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Inhibition of Enzymatic Browning in Mango Juice Using Antioxidant-Rich Eggplant Peel Extracts: A Kinetic and Preservation Study

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Abstract

Enzymatic browning processes negatively influence many food products, such as fresh juices, particularly their sensory properties and nutritional value, ultimately leading consumers to reject such products. Natural antioxidants have been used to reduce this phenomenon, preserving these products and preventing quality deterioration. This research investigates the antioxidant and enzyme inhibitory activities of eggplant peel ethanolic (EEPE) and aqueous (AEPE) extracts, by scavenging DPPH radicals method in mango juice (MJ) during cold storage. EEPE had greater DPPH radical scavenging capacity (0.66 mg/g) than AEPE (0.52 mg/g), although both extracts were weaker than vitamin C (0.73 mg/g). Enzymatic browning assays revealed that EEPE suppressed PPO and POD activities in mango juice (MJ), contributing to decreased browning intensity and better preservation of phenolic content after 15 days storage at 4°C, more than AEPE. Purified mango pulp PPO was of approximately 54 kDa molecular weight and was effectively extracted and purified using ammonium sulfate precipitation, dialysis, and CM-Cellulose ion exchange chromatography with a purification fold value of 15.07. Kinetic studies revealed that EEPE acted like a competitive inhibitor of PPO, while AEPE exhibited non-competitive inhibition. This finding reflects the potential of eggplant peel,as an agricultural waste, is a potent natural substance to be used in food preservation due to its antioxidant and anti-browning activities.

Keywords: Antioxidant activity, Eggplant peel extract, Enzymatic browning, Food preservation, Mango juice, Peroxidase, Polyphenol oxidase

Introduction

Fruit juice (FJ) is considered as nutrient-dense beverage, containing micronutrients, minerals, and antioxidants (Buvé, Pham, Hendrickx, Grauwet, & Van Loey, 2021). It shares the composition of fruits and is made from whole fruit (edible parts), with natural sugars, making it low-in-energy (Walton & Kehoe, 2025). It's convenient to consume and has a longer shelf life without chemical preservatives. The global juice market, was valued at USD 145.2 billion in 2022. The market is projecting to grow due to health-conscious consumers seeking natural, preservative-free beverages (Konar, Gunes, Palabiyik, & Toker, 2022). The market is expected to expand at a CAGR of 5.60% by 2030. The variety of juice products has increased efficiency in quality control and maintenance, facilitated by minimal processing

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(Mieszczakowska-Frąc, Celejewska, & Płocharski, 2021).

Mango (*Mangifera indica* L.) belonging to the family Anacardiaceae is one of the most important fruits consumed in fresh or processed form globally (Singh, Singh, Sane, & Nath, 2013). It is extensively cultivated in tropical and subtropical climates worldwide because of its high nutritional value, flavor, and aroma (Dhanyasree, Rafeekher, & Premachandran, 2025). This fruit is a good source of minerals, dietary fiber, vitamins, and antioxidants like polyphenols, which have been connected to several health advantages (Wu *et al*., 2018). Like many fruits, mango juice turns brown when exposed to air, which changes the color and taste, thus spoiling the product and making it undesirable to drink.

Browning in food products like FJ occurs during processing and storage, resulting in color changes due to two key chemical reactions: non-enzymatic browning (NEB) and enzymatic browning (EB). Nonenzymatic chemical reactions like caramelization, ascorbic acid degradation, and the Maillard reaction are common quality issues in concentrated FJ, leading to nutrient loss and undesirable compounds (Abdul Kareem & Ali Al-Jammaas, 2024). On the other hand, EB is mainly caused by polyphenol oxidase (EC 1.14.18.1, PPO) (Al-abbasy, Alsarraf, Mahdi, & Al-barwari, 2025). This enzyme oxidizes phenolic compounds to quinones that spontaneously polymerize with other compounds (phenolics, sugars, soluble amino proteins), to generate brown pigments (Bobo-García, Arroqui, Merino, & Vírseda, 2020). Several studies have focused on preventing or inhibiting PPO activity in food products. Numerous methods and mechanisms have been suggested over the years for controlling this enzyme activity. Analysis revealed that most chemical or synthetic inhibitors were hazardous compounds that might react with cellular constituents to produce toxicity. Hence, the most important and safest method is using natural sources in products that are minimally processed (AL-BURGUS, Ali, & AL-ABBASY, 2024; Weerawardana, Thiripuranathar, & Paranagama, 2020). For example, studies have demonstrated that, aloe vera (Alberio, Muratore, Licciardello, Giardina, & Spagna, 2015), whey protein (Yi & Ding, 2014), and rice bran extracts (Sukhonthara, Kaewka, & Theerakulkait, 2016) inhibit browning and maintain quality.

Eggplant (*Solanum melongena* L.) is a widespread vegetable crop in temperate and tropical regions (Mat Sulaiman *et al*., 2020). Eggplant fruit is included in the basic diets of many local communities (Davidar *et al*., 2015). It contains many active compounds compared to other crops of the same family. The largest part of these active compounds is particularly abundant in the peel (approximately 30-60%), and is represented by polyphenols, flavonoids, and phenolic acids (Niño-Medina, Urías-Orona, Muy-Rangel, & Heredia, 2017). Anthocyanins and chlorogenic acid are the main and most important compounds in the peel (Yarmohammadi, Rahbardar, & Hosseinzadeh, 2021). These compounds have a high antioxidant capacity .

The main objective of this research was to develop PPO inhibition in mango juice using eggplant peel extracts as natural inhibitors. Thus, the project aimed to reduce browning and increase the shelf life of mango juice.

Materials and Methods

Chemicals such as Folin-Ciocalteu reagent, bovine serum albumin, Triton X-100, ammonium sulfate, and ninhydrin were purchased from Merck Co. Additionally, catechol, polyethylene glycol, polyvinylpyrrolidone, gallic acid, guaiacol, petroleum ether, ethanol, and carboxymethyl (CM) cellulose were obtained from Sigma-Aldrich Co.

