Effect of Different Stabilizers and *Rebaudioside A* (Reb A) Sweetener on Quality Characteristics of Low-Calorie Orange Marmalade

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Low-calorie orange marmalade was produced using sugar and reb A (0.08%) or reb A-sugar free recipes with pectin (1%) and agar agar (AA) (1%) stabilizers. The physical, chemical, and sensory properties of the marmalades were evaluated. AA stabilizers increased the viscosity of the marmalades compared to pectin, but they did not affect samples with reb A. Low-calorie samples had higher *L** and *b** values and lower *a** values than sugar-containing samples. Total sugar content in reb A samples was 8.30 to 9.28 g/100 g, and it was lower in agar samples produced in parallel with pectin. The hydroxymethyl furfural (HMF) value was lower in sugar-free recipes (3.03 to 3.62) than in sugar-containing samples. Pectin-reb A samples had favorable taste, while AA-reb A samples favored consistency among reb A-containing samples.

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INTRODUCTION

Marmalades, which are known to provide high energy because of their high sugar content, have been prepared in different recipes for many years and are widely consumed. However, today consumers have begun avoiding this type of food because of the problems resulting from an excess of calories because of changing living conditions (sedentary lifestyle and inadequate, unbalanced nutrition, *etc*.). Therefore, researchers are working on low energy recipes (dietetics) by reducing the sugar ratio to prevent obesity and some health problems such as diabetes. However, when preparing these recipes, it is essential to consider the potential quality losses (flavor, sweetness, color, viscosity, *etc*.) that may arise from the absence of sugar in the product content. Sugar is used as a natural preservative and sweetener in marmalades and jams. Furthermore, sugar can lower the water activity of fruits, inhibiting the growth of microorganisms (Monaco *et al.* 2018).

Marmalade and jam ensure the sweet and delicious preservation of fruits harvested post-harvest. The difference between marmalade and jam is based on the sizes of the fruit pieces. Jam is a product that is prepared with or without adding sugar to fruit pieces (whole, half, or smaller). By contrast, Marmalade is a consistent product prepared with or without adding sugar to fruit pulp (Nistor *et al.* 2021). In the market, marmalades are sold as normal, low sugared, and light (for diabetes). Artificial sweeteners are widely utilized for the production of low sugared and light marmalade (Yüksel 2019).

Marmalade is produced from various fruits both traditionally and technologically. Especially, traditional marmalades are produced in small-scale enterprises and houses with fresh and dried fruits. For example, they are produced by the people themselves or bought from the market, by using the open boiling cooking technique. Because dietary products are often hard to find in stores, and they may not be easily accessible in certain locations, those people on a diet have an option to produce their own dietary products. For this purpose, various sweeteners are sold in the market (sorbitol, mannitol, xylitol, *etc*.) (Souza *et al.* 2022). Stevia sugar is one of those sweeteners. It is utilized by consumers not only in marmalade but also in cakes, pastries, and many other products. In addition to reducing the sugar content in the production of marmalade, which is produced through using reb A, its many other positive or negative effects are a matter of interest.

In this study, reb A, which is a natural, calorie-free sweetener with high sweetness level and is obtained from the leaves of *Stevia rebaudiana* (Bertoni), was used for the production of low-calorie marmalade (Ahmada *et al.* 2020). High purity reb A is highly stable in food as well as having a pure, clean taste. It demonstrates better stability than the intense sweeteners, which are widely used in acidic beverages. Dry powder reb A is stable for at least 2 years under appropriate temperature and humidity conditions. While showing stability in the pH range of 4 to 8 in its solutions, it shows less stability below pH 2 (Prakash *et al.* 2008). When considering all of these properties, it can be concluded that using Reb A as a sweetener in products like orange marmalade is feasible.

A stabilizer is not generally used in traditionally produced marmalades. However, even though natural pectin is present in orange fruit, it is anticipated that marmalades made with sugar-free sweeteners will not achieve the viscosity and gel-like structure that sugar provides. Additionally, it is expected that the protection against microorganisms through increased osmotic pressure, a function of sugar, will be lacking. Therefore, in this study, agar agar and pectin stabilizers were used to identify and resolve some of the problems that arise due to the absence of sugar. It seems technically possible to produce various auxiliary products such as “make marmalade-jam” with the help of this study for those who are dieting for the future, and this study is thought to be helpful at that point. Ruiz and Campos (2019) stated this in their study with stevia extract and nopal-pineapple marmalade. In the studies, it is observed that pectin is the most widely used stabilizer in products like jams, marmalades, jellies, fruit spreads, and fruit mixtures as well as carrageenan, guar gum, agar agar, locust bean gum, and xanthan gum, which are also used (Hubbermann *et al.* 2006).

The aim of this study was to obtain low-calorie orange marmalade recipes using the sweetener (reb A) obtained from the stevia plant, whose usage is increasing globally with various stabilizers instead of sucrose, and to examine the produced marmalades in terms of physical, chemical, and sensory aspects.

EXPERIMENTAL

Materials

In this study, orange (Finike, Turkey), sugar, pectin (high methoxyl, citrus pectin HM5 mrs gelling at the medium speed, Benosen, Turkey), agar agar (Iceland seaweed extract, E406, Benosen, Turkey), citric acid (Tito SSA30 Liquid, 25%, E330 food type, Izmir, Turkey), and reb A at 98% purity (stevia pura, 98%, Köln, Germany, sweetener) were obtained for use in the marmalade production.

Pre-treatments

The preparation of orange pulp: Oranges were dried after washing. Afterwards, the mesocarp on the surface along with the peel was removed using a knife, and then the fruit was separated into segments without a mesocarp. A homogeneous pulp was formed through blending these segments in a laboratory mixer.

