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QUALITY PARAMETERS OF REFRIGERATED STORED APPLE FRUITS BASED ON SELECTED ENZYMES AND MICROBIAL LOAD

[47]

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ABSTRACT

Quality parameters with respect to microbial load and activity of selected enzymes were stressed upon apple fruits. These two main approaches were considered through storage at 4° and 8°C for 150 days. The responded microflora included aerobic counts, yeasts and molds, psychrotrophic organisms, salmonella, lactic acid bacteria, coliform bacteria and staphylococci, while the tested enzymes were POD, PPO and PME. Experimental results proved that the tested apple samples were free of salmonella, lactic acid bacteria, coliform group and staphylococci; while psychrotrophic organisms recorded a value of $1.3x10^3$ before storage at 4°C and $7.6x10^5$ by the end of storage periods. Similar trend was found for the apple samples stored at 8°C. It is of importance to notify that total count of microflora showed a fluctuated pattern within the aforementioned storage periods. Regards the activity of the tested enzyme (POD, PPO and PME); the obtained data proved a proportional relation between their velocity and storage periods up to 90 days whether the temperature was 4° and 8°C. On prolonging storage period for 90 to 150 days a noticeable downward trend was found for the kinetic aspectics of the investigated enzymes. Statistical analysis in terms of R^2, S.D and slope of reaction confirmed the previous conclusion.

Key words: Apple fruits (Red delicious), Peroxidases (POD), Polyphenoloxidase (PPO), Pectinmethylesterase (PME), Microflora, Storage (4°, 8°C)

INTRODUCTION

Fruits and vegetables are considered to be a significant function in human diets. Apples, on the other hand, are now being one of the most important temperate fruit of the world and usually sold fresh, although some are processed into pies, fillings, flavorings, juices and cider. Other types such as crab apples are still used to make crab- apple jelly, usually from trees in home garden. (**Sharma *et al* 1998 and Taiwo *et al* 2001**).

Products such as apples are intrinsically variable in quality and storage potential at harvest. It is particularly impor-

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tant therefore that storage conditions and/or duration of storage are compatible with achievement of the required quality under the influence of low storage temperatures (**Johnson and Ridout 2000**). They also proved that during cold storage, apples may gradually soften with subsequent decrement in their acid content. The extent of development of the aromatic flavor is highly dependent on cultivar, harvest maturity and duration and regime of storage.

Polyphenoloxidase (PPO), member of the oxidoreductase group, catalyze the hydroxylation and oxidation of phenolic compounds in fruits and vegetables. Enzymatic browning due to this enzyme occur in plant cells once tissue is damaged by slicing, or cutting as given by **Oktay *et al* (1995)**. Enzymatic browning causes severe quality defects during handling of light-colored vegetables and fruits from which apples are highly susceptible towards such phenomena, (**Lozano-De-Gonzales *et al* 1993**). Other enzymes going in parallel with polyphenoloxidase activity are peroxidases (POD), which perform single-electron oxidation on a wide variety of compounds in the presence of hydrogen peroxide. It has been proposed that PPO could act as a promoter of POD activity, which could be due to the generation of hydrogen peroxide during the oxidation of phenolic compounds in PPO catalyzed reaction (**Richard and Gualliard, 1997 and Subramanian *et al* 1999**).

Pectinmethylesterase (PME) is one of the hydrolyzing enzymes realizing prime importance to the food industry. It has a great impact on fruit and vegetable processing technology because of its potential effect on the quality of the finished products. It also plays a central role in the process of fruit softening during ripening. The control of its activity, through knowledge of dependence parameters as the temperature and pH, is of great practical importance in the food industry for protecting and improving the texture and firmness of several fresh or processed fruits and vegetables (**Fayyaz *et al* 1995**).

Microbial contamination of fruits and vegetables has become of increasing concern in recently years as new outbreaks of foodborne illnesses traced to the consumption of fruits and vegetables or their products have been reported by **Tauxe, (1997)**. This is a concern to the fruit and vegetable industry particularly to the rabidly expanding use of fresh-cut fruits and vegetables where large areas of freshly cut uncolonized tissue are exposed. New Food and Drug Administration food safety regulations are expected for producers and handlers of fruits, vegetables, and their products to reduce the risk of future outbreaks (**Warner, 1997**).

The problem at hand was dealing with the pattern of changes that may occur in quality of apple fruits during storage at 4° and 8 °C with respect to the activities of POD, PPO and PME. Microflora in terms of total aerobic plates counts, yeasts, molds, psychrotrophic, lactic acid bacteria, salmonella, staphylococci and coliform bacteria was also considered.

MATERIAL AND METHODS

A- Material: About 50 Kg of Red Delicious Apples *(Malus domestica Borkh,)* that obtained from a local market, Cairo, governorate were divided into two portions and stored in perforated carton boxes (40 x 30 x 10 cm) at 4° and 8 ±1°C for 150 days.

B- Velocity of enzymes

B-1- Enzymes preparations: Extracts of Peroxidase (POD) and Polyphenoloxidase (PPO) were prepared by using 10 g of apple sample, homogenized for 2.5 min with 100 ml sucrose phosphate buffer pH 6.5. The homogenate was filtrated and centrifuged at 10000 rpm at 4°C for 15 min. The supernatant was filtrated and used for analyzed the activity of POD and PPO as described by (**Oktay *et al* 1995**).

B-2-Substrate preparations

B-2-1: Guaiacol solution that is the substrate of POD was prepared by mixing 0.5 ml of guaiacol with 0.1M K_2HPO_4 of pH 6.0 and diluted to 100 ml with the same buffer solution. During assaying. 2.5 ml of guaiacol substrate was added to 0.3 ml of POD extract in the presence of H_2O_2 (80 µl) that was added immediately before use.

B-2-2: Catechol solution that is the substrate of PPO was prepared by dissolving 1.3764 g of catechol in 25 ml McIlvain's buffer of pH 6.5; and during assaying 2.8 ml catechol substrate was added to 0.3 ml of PPO extract.

