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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Investigation of the Bioaccessibility of Functional Ice Cream with Blueberry Enriched with Whey Protein Gel

Peynir Altı Suyu Protein Jeli ile Zenginleştirilmiş Yaban Mersinli Fonksiyonel Dondurmanın Biyoerişilebilirliğinin Araştırılması

Halime PEHLİVANOĞLU[1*], Zeynep SUNAL[2], Mustafa YAMAN[3], Aslı AKSOY[4]

Abstract

Ice cream is a complex product obtained by blowing air through special equipment and then freezing a physicochemical mixture consisting of milk, sugar, emulsifier, stabilizer, oil, color and flavoring substances. Recently, especially with the pandemic experienced all over the world, consumers have begun to turn to functional foods that have high nutritional value and are important for health. Functional foods, in addition to its nutritional effects, are defined as foods that have health protective, corrective and/or disease risk reducing effects, depending on one or more effective ingredients, and these effects are scientifically and clinically proven. In order for a product to have functional properties, it must contain bioactive ingredients, probiotic microorganisms and also have a prebiotic effect. For this reason, our study aimed to provide functional properties to ice cream with blueberries, which are rich in phenolic compounds, and to examine the phenolic substance bioaccessibility of this functional ice cream under mouth, stomach and small intestine conditions simulated with the *in vitro* gastrointestinal digestion model system. In this context, functional ice cream was produced by trapping the phenolic rich blueberry fruit in six different concentrations of whey protein gel, and the amount of phenolic substance and protein amount were determined after *in vitro* digestion. While the phenolic substance content of protein gel ice cream in the small intestine environment was between 261-485 µg/100 g and an average of 114 µg/100 g in the control sample, in the oral environment these values were determined as 85-251 µg/100 g in protein gel ice cream and 291 µg/100 g in the control sample. As a result of our study, it was determined that the amount of gallic acid phenolic substance and bioaccessibility of ice cream samples produced with protein gel increased from the oral environment to the small intestine. In the control sample (blueberry ice cream without protein gel), it was observed that the amount of phenolic substance was highest in the oral environment and decreased as it went to the small intestine environment. According to the FAO Guidelines for Use of Nutrition Claims, samples with a whey protein gel ratio of 16%, 18% and 20% can be considered as "protein sources". Thus, in this study, functionalized in terms of protein content and phenolic substance, increased bioaccessibility and high protein ice cream production was carried out.

Keywords: Ice cream, Phenolic, *In vitro*, Digestion, Blueberry, Whey, Protein gel

[1*] **Sorumlu Yazar/Corresponding Author:** Halime Pehlivanoğlu, Department of Food Hygiene, Faculty of Veterinary Medicine, Tekirdağ Namık Kemal University, Tekirdag, Türkiye. E-mail: hpehlivanoglu@nku.edu.tr OrcID: 0000-0003-3138-9568
[2]Zeynep Sunal, Department of Nutrition and Dietetics, Faculty of Health Sciences, Istanbul Sabahattin Zaim University, Istanbul, Türkiye. E-mail: zeynep_sunal123@hotmail.com OrcID: 0000-0001-6186-6594.
[3]Mustafa Yaman, Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Istanbul Sabahattin Zaim University, Istanbul, Türkiye. E-mail: mustafa.yaman@izu.edu.tr OrcID: 0000-0001-9692-0204
[4]Aslı Aksoy, Department of Gastronomy and Culinary Arts, Faculty of Fine Arts, Haliç University, Istanbul, Türkiye. E-mail: asliaksoy@halic.edu.tr OrcID: 0000-0002-7775-6514
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Öz

Dondurma süt, şeker, emülgatör, stabilizatör, yağ, renk ve aroma maddelerinden oluşan fizikokimyasal karışımın, özel ekipmanlar aracılığıyla hava verilmesi ve ardından dondurulması ile elde edilen kompleks bir üründür. Son zamanlarda özellikle tüm Dünyada yaşanan pandemi ile birlikte tüketiciler, besin değeri yüksek ve sağlık açısından önem arz eden fonksiyonel gıdalara yönelmeye başlamışlardır. Fonksiyonel gıda, besleyici etkilerinin yanı sıra bir ya da daha fazla etkili bileşene bağlı olarak sağlığı koruyucu, düzeltici ve/veya hastalık riskini azaltıcı etkiye sahip olup, bu etkileri bilimsel ve klinik olarak ispatlanmış gıdalar olarak tanımlanmaktadır. Bir ürünün fonksiyonel özelliğe sahip olabilmesi için, biyoaktif bileşenler, probiyotik mikroorganizmalar içermesi ve bununla birlikte prebiyotik etkiye sahip olması gerekmektedir. Bu nedenle çalışmamızda fenolik bileşiklerce zengin yaban mersini ile dondurmaya fonksiyonel özellik kazandırılması ve bu fonksiyonel dondurmanın *in vitro* gastrointestinal sindirim model sistemi ile simüle edilen ağız, mide ve ince bağırsak koşullarında fenolik madde biyoerişilebilirliğinin incelenmesi amaçlanmıştır. Bu kapsamda fenolik madde açısından zengin yaban mersini meyvesi 6 farklı konsantrasyondaki peynir altı suyu tozu protein jeli içerisine hapsedilerek fonksiyonel dondurma üretimi gerçekleştirilmiş ve *in vitro* sindirim sonrası fenolik madde miktarı ve protein miktarı belirlenmiştir. Protein jelli dondurmanın fenolik madde miktarı ince bağırsak ortamında 261-485 µg/100 g arasında ve kontrol numunesinde ortalama 114 µg/100 g iken, ağız ortamında bu değerler protein jelli dondurmalarda 85-251 µg/100 g ve kontrol örneğinde 291 µg/100 g tespit edilmiştir. Çalışmamız sonucunda, protein jeli ile üretilen dondurma örneklerinin, ağız ortamından ince bağırsağa doğru gidildikçe gallik asit cinsinden fenolik madde miktarının ve biyoerişilebilirliğinin arttığı tespit edilmiştir. Kontrol numunesinde (protein jeli içermeyen yaban mersinli dondurma) ise fenolik madde miktarının en fazla ağız ortamında bulunduğu, ince bağırsak ortamına gidildikçe azaldığı gözlenmiştir. FAO Guidelines for Use of Nutrition Claims'e göre peynir altı suyu tozu protein jeli oranı %16, %18 ve %20 olan numuneler "protein kaynağı" olarak nitelendirilebilmektedir. Böylece bu çalışmada protein içeriği ve fenolik madde bakımından fonksiyonelleştirilmiş, biyoerişilebilirliği arttırılmış dondurma üretimi gerçekleştirilmiştir.

