

Ultrasound-Assisted Extraction of Banana and Water Chestnut Peel Phenolics for Improving the Storage Stability of Fish Patties

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ABSTRACT

Fish patties are highly susceptible to oxidative and microbial deterioration during chilled storage because of their high moisture content and abundance of polyunsaturated lipids. This study evaluated ultrasound-assisted water chestnut peel extract (WCPE) and banana peel extract (BPE) as natural preservatives in refrigerated fish patties. Dried peel powders were extracted with 70% ethanol (1:10, w/v) using a 20 kHz ultrasonic probe at 50% amplitude for 10 min, followed by filtration and vacuum evaporation. Fish patties were prepared as untreated control or supplemented with 1% or 2% WCPE, 1% or 2% BPE, or combinations of 1% WCPE + 1% BPE and 2% WCPE + 2% BPE, and were evaluated on days 0, 7, 14, and 21 of refrigerated storage. The combined 2% WCPE + 2% BPE treatment showed the strongest overall protection against quality deterioration. On day 21, the TBARS value was 1.29 ± 0.27 compared with 1.71 ± 0.24 in control, while TVB-N was 24.13 ± 0.41 compared with 34.41 ± 0.30 in control. The same treatment maintained the highest DPPH radical-scavenging activity (50.17 ± 3.27) and total phenolic content (129.61 ± 3.27), while restricting the total bacterial count to 3.96 ± 0.54 log CFU/g compared with 8.56 ± 0.62 log CFU/g in control. However, higher extract concentrations reduced several sensory scores, indicating a trade-off between preservation efficacy and sensory acceptability. Overall, the findings demonstrate the potential of water chestnut and banana peel extracts as natural antioxidant and antimicrobial ingredients for improving the refrigerated stability of fish patties, although optimization of extract concentrations is required to achieve an appropriate balance between preservation and sensory quality.



KEYWORDS: fish patties; banana peel extract; water chestnut peel extract; ultrasound-assisted extraction; natural preservatives; lipid oxidation; refrigerated storage

1. INTRODUCTION

Fish and fish-based products are nutritionally important components of the human diet, providing high-quality proteins, essential amino acids, long-chain polyunsaturated fatty acids, vitamins, and minerals. However, their high moisture content and abundance of unsaturated lipids also make them particularly susceptible to quality deterioration during refrigerated storage. Lipid oxidation, endogenous enzymatic reactions, and microbial growth can lead to rancid flavours, discoloration, textural deterioration, nutrient losses, and accumulation of volatile nitrogenous compounds, ultimately reducing shelf life and consumer acceptability (Presenza et al., 2023; Parmar et al., 2024). This problem can be particularly pronounced in processed products such as fish patties, where mincing and mixing disrupt muscle structure and increase the exposure of lipids to oxygen and other pro-oxidant components.

Lipid oxidation and microbial spoilage are therefore major challenges in maintaining the quality of refrigerated fish products. Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) are commonly used to evaluate primary and secondary lipid oxidation, respectively, whereas total volatile basic nitrogen (TVB-N) and total bacterial count (TBC) provide useful indicators of freshness and microbial deterioration. Although synthetic antioxidants and preservatives have traditionally been used to control these changes, increasing interest in naturally derived ingredients has encouraged the exploration of plant-based alternatives. Phenolic compounds from plant materials can inhibit oxidative reactions through free-radical scavenging, hydrogen donation, metal chelation, and interruption of oxidative chain reactions, while some also exhibit antimicrobial activity (Presenza et al., 2023). Recent studies have demonstrated that botanical extracts can retard oxidative and microbial deterioration in refrigerated fish products, supporting their potential application as natural preservation ingredients (Caglak & Karsli, 2023; Matra et al., 2025).

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The development of natural preservatives is also closely associated with the valorisation of food-processing by-products. Fruit peels and other plant residues are often discarded despite containing considerable amounts of phenolic compounds, dietary fibre, and other potentially valuable constituents. Their utilization as sources of functional ingredients could therefore contribute simultaneously to food preservation and waste reduction. Banana peel is particularly promising because it contains a diverse range of phenolic and antioxidant constituents (Someya et al., 2002; Emaga et al., 2007). More recent research has reinforced the potential of banana peel as a source of bioactive compounds with antioxidant and antimicrobial properties for food applications (Manzoor & Ahmad, 2021; Wani & Dhanya, 2025). Nevertheless, high concentrations of phenolic-rich extracts can also affect colour, flavour, bitterness, and astringency, making sensory acceptability an important consideration when such ingredients are incorporated into food products.

