Pomegranate Peel Powder as a Natural Preservative Enhances the Microbiological Quality, Oxidative Stability and Shelf-Life of Aquafeed During Ambient Storage

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Abstract

This study was conducted at the Aquaculture Unit, College of Agriculture, University of Basrah, to evaluate the effect of dietary pomegranate peel powder (PPP) supplementation 0, 10, 20, and 30 g/kg feed treatments (T1–T4) on the microbiological quality and shelf-life of fish feed stored at ambient temperature (25 ± 2°C) for eight weeks. Results showed that total bacterial count in the control (T1) increased from 0.12 × 10^3 to 9.8 × 10^4 CFU/g by week 8, while T4 remained as low as 2.1 × 10^3 CFU/g; mold and yeast counts decreased from 3.2 × 10^3 CFU/g (T1) to 4.3 × 10^1 CFU/g (T4), a reduction exceeding 99% and coliform bacteria, detected in T1 from week 3 and reaching 14.00 × 10^2 CFU/g by week 8, remained below detection in T4 until week 4 and never exceeded 0.05 × 10^2 CFU/g. Regarding oxidative deterioration, free fatty acid content rose to 4.87 ± 0.18% in T1-surpassing the 4.0% EFSA/FAO limit, while T4 maintained a significantly lower value of 1.80 ± 0.08%, 63% reduction; similarly, TBA values increased from 0.25 to 3.21 ± 0.12 mg MDA/kg in T1, exceeding the 3.0 mg/kg acceptable limit, whereas T4 stayed at 0.74 ± 0.03 mg MDA/kg, a ~77% reduction relative to control. Overall, dietary supplementation with 20–30 g/kg pomegranate peel powder effectively improved the microbiological quality and oxidative stability of aquafeeds during storage, supporting its potential as an economical, sustainable, and environmentally friendly natural preservative that could replace synthetic preservatives in aquafeed formulations.

Keywords: Fish diets, Pomegranate peel, Shelf life, Aspergillus spp.

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Introduction

Aquaculture is the fastest-growing animal food production sector and plays a pivotal role in meeting the increasing global demand for high-quality aquatic protein (FAO, 2022). The sustainability and economic viability of this sector largely depend on the quality of formulated aquafeeds, which account for approximately 60 – 70 % of total production costs (Tacon and Metian, 2008). Therefore, maintaining feed quality and stability during storage is a critical prerequisite for enhancing sustainable production and minimizing economic losses (Al-Noor, 2024). During storage, aquafeeds are subjected to two major forms of deterioration. The first is microbial spoilage, resulting from the proliferation of bacteria, molds, and yeasts, which accelerates nutrient degradation and leads to the production of harmful metabolites that may adversely affect the health and performance of cultured fish (Al-Noor *et al.*, 2023). The second is lipid autoxidation, a process that generates secondary oxidation products such as aldehydes and ketones, thereby reducing the nutritional value, palatability, and overall quality of the feed (Albadran *et al.*, 2018). In recent years, increasing regulatory restrictions on the use of synthetic preservatives due to concerns regarding their potential health and environmental risks have intensified the search for safe and sustainable natural alternatives (Najim and Al-Noor 2026; Al-Noor *et al.*, 2025). Among the promising candidates, pomegranate peel (*Punica granatum* L.) has attracted considerable attention because of its high content of phenolic compounds, tannins, flavonoids, punicalagins, ellagic acid, and anthocyanidins. These bioactive constituents exhibit strong antioxidant activity and broad-spectrum antimicrobial properties against both Gram-positive and Gram-negative bacteria (Rosas-Burgos *et al.*, 2017; Liu *et al.*, 2025). Furthermore, pomegranate peel constitutes approximately 50 % of the fruit's total weight and represents an abundant agricultural by-product. Its utilization as a natural feed additive not only provides a cost-effective preservative but also promotes the valorization of agricultural waste, thereby supporting the principles of the circular economy and environmental sustainability (Topuz *et al.*, 2015). Several studies have demonstrated the effectiveness of pomegranate peel in preserving aquafeeds. Ghasemi-Sadabadi *et al.* (2022) reported that incorporating 2 % pomegranate peel powder into fish diets reduced the total microbial count by 68% compared with the untreated control. Similarly, Mitsagga *et al.* (2021) found that an aqueous extract of pomegranate peel at a concentration of 250 µg mL^{-1} completely inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*. Moreover, Hu *et al.* (2025) showed that aquafeeds supplemented with pomegranate peel maintained significantly lower levels of free fatty acids (FFA) and thiobarbituric acid (TBA) values throughout eight weeks of storage at ambient temperature, indicating enhanced oxidative stability. Despite these encouraging findings, information regarding the effects of different inclusion levels of pomegranate peel powder on aquafeed quality under the storage conditions and climatic environment prevailing in southern Iraq, particularly Basrah Province, remains limited. This knowledge gap highlights the need for locally conducted studies to evaluate the effectiveness of this natural additive in improving feed

