STABILIZATION OF AVOCADO PUREE BY USING EMBLICA FRUIT EXTRACT DURING CHILLING AND FREEZING STORAGE

PLOYPAILIN SUWANNATE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE
PROGRAM IN MASTER OF SCIENCE (APPLIED BIOLOGY)
FACULTY OF SCIENCE AND TECHNOLOGY
RAJAMANGALA UNIVERSITY OF TECHNOLOGY THANYABURI
ACADEMIC YEAR 2022

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Thesis Title	Stabilization of Avocado Puree by Using Emblica Fruit Extract During Chilling and Freezing Storage
Name-Surname	Miss Ploypailin Suwannate
Major Subject	Applied Biology
Thesis Advisor	Assistant Professor Arranee Chotiko, Ph.D.
Academic Year	2022

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ABSTRACT

Avocados are one of the potential economic fruits, gaining huge global attention. They have high nutritional values and can provide several health benefits. However, they are easily perishable and susceptible during processing and storage. This research aimed to evaluate the effect of pretreatment methods combined with emblica fruit extract and anti-browning agents on the quality of avocado puree during chilling and freezing storage.

The ripe avocado was selected for this study. For pretreatment, the avocados were divided into two groups to be blanched in 1) tap water and 2) alkaline water (pH: 10.6) at 85 °C for 3 min. Fresh and non-blanched avocado was used as a control group. The blanched avocado was then homogenized by a blender for 2 min. During blending, anti-browning agents including ascorbic acid, citric acid, mixture of ascorbic acid and citric acid, and emblica fruit extract were separately added into the puree prior to be stored at 4 °C and -20 °C for 5 days and 7 weeks, respectively. Avocado puree samples were determined for color and pH during storage, while polyphenol-oxidase (PPO) activity and total bacterial plate counts were analyzed after storage.

The results showed that blanching avocado in alkaline water helped delay color changes and browning reaction during storage. Furthermore, PPO was completely inhibited. After storing at 4 °C for 5 days and -20 °C for 7 weeks, the color of avocado blanched in alkaline water showed the highest value of lightness and greenness. Browning reaction in avocado puree could be controlled by adding ascorbic acid combined with alkaline water. Emblica fruit extract caused the puree to become darker during storage; however, its color depended on the extract concentrations. The pH of all treatments tended to decrease, and less than 1 log CFU/g of total bacteria plate counts were detected in all samples except fresh avocado in the control group which had more than 4 log CFU/g. This study indicated that the stabilization of avocado puree by blanching in alkaline solutions combined with the addition of ascorbic acid could maintain the puree quality during storage under both chilling and freezing conditions.

Keyword: Avocado, Browning reaction, Alkali water, Emblica fruit, Acidulants

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Ploypailin Suwannate

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CHAPTER 1
INTRODUCTION

1.1 Important and background of thesis

Avocados are a member of the family Lauraceae, called in scientific name as *Persea americana Mill.*. They are one of the potential economic fruit, gaining huge global attention. In Thailand, several varieties of avocados are grown in the North and the Northeast spreading the fruit throughout the year. Department of Agricultural Extension reported that in 2018 fresh avocados were imported 762 tons/year, costing 154,399,344 Baht, while 800-1,200 tons of avocado were produced in Thailand, however they could not meet the requirement or standard of the traders and customers. This could be probably because the avocado has been grown and reproduced in Thailand for more than 40 years and their characteristics have been changed from the avocado breeder, causing avocados from local farmers to not meet the market requirement, have a lower price and be rejected.

Avocados are considered as a healthy fruit, which are beneficial to human health. They have relatively high in good fat (8-30 %), which is mostly unsaturated (85-87% of total fat), and contain high levels of vitamin E and A. This causes avocados to be popular in health-conscious consumers. However, they are one of the climacteric fruits, having a high respiratory rate, resulting in short shelf-life due to physicochemical changes and microorganism spoilages [1]. They can only be preserved for 4-5 days after ripening. Sometimes avocado producing rates have been exceeding the customer demand, causing economic losses to the farmer. Therefore, it is necessary to search for a processing method or technology that could develop a new product from avocados in order to help the local farmers from the mentioned problem, while the technologies need to be simple and safe.

Avocado puree could be one of the avocado products that can be developed with a simple technology. However, they are very sensitive to oxygen, which could cause browning reaction during processing [2]. This directly affects the sensory perception and consumer acceptance. There are many ways to control and inhibit the browning reaction by halting the activity of polyphenol oxidase (PPO), a major factor causing browning reaction, via heating

or using browning inhibitors [3]. The heat causes PPO denature until it cannot catalyze [4]. Alternatively, PPO could be inhibited when the pH is lower than 4. These inhibitors could be ascorbic acid and citric acid [5]. However, inappropriate amount of the browning inhibitor used may result in the avocado puree having an unusual color, odor, and taste [6] that is not acceptable to consumers. Emblica extract could be used to inhibit the browning reaction and prolong the puree shelf-life. Emblica contains very high vitamin and tannin, providing antioxidant and antimicrobial activities, respectively [7]. Several studies were also suggested that emblica extract had enzyme-inhibiting properties [8]. The application of embica extract may help to not only preserve the avocado puree from browning reaction but also protect the puree from microbial spoilage.

Therefore, this research is aimed to investigate the effects of combination effects of heating and browning inhibitors including embilca extract that could stabilize the properties of avocado puree during storage in chilling and freezing state. The study could contribute to the avocado farmers and the food industry to develop a new stable avocado product.

1.2 Purpose of the Study

Overall goal of this research proposal is to develop stable avocado puree during chilling and freezing storage. This research is consist of three internal studies. The objectives of each study are following:

1.2.1 To study the effect of blanching combining pH adjustment as pretreatment method on the inhibition of PPO activity and their physicochemical changes of avocado puree.

1.2.2 To determine the effects of emblica extract on inhibiting browning reaction and antimicrobial activity of avocado puree.

1.2.3 To determine the stability of avocado puree during freezing storage.

CHAPTER 2
LITERATURE REVIEWS

2.1 Avocado

Avocado is native from central America and Mexico, where it has been a staple dietary component for at least 9000 years [9]. Avocados are possible to differentiate three different ecological races: Mexican, Guatemalan, and West Indian (or Antillean). Each race presents typical characteristics in terms of leaves, fruits, flowering period, etc., which have been summarized in Table 1 [10]. There are no sterility barriers among the three races or among any taxonomic category classified under *P. americana*. Hence, hybridization readily occurs wherever trees of different races are growing in proximity. For this reason, most commercial avocado cultivars are interracial hybrids, developed from chance seedings, with different degrees of hybridization, showing different characteristics such as size, shape and color [11].

2.1.1 Botanical description

Avocado tree is a dense polymorphic broad-leaved aromatic evergreen tree species of the genus Persea, classified in the division Magnoliophyta, class Magnoliopsida, order Magnoliales of the flowering plant family Lauraceae (Myrtle). Camphor (*C. camphora*), cinnamon. It is fast growing plant and reaching a height of 20 m with age whilst grafted trees are usually 8-10 m tall [12]. The leaves are alternate, glossy, elliptic to obovate-oblong, 10–30 cm in length, 4–10 cm in width, leathery, upper surface dark green, lower surface glaucous and sparsely hairy; secondary veins prominent, reticulum coarsely areolate; petiole 2–7 cm in length [12]. The trees produce an abundance of flowers, usually less than 0.1% of the flowers set fruit and most of these fruits abscise within 6 weeks from full bloom [13]. Avocado flowers are inconspicuous and appear in terminal panicles of 200–300 small yellow-green blooms. Each panicle will produce only one to three fruits. Calyx is not persistent in fruit. The flowers are 6–7 mm in length [12]. plants normally produce fruit within one to two years compared to 8–20 years for seedlings. Fruits are 7-20 cm long and 7-10 cm in diameter, weighs between 100 and 1000 grams, and has a large central seed 5–

6.4 cm in length. The avocado comes in a variety of shapes, some of which are depicted in Figure 1. Avocado fruit is consists of a large central seed and pericarp, which is the sum of the skin (exocarp), the edible portion (mesocarp) and the inner layer surrounding the seed (endocarp) as shown in Figure 2 [14]. Avocado fruit development is the result of complex metabolic activities that can be affected by environmental factors such as temperature, plant water and nutrient status, light quality, and quantity, etc. [15].

Table 1 Comparison of the three different horticultural races of avocado fruit

Traits	Race		
	Guatemalan (G)	Mexican (M)	West Indian (WI)
Climate	Subtropical	Semitropical	Tropical
Cold tolerance	Intermediate	Most	Least
Salinity tolerance	Intermediate	Least	Most
Leaf anise	Absent	Present	Absent
Young leaf color	Green with red tinge	Green	Pale yellow
Mature leaf color	Dark green	Dark green	Pale green
Blooming season	March-April	January-February	February-March
Size	Small to large	Tiny to medium	Medium to very large
Shape	Mostly round	Mostly elongate	Variable
Color	Green	Often dark	Green or reddish
Skin thickness	Thick	Very thin	Medium
Skin surface	Rough	Waxy bloom	Shiny
Skin peelability	Rigid	Membranous	Leathery
Seed size	Small	Large	Variable
Seed cavity	Tight	Loose	Variable
Seed surface	Smooth	Smooth	Rough
Oil content	High	Highest	Low
Pulp flavor	Rich	Anise-like, rich	Sweeter, milder

SOURCE: [16]

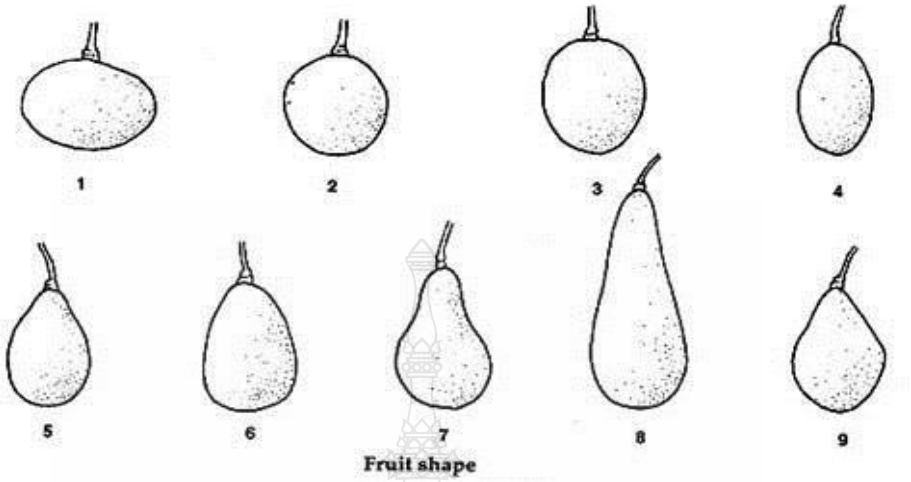


Figure 1 Fruit shape of different avocado varieties (1. oblate, 2. spheriod, 3. high spheroid, 4. ellipsoid, 5. narrowly obovate, 6. obovate, 7. pyriform, 8. clavate, 9. rhomboidal)

SOURCE: [17]

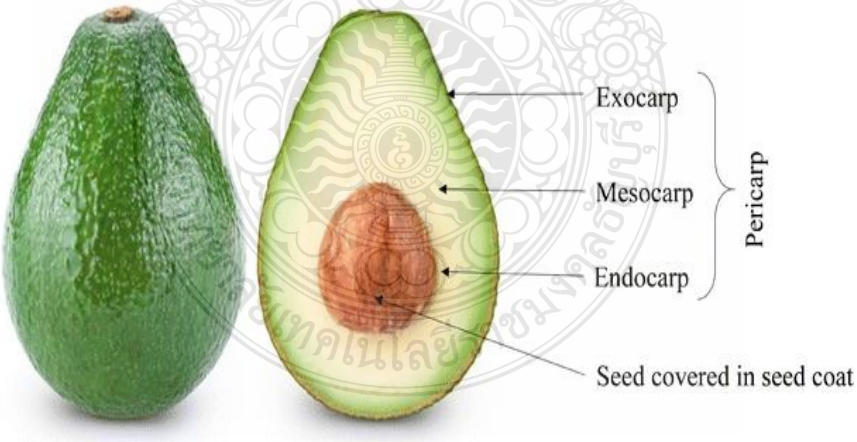


Figure 2 The different parts of the avocado fruit.

SOURCE: [18]

2.1.2 Ripening of avocado

Ripening is the process by which fruits attain their desirable color, flavor, palatable nature, and other textural properties that make the fruit acceptable for consumption. It is associated with change in biochemical compositions, such as conversion of starch to sugar. Avocados, as a climacteric fruit, continue to ripen after harvest, which is associated with increased ethylene production and a rise in cellular respiration [19]. However, a prerequisite is that the avocado is mature when it is harvested, in order to ripe properly after it comes off the tree. This would affect flavors and colors of the avocado when they are used as food ingredients.

The changes in the appearance of an avocado as it ripens vary depending on the cultivar. For example, the Hass cultivar has the color of the change from green to purple (Figure 3), or Boost species that when ripe, the color of the peel will have a darker color. Moreover, another important measure of ripeness is firmness [20]. Firmness and oil content is used as an indicator of fruit maturity and, thus, commonly defines the optimum harvest period. Lipids accumulate during avocado fruit development and constitute ca. 70% of dry matter at maturity. The composition of fatty acids of avocado oil has been shown to remain relatively unchanged during postharvest ripening. The increased oil concentration reported during storage and ripening were related to postharvest dehydration and the increased oil concentration during ripening was attributed to increased lipid recovery due to partial cell wall breakdown [21].

Figure 3 Stage of avocado ripening

SOURCE: [22]

2.1.3 Chemical compositions and nutritional values

The first information about the nutritional composition of avocado fruits dates back to 1922. Data about the macro and micronutrients found in avocado have been compiled in different food composition tables, although the nutrient content of the edible portion of the fruit (pulp or mesocarp) highly varies depending on factors such as variety, ripening degree and cultivation conditions [16]. Avocados have very complex matrix formed by a wide variety of compounds. They are greatly appreciated for being an excellent source of energy, fatty acids, and vitamins. Table 2 shows the nutritional composition of 100 g of avocado fruit according to the USDA National Nutrient Database for Standard Reference [23].

One of the main components of avocado is the fat. In general, the oil content of avocado increases with the ripening process [24]. Monounsaturated fatty acids are the predominant ones; oleic acid stands out within this group as one of the most characteristic. Other important fatty acids of avocado fruit, although less abundant, are linoleic (polyunsaturated) and palmitic (saturated) acids, while the avocado protein level is higher than the protein concentrations of other fruits, reaching values about 2%, whereas most of the fruits present a protein content of around 1% [10]. Avocado is also a very important source of vitamins (especially vitamins E and C), pigments (anthocyanins, chlorophylls, and carotenoids) [25], sterols [26], phenolic compounds [27], and seven-carbon sugars and its related alcohols (D-mannoheptulose and perseitol) [28].

Table 2 Nutritional content of 100 g of avocado fruit

Ingredients	Contents
Energy	160 kcal
Water	73.23 g
Protein	2.00 g
Total lipids	14.66 g
Carbohydrates	8.53 g
Total dietary fiber	6.70 g

Table 2 Nutritional content of 100 g of avocado fruit (Cont.)

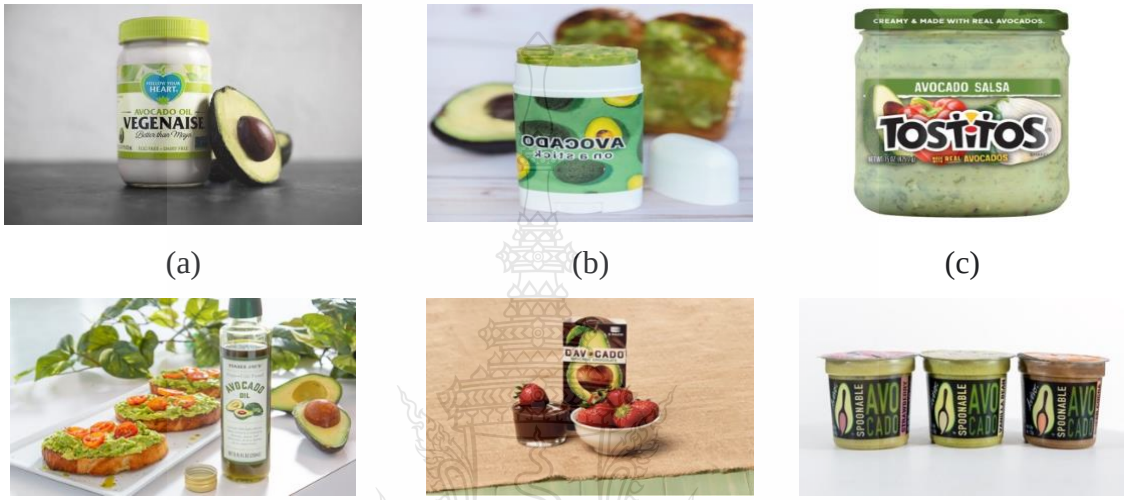
Ingredients	Contents
Sugars	0.66 g
Saturated fatty acid	2.13 g
Monounsaturated fatty acids	9.80 g
Unsaturated fatty acids	1.82 g
Ascorbic acid, Vitamin C	10.00 mg
Thiamine, Vitamin B_1	0.07 mg
Riboflavin, Vitamin B_2	0.13 mg
Niacin, Vitamin B_3	1.74 mg
Pyridoxine, Vitamin B_6	0.26 mg
Folate, DFE	89 μg
Vitamin A, RAE	7 μg
Vitamin E, α-tocopherol	2.07 mg
Vitamin K, phylloquinone	21 μg
Calcium, Ca	12 mg
Iron, Fe	0.55 mg
Magnesium, Mg	29 mg
Phosphorus, P	52 mg
Potassium, K	485 mg
Sodium, Na	7 mg
Zinc, Zn	0.64 mg

SOURCE: [16]

2.1.4 Applications of Avocado in food products

Avocado-based products are gaining traction across the world and being used in most food and beverage products. Avocado is presumed to be a superfood that contains numerous health and nutritional benefits which has further established the proper market set up for avocado-based products such as avocado puree, avocado oil, and others.

They are increasingly becoming favourable among the food and beverage manufacturers [1]. The avocado-based products are utilized in infant foods, snacks, dairy products, nutraceuticals and cosmetics [29]. Examples of products containing avocados are shown in Figure 4.



(a) (b) (c)

(d) (e) (f)

Figure 4 Avocado products (a)=Mayonnaise, (b)=spreadable avocado sticks, (c)=creamy avocado salsas, (d)=cold pressed avocado oils, (e)=chocolate avocado pudding, (f)=yogurt

SOURCE: [30]

Avocado puree is one of the popular avocado products that has been proceeded as an ingredient base for several products such as ice-cream, guacamoles, and salsa. The general process of producing avocado puree is by taking fresh avocados, peeling them, removing the seeds, and bringing it to the grind. However, during processing, the color of the avocado puree was typically changed to become darker, which resulted in consumer unacceptability. Therefore, pretreatment processes of avocado puree production such as heating, adding of anti-browning agents, or their combination have been developed. Heat treatment could destroy microorganisms and inactivate enzymes [31]. Blanching is used to inactivate polyphenol oxidase (PPO), an enzyme causing browning reaction in avocado. To destroy it's

catalytic activity, avocado is typically heated at 70–90°C, but the time required to inactivation depends on the product. Chutintrasri and Noomhorm. [32] revealed that the enzyme activity of PPO was reduced approximately 60% after exposure to 40–60°C for 30 min. In addition, ascorbic acid, sodium sulfite and/or citric acid have been mixed with the avocado during processing to prevent the puree from color changes. Gómez-López. [33] found that citric acid (1%) and ascorbic acid (1%) showed excellent anti-browning activity in avocado halves during storage at 7 °C. Its effectiveness was due solemnly to the low pH. Besides, Ospina *et al*. [34] aimed to produce a minimally processed avocado puree that retain the color during 6 months of frozen storage by combining microwave heating with anti-browning agents. Mashed avocado pulp mixed with lemon juice was treated with citric acid or exposed to a microwave treatment prior to storage in frozen stage for 30 days. It was showed that all samples were preserved for their original color, while microwave heating could completely inhibited polyphenol oxidase.

