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Research Article

From waste to shelf life: Olive pomace extract as a natural preservative for mangoes

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Abstract

The escalating challenge of agricultural and industrial waste, exemplified by olive pomace (OP), presents a unique opportunity to recover valuable bioactive compounds. This study pioneers a sustainable solution by transforming OP extract (OPE) into a potent natural preservative for mangoes. Our findings reveal that OPE exhibits exceptional antioxidant prowess (92% DPPH scavenging) and robust antimicrobial activity against foodborne pathogens. To translate these properties, we developed Arabic gum (AG) coatings enriched with OPE and applied them to mangoes. Over a 45-day storage period, these bio-integrated coatings significantly preserved fruit quality by retarding ripening, minimizing weight loss, and inhibiting decay. Furthermore, OPE not only fortified the antioxidant defence system of the mangoes but also demonstrated anti-inflammatory potential by suppressing pro-inflammatory cytokines (IL-6 and TNF-α) in the fruit rinds. This research underscores the compelling utility of OPE as a natural, multifunctional agent for enhancing mango shelf life, nutritional value, and potentially offering added health benefits. Future investigations will delve into the precise mechanisms of action and optimize OPE-based formulations for wider applications across diverse fruits and vegetables.

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Keywords: agricultural waste; antioxidant; antitumor; food preservation; natural preservative; olive pomace; postharvest treatment; shelf life extension

Introduction

Global population is projected to grow significantly, reaching an estimated 9.1 billion by 2050, up from 7.6 billion in 2011 (Nations, 2017). This necessitates a corresponding increase in food production, placing pressure on agricultural sustainability (Penuelas *et al.*, 2023). Sustainable agriculture offers a solution by meeting current food demands while minimizing environmental impact and preserving resources for future generations (Boix-Fayos and de Vente, 2023). Conventional agricultural practices, reliant on heavy use of chemical fertilizers and pesticides, are increasingly concerning due to their potential harm to human health and the environment (Çakmakçı *et al.*, 2023). Changes towards sustainable agricultural practices are crucial, as these methods aim to balance food security with environmental protection through techniques like crop rotation, integrated pest management, and soil conservation (Jararweh *et al.*, 2023).

The olive oil industry is a growing sector, generating a substantial byproduct: olive pomace (OP). While rich in bioactive phenolics, OP poses disposal challenges. These same phenolics, however, offer potential health benefits and represent a valuable resource for the food and cosmetics industries (Sun and Shahrajabian, 2023). Extracting these compounds with eco-friendly technologies is crucial for maximizing yield, minimizing costs, and reducing environmental impact (Sorrenti *et al.*, 2023). Utilizing OP as a natural ingredient aligns with circular economy principles by minimizing waste and promoting resource efficiency. Transforming OP into a valuable resource reduces the environmental footprint of olive oil production and creates economic opportunities. Bioactive compounds from OP have diverse applications in food production, acting as antioxidants, flavor enhancers, or preservatives, ultimately contributing to healthier and more sustainable food products (Martins *et al.*, 2024).

Postharvest losses significantly impact fresh produce export potential and economic security. Citrus fruits, valuable for their beneficial compounds, can experience decreased bioactive activity during cold storage, leading to food waste (Ehlers, 2016). Similarly, mangoes, despite their relatively long shelf life, are susceptible to spoilage caused by microbial growth. Traditional approaches using synthetic fungicides pose health and environmental risks. Natural edible coatings, such as Arabic gum (AG), offer a sustainable alternative to synthetic waxes. However, AG exhibits limited water vapor barrier properties, potentially reducing shelf life (Kotiyal and Singh, 2023).

The reliance on chemical fungicides like imazalil and thiabendazole in traditional approaches to postharvest disease control presents a significant challenge because they pose risks to human health and the environment (Rani *et al.*, 2021). Applying waxes on citrus fruits successfully inhibits water loss and gas exchange, enhancing the fruits' shelf life and maintaining their quality. Traditionally, these synthetic waxes served as carriers for synthetic fungicides that have been banned globally due to their detrimental effects on human and the environment (Rani *et al.*, 2021). To prolong the shelf life of fruits, a variety of natural edible coatings, i.e., carnauba wax, chitosan, CMC, and beeswax, promise a sustainable alternative to synthetic waxes.

Natural polysaccharides, i.e., Arabic gum extracted from acacia trees, possess a unique molecular structure rich in hydrogen bonds, which enables it to form a tight barrier that restricts both the passage of O_2 and CO_2, thus increasing the shelf life and maintaining the quality of fruits (Manzoor *et al.*, 2022). However, despite its merits, Arabic gum exhibits limited ability to block water vapor transmission. This property can potentially lead to moisture loss and reduced shelf life of coated fruits (Kocira *et al.*, 2021).

