and 6 in terms of G’ values. Due to the highest sucrose content, formulation 6 had the higher G’ value compared to the formulation 1. Thus, the gel strength of the formulation 6 was higher.

The increase in the sucrose concentration increased the G’ and G” values and decreased the water availability to form a hydrogen bond between the mixture of pectin, sucrose, and acid. Although the sucrose provided the stabilization to the structure of junction zones, over a certain concentration of sucrose reduced the gel quality and become a weaker gel structure of the pectin. The observation was supported by (Basu et al., 2011). In their mango jam samples containing sorbitol, the sucrose concentration increased to above 60% resulting in an unstable structure in a firmer gel network of the pectin and a softer jam because of releasing more water molecules in the jam. In the study conducted by Löfgren et al. (2002), the high-methoxyl (HM), low-methoxyl (LM) pectin and their mixture gel structure rheologically were investigated and determined the viscoelastic properties. They expressed that changes in the sucrose concentration affected the gel strength between the HM and LM pectin, as well as the structure of the network. Torres et al. (2013) studied the effect of the addition of sucrose, xylitol and stevia to the prepared chestnut and rice flours gel and evaluated the rheological properties of the formulation. The authors found that the addition of sucrose changed the viscoelastic properties of gels. On the other hand, xylitol addition did not change those properties significantly. By the temperature, time and frequency sweep tests, addition of stevia to the formulations did not change the viscoelastic properties of the formulations.

To characterize the flow behavior of the low sugar apple marmalade samples, specific torque values ranging from 250-2500 μN.m were selected to determine shear stress and shear rate data for each marmalade sample. The torque ranges were as follows: i) Formulation 1: 250- 2500 μN.m ii) Formulation 2: 250-1750 μN.m iii) Formulation 3: 250-1250 μN.m iv) Formulation 4: 250-1750 μN.m v) Formulation 5: 250- 1250 μN.m vi) Formulation 6: 250-2000 μN.m vii) Formulation 7: 25-1500 μN.m viii) Formulation 8: 250-1250 μN.m ix) Formulation 9: 250-1750 μN.m x)

Formulation 10: 250-1500 μN.m. Power Law and Herschel-Bulkley (HB) models were applied and the results of the model parameters for the samples were presented in Table 5.

Table 5. Power Law and HB model parameters of the RSAM formulations

	Power Law Model				Herschel-Bulkley Model				
Formulation	K, Pa.s^n	n	R^2	RMSE	τ_0, Pa	K, Pa.s^{nHB}	n_{HB}	R^2	RMSE
1	410.31±69.26^a	0.10±0.03^a	0.91±0.03	0.01±0.00	406.49±64.29^a	25.85±5.36^a	0.56±0.07^a	0.89±0.03	0.09±0.03
2	275.10±37.12^{ab}	0.09±0.04^a	0.93±0.02	0.01±0.00	304.08±56.85^{abc}	8.18±4.85^b	0.61±0.13a	0.88±0.04	0.10±0.04
3	229.15±53.28^b	0.09±0.04^a	0.87±0.11	0.01±0.01	242.97±36.57^c	6.46±2.23^b	0.73±0.07^a	0.91±0.03	0.08±0.03
4	325.31±27.12^{ab}	0.10±0.00^a	0.94±0.05	0.01±0.01	372.58±53.88^{ab}	11.18±4.70^b	0.57±0.10^a	0.84±0.04	0.11±0.04
5	245.88±21.26^b	0.09±0.04^a	0.87±0.14	0.01±0.01	271.50±20.41^{bc}	7.38±4.32^b	0.64±0.27^a	0.91±0.03	0.08±0.03
6	333.53±36.58^{ab}	0.11±0.03^a	0.94±0.03	0.02±0.00	353.88±11.02^{abc}	24.91±4.81^a	0.46±0.04^a	0.90±0.06	0.09±0.03
7	262.14±44.65^b	0.16±0.03^a	0.97±0.01	0.01±0.00	270.06±51.79^{bc}	16.52±4.51^{ab}	0.80±0.10^a	0.90±0.02	0.08±0.01
8	213.86±18.04^b	0.13±0.01^a	0.95±0.03	0.01±0.00	269.12±16.58^{bc}	3.32±0.92^b	0.82±0.09^a	0.86±0.05	0.09±0.01
9	285.62±98.99^{ab}	0.10±0.04^a	0.89±0.09	0.02±0.01	346.72±56.55^{abc}	10.49±7.61^b	0.52±0.14^a	0.87±0.05	0.10±0.02
10	301.98±23.26^{ab}	0.10±0.06^a	0.92±0.06	0.01±0.01	315.65±31.35^{abc}	13.98±4.37^{ab}	0.57±0.25^a	0.93±0.06	0.07±0.04

Results were reported as mean±standard deviation of 3 replicates. Means±standard deviation within a column followed by different letters is significantly different (*P < 0.05*).

