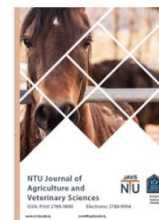




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## Effect of conventional blanching of apple slices on quality characteristics of apple juice after storage at 4°C for 30 days.

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keyword1, PPO  
keyword2, Blanching  
keyword3, Browning Index  
keyword4, Total Soluble Sugars  
keyword5. Ascorbic Acid  
keyword6. Total Phenolic Compounds

### A B S T R A C T

This study showed to extract A.J from G.A fruits that had been treated by blanching. The usual thermal process, before keeping the juice at a of (4)°C, for (1) month, and comparison it with the juice of untreated fruits in order to measurement the effect of inhibiting the enzyme (PPO) in G.A with the treatments which mentioned in some properties of PPO (Polyphenol oxidase). A.J which Prepared from these G.A, and the characteristics studied are: Residual Enzymatic Activity, (T.S. S), (A A) content, (T. P.C), and (B.I), as blanching the fruits in the usual way had a Significantly affected in values of the remaining Activity Enzymatic and (A.A) values of the juice of the treated fruits, While the (T.S.S) and (T.P.C) had a increase in their values after one month storage, as for the (B.I) values . There was no obvious effect at the end of the storage.process

**Abbreviations** [Ascorbic Acid (A.A)Apple juice (A.J) - Browning Index(B.I)- Total Soluble Sugars(T.S.S)- Total Phenols content(T.P.C)- Polyphenol oxidase (PPO)- Green Apple(G.A)- water bath(W.B)- Folin Reagent Solution (F.R.S)- Residual Enzymatic Activity(R.E.A)]



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## Introduction

Apples is one of the strategically important crops and is included in the field of many important food industries, such as juices and vinegar. Its scientific name is *Mauls domestic*. It belongs to the family Rosacea. The apple tree is a deciduous tree that reaches a height of (5-12) meters. Apples were known to the ancient Greeks since the third century BC, and there are many varieties and types of apple fruit, It was transferred to all parts of Europe and Asian countries by the Romans, [1].

Polyphenol oxidase is a type of redox enzymes that is found naturally in fruits and vegetables such as bananas, apples, peaches, avocados and potatoes. This enzyme plays an important role in the occurrence of enzymatic browning reactions, leading to changes such as color and other problems manufacturing, [2].

The enzyme polyphenol oxidase causes enzymatic browning during chopping of fruits and vegetables or during storage, leading to changes in color, flavor, and nutritional value, and thus, Reducing their shelf life. This enzyme works to oxidize phenolic compounds and form Orthoquinones , [3]

These enzymes were classified according to their ability to oxidize according to what was mentioned by [4].

### 1- E.C.1.14.18.1 (Monophenol Oxidases)

These enzymes only oxidize monophenols such as (Tyrosine, p-Cresol) and do not have the ability to oxidize diphenols.

### 2- E.C. 1.10.3.1-(Diphenol Oxidases)

These enzymes perform two types of reactions, the first is adding a hydroxyl group to monophenols, and the second reaction involves removing hydrogen from diphenols to obtain Quinone compounds.

These enzymes are usually found in the plant kingdom, especially in apples and peaches. Other names for these enzymes are called (Phenolase, Catecholase, Polyphenolase).

### 3- (Laccase) E.C. 1.10.3.2

These enzymes are often found in microorganisms (fungi), where they work to oxidize mono- and di-phenols, amino phenols, and diamine aromatic compounds.

**Table 1.** Some sources from which the urinary enzyme was isolated and studied

Source type	Examples
vegetarian	Apples - potatoes - eggplant - bananas - peaches – grapes - quince - apricots
animal	Crayfish - shrimp - crayfish - insects
Microbiology	Aspergillus-Russula-Glomerella in some types of fungi

PPO enzymes cause damage and losses in crops amounting to more than 50% in fruits and vegetables. These enzymes work to deteriorate the quality of the products, changes in weight and flavor, and loss of nutritional value. Brown discoloration does not only lead to changes in fruit,

vegetable and food products, but rather the effect works on., Its type and quality also influence consumer choices and decisions, so enzymatic browning must be controlled to maintain quality Foods, especially juices [5],[6].

