



Reinforcement of Yoghurt Characteristics with Different Pomegranate Peel Powder



Ebtsam O. Ahmed¹ and Mohamed A. Elhafez^{*2}

¹Food Hygiene Department, Animal Health Research Institute (AHRI), Dakhla Lab-New Valley, Agriculture Research Center (ARC), Egypt.

²Food Hygiene Department, Animal Health Research Institute (AHRI), Tanta Lab, Agriculture Research Center (ARC), Egypt.

Abstract

WHILE nutritionally beneficial, yogurt lacks certain bioactive compounds like polyphenols and natural antioxidants. This study investigated the effects of pomegranate peel powder (PPP) incorporation on yogurt quality, focusing on chemical composition, bacterial viability, shelf-life extension, antioxidant properties, and sensory characteristics. Yogurt samples were prepared with different concentrations (0.5%, 1%, and 1.5%) of PPP. Chemical properties (pH, titratable acidity), microbiological analysis (total bacterial count, coliform count, yeast and mold count), antioxidant constituents (total phenolic and flavonoid content), and sensory evaluation were assessed over 21 days of storage. PPP incorporation significantly affected yogurt properties ($p < 0.05$). Higher PPP concentrations (1-1.5%) demonstrated superior antimicrobial effects, eliminating coliforms and reducing total bacterial counts. The outer peel exhibited higher bioactive compound content (224.42 μg GAE/mg phenolics, 64.86 μg RE/mg flavonoids) than the inner peel ($p < 0.05$). pH values decreased during storage across all samples, while titratable acidity showed varying trends. Sensory scores decreased with increasing PPP concentration and storage duration. In conclusion, PPP effectively enhanced yogurt's functional properties, particularly at 1-1.5% concentrations, by providing antimicrobial effects and significant bioactive compounds. While higher PPP concentrations slightly decreased sensory acceptance, the study confirms PPP as a promising natural preservative that enhances yogurt's bioactive properties while extending shelf life.

Keywords: Yogurt, pomegranate peel powder, antimicrobial activity, antioxidants, functional foods, shelf life.

Introduction

Yogurt, produced through the fermentation of milk by lactic acid bacteria, stands as one of the most widely consumed dairy products globally [1]. Its popularity stems from documented health benefits, particularly in improving intestinal microbiota and enhancing immune system function [2].

While yogurt offers numerous nutritional advantages, it is generally poor in certain bioactive compounds, specifically polyphenols and natural antioxidants [3]. This limitation has catalyzed interest in improving the functional properties of yogurt by incorporating natural additives, while concurrently, the food industry is under increasing pressure to adopt sustainable practices by utilizing agricultural by-products, which frequently contain

substantial concentrations of valuable bioactive compounds [4, 5]. Pomegranate (*Punica granatum* L.) peels, traditionally used in Middle Eastern folk medicine and textile dyeing due to their high tannin content, represent a promising source of bioactive compounds. Research has shown that these peels contain up to ten times higher phenolic concentrations than fruit pulp [6]. Their documented benefits include antimicrobial properties that can protect against food spoilage organisms and minimize the occurrence of foodborne illnesses [7]. The antioxidant capabilities of pomegranate peel phenolic compounds have been particularly noted for their ability to inhibit lipid oxidation [8]. These characteristics and antimicrobial properties suggest potential applications in extending food product shelf life [9]. Previous research has successfully

*Corresponding authors: Mohamed A. Elhafez, E-mail: Hafez.2310@gmail.com Tel.: 01020266868
orcid.org/0000-0003-0299-6828

(Received 30 November 2024, accepted 12 January 2025)

DOI: 10.21608/EJVS.2025.340462.2532

©National Information and Documentation Center (NIDOC)

demonstrated the efficacy of pomegranate peels in enhancing antioxidant activity in dairy products [10].

Incorporating antioxidant-rich natural additives into yogurt aligns with growing consumer interest in functional foods that combine traditional nutritional benefits with enhanced health-promoting properties [3]. Furthermore, utilizing pomegranate processing by-products in food applications presents a sustainable solution to waste management while potentially reducing environmental impact and disposal costs [11].

This study aims to investigate the effects of pomegranate peel powder (PPP) incorporation on yogurt quality, focusing specifically on chemical composition, bacterial viability, shelf-life extension, antioxidant properties, and sensory characteristics.

Material and Methods

Materials

Pomegranate (*Punica granatum* L.) fruits were sourced in October 2024 from a local market in Tanta City, Egypt. Fresh raw cow's milk was collected from the Faculty of Agriculture, Tanta University, Tanta, Egypt dairy station. Traditional yogurt starter cultures, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus* were procured from Danisco, Egypt. Reagents and chemicals used in this study included phenolphthalein (1%) from Biopharm (USA), standard sodium hydroxide solution (0.1 mol/L, 0.1 N) from Thermo Fisher Scientific (USA), and microbiological media including peptone water, Plate Count Agar, Violet Red Bile Agar, and Dichloran Rose Bengal Chloramphenicol Agar, all sourced from Himedia (India). Additional chemicals included ethanol (70%), gallic acid, rutin, aluminum chloride, sodium carbonate, sodium acetate, and Folin-Ciocalteu reagent obtained from Sigma Aldrich (USA), while methanol was acquired from Fine-Chem (India).

Preparation of PPP

Pomegranate fruits were thoroughly washed with distilled water, and the peels were separated into two distinct layers: the outer layer and the inner white layer. Samples comprising the outer peels, inner peels, and whole peels were subjected to drying using an oven-based method. The peels were air-dried in a ventilated oven at 40°C for 48 hours, as mentioned by [12], after which they were ground into a fine powder to obtain PPP by using a high-speed coffee grinder (model HC-500Y, DAMAI, China), to grind the dried peels, grind in small batches to ensure a fine texture, pass the ground peels through a fine mesh sieve to remove larger particles, then regrind any leftover coarse bits until the desired fineness is achieved.