To enhance the shelf-life of strawberries during cold storage Khalifa *et al.* (2016) coated a chitosan-based edible covering with olive pomace-derived polyphenolic compounds. The strawberries coated with various formulas containing olive pomace were compared to commercial wax coating treatments. The effect of the coating on AsA, antioxidant content, and MDA levels was evaluated. Incorporating olive pomace extracts promoted an accumulation of phytogenic compounds and enhanced antioxidant status, effectively reducing

MDA levels and decay area compared to control fruits. Sar *et al.* (2023) also determined the antimicrobial potential of OPE in an edible chitosan coat for apple and strawberry fruits, comparing their inhibitory efficacy on selected microorganisms to those of olive leaf extract. While both extracts exhibited antimicrobial properties, olive leaf extract demonstrated more potent antimicrobial activity against foodborne pathogens. These findings indicate the potential of incorporating these extracts into edible coatings as a beneficial method for preserving the quality of fruits and prolonging their shelf life (Pham *et al.*, 2023).

While Arabic gum (AG) is a well-established fruit coating, its synergistic potential with olive pomace extract (OPE) for enhanced postharvest preservation is a significant unexplored area. Crucially, no prior research has documented the impact of incorporating OPE within an AG coating on the shelf life and quality of mangoes. This study addresses this critical gap by rigorously testing the hypothesis that combining OPE with AG will synergistically extend mango shelf life and maintain quality attributes during storage. To achieve this, we first comprehensively characterize OPE's phenolic profile, antimicrobial, and antioxidant properties. We then evaluate the efficacy of OPE-enriched AG coatings in preserving mango quality during storage, providing novel insights into this promising sustainable approach.

Materials and Methods

Aqueous extraction of olive pomace extract (OPE)

Olive pomace was dried and ground into fine particles to enhance solvent penetration during extraction. Ten grams of OPE were mixed with 150 mL of deionized water (dH_2O) and stirred for 2 hours. The mixture was then centrifuged at 4000×g for 20 minutes. The supernatant (approximately 100 mL) was collected and stored for further analysis.

Determination of phenolic compounds in OPE

The OPE extract was filtered through a 0.2-µm filter to remove particulates. A 1-3 mL aliquot of the filtered extract was analyzed using a Shimadzu HPLC system (LC-10AS, Japan) equipped with a C18 column (50 x 2.1 mm, 3 µm), autosampler, quaternary pump, and solvent degasser. The mobile phase, an aqueous acetonitrile gradient, was delivered at a flow rate of 1 mL/min, with a linear gradient from 5% to 60% acetonitrile. Phenolic compounds were identified and quantified using a triple-quadrupole mass spectrometer (MS/MS) with electrospray ionization (ESI) in negative ion mode. MS/MS analysis, with a collision energy of 35%, provided detailed structural information based on the fragmentation patterns of parent ions. A mass scanning range of 100-1500 m/z was employed to capture the m/z values of all ionized compounds.

Biological activity of OPE

Antioxidant activity

The antioxidant activity of OPE was evaluated using the DPPH radical scavenging assay (Jia *et al.*, 2012). Various concentrations of OPE (50-250 µg mL^{-1}) were incubated with a DPPH solution for 30 minutes at room temperature in the dark. The absorbance of the resulting mixture was measured at 517 nm. The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\% \text{ Scavenging activity} = [(Absc - Abs) / Absc] \times 100 \quad (1)$$

where: Absc: Absorbance of the control. Abs: Absorbance of the sample

Antimicrobial activity

The antimicrobial activity of OPE was assessed using the disc diffusion method (Singh *et al.*, 2016) , A McFarland 0.5 standard solution was prepared to obtain a bacterial or fungal cell density of approximately 1.5 × 10^8 CFU/mL. 200 µL of each microbial culture (*L. monocytogenes*, *S. aureus*, *C. jejuni*, *S. typhi*, *C. glabrata*, *C. rugosa*, *and C. stellata*) was spread on nutrient agar or potato dextrose agar plates. 6 mm sterile filter paper

discs impregnated with various concentrations of 10 µL OPE (50, 100, 150, 200, and 250 µg mL^{-1}) were placed on the inoculated plates. The plates were incubated at 37 °C for 24 hours. The antimicrobial activity was determined by measuring the diameter of the inhibition zones around the discs and comparing them to a chloramphenicol control.

Preparation of functional AG-OPE coating

Preparation of Arabic gum (AG) solution

Arabic gum (AG) dispersions (0.4%, 0.8%, and 1.6% w/v) were prepared in deionized water. The AG was dissolved at 50 °C for 1 hour with constant stirring. Glycerol (30%) was added to the solution to enhance film flexibility. The pH of the solution was adjusted to 9 using sodium hydroxide.

Preparation of AG-OPE composite coating

To prepare the AG-OPE composite coating, an 0.8% (w/w) AG solution was prepared as described in the previous section. Olive pomace extract (OPE) solutions (1.5% and 2.5% w/w) were gradually added to the AG solution under constant stirring and sonicated for 20 minutes to ensure uniform dispersion of the OPE within the AG matrix. The resulting AG-OPE composite coating was stored for further use.

Coating experiment and physiochemical analysis of mango fruits

Fruit selection and preparation

Mango fruits were carefully selected based on their uniformity in size, color, and absence of defects. The fruits were disinfected by immersing them in a 0.04% sodium hypochlorite (NaClO) solution for 2 minutes, followed by air-drying at room temperature.

Coating application

Four groups of mango fruits were established: a control group (dipped in water) and three treatment groups. The treatment groups were dipped in either the AG solution (0.8%) or the AG-OPE composite coating solutions (1.5% and 2.5%) for 3 minutes. After dipping, the fruits were air-dried at room temperature and stored under identical conditions.

