Changes of Physicochemical, Antioxidative and Sensory Properties of Pre-treated Slice Pear Cultivar During Frozen Storage

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DECLARATION

I hereby declare that the work embodied in this report is the result of my own work except for the excerpts and summaries which I have just described the sources.

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DPPH	2, 2'-diphenyl-1 picrylhydrazyl
Abs	Absorbance
FCR	Folin Ciocalteu reagent
DCIP	2, 6-dichlorophenolindophenol
Na_2CO_3	Sodium carbonate
HPO_3	Metaphosphoric acid
TPC	Total phenolic content
L*	Lightness
a*	Redness
b*	Yellowness
g	Gram
mg	Milligram
mm/s	Millimeter per second
w/v	Weight per volume
ml	Mililitre
µg	Microgram
µl	Microliter
°C	Degree celcius
±	Plus minus
SD	Standard deviation
≤	Less than or equal to
≥	More than or equal to

Changes of Physicochemical, Antioxidative and Sensory Properties of Pre-treated Slice Pear Cultivar During Frozen Storage

ABSTRACT

Pear is a typical fruit of temperate zones with high nutritive values and organoleptic properties. Pears were pre-treated with blanching, osmotic and both blanching and osmotic solution prior to freezing. Two varieties of pear fruit which are Asian pear and Packham pear were analyzed for physicochemical (colour and texture), antioxidative properties (ascorbic acid content, antioxidant activity and total phenolic content) and sensory acceptability. The pears were subjected to different pre-treatments prior to freezing such as blanching and osmotic solution during storage. The results show that colour attributes of frozen pears were significantly affected by treatment which in contrast to texture properties of frozen pears during storage. Ascorbic acid content of frozen Packham pear was recorded higher (6.38 ± 0.96 mg/100) after treated in osmotic solution compared to frozen Asian pear (4.62 ± 1.15 mg/100) in blanching treatment during storage. The antioxidant activity of frozen Packham pear using DPPH assay exhibited (84.61 ± 0.69 %) contains high scavenging activity on day 1 when treated in blanching treatment while for Asian pear (67.63 ± 2.37 %) in blanching treatment on day 3. Total phenolic content of the frozen pears were found higher in Packham pear (14.77 ± 0.00 mg GAE/g) in control sample whereas Asian pear (3.79 ± 0.00 mg GAE/g) in blanching treatment during storage. Sensory acceptability shows that frozen Asian pear treated in osmotic solution was highly rated among consumers with overall acceptance of 5.80 ± 0.85. Thus, combination of pre-treatment which were osmotic and blanching and freezing helps to retain the quality of frozen pears during period of storage.

Keywords: Antioxidant, ascorbic acid, frozen, osmotic solution, pear.

Perubahan Fizikokimia, Antioksida dan Ciri Deria Pra-rawatan pada Potongan Kultivar Pear Semasa Penyimpanan Beku