B-3- Activity of POD and PPO: were assayed using Shimadzu UV-100-02 and calculated on the basis of the slope from the linear portion of the curve at 470 and 420 nm against time up to 360 and 300 sec. respectively. Activity unit was given by **Yang *et al* (2001)** as follows:

One unit of POD activity was defined as a change of 0.1 ΔA_{470} min^{-1} gm^{-1} of enzyme extract at 25 ± 2°C and pH 6, while one unit of PPO was defined as a change of 0.1 ΔA_{420} min^{-1} gm^{-1} of enzyme extract at 25 ± 2 °C and pH 6.5.

B-4. Kinetics aspects

Km and Vmax of the two tested enzymes were carried out as described by (**Massri 2000 and Rodriguez-Lopez *et al* 2000**).

B-5. Activity of PME

Pectin methylesteras (PME) activity was measured by continuous recording of the titration of methoxy group released from a pectin solution, using a potentiometric method given by (**Fayyaz, *et al* 1995**). Unit of activity was expressed as mg of methoxy group (CH_3O) liberated at 30°C for 30 min per 100g sample, as described by the equation given by **Abd-Allah (1966)** and **Foda *et al* (1970)**

C- Microbial analysis

Total aerobic count (APC), yeast, mold, lactobacilli, psychrotrophic, salmonella, staphylococci, and coliform bacteria were enumerated at each sampling time. Samples were prepared by removing 25 g of apple fruits and blending for 2 min with sterilized peptone water (0.1%). The obtained homogenate was then diluted serially in sterilized peptone water to obtain dilutions for counting, (**Swanson *et al* 1992**). Samples were analyzed by pour plate method for APC using nutrient agar, and incubation at 37°C for 2 days. On the other hand, counts of yeasts and molds were performed on potato dextrose agar (PDA) acidified with 10% tartaric acid solution and incubated for 5 days at 25°C (**Mislivec *et al* 1992**). Lactobacilli was as-

sayed by the pour plate method using Man Rogosa Sharp (MRS) agar incubated anaerobically at 35°C for 3 days, **(Vedamuthu *et al* 1992).** Staphylococci counts were carried out by the pour plate method using Baird - Parker agar, incubated at 37°C for 2 days, **(APHA, 1992)**, while Salmonella was considered by the pour plate method using Bismuth Sulfite agar and incubated at 37°C for 2 days as described by **(Ingham and Tautorus, 1991).** Coliform bacteria was detected by the pour plate method using violet red bile agar and incubated at 37°C for 2 days **(Silk *et al* 1997),** while psychrotrophic organisms was determined by the pour plate method using nutrient agar and incubated at 5°±1°C for 7-10 days **(APHA, 1992).**

RESULTS AND DISCUSSION

1- Activity of the enzymes under investigation

1-1. POD activity

The POD activity was measured in the investigated Apple fruits before and during storage period at 8°C for 150 days. The velocity of the enzyme before storage that measured as O.D through a reaction time of the 360 sec, is given in terms of slope of reaction; i.e.2.5×10^{-4} Δ O.D x Δ sec^{-1}. Such result realizing strong correlation within the experimental condition since the calculated correlation coefficient (R^2) value reached 95.52×10^{-2} in such latter case, standard deviation (SD) and standard error (SE) were found to be 0.0284 and .0062, respectively. However, the aforementioned slope of the tested enzyme before storage was converted to Unit of enzyme activity with a corresponding value of 92.49×10^{-3} Δ O.D x Δ sec^{-1} x g^{-1} source enzyme, as seen in Table (1).

After 3 days of storage under the same condition, velocity of POD in terms of slope of activity unit reached 187.5×10^{-3} Δ O.D x Δ sec^{-1} x g^{-1} source enzyme a pattern which proved the presence of higher activity of the enzyme. The obtained R^2 98.42×10^{-2} confirmed the aforementioned pattern and SD as well as SE were only 0.0567 for the former and 0.0072 for the latter. On contrary to the previous trend, the velocity of the POD in Apple sample stored for 8 days at 8°C was extremely lower than the control as seen in Table (1), from which the unit of activity that concluded from slope of reaction was $32.2 \times ^{10-3}$ Δ O.DxΔ sec^{-1} x g^{-1} source enzyme. Such inhibition could be explained by the physiological pathways that occurred inherently leading to the formation of specified components retarding the velocity of POD. However, such trend was still noticed by extending storage period to 13 days since the enzyme activity recorded of 436.9×10^{-3} unit as Δ O.D x Δ sec^{-1} x g^{-1} source enzyme with an R^2 equal to 99.80×10^{-2}. Such pronounced increment may be due to the partial disappearance of the inhibitory components beside the capability of the enzyme to get rid of the inhibition effect. Through the other storage periods, that extended up to 90 days at the same temperature, the activity of the POD was relatively higher and giving linearity with the latter periods of storage as seen in Fig. (1) and Table (1). Such linearity pattern of the tested enzyme confirmed the release of any inhibition factor as well as the presence of a steric concentration of the responded substrate with the active center of the enzyme. The calculated

Table 1. POD activity as O.D within 360 sec.at 470 nm in Apple fruits stored at 8°C for 150 days

Responded values	Unsorted sample	Storage periods (days)							
		3	8	13	23	30	90	120	150
Slope	2.5E-04	$5E^{-04}$	$8.7E^{-05}$	$11E^{-04}$	$3E^{-04}$	$4E^{-04}$	$9E^{-04}$	$2E^{-04}$	$2E^{-04}$
Units	0.0924	0.1875	0.0322	0.4369	0.1126	0.1579	0.3536	0.0742	0.0836
R^2	0.9552	0.9842	0.9801	0.9980	0.9852	0.9890	0.9661	0.8551	0.7963
SD	0.0284	0.0567	0.0097	0.1313	0.0340	0.0476	0.1080	0.0241	0.0281
SE	0.0062	0.0072	0.0014	0.0059	0.0042	0.0050	0.0203	0.0093	0.0129
Km	$3.03E^{-2}$	$2.00E^{-2}$	$2.20E^{-2}$	0.7092	$4.16E^{-2}$	$1.06E^{-2}$	0.0970	$5.67E^{-3}$	$-1.87E^{-3}$
Vmax	2.065	10.85	5.502	-----	7.005	5.688	14.685	1.294	0.6212