quality and extending storage stability under regional environmental conditions. Such investigations could contribute to reducing reliance on synthetic preservatives while promoting sustainability in aquafeed production. Accordingly, this study investigated whether increasing dietary levels of pomegranate peel powder could improve the microbiological quality and oxidative stability of aquafeeds during prolonged storage. The findings provide new evidence supporting the use of pomegranate peel as a sustainable natural preservative in aquaculture feed production.

Materials and Methods

Study Site

The experiment was carried out at the Aquaculture Unit, College of Agriculture, University of Basrah, Iraq. The study was performed under controlled environmental conditions, with ambient temperature maintained between 23 and 27 °C and relative humidity ranging from 55 to 70 %. These conditions closely simulated those commonly encountered in aquafeed storage facilities in fish farms across southern Iraq, thereby enhancing the practical relevance and applicability of the findings to comparable production environments (Salman *et al.*, 2022).

Preparation of Pomegranate Peel Powder

Ripe pomegranate (*P. granatum* L., cv. *Wonderful*) peels were collected from local markets in Basrah, Iraq. The peels were washed three times with distilled water to remove dust and surface contaminants. They were subsequently dried in a forced-air oven at 45°C for 48 h to minimize enzymatic degradation of bioactive compounds, following the procedure described by Sweidan *et al.* (2023). The peels were then further air-dried at ambient temperature in a thin layer until a constant weight was achieved. The dried peels were ground using an electric grinder (Waring Commercial Blender, Model HGB150), and the resulting powder was passed through a 0.5-mm mesh sieve to obtain a fine and homogeneous powder. The proximate chemical composition of the pomegranate peel powder (dry-matter basis) was 8.2 % moisture, 4.1 % crude protein, 2.3 % ether extract, 39.6 % crude fiber, 4.8 % ash, and 41.0 % nitrogen-free extract, in addition to the phenolic and flavonoid contents reported below. The powder was stored in sterilized airtight glass containers at 4 °C, protected from light, until use. The total phenolic content (TPC) of the pomegranate peel powder was determined using the Folin–Ciocalteu method and measured 312 ± 8.5 mg gallic acid equivalents (GAE)/g dry weight, while the total flavonoid content (TFC) was 87.4 ± 3.2 mg quercetin equivalents (QE)/g dry weight, as reported by (Chen *et al.*, 2020).

Diet Preparation

Four experimental diets were formulated to be isonitrogenous and isoenergetic, containing 30% crude protein and 8 % crude lipid, according to the nutritional requirements recommended for fish during the grow-out stage (Abdel-Tawwab *et al.*, 2022). The experimental diets differed only in the level of pomegranate peel powder

supplementation. The control diets (T1) contained no pomegranate peel powder, whereas the experimental diets were supplemented with % 1 (T2), 2 % (T3), and 3 % (T4) of pomegranate peel powder, respectively. The diets were manufactured using a laboratory pellet mill (Lab Pellet Mill, Model MKL-100) fitted with a die producing pellets of 3 mm in diameter. The pellets were dried in a forced-air oven at 60°C for 2 h, allowed to cool gradually to ambient temperature, and then packed in double-layer polyethylene bags (500 g per bag) and stored in a dark, dry storage room at ambient temperature (25 ± 2 °C) with relative humidity maintained at 55 – 70 %. The bags were tightly sealed after each sampling to prevent moisture uptake and were protected from direct light exposure throughout the eight-week storage period, until use according to the method described by (Dogra and Gandotra, 2022). Each of the four experimental diets (T1–T4) was prepared and evaluated in triplicate, with three independent 500 g polyethylene bags (replicates) per treatment, giving a total of 12 independent experimental units. Each bag represented one independent experimental (sampling) unit and was stored and sampled separately throughout the trial. Weekly samples for microbiological and chemical analyses were drawn from each replicate bag following a randomized sampling scheme, and the mean of the three replicates was used for statistical analysis.

