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Some Characteristics of Breakfast Spreadable Cornelian Cherry (*Cornus mas* L.) Nectar Produced Using Potentially Probiotic Starter Culture

Duygu Alp[1,a,*], Samet Mısır[1,b]

[1]*Department of Gastronomy and Culinary Arts, School of Tourism and Hotel Management, Ardahan University, Ardahan, Türkiye*
[*]*Corresponding author*

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ABSTRACT

In this study, it was aimed to make a breakfast spreadable Cornelian cherry nectar similar to marmalade. *Lactiplantibacillus plantarum* DA100 strain, which has potential probiotic properties, was added to this nectar. It was investigated whether this strain has an effect on various sensory properties of nectar. In the study, sensory and various chemical properties of nectar produced without probiotic microorganism were determined. Since the breakfast spreadable Cornelian cherry nectar produced in our study was not exposed to high temperatures for a long time, a very low HMF of 4.290 mg/kg was determined. Vitamin C was determined as 716.800 mg/L and total phenolic content was determined as 3156.64 mg GAE/100g. In the probiotic group, the microorganism viability, which was 1.5×10^{10} Log CFU/mL initially, was determined below approximately 1.5×10^{6} Log CFU/mL at the end of 5 days. In addition, coliform bacteria and *E. coli* were not detected in any group during this period. Contrary to the texture score, the difference between the sensory evaluation results of the probiotic-added and the control group of breakfast spreadable Cornelian cherry nectar was significant in taste, bitterness, odor and sourness. An alternative to jams and marmalades, rich in vitamin C and phenolic content, limited HMF production, and a functional product with low sugar content were tried to be obtained, and when the results obtained are evaluated, the product is thought to be promising.

[a] *duygualp@ardahan.edu.tr* *http://orcid.org/0000-0003-3268-9677* | [b] *sametmisir@ardahan.edu.tr* *https://orcid.org/0000-0002-5502-5497*

Introduction

Increasing consumer interest and awareness towards healthy living and nutrition in recent years encourages food manufacturers to seek lesser-known and preferred plant species that have various functional and biological activities thanks to produce the secondary metabolites (Tarko et al., 2007; Sevindik et al., 2017; Kazimierski et al., 2018; Mohammed et al., 2020; Kaya and Koca 2021; Pehlivan et al., 2021). As such, many wild fruits containing high levels of phytochemicals are becoming increasingly important (Pehlivan et al., 2018; Savaş et al., 2020). Various parts of some plants such as seeds, roots, leaves, fruits, flowers may have therapeutic properties and bioactive substances (Pehlivan et al., 2018; Mohammed et al., 2020). Especially fruit peels which are rich in phenolic compounds, minerals, vitamins and fibers have started to be preferred by producers frequently in terms of increasing the value and functionality of products because they are natural sources (Haghani et al., 2021; Pehlivan et al., 2021). One such fruit is the cornelian cherry (*Cornus mas* L.), which was being used in ancient times. It is known that there are about 50 species of Cornelian cherry in the world (Kazimierski et al., 2018). Mostly, Cornelian cherry are grown in central and southwestern Europe such as France, the Crimea, the Caucasus and Türkiye, as well as southern Sweden and England (Ercişli et al., 2011; Kazimierski et al., 2018). Cornelian cherry is becoming popular again today due to the development of cultivars with a more attractive color and larger fruit, thanks to the progress in cranberry cultivation (Bijelić et al., 2016). In Türkiye, approximately 15.000 tons of Cornelian cherry fruit is produced yearly, it is known that this plant, which can also be consumed fresh, is processed and preserved with different methods for out-of-season consumption (Ercişli et al., 2011; Topdaş et al., 2017; Şengül et al., 2018). One of these methods is to concentrate the fruit pulp (Savaş et al., 2020). Jam and marmalade production, which is one of the concentration methods, is one of the long-term preservation methods for these products with antioxidant properties (Rosa et al., 2015).

According to the Turkish Food Codex; Marmalades; It is defined as a mixture of pulp, puree, fruit juice, aqueous extracts and peels obtained from citrus fruit, alone or mixed, with water and sugars, brought to a suitable gel consistency. In addition, in traditional jams, the amount of soluble dry matter determined by refractometer should not be less than 68%, the soluble dry matter content determined by refractometer should not be less than 55%, and the pH range should be between 2.8 - 3.5 (Anonimos 2006).