The preparation of pectin and agar agar solutions: Pectin and agar agar must be completely dissolved to form a good gel and avoid any pulp forming. Therefore, 4% solutions were prepared for both stabilizers. For the preparation of the solutions, solutions were placed in a magnetic stirrer with a water boiler and first heated to 75 °C. Pectin or agar agar was added in small numbers to the water and it was mixed for 10 min until they completely dissolved (Benzer Gürel 2016). The prepared solutions (4% agar agar or 4% pectin) were added to the marmalades, with the total stabilizer content in the marmalades being 1%.

Production of Marmalades and Transferring to Jars

A traditional marmalade was produced as a preliminary test to confirm the standard in the recipes in the production of the marmalade. Raw materials, processing time, and additives that would be used in recipes were estimated through the traditional marmalade production. In the preliminary test of traditional marmalade production, the brix value was set to 55 and the cooking time was 22 min. Because the heat treatment time and pH value can be effective on HMF, the same cooking time and citric acid amounts are used in all recipes (Sarıtepe 2018). The amounts used in the marmalade recipes are shown in Fig. 1.

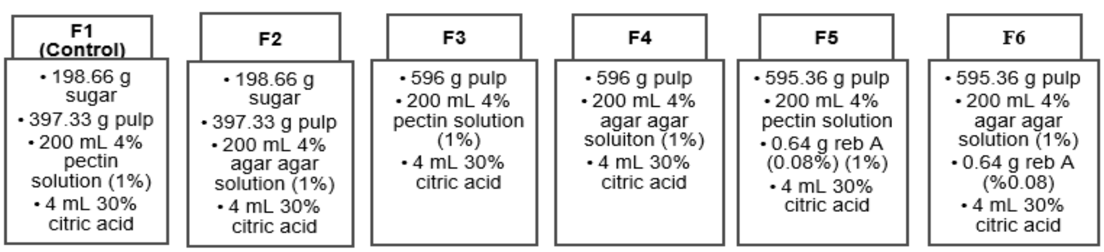

Fig. 1. Marmalade formulations used in the experiment

The production of sugar-containing orange marmalade: Sugar was added to the pulp in the amount corresponding to 50% of the weighted orange pulp. Then, it was put on the stove and cooked. At the 14th minute of the cooking process, 4% (200 mL) stabilizer (pectin or agar agar solution) was added. After mixing the solution and pulp mixture for 3 more minutes (17th min), the pH value was adjusted to ≤ 3.6 with 30% citric acid solution. The cooking process took 22 min in total. The marmalade samples, whose cooking process was completed, were filled into jars with the hot filling method and their lids were firmly closed. Afterwards, the jars were turned upside- down and they were kept in this position for 10 min, and then the jars were left to chill off by bringing them to an upright position (Fig. 2) and waiting (Kaya *et al*. 2019).

The production of low-calorie marmalades: For the production of low-calorie orange marmalade, the sugar addition was eliminated from the recipes. Recipes were formed with i) added sweetener and ii) sweetener and sugar-free formulas.

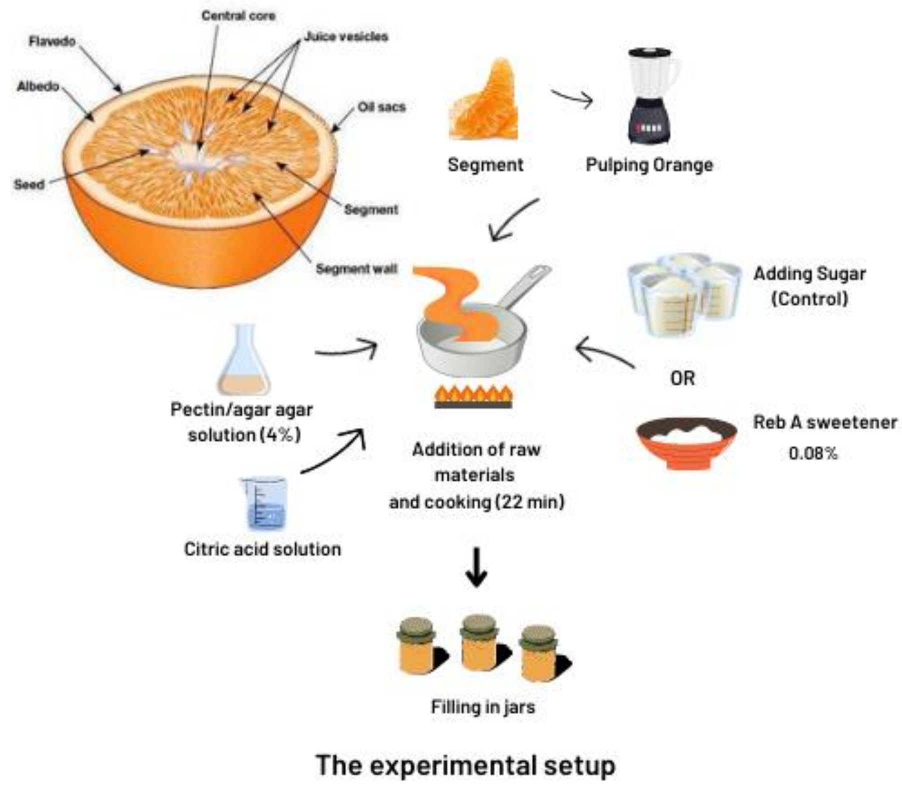

Fig. 2. Experimental setup and production flow

The weighed orange pulp was put on the stove and mixed for 14 min over medium heat for the production of orange marmalade containing reb A. Afterwards, 4% pectin/agar agar solution was added to form the gel structure. After mixing the pulp with the stabilizer for 3 min (17^{th} min), the pH value was adjusted to ≤ 3.6 using 30% citric acid solution. At the 18^{th} min of the cooking process, 0.08% reb A was added for the different recipes. Reb A addition was not performed at the beginning as it is in standard marmalade production. This was because researchers had stated that decreased stability of reb A is observed with increasing temperature (Prakash *et al.* 2008). Thereby, it was ensured that reb A was not exposed to heat for a long time. The cooking process was ceased at the 22^{nd} min. Marmalade samples were filled into jars with the hot filling method and their lids were tightly sealed (Jars and lids had been pre-washed and cleaned before filling and disinfected with boiling water.). Afterwards, they were turned upside down and kept in this position for 10 min; then the jars were put to an upright position and left to cool (Kaya *et al.* 2019).

