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The Inhibitory Effect of *Prosopis juliflora* Pods Protein Hydrolysate on Polyphenol Oxidase and Browning of Apple Slices with Refrigerated Storage Stability

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ABSTRACT

The study was conducted exploit the proteins of the pods of some types of plants, such as *Prosopis juliflora* pods, that are considered by-products in many countries and considered a good source of protein in the preparation of protein hydrolysers and evaluating their effect on inhibiting brown discoloration of apple slices and compared with anti-browning agents (ascorbic acid, acetic acid, and sodium chloride) when stored in the refrigerator for 0, 5 and 8 days. The chemical composition of the moisture, protein, fat, ash, and carbohydrate of the *P. juliflora* pods was estimated, then the hydrolysis process was carried out using the enzymes trypsin and papain for 300 minutes. Amino acids and FTIR analysis of protein hydrolysates were determined. Significant changes ($p\leq0.05$) in pH, total soluble solids, and non-significant changes in titration acidity of apple slices treated with protein hydrolysis and anti-browning agents were studied and significantly decreased ($p\leq0.05$) in the activity of polyphenol oxidase until the end of the storage. The brown coloration decreased when treated with protein hydrolysates compared to other treatments, but non-significant changes. As a result, apple slices can be preserved with protein hydrolysers for several days in the refrigerator.

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1- Introduction

Consumers now choose to eat fresh foods with high nutritional content, low processing, and no synthetic additives due to the increased interest in healthy eating (Wibowo *et al*., 2019). Vegetables' appearances can influence both consumer acceptance and the decision to buy. (Carneiro *et al*., 2022). Apple contains bioactive components, particularly phenolic compounds like hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols, dihydrochalcones, and anthocyanins. These compounds are linked to several health benefits, including antioxidative activity, anti-inflammatory activity, hypocholesterolemic effect, cardiovascular protective effect, antidiabetic activity, and anticancer activity (Patocka *et al*. 2020).

One of the biggest issues with minimally processed meals, such as fresh-cut fruits and vegetables (lettuce, potatoes, eggplant, etc.), fruit juices, and so on, is enzymatic browning (Moon *et al*., 2020). Polyphenol oxidase (PPO) and peroxidase (POD) are two essential enzymes that cause it (Zhu *et al*., 2022). Enzymatic browning is responsible for about 50% of fresh fruit loss; giving fruits an unattractive brown colour also lowers their nutritional value and sensory qualities (Moon et al., 2020). As a result, scientists and the food industry have recently started looking into the best way to reduce enzymatic browning in fruits that have had minimal processing. Other fruits and vegetables, rather than apples, were the topic of earlier studies that discussed how to reduce enzymatic browning (Moon *et al*., 2020; Tinello and Lante, 2018; Xu *et al*., 2020). Zhu *et al*. (2022) also investigated various fruit juices for both enzymatic and nonenzymatic browning. Synthetic and natural anti-browning agents were utilized to prevent enzymatic browning in apple products. The utilization of sulfates as anti-browning agents in the food sector has been limited because of their impact on human health (Oliphan *et al*., 2012, Stohs and Miller, 2014). Barbagall *et al*. (2012) utilized natural inhibitors in place of sulfites to hinder browning. Recent research indicates that amino acids possessing antioxidant properties, like sulfhydryl groups, are effective in inhibiting PPO enzymes (Altunkaya, 2011; Moon *et al*., 2020). Instead of using sulfates, proteins and peptides can be utilized as natural sources of anti-browning PPO activity according to Schurink *et al*., 2007. Hydrolyzed proteases are made from various sources like animal, plant, and fungal materials with increased PPO inhibitors, creating bioactive peptides and proteins that may eventually replace synthetic compounds on the market in the future (Zamora-Sillero *et al*., 2018). Most of the 44 nitrogen-fixing tree species in the Prosopis genus are found in the arid or semiarid regions of the Americas. Prosopis pods consist of 25%–30% seeds (episperm, endosperm, and cotyledons) and 70%–75% pericarp (epicarp, mesocarp, and endocarp), with a sweet taste. Mesquite or carob flour is produced by grinding entire, mature mesquite pods, and is characterized by its brown hue and scent reminiscent of coffee (Felker *et al*., 2003). The nutritional content and digestibility of protein in mesquite flour differ based on the type. Gonzales-Barron *et al*. (2020) have also found sulfur-containing amino acids (E, R, D, and L), lysine, and high antioxidant activity total phenolic compounds in mesquite flour. The examined of nutritional content of the skipy fruits of this plant by many of researches (Kingòri *et al.* 2011).The *P. juliflora* pods protein levels ranging from 7.1% to 18.7%, fat levels ranging from 0.4% to 7.3%, and ash levels ranging from 1.4% to 7.15%. Al-Harthi and colleagues' (2018) research determined that the entire mesquite plant pods contain 15.2% protein. The research discover on examining how plant by products could be utilized as protein sources to make protein hydrolysate using

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different proteases such as trypsin and papain. It also investigated how *P. juliflora* pods protein hydrolysates could minimize changes to the physical and chemical qualities of cut apples and other fruit while blocking polyphenol oxidase activity and browning.

2-MATERIAL AND METHODS

All of the chemicals and enzymes were purchased from (Sigma- Aldrich, Steinheim, Germany) and (Sigma- Aldrich Co., St. Louis, Mo., USA).

Approximate composition *P. juliflora* pods

The collected *P. juliflora* pods, which were dry and ripe, came from a gardening field in Basrah District, Al-Haritha Division, Iraq. The approximate composition of *P. juliflora* pods (moisture, protein, fat, ash, and carbohydrate was analyzed using the AOAC (2012) standard method.

Preparation *P. juliflora* Pods

The pods were milled in an electric mill, sieved, and defatted by using hexane solution as described by Kim *et al. (*2021) with minor modifications. For defatting, the ratio of ground and solvent was 1:2 w/v at room temperature. The solvent was removed. To get a clear solvent, repeat the process five times. The solvent was evaporated.