Structural interactions are divided into two main types:

### 1 - Non-enzymatic structural reactions:

They are non-enzymatic chemical reactions that produce dyes and brown colors such as Maillard reactions, caramelization and catabolism of ascorbic acid.

### 2 - Enzymatic structural reactions:

The reason for these reactions is due to the activity of enzymes, which leads to large economic losses for manufacturers and farmers. This browning often occurs after the process of harvesting crops, during their handling, or during storage and transportation. Enzymatic browning occurs mainly due to the enzymes polyphenol oxidase ( EC 1.10.3.1 PPO) and peroxidase ( EC 1.11.1.7. POD ), Tyrosnase EC 1.14.18.1 [7].

The use of heat treatments at (80)°C for (20) minutes or at (60)°C for (40) minutes is sufficient to eliminate enzyme activity by (90)%. The optimal temperature for enzyme action is (30-35)°C and retardation. This temperature varies from one type to another and depends on the material to be heated. If the temperature is higher or lower than the optimum temperature for the enzyme to work, it affects the activity, effectiveness and work of the enzyme [7].

Blanching is one of the thermal treatments that take place food products. The purpose of these processes is to eliminate on microorganisms and inhibiting and deactivating the enzymes responsible for the appearance of brown color, such as PPO and other enzymes. The temperatures used in the Blanching process vary from one product to another, but in general the temperatures used in the Blanching process, it is between (70-90)°C [8].

## Material and Methods

### Treatments:

The green apple fruit was washed well with water, then cut into slices, boiled in water, then the juice was extracted from the slices, and the resulting juice was pasteurized at (85) °C for (5) minutes in a water bath [9]. After that, it was filled into bottles in special opaque glass containers, leaving a vertical space, and the containers were closed, and the temperature of the bottled juice was cooled to normal room temperature using tap water (comparison sample).

### Boil green apple fruits using the usual thermal method before extracting their juice And its packaging

After selecting well-ripened green apple fruits, they were washed, cleaned, and then cut, before being treated with conventional thermal

boiling at a temperature of (90)°C for (8) minutes in a water bath [10], followed by extracting their juice using an electric juicer, after which the resulting juice was filtered, then heated at a temperature of eighty-five degrees Celsius for ten minutes, and filled while hot in containers, leaving a small vertical space, after which the containers were closed, and the juice was cooled with cold water to about (25)°C.

#### **Storage**

The packages were kept in the refrigerator at Four degrees Celsius for thirty days, and different assessments were made every seven days of storage.

#### **Enzymatic activity estimation**

By measuring the change in absorption, the activity of polyphenol oxidase enzyme was measured according to what was mentioned by [11], using a wavelength of (420) nm as follows:

1- Place five ml of the buffer solution in a test tube that was prepared in advance and add five ml of juice to it and close the tubes and place in the mixing device for one minute.

2- Centrifugation was carried out for sixty minutes using a speed of four thousand revolutions per minute and using filter paper and a thin layer to filter the samples.

3- Place the filtered juice in test tubes in a water bath at a temperature of thirty degrees Celsius.

4- A suitable amount was taken and placed in the cell of the spectrophotometer to record the absorption values after adjusting the device to a wavelength of (420) nm, The remaining enzyme activity (%) was calculated by applying the following law

$$B / A \times 100$$

Since

A = enzyme activity in juice extracted from untreated fruits.

B = Enzyme activity in juice extracted from treated fruits.

#### **Estimating the concentration of total dissolved sugars**

Endogenous total sugars were measured according to the method described in [12].