Analytical Methods

pH value

The pH value of marmalades was determined potentiometrically with the help of a digital pH meter (Starter 3000, Ohaus, Parsippany, NJ, USA) (Cemeroğlu 2010).

Total acidity

A total of 5 g were extracted from the samples and made up to 15 mL by adding distilled water. The diluted solution was titrated with 0.1 N NaOH until pH reached 8.1. The result was determined in terms of citric acid (0.006404) g/100 mL (Cemeroğlu 2010).

Soluble solids (Brix)

Samples were measured with a digital abbe refractometer (Abbe WYA-S, Optic Ivymen System, Barcelona, Spain) and were indicated as °Brix. °Brix is measured as a percentage and represents the current total soluble solids (Cemeroğlu 2010).

Viscosity

Flow behavior analyses were completed using a rheometer device (MCR 102, AntonPaar Co., Graz, Austria) to determine the viscosity of marmalade samples at different temperature values. To form flow behavior graphs, marmalade samples were placed on the plate of the rheometer (diameter 35 mm, spacing 1.000 mm) at the constant temperature (25 °C). Flow behavior graphs were acquired by measuring the shear stress between the range of 0 to 100 s^{-1} shear velocity. Using the obtained data and graphics, the apparent viscosity values of the samples were determined at the shear velocity of 50 s^{-1} (Çevik *et al*. 2016).

Color

After the colorimetry device was calibrated, the L^* (0 = black, 100 = white darkness/lightness), a^* (+a red, -a green), and b^* (+b yellow, -b blue) values of the marmalades were measured at three different points (Minolta, CR-300, Osaka, Japan). Following the color measurement process, L^*, a^*, and b^* values, chroma (C) and hue saturation (h^*) values that appeal to people's color perception were estimated (Eqs. 1 through 3). Moreover, the E value, which gives information about the total color change tendency of the products, was calculated according to the formulas below. The ΔE value in the formula is an assessment of the color difference. A standard observer accepts the color difference results as follows: "$0 < \Delta E < 1$ -the observer does not notice the difference, $1 < \Delta E < 2$ -only the experienced observer can notice the difference, $2 < \Delta E < 3.5$ -the inexperienced observer also notices the difference, $3.5 < \Delta E < 5$ -the clear color difference is noticeable, 5 < observer ΔE - observer notices two different colors (Mokrzycki and Tatol 2011). Calculations have been made based on the L^*, a^*, and b^* values of the control sample (Vega-Gálvez *et al*. 2012).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{1}$$

$$C = \sqrt{(a^*)^2 + (b^*)^2)} \tag{2}$$

$$h^\circ = 180 + tan^{-1}\left(\frac{b}{a}\right)\ (if\ a* < 0 \text{ and } b* \geq 0) \tag{3}$$

Determination of hydroxymethylfurfural (HMF)

The samples were weighed to be 5 g and transferred to a 50-mL volumetric flask. The sample was dissolved by adding 25 mL distilled water. A total of 0.5 mL of Karrez I and 0.5 mL of Karrez II solutions were added to avoid degradation of HMF. The prepared sample was filtered with the help of a funnel. The solution was taken into vials by passing through a 0.45-micron filter and they were injected into the conditioned high performance liquid chromatography (HPLC) system. The following conditions were used: mobile phase of 90 to 10 (water -methanol), flow rate of 1 mL/min, wavelength of 285 nm; 1.0, 2.0, 4.0, 8.0, and 12.0 mg/L HMF standards were used in the preparation of the calibration curve (Baltaci and Aksit 2016).

Determination of total and individual sugars

The sugar analyses of the samples were completed according to the Baltaci *et al.* (2016) method. The Thermo Finnigan spectra system liquid chromatography system was used in a HPLC-RID device (ThermoFinnigan, San Jose, CA, USA) that had a quad pump and auto injector. A total of 2.5 g were taken from the sample, 25 mL ultrapure water was added and homogenized, and 12.5 mL methanol was added and mixed. The homogenized samples were rounded out to 50 mL with ultrapure water and filtered through filter paper. The filtrates were taken into vials by passing through a 0.45-micron filter and placed in the HPLC device. The HPLC conditions applied for sugar analysis were as follows: HPLC with Thermo Finnigan RID Detector, Column: Supercoil LC-NH_2 HPLC column 5 µm particle diameter, L × I.D. 25 cm × 4.6 mm, Mobile system: Gradient, Mobile phase: Acetonitrile – H_2O (80 to 20) Column temperature: 50 °C, Injection volume: 20 µL, Flow Rate: 1.3 mL/min. The amount of analyzed fructose, glucose, and sucrose in the sample was calculated using the calibration graph method (y= 88.4 x + 120 for fructose, y= 53.2 x + 85 for glucose, y= 13.4 x + 37 for sucrose) (Baltaci *et al.* 2016). For total sugar, the obtained sugars were collected to perform the % total sugar content analysis.

Sensory evaluation

The sensory evaluation was performed according to the form that was prepared by making changes in the evaluation form in Aslanova (Table 1). The sensory evaluation questionnaire was conducted by 7 trained panelists (Addinsoft 2023; Aslanova 2005), XLSTAT statistical and data analysis solution, New York, NY, USA).

