

Production of plant-based bioactive milk from germinated oat seeds

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Manuscript received: 25.06.2026

Accepted online: 06.09.2026

Published online: 30.09.2026

Abstract: The growing demand for sustainable dairy alternatives has increased interest in oat milk with improved nutritional, functional, and sensory properties. This study investigated the effects of germination duration on the physicochemical characteristics, colloidal stability, bioactive profile, and sensory acceptability of oat milk. Oat seeds (*Avena sativa* L.) were germinated for 24, 48, and 72 h, and oat milk was prepared from germinated and ungerminated seeds under identical processing conditions. Samples were evaluated for pH, total soluble solids, particle size, zeta potential, physical stability, soluble protein content, total phenolic content (TPC), and DPPH radical scavenging activity. Sensory evaluation assessed appearance, color, smell, taste, mouthfeel, and overall acceptability. Germination improved colloidal stability, with zeta potential becoming more negative, shifting from -15 mV in the ungerminated control to -20 mV after 72 h. TPC increased from 7.83 to 11.45 mg GAE/100 mL, accompanied by an increase in DPPH radical scavenging activity from 6.31% to 8.33%. Soluble protein content also increased moderately, from 2.1 to 3.2 ppm, with increasing germination duration. Although germination improved appearance and color scores, it reduced smell, taste, and mouthfeel scores, indicating differences between visual appeal and other sensory attributes. Overall acceptability decreased after 24 and 48 h of germination, whereas the sample germinated for 72 h (YS72) remained comparable to the ungerminated control. These findings demonstrate the potential of germination as a pretreatment for improving oat milk stability and bioactive properties. The results also highlight the importance of selecting an appropriate germination duration to balance physicochemical improvements with sensory acceptability.

Keywords: Oat, plant milk, bioactive, germination, functional milk.

Filizlenmiş yulaf tohumlarından bitki bazlı biyoaktif süt üretimi

Özet: Sürdürülebilir süt alternatiflerine yönelik artan talep, besinsel, fonksiyonel ve duyuşal özellikleri geliştirilmiş yulaf sütüne olan ilgiyi artırmıştır. Bununla birlikte, bu ürünlerde fiziksel stabilitenin korunması ve duyuşal kabul edilebilirliğin sağlanması, ürün geliştirme çalışmalarında önemli konular arasında yer almaktadır. Bu çalışmada, çimlendirme süresinin yulaf sütünün fizikokimyasal özellikleri, kolloidal stabilitesi, biyoaktif profili ve duyuşal kabul edilebilirliği üzerindeki etkileri incelenmiştir. Yulaf taneleri (*Avena sativa* L.) 24, 48 ve 72 saat süreyle çimlendirilmiş; çimlendirilmiş ve çimlendirilmemiş tanelerden aynı işlem koşullarında yulaf sütü hazırlanmıştır. Örnekler; pH, toplam kuru madde, partikül boyutu, zeta potansiyeli, fiziksel stabilite, çözünür protein içeriği, toplam fenolik madde içeriği (TFM) ve DPPH radikal süpürme aktivitesi açısından değerlendirilmiştir. Duyusal değerlendirmede görünüş, renk, koku, tat, ağız hissi ve genel kabul edilebilirlik özellikleri incelenmiştir. Çimlendirme süresinin uzamasıyla ortalama partikül boyutu, çimlendirilmemiş kontrol örneğinde 45.682,5 nm iken 72 saat sonunda 7.421,2 nm'ye düşmüştür. Zeta potansiyeli ise kontrol örneğinde -15 mV iken 72 saatlik çimlendirme sonrasında -20 mV'ye ulaşmıştır. Fiziksel stabilite, çimlendirme süresine bağlı olarak doğrusal olmayan bir değişim göstermiş; 48 saat sonunda azalırken 72 saat sonunda %91,67'ye yükselerek kontrol örneğinin üzerinde bulunmuştur. TFM, 7,83'ten 11,45 mg GAE/100 mL'ye yükselirken DPPH radikal süpürme aktivitesi %6,31'den %8,33'e artmıştır. Çözünür protein içeriği de çimlendirme süresinin uzamasıyla 2,1'den 3,2 ppm'e yükselmiştir. Çimlendirme, görünüş ve renk puanlarını iyileştirmesine rağmen koku, tat ve ağız hissi puanlarını düşürmüştür; görsel özelliklerdeki iyileşmenin bütün duyuşal özelliklere aynı biçimde yansımadağı görülmüştür. Genel kabul edilebilirlik, 24 ve 48 saatlik çimlendirme sonrasında azalırken 72 saat çimlendirilen örnek (YS72), çimlendirilmemiş kontrolle benzer düzeyde kalmıştır. Bu bulgular, özellikle 72 saatlik çimlendirmenin yulaf sütünün fiziksel stabilitesini ve biyoaktif özelliklerini geliştirmeye yönelik bir ön işlem olarak potansiyelini ortaya koymaktadır. Sonuçlar, fizikokimyasal iyileşmeler ile duyuşal kabul edilebilirlik arasında denge sağlamak için uygun çimlendirme süresinin seçilmesinin önemini vurgulamaktadır.

Anahtar Kelimeler: Yulaf, bitkisel süt, biyoaktif, filizlenme, fonksiyonel süt.

Research Article

Reference: Dolutas, D., Tekin, G. K., Karabulut, Z., & Karabulut, G. (2026). Production of plant-based bioactive milk from germinated oat seeds. *ITU Journal of Food Science and Technology*, 4(2), 30–40.

1. Introduction

The global transition toward more sustainable food systems have increased interest in plant-based milk alternatives, driven by health, environmental, and ethical considerations. Lactose intolerance, cow's milk protein allergy, concerns regarding dietary cholesterol, and the increasing adoption of vegan and flexitarian diets have further stimulated the demand for dairy-free beverages. In parallel, the environmental impacts associated with conventional dairy production, including greenhouse gas emissions and extensive land and water use, have encouraged the development of more sustainable alternatives (Chalupa-Krebzdak et al., 2018). Consequently, plant-based milk alternatives derived from legumes, nuts, oilseeds, and cereals have gained considerable commercial and nutritional importance.

Among cereal-based alternatives, oats (*Avena sativa* L.) are particularly promising because of their favorable nutritional composition, mild flavor, and technological versatility (Patra et al., 2022). Oats contain soluble dietary fiber, especially β -glucans, as well as proteins, unsaturated fatty acids, essential minerals, B-complex vitamins, and various phytochemicals (Singh et al., 2025). They are also distinguished by the presence of avenanthramides, a group of phenolic compounds associated with antioxidant, anti-inflammatory, and cardioprotective activities (Tang et al., 2023). These nutritional and bioactive constituents make oats a suitable raw material for developing functional plant-based beverages.