Preparation of Soxhlet Extraction

Eggplant fruit was purchased freshly from a local market in Mosul City, Iraq, in October 2024 (peak season). The plant was washed with a tap water and then manually peeled and the peel was chopped into small pieces. The pieces

were dried at 40ºC for 72 h until a constant weight was acheived , using a digital forced -air convention oven (5-200 °C, Model UN55, Memmert GmbH Co. KG, Schabach, Germany). The dried pieces were ground to obtain fine homogeneous powder in a high-speed analytical laboratory grinder fitted with 180W motor drive (Model A11 Basic, IKA-WERKE GmbH & Co. KG, Staufen, Germany). For extraction, 25g powder was subjected to Soxhlet extraction with 250 ml petroleum ether for 8 hours at 60-80°C for defatting. The extract was evaporated using a vacuum rotavapor. The defatted peel was macerated in ethanol (70%) for 24 h., extracted by Soxhlet for 6 h., evaporated at 50°C, and kept at -20ºC (Al-Abbasy, Ali, & Younis, 2020). This extract is defined as ethanolic eggplant peel extract (EEPE).

Preparation of Aqueous Eggplant Peel Extract

Twenty-five grams of eggplant peel powder were mixed with 250 mL of distilled water and the mixture was incubated on a rotating shaker at 1,500 rpm at 35ºC for 2 days. The extract was then filtered through a cheesecloth and centrifuged at 10,000×*g* for 15 min. The supernatant was evaporated on a vacuum rotavapor at 70°C (Taqi, Al-Shahery, & Al-Abbasy, 2025). This extract is defined as aqueous eggplant peel extract (AEPE) and kept at -20ºC.

Preparation of Mango Juice

Mango was acquired freshly from a local market in Mosul City, Iraq, in May 2025 to ensure maximum enzymatic activity. After washing and peeling, the mango pulp was homogenized with DW at a ratio of 1:10 (W/V), using blender powered with a 370-W motor driver (Model 8011G, Waring Commercial, Torrington, CT, USA). The crushed mango was filtered through many layers of muslin cloth. The produced juice was centrifuged at 3,000×*g* for 10 min. This was defined as mango juice (MJ). The MJ was divided into two groups treated with 5% of each EEPE and AEPE separately. For comparison, the control was MJ without any extract (Hasan Ali AL-Jammaas, 2023).

Antioxidant Activity Measurement by DPPH Radical Scavenging

According to Matthäus (Matthäus, 2002), two milliliters of EEPE were mixed with three milliliters of DPPH solution (0.5 mg/mL), and twenty-five milliliters of methanol. After thorough shaking, the mixture was kept in the dark for forty-five minutes. The decrease in absorbance was measured at 515 nm. Vitamin C (1%) was used as an antioxidant control. The following formula is used to calculate the percentage:

Inhibitory % = $[(A_c - A_s) \div A_c] \times 100$

When: A_c: Control reaction absorbance, A_s: Testing specimen absorbance.

Intermediate Compounds and Browning Intensity Monitoring

By using spectrophotometry, a colorless intermediate at A_{294} nm was recorded. The brown intensity of a hue was measured at A_{420} nm (Tsai, Yu, Chen, Liu, & Sun, 2009).

PPO Assay

Spectrophotometrically, PPO activity was monitored. One milliliter of 0.01M catechol (substrate), 0.9 ml buffer (pH 7.2), and finally 0.1 ml of enzyme were mixed. The absorbance was measured at 420 nm with a ten-second interval for five minutes (Oktay, Küfreviolu, Kocaçalişkan, & Şaklrolu, 1995).

Peroxidase Assay

To assess POD activity, a 100 µL aliquot of the extracted enzyme was added to the reaction mixture along with 1.5 mL of 0.1 M phosphate buffer (pH 7.0), 0.5 mL of guaiacol (1.68%), and 0.5 mL of H_2O_2 (1.8%). The total volume was then adjusted to 3 mL with distilled water. All components of the reaction mixture, except the enzymatic extract, were replaced with water to create blanks. The absorbance was measured at 470 nm at 30 °C (Lagrimini, Gingas, Finger, Rothstein, & Liu, 1997).

Content of Total Phenols

To a 25 ml volumetric flask, one ml of the sample, one ml of Na_2CO_3 (20%), and 0.75 ml of Folin-Ciocalteu reagent were added. Distilled water was then added to make the total amount of 25 ml. Using spectrophotometer (PU 8800 UV/VIS, Shimadzu, Double Beam), the absorbance at 765 nm was measured following a 30-minute incubation period in a 55 °C water bath. A standard curve for gallic acid (GA) was utilized to quantify the total phenolic content. The result was reported as GA equivalents per gram of fresh weight (mg/g) (Girgžde, Samsone, & Gailis, 2019).

Determination of Protein Concentration

The protein concentration was assessed using bovine serum albumin as a stock solution. The absorbance was monitored at 650 nm (Scharcterle & Pollack, 1973).

Enzyme Extraction

To purify PPO, a procedure described by (Rashan & Al-Abbasy, 2021) with some modifications was used. After washing and peeling, 50 grams of fresh mango fruit were homogenized with 150 ml of cold phosphate buffer (0.1 M, pH 7.2), including (10 mM ascorbic acid, 3% polyvinylpolypyrrolidone (PVPP), 2% PEG, and 1% Triton X-100), using a blender for 2 minutes. The homogenate was frozen using liquid nitrogen to ensure disruption of the cell wall. After that, the extract was filtered through cheesecloth in an ice bath and then centrifuged at 10,000 × g for 15 minutes at 4 °C. The supernatant was collected and considered as crude enzyme, then kept at 4ºC for further analysis.

Precipitation and Dialysis

The crude enzyme solution was precipitated by adding $(NH_4)_2SO_4$ to give a saturation of 30-80% and left overnight. The centrifugation for 30 min at 3000 ×g was achieved. The precipitated fractions were resolubilized in a minimum volume of phosphate buffer (0.1M, pH 7.2) . The dialysis procedure via a 14 kDa cut-off membrane for 24 h. was done against the same buffer at 4˚C with six buffer changes. The centrifugation for 5 minutes to obtain a clear supernatant (Mahdi, Younus, Al-Burgus, & Al-Abbasy, 2025).

Ion Exchange Chromatography

On a column containing CM-cellulose resin, the dialyzed solution was loaded. A protein was eluted using phosphate buffer at a flow rate of 0.8 ml/min (three milliliters/fraction) and monitored at 280 nm. Following detection, pooling, and lyophilization, the fractions with PPO activity were employed in the subsequent studies (Alrushdi *et al*., 2025).