Preparation of Enzymatic Hydrolysates:

The enzymatic hydrolysis was prepared in water deionized at a ratio of 1:6 w/v. The mixture was heated in a water bath at 85 °C for 20 minutes (Guerard *et al*., 2002). The optimum conditions of enzymes from pH 7 and 8, temperature 65 and 37 °C for each papain and trypsin, were adjusted respectively Sun *et al.* (2021). The ratio of enzymes to the substrate was 0.3% added. The hydrolysis reaction continued for 300 min, and the enzyme inhibition at a temperature of 90 °C for 15 min. Then, the mixture was centrifuged at 500xg/15 min, separated the supernatants from insoluble materials, and adjusted pH to 7. Lyophilized hydrolysates were kept at 18-°C until use.

Degree of Hydrolysis Measurement (DH)

The degree of hydrolysis was calculated using the method of Hoyle and Merrit (1994), as reported by Morais *et al*. (2013), by calculating the percentage of soluble protein in 10% Trichloroacetic acid (TCA) 1ml of hydrolysate mixed with 1ml of 10% TCA was left for 30 min before being centrifuged at 4000 x g for 15 min, The Buriet method was used to quantify the dissolved protein by using Kit Buriet. Degree hydrolysis was calculated using the equation below.

$$DH\% = \frac{soluble\ protein\ in\ TCA\ \ 10\%}{total\ protein\ in\ substrate\ mg/ml}$$

Characteristics of Protein Hydrolysates

High Performance Liquid Chromatographic (HPLC) amino acids analysis

Levin and Grushka (1985) method was employed to determine the amino acids of protein hydrolysate. Using a High-Performance Liquid Chromatographic.

Fourier Transform Infrared (FTIR)

FTIR spectra were used to describe the structures of protein hydrolysis in dry form, which was combined with KBr, ground, and formed into a pellet. FTIR spectra were obtained using an FTIR spectrometer (Shemadzu Affinity-1. USA) with a 400–4000 cm–1 wave number range.

Apple Slices Preparation and Dipping Solutions

Red apples were obtained from the local markets in Basrah. Fruits that were

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uniformly sized were chosen, and any that had been bruised or infected were discarded. The selected apples were drained at room temperature after being washed gently with tap water. A stainless-steel knife was then used to cut the apples into 1 cm thickness ×3diameter slices. Apple slices were then dipped in water (control: T0) and other slices of apples were treated with protein hydrolysate T1: Trypsin, T2: Papain, T3: Acetic acid, T4: Ascorbic acid and T5: sodium chloride at concentration 0.5%, for 15 min then samples were allowed to dry on surface (at around 25°C); the Samples were stored in plastic bags in a refrigerator at 4°C for 0, 5 and 8 days.

Preparing Crude PPO extract and Assaying Enzyme Activity:

According to Matsuno and Uritani's (1972) method, the enzyme was extracted by homogenizing 20 grams of sliced fruit in 250 mL of cold potassium phosphate buffer (0.2 M, pH 7). The cheese cloth was used to filter the homogenate, which was then centrifuged for 10 minutes at 5000 xg. The enzymatic activity was determined using the supernatant**.**

Polyphenol oxidase activity was evaluated using the Ying and Zhang (2008) method, which involved detecting the increase in absorbance at 420nm with a spectrophotometer. 2.0 mL of catechol combined with 0.9 mL of 0.2M sodium acetate buffer pH 4.0 and 0.1 mL of enzyme solution. The (blank) contained 2.0 ml of the same substrate solution as well as 1.0 ml of 0.2M sodium acetate buffer. Each sample was tested three times. The formula for calculating enzyme activity inhibition was:

Inhibition (%) = (1-PPOtreatment)/PPO control × 100

Browning Determination

To calculate the browning, a 5 g apple slice was extracted in 100 mL of 65% (v/v) ethanol. One hour at room temperature was spent stirring the mixture. Filter paper Whatman No. 1 was used to filter the extract. A spectrophotometer was used to detect 420 nm absorbance (Supapvanich *et al*., 2 011).

Physio-chemical analysis:

The juice of apple slices was extracted mechanically. Physicochemical properties such as total soluble solids (TSS), pH-value, and titration acidity (TA) were analyzed. The pH of the juice was measured by using a pH meter. TSS was measured by using an Abbe Refractometer. According to AOAC (2012), titration acidity (TA) was measured by Rangana (2010).

Statically analysis:

Statistical techniques were used to analyze variance in the data Gen Stat Release 12.1. At a level (0.05) threshold of significance, all comparisons were conducted by least significant difference, which was used to assess specific differences (LSD).

3- Result and discussions

Proximate composition

Table 1 shows the proximate chemical composition of *P. juliflora* pods. Moisture, protein, fat, ash, and carbohydrate content were recorded at 8.37%, 11.43%, 3.27%, 5.32%, 71.53% respectively. According to Kingòri *et al*. (2011), the protein content of *P. juliflora* is 7.1–18.5%, the fat content is 0.4–7.3%, and the ash content is 1.4–7.15%. Al-Harthi *et al*. (2018) found that the proximate composition of *P. juliflora* contained 8.90% moisture**.**

Table 1: Proximate chemical composition of *P. juliflora* pods

composition %	
moisture	8.45
protein	11.43
fat	3.27
ash	5.32
carbohydrate	71.53

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Degree hydrolysis (DH)

Fig.1 shows the degree of hydrolysis (DH) of *P. juliflora* pods by using Trypsin and Papain enzymes. Significant increased DH with increased time hydrolysis; the highest DH was recorded at 41% for trypsin compared to 40% for papain enzyme at 300 min of hydrolysis. The results agree with Arteagaa *et al*. (2020), who recorded the highest DH to trypsin enzymes compared with DH Papain enzyme after 120 min of pea protein isolated. From the results observed, DH depended upon the period and type of hydrolysis enzymes.

