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Effects of Emblica Extract (*Phyllanthus emblica*) on Color and Antimicrobial Quality of Avocado Puree During Freezing Storage

Ploypailin Suwannate[a], Utthapon Issara[b], Kittikoon Wannasawad[b] & Arranee Chotiko[b]*

[a] *Division of Biology, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Pathum Thani, 12110 Thailand*
[b] *Division of Food Science and Technology Management, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Pathum Thani, 12110 Thailand*

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Abstract

Emblica (*Phyllanthus emblica*) fruits have both antioxidant and antimicrobial activities as well as enzyme-inhibiting properties. They contain high levels of ascorbic acid and citric acid, preventing the browning reaction in minimally proceeded fruits and vegetables. This research investigated the effect of emblica fruit extract on the qualities of avocado puree during freezing storage. Emblica fruits were ethanolic extracted and used as anti-browning and anti-microbial agents. Ripe avocados were selected and blanched in carbonate buffer (pH 10.6). Acidulants, including ascorbic acid and emblica fruit extract, were then added prior to blending to become homogenous. The puree was packed and stored at -20°C for 4 weeks. During storage, the puree was determined for their color, pH and total bacterial plate counts. The result showed that the emblica fruit extract had antioxidants activity and could inhibit *Staphylococcus pasteuri,* spoilage bacteria isolated from avocado puree. The emblica fruit extract could delay the browning reaction during storage. However, the extract concentration had an influence on the puree color, causing the puree lightness to decrease. Blanching helped destroyed some contaminated bacteria in the avocado. After storage at -20°C for 4 weeks, less than 1 log CFU/g were detected in all avocado puree treatments, which were blanched. This study indicates that the emblica fruit extract could be used as an acidulant to prevent the blanched avocado from the browning reaction.

Introduction

Avocados are considered as potential economic fruits, gaining huge global attention. They are relatively high in nutrition, including good fat, low sugar and high antioxidants. They are well-known for their health benefits, mainly associated with hypoglycemic, antihypertensive, anti-obesity and hepatic-protective effects. Although avocados originated from Mexico, they began to be cultured in Thailand in 1978 and have been continuously grown in the North of Thailand for more than 40 years, producing approximately 1,200 tons/year.

* Corresponding Author
e-mail: arranee_c@rmutt.ac.th

Unfortunately, the Thai-grown avocados usually fail to meet the food industry requirements and are rejected or cut-priced, as their characteristics have been changed from the originated avocado breeder. Yearly, more than 25% of the Thai avocados were disposed, causing huge economic losses to Thai farmers. To overcome the problem, avocados could be minimally processed to avocado-based products such as puree, frozen pieces, or powder. However, due to their high perishability and susceptibility, they tend spoil rapidly and become brown during processing and storage (López-Ramírez & Duarte-Sierra, 2020). Polyphenol oxidase, known as PPO, plays an important role in catalyzing browning reaction in avocados. Several technologies have been successfully introduced to reduce or halt those reactions such as high hydrostatic pressure, gamma radiation, flash vacuum-expansion and microwave; however, they require advance equipment, technicians and a high cost of investment (Stephen & Radhakrishnan, 2022).

It has been well-documented that heat causes PPO to be denatured until it cannot catalyze (Lv et al., 2017) and inhibited when the pH is lower than 4. The addition of acidulants such as ascorbic acid, citric acid (Ali et al., 2014) or juice such as lime and onion juice (Bustos et al., 2015) were reported to be able to halt the activity of PPO. Emblica, a fruit originated from India, has the potential to preserve avocados from browning reaction. It contains high levels of ascorbic acid, citric acid and tannin (Liu et al., 2008). Emblica fruits have both antioxidant and antimicrobial activities as well as enzyme-inhibiting properties (Priya et al., 2012; Majeed et al., 2020). It was reported that emblica extract (1%) was more effective in reducing oxidation in biscuit than BHA (200 ppm), synthetic antioxidant, during storage for 6 weeks at ambient temperature and had no influence on the surface color and texture of the biscuit (Reddy et al., 2005). Similarly, less than 7% of emblica juice added into enriched vitamin C aloe vera-apricot beverage was accepted from panelists in terms of appearance, flavor and overall acceptability (Sharma et al., 2022). In addition, emblica extract was successfully used as a natural preservative in raw ground pork during refrigerated storage at 4°C. It helped effectively decrease the number of total viable counts and total Pseudomonas in raw ground pork after 12-day storage as well as lowering lipid oxidation (Nanasombat et al., 2012). Therefore, emblica could be used to not only preserve the avocado puree from the browning reaction, but also protect the puree from microbial spoilage. However, inappropriate amount of heating time, temperatures and concentrations of acidulants may result in unusual and unacceptable color, odor and taste of the avocado.