Physiochemical analysis

To analyze the physiochemical properties of the treated and untreated mango fruits, 10 mL of juice was extracted from each fruit and homogenized. The homogenate was centrifuged to separate the liquid phase. The pH of the clarified juice was measured using a pH meter. Titratable acidity (TTA) was determined as citric acid equivalent using the AOAC standard method 942.15. Total soluble solids (TSS) were measured using a refractometer.

Antioxidant activity

The total phenolic content (TPC) and total flavonoid content (TFC) of the olive pomace extract (OPE) were determined using the Folin-Ciocalteu and aluminum chloride colorimetric assays, respectively. Gallic acid and quercetin were used as standards for TPC and TFC quantification (Singleton and Rossi, 1965; Chang *et al.*, 2002). The free radical scavenging activity of the mango fruit juice was assessed using the DPPH radical scavenging assay, as described in section 2.3.1. Malondialdehyde (MDA) content, a marker of lipid peroxidation, was measured using the thiobarbituric acid (TBA) assay.

Antioxidant enzymes activities

Catalase activity

Catalase (CAT) activity was determined by measuring the decomposition of hydrogen peroxide. Mango rind tissue was homogenized in a phosphate buffer solution containing urea. The reaction mixture containing the enzyme extract, hydrogen peroxide, and phosphate buffer was incubated, and the decrease in absorbance at 240 nm was monitored spectrophotometrically (He *et al.*, 2020). Catalase activity was expressed as units per milligram of protein.

Peroxidase activity

Peroxidase (POD) activity was measured by monitoring the oxidation of guaiacol in the presence of hydrogen peroxide. Mango rind tissue was homogenized in a phosphate buffer solution containing EDTA and PVPP. The reaction mixture containing the enzyme extract, guaiacol, and hydrogen peroxide was incubated, and the increase in absorbance at 470 nm was measured spectrophotometrically (Hussein *et al.*, 2019). POD activity was expressed as units per milligram of protein.

Superoxide Dismutase (SOD) activity

Superoxide dismutase (SOD) activity was assessed by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture containing the enzyme extract, NBT, methionine, and riboflavin was exposed to light, and the formation of the coloured formazan product was monitored at 560 nm. SOD activity was expressed as units per milligram of protein (He *et al.*, 2020).

Anti-inflammation activity

The anti-inflammatory activity of the mango rind extract was evaluated by measuring the levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) using an enzyme-linked immunosorbent assay (ELISA). Fresh mango rind tissue was homogenized in a phosphate buffer solution, and the extract was used to treat cells. The levels of TNF-α and IL-6 in the cell culture supernatant were quantified using a commercial ELISA kit.

Statistical analysis

One-way analysis of variance (ANOVA) followed by a post-hoc test, such as Fisher's Least Significant Difference (LSD) test, was performed to identify significant differences ($p < 0.05$) between the mean values of the data using SPSS 23.0 software.

Results

Polyphenols profile of olive pomace aqueous extract

Liquid chromatography-mass spectrometry (LC-MS) analysis in negative ion mode was employed to identify and quantify phenolic compounds within the olive pomace aqueous extract (OPE). Phenolic acids constituted the most abundant class of compounds, comprising 61% of the total phenolic content. Flavonoids followed, accounting for 26%, while other phenolic compounds, such as lignans, made up the remaining 12%. Caffeic acid emerged as the predominant phenolic acid, with a concentration of 63.6 mg g^{-1}, followed by chlorogenic acid (19.5 mg g^{-1}) and p-coumaric acid (20.3 mg g^{-1}). The identity of caffeic acid was confirmed by accurate mass measurement using a Q-TOF mass spectrometer, which yielded a molecular ion at m/z 179 and a characteristic fragment ion at m/z 135. The obtained mass spectrum was matched against the METLIN database for further verification. Luteolin was the primary flavonoid, detected at m/z 267 with a fragment ion at m/z 132. Verbascoside was also identified, exhibiting a molecular ion at m/z 623. Hydroxytyrosol (6.9 mg g-

[1]) and tyrosol (9.2 mg g^{-1}) were the major simple phenols, characterized by molecular ions at m/z 139 and 199, respectively. Q-TOF mass spectrometry was used to confirm the identity of these compounds.

Antioxidant activity of OPE

The substantial content of phenolic compounds, including flavonoids and phenolic acids, identified in OPE (Table 1) likely contributes to its pronounced antioxidant activity.

Table 1. Polyphenolic profile of olive pomace aqueous extract

Polyphenols		Content (mg g^{-1})	*m/z*	FI
Phenolic compounds	Hydroxytyrosol	6.9	139	111
	Tyrosol	9.2	199	119
	1-acetoxypinoresinol	2.3	416	204
	Pinoresinol	3.5	364	175
	Oleuropein	2.5	376	156
Flavonoids	Apigenin	6.5	284	133
	Rutin	5.6	178	112
	Luteolin	15.3	267	132
	Apigenin-7-Oglucoside	9.2	449	193
	Luteolin-7-*O*-glucoside	6.3	593	387
	Verbascoside	10.3	623	477
Phenolic acids	Chlorogenic	19.5	353	163
	(*E*)-Cinnamic	12.6	147	106
	Ferulic	3.9	193	150
	p-Coumaric	20.3	162	120
	Caffeic	63.6	179	135
	Syringic	5.8	483	186

All identifications in Table 1 were confirmed similarly

At a concentration of 250 µg/mL, OPE demonstrated a higher DPPH free radical scavenging capacity (92%) compared to the well-known antioxidant ascorbic acid (AsA). A concentration-dependent increase in the antioxidant activity of OPE was observed in the DPPH assay (Figure 1). As illustrated in Figure 1, a minimum concentration of 100 µg mL^{-1} of OPE was required to scavenge 50% of DPPH free radicals (IC50 = 100 µg mL^{-1}).