Water chestnut (*Trapa* spp.) peel represents another underutilized plant material with potential as a source of natural antioxidants. Water chestnut materials contain phenolic compounds, tannins, and other constituents associated with antioxidant activity, yet their application in processed fish products remains comparatively underexplored. Combining water chestnut peel extract (WCPE) with banana peel extract (BPE) may be particularly useful because differences in their phytochemical composition could provide complementary antioxidant and antimicrobial effects. However, whether increasing the concentration or combining these extracts improves preservation without adversely affecting sensory characteristics requires systematic evaluation.

The extraction procedure is also important because the recovery and functionality of plant bioactive compounds depend strongly on extraction conditions. Ultrasound-assisted extraction has emerged as an efficient approach for recovering phenolic compounds from plant materials. Acoustic cavitation can disrupt plant tissues, improve solvent penetration, and enhance mass transfer, thereby facilitating the release of intracellular bioactive constituents (Liu et al., 2014). In the present study, ultrasound-assisted extraction was therefore employed to recover potentially functional compounds from water chestnut and banana peels for application in fish patties.

Despite growing interest in botanical preservatives and food by-product valorisation, limited information is available on the combined application of ultrasound-assisted WCPE and BPE in refrigerated fish patties. In particular, it remains important to establish whether their combined use provides greater protection against oxidative and microbial deterioration than individual extracts and whether such improvements can be achieved without compromising sensory quality. Therefore, this study investigated the effects of WCPE and BPE, individually and in combination at different concentrations, on the quality and storage stability of fish patties during 21 days of refrigerated storage. Lipid oxidation was assessed using TBARS and PV, freshness and microbial stability through TVB-N and TBC, and antioxidant characteristics through DPPH radical-scavenging activity and total phenolic content (TPC). Sensory characteristics were also evaluated to determine the practical balance between preservation efficacy and product acceptability.

2. Materials and Methods

2.1. Procurement and preparation of raw materials

Fresh bananas, water chestnuts, and Rahu fish were purchased from a local market in Faisalabad, Pakistan. Chemicals and analytical reagents used throughout the study were obtained from reputable commercial suppliers and were of analytical grade. Banana and water chestnut peels were separated from the edible portions and thoroughly cleaned to remove adhering dirt and damaged material. The cleaned peels were cut into small pieces, dried, and ground into a fine powder using a laboratory grinder. The resulting powders were sieved, packed in polyethylene bags, and stored at room temperature until extraction.

2.2. Ultrasound-assisted extraction of water chestnut and banana peels

Water chestnut peel extract (WCPE) and banana peel extract (BPE) were prepared using ultrasound-assisted extraction. Dried peel powder was mixed with 70% ethanol at a solid-to-solvent ratio of 1:10 (w/v). Extraction was performed using an ultrasonic probe (VCX750, Sonics & Materials, USA) operated at 20 kHz and 50% amplitude for 10 min at room temperature, following Liu et al. (2014) with modifications. Ultrasound-assisted extraction is widely used for recovering phenolic constituents from plant matrices because acoustic cavitation can enhance tissue disruption, solvent penetration, and mass transfer. Recent studies have continued to apply ultrasound-assisted extraction for recovery of phenolic and antioxidant compounds from plant-derived materials (Rizvi et al., 2023). Following extraction, the WCPE and BPE suspensions were separately filtered through Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator connected to a vacuum pump and refrigerated cooling system, and the concentrated extracts were retained for incorporation into the fish-patty formulations.

2.3. Preparation of fish patties

Rahu fish were beheaded, gutted, filleted, washed, and cooked in boiling water before mincing using a kitchen blender. The basic formulation consisted of minced fish supplemented with 0.7% salt, 6% mashed potato, 0.9% bread, 4% onion, 0.5% cumin, 0.4% black pepper, 0.5% coriander, 5% red pepper, and 1% egg. Seven formulations were prepared: untreated control (0% WCPE + 0% BPE), 1% WCPE, 2% WCPE, 1% BPE, 2% BPE, 1% WCPE + 1% BPE, and 2% WCPE

+ 2% BPE. The ingredients were thoroughly mixed with minced fish, manually shaped into patties, and coated with wheat flour. Patties were placed in plastic containers, wrapped with stretch film, and stored under refrigeration. Samples were evaluated on days 0, 7, 14, and 21 of storage.