#### **Estimating steps**

1- Juice samples were placed in test tubes and distilled water was added to dilute them, then phenol solution with a concentration of (5)% and concentrated sulfuric acid were added.

2- Samples were placed at normal room temperature for five minutes with shaking for color development, then placed in a water bath at a temperature of twenty-five for five minutes for cooling.

3- Using a spectrophotometer at a wavelength of (490) nanometers, the readings were recorded.

4- The previous steps were repeated by replacing the juice sample with distilled water.

5- The standard curve was prepared using glucose solution at different specific concentrations.

#### **Determination of ascorbic acid content**

The ascorbic acid content of apple juice samples was estimated using the burette method

Ascorbic acid was determined in the samples according to the method used by [13] by titration with iodine solution until the blue color appeared and calculating the volume of iodine used according to the following equation:  $A=B*C*D*E$

A= Amount of Ascorbic acid

B = Represents the concentration of the prepared iodine solution (0.005) molar

C = The volume of iodine solution consumed in desquamation

D = fixed number (2)

E = Molecular weight of ascorbic acid (176.12) g/mol

#### **Determination of total phenolic compounds**

Following the method of [14], The concentration of total phenolic compounds was estimated.

#### **Estimation solutions**

The total phenolic compounds concentration was estimated by following the method of [14].

1- One ml of juice sample was taken and one ml of Folin's reagent solution was added to it (10 ml was prepared by taking 1 ml of the reagent and adding 9 ml of distilled water) with continuous shaking using the mixer for five minutes.

2- (1.5) ml of distilled water and (1) ml of Na<sub>2</sub>CO<sub>3</sub> solution with a concentration of (7.5)% were added to the mixture obtained from the previous step with the shaking process repeated for an hour at (25) °C.

3- Using filter paper, the samples were filtered after which their absorption was measured at a wavelength of (760) nm.

4- By replacing the juice samples with distilled water, comparison samples were prepared.

5- The standard curve was prepared using gallic acid solution dissolved in ethanol at different concentrations.

#### **Estimate The Browning Index**

The values of the browning index were estimated according to the method described in [15], as follows: 1- Five ml of the juice sample is taken and a tube is placed in a centrifuge at a speed of (2500) rpm for a quarter of an hour, then using a filter paper to filter the samples. 2- (2.5) ml of the filtered juice is withdrawn, (2.5) ml of ethanol alcohol at a concentration of (95)% is added, the tubes are left in an ice water bath for a quarter of an hour. 3- The centrifugation process is repeated again as mentioned in the first step. 4- The wavelength is set to (420) nm for the spectrophotometer, an appropriate volume of the final product is placed in the device cell, and the absorption readings are recorded.

#### **Statistical analysis**

The statistical analysis of the study results was conducted using a completely randomized design in a factorial experiment SAS Proc [16]., GLM., (Proc., GLM.) and using the ready-made statistical analysis program Factorial CRD (and Duncan's

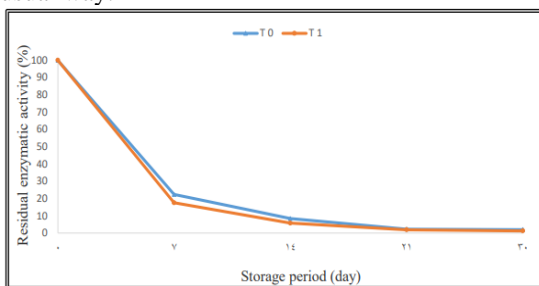
multiple range test was used to compare the means at the level of Significant ( $P > 0.05$ ), as different alphabetical letters indicate between the coefficients until there are significant differences between them

## RESULTS AND DISCUSSION

### Residual enzymatic activity of polyphenol oxidase

Attached table and Shape (1) found that the values of the (R.E.A) % for the samples extracted from treating the fruits by boiling in the usual way were equal to the values of the (R.E.A) values for the juice samples extracted from the untreated fruits, as they amounted to (100.00) % during the storage period (zero) days.