Table 1. Formulation (%) of the experimental diets supplemented with Pomegranate peel powder

	Feed ingredients	**T1 (0%)**	**T2 (1%)**	**T3 (2%)**	**T4 (3%)**
1	Fish meal	35	35	35	35
2	Soybean meal	25	25	25	25
3	Yellow corn	20	20	20	20
4	Wheat bran	12	11	10	9
5	Fish oil	5	5	5	5
6	Premix	3	3	3	3
7	Pomegranate peel powder	0	1	2	3
Total		100	100	100	100

Microbiological Analysis

Weekly samples (10 g) were aseptically collected from each dietary treatment in accordance with the standard procedures recommended by the American Public Health Association (APHA, 2017). Each sample was homogenized with 90 mL of sterile physiological saline solution (0.85 % NaCl) to obtain an initial 1:10 dilution, followed by a series of ten-fold serial dilutions up to 10^{-6}. Appropriate dilutions were inoculated onto selective and differential culture media. Plate Count Agar (PCA) was used for the enumeration of total aerobic bacteria following incubation at 37 °C for 48 h. MacConkey Agar (MCA) was employed for the detection and enumeration of coliform bacteria after incubation at 37°C for 24 – 48 h. Acidified Potato Dextrose Agar (PDA, pH 3.5) supplemented with tartaric acid was used for the isolation and enumeration of yeasts and molds, with plates incubated at 25 °C for 5 – 7 days. Microbial counts were expressed as colony-forming units per gram (CFU/g) and calculated according to the procedures described by APHA (2017). The bacterial isolates were identified using a combination of conventional biochemical tests and the automated VITEK® 2 Compact system (bioMérieux, France). All identification procedures were carried out at the Central Laboratory, College of Veterinary Medicine, University of Basrah.

$$\mathbf{CFU/mL} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated (mL)}}$$

Chemical Analyses of Oxidative Deterioration

The free fatty acid (FFA) content was determined by the standard titrimetric method described in AOAC (2019), and the results were expressed as the percentage of oleic acid equivalents. Lipid secondary oxidation was evaluated by determining the thiobarbituric acid (TBA) value using the spectrophotometric method at 532 nm. The results were expressed as mg malondialdehyde (MDA)/kg diets, following the modified Vyncke method described by (Geng *et al.*, 2023). All chemical analyses were performed in triplicate, and the arithmetic mean of the three determinations was used as the final value for each measured parameter.

Statistical Analysis

The experimental data were analyzed using Two-way analysis of variance (Two-Way ANOVA) with the Statistical Package for the Social Sciences (SPSS version 26.0, IBM Corp., Armonk, NY, USA). Differences among treatment means were evaluated using the Least Significant Difference (LSD) test at a significance level of ($P<0.05$).

Results and Discussion

Total Bacterial Count (TBC)

The changes in the total aerobic bacterial count (TBC) of the experimental diets during eight weeks of storage are presented in Table 2. Bacterial counts increased progressively

in all treatments throughout the storage period; however, the rate of increase differed significantly among treatments ($P<0.05$). The control diets (T1) exhibited the highest bacterial proliferation, increasing from 0.12×10^3 CFU/g in the first week to 98.00×10^3 CFU/g (9.8×10^4 CFU/g) after eight weeks. Although this value remained below the maximum permissible limit for fish feeds (10^5 CFU/g) established by the European Food Safety Authority (Hernández-Contreras *et al.*, 2023), it indicates that the control diets approached the acceptable microbiological spoilage threshold by the end of storage.

In contrast, supplementation with pomegranate peel powder (PPP) significantly suppressed bacterial growth in a dose-dependent manner. The diets containing 30 g/kg^{-1} PPP (T4) recorded the lowest bacterial count (2.10×10^3 CFU/g) at the end of the storage period, representing a 97.9% reduction compared with the control ($P<0.05$). Maintaining bacterial populations at approximately 10^3 CFU/g is considered advantageous for minimizing microbial spoilage and reducing the risk of bacterial toxin production, thereby extending feed shelf life (Al-Noor and Al-Waely, 2024), The statistical analysis confirmed significant differences ($P<0.05$) among treatments throughout the experimental period. The inhibitory effect became more pronounced as storage progressed, indicating that the antimicrobial efficacy of PPP was both concentration- and time-dependent.

