Turkish Journal of Agriculture - Food Science and Technology, 11(10): 1917-1925, 2023
DOI: https://doi.org/10.24925/turjaf.v11i10.1917-1925.6217

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

The Effect of Different Cooking Methods and Addition of Different Sweeteners on the Physicochemical and Antioxidant Properties of Aronia Marmalade

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ARTICLE INFO

Research Article

Received : 05-07-2023
Accepted : 13-10-2023

Keywords:
Aronia marmalade
Cooking method
Sweetener type
Antioxidant activity
Sensory evaluation

ABSTRACT

The present study examined the physicochemical properties, antioxidant activity (DPPH, ABTS, and FRAP), and sensory properties of aronia marmalades prepared with different cooking methods (CM) (boiled (B) and pressure-boiled (PB)) by adding sugar (S) and stevia prebiotic fiber sweetener (SP). Ash, reducing sugar, sucrose, viscosity, L*, a*, b*, C*, and H° values, and total sugar content of aronia pulp and marmalades differed significantly by cooking method and sweetener type (ST). Hydroxymethylfurfural could not be detected in aronia pulp and marmalades. Concerning CM, TPC (total phenolic content) and TMA (total monomeric anthocyanin) values were found to be significantly higher in PB cooking than in the B cooking method. On the other hand, TFC (total flavanoid content) was statistically higher in boiled marmalades. According to CM, the DPPH antioxidant activity of marmalades was significantly higher in B marmalades. The TPC, TMA, TFC, and antioxidant properties of marmalades differed sigficantly by ST. The TPC of marmalades prepared with SP addition was higher than that of S-added marmalades and control. According to ST, whereas the antioxidant activities (DPPH, ABTS, and FRAP) of S and SP-added marmalades were lower compared to the control, the antioxidant activities determined by DPPH and ABTS among S and SP-added marmalades were higher in SP-added marmalades. The panelists gave the highest scores to BSC (boiled S-added marmalade). Considering the overall acceptance scores, the second highest score was given to BST (boiled SP-added marmalade). In other words, in terms of sensory evaluation, boiled marmalades received higher overall acceptance scores, while PBST (PB SP-added marmalade) received the lowest scores. According to these results, astringency components decrease with cooking in an open vessel. Furthermore, it can be said that sugar masks this astringent taste.

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Introduction

Aronia berry, which belongs to the Maloideae subfamily of the *Rosaceae* family and is also known as chokeberry, is widely cultivated worldwide, particularly in Europe and North America (Kokotkiewicz et al., 2010; Huang et al., 2022). Two species of the *Aronia* genus can be distinguished: *Aronia melanocarpa (Michx.) Elliot*, known as black chokeberry, and *Aronia arbutifolia* (L.) Pers. (red chokeberry). The third type is a hybrid of the two mentioned species, *Aronia prunifolia*, purple chokeberry (Jurendić and Ščetar, 2021). Native Americans traditionally used the berries of *A. melanocarpa* to cure the common cold. Nowadays, it is mainly used as an ornamental plant and in the production of fruit juice, jam, liqueurs, and wine (Kokotkiewicz et al., 2010). Aronia (*Aronia melanocarpa*) berries are typically deep purple and have high anthocyanin contents and health-promoting properties such as antioxidant, anti-inflammatory, and antibacterial activity (Jang and Koh, 2023). The polyphenol profile of aronia berries is well-structured, with cyanidin glycosides (cyanidin-3-*O*-galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, and cyanidin-3-*O*-xyloside) as the main anthocyanins. The anthocyanin content of chokeberry (737 mg/100 g FW) is considerably higher compared to other anthocyanin-rich berries (such as blueberry, strawberry, and raspberry) (Gao et al., 2022). It contains highly polymerized B-type proanthocyanidins, chlorogenic acids, and quercetin glycosides. Aronia berries contain high amounts of anthocyanin and proanthocyanidin polyphenols that may help reduce the risk of cardiovascular, and gastrointestinal diseases,

diabetes, and cancer. However, aronia berries have an astringent and bitter taste. Hence, their appeal to consumers is primarily due to their bioactive compounds and health benefits. (King et al., 2021; Jurendić and Ščetar, 2021).

Fruits and vegetables are perishable, and their fresh consumption is seasonal. Therefore, they need to be processed with different preservation techniques, such as jam and marmalade making. Accordingly, processing aronia berries into marmalademay be a good alternative to meet these need, apart from their fresh consumption. In the Turkish Food Codex Communiqué on Jam, Jelly, Marmalade and Sweetened Chestnut Puree, "traditional marmalade" is defined as "a mixture of fruit pulp, puree, fruit juice, and aqueous extracts or edible parts of plants, such as roots, leaves, and flowers brought to a spreadable consistency by adding sugars and water when necessary" (Anonymous, 2006). Cooking in an open vessel or under vacuum can be applied to make products such as jam and marmalade spreadable. It is reported that since a lower temperature is applied in vacuum cooking than in open cooking, color and aroma properties will improve, and nutrient loss will decrease (Özdemir et al., 1998). Moreover,due to increased rates of diabetes and obesity, today's consumers gravitate to foods prepared from raw materials that are low in calories, appealing to the palate, mostly grown naturally, and have health benefits.

