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Research Article

Effect of Freeze-Drying on the Nutritional Value and Antioxidant Activity of Goat Milk Yogurt Casein

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Abstract

Goat milk yogurt contains bioactive peptides derived from casein during fermentation by lactic acid bacteria, which contribute to its antioxidant properties. However, yogurt's high moisture content limits its shelf life and stability. This study aimed to evaluate the effect of freeze-drying on the nutritional composition and antioxidant activity of goat milk yogurt casein. A laboratory experimental study with two treatments (fresh casein and freeze-dried casein) and three replications was conducted. Nutritional composition was analyzed using proximate methods, including protein, fat, moisture, ash, and carbohydrate contents, while antioxidant activity was determined using the DPPH radical scavenging assay. Freeze-drying markedly reduced moisture content from 78.85% to 4.98% and increased protein (4.85% to 31.59%), fat (9.32% to 31.87%), ash (0.73% to 2.90%), and carbohydrate (6.25% to 28.67%) concentrations. Antioxidant activity increased significantly after freeze-drying, as indicated by a lower IC_{50} value (4.52 mg/mL) than fresh casein (25.43 mg/mL). These findings demonstrate that freeze-drying effectively preserves and concentrates nutrients while improving the antioxidant potential of goat milk yogurt casein. Therefore, freeze-dried goat milk yogurt casein has promising potential as a stable functional food ingredient with enhanced nutritional and antioxidant properties.

Keywords *Antioxidant activity; casein; freeze-drying; functional food; goat milk yogurt*

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INTRODUCTION

Yogurt is one of the most widely consumed fermented dairy products owing to its high nutritional value, desirable sensory characteristics, and potential health benefits. Yogurt is produced by fermenting milk with lactic acid bacteria (LAB), primarily *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, with probiotic strains such as *Lactobacillus acidophilus* frequently added to enhance its functional properties (Sarıtaş *et al.,* 2024). During fermentation, LAB proteolytic systems hydrolyze milk proteins into smaller peptides and amino acids, improving protein digestibility and generating bioactive peptides with antioxidant, antihypertensive, antimicrobial, and immunomodulatory effects (Gedik & Karahan, 2023).

Goat milk has gained increasing attention as a functional food ingredient because of its superior digestibility and unique protein composition compared with cow milk. The smaller fat globules, lower αs1-casein content, and softer curd formation contribute to improved digestibility and bioavailability of nutrients and bioactive compounds. Among milk proteins, casein accounts for approximately 80–83% of total protein and serves as an important precursor of bioactive peptides released during enzymatic hydrolysis and fermentation. Casein-derived phosphopeptides have attracted considerable interest because of their antioxidant activity and potential role in reducing oxidative stress-related damage (Dhasmana *et al.,* 2022).

Despite its nutritional and functional advantages, yogurt is highly perishable because its high moisture content and nutrient-rich composition favor microbial growth and biochemical deterioration during storage (Mehra *et al.,* 2022). Consequently, yogurt's short shelf life may limit the preservation, utilization, and commercialization of bioactive compounds in yogurt-derived casein. Therefore, an effective preservation strategy is required to maintain the nutritional quality and biological activity of casein while extending product stability (Moatsou, 2024).

Freeze-drying is considered one of the most suitable preservation techniques for heat-sensitive food components. This process removes water through sublimation under low-temperature, low-pressure conditions, minimizing thermal degradation and preserving the physical, chemical, and biological characteristics of food constituents. In addition, freeze-dried products generally exhibit lower moisture content, improved storage stability, reduced transportation costs, and rapid rehydration properties (Ismail *et al.,* 2020). Although freeze-drying has been extensively applied to dairy products, information regarding its effects on the nutritional composition and antioxidant activity of goat milk yogurt-derived casein remains limited. Therefore, this study aimed to compare the nutritional composition (protein, fat, moisture, ash, and carbohydrate contents) and antioxidant activity of goat milk yogurt casein before and after freeze-drying. The findings are expected to provide scientific evidence regarding the potential application of freeze-drying for preserving and enhancing the functional value of goat milk yogurt casein as a functional food ingredient.