Characteristics of Protein Hydrolysis

Amino acids HPLC analysis

Major amino acids were determined in trypsin *P. juliflora* pods hydrolysis figure (2), 0.5% Aspartic acid, 11.4% Glutamic acid, 2.8% Serine, 5% Histiden, 2.6% Tyrosine, 4.9% Cystine, 27.1% Valine, 39.9% Methionine , 5.7% Isoleucine and 0.1% Leucine, respectively. Papain *P. juliflora* pod's hydrolysis figure (3), 30% Aspartic acid, 2.1%Glutamic acid, 5.8% Serine, 5% Histiden, 7.7% Cystine, 9.2% Phenylalanine 18.1% Isoleucine, 12.8% leucine and 3.1% lysine respectively**.**

Fourier Transform Infrared (FTIR)

FT-IR spectroscopy can be used to determine the chemical structure of biological material based on the wavelength and intensity of IR radiation absorbed by the sample. This method is frequently used to characterize the secondary structure of polypeptides, proteins, and their hydrolysates. The impact of different enzymes on the structural modifications of hydrolysis was investigated using FT-IR spectroscopy (Meutter and Goormaghtigh, 2021). Figures 1 and 2 displays the FTIR spectra of P. juliflora pods that were hydrolyzed using pepsin and papain enzymes. The FTIR spectra of the hydrolyzed P. juliflora barks showed specific absorption peaks near amide bands A and B, as well as I, II, and III. When the NH group of the peptide participates in hydrogen bonding, the amide band A, found in Figures 4 and 5, is linked to N-H stretching vibrations according to Purna Sai and Babu (2001). According to Payne and Veis (1988; Jackson *et al*. 1995), the amide bands II (1419.35–1414.53 cm–1) and III (1083.8–1014.37 cm–1) represented N–H flexural vibrations associated with C–N stretch vibration.

Physiochemical analysis

pH-value

Figure 6 shows that the pH values of apple slices increased significantly ($p \leq 0.05$) during refrigerator storage. The pH value differed according to the treatment. T0 recorded a lower pH of 3.09, followed by T2 and T1, while T5 had the highest pH of 4 at 0 day storage. The pH value for T0 increased when storage time increased compared with the pH value for T3, T4, and T5 treatments. The pH values of T1 and T2 remained constant during storage. This may be due to the role of amino acids, which behave as buffer solutions and help stabilize pH. Additionally, the type and effectiveness of anti-brown agents lead to the variation in pH values between the T3-T5 treatments.

Titration Acidity (TA)

Figure 7 shows a non-significant effect in the titration acidity (TA) of apple slices during refrigerator storage. The TA differed according to the treatment. The T0 recorded less TA at 0.0402 %, followed by T1 and T2, while T4 had the highest TA at 0 day storage. TA decreased when storage time increased of T0, T1, and T2, but the decrease in TA of T1 was lesser than T0 and T2, while observed increase in TA of T3, T4, and T5 at treatments after 8 days from storage. The increase in TA values may be

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due to a decrease in pH values, and the breakdown of amino acids and their conversion into other acids may be responsible for the increase in titration acidity. The results agreed with Supapvanich *et al.* (2011) when they studied the effect of cold storage in fresh-cut wax apples during cold storage; they did not notice significant differences in the values of TA.

Total Soluble Solids (TSS)

The results of total soluble solids (TSS) of apple slices are presented in figure 8**.** The TSS value increased significantly ($p \leq 0.05$) during refrigerator storage. The initial TSS of T0= 10.5, T1= 10.5, T2= 10.5, T3=11, T4= 11, and T5= 11%, respectively. TSS value decreased from 0 day at end period storage. T1 treatment had a higher TSS value at 8 days' storage was recorded. 9.1% compared to T0 and other treatments. The decrease in TSS content may be due to breaking down or precipitating some of the solid materials into other products, including the cracking of pectin into other acidic components. The results agreed with Supapvanich *et al.* (2011), who found that the TSS of fresh-cut wax apples decreased during cold storage.

Polyphenol Oxidase (PPO) Enzyme Activity

Figure 9 shows the polyphenol oxidase (PPO) enzyme activity of the apple slices significantly at $p \leq 0.05$. The maximum activity inhibitory of PPO was observed at T1: 84.67 % and T2: 86.42 compared to other anti-browning agents, and T0 was recorded at 98.55% in 0 day. After 8 days, the PPO activity decreased in all of the samples. Still, the highest effect was when treated with protein hydrolysis, especially T1 and T2, which were found to be more effective than other anti-browning agents, and less inhibitory activity was recorded for T0 treatment. The highest reduction of PPO activity by protein hydrolysis may be the role of amino acids and peptidase in chelating the necessary copper of the PPO's active site and interacting with the O-quinines (Girelli *et al* 2004). Furthermore, several amino acids, particularly sulfur-containing amino acids (methionine and cysteine), can reduce PPO activity (Brosnan and Brosnan,2006) or contain hydroxyl groups (Paungphet *et al*., 2015). Trypsin hydrolysis contains a high concentration of methionine, Cystine, and histidine, which can inhibit PPO activity and act as an antioxidant. So, the low storage temperature and loss of moisture content from the PPO slice can reduce PPO activity. According to Puangphet *et al*. (2015), sericin hydrolysate had the greatest ability to reduce PPO activity when compared to citric acid, sodium chloride, and ascorbic acid in fresh-cut apples, and Silva *et al*. (2018) discovered buffalo whey hydrolyzed inhibitor of PPO activity in sliced apples.

Browning:

Figure 10 depicts the browning effect of protein hydrolysis and anti-browning agents. The optical density of 420 nm (OD420) was used as the browning, with no significant difference at ($P > 0.05$). On 0 day, there was a slightly different in OD420 between the treatments compared to T0, which had a higher OD420. After 5 days, the T1 and T2 showed a lower value in OD420. This continued until the end of the storage period, 8 days compared to control and other anti-browning. This might be returned to amino acids and peptides, which inhibit browning. The result agreed with Silva *et al*. (2018) when they noticed inhibitor browning of apple slices treated with buffalo cheese whey hydrolyzed compared with the control.