Anahtar Kelimeler: Dondurma, Fenolik, *İn vitro,* Sindirim, Yaban mersini, Peynir altı suyu, Protein jel

1. Introduction

Balanced nutrition is important for maintaining health and sustaining life. Therefore, the nutrients the body needs should be consumed daily in appropriate proportions. Milk and dairy products, which have an important place in daily nutrients, are a good source of calcium, phosphorus, protein, vitamin B_{12} and vitamin B_2 (Engindeniz et al., 2021). Milk is an essential nutrient source that is secreted after birth in mammals and is important in terms of carbohydrates, proteins, fats, vitamins and minerals. It contains all the nutrients necessary for living things to develop and continue their lives. However, due to the high amount of water in the composition of milk, its rapid deterioration and the difficulty of transportation and storage conditions have made it necessary to transform milk into a more durable product. Food manufacturers have turned to products such as cheese, yoghurt and ice cream, which are easier to evaluate instead of milk. In this context, ice cream, which is a dairy product, that composition can be changed easily stands out as a delicious and generally preferred alternative by consumers of all ages (Fedakar and Turgay, 2020).

Ice cream is a product that is frequently consumed by people, giving energy, easy to digest and has a high nutritional value. Ice cream was first produced by the Chinese, and the first commercial ice cream production took place in Baltimore in 1851 and in Istanbul and Kahramanmaraş in the 1900s in Turkey (Badıllı, 2020). The composition of ice cream generally varies according to the ingredients in its composition and the demands of the market. Depending on the content of the mixture, ice cream contains 3-4 times more fat, carbohydrates and approximately 12-16% more protein compared to milk. The presence of additives such as nuts and fruit increases the nutritional value of ice cream. A standard ice cream contains 8-20% fat, 13-20% sugar, 36-43% total dry matter, 0-0.7% stabilizer and emulsifier (Legassa, 2020).

Functional foods are foods that have positive effects on human health due to the bioactive substances they contain such as phytochemicals, antioxidants, prebiotics, probiotics, proteins, dietary fibers, cholines and oligosaccharides (Mehmetoğlu and Tarakçı, 2023). Today, consumers who are conscious about healthy living have turned to functional foods. With the increase in this demand, the concept of functional food has become very important. When the most consumed functional food types are examined, it is seen that milk and dairy products take the first place (Ürkek et al., 2021).

It is reported that the blueberry fruit used in the production of ice cream has antimutagenic, anticarcinogenic, antibacterial and antioxidant effects and the ice cream produced positively affects human health (Salihler, 2020; Arslan, 2021). However, the fact that foods have functional properties is not enough on its own in terms of benefits for human health. In a food containing a high amount of phenolic substances, losses are observed in the amount of phenolic substances after digestion. For this reason, the important thing is the amount of phenolic substance remaining in the body after digestion. This refers to bioaccessibility. Bioaccessibility is defined as the amount of nutrients in the food that can be removed from the food matrix and ready for absorption in the small intestine after the food is digested (İnce and Çağındı, 2020).

Protein ratio of products can be increased in order to add functional properties to foods. Whey protein is commonly added to products to increase the amount of protein, which is one of the most important types of macromolecules. Whey is a very valuable product containing approximately 20% of milk proteins and has a high nutritional feature. β-Lactoglobulin (β-Lg), α-Lactalbumin (αLa), Immunoglobulin (Ig) contained in whey protein support immunity. In addition, lactoferrin is known to have antifungal, antiviral and antibacterial properties. Also vitamins (riboflavin, folic acid and cobalamin) and minerals (calcium and phosphorus) in whey are also important for health (Yerlikaya et al., 2010).

