

Investigation of Total Protein and Casein Contents of Fruit Yogurts by Electrophoretic Method

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Abstract

Fruit yogurt is consumed fondly as a healthy snack. The combination of both fruit and yogurt brings together the benefits of both ingredients. Proteins are essential for a healthy life. There is limited data on the protein content of fruit yogurts. This study described the total protein and casein fraction in different fruit yogurts, mostly produced and consumed: strawberry, forest fruit, fig-walnut, mango, blueberry, peach, raspberry, and pineapple. We also provided detailed information about fruit yogurts. We employed a nanodrop spectrophotometer to determine total protein content and SDS-PAGE, a widely recognized and effective protein identification method, though it has not yet been applied to fruit yogurt. Statistical evaluation was performed with an analysis of variance. The highest total protein amount was observed in pineapple (3.51 mg/mL) and fig-walnut yogurts (3.25 mg/mL), and the lowest in forest fruit (1.21 mg/mL) and strawberry yogurts (1.27 mg/mL). The highest casein density was determined in pineapple (41.05%) and fig-walnut yogurts (36.56%) and the lowest in mango (12.94%) yogurts. The remaining fruit yogurts had OD (Optical Density) values of 27.61% for strawberry, 22.40% for peach, 19.85% for raspberry, and 19.04% for blueberry, respectively. In addition, the casein of the fruit yogurts was determined around 25 kDa.

The statistical differences were significant between fruit yogurts ($P < 0.001$). As a result, yogurt producers must carefully consider their choice of starter culture to optimize both sensory attributes and health benefits, ensuring that consumers receive a product that aligns with their expectations for quality and nourishment.

Keywords: Casein, fruit yogurt, nanodrop, protein, SDS-PAGE

Introduction

Milk and dairy products have an important place in human nutrition. These products, which have high nutritional value, contain nutrients such as protein, calcium, phosphorus, B vitamins, and fatty acids. Milk contains many basic nutrients that the human body needs [1]. Being especially rich in calcium is extremely important for bone health. On the other hand, milk is one of the quality protein sources. The milk proteins and casein it contains are well evaluated by the body. It is rich in vitamins A, D, B12, and minerals such as zinc and magnesium [2]. Milk and dairy products are widely consumed in different cultures around the world [3]. Regular consumption of milk and dairy products provides benefits such as reducing the risk of osteoporosis, preserving muscle mass, and supporting the immune system [4].

Yogurt is obtained by bacterial fermentation of milk. Probiotic bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are usually used in yogurt production [5]. Yogurt supports intestinal health, facilitates digestion, and helps balance intestinal flora thanks to its probiotic content. Yogurt is rich in protein, calcium, and vitamins, similar to milk. Additionally, thanks to the lactase enzyme, it can be digested more easily by individuals with lactose intolerance. Yogurt can help with weight control by providing a feeling of fullness. Its high protein content helps people feel full for longer. Milk and dairy products are indispensable for a balanced diet. Yogurt in particular is

recommended to be included frequently in diets due to its health benefits and nutritional content [6,7].

Fruit yogurt is a dairy product obtained by mixing yogurt and various fruits. It is usually preferred as a sweet snack or a healthy snack. Natural fruits (fresh or frozen) or fruit puree are used as a sweetener and nutritional source for fruit yogurt. Commonly used fruits include strawberries, bananas, blueberries, peaches, and mangoes. Some fruit yogurts may contain sweeteners (sugar, honey, or artificial sweeteners); however, using natural fruit can provide flavor without the need for sweetening. Fruit yogurt generally provides the protein, calcium, and probiotics that yogurt provides, as well as the vitamins and minerals that fruits have. Fruits also provide fiber that supports digestive health, thus enriching the beneficial properties of yogurt [8,9]. FAO (Food and Agriculture Organization) and WHO (World Health Organization) recommend that yogurt products should contain 5-15% fruit by weight [10].

