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Efficiency of Turmeric, Hibiscus and Beet Root Extracts as an Eco-Friendly Edible Coating to Extend storage Life of Hass Avocado Fruit

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Abstract
Avocado (*Persea americana* Mill.) is a tropical and subtropical climacteric fruit of Central America. Avocados fruits are increasingly consumed in Europe due to their organoleptic, functional properties and nutritional value. It is necessary to maintain high quality of the fruit after harvest for the distant imported market through sustainable solutions. Therefore, this experiment was aimed to increase the quality and storage potential of "Hass" avocado fruits through natural and water-based edible coatings. The treatments include aqueous extracts of beet root, hibiscus, turmeric and control treatments (water) during cold storage (8 ±1°C). The results indicated that, after six weeks of Hass avocado storage, both turmeric extract and beet root extract significantly decreased decay percent, weight loss percent as well as maintained higher TSS and vitamin C content compared to the control. Turmeric extract maintained high titratable acidity content, total chlorophyll of fruit pulp, carotenoid content and total phenols content compared to the control treatment. In addition turmeric acid recorded the lowest significant polyphenol oxidase activity followed by beet root extract, while the control treatment recorded the highest activity. This study supports the efficacy of turmeric extract and beet root extract in extending storage life of avocado Hass fruit via natural tools.

Keywords: hibiscus; polyphenoloxidase; phenols; pigments; Persea Americana

1. Introduction

Avocado (*Persea americana* Mill.) is a climacteric fruit with high ethylene production and high fruit respiration rate, which stimulates the initiation of ripening during post-harvest storage, resulting in a storage life of only 3-4 weeks [1]. Avocado fruit is a good source of bioactive phytochemicals (carotenoids, phenolic compounds, phytosterols fatty acids), minerals, vitamins (B and E), fiber and unsaturated fatty acids [2, 3, 4]. Avocado extracts has a good medicinal effects on human heath such as cancer [5] and Alzheimer's disease control [6, 7]. Europe is the largest importer for avocados due to its organoleptic, functional attributes and nutritional values [8]. The fruit susceptibility to postharvest diseases infection and high rate biological activity led to huge magnitudes losses in post-harvest fruits [9]. Approximately, 80% of fruit and vegetables are wasted in developing countries [10], with total of 1.3 billion tons of vegetables and fruits wasted worldwide [11].

Nowadays, synthetic wax is not used to improve fruit appearance and reducing fruit moisture loss for fruit export to Europe [9]. Various post-harvest treatments have been studied to reduce fruit loss, improve quality and extend the shelf life of the fruits. The use of fungicides during postharvest period continues to be a pressure to comply with safety regulations [12]. There is a high priority for using a less hazardous alternative substance to comply with safety regulation and facilitates effective protection against postharvest diseases [9]. Due to these facts, emerging alternatives, such as eco-friendly coating materials for extending shelf life, represent a sustainable solution to overcome the excessive food waste [12]. Therefore, it ensures that there is a less harmful alternative substance to edible coating technology is designed for many purposes such as protecting fruits from water loss, texture enhancer carrier, antioxidants carrier and nutrients carrier [12, 13, 14]. Hibiscus or Roselle (*Hibiscus sabdariffa* L.) calyx is rich in polysaccharides and pectin content; it

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is among the main plant sources that can be used to make edible fruit coating [15]. Recently, Roselle extract as an edible coating reduced decay, microbial growth, anthocyanin degradation and enzyme activities of blueberry, while improving the total phenolic content [16]. Moreover, Hibiscus mucilage (2%) as edible coating for soursop fruits (Annona muricata L.) stored at 15°C showed the lowest titratable acidity and weight loss as well as increased vitamin C, total phenolic content and antioxidant activity [15].

Beetroot (*Beta vulgaris* L.) is a rich source of betalains [17], dietary fiber [18], phytochemical compounds, antimicrobial potential [19] and antioxidant compounds [20], which has been supported to be an edible coating [21].

Curcumin is a natural and bioactive polyphenolic compound isolated from dry (*Curcuma longa*) turmeric rhizomes [22, 23]. For human health, turmeric rhizomes shows anti-inflammatory, antimicrobial, antioxidant, anticancer and antiviral effects [24, 25, 26, 27]. Treating apples with compounds contain curcumin as edible coating during postharvest recorded a biodegradable antifungal effect [28, 29]. Also, curcumin increased storability of many fruit species like banana [30], tomato [31], dragon fruit [32]. Curcumin is also a promising bioactive component, highly biocompatible, and biodegradable component, allowing it to be easily metabolized [33].

