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Vanillic Acid: An Antioxidant used in Preventing Browning Process in Pear (*Pyruscommunis*l.) Juice

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Abstract:In this study, Polyphenol Oxidase (PPO) was partially purified from pear fruit pulp which was organically grown in Mosul city of Iraq. The crude enzyme-specific activity was (222.42) Unit/mg protein. After applying precipitation by ammonium sulfate and dialysis, the specific activities have been improved to be (211.66) and (549.60) Unit/mg protein, and the purification folds were (0.95) and (2.47) respectively. After ion-exchange chromatography using CM - Cellulose, the final result revealed one peak of PPO with a specific activity of (11306.1) Unit/mg protein and purification fold of (50.83). Characterization of the partially purified enzyme showed that optimum pH and temperature were 7.5 and 35°C respectively. Polyphenol Oxidase activity and browning intensity in pear juice were inhibited by adding different concentrations of antioxidant compound, vanillic acid. The maximum inhibition percentage of the enzyme was 71.1% and the maximum inhibitory effect of browning intensity was 30.9 %. The inhibition mode of purified PPO was competitive by using 3.3 mMvanillic acid. Michaelis-Menton (Km) value increased from (1.43) to (3.33) mM, with unchanging in Maximum velocity (Vmax)value (123.45) Unit/ml.min. The inhibition constant (Ki) value was 1.846 mM. The results of untreated pear juice indicated decreasing in phenolic compound contents, reducing sugar and free amino group but, increasing in intermediate compounds, browning intensity, and reducing power. After treating with (3.3mM) vanillic acid, decreasing of intermediate compounds, browning intensity, and increasing in reducing power were revealed with comparing to control. Contrariwise, the addition of vanillic acid to pear juice obtained increasing in reducing sugar, free amino acid, and phenolic contents compared to control.

Keywords*: Pear, Polyphenol oxidase, Maillard reaction,Vanillic acid*

1. Introduction

Pear (*Pyruscommunis* L.) is a coastal fruit of moderate regions. The size of pear tree is medium, reaching 10-17 meters (1). Economically, it is the fifth most important fruit in the world. The annual production depends on many countries being in China mainly followed by Europe and the United States. Besides apple and quince, pear belongs to the rosaceous family but has a different delicate flavor (2). Among common fruits, fresh pear considers as a food of moderate energy value which has low calorie. As well as pear is very rich in vitamin C, it contains fibers, carbohydrates, enzymes, phytosterols, and minerals such as calcium, zinc, magnesium, manganese, iron, copper, potassium, and phosphorus [3,4]. Polyphenol oxidase (PPO; EC 1.14.18.1) is a natural multi-copper protein that oxidizes phenolic compounds to create finally black pigments. For this reason, PPO is responsible for the enzymatic browning reaction that takes place when fresh fruits and vegetables are wounded or

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smashed [5]. Several studies demonstrated that enzymatic browning is undesirable andcan be led to an unpleasant appearance, flavor and aroma [6]. The rate and magnitude of browning of pear flesh varies by cultivar, and may be affected by total phenolic content of fruit together with polyphenol oxidase activity level.

The oxidation of colorless monophenols to colorless diphenols is the basic enzymatic browning reaction. Further oxidation reaction forms slightly colored o-quinones. The o-quinoneswill be polymerized into complex brown pigments in the attendance of amino acids and other proteins [7]. Additionally, the quinones, which are produced in the initial browning reaction, may contribute tocoupled oxidation reactions. This will be enabled them to oxidize other polyphenols that cannot be enzymatically oxidized directly. Nonenzymatically brown products are generated due to the reaction of ascorbic acid oxidation, caramelization, lipids oxidation [8, 9], and Maillard reaction which happens via a combination of amino acids and reducing sugars. Many inhibitors such as phytic acid, cinnamic acid, ascorbic acid, and glutathione have been reported as non-toxic compounds for preventing enzymatic browning via inhibiting PPO [10].

Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is a phenolic compound used broadly in food manufacturing as a flavoring, preservative, and food ingredients. It can be found in numerous grains, cereals, fruits, herbs, wines, and juices [11]. It exhibits strong antioxidant action and can act as a functional food by donating to the welfare and health of the population [12]. The antioxidant properties of vanillic acid may contribute to controlling the Maillard reaction and inhibition of polyphenol oxidase. The adding of this compound to foods during processing and storage may help improvethe manufacture of fruit and vegetable. In some plant sources, this compound has occurred as a natural compound, therefore more accepted as food ingredients than synthetically manufactured compounds.
Our current research aim is to purify and characterize PPO enzyme from pear fruits. Besides, vanillic acid was used to inhibit PPO and Maillard reaction to reduce the harmful influence of enzymatic and nonenzymatic browning reaction which lead to food damage then negatively impact the quality of commercial products.

2. Materials and Methods

2.1. Chemicals and Fruit

In our study, we used chemicals gained from either Sigma Aldrich or Fluka which were of high analytical grade. Fresh Iraqi pear (*Pyruscommunis* L.) were acquired from trees of Mosul city in September.

2.2. PPO Extract Preparation

Directly after harvested and washed, 150 gm of fresh pear pulp was weighed and homogenized in 200 ml phosphate buffer (0.1 M, pH 7.5) containing 10 mM ascorbic acid, 1% Triton-X 100, and 2% polyethylene glycol in a blender for 25-sec x 8 at 4°C. By cheesecloth, the supernatant containing PPO was isolated from the pulp, centrifuged at 6,000 rpm for 20 minutes, and used as crude extract [13].

2.3. Ammonium sulfate

For protein precipitation, solid ammonium sulfate 20-80% was used by following these steps: Pouring extracted sample into a graduated beaker, placing on a magnetic stirrer in the refrigerator, and adding ammonium sulfate to protein solution over a four-step period. After obtaining complete sediment, a solution was centrifuged at 6000 rpm for 20 min at 4˚C, then the supernatant was decanted and protein precipitation was dissolved in the smallest volume of buffer [14].

2.4. Dialysis

To isolate proteins with low molecular weights (less than 12 kDa) and to remove ammonium sulfate salt, a dialysis tube was used. Dialysis was carried out by putting 11 ml of a sample against phosphate

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buffer (100 mM, pH 7.5) on a stirrer at 4˚C with four-time buffer changes.

2.5. CM-Cellulose Chromatography
A cation-exchange chromatography was used for the purification of PPO. The dialyzed solution was loaded onto the CM-cellulose column which was pre-equilibrated with two types of phosphate buffers, a (100 mM, pH 7.5) and B (200 mM, pH 7.5) with a flow rate regulated to be 1ml/ min. The monitoring of protein concentration and PPO activity were achieved in each fraction (5 ml) at 280nm and 408 nm respectively.

2.6. Protein determination
Protein content was determined according to the method of Lowry et al. (1951) (15) using bovine serum albumin as standard.

2.7. Determination of Polyphenol Oxidase Activity
The activity of PPO was assayed spectrophotometrically using catechol 11.01 mg/mL phosphate buffer (100 Mm, pH 7.5) as substrate. The reaction cuvette contained 0.3 mL of enzyme solution and 2.7 mL of a substrate. A blank contained only 3 mL of a substrate. A change in absorbance at 408 nm was followed for 3 min to measure quinone formation after incubation for 10 minutes at room temperature (16). A unit of PPO activity was defined as the change in absorbance of 0.001 per minute.