Casein is a globular protein with low molecular weight (19-25 kDa). Casein does not contain disulfide bonds and is generally found in a secondary structure. 80% of the total milk protein consists of casein proteins and 20% of whey proteins, and casein plays a role in calcium transport. Caseins are synthesized in the mammary gland. Casein is divided into four main components: alpha, beta, kappa, and gamma. The alpha is the most abundant among the other caseins. During the fermentation process of the yogurt formation stage, casein changes the structure of the milk, causes it to coagulate, and increases the consistency of the yogurt. Casein is taken into the body together with the calcium mineral provided by milk and dairy products [11,12]. Casein is an important protein source for muscle development and repair. It helps maintain and develop muscle

mass, especially in athletes. Casein provides a feeling of fullness for a longer period due to its slow digestion [13].

In this context, the current research aimed to determine total protein content and observe the changes in the electrophoretic picture of casein in fruit yogurts (strawberry, forest fruit, fig-walnut, mango, blueberry, peach, raspberry, and pineapple).

Methods Preparation of yogurt samples

Fruit yogurt samples were obtained from national supermarkets in Türkiye. All fruit yogurts (strawberry, forest fruit, fig-walnut, mango, blueberry, peach, raspberry, and pineapple) were brought to the laboratory in the cold chain (in an icebox) and immediately analyzed. Each fruit yogurt was homogenized with 25 mM Tris HCl (pH: 7.4) solution at 1:10 (w/v) in an ice bath for 30 seconds. Samples were aliquoted for total protein and SDS-PAGE analyses.

Total protein concentration

A nanodrop Spectrophotometer (NanoDrop 2000c, Wilmington, DE, USA) was used to determine total protein concentration. Before the measurement, 25 mM Tris HCl was given as a blank solution to the device, and the device was read against the blank. Then, two-microliter samples were measured on the device at 280 nm absorbance and the concentration was calculated (mg/mL).

SDS-PAGE (Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis) analysis

Electrophoresis is a laboratory technique utilized to separate DNA (Deoxyribose nucleic acid), RNA (Ribonucleic acid), or protein molecules based on their size and electrical charge. An electric current is used to move the molecules through a gel or other matrix.

SDS-PAGE is a very powerful method for protein analysis. First described by Laemmli (1970). It can be used to separate all proteins, including those that are insoluble in water. In this study, each yogurt sample was boiled for 5 minutes with 4X sample buffer (Tris HCl, 2-mercaptoethanol, and bromophenol blue) added to a final volume of 30 $\mu\text{g}/25 \mu\text{L}$ and loaded onto a 10% resolving gel. A molecular weight marker was used to determine casein. Electrophoresis was performed according to the method of Laemmli [14]. At the end of the SDS-PAGE process, the gel was kept in 1% Coomassie brilliant blue solution for 45 minutes to make the proteins visible and then Coomassie brilliant blue was removed from the parts that did not bind to the proteins with a destaining solution (acetic acid, methanol, distilled water). The gel was photographed at 600 dpi quality and the casein density of the bands was determined with the NIH Image software (National Institutes of Health, California, USA). Casein density in the bands was determined as optical density (OD, %) by measuring from ten different points of each band [15].

Statistical analysis

All data obtained from each fruit yogurt were subjected to the analysis of variance (One-Way ANOVA) in the IBM[®]SPSS30 package program. The Tukey HSD test was utilized as a post-hoc test for multiple comparisons between yogurts. Statistical significance level was accepted when $P < 0.05$ [16].

Results

The total protein concentration of the fruit yogurts is presented in Table 1. The highest total protein amount was observed in pineapple (3.51 mg/mL) and fig-walnut yogurts (3.25 mg/mL), and the lowest in forest fruit (1.21 mg/mL) and strawberry yogurts (1.27 mg/mL). Other fruit yogurts had a total protein of 1.83 mg/mL for peach, 1.48 mg/mL for

blueberry, 1.44 mg/mL for mango, and 1.33 mg/mL for raspberry, respectively. The OD (%) values of the fruit yogurts are illustrated in Figure 1. The highest casein density was determined in pineapple (41.05%) and fig-walnut yogurts (36.56%) and the lowest in mango yogurts (12.94%). The remaining fruit yogurts had OD values of 27.61% for strawberry, 22.40% for peach, 19.85% for raspberry, and 19.04% for blueberry, respectively. The statistical differences were significant between fruit yogurts in terms of OD values ($P < 0.001$). The gel image of the bands belonging to the fruit yogurts is shown in Figure 2. The molecular weight of casein in the fruit yogurts was determined to be around 25 kDa.

