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CLEAN-LABEL PRESERVATION OF APPLE SLICES BY APPLYING AQUEOUS SUMAC EXTRACT: ANTIFUNGAL AND ANTIBROWNING ACTIVITIES

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Abstract: Spoilage of food, particularly fruits like apples, is a global problem that causes economic losses and public health concerns. Fungal contamination and enzymatic browning are two main causes of spoilage. Aqueous sumac extract (ASE) as a natural inhibitor against the growth of *Aspergillus flavus* and enzymatic browning induced by polyphenol oxidase (PPO) in apples was investigated in this study. Iraqi sumac (*Rhuscoriaria* L.) fruits were treated to obtain oil and aqueous extracts, and the phytochemical composition was analyzed by Soxhlet extraction and high-performance liquid chromatography (HPLC). The aqueous extract contained phenolic compounds, including gallic acid and ferulic acid, which are known for their antioxidant and antimicrobial properties. Antifungal assays indicated that only ASE (10%) inhibited *A. flavus* growth, whereas oil extracts proved to be non-inhibitory. Molecular identification and DNA sequencing confirmed the fungal isolate to be *A. flavus*. Apple slices treated with 5% and 10% aqueous extracts were monitored for 15 days at 4°C to assess the inhibition of browning. Spectrophotometric analysis revealed that browning intensity and PPO activity decrease in a concentration-dependent manner. At the same time, there was enhanced retention of total phenolic content in treated samples, especially at the 10% level. The results demonstrate that ASE, especially at higher concentrations, effectively suppresses fungal infestation and mitigates enzymatic browning in apples. The study identifies sumac as a natural, safe preservative with the potential to boost shelf life and quality of fresh fruits and vegetables.

Key words: *Rhuscoriaria*, *Aspergillus flavus*, enzymatic browning, polyphenol oxidase, natural preservatives, shelf life

INTRODUCTION

Food spoilage is a major global problem, resulting in substantial economic losses and even public health risks. Spoilage is marked by undesirable food quality alterations, such as appearance, taste, or smell, that render the food unfit for consumption (Azad, Ahmad & Siddiqui, 2019). Spoilage can result from physical, chemical, and biological factors, but the most prevalent are microbial contamination and enzymatic reactions (Moral, Nagar, Maan & Kaur, 2017; Sonwani, Bansal, Alroobaea, Baqasah & Hedabou, 2022). Fungi represent one of the most pervasive biological contributing agents of food spoilage, as they can infect a wide range of food commodities, particularly fruits, through air-

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borne spores or surface contact (Amubieya & Olawepo, 2024). Apart from accelerating the decomposition process, fungal infection also causes the production of mycotoxins, toxic secondary metabolites harmful to humans and animals. *Aspergillus, Penicillium,* and *Fusarium* species are frequently associated with the decay and contamination of fresh fruits and vegetables during postharvest storage and handling (You, Zhou, Duan, Mao & Li, 2023).

Other than microbial degradation, enzymatic browning is the other major reason for food quality degradation, especially for fruits and vegetables. It is primarily catalyzed by the enzyme polyphenol oxidase (PPO), which oxidizes natural phenolic compounds to quinones (Al-abbasy, Mahdi, Younus, Sheej Ahmad & Al-Azzawi, 2025; Younus, Mahdi, Al-Abbasy & Sheej Ahmad, 2025). The latter are further polymerized to produce dark pigments that are responsible for the discoloration. Enzymatic browning not only lowers the fruit's visual appeal but can also affect its flavor and nutritional content (Moon, Kwon, Lee & Kim, 2020; Arnold & Gramza-Michałowska, 2022). Apples (*Malus domestica*) are among the most widely consumed fruits in the world and are esteemed for their flavor, nutritional value, and medicinal use (Wibowo et al., 2019). They are, nevertheless, highly susceptible to fungal infection and enzymatic browning, especially upon cutting or bruising. Both conditions significantly reduce their market value and consumer acceptability. Therefore, it is necessary to devise effective methods of preventing fungal infection and browning in apples to increase their shelf life and maintain their quality (Rashan & Al-abbasy, 2021; Oyenihi, Belay, Mditshwa & Caleb, 2022).

Among the safe and natural alternatives to chemical preservatives, Sumac (*Rhuscoriaria* L.) is a promising bioactive plant. Sumac has been traditionally used as a spice and medicinal herb in the Mediterranean and the Middle East (Batiha et al., 2022; Sadoon, 2023). It is a rich source of phytochemicals like flavonoids, tannins, anthocyanins, phenolic acids, and organic acids like gallic acid and malic acid. These phytochemicals possess strong antimicrobial, antioxidant, and enzyme-inhibiting properties (Aili et al., 2024; Mikulic-Petkovsek, Ravnjak & Rusjan, 2024). Recent research has revealed the potential of Sumac extracts as natural preservatives. The use of these plant-based alter atives aligns with consumers' demand for cleaner foods and is supportive of developing sustainable preservation technologies (Alsamri, Athamneh, Pintus, Eid & Iratni, 2021; Nxumalo, Aremu & Fawole, 2021).

The study aimed to examine the use of aqueous Sumac extract (ASE) for the simultaneous inhibition of fungal growth and enzymatic browning in apples. By assessing its dual function, the research seeks to demonstrate the potential of Sumac as a natural preservative to enhance the quality and shelf life of apples.

MATERIALS AND METHODS

Chemicals and instruments

Folin–Ciocalteu reagent, catechol, and other analytical standards, as well as phenolic compounds and fatty acids, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Potato dextrose agar and potato dextrose broth were obtained from HiMedia Laboratories (Mumbai, India), for antifungal assays. Fresh Aspergillus flavus isolates were supplied by the Department of Biology, and all microbiological work was performed under sterile conditions using a Class II biosafety cabinet (ESCO, Singapore).

Centrifugation steps were performed using a Hettich EBA 20 centrifuge (Hettich GmbH, Germany), and pH was adjusted using a Hanna Instruments pH meter (Woonsocket, RI, USA). The extracts were filtered using Whatman No. 1 filter papers (Cytiva, UK). Color measurements for browning assessment were taken using a UV-1800 UV-Vis spectrophotometer (Shimadzu, Japan).

Plant collection

Iraqi Sumac berries were collected fresh from a farm in Duhok, Iraq, in September 2024. The material was air-dried in the shade for 48 hours, followed by oven-drying at 50 °C for one hour. The dried berries were subsequently ground into a fine powder for extraction.

Apple collection

In this study, 30 fresh apples *(Malus domestica)* were used, selected according to standardized criteria that minimized variability in enzymatic browning. Fruits that showed uniform ripeness, color, firmness, size, and weight

(200 ± 20 g) were preferred to maintain a comparable surface area after being cut. Apples presenting any visible defects, such as bruises, mechanical injury, or fungal contamination, were rejected. The fruits were pre-experimentally maintained under identical conditions (4 °C, 90–95% RH) for no longer than 48 hours to keep similar physical and chemical characteristics among samples.

Oil extraction

Oil Sumac extract (OSE) was prepared by placing 44 g of powdered Sumac berries in a filter paper bag and performing Soxhlet extraction with 125 ml of petroleum ether at 40-60 °C for 48 hours. The solvent was subsequently evaporated using a rotary evaporator to concentrate the extract, which was stored in sealed vials at low temperature for future analysis (Al-Abbasy, Ali & Younis, 2020).