The results of the statistical interaction at the level of significance ( $P < 0.05$ ) in Shape (1) and Table (1) showed that there were significant differences for the samples extracted from the treatment of apple fruits by blanching for the periods (30, 21, 14, 7) days for storage, while there were no significant differences between the samples for the period The time period is (zero) day, as the values of the (R.E.A) (%) in the samples of apple juice obtained from treating apple fruits with normal thermal blanching were significantly lower compared to the samples of the same juice obtained from untreated fruits, and this result continued throughout the storage period on (4) °C, for a period of (1) month. the value of the (R.E.A) (%) decreased sample of juice extracted from fruits treated by blanching in the usual way during the storage period (1) month was (1.24) %, while the value of the (R.E.A)% for the sample of juice extracted from the fruits was untreated (1.94)% and for the same time period mentioned above, [17]. Mentioned the (E.A) values of blackberries decreased after being treated with blanching in the usual way.



**Shape 1.** The effect normal thermal blanching in remaining enzymatic activity values of their juice stored at (4)°C for (30) days.

So ,

T 0- fresh apple juice sample and the control sample)

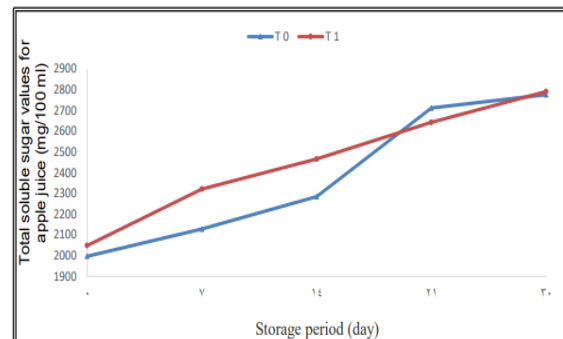
T 1- apple juice sample subjected to the thermal blanching process

### Sugar's total soluble

It was observed from Shape (2) and Table that treating the fruits with normal thermal blanching had a significant effect in increasing (T.S.S) values in the juice of the aforementioned fruits, compared to

the same values in samples of juice extracted from untreated fruits. The increase in the values of total soluble sugars continued for both treatments until the end of the storage period.

Show in Shape (2) and Appendix Table (1) that there were significant differences for samples of juice extracted from treating the fruits by thermal blanching in the usual way, as the values of (T.S.S) increased throughout the storage period and reached their highest values (2790.57) mg /100 ml after (30)days, at (4)°C. As for this values , for the juice extracted from the untreated fruits was (2775.42) mg /100 ml and for the same period of time. [18], treated apple fruits by blanching using hot water, blanching using a microwave oven, and blanching using steam, as observed through the results obtained before. The researcher reported an increase in (T.S.S) values for the samples, for all samples, and for all treatments .This increase was consistent with our results.



**Shape 2.** The effect normal heat blanching on the values of T.S.S (mg/100 ml) of its juice stored at (4)°C for (30) days.

So ,

T 0- fresh apple juice sample and the control sample)

T 1- apple juice sample subjected to the thermal blanching process

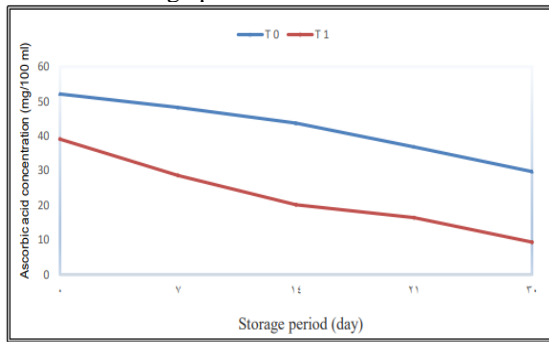
### Ascorbic acid

In Figure (3) indicated that the values of (A.A) in juice samples extracted from apple fruits treated with normal thermal blanching were lower than the (A.A) values in juice samples from untreated fruits, as the values of (A.A) reached for the juice extracted from fruits treated with thermal blanching. The normal rate was 39.14 mg per 100 ml for zero days, while it was (52.17) mg /100 ml for the same period of time.