MATERIALS AND METHODS

Ethical Approval

Ethics approval was not required because no live animals were involved. The study was conducted exclusively on goat milk yogurt casein and freeze-dried goat milk yogurt casein samples obtained for laboratory analysis.

Study Period and Location

This study was conducted over a four-month period, from January to April 2019. Goat milk yogurt was produced at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia. Freeze-drying of yogurt-derived casein was carried out at the Nanomaterials and Kinetics Laboratory, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia. Proximate composition analysis and antioxidant activity determination using the DPPH radical scavenging assay were performed at the Food Quality and Safety Assessment Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia.

Study Design

This study employed a comparative laboratory experimental approach to investigate differences in nutritional composition and antioxidant activity between goat milk yogurt casein and freeze-dried goat milk yogurt casein. Each treatment was replicated three times, yielding a total of six experimental units.

Evaluation Procedures

Fresh goat milk was pasteurized using the High Temperature Short Time (HTST) method at 72°C for 15 seconds, then cooled to 45°C. A commercial starter culture (Yogourmet®) containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* was added at 0.5% (w/v) to produce a working mother culture. The mixture was incubated at 45°C for 4–6 hours until it reached a pH of 4.4–4.5. For yogurt production, 3% (v/v) of the working mother culture was inoculated into pasteurized goat milk and incubated at 45°C for 2–4 hours until the pH reached 4.5–5.0. The resulting yogurt was transferred into 15-mL Falcon tubes and centrifuged at 5°C and 1,200 rpm for 10 minutes. Following centrifugation, the solid fraction (casein) was separated from the liquid fraction. Part of the casein was analyzed directly, while the remaining portion was freeze-dried to obtain freeze-dried goat milk yogurt casein.

Data Collection

Proximate Analysis

The proximate composition of goat milk yogurt casein and freeze-dried goat milk yogurt casein was determined according to the Indonesian National Standard for food and beverage testing (SNI 01-2891-1992) and procedures recommended by the Association of Official Analytical Chemists (AOAC, 2005). Protein content was determined using the Kjeldahl method, fat content by the Weibull extraction method, moisture content by the oven-drying method, and ash content by dry ashing in a muffle furnace. Carbohydrate content was calculated by difference according to the following equation:

$$\text{Carbohydrate (\%)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Protein (\%)} + \text{Fat (\%)}]$$

Determination of Antioxidant Activity

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to the method described by Kedare & Singh 2011). with minor modifications. Briefly, sample solutions at different concentrations were mixed with DPPH solution and incubated in the dark at room temperature for a specified period. The absorbance was measured spectrophotometrically at 517 nm.

The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_s) / A_0] \times 100$$

where A_0 is the absorbance of the control and A_s is the absorbance of the sample.

The percentage inhibition values were plotted against sample concentrations to generate a calibration curve. Linear regression analysis was subsequently performed, and the IC_{50} value was calculated as the concentration required to inhibit 50% of DPPH radicals. Lower IC_{50} values indicate stronger antioxidant activity.

Data Analysis

Data obtained from all measurements were processed using Microsoft Excel 2010. Results were expressed as mean values of three replications and presented in tables and figures. Nutritional composition and antioxidant activity were compared descriptively between goat milk yogurt casein and freeze-dried goat milk yogurt casein.

RESULT AND DISCUSSION

Nutritional Composition of Goat Milk Yogurt Casein and Freeze-Dried Goat Milk Yogurt Casein

The nutritional composition of goat milk yogurt casein and freeze-dried goat milk yogurt casein is presented in **Table 1.**

Table 1. Nutritional Composition of Goat Milk Yogurt Casein and Freeze-Dried Goat Milk Yogurt Casein

Component (%)	Casein	Freeze-Dried Casein
Protein	4.85	31.59
Fat	9.32	31.87
Moisture	78.85	4.98
Ash	0.73	2.90
Carbohydrate	6.25	28.67

The results showed marked differences in nutritional composition between goat milk yogurt casein and freeze-dried goat milk yogurt casein. Moisture content decreased substantially from 78.85% to 4.98% following freeze-drying. In contrast, the concentrations of protein, fat, ash, and carbohydrate increased after the drying process. Protein content increased from 4.85% to 31.59%, fat content increased from 9.32% to 31.87%, ash content increased from 0.73% to 2.90%, and carbohydrate content increased from 6.25% to 28.67%. Overall, freeze-drying reduced moisture content by approximately 73.87 percentage points while increasing the relative concentrations of the remaining nutrients in goat milk yogurt casein.