Table 1. Sensory Evaluation Questionnaire

Sensory Evaluation Questionnaire		
Evaluated Feature	Score	Marmalade Features
Color	5	- Lively, the color of the fruit is dominant
	4	- Very slight discoloration or color darkening
	3	- Slight oxidation or darkening
	2	- Noticeable darkening
	1	- Very noticeable darkening
Consistency	5	- Appropriate gel-like structure
	4	- Very slightly consistent or juicy
	3	- Slightly consistent or juicy
	2	- Noticeably consistent or juicy
	1	- Very noticeable consistent or juicy
Odor	5	- Fruit scent or odorless
	4	- Very slight foreign odor
	3	- Slight foreign odor
	2	- Noticeable burnt and other foreign odors
	1	- Very noticeable burnt and other foreign odors
Taste	5	- Fruity, compatible pleasant
	4	- Very slight foreign taste and compatible, pleasant
	3	- Slight foreign taste, compatible
	2	- Noticeable foreign taste and very sour or sweet
	1	- Very noticeable foreign taste and very sour or sweet

Data analysis

Data obtained from physical and chemical analyses were evaluated with one-way analysis of variance (ANOVA) and their significance levels between means were determined with Tukey's multiple comparison test at the 95% confidence interval. For this, XLSTAT statistical package program (Addinsoft (2023), XLSTAT statistical and data analysis solution, New York, NY, USA) was used.

RESULTS AND DISCUSSION

Soluble Solids (Brix)

The brix measurement was performed for all samples after cooling, following the cooking time that was determined as 22 min in the preliminary tests of traditional (F1) marmalade. The Brix value of traditional marmalade (F1) was determined as 56.0. The Brix value of marmalades (F3 through F6) containing reb A in their recipes and those without sweetener-sugar ranged between 14.01 and 15.58. As can be deducted from the data of the F1 and F2 samples in Table 2, the sugar used in the recipes increased the brix amount ($p \leq 0.05$). The reason for the variations between the samples without sugar addition is thought to be due to the differences in the sugar content of the marmalades (Table 4). In a related study, low-calorie blackberry jam production was conducted by cooking in an open boiler for 20 min. As a result, it was stated that as the more sugar added to the jam samples, the brix rate has also increased, and the sample with the lowest sugar amount (cooking time 20 min, 0.0400 stevia, 0.75 pectin, 3.0686 sugar) had the lowest rate with 15% (Benzer Gürel 2016). Broomes and Badrie (2010) conducted a study on low-energy jam using sucralose instead of sugar. They determined the brix value of jams made with 1.5% low methoxyl pectin as 16 °Brix.

Table 2. Brix, pH, Total Acidity, and Viscosity Values of Orange Marmalades

Recipes	Brix° (%)	pH	TA (%)	Viscosity (s^{-1})
F1	55.98 ± 0.29^{e}	3.66 ± 0.07^{b}	1.20 ± 0.20^{ab}	1782.48 ± 152.84^{bc}
F2	53.41 ± 0.62^{d}	3.63 ± 0.02^{b}	1.10 ± 0.05^{a}	$-^{d}$
F3	15.50 ± 0.82^{c}	3.52 ± 0.02^{a}	1.59 ± 0.08^{c}	1079.40 ± 131.70^{ab}
F4	14.01 ± 0.75^{ab}	3.59 ± 0.02^{b}	1.37 ± 0.10^{bc}	2018.43 ± 16.94^{c}
F5	15.16 ± 0.41^{b}	3.50 ± 0.01^{a}	1.57 ± 0.01^{c}	916.80 ± 196.35^{a}
F6	15.58 ± 0.29^{bc}	3.42 ± 0.08^{a}	1.91 ± 0.19^{d}	670.97 ± 103.51^{a}
*a,b,c,d… shows the statistical difference in the samples at the same column (n = 6)				

Total Acidity (TA) and pH Value

The pH and TA values of all recipes are given in Table 2. The pH value of marmalades with the added sugar ranged between 3.66 and 3.42. The pH value of other recipes was between 3.42 and 3.59. The TA value of marmalades with the added sugar was between 1.10 and 1.20; TA value of other recipes varies between 1.37 and 1.91. TA value of marmalades with the added sugar was lower ($p \leq 0.05$). Moreover, when the effects of different stabilizers on the total acidity of marmalades were studied, no difference was observed between F1 and F2 samples, and between F3 and F4 samples ($p > 0.05$), while a difference was observed between F5 and F6 samples ($p \leq 0.05$). Further, the pulp ratio used in the recipe was lower than other recipes. The addition of sugar might be the reason why the pH value of the samples with the added sugar was high and the total acidity value was low ($p \leq 0.05$). Monju (2013) found the pH value of low-calorie mango jam samples prepared by using stevia in different ratios as a sweetener to be in the range of 2.90 to 3.0. The highest pH value, 3.03 was stated to belong to the sample prepared using sucrose. The acidity was reported in the range of 0.62 to 0.68% in low-calorie mango jams. Carvalho *et al*. (2013) stated that the pH value of diet blueberry jam samples sweetened with reb A was 3.66, and the pH value of jam samples made with sucralose was 3.73. In another study, it was observed that the pH values of the low-calorie strawberry jam samples prepared with reb A were in the range of 3.57 to 3.72. The pH value of the control sample was also determined as 3.70. It was determined that the total acidity of low-calorie jam samples was between 0.53 to 0.57% and the total acidity of the control sample was 0.54% (Yılmaz 2016). In a study on the substitution of sweeteners (sucralose, stevia, and fructose) instead of sucrose in the production of strawberry jam, this substitution had a significant effect on jam pH, acidity, and soluble solids (Brix), and textural properties (hardness, stickiness) depending on the sweeteners used. It was stated that stevia could not only be a natural food sweetener used in jams, but also a relatively healthy choice (Jribi *et al.* 2021).