Marmalade production can be made from fresh or dried fruits. Soft fruits such as peaches and apricots can be made directly into marmalade. However, it is recommended that fruits with no soft flesh such as apples and pears be boiled first so that they can be easily crushed. When making marmalade from dried fruits, the fruits should be kept in water for 8-10 hours and then boiled until soft (Coşkun, 2014). Cornelian cherry, which is a frequently preferred fruit in making marmalade and similar products, is processed more in some local cuisines and has many different traditional productions (Coşkun, 2014; Kazimierski et al., 2018). In addition to these products, traditionally produced products with *Cornus mas L*. include; There are Cornelian cherry syrup, ravioli, sour green lentils, sauce (Ekinek, 2022), tarhana (Işık et al., 2014) and pulp (Topdaş et al., 2018).

The primary aim in this study is to develop an alternative product to marmalades, which are usually produced by applying long-term high heat treatment. For this purpose, breakfast spreadable Cornelian cherry nectar that no long-term and high heat treatment was made. It is aimed to minimize the decrease in the nutritional value of the product, to prevent unwanted color, taste and odor components and the formation of hydroxymethylfurfural.

As a second aim, it is aimed to produce breakfast spreadable Cornelian cherry nectar by using bacterial strain known to have various probiotic properties in order to increase the functional properties of the product. For this purpose, various the physicochemical analysis, sugar profile, total phenolic content, vitamin C and hydroxymethylfurfural content of the product were examined.

Material and Methods

Raw Material

In this study; breakfast spreadable Cornelian cherry nectar were produced using fresh Cornelian cherry fruits were obtained from Ardahan, Türkiye, in September, 2022.

The strain used in the study was isolated from naturally fermented pickles obtained from farmers' markets and identified (Alp, 2018). Also, their cultural and various probiotic properties were determined by (Alp, 2018; Alp and Kuleaşan 2020).

Production of Breakfast Spreadable Cornelian Cherry Nectar

Cornelian cherry fruits were collected from Türkiye's Ardahan province. The method used by Savaş et al. (2020) was modified to produce breakfast spreadable Cornelian cherry nectar. In production, first the stems and leaves of the fruits are removed, then washed and boiled in 400 milliliters of water for 15 minutes. After this process, the shell and core were separated and pulp was obtained. The concentrated product was obtained by adding 85 g/L sugar and boiling for a second time for 10 minutes and placed in bottles in equal grams under aseptic conditions.

In the study, there are 2 groups in total, control group (without any microorganisms) and the probiotic group (*Lactiplantibacillus plantarum* DA100 were inoculated 1.5×10^{10} Log CFU/mL test bacteria). A total of 2 different groups of Cornelian cherry breakfast spreadable nectar were prepared, no preservatives were used in the process. In Figure 1 showed the production of breakfast spreadable cornelian cherry nectar with the probiotic microorganism.

First, the fruits were washed and sorted, then 400 milliliters of water were added and boiled for 15 minutes. After this process it was made into pulp by passing through a wire strainer, sugar was added to the pulp and boiled for 10 minutes. Subsequently they placed in containers in equal grams under aseptic conditions and cooled to room temperature. It was stored at +4°C. In the probiotic added sample, after the nectars came to room temperature, they were inoculated with 1.5×10^{10} Log CFU/mL *Lbp plantarum* DA100 under aseptic conditions.

Determination of Viability in Breakfast Spreadable Cornelian Cherry Nectar of Lactiplantibacillus plantarum DA100 Strain and Other Microorganisms

For the microbiological analysis of breakfast spreadable Cornelian cherry nectar, samples were taken at 0, 72, and 120 hours, and the viability of culture was determined by serial plating on MRS agar. The percent cell survival was calculated according to the formula (1) below (Tokatlı et al., 2015).

$$\%\ \text{survival} = \frac{\text{logcfu of viable surviving cells}}{\text{log cfu of initial viable inoculated cells}} \times 100 \quad (1)$$

For total bacterial counts, Plate Count Agar (PCA) was used while, for Lactic acid bacteria (LAB) counts De Man Rogosa Sharpe (MRS) agar was used, Eosin Methylene Blue (EMB) agar was used for Enterobacteriaceae and Potato Dextrose (PD) agar was used for yeasts. All microbiological analyses were made in triplicate and the mean values and standard deviations were calculated (Beganovic et al., 2011).

Physicochemical Analysis of Breakfast Spreadable Cornelian Cherry Nectar

The total soluble solids (Brix) in breakfast spreadable nectar samples were measured using a digital refractometer. The pH was measured using a digital pH meter (InoLab, pH Level 1)

Determination of Total Phenolic Content (TPC)

Total phenolic content was analyzed according to the method by Slinkard and Singleton (1977) at Ardahan University Central Research Laboratory Application and Research Center.