ABSTRAK

Pear adalah buah-buahan biasa dari zon sederhana dengan nilai khasiat yang tinggi dan sifat organoleptik. Pear telah dirawat dahulu dengan celur, osmotik, dan kedua-dua celur dan larutan osmotic sebelum pembekuan. Dua jenis buah pear iaitu pear Asia dan pear Packham dianalisis untuk fizikokimia (warna dan tekstur), sifat antioksida (kandungan asid askorbik, aktiviti antioksidan dan jumlah kandungan fenolik) dan kebolehterimaan deria. Pear telah mengalami pra-rawatan yang berbeza sebelum pembekukan seperti celur dan larutan osmotik semasa penyimpanan. Hasilnya menunjukkan bahawa sifat warna pear beku telah terjejas dengan ketara disebabkan rawatan yang mana bertentangan dengan sifat tekstur pear beku semasa penyimpanan. Kandungan asid askorbik beku Packham pear direkodkan lebih tinggi (6.38 ± 0.96 mg/100) dirawat dalam larutan osmotik berbanding pear Asia beku (4.62 ± 1.15 mg/100) dalam rawatan celur semasa penyimpanan. Aktiviti antioksidan Packham pear beku menggunakan assay DPPH mempamerkan (84.61 ± 0.69 %) mengandungi aktiviti penipisan yang tinggi pada hari pertama apabila dirawat dalam rawatan celur manakala untuk pear Asia (67.63 ± 2.37 %) dalam rawatan celur pada hari ke-3. Jumlah fenolik kandungan pear beku didapati lebih tinggi dalam pear Packham (14.77 ± 0.00 mg GAE / g) dalam sampel yang tidak dirawat manakala pear Asia (3.79 ± 0.00 mg GAE / g) dalam celur rawatan semasa penyimpanan. Penerimaan sensori menunjukkan bahawa pear Asia beku yang dirawat dalam larutan osmotik sangat diberi nilai di kalangan pengguna dengan penerimaan keseluruhan sebanyak 5.80 ± 0.85. Oleh itu, gabungan pra-rawatan seperti larutan osmotic dan celuran serta pembekuan membantu untuk mengekalkan kualiti pear beku dalam tempoh simpanan.

Kata kunci: Antioksidan, asid askorbik, beku, larutan osmotik, pear.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Pears are a member of Rosaceae (Rose) family, and are commonly called as pome fruits which is a fruit with featured compartmented core and Asian pears are called as "apple pears" because of their apple-like texture (Reiland & Salvin, 2015). Pears are rich in dietary fiber and a have high quality of vitamin C (Reiland & Salvin, 2015). Improvement of different techniques for preservation of food is very crucial as increasing demand for more natural and healthy products. Fruits also are acknowledge as a good and great source of antioxidants in human diet (Gebczynski, 2017).

Short shelf life of fresh produce often associated with enzymatic reaction that could lead to economic loss. Deterioration of fresh produce will lead to economic loss. Economy loss often occur resulting from spoilage of fresh produce during harvesting, transportation and storage. Besides, inappropriate processing method results in fast deterioration in fresh produce. During processing of food, nutrient value of food always altered and also will be reduced thus will lead to fast deterioration. Thus, pre-treatment of the fruits and freezing process will be one of the way to lengthen the shelf life of the products.

Frozen fruits also have gained popular owing to high quality and easily prepared (Suzanne, 2014). Freezing could be as a preservative method to maintain quality attributes (colour, texture) of fresh produce that change over time. Influence of pre-treatments on the quality attributes of frozen products will help to develop commercialized frozen fruit products. Plus, frozen products need to be stored below -18°C in order to decrease the rate of deteriorative reactions during storage time (Tsironi & Taoukis, 2017).

The objectives of this study are to determine physicochemical properties of frozen fruit treated with osmotic solution. Next is to determine antioxidative properties of frozen fruit treated with osmotic solution and to determine sensory acceptability of frozen fruit treated with osmotic solution.

The study will identify relationship between different pre-treatment prior to freezing with the nutritional composition of frozen fruit during storage at -21°C. The quality attributes of the final frozen product will be evaluated based on texture analysis and colour properties during storage. Then, determination of ascorbic acid content, antioxidant activity and sensory acceptability of the frozen pear will be conducted.

Pre-treatment of fruit will help to preserve fruits for longer time. Preservation method of fruits also is vary such as drying, freezing or canning. The other method used to preserve pear was canning. The purpose of this work is to enhance the fresh produce shelf life by using freezing as preservation method. Freezing preservation helps to retain the quality of products over long period of storage. Freezing also consist of combination effect of low temperature at which no growth of microorganism, reduction of chemical reaction caused by enzyme and delay the cellular metabolic reactions.

However, commercial application of frozen pear to be exported is limited (Agricultural and Processed Food Products Exports Development Authority [APEDA], 2007). The market for the frozen fruit is low compared with canned fruits. Besides, there are fewer studies on the impact of process condition on quality attributes of the final fruit product especially on pear.