Viscosity

The samples studied showed non-Newtonian flow. Non-Newtonian behavior is when there is no linear relationship between the shear velocity and the shear stress of the fluid. It is thought that this condition may arise from the marmalades having gel and particle structures. Álvarez *et al.* (2006) determined the effects of temperature and stabilizer agents on the rheological properties of prune, peach, apricot, strawberry, raspberry, and strawberry-raspberry mixture jams using a cylindrical rotary Haake VT550 viscometer. They drew graphs between shear stress and shear velocity to determine the type of flow and determined that all samples had non-Newtonian flow and pseudoplastic properties. Literature data also shows that marmalades have non-Newtonian flow characteristics, as can be deducted from the rheological analyzes conducted on marmalade and similar products (Estajia *et al.* 2020).

In addition to providing a sweet taste, sugar increases the water-soluble dry matter and affects color, texture, and viscosity. Sutwal *et al.* (2019) reported that low-calorie jam caused problems in appearance and texture due to the lack of sugar substitute (Siso´ *et al.* 2022). Therefore, in this study, different pectin and agar agar stabilizers were studied. Regarding this, when the viscosity values were examined, the viscosity value of the F1 sample, whose recipe contains 1% pectin stabilizer, was 1780 (Table 2). Unlike F1, the viscosity value of the F2 sample, which was done by adding 1% agar agar to its recipe instead of pectin, could not be determined because of its solid consistency. The consistency of the F2 sample produced with agar agar was more solid than the consistency of the F1 sample. Similarly, the viscosity of the F4 sample was higher than the viscosity of the F3 sample ($p \leq 0.05$). However, this was not observed in the group containing reb A. Reb A may have reduced the effect of agar or acidity may have affected it. Under this circumstance, it is observed that agar agar is more effective than pectin on viscosity. However, Ma'rquez *et al.* (2016) developed a blackberry jam made with sucralose, stevia and agar-agar and stated that agar-agar can be used as a gelling agent in low-calorie jams.

When the F1 sample containing sugar and pectin in its recipe is compared with other pectin-containing recipes, the viscosity of F1 is significant. A similar situation was also observed in the F2 sample whose recipe contains sugar and agar agar ($p \leq 0.05$). This was because sugar addition and stabilizer type affect viscosity. Hence, Şirin (2019) stated in his study that the firmness of apple marmalades decreased with the added sweetener due to the decrease in total soluble solids, while the consistency index decreased with the increase in sweetener substitutes, the flow behavior index showed an increasing inclination with the increase in sweetener content. Basu *et al.* (2013) produced reduced-calorie mango jams using stevioside and sucralose as the sugar substitutes. It was stated that the total soluble solid content decreased with the increase in the amount of stevioside and sucralose, and therefore the consistency index and yield stress decreased.

No difference was observed between the sweetener/sugar-free recipes and the reb A-containing recipes in the pectin used recipes. Under these circumstances, adding reb A did not significantly affect the viscosity. However, the difference between the sweetener/sugar-free recipes and the recipes containing reb A was significant in the recipes that used agar agar ($p \leq 0.05$).

Color Analysis

Among marmalades containing 1% pectin and 1% agar agar, the *L** value for marmalades produced with only sugar was determined as 34.8 (F1) and 38.4 (F2), respectively. For marmalades containing 0.8% reb A, the *L** value was 49.4 (F5) and 53.0 (F6), respectively; *L** values for sugar-free marmalades were 51.8 (F3) and 52.7 (F4), respectively. As can be understood from these values, the sugar amount decreased the *L** value, which determines brightness ($p \leq 0.05$). (Table 3, Fig 3). When the recipes containing pectin and agar agar were studied separately, the samples containing reb A were lighter than the ones containing sugar, and there was no difference with marmalades whose recipes did not contain reb A or sugar. Similarly, Kaya *et al.* (2019) studied the color values of marmalade samples produced according to 4 different formulations. The highest *L** value was 23.3 in the sample produced with the 4th recipe (375 g of commercial stevia sugar), the lowest *L** value was determined as 21.7 in the sample produced with the 1st recipe (750 g of sugar). The *L** value shows the changes in the brightness of the color. It is expressed as dark (black) when it approaches 0, and as light (white) when it approaches 100. Studies have shown that the *L** value is a measure of caramelization (Koç and Yolcı 2019).

When the marmalades containing the same amount of sugar (F1, F2) or 0.8% reb A (F5, F6) but prepared with different stabilizers were studied, the *L** value of the marmalades prepared with agar agar was higher than the marmalades containing pectin ($p \leq 0.05$). In other words, marmalades containing agar agar were brighter than those containing pectin.

Fig. 3. Pictures of marmalade samples containing pectin (A: F1, B: F5, C:F3)

Positive values of *a** value show the color red and negative values show the color green (Turfan 2008). When Table 3 is examined, the *a** values of all marmalades are observed to be negative. The *a** values of 1% pectin F1 marmalade containing sugar and F2 marmalade with 1% agar agar were determined respectively as -1.05 and -1.37. The *a** values of samples containing 0.08% reb A were determined as -3.00 and -4.04 for samples containing pectin (F5) and agar (F6), respectively. No difference was observed between the samples containing reb A with the same stabilizer and those without any sweetener.

Adding sugar affected *a** value and increased *a** value ($p \leq 0.05$). This is considered to be the result of the caramelization of the sugar. Because the *a** value in the color results is an indicator of the intensity of the red color in fruits and vegetables, it is an undesirable color that is formed and increases with the caramelization of sugar in products such as jam and marmalade. In other words, a decrease in the redness value and an increase in the *L** value are desired features for jam and marmalade production (Zor 2007). Kaya *et al.* (2019) determined that the *a** values of the marmalade samples varied in the range of 0.76 to 0.97. The highest *a** value was 0.97 in the sample produced with the 2nd recipe (375 g sugar + 1.125 g stevia reb D), and the lowest *a** values were 0.76 in the marmalade samples produced with the 1st recipe (750 g sugar) and the 3rd recipe (2.25 g stevia reb D). Igual *et al*. (2010) stated that caramelization may occur in sucrose after the high heat treatment in the jam production, and therefore a darker color may occur.