2.8. Effect of pH and temperature on PPO activity
PPO activity determination was achieved at temperatures between (25 to 60°C intervals 5 degrees) with 100 mM of catechol as substrate. By using Buffer systems of 100 mM which were acetate buffer for pH (4-5), Phosphate buffer for pH (6-7.5), Tris-HCl buffer for (8), and glycine-NaOH buffer for pH (9-10), optimum pH was estimated at 35 °C.

2.9. Preparation of juice
Healthy harvested pears (100g) were peeled after washing then cut and blended with 125ml phosphate buffer. Using a muslin cloth, the slurry was filtered and centrifuged at 6,000 rpm for 15 minutes to acquire a clear juice (17).

2.10. The vanillic acid effect on PPO activity and browning intensity
The mixture which contained 8 milliliters of juice and 2 milliliters of 1-5 mM vanillic acid was used to measure PPO activity at 408nm and browning intensity at 420 nm. A control tube contained juice only. The IC50 value of vanillic acid was obtained according to the equation: IC50= 50-b/a. a and b are slope and intercept respectively of the straight line.

2.11. Time effect on PPO activity and browning intensity
We used six tubes in this experiment. In each one, 8ml of pear juice and 2ml of 3.3mM vanillic acid were added successively and the first tube was kept as a control. At 0-60 minutes, PPO activity and browning intensity were measured.

2.12. Inhibition mode of PPO
The inhibition of purified PPO was designed by using vanillic acid (3.3 mM), and the activity was followed using (0.25-10 mM) of substrate after incubation of 0.2 ml enzyme with 0.1 ml of inhibitor (18).The inhibition constant (Ki) value was calculated by $Km' = Km(1+I/Ki)$ (19).

2.13. Maillard reaction products preparation
Two milliliters of 3.3mM vanillic acid was added to 8 ml of pear juice. Then solution in tubes was transmitted, locked tightly, and heated in a water bath at 100^{o}C. Analysis of sample was done every

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one hour for 5 hours after cooling. The first hour was measured without heating and the control container was kept without inhibitors.

2.14. Colorless intermediate and browning intensity measurement
To evaluate the colorless intermediate and browning intensity, absorbance at 294 and 420 nm was measured respectively (20).
2.15. Reducing sugars content estimation
Garriga*et al* (2017) method (21) was done to determine reducing sugars in juice using Di-nitro salicylic acid as a reagent at 540 nm. Glucose solutions (10-100mg/ml) were used to prepare the standard curve.

2.16. Free amino group content estimation
The method was used for the estimation of a free amino group using ninhydrin reagent. The absorbance was measured at 570 nm. The solutions of amino acid lysine were used between (5-50mg/ml) to prepare the standard curve (22).

2.17. Total phenolic compounds content estimation
By using the Folin-Ciocalteu reagent, total phenolic compounds were evaluatedspectrophotometrically at 765 nm (23). For the standard curve, gallic acid (10-100 μg/mL) was used.

2.18. Reducing power estimation
According to the method described by (24), reducing power was estimated by following the increase of absorbance at 700 nm.

3. Results and Discussions

After CM- Cellulose cation exchange procedure, the enzyme was purified about 50.83 fold with a final yield of 75.69% (Table 1). Purification of enzyme exhibited early a single peak in fractions 9 to 20 (Fig.1) with specific activity 11306.1 U/mg protein.
By using DEAE – Cellulose, the specific activity of one peak purified PPO (EC 1.14.18.1) from quince fruit which was grown in Mosul-Iraq was 11406.77 U/mg. with purification fold of 33.34 (17). The single peak of polyphenol oxidase was also obtained from Hemşin Apple after purification by ion-exchange and gel filtration techniques with specific activity 95.16 EU/mg and 28.80 fold purification (13), while fold purification of Whangkeumbae pear PPO using DEAE-Sepharose and Sephadex G-75 was 32.93 (16).

Table 1 PPO purification steps from pear (*Pyrus communis* L.)

Purification steps	Volume (ml)	Total protein (mg)	Total activity U*	Specific activity (U/mg protein)	Yield %	Purification Fold
Crude	84	12.76	2838.1	222.42	100	1
Ammonium sulfate	11	4.92	1041.41	211.66	36.69	0.95
Dialysis	10.5	1.71	939.82	549.60	33.11	2.47
CM-Cellulose chromatography	58	0.19	2148.16	11306.1	75.69	50.83

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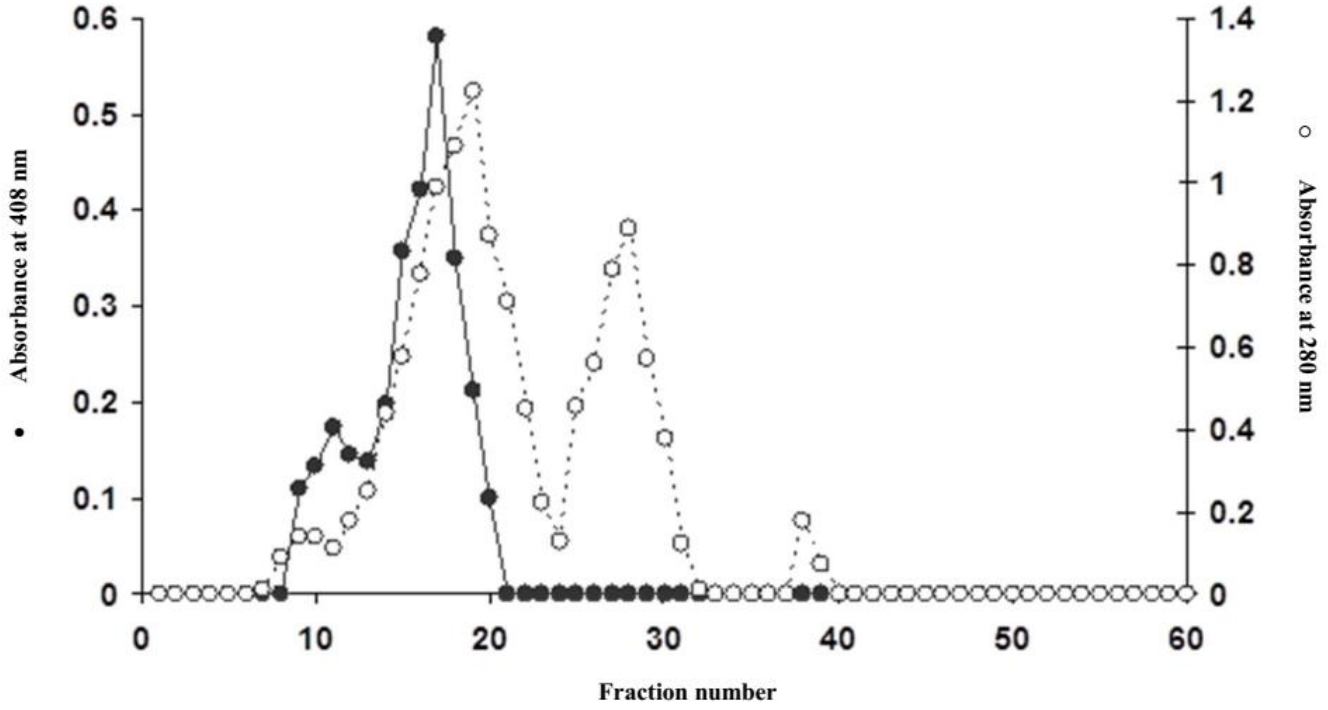

Fig. 1: Elution pattern of pear PPO on CM- cellulose column (40×2.5 cm).