Manufacture of Yogurt Fortified with PPP

Raw cow's milk was heated to 90°C for 5 minutes, then cooled to 40°C. The whole PPP was incorporated into the milk at concentrations of 0.5%, 1%, and 1.5%. Traditional yogurt starter cultures were added to the mixtures, which were then incubated at 45°C until the formation of a gel structure. Once the gel was formed, it was stirred and stored under refrigeration at $6 \pm 2^\circ\text{C}$. A control yogurt sample, prepared without the addition of PPP, was subjected to microbiological testing conducted using standard ISO methods to ensure the absence of *Listeria* [13], *Salmonella* [14], and *Escherichia coli* [15]. These methods involve selective plating on specific agars and confirmation tests. The control and fortified yogurt samples were manufactured following the technique outlined by [16].

Determination of pH

The pH of the sample was measured following the procedure outlined by [17]. A 10 g portion of the sample was dissolved in 100 mL of distilled water and equilibrated for three minutes at ambient room temperature. The pH was subsequently determined by immersing the electrode of a calibrated pH meter (Biolab, Spain) into the prepared solution, and the value displayed on the instrument was recorded.

Determination of Total Titratable Acidity

The total titratable acidity (TTA) was assessed using the protocol established by [18]. The sample was dissolved in distilled water and mixed thoroughly. Subsequently, 1 mL of phenolphthalein indicator was added to a 10 mL aliquot. The solution was titrated with standard sodium hydroxide (NaOH) solution until a persistent pink color, lasting approximately 10–15 seconds, indicated complete neutralization. The acidity was expressed as a percentage and calculated using the following formula: Acidity (%) = Volume of N/9 NaOH (mL) × 0.4.

Microbiological analysis

Sample preparation for serial dilution

Sample preparation was conducted following [19]. A 25 g sample was transferred to a sterile flask containing 225 mL of 0.1% sterile peptone water. The resulting mixture, representing the initial 10^{-1} dilution, was homogenized by shaking. Subsequently, 1 mL of this mixture was aseptically transferred using a sterile pipette to a sterile test tube containing 9 mL of 0.1% sterile peptone water. Serial tenfold dilutions were prepared up to 10^{-6} .

Determination of total bacterial count

The total bacterial count (TBC) was determined following [20]. From each prepared dilution, 1 mL was pipetted into sterile Petri dishes in duplicate. Approximately 15 mL of sterile, tempered Standard Plate Count Agar (SPC agar) was poured into each dish, mixed thoroughly, and allowed to solidify. The

plates were incubated at 32°C for 48 hours. Colony-forming units (CFU) were counted on plates with 25–250 colonies, and the average colony count was recorded as TBC/g.

Determination of coliform count

The coliform count was evaluated following [21]. From each prepared dilution, 1 mL was pipetted into sterile Petri dishes in duplicate. Approximately 15 mL of sterile, tempered Violet Red Bile Agar (VRB agar) was poured into each dish, thoroughly mixed, and solidified. After solidification, an additional overlay of 3–4 mL of VRB agar was applied. Plates were incubated at 32°C for 18–24 hours, after which colonies characteristic of coliforms were enumerated.

Determination of yeast and mold count

Yeast and mold counts were determined following [22]. From each prepared dilution, 1 mL was inoculated onto the surface of solidified Dichloran Rose Bengal Chloramphenicol (DRBC) agar plates and spread uniformly using a sterile bent glass rod. The plates were incubated at 22–25°C for 3 days. Colonies within the 15–150 CFU range per plate were counted, and the results were expressed as yeast and mold count per milliliter (CFU/mL).

Quantification of antioxidant constituents: total phenolic and flavonoid content determination

Reagent preparation and sample processing

Standards and chemical reagents were prepared with high-precision methodological controls. A stock solution of gallic acid (for total phenolic content) and rutin (for total flavonoid content) was prepared in methanol at 2000 µg/mL. Serial dilutions were systematically generated to create standard calibration solutions at concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. Experimental samples were dissolved in 70% ethanol for a standardized final concentration of 1.5 mg/mL.

Total phenolic content (TPC) quantification

The TPC was quantified utilizing the Folin–Ciocalteu spectrophotometric method, following the protocol established by [23] with precise microplate adaptation. The analytical procedure involved a sequential reagent addition protocol: 10 µL of the sample or standard solution was combined with 100 µL of 1:10 diluted Folin–Ciocalteu reagent in a 96-well microplate format. Subsequently, 80 µL of 1 M sodium carbonate was incorporated, and the reaction mixture was incubated for 20 minutes at an ambient temperature (25°C) in the dark. The chromogenic reaction resulted in a characteristic blue complex, whose spectral absorbance was measured at 630 nm using an Omega microplate reader (BMG Labtech Fluostar, Germany). The spectrophotometric quantification allows for precise determination of

phenolic compound concentrations through established spectroscopic principles.

Total flavonoid content (TFC) quantification

The TFC was determined employing the aluminum chloride colorimetric method, adapted from [24] with microplate-specific modifications. The analytical protocol consisted of a carefully controlled reagent addition sequence: 15 µL of sample or standard solution was combined with 175 µL methanol in a 96-well microplate. Subsequent additions included 30 µL of 1.25% aluminum chloride and 30 µL of 0.125 M sodium acetate. The reaction mixture was incubated for 5 minutes at room temperature, facilitating complex formation. The resulting yellow chromogen was quantified spectrophotometrically at 420 nm using the same microplate reader, enabling precise quantification of flavonoid content.