Since *in vivo* studies are faced with problems such as ethics, time, and cost, studies on bioaccessibility of nutrients are generally examined using *in vitro* systems (Lee et al., 2016; Minekus et al., 2014; Sopade and Gidley, 2009). Structural changes, absorption and digestion of foods can be studied using simulated mouth, stomach and small intestine model systems (Menard et al., 2014).

The primary purpose of this study is to produce ice cream by trapping the phenolic rich blueberry fruit in six different concentrations of whey protein gel: 10%, 12%, 14%, 16%, 18% and 20%. The second aim was to examine the bioaccessibility of these ice creams after *in vitro* digestion.

The pandemic experienced all over the world since 2020 has provided a better understanding of the importance of functional products. In this respect, functional ice cream developed in our study is thought to be important in terms of healthy nutrition. It is thought that the method used in product development will shed light on different studies.

2. Materials and Methods

2.1. Material

Cow's milk, cream, sugar and blueberries used in the production of functional ice cream were supplied from the markets in the Istanbul market. Guar gum, soy lecithin, carboxy methyl cellulose (CMC), sodium chloride (NaCl), pancreatin, sodium hydroxide (NaOH), hydrochloric acid (HCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), lipase, serum albumin etc. digestive solutions were obtained from Sigma (Sigma, St. Louis, MO, USA).

2.2. Preparation of whey protein gel

Studies were designed on optimum concentrations by conducting preliminary experiments at concentrations between 2-20%. 10-12-14-16-18% and 20% whey protein gels were prepared with water, respectively. Freeze dried 10% blueberry in a lyophilizer was added to the protein gel (the ratio of blueberries was kept constant in all whey protein gels). The whey protein gel mixture was mixed first in a magnetic stirrer and then in an ultrasonic stirrer for about 15 min. It was kept in a water bath at 85 °C for 30 min and then cooled to 4 °C.

2.3. Drying

Blueberries supplied frozen were freeze dried in a laboratory scale lyophilizer (Martin Christ, Beta 1-8 LSC plus, Germany) at -60 °C and 1 hPa pressure for 36 h (lower than 2% moisture content on dry basis).

2.4. Production of ice cream

In this study, protein gel was used as a carrier. An ultrasonic mixer (SELECTA® (Spain), 5 min) was used for homogenization using the batch system procedure specified in the study of Goff (2006). For the production of functional ice cream, stabilizer (guar gum, CMC), emulsifier (soy lecithin), sugar, cream and fat were weighed and added to the pasteurized milk sample. The mixture was then mixed until a homogeneous appearance was obtained. This prepared mixture was pasteurized by heating at 85 °C for 5 min. After pasteurization, cooling was carried out at 4 °C and the mixture was left to mature for 2 h at 18 °C. After ripening, protein gel was added to the mixture with vanilla flavor and the mixture was mixed in the ice cream maker (Württembergische Metallwaren Fabrik, Germany) for 15 min. The final product obtained was shaped by pouring into molds and then stored at -20 °C.

2.5. In vitro digestion analysis

The total amount of phenolic substance in functional ice cream was calculated in gallic acid before digestion and after *in vitro* digestion. The method suggested by Yaman et al., (2019) was used in the *in vitro* digestion method. The preparation of digestive solutions is shown in *Figure 1*.

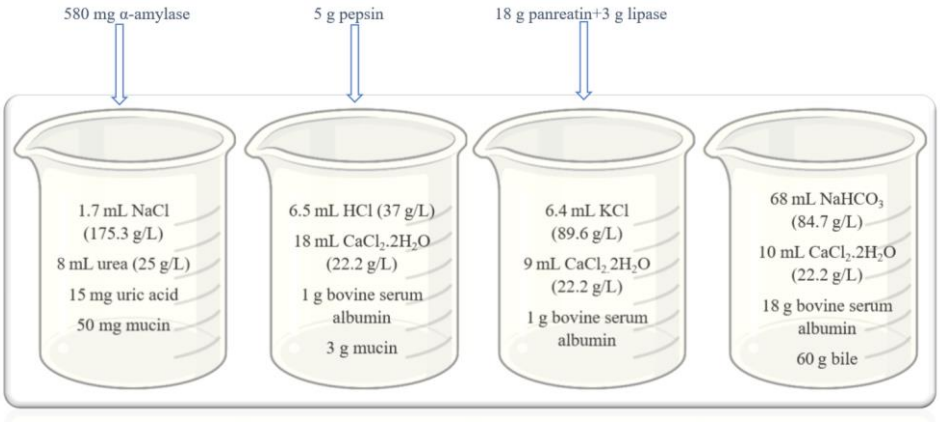

Figure 1. Preparation of digestion solutions

2.6. Digestion simulations

In vitro mouth digestion: 5 g of ice cream sample was weighed in a 100 mL beaker, 10 mL of distilled water was added along with 5 mL of mouth solution, and it was mixed for 30 seconds to make it homogeneous. Homogeneous solutions were incubated in a shaking water bath at 37 °C for 5 min (Yaman et al., 2019).