The marked reduction in bacterial growth can be attributed to the high concentration of bioactive polyphenols present in pomegranate peel, particularly punicalagin, ellagitannins, and ellagic acid. These compounds disrupt bacterial cell membrane integrity through interactions with membrane proteins and phospholipids, leading to increased membrane permeability, leakage of intracellular constituents, and ultimately cell death (Daglia, 2012). Moreover, ellagitannins inhibit essential bacterial metabolic enzymes, including ATP synthase and β-lactamase, thereby impairing energy production and reducing bacterial survival. Covalent interactions between ellagic acid derivatives and these enzymes have recently been confirmed using chromatographic and molecular analyses (Mihaylova *et al.*, 2025).

The antimicrobial activity of pomegranate peel is further enhanced by the synergistic interaction among its phenolic constituents, resulting in greater antibacterial efficacy than that achieved by individual compounds alone (Youssef *et al.*, 2021). Likewise, Rosas-Burgos *et al.* (2017) reported that punicalagin-rich pomegranate peel extracts effectively inhibit aerobic microorganisms in stored foods by interfering with membrane-associated enzymatic systems. In addition, the broad-spectrum antibacterial activity of pomegranate phenolics against both Gram-positive and Gram-negative bacteria has been attributed to their ability to penetrate the peptidoglycan layer and destabilize the outer lipopolysaccharide membrane (Topuz *et al.* 2015).

These findings are further supported by scanning electron microscopy observations, which demonstrated severe membrane disruption and irreversible structural damage in bacterial cells exposed to pomegranate polyphenols Araújo and de Aquino Santana,

(2018). Overall, the present findings demonstrate that dietary supplementation with 30 g/kg^{-1} pomegranate peel powder provides effective microbiological protection during storage and represents a promising natural alternative to synthetic preservatives for improving the microbial stability and shelf life of aquafeeds.

Table 2. Total bacterial count ($\times 10^3$ CFU/g) in the experimental diets during the storage period.

Storage period	T1 (0%)	T2 (1%)	T3 (2%)	T4 (3%)
Week 1	0.12^a	0.09^a	0.07^a	0.05^b
Week 2	0.38^a	0.22^b	0.16^b	0.11^c
Week 3	1.25^a	0.71^b	0.45^c	0.29^d
Week 4	5.10^a	2.35^b	1.12^c	0.68^d
Week 5	18.40^a	7.90^b	3.50^c	1.45^d
Week 6	42.60^a	15.20^b	6.80^c	1.95^d
Week 7	71.80^a	25.50^a	9.30^b	2.05^c
Week 8	98.00^a	38.40^b	12.70^c	2.10^d

- Different letters within columns indicate significant differences among treatments at ($P<0.05$)

Yeast and Mold Count

The changes in yeast and mold counts during the eight-week storage period are presented in Table 3. Significant differences among treatments ($P<0.05$) were observed throughout the experiment. The control diets (T1) showed the highest fungal proliferation, with counts increasing from 0.08 × 10^2 CFU/g in the first week to 32.00 × 10^2 CFU/g (3.2 × 10^3 CFU/g) after eight weeks. In contrast, supplementation with pomegranate peel powder (PPP) significantly suppressed fungal growth in a dose-dependent manner. The 30 g/kg PPP treatment (T4) exhibited the greatest antifungal activity, with yeast and mold counts remaining as low as 0.43 × 10^2 CFU/g (4.3 × 10^1 CFU/g) at the end of storage, representing a reduction of more than 99% compared with the control ($P<0.05$). The substantial reduction in fungal contamination demonstrates the effectiveness of pomegranate peel powder as a natural preservative during feed storage. The absence of antimicrobial compounds in the control diets likely promoted fungal proliferation under ambient storage conditions, consistent with the findings of Magan and Aldred (2007), who reported that unprotected compound feeds are highly susceptible to fungal contamination during post-manufacturing storage. The antifungal activity of pomegranate peel has been primarily attributed to its high content of phenolic compounds, particularly punicalagin, ellagic acid, and hydrolysable tannins, which