*R^2 values are given from the polynomial equation

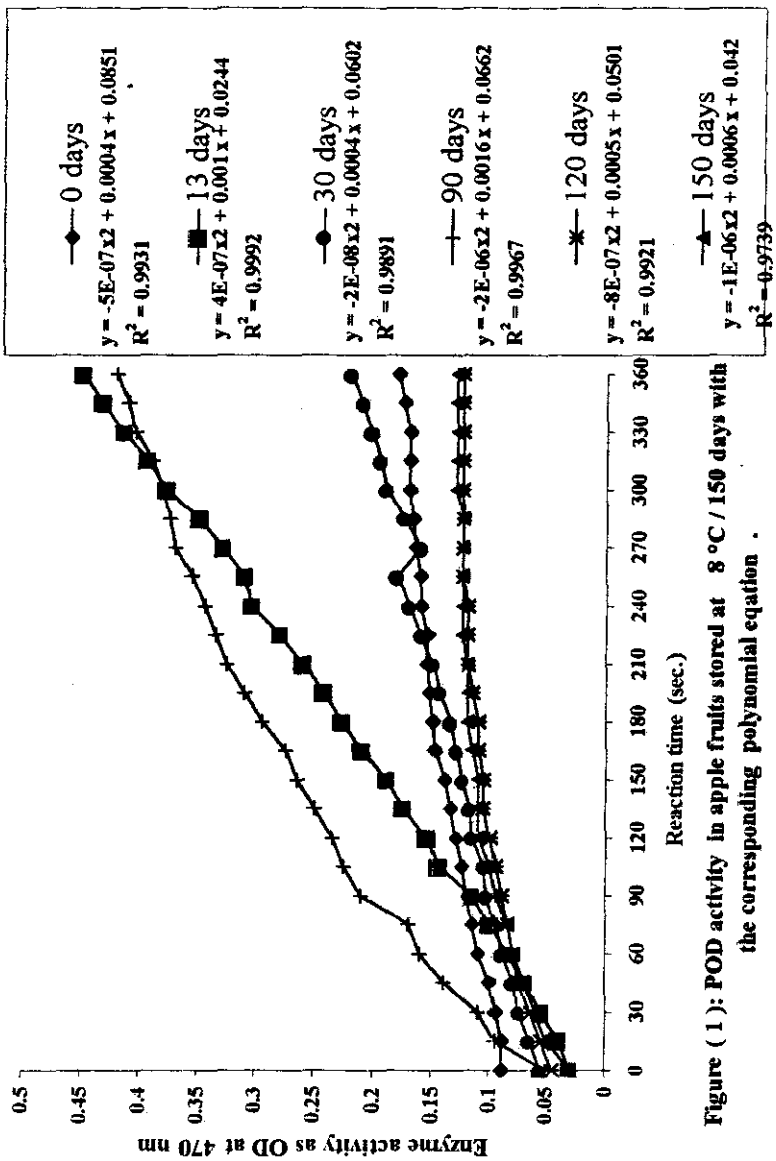

Figure (1): POD activity in apple fruits stored at 8 °C / 150 days with the corresponding polynomial equation .

R^2 values ($96.61x10^{-2}$) matched the previous conclusion. After 90 days up to the end of storage period that extended to 150 days, there was a fluctuated pattern of activities, a trend that may be related to the role of physiological function of living cells. In such latter case, the Vmax reached 62.12 x 10^{-2} Δ O.D x min^{-1} x g^{-1} source enzyme.

The POD activity was also measured during storage at 4°C for 150 days through which unit of activity was calculated from the slope of O.D values in relation to time of reaction. The data given in Table (2) proved the following trends:

* Units of activity of the POD enzyme were 92.4 x 10^{-3}Δ O.D x Δ sec^{-1} x g^{-1} source of enzyme before storage under the aforementioned condition.
* It seems evidence the presence of slow down trend in the activity of POD as a result of storage, since the activity units that were 52.1x 10^{-3} after storage for 3 days showed a down ward trend up to 90 days under similar condition of storage. The obtained unit activity in such latter case was found to be 76.2 x 10^{-3} Δ O.D x Δ sec^{-1} x g^{-1} source enzyme in relation to 121.5 x 10^{-3} Δ O.D x Δ sec^{-1} x g^{-1} source enzyme that reached for the sample stored for 13 days at 4°C as seen in the same table.
* On calculating the correlation coefficient of "R^2", experimental data showed a strong relation between O.D values and time of reaction. All of the given "R^2" values exceed $80.14x10^{-2}$ / 150 days at 4°C with the exception of the POD activity in the Apple sample stored for 90 days at 4°C.

The aforementioned results were figured in Fig. (2) with the corresponding exponential equations from which the POD activity of the unstored apple fruits (0 days) was higher in relation to the other storage periods up to 150 days. In such cases the R^2 value was found to be $99.31x10^{-2}$ for the former sample while it was $94.05x10^{-2}$ for latter one. **Gasik and Horubala (1990)** determined the concentration of total polyphenol bonds or that of individual groups (catechins and leucoanthocyanidins) and the polyphenoloxidase (PPO) and peroxidase (POD) activities on the rate at which apple pulp of 7 varieties darkens. Results indicated that total polyphenols, catechins and leucoanthocyanidins and PPO and POD activities are not sufficient to establish the rate of enzymatic browning of apples. It is considered that the splitting of the polyphenol bonds is a better indicator of the susceptibility of apple pulp to enzymic browning.

1-2. PPO activity

Through the aim of the study, activity of the enzyme polyphenoloxidase (PPO) was considered also as a measure of quality level. With this viewpoint, velocity of the enzyme activity was measured in raw apple and during storage for 150 days at 8 °C and 4 °C. With such viewpoints, the PPO activity in apple sample stored at 8 °C, was considered through the slope of reaction between changes in O.D at 420 nm and time of reaction. The obtained slope values were expressed also in unit of activity as seen in Table (3).