Considering all the experimental results, the RSAM samples containing different formulations had a shear thinning behavior (pseudo-plastic), because viscosity decreased with increasing shear rate applied. A minimum stress value of about 242.97 Pa is required for initiating the flow, indicating the yield stress. Yield stress was obtained for all the marmalade formulations and depicted in Table 5. The addition of sweeteners was obviously effective on the yield stress of the formulations. The yield stress values decreased with increasing sweeteners concentrations. This could be related to the sucrose content. Reduction of the sucrose concentration resulted in a decrease in the resistance to flow. Thus, mechanical forces applied to the marmalade samples were also decreased by the decreased sucrose. Tan et al. (2014) emphasized that the starch concentration which was increased from 15% to 25% led to an increase in the shear stress values because of the effect of sugar and starch as a thickening agent in the apple jam. In addition to this, yield stress values were highly affected by the addition of these agents to the formulations. Similar results were also obtained by Koocheki et al. (2009) in ketchup. The yield stress values provided increase with the increase in the concentration of hydrocolloid in the product. The data of the relationship between shear rate and shear stress fitted well to the Herschel-Bulkley model to describe the flow behaviors of the low sugar apple marmalade exhibiting certain yield stress. In all cases, the coefficient of determination (R^2) was higher than 0.85 and root mean square error (RMSE) were lower than 0.11 (Table 5). The small RMSE values indicate the model better fit for the data (Unluturk et al., 2010). Since the RSAM samples exhibited the yield stress, the Power Law model was not suitable for describing the sample behavior (Table 5). Additionally, the Power Law model resulted in very low flow behavior index (n) values. Therefore, the selected HB model was adequate to describe the flow behavior of RSAM samples having yield stress within the specified range. The determination coefficient between 0.80-0.90 was expressed as a good prediction. The rheological behavior of the RSAM samples was predicted well by the HB model parameters in the range of given shear rate with a determination coefficient of $R^2>0.85$. For only formulation 4, this value was determined as 0.84. The flow behavior index (n) of all the apple marmalade samples determined by the model was observed to vary from 0.46 to 0.82. The flow behavior index was increased by an increase in the concentration of sweeteners substitutions. Since the magnitude of the n_{HB} was smaller than 1 and the coefficient of determination (R^2) was higher than 0.85, it could denote that the RSAM samples exhibited a shear thinning behavior and described as non-Newtonian fluids. The consistency index (K_{HB}) of all formulations also ranged from 3.32 to 25.85. Consistency is a major quality factor in many semisolid foods such as purees and pastes. It indicates a strong interaction between the molecules in the sample structure and stability (Dogan and Kokini, 2006). The observation of this study was supported by Barbieri et al. (2018). They found that the consistency index was 39.40 Pa.s^n for the gabiroba jam. Also, the consistency index was determined between 21-73 Pa.s^n for the peach jam, as given by Falguera et al. (2010). Sagdic et al. (2015) stated that the value of consistency index was found as 17.6 Pa.s^n for the rose hip marmalade at 25 °C. In other words, the consistency index varies depending on the components of jam formulations. Similarly, the consistency index decreased when the sweetener concentration was increased. The effect of the sweeteners addition on the formulations yielded lower values for the index. In other words, the consistency index decreased with a decrease in (TSS). These findings confirm the results of the mango jam made with stevioside and sucralose sweeteners. Basu et al. (2013) reported that the Herschel-Bulkley model explained the rheological behavior of the mango jam samples containing those sweeteners very well. Also, changes in the TSS affected the parameters of the model. The flow behavior index showed an increasing trend with a decrease in the TSS; moreover, the consistency index decreased when the TSS values of the jam decreased, as is seen in the apple marmalade results. In the study conducted by Peinado et al., (2012), the strawberry products containing isomaltulose (30 °Bx) and a blend of isomaltulose and fructose (50 °Bx) caused a lower yield stress and consistency index, compared to other formulations containing sucrose or sucrose glucose blend.