possess both antioxidant and antimicrobial properties. These bioactive constituents disrupt fungal cell wall integrity, alter membrane permeability, and inhibit essential enzymatic pathways required for fungal growth and metabolism (Naseer *et al.*, 2018). Similar findings were reported by Sweidan *et al.* (2023), who demonstrated that pomegranate peel extract effectively inhibited the growth of *Aspergillus flavus* and *Penicillium citrinum* in stored feed products. The observed inhibition is of particular importance because fungal contamination of aquafeeds is frequently associated with the production of mycotoxins, especially those produced by *Aspergillus* spp. and *Fusarium* spp., which adversely affect liver function, immune competence, and growth performance in cultured fish while posing potential food safety risks through bioaccumulation (Hu *et al.*, 2025). In agreement with the present findings, Khanzadeh *et al.* (2025) reported that dietary supplementation with pomegranate peel powder significantly reduced fungal contamination and improved fish health and feed utilization efficiency. Furthermore, Tran *et al.* (2023) demonstrated that pomegranate peel not only suppresses fungal growth but also reduces mycotoxin production, thereby enhancing feed safety during prolonged storage. The superior performance of the 30 g/kg supplementation level suggests that this concentration provides sufficient bioactive compounds to exceed the minimum inhibitory concentration (MIC) for common storage fungi, resulting in sustained antifungal activity throughout the storage period. Nevertheless, further investigations are warranted to determine the precise MIC values against individual fungal species and to evaluate potential interactions between pomegranate polyphenols and feed ingredients under different storage conditions.

Table 3. Mold and yeast count ($\times 10^2$ CFU/g) in the experimental diets during the storage period.

Storage period	T1 (0%)	T2 (1%)	T3 (2%)	T4 (3%)
Week 1	0.08^a	0.05^b	0.03^b	0.01^c
Week 2	0.31^a	0.17^a	0.09^b	0.04^c
Week 3	0.91a	0.43b	0.20c	0.08d
Week 4	2.70^a	1.10^b	0.45^c	0.18^d
Week 5	7.37a	2.89b	1.02c	0.26d
Week 6	20.10^a	7.60^b	2.30^c	0.37^d
Week 7	25.36a	9.30b	2.96c	0.40d
Week 8	32.00^a	11.40^b	3.80^c	0.43^d

- Different letters within columns indicate significant differences among treatments at ($P<0.05$).

Coliform Bacteria Count

Coliform bacteria were not detected in any treatment during the first two weeks of storage, indicating either the absence of these microorganisms in freshly manufactured diets or bacterial populations below the detection limit. However, significant differences among treatments ($P<0.05$) became evident from the third week onward (Table 4). The control diets (T1) exhibited the highest coliform counts, which increased progressively from 0.80×10^2 CFU/g in week three to 14.00×10^2 CFU/g at the end of the eighth week. In contrast, pomegranate peel powder (PPP) supplementation significantly reduced coliform proliferation in a concentration-dependent manner. The 30 g/kg PPP treatment (T4) demonstrated the greatest antimicrobial efficacy, with coliform bacteria remaining below the detection limit until the fourth week and reaching only 0.05×10^2 CFU/g by the end of storage, representing a highly significant reduction compared with the control ($P<0.05$). The pronounced suppression of coliform bacteria observed in PPP-supplemented diets is primarily attributed to the high concentration of phenolic compounds, including punicalagin, ellagic acid, anthocyanins, and hydrolysable tannins, which exhibit potent antimicrobial properties. These compounds disrupt bacterial membrane integrity, increase membrane permeability, inhibit respiratory enzymes, and interfere with bacterial cell wall biosynthesis, ultimately suppressing cell growth and proliferation (Aviram *et al.*, 2000; Bakkiyaraj *et al.*, 2013). Their effectiveness appears particularly pronounced against Gram-negative bacteria, such as coliforms, whose outer membrane is highly susceptible to phenolic compounds despite the protective lipopolysaccharide layer (Taguri *et al.*, 2006). The present findings are consistent with those reported by Mitsagga *et al.* (2021), who demonstrated that pomegranate peel extract effectively inhibited *Escherichia coli* and *Salmonella typhimurium* in stored aquafeeds, with antimicrobial activity increasing proportionally to extract concentration. Similarly, Kupnik *et al.* (2003) reported broad-spectrum antibacterial activity of pomegranate peel against several Gram-negative pathogens, including *Salmonella enterica* and *Shigella dysenteriae*, attributing this effect to the synergistic interaction among multiple bioactive phytochemicals rather than the activity of a single compound. From a practical perspective, controlling coliform contamination is particularly important for maintaining the microbiological quality and safety of aquafeeds during storage. Elevated coliform counts are commonly regarded as indicators of microbial deterioration and poor hygienic quality, while their effective suppression reduces the risk of enteric bacterial infections in cultured fish, thereby minimizing production losses in aquaculture systems (Bondad-Reantaso *et al.*, 2005). Furthermore, reducing the microbial load in stored feeds may decrease the likelihood of transmitting foodborne pathogens through the aquaculture production chain, supporting the One Health approach to food and feed safety. Overall, the present results demonstrate that supplementation with 30 g/kg pomegranate peel powder provides effective long-term control of coliform bacteria during feed storage and highlights its potential as a natural alternative to synthetic antimicrobial preservatives in aquaculture feeds.