Sensory evaluation

The experimental design incorporated a rigorously selected sensory evaluation panel comprising ten trained assessors from the Agricultural Research Center, Egypt, who underwent an intensive two-week training protocol to develop proficiency in evaluating the organoleptic properties of yogurt products. The assessment methodology employed a comprehensive 100-point quality rating system that systematically evaluated three primary sensory attributes: flavor (50 points), body and texture (40 points), and appearance (10 points), following standardized evaluation criteria. The sensory analysis protocol adhered to established methodological frameworks, explicitly implementing the validated assessment procedures delineated by [25], ensuring consistency and reliability in the organoleptic evaluation process.

Statistical Analysis

Statistical analysis was performed using SPSS v27 (IBM©, Armonk, NY, USA). Data was described using the mean and standard deviation (SD) and analyzed through the one-way ANOVA followed by post hoc Tukey's test. A P value < 0.05 was considered statistically significant.

Results

Chemical properties of yogurt samples

The pH values of yogurt samples fortified with different concentrations of pomegranate peel powder (PPP) were monitored over a 21-day storage period. At day 0, the control sample exhibited a significantly higher pH (4.42 ± 0.05) compared to all PPP-fortified samples ($p < 0.05$), which showed similar pH values ranging from 4.33 to 4.34. As storage progressed, pH values generally decreased across all samples. By day 7, no significant differences were observed among the samples. At day 14, the control maintained a significantly higher pH (4.32 ± 0.06) compared to the

0.5% and 1% PPP samples ($p < 0.05$), while the 1.5% PPP sample did not differ significantly from either group. By day 21, pH values converged, with no significant differences observed among the samples, ranging from 4.24 to 4.29 (Table 1).

Titrate acidity, measured as a percentage, showed distinct trends among the yogurt samples over the storage period. Initially (day 0), acidity decreased with increasing PPP concentration, with significant differences observed between all groups except the control and 0.5% PPP ($p < 0.05$). This trend persisted at day 7, with the control showing the highest acidity ($1.81 \pm 0.12\%$) and the 1% and 1.5% PPP samples showing the lowest ($1.28 \pm 0.09\%$ and $1.23 \pm 0.08\%$, respectively). Interestingly, by day 14, no significant differences were observed among the samples. However, at day 21, significant differences re-emerged, with the 1.5% PPP sample showing the highest acidity ($2.12 \pm 0.15\%$) and the 0.5% PPP sample showing the lowest ($1.41 \pm 0.10\%$) ($p < 0.05$) (Table 2).

Microbial analysis of yogurt samples

TBC

The TBC in yogurt samples varied significantly with both PPP concentration and storage time. At day 0, the control and 0.5% PPP samples had the highest bacterial counts (3.98×10^6 and 1.26×10^6 CFU/g, respectively), significantly higher than the 1% and 1.5% PPP samples ($p < 0.05$). As storage progressed, bacterial counts generally decreased across all samples. By day 21, the control sample maintained a significantly higher bacterial count (6.45×10^5 CFU/g) compared to all PPP-fortified samples, which showed similar counts ranging from 3.45×10^4 to 5.05×10^4 CFU/g ($p < 0.05$) (Table 3).

Coliform Count

Colonies exhibiting a purple-red coloration with a diameter of approximately 0.5 mm, surrounded by a zone of precipitated bile acids, were identified, enumerated, and recorded as the total coliform count.

Coliform counts showed a clear reduction trend with increasing PPP concentration and storage time. At day 0, the control sample had the highest coliform count (1.25×10^4 CFU/g), followed by the 0.5% PPP sample (6.15×10^3 CFU/g), while no coliforms were detected in the 1% and 1.5% PPP samples. By day 7, coliforms were only detected in the control and 0.5% PPP samples, and by day 14, only in the control sample. At day 21, no coliforms were detected in any of the samples, indicating complete elimination of these bacteria (Table 4).

Yeast and mold counts

Yeast and mold counts fluctuated over storage, showing complex interactions with PPP concentration. At day 0, the 0.5% PPP sample had a significantly higher count (2.00×10^3 CFU/g)

compared to other samples ($p < 0.05$). By day 7, the 1% and 1.5% PPP samples showed higher counts than the control and 0.5% PPP samples. At day 14, the 1.5% PPP sample exhibited the highest count (24.00×10^2 CFU/g), significantly higher than all other samples ($p < 0.05$). Interestingly, by day 21, the trend reversed, with the 1.5% PPP sample showing the lowest count (2.00×10^2 CFU/g), significantly lower than other samples ($p < 0.05$) (Table 5).

Sensory properties

The sensory properties of yogurt supplemented with varying ratios of PPP were evaluated, and the results were recorded. Sensory scores generally decreased with increasing storage duration and higher PPP concentrations. The flavor scores of the control yogurt samples were consistently higher than those of the supplemented samples. This suggests that the addition of PPP negatively impacted the flavor profile of the yogurt products. There were little differences in body and texture scores along all treatments. The addition of PPP had a significant decrease in the yogurt appearance score. Treatments with a high concentration of PPP (1.5%) had lower scores of appearances among other treatments and the control (Table 6).

Antioxidant activity of PPP extract

The TPC of the oven-dried outer peel was 224.42 ± 11.98 μg gallic acid equivalent (GAE) per mg of sample, significantly higher than that of the white inner peel ($p < 0.05$), which recorded a TPC of 146.19 ± 7.90 μg GAE per mg of sample (Fig. 1).

The TFC values are expressed as μg rutin equivalent (RE) per mg of sample. The outer peel exhibited a significantly higher flavonoid content (64.86 ± 4.76 μg RE/mg) compared to the white inner peel (53.43 ± 2.69 μg RE/mg) ($p < 0.05$). These results indicate that the outer peel of pomegranate contains higher levels of phenolic and flavonoid compounds, contributing to its antioxidant activity (Fig. 2).