Table 1. The total protein amounts of the fruit yogurts

Groups	Total protein
Strawberry	1.27
Forest fruit	1.21
Fig-walnut	3.25
Mango	1.44
Blueberry	1.48
Peach	1.83
Raspberry	1.33
Pineapple	3.25

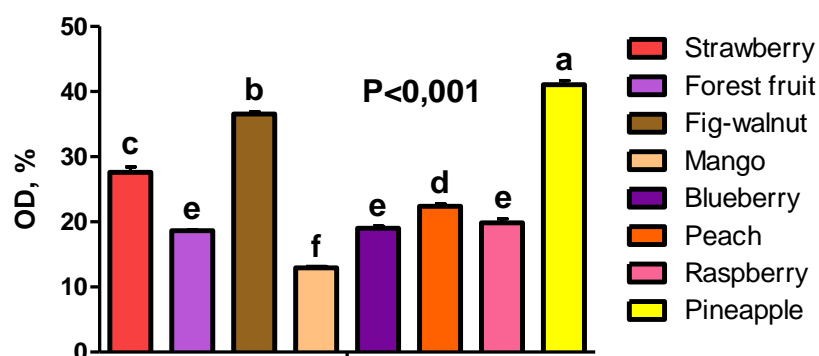


Figure 1: The OD (%) values of the fruit yogurts. **a, b, c, d, e, f:** Different letters show the differences between the fruit yogurt samples.

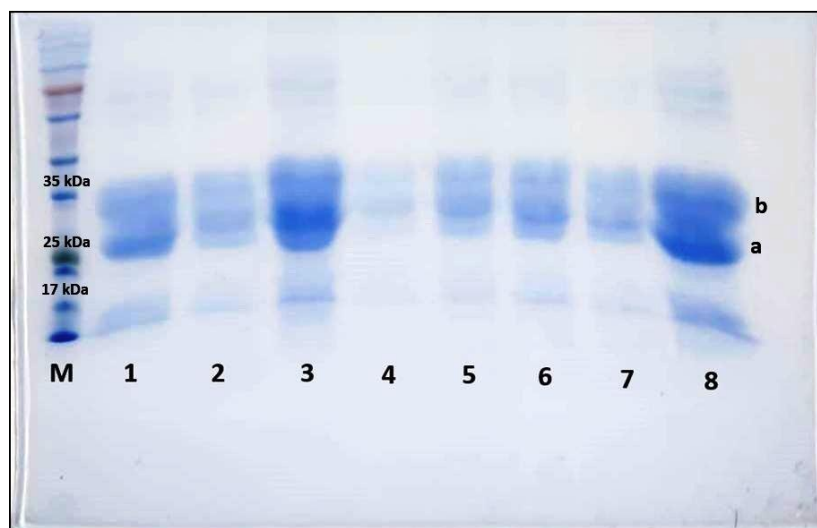


Figure 2: The gel image of the bands belonging to the fruit yogurts. **M:** Protein weight marker. **1:** Strawberry **2:** Forest fruit **3:** Fig-walnut: **4:** Mango **5:** Blueberry **6:** Peach **7:** Raspberry **8:** Pineapple **a:** alpha-casein **b:** beta-casein

Discussion

Fruits are low in energy density and serve as an excellent source of antioxidants, prebiotic fibers, and polyphenols, all of which can enhance digestive health. In contrast, yogurt is a nutrient-dense food that provides a good amount of dairy protein, calcium, magnesium, vitamin B-12, conjugated linoleic acid, and other essential fatty acids [17].