Texture Profile Analysis

The texture of the product is strongly dependent on the changes in its structural history throughout the processing (Sikorski, 2006). Texture analysis can be regarded as a mimic of mastication in the mouth, and it can be used to provide information on the oral processing behavior of semi-solid food for objective measurement of its textural characteristics (Naknaen and Itthisoponkul, 2015). Results of the texture analysis were represented in Table 6.

Table 6. Textural parameters of RSAM products

Formulation	Hardness, N	Adhesiveness, J	Cohesiveness	Springiness, m	Gumminess, N	Chewiness, J
1	2.25 ± 0.10^b	6.84 ± 0.43^b	0.69 ± 0.05^a	0.96 ± 0.03^a	1.55 ± 0.04^b	1.48 ± 0.04^b
2	2.00 ± 0.13^b	4.90 ± 0.89^{bcd}	0.68 ± 0.06^a	0.95 ± 0.02^a	1.37 ± 0.16^b	1.30 ± 0.17^b
3	1.99 ± 0.09^b	4.84 ± 0.91^{cd}	0.68 ± 0.06^a	0.94 ± 0.03^a	1.36 ± 0.16^b	1.28 ± 0.20^b
4	1.80 ± 0.27^b	4.83 ± 0.79^{cd}	0.67 ± 0.05^a	0.92 ± 0.05^a	1.21 ± 0.24^b	1.11 ± 0.21^b
5	1.79 ± 0.05^b	4.54 ± 0.14^{cd}	0.66 ± 0.04^a	0.92 ± 0.04^a	1.19 ± 0.04^b	1.10 ± 0.08^b
6	2.99 ± 0.31^a	9.96 ± 0.83^a	0.75 ± 0.02^a	0.99 ± 0.00^a	2.25 ± 0.23^a	2.22 ± 0.24^a
7	1.75 ± 0.41^b	4.78 ± 0.97^{cd}	0.72 ± 0.05^a	0.96 ± 0.02^a	1.27 ± 0.35^b	1.22 ± 0.33^b
8	1.73 ± 0.13^b	4.03 ± 0.54^d	0.69 ± 0.05^a	0.94 ± 0.01^a	1.19 ± 0.09^b	1.12 ± 0.08^b
9	2.11 ± 0.02^b	6.17 ± 0.41^{bc}	0.69 ± 0.03^a	0.96 ± 0.03^a	1.45 ± 0.06^b	1.39 ± 0.11^b
10	2.00 ± 0.19^b	5.39 ± 0.46^{bcd}	0.68 ± 0.03^a	0.93 ± 0.01^a	1.36 ± 0.19^b	1.26 ± 0.17^b

Results were reported as mean±standard deviation of 3 replicates. Means±standard deviation within a column followed by different letters is significantly different ($P < 0.05$).

Hardness parameter of the low sugar apple marmalade, which is the maximum force, ranged from 1.73 to 2.99 N. The highest values were obtained when the marmalade was prepared with 600 g sucrose only (formulation 6). During cooking of the marmalade, acid, sugar, and pectin formed a strong gel structure. Due to having the maximum amount of sucrose in comparison with other formulations, the highest degree of hardness was observed in the formulation 6. Adhesiveness as a textural characteristic, shows a negative force area in the curves of texture profile analysis. It is the work required to overcome the sticky forces between the sample and the probe. The adhesiveness results of the low sugar apple marmalade samples were obtained in a wide range from 4.03 to 9.97 J. For marmalade formulations containing sucrose only, it was observed that the formulations are significantly ($P < 0.05$) different from each other. Similar results were obtained from the Cantaloupe jam prepared by substituting sucrose with different xylitol concentrations. Naknaen and Itthisoponkul (2015) observed that the increased xylitol concentration slightly reduced the stickiness/adhesiveness values in the cantaloupe jam. Another texture parameter, cohesiveness, which is expressed as a ratio of the areas of positive forces under the compressions, gives how well the product resists a second deformation, compared to under the first deformation behavior. It indicates the strength of internal bonds in the sample. In terms of cohesiveness parameter, there were not any significant differences among all formulations. Springiness is a parameter for determining the texture profile of the products. It is closely related to the elasticity of the samples. After a deformation occurs during the first compression, springiness demonstrates how well the sample physically spreads back. It was found that there were no significant differences in the springiness properties of all formulations. Another parameter of texture examined in this study was gumminess, which is defined as the product of the values of hardness and cohesiveness. It is the energy needed to disintegrate a semisolid food until it is ready to swallow. The results of gumminess parameter for the low sugar apple marmalade samples ranged from 1.19 to 2.25 N, but no significant difference was observed except for formulation 6. The last parameter of the textural characteristics is chewiness, which is expressed as the product of the values of gumminess and springiness. In other words, it can be described as an energy required for masticating the food. The chewiness results of the low sugar apple marmalade ranged from 1.10 to 2.22 J, and no significant change observed except for formulation 6.