In vitro gastric digestion: After oral digestion, 10 mL of gastric solution was added and mixed for 30 seconds to make it homogeneous. Homogeneous solutions were incubated in a shaking water bath at 37°C for 2 h.

In vitro intestinal digestion: After gastric environment, 2 mL of 70% $NaHCO_3$ was added to the solution and the pH was maintained to be 7.0. Then, 10 mL of small intestine solution + 5 mL of bile solution were added and the pH was maintained to be 8.0 with 0.1 M HCl and 1 M NaOH. Finally, the samples were mixed in a shaking water bath at 37 °C for 2 h (Yaman et al., 2019).

After digestion, the final volume was diluted to 50 mL with deionized water and centrifuged at 8000 rpm for 5 min at 25 °C and filtered through a 0.45 µm filter (ChromXpert® (CA)) and total phenolic substance was determined by using HPLC.

2.7. Total phenolic substance analysis

Total phenolic substance in blueberry fruit and ice cream samples was extracted with 99.99 purity methanol using ultrasonic extraction method. Then, the amount was determined by HPLC (Bujor et al., 2016).

Extracts were stored at 4 °C until analysis. The extracted samples were mixed in an ultrasonic mixer (Selecta ultrasounds HD) at 4 °C for 15 min. The mixed samples were transferred to 50 mL falcon tubes, centrifuged at 25 °C, 8000 rpm for 5 min and filtered through a 0.45 µm cellulose acetate filter (ChromXpert® (CA)).

The filtrate was injected into the HPLC device using the ultraviolet (UV) detector. The device was adjusted to have a column temperature of 40 °C, a wavelength of 280 nm, an injection volume of 20 µL, and a flow rate of 1 mL/min. The amount of phenolic substances in gallic acid was calculated with the help of the calculation of the peak areas obtained as a result of the analysis (Yaman et al., 2019).

2.8. Protein analysis and energy calculation

Protein determination was made according to AOAC (Anonymous, 1994), energy calculation was made according to FAO (Anonymous, 2003). An evaluation was made regarding the protein and energy amounts of the developed ice creams using the FAO (Food and Agriculture Organisation) Guidelines for Use of Nutrition Claims. Accordingly, the product is defined as a "protein source" if it meets 5% of the nutritional reference value (50 g for protein) per 100 kcal (Anonymous, 2023).

2.9. Statistical analysis

Analyzes were performed in two replications and two parallels. It was evaluated statistically at the 0.05 significance level by applying the Kruskal Wallis Test ($p<0.05$).

3. Results and Discussion

3.1. The effect of whey protein gel on the amount of phenolic substances in ice cream in in vitro environment

Total phenolic content (µg/100g) of functional ice creams prepared with blueberry and different whey protein concentrations are given in *Table 1*. The total amount of phenolic substances for blueberries was determined as 12311 µg/100g.

As seen in *Table 1* and *Figure 2*, the phenolic substance amount of ice cream samples in the oral environment after in-vitro digestion was detected as the lowest in 20% protein gel ice cream as 85 µg/100gr and the highest in the control ice cream sample as 291 µg/100g. In the stomach environment, the lowest value was determined as 117 µg/100g in the protein gel ice cream sample prepared with 20% whey protein, and the highest was 293 µg/100g in the protein gel ice cream sample prepared with 10% whey protein. In the small intestine, the lowest was determined as 114 µg/100g in the control ice cream sample, and the highest was 485 µg/100g in the protein gel ice cream sample prepared with 20% whey protein.

When the results were evaluated statistically, the increase in protein concentration caused a significant decrease in the amount of phenolic substances in the oral environment and a significant increase in the small intestine environment ($p<0.05$). It is thought that these values are due to the fact that the increased protein gel concentration makes the gel structure more stable and stronger, and the gel acts as a carrier by keeping the phenolic substances in the oral environment and transporting them to the small intestine.

Table 1. Phenolic substance amounts of protein gel ice cream prepared with whey protein at different concentrations in in vitro digestion (µg/100g)

Phenolic Substance Amounts (µg/100g)							
	Type of Ice Cream						
	Control	**10%**	**12%**	**14%**	**16%**	**18%**	**20%**
Mouth Digestion	291±17^{a}	251±12^{b}	256±9^{b}	248±15^{b}	176±8^{c}	96±6^{d}	85±6^{d}
Gastric Digestion	286±6^{ab}	293±6^{a}	283±1^{ab}	270±4^{b}	192±4^{c}	132±10^{d}	117±5^{d}
Small Intestine Digestion	114±7^{e}	261±4^{d}	369±14^{c}	442±20^{b}	436±10^{b}	432±15^{b}	485±14^{a}

10%: Sample with protein gel prepared with 10% whey protein, 12%: Sample with protein gel prepared with 12% whey protein, 14%: Sample with protein gel prepared with 14% whey protein, 16%: Sample with protein gel prepared with 16% whey protein, 18%: Sample with protein gel prepared with 18% whey protein, 20%: Sample with protein gel prepared with 20% whey protein.