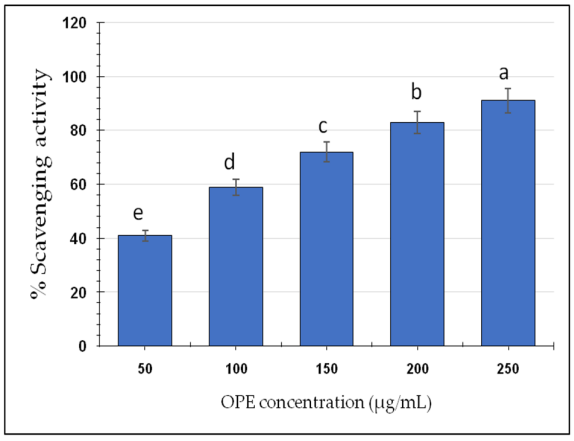

Figure 1. % DPPH radicals scavenging activity of olive pomace aqueous extract, IC50=100 µg mL^{-1}
a-e letters above the column indicate significant differences at $p < 0.05$

Antimicrobial activity of OPE

Figure 2 illustrates the varied susceptibility of the tested microorganisms to the antibacterial properties of OPE. Inhibition zone diameters (IZDs) displayed a concentration-dependent effect, ranging from 10 to 32 mm for bacteria and 11 to 29 mm for *Candida* species. *Staphylococcus aureus* (SA) exhibited the greatest susceptibility to OPE at higher concentrations, with an IZD of 32 mm. Conversely, *Clostridium jejuni* (CJ) demonstrated the strongest resistance, with an IZD of only 25 mm. Within the *Candida* species, *Candida gelberta* displayed the highest resistance to OPE (IZD of 23 mm), followed by *Candida rugosa* (CR) (IZD of 26 mm). The minimum inhibitory concentration (MIC) of OPE against the tested microorganisms ranged from 25 to 45 μg mL^{-1}.

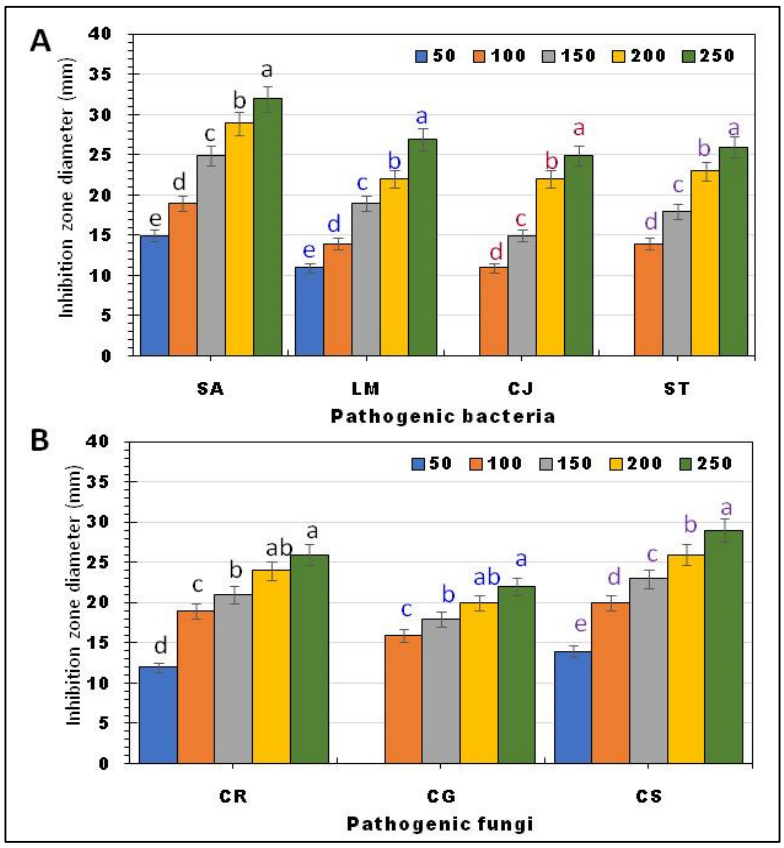

Figure 2. Antimicrobial activity of olive pomace extract against tested pathogenic microorganisms. *L. monocytogenes* (LM), *S. aureus* (SA), *C. jejuni* (CJ), *S.typhi* (ST), *C. glabrata* (CG), *C. rugosa* (CR), and *C. stellata* (CS)

a-e letters above the column indicate significant differences at $p < 0.05$

Effect of coating on physicochemical properties and antioxidant status of mangoes during cold storage

