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RESEARCH ARTICLE

Incorporation of propolis as a natural additive to enhance rheological, physicochemical and sensorial properties of yoghurt

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Abstract

This study aimed to evaluate the potential of propolis as a natural additive to enhance the physicochemical, rheological, and sensory properties of buffalo milk yoghurt. Fresh yoghurt was supplemented with two concentrations of ethanol propolis extraction (0.1% and 0.3% (w/v)) and compared against a control sample without propolis. The samples were stored at refrigeration temperature (4 ± 2°C) for 14 days. Key parameters including pH, titratable acidity, hardness, viscosity, and sensory attributes were monitored throughout the storage period. Results showed that propolis significantly influenced yoghurt characteristics. PH levels decreased while titratable acidity increased in treated samples, indicating enhanced fermentation activity. Hardness improved over time, particularly in the 0.1% propolis treatment, which reached 0.50 N by day 14. Viscosity remained stable across all treatments, suggesting no adverse effects on texture consistency. Sensory evaluation revealed lower initial acceptability for the 0.3% propolis yoghurt due to taste and color changes. However, overall acceptability improved during storage, especially for the 0.1% concentration, which maintained scores above 94 out of 100. These findings suggest that propolis, particularly at 0.1%, can improve yoghurt quality by enhancing acid development, texture firmness, and preservation without compromising sensory appeal after short-term storage. This supports its potential as a clean-label alternative to synthetic preservatives such as potassium sorbate in functional dairy products.

***Keywords*:** Propolis, Yoghurt, Rheology, Natural Preservative, Functional Food

1. Introduction

Dairy products, particularly fermented ones such as yoghurt, are highly perishable and susceptible to spoilage caused by microbial contamination and oxidative degradation. To extend shelf life and maintain quality, the dairy industry often relies on chemical preservatives like potassium sorbate. However, concerns over the safety and side effects of synthetic additives have prompted investigations into safer, naturally derived alternatives (Skandamis et al. 2001; Bhutani, 2003). Several studies have explored the incorporation of natural extracts including essential oils, herbal infusions, and bee products in dairy foods to enhance stability while preserving sensory and nutritional attributes (Negi, 2012; Soliman and Badeea, 2002; Thamnopoulos et al., 2018). Yoghurt is not only a widely consumed dairy product valued for its nutritional profile but also a suitable carrier for functional ingredients. The addition of bioactive components such as prebiotics, probiotics, and natural antioxidants has become a common practice to enhance their health benefits and technological properties (Noshadi et al., 2025). However, modifications to the formulation must be carefully evaluated to ensure they do not compromise texture, viscosity, flavor, or overall acceptability. In recent years, there has been a