Oat milk is generally well accepted because of its creamy consistency, relatively mild sensory profile, and fiber content. Nevertheless, its composition and quality are strongly affected by the characteristics of the oat grains and the processing conditions used during soaking, milling, extraction, filtration, and thermal treatment. Conventional processing may restrict the transfer of proteins and bound phenolic compounds into the aqueous phase. In addition, incomplete starch hydrolysis and the presence of suspended particles may adversely affect viscosity, particle-size distribution, colloidal stability, sedimentation behavior, and sensory quality (Babolanmogadam et al., 2023; McCarron et al., 2024). Therefore, appropriate pretreatment strategies are required to improve the nutritional, functional, and physicochemical properties of oat-based milk alternatives.

Germination is a natural and sustainable pretreatment that induces extensive biochemical and structural changes in cereal grains. During germination, endogenous amylases, proteases, phytases, and cell-wall-degrading enzymes are activated. These enzymes promote starch hydrolysis, protein mobilization, reduction of antinutritional factors, and the release or de novo synthesis of bioactive compounds, including phenolic acids, γ -aminobutyric acid (GABA), vitamins, and bioactive peptides (do Nascimento et al., 2022; Karabulut et al., 2024). In oats, germination has been reported to alter carbohydrate metabolism and improve protein digestibility, phenolic content, and antioxidant capacity (Aparicio-García et al., 2021). Because these transformations modify the quantity and characteristics of soluble and dispersed components transferred into the aqueous phase, germination may affect not only the nutritional and bioactive properties of oat milk but also its particle characteristics, colloidal stability, and sensory acceptability.

Recent studies have demonstrated the potential of germination to improve the nutritional, functional, and sensory properties of oat-based products. Babolanmogadam et al. (2023) compared germination with acidic, alkaline, and α -amylase treatments, applied individually or in combination, in oat-milk production. Germinated samples resulted in the highest oat-milk yield (91.70%), while the combination of germination and acidic treatment produced the highest protein extraction yield

(82.74%). The authors also reported acceptable sensory scores for germinated oat milk and particularly favorable results for treatments combining germination with α -amylase. However, only a single germination duration of 96 h was evaluated. Alsadiq and Ilowefah (2024) investigated the germination of two oat samples for 24, 48, and 72 h and observed time- and sample-dependent changes in total dietary fiber and phenolic content. Milk fortified with germinated oat flour showed greater sensory acceptance than milk fortified with ungerminated oat flour, although the control milk remained the most preferred. Nevertheless, that study focused on the use of germinated oat flour as a fortifying ingredient rather than on an oat-based milk produced directly from germinated grains. More recently, Wei et al. (2024) selected oats germinated for 48 h to produce whole germinated oat milk and reported increases in total protein, GABA, total phenolics, and reducing sugars during germination. They further demonstrated that high-energy fluidic microfluidization reduced particle size and improved the viscosity and physical stability of the resulting beverage. However, because germination was combined with enzymatic treatment and high-pressure microfluidization, the contribution of germination time alone to the physicochemical stability of oat milk could not be clearly distinguished.

These studies demonstrate the nutritional and technological potential of germinated oats but leave several important questions unresolved. Previous research has either examined a single germination duration, used germinated oat flour to fortify milk, or combined germination with intensive enzymatic and mechanical processing. Therefore, the time-dependent effects of germination alone on oat milk produced under otherwise identical processing conditions remain insufficiently characterized. In particular, few studies have simultaneously examined physicochemical properties, particle size, zeta potential, phase stability, soluble protein content, phenolic compounds, antioxidant activity, and sensory acceptance across different germination periods. Accordingly, the present study aimed to determine the effects of 24, 48, and 72 h of germination on the physicochemical stability, bioactive properties, and sensory characteristics of oat milk relative to an ungerminated control.

2. Materials and Methods

2.1 Materials

Oat seeds (*Avena sativa* L.) were obtained from a certified local grain supplier in Sakarya, Türkiye. Prior to the experiment, the seeds were stored in airtight containers under cool and dry conditions to minimize moisture uptake and microbial contamination. Damaged, discolored, or broken grains were manually removed by visual inspection. To preserve the native biochemical composition of the seeds, no thermal or chemical pre-treatment was applied before germination.

All reagents and analytical-grade chemicals used in the study, including Folin–Ciocalteu reagent, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium carbonate, and bovine serum albumin (BSA), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Distilled water used for soaking, extraction, dilution, and analytical procedures was obtained from an ELGA PURELAB® water purification system (Veolia Water Technologies, UK).

2.2 Methods

2.2.1. Germination of oat seeds

The germination process of oat seeds (*Avena sativa* L.) comprised four sequential steps: surface sterilization, soaking, germination, and sample collection. To minimize microbial contamination, approximately 100 g of whole oat

seeds were surface-sterilized by immersion in 0.002 N sodium hypochlorite (NaOCl) solution for 20 min at room temperature under gentle agitation. Following sterilization, the seeds were rinsed three times with tap water and finally with distilled water to remove residual chlorine.

The sterilized seeds were then soaked in distilled water at a seed-to-water ratio of 1:10 (w/v) and incubated in the dark at room temperature (24 ± 1 °C) for 12 h to ensure complete hydration and activation of germination-related metabolic pathways. After soaking, excess water was drained, and the hydrated seeds were evenly distributed in a single layer between two sterile, moistened Whatman No. 1 filter papers placed in 150 mm sterile glass Petri dishes. To maintain adequate humidity, 10–12 mL of distilled water was added to each dish, and moisture was replenished daily.

Germination was carried out in the dark at 25 ± 1 °C for 24, 48, or 72 h to evaluate time-dependent effects on the physicochemical and functional properties of oat milk. Seeds were periodically inspected for radicle emergence and microbial contamination. At the end of each germination period, germinated seeds were collected, gently blotted to remove surface moisture, and non-germinated or contaminated seeds were discarded. Germinated samples were either processed immediately or stored at -20 °C in airtight polyethylene bags prior to oat milk preparation.

2.2.2. Proximate composition of germinated oat seeds

The proximate composition of germinated oat seeds was determined according to standard AOAC methods. Moisture content was measured by oven-drying the samples at 100 °C to constant weight (AOAC, 2000). Ash content was determined by dry ashing in a muffle furnace at 525 °C until complete incineration was achieved (AOAC Official Method 923.03). Crude fat content was analyzed using Soxhlet extraction with chloroform as the solvent (AOAC Official Method 920.39).

Protein content was determined using the Dumas combustion method in accordance with AOAC Official Method 992.23 (AOAC, 2000) using a VELP NDA 701 nitrogen analyzer (VELP Scientifica, Italy). Crude protein content was calculated by multiplying the measured nitrogen content by a nitrogen-to-protein conversion factor of 5.83, which is appropriate for oat-based matrices.