Moreover, it was also found that heating and acids could influence the structure of chlorophylls and the pigment retention. Heating converted chlorophylls to pheophytins and pyropheophytins, causing color change from light green to an unpleasant olive green, while in acidic conditions, the color could change from bright green to olive brown due to loss of Mg in the porphyrin ring which was replaced by hydrogen ion (Koca et al., 2007). On the other hand, it was found that alkaline conditions did not affect the chlorophyll structure by inducing oxidation of the isocyclic ring and de-esterification of phytol in chlorophylls. This helped retain the basic structure of the chromophore group with Mg linked to the porphyrin ring (Koca et al., 2007). Therefore, to apply simple techniques as heating or the addition of acids to avocado products, such as avocado puree, it is necessary to ensure that the structure of chlorophylls is stabilized, while simultaneously, the puree is prevented from a browning reaction. This research investigated the effects of emblica fruit extract combined with alkaline water boiling on quality changes of avocado puree during freezing storage. The study could contribute to the avocado farmers and the food industry by developing a stable avocado puree using a simple method.

Materials and methods

1. Isolation of spoilage bacteria from avocado puree

The fresh avocado was cleaned and halved lengthwise. The seed was then pitted after cleaning and sanitation. The pulp was cut into small pieces and ground for 2 min in a blender (MX-AC400, Panasonic, China). The puree was immediately packaged in polypropylene zip-lock bags after blending and kept at 4°C until it spoiled. Serial dilution from 10^{-1} to 10^{-6} dilutions was performed to isolate bacteria from the spoiled avocado puree. A 100 µL of each of diluted suspension was spread on nutrient agar medium. The plates were incubated at 37°C for 48 h. Morphological analysis of the isolated bacteria was observed and identified by microscopic examination. A 16S ribosomal DNA sequencing method was also used to confirm the identity of these bacteria (Ananchaipattana et al., 2012). The bacterial isolate was sub-cultured and stored on nutrient agar slants at 4°C for the next experiment.

2. Preparation of Emblica fruit extract

Dried emblica fruits were purchased from Popaya Natural Products Co. Ltd. in Pathumthani, Thailand. The dried fruit was pulverized until becoming a powder. The powder (100 g) was soaked in 95% of ethanol (1,000 mL) for 24 h. The extract was filtered through Whatman filter paper No. 1 and evaporated to remove ethanol under a vacuum using a rotary evaporator (R-300, Buchi, Germany) (Patel et al., 2009). The crude extract was then freeze dried and stored at 4°C in storage vials for experimental use. The extract was used to determine the total phenolic contents, scavenging activity and antimicrobial activity.

3. Determinations of Emblica fruit extract

3.1 Total phenolic contents

Total phenolic contents of emblica extract powder were determined by a modified method as described by Hatami et al. (2014). The solution consisted of 0.5 mL of emblica extract solution (0.1 mg of emblica extract powder dissolved in 1 mL of methanol) mixed with 2.5 mL of 10%v/v diluted Folin–Ciocalteu phenol reagent and 2 mL of 7%w/v sodium carbonate. The samples were incubated in the dark for 2 h at room temperature. One hundred microliter of the solution was then added into 96 wells plates and absorbance at 760 nm was determined using a Microplate Reader (EPOCH 2, Bio Tek, USA). Total phenolic content was expressed as mg gallic acid/g using the equation obtained from a calibration curve of gallic acid at the concentrations of 0-120 mg/L. All samples were measured in triplicate.

3.2 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical- scavenging activity

DPPH scavenging activity was analyzed using a spectrophotometric method described by Jan et al. (2013). A solution of DPPH in methanol was prepared freshly. To measure the scavenging capacity of a single antioxidant, a 2.9 mL aliquot of DPPH solution was mixed with 0.1 mL of sample solutions (100 mg/mL), shaken well and incubated in the dark for 30 min at room temperature. The decrease in absorbance was measured at 517 nm. The percentage inhibition of the radicals due to the antioxidant property was calculated using the equation 1 shown below.

$$\% \text{ inhibition} = [(Abs_{blank} - Abs_{sample})/Abs_{blank}] * 100 \ldots\ldots (1)$$

where, Abs_{sample} = absorbance of 1 mmol DPPH with sample in methanol and Abs_{blank} = absorbance of methanol solvent in absence of DPPH and sample.