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growing global interest in the use of natural compounds as alternatives to synthetic additives in food systems. Consumers are increasingly aware of the potential health risks associated with artificial preservatives and are showing a strong preference for products labeled as "natural," "clean label," or "free from chemical additives." This shift in consumer behavior has driven researchers and food technologists to explore bioactive substances derived from plant and bee-derived sources that can serve both functional and preservative roles in food matrices (Narimane et al., 2023). Among natural products, propolis a resinous substance collected by honeybees from tree buds and other botanical sources has attracted considerable attention due to its broad spectrum of biological properties. Propolis is rich in polyphenols, flavonoids, and other phenolic compounds, which contribute to its antimicrobial, antioxidant, anti-inflammatory, and antifungal activities. These characteristics have led to its historical use in traditional medicine and more recently, its application in food preservation and functional food development (Burdock, 1998; Tzima et al. 2015). Several bioactive compound classes have been identified in Brazilian red propolis, including bioflavonoids, pterocarpans, chalcones, flavanones (e.g., liquiritigenin), prenylated benzophenones, terpenes, and tannins (Lopez et al. 2015). Among these, flavonoids are considered key contributors to the biological activity of propolis, being primarily responsible for its anti-inflammatory, antithrombotic, Vaso protective, gastroprotective, and free radical scavenging effects. Additionally, exhibit modulatory activity in allergic responses (Freires, De Alencar, and Rosalen, 2016). Red propolis is widely recognized for its beneficial effects on human health, with particular interest in its phenolic constituents due to their well-documented antioxidant and antimicrobial properties (Jansen-Alves et al., 2019). The antimicrobial and antioxidative characteristics of propolis make it a highly valuable natural additive in the food industry, offering potential applications in delaying lipid oxidation and enhancing the stability and shelf life of food products (Silva et al. 2013). Thamnopoulos et al. (2018) have demonstrated the ability of propolis to inhibit the growth of spoilage and pathogenic microorganisms in milk and dairy-based products, suggesting its potential as a multifunctional ingredient in food preservation. Moreover, its antioxidant activity may help delay lipid oxidation and improve the overall stability of dairy products during storage. Despite these benefits, limited information exists regarding the impact of propolis on the physical and sensory properties of yoghurt, particularly at low concentrations and under refrigerated conditions. Therefore, this study aims to investigate the effect of incorporating two concentrations of ethanolic propolis extract (0.1% and 0.3%) on selected physicochemical, rheological, and sensory properties of buffalo milk yoghurt during cold storage. Specifically, the research focuses on how propolis influences pH, titratable acidity, hardness, viscosity, and sensory evaluation over a 14-days period.

2. Materials and Methods

2.1. Materials

2.1.1. Raw materials

Fresh buffalo milk (7%fat) was obtained from local farms in Qena, Egypt. The milk was transported under refrigerator conditions (4–6°C) to the laboratory and stored at 4°C until further processing. Propolis used in this study was supplied by the Department of Plant Protection, Faculty of Agriculture, South Valley University, Egypt. It was stored at room temperature in a dark, sealed container until extraction and preparation.

2.1.2. Chemicals and reagents

All chemicals and reagents used were of analytical grade and purchased from reputable suppliers including Alfa Aesar (a Johnson Matthey Company), El-Nasr Pharmaceutical, El-Goumhouria, and PioChem. Distilled water was used throughout all experimental procedures. Glassware was made of Pyrex to ensure chemical resistance and accuracy.

2.1.3. Starter

Streptococcus thermophiles and *Lactobacillus bulgaricus*, is used to manufacture buffalo yoghurt and formulated yoghurt, it was obtained from

Hansen Corporation, Denmark, and stored at -20°C for future use.

2.2. Methods of analysis

2.2.1. Propolis extraction procedure

prepare the propolis extract, 50 g of raw propolis was ground into a fine powder using a mortar and pestle under sterile conditions. The powdered propolis was then mixed with 250 mL of 70% (v/v) ethanol in distilled water. The mixture was stirred continuously at room temperature for 48 hours in the dark to ensure maximum extraction of bioactive compounds. After extraction, the solution was filtered through Whatman No. 1 filter paper to remove any residual wax and insoluble particles. The resulting ethanolic propolis extract (EPE) was concentrated under reduced pressure at 40°C using a rotary evaporator to remove ethanol. The semi-solid residue was freeze-dried to obtain a dried propolis extract, which was stored in a sealed amber glass vial at 4°C until use. In recent years, there has been a growing global interest in the use of natural compounds as alternatives to synthetic additives in food systems. Consumers are increasingly aware of the potential health risks associated with artificial preservatives and are showing a strong preference for products labeled as "natural," "clean label," or "free from chemical additives." This shift in consumer behavior has driven researchers and food technologists to explore bioactive substances derived from plant and bee-derived sources that can serve both functional and preservative roles in food matrices. (Chemical characterization of propolis extract (Fikry, et al., 2022). To ensure consistency and understand the active components responsible for the observed effects in yoghurt, the propolis extract was subjected to preliminary chemical analysis: Total Phenolic Content (TPC): Determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE) per gram of extract. (Prior et al., 2005). Total Flavonoid Content (TFC): Measured using the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE) per gram of extract. (Chang et al., 2002). Antioxidant Activity: Evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay to assess the antioxidant potential of the extract. (Molyneux, 2004). These analyses were performed in triplicate to ensure accuracy and reliability.

