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Inhibition of Polyphenol Oxidase Enzyme in Apple Slices using Aqueous Extract of Apple Peels

Zakaria Malik Abdul Al-Kareem[1] and Oday Hasan Ali Al-Jammaas[2]

[1,2]Department of Food Sciences, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.

[1]E-mail: zakariamalik1985@gmail.com

Abstract. This study aimed to extract apple juice from green apple fruits previously treated with aqueous extract of apple peels, before storing the extracted juice at a temperature of (4)°C, for a period of (30) days, and comparing it with the juice of untreated fruits in order to determine the effect of inhibiting the polyphenol enzyme. Polyphenol oxidase (PPO) in apple fruits according to the treatments mentioned in some characteristics of apple juice extracted from those fruits. The studied characteristics are: residual enzymatic activity, total soluble sugars, ascorbic acid content, total phenols content, and browning index, as they were immersed in Fruits treated with aqueous extract of apple peels had a significant effect in reducing the residual enzymatic activity and ascorbic acid values of the treated fruit juice, while the total soluble sugars and total phenols content had a significant increase in their values after the end of the storage period, while the browning index values were not affected at the end of storage.

Keywords. Aqueous extract of apple peels, Browning index, Ascorbic acid, Residual enzymatic activity.

1. Introduction

Over the years, researchers have worked to find and discover natural extracts to replace artificial food additives in order to maintain the quality of food and prolong its storage period because these extracts are non-toxic and have no side effects [1]. Extracts are substances extracted from specific plant parts such as flowers, leaves, roots and seeds. These extracts have the ability to control and prevent the appearance of browning and fortify foods to obtain high quality because they contain antioxidant compounds, [2], Interest has increased in recent years in the use of natural inhibitors of the polyphenol oxidase enzyme extracted from certain food products such as green tea, rice bran, and others, as they were effective in inhibiting the PPO enzyme and preventing juice discolouration in apple puree juice [3,4]. Natural plant or animal extracts are used to inhibit the activity of the PPO enzyme. The fruits, either whole or in the form of cut slices, are immersed in water extracts for a period of time and then the juice is extracted from the fruits. These extracts play an important role in effectively inhibiting the activity of the PPO enzyme. [5]. Mango peel extract also has the ability to delay the appearance of browning in potatoes, as mango peels contain phenolic compounds such as anthocyanins and gallic acid, which have the ability to act as antioxidants. This extract was found to have the ability to inhibit

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the polyphenol oxidase enzyme [6,7]. Use tomato peel extract to prevent browning in fresh apple slices due to its ability to act as an antioxidant and the extract contains lycopene, when treating apple slices with a lycopene solution at a rate of (2) g/L, obtained from treating tomato peels at (75)°C for a period of time. A full hour and storing the slices at (5)°C for (9) days increased the percentage of chlorogenic acid and led to the inhibition of [8]. Pineapple juice is used to inhibit enzymatic activity and effectively reduces the occurrence of browning in fresh apple slices, as the brown color did not appear in apple slices treated with the extract for (6) days at a temperature of (4)°C, in addition to no changes in the physical and chemical properties, which is attributed to The reason for this is that the juice contains phenolic compounds that have an important role in addition to their function as antioxidants [9,10].

2. Materials and Methods

2.1. Treatments

2.1.1. Extracting Fresh Apple Juice and Bottling It

After selecting well-ripened and sound green apple fruits, they were washed and cleaned with running water, then cut, before extracting their juice using a juicer. The juice was then filtered, then pasteurized at (85)°C for (5) minutes in a water bath. (Parfait et al., 2022) before filling it while hot in opaque glass bottles (Amber glass bottles), leaving a slight headspace, then the bottles were closed with plastic stoppers, and cooled immediately with cold running water to (25)°C, before various estimates were made on them (comparison sample).