Microstructural Properties of Low Sugar Apple Marmalade

The morphological differences of low sugar apple marmalade formulations which were prepared by using stevia and sucralose sweeteners were compared with SEM. Images examined at 500× magnifications were shown in Figures 1 and 2. Micrograph of the freeze-dried marmalade samples stevia pectin, acid, sucrose gel mixture (Figure 1a). While the content of the marmalade was changed by substitution of 25% sucrose with stevia (formulation 2), the pectin network structure slightly disappeared and became more homogenous (Figure 1b). By increasing the stevia concentration, i.e., replacing 50% of sucrose with stevia, the surface roughness increased. On the other hand, the addition of sucralose sweeteners (formulation 4), a rough surface occurred with pores. As increasing sweeteners concentrations, the formation of porous structure increases (formulation 5). Compared to the formulation 1, both sweeteners increased the surface roughness but the increase in the concentration of sucralose led to more surface deformation than stevia. As the amount of sucrose increases, it is thought that a better pectin network is formed. Therefore, a smoother surface appearance is obtained. In Figure 2a, the formation of the network structure was observed more clearly, compared to Figure 1a. The SEM images of low sugar apple marmalade in the figures agreed with the results of the apple jam which was reported by Tan et al. (2014). The authors prepared apple jam by using both 15 g sucrose and cross-linked acetylated starch (CAS). SEM micrograph of sucrose containing apple jam showed a smoother surface. Further, porous structures were obtained by addition of a varied amount of CAS in the apple jam. When stevia concentration was increased, i.e., 25% and 50% sucrose were substituted with stevia sweeteners, the images showed the formation of porous structure due to the loss of the mesh structure of pectin (Figure 2b, c). On the other hand, the increase in the amount of sucrose from 500 g to 600 g contributed to the pectin network formation in the presence of sucralose (Figure 2d, e). It was observed that formulation 10 remained very similar to the formulation 6 in terms of surface homogeneity and formation of the pectin network (Figure 2e).
Figures 1 and 2

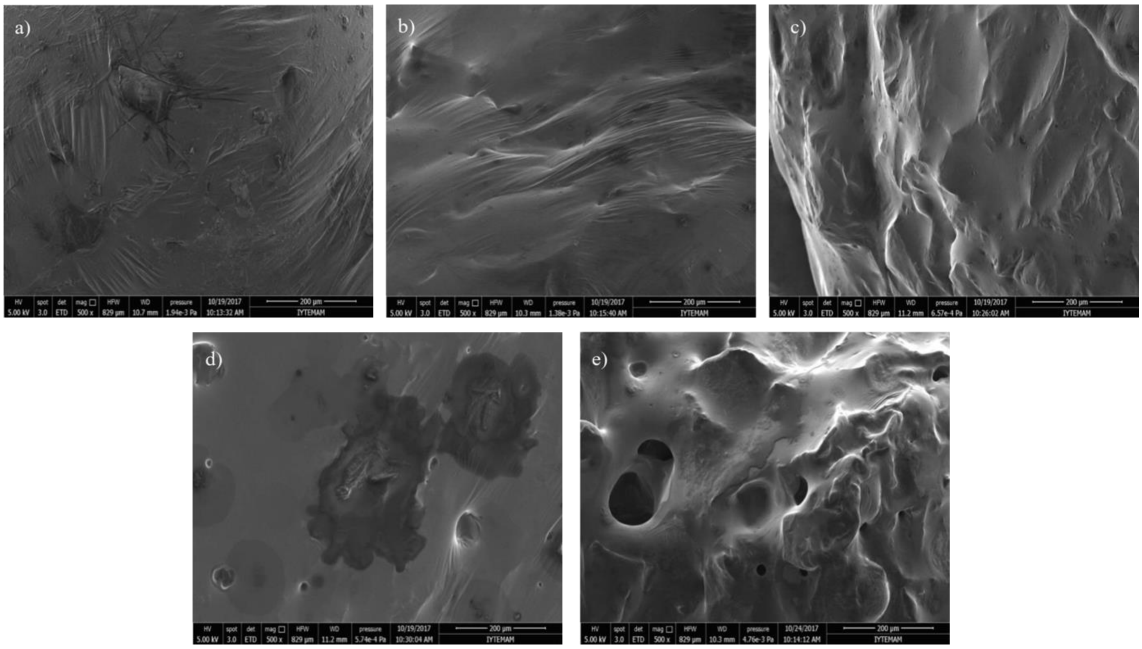

Figure 1. SEM micrographs or RSAM formulations (500 g sucrose base) at 500× magnifications a) formulation 1, b) formulation 2, c) formulation 3, d) formulation