2.2.2. Preparation of yoghurt samples

Yoghurt was prepared using standardized procedures with slight modifications as described below: Fresh buffalo milk was heated to 85°C for 10 minutes to ensure pasteurization and denaturation of whey proteins. Then, the milk was then rapidly cooled to 40°C, after which it was inoculated with 3% (v/v) commercial yoghurt starter culture containing *Lactobacillus bulgaricus* and *Streptococcus* thermophilus. After that, the inoculated milk was divided into three equal portions: (Noshadi et al., 2025).

Control: no addition (plain yoghurt).

Treatment 1: supplemented with 0.1% (w/v) propolis extract.

Treatment 2: supplemented with 0.3% (w/v) propolis extract.

Each batch was poured into sterile plastic cups and incubated at 42°C for 3–4 hours until coagulation occurred. After fermentation, samples were cooled to 15–20°C, then stored at 4 ± 1°C for up to 14 days. Samples were analyzed fresh (0 day), 7 and 14 days of refrigerated storage for physicochemical, rheological, and sensory properties.

2.2.3. Physicochemical and rheological analyses

2.2.4. PH And Titratable Acidity - PH Measurement

The pH of yoghurt samples was determined using a calibrated digital pH meter (ADWA Instruments Laboratory, Hungary). Titratable Acidity: Acidity was measured by titration with 0.1N NaOH using phenolphthalein as an indicator. Results were expressed as a percentage of lactic acid (% LA) (Alamprese et al. 2002; Akalin and Erisir,2008)

2.2.5. Hardness measurement

Hardness was assessed using a digital force gauge (IIAXIS, Model FB 200 S/N 344, Poland) equipped with an internal sensor. Testing parameters included: Penetration depth: 15 mm, Probe speed: 1.0 mm/s, and Force range: 5.0 N.

Hardness was defined as the maximum force (in Newtons, N) required to penetrate the yoghurt sample. All samples were tempered to room temperature for 5 minutes prior to testing. (Muse andHartel, 2004).

2.2.6. Viscosity measurement

Viscosity was measured using a Brookfield Digital Viscometer (Model DV-I+, Brookfield Engineering Laboratories, MA, USA). Conditions were Spindle type: No. 1 and No. 4, Shear rate: Constant, Temperature: 25°C, Speed: 30 rpm, and Duration: 30 seconds per reading. Observations were recorded in centipoise (cP). (Assis et al., 2014).

2.2.7. Sensory evaluation

Sensory analysis was conducted by a trained panel of 10 assessors selected based on their experience in evaluating dairy products. Panelists evaluated the following attributes on a 100-point hedonic scale: Taste (35%), Texture (25%), Appearance (25%), and Odor (15%). (Ibtesam et al.2017) Yoghurt samples were served in coded plastic cups at room temperature. Water was provided for palate cleaning between samples. Evaluations were conducted immediately after production (day 0), and after 7 and 14 days of cold storage. Total acceptability was calculated as the sum of all attribute scores.

3. Results

3-1- Chemical analysis of propolis

Chemical analysis of the bee propolis used in this study revealed a high content of bioactive compounds such as pinocembrin (6.45%), galangin (4.52%), chrysin (3.01%), and caffeic acid (1.47%), which are known for their strong antimicrobial and antioxidant properties Table 1.
These components contributed to the observed decrease in pH and increase in titratable acidity in propolis-enriched yoghurt samples during storage, suggesting enhanced lactic acid bacteria activity or preservation effects. Additionally, linoleic acid (2.12%), a polyunsaturated fatty acid, may have influenced texture improvement, while vanillin (1.04%) could have affected flavor characteristics, explaining the initial sensory differences between control and treated yoghurts. Despite the lower initial sensory scores especially for the 0.3% propolis treatment acceptability improved over time, indicating possible stabilization of flavor compounds or consumer adaptation. These findings support the role of propolis as a natural additive with functional benefits, particularly at 0.1% concentration, which showed the best balance between preservation enhancement, texture improvement, and sensory acceptability without compromising yoghurt quality.