However, natural extracts from beet root and hibiscus have not been studied compared to turmeric extract and its effect as a coating on avocados. So, the aim of this study to evaluate the efficiency and mechanisms of previous natural extract on maintaining avocado fruit quality and extend Shelf Life of Hass Avocado Fruit during cold storage.

2. Material and methods

2-1- Fruit sampling

The present study was carried out at the laboratory of department of Horticultural crops technology, National research center, Giza, Egypt through two easons (2020-2021). Fine-quality avocado fruits of "Hass" cultivar were purchased from PICO exporting commercial farm (30°41'42"N and 30°23'16"E, elevation 9 m) at El Behera Governorate, Egypt. At 20% dry matter mainly as maturity index for harvesting Hass avocados fruits [34]. Full green color, uniform size (150 g), firm stage (35 kg/cm3), free from blemishes and mechanical damage were harvested. Harvested fruit were packed in open display boxes and transported to the Postharvest Laboratory. Then the fruits were then distributed into four postharvest treatments control (tap water) with three aqueous extracts includes hibiscus 10%, turmeric 10% and beet root 10%. This experiment was repeated trough two seasons with three replicates for each one.

2-2-Preparation of plant extracts:

Extracts of each hibiscus, turmeric, beet root were prepared according to Brand *et al.* [35] with some modifications. Natural powder of hibiscus, turmeric, beet root was soaked overnight in distilled water at ratio 1:1 (w/v) and then were boiled at 90 °C [36]. The extracts were filtered by a Whatman No. 41 and kept in a dark container at 4 °C. Before treatment each solution were diluted (1:10) to prepare one liter and 3% glycerol were added to the solution.

2-3- Treatments:

A total of four treatments i.e., hibiscus, turmeric, beet root and control (tap water) were used in this experiment. Forty five fruits for each treatment were dipped for 5 minutes in the previously prepared solutions, then allowed to dry at room temperature (23 ± 1 C) for 20 min, packed in perforated carton boxes and stored up to 6 weeks at 8 ± 1◦ C and 90% RH. During cold storage, the following physical and chemical fruit quality parameters were recorded weekly.

2-4- Determination of fruit physical quality:

2-4-1- Weight loss %

Weight loss % of "Hass" avocado fruit was measured using a digital balance by the following formula: weight loss% = (Initial weight - weight in the specific time of storage)/ initial weight) × 100 [37].

2-4-2- The fruit decay %

The fruit decay % was determined visually and calculated by the following formula: Fruit decay % = (the number of rotten fruit / the total number of fruit) × 100. Rotten fruit were classified as having any visible rotten spot on the surface [37].

2-4-3- Fruit Firmness (kg/cm3)

Fruit Firmness was measured as kg/cm3 on the two opposite sides of each fruit (penetrometer occupied by 8 mm diameter probe, FT 327).

2-4-4- Fruit peel color

Fruit peel color was determined via one trained assessor according to Cox *et al.*[38]. The color turned to score through classified the color using the following scale: 1, emerald green; 2, forest green; 3, olive green; 4, purple; and 5, black.

2-5- Determination of biochemical fruit quality

2-5-1- Total titratable acidity%

In the extracted fruits juice, total titratable acidity was expressed as citric acid % by titration with 0.1 N NaOH using phenolphthalein as indicator [39].

2-5-2- Ascorbic acid (mg/g FW)

In the previous extracted fruits juice, ascorbic acid content was determined using 2,6 dichlorophenol indophenol titrimetic method [40].

2-5-3- Fruit TSS percent

Digital hand refractometer (PR32, Atago Palete

ATago CO .LTD. Japan) expressed as ◦Brix was used to TSS determination [41].

2-5-4- Pulp pigments analysis

Total chlorophyll (Chlorophyll a and chlorophyll b) and carotenoid contents of "Hass" avocado fruit pulp were determined according to the method described by Lichtenthaler and Wellburn [42]. One gram of fresh fruit pulp was placed in a test tube with acetone (80%) for 24 h at 4 °C. Next day, the extracted material was centrifuged for 10 min at 8000g. absorbance of the supernatant was read at a wavelength of 663, 645 and 470 nm, using an UV-B a spectrophotometer (UV-B2501, Shimadzu, Kyoto, Japan) for determine Chlorophyll A, B and carotinoid, respectively. Chlorophyll (a, b and total) and carotenoid contents (mg 100 mL−1) were calculated according to Lichtenthaler and Wellburn [42].