2.2.3. Preparation of germinated oat milk

Following germination, oat seeds from each time point (24, 48, and 72 h) were evenly spread in a single layer on stainless steel trays lined with blotting paper and dried in a hot air oven at 50 °C to constant weight. The dried samples were ground for 5 min using a commercial blender (Electrolux, Sweden) to obtain a fine oat flour. Oat milk was prepared by dispersing the flour in distilled water at a seed-to-water ratio of 1:10 (w/v), followed by thorough blending to facilitate hydration and extraction of soluble components. The slurry was filtered through four layers of muslin cloth to separate the liquid fraction (oat milk) from the solid residue, with gentle filtration applied to minimize foam formation and preserve colloidal stability.

Four oat milk formulations were prepared and analyzed on the same day: Control (non-germinated), YS24 (24 h germination), YS48 (48 h germination), and YS72 (72 h germination). All samples were stored at 4 °C and subjected to physicochemical, functional, and bioactive analyses within 12 h of preparation to minimize oxidative and microbial degradation (Figure 1).

2.2.4. Physicochemical characterization of oat milk samples

2.2.4.1. pH measurement

The pH of oat milk samples was measured using a calibrated digital pH meter (Mettler Toledo, Switzerland). The instrument was calibrated with standard buffer solutions at pH 4.00 and 7.00 prior to analysis. Measurements were performed at room temperature (≈ 22 °C), and all samples were analyzed in triplicate.

2.2.4.2. Total soluble solids content

Total soluble solids content was determined according to AOAC Official Method 925.23 (AOAC, 2000). Approximately 5.00 ± 0.01 g of each oat milk sample was dried in a hot air oven at 105 °C to constant weight. Total solids were calculated gravimetrically and expressed as a percentage of the initial sample mass (Eq. 1).

$$\text{Total Soluble Solids (\%)} = \left(\frac{A_1 - A}{A_2 - A} \right) \times 100 \quad (1)$$

where A is weight of empty crucible (g), A_1 is weight of crucible + dried sample (g) A_2 is weight of crucible + fresh sample (g).

2.2.4.3. Soluble protein content

Soluble protein concentration was determined using the Bradford colorimetric method. Oat milk samples were diluted (1:10, v/v) in 0.2 M sodium phosphate buffer (pH 7.0) and centrifuged at $13,000 \times g$ for 25 min at 25 °C. An aliquot of the supernatant (150 μ L) was mixed with Bradford reagent (Sigma-Aldrich, USA), incubated for 10 min at room temperature, and absorbance was measured at 595 nm using a spectrophotometer (Thermo Spectronic Genesys 5, USA). A buffer blank was used for baseline correction. Protein concentration was quantified using a bovine serum albumin (BSA) calibration curve ($R^2 = 0.9824$).

2.2.4.4. Particle size and zeta potential

The mean particle size and zeta potential of the oat milk samples were determined using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK), based on dynamic light scattering (DLS). Samples were diluted 1:100 in 0.01 M sodium phosphate buffer (pH 7.0) prior to analysis to prevent multiple scattering. Zeta potential measurements were performed at 23 °C, with a fixed scattering angle of 90° and a laser wavelength of 658 nm. The absorption and refractive indices used for the calculations were 0.001, 1.33, and 1.45, respectively.

2.2.4.5. Physical stability

Physical stability of oat milk samples was evaluated based on volume separation and expressed as physical stability (%). Approximately 15 mL of freshly prepared oat milk was transferred into a graduated cylinder and left undisturbed at room temperature. After 2.5 h of storage, the volume of the sedimented phase at the bottom of the cylinder was recorded, and visual phase separation was documented.

Physical stability (%) was calculated according to the ratio of the non-sedimented (stable) phase volume to the total sample volume using the following Eq. 2:

$$\text{Physical stability (\%)} = \left(1 - \frac{V_s}{V_t} \right) \times 100 \quad (2)$$

where V_s represents the volume of the sedimented phase and V_t denotes the total volume of the oat milk sample. Higher physical stability values indicate greater resistance to sedimentation and phase separation.

2.2.4.6. Color measurement

Color characteristics of the oat milk samples were determined using a colorimeter (ColorFlex EZ, HunterLab, USA). The

device was calibrated using a standard white calibration tile prior to each measurement. Color was expressed in terms of L^* (lightness), a^* (red-green), and b^* (yellow-blue) values. Total color difference (ΔE^*) was calculated using Eq. 3:

$$\Delta E^* = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2} \quad (3)$$

where subscripts 1 and 2 represent the values for control and test samples, respectively.

2.2.4.7. Bioactive properties

The total phenolic content (TPC) of the oat milk samples was determined using the Folin–Ciocalteu colorimetric method according to Singleton and Rossi (1965), with minor modifications. Briefly, 100 μ L of oat milk sample was mixed with 7.9 mL of distilled water and 500 μ L of Folin–Ciocalteu reagent. Subsequently, 1.5 mL of 20% (w/v) sodium carbonate solution was added, giving a final reaction volume of 10 mL. The reaction mixture was vortexed and incubated in the dark at room temperature for 1 h. Absorbance was measured at 765 nm using a UV–visible spectrophotometer (Shimadzu UV-1240, Japan). A reagent blank containing distilled water instead of the sample was prepared under identical conditions. TPC was quantified using a gallic acid calibration curve (0–200 μ g/mL; $R^2 = 0.991$) and expressed as mg gallic acid equivalents (GAE) per 100 mL of oat milk.

The antioxidant activity of the oat milk samples was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay according to Brand-Williams et al. (1995), with minor modifications. Briefly, 100 μ L of sample extract was mixed with 3.9 mL of freshly prepared 0.1 mM DPPH solution in methanol. The reaction mixture was vortexed and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a spectrophotometer (Thermo Spectronic Genesis 5, USA). A control containing 100 μ L of methanol and 3.9 mL of 0.1 mM DPPH solution was prepared under identical conditions. DPPH radical-scavenging activity was calculated using Equation 4.

$$\text{DPPH Scavenging Activity(\%)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad (4)$$

Where A_{control} is absorbance of the control, A_{sample} is absorbance of the sample.

2.2.4.8. Sensory evaluation

A preliminary sensory evaluation was conducted to assess the organoleptic characteristics of germinated and ungerminated oat milk samples. All samples were served at 25 °C. A panel of 10 semi-trained individuals (students of food engineering department) was asked to evaluate the samples based on appearance, color, smell, and overall acceptability, using a 9-point hedonic scale (1 = very poor, 9 = excellent). Samples were tasted for evaluation purposes and then expectorated rather than swallowed.

2.2.5. Statistical analysis

All results are expressed as mean $\pm$ standard deviation (SD) from at least three independent replicates per treatment group. Statistical analyses were performed using R software (Version 3.6.3, R Foundation for Statistical Computing, Vienna, Austria). One-way analysis of variance (ANOVA) was conducted to determine statistically significant differences between groups, followed by Duncan's multiple range test at a 95% confidence level ($p < 0.05$).