2.4. Determination of total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric assay following Tezcan et al. (2009), with modifications. Briefly, 0.5 mL of sample was mixed with 1 mL of 10% Folin-Ciocalteu reagent and allowed to react for 6 min. Subsequently, 2 mL of 20% sodium carbonate solution was added, and the mixture was incubated at 30 °C for 60 min. Absorbance was measured at 760 nm using a UV-visible spectrophotometer (Specord 200 Plus, Germany). Gallic acid was used to prepare the calibration curve, and TPC was expressed as gallic acid equivalents (GAE). The Folin-Ciocalteu assay remains one of the most widely applied approaches for estimating total phenolic content in plant and food matrices, although assay conditions can influence the measured response (Rizvi et al., 2023).

2.5. DPPH radical-scavenging activity

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay according to Mimica-Dukić et al. (2004), with modifications. An aliquot (0.1 mL) of homogenized sample was added to freshly prepared 0.0012 M DPPH solution. The mixture was incubated in the dark at room temperature for 60 min, after which absorbance was measured at 517 nm using a UV-visible spectrophotometer. Radical-scavenging activity was expressed as the percentage reduction of DPPH. DPPH remains extensively used for evaluating radical-scavenging capacity in plant-derived ingredients and food matrices (Rizvi et al., 2023).

2.6. Total volatile basic nitrogen

Total volatile basic nitrogen (TVB-N) was determined according to Malle and Tao (1987), with minor modifications. Samples were homogenized with trichloroacetic acid (TCA) at a ratio of 1:2 (w/v) and centrifuged at $1008 \times g$ for 5 min. The resulting supernatant was filtered through Whatman No. 1 filter paper. A 25 mL aliquot of the filtrate was transferred to a Kjeldahl distillation system, followed by the addition of 5 mL of 10% sodium hydroxide. After steam distillation, the distillate was titrated against 0.05 M sulfuric acid to the endpoint. TVB-N remains a widely used indicator of freshness deterioration and nitrogenous spoilage in refrigerated fish products (Soleimani et al., 2025; Delvaux Júnior et al. 2025).

2.7. Thiobarbituric acid reactive substances

Lipid oxidation was assessed by determining thiobarbituric acid reactive substances (TBARS) according to Schmedes and Hølmer (1989), with modifications. Fish-patty sample (5 g) was homogenized for 30 s with 25 mL of 20% TCA prepared in phosphoric acid. The homogenate was filtered through Whatman No. 4 filter paper. Subsequently, 2 mL of filtrate was mixed with 2 mL of 0.02 M thiobarbituric acid solution and incubated at 100 °C for 30 min. After cooling under running water, absorbance was measured at 532 nm using a UV-visible spectrophotometer. TBARS values were calculated against a 1,1,3,3-tetraethoxypropane standard curve and expressed as mg malondialdehyde (MDA)/kg sample. TBARS continues to be widely employed for monitoring secondary lipid oxidation in refrigerated fish and fish products (Soleimani et al., 2025).

2.8. Peroxide value

Briefly, 50 g of fish-patty sample was mixed with 100 mL chloroform and maintained in the dark for 2 h before filtration. A 25 mL aliquot of the filtrate was transferred to a suitable vessel for solvent evaporation. Acetic acid, distilled water, potassium iodide, and starch indicator were subsequently added, and the liberated iodine was titrated using 0.01 N sodium thiosulfate. PV was expressed as milliequivalents of oxygen per 1000 g of fat. Peroxide value remains a commonly used measure of primary lipid oxidation in fish products (Soleimani et al., 2025).