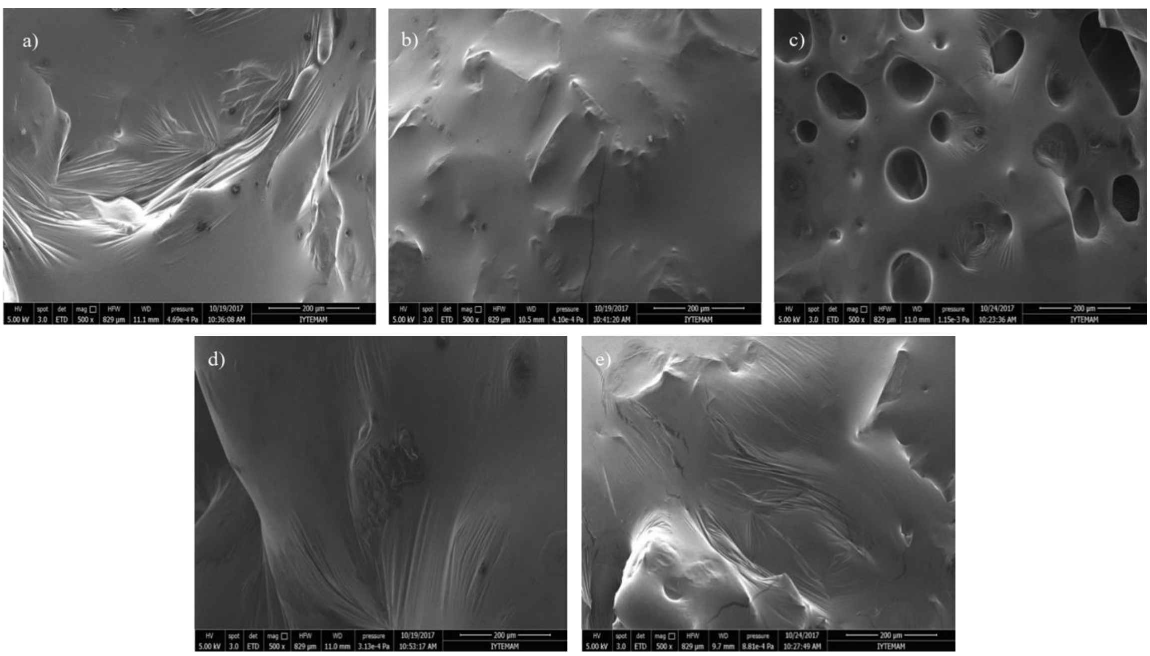

Figure 2. SEM micrographs or RSAM formulations (600 g sucrose base) at 500× magnifications a) formulation 6, b) formulation 7, c) formulation 8, d) formulation 8, and e) formulation 10