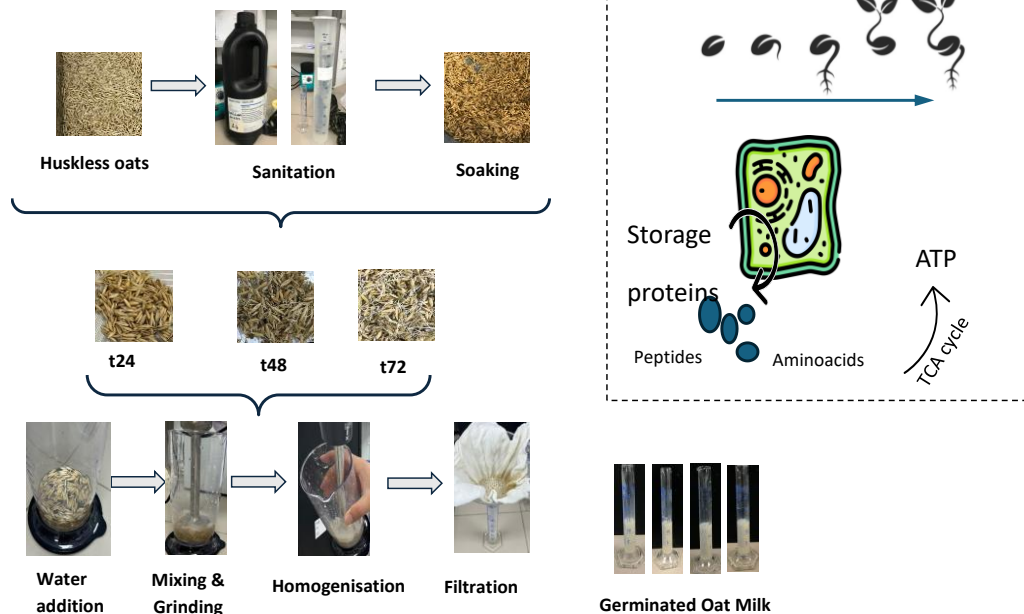


Figure 1. Schematic illustration of the germination process and oat milk preparation. Control (non-germinated), YS24 (24 h germination), YS48 (48 h germination), and YS72 (72 h germination).

Şekil 1. Çimlenmemiş yulafın çimlenme sürecini ve yulaf sütü hazırlama işlemini şematik olarak gösteren çizim. Kontrol (çimlenmemiş), YS24 (24 saat çimlenme), YS48 (48 saat çimlenme) ve YS72 (72 saat çimlenme).

3. Results and Discussion

3.1. Proximate composition of germinated oat samples

Changes in the proximate composition of oat seeds during germination (24, 48, and 72 h) are summarized in Table 1.

Germination induced compositional changes. Fat content exhibited a non-monotonic response to germination. While no significant difference was observed between the ungerminated and YS24 samples, fat content increased significantly at 48 h of germination ($p < 0.05$). The YS72 sample showed intermediate values and did not differ significantly from either the ungerminated or YS48. These

results indicate limited lipid mobilization during early germination, with a transient increase in extractable fat at 48 h, possibly associated with structural loosening of lipid–matrix interactions rather than net lipid biosynthesis.

Table 1. Proximate composition of germinated and ungerminated oat seeds.

Tablo 1. Çimlenmiş ve çimlenmemiş yulaf tohumlarının temel kimyasal bileşimi.

%dm	Fat	Ash	Protein	Carbohydrate
Control	7.09 ± 0.29 ^{b*}	4.51 ± 0.12 ^a	17.60 ± 0.54 ^b	63.00 ± 2.14 ^a
YS24	7.17 ± 0.05 ^b	4.16 ± 0.45 ^a	19.60 ± 0.74 ^a	60.23 ± 3.54 ^b
YS48	7.99 ± 0.11 ^a	4.45 ± 0.78 ^a	18.20 ± 0.81 ^{ab}	60.45 ± 5.41 ^b
YS72	7.40 ± 0.40 ^{ab}	4.65 ± 0.12 ^a	18.80 ± 0.64 ^{ab}	60.61 ± 4.11 ^b

* Control (non-germinated), YS24 (24 h germination), YS48 (48 h germination), and YS72 (72 h germination). Values are expressed as mean ± standard deviation, n = 3.

Crude protein content increased significantly after 24 h of germination compared to the ungerminated. Prolonged germination (48 and 72 h) resulted in intermediate protein levels that were not significantly different from either the ungerminated or YS24 samples. This pattern suggests that the observed increase in protein percentage is primarily due to a relative concentration effect associated with carbohydrate degradation rather than de novo protein synthesis (Wei et al., 2024). Importantly, the absence of protein loss across germination times indicates that germination mainly influences protein quality and structural characteristics, rather than total protein quantity (do Nascimento et al., 2022). Ash content remained statistically unchanged throughout germination. This stability confirms that germination did not alter the total mineral content of oat seeds. Carbohydrate content decreased significantly in all germinated samples compared to the ungerminated ($p < 0.05$), whereas no significant differences were observed among the germinated groups themselves. This reduction reflects partial starch hydrolysis and its utilization as an energy source during germination. The relatively limited magnitude of decrease indicates that carbohydrate degradation was moderate and did not severely disrupt the carbohydrate matrix of the grain (Wei et al., 2024).

3.2. Physicochemical properties of oat milk

3.2.1. pH

Changes in the pH of oat milk samples produced from ungerminated and germinated oat seeds (24, 48, and 72 h) are shown in Figure 2a. The ungerminated oat milk exhibited the lowest pH value (6.33). Germination led to a significant increase in pH ($p < 0.05$), with the highest values observed in the YS24 and YS48 samples (approximately 7.0–7.1), which did not differ significantly from each other.

In contrast, the YS72 sample showed a significantly lower pH than YS24 and YS48, although it remained significantly higher than the ungerminated. The increase in pH during the early and intermediate stages of germination (24–48 h) can be attributed to metabolic and biochemical transformations in the seed, including the activation of proteolytic and phytase enzymes, leading to the release of amino acids and mineral ions that enhance the buffering capacity of the system. The subsequent decrease observed after prolonged germination (72 h) may be related to intensified metabolic activity and the partial accumulation of organic acids, which counteract the initial buffering effects (Saxena et al., 2023). The elevated pH values obtained after 24 and 48 h of germination may be advantageous for oat milk systems, as they can enhance electrostatic repulsion between dispersed components and contribute to improved colloidal stability.