Optimum pH

Ionized R groups of amino acids give the electrical charge of an enzyme, and this charge influences by varying pH which leads to a decrease, increase or prevent conjugation of enzyme active side with a substrate. Therefore each enzyme has special optimum pH.Thus denaturation of the three-dimensional structure of enzymes can be caused by extreme pH values (25-26). Fig.2 shows the optimum pH of PPO which is found at pH 7.5. The optimum pH of borage PPO was 7.5 using pyrogallol as substrate (27). It was reported that the optimum pH of PPO varied widely from various plants. Rosemary, tomatoes, and water yam had optimum activities at 7, 6.8, and 6 respectively using catechol as substrates (28-30). The optimum pH for PPO of Cape gooseberry was 5.0 for substrate, chromogenic acid (31), whereas for tea leaf was found to be 8.0 (32). It was found that the maximum activity of fruits and vegetables were often near to neutral pH.

Optimum temperature:

Generally, the reaction between enzyme and substrate was speeded by elevating temperature due to the increased kinetic energy of the enzyme. Nevertheless, enzyme denaturation happens at great high temperatures and the working will be stopped (33). The optimum activity of purified tyrosinasewas noticed at 35˚C (Fig. 3). After this temperature, the activity was started to decline. This result similar to temperatures for water yam (30). The peak temperature of purified PPO from corn tassel and Chinese parsley was 40°C (34-35), whereas it was been 45°C for mulberry, eggplant, and apricot, using catechol as substrate (36-38). Also, it was reported 20°C for apples and 22°C for potato (39), 30°C for banana pulp and peel (40).

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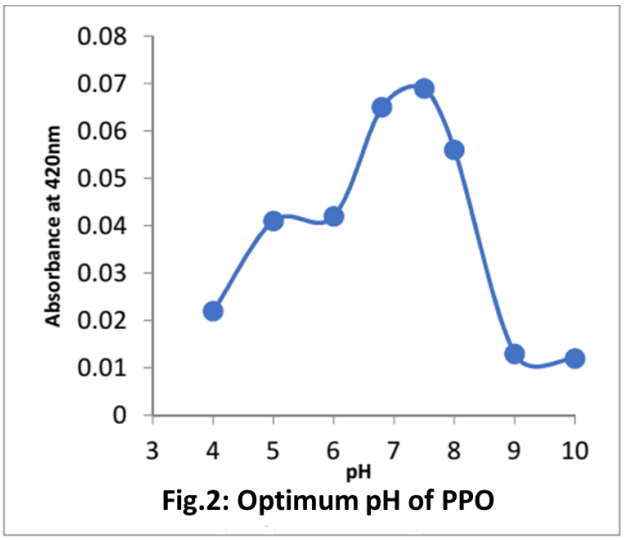

Fig.2: Optimum pH of PPO

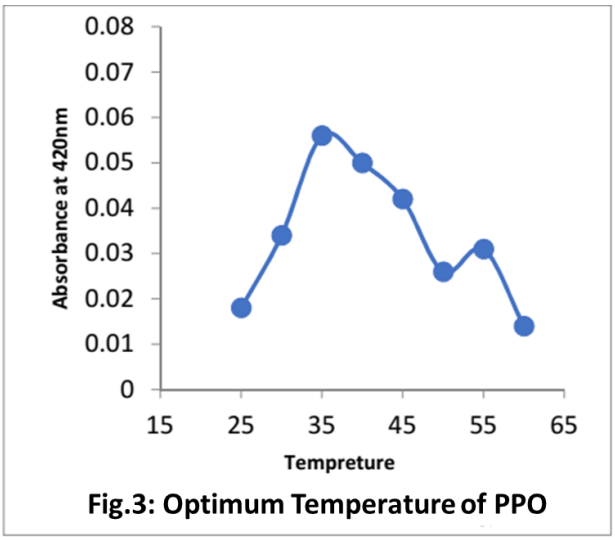

Fig.3: Optimum Temperature of PPO

Vanillic acid effect on PPO activity and browning intensity in pear juice

Inhibition percentage of PPO activity and browning intensity of Iraqi pear juice, which was treated with different concentrations of vanillic acid at different time, are shown in table (2 and 3). Increasing in both results is shown compared to control. In apple juice, increasing anti-browning agents' concentration (cinnamic acid and glutathione) reported increasing in inhibition percentage and PPO inhibition slowed down efficiently the browning reaction rate (41).

Table 2 Inhibitory effect of vanillic acid on PPO activity and browning intensity

Vanillic acid conc.(mM)	Inhibitory effect of browning %	PPO inhibition %
Control	0	0
1	14.6	11.8
2	16.6	27.5
3	24.5	33.5
4	28.1	60.5
5	30.9	71.1

Table 3 Time Effect on PPO activity and Browning intensity using (3.3mM) vanillic acid

Time min.	Inhibitory effect of browning %	PPO inhibition %
0	18.5	14.4
10	24.5	22.1
20	25.1	45.9
30	40	66.6
40	42.6	76.2
50	44	100
60	49.5	100

PPO inhibition:

Competitive inhibition of vanillic acid (3.3mM) on purified PPO was obtained (Fig. 4). Increased Km value from 1.43 to 3.33 mM and unchanged Vmax value 123.45 U/ml.min indicates the competitive inhibitor. Inhibition constant K*i* value was 1.846 mM. In banana, citric and malic acids played an important role in inhibiting PPO(42).Rashan (2020) illustratedcompetitive inhibition ofquince purified

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PPO using vanillin with Ki value 2.312 (17). Apple juice PPO also observedcompetitive inhibition with cinnamic acid as an inhibitor (41).