Sensory Evaluation

The sensory properties of low sugar marmalades were presented in the spider chart (Figure 3). The formulation 1 prepared using 500 g sucrose and the formulation 3 made by replacing of 50% of sucrose with stevia sweetener achieved the highest scores. While the formulation 1 was selected as the most favorable one in the texture, the formulation 6 had the least acceptance due to a highly firmer and more granular structure. Since the sucrose content was extremely high in the formulation 6, the water molecules were bound to the sucrose and the network of pectin, acid, sucrose was strongly interconnected. This caused a highly apparent increase in the hardness of the structure. The results agreed with the textural properties. According to the test scores, it was observed that formulation 1 was the most favorable sample for the appearance parameter. Formulation 2, 3, 4, 6, 7, and 8 are quite different from the formulation 1 ($P < 0.05$). On the other hand, the lowest appearance scores were observed in the formulations prepared by replacing 50% of sucrose with sucralose. There was no significant difference between the formulations 5 and 10. The sensation of a taste is associated with personal impressions and taste experiences, depending on the age, preferences, habits, and environmental conditions (Guiné et al., 2016). The taste parameter results of the low sugar apple marmalade were found to range from 6.86 to 5.76. The spider plot (Fig. 3) also showed that the highest score of marmalades was determined in formulation 1, whereas the formulation 6 had the lowest one. This is because the formulation 6 was extremely sweet due to higher sucrose content. The addition of sweeteners to the formulations did not significantly affect the taste parameters of all formulations. In the study conducted by Gwak et al. (2012) the samples with different concentration levels were prepared by using eight bulk sweeteners and four intense sweeteners and they investigated whether the sweeteners had similar sensory qualities to sucrose. They found that sucralose followed a similar pathway with sucrose and showed a lower bitterness with respect to stevia. Figure 1 demonstrated that the addition of 50 percent sucralose resulted in the lowest formulation scores for formulations 5 and 10. The least acceptance might be associated with the highly light color of the formulations containing a lower amount of sucrose. While formulation 1 had the highest scores of texture results, the formulation 6 had the lowest scores, as depicted in Figure 1. This is because the formulation 6 contains the highest amount of sucrose. Thus, its structure is highly firm and stiff, compared to the formulation 1. The characteristics including taste, flavor, shape/size, color, odor and texture are considered as the parameters that affect the quality acceptability of the product by the consumers. No statistically significant difference was observed in the overall acceptance of different formulations containing stevia and sucralose sweeteners.

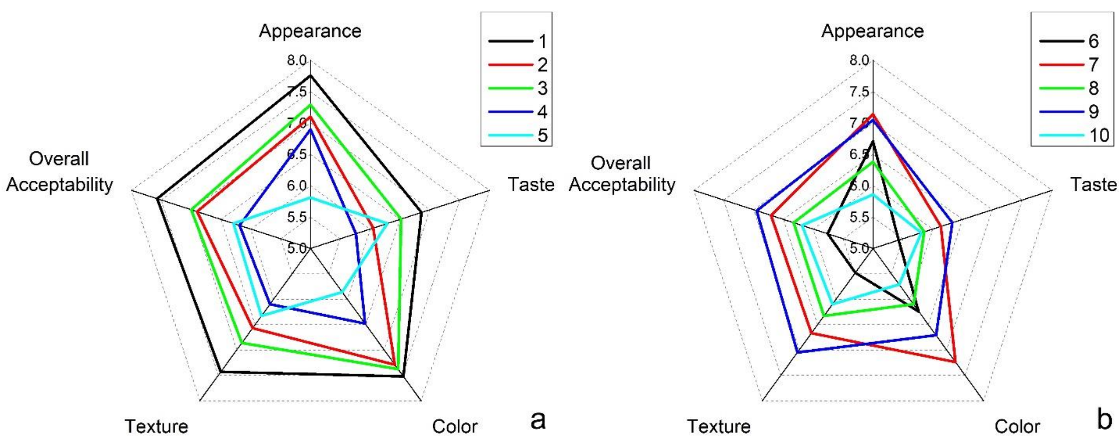

Figure 3. Sensory attributes of RSAM products having (a) 500 g and (b) 600 g sucrose basis

CONCLUSION

In this study, reduced sugar apple marmalade formulations were produced by using two types of sweeteners (stevia and sucralose) with different concentrations. It was aimed to reveal the best marmalade formulation containing sweeteners like the control samples with respect to their physicochemical, textural, rheological, and microstructural properties. The addition of the sweeteners to the formulations had a significant effect on most of them. The rheological behavior of the formulations was described with Herschel-Bulkley model, best. It was concluded that the solid-like behavior was observed from the oscillatory tests due to dominant elastic modulus (G'). Except for formulation 6, there was no significant effect observed between the formulations in terms of texture profile analysis results. This result brings the study to choose the optimum formulation is 3 or 5 due to lowest sugar content with no significant physical properties. 420 g of formulation 3 and 520 g of formulation 5 are maximum daily intake for a 70 kg person. According to SEM micrographs, it was observed that the surface changed depending on the increase of substitutions and sucrose content.

CONFLICT OF INTEREST

Authors declare that they have no known conflict of interest.

CONTRIBUTIONS

B. Berk is responsible for the data analysis, interpretation the results, and writing the manuscript. P. Şirin is responsible for carrying out the experiments, interpretation of the results, and writing the manuscript. S. Ünlütürk is responsible for data analysis, interpretation of the results, planning the analysis and providing research infrastructure.

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