Discussion

The pH and titratable acidity dynamics

The present study demonstrated significant variations in pH values between control and PPP-fortified yogurt samples throughout the 21-day storage period. Initially, the control sample exhibited a higher pH (4.42 ± 0.05) than PPP-fortified samples (4.33-4.34), decreasing pH values across all samples during storage. These findings align with [26], who reported similar pH reduction patterns in pomegranate peel extract-fortified yogurt, with values decreasing from 4.55 to 4.21 over the storage period. The observed pH decline can be attributed to the continued fermentation

process and organic acid production by lactic acid bacteria, as suggested by [27].

The titratable acidity results revealed an interesting pattern, with initial decreases in acidity, as PPP concentration increased, followed by fluctuations throughout storage. This trend corresponds with the findings of [28], who observed acidity increases from 0.20 to 0.35 in their pomegranate peel-enriched yogurt study. The final acidity values in our study (1.41-2.12%) were notably higher than those reported by [29] in commercial yogurt (0.70±0.024%), suggesting that PPP addition may influence acid production dynamics during fermentation and storage.

Microbiological profile analysis

The TBC analysis demonstrated that increasing concentrations of PPP significantly reduced bacterial populations, attributable to its high content of bioactive compounds—such as phenolic acids and flavonoids—which exhibit potent antimicrobial properties by disrupting bacterial cell membranes, inhibiting enzymatic activity, generating reactive oxygen species that damage cellular components, and destabilizing cell walls, thereby overwhelming bacterial defense mechanisms and leading to cell death [30, 31]. This finding is particularly noteworthy when compared to [32], who demonstrated that pomegranate peel extract exhibited significant inhibitory effects on lactic acid bacteria while maintaining sufficient viability ($>30 \times 10^6$ CFU/mL) at 1% concentration. The progressive decrease in bacterial counts during storage aligns with [33] observations, though our initial counts were lower, suggesting enhanced antimicrobial activity of PPP.

Coliform control and food safety

A remarkable finding was the complete elimination of coliforms in 1% and 1.5% PPP samples from day 0, with all samples showing no coliform presence by day 21. The acidic environment of yogurt, resulting from lactic acid fermentation, weakens coliform bacteria, an effect amplified by the addition of PPP, which provides polyphenols that induce oxidative stress and damage bacterial cellular components. At the same time, its gradual release of active compounds sustains antimicrobial activity and preserves yogurt by reducing spoilage and microbial contamination throughout storage [30, 34]. Furthermore, our findings align with those of [35], who demonstrated that a mixed probiotic culture's growth was significantly enhanced when pomegranate peel was utilized in yogurt. This demonstrates superior antimicrobial efficacy compared to the findings of [36], who reported persistent coliform contamination in commercial yogurt samples. The results support [11] assertion regarding the potent antimicrobial properties of

pomegranate peel, making it a valuable natural preservative in dairy products.

Fungal growth patterns

The complex interactions between PPP concentration and yeast/mold counts present an interesting phenomenon, particularly the final reversal, where 1.5% of PPP samples showed the lowest counts. This contrasts with [29], who reported higher yeast and mold contamination in traditional dairy products. The fluctuating patterns suggest that PPP's antifungal activity may be concentration and time-dependent, a finding that warrants further investigation.

Sensory characteristics and consumer acceptance

The gradual decrease in sensory scores with increasing PPP concentration was attributed to polyphenols' inherent bitterness and astringency, which are known to impact flavor perception. Additionally, the darker coloration and grainy texture may have negatively influenced appearance scores. These findings align with [37], who observed declining overall acceptability with increased PPP levels. However, our results differed from those of [38], who reported improved sensory attributes in their pomegranate pomace-enriched products.

Bioactive compound profile

The significant differences in total phenolic and flavonoid content between outer and inner peels (224.42 vs. 146.19 µg GAE/mg for TPC; 64.86 vs. 53.43 µg RE/mg for TFC) demonstrate the superior bioactive potential of the outer peel. These values are comparable to those reported by [10], who found PPP to contain 26.19 ± 0.23 mg GAE/g of total phenolics. The higher concentrations in our study suggest potentially enhanced antioxidant benefits, supporting [39] findings on the significant bioactive potential of pomegranate peel extracts in yogurt applications.

This comprehensive analysis demonstrates that PPP incorporation significantly influences yogurt's physicochemical, microbiological, and sensory properties, with optimal effects observed at moderate concentrations. The findings contribute valuable insights to the growing research on functional dairy products enhanced with natural bioactive compounds.

Limitations and Recommendations

This study is subject to several limitations that warrant acknowledgment. Firstly, the investigation was confined to a single pomegranate variety, which may restrict the generalizability of the findings to other cultivars with potentially differing bioactive profiles. Secondly, the study did not systematically optimize the concentration of PPP concerning sensory acceptance, which could influence consumer preference and product viability. Additionally, the

scope of antioxidant analysis was limited to the quantification of TPC and TFC, providing only a partial assessment of the antioxidant potential of PPP. These constraints highlight the need for a more comprehensive approach in future research.

Future studies should explore using multiple pomegranate varieties to evaluate potential genotype-dependent variations in bioactive compounds and functional properties. Investigations into methods to enhance the sensory properties of PPP-fortified yogurt, such as encapsulation techniques or blending with natural flavor enhancers, are also recommended to improve consumer acceptance. Furthermore, long-term storage studies and in vivo trials should be conducted to validate PPP's functional benefits and stability in yogurt. To strengthen the claims of enhanced antioxidant properties, additional assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and FRAP (Ferric Reducing Antioxidant Power) assays, should be incorporated into future analytical frameworks. These steps will provide a more robust and comprehensive understanding of PPP's functional and sensory implications in yogurt.