1.2 Problem Statement

Short shelf life of fresh produce for example fruits and vegetables and fruits often associated with enzymatic reaction that cause browning eventually spoilage. When fresh fruits being harvested, it will undergo changes of chemical which can be the reason of spoilage and deterioration of the products. Presence of enzyme in fresh produce need to be inactivated in order to prevent deterioration of the products (Scanlon, Henrich, Whitaker, 2018). Thus, shelf life can be prolong.

In addition, deterioration of fresh produce also lead to economic loss. This is because fresh produce cannot be last longer and will degrade over time. Therefore, economy loss often occur resulting from spoilage of fresh produce during harvesting, transportation and storage.

Besides, inappropriate processing method results in fast deterioration in fresh produce. During processing of food, nutrient value of food always altered and also will be reduced thus will lead to fast deterioration. While the combination of pre-treatments and freezing storage could keep the quality of frozen pears, there is no commercialized frozen pear found in the Malaysia market yet.

1.3 Hypothesis

H_0: There are no effects of pre-treated frozen pears on physicochemical properties, antioxidative properties and sensory acceptability during storage.

H_1: There are effects of pre-treated frozen pears in osmotic solution on physicochemical properties, antioxidative properties and sensory acceptability during storage.

1.4 Objectives

1.3.1 To determine physicochemical properties (colour and texture) of pre-treated frozen pears during storage.

1.3.2 To determine antioxidative properties of pre-treated frozen pears treated during storage.

1.3.3 To determine sensory acceptability of pre-treated frozen pears.

1.5 Scope of Study

The study will identify relationship of having different pre-treatment prior to freezing with the nutritional composition of frozen pears during storage at -21°C. The quality attributes of the final frozen product will be evaluated based on texture analysis and colour properties during storage. Then, determination of ascorbic acid content, antioxidant activity, total phenolic content and sensory acceptability of the frozen pear will be conducted.

1.6 Significance of Study

Pre-treatment of fruit will aid to preserve fruits for a longer time. Preservation method of fruits also is vary such as drying, freezing or canning. The purpose of this study is to enhance the fresh produce shelf life by using suitable pre-treatment and freezing as preservation method. Freezing preservation helps to retain the quality of products over long period of storage. Freezing also consist of combination effect of low temperature at which no growth of microorganism, reduction of chemical reaction caused by enzyme and delay the cellular metabolic reactions. Therefore, this frozen fruit may become commercialized in the market.

CHAPTER 2

LITERATURE REVIEW

2.1 Pears

2.1.1 Physiology characteristics of pear fruits.

Pear is a climacteric fruit that belong to the genus of *Pyrus L.* (pear) of the family *Rosaceaea* (Rose family). European pear (*Pyrus communis* L.) and Asian pear (*Pyrus pyrifolia)* or 'nashi' are the two main type of pear cultivated around the world (United States Department of Agriculture [USDA], 2018). There are about 3000 varieties of pears but not all pears are pear shaped, refer to figure 2.1 and 2.2.

Packham pears are an Australian variety and also known as Packham Triumph and called as Barlett in United States. It has very long season and known for their sweet flavor and smooth consistency (Orange, 2018). Packham pear have white firm flesh but juicy with rich flavor. It will turn from bright green to golden yellow as it ripen.

Meanwhile Asian pears are uniform in colour (yellowish –tan) and shape more like apples and it is not a hybrid or mix of the two fruits, but it get their name due the shape and texture of an apple but the flavour of a pear (Huang, n.d). Asian pears do not change colour after being harvested, whereas some European pear do.

Figure 2.1: Asian pear

(Source: Wiktionary, 2018)

Figure 2.2 Packham pear

(Source: Miss Melons, 2018)

The pears are characterized by attractive flavor, aroma and juiciness and high content of potassium, fibre, vitamin C and iodine. Pear cause less allergic reaction compared to apples. Storage of pears are influenced by cultivar, condition of storage and fruit harvest maturity. Fruit quality associated with external condition and internal factors related to fruit structure (Konarska, 2013).