There is no study in the literature on marmalade production with aronia berries using different sweeteners and different cooking methods. Hence, it was aimed to determine changes in various physicochemical and antioxidant properties of aronia berries during their processing into marmalade using different sweeteners and different cooking methods. Furthermore, finding a practical way to reduce the astringencyof products produced with aronia berries is among the most important points to open the aronia industry to the market. Therefore,panelist evaluations, aimed to determine the impacts of different sweeteners on the astringent taste and overall acceptance during the processing of aronia into marmalade to reduce astringency.

Materials and Methods

Material

The aronia marmalades used in the study were produced with fresh aronia (*Aronia melanocarpa*) berries grown in Kırklareli, Vize, Türkiye. Crystal white granulated sugar (S) and stevia prebiotic fiber sweetener (SP) (Fibrelle, Türkiye) used in marmalade production were purchased from a local market in Erzurum.

Marmalade Production

Aronia marmalade formulation was determined by preliminary trials. During marmalade preparation, 400 ml of water was added to 400 g of aronia berries, whose stems were separated and removed, and they were crushed homogeneously for 5 minutes with a laboratory-type grinder (Waring HGB2WTS3, USA). Then sweeteners (sucrose (S) and stevia prebiotic fiber sweetener (SP)) were added separately at the rate of 40%, and marmalade was cooked under vacuum with an evaporator (Heidolph Laborota 4000 Efficient, Germany), as described by Korus et al. (2015). In the study, aronia pulps prepared without adding sweeteners were accepted as a control. It was prepared by first mixing 2.35 g pectin with water, and its mixing was ensured by adding it toward the end of cooking. In both cooking methods (boiled (B) and pressure-boiled (PB)), cooking was stopped when the water-soluble dry matter content of marmalades measured with a refractometer (Abbe Zeiss, Germany) was 55-60% and the pH measured with a pH meter (Ohaus, starter 3100, USA), as specified by Kaya et al. (2016), was between 2.8 and 3.5. Before the end of heat treatment, 10 g of citric acid was added and boiled for another 1-2 minutes. The hot marmalade was placed in glass jars with sealed twist-off lids as, then turned upside down for a while and left overnight. Afterward, the jars were brought to a straight position and stored at room temperature and in a dark place until the analysis.

Marmalades were coded as follows: BSC: Boiled sucrose-added marmalade, PBSC: pressure-boiled sucrose-added marmalade, BST: Boiled stevia prebiotic fiber-added marmalade, PBST: Pressure-boiled stevia prebiotic fiber-added marmalade.

Determination of Dry Matter

3-5 g samples taken from the homogenized aronia marmalade were kept in an oven at 100-105 °C until they reached a constant weight. After the samples were cooled in a desiccator, they were weighed. The total dry matter value was determined as a percentage using the values obtained (Cemeroğlu, 2013).

Ash Determination

After the moisture in the homogenized marmalade samples (3.0±0.1 g) was evaporated in the oven, a few drops of ethyl alcohol were dripped onto the samples, and they were burned in the muffle furnace (Karl Kolb M011, Gerhardt, Germany) at 550±25 °C until white ash was formed. After the crucibles were cooled in the desiccator as a result of combustion, the remaining ash was weighed, and its amount was determined in g/100 g (Cemeroğlu, 2013).

Determination of Total Sugar, Reducing Sugar, and Sucrose

The Lane-Eynon volumetric method was employed to determine sucrose, reducing sugar, and total sugar contents of the marmalade samples. The samples were analyzed before and after inversion. For the inversion procedure, 5 ml of 37% HCl was added to 50 ml of clear filtrate taken into a 250 ml volumetric flask, the temperature was brought to 67 °C in a water bath, and the samples were kept for 5 minutes. Afterward, phenolphthalein was added to the cooled flask and titrated with 4 N NaOH, and titration was terminated when the solution color became light pink. The titrated solution was completed to 250 ml with distilled water, the analysis was continued, and the amounts spent in the end were recorded. Calculations were performed with the recorded pre- and post-inversion amounts, and the total sugar, reducing sugar, and sucrose contents were determined (Cemeroğlu, 2013).

Hydroxymethyl Furfural Analysis

When determining hydroxymethyl furfural (HMF) content, first, 5 g of the sample was weighed and dissolved

in 10 ml of distilled water. Two ml of the prepared solution was taken, and 5 ml of p-toluidine solution was added to it. One ml of barbituric acid solution was added to one of the two test tubes prepared in this way, and 1 ml of distilled water was added to the other, and the contents were mixed by vortexing. The absorbance value of the solution was determined by reading in the spectrophotometer at a 550 nm wavelength. The HMF content was calculated using the formula below (Cemeroğlu 2013):

HMF (mg/kg) = A x 162

A: Absorbance value

Viscosity determination

The viscosity values of marmalade samples were measured in the range of 0.3-10,000 mPa.s at 20 °C using an SV-10 Viscometer (A&D Company, Japan). The results were determined in mPa.s (Wang et al., 2016).