Aqueous extraction

Sumac powder (50 g) was mixed with 100 ml of distilled water and sonicated for 15 minutes using an ultrasound device. Then, the mixture was stirred for 6 hours with a magnetic stirrer and stored at -18 °C for 24 hours. Afterward, the mixture was thawed, filtered, and centrifuged. The supernatant was lyophilized for antibrowning and antifungal testing (Alrushdi et al., 2025).

Phytochemicals analysis

The chemical composition was determined using high-performance liquid chromatography (HPLC). Quantification focused on phenolic compounds, gallic, syringic, hydroxybenzoic, caffeic, vanillic, ferulic acids, besides rutin, and quercetin in the aqueous extract. However, linoleic, oleic, palmitic,and stearic acids in the oil extract. Identification was based on comparison of retention times and UV spectra with analytical standards. For HPLC analysis, the aqueous extract was centrifuged at 5000 rpm for 10 min, the supernatant filtered (Whatman No. 1), diluted with HPLC-grade methanol, and passed through a 0.45 µm PTFE filter. The oil extract (100 mg) was dissolved in HPLC-grade methanol, sonicated for 10–15 min, centrifuged at 6000 rpm for 10 min, and the resulting supernatant filtered through a 0.22 µm PTFE filter. All prepared samples were transferred into amber vials for injection (Sadoon & Ahmed, 2020). High-performance liquid chromatography (HPLC) was used to identify and quantify compounds in oil and aqueous extracts. Quantification was performed using reversed-phase HPLC analysis (SYKAM GmbH, Germany). The column used was a C18-OSD (25 cm, 4.6 mm). The column temperature was kept at 30 ºC. A gradient elution method was employed, with eluent A (methanol) and eluent B (1% formic acid in water (v/v)), as follows: initial 0-5 min, 40% B; 5-15 min, 50% B; at a flow rate of 0.9 mL/min. The injection volumes of the standards and samples were 100 µL, automatically handled by an autosampler (Radovanović, Mladenović, Radovanović, Pavlović & Nikolić, 2015; Alabbas, Salih & Al-Abbasy, 2022).

Isolation of *Aspergillus flavus*

Seven days of incubation in the dark at room temperature were needed to monitor for the presence of fungal growth on ripened apple fruits. Infected tissue samples were aseptically cut, and the surface was sterilized for one minute in 0.3% sodium hypochlorite. For the removal of any residual disinfectant, samples were rinsed twice with sterile distilled water. Chloramphenicol (50 mg/L) was added to Potato Dextrose Agar (PDA) plates after sample sterilization to prevent bacterial growth. Incubation on plates lasted for seven days at 25 ± 2 °C. Pure isolates were obtained by subculturing established fungal colonies onto fresh PDA prepared in advance, which showed morphological features typical of *A. flavus*. The pure cultures were preserved in glycerol stock at -20 °C for long-term storage and on PDA slants at 4°C for short-term storage while waiting for further morphological and molecular characterization (Al-Hindi, Al-Najada & Mohamed, 2011).

Determination of antifungal activity

Previously cultivated on PDA, an actively growing margin of a 7-day-old colony of *A. flavus* was used to harvest fungal hyphae. These were aseptically inoculated onto Mueller-Hinton agar (MHA) plates. Using sterile 6 mm paper discs, ASE was applied at concentrations of 5% and 10% to the center of each plate. The plates were incubated for seven days at 25 °C. Antifungal activity was assessed by quantifying the diameter (in millimeters) of a clear inhibitory zone surrounding the site of the application of the extract. Three replicates per test were carried

out, and the mean inhibition zone diameter was determined (Hassan, Haddad & Sultan, 2020; Al-Burgus, Thanoon-Ali & Al-Abbasy, 2024).

Molecular diagnosis

Genomic DNA from the fungal isolates of apple was extracted using a commercial kit (Gene All, South Korea) with minor modifications to enhance yield and purity according to the manufacturer's instructions. The process began with a pre-lysis step, in which finely powdered fungal samples were treated with Proteinase L to initiate protein digestion. This was followed by cell lysis with Proteinase D and RNase A to remove any contaminating RNA and proteins. The DNA was then precipitated with cold 100% ethanol, bound to a silica-based spin column, and washed with a DNA wash buffer. Finally, the DNA was eluted with pre-warmed elution buffer for better recovery and stored at -20 °C until use (Al-abbasy, 2024).

The quality and integrity of the extracted genomic DNA were first checked using a NanoDrop spectrophotometer. A high-purity A260/A280 ratio of 1.8 to 2.0 was obtained. Confirmation of integrity was performed by agarose gel electrophoresis on 1.5% agarose in 1× TBE buffer; sharp, intact bands were clearly visualized under UV light at 336 nm after staining with RedSafe™ dye.

Using (White, Bruns, Lee & Taylor, 1990) Protocol, molecular identification was performed by PCR amplification of the ITS1–ITS4 region using specific primers obtained from Macrogen (South Korea) (Table 1). The reactions were carried out at a final volume of 100 µL. Thermal cycling was performed with a programmable thermocycler under appropriate conditions. After separation on agarose gel and UV visualization, the PCR products were purified and sent to Macrogen for sequencing to identify the species. The sequences were analyzed using the NCBI BLAST database. Multiple sequence alignments were performed in BioEdit software to evaluate intraspecific genetic variation among *A. flavus* isolates (Table 2). Genetic diversity, potential point mutations, and phylogenetic relationships were examined through comparisons of nucleotide sequences (Tuo et al., 2024).

Experimental design on the effect of ASE on apple slices

Ripe apples were washed with water and left to dry at room temperature. They were then sliced into equal pieces using a sharp knife and grouped as follows:

1. Untreated apple slices (control).
2. Apple slices were soaked in a 5% and 10% (w/v) ASE solutions for two minutes.
3. Apple slices were soaked in 5% and 10% (w/v) OSE solutions for two minutes.

Photographs of the slices were taken and monitored for 13 hours to observe changes in their color.

Determination of the effect of ASE on apple browning during storage

Fresh apples were brought, washed, and sliced into 2-3mm thickness using a sharp knife, and separately soaked in 5% and 10% ASE for two minutes. They were then placed at 4 °C. To monitor visual appearance changes during storage, photographs were docu-

Table 1.
The sequencing of the PCR primer

Gene	PCR Primers	Amplicon length
ITS1-ITS4	F5`- TCCGTAGGTGAACCTGCGG-3` R5`- TCCTCCGCTTATTGATATGC-3	550

Table 2.
Thermal cycle program

Steps	Function	Temperature °C	Cycles	Times
1	Initial denaturation	95	1	300
2	DNA denaturation	95	35	30
3	Primer annealing	60	35	30
4	Template elongation	72	35	45
5	Final elongation	72	1	300

documented, and biochemical parameters concerning browning were analyzed every three days for 15 days. For biochemical analysis, the treated slices were homogenized in distilled water at a ratio of 1:3, filtered, and stored in glass containers at 4 °C for the following analysis:

- *Browning intensity:* The browning intensity was estimated by monitoring the absorbance at 420 nm. The absorbance was measured using a spectrophotometer as described by (Tsai, Yu, Chen, Liu & Sun, 2009).
- *Total Phenolic Compounds (TPCs):* To 0.1 mL of each apple sample supernatant, 0.5 mL of diluted Folin–Ciocalteu reagent (1:10 with distilled water) was added, mixed for 3–5 min, then 1.5 mL 7.5% Na_2CO_3 was added and made up to 5 mL with distilled water, mixed thoroughly, and incubated at room temperature in the dark for 30 min. Absorbance recorded at 765 nm against a reagent blank. The calibration curve was prepared using gallic acid (1 mg/mL stock solution) (Anesini, Ferraro & Filip, 2008).
- *Polyphenol oxidase determination (PPO assay):* PPO activity was measured by a spectrophotometric assay in which the reaction mixture contained 1 mL of 50 mM catechol as substrate, 0.9 mL of 0.2 M phosphate buffer, pH 7.2, and 0.1 mL of the sample containing PPO. The change in absorbance at 420 nm was recorded for 5 min at 10-second intervals using a spectrophotometer. One unit of PPO activity was defined as the change in absorbance of 0.001/min/mL of enzyme (Saleh & Al-azzawi, 2023).