3.3 Antimicrobial activity

The bacteria isolated from avocado puree were then used to determine the antimicrobial activity of emblica fruit extract by agar well diffusion method. The isolate was grown in Muller Hinton broth. The turbidity of the isolate was adjusted to 0.5 McFarland standards. An 0.1 mL of the isolate was then inoculated on Muller-Hinton agar. The plates were dried for 15 min prior to being punched by using sterile cork borers to form wells. A 100 µL of the extracts, prepared by dissolving the extract powder in distilled water to obtain the solution at concentration of 100-500 ppm, was added into the wells and distilled water was used as control. The plates were then incubated for 24 h at 37°C. The diameters of the clear zone of inhibition were measured in millimeters. An agar well showing no clear zone was determined as having no antimicrobial activity. All experiments were done in triplicate.

4. Avocado puree production

Avocados were kindly provided by Boriboon Farm located in Nakhon Ratchasima Province, Thailand. Avocados in stage 5 were selected to be processed by measuring the avocado firmness by a texture analyzer (TA.XT plus C, Stable micro system, United Kingdom) which had to be between 200-300 N. The selected avocados were cleaned, halved lengthwise and pitted to separate the pulp. They were then cut into pieces and scalded in sodium bicarbonate buffer (pH 10.6) at 85°C for 3 min After that, the pulp was ground with a blender for 2 min. Emblica extract prepared by dissolving emblica extract powder in distilled water to obtain the concentrations of 300 ppm and 500 ppm (AAP/E300 and AAP/E500), was then added. Ascorbic acid was used as the positive control (AAP/AA). Blanched avocado puree in alkaline solution (AAP) was used as the blank. The puree was mixed until homogenous, packaged in polypropylene zip-lock bags and stored at -20°C for 4 weeks. During storage, the frozen avocado puree was separately determined for their color and pH, while total bacteria plate count was conducted after storage.

5. Determination of avocado puree

5.1 Color and pH

The colors of avocado puree were monitored using a colorimeter with LED white light (D65) and CIE 10° standard observer (NR 200, 3nh, China). Each sample were measured in the CIE Lab scale for triplicates. Three replicates were carried out. All data were averaged. Data collected included lightness (L*), redness (a*), yellowness (b*) and the total color difference (ΔE) was

calculated according to the equation 2 shown below. The pH of avocado puree was determined by using a pH meter (pH700, Eutech, Singapore) with triplicates.

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad \text{..... (2)}$$

where L_0^*, a_0^* and b_0^* were the values of the sample at day 0, while L^*, a^* and b^* were the values of the sample during storage.

5.2 Total bacterial plate counts

Twenty-five grams of chilled avocado puree was added into a stomacher bag containing 225 mL of saline solution. The sample was then homogenized with a stomacher for 2 min. The serial dilution from 10^{-1} to 10^{-6} was performed prior to transferring 1 mL of suspension of each dilution to 3M Petrifilm Aerobic Count Plate. The plates were then incubated at 37°C for 24-48 h. The number of colonies was counted and expressed as log CFU/g. Fresh avocado puree (FAP) was used as the positive control.

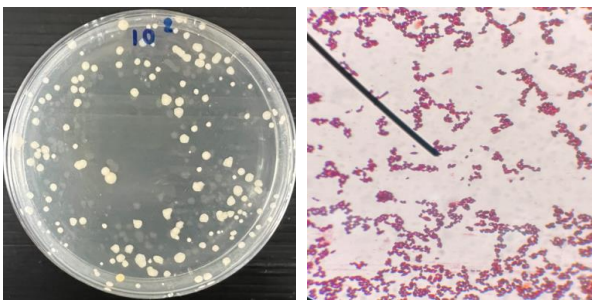
6. Statistical analysis

Each experiment was repeated three times. The influences of the various parameters were assessed by analysis of variance (ANOVA) and Duncan's new multiple range test (DMRT) for mean discrimination or mean comparison, depending on the data. Differences were considered significant at a confidence level superior to 95%. The SPSS statistical program version 16.0 (SPSS Inc., Armonk, NY, USA) was used for the analyses.

Results and discussion

1. Isolation of spoilage bacteria from avocado puree

Spoiled avocado puree was isolated for its microorganisms. The result showed that only one isolate was observed. As shown in Fig. 1, the isolate had yellow, glistening and smooth colonies with regular edge shapes. When observed under light microscope, the isolate was gram-positive bacteria, round shape, nonmotile and nonsporulating. After 16S ribosomal DNA sequencing, the phylogenetic trees of the isolate are shown in Fig. 2. The isolate belonged to the genus of *Staphylococcus* which was identified as *Staphylococcus pasteuri* with the sequence identity of 99%. The bacteria was coagulase-negative which could be found in food products including goat milk, Italian sausage, sea fish and retail beef (Wainwright et al., 2003) or on the surface of drinking water, as well as naturally occurring in the air (Faria et al., 2009). *S. pasteuri* could grow at 15 to 45°C on agar with or without 5 to 15% sodium chloride supplementation. It was able to utilize D-glucose, glycerol, D-fructose and sucrose and produced catalase and urease (Chesneau et al., 1993).