3.2.2. Total soluble solids

Changes in the total soluble solids (TSS) content of oat milk samples produced from ungerminated and germinated oat seeds (24, 48, and 72 h) are shown in Figure 2b. The ungerminated oat milk exhibited a significantly lower TSS value (approximately 1.6%) compared to all germinated samples ($p < 0.05$). In contrast, oat milk samples obtained from germinated seeds (YS24, YS48, and YS72) showed similar TSS values (approximately 1.8%), indicating no significant differences among germination times ($p > 0.05$). The increase in TSS observed after germination can be attributed to enzymatic starch hydrolysis and the release of low-molecular-weight soluble compounds, primarily sugars, into the aqueous phase (Karabulut et al., 2024).

The absence of significant differences among the germinated samples suggests that the major contribution of germination to soluble solid formation occurs during the early stages (24 h), after which a dynamic balance between sugar release and metabolic utilization is established. These findings are consistent with previous reports showing that germination enhances extractable soluble solids in cereal-based systems through enzymatic depolymerization of starch reserves, while prolonged germination does not necessarily result in further increases in TSS (Saxena et al., 2023). The elevated TSS values observed in germinated samples may contribute to improved body and mouthfeel of oat milk without the need for extended germination times.

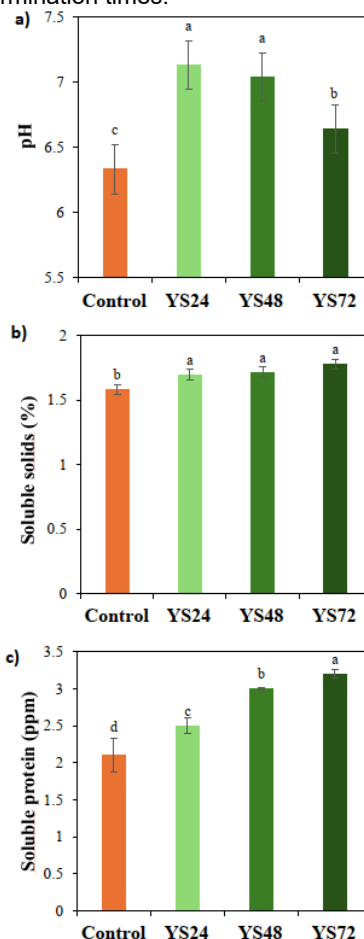


Figure 2. Effects of germination time on basic physicochemical properties of oat milk. a) pH, (b) total soluble solids (%), and (c) soluble protein content of oat milk samples produced from ungerminated (control) and germinated oat seeds for 24 (YS24), 48 (YS48), and 72 h (YS72).

Şekil 2. Çimlenme süresinin yulaf sütünün temel fizikokimyasal özelliklerine etkileri. Çimlenmemiş (kontrol) ve 24 (YS24), 48 (YS48) ve 72 saat (YS72) süreyle çimlendirilmiş yulaf tohumlarından üretilen yulaf sütü örneklerinin pH'ı, (b) toplam çözünür katı madde (%) ve (c) çözünür protein içeriği.

3.2.3. Soluble protein content

Changes in soluble protein content of oat milk samples produced from ungerminated and germinated oat seeds (24, 48, and 72 h) are presented in Figure 2c. Germination resulted in a moderate and time-dependent increase in soluble protein content. The soluble protein concentration increased from 2.1 ppm in the ungerminated control to 2.5, 3.0, and 3.2 ppm in the YS24, YS48, and YS72 samples, respectively. Thus, the YS72 sample exhibited an approximately 52% higher soluble protein content than the ungerminated control. These results demonstrate that germination increased the amount of protein detected in the soluble fraction of the oat milk, with the greatest value observed after 72 h of germination. Similar moderate increases in soluble protein availability following germination have also been reported in germinated oat-based systems (Babolanmogadam et al., 2023).

3.2.4. Particle size and zeta potential

Changes in the particle size and zeta potential of oat milk samples produced from ungerminated and germinated oat seeds are presented in Figures 3a and 3b, respectively. The ungerminated oat milk exhibited a mean particle size of 45,682.5 nm. Germination resulted in progressively lower particle sizes, with values of 15,285.1, 10,285.1, and 7,421.2 nm for the YS24, YS48, and YS72 samples, respectively. The smallest mean particle size was therefore observed in the YS72 sample. Although the numerical particle size decreased

after 24 h, the YS24 sample did not differ significantly from the ungerminated control, whereas more pronounced reductions were observed after 48 and 72 h. The reduction observed with prolonged germination may be associated with the activity of endogenous amylolytic, proteolytic, and cell-wall-degrading enzymes, which can partially disrupt starch–protein aggregates and structural components of the oat matrix (Karabulut et al., 2024).

Germination also altered the zeta potential of the oat milk particles (Figure 3b). The zeta potential changed from –15 mV in the ungerminated control to –18, –22, and –20 mV in the YS24, YS48, and YS72 samples, respectively. These shifts indicate changes in the surface charge characteristics of the dispersed particles, potentially resulting from the exposure or release of ionizable groups during germination. Although more negative zeta potential values may promote electrostatic repulsion, the measured values correspond to moderate rather than strong electrostatic stabilization. Moreover, the YS48 sample exhibited the most negative zeta potential but did not show the highest macroscopic physical stability, as discussed in Section 3.2.5. Therefore, zeta potential and particle size alone were not sufficient to predict the phase-separation behavior of the oat milk samples. Other factors, including continuous-phase viscosity, particle-size distribution, biopolymer hydration, and steric or macromolecular interactions, may also have influenced the observed stability.