Determination of Some Sugar Profile

The analysis of sugar profile was conducted at Central Research Laboratory Implementation and Research Center of Ardahan University by an LC20AD HPLC (Shimadzu) equipped with RID 10A dedector and C18 column (250 × 4,6 mm × 5 µm). Also, the mobile phase was acetic acid: water and water: acetonitrile:acetic acid, with a flow rate of 1.3 mL/min.

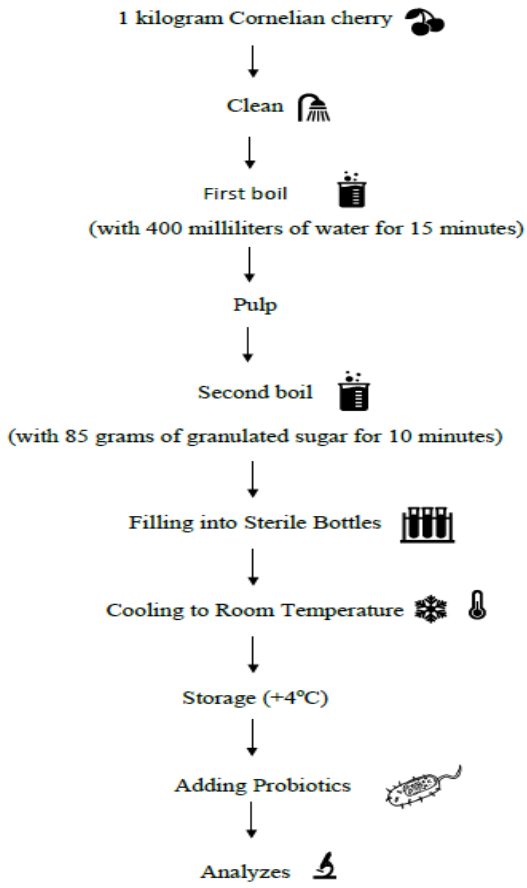


Figure 1. Breakfeast Spreadible Cornelian Cherry Nectar Production with Probiotic Microorganism Scheme

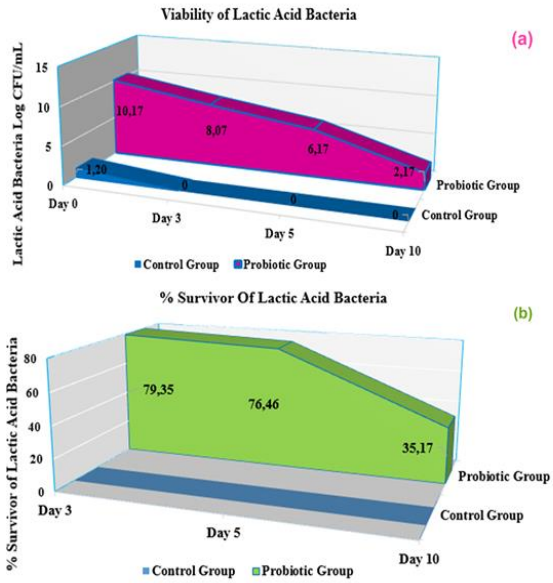


Figure 2. LAB viability (a), % survivor (b) results in the Control Group and Probiotic Group

Determination of Hydroxymethylfurfural (HMF)

The analysis of HMF were determined at Central Research Laboratory Implementation and Research Center of Ardahan University by an LC20AD HPLC (Shimadzu), Intersil OD-3 C18 column (250 mm × 4.6 mm × 5 µm) at ambient temperature and a linear gradient with methanol and water.

Determination of Ascorbic Acid (vitamin C)

The analysis of Ascorbic acid (vitamin C) was determined at Central Research Laboratory Implementation and Research Center of Ardahan University by LC20AD HPLC (Shimadzu) device equipped with SPD-M20A Dedector and using an Ultra Aqueous C18 column (250 mm × 4.6 mm × 5 µm).

Sensory Evaluation

A panel of ten subjects evaluated the sensory properties of breakfast spreadable cornelian cherry nectar, and gave scores for smell, color, consistency, flavor and overall acceptance on a hedonic scale (Onogur and Elmacı 2011). A 5-point hedonic scale was used to rate different attributes of the cotrol group and probiotic group. Acceptance testing was used to determine how much each sample was liked based on the 5-point hedonic scale for a set of attributes. The scale was interpreted where 5 =Very good, 4=Good, 3=Middle, 2=Bad, 1=Too bad.