4-Conclusion

The use of protein hydrolysis in controlling the polyphenol oxidase enzymes and browning

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reaction of apple slices helped improve the physicochemical properties as it reduced the decrease in total dissolved solids, stability in pH, and titration acidity. It is one of the substances used to inhibit the enzymatic brown reaction, but it was better than others, such as citric acid and sodium chloride, if the results showed the possibility of using hydrolysates as a natural alternative to the additives used to inhibit brown coloration in foods, in addition to containing amino acids that enhance the nutritional value of the products**.**

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7-Contributions

Alia Zyara Hashim: conceptualization, data curation, visualization, writing—original draft, writing—review and editing; Faleeha Hasan Hussein: conceptualization, supervision, writing—review and editing; Alaa M.S. Al-Baidhani: conceptualization, supervision, writing—review and editing.

Seyed Hadi Razavi, Correspondence and supervision, writing—review and editing.

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Competing Interests

The authors declare no competing interests.

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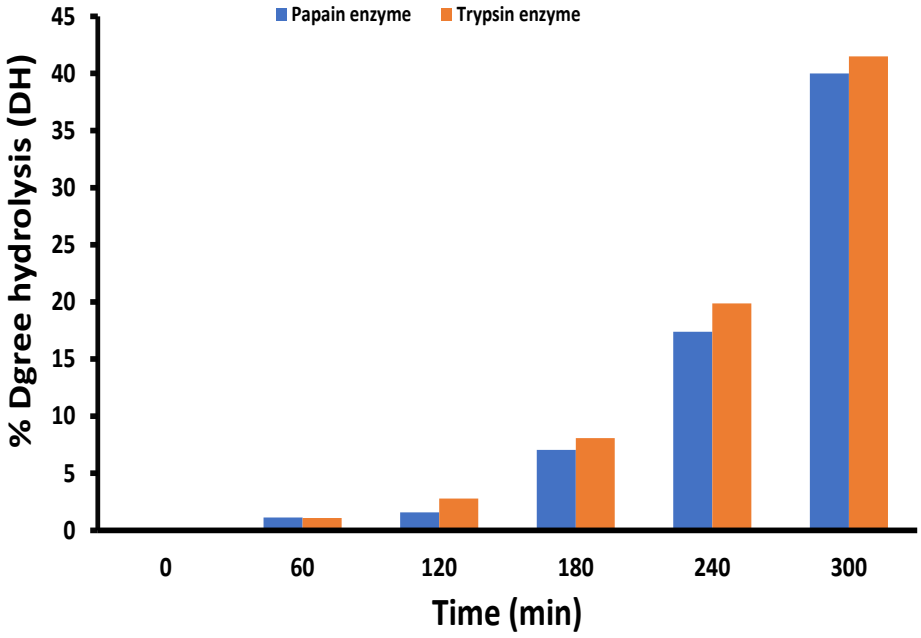
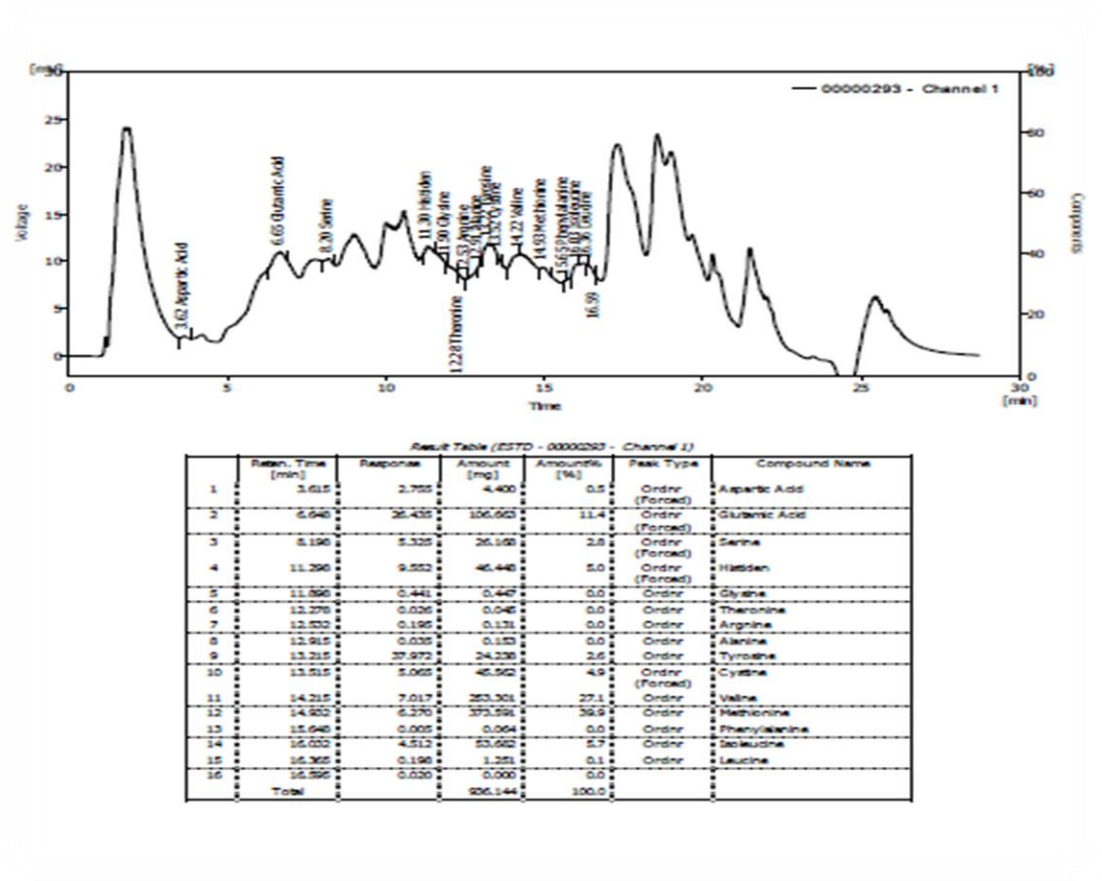


Fig. 1: Degree hydrolysis (DH %) of *P. juliflora* pods using Trypsin and Papain enzymes for 300 min.



