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Modern Teknikler ve α -Amilaz Uygulaması ile Üretilen Yulaf Sütünün Yapısal ve Fizikokimyasal Özellikleri

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ÖZET

Yulaf sütü, besin değerleri ve fonksiyonel özellikleri sayesinde süt ürünlerine bitki bazlı bir alternatif olarak giderek artan bir ilgi görmektedir. Bu çalışma, α-amilaz uygulamasının geleneksel ısıtma, mikrodalga ve ultrason gibi farklı işleme teknikleriyle birlikte kullanımının yulaf sütünün fizikokimyasal özellikleri üzerindeki etkilerini incelemeyi amaçlamıştır. Elde edilen sonuçlar, her bir işleme yönteminin yulaf sütünün yapısal ve fizikokimyasal özellikleri üzerinde önemli etkiler oluşturduğunu göstermiştir. Özellikle, α-amilaz ile mikrodalga işleminin birlikte uygulanması en uygun sonuçları sağlamış, ürünün kremsi yapısını artırırken istenmeyen tatların azalmasına katkı sağlamıştır. Sonuçlar ayrıca enzimatik işlemin pH, toplam çözünür katı madde miktarı ve genel stabilite gibi temel kalite parametrelerini önemli ölçüde etkilediğini ortaya koymuştur. Özellikle α-amilaz enzimi, nişasta moleküllerini parçalayarak çözünürlüğü artırmış ve bu durum aynı zamanda renk özelliklerinde belirgin değişikliklere yol açmıştır. İşleme teknikleri arasında belirgin farklılıklar gözlemlenmiştir. Mikrodalga işlemi en yüksek stabiliteyi sağlamış; dispersiyonu artırma ve faz ayrışmasını azaltma konusunda etkili bir yöntem olduğunu göstermiştir. Ultrason işlemi ise; partikül kümelerinin parçalanması ve mikro yapısal düzeyde daha iyi homojenizasyon sağlanması sayesinde protein çözünürlüğünü artırmış ve çökelmeyi azaltmıştır. Geleneksel ısıtma yöntemi yulaf sütünde bazı iyileştirmeler sağlasa da diğer yöntemlere kıyasla daha düşük performans göstermiştir. Genel olarak, enzimatik hidroliz ile gelişmiş işleme teknolojilerinin kombinasyonu, yulaf sütü kalitesinin artırılmasında önemli bir potansiyele sahiptir. Bu yöntemler fizikokimyasal stabiliteyi iyileştirmekte, çözünürlüğü artırmakta ve ürünün görsel ile fonksiyonel özelliklerini geliştirmektedir. Böylece, tüketici kabulünün artırılmasına ve yüksek kaliteli bitki bazlı süt alternatiflerinin geliştirilmesine önemli katkılar sunmaktadır. Bu tür yenilikçi uygulamalar, gıda endüstrisinde sürdürülebilir üretim yaklaşımlarını da destekleyebilir.

Structural and physicochemical properties of oat milk produced by modern techniques and α-amylase treatment

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ABSTRACT

Oat milk has gained growing popularity as a plant-based alternative to dairy milk due to its nutritional benefits and functional properties. This study investigated the effects of α-amylase treatment combined with different processing techniques—namely traditional heating, microwave processing, and ultrasound on the physicochemical characteristics of oat milk. The results indicated that each processing method significantly influenced the texture and physicochemical properties of the oat milk. The combination of α-amylase treatment with microwave processing yielded the most favorable outcomes, enhancing the creaminess while reducing undesirable off-flavors. The results showed that the enzymatic treatment significantly impacted key quality attributes, including pH, total soluble solids, and overall stability. In particular, α-amylase improved solubility by breaking down starch molecules, which also led to noticeable changes in color. The processing methods demonstrated clear differences in their effectiveness. Microwave treatment resulted in the highest stability, indicating its strong ability to enhance dispersion and reduce phase separation. Ultrasound processing, on the other hand, improved protein solubility and minimized sedimentation, likely due to its capacity to break down particle clusters and promote better homogenization at the microstructural level. Although traditional heating contributed to some improvements, it was less effective compared to the other techniques. Overall, the combination of enzymatic hydrolysis and advanced processing technologies shows considerable promise in enhancing oat milk quality. These methods improve physicochemical stability, increase solubility, and enhance visual and functional properties. As a result, such approaches can improve consumer acceptance and support the production of high-quality plant-based milk alternatives in the food industry. Such innovative applications can also support sustainable production approaches in the food industry.

