

## Determination of Some Essential Oils Effects on the Quality Traits of the Egyptian Anna Apple Fruit During its Shelf Life

Ayman Amin

Department of Plant Physiology, Faculty of Agriculture, Cairo University, Giza, Egypt

**Abstract:** Fruits are considered one of the most important components of the human diet and there are several issues that affect their shelf-life span. This study was conducted to examine the effect of spraying the essential oils from celery, basil and peppermint on Egyptian Anna apples (*Malus Domestica Borkh.*), qualitative and quantitative traits were analyzed during storage. The Fruits were sprayed with celery, basil and peppermint essential oils of (1 %, 2.5% and 5% each). The sprayed fruits were stored for 3 weeks at room temperature. The traits tested in this experiment included the percentage of weight loss, percentage of decayed fruits, fruit firmness, acidity, total soluble sugars, total soluble solids (TSS), total soluble pectin, peroxidase enzyme activity, pectinase enzyme activity and polyphenol oxidase enzyme activity. Results revealed that treated fruits with essential oils showed a longer preservative effect than the control samples with no natural additives. Lowered pectinase activity, decreased weight loss and improved firmness were amongst the qualities viewed particularly with peppermint and celery essential oils.

**Key words:** Anna apples (*Malus domestica Borkh.*) • Pectinase • Peroxidase • Polyphenol oxidase • Essential oil • Peppermint • Basil • Celery • Storage • Room temperature

### INTRODUCTION

Apples are one the members of Rosaceae family which is distributed all over the world [1]. In Egypt, Anna apple (*Malus Domestica Borkh*) is one of the most important cultivars that are planted which are characterized as large fruits having light yellow with a red blush skin [2]. Consequently, fruits should preserve their stable metabolism aligned with their cell integrity to produce high quality fruits that meets up with the customers desire. Moreover, the preservation of the freshness quality of fruits is important due to their high economic impact [1].

Generally, the enzymes, present in fruits, have the ability of causing undesirable changes. Consequently, the management of these naturally occurring enzymes is important to food technology. Enzymatic browning in fruits tissues usually causes the undesirable quality changes [3]. Therefore, the control of the enzymatic browning is important to the horticulture industry, due to the fact that this reaction occurs in many fruits; it negatively affects the attributes of color, taste, flavor and nutritional value [4]. According to Whitaker and Lee [4],

more than 50% of fruit market loss was estimated as a result of enzymatic browning. Some of the enzymes involved in fruit deterioration are peroxidase and polyphenol oxidase (PPO) that are involved in browning of fruits and pectinase that is involved in fruit softness. Peroxidase is a plant enzyme associated with off-flavors in fruits. Peroxidase activity increases in apples during ripening [5]. Therefore, inactivation of the peroxidase enzyme is considered necessary to minimize the possibility of deterioration and in the preservation of fruits [6]. Inhibition of PPO and prevention of enzymatic browning are often treated as one and the same subject. However, browning is prevented by reacting with the products of enzyme action to inhibit the formation of the colored compounds produced in secondary, non-enzymatic reaction steps. Unfortunately, during storage, apples are exposed to different levels of microbial decay, by pathogenic fungus, resulting in fruit decay which shortens their shelf-life span [7].

Considering that, 20% reduction of the major food is due to pathogenic fungi; several post-harvest technologies are used to control this loss [8, 9]. The use of browning inhibitors in food processing is restricted by

special requirements such as non-toxicity, effect on taste, flavor and texture [5]. In most countries, fungicides chemicals usages as postharvest treatment are restricted in most countries. Moreover; consumer demands for free pesticide agricultural commodities are increasing [9]. Therefore, human safe and environmentally friendly new preservation techniques are developed [10]. Natural plant products as essential oils, among the numerous alternatives are catching the attention of researchers worldwide, due to their biodegradability [8]. Essential oils are extracted from plant materials, which can be used as natural food additives, due to their antifungal, antibacterial and antioxidant properties [1, 11]. Generally, The usage of plants essential oils have been broadly used as post-harvest treatment that are applied on fruits [12]. Rabiei *et al.* [13] reported that a reduction on ethylene production, that causes fruit deterioration, was observed after the application of essential oils on apple fruit. For instance spraying “Embul” bananas with basil oil emulsion controlled the crown rot effect and increased its storage life [1].

The objectives of this study aimed to assess the efficiency of spraying the essential oils of (celery, basil and peppermint) on postharvest quality parameters during the storage of Anna apples cultivar for 3 weeks at room temperature.

## MATERIALS AND METHODS

**Plant Material:** Mature fruits of 110 days age from full bloom were picked from a private farm located on Cairo-Alexandria Desert road, which were immediately taken to the laboratory. Three replicates from each treatment were used for determination of fruit physical and chemical properties, sprayed with three concentrations (1, 2.5 and 5%) of essential oils of Celery, Basil and Peppermint on apple fruits and left at ambient temperature (20-23°C) for 3 weeks. This experiment was carried out between 2014 and 2015.