	Reten. Time [min]	Response	Amount [mg]	Amount% [%]	Peak Type	Compound Name
1	3.615	2.755	4.400	0.5	Ordnr (Forced)	Aspartic Acid
2	6.640	26.435	106.663	11.4	Ordnr (Forced)	Glutamic Acid
3	8.190	5.335	26.168	2.8	Ordnr (Forced)	Serine
4	11.296	9.552	46.448	5.0	Ordnr (Forced)	Histidin
5	11.896	0.441	0.447	0.0	Ordnr	Glycine
6	12.276	0.026	0.046	0.0	Ordnr	Theronine
7	12.530	0.195	0.131	0.0	Ordnr	Arginine
8	12.915	0.035	0.153	0.0	Ordnr	Alanine
9	13.215	37.972	24.238	2.6	Ordnr	Tyrosine
10	13.515	5.065	45.562	4.9	Ordnr (Forced)	Cystine
11	14.215	7.017	253.301	27.1	Ordnr	Valine
12	14.953	6.370	373.591	39.9	Ordnr	Methionine
13	15.648	0.005	0.064	0.0	Ordnr	Phenylalanine
14	16.003	4.513	53.662	5.7	Ordnr	Isoleucine
15	16.365	0.196	1.251	0.1	Ordnr	Leucine
16	16.596	0.030	0.000	0.0		
	Total		936.144	100.0		

Fig. 2: Amino acids in Trypsin *P. juliflora* pods hydrolysis

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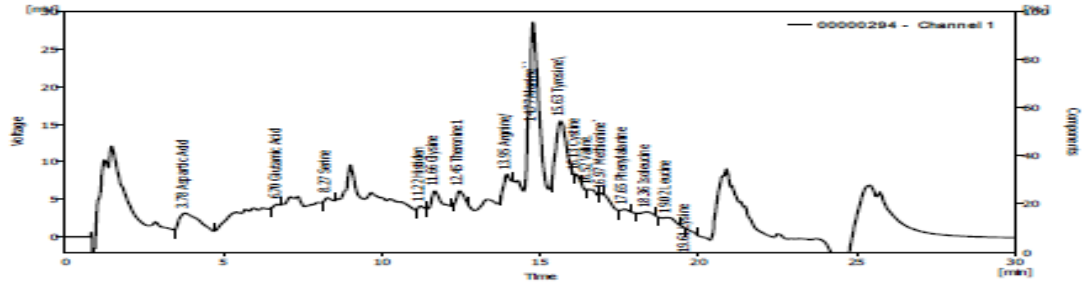

Result Table (ESTD - 00000294 - Channel 1)

	Reten. Time [min]	Response	Amount [mg]	Amount/Wt. [%]	Peak Type	Compound Name
1	3.782	93.901	149.983	30.0	Ondrv (Forced)	Aspartic Acid
2	6.696	3.607	10.519	2.1	Ondrv (Forced)	Glutamic Acid
3	8.268	5.919	29.083	5.8	Ondrv (Forced)	Serine
4	11.215	5.115	24.887	5.0	Ondrv (Forced)	Histidin
5	11.665	37.947	38.461	7.7	Ondrv (Forced)	Glycine
6	12.446	24.021	0.000	0.0		Threonine1
7	13.948	22.173	0.000	0.0		Arginine/
8	14.765	169.556	0.000	0.0		Alanine/
9	15.632	169.607	0.000	0.0		Tyrosine\
10	16.132	3.411	30.686	6.1	Ondrv (Forced)	Cystine
11	16.515	5.963	0.000	0.0		Valine,
12	16.965	1.350	0.000	0.0		Methionine
13	17.646	3.568	46.136	9.2	Ondrv (Forced)	Phenylalanine
14	18.365	7.559	90.296	18.1	Ondrv (Forced)	Isoleucine

Figure 3: Amino acids in Papain P. *juliflora* pods hydrolysis

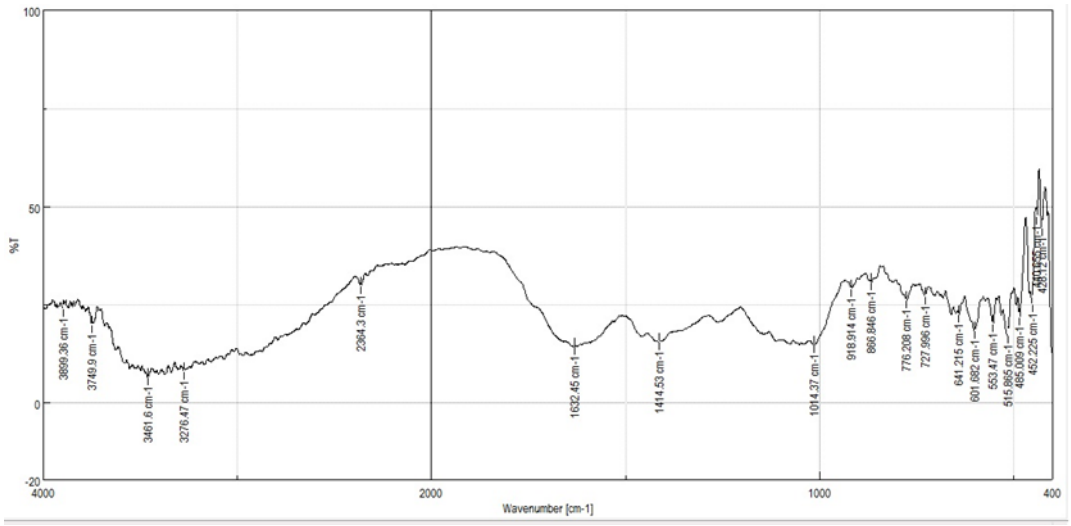

Fig. 4: FTIR spectra of *P. juliflora* pods hydrolysates using Trypsin enzymes**.**