3.2. Physicochemical and Rheological properties

The incorporation of propolis slightly influenced the physicochemical and rheological properties of buffalo milk yoghurt during a 14-days refrigerated storage period. As shown in Table 2, all samples exhibited a progressive decrease in pH over time, with the most pronounced decline observed in the 0.3% propolis treatment (from 4.93 at day 0 to 4.64 after 14 days). This trend was accompanied by an increase in titratable acidity, particularly in the 0.1% propolis sample, which reached 2% lactic acid equivalent by the end of the storage period—indicating enhanced fermentation activity or preservation effects. Hardness also improved with propolis addition, especially at the 0.1% concentration, which showed an increase in firmness from day 0 (0.30 N) to day 14 (0.50 N). The 0.3% propolis treatment also showed improved hardness (0.45 N at day 14), suggesting that propolis may interact with casein micelles or whey proteins, reinforcing the yoghurt gel structure. In contrast, viscosity remained relatively stable across all treatments and time points, ranging between 179–181 cP, indicating that propolis did not negatively affect the flow behavior or mouthfeel of the product.

Table 1. Chemicals composition of bee propolis

Active component	Class	Composition (%)
Flavonoids	Polyphenols	
Pinocembrin	Flavonoid	6.45
Galangin	Flavonoid	4.52
Chrysin	Flavonoid	3.01
Kaempferol	Flavonoid	1.53
Quercetin	Flavonoid	1.33
Phenolic Acids	Polyphenols	
Caffeic Acid	Phenolic Acid	1.47
Ferulic Acid	Phenolic Acid	1.46
p-Coumaric Acid	Phenolic Acid	1.01
Terpenes	Terpenoids	
Beta-Caryophyllene	Sesquiterpene	2.12
Aromadendrene	Sesquiterpene	1.03
Aromatic Aldehydes	Aromatics	
Vanillin	Aromatic Aldehyde	1.04
Benzaldehyde	Aromatic Aldehyde	0.83
Fatty Acids	Lipids	
Linoleic Acid	Polyunsaturated Fatty Acids	2.12
Palmitic Acid	Saturated Fatty Acids	1.65
Esters	Esters	
Benzyl Benzoate	Ester	1.60
Cinnamyl Cinnamate	Ester	1.02
Sterols	Steroids	
Beta-Sitosterol	Phytosterol	0.95
Other Compounds	Various	
Benzyl Alcohol	Alcohol	0.61
Tetracosane	Alkane	0.69

These findings suggest that propolis can enhance yoghurt texture and acid development without compromising its physical consistency, particularly when used at a concentration of 0.1%. In support of its functional role, Figure 1 presents the antioxidant profile of the ethanol propolis extract used in this study. The extract exhibited high total phenolic content (185.2 mg GAE/g), total flavonoid content (92.6 mg QE/g), and strong DPPH radical scavenging activity (84.3%). These results validate the presence of potent antioxidant compounds, which likely contributed to the improved physicochemical stability and extended shelf-life of the yogurt formulations. The bioactivity of the propolis extract justifies its application as a multifunctional additive in dairy products, enhancing both preservation and nutritional quality.

Table 2. Effect of addition Propolis on pH, titratable acidity, hardness, and viscosity of yoghurt during storage for 14 days at 4°C.