2.2 Indian gooseberry

Phyllanthus emblica L. (Synonym: *Emblica officinalis*) is a medium sized deciduous tree belonging to the family Euphorbiaceae, commonly known as Indian gooseberry, emblic myrobalans, and Amla (in Hindi). The plant species are native to India, also growing in Sri Lanka, Uzbekistan, South East Asia, and China nowadays [35]. It is a dietary globular fruit, yellowish-green in color with obtusely triangular six-celled nut Figure 5, which is used in various folk as Indian traditional medicinal systems [36].

Figure 5 Indian gooseberry
SOURCE: [37]

2.2.1 Botanical description

P. emblica L is a monoecious, small to medium-sized tree up to 15-25 m tall with phyllanthoid branching. The bole often crooked and gnarled, up to 35 cm in diameter, bark thin, grey, smooth, and flaking. The cataphylls are inconspicuous and scarious. Their stipules are triangular-ovate [38]. It has deciduous branchlets that are 10-25 cm long, with 30-100 leaves. The leaves are subsessile, narrowly oblong, measures of 12-20 mm x 2-5 mm, slightly oblique and subcordate at base. The margin and tip are almost inflexed, stipules triangular; proximal axils of deciduous branchlets with reduced leaves and cymules of male flowers followed by cymules of 1-2 female flowers surrounded by several male ones, distal part sterile or rarely reduced [38]. The flowers are pale green, with 6 calyx-lobes; male flowers with 6 disk segments, stamens 3, filaments entirely connate, anthers free, minutely apiculate, dehiscing vertically; female flowers subsessile, with cup-shaped, 6-ribbed disk enclosing the ovary, styles shortly connate, mostly twice bifid [38]. The fruit is drupaceous, tardily dehiscent, depressed globose, in wild plants 13-25 mm x 23-30 mm, in cultivated ones up to 42 mm in diameter, pale green becoming yellowish-white, smooth and the seeds are smooth [38].

2.2.2 Chemical compositions and their functions

The fruit of emblica is rich in vitamin C (ascorbic acid) and contains several bioactive phytochemicals, of which majority are of polyphenols (ellagic acid, chebulinic acid, gallic acid, chebulagic acid, apeigenin, quercetin, corilagin, leutolin, etc.) as described by various researchers [39]–[41]. Sugar-substituted phenolics such as flavone glycosides, phenolic glycosides, and flavonol glycosides [40], as well as tannins, such as emblicanin A, emblicanin B, phyllaemblicin B, and punigluconoin, are reported in fruit's pulp [41]. The fresh fruit pulp has been analyzed, roughly including 81-84% of moisture, 14.0-14.3% of carbohydrates, 3.4% of fiber, 0.5-0.7% of protein, and 0.1-0.5% of fat. Emblica has been well known for their antioxidant activity due to high level of vitamin C and polyphenols. Vitamin C functions as radical scavengers, providing one or two electrons to attach with the radicals resulting in a stable mono-oxidized form, ascorbyl radical. Subsequently, the ascorbyl radical converts to dehydroascorbate (DHA), which is a fully oxidized form.

Ascorbyl radical and DHA are a less active free radical. They are harmless to cells or tissues due to the resonance hybrid structure, making the electrons more stable [42] *P. emblica* extracts were evaluated for their antioxidant capacity by DPPH and ATBS assays. It was found that the extract could inhibit DPPH and ABTS free radicals in a dose-dependent manner. The extract showed the most potent ability to scavenge DPPH and ABTS free radicals, which was comparable with ascorbic acid. All 50% inhibition concentrations (IC_{50}) against ABTS were higher than that of DPPH [43].

P . emblica is also often used as traditional botanical drug or functional food due to its multiple biological effects, including anti-inflammatory and antimicrobial activity [44]. Gandhi *et al.*[45] evaluated the anti-microbial activity of the extracts against *Staphylococcus aureus*, *Escherichia. coli*, and *Candida albicans*. The result showed that the extracts could exhibit potent antibacterial and antifungal activities against all the selected bacterial and fungal species. The extracts exhibited the growth inhibitory activity in a dose-dependent manner. Two extracts of emblica (*Phyllanthus emblica* L.) obtained by supercritical fluid extraction (SFE) and methanol extraction were evaluated for their antimicrobial activities against gram-negative bacteria including *E. coli* GIM 1.42, *Pseudomonas aeruginosa* GIM 1.46 and *Salmonella typhi* GIM 1.237, as well as the gram-positive bacteria namely *S. aureus* GIM 1.55, *Bacillus subtilis* GIM 1.136, *B. cereus* GIM 1.3, and three fungi, *C. albicans* GIM 2.169, *C. tropicalis* GIM 2.147 and *Aspergillus niger* GIM 3.24. It was found that SFE extract showed strong antimicrobial activity against all microorganisms tested, whereas the methanolic extract showed low activity against *Staphylococcus aureus* and fungi [46]. The inhibition mechanism of gram-positive and gram-negative bacteria could be because of a process of inhibiting enzyme activity and causing cell membrane dysfunction [47].

2.3 Enzymatic browning reaction

Browning reaction could be widely observed in many fruits and vegetables, as well as some seafood products. It is initiated by the enzymatic oxidation of phenolic compounds, resulting in the formation of brown colored substances. Polyphenol oxidase (PPO) is a group

of copper proteins that catalyzes the oxidation of phenolics to quinones, which produce a series of brown pigments [48]. These reactions are thought to be the major cause of the enzymatic browning of many fruits and vegetables during handling, stor- age, and processing [49]. Avocado flesh is usually turned to brown rapidly when it exposes to the air. In the presence of oxygen, PPO aids the conversion of phenolic compounds to another class of compounds, quinones. Quinones are capable of polymerising, taking the smaller molecules and joining them together to form a long chain, to produce polymers called polyphenols.

This polymerisation manifests itself as a brown colouration to the flesh. The browning doesn't happen in the intact avocado, not only because the flesh isn't exposed to oxygen, but because the phenolic compounds are stored in the vacuole of the plant cells, whilst the enzymes are found in the surrounding cytoplasm. So, both damage to these cell structures and exposure to oxygen is required for browning to occur [49].

PPO, a Cu-containing enzyme, is also referred to as catechol oxidase, tyrosinase, phenolase, catecholase, diphenol oxidase, or o-diphenolase. PPO is found in almost all higher plants, including fruits and vegetables PPO is synthesized as preproteins and contains putative plastid transit peptides at the N-terminal region, which target the enzyme into chloroplast and thylakoid lumen in plants [50]. In addition to its existence in higher plants, PPO is also found in some seafood products, such as shrimp and lobster. Its role in browning reactions causes deterioration in food quality by changing the nutritional and organoleptic properties and hence consumer acceptability [51]. The action mechanism of PPO is based on its capacity to oxidize phenolic compounds. When the plant tissue is damaged, resulting in the rupture of plastids, the cellular compartment where PPO is located leads to the enzyme contact with the phenolic compounds released by rupture of the vacuole, the major storage organelle of these compounds [50]. It is considered that the active site of PPO contains two copper atoms and the enzyme catalyzes two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity) Figure 6. This reaction is generally followed by nonenzymatic polymerization of the quinones giving rise to the formation of melanins, pigments of high molecular mass, and dark color [52]. It was reviewed that the sequence of

biochemical reactions leading to the formation of melanin from the oxidation of phenolic amino acid tyrosine [53]. The rate of enzymatic browning of fruit and vegetables depends largely on specific activity of PPO and concentrations of phenolic compounds, the pH, and temperature [49].

The optimum pH of PPO activity varies with enzyme source and with the substrate over a relatively wide range. In most cases, the optimum pH range of PPO is between pH 4 and 7. The adjustment of pH with acids to 4 or below can be used to control browning as long as the acidity can be tolerated taste wise. The temperature stability of PPO varies with species and with cultivars. The enzyme is relatively heat labile and activity is completely destroyed at 80 °C [54]. Heat inactivation of PPO is feasible by applying temperatures of more than 50 °C but may produce undesirable colors and/or flavors as well as undesirable changes in texture.

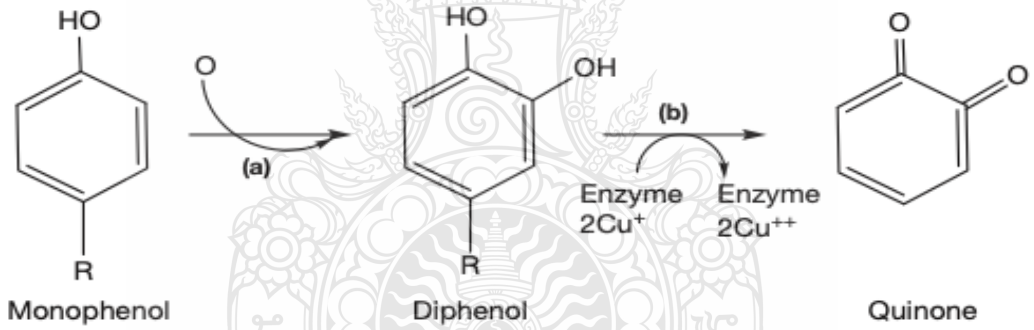


Figure 6 Browning reaction of enzymes (PPO)
SOURCE: [49]

Besides, the relative significance of PPO activity is obscured somewhat by the presence of peroxidase (POD), a similar oxidative enzyme. It is relatively difficult to ascribe a significant role to POD in enzymatic browning when one of its substrates, hydrogen peroxide (H_2O_2), is generally present at very low concentrations in plant cells. Recent evidence collected indi- cates clearly that POD could enhance browning reactions in the presence of ongoing PPO-mediated browning reactions [55]. While the mechanism of this

PPO-coupled browning is not well understood, it is possible that the PPO-mediated generation of quinones can lead to H_2O_2 accumulation, providing a higher concentration of this free radical species, thus enabling the occurrence of the POD-mediated oxidation of polyphenols [56]. The profile and concentration of polyphenol substrates in plant tissues will also influence the potential for the POD-mediated polyphenol browning. Furthermore, the POD related browning can be distinguished by the addition of an H_2O_2 quenching agent such as catalase, which will prevent browning caused by the POD-mediated reactions [57].

As mentioned earlier, PPO catalyzes the oxidation of phenols into 0-quinones. The 0-quinones are highly reactive compounds, which can polymerize spontaneously to form high-molecular-weight compounds or brown pigments (melanins), or react with amino acids and proteins that enhance the brown color produced. Preventing enzymatic browning could be accomplished in many ways. The methodology is to eliminate from the reaction one or more of its essential components: oxygen, enzyme, copper, or substrate. Chemical compounds have also been used to prevent PPO action. Various techniques and mechanisms have been developed over the year to prevent PPO action in food products [58]. Extensive research has focused on control of browning and several approaches to browning inhibition have been explored. Inhibitors of enzymatic browning can be divided into six groups according to their different modes of action, including reducing agents, chelating agents, complexing agents, acidulants, enzyme inhibitors, and enzyme treatments [59].

1) Reducing agents/Antioxidants

Reducing agents play a role in the prevention of enzymatic browning either by reducing o-quinones to colorless diphenols, or by reacting irreversibly with o-quinones to form stable colorless products . Reducing compounds are very effective in the control of browning. Sulfiting agents are the most widely applied reagents for the control of browning in the food industry. Sulfites are the most widely used inhibitors of enzymatic browning. Sulfiting agents include sulfur dioxide (SO) and several forms of inorganic sulfite that liberate SO_2 under the conditions of their use [60]. SO_2 and sulfite salts form sulphurous acid (H_2SO_3) and exist as a mixture of the ionic species, bisulfite (HSO_3) and sulfite (SO_3^{2-}) anions

in aqueous solution. The predominant ionic species varies in accordance with pH, ionic environment, water activity, presence of non-electrolytes, and concentration of the medium in which they are dissolved. Increased concentrations of sulfite at pHs of less than 5 were observed to enhance the inhibition of PPO-catalyzed browning [61]. Sulfites serve a multifunctional role in foods. They possess antimicrobial activity and inhibit both enzymatic and non-enzymatic browning reactions. [62] proposed that bisulfite exerted a competitive inhibitory effect on PPO by binding a sulfhydryl group at the active site of the enzyme. On the other hand [63] proposed that PPO inhibition was due to the reaction of sulfites with intermediate quinones, resulting in the formation of sulfoquinones, which irreversibly inhibited PPO, causing complete inactivation. Although sulfites are very effective in controlling browning, they are subject to regulatory restrictions owing to their potentially adverse effects on health. Many reports have described allergic reactions in humans, following the ingestion of sulfite-treated foods by hypersensitive asthmatics. The use of sulfiting agents in food processing is based on sulfur dioxide equivalence [64]. The Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommend an acceptable sulfite daily intake of 0.0-0.7 mg sulfur- dioxide per kg of body.

2) Chelators

Enzymes generally possess metal ions at their active sites. Removal of these ions by chelating agents can therefore render enzymes inactive. Chelating agents complex with prooxidative agents, such as copper and iron ions, through an unshared pair of electrons in their molecular structures [65]. Chelators used in the food industry include sorbic acid, polycarboxylic acids (citric, malic, tartaric, oxalic, and succinic acids), polyphosphates (ATPand pyrophosphates), macromolecules (porphyrins, proteins), and EDTA. Other non-GRAS chelating agents which are capable of inhibiting PPO include cyanide, diethyldithiocarbonate, sodium azide and 2-mercaptobenzothiazole, carbon monoxide, mercaptobenzthiazol, dimercaptopropanol, and potassium methyl xanthate . EDTA is a chelating agent permitted for use in the food industry as a chemical preservative. Calcium disodium EDTA (21 CFR 172.120) and disodium EDTA (21 CFR 172.135) have been

approved for use as food additives by the United States Food and Drug Administration [66]. Highly stable complexes are formed by the sequestering action of EDTA compounds on iron, copper, and calcium. Maximum chelating efficiency occurs at the higher pH values where carboxyl groups exist in a dissociated state [67]. EDTA is generally used in combination with other chemical treatments for the prevention of enzymatic browning in foods.

3) Complexing agents

Complexing agents are capable of entrapping or forming complexes with PPO substrates or reaction products. Results vary with specific cyclodextrin and more complex mixtures of phenolics. Common complexing agents normally used in the food industry are Cyclodextrins sugar molecules in a ring formation, and cyclic non-reducing oligosaccharides including cyclodextrins and chitosan, where Cyclodextrins binds to L-tyrosine prevents the formation of melanin-induced black spotting and chitosan is a large positive ion substance able to bind to PPO, thus causing PPO to lose efficiency [68].

4) Acidulants

Ionizable groups of the protein structure of enzymes are affected by the pH of the food medium. These groups must be in the appropriate ionic form in order to maintain the conformation of the active site, bind substrates, or catalyze the enzymatic reaction [69]. Changes in the ionization status of enzymes are generally reversible. Irreversible denaturation can however occur under conditions of extreme pH. The stability of the substrate is also affected by changes in pH, since substrates can undergo chemical breakdown under extreme conditions of pH. Degraded substrates often behave as enzyme inhibitors, since they share the molecular features of the substrate [70]. Acidulants are generally applied in order to maintain the pH well below that required for optimum catalytic activity of an enzyme. Acidulants such as citric, malic, and phosphoric acids are capable of lowering the pH of a system, thus inactivating PPO [71]. Acidulants are often used in combination with other antibrowning agents. Citric acid exerts its inhibitory effect on PPO by lowering the pH as well as by chelating the copper at the active site of the enzyme [71]. The use of acidulants is another approach widely used in food processing to reduce enzymatic browning. Acids naturally present in some edible food products, such as ascorbic acid, citric acid, and malic

acid, shift the food pH to 3 or lower. In general, PPO present in fruits and vegetables is more active in the pH range of 4.0–8.0 and thus, the enzyme activity drastically decreases at a higher acid environment. Acidity may reduce the strong binding of the enzyme to its active site of copper. In fact, acidulants only partially prevent enzymatic browning of fresh fruits and vegetables compared with bisulfite; in combination with edible coatings, such as chitosan, enhanced inhibition of enzymatic browning may result [49].

5) Enzyme inhibitors

Enzyme inhibitorsare molecules that interact with enzymes (temporary or permanent) in some way and reduce the rate of an enzyme-catalyzed reaction or prevent enzymes to work in a normal manner. The important types of inhibitors are competitive, noncompetitive, and uncompetitive inhibitors. Besides these inhibitor types, a mixed inhibition exists as well. Competitive enzyme inhibitors possess a similar shape to that of the substrate molecule and compete with the substrate for the active site of the enzyme. This prevents the formation of enzyme-substrate complexes. One of inhibitors with the most potential is 4-hexyl resorcinol. 4-hexylresorcinol (4-HR) is a substance that structure is similar to L-tyrosine, causing it to compete with L-tyrosine in the process of melanin black spot formation in humans, 4-HR is used in medicine and cosmetics to reduce melanin that causes dull skin later, there was an experiment used in shrimp, effective in norway lobster [72], white shrimp, and pink shrimp [73] substitute 4-HR substance. The sulphite region has received widespread attention because 4-HR is a safe, highly stable substance. Decomposes to create toxic fumes, does not bleach and does not adversely affect the color. and the sensory taste of shrimp [74]. In addition, 4-HR was highly effective even will use a small amount. The immersion method uses only a hundred concentrations only 0.25 each, but the disadvantage of 4-HR is that it has a price up to 2,500 baht/ kg.

6) Enzymatic treatments

Enzymatic treatment involves the use of lytic enzymes that attack and degrade the cell wall to allow release of proteins [49]. The use of enzymes to degrade cell wall polysaccharides is considered a gentler alternative method to alkaline or acidic approaches

to protein solubilization and extraction. This is in contrast to most chemical-based protocols that often result in protein denaturation [75]. Enzymatic treatment is usually done prior to conventional solvent extraction as a pretreatment normally uses hydrolytic enzymes in plant extraction such as pectinase and cellulase. These enzymes are used to break down the structure of the cell wall for allowing efficient extraction and the release of bioactive compounds [76].

2.4 Related research

Ospina *et al*. [34] investigated avocado puree color changes during storage. The frozen avocado puree was prepared by mixing 1% lime juice and 0.5% citric acid. The avocado was then heated in microwave for 80 s prior to storage at -10 °C, -14 °C, and -18 °C for 6 months. The activity of PPO and POD and color changes were analyzed. The results showed that PPO and POD activity were inhibited from the preparation of both trials for 98.9% and 94.46%, respectively. The color effect was found in all samples with $\Delta E \leq 3$, which was slightly noticeable to the naked eye.

Koca *et al*. [77] determined effects of pH on the chlorophyll degradation and visual green color loss in blanched green peas were studied at 70, 80, 90 and 100 °C in buffered solutions of pH 5.5, 6.5 and 7.5. It was found that when the pH was lowered, the decomposition of chlorophyll occurred faster. From the experiment, pH 5.5 combined with 100 °C showed the highest chlorophyll degradation, equal to 0.0053 K/min.

Gandhi *et al.* [45] evaluated the anti-microbial activity of *Phyllanthus emblica* extracts against Gram- positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*E. coli*), and Fungal (*Candida albicans*). The agar well diffusion method at concentration level of 25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml was conducted. It was found that *P. emblica* extracts exhibited potent antibacterial and antifungal against all the selected bacterial and fungal species. The extracts exhibited the growth inhibitory activity in a dose-dependent manner. The study reveals *P. emblica* shows good antimicrobial activity.

Gbouri and Hamzah [78] studied antibacterial effects of *Phyllanthus emblica* extract, prepared by solvent extraction (ethanol:methanol was 1:1), *against P. aeruginosa, S. aureus* and *E. coli* at different concentration started with 20, 10, 5, 2.5, 1.25 and 0.625 mg/ml were studied. The antibacterial activity was determined by the agar well diffusion method to investigate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). It was found that the alcoholic extract of *P. emblica* had the highest antibacterial activity at 20 mg/mL and 5 mg/mL except in *Pseudomonas aeruginosa* where the value of inhibition was between 20 mg/mL and 10 mg/mL whereas the MIC concentrations were mostly very high and ranged from 5 to 1.25 mg/ml.

Juree *et al*. [79] investigated effect of the water extract from fruit of *P. emblica* was prepared according to Thai Herbal Pharmacopoeia (THP). Antioxidant activity of the extract was investigated by several different methods, including DPPH and ABTS•+ radical scavenging assays as well as ferric reducing antioxidant power (FRAP) assay. The results showed that the extract has an ability of scavenging radicals generated by both DPPH and ABTS•+. Similar to trolox, the water extract of *P. emblica* fruit also had a ferric reducing property.