The substantial reduction in moisture content observed in freeze-dried goat milk yogurt casein is attributable to the mechanism of freeze-drying, which removes water through sublimation under low-temperature and low-pressure conditions (Nowak & Jakubczyk, 2020). The process generally consists of three stages: freezing, primary drying, and secondary drying.

During primary drying, frozen water is removed by sublimation, whereas secondary drying eliminates residual bound water, resulting in a highly stable dried product (Adams *et al.,* 2015).

The moisture content decreased from 78.85% in fresh yogurt casein to 4.98% after freeze-drying, indicating that more than 90% of the original water was removed. Similar findings have been reported in freeze-dried dairy products, including camel milk powder, where moisture reduction improved storage stability and product quality. Low moisture content is a critical factor in extending shelf life because it limits microbial growth and reduces the rate of chemical and enzymatic deterioration during storage (Gallardo-Rivera *et al.,* 2021).

The increases observed in protein, fat, ash, and carbohydrate contents are primarily associated with the concentration effect resulting from water removal rather than the formation of additional nutrients. As water is removed, the proportion of dry matter increases, leading to higher relative concentrations of nutrients in the final product. Similar increases in nutrient concentrations following freeze-drying have been reported in milk and dairy products (Ismail, 2021).

Protein content increased from 4.85% to 31.59%, while fat content increased from 9.32% to 31.87%. Ash content increased from 0.73% to 2.90%, indicating greater mineral concentration in the dried product. Likewise, carbohydrate content increased from 6.25% to 28.67%. These findings suggest that freeze-drying effectively preserves milk solids and minimizes nutrient losses because the process occurs at low temperatures, thereby reducing thermal degradation compared with conventional drying methods (Hazkhirie *et al.,* 2025).

The preservation of nutritional components observed in this study highlights the suitability of freeze-drying as a processing method for yogurt-derived casein. In addition to extending shelf life, freeze-drying produces a stable, lightweight product with reduced storage and transportation requirements while maintaining the nutritional quality of goat milk yogurt casein. These characteristics support its potential application as a functional food ingredient and as a source of bioactive compounds for future food formulations (Ismail, 2021; Akhsit *et al.,* 2024).

Antioxidant Activity of Goat Milk Yogurt Casein and Freeze-Dried Goat Milk Yogurt Casein

The antioxidant activity of goat milk yogurt casein and freeze-dried goat milk yogurt casein was evaluated using the DPPH radical scavenging assay and expressed as IC_{50} values. The results are presented in **Figure 1.**