2.1.2. Immersing Green Apple Fruits in Aqueous Extract of Apple Peels Before Extracting Their Juice and Bottling It

A well-ripened and sound type of fruit was selected, then washed and cleaned with running water, then prepared, then treated by adding a water extract from the peel of one of the types of fruit (under conditions determined after preliminary experiments) to the juice extracted using a blender, after which it was Filtering the juice, before filling it in opaque glass bottles (amber glass bottles), leaving a slight head space, then closing the bottles with plastic stoppers, then various estimates are made on them. 3- Preparing the aqueous extract To prepare the aqueous extract of apple peels, I followed the method mentioned in [11], with some modifications, as follows:

- Apple fruits were purchased from the local market.
- I washed the fruits well with running water, then separated the fruit peels from their seeds.
- Cut the peels of the fruits into small pieces using a sharp knife.
- The cut fruit peels were dried using a drying oven at a temperature of (60)°C, until the weight was constant.
- The cut and dried fruit peels were cooled before being ground using a laboratory grinder.
- The resulting powder was sifted using a sieve.
- The resulting powder was then packed into plastic containers, then the containers were stored in freezer until extraction.
- The process of mixing (20) grams of fruit peel powder obtained after step (7) with (500) milliliters of boiling distilled water and leaving the aforementioned mixture for (5) minutes
- Filter the product of step (8) using Whatman filter paper No. (1) to obtain the apple peel extract, which was stored at - (18)°C, until use.
- Centrifuge the product of step (8) without proceeding to step (9) using a centrifuge at a speed of (12,000) rpm for (20) seconds.

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2.1.3. Prepare Fruit Juice Immersed in Aqueous Extract of Apple Peels

After selecting well-ripened and sound apple fruits, they were washed and cleaned with running water, then each fruit was cut into four pieces, before being immersed in the water extract of apple peels at a temperature of (30)°C for (3) hours, then Carrying out the process of extracting apple juice using a juicer, then filtering the juice, then pasteurizing it under the aforementioned thermal conditions before filling it in opaque glass bottles (Amber glass bottles), leaving a slight headspace, then closing the bottles with plastic stoppers, before making the various estimates.

2.2. Storage

After preparing the juice of the fruits treated with the methods mentioned above, the different juice samples were stored at a temperature of (4)°C, for a period of (30) days, and various estimates were made on them every (7) days of storage.

2.3. Estimation of Enzymatic Activity

The activity of the polyphenol oxidase enzyme was estimated according to [12], by measuring the change in absorption at the wavelength (420) nm, as follows: Estimation steps

- (5) ml of the juice sample was taken and (5) ml of the previously prepared buffer solution was mixed with it, continuing mixing using the mixing device for one minute.
- The samples were placed in the centrifuge for an hour at a speed of (4000) rpm, after which the samples were filtered using filter paper.
- Incubate the filtered mixture after placing it in test tubes in a water bath at a temperature of (30)°C. 4- Absorption values were recorded after setting the device to the wavelength (420) nanometers.

The enzymatic unit of polyphenol oxidase is defined as the amount of enzyme that leads to a change in optical absorption of (0.001) per minute. The residual enzymatic activity (%) was calculated by applying the following formula B/A x 100 Since A = enzyme activity in juice extracted from untreated fruits. B = Enzyme activity in juice extracted from treated fruits.

2.4. Estimating the Concentration of Total Dissolved Sugars

Total soluble sugars were determined according to the method described in [13].

Estimation steps

- The juice samples were diluted with distilled water in test tubes before adding a 5% phenol solution, concentrated sulfuric acid, and distilled water, respectively.
- The test tubes were left at normal room temperature for (5) minutes with shaking in order for the color to develop, then they were placed in a water bath at a temperature of (25)°C for (5) minutes for the purpose of cooling them.
- The absorbance reading was recorded using a spectrophotometer previously set to a wavelength of (490) nanometers.
- I repeated the previous steps by replacing the juice sample with distilled water.
- Use glucose solution with different concentrations to prepare the standard curve.