(a) (b)

Fig. 1 Morphology of *Staphylococcus pasteuri* on a nutrient agar plate (a) and under microscope (b), isolated from spoilage avocado puree

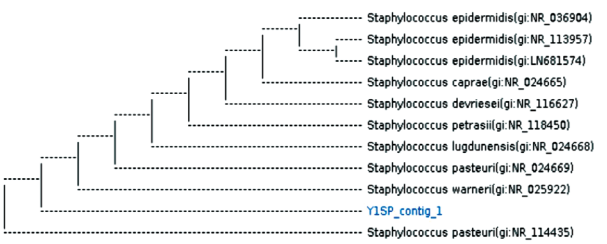


Fig. 2 Phylogenetic tree of bacterial isolate obtained from spoiled avocado puree by 16S rRNA gene sequence method.

2. Antioxidant and antimicrobial activities of Emblica fruit extract

Emblica fruit has been well-known for their rich phenolic compounds and antioxidant activity. In this study, the result showed that the extract had total phenolic content at 167.22 mg GAE/g of emblica fruit extract and its free radical scavenging activity was 37.45%. Sirichai et al. (2022) found that freeze dried emblica fruit extract contained 193.51 mg GAE/g dry weight and had 0.064 µmol Trolox equivalent/g dry weight of radical-scavenging activity investigated by DPPH assay. The presence of quercetin, kaempferol, naringenin were found in the emblica extract, as well as gallic acid which was four-times higher than *Careya arborea Roxb* (Sirichai et al., 2022). Phenolic compounds contributed to the overall antioxidant activities by inactivating lipid free radicals and preventing decomposition of hydroperoxides into free radicals (Sirichai et al., 2022). Alsahli et al. (2021) reported that the ethanolic *P. emblica* fruit extract from Thailand

exhibited strong antioxidant activities. Addition of emblica extract in food products could help enrich vitamin C and increase antioxidant activities of the products (Reddy et al., 2005; Nanasombat et al., 2012: Sharma et al., 2022). However, the activity could be different, depending on the plant variety, extraction methods and drying methods. Besides, phenolic compounds tend to have an important role in antimicrobial activities of plant extracts. *Salmonella* sp. and *Staphylococcus* sp. were inhibited by phenolics and organic acids in cranberry extracts (Puupponen-Pimiä et al., 2005).

As shown in Fig. 3, emblica fruit extract had antimicrobial activity against the spoilage bacteria isolated for avocado puree. The diameters of the clear zone expressed the inhibition activity. They were significantly increased according to the extract concentrations, which was 0.45±0.07 mm, 0.24±0.07 mm, 0.20±0.00 mm, 0.16±0.07 mm and 0.05±0.07 mm, for the emblica fruit extract at concentrations of 500, 400, 300, 200 and 100 ppm, respectively. Gandhi et al. (2020) reported that an ethanolic extract from *P. emblica* showed higher inhibition against gram-positive bacteria (*Staphylococcus aureus*) than gram-negative bacteria (*Escherichia coli*) and fungal (*Candida albicans*). Antimicrobial activity of the emblica fruit extract contributed to Emblicanin A and B, two major tannins which could inactivate enzyme activity and cells envelop transport proteins as well as blocking microbial adhesions (Wang et al., 2017).

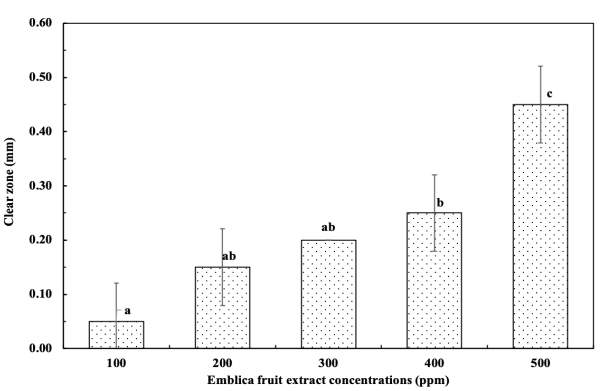


Fig. 3 The clear zone inhibition of emblica fruit extract against *S. pasteuri*
Remark: $^{a\text{-}c}$Mean±S.D. with different letters showing significant difference within the same treatment (p<0.05)