Color determination

The color values of marmalade samples were measured with a colorimeter (Konica Minolta CR-400, Korea). The samples' color intensities were determined with a colorimeter making three-dimensional measurements in the CIE (L*, a*, b*, C*, H°) system. H° (Hue angle), L* (bright: 100, dark: 0), C* (Chroma, color saturation), a* (red: +60, green: -60), and b* (yellow: + 60, blue: -60) values were determined as a result of the readings, and all readings were performed on a white background at 20±2°C (Zor and Şengül, 2022).

Total Phenolic Content (TPC) Analysis

While preparing the aronia marmalade extracts to be used throughout the study, 3 grams of each of the marmalade samples were weighed for extraction, and 30 ml of methanol was added to them. They were kept in an ultrasonic water bath for 45 minutes, and this procedure was repeated 3 times at 15-minute intervals. Afterward, the extracts taken into the tubes were centrifuged at 6000 rpm at 4 °C for 15 minutes and filtered through Whatman No 2 filter paper. The acquired filtrates were used for total phenolic content (TPC), total monomeric anthocyanin (TMA) content, total flavonoid content (TFC), and antioxidant activity analyzes (Zor et al., 2022).

To the total phenolic content in the marmalade samples, 100 µl of the extracts were taken, 0.2 N 2.5 ml FCR (Folin-Ciocalteau reagent) was added to them, and they were left for 3 minutes. After the waiting period, 2 ml of 7.5% sodium carbonate (Na_2CO_3) solution was added to the mixture and incubated for 2 hours. At the end of the period, the samples' absorbance values were read in a spectrophotometer (PG Instruments T60V, UK) at a 760 nm wavelength. To determine the samples' phenolic content, the equation acquired from the graph prepared using the gallic acid standard was used, and the total phenolic content was calculated as gallic acid equivalent (mg GAE/g) (Meda et al., 2005).

Determination of Total Monomeric Anthocyanin (TMA) Content

The monomeric anthocyanin (TMA) content of marmalade was determined according to the pH differential method. The anthocyanin concentration was calculated with the difference of the measured absorbance values when the ambient pH was 1.0 and 4.5. The principle of this method is based on the fact that samples are in the form of colored oxonium when the ambient pH is 1.0 and samples are in the form of colorless carbinol pseudobase when the ambient pH is 4.5 (Cemeroğlu, 2013). The sample extracts were diluted with potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5), and absorbance was measured with a spectrophotometer (PG Instruments T60V, UK) at 515 and 700 nm wavelengths after 30 minutes. The equation below was used when calculating the total monomeric anthocyanin content (Sun et al., 2009).

A=(A510-A700) pH 1.0-(A510-A700) pH 4.5

A: Absorbance difference

A515: absorbance value at 515 nm

A700: absorbance value at 700 nm

The formula used to calculate the monomeric anthocyanin content as cyanidin-3-glucoside equivalents is as follows:

TMA (mg/l)=A(MA)(Sf)1000/(ε)ʃ

MA=449.2 (molecular weight of cyanidin 3-glucoside)

Sf=Dilution factor,

ε=Molar absorptivity

ʃ=Layer thickness of the cuvette used in the spectrophotometer (ʃ=1 cm)

***Total Flavonoid Content* (TFC)**

Total flavonoid content was determined spectrophotometrically according to the method suggested by Koçak et al. (2018). First, 0.25 ml of the sample extracts were taken and 1.25 ml of distilled water was added to it. Then, 0.075 mL of 0.05g/mL $NaNO_2$ was added and incubated for 6 minutes after vortexing. At the end of the period, 0.15 mL of 0.1 g/mL $AlCl_3 \cdot 6H_2O$ was added and vortexed, then left for 5 minutes. Finally, 0.5 mL of 1 mol/L NaOH was added, mixed and then incubated for 15 minutes. At the end of the period, the samples' absorbance values were read in the UV-visible spectrophotometer (PG Instruments T60V) at a wavelength of 510 nm. In the calculations, the equation obtained from the calibration curve drawn as a result of the measurements made by preparing 10-250 mg/L quercetin was used. The total flavonoid content is given as quercetin equivalent (QE)/g.

Determination of Antioxidant Activity

Determination of Antioxidant Activity with the DPPH Method

To analyze DPPH radical scavenging activity in marmalade samples, 10, 20, and 30 µg/ml were taken from the sample extracts and then made up to 2 ml with ethanol. 500 µl of DPPH solution was added to the samples, completed to 2 ml with ethanol, mixed homogeneously by vortexing (Heidolph Reax Top, D-91126 Schwabach, Germany) and incubated for 30 minutes in a dark environment at room temperature. At the end of the incubation period, the samples' absorbance was read in the spectrophotometer at a 517 nm wavelength (Popović et al., 2012). The %inhibition (%I) of the extracts was calculated according to the formula using the absorbance values:

$\%I = ((A_c - A) \times 100) / A_c$

(A_c: Absorbance of the control, A: Absorbance of the extract)

IC_{50} values were calculated from the equation acquired using percentage inhibition values versus different concentrations of the samples.