Statistical Analysis

Microbial enumerations were done in triplicates and the results are presented as mean ± standard deviation. The Minitab 18 statistical software (Minitab, Inc, State College, PA, USA) was used. Sensory data was performed using SPSS software 29 (IBM Corporation, Somers, NY) and the differences were determined by ANOVA with a significance level of $P<0.05$.

Result and Discussion

Viability in Breakfast Spreadable Cornelian Cherry Nectar of Lactiplantibacillus plantarum DA100 Strain and Other Microorganisms

Recently, there has been an increasing interest in the development of products obtained from fruits and vegetables as functional foods with probiotics. Probiotics are consumed by a wide audience because they are naturally healthy (Nematollahi et al., 2016). Lactic acid bacteria known as have various properties are used as starter cultures to add functional properties to products and to improve the quality of the rune (Xiong et al., 2014). In this study, two different marmalades were produced using a control group and a strain determined by previous studies to have probiotic properties. In the initially of the study, the control group had less than 1 logarithmic of lactic acid bacteria, while the experimental marmalade group initially had 1.5×10^{10} Log CFU/mL test bacteria. In the control group LAB and other microorganisms were below the detection limit (<1 Log CFU/g) on hours 0, 72, and 120 during cold storage (4℃). In the probiotic supplemented group, this number was below approximately 1.5×10^{6} Log CFU/mL at the end of 5 days (Figure 2). In addition, coliform bacteria and *E. coli* were not detected in any group during this period.

Nematullahi et al. (2016) stated that the viability of *Lbp. rhamnosus* and *Lbp. plantarum* probiotic strains added to cranberry juice decreased from 8.00 log CFU/mL at the beginning to 4.24 and 4.20 CFU/mL at the end of 7 days. Sheehan et al. (2007) determined the viability of probiotic microorganisms in orange juice, pineapple juice and cranberry juice stored in the refrigerator for 12 weeks. They reported that the number of viable probiotics in

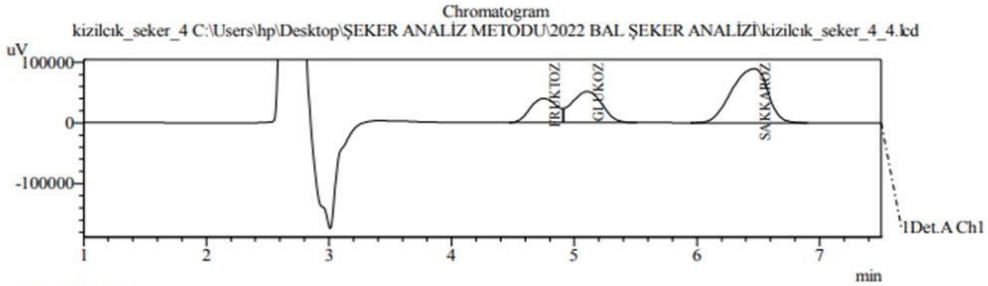
cranberry juice decreased by 3 logs after 2 days, and by the end of the 9th day, there were no viable cells left. As for our study, at the end of the 5th day, our marmalade has completed its shelf life (during cold storage (4°C)) in terms of containing a minimum number of probiotic microorganisms.

Physicochemical Analysis of Breakfast Spreadable Cornelian Cherry Nectar

Cemeroglu et al. (2004) stated that for a good gel formation in products such as jam, marmalade and jelly, the pH value should be between 2.8–3.2 depending on the dry matter content of the product. In addition, when the pH level drops below 3.5, an increase in gel consistency, that is, solidification, can be seen; They stated that when the pH level drops below a certain point syneresis may occur. The pH values, one of the physicochemical samples of breakfast spreadable cornelian cherry nectar, were determined as 3.60 at the beginning, 3.50 on the 3rd day and 3.45 on the 5th day in the control group during the storage period in which the samples were kept in cold storage (4°C). This value was determined as 3.62 at the beginning, 3.48 on the 3rd day and 3.40 at the end of the 5th day in the probiotic supplemented Group. Özdemir et al. (1997) reported that the pH values of marmalades obtained from rosehip fruits, which have similar properties with cornelian cherry, without heat treatment, were between 3.25 and 3.27 at the beginning of storage. Şengül et al. (2018) determined some physical and chemical properties of different marmalade varieties traditionally produced in Artvin province. They stated that the pH values of cornelian cherry marmalade were below 3.5 and that this marmalade was more solid and viscous compared to rosehip and Ahlat pear marmalades. In the Turkish Food Codex; Marmalades; are stated that the water-soluble dry matter content in traditional marmalades should not be below 55% (Anonimos 2006). In our study, the brix value of our products was determined as 33.40, and this value does not comply with the Turkish Food Codex. However, we did not aim to the production of marmalade. Although the pH level of the product is below 3.5, it was observed that the spreadable nectar was more fluid than the marmalades. This situation was attributed to the inability of pectin to form a strong gel depending on the sugar ratio and pH level stated by Cemeroğlu et al. (2004).