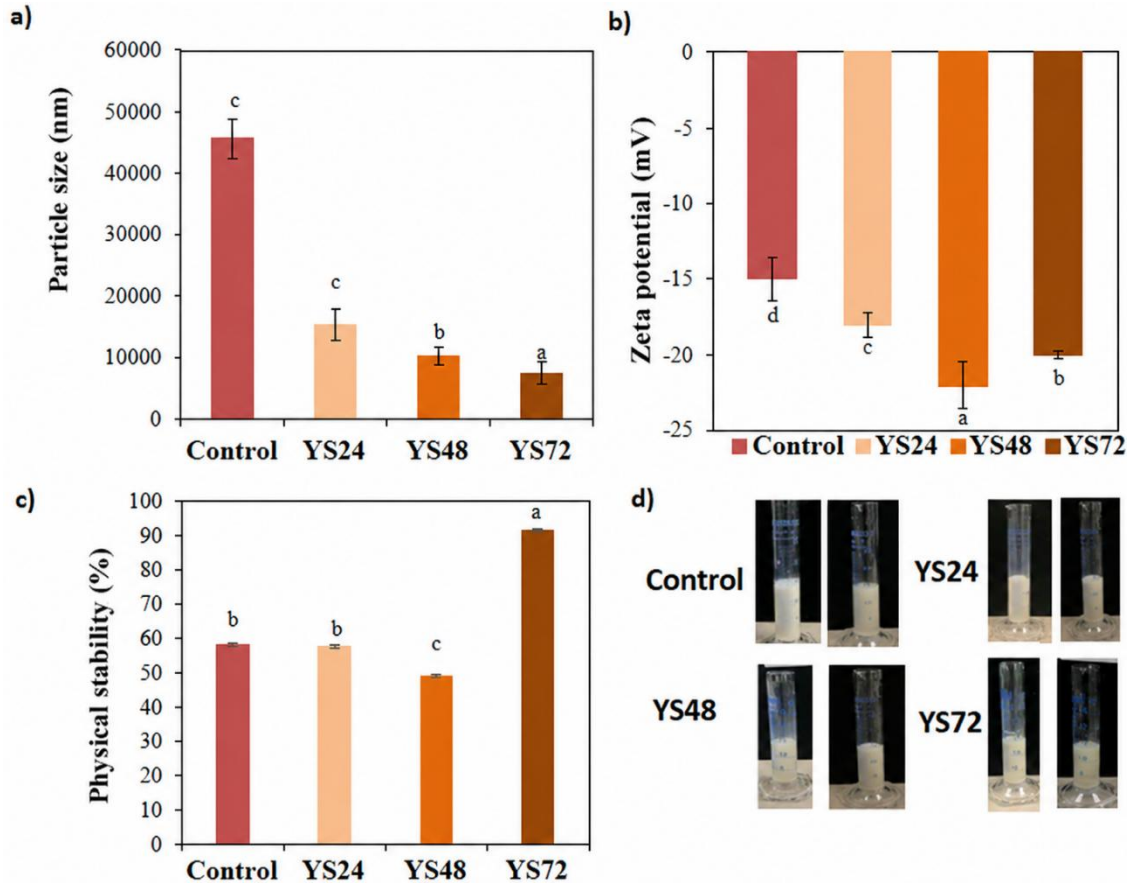


Figure 3. Effect of germination time on particle size and zeta potential of oat milk. (a) Mean particle size, (b) Zeta potential, (c) Physical stability (%) of oat milk samples produced from ungerminated (control) and germinated oat seeds for 24, 48, and 72 h. (d) Representative photographs of oat milk samples after standing 2.5 h, illustrating phase separation behavior.

Şekil 3. Çimlenme süresinin yulaf sütünün partikül boyutu ve zeta potansiyeli üzerindeki etkisi.

(a) Ortalama partikül boyutu, (b) Zeta potansiyeli, (c) Çimlenmemiş (kontrol) ve çimlenmiş yulaf tohumlarından 24, 48 ve 72 saat süreyle üretilen yulaf sütü örneklerinin fiziksel stabilitesi (%). (d) 2,5 saat bekletildikten sonra yulaf sütü örneklerinin temsili fotoğrafları, faz ayrışma davranışını göstermektedir.

3.2.5. Physical stability analysis

Changes in the physical stability of oat milk samples produced from ungerminated and germinated oat seeds (24, 48, and 72 h) are presented in Figure 3c-d. Physical stability differed significantly among the samples ($p < 0.05$) and exhibited a non-linear dependence on germination time.

Physical stability values were 58.33%, 57.50%, 49.17%, and 91.67% for the control, YS24, YS48, and YS72 samples, respectively. The control and YS24 samples exhibited comparable stability, whereas the value decreased to 49.17% after 48 h of germination. In contrast, extending germination to 72 h increased physical stability to 91.67%, corresponding to an approximately 57% improvement relative to the ungerminated control. These results demonstrate a non-linear effect of germination time, characterized by transient destabilization at 48 h followed by pronounced stabilization at 72 h. Notably, prolonged germination (72 h) resulted in a pronounced improvement in physical stability, with the YS72 sample reaching the highest value (approximately 92%). The temporary reduction in stability observed at 48 h may be associated with germination-induced microstructural rearrangements, including partial degradation and redistribution of starch and cell wall polysaccharides, which can lead to less homogeneous particle populations and reduced resistance to sedimentation (Karabulut et al., 2024). Conversely, extended germination (72 h) appears to promote the formation of a more stable colloidal network, likely due to improved dispersion homogeneity, enhanced hydration of biopolymers, and stronger interparticle repulsion. Unlike many commercial oat milk formulations relying on added stabilizers, the improved stability observed in the 72 h germinated sample was achieved solely through germination-induced structural modifications (McCarron et al., 2024; Tuncel et al., 2024). The markedly higher physical stability of the YS72 sample is consistent with the more negative zeta potential values observed at prolonged germination time. These findings demonstrate that germination time is a critical factor governing the physical stability of oat milk, with extended germination (72 h) effectively enhancing resistance to phase separation, while intermediate germination may induce a short-lived destabilization.

3.2.6. Color

The total color difference (ΔE^*) of oat milk samples produced from ungerminated and germinated oat seeds (24, 48, and 72 h) are presented in Figure 4a. Germination induced measurable changes in all color coordinates, with ΔE^* values between 0.30 and 2.31.

These color changes can be attributed to biochemical transformations occurring during germination, including the release of phenolic compounds, enzymatic browning reactions, and redistribution of pigments and soluble solids in the aqueous phase (Liu et al., 2021). Despite these trends, ΔE^* values remained relatively low (< 3), suggesting that the overall color differences between germinated and ungerminated samples were minor and likely below the threshold of strong visual perception. This indicates that germination-induced improvements in nutritional and functional properties do not cause undesirable color alterations, supporting consumer acceptability of germinated oat milk formulations.

3.3. Bioactive properties of oat milk

Changes in TPC of oat milk samples produced from ungerminated and germinated oat seeds (24, 48, and 72 h) are presented in Figure 4b. The ungerminated sample exhibited a TPC of 7.83 mg GAE/100 mL, whereas

germination resulted in a significant and time-dependent increase, reaching 9.95, 10.59, and 11.45 mg GAE/100 mL after 24, 48, and 72 h, respectively, with the highest value observed in the YS72 sample. This progressive increase can be attributed to both *de novo* biosynthesis and enhanced release of phenolic compounds during germination. Activation of phenylalanine ammonia lyase (PAL) stimulates phenolic biosynthesis, while enzymatic degradation of cell wall components and macromolecules facilitates the liberation of phenolics previously bound to polysaccharides and proteins (Domínguez-Arispuro et al., 2017). In addition, germination-induced shifts in pH and nitrogen metabolism may further modulate enzyme activity and phenolic stability, promoting their accumulation. Similar germination-dependent increases in phenolic content have been widely reported for cereal and legume systems and are strongly influenced by germination duration and processing conditions (Domínguez-Arispuro et al., 2017; Merenkova et al., 2022; Lopez-Martinez et al., 2017). The increase in antioxidant capacity following germination may be attributed to enhanced extractability of phenolic compounds rather than excessive accumulation to levels affecting sensory perception (McCarron et al., 2024).