Treatment	Parameter				
	Hardness	Viscosity	PH	Acidity	Storage period
Control	0.30	180	5.3	1.17	
0.1%Propolis	0.30	180	4.97	1.36	Zero time
0.3%Propolis	0.35	181	4.93	1.39	
Control	0.25	179	5.3	1.25	
0.1%Propolis	0.45	180	4.81	1.8	One week
0.3%Propolis	0.45	180	4.91	1.5	
Control	0.30	180	4.96	1.47	
0.1%Propolis	0.50	180	4.57	2	Two weeks
0.3%Propolis	0.45	180	4.64	1.66	

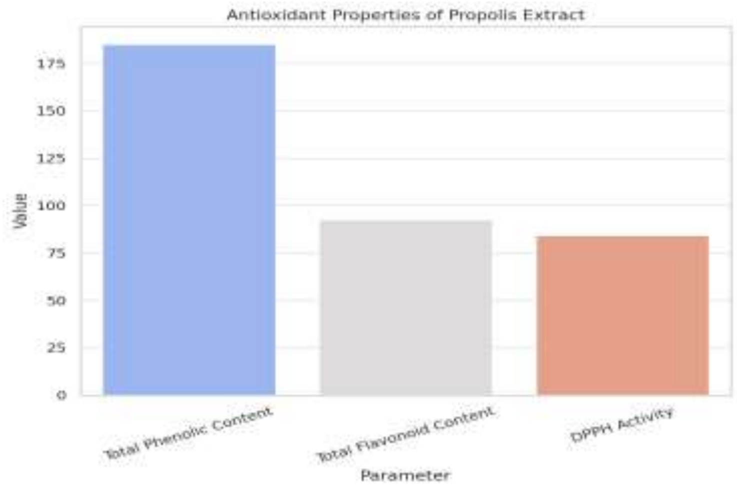

Figure 1. Antioxidant capacity of ethanol Propolis extract used in yoghurt fortification

Sensory evaluation data presented in Table 3 reveal that the initial sensory acceptability of propolis-enriched yoghurt was lower compared to the control, especially for the 0.3% propolis sample. On day 0, the yoghurt received the highest total acceptance score (95.3 out of 100), while the 0.3% propolis yoghurt scored the lowest (77.0). This difference was mainly attributed to changes in taste and appearance, likely due to the phenolic compounds in propolis that impart bitterness or darker coloration. However, sensory scores improved significantly during storage. By week 1, both propolis-treated yoghurt achieved acceptance scores above 95, comparable to the control (95.8). After 14 days of storage, the 0.1% propolis yoghurt maintained a high overall acceptability score (94.4), whereas the 0.3% formulation scored slightly lower (90.5), although still within acceptable limits. Notably, no significant differences were observed in texture desirability among any of the treatments throughout the storage period, indicating that propolis addition did not compromise the textural perception of the yoghurt. These results support the conclusion that propolis can be successfully incorporated into yogurt at low concentrations (particularly 0.1%) without adversely affecting sensory quality after short-term storage. Additionally, Figure 2 provides a comparative visualization of sensory attributes at the end of the 14-days storage period. The radar chart shows that the yoghurt supplemented with 0.1% propolis closely mirrors the sensory profile of the control sample, maintaining high scores across taste, texture, appearance, and odor. In contrast, the 0.3% treatment exhibits lower scores in taste and odor, likely due to the bitterness and stronger aromatic compounds from higher polyphenol levels. Nevertheless, all treatments preserved textural integrity. This underscores the suitability of 0.1% propolis concentration as an optimal level for functional yoghurt production without compromising consumer sensory acceptance.

Table 3. Sensory Evaluation of Yoghurt Supplemented with Ethanolic Propolis During Storage for 14 days at 4 °C

Treatment	Parameter					
	Taste 35%	Texture 25%	Look 25%	Odor 15%	Sum 100%	Storage period
Control	31.7	24.2	24.6	14.8	95.3	Zero time
0.1%Propolis	31.6	23	22.9	10.1	87.6	
0.3%Propolis	24.5	21.5	18.9	12.1	77	
Control	30.8	25	25	15	95.8	One week
0.1%Propolis	32.6	24.5	24.5	14.1	95.7	
0.3%Propolis	32.4	25	24.06	14.8	96.26	
Control	35	25	25	15	100	Two weeks
0.1%Propolis	34.4	25	22.5	12.5	94.4	
0.3%Propolis	28	24.5	24	14	90.5	