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INTRODUCTION

In recent years, with the increase in alternative foods, consumers have become more interested in products that concern their health. Functional foods, which are obtained by differentiating compounds in foods and increasing their bioavailability, create additional benefits for human health, provide defense against diseases and provide a more comfortable life. Plant-based milk drinks used instead of milk stand out in the food market because they do not contain lactose and cholesterol; in addition, plant-based milk drinks offer a visual appearance similar to animal milk, different sensory properties, kinetics, stability and nutritional composition [1,2].

Oat (*Avena sativa.L*) belongs to the *Aveneae* subfamily of the *Poaceae/Gramineae* family. It is a cereal grain used for food, animal feed, and industrial purposes. Economically, oat is the seventh most important cereal after maize, wheat, rice, sorghum, barley, and millet. Oat grain consists of hull (25%), pericarp, testa, and aleurone (9%), starchy endosperm (63%), and embryo (3%). Starch, which constitutes approximately 50% of oat, is the most abundant component. In the food industry, oats are used in various forms, including noodles, oat flakes, porridge, bread, cookies, oat milk and yogurt. Oat milk, a water extract of oat, is considered a novel nutritional and bioactive food [3]. As a cereal grain, oat (*Avena sativa L.*) has gained increasing interest due to its excellent nutritional profile, which includes a balanced amino acid profile, proteins, unsaturated fatty acids, and dietary fiber, particularly β-glucan. Additionally, it is a good source of bioactive phytochemicals, vitamins, phenolic acids, and other micronutrients [3, 4]. Oat exhibits various biological and health benefits, including antioxidant, anticancer, and antiallergic properties.

When an oat-water mixture is heated, the starch gelatinizes, forming a highly viscous gel, which decreases acceptability. To ensure fluidity and prevent gelatinization, a pre-hydrolysis process is recommended [5, 6]. The composition of oat milk varies depending on the oat grain variety and processing factors [7, 8]. The production process of oat milk substitute was improved by using enzymatic hydrolysis in oat milk production, and it was reported that the production of water-soluble extract in oats increased as a result of the starch liquefaction process by increasing the enzyme concentration [6] .

Relatively new technologies such as ultrasound and microwave are possible alternative processing methods that are being investigated for liquid foods compared to traditional methods. Ultrasound (US) processing is a non-thermal technology commonly used for a wide range of applications, including homogenization, emulsification, microbial inactivation, and extracting chemical compounds from plant materials through US irradiation [9].

Microwave heating among innovative thermal technologies for processing plant-based beverages, microwave heating is an efficient method for reducing microbial load and extending shelf life. It operates on electromagnetic radiation (103–104 MHz), which interacts with water molecule dipoles, generating heat through intermolecular friction. Additionally, ionic conduction enhances heating as the electromagnetic field moves ions toward opposite charges, increasing collisions and disrupting hydrogen bonds in water [5]. However, despite its growing popularity, oat milk production still faces several technological challenges. Conventional thermal processing methods can lead to undesirable changes in sensory and nutritional qualities, such as off-flavors, reduced protein solubility, sedimentation of insoluble particles, and viscosity issues due to starch gelatinization. These drawbacks can compromise consumer acceptance and limit shelf stability.

Additionally, the variability in raw material composition and lack of optimized processing parameters further complicate the standardization of oat milk quality. Therefore, there is a need to explore innovative and more precise processing technologies, such as enzymatic treatments and modern techniques like ultrasound and microwave, to improve the functional and physicochemical properties of oat milk without compromising its nutritional value or sensory attributes.

This study aimed to evaluate the properties of oat milk subjected to different modern processing techniques microwave and ultrasound alongside traditional methods, with and without the addition of α-amylase. The research focused on the impact of these treatments on oat milk's physicochemical properties, including pH, stability, solid particle sedimentation, soluble solids content (brix), protein solubility, color, and microstructure.