The overall velocity trend of activity possessed a decline patterns through the whole storage period that extent up to 150 days at the same 8°C. For instance, velocity of PPO was 44.9 x 10^{-3} before storage and minimized to 17.0 x 10^{-3} Δ O.D x Δ sec^{-1} x g^{-1} source enzyme after

Table 2. POD activity within 360 sec as O.D at 470 nm in Apple fruits stored at 4°C for 150 days

Responded values	Unsorted sample	Storage periods (days)							
		3	8	13	23	30	90	120	150
Slope	$2E^{-04}$	$1E^{-04}$	$5.5E^{-05}$	$3E^{-04}$	$2E^{-04}$	$2E^{-04}$	$2E^{-04}$	$3E^{-04}$	$1E^{-04}$
Unit	0.0924	0.0521	0.0202	0.1215	0.1045	0.0801	0.0762	0.1396	0.0511
R^2	0.9551	0.9481	0.9947	0.9936	0.9806	0.9854	0.5061	0.9477	0.8014
SD	0.0284	0.0160	0.0061	0.0366	0.0317	0.0242	0.0321	0.0430	0.0171
SE	0.0061	0.0037	0.0004	0.0029	0.0045	0.0029	0.0409	0.0100	0.0078
Km	$3.032E^{-2}$	$-1.64E^{-3}$	$2.96E^{-2}$	0.2554	$4.54E^{-2}$	$1.74E^{-3}$	$-9.64E^{-4}$	-1.21^{-2}	$1.55E^{-3}$
Vmax	2.065	16.103	2.560	26.203	9.139	2.928	0.6464	0.2488	0.9074

*R^2 values are given from the polynomial equation

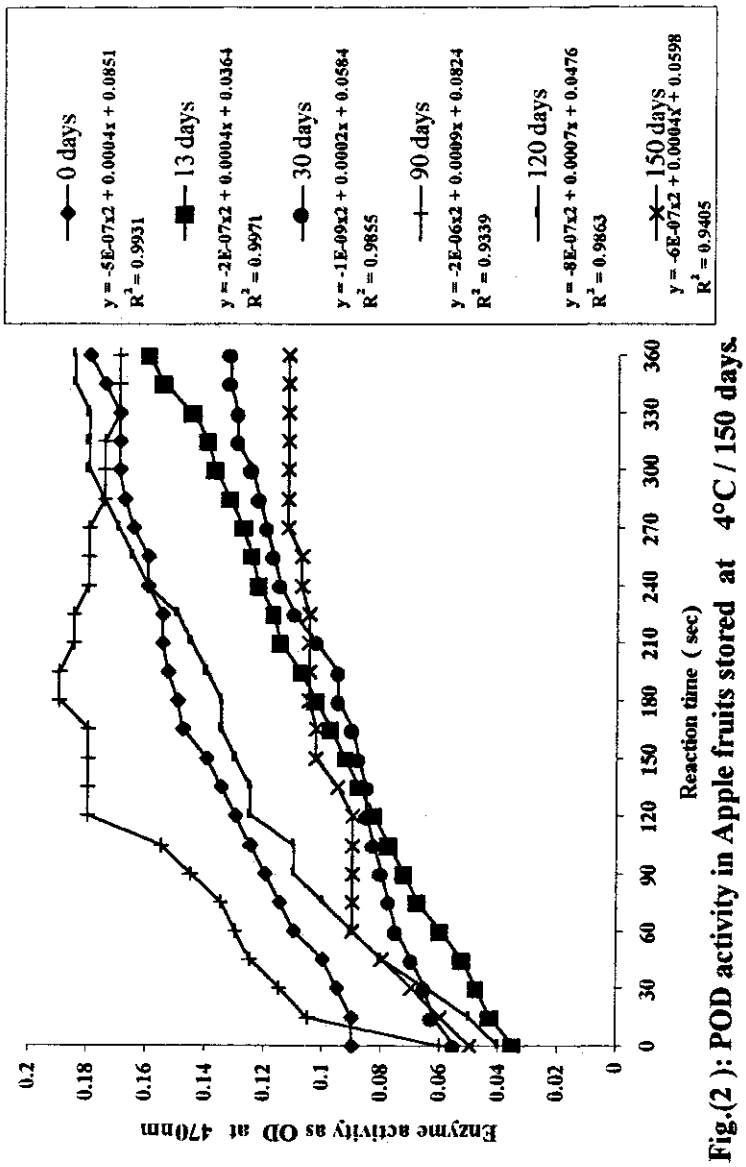

Fig.(2): POD activity in Apple fruits stored at 4°C / 150 days. With the corresponding polynomial eqation

Table 3. PPO activity within 300 sec. as O.D at 420 nm in Apple fruits stored at 8°c for 150 days

Responded values	Unsorted sample	Storage periods (da\ys)							
		3	8	13	23	30	90	120	150
Slope	$1E^{-04}$	$7.7E^{-05}$	$4.1E^{-05}$	$9.1E^{-05}$	$1.1E^{-05}$	$3.7E^{-05}$	$4.6E^{-05}$	$1.7E^{-05}$	$6.7E^{-05}$
Unit	0.0449	0.0284	0.0152	0.0336	0.0043	0.0138	0.0170	0.0063	0.0246
*R^2	0.8201	0.8626	0.9289	0.9574	0.6825	0.7646	0.8201	0.824	0.9113
SD	0.0150	0.0092	0.0058	0.0109	0.0025	0.0070	0.0066	0.0043	0.0071
SE	0.0101	0.0059	0.0044	0.0070	0.0023	0.0062	0.0052	0.0041	0.0036
Km	$3.629E^{-3}$	$1.289E^{-3}$	$3.51E^{-3}$	$-3.79E^{-3}$	$2.95E^{-2}$	$3.08E^{-2}$	$3.55E^{-3}$	$-2.70E^{-3}$	$2.84E^{-2}$
Vmax	0.1038	0.2010	0.1663	0.3753	-0.5390	$6.150E^{-2}$	0.6296	0.6168	2.060

*R^2 values are given from the polynomial equation

90 days of storage then continued to $6.3x10^{-3}$ after 120 days of storage under similar condition as seen in the same Table. Km and V max within the different days of storage proved the tendency of the enzyme to lose partial level of its activity.