2.5. Determination of Ascorbic Acid Content

The ascorbic acid content of apple juice samples was estimated by following the method of testing mentioned by [14], which ends when the dark blue color appears after testing the samples with an iodine solution with a concentration of (0.005) standard. By knowing the consumed volume of the iodine solution, the equation can be applied. The following is to calculate the ascorbic acid content (mg/100 ml).

$$A= B\times C\times D\times E$$

A = amount of ascorbic acid

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

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C = volume of iodine solution consumed in desorption
D = fixed number (2)
E = molecular weight of ascorbic acid (176.12) g/mol

2.6. Determination of Total Phenolic Compounds

The concentration of total phenolic compounds was estimated according to the method of [15], Estimation steps

- (1) ml of juice sample was mixed with (1) ml of diluted Folin reagent solution with continuous shaking for (5) minutes.
- Add (1.5) ml of distilled water and (1) ml of sodium carbonate solution to the previous mixture, and repeat the shaking process for (60) minutes at (25)°C.
- The samples were filtered using filter paper, after which their absorbance was measured at a wavelength of (760) nanometers.
- Comparison samples were prepared by replacing the juice samples with distilled water.
- Different concentrations of a solution of gallic acid dissolved in ethanol were used to prepare the standard curve.

2.7. Browning Index

Browning index values were estimated according to the method described in [16], and are as follows: Estimation steps

- Place a volume of (5) ml of the juice sample in a test tube and centrifuge it at a speed of (2500) rpm for (15) minutes, after which the clear portion is filtered using filter paper.
- (2.5) ml of the product of the previous step was mixed with (2.5) ml of ethanol at a concentration of (95)%, and the samples were then placed in an ice water bath for (15) minutes.
- The centrifugation step was repeated a second time for (15) minutes and at a speed of (2500) r/min.
- Place an appropriate volume of the final product in the spectrophotometer cell and set the wavelength to (420) nanometers, before recording the absorption readings.

3. Results and Discussion

3.1. Residual Enzymatic Activity Values (%)

The effect of treating apple fruits by immersing them in aqueous extract of apple peels before extracting the juice from them and storing them at a temperature of (4)°C for a period of (30) days on the residual enzymatic activity values (%).

Figure (1) and Appendix Table (1) indicated that the residual enzymatic activity values (%) for juice samples extracted from fruits treated with aqueous extract of apple peels were equal to the remaining enzymatic activity values (%) for juice samples extracted from untreated fruits at the period (Zero) day. It was noted from the above-mentioned appendix table and figure that there were significant differences between the remaining enzymatic activity values (%) for samples of juice extracted from treating the fruits with the aqueous extract of apple peels. They were less than the remaining enzymatic activity values (%) for samples of juice extracted from non-dairy fruits. The transaction is for the time periods (30, 21, 14, 7) days.

It was found from the results obtained in Figure (1) and Appendix Table (1), that the residual enzymatic activity values (%) for juice samples extracted from treating the fruits with aqueous extract of apple peels were significantly lower ($P<0.05$), compared to the same untreated samples. And stored under the same conditions, this decrease in values continued throughout the storage period, as these values for samples of fruit juice boiled in the usual way reached (9.78, 4.40, 1.34, 0.56) for the time periods (30, 21, 14.7) days, as for samples The values of untreated fruit juice reached (22.33, 8.36, 2.22, 1.94) during the various storage periods mentioned above and at (4)°C, according to Azevedo, et

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al., (2018) when apple slices were treated with a solution consisting of whey with citric acid and preserved. In special membranes, this treatment effectively reduced the enzyme activity.