2-5-5- Total phenols (mg/g FW)

Total phenols were determined using Folin-ciocalteu method [43]. One gram of fruit pulp sample was homogenized after grinding in a pestle and mortar using 20 ml methanol (80%). Then 0.2 ml of Folin-Ciocalteu phenol reagent was added to the methanolic extract mixture and shaken. After two minutes, 1 ml of 20% Na_2CO_3 solution was added to the mixture. After incubation for 30 minutes in dark at room temperature, the absorbance against the reagent blank was determined at 760 nm with an UV/Vis spectrophotometer. Total phenolics content was expressed as mg gallic acid equivalents.

2-5-6- Polyphenoloxidase activity (PPO activity (mg/g FW)

Twenty gram of avocado fruit pulp was homogenised with 100 ml 0.1 mol/l sodium phosphate buffer (pH 6.5) containing 1% polyvinylpolypyrrolidone, then centrifuged for 10 minutes at 10000 g at 4°C [44]. The enzyme assay was determined in the supernatant according to Liu *et al.* [45]. The standard reaction mixture contained 2.3 ml of 0.1 mol/l phosphate buffer (pH 6.5) and 1.5 ml of 40 mmol/l catechol in test tube, and was placed in water bath for 5 minutes at 25°C. Then, crude enzyme (0.2 ml) was added to the test tube and mixed. Immediately, the increase in absorbance was measured at 420 nm with a UV-spectrophotometer (UV-B2501, Shimadzu, Kyoto, Japan). The activity of PPO was expressed as PPO reaction velocity per mg PPO in avocado fruit and the reaction time for PPO was 2 min.

2-6- Statistical Analysis:

The experiment design was the complete randomized block design with two factors (postharvest material and storage periods), and three replicates for each treatment. Differences significances between treatments were analyzed using MSTAT-C statistical package [46]. The data of both seasons statistically subjected to homogeneity test (Levene's test). After there is no significant differences between two seasons the average of both seasons were presented. The means of the treatments were compared by least significant difference (LSD) values were calculated at 0.05 [47]. The presented data in figures are means ± standard error (SE).

3- Results and Discussion

3-1-Fruit physical quality

Data in figure (1A, B, C and D) show the effect of different natural aqueous extract on decay percent, weight loss and fruit firmness of Hass avocado fruits, respectively. It can be observed that avocado fruit still without decay till 3 weeks during cold storage. Also, turmeric extract recorded the lowest significant fruit decay percent (4%) followed by beet root extract (8%) compared to the control (18%) and Hibiscus extract (16.33%) at the end of storage period. Also, fruit weight loss percent during ‘Hass’ avocado storage is presented in Figure (1B). Fruit weight loss increased gradually with increasing storage periods, at 6 weeks of cold storage it can be concluded that, turmeric extract followed by beet root extract recorded the lowest weight loss percent while hibiscus extract and control treatments recorded the highest values. In addition, fruit firmness decreased gradually through storage periods (figure 1C). The fruit firmness recorded higher value (13-18.63kg/cm3) at the first three weeks of storage. At 35 days of storage, beet root treatment recorded the highest firmness values (7 kg/cm3) followed by turmeric extracts. All treatments recorded low fruit peel color development at the five weeks of storage compared to the control treatment. At the end of storage period hibiscus extract recorded the lowest significant fruit color development. These findings were in line with Yilmaz *et al.* [28] who mentioned that, curcumin as edible coating during storage of apples recorded a biodegradable antifungal effect. Also, curcumin increase storability of banana [30], tomato [31], dragon fruit [32]. Moreover, it has natural antifungal, promising bioactive component, highly biocompatible, and biodegradable component, allowing it to be easily metabolized [24, 25, 26, 27; 33].

Recently, curcumin inhibited postharvest decay of kiwifruit [48]. In addition, Gutiérrez *et al.* [21] reported that, beet root extract has been supported to be an edible coating due to it contains antioxidant compounds [20], betalains [17], dietary fiber [18], phytochemical compounds, antimicrobial potential [19]. Moreover, curcumin-based photodynamic sterilization can effectively in maintaining firmness and water content of fresh-cut Hami melon. [49]. Curcumin-based photodynamic inactivation was proven to prevent weight loss of the treated apples [50].