MATERIALS AND METHODS

The oat used as the raw material for plant-based milk production was sourced from the local market in Konya, Turkey. The amylase enzyme used in oat milk production was procured from a commercial supplier Vatan Chemistry and Machinery Industry Istanbul, Turkey. All other reagents were of analytical grade.

Oat milk production

Whole oat grains were first tempered in water (1:3 v/v) at boiling temperature for 5 minutes and then drained. The tempered oats were subsequently soaked in 1:3 sterilized water at room temperature for 12 hours. The soaked oats were blended BioChef Living Food Blender, Arizona Sol, New South Wales, AU) with water at a (1:6 v/v) ratios at medium speed for 3 minutes. The resulting oat slurry was then filtered and extracted using a 150 µ thick muslin cheesecloth strainer until oat milk was obtained. The oat milk obtained at this stage was used (No Enzyme).

Enzyme treatment

Alpha amylase enzyme was added to the oat milk obtained as described above before the filtration process.The oat slurry treated by enzyme-samples, 0.75 mg/kg α-amylase enzyme was activated for 50 minutes in a water bath at 80°C, followed by enzyme inactivation at 100°C for 5 minutes [11].The enzyme-treated slurry was subsequently filtered through a 150 µm muslin cloth [12]. Following the mixtures was divided into four samples: one remained untreated (Control samples), while the others were subjected to different processing methods traditional heating, microwave treatment, and ultrasound treatment.

Processing Methods Applied to Oat Milk

Traditional method

In the traditional method, oat milk samples were subjected to heat treatment by placing them in a 100°C water bath for 20 minutes. After heating, the samples were allowed to cool to room temperature. The oat milk was subsequently homogenized using a homogenizer (IKA T25 digital, Staufen im Breisgau, Germany) at 4,000 rpm for 3 minutes and then stored at 4°C until further analysis [12].

Microwave method

The microwave method was conducted at 950 W and 2.45 GHz frequency for 3 minutes at 95°C. Following the treatment, the samples were stored at 4°C for further analysis [7, 8].

Ultrasound method

The samples were subjected to ultrasound pre-treatment using an ultrasonic probe (20 kHz, 100 W - Bandelin UW 2200, Berlin, Germany) at 100% amplitude for 10 minutes at a constant 20 kHz frequency [14].

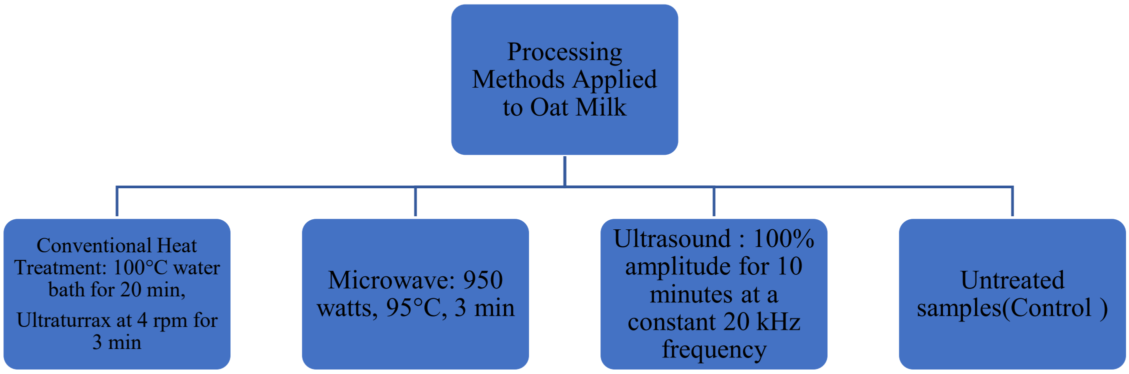

Figure1

Processing Methods Applied to Oat Milk

Soluble solids content (Brix) and pH

The total soluble solids content of the oat milk samples was determined at room temperature (25°C ± 1) using a refractometer following AOAC (2000) guidelines [15]. The pH values samples were measured at 25°C using a digital pH meter (Mettler Toledo Seven Compact S210).

Stability

To assess assess precipitation – sedimentation –floating -stability stability, oat milk samples were placed in 100 mL bottles and stored at 5°C for 8 hours. The sediment layer (volume) was measured, and the phase separation percentage (v/v) was calculated [16].