Albahr *et al*. [80] developed a shelf stable avocado puree by using pressure-assisted thermal sterilization (600 MPa at 90°C for 5 min) combined with high barrier polymeric containers, including Film A (PE/PA6//EVOH) and film B (AlOx-coated PET//AlOx-coated PET//AlOx-coated PET//ONy//CPP). The puree was added with ascorbic acid. The results showed that during storage at 23 °C for 104 days, the puree had weight loss in Package B (0.5%) lower than in Package A (4–5%) as well as the overall color change. No significant degradation of chlorophyll concentrations of PATS-processed avocado was found during storage.

Soliva-Fortuny *et al*. [81] investigated effects of combining techniques such as addition of sorbic acid, modification of water activity (a_w), reduction of pH, modification of the packaging atmosphere and control of the storage temperature on the microbiological shelf life of avocado puree. Results showed that the addition of 300 mg/kg of sorbic acid could

extend the stability of the product from native microflora for storage periods of up to four months. Vacuum packaging had a significant influence over the control of yeast and mould populations and could preserve avocado puree during 112 days without addition of antimicrobial. The addition of maltose to reduce a_W to 0.96 resulted in slightly more stable puree but would impart important changes to the product palatability.

Rodríguez-Campos *et al*. [82] evaluated the effect of adding natural extracts on the antioxidant capacity, color and consumer acceptance of avocado purees. Avocado pulp was mixed with lemon and onion extracts and storage at 4 °C for 7 days. At the beginning of the storage, both reducing power and antioxidant capacity were affected by the proposed formulations, showing their maximum values (710 ± 9.0 mg AAE/100 g and 170.4 ± 8.6 Trolox/100 g, respectively) with the highest lemon extract level (10%). Browning process was detected in all formulations. However, it was reduced when formulated the avocado puree with 90% of avocado, 5% of lemon and 5% of onion extract or 90% of avocado, 7.5% of lemon and 2.5% of onion extract. The latter formulation had the highest acceptant scores by the consumers at the beginning and end of the storage.

Mango puree, at different pH (3.5, 4.0, or 4.4), was mixed with 4-hexylresorcinol (4-HR) (40, 60, or 80 mg/kg), cysteine (Cys) (100, 200, or 300 mg/kg), or ascorbic acid (AA) (250, 500, or 1000 mg/kg) to assess their effects on PPO activity. The darkening effect of puree was slowed down as Cys and AA concentrations were increased in combination with 4-HR. No significant differences were observed for PPO activity among anti-browning agents and concentrations used separately. Similarly, no significant differences in PPO activity were observed for combinations of AA or 4-HR.

Magri *et al*. [83] investigated the effectiveness of melatonin (1 mM) and ascorbic acid (20 mM) treatments, alone or in combination, on qualitative traits and antioxidant systems of fresh-cut avocado fruits during 14 days of cold storage (4 ± 0.5 °C and RH 95 ± 0.5%). The results showed that the combined melatonin and ascorbic acid treatment delayed color changes, thereby retarding the ripening process and weight loss compared with separate melatonin and ascorbic acid treatments. Furthermore, melatonin and ascorbic acid treatment

showed a synergistic effect on fresh-cut avocado, improving the enzymatic and non-enzymatic antioxidant system during cold storage. Compared with the control, the total polyphenol and flavonoid contents in the treated avocado were increased, and a higher antioxidant activity was observed. The combined treatment improved catalase, ascorbate peroxidase, and superoxide dismutase activities; decreased polyphenol oxidase and guaiacol peroxidase activities; and effectively reduced membrane damage by influencing lipoxygenase activity.

CHAPTER 3
MATERIALS AND METHODS

3.1 Chemicals and apparatus

3.1.1 Chemical

Name	Brand/Borough/Country
1. Emblica extracts	Popaya natural products company limited/ Pathumthani/Thailand
2. Citric acid	KEMAUS/Cherry brook/Australia
3. Ascorbic acid	Sigma-aldrich/Germany
4. Ethanol absolute	VWR/France
5. Methanol	RCILabscanlimited/Bangkok /Thailand
6. Folin-ciocalteus phenol	SRL/Maharashtra/India
7. 2,2-diphenyl-1picryl-hydrazyl	Sigma-aldrich/Germany
8. Ammonium sulfate	KEMAUS/Cherry brook/Australia
9. Catechol	TCI/Japan
10. Sodium carbonate	KEMAUS/Cherry brook/Australia
11. Sodium bicarbonate	Mcgarrett/Thailand
12. Agar	Himedia/India
13. Nutrient broth	Himedia/India
14. Mueller hinton broth	Himedia/India

3.1.2 Apparatus

Name	Model/Brand/Country
1. Freeze dry	FDB-5503/Operon Korea
2. Vortex	G560E/Scientific Industries/U.S.A
3. Centrifuge	Combi514R/Hanli/Korea

4. colorimeters	NR200/3nh
5. Incubator	JSGI-250T/JSR
6. Mixer grinder	MX-AC400/Panasonic/India
7. Micropipettes P1000, P200	WITOPET PREMIUM/witeg Lebortechnik GmbH/Germany
8. Micropipettes P 5 ml	LKA PETTE
9. pH meter	pH700/Eutech
10. evaporator	R-300/BUCHI
11. Scanning electron microscope	JSM-5410L/JEOL/Japan
12. Microplate reader	EPOCH 2/Bio Tek
13. Texture analyzer	TA.XT plusC/Stable micro system/United Kingdom

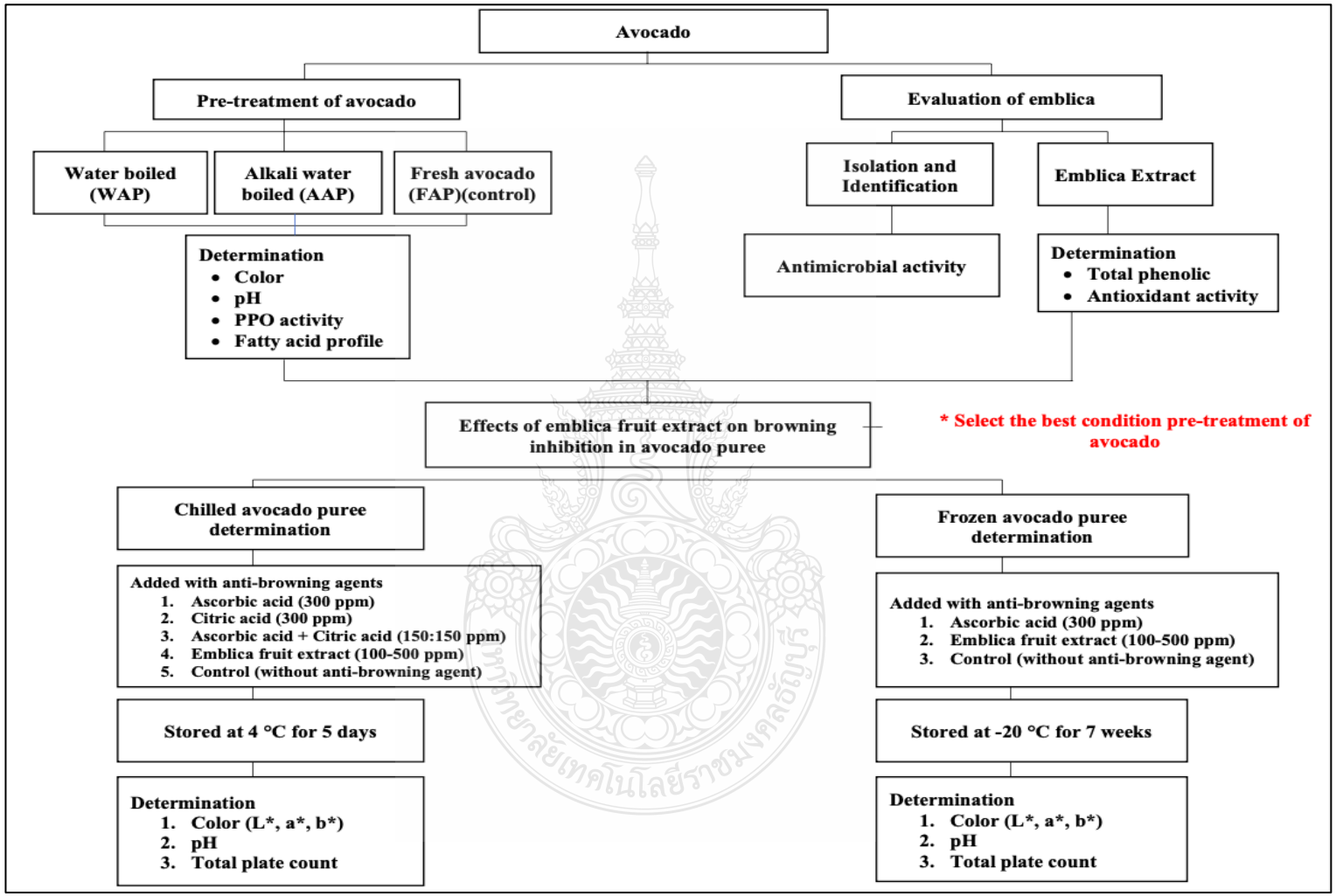


Figure 7 Schematic diagram of research methodology

3.2. Methods

Completely Randomized Design (CRD) was used in this experiment. The study was divided into three parts, including evaluation of emblica fruit extract, pre-treatments of avocado, and effects of emblica fruit extract on browning inhibition in avocado puree during chilling and frozen stages. The schematic diagram of the research methodology was shown in Figure 7.

3.2.1 Evaluation of emblica fruit extract

3.2.1.1 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 as powders. The powder (100 g) was soaked in 95% of ethanol (1000 mL) for 24 h. The extract was filtered through Whatman filter paper No. 1 and was evaporated to remove ethanol under a vacuum using a rotary evaporator (R-300, Buchi, Germany). The crude extract was then freeze dried and stored at 4 °C in storage vials for experimental use. The extract was determined for their total phenolic contents, scavenging activity and antimicrobial activity.

3.2.1.2 Isolation and identification of bacteria from spoiled avocado

The fresh avocado was halved lengthwise and pitted to separate the pulp after cleaning and sanitation. The pulp then was pieced and ground for 2 min in a blender (MX-AC400, Panasonic, China). The puree was immediately packaged in polypropylene zip-lock bags after blending. The purees were kept at 4 °C for 7 days or until it was spoiled. Serial dilution method was carried out to isolate bacteria from the spoiled avocado puree. The puree was serially diluted from 10^{-1} to 10^{-6} dilutions. A 100 µL of each of diluted fruit suspension was spreader over specifically labeled nutrient agar medium bacterial growth. Morphological analysis was observed to determine based on different bacterial colonies and morphology variation of bacteria growth. Consequently, bacterial isolates were sub-cultured, maintained and stored on nutrient agar slants at 4 °C for further use [84]. Development typical colonies on the agar plates were identified by microscopic

examination. A 16S ribosomal DNA sequencing method was also used to confirm the identity of these bacteria [85].

3.2.1.3 Determination of emblica fruit extract

The emblica fruit extract was measured for total phenolic contents, scavenging activity, and antimicrobial activity.

1) Total phenolic content

Total phenolic contents of emblica extract powder were determined by a modified method as described by [86]. 0.5 mL of emblica extract solutions (100 mg/mL) was mixed with 2.5 mL of 10%v/v diluted Folin–Ciocalteu phenol reagent and 2 mL of 7%w/v sodium carbonate, and incubated at room temperature in the dark for 2 hour. 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. All samples were measured in triplicate.

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

DPPH scavenging activity was analyzed using a spectrophotometric method [87]. 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). The solutions in the test tubes were 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 3.1.

$$\% \text{ inhibition} = [(Abs_{blank} - Abs_{sample})/Abs_{blank}] * 100 \quad (3.1)$$

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) Antimicrobial activity

The antimicrobial activity of the extract was evaluated by the agar well diffusion method. Bacteria isolated from the avocado puree were grown in Muller Hinton broth. The concentration of the bacteria was adjusted to match the turbidity of 0.5 McFarland standards prior to be inoculated on Muller-Hinton agar. After inoculation, plates were dried for 15 min, and the wells were punched using sterile cork borers. Once wells were formed, they were filled with 100 µL of the extracts (100-500 ppm) and blanks. Plates were incubated for 24 h at 37 °C to allow the extracts to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition were measured in millimeters. An agar well (6 mm) showing no zone of inhibition was considered as no antimicrobial activity. All experiments were done in triplicate and the average values were used for drawing bar diagrams.

3.2.2 Pre-treatments for avocado puree production

3.2.2.1 Preparation of avocado

Avocados were kindly provided by a local supplier in Nakhon Ratchasima. Avocados in stage 5 were selected to be processed. The firmness of the selected avocado must be between 200-300 N, measured by using a texture analyzer (TA.XT plusC, Stable micro system, United Kingdom). After cleaning and sanitation, the fruit was halved lengthwise and pitted to separate the pulp [15]. The avocados were divided into two experiments 1) avocado that was boiled at 85 ◦C for 3 min (WAP) and 2) avocado that was boiled with alkaline water (sodium bicarbonate buffer, pH 10.6) at 85 ◦C for 3 min (AAP). Then, the pulp was pieced and ground with a blender model (MX-AC400, Panasonic, China) for 2 min. After blending, one-hundred grams of the puree were packaged in PE zip-lock plastic bags and stored at 4 °C for 5 days and analysis their physicochemical changes. Fresh avocado was used as a control (FAP).

3.2.2.2 Chilled avocado puree determinations

1) pH and color

During storage, the avocado puree samples were determined for color, pH, and polyphenol oxidase (PPO) activity. The pH was determined by a pH meter (pH700, Eutech, Singapore) with triplicates and average values calculated. For color determination, instrumental color parameters were monitored using a (NR 200, 3nh, China) colorimeter in the CIE Lab scale. For each sample, three measurements were made, and three readings were taken for each measurement and averaged. All experiments were repeated on separate days. Data collected included lightness values (L*), redness values (a*) and yellowness values (b*) and the total color difference (ΔE) was calculated as below:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \qquad (3.2)$$

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.

2) PPO activity

To extract PPO from chilled avocado puree, FAP, WAP, or AAP (300 g) were added with distilled water (100) ml and mixed with 200 ml of 0.2 M of phosphate buffer pH 7.0 solution. The mixture was blended using a blender (MX- AC400, Panasonic, China) for 60 s and was centrifuged at 4°C with 13,500 rpm for 10 minutes. The supernatant was precipitated with ammonium sulphate 80%w/v saturated at 0°C, then centrifuged at 13,500 rpm, at 4°C (Combi514R,Hanli,Korea), for 10 min. The precipitate was dissolved in 0.2 M phosphate buffer solution pH 7.0 (4 mL) and stored the crude PPO at 0°C.

PPO activity was determined [88] by placing 200 μL of the crude PPO, reacted with 20 mM catechol solution in 0.2 M phosphate buffer solution, pH 7.0 (2.8 ml) at 25°C. The decrease in absorbance was measured at 420 nm by using a microplate reader (EPOCH 2). The PPO activity was measured from the slope of the graph of the relationship between the absorbance and reaction time. The activity of PPO was calculated

by giving 1 enzyme unit equivalent to a change in absorbance of 0.001 units per minute. It was calculated as percentage inhibition (% inhibition).

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100 \qquad (3.3)$$

where A_0 was the activity of PPO from fresh avocado and A_1 was the activity of PPO from avocado pretreatment, respectively.

3) Fatty acid profile

Avocado oil was extracted from the puree (100 to 200 mg) according to the method of AOAC official method 996.6. The oil was weighed into 100 mL corked centrifuge tubes. The internal standard, TAG 13:0 and TAG 11:0, solutions (2 mL) were added (5mg mL-1 in n-hexane) as well as 2 mL of ethanol 95%. Samples were hydrolyzed with 8.3 mol L-1 HCl (10 mL) in a warm-bath (70 to 80 °C) for 40 min. Ethanol 95% was added (10 mL). The tubes were vortex mixed and cooled in water-bath to room-temperature. The contents of the tubes were transferred to a 250 mL separator funnel and fat extracted with 3 aliquots of ethyl ether and petroleum ether (1:1). Ether extract was filtered through fat-free paper in a 150 mL glass. The solvent was dried with nitrogen and the extracted fat was methylated and analyzed by CG/FID. TF was calculated as the sum of individual fatty acids expressed as equivalent TAG. Fatty acid methyl esters (FAMEs), prepared by Hartman and Lago modified method [89], were analyzed by gas chromatography on a Shimadzu gas chromatograph (GC 17A model), with flame ionization detector (FID), using a capillary fused silica column with a cyanopropyl polysiloxane stationary phase (SP^{TM}- 2560, 100 m x 0.25 mm id, 0.20 μm film thickness–Supelco Inc. Bellefonte, PA, USA). The chromatographic conditions were optimized through analyses of vegetable oil and partially hydrogenated vegetable fat (PHVF) samples, with reference values for fatty acids, including for *trans* fatty acids. The determined optimal conditions were as follow: programmed column temperature from 45 up to 175 °C (13 °C min^{-1}); then up to 215 ^{o}C (4 °C min^{-1}); stationary at 215 °C for 35 min; injector and detector temperature: 250 °C; carrier

gas: hydrogen; column pressure: 175 kPa. The compounds were identified by standards co-injection and relative retention time to FAME 13:0 (internal standard).

3.2.3 Effects of emblica fruit extract on browning inhibition of avocado puree

3.2.3.1 Preparation of avocado puree

Avocados were kindly provided by a local supplier in Nakhon Ratchasima. Avocados in stage 5 will be selected to be processed. The firmness of the selected avocado must be between 200-300 N, measured by using a texture analyzer (TA.XT plusC, Stable micro system, United Kingdom) selected to be processed. After cleaning and sanitation, the fruit was halved lengthwise and pitted to separate the pulp. The pretreated avocado as section 3.2.2.1 (AAP) was prepared. After blending, one-hundred grams of the puree were added with citric acid (300 ppm) (AAP/CA), ascorbic acid (300 ppm) (AAP/AA), citric acid (150 ppm)+ascorbic acid (150 ppm) (AAP/CAA), and emblica extract (100-500 ppm) (AAP/E100, AAP/E200, AAP/E300, AAP/E400, and AAP/E500) and mixed until they become homogenous. The sample was packaged in PE zip-lock plastic bags and stored at 4 °C for 5 days and -20 °C for 7 weeks for future analysis.

3.2.3.2 Chilled and frozen avocado puree determinations

During storage, the chilled and frozen avocado puree samples were seperately determined for their color, pH, and PPO activity as mentioned in 3.2.2.2 as well as total bacteria plate count (TPC) and cellular microstructure by scanning electron microscope.

To analyze for TPC, 25 g chilled or thawed avocado puree were placed in a stomacher bag. A total of 225 ml of sterile phosphate buffer (PBS) solution was added to the sample, and then homogenized with stomachers for 2 min. This was a solution with 10^{-1} dilution. One ml of the suspension with 10^{-1} dilution was transferred with a sterile pipette to solution of 9 ml PBS to obtain 10^{-2} dilution. Dilution were carried out to 10^{-3}, 10^{-4}, 10^{-5} as needed. A total of 1 ml of suspension of each dilution was introduced into the petri dish in plate count agar (PCA) The media were incubated at 37 °C for 24-48 h.

3.2.4 Statistical analysis

Each experiment was repeated three times. The influences of the various parameters were assessed by analysis of variance (ANOVA) and Duncan test 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 was used for the analyse.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Isolation and identification of spoilage microorganisms in avocado puree

Avocado puree was prepared and stored at 4 °C until it became spoiled. The sample was taken to isolate spoilage microorganisms. The result showed that there was only one isolate found from the avocado puree. As Figure 8, the isolate had yellow, glistening, and smooth colonies (Figure 8a.) The shape was regular edges and raised with slightly elevated centers. The isolate was gram-positive bacteria, nonmotile and nonsporulating (Figure 8b). After 16S ribosomal DNA sequencing, the phylogenetic trees of the isolate were shown in Figure 9. The isolate belonged to the genus of *Staphylococcus* which was identified as *Staphylococcus pasteuri* with the sequence identity of 99%. The isolate was identified as ***Staphylococcus pasteuri***, which could be found in food products as well as naturally occurring in the air and on surfaces such as drinking water [90]. The strain is coagulase-negative coccus bacteria, generally grow at 15-45 °C, and exhibits catalase, urease, glucosidase and glucuronidase activities, produces acid aerobically from glycerol, glucose, sucrose, and fructose and commonly exerts resistance to bacitracin. Many studies reported that number of foods including goat milk, Italian sausage sea fish and retail beef were contaminated with *S. pasteuri* [91]. Moreover, the bacteria could be overrepresented in the gastrointestinal tract in children with active celiac disease [92] and sometimes could be found in platelet transfusions [93].