2.1.2 Chemical characteristics of pears

According to the US Department of Agriculture National Agriculture Statistics Service, US per-capita consumption of fresh pears was 2.8 lb in 2012. In 2010, all pear products consumption per capita was 7 lb. As cited in Reiland & Slavin (2015), about 60% of the US pear crop is sold freshly and 40% is processed and predominantly in canned product.

Pears are highly rich in nutrients which comprises of vitamin C, fiber, and potassium and source of phytochemicals, especially antioxidants. Pear also are high in dietary fiber and rich in fructose and sorbitol which contribute to laxative properties of the pears (Reiland & Slavin, 2015).

2.1.2.1 Ascorbic acid

According to Rickman (2007), ascorbic acid is a heat- labile and highly water soluble. The term heat-labile of vitamins come from when ascorbic acid loss due to thermal processing, water leaching or oxidation. Vitamin C could be describes as the general term for all compounds showing the biological activity of ascorbic acid (Bulut, 2015). The level of vitamin C in a products could be as indicator to measure degradation of other biologically active substances. Vitamin levels depend on the growing, conditions, cultivars, post-harvest handling, storage conditions and maturity of the edible portion.

Previous study by Skrede (1996) indicated that ascorbic acid degradation is influenced by several factors such as pretreatments, freezing process, time-temperature conditions, type of food and packaging during storage. Heat treatment such as hot water blanching could be done to eliminate ascorbic acid oxidase which cause enzymatic degradation (Leong & Oey, 2012).

Vitamin C level is higher when stored at lower temperature (Gebczynski *et al*, 2018). Furthermore, peeling lead to decrease in ascorbic acid contents as well as total phenolic content for more than 25% (Kevers *et al*, 2011). This suggest that storing of product in low temperature will retain the higher vitamin C content as high temperature could lower the vitamin C content.

As cited by Devi (2015), Paul & Southghate (1978) revealed that vitamin C content of 2.00 mg/100 g flesh of pear fruit was recorded. The literature review found 3.6 mg/100 g of ascorbic acid content in pathernakh reported by Saini & Grewal (1995) while comparatively higher value of 5.56 mg/100 g was given by Anju (1998). It is in contrast to the finding of Kaushal (2002), 125.00 mg/100 g of ascorbic acid in pear fruit was recorded. The ascorbic acid content of two varieties viz. 'Deveci' and 'Santa Maria' of *Pyrus communis* was noted 3.30 and 4.70 mg/100 ml, respectively (Ozturk *et al.*, 2009).

2.1.2.2 Antioxidant activity (DPPH)

Antioxidant capacity is determined by measuring the reaction between antioxidants and free radicals. Generally, there are two mechanism that used to test antioxidant capacity which is hydrogen atom transfer (HAT) or single electron transfer (ET) (Paixao *et al*, 2007). According to Huang, Ou & Prior (2005) HAT-based assays consist of free radical generator, an antioxidant, and a detector molecules that become oxidized in general whereas ET-mechanism involves one redox reaction of an antioxidant and assess antioxidant's reducing capacity (Benzie & Strain, 1999).

In addition, 2, 2-diphenyl-1-picrylhdrazyl (DPPH) assay was one of the assay to determine the antioxidant capacity. DPPH is a dark-colored crystalline powder composed of stable free-radicals molecules (Norma, Virginia, Ral, Jos & Cristbal, 2018). Figure 2.1.2.2 shows the structure of DPPH. Oxidation causes the solution containing DPPH and antioxidant changes colour to fade. This assay quantifies capacity of antioxidant by determining the absorbance decrease after oxidation. Antioxidant capacity was determined by the means of the antioxidant to delay colour loss (Wang, 2013).