[DOI: 10.22034/FSCT.21.150.1]

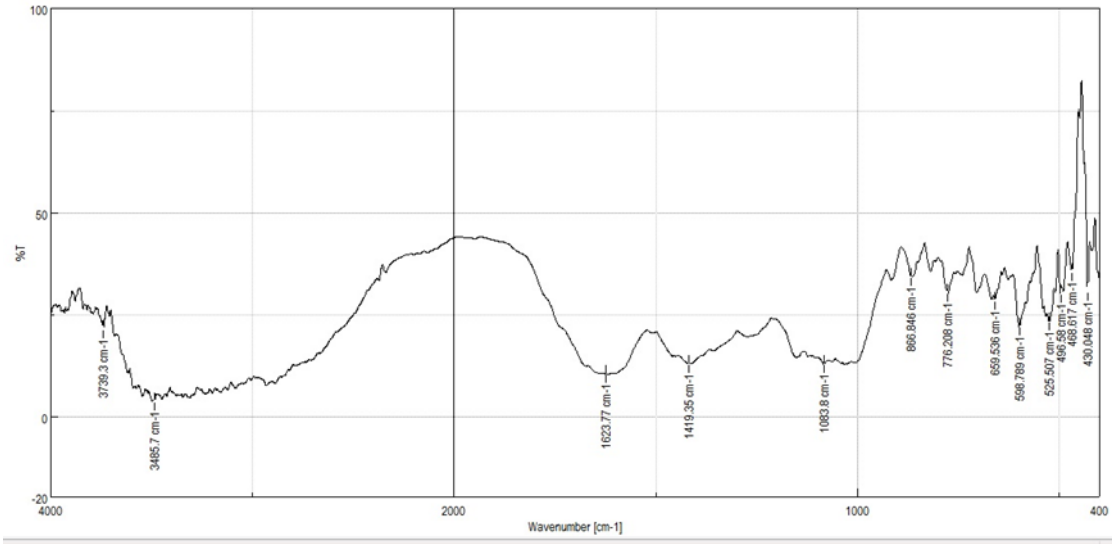

Fig. 5: FTIR spectra of *P. juliflora* pods hydrolysates using Papain enzymes**.**

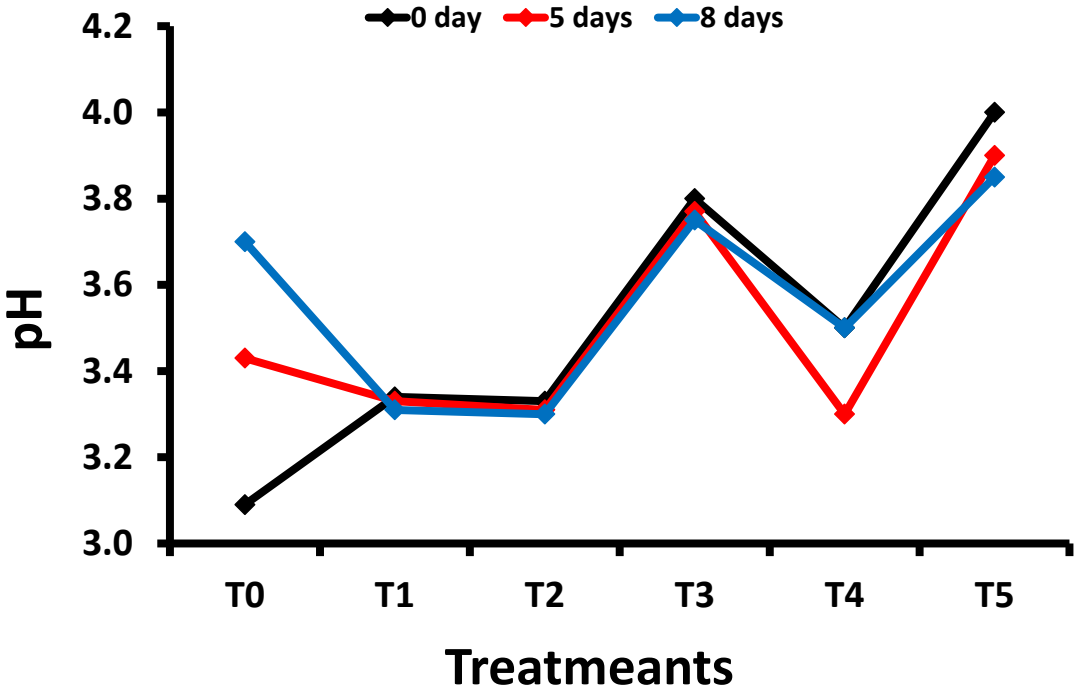

Fig. 6: Effect of treatment with protein hydrolysis and anti-browning agents on the pH values of apple slices during refrigerator storage.