Table 4. Coliform bacteria count ($\times 10^2$CFU/g) in the experimental diets during the storage period.

Storage period (weeks)	T1 (0%)	T2 (1%)	T3 (2%)	T4 (3%)
Week 1	*ND*^{a}	*ND*^{a}	*ND*^{a}	*ND*^{a}
Week 2	*ND*^{a}	*ND*^{a}	*ND*^{a}	*ND*^{a}
Week 3	0.80^{a}	0.38^{b}	0.10^{c}	*ND*^{d}
Week 4	2.10^{a}	0.90^{b}	0.22^{c}	0.04^{d}
Week 5	4.50^{a}	1.80^{b}	0.33^{c}	0.04^{d}
Week 6	7.80^{a}	3.20^{b}	0.42^{c}	0.05^{d}
Week 7	11.50^{a}	5.80^{b}	0.47^{c}	0.05^{d}
Week 8	14.00^{a}	8.60^{b}	0.50^{c}	0.05^{d}

- Different letters within columns indicate significant differences among treatments at ($P<0.05$)
- ND = Not Detected

Free Fatty Acids (FFA)

The changes in free fatty acid (FFA) content during the eight-week storage period are presented in Table 5. FFA values increased significantly in all treatments throughout storage ($P<0.05$), indicating progressive lipid hydrolysis. However, diets supplemented with pomegranate peel powder (PPP) exhibited a markedly lower rate of FFA accumulation than the control. The control diets (T1) showed the highest FFA values, increasing from 0.85 ± 0.04 % in the first week to 4.87 ± 0.18 % after eight weeks. Notably, the FFA level exceeded the internationally accepted quality limit of 4.0% oleic acid, as recommended by the European Food Safety Authority (Hernández-Contreras *et al.*, 2023) and the Food and Agriculture Organization (FAO, 2010), indicating deterioration of feed quality before the end of the normal storage period. In contrast, PPP supplementation significantly delayed lipid hydrolysis in a concentration-dependent manner. The diets containing 30 g/kg PPP (T4) exhibited the greatest protective effect, with FFA values remaining at only 1.80 ± 0.08 % after eight weeks, representing less than 45 % of the maximum recommended limit and demonstrating excellent oxidative stability throughout storage. The reduction in FFA formation can be attributed to the high concentration of phenolic compounds present in pomegranate peel, particularly ellagic acid and hydrolysable tannins, which inhibit lipid hydrolysis through multiple mechanisms. Ellagic acid competitively binds to the active site of microbial lipase, thereby reducing enzymatic

hydrolysis of triglycerides (Chen *et al.*, 2020). In addition, hydrolysable tannins form stable complexes with lipolytic enzymes, leading to enzyme inactivation and reduced free fatty acid formation (Camarda *et al.*, 2026). These findings indicate that pomegranate peel powder effectively preserves lipid quality by limiting enzymatic degradation during prolonged feed storage.

Table 5. Free fatty acid (FFA) content (%) in the experimental diets during storage.

Storage period	T1 (0%)	T2 (1%)	T3 (2%)	T4 (3%)
Week 1	0.25 ± 0.01^{a}	0.18 ± 0.01^{b}	0.14 ± 0.01^{c}	0.10 ± 0.01^{d}
Week 2	0.45 ± 0.02^{a}	0.32 ± 0.01^{b}	0.24 ± 0.01^{c}	0.18 ± 0.01^{d}
Week 3	0.75 ± 0.03^{a}	0.54 ± 0.02^{b}	0.38 ± 0.02^{c}	0.28 ± 0.01^{d}
Week 4	1.09 ± 0.04^{a}	0.78 ± 0.03^{b}	0.58 ± 0.02^{c}	0.41 ± 0.02^{d}
Week 5	1.52 ± 0.06^{a}	1.08 ± 0.04^{b}	0.74 ± 0.03^{c}	0.52 ± 0.02^{d}
Week 6	2.10 ± 0.08^{a}	1.42 ± 0.05^{b}	0.94 ± 0.04^{c}	0.60 ± 0.02^{d}
Week 7	2.65 ± 0.10^{a}	1.85 ± 0.07^{b}	1.15 ± 0.05^{c}	0.68 ± 0.03^{d}
Week 8	3.21 ± 0.12^{a}	2.24 ± 0.08^{b}	1.45 ± 0.06^{c}	0.74 ± 0.03^{d}

- Different letters within columns indicate significant differences among treatments at ($P<0.05$).