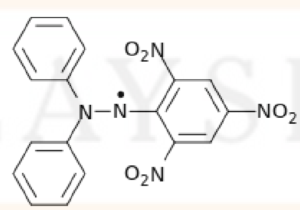

Figure 2.3: The structure of DPPH
(Source: Wikipedia, 2018)

Antioxidant activity of the fruits are based on the type of fruits, cultivars, period of storage and method of measurement (Connor *et al.*, 2002; Kalt *et al.*, 1999). Pear is an excellent source of antioxidant compounds such as phenolic, anthocyanin, and vitamin C (Li, Li, Fan, Tang & Yun, 2012). Previous studies by Poiana *et al.*, (2010) emphasize that during frozen storage (-18°C) of fruits, there was reduction in antioxidant capacity. Gebczynski *et al.*, (2018) found that retention rates for antioxidant activity against DPPH radical were between 45-56%. Meanwhile, study by Li *et al*, (2012), found arbutin and catechin as dominants compound in eight pears diversity.

2.1.2.3 Phenolics

Vitamin A and C, carotenoids and phenolics are the main bioactive compound found in fruits. Phenolic compounds function as reducing agents, hydrogen donor, metal chelators and singlet oxygen quenchers due to redox properties they have (Santiago *et al.*, 2016). From previous studies, antioxidants activity and phenolic content have significant correlation. De Ancos *et al.* (2000) found that total phenolic content in raspberry fruit slightly affected by freezing process. Decreasing reading of total phenolic content during storage occur owing to the enzyme polyphenol oxidase released from the cell wall of the fruit. It was reported in literature that frozen storage of fruit causes a slight decrease in phenolic compound (Rickman *et al.*, 2007). However, they also reported that frozen storage was suitable method to sustain phenolic compounds during long term storage (Hui, 2011) as freezing and long term storage periods did not affect the radical scavenging activity.

A study conducted by Cui, Nakamura, Ma and Kayahara (2005) found out that only a few phenolic compounds have been identified in Asian pear, which includes arbutin and chlorogenic acid compared to what exclusively identified in European pear. The peels part of the pear fruits have higher content of phenolic compound compared to pulp (Cui *et al.*, 2005; Salta *et al.*, 2010). Cui et al., (2005) also state the highest concentration of arbutin was found in the peel (1.20 mg/g FW), which was 3−5 times greater than that found in the core and 10−45 times greater than the level in the pulp.

2.2 Deterioration of fresh produce

2.2.1 Chilling injury

Chilling injury could be defined as a physiological disorder that take places in some plant parts result from low temperatures, above freezing temperature (Parkin *et al.,* 1989). The level of injury customarily a function of the temperature extreme, plant species, duration of exposure and morphological and physiological condition of the plant material at the time of exposure (Lyons *et al.,* 1979). Temperature threshold should be avoided in order to evade chilling injuries. According to Bramlage (1982), the problem is that low intensity of heat is the most efficient way to extend postharvest life of fresh produce as cited in Malik *et al.*, (2014), either by minimizing decay or decelerate the metabolic processes that contribute to deterioration (Couey, 1982). Examples of chilling sensitive temperate-zone crops include asparagus, apples, cranberries, nectarines, plums and peaches (Hardenburg *et al.,* 1986).

Morris (1982) studied a few signs of chilling injury. There are abrasion of surface which is large sunken areas, hole and discoloration, browning of pulp, vascular strands and seeds, increased susceptibility to decay and compositional changes, associated to flavor and taste. Pears are climacteric fruit and the level of maturity at harvest was very crucial (Martin, 2018). The time during ripening with the best flavour and eating quality was attained after cold storage of pears (Ben-Arie & Sonego, 1979).

Crisosto (2018) stated that the main worldwide consumer complaint is the internal browning of Asian pear. During storage, brown to dark brown water-soaked areas in the core or flesh occurred (Thompson, 2015). Occurrence of internal browning in Asian pear increased when cooling is delay, therefore immediate cooling was suggested. Storage life of Asian pear was limited to development of one or more maturity-related disorder. Therefore, it is important to keep high humidity (greater than 90%) in the storage atmosphere due to fruits are susceptible to water loss. Fruit become dehydrated and have a shriveled look when water loss has been greater than 5-7% (Crisosto, 2018).