Solid Particle Sedimentation

10 g sample was put in a centrifuge tube and centrifuged at 10,000 rpm for 10 minutes at 20°C. The supernatant was transferred to a new 15 mL centrifuge tube for protein solubility testing. The residual sediment was weighed, and the solid particle sedimentation percentage was calculated using Equation 1.

$$\text{SPS } \% = \frac{\text{Wsi} - \text{Wpr}}{\text{Wsi}} * 100 \quad (1)$$

where W_{si} is the initial sample weight and W_{pr} is the weight of the precipitate.

Protein Solubility

Protein solubility was analyzed using the supernatant obtained from the sedimentation test. 2 mL supernatant was mixed with 2 mL Biuret reagent and incubated for 20 minutes before filtration through 0.45 µm PFTE syringe filters. Absorbance was measured at 550 nm using a spectrophotometer (Libra S60, Biochrom Ltd., Cambridge, UK). Protein solubility was determined using a standard bovine serum albumin (BSA) calibration curve and expressed as a percentage [9].

Color Analysis

The color parameters of oat milk samples were measured using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan) based on L* (lightness: 0 = black, 100 = white), a* (red-green axis), and b* (yellow-blue axis) values. Chroma © and hue angle (H*) was calculated using the following formulas 2 and 3:

$$C = \sqrt{a^2 + b^2} \tag{2}$$

$$H = \tan^{-1}\frac{b}{a} \tag{3}$$

Microstructure Analysis

The microstructure of samples was examined using an inverted optical microscope (40X COIC XJP-3A Optical Microscope). A homogenized drop of each sample was placed on a microscope slide and covered with a cover slip. Images were captured at least three times per sample using the microscpe's imaging software [9].

RESULTS AND DISCUSSION

pH

pH is a convenient indicator of quality milk and is widely used. The determination of pH and titration acidity is crucial for assessing microbial growth in food products. pH significantly influences microbial formation and distribution [13,14].

The pH values of oat milk samples ranged between 5.03 and 5.84 presented in Table 1. Oat milk samples with added amylase enzyme exhibited lower pH values compared to those without enzyme addition. Among different processing methods, the higher pH values were recorded in the control samples, while the lower pH values were observed in samples produced using the traditional method. Starch, being a large polymeric polysaccharide confined or exist in granules in native form, tend to be insoluble, exhibits a neutral or slightly alkaline pH when dissolved. It does not release free hydrogen ions (H^+) unless hydrolyzed. Amylase enzyme hydrolyzes starch into glucose units, and glucose, as a monosaccharide, can exhibit a slightly acidic pH when dissolved in water. This is due to structural changes in glucose and the release of free H^+ ions affecting the solution's pH [19].

Soluble solids content (Brix)

The results of soluble solids content shown in Table 1 were significantly different between the samples. Oat milk samples with added α-amylase enzyme exhibited higher Brix values compared to those without enzyme addition. Among different processing methods, the lowest Brix values were observed in the control samples, followed by samples produced using microwave, ultrasound, and traditional methods. Enzyme enhance solubility and flavor profile by breaking down starch and other oat components. Amylase, in particular, converts, resulting in improved consistency, and increased yield. This process also increases the total solid content by promoting maltodextrin production. The application of ultrasonic waves in liquid environments induces cavitation, leading to the formation, expansion, and sudden collapse of microscopic bubbles. This collapse generates high-temperature and high-pressure waves that exert mechanical stress on cell walls, facilitating their breakdown and the release of intracellular contents[16, 17] The combined effect of ultrasound amplitude and cavitation enhances polysaccharide hydrolysis and reduces particle size [22]. A study examining the effect of temperature and soluble solids concentration on the apparent viscosity of oat milk reported a strong dependence on these factors [10].