Raspberries are a diverse group of flowering plants that are closely related to blackberries. Both raspberries and blackberries belong to the genus *Rubus*. The genus *Rubus* is a member of the rose family (*Rosaceae*), which also includes important fruit crops such as apples, pears, cherries, peaches, plums, and strawberries. More than 200 species have been identified, but only a few are important commercially among raspberries. These include the European red raspberry (*R. idaeus* subsp. *vulgatus* Arrhen.), the North American red raspberry (*R. idaeus* subsp. *strigosus* Michx.), and the black raspberry (*R. occidentalis* L.) of the eastern USA. Raspberries are low in calories

(69 calories in 123 grams) and protein (1.5 grams in 123 grams). However, they are rich in vitamin C and a good fiber source [18]. Being a source of vitamin C, it suggests that the antioxidant properties of raspberries are more prominent. Antioxidants may reduce your risk of certain chronic diseases. In a study, it was found that ellagic acid, one of raspberries' antioxidants, may not only prevent oxidative damage but also repair damaged DNA [19]. They contain other vitamins and minerals such as vitamin A, thiamine, riboflavin, B6, calcium, and zinc. In addition, high fiber and tannin content may benefit blood sugar control [20,21]. This is also connected to less fatty liver disease.

Another important fruit from the rose family is strawberry. Its protein (0.7 grams in 100 grams) and calorie (32 calories in 100 grams) content are similar to raspberries as well as other nutritious ingredients such as vitamins, minerals, and fibers. In addition to ellagic acid, strawberries contain pelargonidin, ellagitannins, and procyanidins which are linked to numerous health benefits. Phenolic antioxidant content is 2–11 times greater than other fruits [22,23].

Peach (*Prunus persica* L. Batsch) is a highly valued deciduous fruit tree, notable for its sweet flavor, juicy texture, and rich nutrient profile. Peaches are believed to have originated in China over 2,000 years ago before spreading to various parts of the world. Peaches are low in calories (100 g just provides 39 calories) and contain no saturated fats. The protein content of a peach is 1 gram in 147 grams. Peaches are not only delicious but also offer several health benefits due to their nutritional composition. It provides 6% of your daily vitamin A needs and 15% of daily vitamin C needs [24].

Mango (*Mangifera indica* L.) is a climacteric fruit, capable of developing characteristic ripening-associated changes such as color, aroma, and taste, before or after harvest [25]. Mangoes are primarily composed of carbohydrates, mainly in the form of sugars like

glucose, fructose, and sucrose, which provide quick energy. While low in total fat content (about 0.5 g per 100 g), mangoes do contain small amounts of beneficial fatty acids. Mangoes have low protein content (approximately 0.8 g per 100 g). Mangoes are associated with various health benefits due to their rich antioxidant profile. They may protect against certain cancers due to high levels of polyphenols and support heart health through potassium content which helps maintain normal blood pressure levels [26,27].

Pineapple (*Ananas comosus*) is an exotic fruit known for its distinctive sweet and tangy flavor, aromatic scent, and juicy texture. Pineapple primarily consists of carbohydrates (approximately 13 g per 100 g), mostly in the form of sugars such as fructose, glucose, and sucrose that provide energy. It has a low protein content (around 0.5 g per 100 g) and is extremely low in fat (about 0.1 g per 100 g). One of the most important features of pineapple is containing bromelain. Bromelain is a unique enzyme found in pineapple that may aid digestion by helping to break down proteins. Some studies suggest pineapple may assist with weight management due to its hydrating properties combined with natural sweetness. Pineapple is not just a delicious tropical delicacy but also provides essential nutrients that contribute positively to overall health [28,29].

Fruit yogurt is a healthy snack that is consumed with pleasure. A study stated that the physical, chemical, and sensory properties of yogurt with fruit added were different from those of plain yogurt. It reported that yogurts with added fruit were preferred more by consumers and positively contributed to yogurt consumption [30]. A diet high in fruits, vegetables, and low-fat dairy products has been effective in reducing blood pressure in American adults [31]. Yogurt and fruit have been recognized individually for their protective effects against certain diet-related diseases, including type 2 diabetes