Gel Electrophoresis

SDS-PAGE was carried out in a mini gel equipment (Bio-Rad Mini-PROTEIN Tetra Cell, Bio-Rad) as mentioned by Laemmli (Laemmli, 1970). Protein solutions were denatured by heating for 5 minutes at 99 °C (Eppendorf Thermomixer Comfort). The samples were then put in 5% stacking and 9% resolving polyacrylamide gels before being run at 120 V. The gel was stained using Coomassie Brilliant Blue G-250 (Al-Taie, Alaboo, Al-Abbasy, & Taqi, 2026). The protein markers were used to calculate the molecular weight of the purified PPO.

Inhibition Mode of PPO

The inhibition of purified mango PPO was examined in the presence of EEPE and AEPE as inhibitors. The activity was monitored at 420 nm using (0.25-3 mM) of substrate after incubation of 0.2 ml of enzyme with 0.1 ml of inhibitor for 30 minutes (Alabbas, Salih, & Al-Abbasy, 2022).

Statistical Analysis

All acquired data were represented by mean ± S.D. The data were processed using the SPSS Statistics programme for one-way ANOVA analysis followed by Duncan's Multiple Range test. The comparisons between various groups were carried out, and a p-value of <0.05 indicated the statistically significant differences.

Results and Discussion

Table 1 shows the scavenging impact of eggplant peel extracts on DPPH radicals. Both extracts were found to have antioxidant properties. EEPE showed higher scavenging properties (0.66 mg.gm^{-1}) than AEPE **(**0.52 mg. gm^{-1}) compared to vitamin C (0.73 mg. gm^{-1}).

Table 1- The scavenging effect of eggplant peel extracts (mg.g^{-1}) for quenching DPPH radicals

Solution	mg/g^{-1}
Vitamin C	0.73± 0.015
EEPE	0.66± 0.021
AEPE	0.52± 0.035

The DPPH free radical scavenging activity of the ethanolic extract of eggplant pulp powder was evaluated using the common antioxidant ascorbic acid as a standard. The antioxidant activity was observed to be concentration-dependent and to be significantly greater than that of ascorbic acid (Nasraldeen Majeed, 2024; Salih & Sadoon, 2026). The antioxidant activity of ethanolic and water extracts from different parts of eggplant was evaluated. The extracts of peel and calyx parts exhibited a higher DPPH radical scavenging activity in both extracts (Jung, Bae, Jo, Jo, & Lee, 2011). The biological process underlying the antioxidant property is linked to the scavenging of free radicals, specifically hydroxyl radicals, and metal chelation (Akanitapichat, Phraibung, Nuchklang, & Prompitakkul, 2010). Eggplant peel is frequently discarded as biological waste, resulting in significant losses of organic matter that typically contains high levels of bioactive compounds (Agnello *et al*., 2020).

Several studies have revealed that plant extracts are accomplished of inhibiting PPO activity in foods. The ability of extracts to inhibit PPO varies depending on the phytochemicals they contain and the antioxidant potential of the compounds. Further, Fig. 1Fig. 1 shows an evaluation of the inhibitory effects of EEPE and AEPE extracts compared to vitamin C on PPO.

Compared to the untreated juice sample, EEPE showed an inhibitory effect of 45.87%, which is better than AEPE, which inhibited the enzyme by 16.84%. However, both extracts were less effective than vitamin C, considered a potent antioxidant, with a 56.79% inhibitory effect. The ability of these extracts to inhibit oxidation processes may rely on several mechanisms, involving reduction activity, radical scavenging, and chelating of metal ions (Billaud *et al*., 2005).

Effect of Extracts on Browning Intensity and Intermediate Compounds in MJ during Storage

According to Ali *et al*. (Ali, Al-Burgus, Al-Abbasy, & Haddad, 2026) absorbance at 294nm and 420nm indicates the existence of colorless intermediate particles and browning compounds. The browning intensity and intermediate compound values of MJ after treatment with 5% of EEPE and AEPE and storing for 15 days at 4°C are shown in Fig. 2 and Fig. 3. The values of MJ-treated AEPE were higher than those of EEPE-treated at all storage times. These results suggest that EEPE could diminish the increase in absorbance at 420 and 294 nm in MJ during storage more than AEPE. The EEPE might contain phenolic compounds capable of inhibiting the PPO isozyme in MJ, with each phenolic compound potentially having different specific inhibitory activities on various PPO isozymes. These compounds have structures similar to the substrate and might inhibit browning (Ajila, Rao, & Rao, 2010). Regardless of the type of natural anti-browning chemical utilized, the findings correspond with those documented by others (Jirasuteeruk & Theerakulkait, 2020; Shomodder, Hossain, Das, Ranganathan, & Mazumder, 2021).

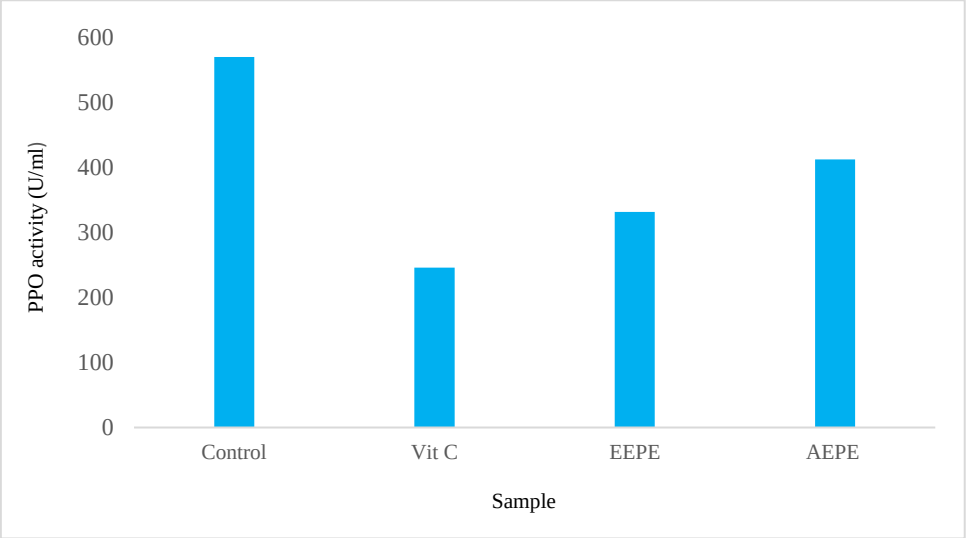

Fig. 1. Comparison the effects of eggplant peel extracts and vitamin C on purified PPO from Mango pulp