Table 1

Physicochemical characteristics of the oat milk obtained among various processing methods

Factors	pH	SDM (%)	Stability (%)	SPS (%)	Protein solubility (%)
Enzyme Addition					
α-amylase	5.23[b]	4.00[a]	86.96[a]	24.90[a]	24.90[a]
No Enzyme	5.26[a]	3.62[b]	76.29[b]	20.86[b]	20.86[b]
Methods					
untreated (Control)	5.84[a]	3.33[b]	88.97[a]	33.89[a]	33.89[a]
Ultrasonic	5.05[bc]	4.01[a]	72.09[c]	29.96[b]	29.96[b]
Microwave	5.07[b]	3.97[a]	90.02[a]	19.72[c]	19.72[c]
Traditional	5.03[c]	3.95[a]	75.41[b]	7.95[c]	7.95[c]

Means in the same column marked with the same letter are not statistically different from each other ($p < 0.05$). SDM: Soluble dry matter. SPS: Solid particle sedimentation."

Stability

The stability of plant-based milk alternative beverages depends on the particle size of the dispersed phase. These products are colloidal systems composed of dispersed particles such as fat globules, raw material particles, proteins, and starch granules [23]. Including partially or fully swollen granules, gelatinized (solubilized amylose and amylo-pection pars to starches, partially of fully denatured proteins and other particles. Due to particle heterogeneity and varying density, these products are susceptible to sedimentation and phase separation, leading to instability [24]. Instability in milk alternatives may also contribute to a sandy or chalky mouthfeel. Furthermore, the lack of creaminess due to fat separation is a sensory factor perceived by consumers. The stability of plant-based milk alternatives is influenced by three primary factors: particle size, emulsion formation, and protein solubility. Various physicochemical attributes were shown in as shown in Table 1 were significantly different between the samples, oat milk samples with added amylase enzyme exhibited higher stability values than those without enzyme addition. Among different processing methods, the highest stability values were observed in untreated (control) samples and those processed using the microwave method, followed by samples produced using the traditional method. Oat starch, comprising 50-60% of the grain, has a gelatinization temperature ranging between 44.7°C and 73.7°C. During thermal processing, enzyme addition may interfere with oat milk gelatinization [25]. This enzymatic liquefaction of starch not only improves solubility but also influences the interaction between solubilized starch, proteins, and other oat milk constituents, thereby contributing to the colloidal stability and textural characteristics of the final product.

Sedimentation of solid particles

Plant-based beverages are emulsified products undergoing multiple processing steps that influence component distribution. Dispersed particles in the liquid phase vary from natural protein aggregates to fat droplets and other aggregates formed by proteins, fats, or polysaccharides. The most common stability issues in plant-based beverages are sedimentation of solid particles and creaming of fat droplets, both of which depend on particle size distribution [26]. Centrifugation-induced sedimentation does not directly measure physical stability but provides insight into the potential stability of the colloidal system. A high sedimentation value indicates low stability due to particle settling [27].

The sedimentation values of oat milk samples ranged between 7.95% and 33.89%. According to the Tukey HSD comparison test results in Table 1, samples with added amylase enzyme exhibited higher sedimentation values than those without enzyme addition. Among different processing methods, the highest sedimentation values were observed in control samples, while the lowest values were determined in samples processed using microwave and traditional methods. There was a notable difference between enzyme-treated and untreated samples. Enzymes facilitate the breakdown of complex compounds, and different processing techniques (ultrasound, microwave, and traditional methods) promote a more homogeneous structure. The ultrasonic method enhances particle stability and uniform distribution, reducing sedimentation rates. The microwave method improves protein solubility and binding properties, further decreasing sedimentation. Previous studies reported that almond milk sedimentation rates were reduced more significantly by pulsed electric field (PEF) treatment compared to thermal processing [28]. Studies on nut milk alternatives demonstrated that homogenization significantly reduced sedimentation values from 51.85% to 24.5% [26].

Protein solubility

Protein solubility is a crucial functional property of proteins, as it plays a significant role in other characteristics such as rheological, hydrodynamic, and surface-active properties. High solubility is essential for many protein-based products [30] .

The protein solubility values of oat milk samples given in Table 1, when examining the impact of amylase enzyme addition in oat milk, it was observed that enzyme-added samples had lower protein solubility values than those without enzyme addition. When analyzed based on production methods, the highest protein solubility values were found in the control sample, followed in decreasing order by ultrasound and microwave methods. The lowest protein solubility values were observed in oat milk samples produced using the traditional method. In a study, the effect of protease and amylase application on proteins and product quality in quinoa-based dairy products was investigated and The solubility of protein in the samples was found to be between 48.02-75.19% [23]. In another study, the functional properties of soy proteins were examined by high pressure homogenization (UHPH) method and it was determined that protein solubility values vary between 21-39% and protein solubility increases [31]. Similarly, Dhakal et al. (2014), the effect of high pressure and temperature on almond milk protein solubility was examined. It was reported that protein solubility decreases with increasing pressure [32].