**Separation of Essential Oils:** Essential oils of celery oil (*Apium graveolens*), basil oil (*Ocimum basilicum*) and peppermint oil (*Mentha piperita L.*) were separated by hydro distillation according to Guenther [14]. The pure volatile oil was injected in Gas Chromatograph Mass Spectrometer (GCMS) model Shimadzu QP-5000 equipped with DB-1 column (30m × 0.25mm × 0.250 Micron film thicknesses) according to Boniface *et al.* [15].

In each treatment, different measurements were carried out starting from zero-time of storage and continued at intervals of one week till the end of three weeks.

**Physical Characteristics:** Weight loss (%), Fruits were periodically weighed and loss in fruit weight was recorded for each replicate and then it was calculated as percentages in relation to the fruits weight at zero time of storage at room temperature. Fruit decay (%), Fruits which were decayed by different physiological and pathological factors were periodically counted and discarded and then percentage of fruit decay was calculated in relation to the total number of fruits. Fruit firmness, Fruit firmness was determined for each replicate using Magness and Taylor-pressure tester with 5/16 inch plunger. Fruit firmness recorded as lb/inch<sup>2</sup>.

**Chemical Characteristics:** Total acidity (%), Total acidity as gm of anhydrous citric acid determined and estimated per 100 ml fruit juice, according to Horwitz *et al.* [16]. Total soluble sugars, Colorimetric determination of total soluble sugars expressed as glucose was determined according to Dubois *et al.* [17]. Total soluble solids percentages (T.S.S.), It was measured in juice using the Hand Refractometer. Total soluble pectin, Pectic substances were determined as gm./100 gm. dry weight according to the procedure of Aina *et al.* [18]. Peroxidase activity, Peroxidase enzyme activity was determined according to Horwitz *et al.* [16] method and the activity was expressed as the optical density (OD). Pectinase Activity, Pectinase enzyme activity was determined using Horwitz *et al.* [16] method and the activity was expressed as the optical density (OD). Polyphenol Oxidase Activity, Polyphenol Oxidase (PPO) activity was determined according to Susana *et al.* [19] and the activity was expressed as the optical density (OD).

**Statistical Analysis:** Data for the physical and chemical characteristics were subjected to analysis of variance (ANOVA). All characteristics were analyzed considering both within groups and between groups.

## RESULTS AND DISCUSSION

**Chemical Composition of (Celery, Basil and Peppermint) Essential Oils:** Results from the GCMS (Table 1) revealed that the major components of the celery essential oil are

Table 1: Fractionation of celery, basil and peppermint essential oils by

Compound name	Peak area (%)		
	Celery oil	Basil oil	peppermint oil
$\alpha$ -pinene	6.94	1.4	1.55
$\beta$ -pinene	8.85	1.9	1.81
Myrcene	4.94	---	---
$\alpha$ -terpinene	0.52	0.96	0.64
$\gamma$ -Terpinene	---	1.89	0.59
p-cymene	3.94	0.25	---
Limonene	33.96	2.54	4.49
Camphor	---	2.32	1.22
1, 8 cineole	---	5.49	6.54
Linalool	4.46	59.37	1.40
$\beta$ -cadinol	---	17.95	---
Geraniol	1.24	1.79	---
Geranyl acetate	10.8	---	0.32
Apiol	7.35	---	---
Citronellol	0.86	---	---
$\beta$ -Ocimene	7.7	---	---
Menthofuran	---	---	9.09
Menthol	---	---	48.29
Menthyl-acetate	---	---	19.38
Terpineol	3.39	3.82	---
Carvone	---	---	3.41
$\beta$ -Caryophyllene	5.05	---	---
$\alpha$ -phellandrene	---	---	---
$\gamma$ -cadinene	---	---	1.27
Caryophyllene	---	0.32	---

limonene followed by Geranyl acetate and  $\beta$ -pinene with percentages (33.96, 10.8 and 8.85 % respectively). Results here are in agreement with those results of Sipailiene *et al.* [20] indicated that the main constituent in the celery essential oil was limonene. Moreover, the major components of the basil essential oil (Table 1) are linalool followed by  $\beta$ -cadinol and 1, 8 cineole with percentages (59.37, 17.95 and 5.49 % respectively). The results are in line with Sajjadi, [21] who revealed that the main constituent of basil essential oil is linalool. Moreover, results obtained from the GCMS indicated that the major components of the peppermint essential oil are Menthol (Table 1) followed by Menthyl-acetate and Menthofuran with percentages (48.29, 19.38 and 9.09 % respectively). These results are in agreement with those of Tsai *et al.* [22] reported that the major component in peppermint essential oil is menthol.