Conclusion

Incorporating PPP enhanced yogurt's functional properties, demonstrating potent antimicrobial

effects, particularly at 1-1.5% concentrations. PPP effectively eliminated coliforms and reduced total bacterial counts while contributing substantial phenolic (224.42 µg GAE/mg) and flavonoid (64.86 µg RE/mg) content from outer peels. Though higher PPP concentrations slightly decreased sensory acceptance, the study confirms PPP as a promising natural preservative that enhances yogurt's bioactive properties while extending shelf life through its antimicrobial activity.

Acknowledgments

Not applicable.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study adheres to the ethical guidelines established by the Faculty of Veterinary Medicine at Kafr El Sheikh University, Egypt (Ethics Approval No. KFS-IACUC/189/2024).

TABLE 1. pH in yogurt samples fortified with different PPP concentrations (n=20 per group, total N=80).

Samples	Storage Period (Days)			
	0	7	14	21
Control	4.42 ± 0.05 ^a	4.25 ± 0.04 ^a	4.32 ± 0.06 ^a	4.29 ± 0.05 ^a
0.5%	4.33 ± 0.04 ^b	4.24 ± 0.05 ^a	4.26 ± 0.03 ^b	4.24 ± 0.04 ^a
1%	4.34 ± 0.06 ^b	4.26 ± 0.05 ^a	4.27 ± 0.04 ^b	4.26 ± 0.05 ^a
1.5%	4.34 ± 0.05 ^b	4.23 ± 0.04 ^a	4.28 ± 0.06 ^{ab}	4.26 ± 0.05 ^a

*Values are presented as mean ± SD. Different superscript letters in the column indicate significant differences (p < 0.05).

TABLE 2. Titratable acidity in yogurt samples fortified with different PPP concentrations (n=20 per group, total N=80).

Samples	Storage Period (Days)			
	0	7	14	21
control	1.61 ± 0.10 ^a	1.81 ± 0.12 ^a	1.91 ± 0.15 ^a	1.92 ± 0.14 ^a
0.5%	1.52 ± 0.09 ^a	1.61 ± 0.11 ^b	1.88 ± 0.13 ^a	1.41 ± 0.10 ^b
1%	1.42 ± 0.08 ^b	1.28 ± 0.09 ^c	1.76 ± 0.12 ^a	1.72 ± 0.11 ^c
1.5%	1.12 ± 0.07 ^c	1.23 ± 0.08 ^c	1.76 ± 0.13 ^a	2.12 ± 0.15 ^d

*Values are presented as mean ± SD. Different superscript letters in the column indicate significant differences (p < 0.05).

TABLE 3. TBC of the examined yogurt samples fortified with different PPP concentrations (n=20 per group, total N=80).

Samples	Storage Period (Days)			
	0	7	14	21
Control	$(3.98 \pm 0.45) \times 10^6$ ^a	$(2.56 \pm 0.30) \times 10^6$ ^a	$(1.20 \pm 0.18) \times 10^6$ ^a	$(6.45 \pm 0.81) \times 10^5$ ^a
0.5%	$(1.26 \pm 0.15) \times 10^6$ ^b	$(2.27 \pm 0.26) \times 10^5$ ^b	$(8.40 \pm 0.22) \times 10^4$ ^b	$(5.05 \pm 0.62) \times 10^4$ ^b
1%	$(4.01 \pm 0.52) \times 10^5$ ^c	$(1.75 \pm 0.23) \times 10^5$ ^b	$(9.30 \pm 0.35) \times 10^4$ ^b	$(4.15 \pm 0.53) \times 10^4$ ^b
1.5%	$(4.66 \pm 0.56) \times 10^5$ ^c	$(3.79 \pm 0.43) \times 10^5$ ^b	$(1.26 \pm 0.17) \times 10^5$ ^c	$(3.45 \pm 0.44) \times 10^4$ ^b

*Values are presented as mean \pm SD. Different superscript letters in the column indicate significant differences ($p < 0.05$).

TABLE 4. The coliform count of the examined yogurt samples was fortified with different PPP concentrations (n=20 per group, total N=80).

Samples	Storage Period (Days)			
	0	7	14	21
Control	$(1.25 \pm 0.15) \times 10^4$ ^a	$(9.00 \pm 1.10) \times 10^2$ ^a	$(7.40 \pm 0.90) \times 10^2$ ^a	0 ^a
0.5%	$(6.15 \pm 0.75) \times 10^3$ ^b	$(5.50 \pm 0.65) \times 10^2$ ^b	0 ^b	0 ^a
1%	0 ^c	0 ^c	0 ^b	0 ^a
1.5%	0 ^c	0 ^c	0 ^b	0 ^a

*Values are presented as mean \pm SD. Different superscript letters in the column indicate significant differences ($p < 0.05$).

TABLE 5. The yeast and mold count of the examined yogurt samples was fortified with different PPP concentrations (n=20 per group, total N=80).

Samples	Storage Period (Days)			
	0	7	14	21
Control	$(3.00 \pm 0.35) \times 10^2$ ^a	$(2.00 \pm 0.25) \times 10^2$ ^a	$(19.00 \pm 2.20) \times 10^2$ ^a	$(6.00 \pm 0.70) \times 10^2$ ^a
0.5%	$(2.00 \pm 0.26) \times 10^3$ ^b	$(2.00 \pm 0.28) \times 10^2$ ^a	$(8.00 \pm 0.95) \times 10^2$ ^b	$(7.00 \pm 0.85) \times 10^2$ ^a
1%	$(2.00 \pm 0.28) \times 10^2$ ^a	$(4.00 \pm 0.43) \times 10^2$ ^b	$(10.00 \pm 1.20) \times 10^2$ ^b	$(8.00 \pm 0.95) \times 10^2$ ^a
1.5%	$(4.00 \pm 0.45) \times 10^2$ ^a	$(4.00 \pm 0.47) \times 10^2$ ^b	$(24.00 \pm 2.80) \times 10^2$ ^c	$(2.00 \pm 0.25) \times 10^2$ ^b

*Values are presented as mean \pm SD. Different superscript letters in the column indicate significant differences ($p < 0.05$).