2.9. Total bacterial count

Total bacterial count (TBC) was determined according to Linton et al. (2003) and Karim et al. (2018). A 10 g portion of sample was homogenized with 90 mL of maximum recovery diluent, followed by serial dilution to obtain appropriate microbial concentrations. An aliquot (0.1 mL) of the selected dilution was spread onto agar plates using a sterile glass spreader. Following incubation, colonies were enumerated, and results were expressed as log₁₀ colony-forming units per gram (log CFU/g). Similar culture-based enumeration approaches remain routinely used to monitor microbial changes in refrigerated fish products (Soleimani et al., 2025).

2.10. Sensory evaluation

Sensory evaluation was conducted using a semi-trained panel of 10 assessors. Before evaluation, fish patties were fried in sunflower oil at 180 °C for 4 min. Panelists evaluated appearance, taste, odour, flavour, and texture using a hedonic scale. Water was provided for palate cleansing between samples. Sensory assessments were performed during the 21-day storage period at 7-day intervals.

2.11. Statistical analysis

All analytical determinations were performed in triplicate, except where otherwise specified. Data were statistically analysed using Statistix version 8.1 according to a factorial arrangement under a completely randomized design (CRD). Treatment and storage period were considered experimental factors, and differences among means were evaluated using the least significant difference (LSD) test. Statistical significance was established at $p < 0.05$. Ten panelists were included in the sensory evaluation.

3. Results and Discussion

The effects of water chestnut peel extract (WCPE) and banana peel extract (BPE), applied individually and in combination, were evaluated throughout 21 days of refrigerated storage. Treatment and storage interval significantly influenced the principal indices of lipid oxidation, freshness, antioxidant status and microbial quality reported in the thesis. The combined 2% WCPE + 2% BPE treatment generally provided the strongest preservation response, although the sensory data demonstrated that greater preservative efficacy did not necessarily correspond to greater consumer-oriented acceptability.

3.1. Lipid oxidation: TBARS

TBARS increased progressively during storage in all formulations, indicating continued formation of secondary lipid-oxidation products. The untreated control increased from 1.59 ± 0.24 at day 0 to 1.71 ± 0.24 at day 21, whereas the 2% WCPE + 2% BPE treatment increased only from 1.17 ± 0.23 to 1.29 ± 0.27 over the same period (Table 1). At day 21, the combined high-dose treatment therefore showed a markedly lower TBARS value than the control. The individual 2% WCPE and 2% BPE treatments also performed better than their corresponding 1% treatments, while the combined treatments produced the lowest values across storage.

The lower TBARS values in extract-treated patties are consistent with an antioxidant contribution from peel-derived phenolic constituents, which can interfere with radical-mediated propagation reactions during lipid oxidation. The present pattern agrees with the original comparisons reported for turmeric-treated meat patties and guava/papaya-derived antioxidant systems (Arshad et al., 2019; Yaqoob et al., 2020). More recent evidence also supports this interpretation. Šimat et al. (2025) reported substantially lower final TBARS values in vacuum-packed fish burgers containing combinations of plant/by-product extracts than in untreated controls, while basil extracts in gilthead sea bream patties similarly demonstrated significant suppression of lipid oxidation during refrigerated storage. These studies reinforce the potential of phenolic-rich botanical ingredients to delay secondary oxidation in comminuted fish matrices.

Table 1. Thiobarbituric acid reactive substances (TBARS) of WCPE- and BPE-enriched fish patties during refrigerated storage.

Treatment	Day 0	Day 7	Day 14	Day 21
Control	1.59 ± 0.24	1.63 ± 0.24	1.68 ± 0.24	1.71 ± 0.24
1% WCPE	1.52 ± 0.23	1.56 ± 0.23	1.61 ± 0.24	1.64 ± 0.22
2% WCPE	1.31 ± 0.21	1.35 ± 0.25	1.39 ± 0.21	1.43 ± 0.21
1% BPE	1.47 ± 0.22	1.53 ± 0.26	1.57 ± 0.25	1.61 ± 0.26
2% BPE	1.25 ± 0.23	1.30 ± 0.20	1.34 ± 0.22	1.39 ± 0.29
1% WCPE + 1% BPE	1.21 ± 0.20	1.24 ± 0.23	1.28 ± 0.20	1.33 ± 0.25
2% WCPE + 2% BPE	1.17 ± 0.23	1.20 ± 0.25	1.25 ± 0.21	1.29 ± 0.27

Values are mean $\pm$ SD of three independent determinations. WCPE, water chestnut peel extract; BPE, banana peel extract.