Physicochemical changes

Figure 3 illustrates the influence of storage duration and coating treatment on the physicochemical properties of mangoes. Total soluble solids (TSS) content exhibited a significant ($p < 0.05$) increase over the storage period, indicative of fruit ripening. Uncoated control mangoes displayed an initial TSS of 11.9%, which progressively increased to 14.1% by day 45. AG-OPE 2.5% coated fruits followed a similar trend, with TSS levels rising from 11.6% on day 15 to 12.9% on day 45. In contrast, titratable acidity (TA) decreased throughout the storage period for coated fruits. The reduction in TA was more pronounced in AG-OPE 2.5%

coated fruits (41%) compared to controls. This decrease may be attributed to a reduced respiration rate caused by the coating, leading to lower acidity. Uncoated mangoes, experiencing higher acidity levels due to the concentration of organic acids.

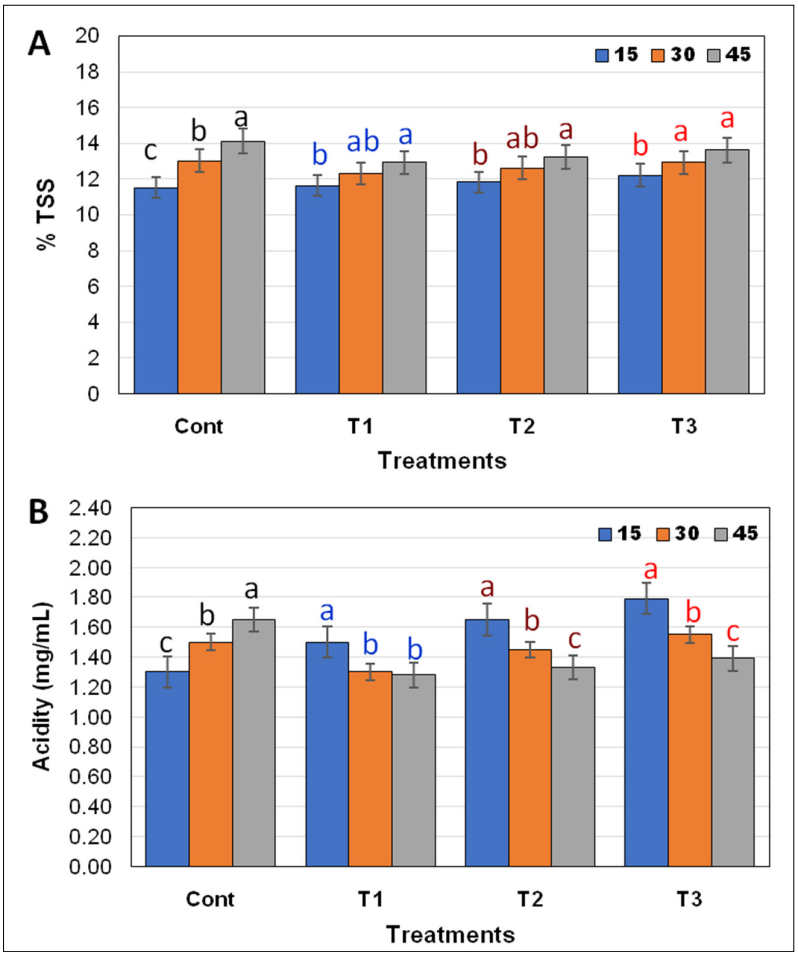

Figure 3. Fluctuation in total soluble solids (TSS) and acidity of mango fruit during 45-day storage at 4°C under coating treatments of T1, Arabic gum coating; T2, Arabic gum-OPE 1.5% coating; T3, Arabic gum-OPE 2.5% coating.

a-c letters above the column indicate significant differences at $p < 0.05$

Antioxidant profile

Figure 4 illustrates the significant impact ($p < 0.05$) of coating treatment and storage duration on the antioxidant content and activity of mango fruits. Total phenolic compounds (TPC) and flavonoid concentrations generally decreased during storage. However, the incorporation of 2.5% olive pomace into the coating (AG-OPE 2.5%) effectively mitigated this decline. By the end of the storage period (Fig. 5A), both coated and uncoated fruits experienced a 21-50% reduction in TPC. Notably, AG-OPE 2.5% coated fruits retained 60% of their initial TPC at day 45 compared to the control. Similarly, the flavonoid content of coated and uncoated fruits decreased by 27-38% by the end of storage. Nevertheless, coated fruits retained 68% of their flavonoids compared to the control (Figure 4B).

Figure 4C highlights the significant impact ($p < 0.05$) of various coatings and storage durations on the antioxidant stability of mango fruits. The superior antioxidant activity observed in AG-OPE 2.5% coated fruits, demonstrated by their ability to scavenge 92% of DPPH free radicals compared to 75% for uncoated fruits, is likely attributable to their higher phenolic and flavonoid content. By the end of the storage period, the

DPPH radical scavenging activity (%AA) decreased to 49% for uncoated fruits and 75% for AG-OPE 2.5% coated fruits. Regarding malondialdehyde (MDA) content, coated fruits exhibited an 88% reduction compared to uncoated fruits at the end of storage.