It was observed in Figure three and Table one that there were essential differences in the values of (A.A) in samples of juice extracted from apple fruits treated with normal thermal blanching from the values of (A.A) in samples of untreated fruits throughout the different storage periods. The results in Figure (3) and Table (1) founded (A.A) values are decreased significantly to (9.38) mg/100 ml in the fruit juice samples after treating them with normal thermal blanching, compared to the same untreated samples (52.17) mg/100 ml. This decrease continues throughout the storage period. The reason for the decrease in Ascorbic Acid values in apple juice samples is the result of the juice being exposed

to high heat treatment, such as pasteurization and blanching, which led to the loss of values Of Ascorbic Acid.

Likewise, the duration of storage had a clear effect on the Ascorbic Acid values and their reduction, and this is what Reported by [19] treated mango fruits by blanching and stored the resulting juice for (18) weeks and compared blanching in the usual way and blanching using a microwave oven, where they found a significant decrease in values (A.A) after the end of the storage process for both treatments.



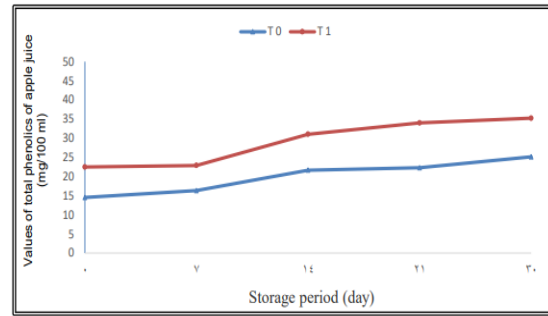
**Shape 3.** The normal heat blanching on the concentration of Ascorbic Acid (mg 100 ml) in their juice stored at (4) C for (30) days.

So ,  
T 0- fresh apple juice sample and the control sample)  
T 1- apple juice sample subjected to the thermal blanching process

### Total phenolic content

It can be seen from Shape number four and Table number one, show increase in the content of (T.P.C) was not significant in the (A.J) samples produced after blanching the fruit in the usual way compared to the samples from apple that were not blanched.

It was found from the results obtained in Table one and Figure four , that the storage conditions of (A.J) treated with normal thermal blanching at four °C for a period of thirteen days did not lead to a significant change in the values of (T.P.C) ,to With untreated (A.J)stored under the same conditions, these values reached (35.20.33.98, 31.01, 22.85) mg /100 ml for juice of apple blanched in the usual way and (25.10, 22.27, 21.62, 16.29) mg per 100 ml for juice of un blanched fruits. During different storage periods (30, 21,14,7), respectively, one of the studies whose results were consistent with the results obtained by us is when [18] treated apple fruits by blanching using hot water, blanching using a microwave oven, and blanching using steam. It was noted, through the results obtained by the researcher, that there was an increase in the values of (T.P.C) for the samples, for all samples, and for all treatments. There was also an increase in (T.P.C) values of when [15] boiled slices of carrots and stored them at (4) °C, and they increased. These values advance the storage period, and this is what was shown by the results of the researchers mentioned above.



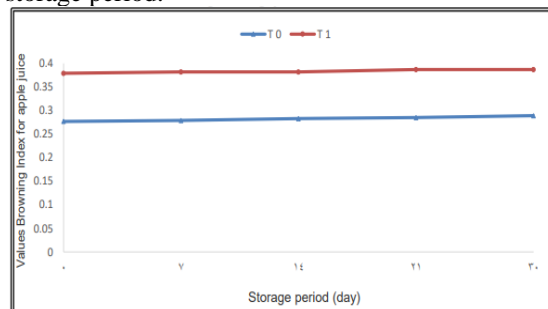
**Shape 4.** The effect normal thermal blanching on the values of total phenolic compounds (mg/100 ml) - their juice stored at (4)°C for (30) days.