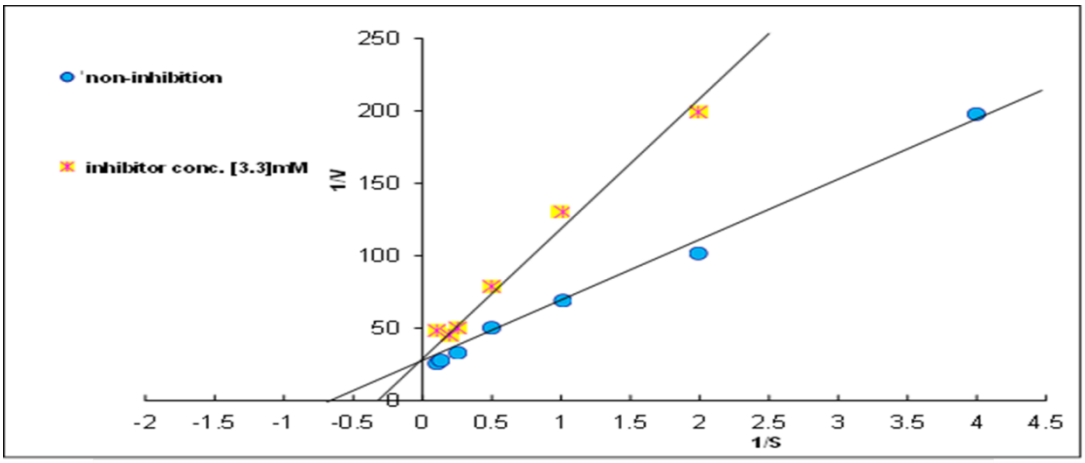

Fig. 4: Lineweaver-Burk plot of purified PPO inhibition by vanillic acid

Changes in UV λmax (AUV λ294) and browning intensity (A420)

Fig. 5 and Fig.6 explain a uniform increase in AUVλmax and Browning intensity (A420) with increasing heating time for untreated pear juice. These results alike to the study was carried out for quince juice (43), heated glucose - glycine mixture (44), and heated chitosan - glucose mixture (45). Before generation brown pigments, the Maillard reaction improves UV-absorbing intermediate complexes. Intermediate products are still during heating, but several others may be changed to ending brown complexes. The previous studies indicate that AUV λ294 and browning intensity (A420) are reflected of intermediate and browning compounds respectively (Ajandouz*et al.*, 2011)(46). Depending on sugar natures, intermediates, and colored polymers which are resulted, the browning intensity (A420) increased for numerous model systems (47).

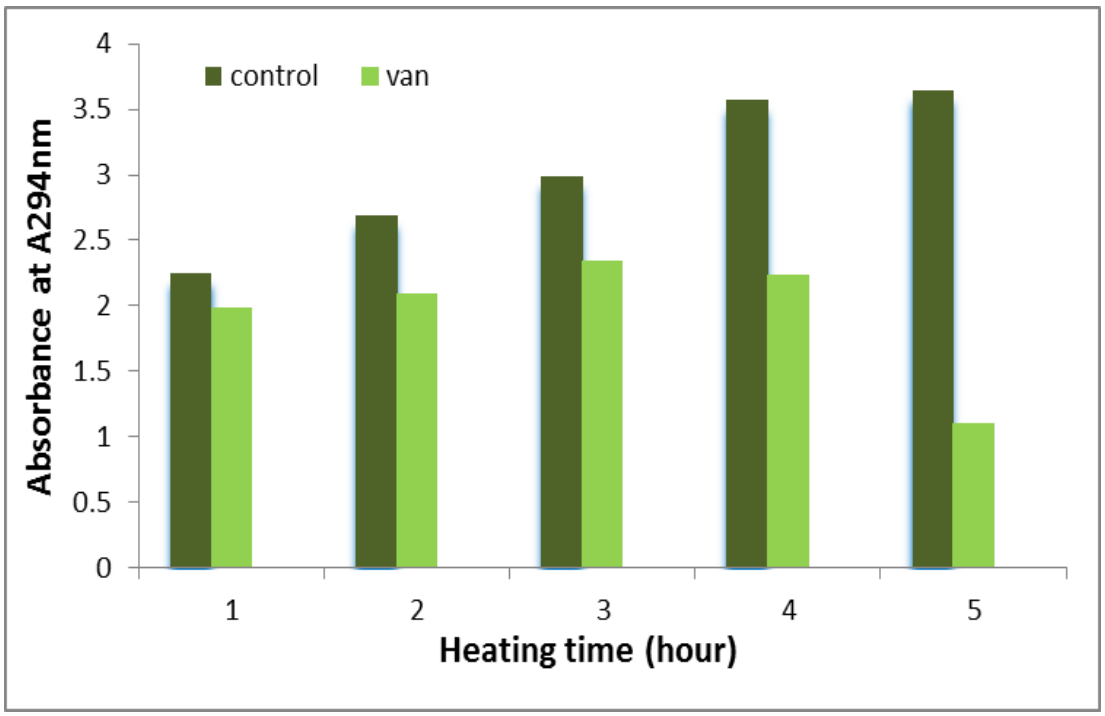

Figure 5: Changes in A294nm of MRPs derived during heating for various times

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A decrease in AUV λmax and browning intensity was observed after treating the juice with vanillic acid as compared to control (Fig 5 and Fig.6). Treated quince juice with vanillin and glutathione revealed decreasing in UV-absorbing intermediate complexes and brown pigments generation with heating (43).

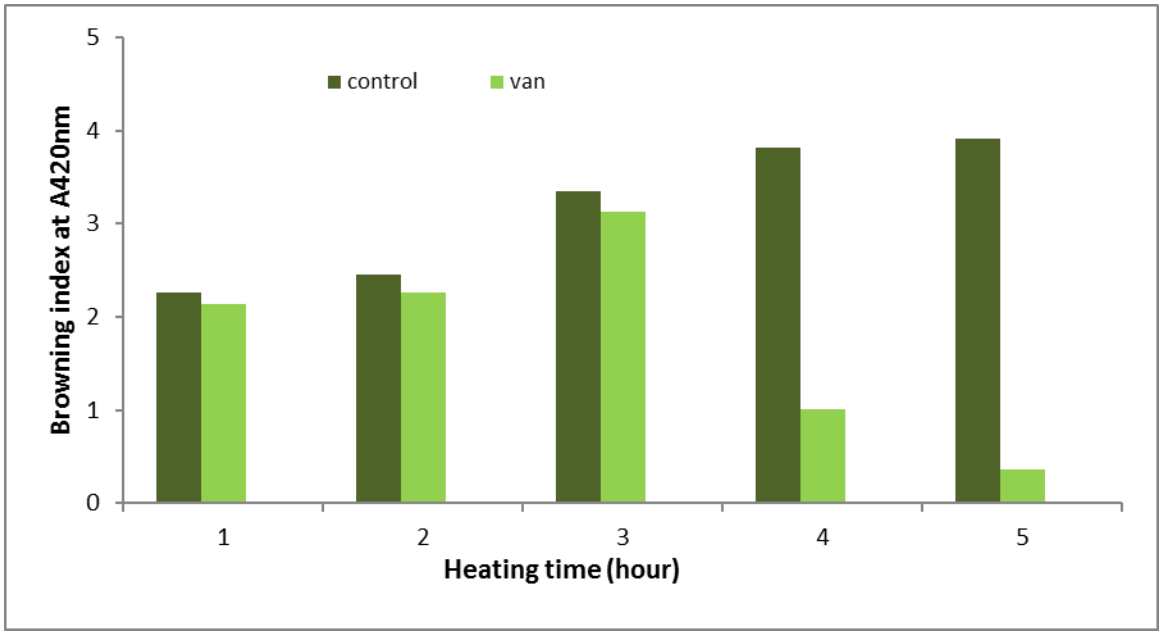

Figure 6: Changes in A420 of MRPs derived during heating for various times

Changes in reducing sugar content:
The lower reducing sugar content of pear juice was found when heated to 5 hours (Fig.7). The decrease in reducing sugar for heated lysine-fructose (48), casein-glucose (49), and chitosan-glucose (50) systems was reported. Whereas, pear juice treatment with vanillic acid exposed increasing in reduced sugar compared to control (Fig.7). This likes the lowering of reduced sugar for treated quince juice with vanillin (43).