When the effect of marmalades prepared with different stabilizers on the *a** values is studied, it was determined that the F4 sample gave a lower *a** value than F3, and the F6 sample showed lower than F5 ($p \leq 0.05$). In other words, the samples containing agar agar gave lower *a** values when compared with the parallel containing pectin.

Table 3. Color Values of Orange Marmalades

Recipe	*L**	*a**	*b**
F1	34.78 ± 3.57^{a}	-1.05 ± 0.24^{e}	12.25 ± 3.13^{a}
F2	38.44 ± 2.69^{b}	-1.37 ± 0.54^{e}	12.93 ± 2.37^{a}
F3	51.76 ± 0.56^{cd}	-3.52 ± 0.16^{cd}	19.77 ± 0.53^{b}
F4	52.73 ± 1.96^{cd}	-4.56 ± 0.24^{ab}	18.96 ± 1.92^{b}
F5	49.36 ± 0.32^{c}	-3.00 ± 0.40^{d}	18.38 ± 1.47^{b}
F6	52.99 ± 0.23^{d}	-4.04 ± 0.29^{bc}	20.60 ± 1.23^{b}
Recipe	*ΔE**	*C**	*h°*
F1	4.33 ± 0.12^{a}	12.31 ± 3.09^{a}	95.41 ± 2.49^{a}
F2	4.33 ± 2.49^{a}	13.00 ± 2.42^{a}	95.86 ± 1.28^{a}
F3	18.75 ± 0.64^{bc}	20.08 ± 0.50^{b}	100.11 ± 0.66^{b}
F4	19.53 ± 2.25^{c}	19.51 ± 1.92^{b}	103.58 ± 0.80^{c}
F5	16.00 ± 0.56^{b}	18.63 ± 1.52^{b}	99.21 ± 0.50^{b}
F6	24.69 ± 1.49^{c}	21.00 ± 1.16^{b}	101.16 ± 1.37^{bc}

*a,b,c,d… shows the statistical difference in the samples at the same column (n = 6)

The *b** value indicates the yellow coordinates if it is +, and the blue coordinates if it is -. Because the *b** value is positive and its value is high, the samples containing reb A in their recipe and the samples without sweetener-sugar were more yellow than those containing sugar in their recipe. As well as having lower *b** values than the other marmalades ($p \leq 0.05$), F1 and F2 values were determined as 12.2 and 12.9, respectively. Kaya *et al.* (2019) determined the highest *b** value; as 6.8 in the sample produced with the 4th recipe (375 g of commercial stevia sugar), the lowest *b** value was determined as 5.8 in the sample produced with the 1st recipe (750 g sugar). In accordance with all these results, it was determined that the *b** value of marmalades decreased after adding sugar to the recipe. No difference was observed between the *b* values of F3 through F6 marmalades. In this case, pectin and agar agar stabilizers had the same effect on the *b** value of the samples.

Table 3 shows the chroma (*C*), the color difference (Δ*E*) and hue angle (*h*) values of marmalades. The *C* value, which addresses the redness and yellowness values together and is defined as the metric color chroma, is one of the most effective factors in the appearance of the products and is effective in the product preference. The *C** value demonstrates the color tone of the products, and in pale colors, the values are low, and in vivid colors the values are high (Çetin 2019). The *C* values of F1 and F2 marmalades containing sugar were determined as 12.3 and 13.0, respectively. Along with varying between 18.6 and 21.0, the other recipes had no significant difference with the sugar-free samples. The data reveals that samples containing reb A in their recipes showed higher *C** values than those containing sugar, which means they appeared livelier. When examining the Δ*E** values, there was no significant color difference between sample F1 and F2. However, there was a statistically significant color difference between the other samples and samples F1 and F2 ($p \leq 0.05$). This can be attributed to the presence of sugar in samples F1 and F2. The F4 and F6 Δ*E** values were found to be significantly different from F5 ($p \leq 0.05$).

In *h** values, 0° represents redness (+*a*), 90° represents yellowness (+*b*), 180° represents green (-*a*), and 270° represents blue (-*b*). Hereby, 0 to 90° shows the region I; (+*a*, +*b*), 90 to 180° shows the region II; (-*a*, +*b*), 180 to 270° shows the region III; (-*a*, -*b*) and finally 270 to 360° shows the region IV in trigonometric functions that are based on the color (+*a*, -*b*) (Ağçam 2011). As a result of the color analysis of the orange marmalade samples, the *a** value was determined as negative (-) and the *b** value was determined as positive (+). This situation indicates that the *h** values of the orange marmalade samples are going to be in the region II. The *h** value ranged between 95.4 and 103.6. Ağçam (2011) also stated that the hue angle values of orange juice samples varied between 98.0° and 101.0°. It was determined that the *h** values of the F1 and F2 samples were lower than the other recipes ($p \leq 0.05$).

Determination of Total and İndividual Sugars

When Table 4 is studied, the total sugar content of the formulas with the added sugar was high, as expected. The fructose value of the F1 sample that was prepared in accordance with the traditional marmalade recipe in the market was 4.51 g/100 g, the glucose value was found as 8.57 g/100 g, the sucrose value was determined as 18.2 g/100 g, and the total sugar value was determined as 31.3 g/100 g. The total sugar content of the F2 marmalade sample, which was produced by adding agar instead of pectin unlike the traditional marmalade, was determined as 29.2 g/100 g. Overall, it was observed that the total sugar and glucose content of the agar samples produced in parallel with pectin was low ($p \leq 0.05$). In the meantime, fructose, glucose, sucrose, and total sugar contents of the samples (F3 through F8) produced with sweetener instead of sugar were statistically different from the F1 and F2 samples ($p \leq 0.05$). This is an expected result because of the sugar addition to F1 and F2 samples. The total sugar content of the sample without the added sugar and sweetener varied between 5.51 and 6.61 g/100 g. The total sugar content of the samples with the added reb A was determined as between 8.30 and 9.28 g/100 g.