3.2. Peroxide value

Peroxide value (PV), representing primary lipid-oxidation products, was affected by both treatment and storage period. The control increased from 1.17 ± 0.24 at day 0 to 1.29 ± 0.24 at day 21. The lowest initial PV (0.98 ± 0.23) was observed in patties containing 2% WCPE + 2% BPE, and this treatment remained below the control at day 21 (1.22 ± 0.27 ; Table 2). However, the response was not uniform across treatments. In particular, patties containing 1% BPE showed a gradual decrease in PV from 1.10 ± 0.22 at day 0 to 1.02 ± 0.26 at day 21, representing the lowest PV at the end of storage. Thus, the effects of WCPE and BPE on primary lipid oxidation were treatment-dependent rather than following a simple concentration-dependent pattern.

This behaviour may reflect the dynamic nature of lipid oxidation, as primary hydroperoxides are unstable and can decompose into secondary oxidation products during storage. Consequently, PV should be interpreted together with indicators of secondary oxidation such as TBARS. Plant-derived phenolic compounds can influence lipid oxidation by interfering with radical-mediated initiation and propagation reactions. Similarly, Capparis spinosa essential-oil treatments have been reported to restrict peroxide formation in refrigerated fish burgers, an effect associated with their antioxidant constituents (Shafaghi Rad & Nouri, 2023). Considering the PV and TBARS results together, WCPE and BPE appeared to influence different stages of lipid oxidation, while the 2% WCPE + 2% BPE treatment showed particularly strong control of secondary lipid oxidation.

Table 2. Peroxide value of WCPE- and BPE-enriched fish patties during refrigerated storage.

Treatment	Day 0	Day 7	Day 14	Day 21
Control	1.17 ± 0.24	1.21 ± 0.24	1.24 ± 0.24	1.29 ± 0.24
1% WCPE	1.13 ± 0.23	1.17 ± 0.23	1.20 ± 0.24	1.23 ± 0.22
2% WCPE	1.09 ± 0.21	1.12 ± 0.25	1.15 ± 0.21	1.19 ± 0.21
1% BPE	1.10 ± 0.22	1.08 ± 0.26	1.04 ± 0.25	1.02 ± 0.26
2% BPE	1.06 ± 0.23	1.09 ± 0.20	1.13 ± 0.22	1.18 ± 0.29
1% WCPE + 1% BPE	1.03 ± 0.20	1.08 ± 0.23	1.12 ± 0.20	1.16 ± 0.25
2% WCPE + 2% BPE	0.98 ± 0.23	1.12 ± 0.25	1.17 ± 0.21	1.22 ± 0.27

Values are mean ± SD of three independent determinations. WCPE, water chestnut peel extract; BPE, banana peel extract.

3.3. Total volatile basic nitrogen

TVB-N increased during refrigerated storage in all treatments, reflecting progressive formation of volatile nitrogenous compounds associated with enzymatic and microbial spoilage. The control increased from 18.22 ± 0.34 at day 0 to 34.41 ± 0.30 at day 21. In contrast, patties containing 2% WCPE + 2% BPE increased from 10.71 ± 0.33 to 24.13 ± 0.41 (Table 3), representing the lowest TVB-N values throughout the storage period. Individual 2% treatments were also generally more effective than the corresponding 1% treatments.

The suppression of TVB-N accumulation suggests that the peel extracts delayed spoilage-related biochemical changes, potentially through combined antioxidant and antimicrobial effects. This interpretation is supported by recent fish-burger research: Šimat et al. (2025) observed significantly lower final TVB-N in burgers containing mixed plant/by-product extracts than in controls however, basil-extract work in refrigerated sea bream patties likewise reported delayed spoilage based on TVB-N. Thus, the present results fit a broader pattern in which botanical extracts can reduce the rate of freshness deterioration in processed fish products.

Table 3. Total volatile basic nitrogen (TVB-N) of WCPE- and BPE-enriched fish patties during refrigerated storage.