3. Effects of emblica fruit extract on quality of avocado puree during freezing storage

3.1 Color

Emblica fruit extract was added into the avocado puree prior to storage at -20°C for 4 weeks. The extract at the concentration of 300 ppm and 500 ppm were selected to be added into the puree to compare its ability with 300 ppm of ascorbic acid, which was generally used to prevent browning in fruits (Singh et.al, 2017; Singh & Mirza, 2018). Regardless of the extract concentration, after mixing AAP/E300 and AAP/E500 had no significant difference in lightness or L* when compared with AAP/AA but lower than AAP. During storage, L* of all treatments trended to decrease. After 4-week storage, ascorbic acid and the emblica fruit extract could help delay the effects of browning reaction in the puree. The L* of AAP/AA was 39.83±0.06, which was significantly greater than AAP/E300 (39.60±0.05) and AAP/E500 (39.43±0.06) (Fig. 4). In regards to the greenness, as shown in Fig. 5, the greater a* was found in AAP/AA (-1.97±0.02), followed by AAP (-1.55±0.04), AAP/E300 (-1.22±0.01) and AAP/E500 (-0.96±0.06). This could be due to the brown color of the emblica fruit extract, lowering greenness of the puree, while increasing the redness. For the yellowness, AAP/AA showed significantly higher b* than AAP/E300, AAP/E500 and AAP, which were 17.02±0.01, 16.45±0.01, 16.23±0.01 and 16.23±0.06, respectively (Fig. 6). Regarding the color change (ΔE), as shown in Fig. 7, after storage for 4 weeks the puree without an acidulant (AAP) had higher color changes than the addition of acidulants, which was 3.31, 0.92, 0.93 and 2.12 for AAP, AAP/AA, AAP/E300 and AAP/E500, respectively. However, the extract could negatively affect the puree color if its concentration was too high. The more extract was added, the more changes were detected. Ospina et al. (2019) reported that the average human eyes could not distinguished any color when the ΔE was less than 3. The results indicating that ascorbic acid and emblica fruit extract could help maintain avocado color during storage.

Emblica fruit extract contained high levels of phenolic compounds and antioxidants as well as ascorbic acid, citric acid and tannin (Koca et al., 2007). It has been well-documented that ascorbic acid behaved where it could reduce instantly the formed color and acted as quinone reducer (Altunkaya & Gokmen, 2008; Dincer et al., 2002; Neves et al., 2009). Ascorbic acid reduced the conversion of O-quinones to diphenols, leading to the formation of colorless compounds (Ding et al., 2002).

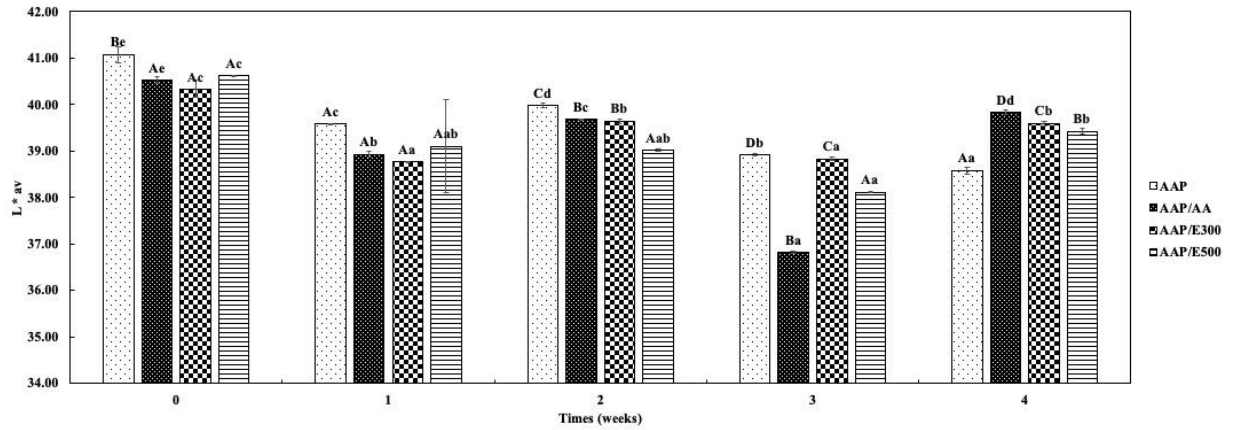


Fig. 4 Average lightness (L*) of avocado puree during storage at -20°C for 4 weeks. (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avoca do puree added with ascorbic acid, AAP/E300 and AAP/500 = alkaline blanched avocado puree added with the emblica fruit extract at the concentration of 300 and 500 ppm, respectively.)

