DEVELOPMENT OF STRAINED YOGURT ICE CREAM WITH ARONIA AND HYDROLYZED COLLAGEN

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ABSTRACT

DEVELOPMENT OF STRAINED YOGURT ICE CREAM WITH ARONIA AND HYDROLYZED COLLAGEN

Yogurt ice cream, made using yogurt cultures *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus,* is integrated with freezing technology, and it is a healthy food that creates cooling effect on consumers. In this study, it was aimed to obtain strained yogurt ice cream that would allow consumption of hydrolyzed collagen and aronia together. Different concentrations of collagen were added to samples with and without aronia. Different amounts of stevia were also added to selected sample due to its antioxidant activity.

The quality of the ice creams in the study were evaluated by analyses as follows; dry matter, protein, fat, ash, carbohydrates, pH, color, viscosity, overrun, melting rate, antioxidant activity, coliform and lactic acid bacteria counts, and sensory analyses.

While aronia decreased pH, collagen increased pH; adding stevia did not cause any significant change on pH. A correct proportion was observed between the viscosity and fat content of formulations. Samples were examined for antioxidant activity by DPPH and total phenolic content analyses. According to DPPH analysis results, sample B containing 20g of aronia had highest antioxidant activity. According to total phenolic content analysis, sample F containing 20g of aronia and 2.5g of hydrolyzed collagen had the highest total phenolic content. After the antioxidant activity was evaluated, stevia was added as a sweetener to sample E, which contained 1g of hydrolyzed collagen. According to sensory analysis results, sample E1 containing 20g of aronia, 1g of collagen and 0.006g of stevia was the most liked yogurt ice cream in terms of taste.

Keywords: *strained yogurt ice cream, collagen, aronia, antioxidant activity*

ÖZET

ARONYALI VE HİDROLİZE KOLAJENLİ SÜZME YOĞURT DONDURMASI GELİŞTİRİLMESİ

Yoğurt dondurması yoğurt kültürleri olan *Lactobacillus delbrueckii subsp. bulgaricus* ve *Streptococcus thermophilus* ile yapılan, dondurma teknolojisi ile bütünleştirilen ve tüketicilerde serinletici bir etki uyandıran sağlıklı bir gıdadır. Bu çalışmada hidrolize kolajen ve aronyanın birlikte tüketimini sağlayacak süzme yoğurt dondurması elde edilmesi amaçlanmıştır. Aronya içeren ve içermeyen örneklere farklı konsantrasyonlarda kolajen eklenmiştir. Antioksidan aktivitesine göre seçilen örneğe farklı miktarlarda stevia eklenmiştir.

Bu çalışmada dondurmaların kalitesi; toplam kuru madde, protein, yağ, kül, karbonhidrat, pH, renk, viskozite, hacim genişlemesi, erime hızı, antioksidan aktivite, koliform ve laktik asit bakteri sayımı ve duyusal analizler ile değerlendirilmiştir.

Aronya pH'yı düşürürken, kolajen pH'yı arttırmıştır, stevia ilavesi pH'da önemli değişiklik yaratmamıştır. Formülasyonların viskoziteleri ve yağ içerikleri arasında doğru orantı gözlemlenmiştir. Örneklerin antioksidan aktivitesi DPPH analizi ve toplam fenolik madde analizi ile belirlenmiştir. DPPH analizine göre 20 g aronya içeren B örneği en yüksek antioksidan aktiviteye sahiptir. Toplam fenolik madde analizi sonuçlarına göre 20 g aronya ve 2,5 g hidrolize kolajen içeren F örneği en yüksek toplam fenolik maddeye sahiptir. Antioksidan aktivite değerlendirildikten sonra, bileşiminde 1 gram hidrolize kolajen olan E örneğine tatlandırıcı olarak stevia eklenmiştir. Duyusal analiz sonuçlarına göre 20 g aronya, 1 g kolajen ve 0,006 g stevia içeren E1 örneği tat olarak en çok beğenilen yoğurt dondurması olarak belirlenmiştir.

Anahtar Kelimeler: *süzme yoğurt dondurması, kolajen, aronya, antioksidan aktivite*

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ABBREVIATIONS

%	Percentage value
°C	Degrees Celsius
g	Grams
L	Liter
mg	Milligram
mL	Milliliter
rpm	Revolution per minute
FC	Folin-Ciocalteu
H_2SO_4	Sulfuric acid
$(NH_4)_2SO_4$	Ammonium sulfate
NH_3	Ammonia
Na_2CO_3	Sodium carbonate
nm	Nanometer
AOAC	International Association of Analytical Chemists
min	Minute
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalents
VRBA	Violet red bile agar
LAB	Lactic acid bacteria

CHAPTER 1

INTRODUCTION

Yogurt ice cream is a dairy product that is very similar to ice cream in structure and is made using cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus*. This dairy product offers us a unique taste by combining the coldness of ice cream with the sharp acidity of yogurt (Tamime and Robinson 2007). Yogurt ice cream is a very nutritious product with its high protein and easily absorbable calcium content. Yogurt has many health benefits, including the development of the immune system, lactose digestion, regulation of intestinal flora and increasing natural killer cell activation, detoxification of harmful substances, reduction of carcinogenic by-products and inhibition of the growth of harmful bacteria. However, it has also been found that yogurt has promising health benefits against some gastrointestinal problems for human health (Rabotyagova et al. 2008). Maintaining the viability of the starter cultures used in the production of yogurt ice cream is of critical importance in ensuring and protecting the health-promoting and healing image of the product (Tamime and Robinson 2007).

Structure, texture, viscosity, crystallization and melting properties are all very important factors in frozen desserts. However, as in every food product, taste is considered the most important quality criterion. The size, shape and distribution of ice crystals and air bubbles play a very important role. In addition, the amount and distribution of unfrozen material is also important in determining the quality of the dessert. A well-prepared formulation, flavor, texture, cooling effect, melting speed, viscosity and whipping ability combine to create a balanced ice cream. As the viscosity of the non-frozen yogurt mixture increases, the melting resistance and smoothness of the structure increase, but the whisking speed decreases. The viscosity of frozen yogurt is a critical factor influencing its characteristics. Research has demonstrated that an increase in viscosity leads to enhanced melting resistance and smoothness of frozen yogurt. Stabilizers such as κ-carrageenan, corn starch, and inulin have been shown to notably elevate the viscosity of frozen yogurt. A higher viscosity is linked to a more stable fat-liquid emulsion, resulting in improved melting stability of the product. In particular, the

addition of inulin has been found to significantly boost viscosity compared to regular frozen yogurt (Skryplonek et al. 2019). Moreover, the incorporation of probiotics, such as *Bifidobacterium*, can substantially raise the viscosity of frozen yogurt, thereby improving its melting resistance (Açu 2014). Additionally, the use of beetroot extracts as an antioxidant source in stirred yogurt has been associated with alterations in viscosity, highlighting the impact of various additives on the rheological properties of yogurt (Flores et al. 2021). In addition, the heat treatment of the product is also affected by these factors (Kaya and Tekin 2001). If sugar is used as a substitute in ice cream, it is important to consider the effects of sugar on ice cream, such as sweetness, freezing point and dry matter content. Additionally, the lower the freezing point, the shorter the shelf life (Işık 2006).

In this study, collagen, a soluble dietary fiber added to yogurt ice cream, offers the opportunity to be used from an innovative perspective to increase quality in the food industry with its nutritional and technological advantages. Additionally, aronia, which has high antioxidant properties, was added to the collagen-containing yogurt ice cream. According to the determined formulation and processing method, that is, Balkan Milk and Dairy Products Company recipe hard type strained yogurt ice cream was obtained. A factorial design was used to examine the effects of different concentrations of aronia and collagen on the sensory, physical and chemical properties of ice cream. Aronia and collagen concentrations were systematically varied to determine the appropriate levels for the desired attributes. Control samples without aronia extract and collagen were also prepared for comparison.

After the product was obtained, the physical and chemical properties of the ice cream were determined using standard analytical methods. Sensory evaluation was performed by a trained panel to evaluate attributes such as appearance, odor, texture, taste and overall acceptability. Coliform and lactic acid bacteria counts were also performed for strained yogurt ice cream samples.

In addition to these experiments, DPPH analysis was performed to investigate the synergistic effect between aronia and collagen. In the presence of collagen, aronia increases the antioxidant activity value by scavenging free radicals. Oxidation occurs as a result of the metabolic activities of living things and causes the formation of free radicals that cause serious diseases such as cancer and heart diseases. In addition, some factors such as exposure to radiation, unhealthy eating habits, and smoking also cause the formation of free radicals. Antioxidant compounds eliminate the harmful effects of free

radicals (Gökpınar et al. 2006). In this master's thesis study, the antioxidant activity of the samples was determined by calculating the total DPPH value.

The aim of this study was to evaluate the effect of aronia and collagen on the sensory, physical and chemical properties of strained yogurt ice cream, as well as to determine the antioxidant activity to investigate the synergistic effect between aronia and collagen. For this purpose, it was investigated whether the antioxidant activity would be increased in the presence of collagen by adding different amounts of collagen to the products obtained with a fixed amount of aronia. The use of stevia as a sugar substitute has also been tried to improve the nutritional profile and quality of ice cream.

CHAPTER 2

LITERATURE REVIEW

2.1. History of Yogurt

Yogurt, a product with a long history, has been consumed for centuries and carries important cultural and nutritional value. The etymology of the word "yogurt" dates back to Turkish scientists, indicating its historical roots in Turkish culture (Durukoğlu 2017). When historical records are examined, it is stated that it is a Turkish invention and has been produced and consumed in countries under the influence of Turks and Turkish culture for centuries. Yogurt first reached Europe through a Turkish doctor. Suleiman the Magnificent sent yogurt to King François I of France for his treatment. The spread of yogurt in Europe coincides with the beginning of the twentieth century, and its introduction to America coincides with the Second World War (Özden 2008). The production and consumption of yogurt has evolved over time, with different types containing probiotics and emphasizing its benefits for gut health (Neuburger 2022). In conclusion, yogurt is a versatile and valuable food product that has been in human consumption for many years due to its historical importance, cultural diversity, nutritional value and potential health benefits.

2.2. Yogurt

Yogurt has nutritional benefits that include essential nutrients such as protein, calcium, vitamins, and beneficial microorganisms (Guo et al. 2020). Yogurt is actually an acid casein gel based on protein interactions promoted by heat treatment. Just as it contains all the nutrients in milk, it also gains some unique properties and becomes more

valuable due to the changes that occur during the formation of the yogurt gel. It is a very rich product in terms of protein, calcium, phosphorus, vitamin B2, vitamin B1 and vitamin B12. Yogurt proteins have very high biological availability. In addition, they are important in terms of nutritional physiology because their digestibility is higher than milk. In addition, yogurt has higher folic acid, niacin, magnesium and zinc values than milk. At the same time, the biological availability of these minerals and vitamins in yogurt is also high (Sarkar 2019). Since lactose, the milk sugar, is broken down during yogurt production, it is made suitable for consumption by people with lactose intolerance. In addition, thanks to its lipolytic enzyme activity, it breaks down milk fat and increases its digestibility and absorption, thus increasing its value. (Szilagyi 2015).