Determination of Antioxidant Activity with the ABTS Method

ABTS (2.2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) solution to be used for $ABTS^{\bullet+}$ radical scavenging activity analysis in marmalade samples was prepared by adding potassium persulfate solution and mixing it for 16 hours in the dark environment. The absorbance value of the prepared solution was measured at a wavelength of 734 nm in the spectrophotometer and diluted to 0.700±0.025. 10 µl of the sample extracts were taken and 990 µl of ABTS radical was added to them. Then they were mixed homogeneously by vortexing and incubated for 6 minutes in a dark environment. The samples' absorbance values were read at a wavelength of 734 nm at the end of the incubation period (Özkan et al., 2007). The %inhibition (%I) of the extracts was calculated according to the following formula using the absorbance values:

$\%I = (A_c - A) \times 100/A_c$

(A_c: Absorbance of the control, A: Absorbance of the extract)

IC_{50} values were calculated from the equation acquired using percentage inhibition values versus different concentrations of the samples.

Determination of Antioxidant Activity with the FRAP Method

It is based on reducing Fe^{3+} ions in the Fe $(TPTZ)^{3+}$ mixture present in the radical to be used in the determination of antioxidant activity in tea samples with the FRAP method to the blue-colored Fe $(TPTZ)^{2+}$ complex in the acidic medium (Koçak et al., 2018). Antioxidant activity was determined withthe FRAP method by making some changes to the method reported by Koçak et al. (2018). The solvents used in the study were prepared as follows;

1-Three solutions were prepared daily as acetate buffer (pH 3.6) by dissolving 3.1 g sodium acetate+16 mL acetic acid in 1 L solution,

2-By dissolving 0.156 g of TPTZ (2,4,6-tripyridyl-s-triazine) in 50 mL of ethanol, and

3-As 0.5404 g $FeCl_3 \cdot 6H_2O$+2 mL HCl (37% m/m) in 100 mL solution.

After the solutions were prepared, 80 mL of the 1st solution, 8 mL of the 2nd solution, and 8 mL of the 3rd solution were taken and mixed. In this way, the FRAP reagent was prepared . 0.9 mL of FRAP reagent was added to 0.1 mL of the sample extract, and the mixture was vortexed. After 4 minutes, the absorbance was measured at 593 nm. A calibration curve was acquired with 5-25 micromole solutions of Trolox prepared with methanol. The results from the calibration curve were calculated as µM Trolox equivalent (TE)/g.

Sensory Analysis

The panelist group, consisting of students and faculty members at the Department of Food Engineering of Atatürk University (Erzurum, Turkey), carried out sensory tests at the Department of Food Engineering at Atatürk University. The panelists evaluated various parameters of aronia marmalade samples (appearance, color, odor, texture, taste, fluidity, and overall acceptance). Marmalades, randomly named with three digit numbers, were presented to each panelist at room temperature in glass plates, allowing them to determine the product features clearly. Semi-expert graduate students and academicians at Atatürk University, Department of Food Engineering, conducted sensory analysis.

Statistical Analysis

The data obtained in triplicate were analyzed with the SPSS 20.0 program. The results were expressed as mean values with standard deviation (±SD). One-way analysis of variance (ANOVA) was carried out to determine the significant group differences between the means ($p \leq 0.05$, $p \leq 0.01$). Duncan's multiple range test was used to compare mean values. Moreover, principal component analysis (PCA) was applied to some data to facilitate the identification of similarities and differences between the samples (SIMCA-P + 14.1, UMETRICS).

Results and Discussion

Table 1 contains some physical and chemical properties of aronia marmalades produced withdifferent sweeteners (S, and SP) and different cooking methods (boiled, and pressure-boiled). Considering the cooking method, dry matter content differed insignificantly ($p>0.05$), while the ash content of pressure-boiled marmalade was significantly higher ($p<0.05$). As seen in Table 1, the dry matter content of S-added marmalades was significantly ($p<0.05$) higher than that of SP-added marmalades and the control, whereas ash content was statistically lower in sweetener-added marmalades. This situation is thought to have been caused by the fact that the fruit ratio in the control and the fruit ratio in marmalades were not equal according to the formulation. Kaya et al. (2019) reported that the dry matter content of the marmalade in which a mixture of sugar and stevia Reb D was used among hawthorn marmalades produced by adding different sweeteners was higher than marmalades prepared by adding sugar alone. The same study stated that ash content was the lowest in hawthorn marmalades with the addition of commercial stevia whereasthe highest ash content was in hawthorn marmalades prepared with stevia Reb D.

It is known that some amount of sucrose in jam and marmalade production undergoes inversion depending on cooking temperature and time (Özdemir et al., 1998). In the study, the reducing sugar content of pulp and marmalades cooked by the pressure-boiled method was higher compared to boiled marmalades. It can be said that less Maillard reaction occurs in pressure-boiled marmalades due to low temperature, and therefore, thereducing sugar content is higher.