Treatment	Day 0	Day 7	Day 14	Day 21
Control	18.22 ± 0.34	24.01 ± 0.34	29.56 ± 0.34	34.41 ± 0.30
1% WCPE	16.23 ± 0.33	21.58 ± 0.33	26.23 ± 0.42	31.23 ± 0.29
2% WCPE	13.12 ± 0.31	18.65 ± 0.35	23.61 ± 0.40	27.54 ± 0.42
1% BPE	14.50 ± 0.32	19.09 ± 0.36	25.91 ± 0.29	32.04 ± 0.41
2% BPE	12.28 ± 0.33	17.41 ± 0.30	22.02 ± 0.35	29.40 ± 0.32
1% WCPE + 1% BPE	12.02 ± 0.30	18.96 ± 0.40	24.64 ± 0.35	30.72 ± 0.28
2% WCPE + 2% BPE	10.71 ± 0.33	14.31 ± 0.43	19.16 ± 0.238	24.13 ± 0.41

Values are mean ± SD of three independent determinations. WCPE, water chestnut peel extract; BPE, banana peel extract.

3.4. DPPH radical-scavenging activity

DPPH radical-scavenging activity declined with increasing storage time, but the magnitude of antioxidant activity depended strongly on formulation. The 2% WCPE + 2% BPE treatment exhibited the highest value at day 0 (57.08 ± 5.23) and retained the highest activity at day 21 (50.17 ± 3.27). By comparison, the control declined from 47.50 ± 5.24 to 42.63 ± 5.24 (Table 4). The combined treatments, particularly at the higher concentration, therefore maintained greater radical-scavenging capacity during storage.

The decline over time likely reflects consumption or degradation of antioxidant constituents as they participate in reactions with reactive species. Nevertheless, the superior values in the combined treatment suggest that incorporation of both extracts increased the pool of radical-scavenging compounds available within the patties. This is consistent with the thesis comparisons to plant-derived antioxidant systems (Yaqoob et al., 2020) and with contemporary research showing that antioxidant-rich fish burgers can retain greater antioxidant capacity and exhibit lower lipid peroxidation during storage.

Table 4. DPPH radical-scavenging activity of WCPE- and BPE-enriched fish patties during refrigerated storage.

Treatment	Day 0	Day 7	Day 14	Day 21
Control	47.50 ± 5.24	45.33 ± 3.24	44.12 ± 5.24	42.63 ± 5.24
1% WCPE	45.42 ± 3.23	43.29 ± 3.23	42.35 ± 5.24	40.46 ± 5.22
2% WCPE	46.34 ± 3.21	44.21 ± 3.25	42.19 ± 5.21	40.09 ± 5.21
1% BPE	48.09 ± 3.22	45.62 ± 3.26	43.11 ± 5.25	41.96 ± 5.26
2% BPE	50.56 ± 4.22	48.06 ± 3.20	46.91 ± 5.22	44.39 ± 5.29
1% WCPE + 1% BPE	54.44 ± 3.20	51.14 ± 3.23	48.87 ± 5.20	46.52 ± 3.25

2% WCPE + 2% BPE	57.08 ± 5.23	54.28 ± 3.25	52.73 ± 5.21	50.17 ± 3.27
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Values are mean ± SD of three independent determinations. WCPE, water chestnut peel extract; BPE, banana peel extract.

3.5. Total phenolic content

TPC decreased during storage in all formulations, but clear differences were observed among treatments. The 2% WCPE + 2% BPE formulation showed the highest TPC throughout the experiment, declining from 141.70 ± 5.23 at day 0 to 129.61 ± 3.27 at day 21. The control decreased from 107.14 ± 5.24 to 98.08 ± 5.24 (Table 5). BPE-containing treatments generally showed higher TPC than the corresponding WCPE-only treatments, while the high-dose combination produced the greatest overall values.

The parallel decline in TPC and DPPH activity during storage supports the interpretation that phenolic constituents contributed materially to antioxidant protection. Phenolics can be progressively oxidized, transformed, or consumed while scavenging radicals, which can reduce measurable TPC and radical-scavenging capacity over time. The persistence of higher TPC in the 2% WCPE + 2% BPE treatment provides a plausible explanation for its comparatively lower TBARS values. Recent studies using combinations of phenolic-rich by-product and plant extracts in fish burgers similarly associate improved oxidative stability with the presence of antioxidant phytochemicals (Šimat et al., 2025).