So ,  
T 0- fresh apple juice sample and the control sample)  
T 1- apple juice sample subjected to the thermal blanching process

### Browning Index

It is noted from Table (1) and Shape (5) that there are significant differences in the samples of (B.I) values in samples of juice extracted from apple treated by blanching in the usual way compared to the (B.I) values of (A.J) extracted from untreated fruits.

Show in Figure (5) and Appendix Table (1) the browning index values for juice samples apple fruits treated with regular thermal blanching were significantly higher (P<0.05) compared to the samples the same untreated and stored under the same conditions, these values reached (0.3870) at the end of the period storage, while the values of the browning index for the untreated juice samples reached (0.2890), which may explain the reason until the use of high thermal coefficients led to its effect on some sensitive components to thermal treatments such as vitamin C or light exposure, and our results were consistent with [20], when he treated fresh sense slices by blanching them using hot water. A slight significant increase during the storage period.



**Shape 5.** The effect blanching using hot water on the (B.I) values and their stored juice.

So ,  
T 0- fresh apple juice sample and the control sample)  
T 1- apple juice sample subjected to the thermal blanching process

### Conclusions

1-Treating Apple fruits with blanching in the usual way had a significant effect in reducing the values

of (R.E.A), better than fruits not treated with blanching.

2- The (T.S.S) increased after treatment with blanching, as the values of Total Soluble Sugars in the juice of fruits treated with blanching in the usual way were higher than the values of Total Soluble Sugars, For juice of untreated fruits.

3- Treatment by blanching using the usual method significantly reduced the (A.A) content compared to the juice of the untreated fruits.

4- There was a significant increase in the values of Total Phenolic content after the end of the storage period for both treatments, but there was a clear superiority for the fruits treated by thermal blanching in the usual way.

5- The values of the (B.I) were not affected after the end of the (30) days period, as it was noted that there was a slight increase in their values for both treatments. The fruits treated by blanching in the usual way were higher in value than the untreated fruits.

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#### Competing Interests

There are no competing interests.