Statistical analysis

All experimental data were analyzed in triplicate. Statistical significance was evaluated using one-way analysis of variance in SPSS software package (version 19, SPSS Inc., Chicago, IL, USA). Significant differences among treatments were identified using Duncan's multiple range test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Plants serve as a significant source of natural phytochemical compounds used across various fields (Rashan, Altaee, Salh, Al-Abbasy & Al-Lehebe, 2023). Extraction is essential for isolating and identifying these active compounds. Many of these compounds display antioxidant, antifungal, and antibacterial properties, and they are also considered safe and environmentally friendly (Chu, 2022; Chen, Xing, Chen, Tian & Li, 2023; Alsaayigh & Al-Azzawi, 2025).

Figure 1 illustrates the results of petroleum ether extraction, showing that the Sumac plant contains some fatty acids. Using HPLC ana-lysis, the main fatty acids were identified and quantified.

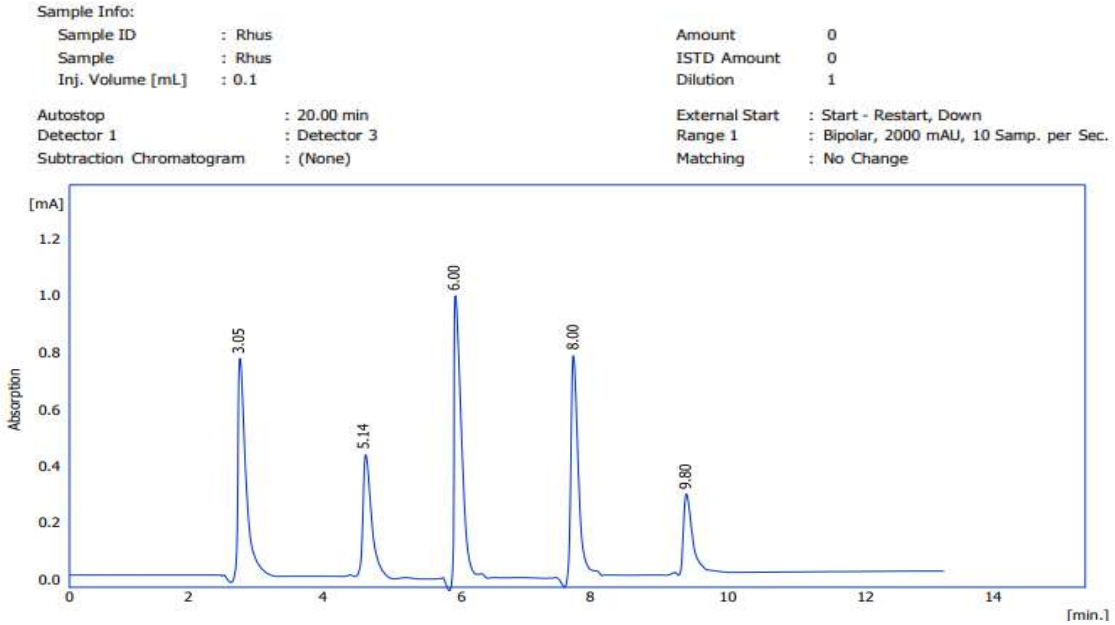
Table 3 presents the relative percentages of these fatty acids, with five compounds successfully identified. Oleic acid, a monounsaturated fatty acid, had the highest concentration at 34.56%, whereas linolenic acid, a polyunsaturated fatty acid, was present at the lowest percentage of 1.08%.

Nonpolar solvents enrich fatty acids, consistent with our detection of polyunsaturated fatty acids and predominantly oleic acid. Fatty acids may contribute to biological effects in two ways: (1) unsaturated fatty acids such as oleic and linoleic can modulate membrane fluidity and might exhibit weak antimicrobial action or synergy with phenolics; (2) they impact the nutritional and physicochemical properties of extracts if these are considered for food applications. The proportions of fatty acids and the total oil yield vary greatly with geographic origin, cultivar, plant part, harvest time, and extraction method (Matthaus & Özcan, 2015). Figure 2 reveals eight distinct phenolic peaks identified in the aqueous extract when compared with the standard.

Table 4 lists the phenolics detected along with their concentrations. Among them, gallic acid exhibited the highest concentration value of 125.6 µg/g while hydroxybenzoic acid showed the lowest concentration of 50.9 µg/g.

Table 3.
The percentage of fatty acids in the OSE

Fatty acid	Palmitic	Stearic	Oleic	Linoleic	Linolenic
% in the petroleum ether extract	20.58	2.66	34.56	21.44	1.08



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.05	9854.08	800.08	25.00	25.00	0.08	
2	5.14	5241.11	408.78	13.00	13.00	0.04	
3	6.00	15426.98	998.41	27.00	27.00	0.10	
4	8.00	9832.14	806.08	25.00	25.00	0.08	
5	9.80	3202.58	310.25	10.00	10.00	0.02	
	Total	43556.29	3323.16	100.00	100.00		

Figure 1. HPLC chromatogram of the OSE

Table 4.
The concentration of polyphenol compounds in the ASE

Phenolic compounds	Caffeic acid	Ferulic acid	Gallic acid	Quercetin	Rutin	Vanillic acid	Syringic acid	Hydroxybenzoic acid
Conc. (µg/g)	78.9	112.4	125.6	78.9	96.5	80.6	84.6	50.9