(a) (b)

Figure 8 Morphology of *Staphylococcus pasteuri* on a nutrient agar plate (a) and under microscope (b), isolated from spoilage avocado puree

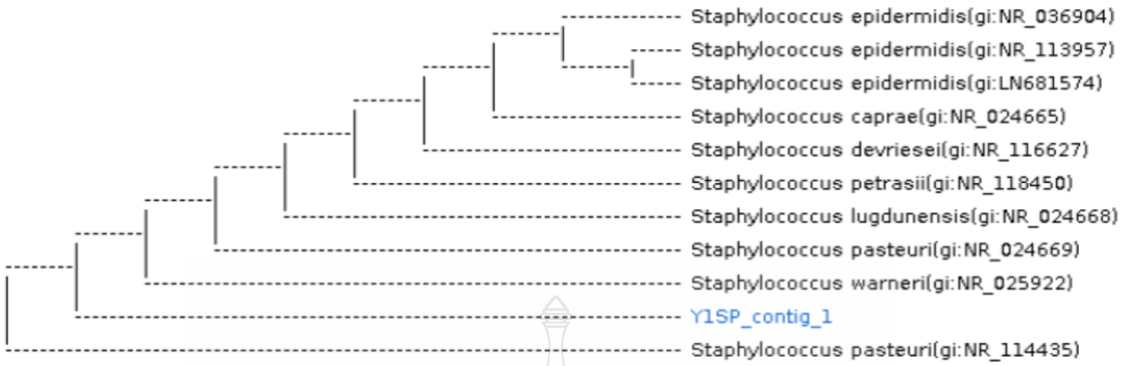


Figure 9 Phylogenetic tree of bacterial isolate obtained from spoiled avocado puree by 16S rRNA gene sequence method.

4.2 Determination of emblica fruit extract

4.2.1 Total phenolic contents and antioxidant activity

Phenolic compounds have been proved to be responsible for the antioxidant activity of emblica fruit [46]. The amounts of total phenolics in emblica fruit extracts were measured in this study. The extract had total phenolic content at 167.22 mg GAE/g of freeze dried emblica fruit extract and its free radical scavenging activity was 37.45%. The emblica fruit extract has been well-known for their high levels of total phenolic contents and antioxidant activity. Luo *et al*. [94] reported that phenolic compounds extracted from *P. emblica* exhibited strong radical scavenging activity, good potency to chelate Fe^{2+}, and good inhibition of lipid peroxidation. Liu *et al*. [46] found that gallic acid and tannic acid, in phenolic fraction, were major antioxidant compounds of *P. emblica*. Moreover, phenolic compounds could be included gallic acid, ellagic acid, mucic acid 1,4-lactone 3-*O*-gallate, isocorilagin, chebulanin, chebulagic acid, and mallotusinin [94]. Chaiyasut *et al*. [95] found that ethanolic extract of *P. emblica* Linn. showed higher phenolic content than other extracts namely *Betula alnoides* Buch Ham., *Terminalia* sp., *Anaxagorea luzonensis* Gray, *Terminalia chebula* Retz., *Caesalpinia mimosoides* Lamk., *Jussiaea repens* Linn., *Gymnema inodorum* Decne., *Manihot esculenta* Crantz., and *Ocimum sanctum* Linn. Phenolic compounds contributed to the overall antioxidant activities by inactivating lipid free radicals

and preventing decomposition of hydroperoxides into free radicals [96]. Besides, antimicrobial activities of plant extracts tended to be correlated well with their phenolic compounds. Puupponen-Pimiä *et al.* [97] reported that bioactive compounds such as phenolics and organic acids had antimicrobial activities against human pathogens, e.g., *Salmonella* sp. and *Staphylococcus* sp.

4.2.2 Antimicrobial activity

The emblica fruit extract with different concentrations was determined for its inhibition of the spoilage bacteria isolated from avocado puree. The results were expressed as diameters of clear zones and showed that the extract had ability to inhibit *S. pasteuri*, isolated from the spoiled avocado puree. The diameters of the clear zone were significantly increased according to the extract concentrations, which 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 (Figure 10). Gandhi *et al.* [45] reported that an ethanolic extract from *P. emblica* showed higher inhibition against gram-positive bacteria (Staphylococcus aureus), followed by gram-negative bacteria (Escherichia coli), and fungal (*Candida albicans*). The results of the antimicrobial and antifungal activities found that when the concentration of emblica fruit extract was increased, the clear zone was increased. Emblicanin A and B were the two major tannins that gived antimicrobial property to emblica fruit extract. The tannins produced antimicrobial actions due to the capability to inhibit microbial adhesions, inactivate enzymes, and cells envelop transport proteins. Antibacterial compounds could destroy cell wall and cytoplasmic membrane of the bacteria, resulting in cytoplasm leakage and coagulation, damage proteins, interfere with enzymatic activities, adversely affect DNA and RNA synthesis, lead to disturb electron transport and nutrient uptake, impair energy production and fatty acids and phospholipid constituents are altered inside the cell [98].

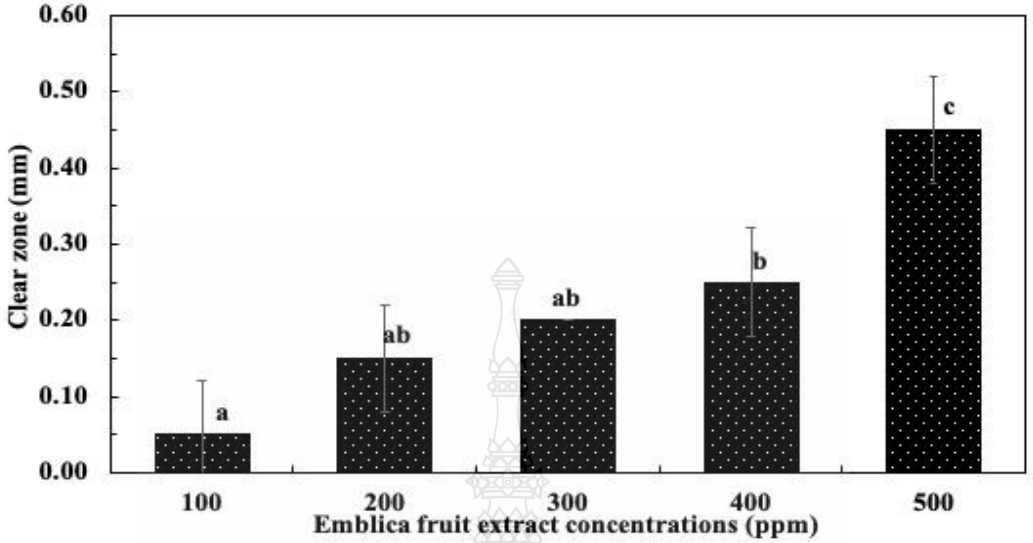


Figure 10 The clear zone inhibition of emblica fruit extract against *S. pasteuri*

[a-c]Mean±S.D. with different letters showed significant difference within the same treatment ($p<0.05$)

4.3 Pre-treatment methods for avocado puree production

4.3.1 Color

Prior to prepare avocado puree, the avocado was pretreated by boiling in water (WAP) or alkaline water (AAP) at 85 °C for 3 min. The effect of pretreatments on avocado puree colors and pH during chilled storage were observed. After boiling, it was obvious that AAP and WAP were significantly darker than the control (FAP). During storage, the avocado puree of all treatments became darker. However, AAP and WAP had color changes apparently more slowly than FAP. At 5th day of storage, WAP and AAP were significantly lighter than WAP and FAP, which were 43.23±0.37, 40.23±0.22, and 36.14±0.38, respectively (Figure 11).

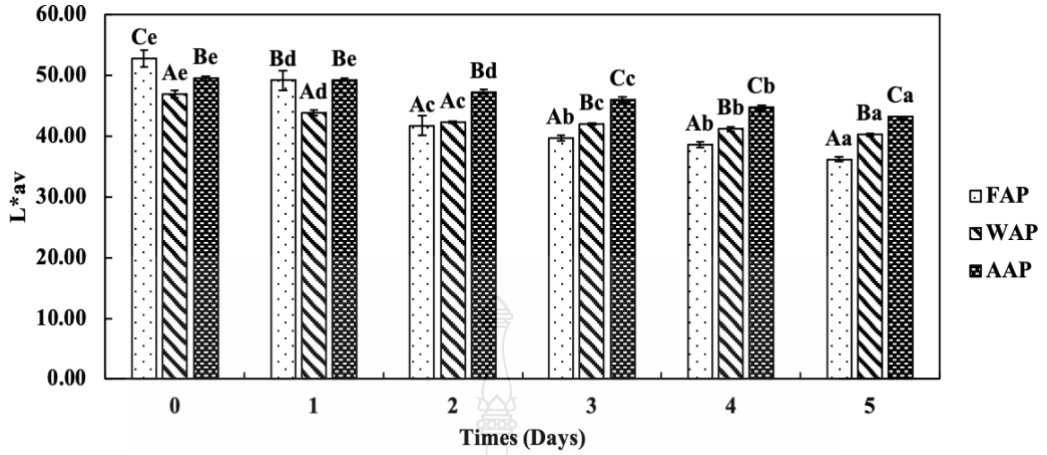


Figure 11 Average lightness (*L**) of pretreated avocado puree during storage at 4 °C for 5 days (FAP = fresh avocado puree, WAP = water blanched avocado puree and AAP = alkaline blanched avocado puree). $^{a\text{-}e}$Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05) $^{A\text{-}C}$Mean±S.D. with different letters showed significant difference within the same time (p<0.05)

When consider the greenness (*a**), after boiling, AAP and FAP had green color, while WAP showed some redness. During storage, it was found that color of FAP turned to red after storage for 2 days. At day 5, AAP was significantly greener than WAP and FAP, with the values of -1.19±0.05, 0.12±0.06 and 1.13±0.19, respectively (Figure 12).

On the other hand, the highest *b** value (yellowness) was found in FAP, followed by WAP and AAP after boiling. During storage, the yellowness was decreased in all treatments. At 5th day, the yellowness of all treatments was significantly different. WAP had the greatest yellowness, which was 19.90±0.26, followed by AAP (19.23±0.14) and FAP (18.19±0.30) (Figure 13). Color changes (△E) during storage for 5 days were shown in Figure 14. AAP was changed more slowly than WAP and FAP, indicating that blanching the avocado in alkaline buffer solution (bicarbonate buffer, pH 10.6) could help preserve the avocado color during storage.

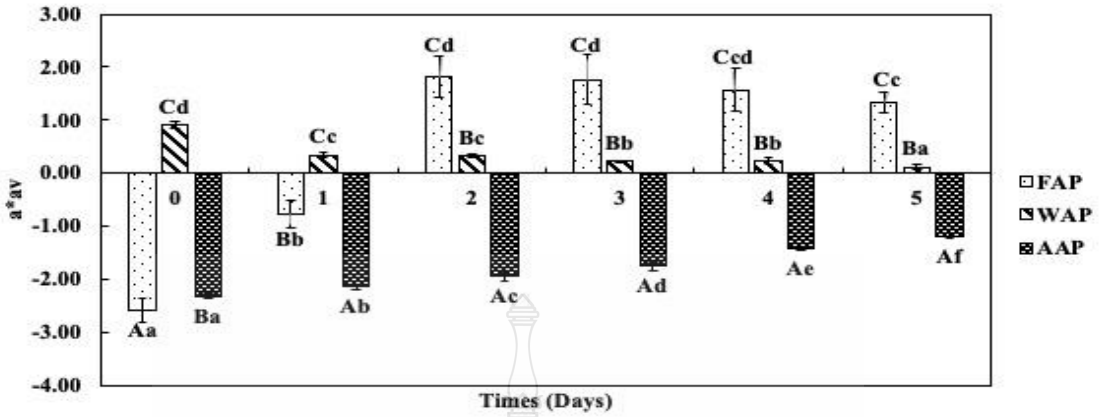


Figure 12 Average greenness (*a**) of pretreated avocado puree during storage at 4 °C storage for 5 days (FAP = fresh avocado puree, WAP = water blanched avocado puree and AAP = alkaline blanched avocado puree) respectively. [a-d]Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05) [A-C]Mean±S.D. with different letters showed significant difference within the same time (p<0.05)

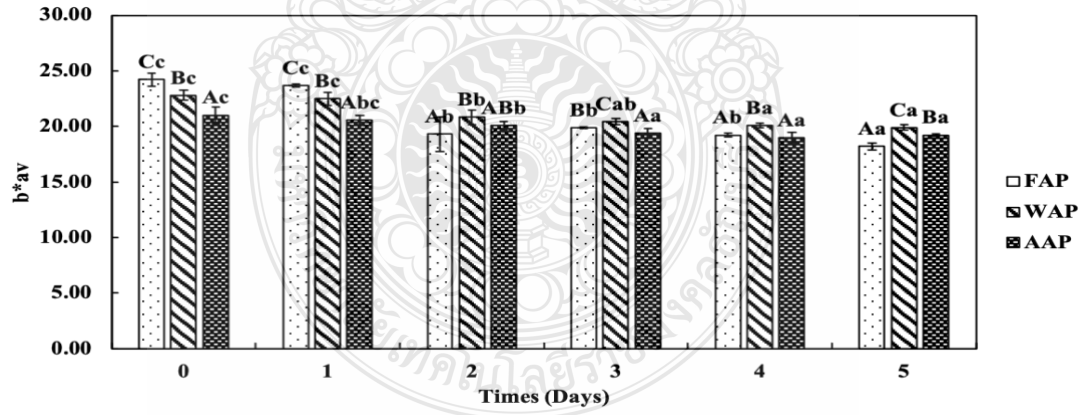


Figure 13 Average yellowness (*b**) of pretreated avocado puree during storage at 4 °C for 5 days [a-c]Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05) [A-C]Mean±S.D. with different letters showed significant difference within the same time (p<0.05)

Colors are important parameters in fruits and vegetables. They are associated with the presence of pigments in plant tissues. Chlorophyll is an important pigment that provides green color to many food products. Salvador-Reyes *et al*. [99] found that people commonly associate an intense and luminous green with a fresh and natural product, while the green-yellow color relates it to a rancid or oxidized product. Chlorophyll could be degraded by thermal processing, causing color in food products changes. From our results, they were showed that blanching the avocado in different solutions had effects on color of avocado. It could lead the avocado to became darker due to the degradation of chlorophyll. Salvador-Reyes *et al*. [99] mentioned that decrease of color huge in the avocado was affected by heat. Thermal process caused the loss of the magnesium atom in chlorophyll, which produced the formation of pheophytin that affected the pulp color, making it a yellow-green tone at the beginning and olive-green if the scalding conditions were increased. Similarly, Schelbert *et al.* [100] reported that heat or acidic substitution caused removal of the magnesium, while cleavage of the phytol chain could be promoted by either chemical hydrolysis or enzymatic action.

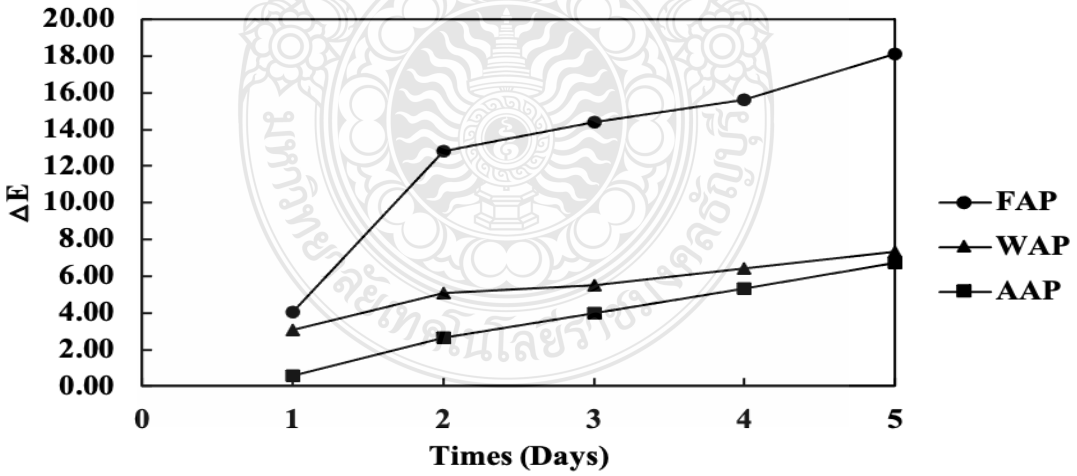


Figure 14 △E of pretreated avocado puree during storage at 4 °C for 5 days (FAP = fresh avocado puree, WAP = water blanched avocado puree and AAP = alkaline blanched avocado puree) respectively.

Moreover, our finding indicated that alkaline buffer solution could maintain avocado greenness during blanching and storage. This was confirmed by Scipioni *et al*. [101], reporting that magnesium carbonate could reduce chlorophyll loss. Higher losses of chlorophyll occurred from blanching in water because during heating the chlorophyll formed new complexes of chlorophyll derivatives without Mg^{2+}. Salt solutions of metal ion such as Zn^{2+} and Cu^{2+} could been used to preserve the green color of vegetables [102]. In addition, a pH between 7.0 and 9.0 is important for color stability. In plant tissues, pH values > 7 helped stabilize chlorophylls due to the effect of positive ions that decreased membrane permeability and led to an equilibrium between positive and negative charges, decreasing chlorophyll degradation [103].

4.3.2 pH

The pretreatment affected on pH of the avocado puree. It was evident that after blanching, AAP showed higher pH compared to the WAP and FAP significantly, while the pH of all treatments was reduced during storage. After 5 days of storage, pH of AAP was 6.83±0.02, followed by WAP and FAP, whose pH was 5.39±0.02 and 5.31±0.01, respectively (Figure 15). Previous works reported that the decline in the pH value attributed to the movement of organic acids from intercellular locations to the avocado puree [29]. Moreover, Salvador-Reyes *et al*. [99] mentioned that avocados scalded at temperatures above 78 °C could generate an acidity because the acids present in the vacuoles could be released because of the dissociation of H^+ and OH^- from the avocado's water. Besides, the increase of acidity could be due to the increase of the concentration of free fatty acids as a result of trigliceride lipolysis [104]. The effect of pH in processed vegetables were related to its color. During processing or heating, Mg could be replaced by hydrogen ions. Losses of chlorophylls could be diminished with an increase of pH [77].

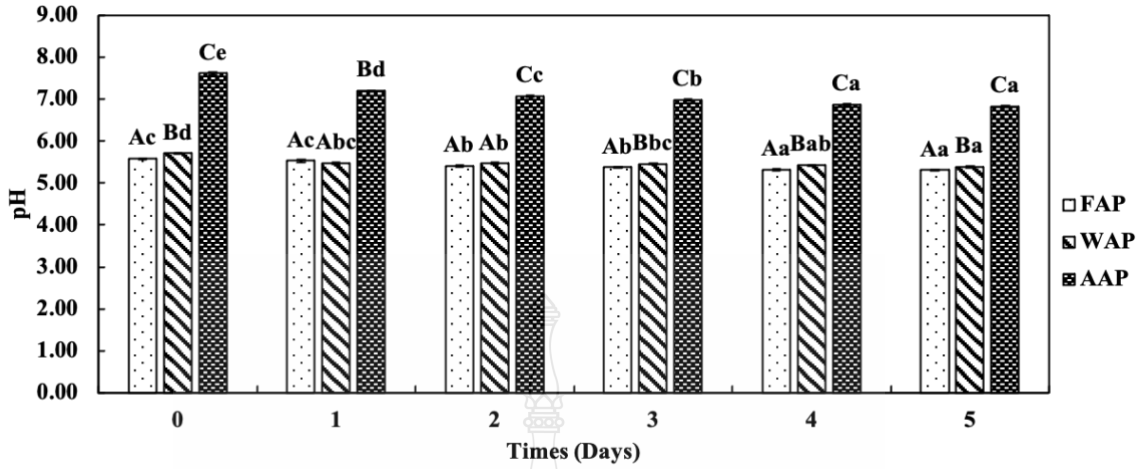


Figure 15 Changes in the pH of pretreated avocado puree storage at 4 °C for 5 days. (FAP = fresh avocado puree, WAP = water blanched avocado puree and AAP = alkaline blanched avocado puree) respectively. [a-e]Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05) [A-C]Mean±S.D. with different letters showed significant difference within the same time (p<0.05)

4.3.3 polyphenol oxidase (PPO) activity

The result of PPO activity of pretreated avocado puree showed that WAP and AAP had completely inhibited PPO activity. Changes in initial PPO activities of treated purees respecting the untreated sample are showed in Table 3. Enzymatic activities were inhibited by both treatments. PPO exhibited a sharped decrease, in which 100% reduction in both treatments. Previous works reported similar results. Salvador-Reyes *et al.* [99] reported that PPO was sensitive to heat. It could be inhibited by scalding processes from 73 °C/ 10 min to 85 °C/ 4.6 min. [99]. Bingol *et al*. [105] showed that PPO in potato can be significantly inactivated with infrared heating when the temperature rose to approximately 70°C. Similarly Palma-Orozco *et al*. [106] found that PPO in mamey fruit can be completely inactivated with microwave at a temperature of 70 °C.

