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Determination of Antioxidant Activities of Rosehip Marmalade Added Kefir During Its Storage Process

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ABSTRACT: Herein, the effect of rosehip marmalade addition to kefir on it antioxidant activities was investigated. Firstly, the production of kefir, having the supplements of semi-skimmed milk, powder kefir ferment, and rosehip marmalade at different proportions (0% (control), 10%, and 15%) was made. Kefirs were stored in the refrigerator on days 1, 7, 14 and 21 at 4 ± 1ºC. It has been determined that it has a highly significant ($p<0.01$) effect on TPC during the storage period. In addition, it has been determined that storage period has a significant ($p<0.05$) effect on Vitamin C, while has a insignificant ($p>0.05$) effect on DPPH. The rosehip marmalade kefirs and control kefirs vitamin C and total phenolic content values were in the range of 7.85-9.04 mg $100g^{-1}$ and 1931,18-2447,11 mg GAE L^{-1}, respectively. On the other hand, the 2.2-diphenyl-1-picrylhydrazyl values of the samples were determined up to 67.85 µg ml^{-1} (EC_{50}). With this research, it was concluded that the low Vitamin C content of kefir, which stands out in dairy products especially with its probiotic character, can be increased and it can be transformed into a fermented product with different sensory characteristics, both probiotic and prebiotic.

Keywords: Kefir, Antioksidant, Vitamin C, TPC, DPPH

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INTRODUCTION

Milk, defined as a porcelain-white liquid, secreted at different times according to the species of female mammals, has sufficient nutritional content to feed the offspring. The main purpose of milk is to ensure that the offspring develop, and protect themselves by gaining immunity against external influences (Metin, 2013). Besides being the source of minerals such as calcium and phosphorus; milk and its products are also an important source of group B vitamins. Hence, milk is a convenient source for obtaining sufficient macro and micro components necessary for human nutrition (Ünal and Besler, 2008). It is stated that the components that increase the biological value of milk and dairy products are essential amino acids and fat composition. Besides, minerals found in milk have been reported to have significant functions such as stimulating muscles and nerves, controlling osmotic pressure and pH (Demirci, 1981).

Kefir, a fermented dairy product, is a slightly acidic product usually obtained by fermentation of cow, sheep, goat, camel or buffalo milk by adding kefir grain (Karaçıl and Tek, 2013; Bengoa et al., 2019; Larosa et al., 2021). As a result of research conducted by Russian scientists in the 1920s, kefir, which dates back to the Caucasus, was found to more richer than yogurt in terms of probiotic bacteria. Kefir is a significant source of protein, probiotic and prebiotic as well as a source of many vitamins and minerals (Ötleş and Çağındı, 2003; Güzel-Seydim et al., 2021). Many factors affect the chemical, microbiological and sensory properties of kefir. Especially, milk type, fat content of milk, kefir yeast, fermentation temperature and time directly affect these (Farnworth and Mainville, 2008; Bulat and Topçu, 2021). Kefir, which is available in many regions of the world today, differs in that it is loved and consumed due to its sour taste. On the other hand, kefir, which is a substantial food in terms of health, has many advantages (Tomar et al., 2017). Especially in studies with kefir; it is reported to have antimicrobial, anti-inflammatory and anticarcinogenic effects. In addition, kefir strengthens the immune system and has an anti-microbial effect on some pathogens (*Bacillus cereus, Escherichia coli, Salmonella* spp. and *Staphylococcus aureus, Listeria monocytogenes)* (Ötleş and Çağındı, 2003; Koyu and Demirel, 2018; Sindi et al., 2020; Vimercati et al., 2020; Buran et al., 2021; Larosa et al., 2021).

Many studies have shown that milk and dairy products have antioxidant activity (Chen et al., 2015; Yilmaz-Ersan et al., 2018). Antioxidants is defined as a substance that directly scavenges ROS or inhibit ROS production. Antioxidants play a vital role in both food systems and the human body. In particular, antioxidants reduce the oxidative processes and harmful effects of ROS, as well as help preserve the flavor, color and texture of food products during storage. Consumption of fruits and vegetables has been associated with a reduced risk of many diseases (Gulçin, 2020). Therefore, today, many studies have been conducted in which fruits, fruit sauces and juices have been used to increase the consumption of functional products such as kefir (Yilmaz-Ersan et al., 2018; Kabakcı et al., 2020) One of the foods added to kefir is rosehip fruit.