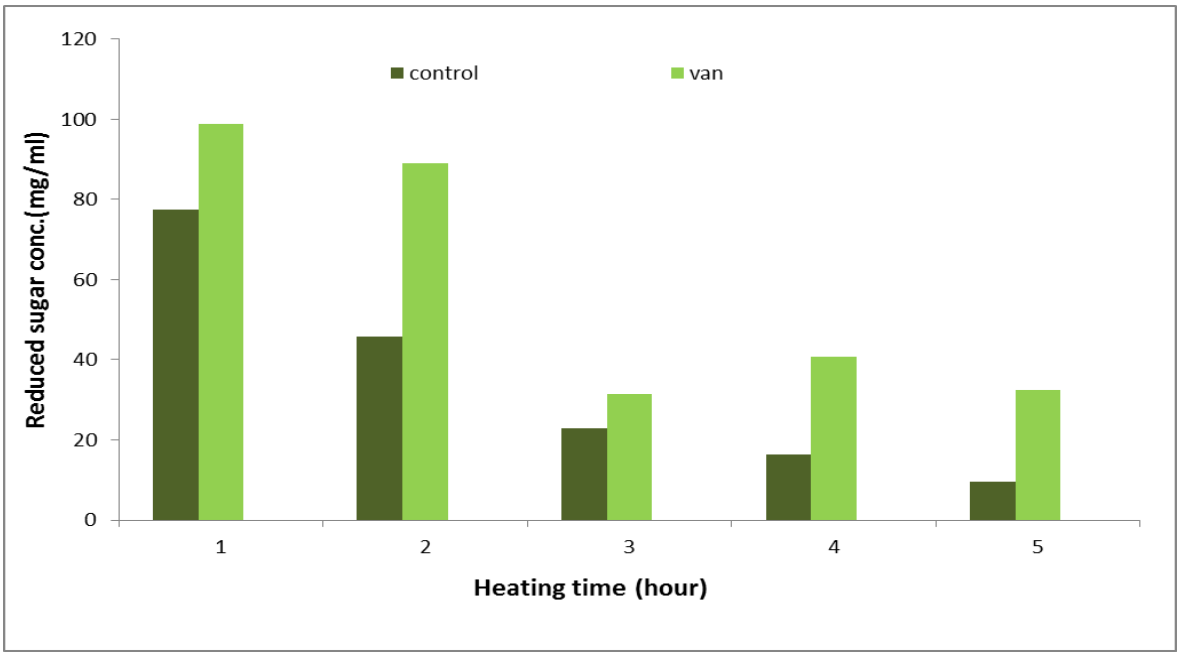

Figure7: Changes in reducing sugar content of MRPs derived during heating for various times

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Changes in free amino group content:

Figure 8 shows a gradually decreasing of free amino group contents of heated pear juice. The reduction in the amino group content was observed as the heating time increased (45). The addition of vanillic acid to pear juice increased amino acid contents compared to control (Fig.8). These results were according to Rashan and Al-abbasy 2020 study (43), who's noticed a higher free amino acid content of heated quince juice by using glutathione.

During long heat treatment, the interaction between the amino group and carbonyl group was catalyzed and contributed to the development of MRPs. The lowering of reducing sugar and free amino group reflect the increment of absorbance at 294nm and 420 nm. The glycation reaction was persuaded by carbonyl electrophilicity (51-52).

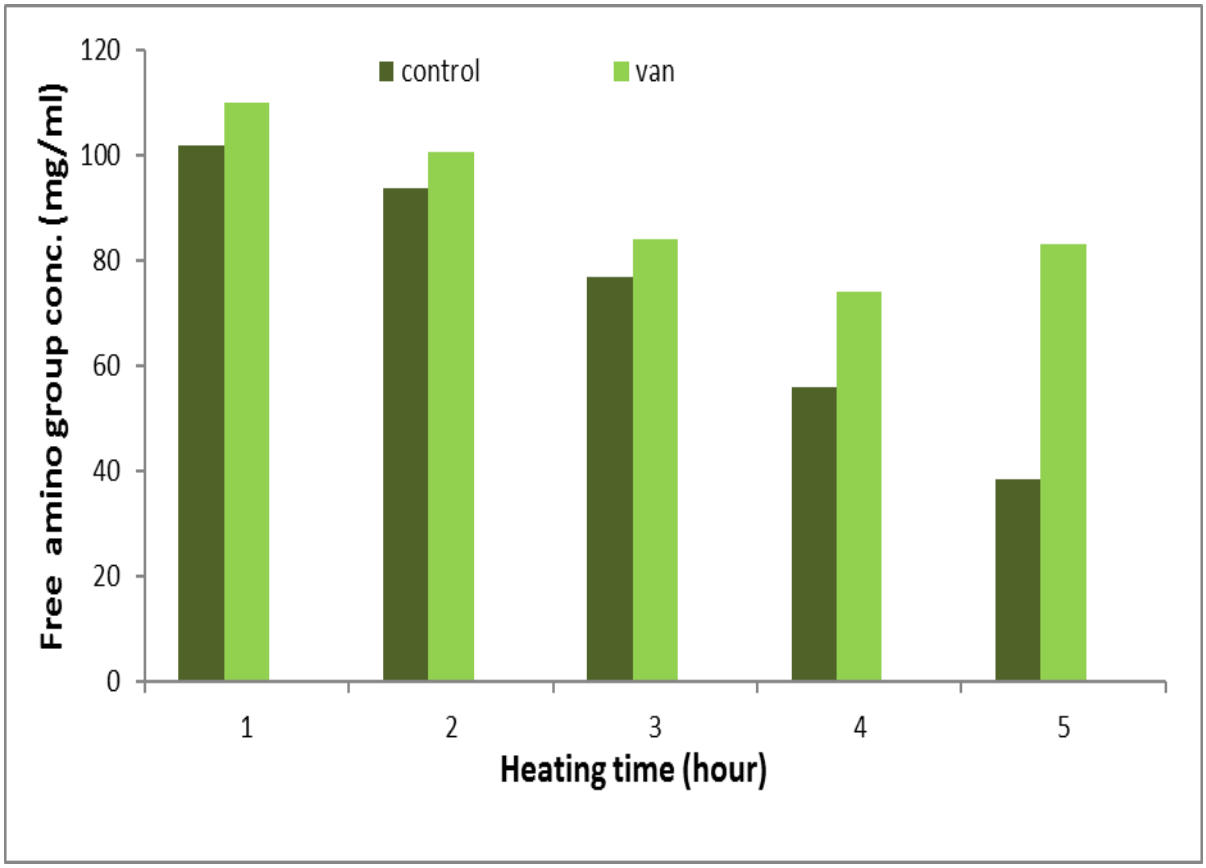

Figure 8: Changes in free amino group content of MRPs derived during heating for various times

Changes in phenolic content:

In the occurrence of oxygen, polyphenol oxidases oxidize phenolic constituents in most eatable plant products such as vegetables and fresh fruits to generate o-quinones. O-quinones polymerization into complex brown pigments carries out in presence of proteins and amino acids (53). Furthermore, coupled oxidation reactions of quinones formed in the initial browning reaction can be contributed, permitting them to oxidize additional polyphenols which cannot be oxidized enzymatically. Conversely to heating time, phenolic content decreased in pear juice, but the addition of vanillic acid, exhibited increasing in phenolic content compared to control (Fig.9). Increasing heating time for quince juice led to a decrease in phenolic contents, but increased after vanillin was added (43).