Table 3 PPO activity of avocado puree pretreatment

Samples	Times (Days)		Times (Days)	
	0		5	
	PPO activity*	% inhibition	PPO activity*	% inhibition
FAP	0.3	-	4.8	-
WAP	0.0	100.0	0.0	100.0
AAP	0.0	100.0	0.0	100.0

Remark: FAP = fresh avocado puree, WAP = boiled avocado puree, and AAP = alkaline avocado puree

4.3.4 Fatty acid profile

The AAP was selected to be determined the effects of the pretreatment on avocado's fatty acid profile. In this experiment, it was found that the number of fatty acids of AAP increased, compared to FAP, however it was not significantly different (Table 4). FAP composed of saturated, monounsaturated and polyunsaturated fatty acids at 28.1%, 43.4%, and 28.6%, of the total fatty acids quantified, respectively. For AAP, saturated and monounsaturated fatty acids were slightly higher than FAP, which were 29.1% and 50.6%, respectively, while the polyunsaturated fatty acids were lower (20.35%). FAP had 28.1% of saturaged fatty acids, 43.4% of monounsaturated fatty acids, and 28.6% of polyunsaturated fatty acids. Thermal process could affect contents of fatty acids, which depends on temperatures and extraction methods of oil [107]. Oleic (C18:1) acid was the major fatty acid in both FAP and AAP, representing 38.1% and 43.79%, respectively. Also, they were rich in palmitic (C16:0) and palmitoleic (C16:1) which were 25.93% and 5.30% for FAP and 27.97%, and 6.78% for AAP, respectively.

The avocado is a good source of lipophilic phytochemicals, containing mono-unsaturated fatty acids, carotenoids, vitamin E and sterols. The main fatty acid in avocado is as oleic acid and relatively contained palmitic (16:0), linoleic (18:2), palmitoleic (16:1), and alpha-linolenic (18:3) acids [108]. Stearic (18:0), tridecanoic (13:0), tetradecanoic (14:0), cis-10-heptadecenoic (17:1) and cis-13-16-eicosenoic (20:2) acids were present in trace amounts [109].

Table 4 Fatty acid composition of avocado puree

Fatty acid	FAP (%)	AAP (%)
Palmitic acid (C16:0)	25.9±1.0	28.0±2.09
Stearic acid (C18:0)	1.1±0.7	1.1±0.6
Lignoceric acid (C24:0)	1.1±0.5	0.00
Saturated fatty acids	**28.1±1.3**	**29.1±2.2**
Palmitoleic acid (C16:ln7)	5.3±1.3	6.8±1.0
cis-9-Oleic acid (C18:ln9c)	38.1±1.8	43.8±1.8
Monounsaturated fatty acids	**43.4±1.3**	**50.6±3.1**
trans-Linolelaidic acid (C18:2n6t)	1.6±0.3	0.9±0.1
cis-9,12-Linoleic acid (C18:2n6c)	23.3±2.1	17.2±1.1
alpha-Linoleic acid (C18:3n3)	3.7±0.3	2.3±0.6
Polyunsaturated fatty acids	**28.6±2.5**	**20.3±2.6**

It was reported that fatty acid contents in avocado could be variable, influenced by the cultivars, maturity stage, anatomical region of the fruit and geographic location for plant growth [42]. The avocado oils (Merah bundar, Ijo bundar, Ijo panjang, Fuerte and Shepard) contained lower amount of oleic acid and unsaturated fatty acids compared to that of Hass variety [110]. During avocado ripening monounsaturated and saturated fatty acids was increased, while the polyunsaturated fatty acid content decreased [1]. Avocados grown in cooler areas presented a higher proportion of monounsaturated fatty acids [111]. It was also reported that the enzymatic reactions such as lipase and lipoxygenase and auto-oxidation played important roles in the release or increase of fatty acids in avocados, especially linoleic and oleic acid, they were oxidized easily [26]. Heat and light reported as physical factors resulting in oil oxidation. They could trigger the autoxidation chain reaction of unsaturated fatty acids by supplying the energy required to activate their reaction with O_2 and to generate the first radicals necessary for their propagation. Oil oxidation could occur even at temperatures as low as at ambient temperature (30 °C) [112].

4.4 Effects of emblica fruit extract on browning inhibition in avocado puree during chilling and freezing storage

4.4.1 Color

For chilled avocado puree, AAP treatments were added with anti-browning agents including ascorbic acid (AAP/AA), citric acid (AAP/CA), citric acid+ascorbic acid (AAP/CAA), and emblica fruit extract at concentrations of 100-500 ppm (AAP/E100, AAP/E200, AAP/E300, AAP/E400, and AAP/E500). At day 0, the lightness (L^*) of AAP/AA and all of AAP/E expect AAP/E500 were not significantly different, when compared to control, while AAP/E500 was noticeably darker influenced by the emblica fruit extract's color. During storage at 4 °C, all of the samples became darker. After 5 days storage, the greater L^* value was found in AAP/AA (45.96±0.52), followed by AAP/CA (40.72±0.01), AAP/CAA (40.17±0.36), AAP/E100 (35.73±0.01), AAP/E200 (35.06±0.29), AAP/E300 (34.81±0.46), AAP/E400 (33.54±0.02), and AAP/E500 (32.47±0.13) respectively (Figure 16). Color of the samples during storage for 5 days at 4°C were shown in Figure 16-18 and Table 5 which shows the change of avocado puree color at the 0 day and the 5th day. Ascorbic acid showed greater abilities in slowing browning reaction than that citric acid, ascorbic acid+citric acid, and emblica fruit extracts.

For greenness, ascorbic acid could help improve the a^* of the puree. This could be observed from the a^* of AAP/AA, which was -0.95±0.23, followed by AAP/CA (-0.2±0.01) and AAP/CAA (-0.08±0.01) after storage for 5 days (Figure 17). On the contrary, the addition of emblica fruit extract made the avocado puree to become slightly red. The more extract was added, the redder of the puree could be observed. The a^* of AAP/E100 to AAP/E500 were 3.30±0.06, 3.91±0.12, 3.63±0.06, 4.18±0.05 and 5.03±0.02, respectively. which were unidirectional with the yellowness (b^*). Figure 18 showed the b* values of AAP/E100 to AAP/E500, which were 19.97±0.06, 17.94±0.03, 16.75±0.31, 15.98±0.09 and 16.61±0.17. To confirm the effects of emblica fruit extract on the puree color, the experiment was then conducted at -20°C and only AAP/AA was selected to compare to AAP/E.

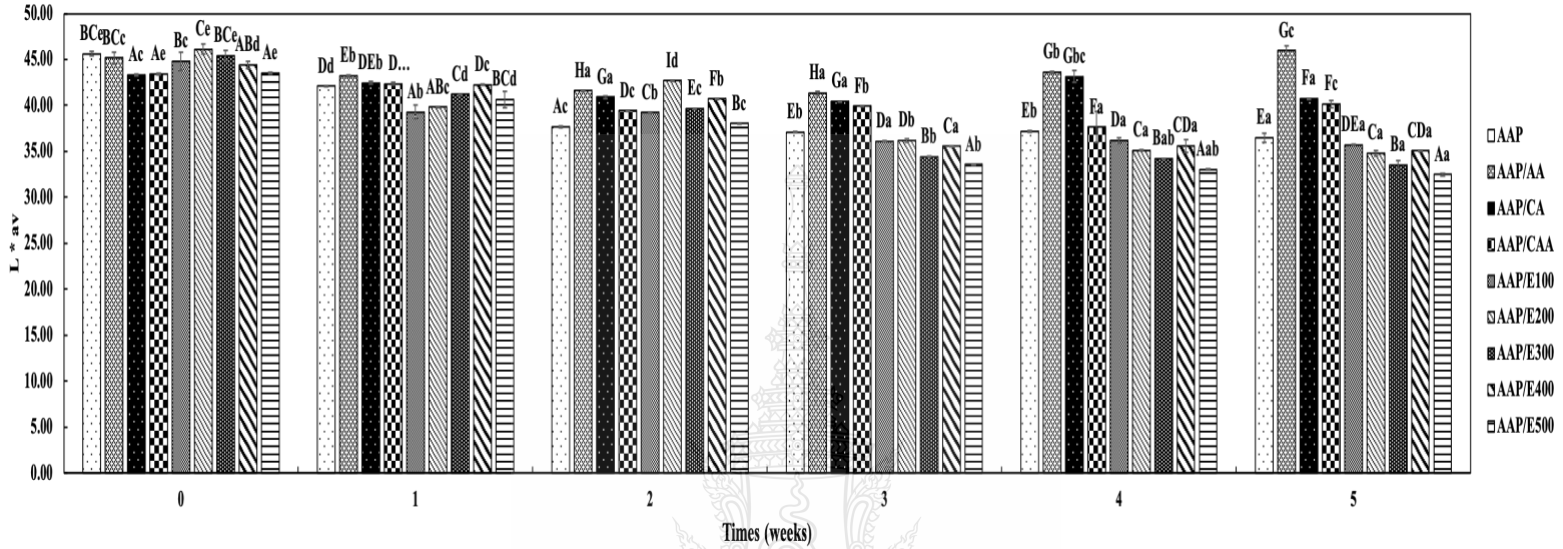


Figure 16 Average lightness (*L**) of pretreated avocado puree combine with anti-browning agent during storage at 4 °C for 5 days (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid, AAP/CA = alkaline blanched avocado puree added with citric acid, AAP/CAA = alkaline blanched avocado puree added with ascorbic acid plus citric acid; AAP/E100-AAP/E500 = alkaline blanched avocado puree added with the Emblica fruit extract at the concentration of 100-500 ppm, respectively.

[a-e]Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05)

[A-I]Mean±S.D. with different letters showed significant difference within the same time (p<0.05)

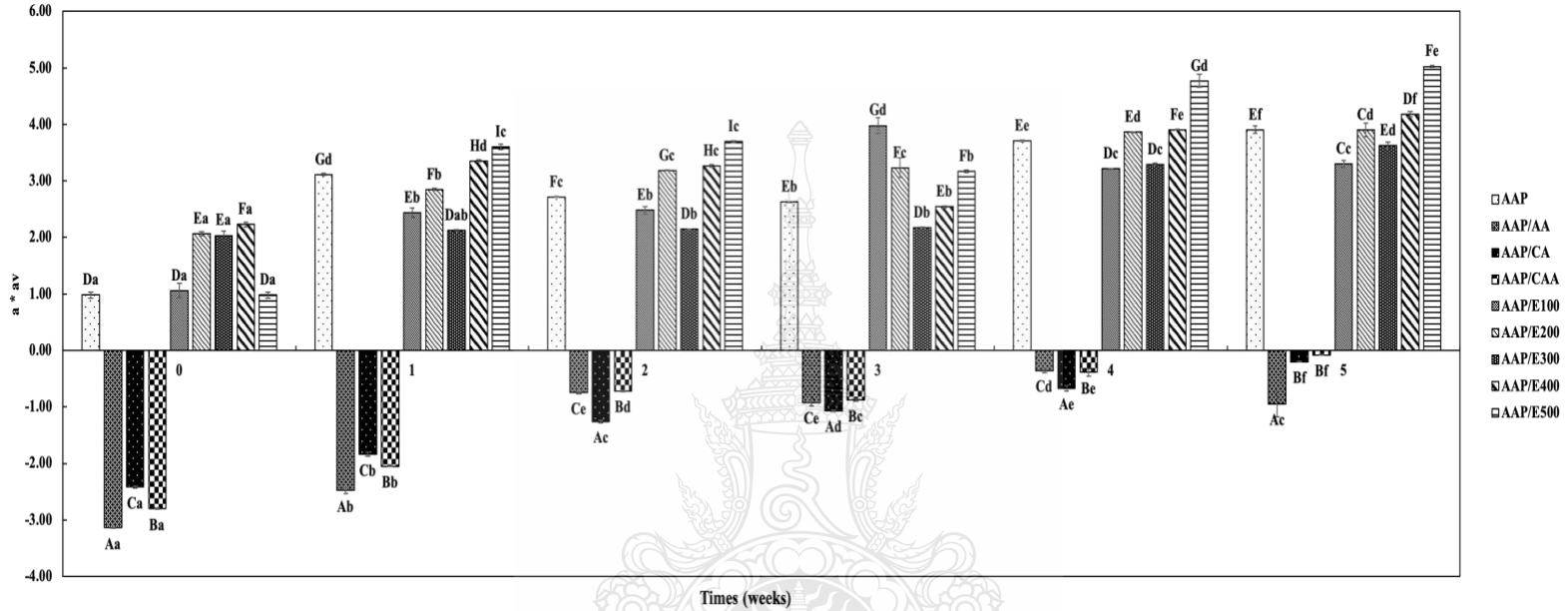


Figure 17 Average greenness (*a**) of pretreated avocado puree combine with anti-browning agent during storage at 4 °C for 5 days. (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid, AAP/CA = alkaline blanched avocado puree added with citric acid, AAP/CAA = alkaline blanched avocado puree added with ascorbic acid plus citric acid; AAP/E100-AAP/E500 = alkaline blanched avocado puree added with the Emblica fruit extract at the concentration of 100-500 ppm, respectively.

[a-f]Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05)

[A-I]Mean±S.D. with different letters showed significant difference within the same time (p<0.05)

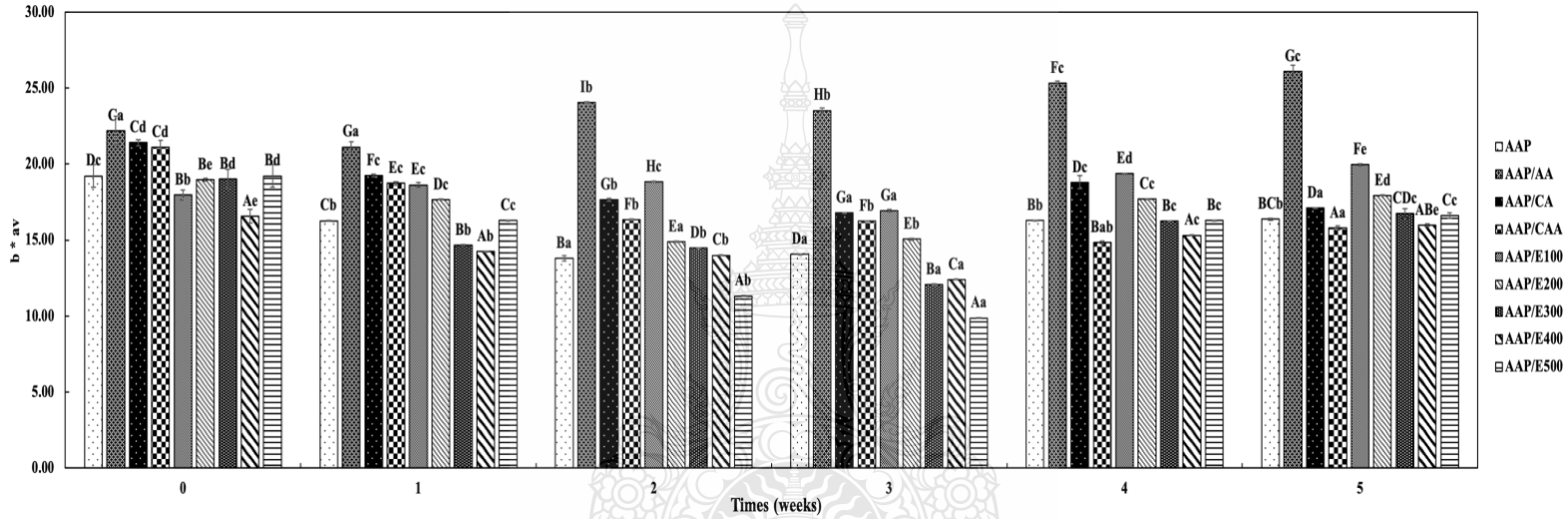
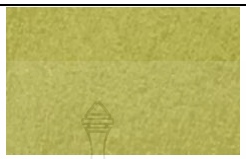
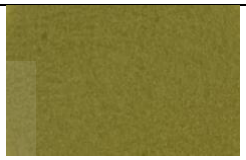
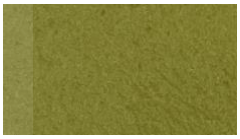
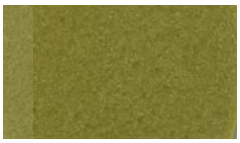
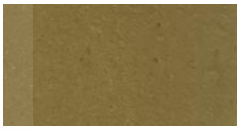
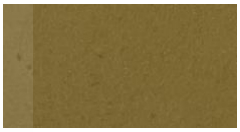
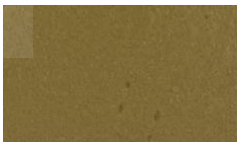


Figure 18 Average yellowness (*b**) of pretreated avocado puree combine with anti-browning agent during storage at 4 °C for 5 days (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid, AAP/CA = alkaline blanched avocado puree added with citric acid, AAP/CAA = alkaline blanched avocado puree added with ascorbic acid plus citric acid; AAP/E100-AAP/E500 = alkaline blanched avocado puree added with the Emblica fruit extract at the concentration of 100-500 ppm, respectively.

[a-e]Mean±S.D. with different letters showed significant difference within the same treatment ($p<0.05$)

[A-H]Mean±S.D. with different letters showed significant difference within the same time ($p<0.05$)

Table 5 Colors of Pretreated avocado puree combined with anti-browning agents during storage at 4 °C for 5 days

Treatments	0 Days	5 Days
AAP (Control)		
AAP/AA		
AAP/CA		
AAP/CAA		
AAP/E100		
AAP/E200		
AAP/E300		
AAP/E400		
AAP/E500		

For freezing storage, similar results were found. During storage for 7 weeks at -20°C, AAP/AA showed greater abilities in slowing browning reaction than emblica fruit extract. The greater *L** value was found in AAP/AA (41.71±0.52), followed by AAP/E200 (39.61±0.01), AAP/E300 (39.61±0.36), AAP/E100 (39.34±0.01), AAP/E500 (39.23±0.29), and AAP/E400 (38.88±0.46), respectively (Figure 19). The reddish to brownish puree was found in AAP/E. The *a** values of AAP/AA were -2.13±0.03, followed by AAP/E200 (-1.73±0.06), AAP/E100 (-1.44±0.04), AAP/E300 (-0.85±0.04), AAP/E400 (-0.61±0.02), and AAP/E500(-0.49±0.06), respectively (Figure 20). AAP/AA showed the highest *b**, 22.46±0.06 followed by AAP/E500 (16.29±0.04), AAP/E100 (15.83±0.03), AAP/E300 (15.64±0.13), AAP/E200(15.49±0.01), and AAP/E400(14.74±0.35), respectively (Figure 21). Table 6 showed the change of avocado puree color after 7-week storage.

Regarding to emblica fruit extract, it negatively affected color of the puree even though low amounts of the extract was added. It was found that although emblica fruits contained high antioxidants as vitamin C, they could turn to be brown easily even they were at low temperature as 25°C or 35°C [113]. Li *et al*. [114] 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 [114]. They also found that the change of PPO activity in *P. emblica* was related to titratable acid during browning.