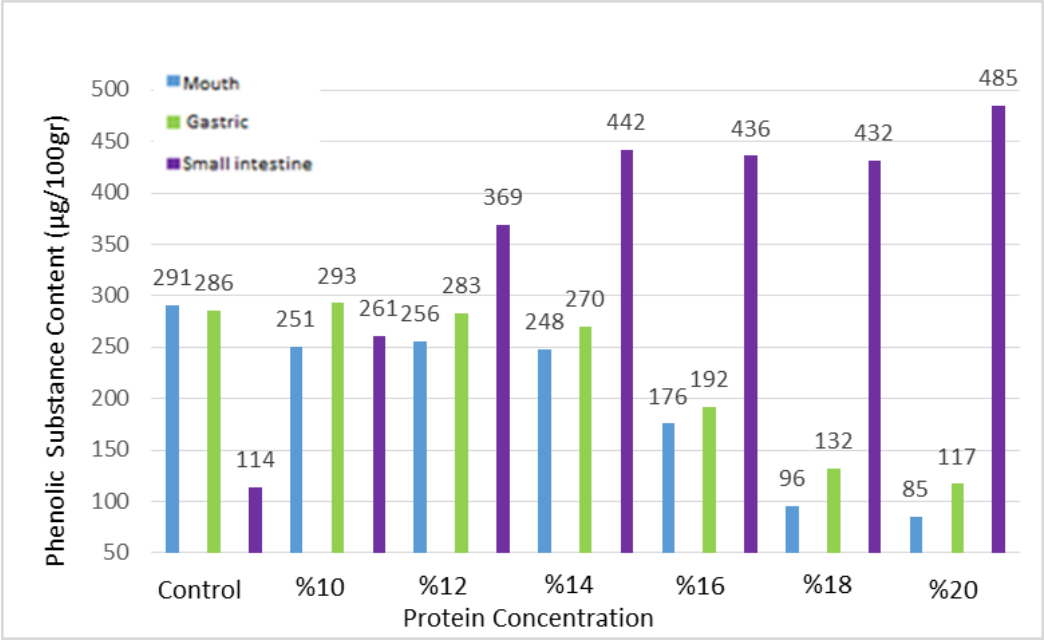

Figure 2. Relationship between protein concentration and phenolic substance content in different digestion stages

Texture analysis was not performed instrumentally, but was interpreted according to the evaluation of the texture parameter in the sensory analysis in the study conducted by Sunal et al., (2022). In the protein gel formation process, it was observed that gelling was better after 20% protein concentration. Because whey protein ~~is whey powder protein, it~~ is resistant to hydrolysis and when it reaches the small intestine, the digestion of proteins takes place. Whey proteins are also known as 'fast' proteins because they empty rapidly from the stomach and are delivered intact to the small intestine (Boirie et al., 1997). For this reason, phenolic substances trapped in the whey protein gel in our samples became free in the small intestine with the controlled release of the gel. In this context, protein gel caused a significant increase in the amount of phenolic substance from the oral environment to the small intestine environment in the *in vitro* system ($p<0.05$) (*Figure 3*). As the gelling power increases, phenolic substances are better kept in the mouth and stomach environment, and due to the gel structure formed, phenolic substances are protected from external factors such as gastric fluid, temperature, pH, enzymes.

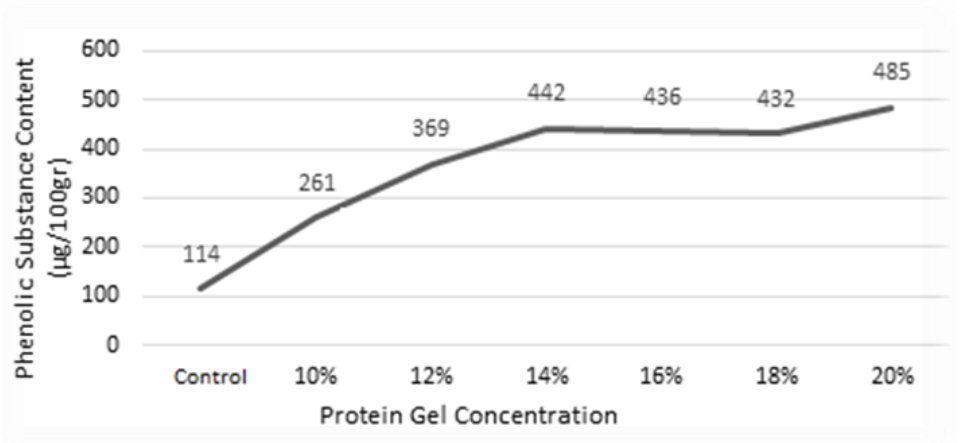

Figure 3. Amount of phenolic substance in in vitro small intestine digestion

Yaman et al., (2019) found that the bioaccessibility of folic acid added to infant formula was affected by gastric pH. The protein gel in our study prevented phenolic substances from being affected by gastric pH. It can be thought that the protein gel retains especially phenolic substances throughout the digestive system and protects them against oxidation.