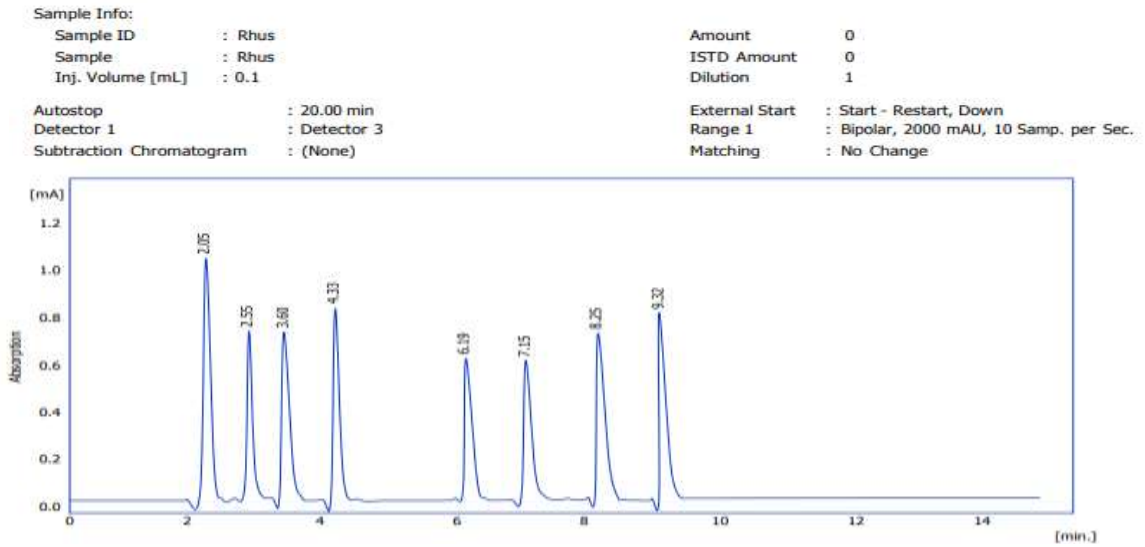
Plants are a rich source of bioactive phytochemicals, and their extracts are still being researched for safe and cost-effective antioxidants and antimicrobial agents (Espíndola et al., 2019). Studies show that simple phenols constitute the main components of Sumac, while more complex tannins and flavonoids emerge when analytical methods are more sensitive. These compounds have demonstrated antioxidant and antimicrobial mechanisms, including free radical scavenging and fungal inhibition through membrane disruption and enzyme interference (Mazzara, Caprodossi, Mustafa, Maggi & Caprioli, 2023; Molski, 2023). This is consistent with the antifungal activity observed in this study. The phenolic content of Sumac varies depending on the plant part, geographic location, and extraction method (Wu et al., 2013).

Comparisons with previous studies indicate that the differences in phenolic concentration largely reflect the extraction solvent and analytical method, rather than biological variation. Hydro-based extraction processes favor the extraction of water-soluble acids and tannin oligomers (Gil-Martín et al., 2022). In general, the phenolic composition and biological activities suggest a possible role for Sumac aqueous extract as a natural food preservative; however, its practical application should be considered in terms of sensory effects, stability, regulation, and safety (Al-Azzawi, Iraqi, Sadoon, Esmaeel & Karomi, 2025).

Identification of fungal pathogens and antifungal assessment

Fungal colonies grown on PDA at 25 °C initially appeared light green, turning dark green as they aged (Fig. 3).

The growth of *A. flavus* was not inhibited by 5% or 10% OSE or 5% ASE (Figure 4). However, 10% ASE showed a distinct antifungal effect, significantly reducing fungal growth by measuring the inhibition zone areas, as depicted in Fig. 5.



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.05	9854.08	1090.14	16.00	16.00	0.10	
2	2.55	7411.14	788.49	12.00	12.00	0.07	
3	3.60	7520.32	787.14	12.00	12.00	0.07	
4	4.33	8011.25	805.65	13.00	13.00	0.08	
5	6.19	6523.65	612.26	11.00	11.00	0.05	
6	7.15	6685.98	611.45	11.00	11.00	0.05	
7	8.25	7124.47	710.56	12.00	12.00	0.07	
8	9.32	8011.65	794.00	13.00	13.00	0.08	
	Total	61142.19	6199.80	100.00	100.00		

Figure 2. HPLC chromatogram of the ASE

The results indicated that maximum inhibition zones were observed for *A. flavus* (27 mm). Previous research has also stated that phenolic acids can damage fungal cell membranes, inhibit sporulation, and scavenge reactive oxygen species (Long & Li, 2024.

In the fresh-cut fruit preservation, control of spoilage fungi is a central goal since fungi infect fruits, produce mycotoxins, and lead to substantial postharvest losses by decreasing the marketability (Gregori et al., 2008).

Sumac (*Rhuscoriaria*) is generally recognized as a natural source of antimicrobial and antioxidant compounds; it causes membrane damage, inhibits sporulation, and scavenges ROS through its phenolics and tannins-mechanisms asso-ciated with the antifungal action reported for 10% ASE (Singh & Corradini, 2022; Sethuraman, Hamad & Raju, 2025).

Some comparative examples from the literature show that extracts differ not only in potency but also in mode of action: for instance, pomegranate-peel extracts have variously been reported to cause substantial in vitro inhibition of *A. flavus*. For instance, ~39% inhibition under the specific conditions of a given assay-

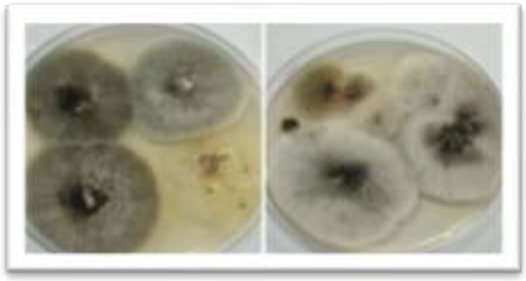


Figure 3. Apple fungus colonies growing on PDA medium

Figure 4. Effect of OSE on inhibiting the growth of *A. flavus*

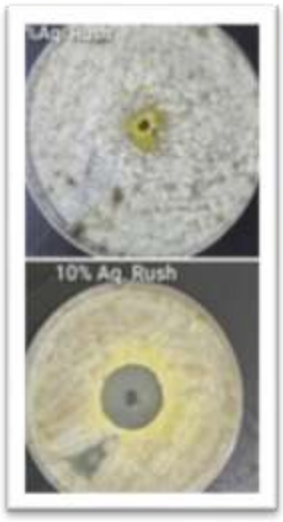


Figure 5. Effect of ASE on inhibiting the growth of *A. flavus*

emphasizing that (1) the magnitude of in vitro growth or toxin inhibition strongly depends on the type of extract, its concentration, and the assay format used, and that (2) even different polyphenol-rich matrices might differ in performance against the same pathogen (Rosas-Burgos et al., 2017). Recent studies continue to confirm Sumac's antimicrobial properties across different extraction methods. They highlighted that the action is dose- and extract-dependent and recommended detailed phytochemical and mechanistic analyses to identify active fractions. Although our findings against *A. flavus* are in line with this evidence, the literature shows significant variation among species and strains, underscoring the need for broader pathogen panels and standardized assays before broader antifungal claims can be made (Mazzara et al., 2023).

Isolation and analysis of fungal DNA

Genomic DNA from *A. flavus* was successfully recovered and verified through agarose gel electrophoresis. The extracted DNA was visualized as sharp, well-defined bands of approximately 550 base pairs (bp) under 254 nm UV illumination, indicating high integrity and purity (Figure 6). PCR amplification with fungal-specific primers yielded clear and distinct bands, confirming the suitability of the DNA for downstream molecular analyses. This is consistent with previous studies indicating that high-quality genomic DNA requires efficient removal of proteins, polysaccharides, and secondary metabolites that interfere with the final molecular action (Abdelwahab et al., 2016).

Furthermore, polymerase chain reaction (PCR) amplification using fungal-specific primers demonstrated the absence of common PCR inhibitors such as polysaccharides and phenolic residues (Schrader, Schielke, Ellerbroek & Johne, 2012). These findings are supported by similar studies, which have proven the importance of high-quality DNA in reliably amplifying the ITS region for species identification and further molecular analysis (White et al., 1990)

However, there are still some limitations. Gel visualization alone cannot determine purity, and spectrophotometric or fluorometric measurements would strongly validate these findings. Given the genetic diversity within *A. flavus*, discriminatory power is reduced because of the use of only a single genetic marker; additional loci such as β-tubulin or calmodulin increase resolution (Geiser et al., 2013). Finally, sequencing of the PCR product would definitively confirm strain identity and primer specificity.