Table 4. Fructose, Glucose, Sucrose and Total Sugar Values of Marmalades

Recipe	Fructose	Glucose	Sucrose	Total Sugar	HMF
F1	4.51 ± 0.26^{c}	8.57 ± 0.49^{f}	18.23 ± 0.40^{e}	31.31 ± 0.40^{f}	9.32 ± 1.38^{b}
F2	4.28 ± 0.42^{c}	7.10 ± 0.67^{e}	17.78 ± 0.30^{d}	29.15 ± 1.36^{e}	8.47 ± 0.59^{b}
F3	1.67 ± 0.10^{a}	3.17 ± 0.19^{b}	1.77 ± 0.13^{ab}	6.61 ± 0.43^{b}	3.20 ± 0.40^{a}
F4	1.53 ± 0.10^{a}	2.49 ± 0.24^{a}	1.53 ± 0.11^{a}	5.51 ± 0.45^{a}	3.62 ± 0.19^{a}
F5	2.54 ± 0.10^{b}	4.53 ± 0.28^{d}	2.21 ± 0.09^{c}	9.28 ± 0.33^{d}	3.40 ± 0.08^{a}
F6	2.68 ± 0.10^{b}	3.71 ± 0.58^{c}	1.90 ± 0.43^{ab}	8.30 ± 0.77^{c}	3.03 ± 0.20^{a}

*a,b,c,d… shows the statistical difference in the samples at the same column (n = 6)

Hydroxymethylfurfural (HMF)

Orange marmalade is generally obtained by adding orange pulp, pectin, acid, sugar, and other additives (colorant, *etc.*). Scientists stated that orange marmalade is an ideal substrate for browning due to its high sugar content and low water activity, and that undesirable compounds, such as HMF, can be formed with storage and high temperature (Sicari *et al.* 2017). For this reason, HMF is one of the compounds that are considered a significant criterion for the quality grading in jam and marmalade. Therefore, HMF analysis was carried out and the HMF values of the F1 and F2 samples containing sugar in their recipe were 9.32 and 8.47, respectively. Although no significant difference was observed between the HMF values of the F1 and F2 samples, their recipes were significantly different from the other recipes that did not contain sugar ($p \leq 0.05$). The HMF value of all sugar-free recipes ranged between 3.03 and 3.62, and the difference between them is not significant ($p > 0.05$). In a conducted study, it was stated that the HMF contents on the dry matter basis of all jam samples ranged from 0.79 to 37.2 mg/kg. The HMF contents of the commercial jam and the jam prepared with sweetener were 28.5 and 71.9 mg/kg solids, respectively. It has been reported that the HMF content of all samples except one sample was quite low during the jam tests when compared with the commercial samples, and the linear effect of the sugar on forming HMF was significant in the variance analysis results on the regression parameters of the model created with the obtained results ($p \leq 0.05$) (Benzer Gürel 2016). In another study, the HMF contents of biscuit samples prepared with different formulations were investigated by adding stevia at different ratios. When the results were studied, a significant decrease was observed in the HMF content of the biscuit samples, whose sucrose ratio was reduced by more than 30% and stevia was added instead (Garcia-Serna 2014).

Table 4 shows that the HMF content of the samples containing sugar in their recipe was high, this was because one of the most significant factors affecting the formation of HMF is the carbohydrate content. Moreover, physicochemical properties, such as pH, total acidity, temperature applications, water activity, long-term storage, use of metallic containers, and generally the use of monosaccharides, including glucose or fructose, are effective. Moreover, disaccharides and many polysaccharides serve as starting materials for the formation of HMF by hydrolyzing to simple sugars. HMF is formed under acidic conditions in the presence of amino groups. Therefore, honey, jam, cereal products, which are products containing simple sugars and acids, and products containing fruits and vegetables are more appropriate for the HMF formation (Jalili and Ansari 2015).

HMF is significant in terms of both giving information about the heat treatment conditions that foods are exposed to during the process and causing the formation of brown colored pigments by being polymerized. The HMF content of the product increases with negative changes in taste and color (Burdurlu and Karadeniz 2002). In the current study, it was observed that the HMF ratio of the samples with reb A addition was lower than those produced with sugar and turned up positive. In this case, healthier and more natural marmalades can be produced by using reb A instead of sugar. In addition, quality losses (flavor change, toxicity, *etc.*) resulting from HMF formation in traditional marmalades can be minimized.

The Sensory Evaluation

Panelists were presented with marmalades containing only reb A and those that did not contain reb A-sugar, and they were asked about the color difference with standard marmalade (darker, lighter, or no difference). All of the panelists stated that the marmalade samples containing only Reb A and those that did not contain reb A-sugar were lighter in color than the samples prepared with sugar ($p \leq 0.05$). They stated that they did not observe any difference in the color of marmalade containing only reb A and those that did not contain reb A-sugar ($p > 0.05$). In this case, the panelists stated that adding reb A was not significant in affecting the color. Moreover, the questions in Table 1 were asked to the panelists and the panelists were asked to make comparisons according to the fruit color. They stated that F1 and F2 marmalades had slight oxidation and discoloration, other marmalades had very slight color loss. In relation to this, in a study conducted on producing homemade low sugar apple marmalade by partially replacing sucrose with alternative sweeteners such as stevia and sucralose (25%, 50%) without using commercial pectin and chemical preservatives, it was determined that the addition of sweetener was effective on the appearance and color parameters. It was stated that the color scores of marmalades decreased with the decrease in total soluble solids concentration, formulation 5 (250 g sugar, 416 mg sucralose) and formulation 10 (300 g sugar, 500 mg sucralose) resulted in the lowest appearance and color score among all formulations by demonstrating a lighter color and less stable structure due to the lower amount of sucrose (Álvarez *et al.* 2006).