Yogurt can be made using various types of milk, including cow's milk, goat's milk and sheep's milk. It can also be made with non-animal milk, such as soy milk or almond milk. The type of milk used affects the taste, texture and nutritional content of the resulting yogurt (Köse and Ocak 2014). In addition to the live bacterial cultures used to ferment milk into yogurt, yogurt may also contain other ingredients such as sugar, fruit and flavorings. These ingredients are generally added after the yogurt is fermented and thickened, and these ingredients can also be added to yogurt that has completed all production stages.

The bacteria used to make yogurt are typically *Lactobacillus bulgaricus* and *Streptococcus thermophilus* species, but other probiotic and/or non-probiotic bacterial species may also be used. The texture of yogurts varies depending on its content. Generally, yogurt is classified according to its texture. Greek yogurt (strained yogurt), for example, is strained to remove most of the liquid whey, resulting in a thicker, creamier texture. Normal homogenized yogurts have a thinner consistency. Some types of yogurt are fortified with additional nutrients such as vitamin D or probiotics. The nutritional content of yogurt may vary depending on the type of milk used and the brand or variety. For example, full-fat yogurt typically has more calories and more fat than low-fat yogurt, and may also contain more protein. Yogurt can be a healthy addition to a balanced diet for many people, although some types of yogurt can be high in added sugar. It is important to read the nutrition label and choose yogurt that is low in added sugar and high in protein and other nutrients. Yogurt has been consumed for thousands of years and evidence of its use dates back to ancient times. It is believed to have originated in Central Asia, where nomadic tribes used animal skins to ferment milk. In addition to the health benefits of its

direct consumption, yogurt is also a versatile ingredient in cooking and baking. It can replace sour cream or mayonnaise in sauces and add moisture and flavor to baked goods.

The fermentation process of milk by lactic acid bacteria converts lactose, or milk sugar, into lactic acid, which lowers the pH of the milk and creates an acidic environment that inhibits the growth of harmful bacteria. This ensures a longer shelf life of yogurt.

2.3. Strained Yogurt

Straining yogurt removes more whey components, resulting in a thicker, creamier product with a higher concentration of solids (Bielajew et al. 2020). In terms of sensory properties, strained yogurt has a tangier taste and thicker texture than regular yogurt (Amirrah et al. 2022). This makes it a popular ingredient in many dishes. Also, strained yogurt has a higher protein content and lower lactose content than regular yogurt, making it a popular choice for those with lactose intolerance. In addition, since its concentration of protein and other nutrients is higher, it is frequently used in diets as a nutritious food that can contribute to healthy nutrition. Removing whey also reduces the water content, resulting in yogurt with a higher dry matter content. It also contains less sugar and carbohydrates than regular yogurt, making it a good choice for people following a low-carbohydrate or low-sugar diet. For this reason, strained yogurt is often referred to as "Greek yogurt" because it is traditionally associated with Greek cuisine. But Greek yogurt is also a popular ingredient in many other cultures, including Turkish and Middle Eastern cuisine.

During the straining process, some water-soluble vitamins such as B1 (thiamine) and B2 (riboflavin) were lost. They are dissolved in the whey, which is removed. Additionally, methods being investigated to minimize whey waste and increase efficiency used as a by-product during Greek yogurt production are an area of research for the dairy industry (Flores et al. 2021). It is still a nutritious food due to the high protein, fat and essential amino acids it contains, as well as fat-soluble vitamins A, D, E and K. Greek yogurt is a good source of calcium, which is important for maintaining strong bones and teeth.

Greek yogurt is an excellent source of protein, containing twice as much protein as regular homogenized yogurt. Protein is very important for building and repairing tissues as well as maintaining muscle mass. Like regular homogenized yogurt, Greek yogurt may contain live cultures of beneficial bacteria known as probiotics. Probiotics help improve intestinal health and digestion (Ahmed et al. 2018). It can be used in a variety of dishes, from salty dips to sweet smoothies, soups and cakes.

As mentioned before, strained yogurt is very beneficial for health. One of its main benefits is that it is a rich source of protein, which is important for building and repairing tissues in the body. Some studies have claimed that Greek yogurt may help with weight management and reduce the risk of type 2 diabetes. However, it is important to note that some products may contain added sugar or other ingredients that may negate their health benefits (Doan et al. 2015). For this reason, it is important to read labels carefully and choose strained yogurt that is as plain, unsweetened and additive-free as possible.

2.4. Strained Yogurt Ice Cream

Frozen yogurt made from strained yogurt, also known as Greek yogurt, is a popular frozen dessert made from yogurt and sometimes other dairy products. It is typically lower in fat than ice cream due to the use of yogurt as the base ingredient. Frozen yogurt can be made in various flavors and may contain additional ingredients such as fruits, nuts, and sweeteners. The freezing process gives frozen yogurt a creamy texture similar to ice cream but with a sharp flavor characteristic of yogurt. It is often served as a refreshing treat and can be enjoyed plain or with toppings such as fresh fruits, granola, or chocolate chips. Frozen yogurt, as a healthier alternative to traditional ice cream, offer a lighter option for those looking to a frozen dessert.

According to previous studies, strained yogurt can be used in ice cream making. In a study, the effect of using yogurt on the viscosity, volume, melting properties, pH, acidity, microbiological properties and sensory properties of ice cream was investigated. It was found that using yogurt instead of milk reduced the viscosity of the ice cream mix and exceeded the ice cream's capacity. However, no negative effect of yogurt on the melting properties of ice cream was observed (Asserin et al. 2015). In another study, ice

cream was listed as one of the dairy products that can be made using yogurt (Clark et al. 2008).

The impact of sweeteners and honey supplementation on the quality and sensory attributes of strained yogurt ice cream has been studied extensively. In this study it was found that the addition of honey increased the b* values of yogurt ice cream sweetened with sucrose, stevia, and honey (Arslaner et al. 2019). In addition, it was reported that complete melting times decreased with the increase in sugar content and fruit concentration in vanilla and fruit ice-cream-type frozen yogurts (Güven and Karaca 2002).

2.5. Aronia

Aronia, also known as black chokeberry, is a shrub native to North America that has recently become more popular for its potential health benefits. This fruit is rich in bioactive compounds such as anthocyanins, carotenoids, fatty acids, flavonoids, phenolic compounds and vitamins. Aronia fruit, as can be seen from its color, has a particularly high anthocyanin content, which contributes to antioxidant, anti-inflammatory and anti-aging effects (Choi et al. 2018). These phenolic antioxidants found in aronia berries have been associated with many health benefits.

One of the health benefits of aronia is its potential anti-obesity effect. According to clinical studies, it has been shown that consumption of aronia berries will reduce the accumulation of body fat around the waist (Konstantinidi and Koutelidakis 2019). In addition, another benefit of aronia is that it has heart-protective effects. It has also been found to prevent hypertension and type 2 diabetes. Polyphenols, including anthocyanins and procyanidins, found in aronia berries are thought to contribute to these beneficial effects (Parzonko et al. 2015).

In addition, aronia consumption promises hope in the prevention and treatment of cancer by inhibiting the activity of cancer cells. One study showed that aronia components can inhibit the formation of breast cancer stem cells and reduce the expression of the cellular myelocytomatosis protein, which is involved in cancer cell proliferation. Additionally, it was found that breast cancer stem cell formation was inhibited when

aronia juice was fermented by lactic acid bacteria (Choi et al. 2018). Based on these findings, it is thought that aronia may have potential as an anti-cancer agent.

In addition to all these health benefits, it is also very valuable due to its high polyphenol and anthocyanin content. Thus, it can be used as a suitable raw material in functional food production. The popularity of aronia stems from both its nutritional value and emerging evidence supporting its health-promoting effects (Denev et al. 2018).

Aronia fruit is generally consumed fresh, but it can also be used as juice, jam, dried fruit, added to another product, or as a supplement (Olas 2018). Aronia berries have been found to have a wide range of bioactivities that may benefit human health (Ren et al. 2022). It should be noted that aronia fruit may cause digestive problems in some people and may interact with some medications. Therefore, it is important to consult a healthcare professional before consuming aronia fruit. However, more research is needed to determine therapeutic doses of different aronia products for the future (Olas 2018).

In addition to its anti-cancer properties, one of the medicinal uses of aronia berries is their potential antidiabetic effect. According to research, aronia polyphenols may provide assistance in regulating blood sugar levels and increasing insulin sensitivity (Denev et al. 2012). In this way, aronia berries become a promising product for people with diabetes or at risk of developing diabetes based on their genetic background and lifestyle.

Aronia berries also have a heart-protective effect. The antioxidant activity of aronia polyphenols helps in reducing oxidative stress and inflammation. This is one of the key factors in the development of cardiovascular diseases (Denev et al. 2012). Additionally, it improves endothelial cell function and increases nitric oxide production, which can improve heart health through healthy blood flow (Varela et al. 2016). Studies have shown that the polyphenols in aronia can protect liver cells from damage caused by toxins and oxidative stress (Denev et al. 2012). This indicates that it may be a beneficial fruit for people with liver disease or at risk of liver damage.

Additionally, aronia berries have antimutagenic and anticarcinogenic effects. Polyphenols in aronia berries have been shown to inhibit the growth of cancer cells and reduce the risk of DNA damage (Denev et al. 2012). These findings suggest that aronia berries may have potential applications in the prevention and treatment of cancer.

In addition to its physical benefits, aronia can also be used to reduce psychological disorders such as anxiety and depression. Various phenolic compounds found in aronia

berries have mood-improving effects as they are anti-inflammatory and antidepressant (Tomić et al. 2016).

In conclusion, aronia berries are suitable for various medicinal uses due to their bioactive compounds and antioxidant and anti-inflammatory properties. It had antidiabetic, cardioprotective, hepatoprotective, antimutagenic and anticarcinogenic effects. Additionally, aronia berries have shown potential in reducing anxiety-like and depression-like behaviors.

Freeze-dried aronia is a product very rich in antioxidant activity (Lachowicz and Oszmiański 2016). High antioxidant activity was detected in freeze-dried pomegranate seeds (Gölükcü 2015). Additionally, the freeze-drying process has shown that olive seed antioxidants have high reducing power and may be protective against uncontrolled oxidation (Nakilcioğlu-Taş and Ötleş 2021). This shows that freeze-dried aronia has a high antioxidant capacity. Additionally, it has been stated that phages can remain stable for many years in lyophilized or freeze-dried form (Aydoğan and Hadimli 2016).