The antioxidant activity of the same samples, evaluated by the DPPH radical scavenging assay (Figure 4c), followed a comparable trend. The ungerminated oat milk showed a DPPH scavenging activity of 6.31%, while germinated samples exhibited gradually higher values, reaching up to 8.33% in the YS72 sample, corresponding to an approximate 2% absolute increase. The relation between TPC and DPPH results indicates that phenolic enrichment is the primary contributor to the observed enhancement in antioxidant activity. In addition to phenolics, germination activates proteolytic pathways that may release amino acids and short peptides with intrinsic antioxidant properties, such as histidine, tyrosine, methionine, and cysteine, which can further contribute to radical scavenging through hydrogen or electron donation (Fadlillah et al., 2023). Previous studies have demonstrated that enzymatic proteolysis and metabolic reorganization during germination promote the formation of bioactive peptides and redistribution of antioxidant metabolites (Merenkova et al., 2022). These results demonstrate that germination promotes a coordinated increase in antioxidant potential through combined phenolic biosynthesis, release of bound phenolics, and metabolic restructuring of seed components. Although the absolute increase in DPPH activity was moderate, the consistent and time-dependent trend suggests germination as an effective and natural strategy to enhance the bioactive functionality of oat milk systems (Lopez-Martinez et al., 2017).

However, antioxidant activity was evaluated using only the DPPH radical scavenging assay, which primarily reflects the hydrogen- or electron-donating ability of antioxidants toward a stable organic radical. Therefore, the results represent DPPH radical scavenging activity rather than the overall antioxidant capacity of the samples. The absence of complementary assays based on different antioxidant mechanisms, such as ABTS radical scavenging or ferric reducing antioxidant power (FRAP), is a limitation of the present study.

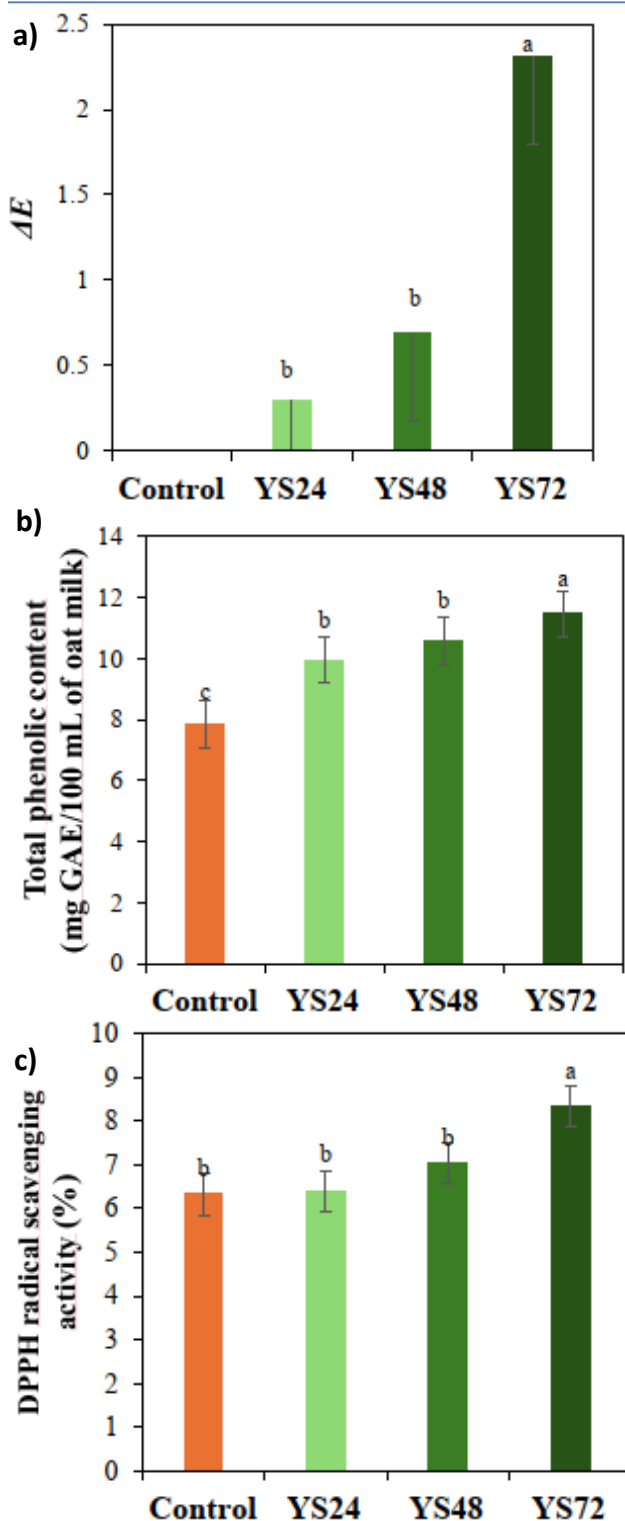


Figure 4. Color and bioactive properties of oat milk as affected by germination time.

Şekil 4. Yulaf sütünün rengi ve biyoaktif özellikleri, çimlenme süresine bağlı olarak değişimi.

3.4. Sensory evaluation

The sensory attributes of oat milk samples produced from ungerminated and germinated oat seeds were evaluated in terms of appearance, color, smell, taste, mouthfeel, and overall acceptability, and the results are presented in Figure 5a–f. Germination significantly influenced all sensory attributes; however, the direction and magnitude of the changes depended on the germination time and the attribute evaluated.

Appearance scores increased from approximately 6.0 in the ungerminated control to 6.1 in YS24 and 6.3 in both YS48 and YS72. The YS48 and YS72 samples received the highest appearance scores and did not differ significantly from each other ($p > 0.05$). Color scores also increased with germination, rising from approximately 5.4 in the control to 5.6, 5.7, and 6.1 in YS24, YS48, and YS72, respectively. The highest color score was observed for YS72. These results indicate that prolonged germination improved the visual attributes of the oat milk. The relatively small instrumental color differences among the samples ($\Delta E^* < 3$) also indicate that the germination-induced color changes remained limited.

In contrast, smell scores decreased from approximately 4.8 in the ungerminated control to 4.5 in YS24 and approximately 4.2 in both YS48 and YS72. The control received the highest smell score, whereas YS48 and YS72 received the lowest scores. Taste scores followed a non-linear pattern and decreased from approximately 4.0 in the control to 3.7 in YS24 and 3.1 in YS48, before increasing to approximately 3.8 in YS72. Nevertheless, all germinated samples received significantly lower taste scores than the control. Mouthfeel was also affected by germination. The control received the highest score of approximately 5.1, while YS24 received the lowest score of approximately 3.9. The mouthfeel scores of YS48 and YS72 recovered to approximately 4.9 but remained lower than that of the control.