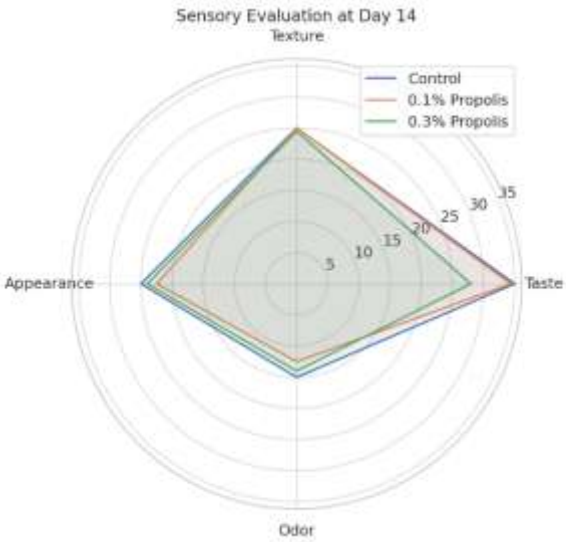

Figure 2. Comparative Radar Plot of Sensory Attributes for Yoghurt Samples Enriched with Ethanolic Propolis Extract After 14 Days of Storage

4. Discussion

The incorporation of propolis into buffalo milk yoghurt slightly influenced its physicochemical, rheological, and sensory characteristics. These changes can be attributed to the presence of various bioactive compounds identified in the chemical composition of the propolis used (see Table 1), particularly flavonoids, phenolic acids, and fatty acids—each of which has well-documented functional properties in food systems. Propolis supplementation resulted in a progressive decrease in pH and a corresponding increase in titratable acidity over the 14-days storage period, especially in the 0.3% treatment group. This observation aligns with previous findings by El-Deeb, (2017), who reported enhanced acidification in raw milk supplemented with propolis extract. The incorporation of propolis into buffalo milk yoghurt significantly altered its physicochemical, rheological, and sensory attributes. These modifications are attributed to the diverse array of bioactive compounds in propolis—including flavonoids, phenolic acids, and fatty acids—which are known to influence microbial behavior, matrix structure, and consumer perception in fermented dairy products. Propolis addition led to a progressive decline in pH and an increase in titratable acidity during storage, most notably in the 0.3% treatment group. These findings mirror the acidification patterns reported in propolis-enriched low-fat yoghurt, where enhanced organic acid production was attributed to intensified microbial fermentation activity (Noshadi *et al*. 2025). Texture analysis showed an increase in hardness and cohesiveness, particularly at the 0.1% propolis level at 14 days, which corresponded with optimal network formation in the yoghurt matrix. This can be attributed to the fatty acid profile of propolis—especially linoleic acid—which may facilitate protein-fat interactions that reinforce the gel structure (Tumbarski *et al*. 2024). Moreover, the formation of polyphenol–protein complexes can enhance casein micelle stability and reduce syneresis, leading to more desirable textural outcomes (Chon *et al*., 2020). However, higher concentrations (e.g., 0.3%) did not further improve texture, potentially due to saturation effects or competition for binding sites on protein molecules, consistent with reports of polyphenol overload disrupting gelation pathways (Korkmaz *et al*. 2021). Interestingly, viscosity measurements remained stable across treatments, suggesting that propolis acts more on microstructure than bulk flow behavior—a desirable trait for maintaining consumer-friendly mouthfeel and pourability. Sensory evaluation of food products is an important indicator of potential consumer preference. The prepared yoghurt as shown in Table (3) and Fig (2) showed that increasing levels of propolis affected slightly on the sensory scores of some properties of yoghurt, yoghurt samples containing propolis gained smaller scores for taste, bodyand texture, odor and overall acceptability than control in fresh and during the storage period till 14 days this contrary with (El-Deeb. 2017). However, yoghurt samples with 0.3% of propolis recorded the lowest scores for most parameter at fresh and during of storage period but it gained the higher score at summed after one week of storage. Also, yoghurt containing 0.1% of propolis recorded the medium values for overall sensory attributes as compared to other treatments at the end of storage period followed by yoghurt with 0.3 % of propolis. These results were similar to (El-Deeb. 2017). An enhancing effect of propolis on yoghurt. Regarding the sensory assessment of the samples, the control was most acceptable and yoghurt with propolis was least acceptable. The sensory features of the yoghurt were affected by the distinct look and odor of propolis, and this could be adjusted by changing the doses of this supplement. Total acceptance of the yoghurt treatments was found no differences in the texture desirability of the yoghurt containing propolis and control sample. In conclusion, propolis supplementation did not have the infidel influence on the investigated sensory criteria. These findings might be related to the concentrations of supplement used in this study. It reduced sensory acceptability slightly. This study comprised of only short-time storage data, and hence, future studies with longer storage periods must be. This results accordance with (Korkmaz *et al*., 2020; Noshadi *et al*., 2025). Regarding to sensory properties, Chon *et al*. (2020) reported that the taste values of market yoghurt evaluated after various propolis addition in sensory properties were similar to the control group or lower. Bilici et al. (2017) reported similar results. Propolis was used to produce yoghurt with improved functional properties. No change was observed in taste, smell, or look. Sensory evaluation showed initial rejection of high-propolis formulations (0.3%), primarily due to bitterness and off-color. These effects are consistent with the presence of volatile phenolic aldehydes like vanillin and aromatic acids, which can strongly influence taste perception even at low levels (Habryka *et al*. 2020). However, palatability improved significantly over storage time, especially at the 0.1% level. This may be due to interaction or entrapment of bitter compounds within the yogurt matrix, or volatilization of some sensory-active compounds during refrigeration (Tumbarski *et al*. 2024). These findings suggest a delayed sensory adaptation that enhances consumer acceptability over time. Crucially, mouthfeel and texture preferences remained unaffected, indicating that propolis's benefits do not come at the cost of tactile sensory quality-important for repeat consumption and market viability (Noshadi *et al*. 2025). According to the acceptability index, the yoghurt produced with the propolis presented a good potential of consumption and commercialization. From the characteristics presented by the product and those inherent to propolis, such as the presence of bioactive compounds, antimicrobial and antioxidant activities, the yoghurt proposed in this work can be classified as a product that is attractive to the public in general, and to the people who seek healthier foods, without the addition of chemical preservatives, this accordance with (Santos et al.2019).