Sugar Profile of Breakfast Spreadable Cornelian Cherry Nectar

Cornelian cherry contains a significant amount of biologically active substances such as easily digestible sugars, glucose, fructose, organic acids, aromatic compounds, phenolic substances, pectin, ascorbic acid and anthocyanins (Tural and Koca 2008; Özbey et al., 2017; Şengül et al., 2018). With an average dry matter content of 14-28%, the main component of cranberry fruit is predominantly glucose and fructose, while the total sugar content is 6-19%, including directly reduced sugars (2-12%) and sucrose (0-3%) (Kazimierski et al., 2018). Sugar content of marmalades, on the other hand, is affected by the proportional changes in the amount of commercial sugar added during their production, as well as the difference of fruits.



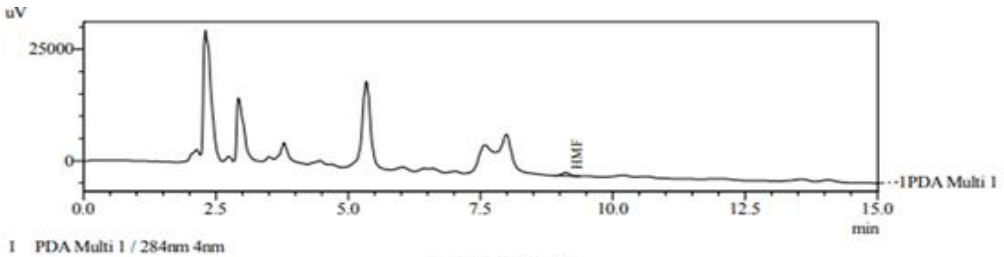
1 Det.A Ch1 /

Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Conc.	Units
1	FRUKTOZ	4.748	607652	4.384	g/100g
2	GLUKOZ	5.102	877965	5.824	g/100g
3	SAKKAROZ	6.463	1823121	13.108	g/100g

Figure 3. The sugar content of breakfast spreadable cornelian cherry nectar



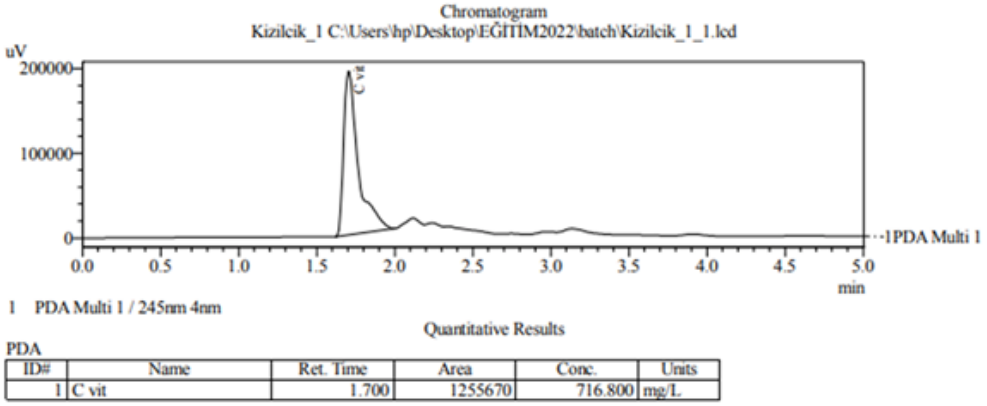
1 PDA Multi 1 / 284nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Conc.	Units
1	HMF	9.110	7764	4.290	mg/kg

Figure 4. HMF results of breakfast spreadable nectar



Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Conc.	Units
1	C vit	1.700	1255670	716.800	mg/L

Figure 5. Ascorbic acid (Vitamin C) results of breakfast spreadable cornelian cherry nectar