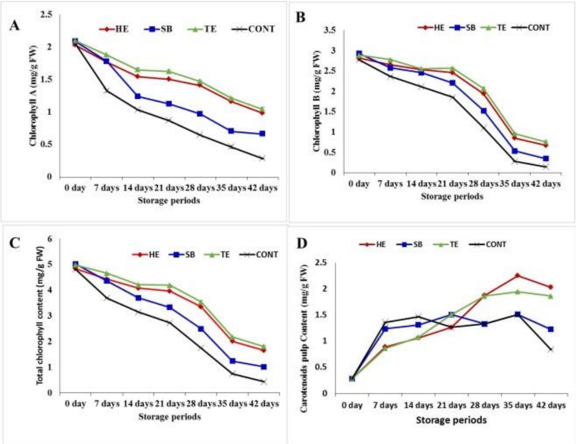

Figure 1. Effect of natural extracts as edible coating of hebiscus extract (HE), beet root (SB), turmeric (TE) and control (cont) treatments on decay percent (A), weight loss (B), fruit firmness (C) and fruit peel color (D) of Hass avocado cv. during 6 week of cold storage at 8 ºC. Noted that TE and SB have the same values in fruit peel color (D), so they presented as one curve. The presented data is average of two seasons (2020-2021)

These results were in line with Santos-Santos *et al.* [15] as they concluded that the coating based on roselle mucilage (2%) prevents weight loss of Soursop fruits. More recently, Sarwar *et al.* [51] found that strawberry fruit photosensitized with 1000 µM curcumin for 10 min, delay firmness reduction and decreased fungal infection by 52% through 12 days of cold storage at 4 °C.

3-2- Fruit chemical quality

Fruit TSS content generally tends to be fixed to somewhat, than increased during storage (Figure 2A). At the 6th weeks of storage, beet root extract followed by turmeric extract recorded the highest significant values compared to hibiscus extract and the control treatment. With increasing storage periods, titratable acidity decreased gradually (Figure 2C). At the end of the storage periods it can be observed that, turmeric extract recorded the highest titratable acidity compared to the control treatment. Also, Ascorbic acid (Vitamin C) content decreased with increasing storage periods (figure 2 B). At the end of storage periods (42 days) turmeric extract followed by beet root extract recorded the highest significant values compared to the control. These results were harmony with Taoa *et al.* [50] who reported that, curcumin can effectively maintaining soluble solids content of fresh-cut Hami melon. [49]. On the other hand, strawberry fruit treated with 1000µM curcumin for 10 min, followed by storage at 4 ± 2 °C did not have an effect on titratable acidity and ascorbic acid content, whereas total soluble solids and total sugar were increased [51]. Also, Santos-Santos *et al.*[15] concluded that coating based on roselle mucilage (2%) and hibiscus mucilage (2%) as edible coating increased vitamin C content of soursop fruits. Furthermore, the pectin, corn flour and beetroot powder based edible coatings treatment have improved shelf life and fruit quality of tomatoes as compared to control [52].

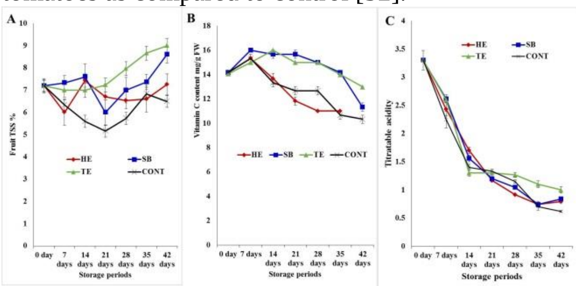

Figure 2. Effect of natural extracts as edible coating of hebiscus extract (HE), beet root (SB), turmeric (TE) and control (cont) treatments on TSS percent (A), vitamin C (B), fruit titratable acidity (C) of Hass avocado cv. during 6 week of cold storage at 8 ºC. The presented data is average of two seasons (2020-2021).

3-3- Fruit pulp pigment content

Chlorophyll pigments (Chl. A, Chl. B and total Chl) decreased gradually with increasing storage periods (Figure 3 A, B, C). Turmeric extract recorded the highest total fruit pulp of chlorophyll content followed by hibiscus extract while control treatment recorded the lowest values. Also, control and beet root extract recorded the highest values while turmeric acid and hibiscus extract recorded the lowest values. In addition, through storage period carotenoid content increased (Figure 3 D). The highest carotene content recorded by hibiscus extract followed by turmeric extract while beet root and control treatments recorded the lowest values. These findings were harmony with Paula *et al.* [30] who found that coatings based on turmeric flour display good antioxidant activity which delay color development in coated bananas as compared to control bananas. The avocado showed higher chlorophyll content, indicating that it was still fresher than the one with lower chlorophyll content and higher carotenoid content which obtained in the TE treatment.