These findings suggest that freeze-dried aronia, in addition to its antioxidant properties, is also effective in other applications. Therefore, freeze-dried aronia may be a potential strategy to preserve the antioxidant properties of aronia and use it in various applications. Freeze-dried aronia is used in this study.

2.6. Collagen

Collagen is a structural protein abundant in the extracellular matrix and connective tissues of vertebrate animals, including humans. Since it is the most abundant protein in the human body, it forms the basis of tissue structure and directs cellular functions. Collagen is a trimeric protein. Large intermolecular structures such as fibers and sheets come together, forming unique triple helices. These enable the extracellular matrix to form its barriers and structures (Deshmukh et al. 2016). Collagens undergo many modifications, including extensive hydroxylation of prolyl and lysyl residues, N- and O-linked glycosylation, and processing of proforms. Collagen is a family of proteins that includes 28 different types, each with a unique structure and function. Type I collagen is the most abundant type of collagen in the human body and is found in skin, tendons,

ligaments, bones and teeth. Type II collagen is found in cartilage, while type III collagen is found in blood vessels and internal organs. Other types of collagen include types IV, V, VI, VII, and VIII, which are found in various tissues of the body (Wallace et al. 2012). All types of collagen have their own unique structure and function. Defects in collagen synthesis or structure can lead to various diseases and disorders. There are different types of collagen. The most common types of collagen are types I, II and III. For example, type I collagen is the most abundant extracellular matrix protein in the human body. Collagen IV acts directly in a number of genetic diseases such as Alport and Goodpasture syndromes (Wallace et al. 2012). Injection of collagen as an acellular treatment for heart attack has demonstrated improved LV geometry and cardiac functionality without improved vascularization compared to saline controls (Khoshnoodi, Pedchenko and Hudson 2008).

Since collagen is a protein composed of amino acids, it can be broken down by the digestive system and returned to the body, meaning it is a digestible type of protein. During digestion, collagen is broken down by enzymes into amino acids, which are used by the body for protein synthesis and other functions. The formulations of collagen supplements are generally in the form of hydrolyzed collagen so that collagen can be digested more quickly and effectively. The hydrolysis process breaks collagen molecules into smaller peptides, making it easier for the human body to digest and absorb collagen (Iwai et al. 2005). Studies have shown that collagen can be broken down into smaller pieces using enzymes such as membrane type 1 matrix metalloproteinase (Ohuchi et al. 1997). These enzymes have the ability to digest various types of collagen, including type I, II, and III collagens. Additionally, studies have found that human digestive endoproteinases cleave the peptide bond between prolyl and hydroxyproline residues in collagen (Asai et al. 2019). Accordingly, it can be thought that the human digestive system has specific enzymes that can effectively break down collagen.

Digesting collagen can have various positive effects on the human body. As an example, collagen hydrolyzate, a form of hydrolyzed collagen, has been observed to alter the composition of hydroxyproline peptides in human blood after long-term ingestion (Shigemura et al. 2018). Accordingly, collagen hydrolyzate may affect protease activity in the digestive tract, with potentially beneficial effects observed.

Collagen digestion also applies in the context of specific tissues and conditions. Depending on age and conditions such as diabetes, the solubility and non-enzymatic glycosylation of collagen in the skin may be affected (Schnider and Kohn 1981).

Activation of procollagenases in cartilage plays a crucial role in collagen degradation (Milner et al. 2001). Collagen digestion is also very important during the isolation of the islets of Langerhans, which contain endocrine hormones in the pancreas. The success of islet isolation is related to the digestion percentage of collagen. This can be determined via colorimetry (Meier et al. 2020). Additionally, during the isolation process, digestion of key matrix proteins in the membrane of islets may occur (Cross et al. 2017). In summary, collagen can be digested by humans by the action of specific enzymes. Digestion of collagen may result in various effects on different tissues and conditions.

In short, it proves that collagen can be digested and used by the body when used in hydrolyzed collagen form. The hydrolysis process makes it easier for the body to use collagen-derived amino acids for protein synthesis and other functions. Additionally, the absorption and distribution of hydrolyzed collagen in tissues such as cartilage and skin further supports its digestibility and use by the body.

Understanding the collagen digestion process is important to studying its role in health and disease. A study conducted on mice investigates the absorption and distribution of gelatin hydrolyzate, a form of hydrolyzed collagen. The results show that orally administered gelatin hydrolyzate is absorbed and accumulates in the cartilage of mice. This proves that hydrolyzed collagen can be digested and used by the body (Oesser et al. 1999). Another review article addresses the beneficial effects of hydrolyzed collagen on skin properties. The effect of using collagen peptides on improving skin health is being studied. The article also included several clinical studies supporting the effectiveness of hydrolyzed collagen in improving skin elasticity, hydration, and reducing wrinkles (Sbilla et al. 2015). One of the studies investigated dose-dependent changes in the levels of free and peptide forms of hydroxyproline, a marker of collagen digestion, in human plasma after ingestion of collagen hydrolyzate. The results showed that the levels of both free and peptide forms of hydroxyproline increased significantly after collagen hydrolyzate intake; this suggests that hydrolyzed collagen is digested and absorbed by the body (Shigemura et al. 2014).

Collagen has many benefits for the body. It protects the integrity of tissues and organs, moisturizes the skin, helps maintain its resistance and elasticity, and reduces the risk of developing degenerative joint diseases. Therefore, it is a very important structural protein (Kazirod 2023). Collagen peptides have been shown in some studies to improve skin barrier function, stimulate collagen and hyaluronic acid synthesis, promote fibroblast growth and migration, and improve skin moisture (Asserin et al. 2015). In addition,

collagen supplementation has also been analyzed to evaluate the improvement in health values and prevention of skin aging (França 2023). In addition, collagen has been examined to have antihypertensive, antioxidant and antidiabetic activities and has been found to be beneficial on bone, joint and skin health (Fu et al. 2018). When collagen derived from fish processing waste is used, several potential benefits are provided, such as lower value-added products, prevention of environmental pollution, and prevention of disease spread of mammal-based collagen, as the waste product is processed (Shen et al. 2019). Collagen therapy has been found to have positive effects in different clinical studies, including skin regeneration, bone defects, muscle wasting, wound healing, dental treatment, gastroesophageal reflux, osteoarthritis and rheumatoid arthritis (Wang 2021). A collagen-enriched diet, especially by adding collagen-derived products such as gelatin or hydrolyzed collagen, may provide benefits for the human skeletal system. Overall, collagen is important for maintaining the structural integrity of every tissue in the body (Słupczyńska 2022).

Vitamin C, also known as ascorbic acid, inhibits the collagenase enzyme, reducing its activity and preventing the breakdown of collagen hydrolyzate. It plays an important role in collagen synthesis. In one study, vitamin C is required for the hydroxylation of lysyl residues and the secretion of different types of collagen and has been shown to contribute to the structural integrity of collagen (Paco et al. 2015). Additionally, vitamin C ensures proper collagen formation by acting as a cofactor for enzymes involved in collagen biosynthesis, such as prolyl- and lysyl-hydroxylase (Kypreos et al. 2000). Additionally, vitamin C has been found to increase collagen synthesis by stimulating mRNA levels and enzymes of collagen types I and III in the human dermis (Nusgens et al. 2001).

The antioxidant effect of vitamin C and its ability to facilitate collagen maturation contribute to its positive effect on wound healing and tissue regeneration (Li et al. 2018).

As a result, vitamin C is a vital nutrient that supports collagen synthesis, tissue repair and skin health. Its role as a cofactor in collagen formation and its antioxidant properties make it an important ingredient in the development of products containing collagen hydrolyzate for a variety of applications in medicine, skin care and wound healing. In the development of new products containing collagen hydrolyzate, the positive or negative effects of other ingredients to be used should be taken into account. It was determined that vitamin C, which supports collagen synthesis, was generally used in the product formulations developed in this context. Due to the mentioned positive effects, the

inclusion of vitamin C in formulations containing collagen hydrolyzate contributes positively to the development of functional products. Many experiments have been conducted on the use of collagen in food products. There are a few examples of this: One study examined the results of mixing collagen hydrolyzate into ice cream. This study showed that collagen supplementation increased the nutritional value of ice cream while also increasing its texture and sensory qualities. A study examined on the effects of collagen hydrolyzate on the texture and nutritional value of ice cream. The study found that collagen hydrolyzate, improved the texture of ice cream, making it smoother and creamier (Li et al., 2015). Additionally, adding collagen hydrolyzate to ice cream provides additional protein and amino acids, increasing its nutritional value. However, further research is needed to gain a more comprehensive understanding of the effects of collagen on ice cream and to determine the optimal dose and composition of collagen for various ice cream flavors.

In addition to ice cream products, another study examined the effect of adding collagen to baked foods such as bread and cakes. The results showed that collagen improved the texture and moisture retention of baked goods and also increased their protein content, similar to its effect in ice cream (Nguyen et al. 2018). Because collagen is an important component of meat, the effects of adding collagen to processed meat products have been examined in certain studies. One study found that adding collagen to sausages made them softer and juicier, improve quality factors (Pereira et al. 2011). In addition, collagen as a potential fat substitute has also been investigated as a possible fat substitute in food products. According to one study, using collagen instead of fat in meat products not only produced a product with less fat than the original product, but also produced a product with similar sensory qualities (Ham et al. 2016). These studies show that collagen, in addition to its positive health effects, can improve the texture, moisture retention, and nutritional content of food products. More research is also needed in different types of products to fully understand the effects of collagen on various types of food products and to determine the most effective techniques for adding collagen to food recipes.

Collagen does not have a significant effect on the taste of ice cream (İlhan 2023). It has the potential to increase the stability and shelf life of the product as it limits the growth of ice crystals, making ice cream gritty and less smooth (Damodaran 2007). The presence of collagen can also help prevent ice cream from melting and keep it in shape during storage and transportation. As a result, collagen is often used as a stabilizer in ice

creams as it helps extend the shelf life of the product and prevent the formation of ice crystals. However, it does not directly affect the taste or quality standards of ice cream.

The ingredients and flavors used in ice cream production are the main factors that affect the taste of ice cream. For example, the quality and type of dairy products, sugar and flavors are important factors that affect the taste of ice cream. In addition, the production processes and storage conditions of ice cream also affect the taste and texture of ice cream. Some ice cream manufacturers may decide to use different stabilizers or thickeners instead of collagen, such as gelatin, guar gum or carrageenan. Aside from its nutritional benefits, collagen is also added to ice cream for functional purposes. There are other sources you can consider consuming collagen for health benefits, such as supporting joint health or improving skin elasticity. These may also include bone broth, collagen supplements, and vitamin C-rich foods, which can help your body produce its own collagen. In this study, it is expected to benefit more from various antioxidants such as vitamin C in aronia by using collagen added to the ice cream product.