Remark: [a-c] Mean±S.D. with different letters showing significant difference within the same treatment (p<0.05)
[A-D] Mean±S.D. with different letters showing significant difference within the same time (p<0.05)

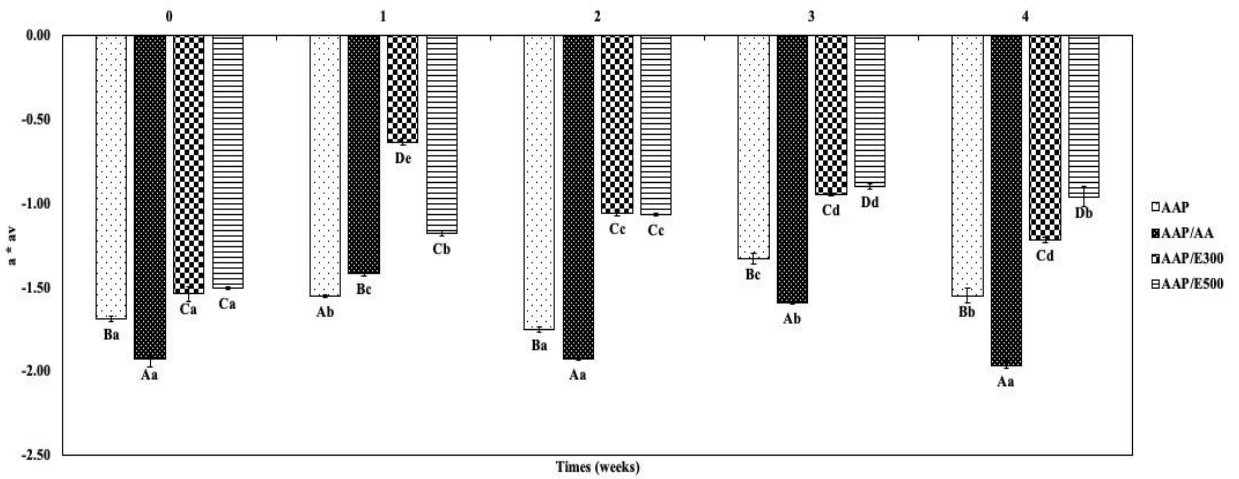


Fig. 5 Average greenness (a*) of avocado puree during storage at -20°C for 4 weeks. (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avo cado puree added with ascorbic acid, AAP/E300 and AAP/500 = alkaline blanched avocado puree added with the Emblica fruit extract at the concentration of 300 and 500 ppm, respectively.)

Remark: [a-c] Mean±S.D. with different letters showing significant difference within the same treatment (p<0.05)
[A-D] Mean±S.D. with different letters showing significant difference within the same time (p<0.05)

However, their effectiveness depends on environmental factors such as pH, water activity, temperature, light and composition of the atmosphere (Lindley, 1998). In addition, citric acid contributed to pH reduction or chelation of the copper at PPO active site, resulting in lowering the PPO activity (Sedaghat & Zahedi, 2012). However, it was found that although emblica fruits contained high antioxidants as vitamin C, they could turn to be brown easily even if they were at low temperature as 25°C or 35°C (Scartezzini et al., 2006). Li et al. (2022) reported that gallic acid, tannic acid, 1,2,3,4,6-penta-O-galloyl-β-D-glucose (β-PGG) and the substrates of *P. emblica* could promote enzymatic and nonenzymatic browning reactions (Li et al., 2022). They also found that the change of PPO activity in *P. emblica* was related to titratable acid during browning. Therefore, appropriate concentration of the emblica extract was needed. The result also indicated that combination of blanching and