3-4- Fruit biochemical analysis

Generally fruit total phenol contents tended to decrease through storage periods (Figure 4 A). At the end of storage, turmeric acid recorded the highest significant phenols content while the control treatments recorded the lowest value. Polyphenol oxidase activity recorded increase and decrease through storage periods (Figure 4 B).

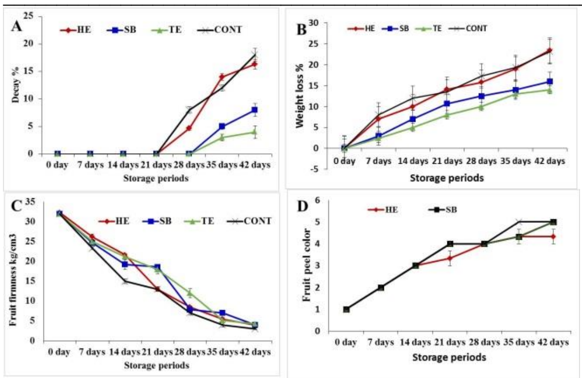

Figure 3. Effect of natural extracts as edible coating of hebiscus extract (HE), beet root (SB), turmeric (TE) and control (cont) treatments on pulp content of chlorophyll A content (A), chlorophyll B content (B), total chlorophyll content (C) and carotenoid content (D) of Hass avocado cv. during 6 week of cold storage at 8 C. The presented data is average of two seasons (2020-2021).

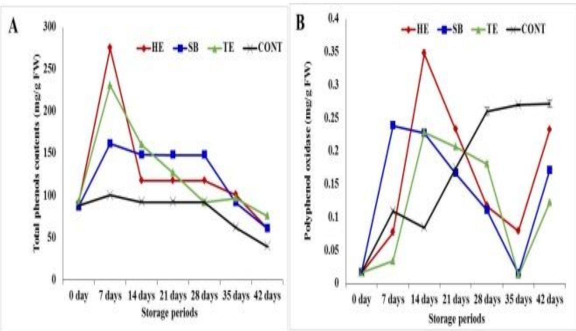

Figure 4. Effect of natural extracts as edible coating of hebiscus extract (HE), beet root (SB), turmeric (TE) and control (cont) treatments on pulp content of total phenol content (A) and polyphenol oxidase activity (B of Hass avocado variety during 6 week of cold storage at 8 ºC. The presented data is average of two seasons (2020-2021).

At the end of storage period turmeric extract followed by beet root extract recorded the lowest polyphenol oxidase activity. While the control treatment recorded the highest polyphenol oxidase activity. PPO is related to the avocado browning process, which can occur from mechanical injuries during harvest and post-harvest storage. Therefore, inactivation of PPO is of great importance in the food industry to prevent the browning process [53]. These results confirm that Curcumin-based coating was significantly reduced the activity of polyphenol oxidase to 48% in fresh cut apple [50]. Also, Santos-Santos *et al.* [15] who concluded that the coating based on roselle mucilage (2%) increased the phenols content of Soursop fruits. In addition, roselle extract as an edible coating reduced enzyme activities of blueberry, while improving the total phenolic content [16].On the other hand, strawberry fruit treated with 1000 μM curcumin for 10 min, followed by storage at 4 ± 2 °C did not have an effect on total phenolic content [51]. Also there were few negative changes in total phenolic, ascorbic acid content and antioxidant activity of the treated apples [50].

4- Conclusion

Both turmeric extract and beet root extract significantly decreased decay percent, weight loss percent as well as maintained higher TSS and vitamin C content of Hass avocado fruit compared to the control. Turmeric extract increased titratable acidity content, total chlorophyll of fruit pulp, carotenoids content and total phenols content compared to the control treatment. In addition turmeric acid recorded the lowest significant polyphenol oxidase activity followed by beet root extract. This study support the effectiveness of turmeric extract and beet root extract in extending the shelf life of avocado Hass fruit via natural and safety tools.

Conflicts of interest

There is no conflict of interest between authors.

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