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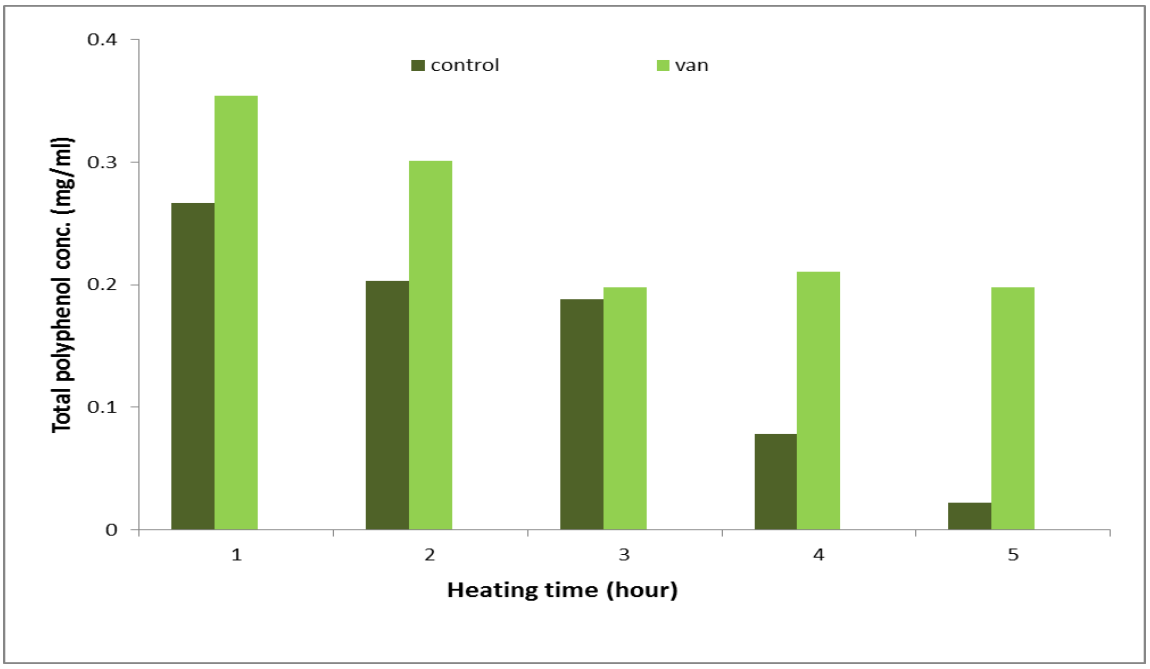

Figure 9: Changes in total polyphenol of MRPs derived during heating for various times

Changes in reducing power:

Fig. 10 explains a consistent rise in reducing power to heating time end. By comparing to control, absorbance at 700 nm was noticed increasing after vanillic acid addition to the juice of pear. Many studies reported increasing absorbance of reducing the power of glucose-glycine (54) and xylose-lysine (55) mixtures. The addition of vanillin and glutathione raises the absorbance of reducing power than control in heated quince juice (43).

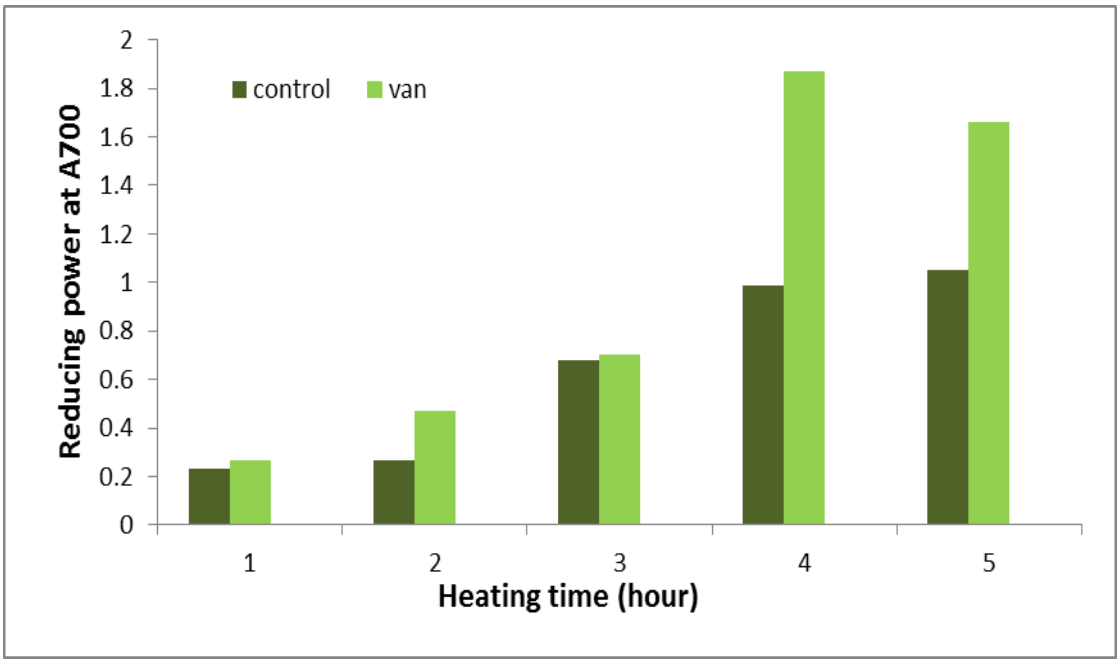

Figure 10: Changes in reducing power of MRPs derived during heating for various time

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For controlling Maillard reactions infoods, many efforts have been accomplished. For example, adding natural or artificial compounds, such as N-acetylcysteine inhibit reactions or generate colorless products by eliminating one of the reactants (56-57) or activating hydroxyl-containing compounds such as gallic acid by trapping α-dicarbonyl compounds (58-59). On the other hand, acetylation of amines residues can be effectively inhibited Millard reaction (60). Generally, antioxidants prevent browning initiation by reacting with oxygen. Also, intermediate products react with antioxidants compounds, thus breaking chain reaction and preventing melanin formation (61).

4. Conclusions

In the current study, one effective PPO peak was purified and characterized from pear (*Pyruscommunis* L.). In the presence of catechol as substrate, the enzyme behaved optimally at pH 7.5 and 35°C. The effect of vanillic acid which has inhibition properties was studied. The IC_{50} value was calculated (3.3 mM). The inhibition mode was competitive. The kinetic parameters Vmax and Ki were found to be (123.45) Unit/ml.min. and 1.846 mM respectively and Km value increased from (1.43) to (3.33) mM. Furthermore, the treatment of pear juice by vanillic acid resulted in a decrease in intermediate compounds and browning intensity, but increasing in reducing power, reducing sugar, free amino acid, and phenolic contents compared to control. Adding antioxidant compounds such as vanillic acid can take part in controlling browning reactions and could be useful for improving the quality of foods during processing and storage.