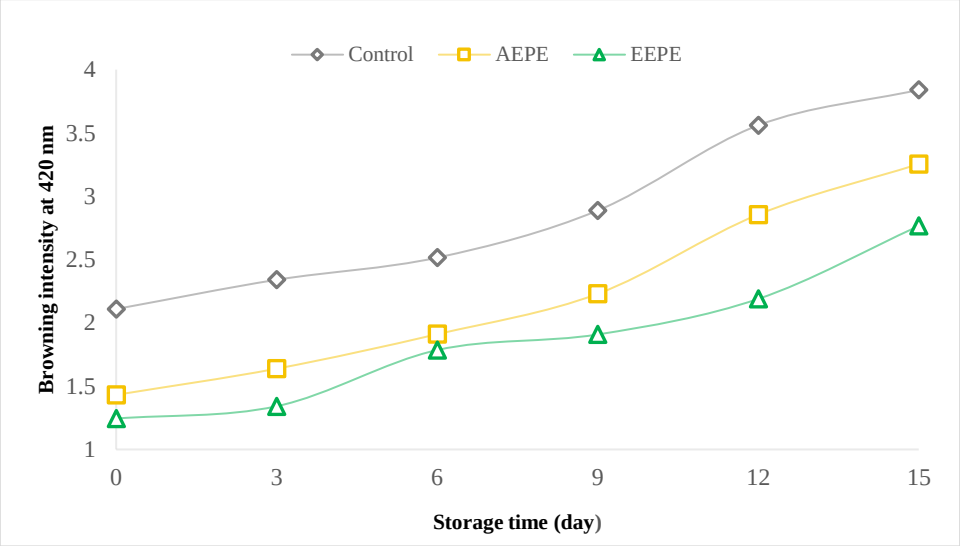

Fig. 2. Extracts effect on browning intensity in MJ during storage

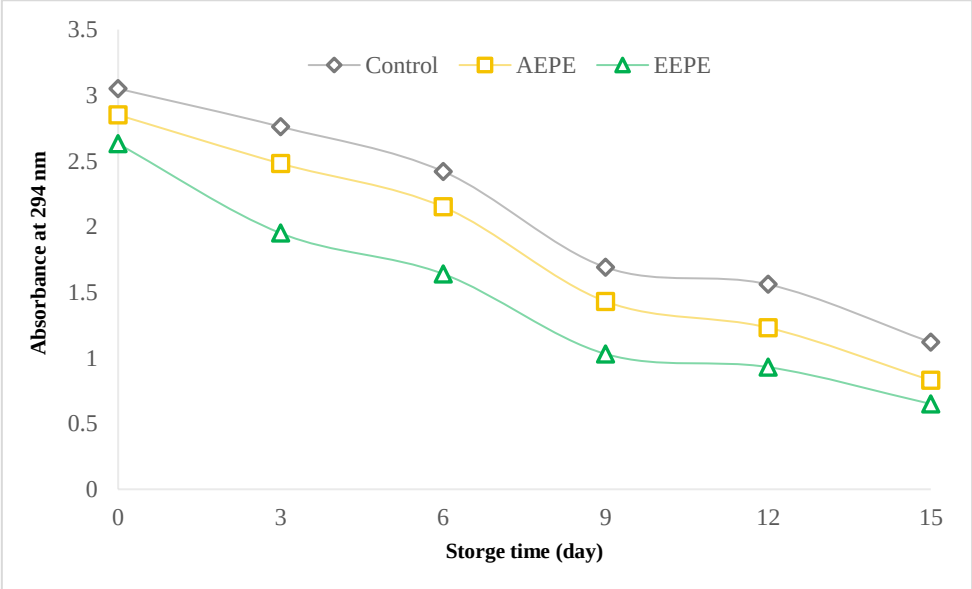

Fig. 3. Extracts effect on intermediate compounds in MJ during storage

Effect of EEPE and AEPE Addition on the PPO Activity during Storage

The impact of EEPE and AEPE on mango juice PPO enzymatic activity during storage for 15 days at 4°C is presented in Fig. 4. The results showed that both extracts could inhibit PPO compared to the control group for all storage times. However, the percentage of enzyme inhibition in the presence of EEPE was stronger than that of AEPE in the treated juice samples.

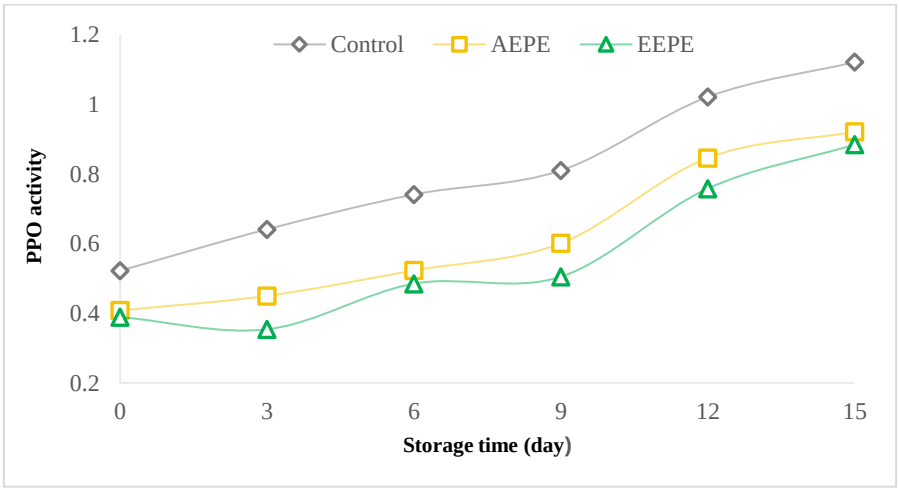

Fig. 4. Inhibitory effect of extracts on PPO in MJ during storage

Several studies have attempted to inhibit PPO activity by utilizing extracts from sources rich in antioxidant compounds. For example, it has been found that aqueous extracts of mango peels of several cultivars inhibited the activity of PPO in potato puree to varying degrees (AL-abbasy, M Mahdi, Younus, Sheej Ahmad, & Al-Azzawi, 2025; Jirasuteeruk & Theerakulkait, 2020). Previous results indicated that the oil extract of cinnamon bark showed 40% inhibition of PPO activity in mushrooms (Marongiu *et al.*, 2007). It was also observed that the aqueous extract of ginger has an anti-tanning effect by reducing the enzymatic activity of PPO of *Annona muricata* and *Musa acuminata* (Weerawardana *et al.*, 2020). Different concentrations of garlic and onion extracts presented a higher inhibitory