TABLE 6. Organoleptic properties of yogurt samples fortified with different PPP concentrations (n=20 per group, total N=80).

Item	Samples	Storage Period (Days)			
		0	7	14	21
Flavor (50)	Control	48	43	38	37
	0.5%	46	40	34	32
	1%	41	37	32	30
	1.5%	40	35	31	28
	Control	40	39	37	35
Body and texture (40)	0.5%	38	37	34	33
	1%	35	34	33	30
	1.5%	34	33	30	28
	Control	10	10	8	7
	0.5%	9	8	7	6
Appearance (10)	1%	7	6	5	4
	1.5%	6	5	5	4
	Control	98	92	83	79
Overall acceptability (100)	0.5%	93	85	75	71
	1%	83	77	70	64
	1.5%	80	73	66	60

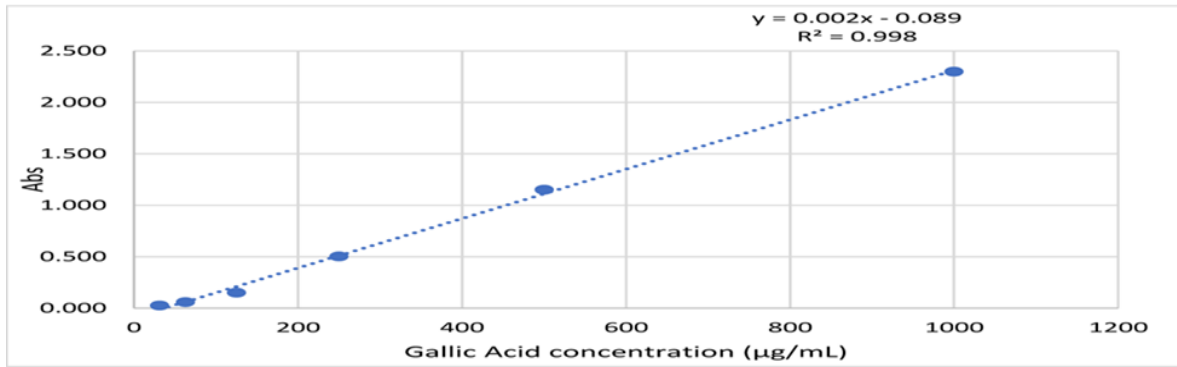


Fig. 1. Gallic acid standard absorbance and calibration curve.

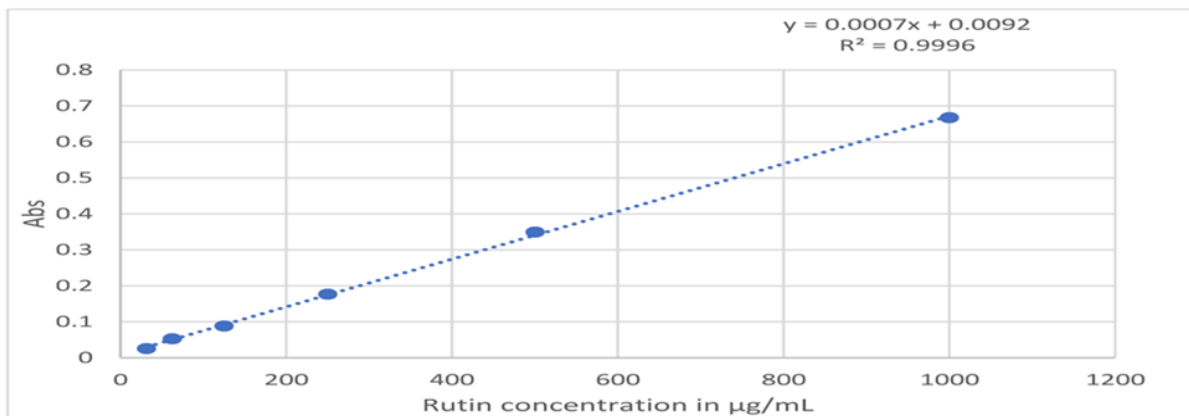


Fig. 2. Rutin standard absorbance and calibration curve.