Rosehip, a fruit belonging to the *Rosaceae* family, is used in varied areas as well as growing naturally in our country. In our country, rosehip, which is one of the indispensable fruits of the winter months, is consumed by marmalade, herbal tea, nectar, dried, and frozen. Rosehip is a fruit rich in phenolic substances and carotenoids as well as high vitamin C content. Carotenoids such as lycopene, β-carotene, and xanthophyll in their texture play a role in the formation of the unique color of rosehips. It also contains various phenolic substances such as hydroxycinnamic acid, catechin, quercetin, and kaempferol (Ercişli, 2007; Koca et al., 2008; Sarıcaoğlu et al., 2019; Atalar et al., 2020). In addition, rosehips are used in the treatment and prevention of many diseases (cold, gastrointestinal disorders, infections and diabetes) due to their rich bioactive compounds (Ercişli, 2007; Sarıcaoğlu et al., 2019).

In order to improve and enhance the quality of health, people should consume nutrients in their dosage, in a certain order and consciously. Because, in recent studies, it is predicted that nutrition is directly pertinent to human health, some diseases can be prevented and some can be cured by regulating the diet. On the other hand, there is no doubt that inadequate and unbalanced nutrition causes many diseases and distracts human beings from quality life. In recent years, there has been a tendency to foods such as kefir, which are natural and have positive effects on health. In this context, there are various studies on the kefir in the literature. However, in our literature review, we did not come across any study on the addition of rosehip marmalade to kefir. For this reason, in our study, it was aimed to determine of kefir prepared by adding rosehip marmalade in different proportions (%10 and %15) vitamin C ve antioxidant activities.

MATERIALS AND METHODS

Materials and Chemicals

Milk and powdered kefir yeast used in the study were obtained from a national market in Erzurum province and fermentation was carried out in a laboratory environment. Rosehip fruits were purchased in dry from a herbalist in Erzurum. Semi-skimmed cow milk (Ultra High Temperature milk) was used in the research. For DPPH; 2.2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich), ethanol (Sigma-Aldrich), TPC for; Gallic acid 3,4,5-Trihydroxy benzoic acid (Sigma Aldrich), sodium carbonate (Merck), folin & ciocalteu's phenol reagent (Sigma Aldrich) and Vitamin C; Vitamin C (Sigma Aldrich), 2,6-Dichlorophenolindophenol sodium salt hyrdate (Sigma Aldrich), Oxalic acid dihydrate (Merck) was used. In addition, DPPH and TPC analysis were performed in spectrophotometry (T60V Spectrometer, PG Instruments Ltd.) while vitamin C analysis was carried out by titrimetric method.

Preparation of Kefir and Rosehip Marmalade

Kefir was prepared by adding powdered kefir yeast (1g powder kefir yeast to 1 liter of semi-skimmed milk) to sterilized semi-skimmed milk obtained from a local market in Erzurum and keeping it under room conditions for 16-24 hours. Rosehip marmalade was prepared by adding sugar (water/sugar, 2/1 w/w) after the rosehip fruit was boiled and separated from its pulp. The present process continued until the rosehip marmalade consistency. Then, the prepared rosehip marmalade was added to kefir in different proportions. Fruit kefirs were produced by adding 10 % and 15 % rosehip marmalade to control kefir.

Extraction for Antioxidant Content of Samples

The extract was prepared according to Gülçin et al. (2002), Gülçin (2005) and Şengül et al. (2020). To determine the total amount of total phenolic content (TPC) and 2.2-diphenyl-1-picrylhydrazyl (DPPH), 25 g of each sample was mixed with 75 ml of ethanol-water (90:10, v/v) using a magnetic stirrer in the dark. Then the mixture was filtered through the filtrate was evaporated at 50°C, the solution was completed to 25 ml with distilled water, and the stock solution was prepared.

DPPH Free Radical Scavenging Activity

DPPH (2.2-diphenyl-1-picrylhydrazyl) was diluted to 3 ml with ethanol for sample extracts (10-30 μg ml^{-1}) for free radical scavenging activity. Then, 1 ml of prepared DPPH solution (1 mM) was added to the samples and mixed thoroughly with the help of vortex and left for 30 minutes incubation at 30 °C in the dark. Absorbance was measured against blank at 517 nm in the spectrophotometer (Ozcelik et al., 2021; Binici et al., 2021).

Total Phenolic Content

1 ml of the samples was taken and 46 ml of distilled water and 1 ml of FCR (Folin–Ciocalteu reagent) were added. After the mixture was retained for 3 minutes, 3 ml of 2 % sodium carbonate (Na_2CO_3) solution was added and mixed in a magnetic stirrer for 2 hours. Then, absorbance values were found in a spectrophotometer at 760 nm wavelength. The phenolic content of the samples was calculated in gallic acid equivalent (mg GAE L^{-1}) with the help of graphics using standards prepared using gallic acid (Ozcelik et al., 2021; Binici et al., 2021).