effect on the yam PPO activity (Yapi, Gnangui, Dabonné, & Kouamé, 2015). Such inhibition may be due to the high total phenolic content in these extracts.

Effect of EEPE and AEPE Addition on POD Activity during Storage

The effect of eggplant peel extracts on POD activity was evident Fig. 5. A gradual increase in POD activity was observed during storage at 4°C for untreated juice, reaching its maximum on the 15th day. When juice samples were treated with EEPE and AEPE extracts (5%), POD activity decreased significantly compared to untreated juice samples. The results demonstrated that EEPE was more effective than AEPE in inhibiting POD.

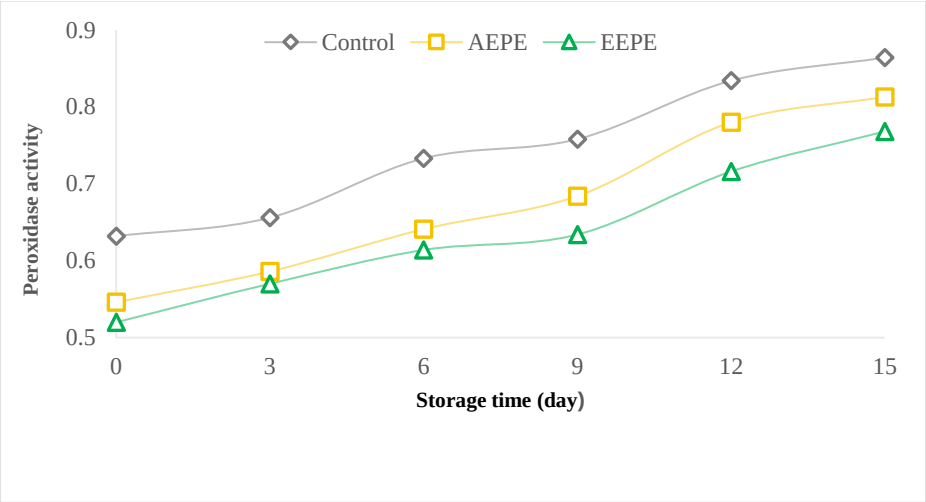

Fig. 5. Inhibitory effect of extracts on POD in MJ during storage

POD is an enzyme that catalyzes the oxidation of phenols by peroxide, which is the major cause of enzymatic browning in fruits and vegetables (Oms-Oliu *et al*., 2010). During litchi fruit storage, POD activity in the pericarp steadily rose in tandem with the skin browning index (Zhang, Pang, Xuewu, Ji, & Jiang, 2005). POD activity increased steadily at 4°C throughout the storage period, with the highest activity level recorded in untreated mandarin fruit. In contrast, enzyme activity at 8°C rose during the first two weeks, followed by a sharp decline for the remainder of the storage period. (Tao, Wang, Luo, & Yan, 2018). Each vegetable substrate under study had a unique reaction of POD activity to the antioxidant treatment, which was contingent upon storage conditions. Freshly cut veggies are reported to be best preserved when stored in a refrigerator (Xu *et al*., 2012). The use of antioxidant solutions, such as tea tree essential oil and propolis extract, at 5°C under optimal storage conditions for 14 days inhibited POD activity in butternut squash throughout the storage period. Furthermore, the use of propolis delayed browning and preserved the product's visual quality for a longer period compared to untreated sample (Alvarez, Moreira, & Ponce, 2015).

Effect of Extracts on Phenolic Content in MJ during Storage

Fig. 6 depicts the total phenolic content (mg/100 ml) in MJ treated with EEPE and AEPE throughout storage. The amount of phenolic content significantly declined during storage at 4°C, reaching its minimum on the 15th day. Treatment of MJ with 5% of EEPE and AEPE showed a significant increase compared to the untreated juice at 0 and 15th day of storage. It is clear from the diagram that the alcoholic extract had a better effect in maintaining the level of phenolic compound content than the aqueous extract. This may be due to high levels of active phenolic

compounds in these extracts, which can reduce PPO activity and thus prevent the oxidation of the phenolic compounds initially present in MJ and preserve them during the storage period.