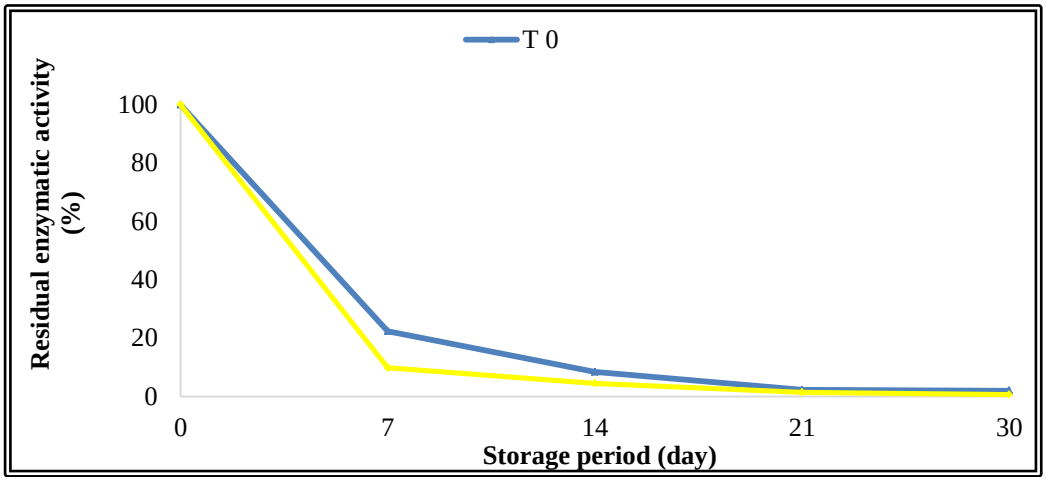

Figure 1. The effect of treating apple fruits with aqueous extract of apple peels on the remaining enzymatic activity of their juice stored at (4)°C for (30) days. (T0 = juice sample of fresh apples (comparison sample), T1 = juice sample of apples to which aqueous extract of apple peels is added).

3.2. Total Soluble Sugars

The effect of treating apple fruits by immersing them in aqueous extract of apple peels before extracting the juice from them and storing them at a temperature of (4)°C for a period of (30) days on the values of total soluble sugars (mg/100 ml).

It was observed from Figure (2) and Appendix Table (1), that the values of total soluble sugars (mg/100 ml) in the juice samples extracted from treating apple fruits with aqueous extract of apple peels were lower than the values of total soluble sugars (mg/100 ml) in the extracted juice samples. Of the untreated fruits, as treating the fruits with aqueous extract of apple peels had a significant effect in reducing the values of total dissolved sugars in the juice of the aforementioned fruits (1974.29) mg/100 ml, compared with the same values in samples of juice extracted from untreated fruits (1997.14). mg/100 ml for storage period of 0 days.

Figure (2) and attached table (1) indicated that the values of total soluble sugars (mg/100 ml) in juice samples extracted from treating apple fruits with aqueous extract of apple peels increased at the end of the storage period (30) days and reached (2082.86) mg. /100 ml, and the results of the statistical analysis in the figure and the attached table showed that there were significant differences between the samples, and our results agreed with [17], when apple slices were immersed in a solution of aloe vera extract at different concentrations and the extracted juice was stored for two months, where it was found that there was an increase in the values of total sugars. During the storage period.

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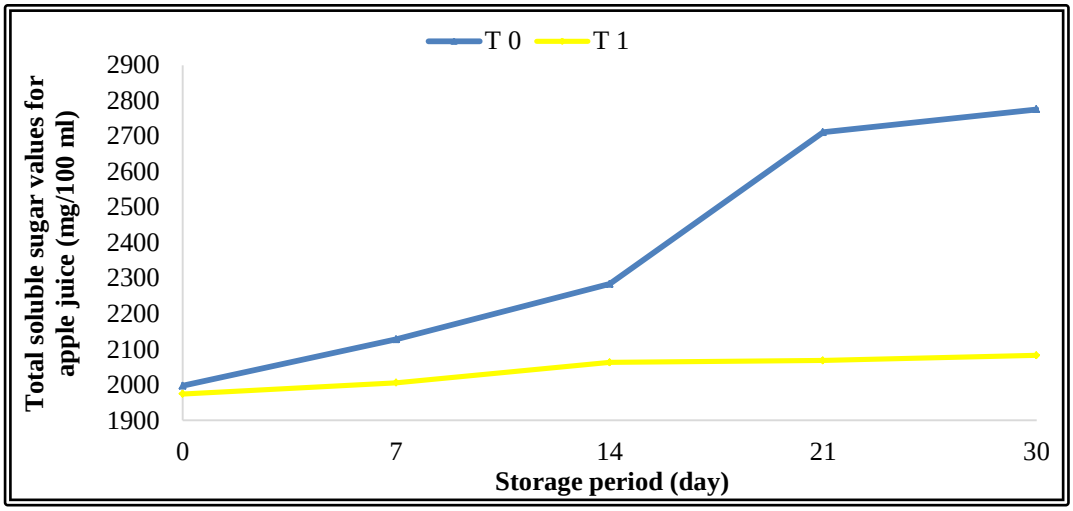

Figure 2. The effect of treating apple fruits with aqueous extract of apple peels on the values of total soluble sugars (mg/100 ml), for their juice stored at (4)°C for (30) days. (T0 = juice sample of fresh apples (comparison sample), T1 = juice sample of apples to which aqueous extract of apple peels is added).