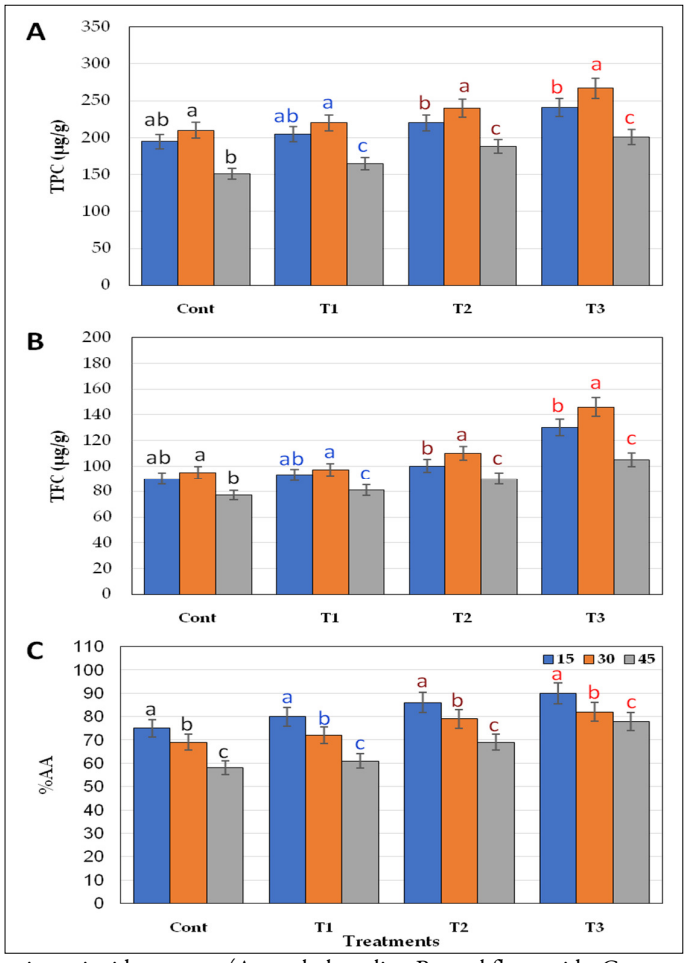

Figure 4. Changes in antioxidant status (A, total phenolics; B, total flavonoids; C, scavenging activity) of mango fruits affected with different coating treatmentsT1, Arabic gum coating; T2, Arabic gum-OPE 1.5% coating; T3, Arabic gum-OPE 2.5% coating. a-c letters above the column indicate significant differences at $p < 0.05$.

Antioxidant enzyme activity

The activity of the antioxidant enzyme superoxide dismutase (SOD) declined with increasing storage duration. However, coated fruits maintained higher SOD activity, with AG-OPE 2.5% coated fruits exhibiting the highest activity after 45 days of storage, reaching 15.1 U /mL/min, a 29% increase compared to uncoated fruits (Table 2).

Peroxidase activity followed a biphasic pattern, initially increasing during the first 30 days of storage and subsequently decreasing to normal levels. AG-OPE 1.5% coated fruits displayed the highest peroxidase activity at 30 days, reaching 20 U/mL/min, a 33% increase over uncoated fruits. This suggests that olive pomace extract (OPE) incorporated into the coating may stimulate peroxidase activity.

Catalase activity decreased over the storage period, as shown in Table 2. In mango fruits coated with AG-OPE 1.5%, CAT activity peaked at 21.6 U/mL/min after 30 days of storage, compared to the control sample (10-16 U/mL/min). Coating treatments (T1 and T2) increased CAT activity (Table 2).

Table 2. Changes in antioxidant enzymes activity of mango rind affected with different coating treatments during 45 storage periods at 4 °C

Storage (d)	Antioxidant enzymes (U/mL/min) / Treatments			
	Cont	T1	T2	T3
SOD				
0	24	23.9	24	23.9
15	17b	17.5b	18.6a	18a
30	15b	16ab	17a	16.8ab
45	11d	13.2c	15.5a	14.2b
POD				
0	13.5b	13.8ab	14.2a	14a
15	16c	17bc	18.3a	17a
30	15d	18b	20.1a	16.2c
45	14b	16a	16.8a	15b
CAT				
0	33a	32.8b	33.3a	33.4a
15	24c	26b	28.1ab	30a
30	16c	17.2b	21.6ab	23a
45	14b	14.5b	17ab	19a

T1, Arabic gum coating; T2, Arabic gum-OPE 1.5% coating; T3, Arabic gum-OPE 2.5% coating. a-c letters above the column indicate significant differences at $p < 0.05$

Regulation of proinflammatory cytokine expression

Proinflammatory cytokines are small signaling molecules primarily produced by activated immune cells, such as macrophages and T lymphocytes. These molecules play a crucial role in initiating and orchestrating the inflammatory response. They function as intercellular messengers, binding to specific receptors on target cells and triggering downstream signaling pathways that activate various immune effector mechanisms.

Table 3 presents the effects of different coating treatments and storage durations on the expression of proinflammatory cytokines in mango rind. Notably, the addition of 2.5% OPE to the arabic gum (AG) coating significantly downregulated the expression of proinflammatory cytokines, with reductions of 30% for tumor necrosis factor-alpha (TNF-α) and 42% for interleukin-6 (IL-6).