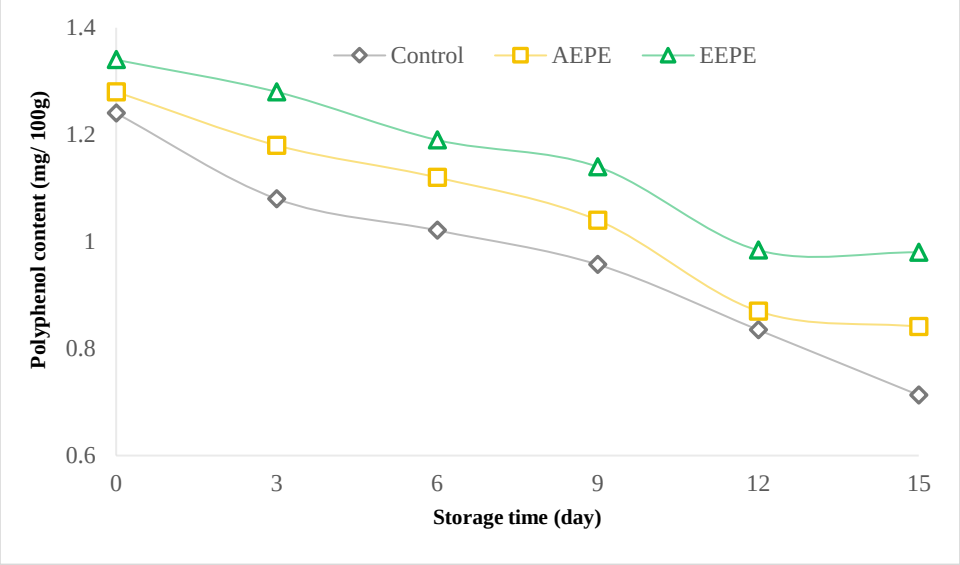

Fig. 6. Extracts effect on polyphenol content in MJ during storage

Similarly, Altunkaya *et al*. (2013), reported a significant increase in the phenolic content of apple juice treated with pomegranate peel extracts (Altunkaya *et al*., 2013). An apparent increase in the concentration of phenolics was also observed, directly proportional to the concentration of the added natural extracts, as in apple juice (Phaiphan, Panichakool, Jinawan, & Penjumras, 2019) and quince juice (Rashan & Al-Abbasy, 2021) during storage.

Enzyme Purification

Precipitation of the enzyme-containing protein with 30-80% ammonium sulfate increased the specific activity of PPO to 2449.11 units /mg protein, with a purification fold of 5.61 compared to the crude. After dialysis to remove salts, peptides, and low-molecular-weight compounds, the results indicated that PPO was more purified by 7.00-fold. The protein concentration decreased after this step, and the specific activity value increased to 3055.9 units /mg protein (Table 2Table 2). To obtain almost completely pure enzyme, the CM-cellulose ion exchange column chromatography was used, and the elution profile showed a single band (Fig. 7Fig. 7) with a specific activity of 6573.42 compared to the crude enzyme, and the purification fold was raised to 15.07.

Table 2- Purification steps of PPO from mango pulp

Purification steps	Volume (ml)	Total protein (mg)	Total activity U*	Sp. Ac. (U/mg protein)	Yield %	Purification Fold
Crude extract	66.5	55.86	24361.61	436.12	100	1.0
Ammonium sulfate	12	6.48	15870.24	2449.11	65.14	5.61
Dialysis	13.2	4.818	14723.28	3055.**9**	60.43	7.00
CM-Cellulose	24.2	1.76	18301.39	10398.51	75.12	23.84

* The unit is defined as the amount of enzyme that oxidizes 1 micromole of catechol per minute at 25 °C.

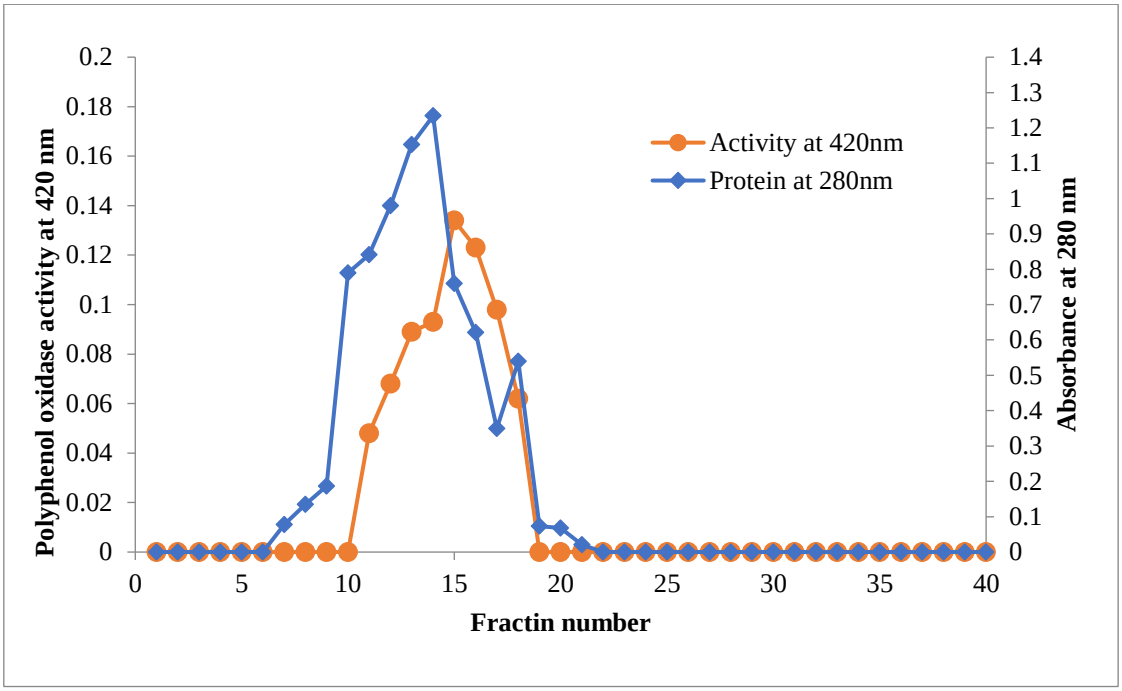

Fig. 7. Elution profile of mango pulp PPO by ion exchange chromatography

Numerous studies have used ion exchange chromatography to purify PPO from various plant tissues. This technique relies on the positive or negative charges present on the exchanger used for separation. The PPO was purified from *Prunus domestica L.* 36-fold using DEAE-cellulose chromatography (Siddiq, Sinha, Cash, & Hanum, 1996). One peak of PPO was obtained from the Mirabelle plum with a purification fold of 26.31 after passing the enzyme extract on a column containing the cationic exchanger, CM-cellulose (Younus, Mahdi, Al-Abbasy, & Sheej Ahmad, 2025). DEAE Sephadex A-50 and DEAE-Sepharose columns were used to purify PPO from the seeds of Melon (Adeseko & Fatoki, 2021) and the leaves of *Cleome gynandra* L. (Gao, Liu, & Xiao, 2011), respectively.