Vitamin C Content

Vitamin C content of samples, 10 ml of the samples were taken and 10 ml of oxalic acid was added and filtered through a simple filter paper (Whatman No1). Oxalic acid was added (1:10 w/w, % 2) again to the filtrate and extraction continued. From the filtrate obtained from the extraction process, 5-25 ml was taken into a 50 ml Erlenmeyer flask. After that, it was titrated with 2.6 dichlorophenolindophenol solution (% 0 .05). The dye solution was standardized by titration with 20 mg/100 ml ascorbic acid standard solution. The titration process was continued until the pinkish color was achieved. Then, the amount spent was recorded and the amount of vitamin C (mg $100g^{-1}$) was determined (Cemeroğlu, 2007; Cemeroğlu, 2010).

Vitamin C, mg $100g^{-1}$ = (V x f x 100) / m_2 (1)

V: Amount of 2.6-Dichlorophenolindophenol solution spent in titration, ml

f: The factor of 2.6-Dichlorophenolindophenol solution, that is, the amount of vitamin c equivalent to 1 ml of this solution, mg

m_2 : Amount of original sample in titrated filtrate, g.

Statistical Analysis

All of the data were represented as mean ± standard deviation. Statistical analysis was performed with two-way ANOVA and Duncan's multiple range test by using SPSS 20 software.

RESULTS AND DISCUSSION

Vitamin C Content

Vitamin C is one of the most powerful natural antioxidants, as well as being a water-soluble vitamin (Gulçin, 2020). The vitamin C values determined during the storage period of rosehip marmalade added kefirs and control kefirs are given in Table 1. It was concluded that the amount of ascorbic acid varied between 7.80 and 9.04 (mg $100g^{-1}$) and the effect of storage time on the amount of ascorbic acid is significant ($p<0.05$). Also, it was determined that the effect of the marmalade rate was highly significant ($p<0.01$). The ascorbic acid amount of rosehip marmalade added kefirs (8.66-14.55 mg $100g^{-1}$) was found higher than the control kefir (1.33 mg $100g^{-1}$). It is stated that ascorbic acid undergoes degradation depending on aerobic, anaerobic, temperature and storage time (Kırca and Cemeroğlu, 2001) There is scarcely any research in the literature to support the current research. In this context, Yıldız and Alpaslan (2012) determined the ascorbic acid content 25.05 mg $100g^{-1}$ in marmalades produced by the classical method. In the study conducted by Zeytun (2007), the level was detected as follows; 8.7 mg $100g^{-1}$ in rosehip marmalade, 2.30 on the 1st day, and 2.00 mg $100g^{-1}$ on the 14th day in plain BiYogurts, 4.21 and 4.09 mg $100g^{-1}$ in the rosehip marmalade BiYogurts.

Table 1. The Vitamin C value of rosehip marmalade added kefirs and control kefirs

	Vitamin C (mg 100g^{-1})
Storage time	
1.day	7.85 ± 5.63[a]
7.day	9.04 ± 6.25[b]
14.day	7.85 ± 5.43[a]
21.day	7.80 ± 5.93[a]
Sign	*
Marmalade rate %	
% 0 (control)	1.33 ± 0.00[a]
% 10	8.66 ± 1.56[b]
% 15	14.55 ±1.06[c]
Sign	**
Interaction	
ST x MR	ns

a-c: means with different letters in the same column are significantly different ($p < 0.05$); Sign: Significance; ns: not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$. (% 0: Control (plain) kefir, %10: Kefir with ten percent rosehip marmalade added, %15: Kefir with fifteen percent rosehip marmalade added).

Total Phenolic Content

The phenolic compunds values determined during the storage period of rosehip marmalade added kefirs and control kefirs are given in Table 2. As seen in the table, it was concluded that the amount of phenolic varied between 1931.18 and 2447.11 mg GAE L^{-1} and the effect of storage time on the amount of phenolic was highly significant ($p<0.01$). TPC values of the samples decreased during storage compared to day 1. Also, it was determined that the effect of the marmalade rate was highly significant ($p<0.01$) and the lowest phenolic content was determined in control kefir. It is understood that the amount of phenolic content are high especially in the samples with rosehip marmalade. It is stated that phenolic content are found in milk with proteins and in soluble form. In addition, pH, type of protein, type and structure of phenolic content, heat treatment temperature and duration can cause protein-polyphenolic to degradation (Yılmaz-Ersan et al., 2018). As for the total amount of phenolic content, many studies have been conducted on kefir using different formulations (fruit, juice and fruit sauces) (Kabakcı et al., 2020; Ozcelik et al., 2021). However, the number of studies using rosehip marmalade is limited. While Satir and Güzel-Seydim (2015) stated that the total amount of phenolic substance in kefir samples made from goat's milk varied between 726.08-1359.32 mg GAE L^{-1}; Çınar (2019) determined as 66.15 mg GAE g^{-1} in plain kefir, 107.49, 127.49, 174.60, 203.05 mg GAE g^{-1} in 5%, 10%, 15% and 20% blueberry kefir samples, respectively. In this context, Yıldız and Alpaslan (2012) determined the total phenolic content 912.4 mg 100g^{-1} in rose hip marmalades produced by the classical method. Ozcelik et al. (2021) determined that the total phenolic content of water kefir produced using different fruit juices (Cornelian cherry, hawthorn, rosehip, pomegranate and red plum) decreased during storage. On the other hand, Yilmaz-Ersan et al. (2018) found that the mean total phenolic content in cow and sheep kefir samples increased during storage and the TPC ranged between 59.09 and 85.69 mg GAE/100 mL and 77.74 and 84.79 mg GAE/100 mL, respectively.