On calculating the correlation coefficient between velocity of the PPO and time of reaction, the polynomial linear equation in terms of: ($Y=-6\ E^{-7}\ X^2 + 0.0002\ X + 0.0555$) showed R^2 to be varied between $68.25x10^{-2}$ (23 days storage) to 95.74x10-2 (13 days storage).

The aforementioned results are also illustrated in Fig. (3) from which the PPO activity during any given period of storage was less than the unstored samples.

The apple samples stored at 4 °C were put forward to check their quality during storage. To reach such goal, the velocity of PPO was considered up to 150 days of storage at 4°C as seen in Table (4). On comparing the reaction velocity of the PPO during storage under the aforementioned condition, the activity of the enzyme through the reaction periods of 300 sec possessed the lowest value in the unstored sample in relation to any velocity given for the stored sample as seen in Fig (4). Such trend was out of order after calculating slope of reaction and unit of enzyme activities. In other words, velocity of PPO in the unstored apple samples was higher than that measured at any given storage period. For instance, PPO activity that was 44.9×10^{-3} in the unsorted apple sample had been minimized to 9.7×10^{-3} unit.g^{-1} enzyme source after 90 days of storage under similar conditions as seen in Table (4). Correlation coefficient of the same data based on the polynomial equation ($Y=-1\ E^{-6}\ X^2 + 0.0004\ X + 0.0301$) indicated a minimal value of R^2 to be $64.64x10^{-2}$ (30 days of storage) up to a maximal value of $98.48x10^{-2}$ (3 days of storage). The calculated SD and SE confirmed the aforementioned conclusion.

On prolonging storage period up to 120 days under similar storage conditions; data of Table (4) showed further decline in the velocity of the PPO ($3.8x10^{-3}$ unit.g^{-1} enzyme source). Within the latter period of storage; i.e. 150 days at 4°C, the enzyme PPO tends to promote a noticeable reactivity reaching $41.2x10^{-3}$ unit.g^{-1} enzyme source as seen in Fig (4). It seems evidence from the data that Vmax was positively correlated with storage periods; being 0.1038 before storage and 0.7144 Δ O.D x Δ min^{-1} x g^{-1} source of enzyme.

Polyphenoloxidases (PPO; catechol oxidases) extracted from apples were characterized by **Weemeas *et al* (1998)** with respect to absorption maximum of the extract, pH optimum, molecular weight isoelectric point and heat stability. Heat inactivation of the apple PPO as well as characterization of enzyme thermostability in terms of kinetic parameter allowed comparison of the heat resistance of PPO in the fruit systems studied. of the apples PPO, maximum enzyme activity was observed at pH 7. A sharp drop in enzyme activity was noticed when pH was increased from 7 to 8 and the enzymes showed very low activity at or below pH 4. These results were matched with the pH applied for measuring the activity of PPO and POD in the tested apple sample.

1-3. PME activity

Activity of PME was assayed in the apple sample stored at 8 and 4 °C for 150

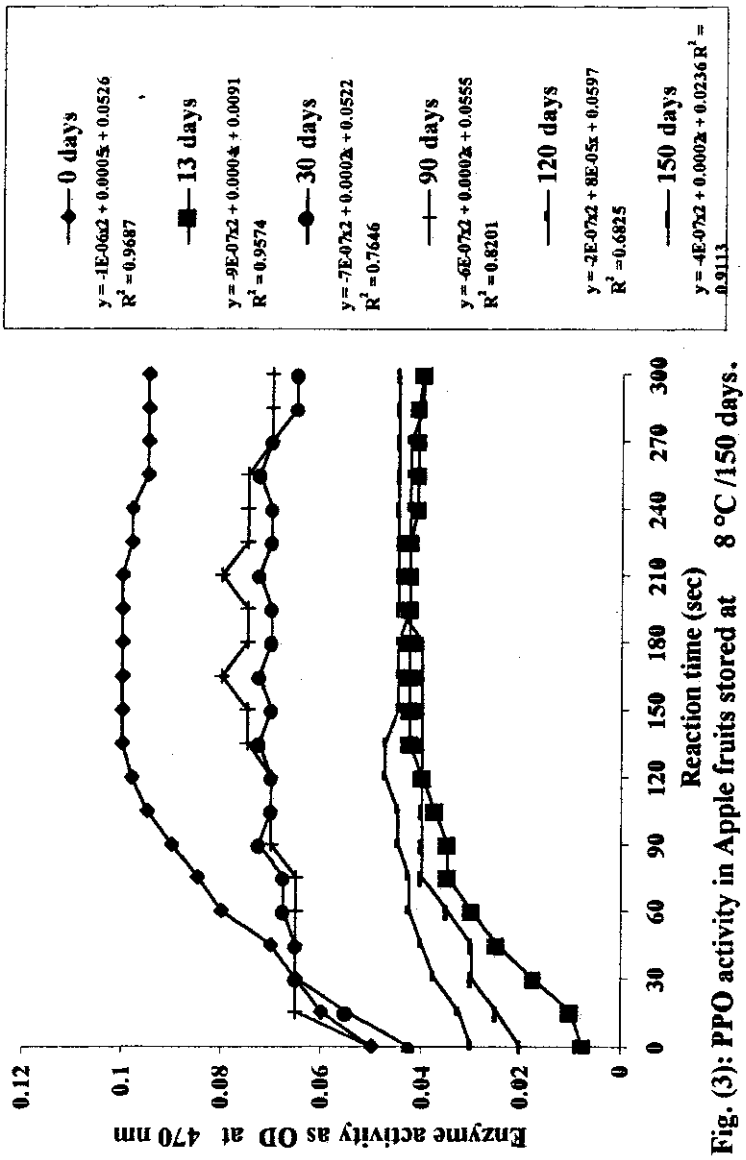

Fig. (3): PPO activity in Apple fruits stored at 8 °C /150 days. With the corresponding polynomial eqation