**Physical Characteristics:** Weight loss, Results revealed that the weight loss percentages were gradually increasing significantly ( $P$  – value < 0.05) (Fig. 1) by increasing the storage period at room temperature. After 3 weeks of storage at room temperature, the lowest weight loss percentages were recorded by the treatment of the apples with 1% peppermint, celery 2.5 % and basil 5% essential oils (4.27, 5.78 and 6.97 % respectively) the highest values of weight losses (%) were recorded by the control, (9.34 %). Whereas differences were noticed among the different essential oils percentages and sources. These results showed similarity with the results of Bidabe *et al.* [23] which, showed that there was a weight loss in apple fruits as the storage period was further extended.

The possible reason for this decrease in weight loss may be that essential oils served as a semi permeable membrane around the fruit surface that resulted in the reduction of the respiration rate and the evapotranspiration. Moreover, our results were confirmed by Wills *et al.* [24] they indicated that a decrease in the metabolic activities of the fruit results in a decrease in carbohydrate depletion rate and in fruit water loss and as a result, it efficiently delays the process of fruit senescence. Moreover, the results of Tzortzakis [8] showed that the essential oil vapour improved the fruit quality. These results are in agreement with those of Abdolahi, *et al.* [25] who showed that essential oils had a great antifungal activity and can be used as a benefit and safe tool for reservation of the grape fruit.

**Decay:** Results showed that the decay percentages were gradually increasing significantly ( $P$  –value < 0.05) by increasing the storage period at room temperature (Fig. 2). Also, indicated that the lowest decay percentage was recorded by the treatment with 5 % of basil essential oil as in basil 2.5% and basil 1% (17, 29 and 33% respectively). On the other hand, the highest decay percentage was recorded by the control and the peppermint 5% (93 % and 75% respectively). The overall observation showed that the basil 5% (Fig. 4) was the best essential oil treatment for keeping the fruit from decay at room temperature as shown in (Fig. 3, Fig. 4 and Fig. 5). These results are supported by the study that indicated that the essential oils reported in various studies exhibit antioxidant, antimicrobial and bio regulatory properties [26]. Moreover, these results are in line with Wilson *et al.* [27] they, revealed that fungi-toxic compounds that are from the plant essential oils can be used to control postharvest disease in fruits and vegetables.

**Firmness:** Results revealed that the firmness value was gradually decreasing significantly ( $P$ –value <0.05) by increasing the storage period at room temperature (Fig. 6). Results showed that the highest values for the firmness values of apples after 3 weeks were for the 5 % of the basil essential oil and basil 2.5% (7.78 and 7.39 % respectively). On the other hand the lowest firmness values were indicated by the control (6.41 %). Data observed was similar to the result of Serrano *et al.* [9] they described that the use of natural antifungal compounds on fruits and storing it increased the fruit firmness. Moreover, the results of this study were matched with previous study that showed that the essential oil had positive effect on the firmness and keeping the quality of fruits [28].

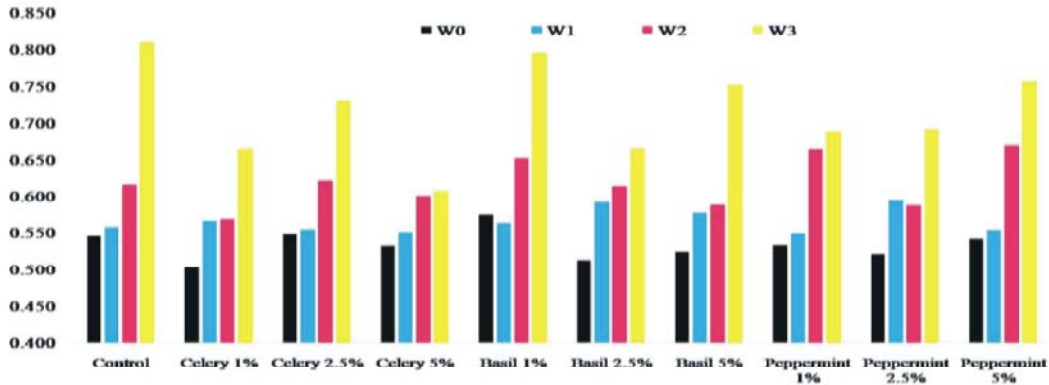


Fig. 1: The effect of spraying celery, basil and peppermint essential oils on the weight loss values of Anna apples fruits during the storage period of 3 weeks