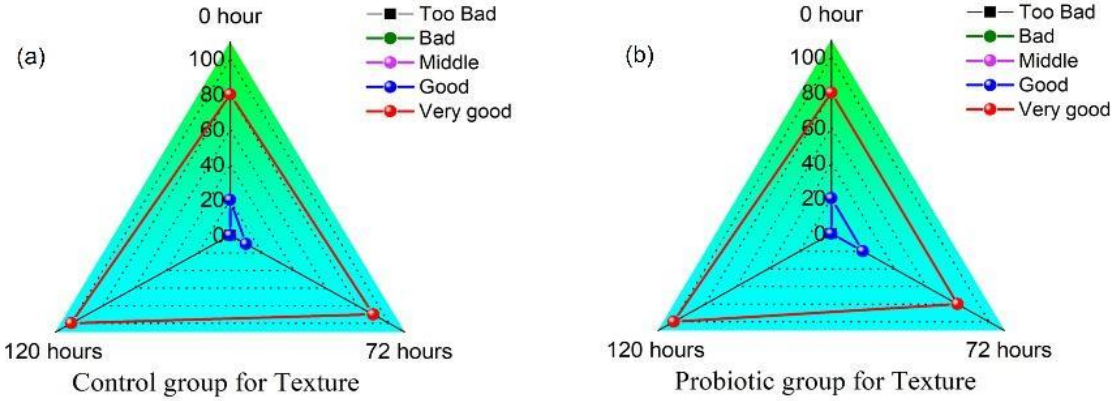


Figure 6. Texture results of the control group and probiotic group

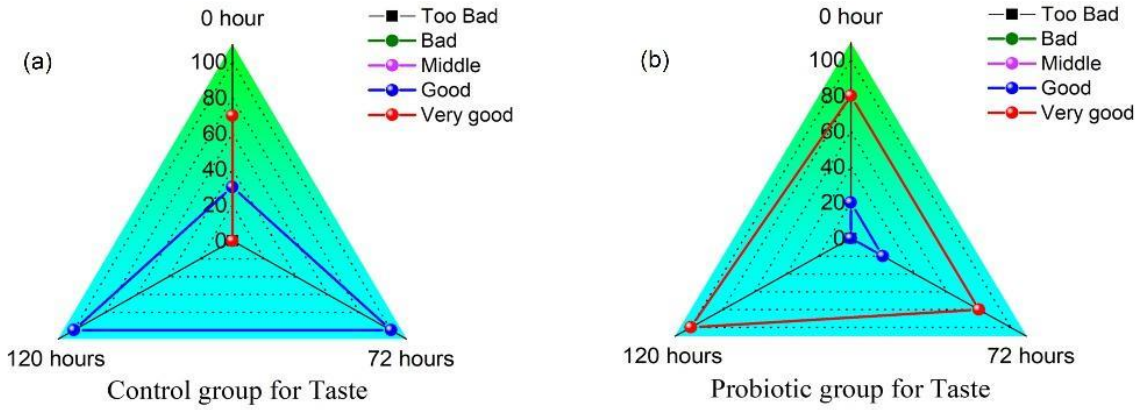


Figure 7. Taste results of the control group and probiotic group

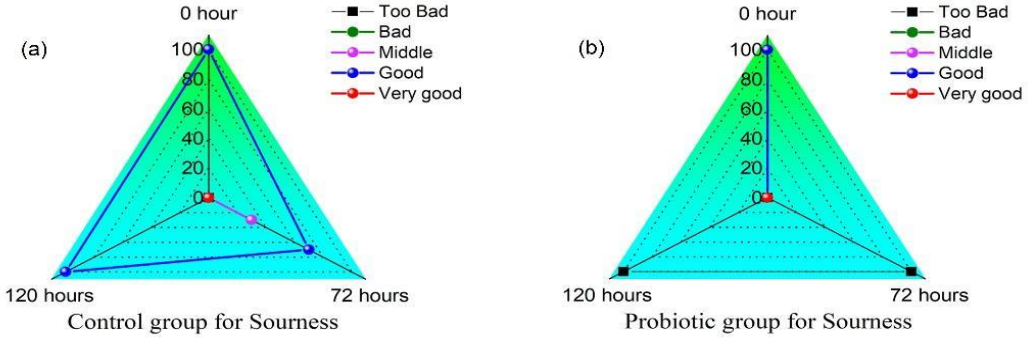


Figure 8. Sourness results of the control group and probiotic group

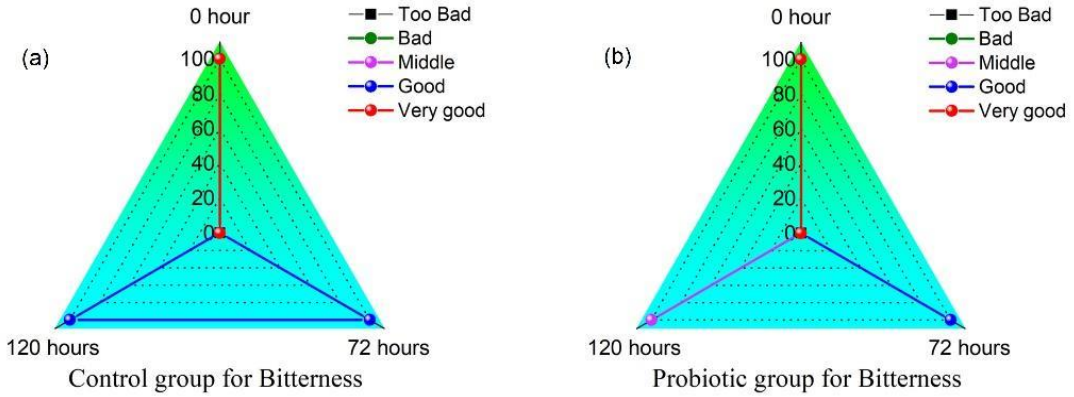


Figure 9. Bitterness results of the control group and probiotic group

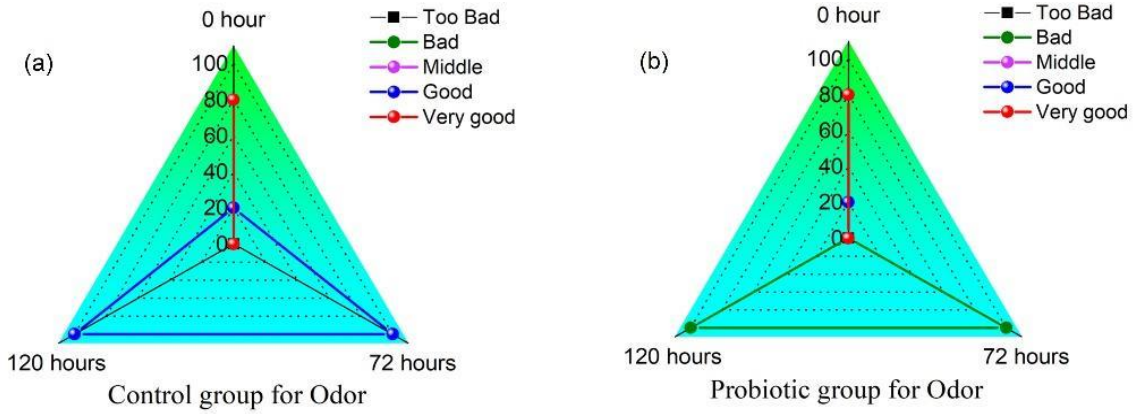


Figure 10. Odor results of the control group and probiotic group