Table 4. PPO activity within 300 sec. as O.D at 420 nm in Apple fruits stored at 4°c for 150 days

Responded values	Unsorted sample	Storage periods (days)							
		3	8	13	23	30	90	12 0	150
Slope	$1E^{-04}$	$3.2E^{-05}$	$2.1E^{-05}$	$7.9E^{-05}$	$2.3E^{-05}$	$-1.5E^{-05}$	$2.6E^{-05}$	$1.0E^{-05}$	$1E^{-04}$
Unit	0.0449	0.0117	0.0079	0.0291	0.0085	-0.0056	0.0097	0.0038	0.0412
*R^2	0.8201	0.9848	0.9671	0.8851	0.6525	0.6464	0.8343	0.5994	0.9365
SD	0.0150	0.0036	0.0029	0.0121	0.0033	0.0096	0.0100	0.0026	0.0112
SE	0.0101	0.0020	0.0021	0.0098	0.0026	0.0097	0.0099	0.0025	0.0042
Km	$3.629E^{-3}$	$4.024E^{-2}$	$4.211E^{-2}$	$1.904E^{-3}$	$-1.14E^{-3}$	$1.595E^{-2}$	$-2.96E^{-3}$	$-4.46E^{-3}$	$-5.52E^{-4}$
Vmax	0.1038	0.8151	0.7205	0.4499	0.5411	0.2417	0.4911	0.3996	0.7144

*R^2 values are given from the polynomial equation

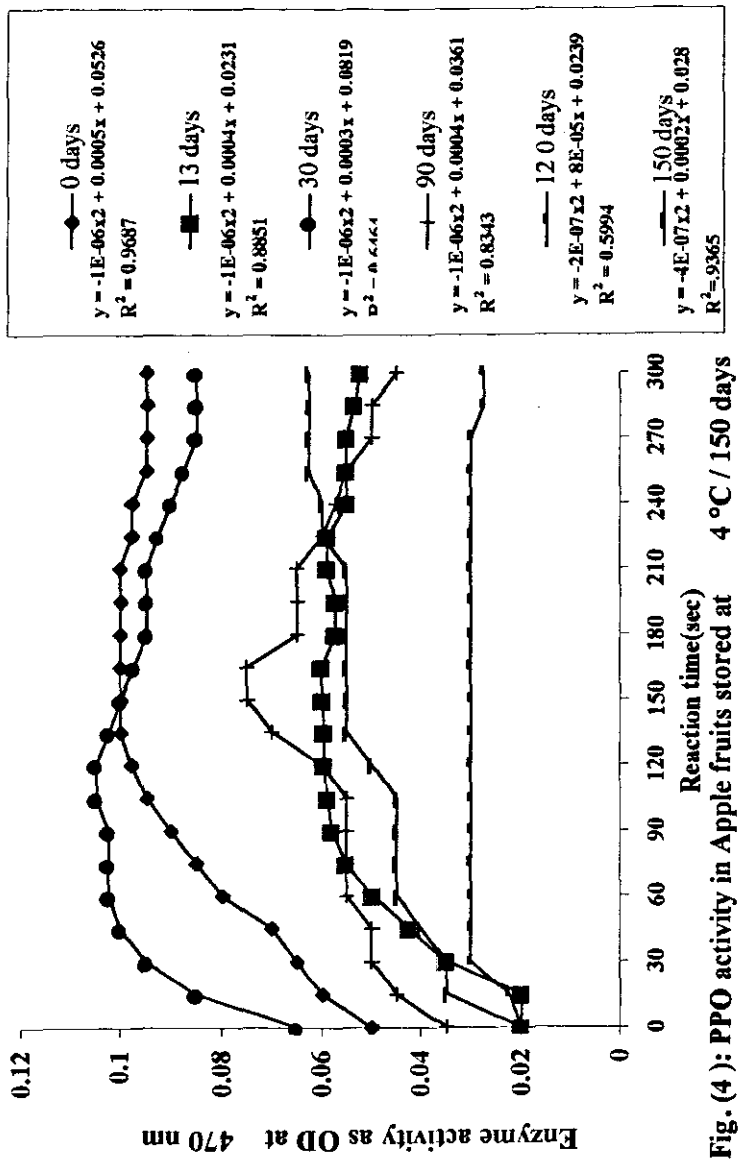

Fig. (4): PPO activity in Apple fruits stored at 4 °C / 150 days With the corresponding polynomial eqation

days as given in Table (5). The overall trend of the PME velocity showed a down ward trend on storage at 4°C for 150 days. R^2 value for the activity of the PME within the storage period was $47.17x10^{-2}$ with a negative slope when storage was performed at 4°C. On the other hand, the PME showed a similar negative trend of the apple on using 8°C for 150 days but with lower value than those of 4°C. So, these results could be explained on the basis that 8°C is suitable than 4°C for the activity of the PME but not matched with the hardness of apple fruits. In other words and on the bases of PME activities, it seems evidence the choice of 4°C rather than 8°C for storing apple fruits.

The noticeable differences in the rate of activity of pectinmethylestrase (PME) in the investigated apple samples during stored could be explained mainly on changes in pectin ester content as well as the increase in free carboxyl groups liberated from the pectin content of the sample. However, the free carboxyl groups are more convenient than a chemical determination of ester content. This procedure is more convenient for measurement of PME activity in pH ranges from 6.5 to 7.5. The presence of a natural buffer in a crude enzyme preparation, however, may interfere with the shift in pH, which in turn affects the enzyme activity and its measurement. **(Lee and Wiley, 1970)**.

Statistical analysis of the Data given in Table (5) concerned with the apple fruit sample stored at 4°C showed the following trends:

* Analysis of Variancea q\ proved the presence of "F" ratio to be 5.36 with a probability level of 0.060,
* The regression equation between activity of PME and storage was found to be

$$Y = 6.07x10^{-4} + (-2x10^{-6}X)$$

Where:

Y = activity of PME as mg $CH_3Oxg^{-1}xmin^{-1}$

X =storage periods in days

When storage was preformed for the apple fruit at 8°C for 150 days, analysis of the data of Table (5) that given as statistical parameters was found to be

$$Y = 6.24x10^{-4} + (-2x10^{-6}X)$$

Where:

Y equal to the activity of PME as mg $CH_3Ox\ g^{-1}x\ min^{-1}$.