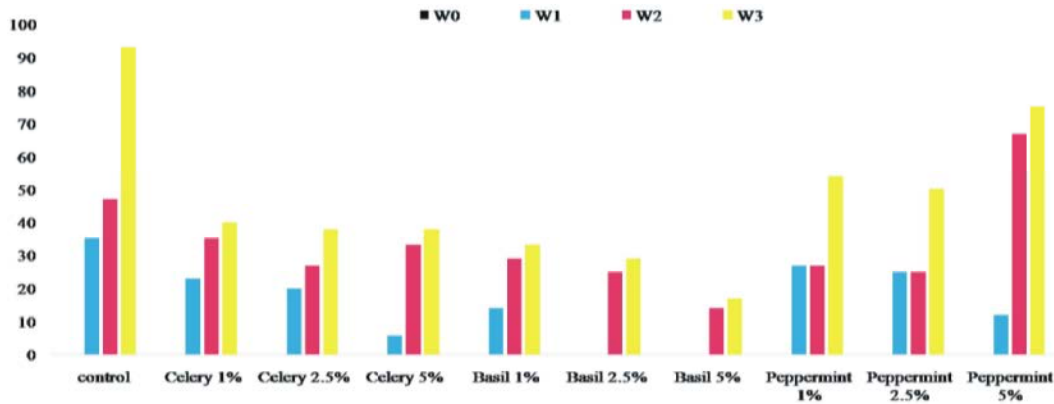


Fig. 2: The effect of spraying celery, basil and peppermint essential oils on the decay values of Anna apples fruits during the storage period of 3 weeks



Fig. 3: (A) The control Anna apples at 0 days storage, (B) Anna apples sprayed with Celery 5% at 0 days, (C) control Anna apples after 3 weeks storage and (D) Anna apples sprayed with celery 5% after 3 weeks storage



Fig. 4: (A) The control Anna apples at 0 days storage, (B) Anna apples sprayed with Basil 5% at 0 days, (C) control Anna apples after 3 weeks storage and (D) Anna apples sprayed with Basil 5% after 3 weeks storage



Fig. 5: (A) The control Anna apples at 0 days storage, (B) Anna apples sprayed with Peppermint 5% at 0 days, (C) control Anna apples after 3 weeks storage and (D) Anna apples sprayed with Peppermint 5% after 3 weeks storage

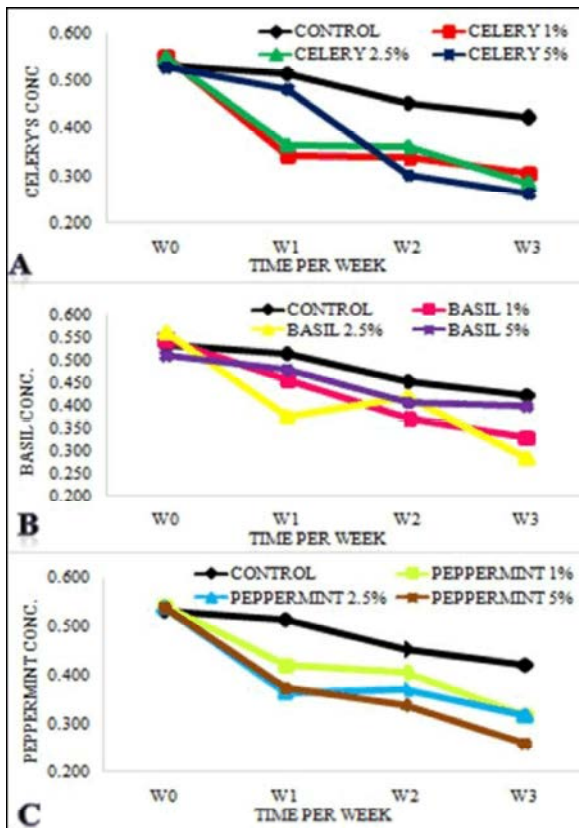


Fig. 6: (A) The Celery concentration vs. the storage time per week according to the firmness values, (B) The Basil concentration vs. the storage time per week according to the firmness values and (C) The Peppermint concentration vs. the storage time per week according to the firmness values

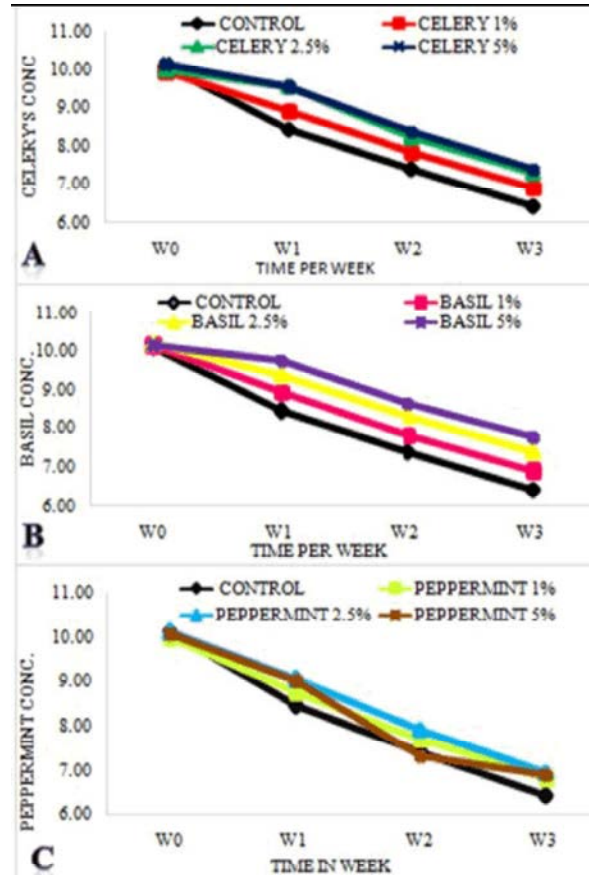


Fig. 7: (A) The Celery concentration vs. the storage time per week according to the acidity values, (B) The Basil concentration vs. the storage time per week according to the acidity values and (C) The Peppermint concentration vs. the storage time per week according to the acidity values