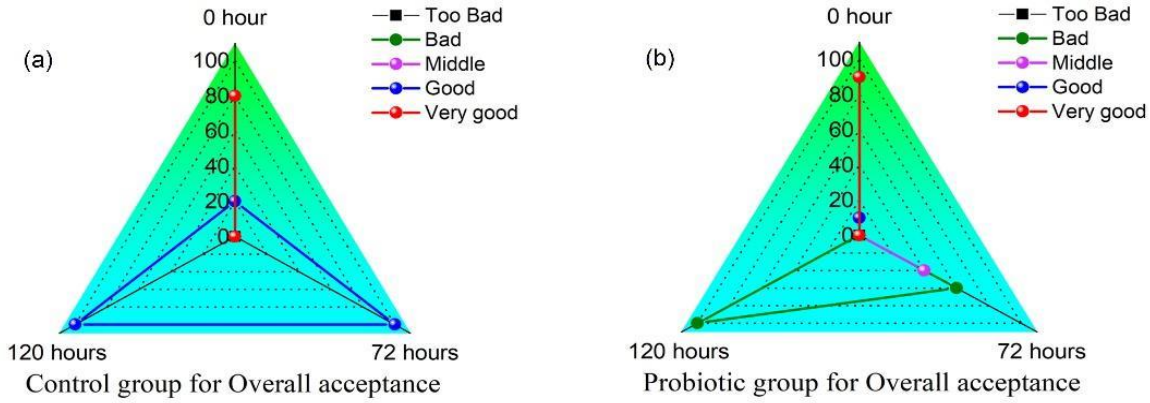


Figure 11. Overall acceptance results of the control group and probiotic group

The sugar content of our breakfast spreadable nectar sample (Control group) (Figure 3) was determined as 4.384 g/100g, glucose 5.824 g/100g and sucrose 13.108 g/100g. The amount of sucrose determined in cornelian cherry marmalade indicated that the sucrose was not completely degraded. The reason for this was attributed to the fact that long-term and high high temperatures were not applied during production and that it was not high acidic.