2.7. Stevia

Stevia is a natural sweetener obtained from the leaves of the *Stevia rebaudiana* plant. It is a popular sugar substitute due to its intense sweetness and minimal calorie content (Iatridis et al. 2022). The most important components of stevia are stevioside and rebaudioside, which are considered safe to use as a sweetener (Li et al. 2015). Stevia consumption has been approved by various food regulatory and safety authorities worldwide, confirming its safety. Despite the potential health benefits of stevia, there is a need for more education about its safety and benefits. Insufficient education about stevia's safety and benefits, as well as concerns about the safety of low-calorie sweeteners in general, may deter health professionals and consumers from recommending or using stevia (Samuel et al. 2018).

In addition to the sensory enhancement of products, that is, its use as a sweetener, the potential health benefits of stevia have also been investigated. It has been found to have antioxidant properties that may help protect against oxidative stress and reduce the risk of chronic diseases (Papaefthimiou et al. 2023). Stevia has been investigated for the

presence of anti-cancer properties, as some of its compounds show antineoplastic activity (Iatridis et al. 2022). Additionally, stevia is also very effective on insulin secretion and blood sugar levels. In this case, it becomes more beneficial for individuals with diabetes (He et al. 2019). It has been shown to have hypoglycemic activity in diabetic rats (Ahmad 2018).

Stevia has been widely used as a natural sweetener as a sugar substitute in the food and beverage industry for many years. The addition of stevia may affect the sensory properties and consumer acceptance of the product depending on the conditions of use. However, it is used for the production of low-calorie products (Reale et al. 2020). Stevia is also suitable for individuals with diabetes and healthy people who want to reduce their sugar intake, as it has a low glycemic index and is calorie-free (Saharudin et al. 2020).

The cultivation and extraction of stevia have been the subject of research as well. Studies have explored the optimization of extraction conditions to obtain bioactive components from stevia leaves (Ameer et al. 2022). Additionally, research has been conducted on the growth and yield of *Stevia rebaudiana* plants, including the effects of fertilization rates and soil water tension on leaf yield and steviol glycoside composition (Mohammed et al. 2019; Parris et al. 2017).

Stevia has potential health benefits, including antioxidant and anti-cancer properties. Like other ingredients to add to ice cream. It is suitable for individuals with diabetes and those who want to reduce their sugar intake. In addition, stevia has been found to have potential health benefits, including antihypertensive, antihyperglycemic, and anti-cavity properties. It may also have antioxidant and antibacterial effects. But more education is needed to raise awareness about the safety and benefits of stevia. Research has also been conducted on the cultivation, extraction and optimization of stevia production.

Research suggests that stevia may have various positive effects on human health. Some of the potential health benefits of stevia include its antihypertensive, antihyperglycemic, and anticavity properties. Stevia has been found to have hypoglycemic effects, making it potentially beneficial for individuals with diabetes. It has also been investigated for its antioxidant properties, which can help protect against oxidative stress and reduce the risk of chronic diseases. Additionally, stevia has been studied for its potential antibacterial effects (Salehi et al. 2019).

The cultivation and extraction of stevia is also a subject of research in itself. Some studies have investigated extraction conditions to obtain bioactive compounds from stevia

leaves (Ameer et al. 2022). In addition, there are also studies on the growth and yield of *Stevia rebaudiana* plants according to the effects of factors such as fertilization and irrigation on leaf yield and steviol glycoside composition (Mohammed et al. 2019; Parris et al. 2017).

Although stevia has many health benefits, more research is needed on its safety and benefits. Today, due to concerns about the safety of low-calorie sweeteners in general, experts and consumers can be very sensitive about their use (Samuel et al. 2018). Moreover, studies have shown that stevia and sucrose loadings significantly lower glucose and insulin levels when compared, making them suitable for use in glycemic control (Iatridis et al. 2022). Products containing stevia have been found to have better sweetening power and maximum consumer acceptability than other sweeteners used instead of sugar (Kamdar et al. 2007). More research is needed to make an accurate decision regarding the use of stevia and/or sugar. Factors such as metabolic health, taste and consumer behavior need to be taken into account during these studies.

One study found that a stevia/sucrose replacement ratio of 1/250 achieved the sweetness of 41.5 grams of sucrose with just 0.166 grams of stevia powder (Zahn et al. 2013). Stevia provides higher sweetness compared to sugar. In the Turkish Food Codex Communiqué on Additives, it is stated that stevia can be used at 100 mg/kg in the flavored fermented dairy products.

CHAPTER 3

MATERIAL AND METHODS

3.1. Materials

3.1.1. Strained Yogurt

Strained yogurt production was made in Balkan Milk and Dairy Products Company (Torbalı, İzmir) according to the company's special recipe. The flow diagram of strained yogurt production is given in Figure 1. Strained yogurt is made by straining yogurt in cloth bags until the desired level of total solids is achieved. This process can be achieved through traditional methods or modern manufacturing techniques. Traditional methods are used in Balkan milk and Dairy Products Company. According to the energy and nutritional elements table, the product composition contains 8% fat, 7.1% carbohydrates and 8% protein. The composition and quality of strained yogurt can be influenced by factors such as heat treatment, straining duration, the use of hydrocolloids, and processing conditions.

Additionally, the production of strained yogurt can impact the viability of microorganisms and potentially reduce the presence of harmful toxins. (Gyawali and Ibrahim 2016). Flow diagrams of strained yogurt and strained yogurt ice cream productions are shown in Figures 1 and 2, respectively.

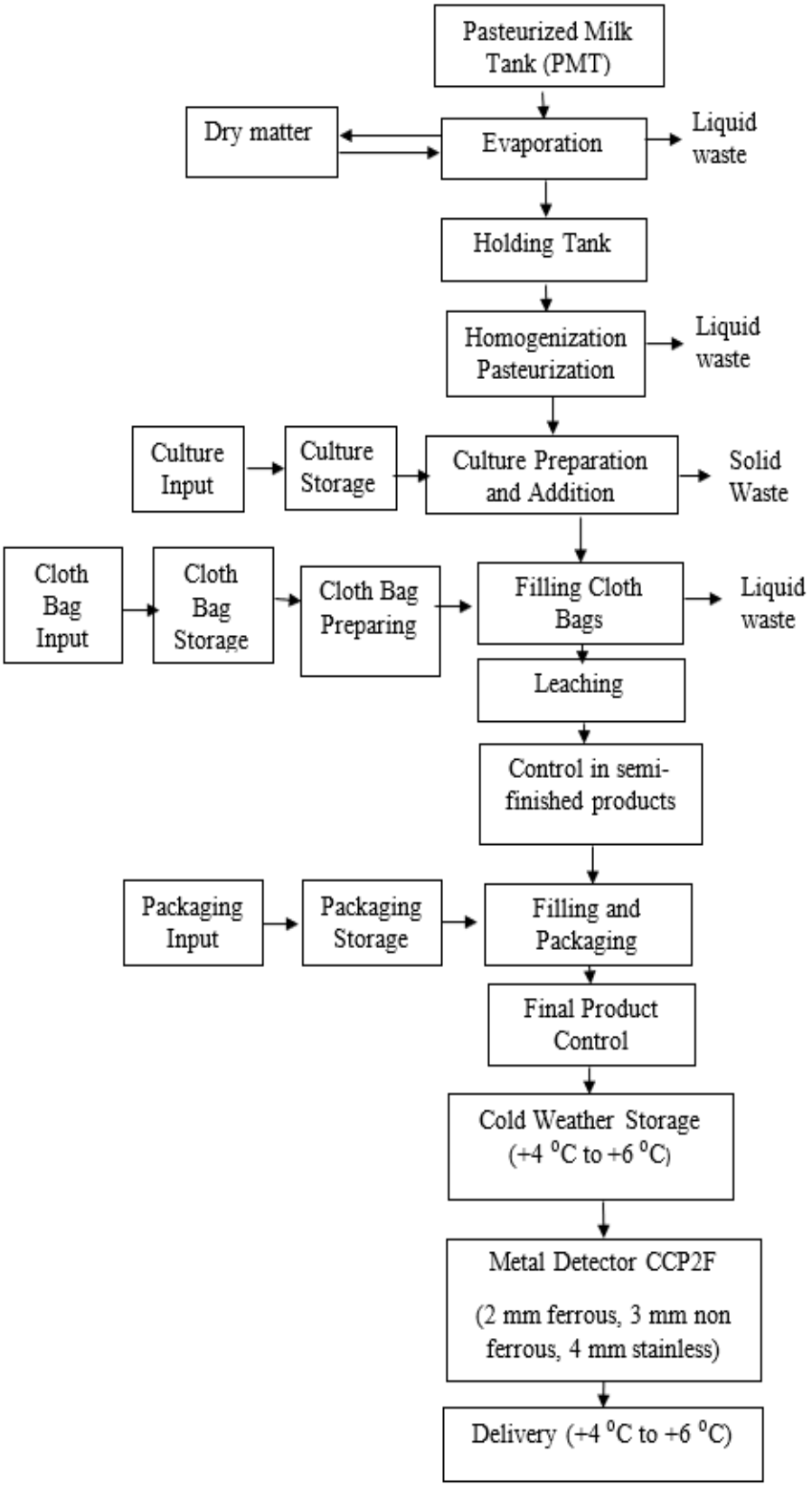

Figure 1. Strained yogurt production flow diagram

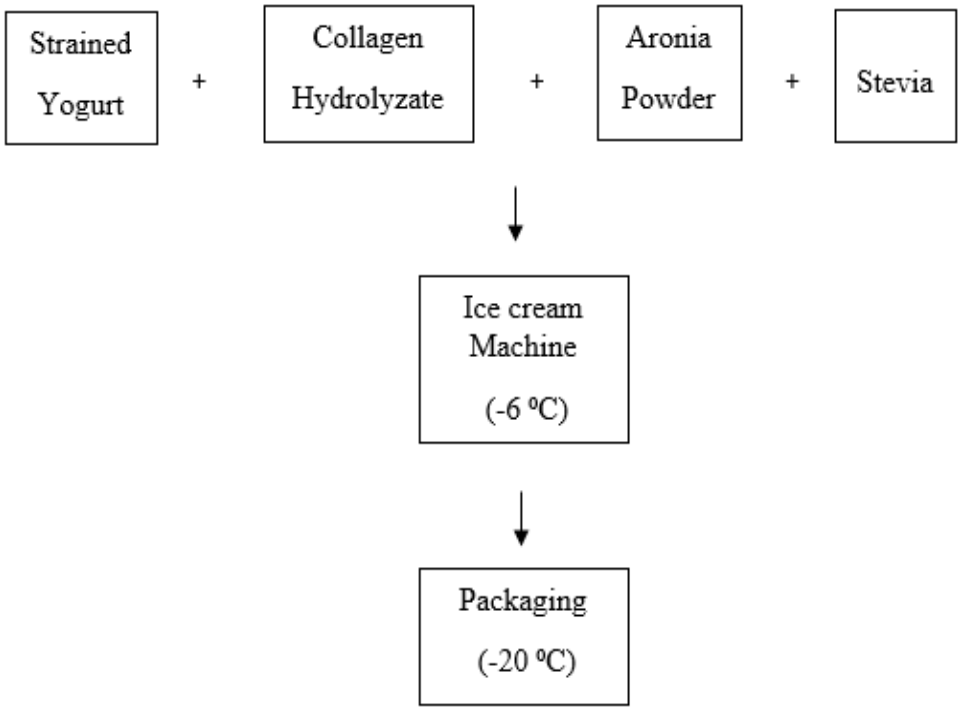

Figure 2. Strained yogurt ice cream production flow diagram

3.1.2. Ingredients Added to Strained Yogurt Ice Cream

Aronia powder, SagaFresh brand, was purchased in freeze-dried powder form from Saga Chemistry in Çorum and it was added directly to the ice cream mixture during production brand.