3.3. Ascorbic Acid

The effect of treating apple fruits by immersing them in aqueous extract of apple peels before extracting the juice from them and storing them at a temperature of (4)°C for a period of (30) days on the values of ascorbic acid (mg/100 ml).

The results of the statistical analysis in Figure (3) and Appendix Table (1) showed that immersing apple fruits with aqueous extract of apple peels led to a significant decrease in ascorbic acid values compared to the juice of untreated fruits, and this decrease continued throughout the storage period.

The above-mentioned figure and attached table indicated that there were significant differences between samples of juice extracted from fruits treated with aqueous extract of apple peels and juice from untreated fruits during the different storage periods, while there were no significant differences at the beginning of the storage period (0) day.

The results of statistical interaction at a significant level ($P<0.05$) in Figure (3) and Appendix Table (1) showed that the values of ascorbic acid in apple juice samples obtained from treating apple fruits with the aqueous extract of apple peels were significantly lower compared to the same juice samples. Obtained from untreated fruits, and this result continued throughout the storage period at (4)°C, for a period of (30) days, and these results were similar to what [18], reported when he added cranberry juice extract mixed with blue honeysuckle. In a certain proportion to apple juice and stored for (4) months, a decrease in ascorbic acid values was observed, and the decrease increased the longer the storage period was. Our results also matched those of [19], when apple slices were immersed in a solution of aloe vera extract at different concentrations and stored. The juice extracted for two months showed a decrease in the ascorbic acid values for all samples.

It was observed in Figure (3) and the attached table (1), that the ascorbic acid values for the apple juice sample treated with the aqueous extract of apple peels reached (22.96) mg/100 ml at the end of the storage period (30) days, while the ascorbic acid value for the fruit juice was not Treatment (29.78) mg/100 ml for the same period of time.

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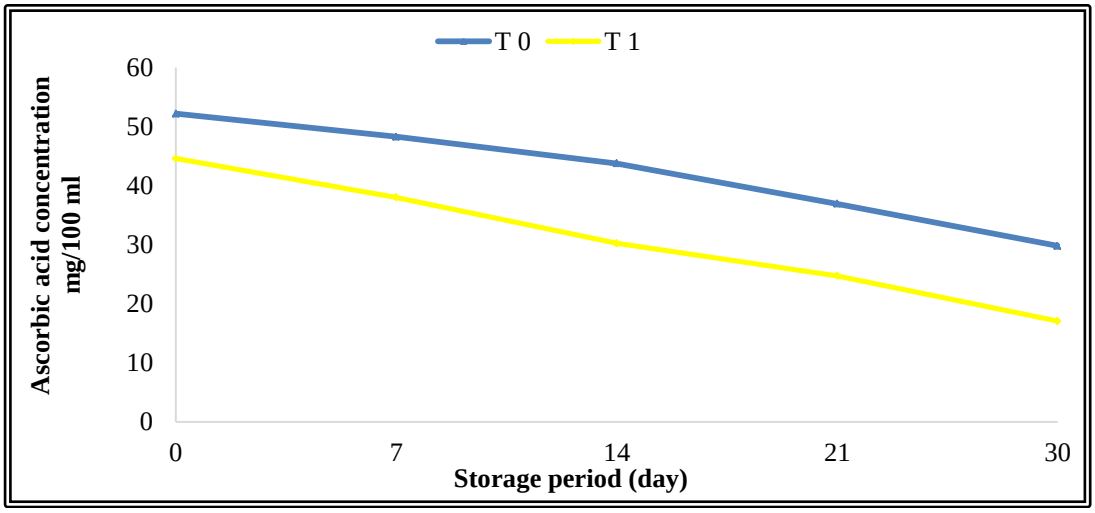

Figure 3. The effect of treating apple fruits with aqueous extract of apple peels on the concentration of ascorbic acid (mg/100 ml) of their juice stored at 4°C for 30 days. (T0 = juice sample of fresh apples (comparison sample), T1 = juice sample of apples to which aqueous extract of apple peels is added).