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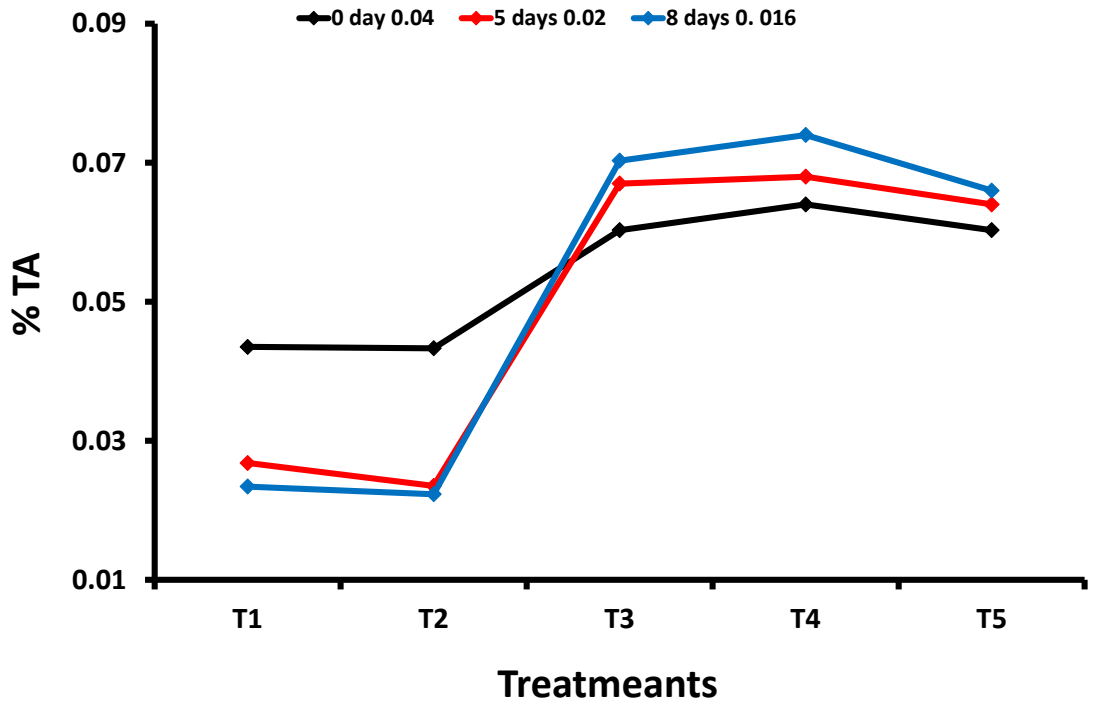

Fig. 7: Effect of treatment with protein hydrolysis and anti-browning agents on the TA% of apple slices during refrigerator storage

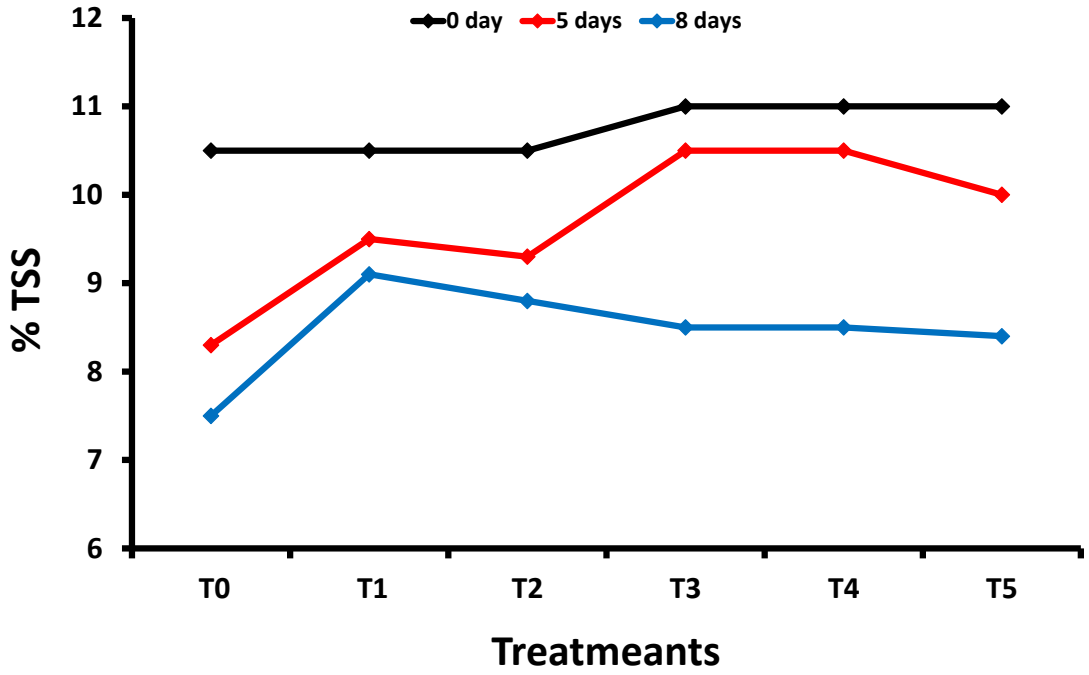

Fig. 8: Effect of treatment with protein hydrolysis and anti-browning agents on the %TSS of apple slices during refrigerator storage