Table 3. Expression of proinflammatory cytokines in mango's rind-affected with different coating treatments after 45 storage periods at 4 °C

Treatment	TNF-α	IL-6
Cont	32.9a	20.5a
T1	31.6ab	19.6ab
T2	30.5b	18.5b
T3	25.8c	14.3c

T1, Arabic gum coating; T2, Arabic gum-OPE 1.5% coating; T3, Arabic gum-OPE 2.5% coating. a-c letters above the column indicate significant differences at $p < 0.05$

Discussion

The food industry generates significant byproducts and waste streams, posing environmental and economic challenges (Goldsmith *et al.*, 2018). Researchers are increasingly focusing on these by-products due

to their potential as renewable resources. Olive pomace (OP), the solid residue remaining after olive oil extraction, is a prime example (Difonzo *et al.*, 2019).

Olive pomace remains a significant agricultural and industrial source of phenolic compounds because only 2% of the polyphenols present in the olive fruit are converted into olive oil, leaving the remaining 98% in olive oil byproducts (Ribeiro *et al.*, 2020). Approximately 45% of the total phenolics in the olive fruit are retained in OP. The dried olive pomace contains approximately 12-15 g/100g of residual oils (Cravotto *et al.*, 2022). Consequently, solvent extraction retrieves the olive pomace oil (Chanioti and Tzia, 2019).

Liquid chromatography-mass spectrometry (LC-MS) analysis revealed a diverse phenolic profile within the olive pomace aqueous extract (OPE). Phenolic acids were the most abundant, with caffeic acid, chlorogenic acid, and p-coumaric acid as the major constituents. Flavonoids, such as luteolin, and simple phenols, including hydroxytyrosol and tyrosol, were also identified. The accurate mass measurements obtained from Q-TOF mass spectrometry confirmed the identities of these compounds, further validating the LC-MS analysis. These findings highlight the potential of olive pomace as a rich source of bioactive phenolic compounds with various health benefits. This aligns with previous researchs highlighting the potential of olive pomace as a valuable source of bioactive compounds, particularly phenolic compounds, fatty acids, and vitamin E (Difonzo *et al.*, 2021; Quero *et al.*, 2022).

The demonstrable and concentration-dependent scavenging of DPPH radicals by OPE, characterized by an impressive IC50 value of 100 μg mL^{-1}, unequivocally establishes its potent antioxidant capacity. This activity notably surpasses that of the widely recognized standard, ascorbic acid. This superior performance is highly likely attributable to the intricate interplay of OPE's diverse and abundant phenolic compounds, including but not limited to various flavonoids and phenolic acids. These findings strongly position OPE as a promising natural reservoir of bioactive antioxidants, suggesting significant potential for its application in health promotion and disease prevention strategies. (Quero *et al.*, 2022). The reported IC50 value of 100 μg mL^{-1} for OPE's DPPH radical scavenging activity is a strong indicator of its potent antioxidant properties. Pikuli and Devolli (2024) investigated olive mill pomace extracts and reported significant antioxidant activity (80-105% antiradical activity) using an ethanol:water (80:20) solvent system, attributing this to high total polyphenol content (0.7-0.8 g gallic acid equivalent/L extract). While an exact IC50 isn't given for direct comparison, their findings corroborate the rich phenolic content and high antioxidant capacity of OPE. Azeez *et al.* (2017) analyzed olive-waste cakes from different varieties and found that Picual variety had the highest free phenolic and flavonoid content, showing the highest antioxidant capacity. This supports the idea that the specific composition of phenolic compounds influences the overall antioxidant effect.

Figure 2 provides a clear illustration of the differential susceptibility of various microorganisms to the antibacterial properties of OPE. A notable concentration-dependent trend is observed, with inhibition zone diameters (IZDs) increasing with higher OPE concentrations. Among the tested bacteria, Staphylococcus aureus (SA) exhibited the highest susceptibility, displaying an IZD of 32 mm at the maximum concentration. Conversely, *Clostridium jejuni* (CJ) demonstrated the strongest resistance, with an IZD of 25 mm. Similarly, within the *Candida* species, *Candida gelberta* showed the highest resistance (IZD of 23 mm), while *Candida rugosa* (CR) exhibited moderate susceptibility (IZD of 26 mm). The minimum inhibitory concentration (MIC) values, ranging from 25 to 45 μg/mL, further support the observed susceptibility patterns. Gołębiewska *et al.* (2022) reported that olive leaf extract showed an IZD of 15 mm against *S. aureus* at a concentration of 25 mg/mL (25,000 μg mL^{-1}), significantly higher than the concentrations implied by the MIC values given in the prompt (25-45 μg mL^{-1}). This further underscores the high efficacy reported for OPE in this text.