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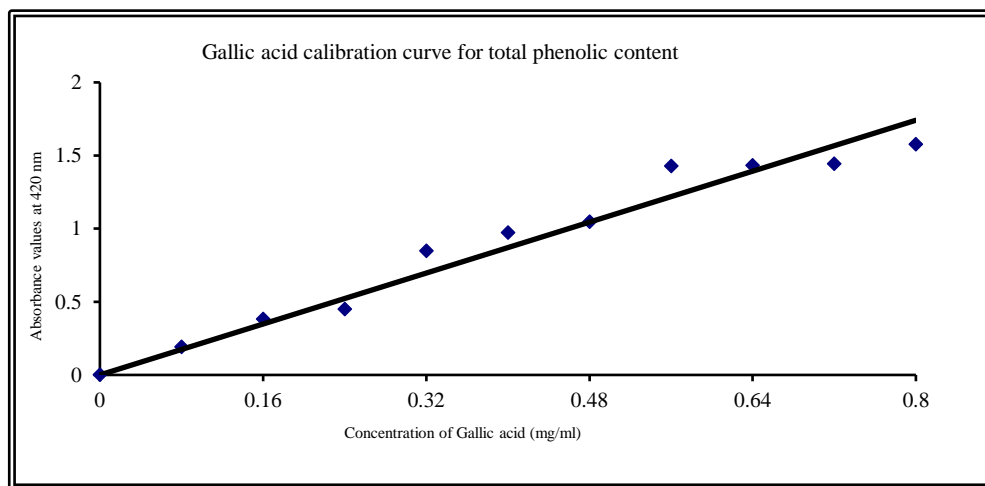
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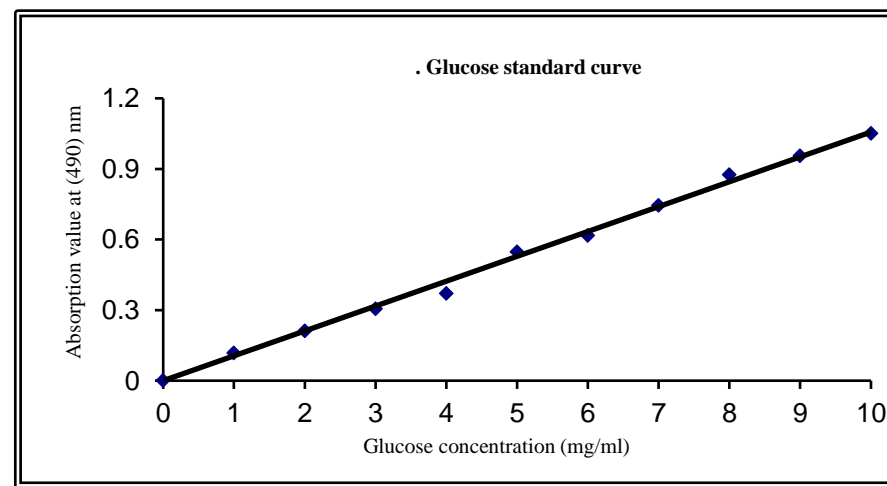
**Table 1.** The effect of storage at 4°C for 30 days on some apple juice ingredients and recipes

Browning Index		Total Phenolic Compounds (mg/100ml)		Ascorbic Acid (mg/100ml)		Total sugar (mg/100ml)		Residual Enzymatic Activity of PPO %		STORGE At (4)°C
T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	Day
0.3790 ± 0.100 a	0.2770 ± 0.015 bc	22.45 ± 7.20 fg	14.50 ± 4.33 g	39.14 ± 1.61 cde	52.17 ± 4.31 a	2048.57 ± 46.59 fgh	1997.14 ± 14.46 gh	100.00 ± 0.00 a	100.00 ± 0.00 a	0
0.3820 ± 0.007 a	0.2790 ± 0.007 bc	22.85 ± 10.59 efg	16.29 ± 5.36 g	28.65 ± 4.12 gh	48.28 ± 2.82 ab	2320.90 ± 31.59 e	2128.00 ± 53.57 f	17.51 ± 2.35 c	22.33 ± 3.96 b	7
0.3820 ± 0.007 a	0.2830 ± 0.013 bc	31.01 ± 4.98 b-f	21.62 ± 3.27 fg	20.20 ± 5.70 ijk	43.74 ± 3.28 bc	2465.23 ± 58.99 d	2284.05 ± 91.93 e	5.72 ± 0.40 f	8.36 ± 2.33 e	14
0.3870 ± 0.005 a	0.2850 ± 0.007 bc	33.98 ± 4.51 a-e	22.27 ± 2.73 fg	16.44 ± 3.17 k	36.91 ± 3.95 de	2642.10 ± 34.81 c	2711.29 ± 50.88 bc	1.85 ± 0.30 g	2.22 ± 0.44 g	21
0.3870 ± 0.002 a	0.2890 ± 0.009 b	35.20 ± 5.93 a-d	25.10 ± 3.07 d-f	9.38 ± 2.24 I	29.78 ± 2.73 fg	2790.57 ± 89.90 ab	2775.42 ± 52.35 ab	1.24 ± 0.57 g	1.94 ± 1.31 g	30

So ,  
 T 0- fresh apple juice sample and the control sample)  
 T 1- apple juice sample subjected to the thermal blanching proces



**Figure 6.** Standard curve for gallic acid



**Figure 7.** Glucose standard curve