The reducing sugar, sucrose, and total sugar contents of marmalades differed significantly by sweetener type ($p<0.05$). The reducing sugar and total sugar contents of SP-added marmalades were lower compared tothe control and S-added marmalades. The lowest sucrose content was found in the control. It is thought that the total sugar detected in SP-added marmalades is the sugar passing from fruit to marmalade.

Table 1. Some physicochemical properties of marmalades according to cooking method and sweetener type

	DM	A	RS	S	TS	HMF
Cooking method (CM)						
Boiled	55.85±18.15	0.78±0.20^{b}	12.41±3.32^{b}	11.53±16.16^{a}	23.94±17.96^{b}	ND
Pressure-boiled	56.61±17.87	0.82±0.19^{a}	28.22±21.85^{a}	1.12±0.94^{b}	29.34±22.77^{a}	ND
Significance	ns	**	**	**	**	-
Sweetener type (ST)						
Control	32.54±0.70^{c}	1.06±0.02^{a}	14.95±0.35^{b}	0.32±0.08^{c}	15.26±0.41^{b}	ND
Sucrose	71.36±1.41^{a}	0.65±0.02^{c}	35.95±23.39^{a}	17.70±16.83^{a}	53.65±6.64^{a}	ND
Stevia prebiotic fiber sweetener	64.79±0.62^{b}	0.69±0.06^{b}	10.06±2.26^{c}	0.95±0.36^{b}	11.01±1.93^{c}	ND
Significance	**	**	**	**	**	-
CMXST	ns	**	**	**	**	-

	V	L*	a*	b*	C*	H°
Cooking method (CM)						
Boiled	7.72±1.57^{a}	26.61±0.70^{a}	2.17±1.35^{a}	2.30±0.23^{a}	3.25±1.13^{a}	50.70±13.14^{b}
Pressure-boiled	5.50±2.11^{b}	26.46±0.43^{b}	1.78±1.12^{b}	2.28±0.21^{b}	2.97±0.88^{b}	55.39±13.11^{a}
Significance	**	*	**	*	**	**
Sweetener type (ST)						
Control	5.93±2.85^{c}	27.25±0.35^{a}	3.62±0.40^{a}	2.58±0.04^{a}	4.45±0.34^{a}	35.59±2.67^{b}
Sucrose	7.01±2.16^{a}	26.06±0.08^{c}	1.14±0.09^{b}	2.16±0.04^{b}	2.45±0.02^{b}	62.17±2.36^{a}
Stevia prebiotic fiber sweetener	6.89±1.36^{b}	26.29±0.07^{b}	1.17±0.17^{b}	2.13±0.05^{c}	2.43±0.12^{b}	61.38±3.09^{a}
Significance	**	**	**	**	**	**
CMXST	**	**	**	**	**	**

DM: Dry Matter (g/100 g); A: Ash (g/100 g); RS: Reducing Sugar (g/100g); S: Sucrose (g/100g); TS: Total Sugar (g/100g); HMF: HMF (mg/kg); V: Viscosity (mPa.s); Note: $^{a-c}$Means with different letters in the same column are significantly different (*p < 0.05. **p < 0.01); Sign: Significance; ns: not significant (p > 0.05), ND: Not detected

Table 2. TPC, TMA, TFC and antioxidant properties of marmalades

	TPC	TMA	TFC	DPPH	ABTS	FRAP
Cooking method (CM)						
Boiled	10.43±1.79^{b}	2217.52±941.74^{b}	31.71±6.93^{a}	364.51±61.90^{b}	192.43±32.00	51.35±13.90
Pressure-boiled	11.93±2.51^{a}	4581.17±1267.34^{a}	29.00±6.37^{b}	431.08±90.95^{a}	192.70±37.41	51.48±12.93
Significance	**	**	**	**	ns	ns
Sweetener type (ST)						
Control	11.35±1.60^{b}	4792.72±1456.78^{a}	37.39±4.10^{a}	324.96±40.49^{c}	157.81±7.14^{c}	68.46±2.03^{a}
Sucrose	9.84±1.03^{c}	2424.98±872.60^{c}	24.08±3.78^{c}	487.93±71.13^{a}	222.78±21.61^{a}	43.75±4.67^{b}
Stevia prebiotic fiber sweetener	12.34±3.13^{a}	2980.33±1566.41^{b}	29.58±3.14^{b}	380.50±13.49^{b}	197.11±28.11^{b}	42.03±5.07^{b}
Significance	**	**	**	**	**	**
CMXST	**	**	**	ns	**	**

TPC: TPC (mg GAE/g); TMA: TMA (mg Cy-3-Gl/kg); TFC: TFC (mg QE/g); DPPH: DPPH (IC_{50} µg/ml); ABTS: $ABTS^{\cdot+}$ (IC_{50} µg/ml); FRAP: FRAP (mM TE/100 g); Note: $^{a-c}$Means with different letters in the same column are significantly different (*p < 0.05. **p < 0.01); Sign: Significance; ns: not significant (p > 0.05

The formation of HMF, which is not present naturally in fruits but is an intermediate product of the Maillard reaction, varies depending on the temperature and duration of heat treatment applied during production and storage and various factors such as pH, dry matter, type and concentration of the reacting compounds (Şengül et al., 2018). HMF could not be detected in aronia pulp and marmalades (Table 1), showing that an appropriate procedure was followed in marmalade production. It may also thought that it may have originated from the absence of amino acids in the compound, required in the HMF formation process.