Molecular Weight Determination Using SDS-PAGE

This technique has been used to document the molecular weights of PPO, with masses ranging from 25 kDa for pineapple (Das, Bhat, & Gowda, 1997) to 120 kDa for beans (Paul & Gowda, 2000). In our analysis, the purified PPO obtained after ion exchange chromatography performed as a single protein band on a gel with a molecular weight of 54 kDa (Fig. 8Fig. 8). This result is very close to the PPO enzyme purified from African bush mango peel, which was observed to have a molecular weight of 53 kDa (Adeseko *et al.*, 2021). It is slightly less than that purified from Mexican Golden Delicious apples (58 kDa) (Macheix, Fleuriet, & Billot, 1990), Turkish Alyanak apricots (57 kDa) (Ünal & Şener, 2016), and potato (50 kDa) (Aksoy, 2020). On the other hand, the molecular weight of several mango parts was reported and found to be significantly lower than what we obtained (Adeseko, Sanni, & Lawal, 2022; Palma-Orozco, Marrufo-Hernández, Sampedro, & Nájera, 2014).

Inhibition Type of PPO

The inhibition type of the purified mango PPO activity was examined in the presence of EEPE and AEPE extracts. It was verified by drawing a Lineweaver-Burk plot using various concentrations of a substrate. The results demonstrated a competitive inhibition type with EEPE (Fig. 9Fig. 9). The value of Vmax was approximately 0.208 units /ml/ min. The Km

value increased from 1.020 to 2.127 mM. When using the AEPE as an inhibitor, Fig. 10 shows that the inhibition type was non-competitive. The Vmax value decreased from 0.208 to 0.116 units/ml/min. The Km value was stable at 1.020 mM.

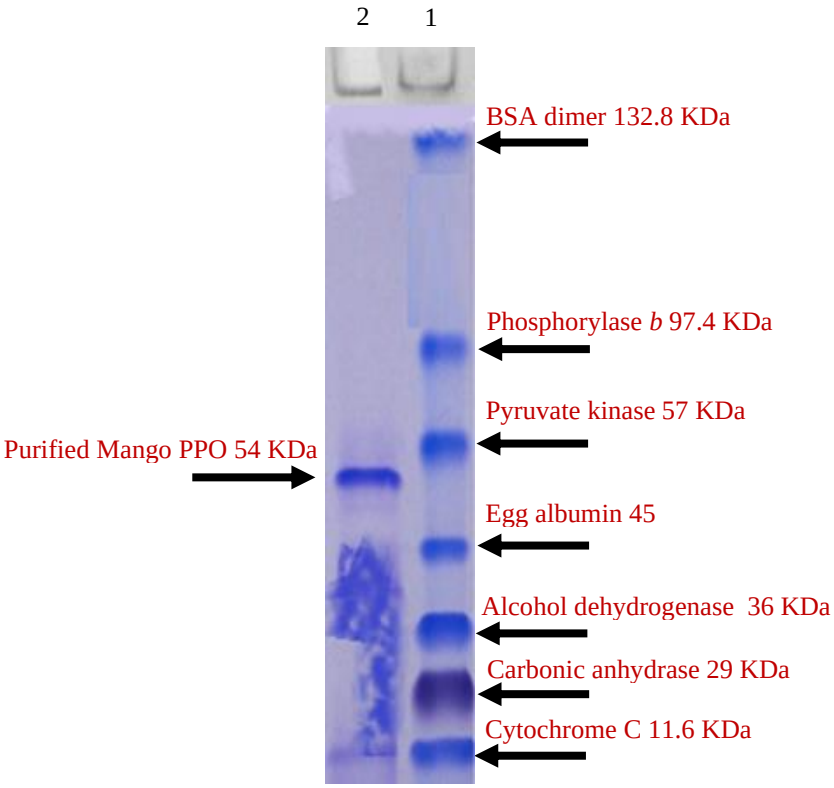

Fig. 8. Photograph of SDS-PAGE electrophoresis. Lane 1: Marker protein bands. Lane 2: Band of purified PPO from the mango of molecular weight approximately 54 kDa