Collagen, pure type 1 and type 3, was supplied by private pharmaceuticals company located in İstanbul, Turkey.

Takita brand stevia, steviol glycoside, was purchased in powder form from Egepak Food and Packaging Industry Inc,. Izmir. It was used in yogurt ice cream during production.

3.2. Methods

3.2.1. Production of Strained Yogurt Ice Cream

Production was carried out in the Pilot Dairy Factory at the Balkan Milk and Dairy Products Company. First, all products were mixed and passed through to Karaca Frozen Healthy Ice Cream machine. The matured mixtures were frozen in a freezing machine (−5°C; Uğur Cooling Inc., Turkey), and stored at −20°C.

3.2.2. Ingredients of Strained Yogurt Ice Cream

The ingredients of the strained yogurt ice cream samples are given in Table 1.

Table 1. Aronia, collagen and stevia contents for each sample

Sample	Aronia (g)	Collagen (g)	Stevia (g)
***A**	-	-	-
B	20	-	-
C	-	1	-
D	-	5	-
E	20	1	-
F	20	2.5	-
G	20	5	-
E1	20	1	0.006
E2	20	1	0.12

*Control sample

The purpose of this experiment was to investigate the effects of addition of collagen and aronia to strained yogurt ice cream on the structural, physicochemical and sensory properties of ice cream. Additionally, the contribution of the amount of collagen added to the product to the antioxidant activity of aronia was desired to examine.

After determining the amounts of collagen and aronia, sample E was selected according to the antioxidant activity in the results of the DPPH analysis. Stevia was added to the selected E sample within the maximum limit in the Turkish Food Codex to increase sensory appeal. Stevia-added samples were also examined in all analyses.

3.3. Analyses

3.3.1. Proximate Analyses

Yogurt ice cream samples were lyophilized for protein, fat and ash contents analyses. The samples were dried at -80 ^{o}C and then kept in a lyophilizer at -55 ^{o}C and 0.125 mbar for 48 hours.

Figure 3. Samples lyophilized in a lyophilizer (Lablonco, USA)

3.3.1.1. Dry Matter Content Analysis

Dry matter and ash determination of ice cream samples was done by gravimetric method, AOAC, 2000 Official Method 941.08 was used to determine the dry matter amounts of ice cream mixes. In the Balkan Dairy and Milk Products chemistry laboratory, after the empty metal plates are numbered, the first weighing is taken. Then the second weighing is taken by weighing the plate and sample together. Petri dishes are kept in 100±5°C drying in the NÜVE brand oven for 24 hours. After this process, the petri dish is taken out of the oven and cooled in the desiccator and and cooled to room temperature, then weighed again were calculated as % dry matter. The result is calculated using the formula below.

$$\%Dry\ matter = \frac{m3-m1}{m2-m1}\ x100$$

m1: Dry empty drying container and weight of the lid (g)

m2: The weight of the drying container and its lid before the drying process (g)

m3: The weight of the test sample, drying vessel and lid after drying (g)

% Moisture= 100 - % Dry matter

3.3.1.2. Protein Content Analysis

Protein analysis was made according to AOAC Method 930.33. The protein contents of the samples were determined by Kjeldahl method and it was calculated by multiplying the total amount of nitrogen found by the Kjeldahl method by the factor 6.38 for yogurt. One gram dry sample was weighed and placed in the Kjeldahl flask. Twenty mL of concentrated sulfuric acid, 2 catalyst tablets and 1 spatula antifoam were put into

the Kjeldahl flask. The sample was burned at 450 °C for 4 hours in the incinerator. The ammonium released by adding 32% sodium hydroxide was distilled into of 3% boric acid solution and pH drops. The amount of nitrogen was determined by titration the distillate with hydrochloric acid.

3.3.1.3. Fat Content Analysis

Fat analysis was made according to AOAC Method 952.06. Fat analysis in the yogurt ice cream was made according to Soxhlet extraction. One gram of dry ice cream sample was weighed and extraction was done for 2 hours 45 minutes to separate the oil phase. Hexane was used as a solvent. Hexane was removed using a rotary evaporator at 180 ^{o}C. The amount of fat is calculated as follows:

Fat = Initial weight of sample and glass (g) – Final weight of glass (g)

3.3.1.4. Ash Content Analysis

AOAC, 2002 Official Method 945.46 was used for ash determination. One gram of dry sample was weighed in a ceramic container and burned in an ash furnace at 550 ^{o}C for 6 hours until constant weighing was obtained. Afterwards is complete, the ceramic vessel and gray ash were cooled and reweighed.

3.3.1.5. Carbohydrate Content Determination

The amounts of carbohydrates were calculated by subtracting the moisture, fat, protein, and ash contents from the sample weight (Işık 2006).

3.3.2. Physical and Chemical Analyses

3.3.2.1. Determination of pH

The pH values of yogurt ice cream samples were measured using a pH meter (WTW pH 3110).

3.3.2.2. Overrun Test

The basic principle in the overrun experiment was to express the ratio of the mass of a certain volume of ice cream to the mass of the mix of ice cream in the same volume as a percentage. It significantly affected the texture and flavor of ice cream. The result was calculated using the formula below (Atsan 2011). The obtained value should be at most 100%.

$$\%\text{Overrun} = \frac{A-B}{B}\ x100$$

A = Weight of a given volume of unfrozen solution (g)

B= Weight of the same volume of sample after freezing (g)

3.3.2.3. Melting Rate Determination

Twenty grams of ice cream sample was weighed and kept at room temperature. The melting rate was calculated by determining the initial dripping time and the amount dissolved after 30 minutes (Kavaz et al. 2016).

3.3.2.4. Viscosity Analysis

Brookfield DC-II+Pro viscometer (Brookfield, Middleboro, MA, USA) was used for rheological measurements of the molten product. Measurements were made at 10 rpm using spindle coded 64 = LV4. The apparent viscosity value was recorded (Kocatürk 2019).

3.3.2.5. Color Analysis

CIE L *, a *, b * values of the products were measured with the aid of a colorimeter (Konica minolta, CR-400 Japan). (L: lightness-darkness, a: redness-greenness, b: blueness-yellowness).

3.3.2.6. DPPH Analysis

Antioxidant activity of strained yogurt ice cream was determined by 2,2-diphenyl-1-picrylhydrazy (DPPH) method and % inhibition values were calculated. DPPH is method used to determine antioxidant capacity. The DPPH radical forms a purple solution. This radical reacts with antioxidant compounds during free radical screening tests. Antioxidant components neutralize DPPH radicals and reduce them, and as a result of this reaction, the color of the solution changes from purple to yellow. This color change indicates that DPPH radicals are neutralized by antioxidant components.

In DPPH analysis, the antioxidant capacity in the sample is determined by the ability to react with DPPH radicals, which in turn neutralizes free radicals. This reaction is measured spectrophotometrically to determine the amount of antioxidant capacity. Neutralizing DPPH radicals indicates the amount and effectiveness of antioxidant components.

This method is important in evaluating the biological activity of antioxidant components and their ability to fight free radicals. DPPH solution was prepared using 0.0045 g DPPH and 100 mL methanol. For antioxidant activity analysis from ice cream samples, samples were prepared at a ratio of 1:5 using 50% ethanol. Antioxidant activity was determined by the color formed by the substances reacting with 2,2-Diphenyl-1-picrylhydrazyl at a wavelength of 517 nm. Two mL of DPPH was added to 0.5 mL of sample solution. After waiting for 20 minutes at 30 °C, the samples were read using the spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan) (Seyhan 2019). The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Inhibiton (\%)} = ((A_{control} - A_{sample}) / A_{control}))x100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

3.3.2.7. Total Phenolic Contents Analysis

In total phenol content analysis, the method suggested by Spanos and Wrolstad (1990) was used by modification. In the extraction of samples; 1 g of sample was diluted with methanol at a ratio of 1:1, and the resulting mixture was centrifuged at 9000 rpm for 20 minutes. After the centrifugation process, the liquid part of the samples was passed through special filters (Whatman No.1) and used for total phenolic substance and antioxidant activity determinations. The amount of total phenolic substances in ice cream samples was determined according to the Folin-Ciocalteu colorimetric method. Phenolic substances reduced the Folin-Ciocalteu reagent and turned into oxidized form. The blue color formed by the reduced reagent at the end of the reaction was measured spectrophotometrically. Blank sample was prepared with 1 mL of purified water. The extraction sample (0.2 mL) was taken into a glass tube, 1 mL of Folin-Ciocalteu (FC) reagent (1 unit FC: 10 units of pure water, v/v) was added and the mixture was mixed in

the vortex. After 5 minutes of vortexing, 0.8 mL of 7.5% Na_2CO_3 solution was added to the tube contents, shaken and left in the dark for 1 hour (Uysal and Tekyiğit 2023).

For the preparation of the gallic acid calibration curve, solutions at concentrations of 20, 40, 60, 80, and 100 mg/L were prepared. At the end of the period, the absorbance of the sample taken from the tube was read at 760 nm with UV-visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan) against the reference sample prepared with distilled water instead of the extract, and the result was calculated as mg gallic acid equivalent/g from the formula obtained with the help of the gallic acid curve prepared at different concentrations from the gallic acid solution.

3.3.3. Sensory Analysis

Sensory analyses of ice creams were evaluated by a panelist group of 25 people. Sensory evaluation of ice cream samples was made using a hedonic scale. Panelists evaluated the samples based on appearance, odor, texture in mouth, taste attributes and overall acceptability of the samples. (1: dislike extremely, 2: dislike, 3: neither like nor dislike, 4: like, 5: like extremely).