2.3 Freezing

In modern society, freezing is a widely used preservation method. Freezing involves the process in which the temperature of a food is lowered below its freezing point and a proportion of water undergoes a change in state to form ice crystals (Fellows, 2000). The use of freezing as preservation methods has been applied since ancient years as great quality of product could be obtained (Li & Sun, 2002). Bolin & Huxsoll (1993) has discussed on application of freezing to fruits in which freezing preservation of fruit is less destructive toward some antioxidant compound compared to other method.

The articles written by Kyureghian *et al*, (2010) made a review on the effects of pre-freezing treatments, freezing step and frozen storage on the nutritional value of fruits and vegetables and their phytochemicals. There are four main important things in freezing process includes pre-treatment, freezing, frozen storage and thawing. Some of the factor that might influence nutritional content of the fruit are origin, harvesting year, storage time, pretreatment, processing time and conditions (Kyureghian *et al.*, 2010). In order to obtain high quality of product, it is importance to store at lowest temperature due to deteriorative process in fruits products are mainly temperature dependent (Harrison & Croucher, 1993).

2.3.1 Mechanism of Freezing

The freezing process composed of four main changes as shown in figure 2.3.1. Alexandre *et al.*, (2013) explain further about the cooling curve where the first stage known as pre-freezing stage, where temperature of the products drop to freezing point by emit heat. Second stage is super cooling stage where temperature drops below the freezing point. Third stage is freezing stage where latent heat is discarded and ice crystals are form. The last stage is sub-freezing stage where the food temperature is reduced to the storage temperature. Sun (2011) stated that rapid freezing methods result in small ice crystal and a better quality of frozen food could be achieved.

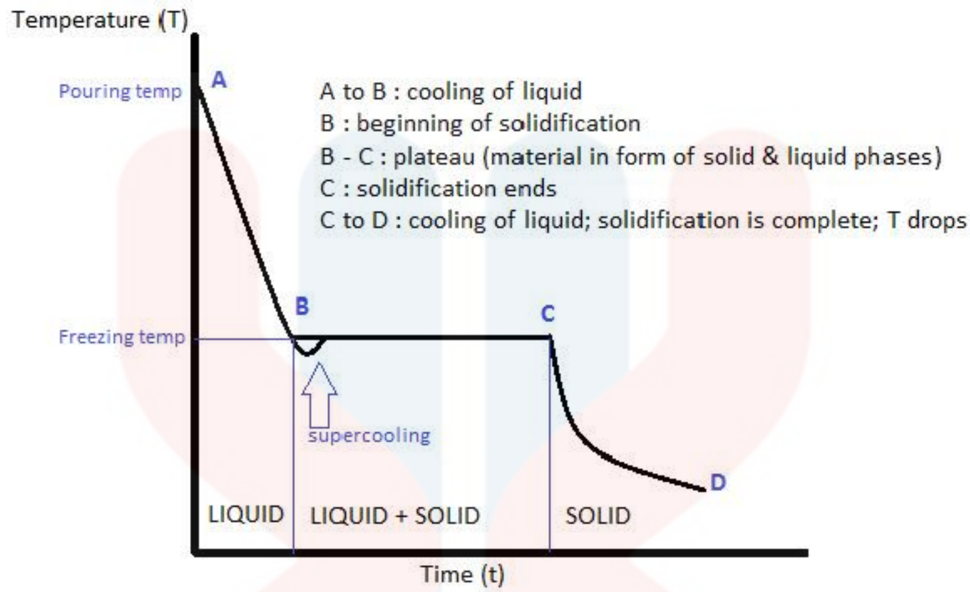

Figure 2.4: Cooling curve of freezing process.