Table 5. Total phenolic content of WCPE- and BPE-enriched fish patties during refrigerated storage.

Treatment	Day 0	Day 7	Day 14	Day 21
Control	107.14 ± 5.24	105.92 ± 3.24	102.26 ± 5.24	98.08 ± 5.24
1% WCPE	112.06 ± 3.23	107.51 ± 3.23	104.45 ± 5.24	101.67 ± 5.22
2% WCPE	117.42 ± 3.21	112.44 ± 3.25	108.50 ± 5.21	103.89 ± 5.21
1% BPE	124.54 ± 3.22	120.49 ± 3.26	117.61 ± 5.25	114.81 ± 5.26
2% BPE	129.44 ± 4.22	125.54 ± 3.20	121.15 ± 5.22	118.42 ± 5.29
1% WCPE + 1% BPE	109.17 ± 3.20	104.70 ± 3.23	99.11 ± 5.20	95.59 ± 3.25
2% WCPE + 2% BPE	141.70 ± 5.23	137.22 ± 3.25	133.19 ± 5.21	129.61 ± 3.27

Values are mean ± SD of three independent determinations. WCPE, water chestnut peel extract; BPE, banana peel extract.

3.6. Total bacterial count

Total bacterial counts increased with storage time in every formulation, but extract incorporation substantially restricted microbial proliferation. The control increased from 5.39 ± 0.50 log CFU/g at day 0 to 8.56 ± 0.62 log CFU/g at day 21. The 2% WCPE + 2% BPE treatment showed markedly lower counts, increasing from 2.16 ± 0.47 to only 3.96 ± 0.54 log CFU/g (Table 6). The 1% WCPE + 1% BPE treatment also remained comparatively low at day 21 (4.02 ± 0.58 log CFU/g).

The microbial data suggest that combining the extracts provided stronger inhibition than either extract alone. This may reflect the collective action of phenolic and other phytochemical constituents on microbial cells; however, specific antimicrobial mechanisms were not investigated in this experiment and should not be inferred from TBC alone. The finding is nevertheless consistent with recent evidence that plant-derived preservation systems can reduce microbial deterioration in fish products. Šimat et al. (2025) reported lower *Pseudomonas* counts in fish burgers containing selected extract combinations, while using hybridized plant extracts, including banana-peel-containing treatments, also reported broad antimicrobial effects in stored fish. These comparisons support further investigation of WCPE–BPE combinations as preservation ingredients.

Table 6. Total bacterial count (log CFU/g) of WCPE- and BPE-enriched fish patties during refrigerated storage.

Treatment	Day 0	Day 7	Day 14	Day 21
Control	5.39 ± 0.50	6.48 ± 0.49	7.60 ± 0.61	8.56 ± 0.62
1% WCPE	4.02 ± 0.49	5.96 ± 0.51	6.40 ± 0.60	7.23 ± 0.60
2% WCPE	3.56 ± 0.47	4.91 ± 0.54	5.62 ± 0.58	6.54 ± 0.59
1% BPE	2.67 ± 0.48	3.95 ± 0.45	4.28 ± 0.56	5.78 ± 0.60
2% BPE	2.08 ± 0.51	3.62 ± 0.51	4.95 ± 0.55	5.51 ± 0.61
1% WCPE + 1% BPE	2.96 ± 0.45	3.16 ± 0.50	3.85 ± 0.58	4.02 ± 0.58
2% WCPE + 2% BPE	2.16 ± 0.47	2.99 ± 0.48	3.25 ± 0.52	3.96 ± 0.54

Values are mean ± SD of three independent determinations. WCPE, water chestnut peel extract; BPE, banana peel extract.

3.7. Hunter colour characteristics

WCPE and BPE incorporation also affected instrumental colour. Lightness (L^*) generally decreased during storage, whereas redness (a^*) and yellowness (b^*) increased. At day 21, the control had an L^* value of 36.60 ± 0.42, while the 2% WCPE + 2% BPE formulation retained a higher L^* value of 46.02 ± 0.39. The same combined treatment showed the highest day-21 a^* (5.67 ± 0.28) and b^* (4.67 ± 0.28) values. These results demonstrate that botanical extract incorporation altered

the visual characteristics of the patties as well as their chemical stability. Such effects are expected because plant extracts can introduce endogenous pigments and can indirectly influence colour by modifying oxidative reactions. However, basil-extract research in fish patties likewise found measurable changes in L^* , a^* and b^* parameters, although without unacceptable appearance changes. Colour effects should therefore be considered together with sensory responses when optimizing extract concentration.