X; equal to storage period that attended for 150 days at 8°C.

* Slope of reaction showed the upward trend of the PME velocity where fruit samples stored at 8°C for 150 days

Effect of storage at 4° and 8 °C on microbial population of apple samples

Experiments were considered to shed light upon the microbial counts of the investigated apple during storage at 4° and 8°C. The present data in Tables (7) and (8) indicated that 4°C of storage was effective relatively in reducing microbial colony forming units (CFU) on nutrient agar medium. For instance, microbial organisms (APC) of the tested samples stored at 8°C were generally higher during the following days 6, 15, 60, and 90 ($3.0x10^2$, $6.6x10^3$, $3.9x10^3$ and $2.3x10^5$, respectively), compared to corresponding 4°C, although the counts of APC were more numerous in apple tissues stored at

Table 5. Activity of PME in Apple fruits stored at 4°C & 8°C / 150 days

Storage days	Activity of Replicates at					
	Mean at 4°C			Mean at 8°C		
	I	II	Average	I	II	Average
0	0.000689	0.000775	7.32×10^{-4}	0.000689	0.000775	7.32×10^{-4}
3	0.000689	0.001033	8.61×10^{-4}	0.000517	0.000517	5.17×10^{-4}
8	0.000413	0.000517	4.65×10^{-4}	0.000517	0.000517	5.17×10^{-4}
13	0.000344	0.000344	4.43×10^{-4}	0.000344	0.000689	5.17×10^{-4}
23	0.000689	0.000689	6.89×10^{-4}	0.000517	0.000517	5.17×10^{-4}
30	0.000344	0.000344	3.44×10^{-4}	0.000689	0.000689	6.89×10^{-4}
90	0.000413	0.000413	4.13×10^{-4}	0.000758	0.00062	6.89×10^{-4}
120	0.000344	0.000344	3.44×10^{-4}	0.000413	0.000413	4.13×10^{-4}
150	0.00031	0.000241	2.76×10^{-4}	0.000344	2.41E-05	2.93×10^{-4}
Slope	-1.754E-06	-2.974E-06	-2.37E-06	-7.9815E-07	-2.9442E-06	-1.51E-06
R2	0.35107453	0.418038	0.471775	0.087433456	0.567528395	0.353253
SD	0.0001670	0.000259	0.000204	0.000152253	0.000220442	0.000151
SE	0.00014387	0.0002117	0.000161	0.000155487	0.000154977	0.000131

Table 6. Statistical analysis of PME activity (mg of CH_3O. $g^{-1}.min^{-1}$) within storage periods

Regression Analysis: PME (4°C) versus 150 days
The regression equation is
PME(4°C) =0.000607 -0.000002 storage days

Predictor	Coef	SE Coef	T	P
Constant	0.00060735	0.00007762	7.82	0.000
storage	-0.00000237	0.00000102	-2.31	0.060

S = 0.0001607 R-Sq = 47.2%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	1.38351E-07	1.38351E-07	5.36	0.060
Residual Error	6	1.54906E-07	2.58176E-08		
Total	7	2.93257E-07			

Regression Analysis: PME (8°C) versus 150 days
The regression equation is
PME (8°C) =0.000624 -0.000002 storage days

Predictor	Coef	SE Coef	T	P
Constant	0.00062395	0.00006339	9.84	0.000
storage	-0.00000151	0.00000083	-1.81	0.120

S = 0.0001312 R-Sq = 35.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	5.64403E-08	5.64403E-08	3.28	0.120
Residual Error	6	1.03332E-07	1.72221E-08		
Total	7	1.59773E-07			

4°C for 21 and 33 days ($1.2x10^2$ and $1.2x10^3$, respectively). In general, counts increased substantially after 90 days of storage for both the two storage temperatures as seen in the same Tables.

The PDA medium (made semi-selective for yeasts and molds by acidification to pH 3.5) showed a noticeable counts differences in relation to results for APC as seen in the same Tables. Upon storage at 8°C, the counts of yeasts and molds decreased two logarithmic cycles; on such a base the mean numbers of the tested samples for 6, 15, 21, 33 and 60 days were ($3.0x10^1$, $7.0x10^1$, $4.5x10^1$, $2.5x10^1$ and $2.0x10^1$, respectively). A similar trend was noticed at 4°C, since the counts of tested samples decreased two logarithmic cycles after 3, 6, 9, 12 and 15 days ($7.0x10^1$, $1.5x10^1$, $7.5x10^1$, $1.5x10^1$and $9.0x10^1$, respectively), whereas decrement pattern after 21, 33 and 90 days, was found to be one logarithmic cycle ($2.7x10^2$, $3.1x10^2$ and $1.4x10^2$, respectively). These results are in agreement with Chand-Goyal and Spotts, 1996 who found that approximately yeast and bacterial populations on the surface of Golden Delicious were $8.0x10^3$ and $9.5x10^4$ CFU/cm^2, respectively.

Data in Tables (7) and (8) showed also the variable counts of psychrotrophic organisms in apple tissue stored under the aforementioned condition. It was clearly found that, at 4°C the psychrotrophic counts after 6 and 15 days showed a down ward trend of two logarithmic cycles; on contrary, counts were slightly reduced in apple tissue in the days 33 and 60 reached ($1.8x10^2$and $2.6x10^2$, respectively).