3.4. Total Phenolic Content

The effect of treating apple fruits by immersing them in aqueous extract of apple peels before extracting the juice from them and storing them at a temperature of (4)°C for a period of (30) days on the values of total phenols (mg/100 ml).

It is noted from Figure (4) and Appendix Table (1) that the values of total phenols in samples of juice extracted from apple fruits treated with aqueous extract of apple peels were higher than the values of total phenols in apple juice extracted from untreated fruits, as they amounted to (33.23) mg. /100 ml.

It appears from Figure (4) and Appendix Table (1) that there are significant differences between the values of total phenols in samples of juice extracted from apple fruits treated with aqueous extract of apple peels compared to the values of total phenols in apple juice extracted from untreated fruits.

Figure (4) and Appendix Table (1) indicated that treating apple fruits with aqueous extract of apple peels led to a significant increase in the values of total phenols (mg/100 ml) in the apple juice extracted from the treated fruits compared to the juice from the untreated fruits. This increase continued until the end of the storage period, when the values mentioned during the period (30) days reached (44.74) mg/100 ml. These results were identical to what was reported by [20] when cut apple slices were immersed in the aqueous extract of tomato peels, there was an increase in the values of phenolic compounds, and this value increased the longer the storage period.

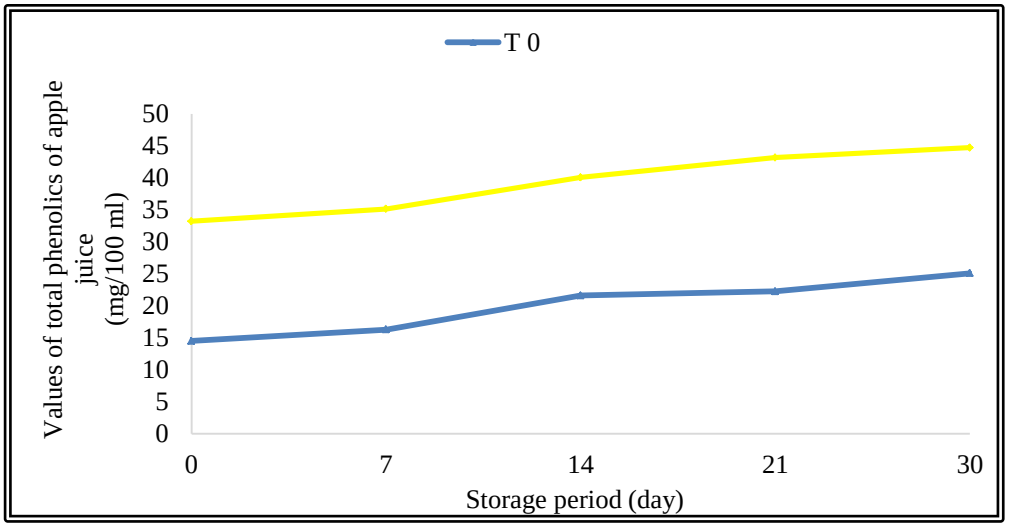

Figure 4. The effect of treating apple fruits with aqueous extract of apple peels on the values of total phenolic compounds (mg/100 ml), of their juice stored at (4)°C for (30) days. (T0 = juice sample of fresh apples (comparison sample), T1 = juice sample of apples to which aqueous extract of apple peels is added).

3.5. Browning Index

The effect of treating apple fruits by immersing them in aqueous extract of apple peels before extracting the juice from them and storing them at a temperature of (4)°C for a period of (30) days on the values of the browning index.

It is noted from the results of Figure (5) and Appendix Table (1), that treating juice samples extracted from fruits treated with aqueous extract of apple peels led to a significant decrease in browning index values compared to samples of juice extracted from untreated fruits at the same storage period.

Between Figure (5) and Appendix Table (1), there are significant differences at ($P<0.05$) for the browning index values in samples of juice extracted from fruits treated with aqueous extract of apple peels, and this value increased during the storage period if it reached its highest value during the storage period. (30) days (0.2850), while the browning index values for samples of juice extracted from untreated fruits (0.2890) for the same time period were the highest.