Molecular identification of *Aspergillus flavus*

The successful extraction and identification of *A. flavus* genomic DNA are attributed to the use of an extraction method tailored to its unique cell wall characteristics.

** 1 Refer to the DNA of A. flavus. *2 is a repeated sample that has been tested for more accuracy.*

Figure 6. DNA amplification products of the ITS1-ITS4 gene for the fungal isolate *Aspergillus flavus*. Horizontal electrophoresis conditions were 5 V/cm3 and 1.5% agarose gel concentration

Filamentous fungi have structurally complex cell walls, mainly comprising chitin, β-glucans, mannoproteins, and melanin, which contribute to mechanical rigidity and resistance to lysis. These features often pose a serious problem during nucleic acid recovery and, thus, require special organism-specific adaptations in the available extraction methods (Pham et al., 2024).

In this study, genomic DNA was successfully isolated from *A. flavus* using PCR amplification of a specific genomic region, commonly the ITS regions (18S rRNA), or specific genes, followed by quality assessment with NanoDrop spectrophotometry, which sho-wed an (A260/A280) ratio between (1.8–2.0), indicating high purity. Integrity was confirmed by agarose gel electrophoresis, revealing sharp bands without degradation.

Molecular identification was performed through PCR amplification and sequencing of the ITS region. The obtained sequence was ana-lyzed using BLAST in the NCBI GenBank database, revealing 100% similarity with the reference strain (*A. flavus*, accession no. PP-937579.1) (Fig. 7). Such high sequence similarity is in line with established standards in molecular systematics, where an ITS similarity of>98–99% is usually sufficient to allow precise identification within Aspergillus and other filamentous fungi (Manogna, Kumar, Kumar & Swathi, 2021; Sharma, Singh & Sarma, 2023).

Compared to classical morphological identification, which may be subjective and influenced by cultural conditions, molecular methods offer speed, reproducibility, and high discrimination. The importance of such methods for *A. flavus*, a species renowned for its morphological variability and close resemblance to other members of the Aspergillus section Flavi (Peterson, 2008).

ITS sequencing proves to be effective for accurate species identification, but multi-locus markers are required for better resolution among the closely related species of Aspergillus. The limitation of this study includes testing only one extraction and sequencing methodology when more could have been added. Overall, the results confirm the reliability of ITS-based diagnostics but also highlight the need for organism-specific strategies of extraction toward the precise identification of *A. flavus* (Schoch et al., 2012).

The overall successful extraction, characterization, and sequencing of DNA from *A. flavus* in this study further underscore the importance of organism-specific DNA isolation strategies and reiterate the pivotal role that molecular techniques play in any accurate fungal diagnostics.

Analysis of the phylogenetic tree of the fungus *Aspergillus flavus*

Based on the complete nucleotide sequence, the analysis revealed that the isolate is most closely related to members of the genus *Aspergillus*, specifically *A. flavus*. The study demonstrated a strong clustering with the reference strain *A. flavus* (GenBank accession number: PP937579.1), with a percentage identity of (~99–100%) and high bootstrap support (>95%), indicating a robust phylogenetic relationship. Fig. 8 illustrates the phylogenetic position of the isolate in comparison with related fungal species.

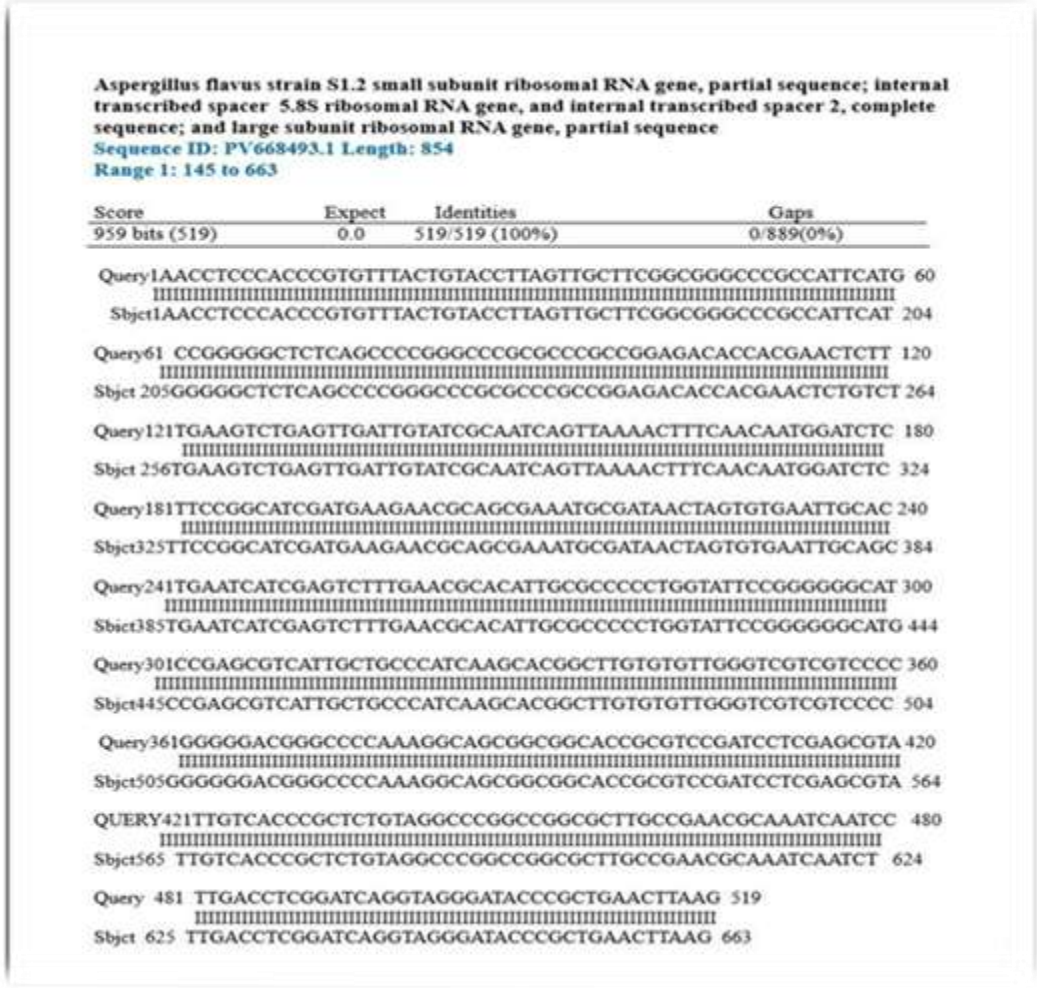
The effect of Sumac extracts on apple slices

Apple slices were treated individually with (5% and 10%) ASE and OSE. Images taken over 13 hours showed that the oil extract was ineffective at preventing the slices from browning.

However, the aqueous extract was effective in reducing the browning of the slices (Figure 9). ASE (10%) was more effective in preventing browning of sliced apples than the 5% extract. The oil extract did not show apparent anti-browning activity compared to the aqueous extract. Therefore, it was excluded in sub-sequent experiments, and the focus was placed only on the aqueous extract (Figure 10).

The more substantial anti-browning effect of 10% ASE compared to 5% indicates that water extracts more of Sumac's active compounds than oil. It is further supported by the fact that phenolics, organic acids, and tannins in *Rhus coriaria* are mostly water-soluble; hence, the aqueous extract shows higher efficacy.