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References

1-BRAIN, A.F., CAMERON, A.G., Nutrition and Health, Fifth Edition. Edward Arnold Publisher, Inc., London, 1995, 266-284.
2-SILVA, G.J., SOUZA, T., BARBIERI, R., OLIVERA, A.C., Domestication, and Dispersing of Pear (*Pyrus*spp.), Hindawi Publishing Corporation Advances in Agriculture, (2014)
3- USDA, National Agriculture Library, National Nutrient Database for Standard Reference, 2012.
4-VADIVEL, V., JANARDHANAM, K., Plant foods for Human nutrition, The wonder of pears, free diets, 2005, 69-75.
5- LE BOURVELLEC, C., LE- QUERE, JM., SANONER, P., DRILLEAU, JF. GUYOT, S., Inhibition of apple polyphenol oxidase activity by procyanidins and polyphenol oxidation products, *Journal of Agricultural and Food Chemistry*, **52**, (2004b),122-130.
6- GAMBARO, A., ARES, G., Gimenez, A., Shelf-life estimation of apple-baby food, *Journal of sensory studies*, **21**(1), 2006, 101-111.
7- MARTINEZ, C., CORZO, M.¸ VILLAMIEL, N., MAR, D., CASTILLO, M., Food Biochemistry and Food Processing, Benjamin K. Simpson (ed.). Wiley-Blackwell, 2012, 56–83.
8-PIZZOCARO, F., TORREGGIANI, D., GILARDI, G., Inhibition of Apple Polyphenol Oxidase by Ascorbic Acid, Citric Acid, and Sodium Chloride, *Journal of Food Processing and Preservation*, **17**, 1993, 21–30.
9- LANGDON, T.T., Preventing Browning in Freshly Prepared Potatoes without the Use of Sulfating Agents, *Food Technology*, **41**, 1987, 64–67.
10- MOON, K.M., LEE, B., CHO,W.K., LEE, B.S., KIM, C.Y., MA, J.Y., Swertiajaponin as an anti-browning and antioxidant flavonoid, Food Chemistry, **252**, 2018, 207–214.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 5272 - 5285
Received 16 February 2021; Accepted 08 March 2021.

11- ALMEIDA, I.V., CAVALCANTE, F.M.L., VICENTINI, V.E.P., Different responses of vanillic acid, a phenolic compound, in HTC cells: cytotoxicity, antiproliferative activity, and protection from DNA-induced damage, *Genetics and Molecular Research*, **15** (4), 2016, 1-12.
12- NAVARRO, S.D., MAURO, M.O., PESARINI, J.R., OGO, F.M., Resistant starch: a functional food that prevents DNA damage and chemical carcinogenesis, *Genet. Mol. Res.,* **14**, 2015, 1679-1691.
13- AYDIN, T., GULCIN, I., ALWASE, S.H., Purification and Characterization of Polyphenol Oxidase from Hemşin Apple (*Maluscommunis* L.), *International Journal of Food Properties,***18**, 2015 2735–2745.
14- SENTURK, M., GULCIN, I., BEYDEMIR, S., KUFREVIOGLU, O.I., SUPURAN, C.T., In Vitro Inhibition of Human Carbonic Anhydrase I and Ii Isozymes with Natural Phenolic Compounds, *Chemical and Biology Drug Design*,**77**, 2011, 494–499.
15- SCHARCTERLE, G.R., POLLACK, R.L., Simplified method quantitative assay for small amounts of proteins in biological materials, *Anal. Biochem*.,**51**, 1973, 645-655.
16-ZHOU, X. R., XIAO, Y. J., MENG, X. H., LIU, B. J., Full inhibition of Whangkeumbae pear polyphenol oxidase enzymatic browning reaction by l-cysteine, *Food Chemistry*, **266**, 2018,1–8.
17- RASHAN, A.I., Inhibitory and Kinetic Study of Partially Purified Tyrosinase from Iraqi Quince (*Cydonia oblonga* Miller) Fruit, M. A. Thesis. *College of Education for pure science*, *University of Mosul, Iraq,* 2020.
18- AL-ABBASY, O.Y., ALI, W.I., YOUNIS, S. A., Study of inhibitory effect of *Rosmarinusofficinalis L*. extracts and quercitin on partially purified cow's brain Polyamine Oxidase, ***Biochem. Cell. Arch.,*** 20(2),2020, 5617-5625.
19- BUGG, T., An introduction to enzyme and coenzyme chemistry. Blackwell Science Ltd, 1997.
20 – TSAI, J.P., YU, Y.T., CHEN, H.S., LIU, C.C., SUN, Y.F., Interactive role of color and antioxidant capacity in caramels, *Food Research International,***42**, 2009, 380-386.
21 – GARRIGA, M., ALMARAZ, M., MARCHIARO, A., Determination of reducing sugars in extracts of Undariapinnatifida (Harvey) algae by UV-visible spectrophotometry (DNS method), *Actas de Ingeniería*, **3**, 2017, 173-179.
22- ROBYT, F.J., WHITE, J.B., Biochemical techniques, theory and Practice, Brookes/Cole publishing company, Monterey, California, 2001, 229-230.
23- ANESINI, C., FERRARO, G.E., FILIP R., Total Polyphenol Content and Antioxidant Capacity of Commercially Available Tea (*Camellia sinensis*) in Argentina, *J. Agric. Food Chem.,* **56**(19), (2009, 9225- 9229.
24- MATMAROH, K., BENJAKUL, S., TANAKA, M., Effect of reactant concentrations on the Maillard reaction in a fructose–glycine model system and the inhibition of black tiger shrimp polyphenol oxidase, *Food Chemistry*, **98**(1), 2006, 1-8.
25- AL FLAYEH, K. A., An Introduction to Biochemistry. Second Edition, Dar Al-Kutub for Printing and Publishing, University of Mosul, (In Arabic), 2006, 155-156.
26- MC KEE, T., MC KEE, J.R., Biochemistry: An Introduction. The McGraw-Hill Companies, Inc. Wm. C. Brown Publishers, Boston, USA, 2001, 184.
27- ALICI, E. H., ARABACI, G., Purification of polyphenol oxidase from borage (*TrachystemonOrientalis* L.) by using three-phase partitioning and investigation of kinetic properties, *International Journal of Biological Macromolecules*,**93**, 2016, 1051–1056.
28- AYDEMIR, T., Selected kinetic properties of polyphenol oxidase extracted *from Rosmarinusofficinalis L., International Journal of Food Properties*, **13**, 2010, 475–485.
29- SHAHRAYAR, S., Partial purification and characterization of polyphenol oxidase from tomatoes *(solanumLycopersicum), International Journal of Advanced Biological and Biomedical Research*, **1**(6), 2013, 637-648.