Thiobarbituric Acid (TBA) Value

The thiobarbituric acid (TBA) assay was used to evaluate secondary lipid oxidation by measuring malondialdehyde (MDA), one of the principal degradation products of lipid hydroperoxides. Because aquafeeds are rich in highly unsaturated fatty acids such as EPA and DHA, TBA is considered one of the most reliable indicators of oxidative deterioration (Esterbauer *et al.*, 1991). As shown in Table 6, TBA values increased significantly in all treatments during storage ($P<0.05$), although the magnitude of the increase was markedly affected by PPP supplementation. The control diets (T1) exhibited the highest degree of lipid oxidation, with MDA levels increasing from 0.25 ± 0.01 mg MDA/kg in the first week to 3.21 ± 0.12 mg MDA/kg after eight weeks, exceeding the generally accepted limit of 3.0 mg MDA/kg for high-quality aquafeeds (De Leon and Borges, 2020). Such elevated MDA concentrations are associated with membrane lipid peroxidation, enzyme inactivation, impaired liver and immune function, and reduced growth performance in fish (Hu *et al.*, 2025). Supplementation with PPP significantly reduced secondary lipid oxidation in a dose-dependent manner. The 30 g/kg PPP treatment (T4) consistently recorded the

lowest TBA values throughout the storage period, reaching only 0.74 ± 0.03 mg MDA/kg at week eight, corresponding to approximately 77% lower MDA levels than the control ($P<0.05$). The superior antioxidant activity of PPP is mainly attributed to its high concentration of polyphenols, ellagitannins, and punicalagin, which function as potent free radical scavengers and metal chelators. These compounds inhibit lipoxygenase-mediated oxidation of polyunsaturated fatty acids, thereby reducing hydroperoxide formation and limiting their subsequent decomposition into secondary oxidation products such as aldehydes and ketones (Hu *et al.*, 2025). Similar observations were reported by Vučina *et al.* (2011), who demonstrated that polyphenol-rich plant extracts effectively suppress the activities of lipoxygenase and cyclooxygenase in lipid-rich food systems. Furthermore, Çam *et al.* (2009) reported that whole pomegranate peel powder provides sustained antioxidant protection because the synergistic interactions among its naturally occurring bioactive compounds are preserved, resulting in greater oxidative stability than isolated phytochemicals. Overall, the present findings demonstrate that supplementation with 30 g/kg pomegranate peel powder effectively inhibits both primary lipid hydrolysis and secondary lipid oxidation, thereby maintaining feed quality and extending the oxidative shelf life of aquafeeds during storage.

Table 6. Table 6. Thiobarbituric acid (TBA) values (mg MDA/kg) in the experimental diets during storage.

Storage period	T1 (0%)	T2 (1%)	T3 (2%)	T4 (3%)
Week 1	0.85 ± 0.04^{a}	0.70 ± 0.03^{b}	0.58 ± 0.02^{c}	0.52 ± 0.02^{d}
Week 2	1.20 ± 0.05^{a}	1.00 ± 0.04^{b}	0.80 ± 0.03^{c}	0.70 ± 0.03^{d}
Week 3	1.75 ± 0.07^{a}	1.45 ± 0.06^{b}	1.10 ± 0.05^{c}	0.95 ± 0.04^{d}
Week 4	2.41 ± 0.09^{a}	1.90 ± 0.08^{b}	1.42 ± 0.06^{c}	1.20 ± 0.05^{d}
Week 5	3.10 ± 0.12^{a}	2.25 ± 0.09	1.65 ± 0.07^{c}	1.38 ± 0.06^{d}
Week 6	3.75 ± 0.14^{a}	2.60 ± 0.10^{b}	1.82 ± 0.08^{c}	1.52 ± 0.07^{d}
Week 7	4.30 ± 0.16^{a}	2.85 ± 0.11^{b}	1.95 ± 0.08^{c}	1.65 ± 0.07^{d}
Week 8	4.87 ± 0.18^{a}	3.12 ± 0.12^{b}	2.10 ± 0.09^{c}	1.80 ± 0.08^{d}

- Different letters within columns indicate significant differences among treatments at ($P<0.05$)

Finally, from an industrial perspective, replacing synthetic preservatives with pomegranate peel powder could reduce feed spoilage, improve feed safety and increase

storage stability while simultaneously promoting the utilization of agro-industrial by-products within a circular bioeconomy framework.