Table 2. The TPC value of rosehip marmalade added kefirs and control kefirs

	TPC (mg GAE L^{-1})
Storage time	
1.day	2447.11 ± 1244.03[b]
7.day	2089.89 ± 982.17[a]
14.day	1931.18 ± 289.53[a]
21.day	1998.59 ± 1097.22[a]
Sign	**
Marmalade rate %	
% 0 (control)	994.1 ± 404.6[a]
% 10	2621.1 ± 627.5[b]
% 15	2734.9 ± 576.5[c]
Sign	**
Interaction	
ST x MR	**

a-c: means with different letters in the same column are significantly different ($p < 0.05$); Sign: Significance; ns: not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$. (% 0: Control (plain) kefir, %10: Kefir with ten percent rosehip marmalade added, %15: Kefir with fifteen percent rosehip marmalade added).

DPPH Free Radical Scavenging Activity Values

DPPH radical scavenging activity is one of the most widely used methods to measure antioxidant activity (Ozcelik et al., 2021). While DPPH free radical scavenging activity (EC_{50}) of the samples was determined in the lowest antioxidant activity control sample, it was determined that the antioxidant activity increased as the fruit ratio increased. As seen in the Table 3, it was concluded that the amount of DPPH varied between 41.64 and 67.85 μg ml^{-1} and the effect of storage time on the amount of DPPH was not highly significant ($p>0.05$). It is stated that the change during storage may be due to post-acidification proteolysis and increased organic acid content (Yılmaz-Ersan et al., 2018). Also, it was determined that the effect of the usage rate was highly significant ($p< 0.01$). When the EC_{50} values are examined, it is seen that the highest value belongs to the control kefir samples with 148.3 μg ml^{-1} and the lowest value belongs to the kefir samples with 15% rosehip marmalade with 11.8 μg ml^{-1}. In other words, while the highest DPPH radical scavenging activity was detected in kefir samples 15% rosehip marmalade, control kefir samples had the lowest antioxidant activity as expected. Turker et al. (2014), was determined that cow milk DPPH value 188. 35 mg ml^{-1}. In the study, Çınar (2019) found the EC_{50} value of plain kefir as 3230.67 μg ml^{-1} on day 1; and reported that in blueberry added kefir samples, it was varied between 93.45–244.17 μg/ml on the 1st day and 93.45–205.67 μg ml^{-1} on the 21st day of storage. In another study, DPPH values of cow and sheep milk kefir were determined as 9.57 mg TE 100 mL^{-1} (Yılmaz-Ersan et al., 2018). Kavaz (2019) determined the EC_{50} value of fresh black rosehip fruit as 9.63 μg ml^{-1}.

Table 3. The DPPH value of rosehip marmalade added kefirs and control kefirs

	DPPH (EC_{50}, μg ml^{-1})
Storage time	
1.day	63.26 ± 82.76[a]
7.day	67.85 ± 87.60[a]
14.day	57.15 ± 77.80[a]
21.day	41.64 ± 73.13[a]
Sign	ns
Marmalade rate %	
% 0 (control)	148.3 ± 59.6[a]
% 10	12.4 ± 1.84[b]
% 15	11.8 ± 1.59[b]
Sign	**
Interaction	
ST x MR	ns

a-b : means with different letters in the same column are significantly different ($p < 0.05$); Sign: Significance; ns: not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$. (% 0: Control (plain) kefir, %10: Kefir with ten percent rosehip marmalade added, %15: Kefir with fifteen percent rosehip marmalade added).

CONCLUSION

As a result, the antioxidant capacities of kefirs with different proportions of rosehip marmalade added to kefir obtained by using semi-skimmed UHT milk and culture yeast were determined. According to the data obtained, it was determined that the antioxidant activity of kefir increased as the addition of rosehip marmalade increased. In addition, it was statistically determined that antioxidant capacities (Vitamin C, TPC) decreased and DPPH values increased during storage. In the present study, it is thought that rosehip marmalade added to kefir increases antioxidant activity and is an important product in terms of new functional products. It was determined that the addition of rosehip marmalade had a positive effect on the taste and appearance of kefir. In addition, as a result of the preliminary trials, it is thought that kefir prepared with the addition of 10% rosehip marmalade can be commercially produced.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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