Hydroxymethylfurfural (HMF)

High heat treatment and storage conditions directly affect the sensory properties and nutritional components of products such as jam and marmalade. This process (excessive heat application, long-term heat treatment times) can significantly reduce nutritional values, even before reaching the desired consistency, they cause undesirable losses in color, flavor and nutritional components and the production of undesirable compounds like hydroxymethylfurfural, furfural and melanoidins (Kuscu and Bulantekin 2021; Şengül et al., 2022). Hydroxymethylfurfural (HMF), an organic compound produced by the Maillard reaction as a result of heating reducing sugars in an acidic environment during processing and storage (Rada-Mendoza et al., 2004; Shapla et al., 2018). HMF results of spreadable nectar at breakfast are given in Figure 4. It is seen that the HMF value of the product obtained in our study is very low due to excessive heat application and the lack of long-term heat treatment time.

Total Phenolic Content (TPC)

The total amount of phenolic substances obtained in the studies varies. Researchers attribute this to the fruit types used, their degree of maturity and different extraction methods (Şengül et al., 2018). Today, it is consumed in the form of cranberry sherbet or by making more marmalade. However, it would be better to improve/change the processing conditions (heat treatment temperature and time) so that the bioactive components are damaged as little as possible during production. In our study, we set out for this purpose and heat treatment was kept at a minimum level. The total phenolic content of our breakfast spreadable nectar was determined as 3156.64 mg GAE/100g on average, and a very high figure was obtained when compared to products such as marmalade or jam. When the results obtained in marmalade studies made with rosehip, which is known to be rich in phenolic substances, are examined; Serious effects of heat treatment were observed (Yoo et al., 2008; Chrubasik et al., 2008; Sagdıc et al., 2015).

Ascorbic Acid (Vitamin C)

Although vitamin C (ascorbic acid), which has an important place in human nutrition, is widely found in nature, its stability is very low compared to other vitamins and is affected by processes such as heating, which are frequently used in food production (Cemeroğlu et al., 2004). Cornelian cherry are known to contain more vitamin C compared to other vitamin C-rich fruits (Skender et al., 2022). Güzel (2021) reported that the average vitamin C in cornelian cherry fruits varied between 29.0–103.3 mg/100 g in Türkiye. Heat-resistant substances, including vitamin C, can be preserved in the product when cranberry berries are used for fresh consumption or processed into various products without heat treatment or with low heat treatment (Skender et al., 2022). Based on this, the amount of vitamin c in the breakfast spreadable cornelian cherry nectar we produced was determined as 716.800 mg/L (Figure 5).

Sensory Evaluation

Sensory evaluation results of breakfast spreadable cornelian cherry nectar indicated that difference in taste, bitterness, odor and sourness were significant ($P>0.05$) contrary to texture score. Texture, which is an important parameter in terms of sensory acceptance, is largely affected by fruit type, fruit amount and sugars used. Jams with a lower elastic phase are more difficult to spread on bread (Kavaya et al., 2019). When the sensory analysis results are examined; it is seen that the probiotic microorganism additive does not have a serious effect on the texture (Figure 6).

However, it was determined that the probiotic additive was effective on these properties of nectar in taste, sourness, bitterness and odour scores. Especially the difference in taste (Figure 7) is due to sourness and bitterness (Figure 8 and Figure 9).

This sour taste increase on hours 72 and 120 is attributed to *Lbp. plantarum* DA100 causing pH reduction. The difference in odor (Figure 10) between the products led us to inferred that the probiotic microorganism may have produced various aroma substances.

As an overall acceptance (Figure 11), nectar without probiotic additives was liked more by the panelists. This situation was attributed to the probiotic microorganism possibly produced the various aroma substances and organic acids (Alp and Kuleaşan 2022) which is caused pH drop during the 120-hour period.

Conclusion

Cornelian cherry contains many components that are beneficial for improving and maintaining health. Therefore, the application of cornelian cherry-derived products in functional foods may be a new focus of research and development. Considering the properties of the product obtained in our study; It has been observed that how effective the short-term application of heat treatment is in terms of the properties of the product during the processing of a fruit with many health-important components such as cornelian cherry. In this study, it has been tried to obtain a functional product that is an alternative to jams and marmalades, rich in vitamin C and phenolic content, limited HMF production, and which can be consumed by diabetic patients, especially thanks to its low sugar content. When the results obtained are evaluated, the product is thought to be promising.

Conflict of Interest

The authors declare that there are no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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