Conclusions

The present study demonstrated that dietary supplementation with pomegranate peel powder (PPP) significantly improved the microbiological and oxidative stability of aquafeeds during eight weeks of storage under ambient conditions. The highest inclusion level (30 g/kg^{-1}) consistently produced the greatest reduction in total bacterial, yeast and mold, and coliform counts while effectively limiting lipid hydrolysis and secondary lipid oxidation. These findings confirm the strong antimicrobial and antioxidant properties of pomegranate peel and highlights its potential as a natural preservative for extending aquafeed shelf life. In addition to improving feed quality and safety, the utilization of pomegranate peel represents an environmentally sustainable strategy for valorizing agricultural by-products and reducing dependence on synthetic preservatives. Future studies should evaluate the long-term effects of this additive on feed palatability, fish growth performance, immune responses and economic feasibility under commercial aquaculture conditions.

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مسحوق قشور الرمان كمادة حافظة طبيعية يعزز الجودة الميكروبيولوجية والثبات التأكسدي ومدة صلاحية الأعلاف المائية أثناء الخزن في درجة حرارة الغرفة

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المستخلص

أجريت هذه الدراسة في وحدة الاستزراع المائي، كلية الزراعة، جامعة البصرة، بهدف تقييم تأثير إضافة مسحوق قشور الرمان الى الأعلاف، بأربع معاملات هي 0 و 10 و 20 و 30 غم/كغم علف (T4 – T1) لغرض متابعة الجودة الميكروبيولوجية ومدة صلاحية أعلاف الأسماك المخزنة لمدة ثمانية أسابيع في درجة حرارة الغرفة 25 °م ± 2، أظهرت النتائج أن العدد الكلي للبكتيريا في معاملة السيطرة (T1) أرتفع من 0.12×10^3 الى 9.8×10^4 CFU/g بحلول الأسبوع الثامن، في حين حافظت المعاملة T4 على مستوى منخفض بلغ 2.1×10^3 CFU/g كما انخفضت أعداد الأعفان والخمائر من 3.2×10^3 CFU/g في معاملة السيطرة (T1) الى 4.3×10^1 CFU/g في المعاملة T4، أي بانخفاض تجاوز 99 %. أما بالنسبة الى بكتيريا القولون فقد ظهرت في معاملة السيطرة (T1) ابتداء من الأسبوع الثالث وبلغت قيمتها 14.00×10^2 CFU/g في الأسبوع الثامن في حين بقيت دون مستوى الكشف في المعاملة T4 حتى الأسبوع الرابع، ولم تتجاوز 0.05×10^2 CFU/g طوال فترة الخزن. وفيما يتعلق بالتدهور التأكسدي، فقد أرتفع محتوى الأحماض الدهنية الحرة (FFA) في معاملة السيطرة (T1) الى 4.87 % ± 0.18 متجاوزاً الحد المسموح البالغ 4.0 % وفقاً للحدود المشار إليها من قبل EFSA/FAO، في حين حافظت المعاملة T4 على قيمة منخفضة ومعنوية بلغت 1.80 % ± 0.08 أي بانخفاض قدره 63 %، وبالمثل ارتفعت قيم حمض الثيوباربيتوريك (TBA) من 0.25 الى 3.21 ± 0.12 ملغم مالونالديهايد/كغم سمك في معاملة السيطرة T1 متجاوزة الحد المقبول البالغ 3.0 ملغم مالونالديهايد/كغم سمك بينما بقيت قيمة T4 عند 0.74 ± 0.03 ملغم مالونالديهايد/كغم سمك أي بانخفاض يقارب 77 % مقارنة بمعاملة السيطرة. وبصورة عامة، أظهرت النتائج أن إضافة مسحوق قشور الرمان بمستويات 20 – 30 غم/كغم علف أسهمت بصورة فعالة في تحسين الجودة الميكروبيولوجية والثبات التأكسدي للأعلاف المائية أثناء الخزن، مما يدعم إمكانية استخدامه كمادة حافظة طبيعية اقتصادية ومستدامة وصديقة للبيئة، ويمكن أن يشكل بديلاً عن المواد الحافظة الصناعية في تركيبة علائق الأسماك.

الكلمات المفتاحية: علائق الأسماك، قشور الرمان، مدة الصلاحية، Aspergillus spp.