30- PENG, X., CHAO, D., HAIYANG, Y., XIAOYU, Z., XIUYUAN, Z., XIAOYU, W., Purification and characterization of polyphenol oxidase (PPO) from water yam (*Dioscoreaalata*), *CYTA – Jornal of food*, **17**(1), 2019, 676–684.
31- BRAVO, K., OSORIO, E., Characterization of polyphenol oxidase from Cape gooseberry (*Physalisperuviana*L.) fruit, *Food Chemistry*, **197**, 2016, 185–190.
32- TENG, J., GONG, Z., DENG, Y., CHEN, L., LI, Q., SHAO, Y., XIAO, W., Purification, characterization and enzymatic synthesis of theaflavins of polyphenol oxidase isozymes from tea leaf (*Camellia sinensis*), *LWT - Food Science and Technology*, **84**, 2017, 263–270.
33- AHMED, T.Y., AL-HILALI, L.A., Biochemistry. Part I, Ibn ALatheer Publishing, Mosul university, Iraq, (In Arabic), 2010, 278.
34- GUL GUEVEN, R., ASLAN, N., GUVEN, K., BEKLER, F.M., ACER, O., Purification and characterization of polyphenol oxidase from corn tassel, *Cell. Mol. Biol.*, **62**(13), 2016, 6-11.
35- LIN, H., NG, A.W., WONG, C.W., Partial Purification, and Characterization of Polyphenol Oxidase from Chinese parsley (*Coriandrumsativum*), *Food Sci. Biotechnol.*, **25**, 2016, 91-96.
36- ARSLAN, O., ERZENGIN, M., SINAN, S., OZENSOY, O., Purification of mulberry (*Morus alba* L.) polyphenol oxidase by affinity chromatography and investigation of its kinetic and electrophoretic properties, *Food Chemistry*,**88**, 2004, 479-484.
37- MADANI, I., LEE, P.M., HUNG, L.K., Partial Purification and characterization of polyphenol oxidase from Hibiscus rosa – Sinensis L, 2nd international conference on Biotechnology and food Science. IPCBEE Vol7 IACSIT Press, Singapore, 2011.
38- DERARDJA, A. E., PRETZLER, M., KAMPATSIKAS, L., BARKAT, M., ROMPEL, A., Purification and Characterization of Latent Polyphenol Oxidase from Apricot (*Prunusarmeniaca* L.), *Journal of agricultural and food chemistry*, **65** (37), 2017, 8203-8212.
39- MAHMOOD, W. A., SULTAN, S. H., HAMZA, S. R., Extraction and characterization of polyphenol oxidase from apricot, apple, eggplant, and potato, Mesopotamia *J. of Agric.*,**37**(4), 2009, 28-36.
40- CHONG, L.C., CHENG, L.H., NOOR AZIZ, A.A., Properties of polyphenol oxidase obtained from *Musa acuminata 6 balbisianaColla cv. ''PisangAwak*'' pulp and peel CyTA, *Journal of Food*, **9**(1), 2011, 25–30.
41- GACCHE, R.N., WARANGKAR, S.C., GHOLE, V.S., Glutathione, and Cinnamic Acid: Natural Dietary Components Used in Preventing the Process of Browning by Inhibition of Polyphenol Oxidase in Apple Juice, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **19** (2), 2004, 175–179.
42- CHAISAKDANUGULL, C., THEERAKULKAIT, C., WROLSTAD, RE., Pineapple juice and its fractions in enzymatic browning inhibition of Banana [Musa (AAA Group) Gros Michel], *J. Agric. Food Chem.*, **55**(10), 2007, 4252–4257.
43- RASHAN, A.I., AL-ABBASY, O.Y., The Effect of Some Antioxidants Compounds on Enzymatic and Nonenzymic Browning of Quince Juice During Thermal Treatment, Journal *of Education and Science*, **29 (4), 2020, 206 - 221**.
44- CHANG, L.H., CHEN, C.Y., TAN, F.J., Antioxidative properties of a chitosan–glucose Maillard reaction product and its effect on pork qualities during refrigerated storage,*Food Chemistry*, **124**(2), 2011, 589-595.
45- PHISUT, N., JIRAPORN, B., Characteristics and antioxidant activity of Maillard reaction products derived from chitosan-sugar solution, *International Food Research Journal*, **20**(3), 2013, 1077-1085.
46- AJANDOUZ, E.H., TCHIAKPE, L.S., ORE, F.D., BENAJIBA, A., PUIGSERVER, A., Effect of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems, *Journal of Food Science*, **66**, 2011, 926-931.
47- VAN BOEKEL, M.A., Effect of heating on Maillard reactions in milk, *Food Chemistry*, **62**, 1998, 403-414.

48- GU, F., KIM, M.J., HAYAT, K., XIA, S., FENG, B., ZHANG, X., Characteristics and antioxidant activity of ultra-filtrated Maillard reaction products from a casein–glucose model system, *Food Chemistry*, **117**, 2009, 48-54.
49- RAO, M. S., CHAWLA, S. P., CHANDER, R., Sharma, A., Antioxidant potential of Maillard reaction products formed by irradiation of chitosan–glucose solution, *Carbohydrate Polymers*, **83**(2), 2011, 714-719.
50- ZENG, Y., ZHANG, X., GUAN, Y., SUN, Y., Characteristics and antioxidant activity of Maillard reaction products from psicose-lysine and fructose-lysine model systems, *Journal of food science*, **76**(3), 2011, C398-C403.
51- GABRIELA, B., NARANJO, G.B., MALEC, L.S., VIGO, M.S., Reducing sugars effect on available lysine loss of casein by moderate heat treatment, *Food Chemistry*,**623**, 1998, 309-313.
52- BUNN, H.F., HIGGINS, P.J., Reaction of monosaccharide with proteins: possible evolutionary significance, *Science,***213**, 1981, 222-224.
53- GROTHEER, P., MARSHAL, M., SIMONNE, A., Sulfites: Separating fact from fiction. U.S. Department of Agriculture, UF/IFAS Extension Service, University of Florida, 2005, 1-5
54- YOSHIMURA, Y., IIJIMA, T., WATANABE, T., NAKAZAWA, H., Antioxidative effect of Maillard reaction products using glucose-glycine model system, Journal *of Agricultural and Food Chemistry*, **45**, 1997, 4106-4109.
55- KIM, S.J., LEE, S.Y., Antioxidant activity of Maillard reaction products derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time, *Food Chemistry*, **116**(1), 2009, 227-232.
56- AMES, J.M., Control of the Maillard reaction in food systems, *Trends Food Sci. Technol.*,**1**, 1990, 150-154.
57- FRIEDMAN, M. J., Nutritional Value of Proteins from Different Food Sources, Agric. *Food Chem.*,**44** (3), 1996, 631−653.
58- NAVARRO, M., MORALES, F.J., In vitro investigation on the antiglycative and carbonyl trapping activities of hydroxytyrosolEur, *Food Res.Technol.*,**242,** 2016, 1101−1110.
59- WU, C. H., HUANGE, S. M., LIN, J. A., YEN, G. C., Inhibition of advanced glycation end-product formation by foodstuffs, *Food & function*, **2**(5), 2011, 224-234.
60- LAKKIS, J., VILLOTA, R., Technological and Bioactive Functionalities of Canola Meal Proteins and Hydrolysates, *Food Chem.*,**43**, 1992, 93−105.
61- LINDLEY, M.G., The impact of food processing on antioxidants in vegetable oils, fruits and vegetables,*Trends in Food Science & Technology*, **9**(8-9), 1998, 336-340.