Fruit freshness is usually provided by the firmness value. The loss of firmness to the cell wall carbohydrate metabolism during storage is associated with increased susceptibility to infection by fungal pathogens Firmness plays a critical element in the decision of purchasing apples by the customers [29, 30]. However, Bai *et al.* [31] and Celikel *et al.* [32] reported that no decrease in the apples firmness was recorded in apples treated and kept at room temperature and a decrease in the firmness of untreated apples was recorded when undergone the same process.

**Chemical Characteristics:** Acidity, Results revealed that the acidity percentage was gradually significantly ( $P$ -value  $<0.05$ ) decreasing (Fig. 7) by increasing the storage period at room temperature. After 3 weeks of storage at room temperature, the lowest percentages of

acidity were recorded by the treated apples with celery 5%, peppermint 5% and basil 2.5% and that showed (0.2604, 0.2604 and 0.2834 % respectively). On the other hand, the highest acidity percentages were indicated by the control that showed (0.4214 %). These results supported the finding of Wills *et al.*, [24] who found that acidity percentage decreased as storage period increased. The study by Gonzalez-Aguilar *et al.* [33], indicated that the methyl jasmonate had increased the organic acids and the postharvest quality of papaya. Moreover, Wang [34] reported that the treated raspberries with natural volatile compounds increase the acidity during storage as increasing the acidity of the raspberries acidity is a sign of increasing the fruit quality. However, those of Rabiei, *et al.* [13] showed that the immersed apples in 200 ppb thyme essential oil had significantly higher acidity than the acidity values of the control apples.

Total Soluble sugar, Results revealed that treatments with essential oils resulted in significant ( $P$ -value  $<0.05$ ) increase (Fig. 8) in the total sugars percentage by increasing the storage period at room temperature. After 3 weeks at room temperature, the highest total sugars percentages were recorded by celery essential oil of 1 %, celery 2.5% and basil 5% that showed (9.98, 9.82 and 9.37 % respectively). On the other hand, the lowest total sugars percentages after 3 weeks at room temperature were recorded by the control that showed (7.90 %). These results are supported by Badshah *et al.* [35] when they found that sugar content of apples increased with increasing shelf life period. As well as, supported by study of Bhadra and Sen [36] on apple fruits. They indicated that a gradual increase in sugar contents when fruits were treated with different coatings.

TSS, Results revealed that the treatments with essential oils resulted in significant ( $P$ -value  $<0.05$ ) increase in the total soluble solids percentage (Fig. 9). After 3 weeks of storage at room temperature, the highest TSS percentages were recorded by basil essential oil of 5 %, celery 2.5% and celery 1% that showed (17.5, 17.4 and 17.0 % respectively). On the other hand, the lowest TSS percentages after 3 weeks of storage period at room temperature were recorded by the control (untreated) that showed (15.6 %). Results are in agreement with the finding by Tzortzakis [8], who reported that the spraying of the essential oil had increased the total soluble solids of the fruits during exposure to the vapor. These results are in line with Rabiei, *et al.* [13], who reported that the essential oil had good efficacy on the TSS in apples.

Total soluble pectin, Results revealed that the total soluble pectin was gradually increasing significantly ( $P$ -value  $<0.05$ ) (Fig.10) by increasing the storage period

at room temperature. Results revealed that after 3 weeks on shelf at room temperature, the total soluble pectin was increasing gradually. The lowest total soluble pectin values were recorded by celery essential oil of 5%, basil 2.5% and peppermint 1% that showed (0.607, 0.665 and 0.689 respectively). On the other hand, the highest total soluble pectin values were recorded by the control (untreated) that showed (0.809 %). This result may be due to the fact that pectin is found in the primary cell walls and it is one of the main components of the middle lamella that helps in attaching cells together. Pectin is a polysaccharide cell wall which helps in the growth of the plant and the extension of the primary cell wall. Pectinase enzyme breaks down the pectin during fruit ripening process in which the softening of the fruits occur and the fruit becomes softer due to the breaking down of the middle lamellae and the separation of the cells from each other [37].