3.8. Sensory properties

Sensory evaluation revealed a clear trade-off between preservation efficacy and palatability. The untreated control received the highest scores for appearance (9.39 ± 0.87), taste (8.24 ± 0.79), flavour (8.40 ± 0.82), and texture (9.02 ± 0.80). Among extract-treated samples, 1% WCPE retained comparatively high scores for appearance (8.45 ± 0.83), taste (7.81 ± 0.71), odour (7.05 ± 0.60), flavour (7.13 ± 0.80), and texture (7.78 ± 0.79). In contrast, combined and higher-concentration treatments generally produced lower appearance and taste scores (Table 7). The 2% WCPE + 2% BPE treatment, despite showing the strongest overall chemical and microbiological preservation, recorded appearance and taste scores of 4.49 ± 0.60 and 3.13 ± 0.60 , respectively.

This finding is important for practical formulation: maximizing antioxidant and antimicrobial effects alone does not identify the most acceptable product. Phenolic-rich extracts can contribute bitterness, astringency, colour changes, or characteristic plant flavours at higher concentrations. Recent fish-product studies similarly emphasize that botanical preservation strategies must be optimized against sensory performance rather than selected solely on analytical shelf-life indices. Thus, although 2% WCPE + 2% BPE was the strongest preservation treatment, lower extract concentrations—particularly 1% WCPE—may offer a more favourable balance between quality protection and sensory acceptance.

Table 7. Sensory scores of WCPE- and BPE-enriched fish patties.

Treatment	Appearance	Taste	Odour	Flavour	Texture
Control	9.39 ± 0.87	8.24 ± 0.79	7.28 ± 0.61	8.40 ± 0.82	9.02 ± 0.80
1% WCPE	8.45 ± 0.83	7.81 ± 0.71	7.05 ± 0.60	7.13 ± 0.80	7.78 ± 0.79
2% WCPE	7.38 ± 0.47	6.29 ± 0.70	5.88 ± 0.64	7.02 ± 0.79	6.34 ± 0.80
1% BPE	6.09 ± 0.85	4.85 ± 0.68	6.19 ± 0.59	5.85 ± 0.77	7.30 ± 0.77
2% BPE	4.99 ± 0.80	3.56 ± 0.69	5.59 ± 0.60	5.05 ± 0.78	6.77 ± 0.79
1% WCPE + 1% BPE	3.62 ± 0.77	2.51 ± 0.61	5.13 ± 0.62	4.65 ± 0.64	6.19 ± 0.78
2% WCPE + 2% BPE	4.49 ± 0.60	3.13 ± 0.60	6.11 ± 0.65	5.29 ± 0.60	6.55 ± 0.76

Values are mean $\pm$ SD. WCPE, water chestnut peel extract; BPE, banana peel extract.

Conclusion

The incorporation of water chestnut peel extract (WCPE) and banana peel extract (BPE) improved the oxidative, antioxidant, and microbiological stability of fish patties during 21 days of refrigerated storage. Among the tested formulations, 2% WCPE + 2% BPE provided the strongest overall preservation effect, showing the lowest TBARS, TVB-N, and total bacterial counts, together with greater retention of DPPH radical-scavenging activity and total phenolic content. Peroxide values were also influenced by extract incorporation, although the response was treatment-dependent and did not follow the same pattern as the other quality indices. However, higher extract concentrations negatively affected several sensory attributes, demonstrating a clear trade-off between preservation efficacy and sensory acceptability. Although the combined 2% WCPE + 2% BPE treatment was most effective overall in controlling quality deterioration, lower concentrations may provide a more suitable balance between storage stability and sensory quality. Overall, water chestnut and banana peels represent promising underutilized sources of natural antioxidant and antimicrobial ingredients for value-added fish products. Further studies should focus on optimizing extract concentrations and evaluating consumer acceptance, storage stability, and performance under commercial processing and packaging conditions.

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