Concerning the viscosity values according to the cooking method, it was found that boiled marmalades had higher viscosity. The use of SP in marmalades significantly increased viscosity compared to the control group (p<0.05). In the study by Öztürk (2023), the panelists evaluating the sensory properties of low-sugar orange marmalade using Reb A stated that the consistency of the samples containing only Reb A was thin in comparison with the standard sample. Therefore, it is thought that the use of stevia prebiotic fiber sweetener in our study increased in viscosity due to the higher water-holding capacity because of the fibrous components in the formulation of SP.

L*, a*, b*, and C* values were statistically significantly higher in boiled marmalades in terms of the cooking method and in the control in terms of the sweetener type than in other marmalades. Since the L* value represents the lightness or darkness of the product color, it is important to determine the quality characteristics. Upon examiningsweetener-added marmalades, it was seen that SP-added marmalades had higher L* value than S-added marmalades. Likewise, Suna et al. (2023) reported that sweetener-added persimmon marmalades had higher L* values than sugar-added marmalades. The a*, b*, and C* values of boiled pulp and marmalades were higher than the values of pressure-boiled samples. Which may be explained by the possible Maillard and non-enzymatic browning reactions during heat

treatment. Accordingly, red pigments may be reduced due to the degradation of the compounds causing the color. The addition of S and SP significantly ($p<0.05$) reduced a*, b*, and C* values (Table 1). The C* value shows the color tone of products, and its values are low in pale colors and high in vivid colors (Öztürk, 2023). It is thought that this decrease in the color values of S and SP-added marmalades compared to the control originates from formulation and heat treatment.

Contrary decreased C* values, H° values, which are important in defining the color of marmalades, were statistically higher in pressure-boiled marmalades. Furthermore, according to the sweetener type, the H° value was similar in sugar and stevia prebiotic fiber sweetener-added marmalades and significantly higher than the control ($p<0.05$). Likewise, Suna et al. (2023) reported that the addition of sweetener in persimmon marmaladesincreased hue values.

Table 2 shows changes in the TPC, TMA, TFC, and antioxidant properties of aronia marmalades by the cooking method and sweetener type. The TPC and TMA content of pressure-boiled samples were significantly higher than those of boiled ones ($p<0.05$). On the other hand, TFC was statistically higher in boiled marmalades. Scibisz and Mitek (2009) reported that the thermal degradation of anthocyanins was a first-order reaction and high temperature increased the adverse effects on anthocyanins in the presence of oxygen and fructose. Moreover, it is reported that nutrient loss can be reduced by cooking products, such as jam and marmalade, under vacuum (Özdemir et al., 1998).

The DPPH antioxidant activity of boiled marmalades was significantly higher ($p<0.05$). However, it was concluded that the effect of the cooking method on the antioxidant activities determined by ABTS and FRAP was insignificant ($p>0.05$).

The TPC, TMA, TFC and antioxidant properties of marmalades differed significantly by sweetener type ($p<0.05$) (Table 2). The TPC of SP-added marmalades washigher than S-added marmalades and control (Figure 1). Similar to our study, studies have reported that the addition of stevia to various marmalades increases TPC (Kaya et al., 2019; Suna et al., 2023). Contrary to our results, Kamiloglu et al. (2015) stated that the use of sweeteners instead of sugar in jams and marmalades, in general, did not cause a significant difference in total phenolic content. Scibisz and Mitek (2009) indicated that blueberry jam with high sugar content had higher TPC compared to jams with low sugar and sweetener (Aspartame and Acesulfame-K) addition.

The TMA and TFC values were higher in the control sample. Additionally, the TMA and TFC of SP-added marmalades were higher in comparison with S-added marmalades (Figure 1). It is thought that one of the reasons for the higher TMA and TFC in the control group is that the added sweeteners reduce the amount of fruits relatively; thus, the TMA and TFC from the fruit decrease. Contrary to the results obtained from our study, Scibisz and Mitek (2009) found that TMA content was higher in sugar-sweetened jams than in sweetener-added jams (Aspartame and Acesulfame-K).