Ascorbic acid behaved where it could reduce instantly the formed color and acted as quinone reducer [115], [116], [117]. It reduced the conversion of O-quinones to diphenols, leading to the formation of colorless compounds [118]. Their effectiveness depends on environmental factors such as pH, water activity (a_w), temperature, light and composition of the atmosphere [119]. Hasan *et al*. [120] treated fresh slices of apple with 1% ascorbic acid solution for one min and hot water with 50°C for two min. The results showed that both heat and ascorbic acid treatments could significantly reduce cut surface browning, they showed inhibitory effects on PPOs and peroxidase (POD) enzymes in related to enzymatic browning in fresh cut browning. Hot water treatments played more important role in suppressing both monophenolase and diphenolase activity of PPOs and POD than ascorbic acid [121]

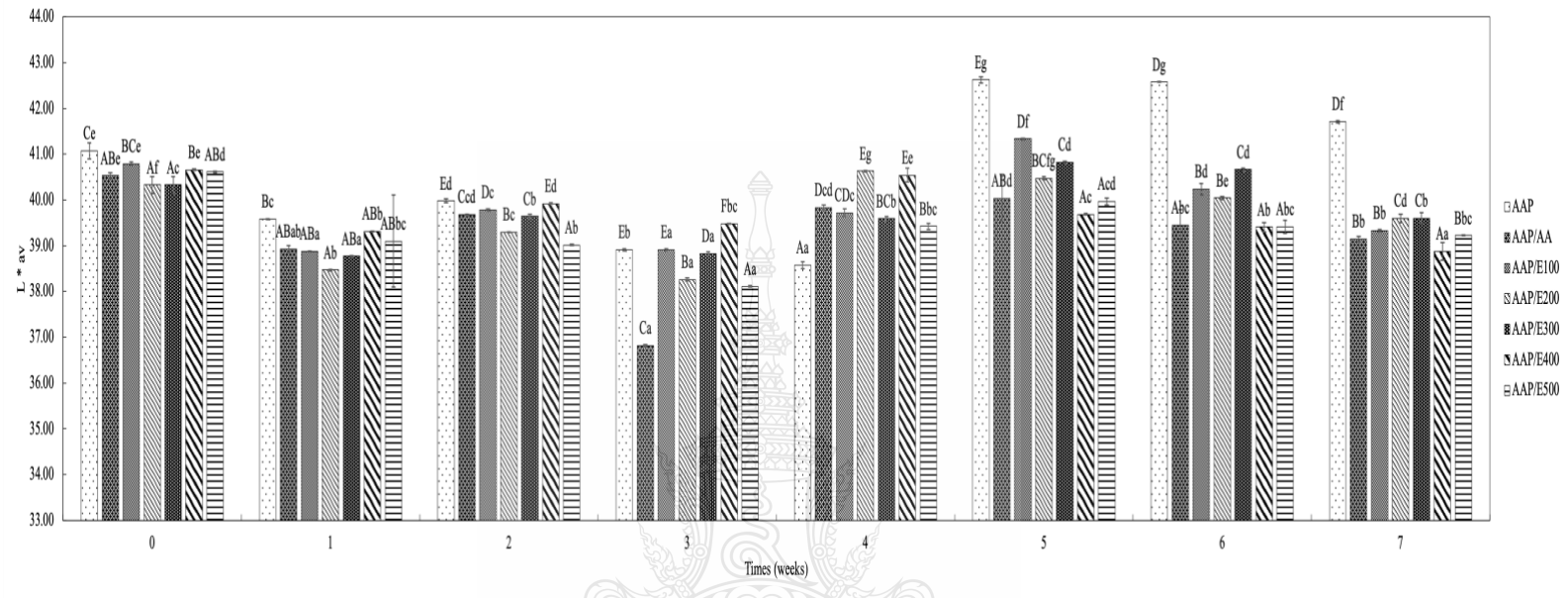


Figure 19 Average lightness (*L**) of pretreated avocado puree combined with anti-browning agent during storage at -20 °C 7 weeks. (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid; AAP/E100-AAP/E500 = alkaline blanched avocado puree added with the Emblica fruit extract at the concentration of 100-500 ppm, respectively.

[a-e]Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05)

[A-F]Mean±S.D. with different letters showed significant difference within the same time (p<0.05)

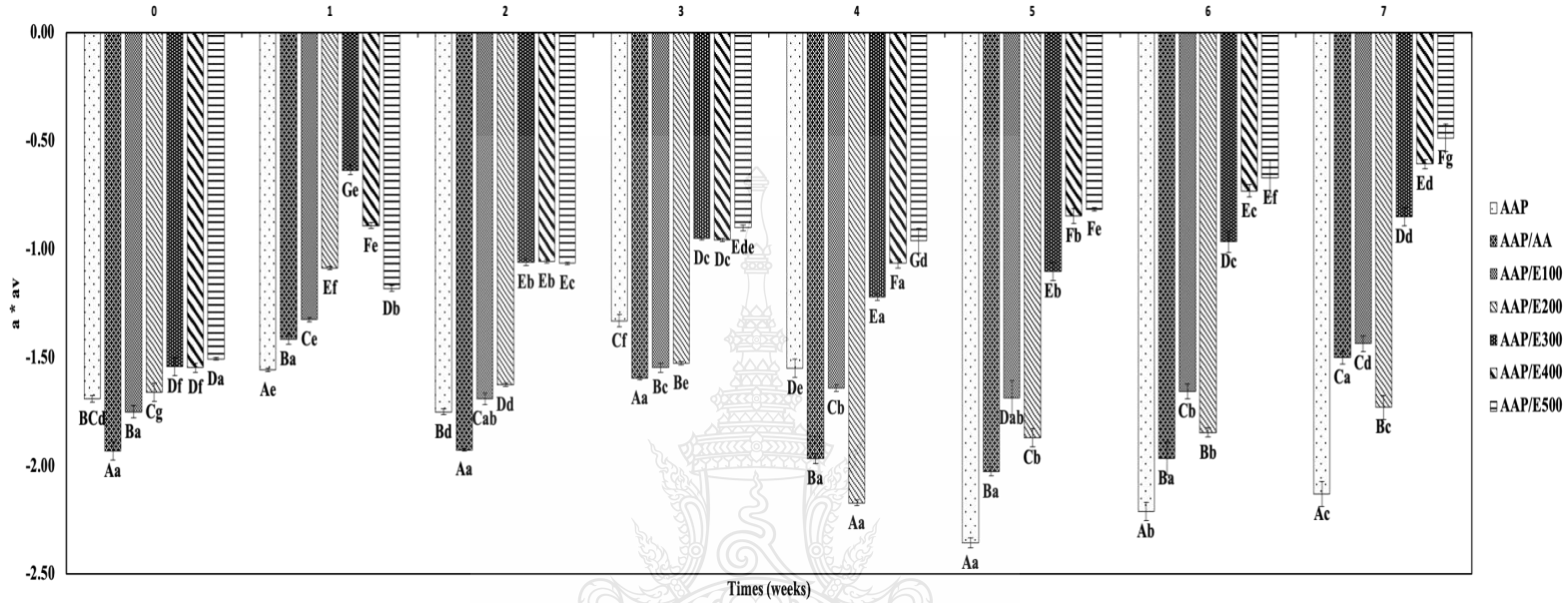


Figure 20 Average greenness (*a**) of pretreated avocado puree combine with anti-browning agent during storage at -20 °C 7 weeks. (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid; AAP/E100-AAP/E500 = alkaline blanched avocado puree added with the Emblica fruit extract at the concentration of 100-500 ppm, respectively.

[a-g]Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05)

[A-G]Mean±S.D. with different letters showed significant difference within the same time (p<0.05)

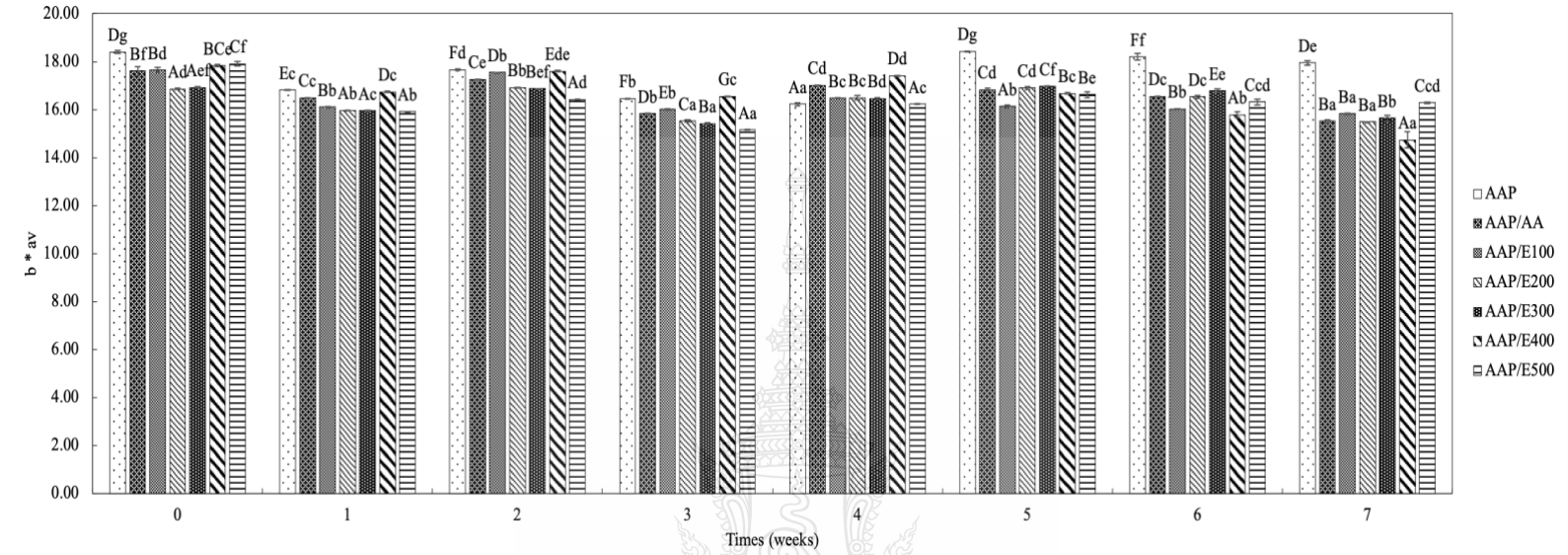
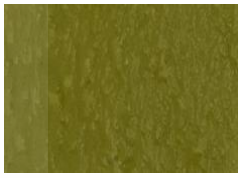
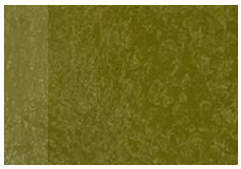
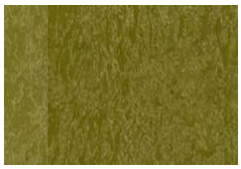
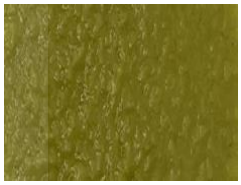


Figure 21 Average yellowness (*b**) of pretreated avocado puree combine with anti-browning agent during storage at -20 °C 7 weeks. (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid; AAP/E100-AAP/E500 = alkaline blanched avocado puree added with the Emblica fruit extract at the concentration of 100-500 ppm, respectively.

[a-g]Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05)

[A-F]Mean±S.D. with different letters showed significant difference within the same time (p<0.05)

Table 6 Colors of pretreated avocado puree combined with anti-browning agents during storage at 20 °C for 7 weeks

Treatments	0 weeks	7 weeks
AAP (Control)		
AAP/AA		
AAP/E100		
AAP/E200		
AAP/E300		
AAP/E400		
AAP/E500		

4.4.2 pH

The anti-browning agents affected on pH of the avocado puree during storage. It was evident that after adding anti-browning agents, all treatment has a lower pH compared to the control. Regarding to chilled avocado puree, APP/AA showed higher pH compared to the AAP/CA, AAP/CAA and AAP/E100-500 significantly. The pH of all treatments was significantly reduced during storage. After 5 days of storage, AAP/AA was 7.83±0.03, which was significantly higher than AAP/CA (7.70±0.03), followed by AAP/E100 (7.74±0.01), AAP/E200 (7.64±0.01), AAP/E300 (7.68±0.04), AAP/E400 (7.74±0.02), and AAP/E500 (7.7±0.03), respectively. AAP/E100 and AAP/E200 were not significantly different as well as, AAP/E300, AAP/E400, and AAP/E500. Similarly, for frozen avocado puree, the emblica fruit extract affected on pH of the avocado puree. APP/E100 showed higher pH compared to other treatments significantly. The pH of all treatments was reduced during storage. After 7 weeks of storage, AAP/100 was 6.57±0.02, followed AAP/E400, AAP/AA, AAP/E200, AAP/E300, and AAP/E500 whose pH were 6.52±0.05, 6.49±0.01, 6.45±0.02, 6.37±0.04, and 6.34±0.01 respectively.

Martinez and Whitaker [122] reported 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 [123]. Guerrero *et al*. [124] found that addition of ascorbic acid (500 ppm) to mango puree adjusted pH to 3.5 showed reduction in browning rate during storage at 3 °C. Moreover, emblica fruits are rich in vitamin C (ascorbic acid) and contains several bioactive phytochemicals, of which majority are of polyphenols (ellagic acid, chebulinic acid, gallic acid, chebulagic acid, apeigenin, quercetin, corilagin, leutolin, etc.) [41]. They are highly acidic (pH 2.85), causing pH of the puree reduced [125].

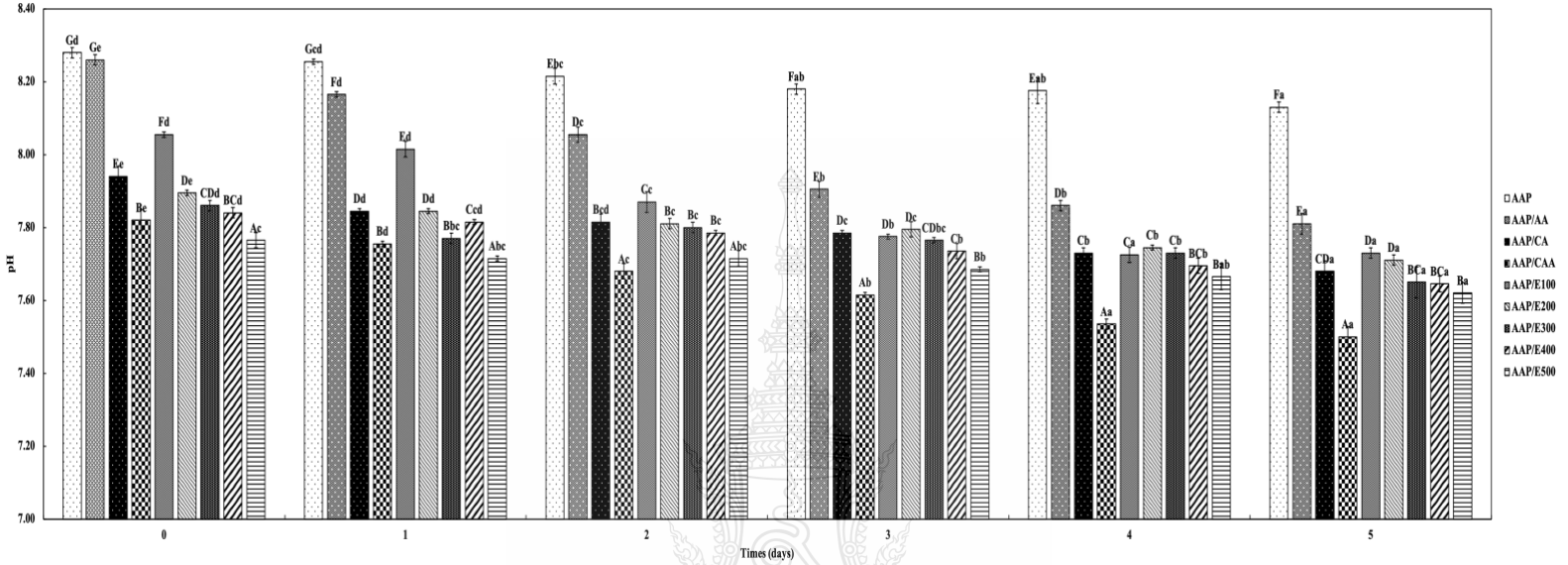


Figure 22 Changes in the pH of the avocado puree combine with anti-browning agent, storage at 4°C for 5 days.

(AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid, AAP/CA = alkaline blanched avocado puree added with citric acid, AAP/CAA = alkaline blanched avocado puree added with ascorbic acid plus citric acid; AAP/E100-AAP/E500 = alkaline blanched avocado puree added with the Emblica fruit extract at the concentration of 100-500 ppm, respectively.

$^{a-e}$Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05)

$^{A-G}$MeanS.D. with different letters showed significant difference within the same time (p<0.05)

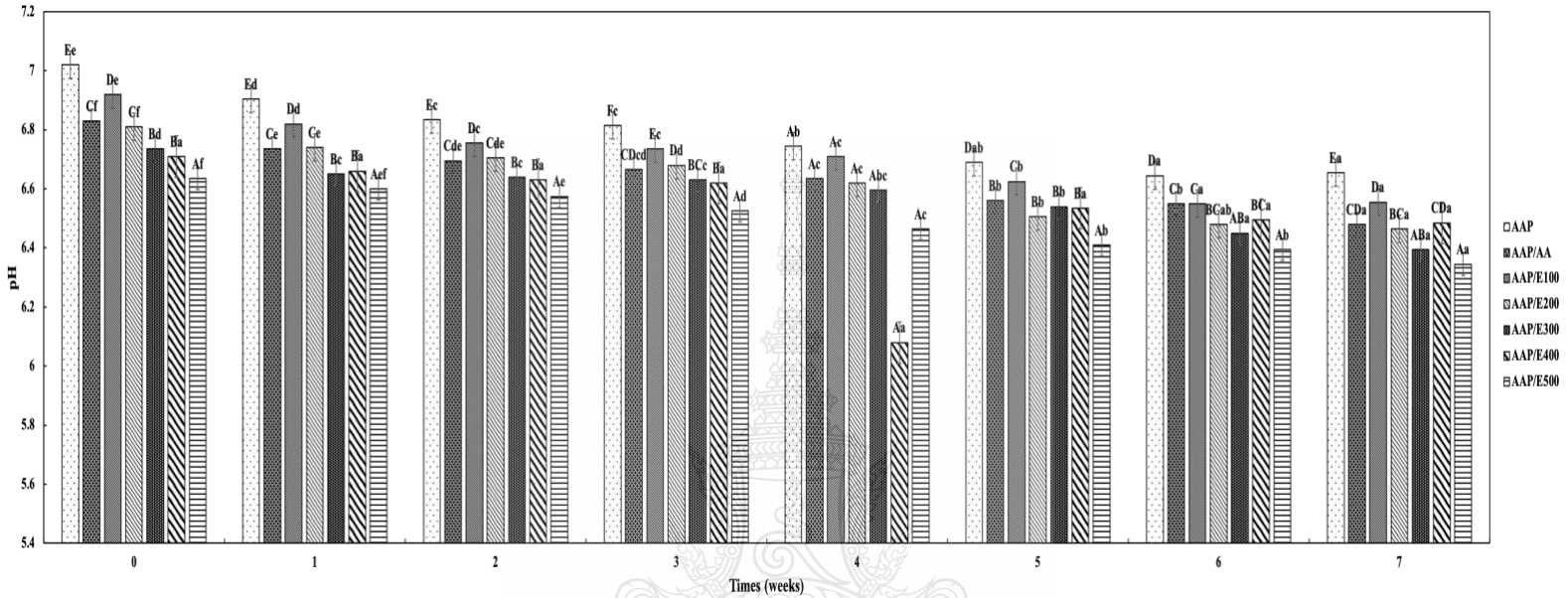


Figure 23 Changes in the pH of the avocado puree combine with anti-browning agent, storage at -20°C for 7 weeks. (AAP = alkaline blanched avocado puree, AAP/AA = alkaline blanched avocado puree added with ascorbic acid; AAP/E100-AAP/E500 = alkaline blanched avocado puree added with the Emblica fruit extract at the concentration of 100-500 ppm, respectively.