Overall acceptability decreased from approximately 4.9 in the ungerminated control to 4.5 and 4.3 in the YS24 and YS48 samples, respectively. The YS72 sample received an overall acceptability score of approximately 4.8 and did not differ significantly from the control ($p > 0.05$). Thus, although intermediate germination periods reduced overall acceptability, extending germination to 72 h restored acceptability to a level comparable to that of the ungerminated oat milk. These findings partially agree with Babolanimogadam et al. (2023), who reported acceptable sensory scores for oat milk produced using germination treatments. Overall, prolonged germination improved appearance and color and maintained overall acceptability, although reductions in smell, taste, and mouthfeel scores revealed attribute-specific sensory trade-offs.

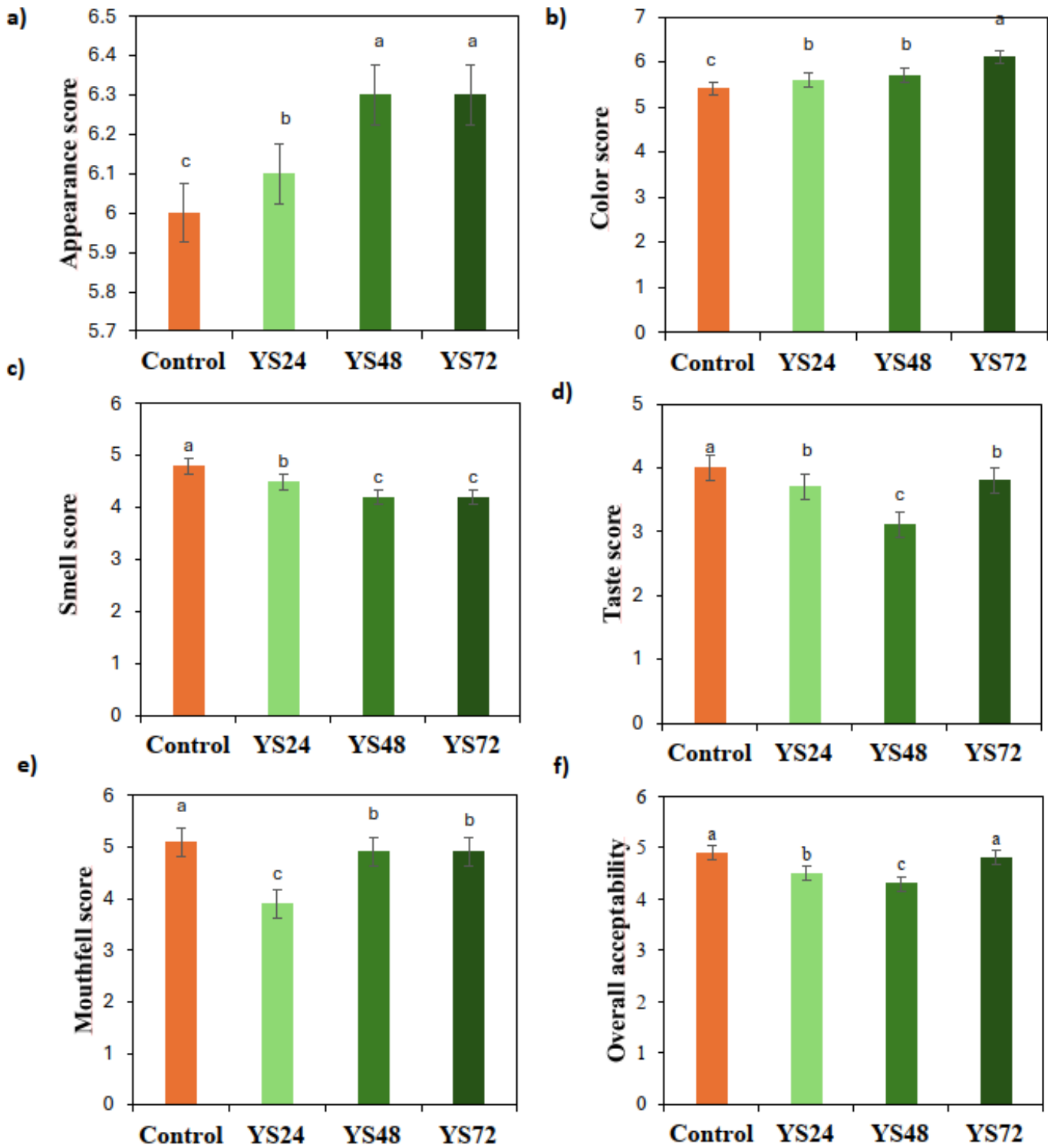


Figure 5. Sensory properties of oat milk as affected by germination time. Control (non-germinated), YS24 (24 h germination), YS48 (48 h germination), and YS72 (72 h germination).

Şekil 5. Yulaf sütünün duyu özelliklerinin çimlenme süresine bağlı olarak değişimi. Kontrol (çimlendirilmemiş), YS24 (24 saat çimlendirme), YS48 (48 saat çimlendirme) ve YS72 (72 saat çimlendirme).

4. Conclusion

This study demonstrated that germination is an effective and sustainable pretreatment for modifying the physicochemical stability and bioactive properties of oat milk. Germination for 24–72 h induced systematic biochemical and structural changes, including increases in pH and total soluble solids and a progressive reduction in mean particle size from 45,682.5 nm in the ungerminated control to 7,421.2 nm after 72 h. Germination also produced more negative zeta potential values, with the highest absolute value observed after 48 h.

The reduction in particle size, together with the increase in the absolute zeta potential, likely contributed to reduced particle aggregation and gravity-driven separation. Soluble protein content increased moderately from 2.1 to 3.2 ppm, indicating partial protein solubilization during germination. However, the relatively limited magnitude of this increase suggests that the observed changes in colloidal stability also involved modifications in starch, other soluble components, and interfacial interactions.

Germination significantly increased total phenolic content in a time-dependent manner, accompanied by an increase in DPPH radical scavenging activity. Sensory evaluation revealed improvements in appearance and color but reductions in smell, taste, and mouthfeel. Overall acceptability decreased in the YS24 and YS48 samples, whereas the YS72 sample remained comparable to the ungerminated control. Overall, controlled germination represents a mild and environmentally friendly strategy for producing oat milk with modified colloidal characteristics and enhanced bioactive potential.

Authors Contributions

All authors: Writing – original draft, Methodology, Investigation.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding Statement

This research was supported by the TUBITAK 2209-A under Grant [1919B012322444].

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Statement

This study, including the preliminary sensory evaluation involving human participants, was approved by the Science and Engineering Ethics Committee of Sakarya University, Türkiye, on 24 May 2024 (Meeting No. 47, Decision No. 02). All procedures involving human participants were conducted in accordance with the ethical standards of the committee and the Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants prior to the sensory evaluation. During the evaluation, participants tasted the samples and then expectorated them without swallowing.

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