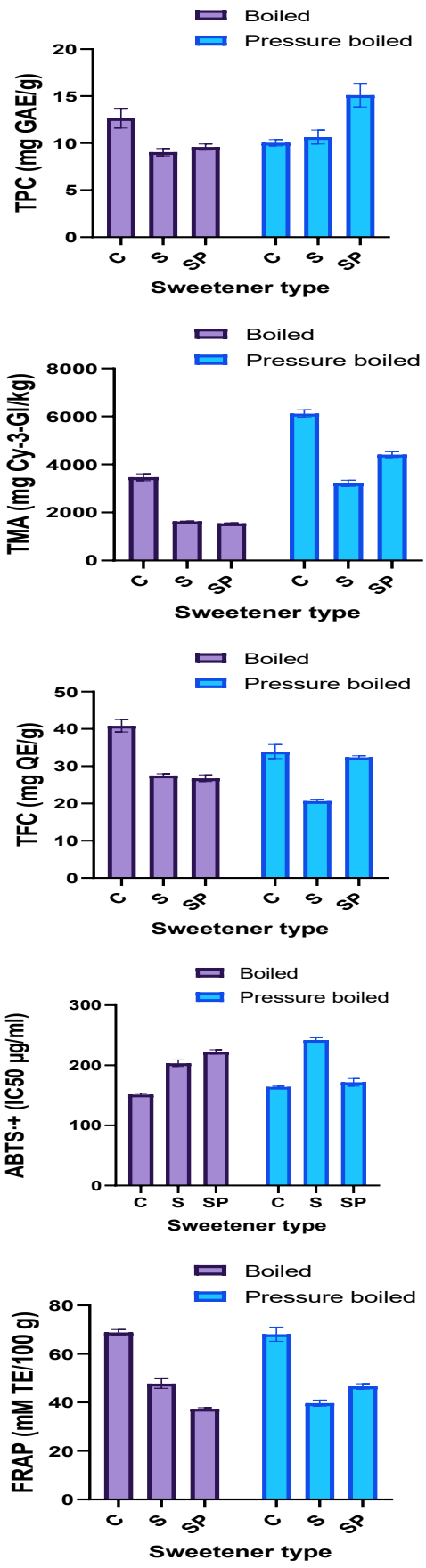


Figure 1. The effect of cooking method x sweetener type interaction on the TPC, TMA, TFC and antioxidant properties of aronia pulp and marmalades

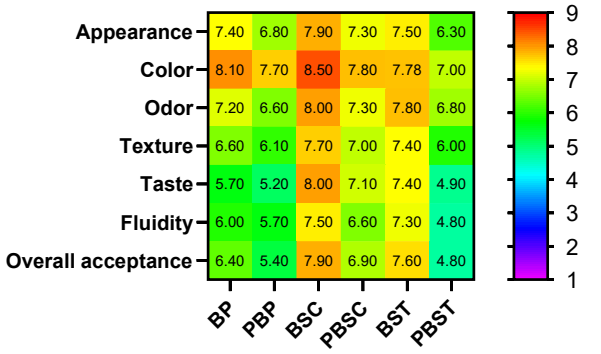
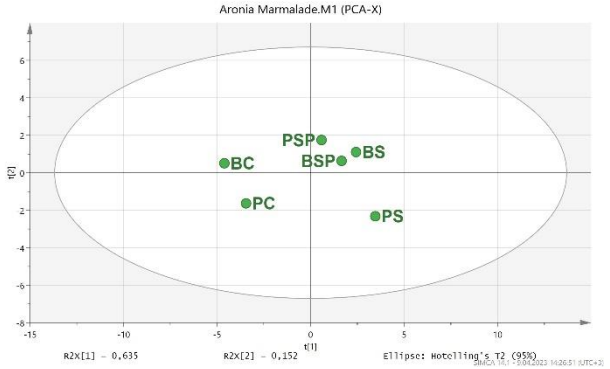
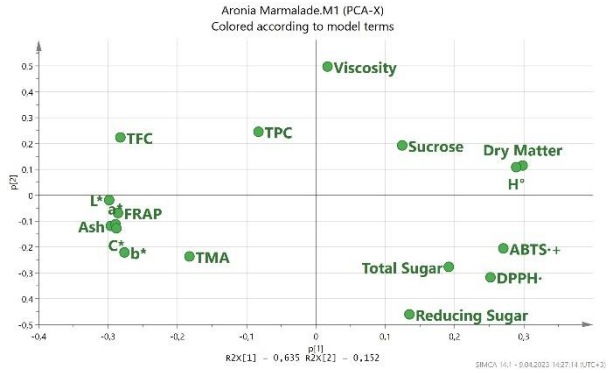


Figure 2. Sensory properties of aronia pulp and marmalades.

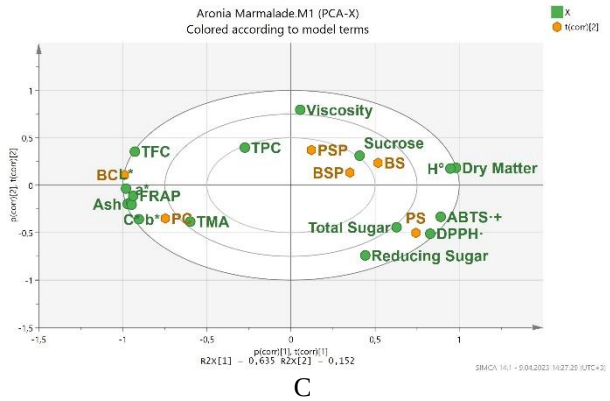
In the figure; BP: Boiled aronia pulp, PBP: Pressure-boiled aronia pulp, BSC: Boiled sucrose-added marmalade, PBSC: pressure-boiled sucrose-added marmalade, BST: Boiled stevia prebiotic fiber-added marmalade, PBST: Pressure-boiled stevia prebiotic fiber-added marmalade.