[32]. There are few studies conducted on fruit yogurts' specific nutritional elements. Studies on the nutritional content of fruit yogurts are mostly based on a general examination and are mixed with sensory properties. This situation causes individual nutrients to be overlooked and can overshadow niche information about fruit yogurts. The protein content of fruit yogurts could not be determined by the electrophoretic method, or this approach was not adopted. Electrophoresis is one of the most effective methods for separating and examining proteins. In particular, the SDS-PAGE method has the advantages of simple operation and good reproducibility in determining protein molecular weight, detecting specific proteins, and identifying strain species among the other electrophoretic methods [33]. However, the combination of the unique properties of fruits and the properties of yogurt needs to be understood more. Proteins are indispensable elements of a healthy life. This study provided us with the information that the protein content of fruits is lower than dairy products. However, the combination of yogurt and fruit makes fruit yogurt a superior snack format in this context. The most important and abundant protein in milk is casein, and casein has an important place in the diet of athletes, especially with its fullness and the benefits it provides to the muscles [13]. Studies have shown that the total protein content of yogurt can be affected by the protein found in fruit or other mixtures [34,35]. Indeed, the highest protein content was found in fig-walnut yogurt in this study. While figs do not contain protein and rich in fiber and other components such as vitamins and minerals, walnuts have an average protein content of 4 grams per ounce (about a quarter cup). This is the highest value of all the fruits examined in this study. On the other hand, raspberry and pineapple yogurts contained similar amounts of protein. This fact may depend not only on the fruit content but also on the starter culture used in yogurt production, which affects the protein content of the yogurt

as well [34]. Moreover, the interaction between the fruit and the specific strains of bacteria in the starter culture can lead to variations in texture and flavor. Different cultures may produce distinct metabolites during fermentation, influencing not only taste but also nutritional profiles. For instance, some cultures might enhance the bioavailability of certain nutrients while others could enhance probiotic benefits.

As a result of the fermentation of yogurt with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles*, a protein network is formed [36]. The proteins formed can be broken down into small molecular peptides and form flavor substances during fermentation [37]. Bioactive peptides released from casein can be formed both in vivo by gastrointestinal processes and in vitro due to enzymatic hydroxylation of lactic acid bacteria such as *Lactobacillus helveticus*, *Lactococcus lactis* subsp. *cremoris*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* [38].

Many parameters such as the amount of starter culture used during yogurt production, usage rate, culture mixture ratio, incubation, cooling temperature, and the filtration and storage room temperature are very important in proteolysis. Especially the enzyme production of *Lb. delbrueckii* subsp. *bulgaricus* with stronger proteolytic activity plays an important role in the breakdown of casein into amino acids [39]. In addition, these enzymes are more effective on β -casein and *as*-casein than serum proteins *Lb. delbrueckii* subsp. *bulgaricus*-derived proteases are more effective on caseins, depending on pH [40]. It can be said that the differences in the protein structures of yogurts are due to many factors such as the raw material milk, the starter cultures used, incubation temperature, dry matter concentration, storage conditions, and duration [38].

It was determined that the casein fractions of the yogurt samples correlated with the yogurt's acid value and total nitrogen of the yogurt [38]. The acid value, an indicator of the degree of lipolysis in milk and its products, can vary depending on the fat content.

Because the correlation coefficient between free fatty acids and acid value is high [41].

In addition, the amount of acidic yogurt can vary depending on the culture used [42]. The homogenization process applied in yogurt production causes an increase in free fatty acids, and the broken fat globule is covered with a membrane layer consisting of serum proteins, casein, and submicelles. This new structure is hydrolyzed by plasma lipase associated with casein increasing the acid value [43].

As a result, yogurt producers must carefully consider their choice of starter culture to optimize both sensory attributes and health benefits, ensuring that consumers receive a product that aligns with their expectations for quality and nourishment.

Author contributions

PD conducted research; YB data analysis and software. PD and YB wrote the paper. All authors read and approved the final manuscript.

Conflict of interest

All other authors report no conflict of interest. This study was published as oral and abstract presentations at the 10th International European Conference on Interdisciplinary Scientific Research, in Zurich, Switzerland, from 27 to 29 August 2024.

Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval by interested.

Ethical Statement

This study was conducted in Elazig with the approval of the Firat University NonInterventional Research Ethics Committee dated 25.04.2024 and protocol number 2024/06-08.

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