Color properties

Color is a visual indicator used to assess the quality of a beverage and plays a critical role in consumer satisfaction. The color variations in plant-based milks result from differences in composition and structure, which are influenced by different ingredients and unit operations used in production.

The color properties of oat milk, including brightness, redness, yellowness, saturation index, and hue angle are influenced by enzyme addition and production methods as shown in Table 2. The L* values of samples ranged between 67.57 and 77.64, with enzyme-added samples exhibiting higher brightness due to improved homogenization and solubility. Color measurements on a number of commercial plant-based milk substitutes (including cashew, hazelnut, hemp, almond, oat, soy and rice milk) have shown that whiteness indices calculated from L* values range from 52 to 76, which is statistically significantly different. This value is also reported to be lower than the value of 82 reported for cow's milk [19, 30]. The a* values of oat milk samples ranged from -6.21 to -5.93, with enzyme addition leading to increased redness, potentially due to Maillard reactions or pigment modifications. Production methods also influenced redness, with the highest a* values in control samples. The b* values ranged between 5.74 and 8.68, where enzyme-added samples had lower values, and traditional methods resulted in the lowest yellowness. Stabilizers and emulsifiers used in plant-based milks can alter the physicochemical properties of the product. For example, the addition of vanilla or other flavors can increase the b* value, as these substances often contain yellowish tones [34]. Another study investigated the effects of pulsed electric field (PEF) treatment on the quality properties and microbial inactivation of soy milk under different PEF parameters. It was reported that the "a" and "L" values increased with PEF duration from 50.34±0.19 to 53.64±0.25, while the "b" value decreased from 5.31±0.04 to 5.17±0.01 [35]. A study on soy milk reported that different temperature and pressure applications altered the b* value. Heat treatment induced Maillard reactions in soy milk, resulting in a yellowish appearance (increased b* values) [36].

The saturation index (SI) and hue angle values of oat milk samples ranged between 8.41–10.20 and 180.29–188.41, respectively. Enzyme-added samples had lower SI and higher hue angle values, while different production methods affected chroma intensity. Research has explored the impact of high-pressure homogenization and heat treatments on the physicochemical properties and stability of hazelnut and almond milk. Results showed that almond milk appeared whiter and lighter, with reduced hue and chroma values, leading to a less saturated reddish color [14, 26] reported that ultrasound treatment had significant effects on the color values of almond milk [14].

Another study developed plant-based milk alternatives using chickpea and coconut as replacements for cow's milk. It was reported that as the coconut milk concentration increased, chroma values decreased [18, 37].

Table 2
Results of Multiple Comparison Tests for The Color Properties of Oat Milk Samples

Factors	L*	a*	b*	SI	Hue angle
Enzyme Addition					
α-amylase	75.06^a	-6.07^a	5.74^b	8.412^b	188.41^a
No Enzyme	73.82^b	-6.17^b	8.68^a	10.20^a	180.29^b
Methods					
Control	77.64^a	-5.93^a	7.88^a	9.86^a	184.26^a
Ultrasonic	76.66^b	-6.20^b	7.00^b	9.43^b	186.29^a
Microwave	75.89^b	-6.14^b	6.85^b	9.25^b	183.25^a
Traditional	67.57^c	-6.21^b	5.98^c	8.68^c	183.59^a

"Means in the same column marked with the same letter are not statistically different from each other ($p < 0.05$)."

Microstructure

Figure 2 presents the micrographs of oat milk samples. These micrographs reveal noticeable differences between homogenized and non-homogenized samples due to the significant reduction in the size of suspended particles caused by homogenization. The general effects of enzymes on microstructure involve the breakdown or modification of proteins and polysaccharides in plant-based milk.