3.2. Effect of whey protein gel on bioaccessibility

The % bioaccessibility values of the functional ice creams produced in our study were 9.3 for control, 21.2 for 10%, 30.0 for 12%, 35.9 for 14%, 35.4 for 16%, 35.1 for 18% and 39.3 for 20%. Bioaccessibility values were calculated based on the values obtained before and after the initial digestion. Bioaccessibility was determined by dividing the value after digestion by the value obtained before digestion. When these values were compared statistically, it was determined that protein gel had a significant positive effect on % bioaccessibility ($p<0.05$). When the effect of protein concentration on bioaccessibility is examined, the ideal protein concentration can be considered to be 20%. Proteins, as biological macromolecules, are reported to be good carriers for bioactive components (Ju et al., 2020). In our study, it was determined that the increased protein concentration provides bioaccessibility by keeping the phenolic substance better in the mouth and stomach environment and delivering it to the small intestine. The protein gel in this study acted as a kind of microencapsulation, so that phenolic substances were transported with an easier method. In a study by Betz et al., (2012), anthocyanin rich blueberry extract (BE) was microencapsulated using whey protein gels and it was determined that the loss of BE compounds of the capsules prepared by the emulsion method reduced TEAC. In another study, it is stated that filamentous gels support intracellular iron absorption and these results show promise for filamentous gels to transport iron and increase its absorption, and therefore should receive great attention in the development of innovative functional foods (Remondetto et al., 2004).

İncedayı (2021) investigated the bioaccessibility of pomegranate syrup and pomegranate sauce after gastrointestinal digestion, and as a result of the study, it was found that the total amount of phenolic substances decreased in these samples after *in vitro* digestion, and the bioaccessibility was 74% for pomegranate molasses and 81% for pomegranate sour sauce. As a result of our study, it was determined that phenolic substances in the gastrointestinal system were adversely affected by pH, enzyme, temperature, etc. and were damaged before it reaches the small intestine. When *Table 2* is examined, it can be seen that the protein gel protects the phenolic substances during *in vitro* digestion and has a positive effect on bioaccessibility. Both our study and the study by İncedayı (2021) show that phenolic substances are adversely affected during *in vitro* digestion.

In a study by Hatamipour et al. (2019), curcumin, bisdemethoxycurcumin, and desmethoxycurcumin were found to decrease in the bioaccessibility values of 89%, 87%, and 87%, respectively, in the small intestine after *in vitro* digestion. It was determined that these rates increased significantly after nanoencapsulation, and encapsulation with nanomicelles increased bioaccessibility as in protein gel ($p<0.05$).

In another study, it was determined that the release of unencapsulated raw propolis in the small intestine environment was quite low under *in vitro* digestion conditions, and more than 95% of propolis encapsulated with alginate biopolymer was released at pH 7.4. In addition, it was determined that propolis capsules coated with alginate completely dispersed in the simulated small intestine environment after 2 hours, releasing all of the active ingredients in it, so that the alginate capsules released at the target point at a high rate as desired (Keskin, 2018).

All studies have shown that the stomach and intestinal environments negatively affect the stability of phenolic substances and cause a decrease in the amount of phenolic substances, and the gelation or encapsulation process applications have positive effects on the digestion of the active ingredients and their releases in the small intestine.

3.3. Effect of whey protein concentration and temperature on gelling

Whey protein is used in many food products due to its water binding, emulsion and gelling properties (Damodaran, 1994).

In our study, it was determined that the gelation time decreased with increasing whey protein concentration. As a result, it was determined that increasing protein concentration contributed positively to gelation, and the shortest gelation time was at 20% protein concentration, and the longest gelation time was at 10% protein concentration ($p<0.05$). Values were given in *Table 2*.

Table 2. Relationship between whey protein concentration and gelation time

Protein concentration	Gelation time
10%	45 min
12%	40 min
14%	35 min
16%	30 min
18%	25 min
20%	20 min

Similarly, in a study by, it was found that the higher the protein concentration, the longer the gelation time in whey proteins prepared at the rate of 12%, 15%, 18% by keeping the pH constant (pH: 10.0) was found to decrease. The values in our study are consistent with the mentioned study (Gunasekaran, et al., 2007).

In another study by Opazo-Navarrete et al., (2018), it was emphasized that when the protein concentration is high enough, aggregation causes a strong gel formation, while aggregation leads to precipitation of isolated proteins when the protein concentration is low. When our study is compared with the previous studies, it can be concluded that the results obtained are similar.

In order to determine the best gelling temperature, preliminary experiments were carried out at different temperatures and it was determined that the best gel structure was formed at 85 °C. Since a strong gel structure does not form at temperatures below 85 °C and the transport of phenolic substances to the small intestine was detected at lower values, it was studied at 85 °C. Similarly, in a study it was determined that heating O/W emulsions stabilized with whey protein isolate (WPI) up to 85°C prevents proteolysis by making whey protein isolate (WPI) more resistant (Li et al., 2013).

3.4. Protein and energy amount of developed ice creams

The protein and energy content of the developed ice creams and the health claims that can be made according to the FAO Guidelines for Use of Nutrition Claims are shown in *Table 3*. According to the FAO Guidelines for Use of Nutrition Claims, if the product meets 5% (2.5 g) of the nutritional reference value (50 g for protein) per 100 kcal, the product is defined as a "protein source", and if it provides 2 times the source value (5 g), the product is defined as "high protein".