The results of this study are consistent with what was reported by [20,21] where the first researcher immersed fresh lettuce slices in the aqueous extract of clove oil and eugenol at a concentration of (0.05)% each, while the researcher The second immersed the cut apple slices in the aqueous extract of tomato peels. There was a slight increase in the browning index values as the storage period progressed, as the use of the aqueous extract of apple peels reduced the browning index because the aqueous extract of apple peels contains phenolic compounds that have the ability to act as antioxidants. To oxidize and reduce the appearance of brown color in juices.

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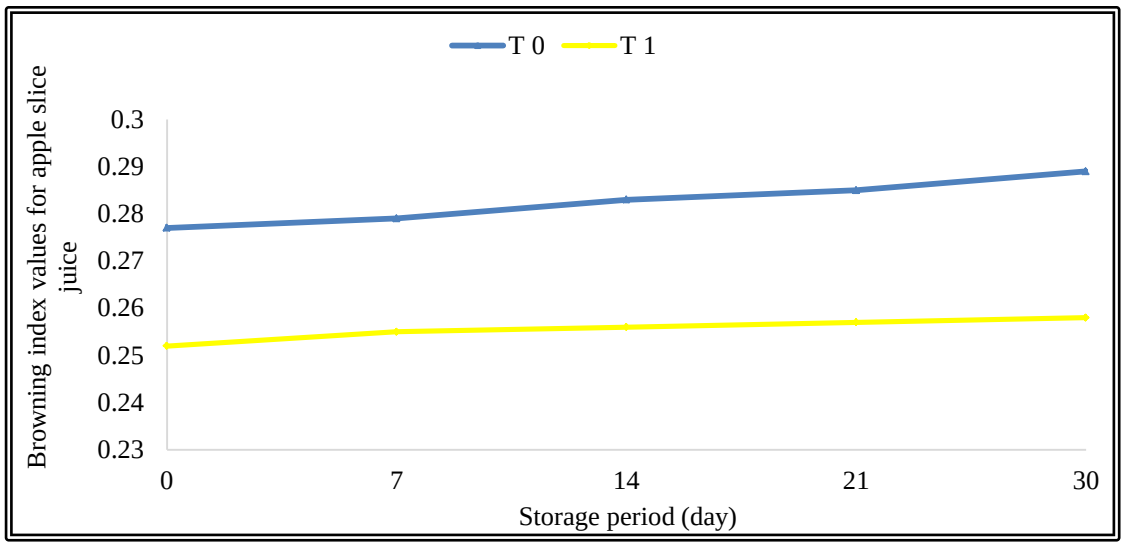

Figure 5. The effect of treating apple fruits with aqueous extract of apple peels on the concentration of browning index values for their juice stored at (4)°C for 30 days. (T0 = juice sample of fresh apples (comparison sample), T1 = juice sample of apples to which aqueous extract of apple peels is added).

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

STORGE At (4)°C	Residual Enzymatic Activity of PPO %		Total sugar (mg/100ml)		ASCORBIC ACID (mg/100ml)		Total Phenolic Compounds (mg/100ml)		Browning Index	
Day	T_0	T_1	T_0	T_1	T_0	T_1	T_0	T_1	T_0	T_1
0	100.00 ± 0.00 a	100.00 ± 0.00 a	1997.14 ± 14.46 gh	1974.29 ± 97.80 a	52.17 ± 4.31 a	47.26 ± 4.32 ab	14.50 ± 4.33 g	33.23 ± 6.72 a-f	0.2770 ± 0.015 bc	0.2520 ± 0.008 f
7	22.33 ± 3.96 b	9.78 ± 0.46 de	2128.00 ± 53.57 f	2005.71 ± 21.60 a	48.28 ± 2.82 ab	42.14 ± 3.28 bcd	16.29 ± 5.36 g	35.14 ± 7.89 a-d	0.2790 ± 0.007 bc	0.2550 ± 0.004 ef
14	8.36 ± 2.33 e	4.40 ± 0.43 f	2284.05 ± 91.93 e	2062.86 ± 41.04 a	43.74 ± 3.28 bc	35.22 ± 2.70 ef	21.62 ± 3.27 fg	40.08 ± 6.61 ab	0.2830 ± 0.013 bc	0.2560 ± 0.006 ef
21	2.22 ± 0.44 g	1.34 ± 0.64 g	2711.29 ± 50.88 bc	2068.60 ± 62.81 a	36.91 ± 3.95 de	29.49 ± 3.50 fg	22.27 ± 2.73 fg	43.19 ± 5.46 a	0.2850 ± 0.007 bc	0.2570 ± 0.009 ef
30	1.94 ± 1.31 g	0.56 ± 0.35 g	2775.42 ± 52.35 ab	2082.86 ± 134.02 a	29.78 ± 2.73 fg	22.96 ± 2.62 hij	25.10 ± 3.07 d-f	44.74 ± 5.21 a	0.2890 ± 0.009 b	0.2580 ± 0.009 def