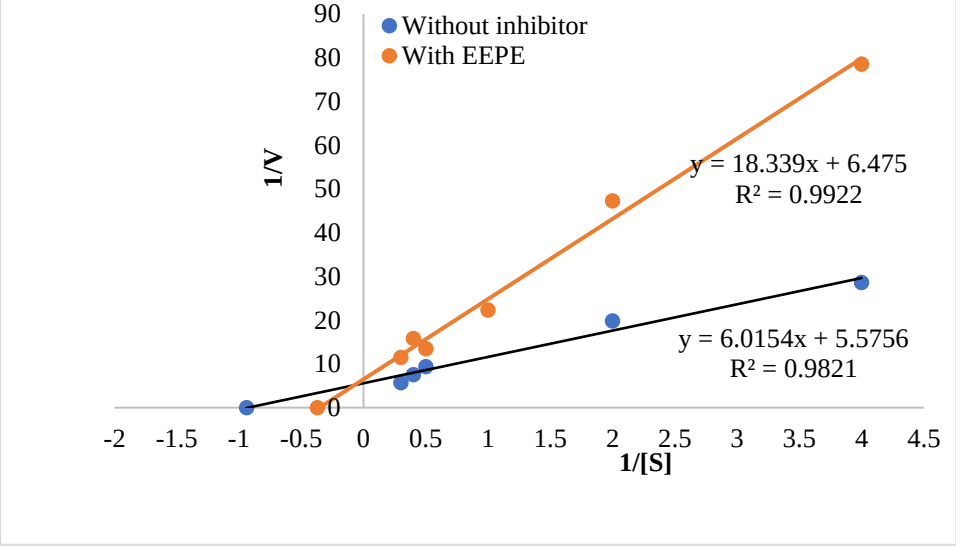

Fig. 9. Inhibition mode of PPO by ethanolic eggplant peel extract

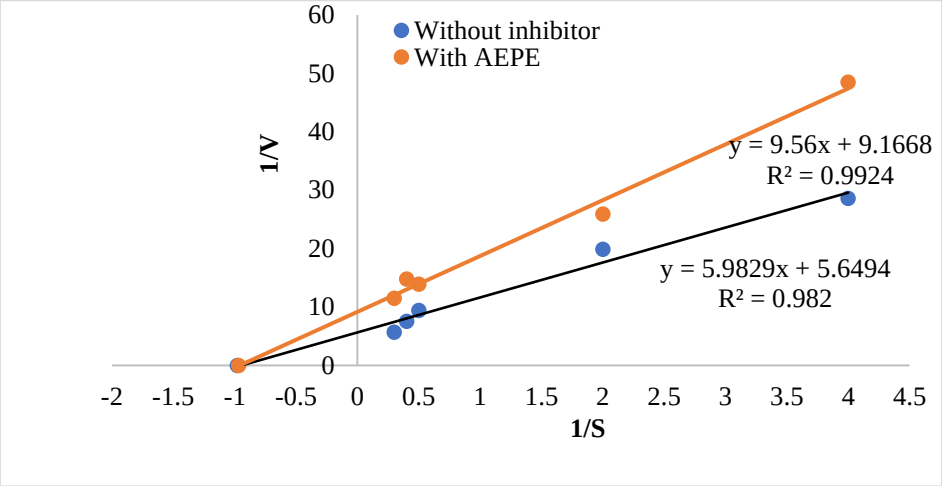

Fig. 10. Inhibition mode of PPO by aqoeous eggplant peel extract

Competitive inhibition may be attributed to the presence of high levels of phenolic compounds in these extracts, as they are structurally similar to the substrate and are able to bind to the active site of the enzyme to hinder the process of enzyme reaction (Sadoon & Al-Abbasy, 2026; Zheng, Shi, & Pan, 2012). Noncompetitive inhibition, on the other hand, may be due to chelation of the metal copper ions present in the enzyme (Al-Barwari & Abbas, 2026; Sun *et al*., 2012). Many studies have indicated the inhibition of polyphenol oxidase purified from plant sources using extracts such as *Rhuscoriaria* L. (Salim, Al, & Sadoon, 2026), *Rosa roxburghii* (Yu *et al*., 2021), chili pepper (Lim & Wong, 2018), onion (Yapi *et al*., 2015), oregano, and lovage extracts (Sierocka & Świeca, 2024).

Conclusion

The findings of this study indicated that eggplant peel extracts, particularly the ethanolic extract (EEPE), exhibit strong antioxidant and anti-browning activities. EEPE showed higher DPPH free-radical-scavenging activity and more effective inhibition of PPO and POD enzymes than the aqueous extract (AEPE). This reduced browning intensity and improved the preservation of phenolic compounds in mango juice during storage. Kinetic analysis confirmed that EEPE is a competitive inhibitor of PPO, whereas AEPE is a non-competitive inhibitor. The successful purification and characterization of mango PPO further confirmed its enzymatic nature. Overall, utilizing eggplant peel, a relatively underutilized agro-industrial by-product, presents an environmentally friendly and efficient approach to preventing EB and oxidative spoilage in fruit juices. These findings justify further research into incorporating such extracts into food preservation programs, aligning with global efforts toward waste valorization and cleaner-label foods.

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Authors Contirbution

M.G.A: Methodology, Resources, Software, Validation, Software, Visualization. **O.H.A:** Conceptualization, Data curation, Formal analysis, Investigation, Writing– original draft. **R.T.A:** Methodology, Project administration, Supervision, Resources, Software, Validation, , Writing–review and editing. **O.Y.A:** Conceptualization, Data curation, Writing–original draft, Investigation.

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Abbreviation

Fruit juice (FJ); Eggplant Peel Ethanolic Extract (EEPE); Eggplant Peel Aqueous extract (AEPE); Mango Juice (MJ); Enzymatic browning (EB); Polyphenol oxidase (PPO); Peroxidase (POD).

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مهار قهوه‌ای شدن آنزیمی در آب انبه با استفاده از عصاره‌های پوست بادمجان غنی از آنتی‌اکسیدان: یک مطالعه سینتیکی و نگهداری

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چکیده

فرآیندهای قهوه‌ای شدن آنزیمی تأثیر منفی بر بسیاری از محصولات غذایی، مانند آبمیوه‌های تازه، به‌ویژه خواص حسی و ارزش غذایی آنها دارند و در نهایت منجر به رد محصولات آسیب‌دیده توسط مصرف‌کنندگان می‌شوند. از آنتی‌اکسیدان‌های طبیعی برای مقابله با این پدیده، حفظ این محصولات و جلوگیری از افت کیفیت آنها استفاده شده است. این تحقیق به بررسی فعالیت‌های آنتی‌اکسیدانی و مهار آنزیمی عصاره‌های اتانولی پوست بادمجان (EEPE) و آبی (AEPE) و همچنین ظرفیت آنها در مهار رادیکال‌های DPPH و مهار آنزیم‌های قهوه‌ای شدن در آب انبه (MJ) در طول نگهداری در یخچال می‌پردازد. EEPE ظرفیت مهار رادیکال DPPH بیشتری (۰/۶۶ میلی‌گرم بر گرم) نسبت به ۰/۵۲ AEPE میلی‌گرم بر گرم) داشت، اگرچه هر دو ترکیب از ویتامین C (۰/۷۳ میلی‌گرم بر گرم) ضعیف‌تر بودند. سنجش‌های قهوه‌ای شدن آنزیمی نشان داد که EEPE فعالیت‌های PPO و POD را در آب انبه (MJ) سرکوب می‌کند و به کاهش شدت قهوه‌ای شدن و حفظ بهتر محتوای فنلی پس از ۱۵ روز در دمای ۴ درجه سانتی‌گراد، بیشتر از AEPE، کمک می‌کند. PPO پالپ انبه خالص‌شده تقریباً ۵۴ کیلو دالتون وزن مولکولی داشت و با استفاده از رسوب سولفات آمونیوم، دیالیز و کروماتوگرافی تبادل یونی CM- سلولز با مقدار خالص‌سازی ۱۵/۰۷ به‌طور مؤثر استخراج شد. مطالعات سینتیکی نشان داد که EEPE مانند یک مهارکننده رقابتی PPO عمل می‌کند، در حالی‌که AEPE مهار غیررقابتی از خود نشان می‌دهد. این پاسخ، پتانسیل پوست بادمجان، ضایعات کشاورزی، را به‌عنوان یک ماده طبیعی برای نگهداری مواد غذایی به‌دلیل فعالیت‌های آنتی‌اکسیدانی و ضد قهوه‌ای شدن آن نشان می‌دهد.

واژه‌های کلیدی: آب انبه، پراکسیداز، پلی‌فنل اکسیداز، عصاره پوست بادمجان، فعالیت آنتی‌اکسیدانی، قهوه‌ای شدن آنزیمی، نگهداری مواد غذایی

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