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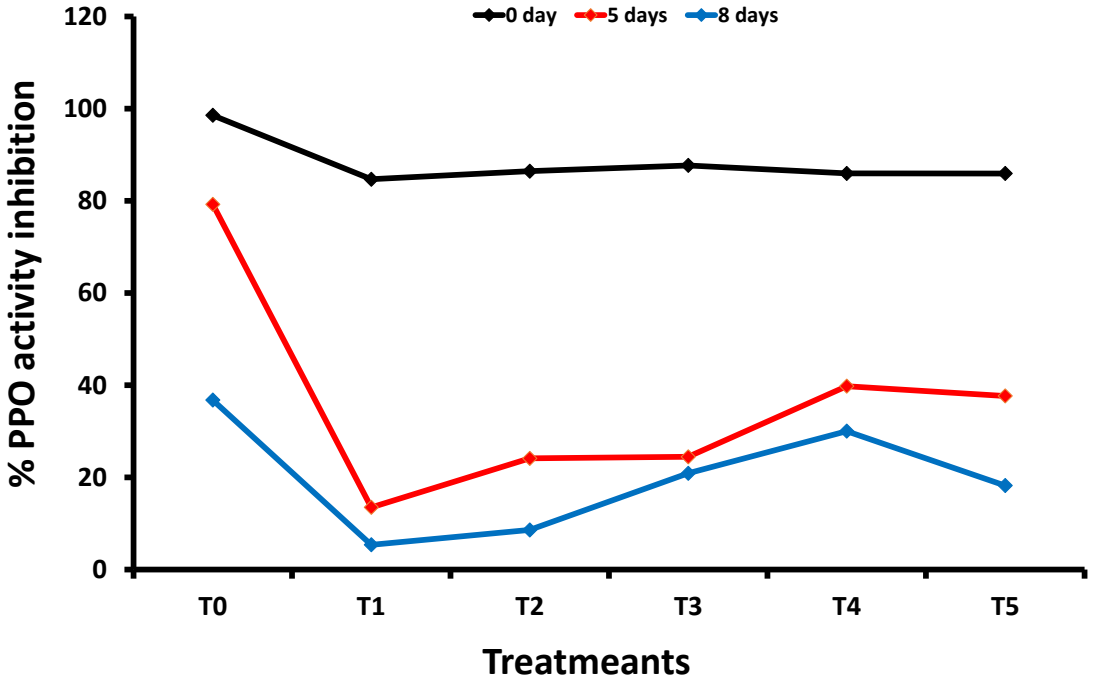

Fig. 9: Effect of treatment with protein hydrolysis and anti-browning agents on the PPO activity inhibitory % of apple slices during refrigerator storage

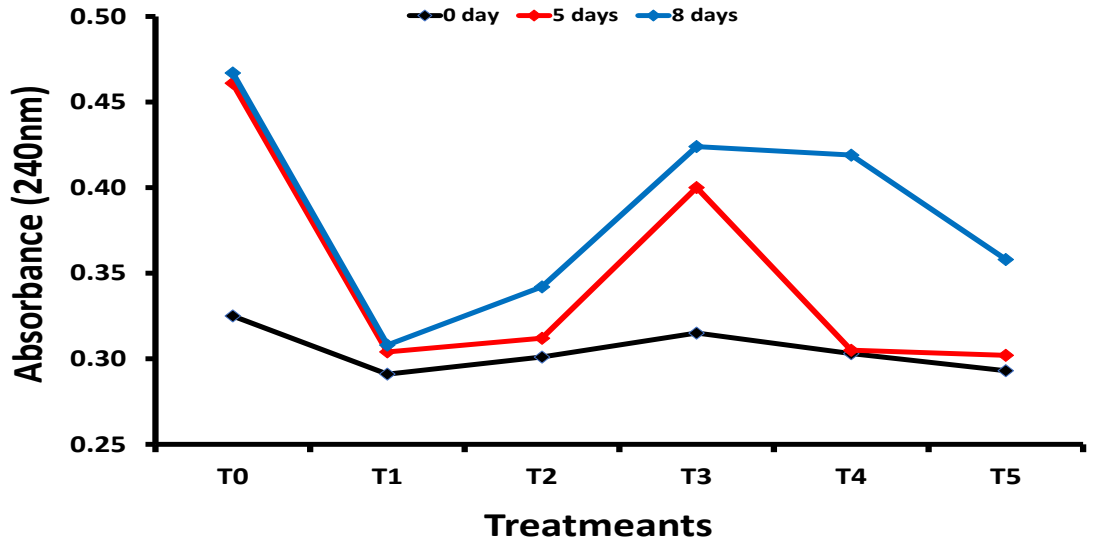

Fig. 10: Effect of treatment with protein hydrolysis and anti-browning agents on the browning of apple slices during refrigerator storage.

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مجله علوم و صنایع غذایی ایران

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مقاله علمی_پژوهشی

اثر مهاری هیدرولیز پروتئین Pods *Prosopis juliflora* بر پلی فنل اکسیداز و قهوه ای شدن برش های سیب با پایداری نگهداری در یخچال

عالیهٔ زیاره هاشم[۱]، فلیحه حسن حسین[۲]، الاء.محمد. البیضانی[۳]، سیدهادی رضوی[۴*].

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چکیده

این مطالعه با بهره‌برداری از پروتئین‌های غلاف برخی از انواع گیاهان مانند غلاف *Prosopis juliflora* که در بسیاری از کشورها محصول جانبی محسوب می‌شود و منبع خوبی از پروتئین در تهیه هیدرولیزهای پروتئینی و ارزیابی اثر آن‌ها بر مهار آن محسوب می‌شود، انجام شد. تغییر رنگ قهوه ای برش های سیب و در مقایسه با عوامل ضد قهوه ای (اسید اسکوربیک، اسید استیک و کلرید سدیم) در زمان نگهداری در یخچال به مدت ۰، ۵ و ۸ روز. ترکیب شیمیایی رطوبت، پروتئین، چربی، خاکستر و کربوهیدرات غلاف *P. juliflora* برآورد شد، سپس فرآیند هیدرولیز با استفاده از آنزیم‌های تریپسین و پاپائین به مدت ۳۰۰ دقیقه انجام شد. اسیدهای آمینه و آنالیز FTIR هیدرولیزهای پروتئینی تعیین شد. تغییرات معنی‌دار ($p\leq0.05$) در pH، کل مواد جامد محلول، و تغییرات غیر قابل توجه در اسیدیته تیتراسیون برش‌های سیب تیمار شده با هیدرولیز پروتئین و عوامل ضد قهوه‌ای مورد مطالعه قرار گرفت و به طور معنی‌داری ($p\leq0.05$) در فعالیت پلی فنل اکسیداز کاهش یافت. تا پایان ذخیره سازی رنگ قهوه ای در هنگام درمان با پروتئین هیدرولیز در مقایسه با سایر تیمارها کاهش یافت، اما تغییرات غیر قابل توجهی داشت. در نتیجه، برش های سیب را می توان با هیدرولیزهای پروتئینی برای چند روز در یخچال نگهداری کرد.

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