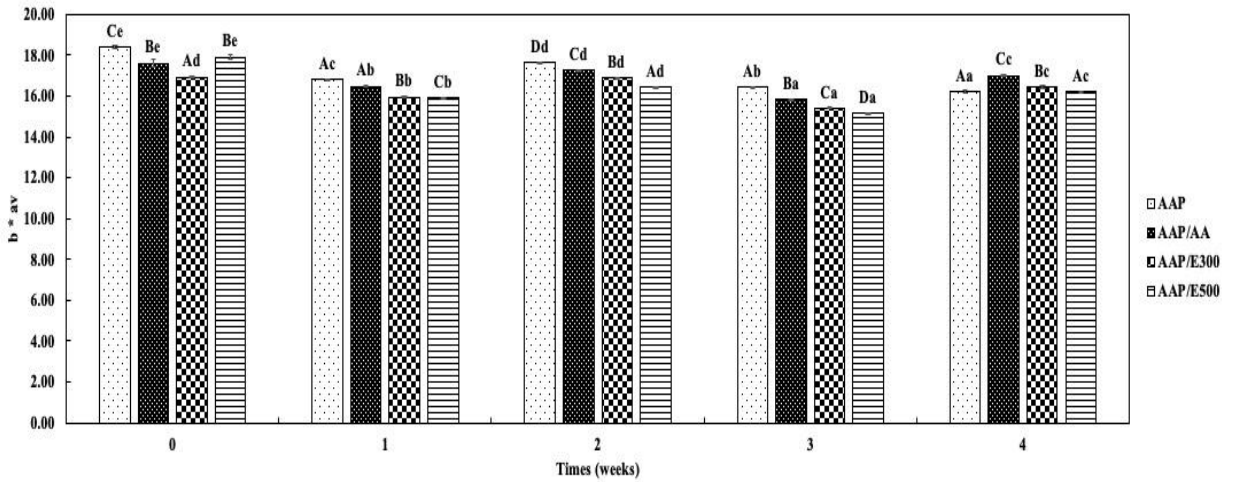


Fig. 6 Average yellowness (b*) of avocado puree during storage at -20°C for 4 weeks. (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched av ocado puree added with ascorbic acid, AAP/E300 and AAP/500 = alkaline blanched avocado puree added with the emblica fruit extract at the concentration of 300 and 500 ppm, respectively.)

Remark: $^{a\text{-}c}$ Mean±S.D. with different letters showing significant difference within the same treatment (p<0.05)
$^{A\text{-}D}$ Mean±S.D. with different letters showing significant difference within the same time (p<0.05)

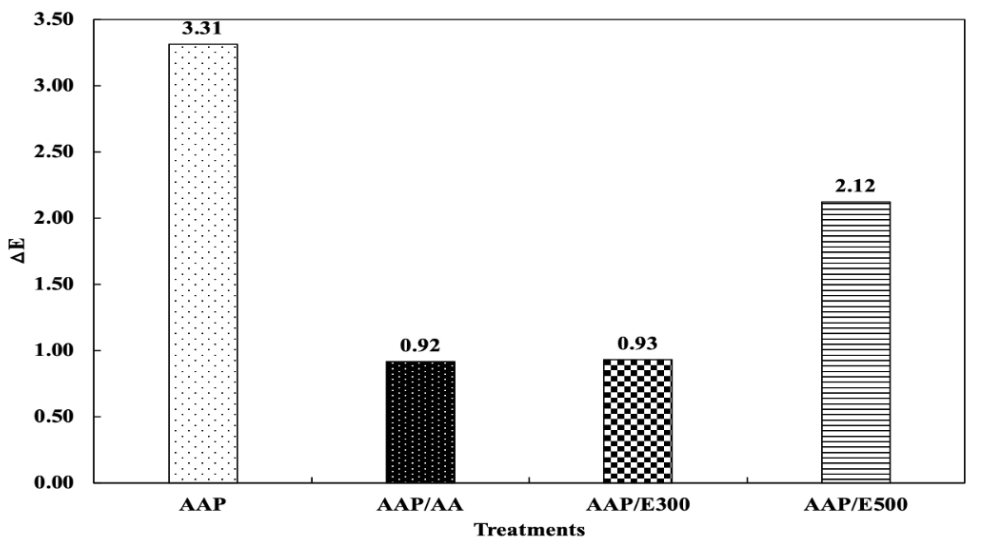


Fig. 7 ΔE of avocado puree during storage at -20°C for 4 weeks (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid; AAP/E300 and AAP/500 = alkaline blanched avocado puree added with the emblica fruit extract at the concentration of 300 and 500 ppm, respectively.)

adding of ascorbic acid or emblica extract helped improve the puree color. Hasan et al. 2017 treated fresh slices of apple with 1% ascorbic acid solution for one min and hot water with 50°C for 2 min. The results showed that both heat and ascorbic acid treatments could significantly reduce cut surface browning. The combination between heating and addition of ascorbic acid showed inhibitory effects on PPOs peroxidase (PODs), which was one of the factors mainly causing enzymatic browning in fresh cut browning. Hot water treatments played a more important role in suppressing both monophenolase and diphenolase activity of PPOs and POD than ascorbic acid.

3.2 pH

The anti-browning agents of both ascorbic acid and emblica fruit extract had an effect on the pH of the avocado puree. It was evident that after adding anti-browning agents, all treatment had a lower pH compared to the control. In regards to the chilled avocado puree, the emblica fruit extract affected the pH of the avocado puree (Fig. 8). APP/E100 showed higher pH compared to other treatments significantly. The pH of all treatments was reduced during storage. After 4 weeks of storage, AAP/E500 was 6.47±0.01, followed by AAP/E300 (6.60±0.02), AAP/AA (6.64±0.02) and AAP (6.75±0.02). Martinez & Whitaker (1995) reported that adding of acidulants such as ascorbic acid, citric acid and acetic acid could control the browning of fruit juices, causing the pH of a system to be lower than 4. Most of the chemical products used to inhibit darkening enzyme has acidifiers in their composition (Mattos et al., 2007). Guerrero-Beltrán et al. (2006) found that the addition of ascorbic acid (500 ppm) to mango puree adjusted pH to 3.5 showing a reduction in the browning rate during storage at 3°C (Guerrero-Beltrán et al., 2006).