As seen in *Table 3* the energy values and protein ratios of the ice cream samples increased depending on the increased whey protein concentration. According to FAO, protein gel ice cream samples prepared with 16%, 18% and 20% whey protein can be defined as protein sources.

Table 3. Health claim that can be made with protein and energy ratios of ice cream samples

Ice cream samples	Energy (kcal/100 g)	Protein (%)	Health claim
Control	215.01	2.12	-
10%	200.72	3.84	-
12%	202.83	432	-
14%	204.95	4.80	-
16%	207.07	5.28	protein source
18%	209.19	5.76	protein source
20%	211.31	6.24	protein source

4. Conclusions

In our study, which we have carried out starting from the concept of functional food, that has gained great importance in line with consumer demands in recent years, ice cream, which is a healthy and delicious dairy product, has been made a more beneficial product in terms of health by gaining functional properties and increasing its bioaccessibility. Within the scope of this purpose, six functional ice creams were developed by trapping the functionally rich blueberry fruit (10%) in the protein gel obtained with 10%, 12%, 14%, 16%, 18% and 20% whey protein. Thus, the functionality of the samples in terms of protein amount and their bioaccessibility in terms of phenolic substances were determined in mouth, stomach and small intestine conditions simulated with the in vitro gastrointestinal digestion model system.

In the blueberry ice cream (control) sample that does not contain whey protein gel, the amount of phenolic substance is at least 114 μg/100g in the small intestine and at most 291 μg/100g in the oral environment. In the protein gel ice cream samples prepared with whey protein, it was determined that the amount of phenolic substance was at least 85 μg/100g in the oral environment, and the maximum was 485 μg/100g in the small intestine.

It is thought that the protein gel protects against oxidation, especially by keeping phenolic substances throughout the digestive tract, thus increasing bioaccessibility. In addition, proteins can be used in functional product formulations due to their gelling properties. Therefore, if this study is supported by *in vivo* and other *in vitro* studies, that it can guide functional product development studies.

The increase in protein concentration caused a significant decrease in the amount of phenolic substances in the oral environment and a significant increase in the small intestine environment ($p<0.05$). It has been determined that increasing protein concentration makes the gel structure more stable and stronger, and plays a carrier role in terms of phenolic substances in the gastrointestinal tract. As the protein gel concentration and temperature increased, the gelation time and capacity increased, and the optimum temperature for gel formation was determined to be 85 °C.

In addition, protein ratios and energy values of the control ice cream sample and the developed ice cream samples were determined. Depending on the increased whey protein concentration, the energy values and protein ratios of the products have also increased. According to FAO, protein gel ice cream samples prepared with 16%, 18% and 20% whey protein can be considered as protein sources.

The protein gel ice cream sample, prepared with 20% whey protein, is the ice cream type with the highest gelation, bioaccessibility and protein content. Therefore, it is thought that this sample, which is the strongest in terms of functionality, can be preferred for industrial production.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

The study was conducted within the scope of Zeynep Sunal's MSc thesis. Concept: Pehlivanoğlu, H., Aksoy, A.; Design: Pehlivanoğlu, H., Aksoy, A.; Data Collection or Processing: Pehlivanoğlu, H., Sunal, Z., Yaman, M.; Statistical Analyses: Yaman, M., Sunal, Z., Pehlivanoğlu, H.; Literature Search: Pehlivanoğlu, H., Aksoy, A., Sunal, Z.; Writing, Review and Editing: Pehlivanoğlu, H., Aksoy, A., Yaman, M.

References

Anonymous (1994). Association of Official Analytic Chemists (AOAC). Nitrogen (total) in milk - Kjeldahl methods (No. 991.20).

Anonymous (2003). Food and Agriculture Organization of the United Nations (FAO). Food and Nutrition paper 77: Food energy—Methods of analysis and conversion factors. FAO: Rome, Italy.

Anonymous (2023). Food and Agriculture Organization of the United Nations (FAO). https://www.fao.org/3/y2770e/y2770e06.htm#fn19 (Accessed Date: 26.02.2023)

Arslan, M. (2021). The place of functional foods in health. Güven Plus Group Inc. Publishing, Istanbul.

Badıllı, A. G. (2020). Investigation of possibilities of chickpea flour usage opportunities instead of milk powder in reduced fat functional ice cream production (MSc Thesis). Harran Üniversity, Sanlıurfa.

Betz, M., Steiner, B., Schantz, M., Oidtmann, J., Mäder, K., Richling, E. and Kulozik, U. (2012). Antioxidant capacity of bilberry extract microencapsulated in whey protein hydrogels. *Food Research International*, 47(1): 51-57.

Boirie, Y., Dangin, M., Gachon, P., Vasson, M. P., Maubois, J. L. and Beaufrère, B. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proceedings of the National Academy of Sciences*, 94(26): 14930-14935.

Bujor, OC, Le Bourvellec, C., Volf, I., Popa, V.I. and Dufour, C. (2016). Seasonal variations of the phenolic constituents in bilberry (*Vaccinium myrtillus* L.*)* leaves, stems and fruits, and their antioxidant activity. *Food Chemistry*, 213: 58-68.

Damodaran, S. (1994). Structure-function relationship of food proteins. In: Protein Functionality in Food Systems, New York, USA.