Peroxidase enzyme activity, Results showed that the peroxidase enzyme activity increases by extending the storage period at room temperature of the apples (Fig. 11). Treatments with essential oils resulted in decrease in the peroxidase enzyme activity. After 3 weeks shelf life, the lowest peroxidase enzyme activity was recorded by the spraying of the apples with peppermint essential oil of 1%, basil 5% and peppermint 5% (0.306, 0.318 and 0.334 respectively). On the other hand the highest peroxidase enzyme activity was indicated by the untreated apples (control) that showed that (0.656 %).

The reason behind this result may be due to the fact that peroxidase is an enzyme which is involved in the process of browning of the fruits. The increasing of peroxidase is a sign of fruit quality deterioration and is relevant to the enzymatic browning process because di-phenols could promote darkening in fruit and vegetable products during processing and preservation and could function as reducing substrate in the enzyme reaction [38]. Results are in agreement with Mousavizadeh and Sedaghatthoor [11]. They reported the effectiveness of natural essential oils to decrease the activity of the peroxidase enzyme of fruits and vegetables. In addition to, Alikhani *et al.* [39] results revealed that thyme essential oil reduced the peroxidase activity and enzymatic browning in fruits. However, Montealegre *et al.* [40], indicated that the peroxidase enzyme activity of the treated apple fruits with biologically active products were higher than the controls. These results indicate that essential oils as respects to antioxidants properties can be used to minimize the peroxidase activity and enzymatic browning in the apples fruits.



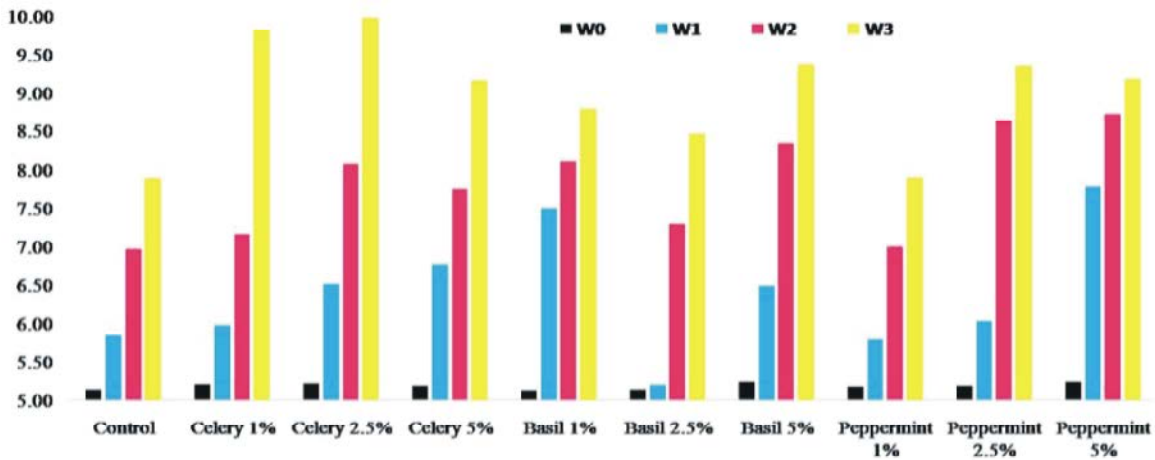


Fig. 8: The effect of spraying celery, basil and peppermint essential oils on the total soluble sugar values of Anna apples fruits during the storage period of 3 weeks

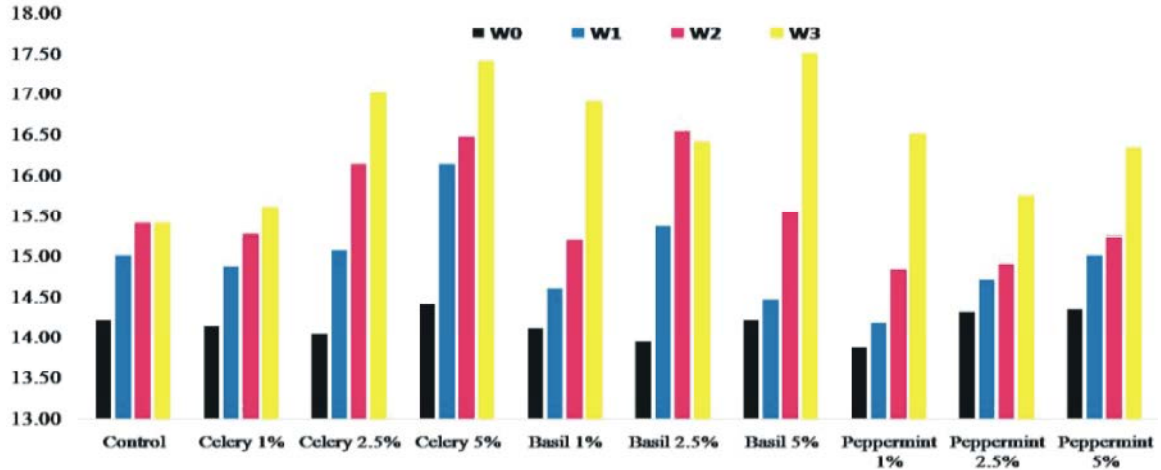


Fig. 9: The effect of spraying celery, basil and peppermint essential oils on the TSS values of Anna apples fruits during the storage period of 3 weeks