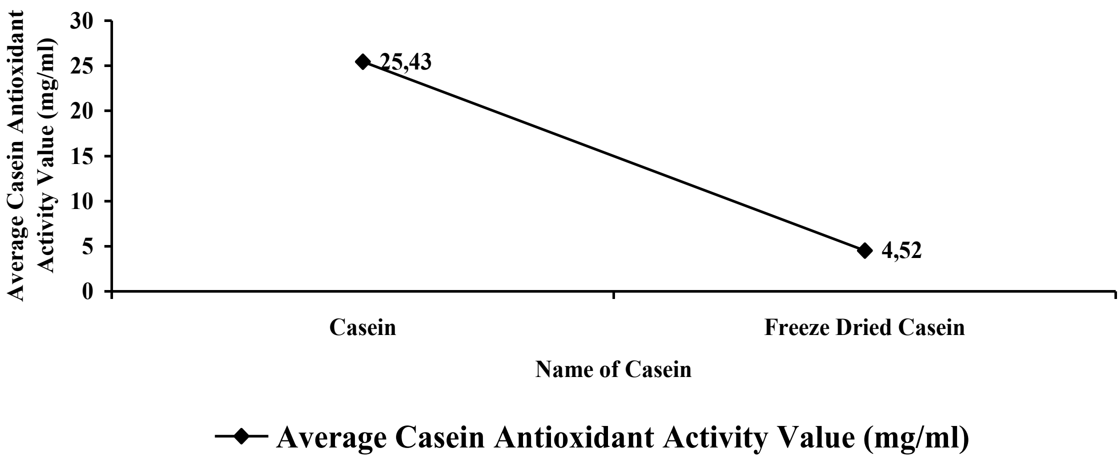

Figure 1. IC_{50} of Goat Milk Yogurt Casein and Freeze-Dried Goat Milk Yogurt Casein

The average IC_{50} value of goat milk yogurt casein was 25.43 mg/mL, whereas freeze-dried goat milk yogurt casein exhibited a substantially lower IC_{50} value of 4.52 mg/mL. The reduction in IC_{50} value after freeze-drying indicates an increase in antioxidant activity. Compared with fresh yogurt casein, freeze-dried casein demonstrated approximately a 5.6-fold greater antioxidant capacity. These findings suggest that freeze-drying affected the antioxidant properties of goat milk yogurt-derived casein and resulted in a product with enhanced free radical scavenging activity. Antioxidants are compounds capable of scavenging free radicals and inhibiting oxidative reactions that may cause cellular damage. In the DPPH assay, antioxidant activity is commonly expressed as the IC_{50} value, which represents the concentration required to inhibit 50% of DPPH free radicals. Lower IC_{50} values indicate stronger antioxidant activity (Gulcin & Alwasel, 2023).

In the present study, freeze-dried goat milk yogurt casein exhibited a considerably lower IC_{50} value (4.52 mg/mL) than fresh goat milk yogurt casein (25.43 mg/mL), indicating enhanced antioxidant activity after freeze-drying. This improvement may be attributed to the concentration of bioactive compounds following moisture removal and the ability of freeze-drying to preserve heat-sensitive antioxidant peptides. Unlike conventional drying methods, freeze-drying operates under low-temperature conditions, thereby minimizing thermal degradation and maintaining the structural integrity of biologically active molecules (Auli *et al.,* 2025; Srivastava, 2015).

The antioxidant activity observed in yogurt-derived casein is closely associated with bioactive peptides generated during milk fermentation. Proteolytic enzymes produced by lactic acid bacteria hydrolyze casein into smaller peptides that possess antioxidant properties through their ability to donate protons, chelate pro-oxidative metal ions, and neutralize reactive oxygen species. Previous studies have demonstrated that casein-derived peptides contribute significantly to the antioxidant potential of fermented dairy products (Şanlıdere Aloğlu & Öner, 2011).

The enhanced antioxidant activity observed in the freeze-dried product is consistent with reports showing that freeze-drying effectively preserves antioxidant compounds in food matrices. Previous studies reported improved antioxidant retention in freeze-dried functional foods, while others observed the preservation of antioxidant compounds and peptides in dried dairy and starter culture products. Similarly, the low-temperature conditions employed during freeze-drying help maintain the functionality of antioxidant peptides and other bioactive constituents in goat milk yogurt casein (Chen *et al.,* 2015). These findings indicate that freeze-drying not only improves the storage stability of yogurt-derived casein but also preserves and potentially enhances its antioxidant functionality, thereby increasing its value as a functional food ingredient.

CONCLUSIONS

Freeze-drying significantly altered the nutritional composition and antioxidant activity of goat milk yogurt casein. Compared with fresh yogurt casein, freeze-dried casein exhibited substantially lower moisture content and higher concentrations of protein, fat, ash, and carbohydrates. In addition, freeze-dried goat milk yogurt casein demonstrated greater antioxidant activity, as indicated by its lower IC_{50} value. These findings suggest that freeze-drying is an effective preservation method for reducing moisture content while maintaining and enhancing the nutritional and functional properties of goat milk yogurt-derived casein. Therefore, freeze-dried goat milk yogurt casein has potential as a shelf-stable functional food ingredient with improved antioxidant capacity.

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