Whereas
T0 = juice sample of fresh apples (comparison sample).
T1 = juice sample of apples subjected to the normal thermal blanching process.

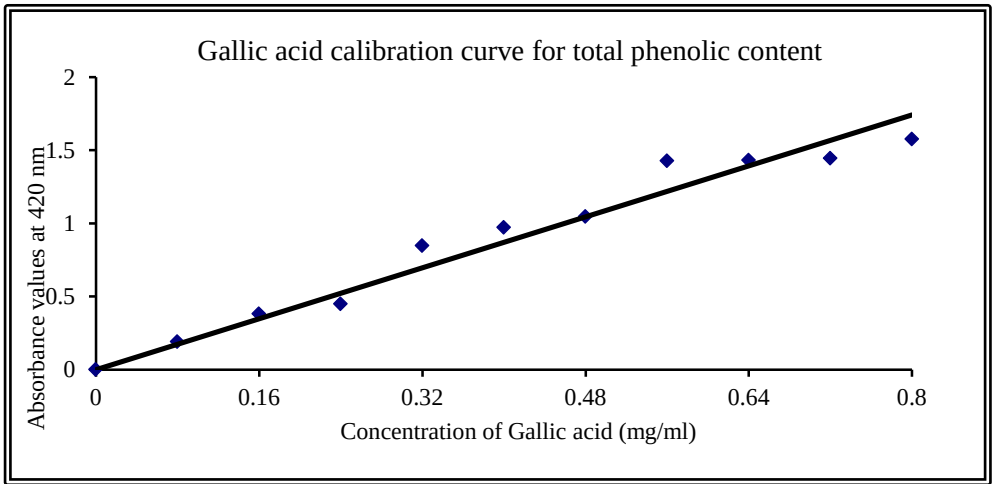

Figure 6. Standard curve for gallic acid.

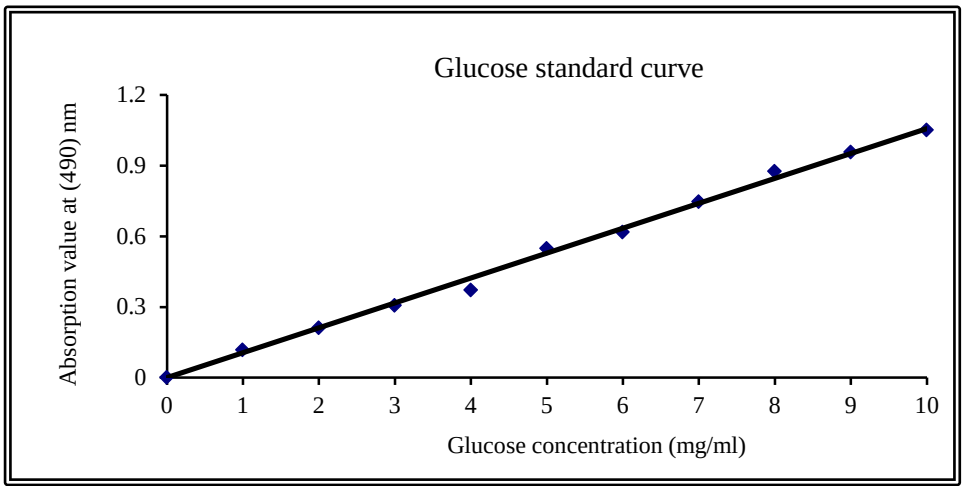

Figure 7. Glucose standard curve.

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