Fig. 10: The effect of spraying celery, basil and peppermint essential oils on the total soluble pectin values of Anna apples fruits during the storage period of 3 weeks

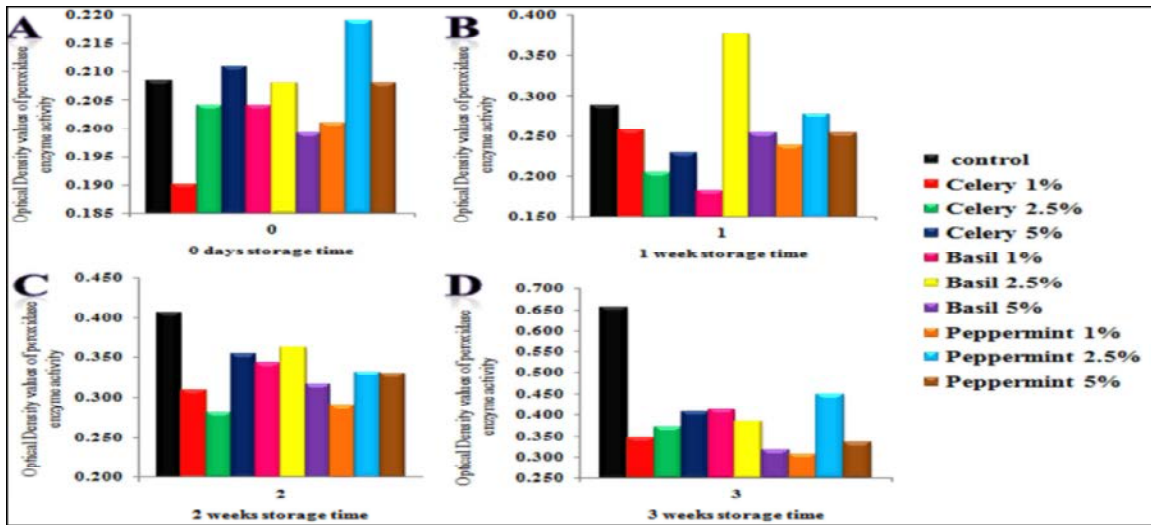


Fig. 11: (A) The optical density values of peroxidase enzyme activity at 0 days storage, (B) The optical density values of peroxidase enzyme activity after 1 week storage, (C) The optical density values of peroxidase enzyme activity after 2 weeks storage and (D) The optical density values of peroxidase enzyme activity after 3 weeks storage

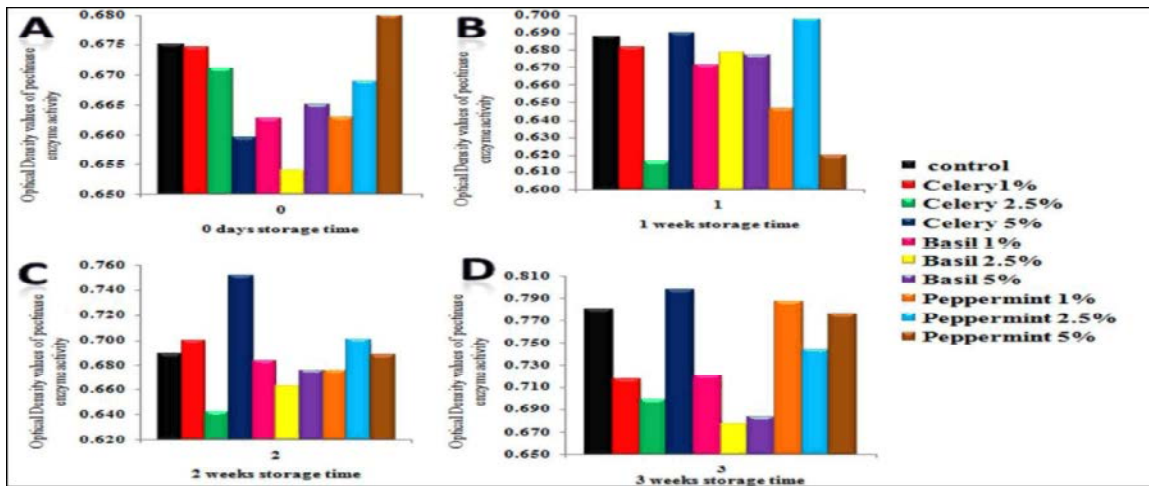


Fig. 12: (A) The optical density values of pectinase enzyme activity at 0 days storage, (B) The optical density values of pectinase enzyme activity after 1 week storage, (C) The optical density values of pectinase enzyme activity after 2 weeks storage and (D) The optical density values of pectinase enzyme activity after 3 weeks storage