3.3.4. Microbiological Analyses

3.3.4.1. Total Coliform Bacteria Count

Total coliform bacteria count was determined with using violet red bile agar (VRBA) by double-layered pouring method. Ten g of the sample to be analyzed is taken under aseptic conditions and placed in a stomacher bag and mixed 90 mL of peptone water. After homogenization, serial dilutions are prepared. Dilutions were prepared from 10^{-1} to 10^{-2}. Petri plates leaving it at 37°C for 24 hours incubation, and then counting the violet-red colonies formed on the petri plates (Feng 2002).

3.3.4.2. Lactic Acid Bacteria Count

MRS agar was used for lactic acid bacteria count. Ten g of ice cream was placed in the stomacher bag and mixed with 90 mL 0.1 % peptone water. Dilutions were prepared from 10^{-1} to 10^{-7}. Cast plate method was used and plating were done in 2 parallels. The petri dishes were incubated at 30°C for 4 days under anaerobic conditions. All procedures were carried out under sterile conditions (Işık 2006).

3.3.5. Statistical Analysis

The results of the physical, chemical, microbiological and sensory analyses of the products were compared statistically using Analysis of Variance (ANOVA). ANOVA was performed at a 95% confidence interval ($p<0.05$) to define the significant terms of the predictive model. Multiple comparisons were made by using Tukey's test. Minitab statistical software program (v.19.1, Minitab Inc., Pennsylvania, USA) was used for this purpose.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Dry Matter, Protein, Fat, Ash and Carbohyrates Results

In general, dry matter content of yogurt ice creams is in between 28.8 and 34.2%, protein content is in between 3.5 and 3.8%, the fat content is in between 1.8 and 5.9%, and ash content is in between 0.7 and 1.0% (Işık 2006; Tamime and Robinson 2007). The dry matter, protein, fat, ash and carbohydrate contents are shown in Table 2.

Table 2. The results of dry matter, protein, fat, ash, carbohyrates (%)

Sample	Dry Matter	Protein	Fat	Ash	Carbohyrates
A	22.8 ± 0.8^{c}	1.05 ± 0.18^{b}	7.4 ± 0.17^{cd}	1.04 ± 0.01^{a}	13.31
B	45.8 ± 0.02^{a}	1.9 ± 0.02^{ab}	8.3 ± 0.38^{abc}	0.98 ± 0.05^{a}	34.62
C	25.1 ± 0.02^{bc}	1.49 ± 0.40^{ab}	7.6 ± 0.09^{d}	1.05 ± 0.06^{a}	14.96
D	26.5 ± 0.08^{b}	1.19 ± 0.001^{b}	7.3 ± 0.01^{cd}	1.00 ± 0.04^{a}	17.01
E	46.2 ± 0.6^{a}	1.43 ± 0.67^{a}	8.2 ± 0.01^{bc}	1.04 ± 0.60^{a}	35.53
F	46.5 ± 0.18^{a}	1.72 ± 1.21^{ab}	9.5 ± 0.07^{a}	0.99 ± 0.03^{a}	34.29
G	47.1 ± 0.007^{a}	1.67 ± 0.35^{ab}	9.5 ± 0.02^{a}	0.99 ± 007^{a}	34.94
E1	46.4 ± 0.22^{a}	1.68 ± 0.05^{ab}	8.1 ± 0.05^{bc}	1.01 ± 0.07^{a}	35.61
E2	46.6 ± 0.1^{a}	1.85 ± 0.60^{ab}	8.8 ± 0.70^{ab}	0.99 ± 0.10^{a}	34.96

a-d: Significantly different results are indicated by various superscripts in columns. ($p<0.05$).

In our study, dry matter contents of strained yogurt ice creams exceeded the value range in samples containing aronia. The fat contents of the samples were also higher than

the values stated in literature, but this may be due to the fat content of strained yogurt. In addition, while the ash contents of the samples were in accordance with the literature values, the protein contents were quite low compared to the values stated in literature. According to the ANOVA results, there were not any significant differences among samples based on the ash contents. The dry matter, protein and fat contents of yogurt ice creams were significantly different from each other ($p<0.05$).

According to the analysis results, the average dry matter content in sample A, was calculated as 22.8±0.8 %. In samples C and D, where only collagen was added, the results increased to 25.1 ± 0.02 and 26.5 ± 0.08%. Considering that samples B, E, F and G were in the same group, the addition of collagen did not cause a significant change.

Carbohydrate values were also calculated to be used in energy calculation. The total energy of the E1 sample selected by the sensory analysis after adding stevia was 222.06 kcal/g by calculation.

4.2. The pH Values

The pH values of the samples are shown in Table 3. While the pH of strained yogurt (Sample A) was 4.23, it decreased to 4.11 when only aronia was added. When only 5 g collagen (Sample D) was added, the pH value increased to 4.32. When the amount of collagen was increased, the pH value increased to 4.32. The pH value decreased when aronia was added. While the pH of strained yogurt (Sample A) was 4.23, it decreased to 4.11 when only aronia was added. When only 5 g collagen (Sample D) was added, the pH value increased to 4.32. When the amount of collagen was increased, the pH value increased to 4.32. The pH value decreased when aronia was added. When E, F and G were examined, it was observed that the amount of collagen increased the pH value. Additionally, when stevia was added, pH values slightly increased. The pH values were significantly different from each other ($p<0.05$).

Table 3. The pH analysis results

Sample	pH
A	4.23 ± 0.03^{abc}
B	4.11 ± 0.08^{c}
C	4.23 ± 0.21^{abc}
D	4.32 ± 0.14^{a}
E	4.17 ± 0.02^{bc}
F	4.22 ± 0.36^{abc}
G	4.28 ± 0.18^{ab}
E1	4.19 ± 0.04^{abc}
E2	4.20 ± 0.26^{abc}

a-c: Significantly different results are indicated by various superscripts in columns. ($p<0.05$).

4.3. Overrun Results

Volume expansion test in ice cream is a test performed to determine how changing ingredients in ice cream or adding new stabilizers affects the volume properties of ice cream. Research indicates that components of ice cream such as pH, dry matter, fat and protein are effective on overrun. Additionally, some studies show that the antioxidant activity, vitamin C content and other physical properties of ice cream may affect overrun (Gün and Şimşek 2021; Topdaş et al. 2017). According to these studies, overrun is inversely proportional to pH, fat, dry matter and protein, and directly proportional to vitamin C ratio. It has also been reported that as the proportions of fruit puree increase, the resistance of ice cream to melting decreases and the overrun increases (Aloğlu et al. 2018). Therefore, volume increase tests emerge as a very important research area in order to improve product quality or develop new products in the ice cream industry.

While excessive overrun creates a profitable structure, insufficient overrun results in the formation of a wet and heavy structure.

The highest overrun was detected in sample E2 with 16.2%, and the lowest overrun was detected in sample C with 11.1% (Figure 4). The higher overrun in aronia-added samples compared to samples A, C and D may be due to the aronia effect.

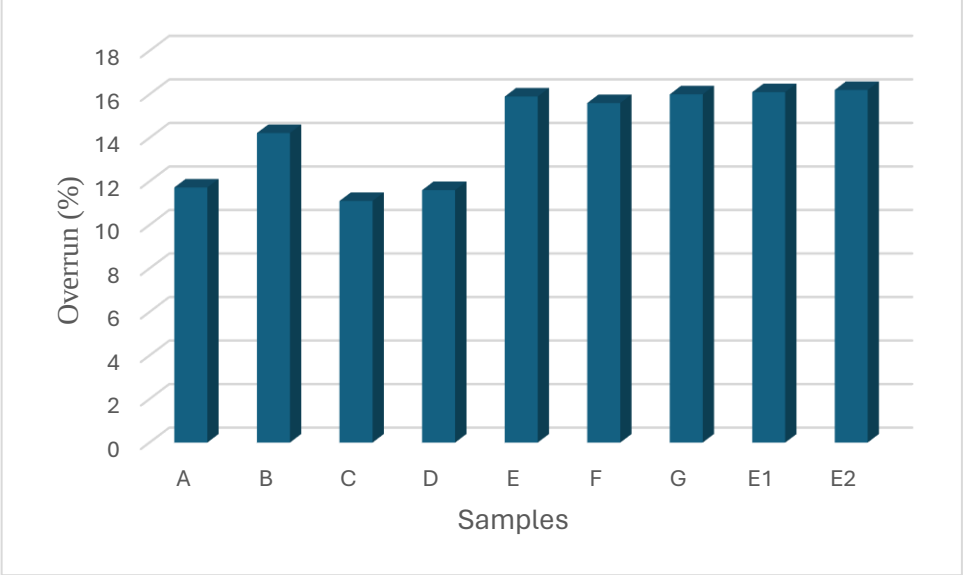

Figure 4. Percentage of overrun results

4.4. Melting Rate Results

Melting property, known as the property most affected by temperature fluctuations, is also considered as a measure of the durability of ice cream during transportation and storage (Erkaya et al. 2012).

In the samples, the dissolution rate at the end of the 30th minute varied between 39.8% and 49.5%. The highest rate was found in sample C and the lowest rate was found in sample F. Ice cream type significantly affected the melting rates of the samples at the 30th minute. The three components that make up the basic structure of ice cream are air cells, ice crystals and fat globules. Although a definitive specific relationship has not yet been established, the physical structure of ice cream affects its melting rate and hardness (Muse and Hartel 2004). It is estimated that the ice crystals formed in samples containing aronia due to the inhibition of the free movement of water have a more stable structure

and, as a result, melt later. It was observed that such an effect did not occur in samples where collagen alone was added.

4.5. Viscosity Results

Apparent viscosity values of ice cream mixes are given in Table 4. According to the results, the ice cream mixture with the lowest apparent viscosity was determined as sample A (324.75 cP), and the ice cream mixture with the highest viscosity was determined as sample F (1444 cP). Apparent viscosity results were found as significantly different from each other ($p<0.05$), but adding stevia did not create any significant change for samples E1 and E2. Powder components added to the product generally increased its apparent viscosity because it binds the water in yogurt. The viscosity of the mix was affected by the composition (especially fat), the mix making process and the total dry matter content (Çeliker 2008). It has been studied that the fat content significantly increased the apparent viscosity (Turgut 2006).

Table 4. Apparent viscosity values (Cp) of ice cream mixes

Sample	Viscosity
A	324.75 ± 1.20^{h}
B	1130 ± 4.24^{e}
C	371 ± 1.41^{g}
D	499 ± 4.24^{f}
E	1304 ± 5.66^{d}
F	1444 ± 1.4^{a}
G	1405.5 ± 0.71^{b}
E1	1362 ± 2.83^{c}
E2	1367 ± 0.07^{c}

a-h: Significantly different results are indicated by various superscripts in columns. ($p<0.05$).

In this study, it was seen that the apparent viscosity values of the mixtures were proportional to the amount of fat they contained (Table 2). The highest viscosity was

detected in samples F and G, which had the highest fat content. Additionally, an increase in viscosity directly proportional to the pH was generally observed.