A



B



C

Figure 3. Score scatter plot (A), loading scatter plot (B), and biplot (C) of the principal component analysis (PCA) (PC1 vs. PC2) for the attributes in marmalade

Whereas the antioxidant activities (DPPH, ABTS, and FRAP) of S and SP-added marmalades were be lower than those of the control, DPPH and ABTS antioxidant activities among S and SP-added marmalades were higher in SP-added marmalades (Table 2, Figure 1).

At this point, the knowledge that carotenoids and phenolics with antioxidant and antimicrobial properties in stevia act as natural substances supporting the antioxidant capacity of the food to which it is added supports our results (Shivanna et al., 2013; Suna et al., 2023). Similar to our results, Kaya et al. (2019) reported a decrease in the antioxidant capacity of hawthorn marmalades determined by ABTS compared to pulp. Cıngoz and Demırdoven (2022) also stated that the addition of stevia and spices increased the total phenolic content and antioxidant capacity values of marmalades. In our study, the FRAP antioxidant activity analysis determined that the antioxidant activity of the control was the highest and the antioxidant activities of S and SP-added marmalades were statistically similar. Kamiloglu et al. (2015) reported that the addition of sugar or sweetener (containing sorbitol and saccharin) to black carrot marmalades caused similar antioxidant activities determined by ABTS and CUPRAC.

In the sensory evaluation of aronia pulp and marmalades, the panelists gave the highest scores to BSC marmalade in terms of appearance, color, odor, texture, taste, fluidity, and overall acceptance. Considering the overall acceptance scores, the second highest score was given to BST marmalade (Figure 2). In other words, in terms of sensory evaluation, boiled marmalades received higher overall acceptance scores, while PBST received the lowest scores. According to these results, astringency components decreased with cooking in an open vessel. Furthermore, it can be said that sugar masks this astringent taste.

Cıngoz and Demırdoven (2022) reported that, according to the sensory evaluation results of pumpkin marmalades prepared with four different recipes by adding spices, stevia, and granulated sugar in different proportions, granulated sugar-added pumpkin marmalades without spices received the highest scores, followed by stevia-added samples. In their study on the effect of usingcarob flour, stevia and cinnamon in the production of diabetic strawberry jam on the product's sensory properties, Mutlu et al. (2021) reported that the control group (industrially produced traditional strawberry jam) had the highest average values according to the general acceptability scores. They also reported that the traditional strawberry jam produced with stevia + cinnamon had the closest mean values to the control group in terms of all parameters. Öztürk (2023) indicated that for low-sugar orange marmalade using Rebaudioside A, panelists stated that they could purchase marmalades produced with Reb-A when on a diet, but they liked the recipes produced with Reb A-sugar mixture more.

Principal component analysis (PCA)

Principal component analysis (PCA) was performed to determine differences between samples by evaluating some physicochemical properties and antioxidant activities, total phenolic, total flavonoid, and total monomeric anthocyanin contents of aronia marmalades prepared withdifferent sweeteners and cooking methods.

Figures 3A-C show the score scatter plot of the marmalade samples, the loading scatter plot, and the two plots of the principal component analysis. The first two principal components (PC1=63.50% and PC2=15.20%) explained 78.70% of the variance.

As a result of the analysis, marmalade samples could be divided into two main groups (Figure 3A). While samples in the control group (BC, PC) were located on the right side of PC1, sweetener-added samples cooked in an open vessel and under pressure were located on the left side of PC1 (Figure 3A). TFC FRAP, TMA, L*, a*, b*, and C* values were located closely to the control samples (Figure 3C), showing that these characteristics of the control samples were higher. Furthermore, the antioxidant activity determined with the DPPH and ABTS methods in marmalade (PS) cooked under vacuum and added with sucrose was in a close position with the IC_{50} values. Since it is known that the IC_{50} value and antioxidant activity are inversely correlated, we can say that the lowest antioxidant activity was in this sample (PS) (Figure 3C). The said results showed that the total ash content, antioxidant activity, TPC, TFC, and TMA contents were the highest in the controlsamples, followed by stevia-added samples cooked under vacuum.

Conclusion

In line with all these results, it was seen thatmarmalade productionunder vacuum with stevia prebiotic fiber sweetener was suitable in terms of preserving the product's nutritional components. The TPC and TMA content of the samples cooked under vacuum were higher than the samples cooked in an open vessel, and the antioxidant activity determined with the ABTS and FRAP methods did not differ statistically significantly. When evaluated in terms of sweetener addition, it was determined that the TPC, TMA, TFC and antioxidant activity determined with the DPPH, ABTS, and FRAP methods of stevia prebiotic fiber sweetener-added marmalades were higher than those of sucrose-added samples. When the cooking method and sweetener parameters were evaluated together, it was seen that cooking under vacuum and the addition of stevia prebiotic fiber sweetener preserved the product's properties better. According to the study results, the use of stevia prebiotic fiber sweetener can be recommended in products sweetened with sugar, such as marmalade.

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