**Pectinase Enzyme Activity:** Results showed that the pectinase enzyme activity increases by extending storage period at room temperature of the Anna apples (Fig. 12). Treatments with essential oils resulted in decrease in the pectinase enzyme activity. After 3 weeks shelf life, the lowest pectinase enzyme activity was recorded by the spraying of the apples with basil essential oil of 2.5%, basil 5% and celery 2.5% (0.677, 0.683 and 0.699 respectively). On the other hand the highest pectinase enzyme activity was indicated by the apples sprayed with celery 5%, the untreated apples (control) and the apples sprayed with peppermint essential oil of concentration 1%

(0.798, 0.780 and 0.787 respectively). Similar to that Dubey *et al.* [41] revealed that the essential oil of the eupatorium cannabinum showed an inhibitory effect on pectinase enzyme activity. The reason behind this is that the pectinases are an enormous group of enzymes which break down pectic polysaccharides of the tissues of the plants into un-compounded molecules as galacturonic acids [42].

Polyphenol oxidase enzyme activity, Results showed that the polyphenol oxidase enzyme activity increases by extending the storage period at room temperature of the apples (Fig. 13). Treatments with essential oils resulted in



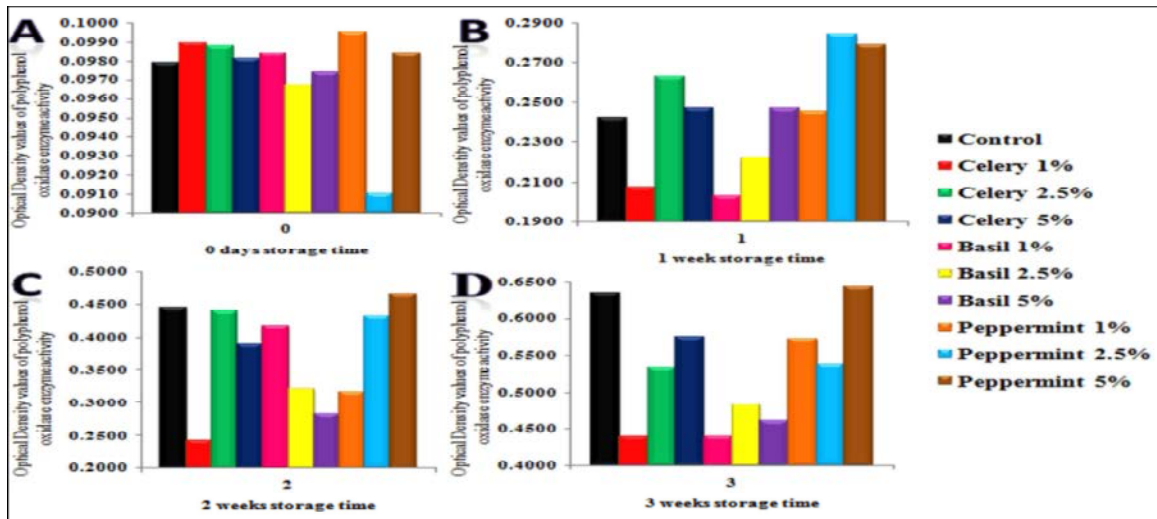


Fig. 13: (A) The optical density values of polyphenol oxidase enzyme activity at 0 days storage, (B) The optical density values of polyphenol oxidase enzyme activity after 1 week storage, (C) The optical density values of polyphenol oxidase enzyme activity after 2 weeks storage and (D) The optical density values of polyphenol oxidase enzyme activity after 3 weeks storage

decrease in the polyphenol oxidase enzyme activity. After 3 weeks shelf life, the lowest peroxidase enzyme activity was recorded by the spraying of the apples with celery essential oil of 1%, basil 1% and basil 5% (0.4397, 0.4400 and 0.4612 respectively). On the other hand the highest pectinase enzyme activity was indicated by the apples sprayed with peppermint essential oil of concentration of 5 %, the untreated apples (control), the apples sprayed with celery essential oil of concentration of 5 % and apples sprayed with basil essential oil of 1% (0.6433, 0.6341 and 0.5747 respectively). This result may be due to the fact that Polyphenol oxidase (PPO) is an enzyme that is involved in the browning process.

PPO catalyses the hydroxylation of monophenols (monophenolase) and the oxidation of o- diphenols into o-quinones (diphenolase), that consequently polymerise originating the undesirable brown pigments when oxygen is present. In agree with the present result, Sivakumar and Bautista-Banos [43] indicated that essential oils have the capability to reduce the PPO activity. Moreover, previous results indicated that Shieh and Kafoor of 0.5 % had reduction effects on the PPO activity during the apple juice preservation [44].

Conclusively, from the results obtained in the present study, spraying the Egyptian Anna apples with essential oils is suggested to be a good recommendation for improving the fresh quality assessments after 3 weeks of room temperature storage.

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