The panelists were asked how the consistency of the samples containing reb A was when compared with the conventional sample (darker, runny). All the panelists stated that all of the samples containing reb A were runnier ($p \leq 0.05$). They stated that the F2 sample was too thick, and they did not like its consistency ($p \leq 0.05$). Addition of 1% agar instead of 1% pectin in sugar marmalades led the product to be very solid. However, this adverse situation was not observed in the samples containing reb A and those that were sugar and sweetener free. The panelists stated the following consistencies of the marmalades: F1 as suitably gel-like, F2 distinctly dark, F3 distinctly runny, F4 very slightly runny, and F5, F6; slightly runny (Fig. 4). In another study, the production of energy-reduced jam was completed using reb A-sugar mixture. The researchers studied sensorially the appearance, color, odor, liquidity, taste and flavor and texture properties of the jam samples and evaluated them statistically. They stated that appearance, color, smell, taste, and flavor are not statistically significant on the sensory properties, but they are significant at a level of $p < 0.05$ on the fluidity properties (Yılmaz 2016). Moreover, they liked the consistency of the F6 sample more than the F5 sample among the samples containing reb A.

Panelists described the smell of marmalades as follows: F1 fruit-scented or odorless, F2 odorless, F3 fruit- scented, F4 fruit-scented or odorless, F5 very slight foreign odor, and F6 fruit-scented or odorless (Fig. 4). Agar agar is thought to suppress more fruit odor and reb A odor. They stated that they smelled a very slightly different odor in the F5 sample. Moreover, they stated that the fruit odor was more pronounced in marmalades that did not contain sugar, and the fruit odor was slightly reduced in the marmalades that contain agar.

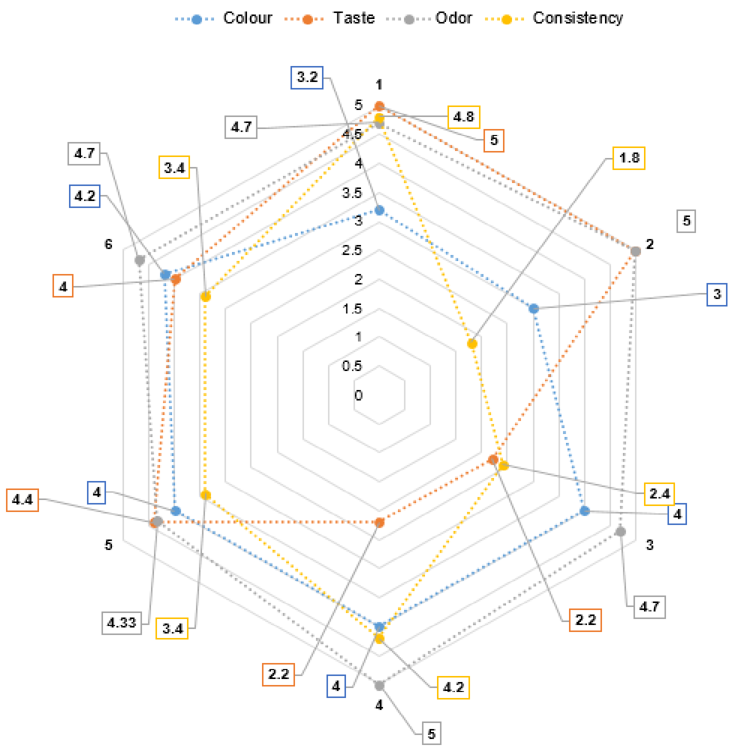

Fig. 4. Radar graph of sensory data of marmalades

Panelists described marmalades as: F1 and F2 fruity compatible pleasant, F3 and F4 sour and tasteless, and F5 and F6 a very mild foreign taste but compatible and pleasant (Fig. 4). Studies have demonstrated that steviol glycosides including stevioside and rebaudioside A exhibit bitter taste and licorice aroma properties (Muñoz-Labrador *et al.* 2020). Moreover, Fujimaru *et al.* (2012) also reported the bitterness and chemical sensation from reb A. Another study reported that consumers rated ice creams containing Reb A as more artificial and chemical (Muenprasitivej *et al.* 2022). They stated that the purer and more concentrated the rebaundiana A, the less bitter and sweeter it is (Ever Stevia 2022). It has been reported by Tao and Cho (2020) that Reb A bitterness persists even 1 minute after consumers taste it. Similarly, Yılmaz (2016) reported that a slightly bitter taste is dominant as a result of the sensory analysis of jam samples containing reb A. Panelists stated that this bitter taste is not so significant, but that it tastes pleasant.

CONCLUSIONS

1. The use of reb A as a sweetener in the production of low sugar marmalades shows potential in providing healthier alternatives for diabetic and obese individuals, as it was well-liked by consumers and resulted in lower levels of total sugar and HMF.
2. The addition of stabilizers such as pectin and agar agar had an impact on the physical properties of low-calorie marmalades, particularly their viscosity, and the type and ratio of stabilizers used resulted in differences in the consumers liking of the marmalades.

3. The absence of sugar in the production of low-calorie marmalades led to lower brix values and affected the brightness of the marmalades. The addition of reb A had a minimal effect on the color of the marmalades.
4. Further research can be conducted to optimize the use of reb A and explore different stabilizer combinations to improve the physical and sensory properties of the marmalades.

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