5. Conclusion

The incorporation of ethanolic propolis extract into buffalo milk yoghurt enhanced its physicochemical stability, rheological behavior, and sensory properties during refrigerated storage. The 0.1% propolis concentration proved to be optimal, achieving improved acidity development, enhanced hardness, and maintained viscosity without compromising organoleptic quality. While the 0.3% propolis treatment initially exhibited lower sensory acceptance due to bitterness and coloration, acceptability improved over time, indicating adaptation or stabilization of flavor compounds. Sensory data confirmed that 0.1% propolis retained a profile closely resembling the control, suggesting high consumer acceptability. These findings support the use of propolis as a natural, multifunctional additive capable of replacing synthetic preservatives like potassium sorbate in functional dairy applications. Future work should focus on extended shelf-life studies, microbiological stability, and probiotic viability in propolis-fortified yoghurts.

Declarations

Conflict of interest

The authors hereby declare that no competing and conflict of interest exists

Ethical considerations and permissions

All procedures in this study followed institutional guidelines for food safety and research ethics. Sensory evaluation was conducted with trained adult panelists who provided verbal consent after being informed of the study purpose. No personal data was collected, and no invasive procedures were involved. Raw milk and propolis extract were used with permission from local suppliers, and no animal or human clinical testing was performed

Consent to participate

Not applicable

Consent for publication

Not applicable

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Author Contributions

The authors of this manuscript contributed equally to the design and/or execution of the experiments described in the manuscript. All authors approved the final manuscript

Data availability statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

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