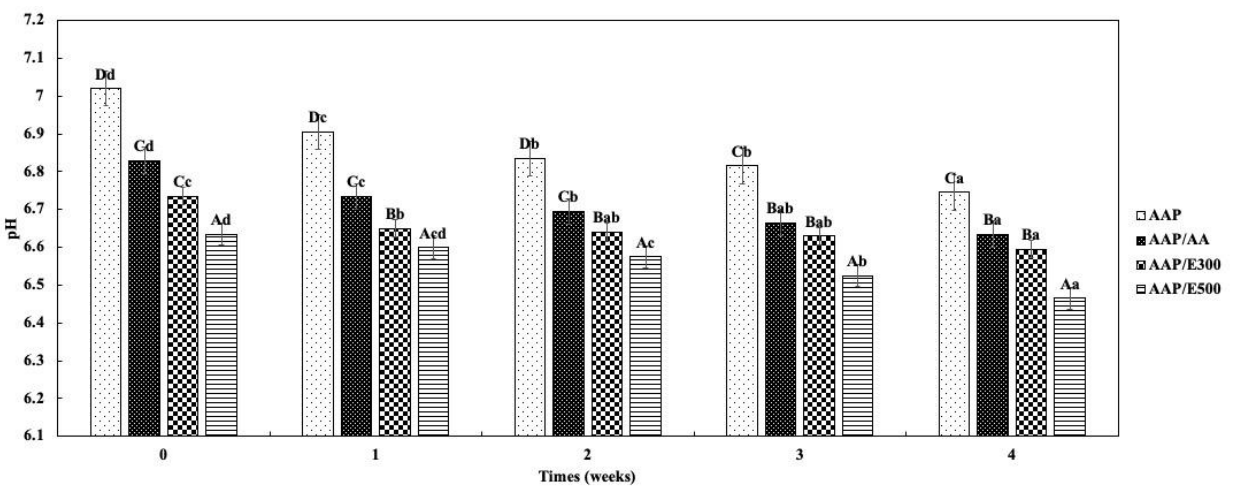


Fig. 8 Changes in the pH of the avocado puree storage at -20°C for 4 weeks (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid, AAP/E300 and AAP/500 = alkaline blanched avocado puree added with the emblica fruit extract at the concentration of 300 and 500 ppm, respectively.)

Remark: [a-d] Mean±S.D. with different letters showing significant difference within the same treatment (p<0.05)
[A-D] MeanS.D. with different letters showing significant difference within the same time (p<0.05)

3.3 Total bacterial counts (TPC)

During storage at -20 °C, less than 1 log CFU/g were found in all AAP treatments at day 0. However, they were detected in FAP, although the avocado was cleaned before processing. For FAP, after processing (at day 0), it was found that FAP had 4.05±0.10 log CFU/g for frozen avocado. After 4-week storage at -20 °C, TPC of FAP was slightly increased, to 4.18±0.01 log CFU/g. These results probably contributed to avocado pretreatment, boiling of the avocado at 85°C for 3 min, which helped destroy some bacteria as well as addition of certain browning agents. Ukuku et al. (2004) demonstrated that immersion of inoculated cantaloupe in hot water at 70°C for 1 min, resulted in up to a 3.8 log CFU/cm^2 reduction in Salmonella. McCann et al. (2006) reported that surface pasteurization with hot water at 76°C for 3 min, resulted in more than 5 log CFU/cm^2 reduction in *Salmonella enterica* and *E. coli.* In addition, the acidulant such as ascorbic acids, citric acid and emblica fruit extract has an acidic pH, making it unsuitable for microbial growth. Ascorbic acid could function as a sanitizer agent inhibiting *C. albicans, S. aureus* and *E. coli* (EliUz, 2020). Emblica fruit extract acted on the cell membrane of microorganisms, resulting in the inability to grow. The minimum inhibitory concentration of emblica fruit extract at 13.97 mg/mL and the minimum biocidal concentration at 13.97 mg/mL could inhibit and destroy *Staphylococcus* aureus, respectively (Mayachiew & Devahastin, 2008).

Conclusion

Avocado puree could be stabilized by blanching and addition of acidulants during storage. Addition of ascorbic acid into avocado puree helped prevent the puree from color changes, while the emblica fruit extract

could cause the puree to become darker, depending on the extract concentrations. The pH of all treatments tended to decrease during storage. Less than 1 log CFU/g of total bacteria plate counts were detected in all samples, while more than 4 log CFU/g was found in the control, fresh avocado, which was unacceptable. This study indicated that stabilizing avocado puree by blanching in alkaline solutions combined with addition of ascorbic acid or emblica fruit extract could help maintain the puree quality during storage at freezing conditions.

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