Such phytochemicals include gallic acid, quercetin derivatives, and hydrolysable tannins, which are known PPO inhibitors acting by chelating copper at the enzyme's active site, competing with natural substrates, and scavenging quinones before they form brown pigments (Huang, Sun, Ma, Sui & Wang, 2021; Chu, 2022). The impact of cinnamon essential oil on PPO activity in apple juice was examined by Xu et al. (2020).



Aspergillus flavus strain S1.2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: PV668493.1 Length: 854

Range 1: 145 to 663

Score	Expect	Identities	Gaps
959 bits (519)	0.0	519/519 (100%)	0/889(0%)

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Query1AACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATG 60
Sbjct1AACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCAT 204

Query61 CCGGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTT 120
Sbjct 205GGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCT 264

Query121TGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTC 180
Sbjct 256TGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTC 324

Query181TTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAC 240
Sbjct325TTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGC 384

Query241TGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCAT 300
Sbict385TGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATG 444

Query301CCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCC 360
Sbjct445CCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCC 504

Query361GGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTA 420
Sbjct505GGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTA 564

QUERY421TTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCC 480
Sbjct565 TTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCT 624

Query 481 TTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAG 519
Sbjct 625 TTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAG 663
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Figure 7. Identical DNA sequence of the *Aspergillus flavus* isolate

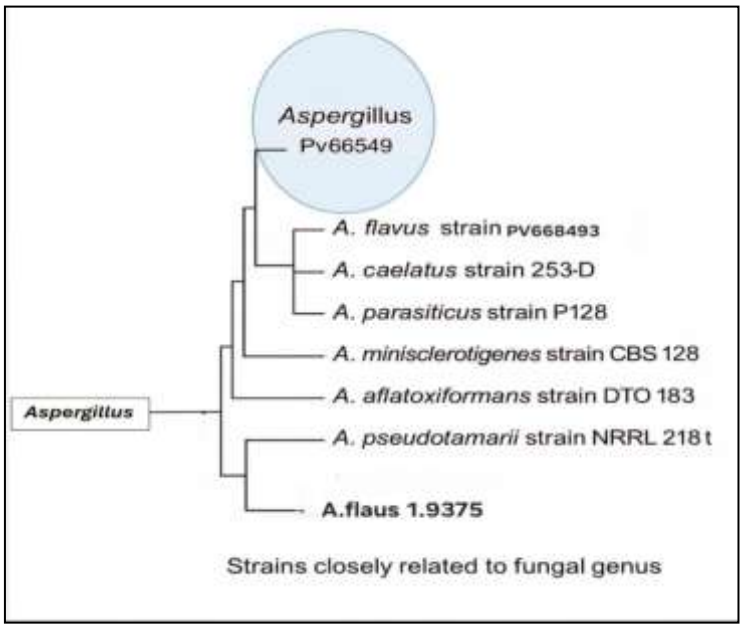

Figure 8.Taxonomic genetic tree of *Aspergillus flavus*

They discovered 80–90% suppression, leading to a significant reduction in color development associated with enzymatic browning. Green tea extract was shown to be a potent inhibitor of PPO activity and apple juice browning color (Klimczak & Gliszczyńska-Świgło, 2017).

Moreover, the enzymatic browning of apple juice, an antioxidant, was significantly affected by the addition of onion extract. The browning hue was found to have decreased (Lee, Seo, Rhee & Kim, 2016).

The results further evidence that the phenolic antioxidants in ASE serve multifunctionally by inhibiting PPO, reducing quinone formation, scavenging radicals, and thereby stabilizing apple tissues after cutting.

By contrast, OSE consists mainly of lipophilic terpenoids, which interact poorly with PPO in the aqueous environment inside the apple and diffuse slowly through the tissue, explaining its limited anti-browning effect, also described for other essential oil studies (Bakkali, Averbeck, Averbeck & Idaomar, 2008; Gallego, Gordon, Segovia, Skowyra & Almajano, 2013).

Overall, ASE emerges as an effective clean-label anti-browning agent, with efficacy comparable to other phenolic-rich natural inhibitors and strong potential for preserving fresh-cut fruit.

Effect of ASE on apple slices browning during storage

Figure 11 shows the effect of ASE on the browning of apple slices stored at 2°C for 15 days. Visual assessment revealed that on day 0, all samples were standard in color and showed no browning. On day 3, color change began to appear, with slices treated with 10% ASE showing significantly less color change than those treated with 5% ASE or untreated slices. This pattern persisted until day 6, when the 10% extract exhibited consistently more potent inhibitory effects. From day 9 to day 15, all samples showed intense browning; however, slices treated with the 10% extract showed significantly less browning than those treated with the 5% extract or the untreated control, demonstrating its strong protective effect. This concentration-dependent trend is consistent with previous studies that have shown more potent PPO inhibition and antioxidant protection at higher levels of extracts of natural anti-tanning agents (Zhang, Meng, Chen & Peng, 2022). This can be attributed to the rich phenolic and antioxidant content of Sumac extract, which reduces quinones, removes ROS, and may inhibit the active copper site in PPO. Those mechanisms are in line with the known pathways of plant anti-tanning inhibitors, which act either by preventing the oxidation of phenolics or by destabilizing PPO (Yousefi, Mirdehghan,