Figure 3 demonstrates the impact of storage duration and coating treatment on the physicochemical properties of mangoes. Over the storage period, a significant ($p < 0.05$) increase in total soluble solids (TSS) content was observed, a clear indication of fruit ripening. Uncoated control mangoes exhibited an initial TSS of 11.9%, which gradually increased to 14.1% by day 45. AG-OPE 2.5% coated fruits followed a similar trend, with TSS levels rising from 11.6% on day 15 to 12.9% on day 45. Conversely, titratable acidity (TA) decreased for both coated fruits during storage. However, the reduction in TA was more pronounced in AG-OPE 2.5%

coated fruits (41%) compared to controls. This decrease is likely due to a reduced respiration rate caused by the coating, leading to slower organic acid degradation. Uncoated mangoes, experiencing higher fruit hydration loss, likely maintained higher acidity levels due to the concentration of organic acids. The general trend of increasing total soluble solids (TSS) and decreasing titratable acidity (TA) during the ripening and storage of climacteric fruits like mangoes is well-established in postharvest physiology. This phenomenon is primarily attributed to the enzymatic conversion of starches into sugars (leading to increased TSS) and the consumption of organic acids during respiration (resulting in decreased TA). Priyadarsani and Kar (2022) observed this pattern in a study on the natural ripening of mangoes, noting that the TSS of mango fruits increased with progressive ripening over 11 days at various temperatures, while firmness simultaneously decreased. This rise in TSS was specifically attributed to enzymatic activities facilitating the conversion of starch to sugars. Similarly, Hossain *et al.* (2014) demonstrated that in Ashwina hybrid mangoes, titratable acidity gradually decreased with extended storage time across different temperatures, concurrent with a gradual increase in total soluble solid content. Both studies provide strong evidence for these common ripening patterns.

The antioxidant profile of mango fruits was significantly influenced by coating treatments and storage duration. Total phenolic compounds (TPC) and flavonoid concentrations decreased during storage, but the AG-OPE 2.5% coating effectively mitigated this decline, preserving 60% of initial TPC and 68% of flavonoids at the end of storage. This coating also significantly enhanced antioxidant activity, as measured by DPPH radical scavenging capacity, reaching 92% compared to 75% for uncoated fruits. Additionally, the coating reduced malondialdehyde (MDA) content by 88% compared to uncoated fruits.

Regarding antioxidant enzymes, superoxide dismutase (SOD) activity declined with storage, but coated fruits maintained higher levels, with AG-OPE 2.5% coated fruits exhibiting the highest activity. Peroxidase activity initially increased and then decreased, with AG-OPE 1.5% coated fruits showing the highest peak activity. Catalase activity also decreased during storage, but coated fruits, particularly those treated with AG-OPE 1.5%, exhibited higher activity compared to uncoated fruits. These findings suggest that olive pomace extract-incorporated coatings can effectively enhance the antioxidant profile and enzyme activity of mango fruits, thereby extending their shelf life.

The addition of olive pomace extract (OPE) to Arabic gum (AG) coatings has been shown to be an effective strategy for reducing the expression of proinflammatory cytokines in mango rind. By incorporating OPE into the AG coating, a significant downregulation of TNF-α and IL-6 was observed, indicating a reduction in the inflammatory response. This reduction in proinflammatory cytokine expression is likely due to OPE's ability to mitigate the negative effects of chemical and microbial fluctuations that occur during prolonged storage. These findings suggest that OPE-treated AG coatings have the potential to improve the quality and shelf life of mangoes by reducing inflammation and oxidative stress.

Conclusions

In conclusion, this study demonstrates the significant potential of olive pomace extract (OPE) as a natural preservative for mangoes. OPE's strong antioxidant and antimicrobial activities suggest its diverse bioactivity. Postharvest treatment of mangoes with AG-OPE coatings effectively slowed ripening, reduced weight loss and decay, and enhanced the fruit's antioxidant capacity. Furthermore, the observed downregulation of pro-inflammatory cytokines suggests potential health benefits. These findings support the use of OPE as a viable and effective natural preservative for extending shelf life and improving the nutritional value of mangoes, offering a promising alternative to synthetic preservatives. Future research should focus on elucidating the specific mechanisms of action and optimizing OPE-based coating formulations for broader application in preserving various fruits and vegetables.

Authorship Contribution

Conceptualization: TKK, MAl-M, MOA, WA, HE, BO, GSE, TME, AS; Methodology: TKK, MAl-M, MOA, WA, HE, BO, GSE, JMA, TME, AS; Software: TKK, MAl-M, MOA, WA, HE, BO, GSE, TME, HIG, AS; Validation: TKK, MAl-M, MOA, WA, HE, BO, GSE, TME, JMA, HIG, AS; Formal analysis: TKK, MAl-M, MOA, WA, HE, BO, GSE, TME, AS; Investigation: TKK, MAl-M, MOA, WA, HE, BO, GSE, TME, AS; Resources: TKK, MAl-M, MOA, WA, HE, BO, GSE, TME, JMA, HIG, AS; Data curation: TKK, MAl-M, MOA, WA, HE, BO, GSE, TME, HIG, AS; Writing-original draft preparation: TKK, MAl-M, MOA, WA, HE, BO, GSE, JMA, TME, AS; Writing-review and editing: TKK, MAl-M, MOA, WA, HE, BO, GSE, TME, JMA, HIG, AS; Visualization: TKK, MAl-M, MOA, WA, HE, BO, GSE, TME, JMA, AS; Supervision: TKK, MAl-M, AS; Project administration: TKMK, MAl-M, AS; Funding acquisition: MAl-M, HIG, AS

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Conflicts of Interest

The authors declare no conflict of interest.

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