References

1. Ayivi, R.D. and Ibrahim, S.A. Lactic acid bacteria: an essential probiotic and starter culture for the production of yoghurt. *International Journal of Food Science and Technology*, **57**(11), 7008-7025 (2022).
2. Michael, M., Phebus, R.K. and Schmidt, K.A. Impact of a plant extract on the viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* in nonfat yogurt. *International Dairy Journal*, **20**(10), 665-672 (2010).
3. Chouchouli, V., Kalogeropoulos, N., Konteles, S.J., Karvela, E., Makris, D.P., and Karathanos, V.T. Fortification of yoghurts with grape (*Vitis vinifera*) seed extracts. *LWT-Food Science and Technology*, **53**(2), 522-529 (2013).
4. Ahmad, I., Hao, M., Li, Y., Zhang, J., Ding, Y. and Lyu, F. Fortification of yogurt with bioactive functional foods and ingredients and associated challenges-A review. *Trends in Food Science and Technology*, **129**, 558-580 (2022).
5. Fan, X., Li, X., Du, L., Li, J., Xu, J., Shi, Z., Li, C., Tu, M., Zeng, X. and Wu, Z. The effect of natural plant-based homogenates as additives on the quality of yogurt: A review. *Food Bioscience*, **49**, 101953 (2022).
6. Suleria, H.A., Barrow, C.J. and Dunshea, F.R. Screening and characterization of phenolic compounds and their antioxidant capacity in different fruit peels. *Foods*, **9**(9), 1206 (2020).
7. Singh, B., Singh, J.P., Kaur, A. and Singh, N. Phenolic compounds as beneficial phytochemicals in pomegranate (*Punica granatum L.*) peel: A review. *Food Chemistry*, **261**, 75-86 (2018).
8. Naveena, B.M., Sen, A.R., Kingsly, R.P., Singh, D.B. and Kondaiah, N. Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. *International Journal of Food Science and Technology*, **43**(10), 1807-1812 (2008).
9. Sandhya, S., Khamrui, K., Prasad, W. and Kumar, M. Preparation of pomegranate peel extract powder and evaluation of its effect on functional properties and shelf life of curd. *LWT-Food Science and Technology*, **92**, 416-421 (2018).
10. El-Batawy, O., Ashoush, I. and Mehanna, N.S. Impact of mango and pomegranate peels supplementation on quality characteristics of yoghurt with or without whey powder. *World Journal of Dairy and Food Sciences*, **9**(1), 57-65 (2014).
11. Ain, H.B.U., Tufail, T., Bashir, S., Ijaz, N., Hussain, M., Ikram, A., Farooq, M.A. and Saewan, S.A. Nutritional importance and industrial uses of pomegranate peel: A critical review. *Food Science and Nutrition*, **11**(6), 2589-2598 (2023).

12. Singh, R.P., Chidambara Murthy, K.N., and Jayaprakasha, G.K. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agricultural and Food Chemistry*, **50**(1), 81-86 (2002).
13. ISO 11290-1:2017 Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. In: Part 1: Detection method, International Standard. *International Organization for Standardization: Geneva, Switzerland*, (2017).
14. ISO 6579-1: 2017 Microbiology of the food chain– Horizontal Method for the Detection, Enumeration and Serotyping of *Salmonella*- Part 1: Detection of *Salmonella* spp. *International Organization for Standardization Geneva*, (2017).
15. ISO 7251:2005 Microbiology of food and animal feeding stuffs – horizontal method for the detection and enumeration of presumptive *Escherichia coli*. *International Organization for Standardization, Geneva, Switzerland*, (2005).
16. Food and Agriculture Organization (FAO)-The technology of traditional milk products in developing countries. *United Nations, Rome, Italy*, **85**, 1-318 (1990).
17. Chandan, R.C. and Kilara, A., *Manufacturing yogurt and fermented milks*. 2013: Wiley Online Library.
18. Association of Official Analytical Chemists (AOAC)- Official Methods of Analysis, 12th ed. (2005).
19. ISO 6887-1:2017 Microbiology of the food chain— preparation of test samples, initial suspension and decimal dilutions for microbiological examination— part 1: General rules for the preparation of the initial suspension and decimal dilutions. *International Organization for Standardization: Geneva, Switzerland*, (2017).
20. ISO 4833-1:2013 Microbiology of the food chain— Horizontal method for the enumeration of microorganisms—Part 1: Colony count at 30 C by the pour plate technique. *International Organization for Standardization: Geneva, Switzerland*, (2013).
21. ISO 4832:2006 Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of coliform colony count technique. *International Organization for Standardization: Geneva, Switzerland*, (2006).
22. ISO 21527-1:2008 Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of yeasts and molds —Part 1: Colony count technique in products with water activity greater than 0.95. *International Organization for Standardization: Geneva, Switzerland*, (2008).
23. Attard, E. A rapid microtitre plate Folin-Ciocalteu method for the assessment of polyphenols. *Open Life Sciences*, **8**(1), 48-53 (2013).
24. Kiranmai, M., Kumar, C.M., and Ibrahim, M. Comparison of total flavanoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **2**(3), 254-261 (2011).
25. El-Said, M.M., Haggag, H., El-Din, H.M.F., Gad, A. and Farahat, A.M. Antioxidant activities and physical properties of stirred yoghurt fortified with pomegranate peel extracts. *Annals of Agricultural Sciences*, **59**(2), 207-212 (2014).
26. Jany, J.F., Nupur, A.H., Akash, S.I., Karmoker, P., Mazumder, M.A.R. and Alim, M.A. Fortification of functional yogurt by the phytochemicals extracted from pomegranate peel. *Applied Food Research*, **4**(2), 100479 (2024).
27. Chen, C., Zhao, S., Hao, G., Yu, H., Tian, H. and Zhao, G. Role of lactic acid bacteria on the yogurt flavour: A review. *International Journal of Food Properties*, **20**, S316-S330 (2017).
28. Al-Hindi, R.R. and Abd El Ghani, S. Production of functional fermented milk beverages supplemented with pomegranate peel extract and probiotic lactic acid bacteria. *Journal of Food Quality*, **2020**(1), 4710273 (2020).
29. Ahmed, L., Morgan, S., Hafez, R., and Abdel-All, A. Hygienic quality of some fermented milk products. *International Journal of Dairy Science*, **9**(3), 63-73 (2014).
30. Chen, J., Liao, C., Ouyang, X., Kahramanoğlu, I., Gan, Y., and Li, M. Antimicrobial activity of pomegranate peel and its applications on food preservation. *Journal of Food Quality*, **2020**(1), 8850339 (2020).
31. Kayed, A.M., Elbayoumi, Z.H., Yassien, N.A., and Shawish, R.R. Antimicrobial activity of pomegranate peel and its applications on food preservation. *Alexandria Journal of Veterinary Sciences*, **83**, p47 (2024).
32. Abd El-Aziz, M., El-Gammal, R.E., Abo-Srea, M., and Youssf, F.I. Antioxidant and antimicrobial activity of pomegranate (*Punica granatum l.*) fruit peels extract on some chemical, microbiological and organoleptical properties of yoghurt during storage. *Journal of Food and Dairy Sciences*, **4**(7), 387-400 (2013).
33. El-Bakri, J. and El-Zubeir, I.E. Chemical and microbiological evaluation of plain and fruit yoghurt in Khartoum State, Sudan. *International Journal of Dairy Science*, **4**(1), 1-7 (2009).
34. Lai, J.X. and Tang, S.S. Utilizing pomegranate extracts for enhancing yogurt quality and preservation. *Food and Humanity*, **3**, 100434 (2024).
35. Ibrahim, A., Awad, S. and El-Sayed, M. Impact of pomegranate peel as prebiotic in bio-yoghurt. *British Food Journal*, **122**(9), 2911-2926 (2020).
36. Ifeanyi, V., Ihesiaba, E., Muomaife, O., and Ikenga, C. Assessment of microbiological quality of yogurt sold by street vendors in Onitsha metropolis, Anambra state, Nigeria. *British Microbiology Research Journal*, **3**(2), 198-205 (2013).
37. Ahmed, M., Ali, A., Sarfraz, A., Hong, Q., and Boran, H. Effect of freeze-drying on apple pomace and pomegranate peel powders used as a source of