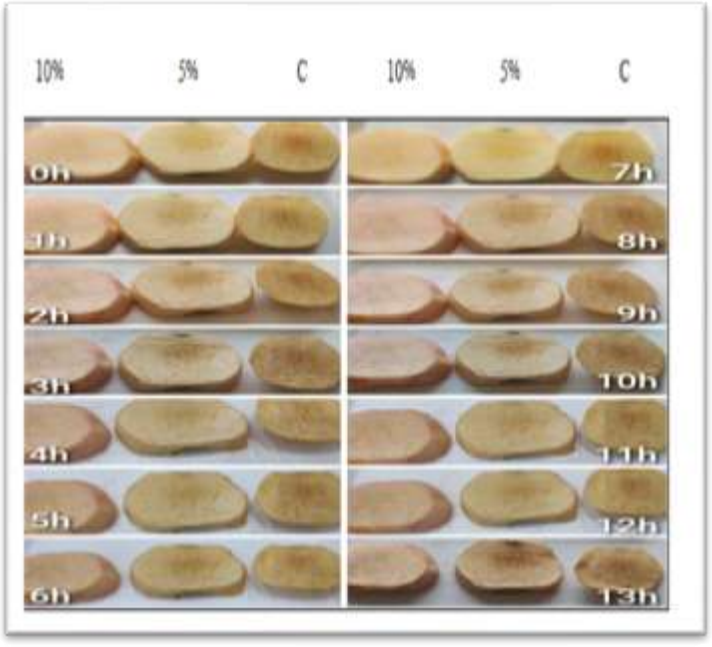

Figure 9. The effect of the ASE on apple slices

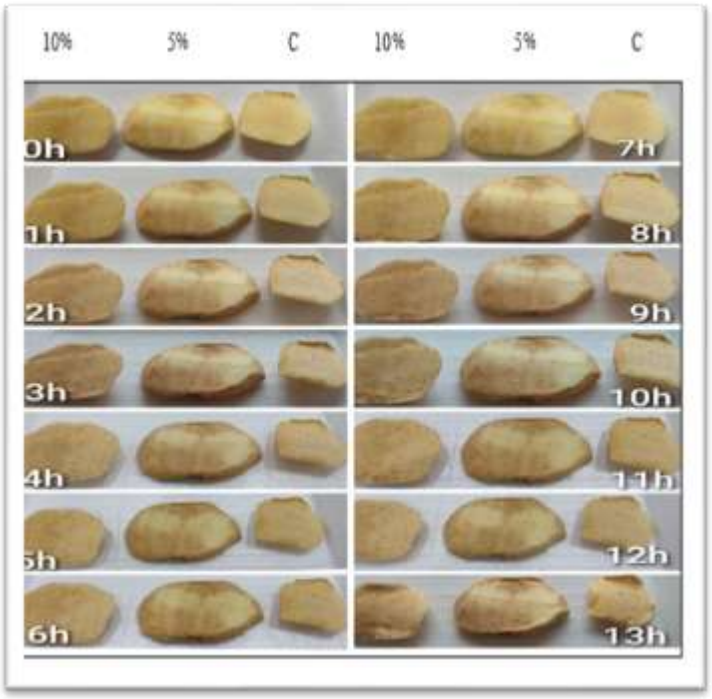

Figure 10. The effect of OSE on apple slices

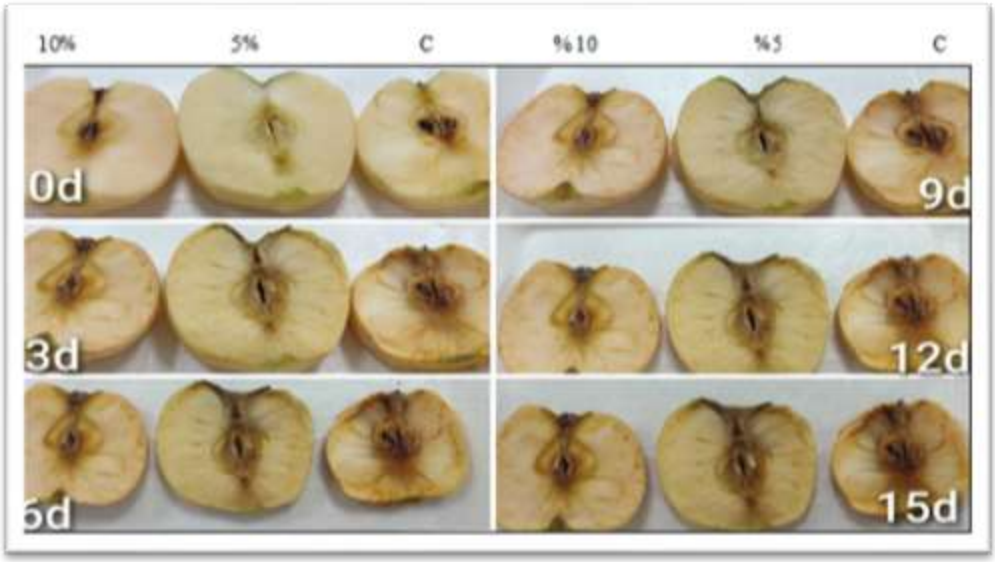

Figure 11. Apple slices treated with ASE during storage

Esmaeilizadeh, Nazoori & Sahhafi, 2025). ASE may also inhibit microbial activity indirectly and, in turn, reduce tissue stress that accelerates browning, as has been described for the action of antimicrobial plant extracts on fresh fruit (Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny & Martín-Belloso, 2009).

In summary, the findings here support the use of 10% ASE as a promising and effective treatment for browning, acting similarly to other phenol-rich extracts reported in the literature.

Change in browning intensity at 420 nm

Spectrophotometric measurements at 420 nm showed that the untreated apple exhibited the highest browning after storage, indicating increased PPO activity and melanoidin accumulation. In contrast, samples treated with ASE showed a significant decrease in ab-

sorbance at 420 nm, especially at 10%, indicating the effectiveness of these extracts in reducing browning, as shown in Figure 12.

This reduction suggests that ASE effectively inhibits PPO or limits the formation of subsequent brown quinones and polymers. The potential of ASE to reduce browning has been reported for natural extracts rich in phenols, lignans, and other antioxidant compounds. These findings also agree with other reports indicating that plant extracts reduce the intensity of browning by inhibiting PPO activity or altering the substrate available for oxidation (Eissa, Mostafa, Bareh & Shouk, 2014). Mechanistically, ASE may function through: a) direct PPO inhibition via interaction with its copper center, b) scavenging free radicals that limit the development of quinone; c) chelation of copper by the phenolic groups; and d) inhibition of pH-induced PPO activity (Queiroz, Mendes Lopes, Fialho & Valente-Mesquita, 2008). Generally, in fruits and vegetables, color is a critical cue that has a significant consumer impact and enables people to gain insight into many other characteristics associated with the final food product (Yildiz, Palma & Feng, 2019).

Findings indicate that ASE treatment (5% and 10%) significantly retained total phenolic content through the 15-day storage period compared to the untreated control (Fig. 13). The phenolic content in all samples was the lowest on day 0, indicating an equivalent initial level across samples. The phenolic content steadily decreased over days 3 to 12 in all samples, but at significantly greater stability in treated slices. By day 15, the control sample had the lowest phenolic content, while the 10% extract-treated sample had the highest, followed by the 5% treatment. This inhibitory effect is attributed to the phenolic antioxidants in Sumac that inhibit oxidative degradation.

The performance of the 10% extract, which was best, shows a concentration-dependent relation between Sumac treatment and retention of phenolics. In contrast, phenolic loss due to oxidation and enzymatic activity occurred in all samples, but to a far lesser extent in samples treated with Sumac. The mechanism corresponds with other studies that stated the phenolic-rich extracts and lignans increase the stability and levels of phenolics present in fresh-cut fruits due to the suppression of PPO activity and limitation of oxidative stress (Altunkaya & Gökmen, 2008).

It was found that storing apples reduced phenolic compounds because of their oxidation by PPO (Ferreira, Ribeiro & Nunes, 2024). Aloe vera gel extract at a 75% concentration was studied for its anti-browning effects in apple fruit. The treatment significantly reduced browning, increased total phenolic content and antioxidant activity, and effectively preserved the sensory qualities of the apple (Supapvanich & Boonyaritthongchai, 2016). Results may vary with cultivar of apple, maturity, and storage conditions.

Effect of ASE on the PPO activity during storage

The impact of ASE on apple PPO activity during storage at 4 °C for 15 days is revealed in Figure 14. The results exhibited that ASE (5% and 10%) could inhibit PPO compared to the control group for all storage times. The results indicate that ASE possesses a concentration-dependent inhibitory effect on PPO activity. The 10% extract is more potent than the 5% in inhibiting PPO activity, presumably because of the higher quantity of phenolic compounds or antioxidants, which can inhibit PPO either through direct inhibition of an enzyme or by reducing quinone formation.

Several studies have used extracts to reduce PPO activity. Aqueous mango peel extracts have been shown ability to inhibit PPO activity in potato puree (Jirasuteeruk & Theerakulkait, 2020). The purified enzyme from Jerusalem artichoke was inhibited by the aqueous extract of rosemary leaves. This natural anti-darkening extract can be used as an alternative to chemical agents that may pose a risk to public health (Al-Abbasy, Ali, Rashan & Al-Bajari, 2021). Moreover, ginger aqueous extract was found to have an anti-browning effect by decreasing the *Annona muricata* and *Musa acuminata* PPO (Weerawardana, Thiripuranathar & Paranagama, 2020). The PPO activity of yams was more inhibited by extracts of garlic and onion at varying doses (Yapi, Gnangui, Dabonné & Kouamé, 2015; Sadoon & Saeed, 2021).