The micrographs in Figure 2 indicate a clear distinction between processed and unprocessed samples. Among the different processing methods, ultrasound treatment significantly reduced the size of suspended particles in the milk. As observed, milk produced using the control (untreated) method primarily consists of irregularly shaped, deformable particles and a few small clusters, which are likely flocculated proteins due to their hydrophobic nature. On the other hand, oat milk samples subjected to traditional and microwave processing exhibited extremely large protein aggregates in both cases. In oat milk, the high starch content may have led to gelatinization due to heat exposure.

In the study the effects of high-pressure homogenization and thermal treatments on the physicochemical properties and stability of almond and hazelnut milk were examined. The influence of homogenization pressure on the product's microstructure was observed [26]. obule size and an increase in protein stability [38]. In their study, Şen and Okur reported that examination of microscopic images of hazelnut milk samples revealed clear differences between the control and thermally treated samples, as well as between the thermally treated and ultrasound-treated samples in both groups[9].

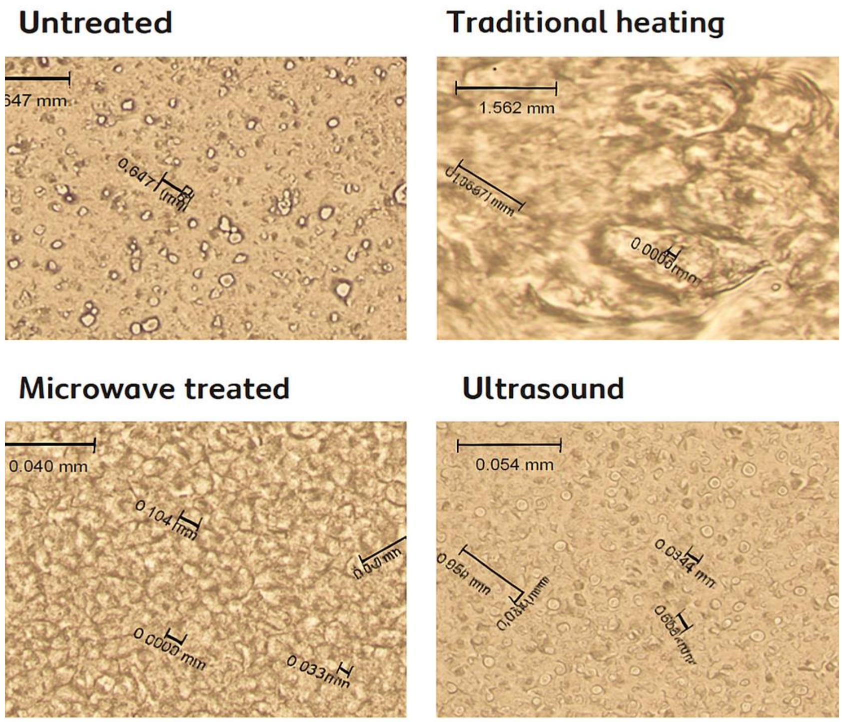

Figure 2
Microstructure of oat milk samples.

CONCLUSION

This study demonstrated that both amylase enzyme addition and different processing methods have a significant impact on the physicochemical and functional properties of oat milk. α-Amylase improved solubility and stability, while influencing pH, color, and Brix values. Microwave treatment yielded the highest stability, ultrasound enhanced protein solubility, and reduced sedimentation. Notably, processing methods altered brightness, redness, saturation index, and hue angle, indicating distinct differences between treated and untreated samples. Microscopic analysis further revealed that ultrasounds reduced the size of suspended particles, while traditional and microwave methods produced large protein aggregates. These results highlight that modern processing technologies, particularly ultrasound and microwave treatments, combined with enzyme application, can effectively enhance the functional qualities of oat milk, increasing its appeal as a plant-based alternative.

Ethical Statement

The present study is an original research article designed and produced by the authors.

Author Contributions

Research Design (CRediT 1) N.E. (70%) – S.I.H.A. (30%)

Data Collection (CRediT 2) N.E. (90%) – S.I.H.A. (10%)

Research- Data Analysis- Validation N.E. (70%) – S.I.H.A. (30%)

Writing the Article (CRediT 12-13) N.E. (80%) – S.I.H.A. (20%)

Revision and Improvement of the Text (CRediT 14) N.E. (50%) – S.I.H.A. (50%)

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Sustainable Development Goals (SDG)

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