4.6. Color Results

Color is one of the important factors affecting consumer liking of foods. L*, a*, and b* values of the samples were significantly different from each other ($p<0.05$) (Table 5).

Table 5. Color values of samples

Samples	**L***	**a***	**b***
A	84.81 ± 0.58^{a}	-6.62 ± 0.35^{c}	12.27 ± 0.60^{a}
B	53.25 ± 0.02^{e}	16.62 ± 0.56^{a}	3.61 ± 0.04^{b}
C	84.96 ± 0.81^{a}	-6.56 ± 0.05^{c}	12.52 ± 0.08^{a}
D	85.29 ± 0.007^{a}	-6.45 ± 0.07^{c}	12.48 ± 0.001^{a}
E	55.81 ± 0.85^{d}	16.06 ± 0.45^{ab}	3.76 ± 0.60^{b}
F	57.35 ± 0.02^{c}	15.96 ± 0.007^{b}	3.95 ± 0.04^{b}
G	58.02 ± 0.04^{b}	15.88 ± 0.07^{b}	3.83 ± 0.03^{b}
E1	55.66 ± 0.24^{d}	15.82 ± 0.33^{b}	3.47 ± 0.40^{b}
E2	56.14 ± 0.46^{d}	15.86 ± 0.35^{b}	3.80 ± 0.05^{b}

a-e: Significantly different results are indicated by various superscripts in columns. ($p<0.05$).

While collagen addition generally increased the L* value, it also slightly decreased the a* and b* values. According to the ANOVA results, adding collagen to strained yogurt ice cream did not cause any changes in L*, a* and b* values since samples A, C, and D were in the same group. When samples B, E, F and G were examined, it was determined that adding collagen to the strained yogurt ice cream samples created a difference in the L* value, but the samples were close or in the same group in terms of a* and b* values. In other words, adding collagen to products containing aronia created a difference in L* values, while there was no difference in L* values in products that did not contain aronia.

Therefore, presence of aronia in samples containing collagen affected the L* values of these samples. Adding stevia did not create any significant change because E1 and E2 were in the same group. Since the L* value indicates lightness, it was detected at the highest level in plain and collagen-only ice creams, while in other samples this value was found to be lower due to the color of aronia.

4.7. The Antioxidant Activity Results

Collagen hydrolysates can exhibit antioxidant activities and increase cellular antioxidant capacity (Wang et al. 2018). Additionally, it is stated that collagen peptides obtained from seafood have antioxidant properties and can reduce cell damage (Wang et al. 2020). These studies show that collagen may contribute positively to antioxidant capacity. On the other hand, some studies have obtained different results regarding antioxidant activity. For example, it has been observed that the antioxidant activity of some protein hydrolysates increases depending on the hydrolyzate concentration, but the activity decreases above a certain concentration (Lima et al. 2015). In this experiment, as the amount of collagen increases, the amount of antioxidant activity decreases (Figure 5). This shows that antioxidant activity is a complex process and concentration is an important factor.

In line with the findings obtained from studies on reducing antioxidant activity in general, it can be thought that some strategies may reduce antioxidant activity. Although a specific reference could not be cited due to the lack of studies on reducing antioxidant activity, it is emphasized that research on reducing antioxidant activity is important and more studies should be done in this field. It is stated that studies focusing on reducing antioxidant activity are important to understand the effects of antioxidant activity on health and to control the biological activities of antioxidant compounds.

Factors that reduce antioxidant activity are generally factors that reduce or inhibit the effect of antioxidant compounds. Oxidation is one of the factors that reduce antioxidant activity. Oxidation or reaction of antioxidant compounds may reduce antioxidant activity. This may reduce antioxidant capacity by reducing the stability of antioxidant compounds. The tendency of phenolic antioxidants to react with free radicals

determines antioxidant activity. Therefore, as the oxidation process progresses, a decrease in antioxidant activity may occur. This may result in a reduced capacity of antioxidants to combat oxidative stress and free radicals. In particular, a decrease in antioxidant activity may occur as a result of the reaction of antioxidants with oxidation products (Atalay and Ocak 2019).

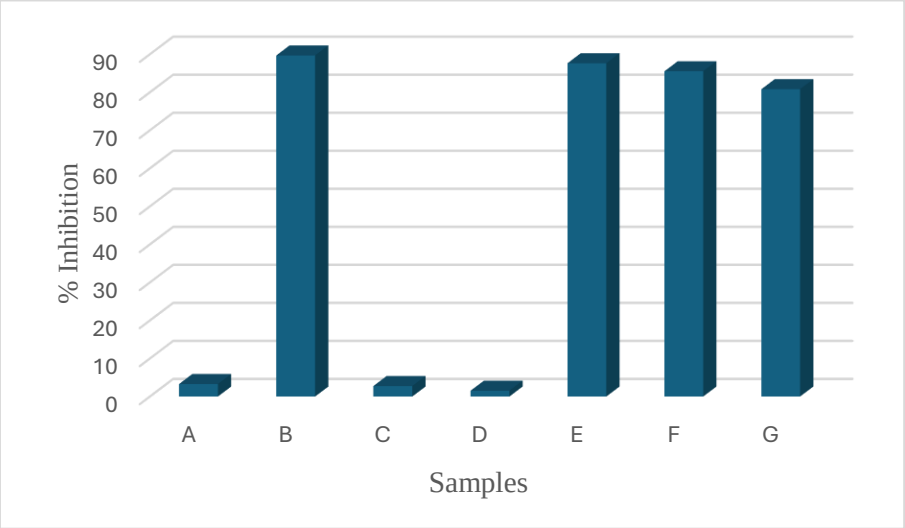

Figure 5. DPPH analysis results

In this study, antioxidant activity decreased as collagen was added (Figure 5). The reason for this may be the oxidation factor. Oxidation is the process where molecules undergo chemical changes as a result of interaction with oxygen. Collagen is a natural protein and can be subjected to oxidation under oxidative stress. For example, Monboisse and Borel (1992) stated that collagen may be subject to oxidative damage. Additionally, Liu et al. (2016) showed in their study with fibroblast cells obtained from human uterosacral ligaments that oxidative stress can lead to collagen metabolic disorders. These studies highlight the susceptibility of collagen to oxidation and the potential effects of this process on tissue health. In this case, collagen molecules may undergo oxidative damage and their structure may change, thus affecting the function and structure of collagen.

Excessive amounts of antioxidants can be shown as the reason for oxidation of collagen. Taking high doses of antioxidants may cause adverse effects in some cases. For example, a study by Bouayed and Bohn (2010) stated that antioxidants may have bidirectional effects on the cellular redox state if antioxidant intake is above physiological

doses. That is, high doses of antioxidants may negatively affect cellular redox balance and lead to detrimental effects on health. As a result, there are some studies in the literature on the harms of excessive antioxidant intake, and it is stated that high doses of antioxidants may affect the cellular redox balance and lead to negative health effects. Therefore, it is important to take antioxidant supplements in a balanced manner and in recommended doses. Additionally, a study stated that taking antioxidant supplements can modulate the antioxidant status and synthesis of hsp70 heat shock proteins, which may create effects at the cellular level (Sari et al. 2021). This supports that antioxidant intake may cause some changes and effects at the cellular level.

Aronia and stevia both show antioxidant properties. When combined in functional fruit and herb beverages, the thermal degradation of anthocyanins in aronia beverages was lower when sweetened with stevia, suggesting a potential synergistic effect between the two in preserving antioxidant compounds (Skąpska et al. 2020). Additionally, the physicochemical and antioxidant properties of aronia marmalades were found to be positively affected by the addition of stevia prebiotic fiber sweetener, further emphasizing the potential benefits of combining these two ingredients (Şengül 2023). Another study revealed a potential interaction between aronia extract and stevia in intestinal health (Vamanu et al. 2023). This indicates a possible interaction between stevia and aronia in affecting the gut microbiota balance. Therefore, it may also have effects on general health.

In conclusion, the combination of stevia and aronia appears to offer a synergistic effect in enhancing antioxidant properties and potentially influencing the gut microbiota.

Although the highest antioxidant activity was detected in sample B, antioxidant activity decreased slightly in sample E to which 1 gram of collagen was added. Therefore, in order to see what effect stevia would have, DPPH analysis was repliacted by adding different amounts of stevia to sample E (Figure 6). Various research studies have highlighted the antioxidant potential of stevia leaves due to the presence of phenolic compounds, flavonoids, and polyphenols (Covarrubias 2018; Gaweł-Bęben et al. 2015; López et al. 2016; Ramya et al. 2021). Additionally, stevia has been found to exhibit antioxidant activity not only *in vitro* but also at the intracellular level, indicating its potential health benefits beyond just sweetness (Bender et al. 2015).

The antioxidant capacity of stevia has been attributed to its polyphenol content, especially chlorogenic acids, which exhibit excellent hydrophilic antioxidant activity (Myint et al. 2020; Xiong et al. 2022). The antioxidant potential of stevia has been demonstrated in different forms such as ethanolic extracts and dry powder extracts,

highlighting its versatility as a natural source of antioxidants (López et al. 2016; Covarrubias-Cárdenas et al. 2018).

Moreover, the antioxidant activity of stevia has been associated with its ability to prevent oxidative DNA damage and lipid peroxidation, indicating its potential in combating oxidative stress (Ghanta et al. 2007; Xiong et al. 2022). In this respect, it is thought that it can prevent the negative effects caused by collagen. Stevia has also been reported to have a synergistic effect with vitamin C, further increasing its antioxidative capacity (Myint et al., 2021). According to this information, stevia has important antioxidant properties that may be beneficial for healthy living.

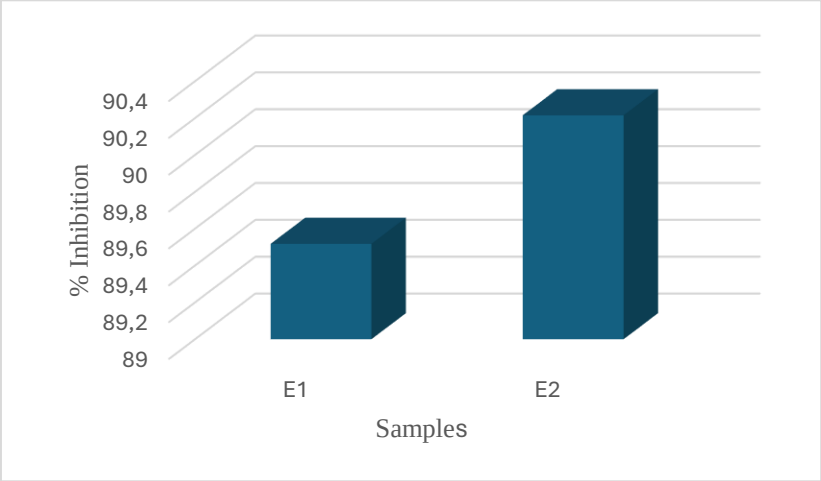

Figure 6. DPPH analysis results after adding stevia

In conclusion, research literature provides significant evidence supporting the claim that stevia increases antioxidant activity due to the presence of various bioactive compounds in its leaves. Consistent with studies in the literature, according to the DPPH analysis performed on sample E, antioxidant activity increases as the amount of stevia added to the product increases (Figure 6). The antioxidant potential of stevia makes it a valuable source of natural antioxidants that can contribute to overall health and well-being.