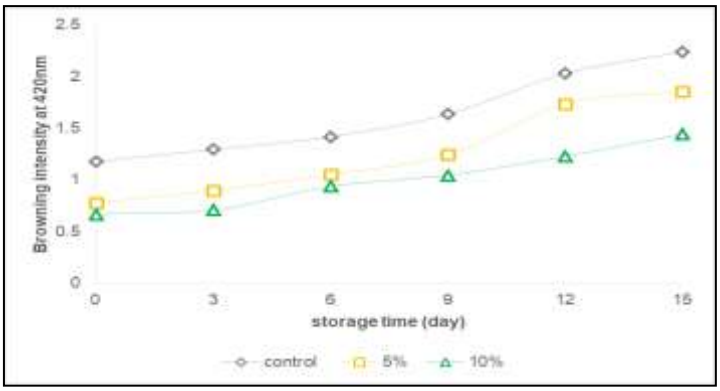

Figure 12.Effect of ASE on browning intensity during storage

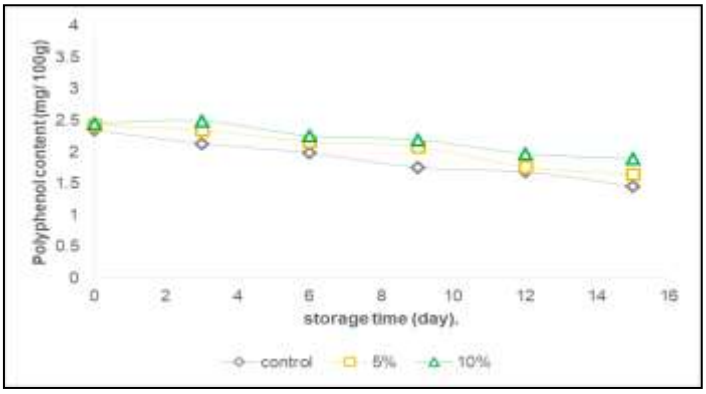

Figure 13.The effect of ASE on the phenolic content of apple during storage

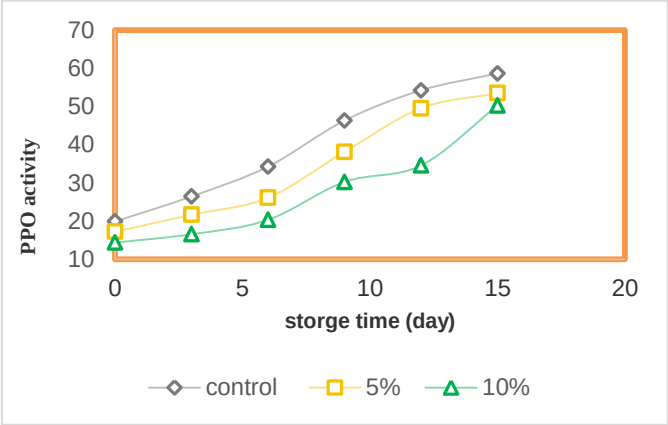

Figure 14. The effect of ASE on the PPO of apple during storage

ASE exhibited concentration-dependent inhibition of PPO, although active compounds and synergistic interactions remain unidentified. Only crude extracts were used for PPO activity determination, which may reduce the accuracy of the data. Assays with a purified enzyme will provide stronger evidence for such properties. This study has its limitation to storage at 4 °C; therefore, the results may be very different at higher temperatures and in varieties other than apple. Finally, since extracts of plants may affect the taste or color, sensory evaluation is necessary before recommending ASE for industrial applications as an anti-browning agent.

CONCLUSIONS

This study reveals the promising potential of aqueous *Rhuscoriaria* L. (Sumac) extract as a natural food preservative that can simultaneously inhibit fungal spoilage and enzymatic browning of apples. The extract was

rich in phenolic constituents, including gallic and ferulic acids, which contributed to its pronounced antioxidant and antifungal activities. Antifungal assays confirmed that the 10% aqueous extract significantly suppressed the growth of the common postharvest pathogen *A. flavus* . ASE treatment of apple slices, especially at 10% level, significantly reduced the severity of browning, PPO activity, and phenolic breakdown during 15-day cold storage. The two-in-one functionality of Sumac extract offers a functional, safe, and environmentally friendly alternative to synthetic chemical preservatives. Its application in fresh-cut fruit preservation holds promise to extend shelf life, improve visual and nutritional quality, and satisfy consumer demand for clean-label and plant-based ingredients. Future research would benefit from examining its sensory impact, commercial processing stability, and efficacy in other vegetable and fruit systems.

AUTHOR CONTRIBUTIONS

Methodology and conceptualization, S.A.S.; Investigation, formal analysis, validation, writing-original draft preparation, O.Y.A; Writing-review, editing, and Supervision, A.M.S.

DATA AVAILABILITY STATEMENT

All data included within the article

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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OČUVANJE JABUKA BEZ ADITIVA (CLEAN-LABEL) PRIMENOM VODENOG EKSTRAKTA SUMAKA: ANTIFUNGALNA I ANTIOKSIDACIONA AKTIVNOST

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Sažetak:: Kvarenje hrane, naročito voća poput jabuka, predstavlja globalni problem koji izaziva značajne ekonomske gubitke i zabrinutost za javno zdravlje. Glavni uzroci kvarenja su gljivična kontaminacija i enzimatsko tamnjenje. U ovoj studiji je ispitivan potencijal vodenog ekstrakta sumaka (ASE) kao prirodnog inhibitora rasta plesni *Aspergillus flavus* i enzimatskog tamnjenja izazvanog polifenol oksidazom (PPO) u jabukama. Plodovi iračkog sumaka *(Rhus coriaria* L.*)* tretirani su radi dobijanja uljanih i vodenih ekstrakata, a fitohhemijski sastav analiziran je Soxhlet ekstrakcijom i tečnom hromatografijom visokih performansi (HPLC). Vodeni ekstrakt je sadržao fenolna jedinjenja poput galne kiseline i ferulinske kiseline, poznate po antioksidativnim i antimikrobnim svojstvima. Antifungalni testovi pokazali su da samo vodeni ekstrakt ASE (10%) inhibira rast *A. flavus*, dok uljani ekstrakti nisu imali inhibitorni efekat. Molekularna identifikacija i sekvenciranje DNK potvrdili su da je izolovana plesan *A. flavus*. Komadi jabuke tretirani sa 5% i 10% vodenog ekstrakta ASE praćeni su tokom 15 dana na 4 °C radi procene inhibicije tamnjenja. Spektrofotometrijska analiza pokazala je da se intenzitet tamnjenja i aktivnost PPO smanjuju zavisno od koncentracije. Istovremeno je zabeležena povećana retencija ukupnog sadržaja fenola u tretiranim uzorcima, naročito u slučaju tretmana s 10% ASE. Rezultati ukazuju da je vodeni ekstrakt sumaka (ASE), posebno pri višim koncentracijama, veoma efikasan u smanjenju gljivične infestacije i enzimatskog tamnjenja jabuka. Studija identifikuje sumak kao prirodni, bezbedan konzervans, sa potencijalom da produži rok trajanja i kvalitet svežeg voća i povrća.

Ključne riječi: *Rhuscoriaria, Aspergillus flavus, enzimsko tamnjenje, polifenoloksidaza, prirodni konzervansi, rok trajanja*

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