- bioactive ingredients for the development of functional yogurt. *Journal of Food Quality*, **2022**(1), 3327401 (2022).
38. Alsubhi, N.H., Al-Quwaie, D.A., Alrefaei, G.I., Alharbi, M., Binothman, N., Aljadani, M., Qahl, S.H., Jaber, F.A., Huwaikem, M. and Sheikh, H.M. Pomegranate pomace extract with antioxidant, anticancer, antimicrobial, and antiviral activity enhances the quality of strawberry-yogurt smoothie. *Bioengineering*, **9**(12), 735 (2022).
39. Temiz, H. and Ersöz, E.B. Determination of some quality characteristics and rheological properties of yoghurts made using cow Milk and soy drink mixture enriched with pomegranate Peel extract. *Journal of Agricultural Sciences*, **29**(2), 561-572 (2023).

تعزيز خصائص الزبادي بمسحوق قشر الرمان

إبتسام عمر أحمد¹ و محمد أحمد عبد الحافظ^{2*}

¹ قسم مراقبة الأغذية، معهد بحوث الصحة الحيوانية، معمل الداخلة - الوادي الجديد، مركز البحوث الزراعية، مصر.

² قسم مراقبة الأغذية، معهد بحوث الصحة الحيوانية، معمل طنطا، مركز البحوث الزراعية، مصر.

*المؤلف المراسل: محمد أحمد عبد الحافظ

البريد الإلكتروني: Hafez.2310@gmail.com

الملخص

يعد الزبادي من المنتجات الغذائية المفيدة من الناحية التغذوية، إلا أنه يفتقر إلى بعض المركبات البيولوجية النشطة مثل البوليفينولات ومضادات الأكسدة الطبيعية. درست هذه الدراسة تأثيرات دمج مسحوق قشر الرمان (PPP) على جودة الزبادي، مع التركيز على التكوين الكيميائي، وحيوية البكتيريا، وإطالة فترة الصلاحية، وخصائصه المضادة للأكسدة، وخصائصه الحسية. تم تحضير عينات الزبادي باستخدام تركيزات مختلفة (0.5%، 1%، و1.5%) من مسحوق قشر الرمان. تم تقييم الخصائص الكيميائية (الرقم الهيدروجيني، الحموضة المعاييرة)، التحليل الميكروبيولوجي (عدد البكتيريا الكلي، عدد القولونية، عدد الخمائر والعفن)، مكونات المضادة للأكسدة (محتوى البوليفينولات والفلافونويدات الكلي)، والتقييم الحسي على مدار 21 يوماً من التخزين. تم إجراء التحليل الإحصائي باستخدام تحليل التباين الأحادي. أظهر دمج مسحوق قشر الرمان تأثيراً كبيراً على خصائص الزبادي. أظهرت تركيزات أعلى من مسحوق قشر الرمان (1-1.5%) تأثيرات مضادة للبكتيريا متفوقة، حيث قضت على القولونية وأدت إلى تقليل عدد البكتيريا الكلي. أظهر القشر الخارجي محتوى أعلى من المركبات البيولوجية النشطة 224.42 ميكروغرام مكافئ/مغ حمض الجاليك /مغ من البوليفينولات، 64.86 ميكروغرام/مغ مكافئ الريتين/مغ من الفلافونويدا مقارنة بالقشر الداخلي. انخفضت قيم الرقم الهيدروجيني خلال فترة التخزين عبر جميع العينات، في حين أظهرت الحموضة المعاييرة اتجاهات متباينة. انخفضت درجات التقييم الحسي مع زيادة تركيز مسحوق قشر الرمان وفترة التخزين. في الختام، يعزز مسحوق قشر الرمان خصائص الزبادي الوظيفية بشكل فعال، خاصة عند تركيزات 1-1.5%، من خلال توفير تأثيرات مضادة للبكتيريا ومركبات بيولوجية نشطة. وعلى الرغم من أن تركيزات مسحوق قشر الرمان العالية قد قللت قليلاً من قبول الحسية، فإن الدراسة تؤكد على أن مسحوق قشر الرمان يعد مادة حافظة طبيعية واعدة تعزز الخصائص البيولوجية للزبادي بينما تطيل فترة صلاحيتها.

الكلمات الدالة: الزبادي، مسحوق قشر الرمان، النشاط المضاد للبكتيريا، مضادات الأكسدة، الأغذية الوظيفية، فترة الصلاحية.