Engindeniz, S., Taşkın, T., Gbadamonsi, A. A., Ahmed, A. S., Seioudy, A., Kandemir, Ç. and Koşum, N. (2021). Analysis of preferences for milk and milk products of consumers. *Journal of Tekirdag Agricultural Faculty*, 18(3): 470-481.

Fedakar, F. and Turgay, Ö. (2020). Some properties of Maras ice cream. *Journal of Food and Feed Science Technology*, (23): 19-24.

Goff, H. D. (2006). Ice Cream. In: Advanced Dairy Chemistry Volume 2 Lipids. Springer, US.

Gunasekaran, S., Ko, S. and Xiao, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. *Journal of Food Engineering*, 83(1): 31-40.

Hatamipour, M., Sahebkar, A., Alavizadeh, S. H., Dorri, M. and Jaafari, M. R. (2019). Novel nanomicelle formulation to enhance bioavailability and stability of curcuminoids. *Iranian Journal of Basic Medical Sciences*, 22(3): 282.

İnce, C. and Çağındı, Ö. (2020). Iron mineral: Functions, effects of food processing on bioavailability and interactions with bioactive components. *Çukurova Journal of Agriculture and Food Sciences*, 35(2): 151-164.

İncedayi, B. (2021). Assessment of antioxidant properties and in-vitro bioaccessibility of some pomegranate products. *Journal of Balikesir University Institute of Science and Technology*, 23(1): 96-110.

Ju, M., Zhu, G., Huang, G., Shen, X., Zhang, Y., Jiang, L. and Sui, X. (2020). A novel pickering emulsion produced using soy protein-anthocyanin complex nanoparticles. *Food Hydrocolloids*, 99: 105329.

Keskin, M. (2018). Comparing the release of alginate-propolis micro capsules in an *in vitro* digestion system with the release of raw propolis. *Uludag Bee Journal*, 18(2): 94-100.

Lee, S. J., Lee, S. Y., Chung, M. S. and Hur, S. J. (2016). Development of novel in vitro human digestion systems for screening the bioavailability and digestibility of foods. *Journal of Functional Foods*, 22: 113-121.

Legassa, O. (2020). Ice cream nutrition and its health impacts. *Journal of Food Nutritional Science*, 7(1): 19-27.

Li, J., Ye, A., Lee, S. J. and Singh, H. (2013). Physicochemical behaviour of WPI-stabilized emulsions in in vitro gastric and intestinal conditions. *Colloids and Surfaces B: Biointerfaces*, 111: 80-87.

Mehmetoğlu, S. and Tarakçi, Z. (2023). Investigation of the physicochemical properties of propolis added ice creams during storage. *Journal of Tekirdag Agricultural Faculty*, 20(2): 361-373.

Ménard, O., Cattenoz, T., Guillemin, H., Souchon, I., Deglaire, A., Dupont, D. and Picque, D. (2014). Validation of a new in vitro dynamic system to simulate infant digestion. *Food Chemistry*, 145: 1039-1045.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function*, 5(6): 1113–1124. https://doi.org/10.1039/c3fo60702j

Opazo-Navarrete, M., Schutyser, M. A. I., Boom, R. M. and Janssen, A. E. M. (2018). Effect of pre-treatment on in vitro gastric digestion of quinoa protein *(Chenopodium quinoa Willd.)* obtained by wet and dry fractionation. *International Journal of Food Sciences and Nutrition*, 69(1): 1-11.

Remondetto, G. E., Beyssac, E. and Subirade, M. (2004). Iron availability from whey protein hydrogels: an in vitro study. *Journal of Agricultural and Food Chemistry*, 52(26): 8137-8143.

Salihler, K. (2020). *Studies on the evaluation of leaves and fruits of Vaccınıum myrtıllus l. (yabanmersini) plant growing in our country by the food and cosmetic industry.* (MSc Thesis) Bezmialem Foundation University, Institute of Health Sciences, Istanbul.

Sopade, P. A. and Gidley, M. J. (2009). A rapid in-vitro digestibility assay based on glucometry for investigating kinetics of starch digestion. *Starch-Stärke*, 61(5): 245-255.

Sunal, Z., Pehlivanoğlu, H., Aksoy, A. and M., Yaman (2022). Determination of sensory acceptability of functional ice cream produced using whey protein gel and blueberry in International Research in Engineering Sciences III, (Edt: M. Kamanlı), 1. Edition, November-December 2022, Eğitim Publishing House, Istanbul.

Ürkek, B., Gürmeriç, H. E. and Şengül, M. (2021). Effect of addition of chia seed (*Salvia hispanica L.*) on physicochemical and sensory properties of ice cream. *The Journal of Food*, 46(1): 180-189.

Yaman, M., Mızrak, Ö. F., Catak, J. and Sargın, H. S. (2019). In vitro bioaccessibility of added folic acid in commercially available baby foods formulated with milk and milk products. *Food Science and Biotechnology*, 28(6): 1837-1844.

Yerlikaya, O., Kınık, Ö. and Akbulut, N. (2010). Functional properties of whey and new generation dairy products manufactured with whey. *The Journal of Food*, 35(4): 289-296.