4.8. Total Phenolic Contents Results

The total phenolic contents of ice cream samples were analyzed spectrophotometrically and calculated using the gallic acid calibration curve (Appendix B.). In this method, the total phenolic content of plants and foodstuffs is expressed as gallic acid equivalent. High amounts of phenolic content have been associated with high antioxidant ability. Therefore, this assay is an important parameter for determining the total antioxidant capacity (Gülçin 2020).

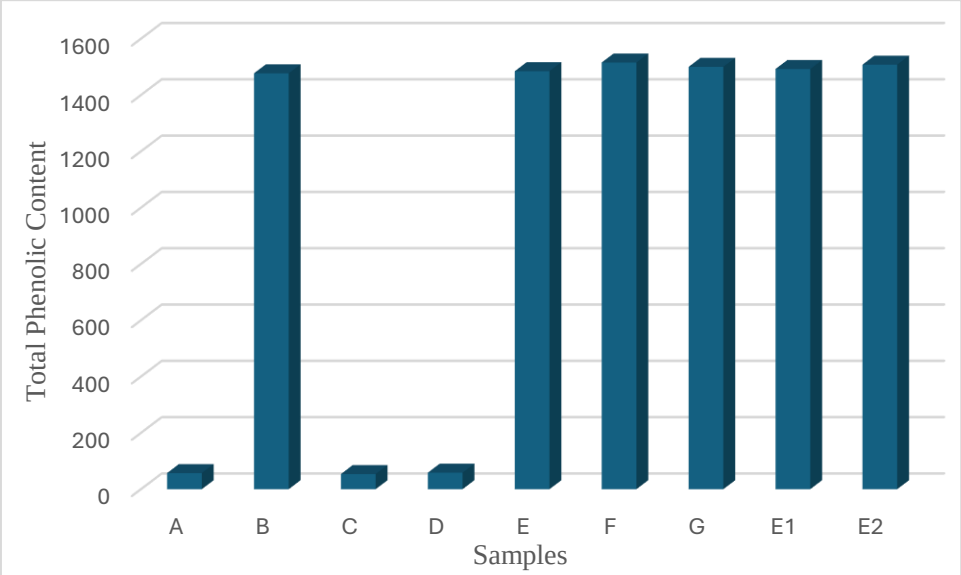

Figure 7. Total phenolic content results

The total phenolic contents determined of ice cream samples varied between 59-1626 mg GAE/100 g in terms of gallic acid equivalent. The total phenolic content and antioxidant activity results of the ice cream samples are shown in Figure 7. It was found that increasing aronia concentration increased the total phenolic content and antioxidant activity of the samples. The presence of collagen did not make a big difference in antioxidant levels, similar to the DPPH results. While the highest antioxidant value according to the DPPH method was in sample B, as a result of this method, the highest antioxidant value was detected in sample F with a value of 1626.53± 0.64 mg GAE/100 g. In terms of total phenolic substance content, sample F was followed by E2 > G > E1 > E > B > D > A > C.

The reason for the detection of phenolic substances in sample A, which is the control yogurt, is not phenolic substances, but due to low molecular weight antioxidant substances, free amino acids and peptides (Akan 2022). In a study, it was observed that in puddings produced using aronia powder and aronia fiber powder, the amount of total phenolic substances increased as the amount of aronia powder increased (Erem 2023). It was observed that with the use of aronia in strained yogurt, which is rich in phenolic substances, the amount of total phenolic content increased approximately 20-25 times compared to the control yogurt.

4.9. Sensory Analysis Results

Sensory analysis results of ice cream produced with the addition of aronia and collagen at different concentrations are given in Table 6. Stevia was added to the selected E sample based on preliminary trials.

Table 6. Sensory analysis results

Samples	Appearance	Odor	Texture in mouth	Taste	Overall Acceptability
A	2.92 ± 1.01^{b}	2.75 ± 0.94^{b}	2.45 ± 1.18^{a}	2.45 ± 1.06^{a}	2.50 ± 0.97^{c}
B	3.83 ± 0.76^{a}	3.0 ± 0.83^{ab}	3.08 ± 1.17^{a}	2.58 ± 1.05^{a}	3.0 ± 1.02^{abc}
C	2.70 ± 1.12^{b}	2.75 ± 0.89^{b}	2.5 ± 1.38^{a}	2.41 ± 1.28^{a}	2.54 ± 1.21^{bc}
D	2.66 ± 1.20^{b}	3.04 ± 0.85^{ab}	4.04 ± 6.2^{a}	2.75 ± 1.19^{a}	2.75 ± 1.11^{abc}
E	3.79 ± 0.78^{a}	3.16 ± 0.63^{ab}	3.08 ± 0.92^{a}	2.62 ± 0.87^{a}	2.96 ± 0.69^{abc}
F	3.79 ± 0.83^{a}	3.33 ± 0.76^{ab}	3.37 ± 1.09^{a}	3.08 ± 1.10^{a}	3.42 ± 0.82^{ab}
G	4.0 ± 0.65^{a}	3.62 ± 0.92^{a}	3.58 ± 0.97^{a}	3.16 ± 0.96^{a}	3.58 ± 0.77^{a}
E1	4.0 ± 0.88^{a}	3.54 ± 0.97^{a}	3.42 ± 0.92^{a}	3.37 ± 1.01^{a}	3.54 ± 0.88^{a}
E2	4.0 ± 0.88^{a}	3.66 ± 0.96^{a}	3.62 ± 1.05^{a}	3.04 ± 1.12^{a}	3.45 ± 1.1^{a}

a-c: Significantly different results are indicated by various superscripts in columns. ($p<0.05$).

A sensory analysis was performed with samples with stevia. According to the general evaluation results, it was determined that the amount of collagen added did not make a significant difference. When looking at the samples, although the amount of added collagen varies, samples A, C, D are in the same group and E, F, G are in the same group in terms of appearance, texture and taste. Secondly, it was determined that aronia was liked by the panelists in terms of appearance, but not in terms of taste. Some panelists commented 'bitter'. Stevia added in different amounts did not make a significant difference because E1 and E2 were in the same group in all parameters. It was determined that the difference between the appearance, odor, taste and overall acceptability properties of the products significantly change ($p<0.05$). The differences in texture properties do not have significant effect ($p>0.05$). The taste properties, which is the purpose of adding stevia, was most appreciated in the E1 sample with 3.37 ±1.01 value.

4.10. Microbiological Analyses Results

In the Turkish Food Codex microbiological criteria communiqué, there is only *Escheria coli* criterion for fermented dairy products. In the study, the number of coliform bacteria in the product was found to be 1 log cfu/g. It is below the limit value ($m< 3$). Consumption of the product produced did not pose any threat to human health against coliform group bacteria. However, since the product is a frozen product, its shelf life must be determined by waiting for a long time and evaluating any sensory or chemical quality losses that may occur in the product.

In the study, lactic acid bacteria counted in strained yogurt was detected as $4.1x\ 10^7$ cfu/g. It has been determined that the number of lactic acid bacteria counted in products stored at -20°C after freezing has decreased, although they maintained their viability. Respectively, the number of LAB determined as $2.3x10^7$ cfu/g, $1.6x10^7$ cfu/g, $1.2\ x10^6$ cfu/g, $1,1\ x10^5$ cfu/g, $1,8x10^7$ cfu/g, $2.1x10^7$ cfu/g, $2.6x10^6$ cfu/g, $1,9x10^6$ cfu/g and $1,1x10^6$ cfu/g. LAB numbers decreased after storage. Similar results have been reported in different studies. One study shows *L. acidophilus* as $1.5x10^8$ cfu/mL and *B. bifidum* as $2.5x10^8$ cfu/mL at the beginning of the storage period in the fermented mix

theprepared by adding *L. acidophilus* and *B. bifidum*. At the end of the 17 week storage period, these numbers decreased to $4x10^6$ and $1x10^7$ cfu/mL (Başyiğit et al. 2005).

One study found that a whole-fat yogurt ice cream sample contained significantly more LAB than reduced-fat samples. The reduction of fat (and therefore total solids) resulted in a significant decrease in the number of LAB (Işık 2006). In another study, ice cream was produced from plain and strawberry yogurts made from cow milk and stored in a deep freezer at -23°C for 12 weeks. When the total LAB count of yogurt ice creams was examined, it was found that strawberry samples had higher values ($6.9x10^7$-$8.70x10^7$ cfu/mL), and in plain ones this value varied between $7.60x10^5$ cfu/mL and $4.0x10^6$ cfu/mL (Var et al. 2000).

CHAPTER 5

CONCLUSION

In recent years, consumer preferences have been towards consuming foods containing antioxidants. Therefore, the demand for functional foods with high nutritional value has increased. While hypotheses suggesting a relationship between chronic diseases and nutrition reveal the positive effects of functional compounds found in fruits and vegetables on health, the tendency to consume these products is becoming increasingly widespread. In this study, yogurt cultures, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, was used in strained yogurt production. Physical, chemical, textural, microbiological, and sensory properties were determined by adding certain concentrations of aronia, hydrolyzated collagen, and stevia to strained yogurt.

Antioxidant activity was also determined in the presence of collagen, which was the main purpose of this study. In this study, we expected collagen to increase antioxidant activity, based on the information in previous studies. However, as a result of DPPH analysis, it was determined that antioxidant activity decreased as the amount of collagen increased in the samples having aronia. Based on studies in the literature, it can be thought that this negative effect can be attributed to the very high doses of antioxidants contained in aronia. Further studies on this subject need to be conducted to understand the full mechanism of action. As a result, ice cream manufacturers can add stevia to products as a sugar substitute, as it both increases antioxidant levels and preferred by consumers. More research is needed to discuss whether collagen should be added or not. In order to contribute to the food industry and literature, further studies can focus on the effects of the presence of collagen by using different antioxidant-rich fruits.

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APPENDICES

APPENDIX A.

SENSORY PANEL FORM

	APPEARANCE	ODOR	TEXTURE IN MOUTH	TASTE	OVERALL ACCEPTABILITY
627					
179					
953					
546					
231					
189					
828					
624					
126					

5-Like extremely 4-Like 3-Neither like nor dislike 2-Dislike 1-Dislike extremely

APPENDIX B.

GALLIC ACID STANDARD CURVE