A similar trend was noticed when storage was performed at 8°C, since within the first 9 days the psychrotrophic counts decreased one logarithmic cycle but in the 15, 21, 27, and 33 days a decrement of two logarithmic cycles was scored. On the other hand, a count increased substantially after 90 days of storage. In such a case the mean number of psychrotrophic counts tends to realize a positive increment of two logarithmic cycles for both of the two storage temperatures, ($7.6x10^5$ for the 4 °C and $2.9x10^5$ for the 8°C), as seen in Tables (7) and (8). Microbiological analysis was discontinued after 120 days due to excessive microbial growth as noticed in the same Tables. Experimented work proved also that all of the tested samples were found to be free of salmonella, staphylococci, coliform bacteria and lactic acid bacteria. It is of importance to clarify that the investigated samples are completely free of coliform group. But **Janisiewicz *et al*** (1999) stated that the *E. Coli* O157:H7 can grow exponentially on freshly cut apple tissue and this should be considered by the industry during the handling and processing of the apples. It is axiomatic that any measures taken to prevent or reduce the establishment of *E. coli* O157:H7 on apple tissue will reduce the risk of potential illnesses due to consumption of the contaminated fruits and their products. It will also reduce the risk of cross-contamination of fruit during post-harvest handling. From the obtained mentioned results, it is obvious that 4 °C could be recommend for preserving apple fruits for about 120 days for inhibiting and controlling of the tested enzymes namely PPO, POD and PME.

Table 7. Effect of storage at (4±1°C) on the growth of microbial populations on cut apple tissue

Storage days at 4°C	Microbial groups as CFU/ml blended cut apple tissue		
	Total aerobic count on plate nutrient agar	Yeasts&Molds on acidified PDA	Psychrotrophic organisms on plate nutrient agar
0	$1.3*10^3$	$3.0*10^3$	$1.3*10^3$
3	$7.6*10^2$	$7.0*10^1$	$3.7*10^2$
6	$4.0*10^1$	$1.5*10^1$	$4.5*10^1$
9	$9.0*10^1$	$7.5*10^1$	$5.4*10^2$
12	$1.0*10^1$	$1.5*10^1$	$8.4*10^2$
15	$8.0*10^1$	$9.0*10^1$	$8.0*10^1$
21	$1.2*10^2$	$2.7*10^2$	$1.3*10^2$
27	$5.0*10^1$	$1.0*10^1$	$6.0*10^1$
33	$1.2*10^3$	$3.1*10^2$	$1.8*10^2$
60	$1.9*10^2$	$7.5*10^1$	$2.6*10^2$
90	$1.4*10^4$	$1.4*10^2$	$7.6*10^5$
120	$2.8x10^8$	$1.4*10^2$	$3.6*10^6$

Table 8. Effect of storage at (8±1°C) on the growth of microbial populations on cut apple tissue

Storage days at 8°C	Microbial groups as CFU/ml blended cut apple tissue		
	Total aerobic count on plate nutrient agar	Yeasts&Molds on acidified PDA	Psychrotrophic organisms on plate nutrient agar
0	$1.3*10^3$	$3.0*10^3$	$1.3*10^3$
3	$8.0*10^1$	$1.4*10^3$	$9.1*10^2$
6	$3.0*10^2$	$3.0*10^1$	$3.4*10^2$
9	$4.7*10^3$	$1.1*10^2$	$9.0*10^1$
12	$4.0*10^1$	$8.5*10^1$	$1.0*10^2$
15	$6.6*10^3$	$7.0*10^1$	$2.0*10^1$
21	$4.0*10^1$	$4.5*10^1$	$8.0*10^1$
27	$1.0*10^1$	$1.0*10^1$	$6.5*10^1$
33	$4.5*10^1$	$2.5*10^1$	$1.5*10^1$
60	$3.9*10^3$	$2.0*10^1$	$8.7*10^2$
90	$2.3*10^5$	$2.0*10^1$	$2.9*10^5$
120	$1.1x10^{10}$	$1.7x10^2$	$1.1x10^8$

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معايير الجودة المرتبطة ببعض الانزيمات والحمل الميكروبي في ثمار التفاح المخزن بالتبريد

[٤٧]

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أخذ فى الإعتبار دراسة معايير الجودة لثمار التفاح والتى تضمنت الحمل الميكروبى ونشاط بعض الانزيمات من خلال اختيار درجتى حرارة ٤ و٨°م خلال فترة التخزين والتى امتدت الى ١٥٠ يوما, وكانت الميكروبات موضع الدراسة التى تم الكشف عنها هي العدد الكلى والخمائر والفطريات والميكروبات المقاومة للبرودة والسلمونيلا وبكتيريا حمض اللاكتيك وبكتيريا القولون وبكتيريا التسمم العنقودي. بينما الانزيمات التى تم تقدير نشاطها هي انزيمات البيروكسيديز والبولي فينول اكسيديز والبكتين ميثيل استريز. ولقد اشارت النتائج المتحصل عليها ان عينات التفاح المختبرة كانت خالية تماما من السلمونيلا وبكتيريا التسمم العنقودي وبكتيريا القولون وبكتيريا حمض اللاكتيك. اما الميكروبات المقاومة للبرودة فقد تراوحت قيمتها من ١٠ X١,٣ [٣] قبل التخزين على ٤°م الى ١٠X٣,٩ [٦] مزرعة ميكروبية/١ مل في نهاية فترة التخزين ولقد وجد نفس الاتجاه لعينات التفاح المخزن على ٨°م. هذا ومن الامور الهامة الجديرة بالذكر ان سلوك الحمل الميكروبي اظهر تذبذبا خلال فترة التخزين المذكورة.

وفيما يتعلق بالنشاط الانزيمى موضع الدراسة(البيروكسيديز والبولى فينول اكسيديز والبكتين ميثيل استريز) فان النتائج المتحصل عليها اشارت الى ان هناك علاقة طردية بين سرعة النشاط الانزيمي وفترة التخزين والتى امتدت الى ٩٠ يوما سواء كان التخزين على ٤ او على ٨° م اما عند اطالة فترة التخزين الى ١٢٠ و١٥٠ يوما وجد ان هناك اتجاها عكسيا في حركيات الانزيمات موضع الدراسة.

ولقد اكدت نتائج التحليل الاحصائي والتى شملت معامل الارتباط وميل التفاعل والانحراف القياسي الاستنتاج الذي تم التوصل اليه والمذكور سابقا.

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