[a-f]Mean±S.D. with different letters showed significant difference within the same treatment (p<0.05)

[A-F]MeanS.D. with different letters showed significant difference within the same time (p<0.05)

4.4.3 Total bacterial

During storage at 4 °C, less than 30 colonies of bacteria were found in all AAP treatments at day 0 and after storage at 4 and 20 °C for 5 days and 7 weeks, respectively. 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 4.72±0.04 log CFU/ml and 4.05±0.06 log CFU/ml for chilled and frozen avocado, respectively. After 5 days of storage at 4 °C, TPC of FAP was slightly increased to 5.2±0.01 log CFU/ml, while 4.27±0.02 log CFU/ml were found in FAP stored at -20 °C for 7 weeks. These results could contribute to avocado pretreatment, boiling of the avocado at 85 °C for 3 min, which could destroy some bacteria as well as addition of certain browning agents. Ukuku *et al.* [126] 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. EliUz [127] reported that surface pasteurization with hot water at 76 °C for 3 min resulted in more than 5 log CFU/cm^2 reduction in *S. 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 and citric acid as sanitizers agent could inhibit *C. albicans*, *S. aureus* and *E. coli*. Emblica fruit extract acted on the cell membrane of microorganisms, resulting them to be unable to grow. The Emblica fruit extract showed anti-microbial activity on *S. aureus*; its minimum inhibitory concentration was 13.97 mg/ml and the minimum biocidal concentration (MBC) was 13.97 mg/ml [128].

CHAPTER 5
CONCLUSION

Avocado puree could be stabilized by blanching and addition of acidulants during storage. Blanching of avocado in alkaline solution helped maintain the brightness and greenness of avocado puree according to customer requirements as well as completely inhibiting PPO. Addition of ascorbic acid into avocado puree helped prevent the puree from color changes better than citric acid, the mix of ascorbic+citric acid, and emblica fruit extract. The fruit extract caused the puree to become darker during storage in both 4 and -20 °C depended on the extract concentrations. The pH of all treatments tended to decrease during stroage. 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 control, fresh avocado, which was unacceptable. This study indicated that stabilizing avocado puree by blanching in alkaline solutions combined with addition of ascorbic acid could help maintain the puree quality during storage at both chilling and freezing conditions.

List of Bibliography

[1] M. L. Dreher and A. J. Davenport, "**Hass avocado composition and potential health effects**," *Crit. Rev. Food Sci. Nutr.*, vol. 53, no. 7, pp. 738–750, Jan. 2013, doi: 10.1080/10408398.2011.556759.

[2] A. M. López-Ramírez and A. Duarte-Sierra, "**Avocado jelly: Formulation and optimization of an avocado gel using hydrocolloids**," *Int. J. Gastron. Food Sci.*, vol. 21, p. 100234, Oct. 2020, doi: 10.1016/j.ijgfs.2020.100234.

[3] J. Macheix, J. Sapis, A. Fleuriet, and C. Y. Lee, "**Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines**," *Crit. Rev. Food Sci. Nutr.*, vol. 30, no. 4, pp. 441–486, Jan. 1991, doi: 10.1080/10408399109527552.

[4] Y. Lv, L. Cai, M. Yang, X. Liu, N. Hui, and J. Li, "**Purification, characterisation, and thermal denaturation of polyphenoloxidase from prawns (*Penaeus vannamei*)**," *Int. J. Food Prop.*, vol. 20, no. sup3, pp. S3345–S3359, Dec. 2017, doi: 10.1080/10942912.2017.1354019.

[5] H. M. Ali, A. M. El-Gizawy, R. E. I. El-Bassiouny, and M. A. Saleh, "**Browning inhibition mechanisms by cysteine, ascorbic acid and citric acid, and identifying PPO-catechol-cysteine reaction products**," *J. Food Sci. Technol.*, Jun. 2014, doi: 10.1007/s13197-014-1437-0.

[6] R. P. Bates, "T**he Retardation of Enzymatic Browning in Avocado Puree and Guacamole**," *Fla. Agric. Exp. Stn. J.*, no. 81, pp. 230–235, 1968.

[7] G. Priya, N. Parminder, and S. Jaspreet, "**antimicrobial and antioxidant activity on emblica officinalis seed extract**," vol. 3, no. 4, Aug. 2012.

[8] M. Majeed *et al.*, "**Standardized *Emblica officinalis* fruit extract inhibited the activities of *α* -amylase, *α* -glucosidase, and dipeptidyl peptidase-4 and displayed antioxidant potential**," *J. Sci. Food Agric.*, vol. 100, no. 2, pp. 509–516, Jan. 2020, doi: 10.1002/jsfa.10020.

List of Bibliography (Continue)

[9] H. Chen, P. L. Morrell, V. E. T. M. Ashworth, M. de la Cruz, and M. T. Clegg, "**Tracing the Geographic Origins of Major Avocado Cultivars**," *J. Hered.*, vol. 100, no. 1, pp. 56–65, May 2008, doi: 10.1093/jhered/esn068.

[10] M. K. V. Carr, "**The water relations and irrigation requirements of avocado (*Persea americana* Mill.)**: A REVIEW," *Exp. Agric.*, vol. 49, no. 2, pp. 256–278, Apr. 2013, doi: 10.1017/S0014479712001317.

[11] B. Schaffer, B. N. Wolstenholme, and A. W. Whiley, "**The avocado: botany, production and uses**," 2nd ed.CAB International, 2013, pp. 268–300.

[12] M. Sedgley, P. B. Scholefield, and D. McE. Alexander, "**Inhibition of flowering of Mexican- and Guatemalan-type avocados under tropical conditions**," *Sci. Hortic.*, vol. 25, no. 1, pp. 21–30, Jan. 1985, doi: 10.1016/0304-4238(85)90073-1.

[13] B. Schaffer and A. W. Whiley, "**Environmental Regulation of Photosynthesis in Avocado Trees** – A Mini-Review," pp. 335–342, 2003.

[14] C. J. Clark, A. White, R. B. Jordan, and A. B. Woolf, "**Challenges associated with segregation of avocados of differing maturity using density sorting at harvest**," *Postharvest Biol. Technol.*, vol. 46, no. 2, pp. 119–127, Nov. 2007, doi: 10.1016/j.postharvbio.2007.05.010.

[15] J. P. Bower and J. G. Cutting, "**Avocado fruit development and ripening physiology**," in *Horticultural Reviews*, J. Janick, Ed., Hoboken, NJ, USA: John Wiley & Sons, Inc., 2011, pp. 229–271. doi: 10.1002/9781118060834.ch7.

[16] E. Hurtado-Fernández, A. Fernández-Gutiérrez, and A. Carrasco-Pancorbo, "**Avocado fruit— Persea americana**," in *Exotic Fruits*, Elsevier, 2018, pp. 37–48. doi: 10.1016/B978-0-12-803138-4.00001-0.

[17] International Plant Genetic Resources Institute, Ed., *Descriptors for Avocado (Persea spp.): = Descriptores para aguacate (Persea spp.)*. Roma: IPGRI, 1995.

List of Bibliography (Continue)

[18] C. A. Jaramillo-Acevedo, W. E. Choque-Valderrama, G. E. Guerrero-Álvarez, and C. A. Meneses-Escobar, "**Hass avocado ripeness classification by mobile devices using digital image processing and ANN methods**," *Int. J. Food Eng.*, vol. 0, no. 0, Sep. 2020, doi: 10.1515/ijfe-2019-0161.

[19] M. Islam, K. Wahid, and A. Dinh, "**Assessment of ripening degree of avocado by electrical impedance spectroscopy and support vector machine**," *J. Food Qual.*, vol. 2018, pp. 1–9, Nov. 2018, doi: 10.1155/2018/4706147.

[20] K. A. Cox, T. K. McGhie, A. White, and A. B. Woolf, "**Skin colour and pigment changes during ripening of 'Hass' avocado fruit**," *Postharvest Biol. Technol.*, vol. 31, no. 3, pp. 287–294, Mar. 2004, doi: 10.1016/j.postharvbio.2003.09.008.

[21] R. Pedreschi *et al.*, "**Impact of postharvest ripening strategies on 'Hass' avocado fatty acid profiles**," *South Afr. J. Bot.*, vol. 103, pp. 32–35, Mar. 2016, doi: 10.1016/j.sajb.2015.09.012.

[22] A. Apelbaum, G. Zauberman, and Y. Fuchs, "**Prolonging storage life of avocado fruits by subatmospheric pressure1**," *HortScience*, vol. 12, no. 2, pp. 115–117, Apr. 1977, doi: 10.21273/HORTSCI.12.2.115.

[23] I. Amadou, "Date Fruits," in *Nutritional Composition of Fruit Cultivars*, Elsevier, 2016, pp. 215–233. doi: 10.1016/B978-0-12-408117-8.00010-6.

[24] F. Ozdemir and A. Topuz, "**Changes in dry matter, oil content and fatty acids composition of avocado during harvesting time and post-harvesting ripening period**," *Food Chem.*, vol. 86, no. 1, pp. 79–83, Jun. 2004, doi: 10.1016/j.foodchem.2003.08.012.

[25] J. Gross, M. Gabai, A. Lifshitz, and B. Sklarz, "**Carotenoids in pulp, peel and leaves of Persea americana**," *Phytochemistry*, vol. 12, no. 9, pp. 2259–2263, Sep. 1973, doi: 10.1016/0031-9422(73)85130-1.

List of Bibliography (Continue)

[26] L. Plaza, C. Sánchez-Moreno, S. de Pascual-Teresa, B. de Ancos, and M. P. Cano, "**Fatty acids, sterols, and antioxidant activity in minimally processed avocados during refrigerated storage**," *J. Agric. Food Chem.*, vol. 57, no. 8, pp. 3204–3209, Apr. 2009, doi: 10.1021/jf900541r.

[27] M. Golukcu and F. Ozdemir, "**Changes in phenolic composition of avocado cultivars during harvesting time**," *Chem. Nat. Compd.*, vol. 46, no. 1, pp. 112–115, Mar. 2010, doi: 10.1007/s10600-010-9541-5.

[28] L. A. Terry, Ed., **Health-promoting properties of fruit and vegetables**. Wallingford, Oxfordshire, UK: Cambridge, MA, USA: CABI, 2011.

[29] T. Juan Camilo Solarte, S. Mariana Ortiz, S. Daissy Lorena Restrepo, and P. Pavel Peroza, "**Influence of products portfolio and process contextualization on the economic performance of small- and large-scale avocado biorefineries**," *ELSEVIER*, vol. 342, 2021, doi: 10.1016/j.biortech.2021.126060.

[30] R. G. Araújo, R. M. Rodriguez-Jasso, H. A. Ruiz, M. M. E. Pintado, and C. N. Aguilar, "**Avocado by-products: Nutritional and functional properties**," *Trends Food Sci. Technol.*, vol. 80, pp. 51–60, Oct. 2018, doi: 10.1016/j.tifs.2018.07.027.

[31] A. B. Woolf *et al.*, "**Effect of high pressure processing on avocado slices**," *Innov. Food Sci. Emerg. Technol.*, vol. 18, pp. 65–73, Apr. 2013, doi: 10.1016/j.ifset.2013.02.011.

[32] B. Chutintrasri and A. Noomhorm, "**Thermal inactivation of polyphenoloxidase in pineapple puree**," *LWT - Food Sci. Technol.*, vol. 39, no. 5, pp. 492–495, Jun. 2006, doi: 10.1016/j.lwt.2005.04.006.

[33] V. M. Gómez-López, "**Inhibition of surface browning, cut avocado**," *J. Food Qual.*, vol. 25, no. 4, pp. 369–379, Oct. 2002, doi: 10.1111/j.1745-4557.2002.tb01032.x.

[34] S. Ospina, D. L. Ortiz, and C. E. Orrego, "**Enzymatic Browning and Color Evolution in Frozen Storage of Two Kinds of Minimally Processed Avocado Puree**," *Int. J. Food Eng.*, vol. 15, no. 11–12, Dec. 2019, doi: 10.1515/ijfe-2018-0431.

List of Bibliography (Continue)

[35] M. S. Baliga and J. J. Dsouza, "**Amla (*Emblica officinalis Gaertn*), a wonder berry in the treatment and prevention of cancer**," *Eur. J. Cancer Prev.*, vol. 20, no. 3, pp. 225–239, May 2011, doi: 10.1097/CEJ.0b013e32834473f4.

[36] S. Mirunalini and M. Krishnaveni, "**Therapeutic potential of *Phyllanthus emblica* (amla): the ayurvedic wonder**," *J. Basic Clin. Physiol. Pharmacol.*, vol. 21, no. 1, Jan. 2010, doi: 10.1515/JBCPP.2010.21.1.93.

[37] H. P. Bhat, R. P. Jakribettu, R. Boloor, R. Fayad, and M. S. Baliga, "**Use of Ayurvedic Medicinal Plants as Immunomodulators in Geriatrics**," in *Foods and Dietary Supplements in the Prevention and Treatment of Disease in Older Adults*, Elsevier, 2015, pp. 143–149. doi: 10.1016/B978-0-12-418680-4.00015-4.

[38] Y. Vimala, K. V. Rachel, Y. Pramodini, and A. Umasankar, "**Usage of Indian Gooseberry (*Emblica officinalis*) Seeds in Health and Disease**," in *Nuts and Seeds in Health and Disease Prevention*, Elsevier, 2011, pp. 663–670. doi: 10.1016/B978-0-12-375688-6.10077-5.

[39] J. R. Patel, P. Tripathi, V. Sharma, N. S. Chauhan, and V. K. Dixit, "***Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review**," *J. Ethnopharmacol.*, vol. 138, no. 2, pp. 286–313, Nov. 2011, doi: 10.1016/j.jep.2011.09.040.

[40] B. C. Variya, A. K. Bakrania, and S. S. Patel, "***Emblica officinalis* (Amla): A review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms**," *Pharmacol. Res.*, vol. 111, pp. 180–200, Sep. 2016, doi: 10.1016/j.phrs.2016.06.013.

[41] S. S. Yadav, M. K. Singh, P. K. Singh, and V. Kumar, "**Traditional knowledge to clinical trials: A review on therapeutic actions of *Emblica officinalis***," *Biomed. Pharmacother.*, vol. 93, pp. 1292–1302, Sep. 2017, doi: 10.1016/j.biopha.2017.07.065.

List of Bibliography (Continue)

[42] J. C. Dekkers, L. J. P. van Doornen, and H. C. G. Kemper, "**The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage**," *Sports Med.*, vol. 21, no. 3, pp. 213–238, Mar. 1996, doi: 10.2165/00007256-199621030-00005.

[43] S. Pientaweeratch, V. Panapisal, and A. Tansirikongkol, "**Antioxidant, anti-collagenase and anti-elastase activities of *Phyllanthus emblica, Manilkara zapota* and silymarin: an *in vitro* comparative study for anti-aging applications**," *Pharm. Biol.*, vol. 54, no. 9, pp. 1865–1872, Sep. 2016, doi: 10.3109/13880209.2015.1133658.

[44] V. Tiwari, A. Kuhad, and K. Chopra, "***Emblica officinalis* corrects functional, biochemical and molecular deficits in experimental diabetic neuropathy by targeting the oxido-nitrosative stress mediated inflammatory cascade: *Emblica officinalis* ameliorates diabetic neuropathy in rats**," *Phytother. Res.*, vol. 25, no. 10, pp. 1527–1536, Oct. 2011, doi: 10.1002/ptr.3440.

[45] A. J. Gandhi, A. Kulkarni, M. Bora, and L. Hiray, "**Antimicrobial activity of *phyllanthus emblica* – a medicinal plant**," *Clin. Med.*, vol. 08, no. 2, p. 6, 2020.

[46] X. Liu, M. Zhao, J. Wang, and W. Luo, "**Antimicrobial and antioxidant activity of emblica extracts obtained by supercritical carbon dioxide extraction and methanol extraction**," *J. Food Biochem.*, vol. 33, no. 3, pp. 307–330, Jun. 2009, doi: 10.1111/j.1745-4514.2009.00220.x.

[47] P. Meesup and S. Buachard, "**Efficiency of plant extracts against Staphylococcus aureus and Escherichia coli**," vol. 2, pp. 485–488, 2018.

[48] V. L. V. Mesquita and C. Queiroz, "**Enzymatic Browning**," in *Biochemistry of Foods*, Elsevier, 2013, pp. 387–418. doi: 10.1016/B978-0-08-091809-9.00010-8.

[49] Y. Jiang, X. Duan, H. Qu, and S. Zheng, "**Browning: Enzymatic Browning**," in *Encyclopedia of Food and Health*, Elsevier, 2016, pp. 508–514. doi: 10.1016/B978-0-12-384947-2.00090-8.

List of Bibliography (Continue)

[50] V. S. Sharov *et al.*, "Selective **fluorogenic derivatization of 3-nitrotyrosine and 3,4-dihydroxyphenylalanine in peptides: a method designed for quantitative proteomic analysis**," in *Methods in Enzymology*, Elsevier, 2008, pp. 19–32. doi: 10.1016/S0076-6879(08)01202-0.

[51] N. P. Nirmal, S. Benjakul, M. Ahmad, Y. A. Arfat, and P. Panichayupakaranant, "**Undesirable enzymatic browning in crustaceans: causative effects and its inhibition by phenolic compounds**," *Crit. Rev. Food Sci. Nutr.*, vol. 55, no. 14, pp. 1992–2003, Dec. 2015, doi: 10.1080/10408398.2012.755148.

[52] F. Solano, "**Melanins: skin pigments and much more—types, structural models, biological functions, and formation routes**," *New J. Sci.*, vol. 2014, pp. 1–28, Mar. 2014, doi: 10.1155/2014/498276.

[53] A. B. Lerner and T. B. Fitzpatrick, "**Biochemistry of melanin formation**," *Physiol. Rev.*, vol. 30, no. 1, pp. 91–126, Jan. 1950, doi: 10.1152/physrev.1950.30.1.91.

[54] L. Vámos-Vigyázó and N. F. Haard, "**Polyphenol oxidases and peroxidases in fruits and vegetables**," *C R C Crit. Rev. Food Sci. Nutr.*, vol. 15, no. 1, pp. 49–127, Sep. 1981, doi: 10.1080/10408398109527312.

[55] Y. Jiang, X. Duan, D. Joyce, Z. Zhang, and J. Li, "**Advances in understanding of enzymatic browning in harvested litchi fruit**," *Food Chem.*, vol. 88, no. 3, pp. 443–446, Dec. 2004, doi: 10.1016/j.foodchem.2004.02.004.

[56] P. Kaewubon, "**Inhibition of browning agents in bisected protocorm-derived callus of pigeon orchid (*Dendrobium crumenatum Swartz*)**," *Signature*, p. 172.

[57] T. Boeckx, A. L. Winters, K. J. Webb, and A. H. Kingston-Smith, "**Polyphenol oxidase in leaves: is there any significance to the chloroplastic localization**," *J. Exp. Bot.*, vol. 66, no. 12, pp. 3571–3579, Jun. 2015, doi: 10.1093/jxb/erv141.

[58] P. Thipnate and S. Sukhonthara, "Control **of enzymatic browning in apple and potato purees by using guava extract**," p. 10, 2015.

List of Bibliography (Continue)

[59] J. J. Sullivan *et al.*, "**Determination of total sulfite in shrimp, potatoes, dried pineapple, and white wine by flow injection analysis: collaborative study**," *J. AOAC Int.*, vol. 73, no. 1, pp. 35–42, Jan. 1990, doi: 10.1093/jaoac/73.1.35.

[60] T. Marshall, S. Simpson, and A. Stevens, "**Health care needs assessment in prisons: a toolkit**," *J. Public Health*, vol. 23, no. 3, pp. 198–204, Sep. 2001, doi: 10.1093/pubmed/23.3.198.

[61] L. A. Sayavedra-Soto and M. W. Montgomery, "**Inhibition of polyphenol oxidase by sulfite**," *J. Food Sci.*, vol. 51, no. 6, pp. 1531–1536, Nov. 1986, doi: 10.1111/j.1365-2621.1986.tb13852.x.

[62] S. George and B. Chaleeda, *Konjac Glucomannan:* **Production, Processing, and Functional Applications**. Taylor&Francis Group, 2020.

[63] V. Brozzoli, S. Crognale, I. Sampedro, F. Federici, A. D'Annibale, and M. Petruccioli, "**Assessment of olive-mill wastewater as a growth medium for lipase production by Candida cylindracea in bench-top reactor**," *Bioresour. Technol.*, vol. 100, no. 13, pp. 3395–3402, Jul. 2009, doi: 10.1016/j.biortech.2009.02.022.

[64] T. D'Amore *et al.*, "**Sulfites in meat: Occurrence, activity, toxicity, regulation, and detection. A comprehensive review**," *Compr. Rev. Food Sci. Food Saf.*, vol. 19, no. 5, pp. 2701–2720, Sep. 2020, doi: 10.1111/1541-4337.12607.

[65] U. Chanasut and N. Rattanapanone, "**Characterization and comparative study of polyphenol oxidases from four cultivars of thai solanum melogena fruits**," p. 16.

[66] Anon, *Applications of Biotechnology to Fermented Foods*. 1992.

[67] Y. Jiang, X. Duan, D. Joyce, Z. Zhang, and J. Li, "**Advances in understanding of enzymatic browning in harvested litchi fruit**," *Food Chem.*, vol. 88, no. 3, pp. 443–446, Dec. 2004, doi: 10.1016/j.foodchem.2004.02.004.

[68] S. Gawborisut and P. Srisakultiew, "**Possibility of controlling melanin black spot in shrimps without using sulfiting agents**," pp. 85–94, 2010.

List of Bibliography (Continue)

[69] M.-M. Wei and J. F. Gregory, "**Organic acids in selected foods inhibit intestinal brush border pteroylpolyglutamate hydrolase in vitro: potential mechanism affecting the bioavailability of dietary polyglutamyl folate**," *J. Agric. Food Chem.*, vol. 46, no. 1, pp. 211–219, Jan. 1998, doi: 10.1021/jf970662g.

[70] R. Yoruk and M. R. Marshall, "**Physicochemical properties and function of plant polyphenol oxidase: a review**," *J. Food Biochem.*, vol. 27, no. 5, pp. 361–422, Nov. 2003, doi: 10.1111/j.1745-4514.2003.tb00289.x.

[71] S. Saeidian, "**Effect of anti-browning agents on partial purified polyphenol oxidase of hawthorn**," *Nternational J. Adv. Biol. Biomed. Res.*, vol. 2, no. 8, p. 11, 2014.

[72] M. E. López-Caballero, Ó. Martínez-Álvarez, M. C. Gómez-Guillén, and P. Montero, "**Quality of Norway lobster (*Nephrops norwegicus*) treated with a 4-hexylresorcinol-based formulation**," *Eur. Food Res. Technol.*, vol. 222, no. 3–4, pp. 425–431, Feb. 2006, doi: 10.1007/s00217-005-0125-8.

[73] O. Martínez-Alvarez, M. E. López-Caballero, M. C. Gómez-Guillén, and P. Montero, "**The effect of several cooking treatments on subsequent chilled storage of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with different melanosis-inhibiting formulas**," *LWT - Food Sci. Technol.*, vol. 42, no. 8, pp. 1335–1344, Oct. 2009, doi: 10.1016/j.lwt.2009.03.025.

[74] M. E. López-Caballero, M. C. Gómez-Guillén, M. Pérez-Mateos, and P. Montero, "**A chitosan–gelatin blend as a coating for fish patties**," *Food Hydrocoll.*, vol. 19, no. 2, pp. 303–311, Mar. 2005, doi: 10.1016/j.foodhyd.2004.06.006.

[75] H. Mæhre, I.-J. Jensen, and K.-E. Eilertsen, "**Enzymatic pre-treatment increases the protein bioaccessibility and extractability in dulse (*Palmaria palmata*)**," *Mar. Drugs*, vol. 14, no. 11, p. 196, Oct. 2016, doi: 10.3390/md14110196.

[76] R. K. Saini and Y.-S. Keum, "C**arotenoid extraction methods: A review of recent developments**," *Food Chem.*, vol. 240, pp. 90–103, Feb. 2018, doi: 10.1016/j.foodchem.2017.07.099.

List of Bibliography (Continue)

[77] N. Koca, F. Karadeniz, and H. S. Burdurlu, "**Effect of pH on chlorophyll degradation and colour loss in blanched green peas**," *Food Chem.*, vol. 100, no. 2, pp. 609–615, Jan. 2007, doi: 10.1016/j.foodchem.2005.09.079.

[78] AL Gbouri and Hamzah, "**Evaluation of phyllanthus emblica extract as antibacterial and antibiofilm against biofilm formation bacteri**," *IRAQI J. Agric. Sci. IJAS*, vol. 49, 2018, doi: https://doi.org/10.36103/ijas.v49i1.217.

[79] J. Charoenteeraboon, C. Ngamkitidechakul, N. Soonthornchareonnon, K. Jaijoy, and S. Sireeratawong, "**Antioxidant activities of the standardized water extract from fruit of *Phyllanthus emblica* Linn.**," p. 6, 2010.

[80] Z. Albahr, S. Al-Ghamdi, J. Tang, and S. S. Sablani, "**Pressure-assisted thermal sterilization and storage stability of avocado puree in high barrier polymeric packaging**," *Food Bioprocess Technol.*, vol. 15, no. 11, pp. 2616–2628, Nov. 2022, doi: 10.1007/s11947-022-02904-2.

[81] R. C. Soliva-Fortuny, P. Elez-Martínez, M. Sebastián-Calderó, and O. Martín-Belloso, "**Effect of combined methods of preservation on the naturally occurring microflora of avocado purée**," *Food Control*, vol. 15, no. 1, pp. 11–17, Jan. 2004, doi: 10.1016/S0956-7135(02)00151-2.

[82] S. V. Rodríguez-Campos, P. Hernández-Carranza, R. Ávila-Sosa, I. I. Ruiz-López, and C. E. Ochoa-Velasco, "**Effect of natural extracts addition on antioxidant, color and sensory properties of avocado (*Persea americana* sp.) puree**," *J. Food Meas. Charact.*, vol. 14, no. 5, pp. 2623–2634, Oct. 2020, doi: 10.1007/s11694-020-00509-x.

[83] A. Magri, D. Cice, G. Capriolo, and M. Petriccione, "**Effects of ascorbic acid and melatonin treatments on antioxidant system in fresh-cut avocado fruits during cold storage**," *Food Bioprocess Technol.*, vol. 15, no. 11, pp. 2468–2482, Nov. 2022, doi: 10.1007/s11947-022-02892-3.

List of Bibliography (Continue)

[84] N. A. Hasan and I. M. Zulkahar, "**Isolation and identification of bacteria from spoiled fruits**," presented at the advances in civil engineering and science technology, Penang, Malaysia, 2018, p. 020073. doi: 10.1063/1.5062699.

[85] C. Ananchaipattana *et al.*, "**Bacterial contamination in retail foods purchased in Thailand**," *Food Sci. Technol. Res.*, vol. 18, no. 5, pp. 705–712, 2012, doi: 10.3136/fstr.18.705.

[86] T. Hatami, S. A. Emami, S. S. Miraghaee, and M. Mojarrab, "**Total phenolic contents and antioxidant activities of different extracts and fractions from the aerial parts of artemisia biennis willd**," p. 8, 2014.

[87] S. Jan, M. R. Khan, U. Rashid, and J. Bokhari, "**Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of monotheca buxifolia fruit**," *Osong Public Health Res. Perspect.*, vol. 4, no. 5, pp. 246–254, Oct. 2013, doi: 10.1016/j.phrp.2013.09.003.

[88] K. Duangmal and R. K. Owusu Apenten, "**A comparative study of polyphenoloxidases from taro (*Colocasia esculenta*) and potato (*Solanum tuberosum* var. Romano)**," *Food Chem.*, vol. 64, no. 3, pp. 351–359, Feb. 1999, doi: 10.1016/S0308-8146(98)00127-7.

[89] "Modifying AOAC Method 996.06 for FAME Analysis in Foods: Faster Throughput Using Hydrogen Carrier Gas".

[90] C. Faria, I. Vaz-Moreira, E. Serapicos, O. C. Nunes, and C. M. Manaia, "**Antibiotic resistance in coagulase negative staphylococci isolated from wastewater and drinking water**," *Sci. Total Environ.*, vol. 407, no. 12, pp. 3876–3882, Jun. 2009, doi: 10.1016/j.scitotenv.2009.02.034.

[91] M. Wainwright, N. C. Wickramasinghe, J. V. Narlikar, and P. Rajaratnam, "**Microorganisms cultured from stratospheric air samples obtained at 41 km**," *FEMS Microbiol. Lett.*, vol. 218, no. 1, pp. 161–165, Jan. 2003, doi: 10.1111/j.1574-6968.2003.tb11513.x.

List of Bibliography (Continue)

[92] E. Sánchez, E. Donat, C. Ribes-Koninckx, M. L. Fernández-Murga, and Y. Sanz, "**Duodenal-Mucosal bacteria associated with celiac disease in children**," *Appl. Environ. Microbiol.*, vol. 79, no. 18, pp. 5472–5479, Sep. 2013, doi: 10.1128/AEM.00869-13.

[93] V. Savini *et al.*, "**Meticillin-heteroresistant *Staphylococcus pasteuri* from an apheresis platelet product**," *J. Med. Microbiol.*, vol. 58, no. 11, pp. 1527–1528, Nov. 2009, doi: 10.1099/jmm.0.008193-0.

[94] W. Luo, M. Zhao, B. Yang, J. Ren, G. Shen, and G. Rao, "**Antioxidant and antiproliferative capacities of phenolics purified from *Phyllanthus emblica* L. fruit**," *Food Chem.*, vol. 126, no. 1, pp. 277–282, May 2011, doi: 10.1016/j.foodchem.2010.11.018.

[95] C. Chaiyasut, P. Kesika, K. Chaiyasut, P. Sittiyuno, S. Peerajan, and B. S. Sivamaruthi, "**Total phenolic content and free radical scavenging activity of representative medicinal plants of Thailand**," *Asian J. Pharm. Clin. Res.*, vol. 10, no. 11, p. 137, Nov. 2017, doi: 10.22159/ajpcr.2017.v10i11.20741.

[96] V. Lobo, A. Patil, A. Phatak, and N. Chandra, "**Free radicals, antioxidants and functional foods: Impact on human health**," *Pharmacogn. Rev.*, vol. 4, no. 8, p. 118, 2010, doi: 10.4103/0973-7847.70902.

[97] R. Puupponen-Pimi, L. Nohynek, H.-L. Alakomi, and K.-M. Oksman-Caldentey, "**Bioactive berry compounds novel tools against human pathogens**," *Appl. Microbiol. Biotechnol.*, vol. 67, no. 1, pp. 8–18, Apr. 2005, doi: 10.1007/s00253-004-1817-x.

[98] L. Wang, C. Hu, and L. Shao, "**The antimicrobial activity of nanoparticles: present situation and prospects for the future**," *Int. J. Nanomedicine*, vol. Volume 12, pp. 1227–1249, Feb. 2017, doi: 10.2147/IJN.S121956.

List of Bibliography (Continue)

[99] R. Salvador-Reyes and L. M. Paucar-Menacho, "**Optimization of the blanching time and temperature in the manufacture of Hass avocado pulp using low quality discarded fruits**," *Braz. J. Food Technol.*, vol. 22, p. e2018244, 2019, doi: 10.1590/1981-6723.24418.

[100] S. Schelbert *et al.*, "**Pheophytin pheophorbide hydrolase (pheophytinase) is involved in chlorophyll breakdown during leaf senescence in *Arabidopsis***," *Plant Cell*, vol. 21, no. 3, pp. 767–785, Apr. 2009, doi: 10.1105/tpc.108.064089.

[101] G. P. Scipioni, B. del V. Argüello, and M. E. Schmalko, "**The Effect of Mg2+, Cu2+ and Zn2+ pre-treatment on the color of yerba maté (*Ilex paraguariensis*) leaves**," *Braz. Arch. Biol. Technol.*, vol. 53, no. 6, pp. 1497–1502, Dec. 2010, doi: 10.1590/S1516-89132010000600027.

[102] T. Ngo and Y. Zhao, "**Retaining green pigments on thermally processed peels-on green pears**," *J. Food Sci.*, vol. 70, no. 9, pp. C568–C574, May 2006, doi: 10.1111/j.1365-2621.2005.tb08306.x.

[103] C. Weemaes *et al.*, "**Pressure-temperature degradation of green color in broccoli juice**," *J. Food Sci.*, vol. 64, no. 3, pp. 504–508, May 1999, doi: 10.1111/j.1365-2621.1999.tb15072.x.

[104] M. Alves-Bezerra and D. E. Cohen, "**Triglyceride metabolism in the liver**," in *Comprehensive Physiology*, R. Terjung, Ed., 1st ed. Wiley, 2017, pp. 1–22. doi: 10.1002/cphy.c170012.

[105] B. Wu, J. Wang, Y. Guo, Z. Pan, and H. Ma, "Effects of infrared blanching and dehydrating pretreatment on oil content of fried potato chips," *J. Food Process. Preserv.*, vol. 42, no. 3, p. e13531, Mar. 2018, doi: 10.1111/jfpp.13531.

[106] G. Palma-Orozco, J. G. Sampedro, A. Ortiz-Moreno, and H. Nájera, "**In situ inactivation of polyphenol oxidase in mamey fruit (*Pouteria sapota*) by microwave treatment**," *J. Food Sci.*, vol. 77, no. 4, pp. C359–C365, Apr. 2012, doi: 10.1111/j.1750-3841.2012.02632.x.

List of Bibliography (Continue)

[107] M. Abbas Ali, M. Anowarul Islam, N. H. Othman, and A. M. Noor, "**Effect of heating on oxidation stability and fatty acid composition of microwave roasted groundnut seed oil**," *J. Food Sci. Technol.*, vol. 54, no. 13, pp. 4335–4343, Dec. 2017, doi: 10.1007/s13197-017-2904-1.

[108] C. P. Carvalho, J. Bernal E., M. A. Velásquez, and J. R. Cartagena V., "**Fatty acid content of avocados (*Persea americana Mill*. cv. Hass) in relation to orchard altitude and fruit maturity stage**," *Agron. Colomb.*, vol. 33, no. 2, pp. 220–227, May 2015, doi: 10.15446/agron.colomb.v33n2.49902.

[109] J. A. Villa-Rodríguez, F. J. Molina-Corral, J. F. Ayala-Zavala, G. I. Olivas, and G. A. González-Aguilar, "**Effect of maturity stage on the content of fatty acids and antioxidant activity of 'Hass' avocado**," *Food Res. Int.*, vol. 44, no. 5, pp. 1231–1237, Jun. 2011, doi: 10.1016/j.foodres.2010.11.012.

[110] Y. N. Manaf, A. P. Rahardjo, Y. A. Yusof, M. N. Desa, and B. P. Nusantoro, "**Lipid characteristics and tocopherol content of the oils of native avocado cultivars grown in Indonesia**," *Int. J. Food Prop.*, vol. 21, no. 1, pp. 2758–2771, Jan. 2018, doi: 10.1080/10942912.2018.1564761.

[111] R. Ferreyra *et al.*, "**Identification of pre-harvest factors that affect fatty acid profiles of avocado fruit (*Persea americana Mill*) cv. 'Hass' at harvest**," *South Afr. J. Bot.*, vol. 104, pp. 15–20, May 2016, doi: 10.1016/j.sajb.2015.10.006.

[112] J. del P. Ramírez-Anaya, A. J. Manzano-Hernández, E. Tapia-Campos, K. Alarcón-Domínguez, and M. C. Castañeda-Saucedo, "**Influence of temperature and time during malaxation on fatty acid profile and oxidation of centrifuged avocado oil**," *Food Sci. Technol.*, vol. 38, no. 2, pp. 223–230, Mar. 2018, doi: 10.1590/1678-457x.33116.

List of Bibliography (Continue)

[113] P. Scartezzini, F. Antognoni, M. A. Raggi, F. Poli, and C. Sabbioni, "**Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblica officinalis Gaertn***," *J. Ethnopharmacol.*, vol. 104, no. 1–2, pp. 113–118, Mar. 2006, doi: 10.1016/j.jep.2005.08.065.

[114] R. Li *et al.*, "**Determination of the content of phenolic compounds and the changes of polyphenol oxidase and each index during browning of phyllanthus emblica at different storage temperatures**," *J. Food Qual.*, vol. 2022, pp. 1–12, Jul. 2022, doi: 10.1155/2022/3472725.

[115] A. Altunkaya and V. Gokmen, "**Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*)**," *Food Chem.*, vol. 107, no. 3, pp. 1173–1179, Apr. 2008, doi: 10.1016/j.foodchem.2007.09.046.

[116] B. Dincer, A. Colak, N. Aydin, A. Kadioglu, and S. Güner, "**Characterization of polyphenoloxidase from medlar fruits (*Mespilus germanica* L., Rosaceae)**," *Food Chem.*, vol. 77, no. 1, pp. 1–7, May 2002, doi: 10.1016/S0308-8146(01)00359-4.

[117] V. A. Neves, D. G. Picchi, and M. A. da Silva, "**Some Biochemical properties of polyphenoloxidase from spearmint (*Mentha arvensis*)**," *Braz. Arch. Biol. Technol.*, vol. 52, no. 4, pp. 1001–1010, Aug. 2009, doi: 10.1590/S1516-89132009000400025.

[118] C.-K. Ding, K. Chachin, Y. Ueda, and C. Y. Wang, "**Inhibition of loquat enzymatic browning by sulfhydryl compounds**," *Food Chem.*, vol. 76, no. 2, pp. 213–218, Feb. 2002, doi: 10.1016/S0308-8146(01)00270-9.

[119] M. G. Lindley, "**The impact of food processing on antioxidants in vegetable oils, fruits and vegetables**," *Trends Food Sci. Technol.*, vol. 9, no. 8–9, pp. 336–340, Aug. 1998, doi: 10.1016/S0924-2244(98)00050-8.

List of Bibliography (Continue)

[120] S. M. K. Hasan, L. Manzocco, K. Morozova, M. C. Nicoli, and M. Scampicchio, "**Effects of ascorbic acid and light on reactions in fresh-cut apples by microcalorimetry**," *Thermochim. Acta*, vol. 649, pp. 63–68, Mar. 2017, doi: 10.1016/j.tca.2017.01.008.

[121] A. Rouf *et al.*, "**Enzymatic browning of apple and its control by chemical treatment: A review**," *Int. J. Food Sci. Nutr.*, vol. 3, no. 3, pp. 81–88, 2018.

[122] M. V. Martinez and J. R. Whitaker, "**The biochemistry and control of enzymatic browning**," *Trends Food Sci. Technol.*, vol. 6, no. 6, pp. 195–200, Jun. 1995, doi: 10.1016/S0924-2244(00)89054-8.

[123] L. M. Mattos, C. L. Moretti, A. B. Chitarra, and M. E. Prado, "**Qualidade de alface crespa minimamente processada armazenada sob refrigeração em dois sistemas de embalagem**," *Hortic. Bras.*, vol. 25, no. 4, pp. 504–508, Dec. 2007, doi: 10.1590/S0102-05362007000400003.

[124] J. A. Guerrero-Beltrán, G. V. Barbosa-Cánovas, G. Moraga-Ballesteros, M. J. Moraga-Ballesteros, and B. G. Swanson, "**Effect of pH and ascorbic acid on high hydrostatic pressure-processed mango puree**," *J. Food Process. Preserv.*, vol. 30, no. 5, pp. 582–596, Oct. 2006, doi: 10.1111/j.1745-4549.2006.00090.x.

[125] P. K. Jain and D. Das, "**Pharmacognostic comparison of bacopa monnieri, cyperus rotundus, and emblica officinalis**," vol. 4, no. 4, 2016.

[126] D. O. Ukuku, V. Pilizota, and G. M. Sapers, "**Effect of hot water and hydrogen peroxide treatments on survival of salmonella and microbial quality of whole and fresh-cut cantaloupe**," *J. Food Prot.*, vol. 67, no. 3, pp. 432–437, Mar. 2004, doi: 10.4315/0362-028X-67.3.432.

[127] E. EliUz, "**Antimicrobial activity of citric acid against *Escherichia coli, Staphylococcus aureus* and *Candida albicans* as a sanitizer agent**," *Eurasian J. For. Sci.*, Sep. 2020, doi: 10.31195/ejejfs.787021.

List of Bibliography (Continue)

[128] P. Mayachiew and S. Devahastin, "**Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts**," *LWT - Food Sci. Technol.*, vol. 41, no. 7, pp. 1153–1159, Sep. 2008, doi: 10.1016/j.lwt.2007.07.019.

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