(Souce: Chemmum, 2012)

During frozen of the fruits, water is crystallized as ice. Quality of the products were determined by the number and size of the crystals (Meryman, 1956; Woodroof & Shelor, 1958). A series of studies by Meryman (1956) indicated that with slow freezing, crystal nucleation usually restricted to the extracellular space and with rapid rates of freezing nucleation occurs throughout the medium in both extracellular and intracellular ice crystal formation (Chambers & Hale, 1932; Gane, 1955; Hanson, 1961; Joslyn & Marsh, 1933). Soluble solid concentration increased and water activity is lowered in the unfrozen region owing to ice formation in extracellular areas. Therefore, higher amount of ionic particles inside the cell due to the higher concentration of non-diffusible ions in cell than the surrounding fluid. As a consequences, freezing point was supposed to be lower in internal region. In the case of high subfreezing temperature, water inside the side may diffuse from the cells due to its high water activity (Sun, 2011).

Large ice crystals particularly result from slow freezing in extracellular area and causes shrinkage of cell. In contrast, fast freezing develop numerous small ice crystals that are uniformly distributed both in intracellular and extracellular spaces. Therefore, a frozen food product that looks alike unfrozen food was acquired (Sun, 2011).

2.3.2 Changes during freezing

Food compound such as protein, carbohydrate, pigments, enzymes and salts could undergo many changes or alteration in physical, biological and chemical when frozen. The level of changes is important to determine the quality of final product. Physical changes that take place during freezing usually depend on ice formation and osmotic action (Joslyn & March, 1933). The temperature decline and transformation of water to ice crystal are the most significant change that could be observed in the frozen food (Zhou, 2016). Freezing can remove water and reduce water activity in the food matrix by forming ice crystal. Low water activity is one of the criteria that ensure food safety.

Chemical changes that happen when preparation, freezing and thawing is significant to retain the good quality of final product. Example of chemical changes are irreversible denaturation of proteins, altered mass action relationship, hydrolysis of pectin and sucrose due to the presence of hydrolytic enzymes, changes in colour due to oxidation and flavor changes. Kramer, Wani, Sullivan & Shomer (1971) pointed out that slow freezing cause greater degree of solute concentration than rapid freezing. Upon freezing at lower temperature, chemical and enzymatic reactions decrease such as microbial growth and oxidation take place, however the product still exposed to deterioration, off-colour and off-flavor production. However, all fruit studies by Joslyn & Marsh (1933) did not show same level of deterioration. They observed that apples, apricots, avocado, peaches, pear and grapes are subjected to more severe color deterioration than berries.

2.3.3 Freezing and thawing

Certain amount of fluid will be lost when food is thawed. Plant tissue usually become soft upon thawing while in animal tissues, the effect is not severe. This might be due to the ruptured cell walls (Woodroof, 1938). He also stated that there is a direct relationship between loss of fluids by cell to the outside, degree of fragmentation of the precipitated protoplasm and the loss of original turgidity. He also reported that water movement in and out of cells upon freezing and thawing is a diffusion phenomenon as the water movement is fast and take place in dead and live cells. Joslyn & Marsh (1948) also added that other factors besides osmotic pressure are involved in loss of weight upon thawing. Despite that, when fruits are frozen without added syrup, the weight loss could be due to mechanical injury of the tissue.

2.4 Pre-Treatment

2.4.1 Blanching

Pre-treatment is widely used in order to inactivate enzyme and improve quality of frozen products (Deng *et al.,* 2017).Blanching is a low- intensive thermal treatment used mainly to destroy enzyme activity in vegetable and some fruits (Mascheroni, 2012). It is not the only method for preservation but as pre-treatment that execute between the raw material preparation and next operation. Usually, application of blanching is to determine the product quality. As mentioned by Suwan (2018) blanching and freezing combination has been proven as the most suitable preservation method for vegetables (Reyes de Corcuera *et al.,* 2004). Blanching is the critical step that will directly affect the quality of fruit. In fruit that have high water content, textural changes will become more clearly after thawing (Suwan, 2015). In contrary with study conducted by Kyureghian *et al.* (2010) stated that differently from vegetable that need to be blanched prior to freezing to inactivate some enzyme, fruits usually not blanched before freezing because of their nature and inherent acidity. Frozen product lose small numbers of nutrients at first due to short heating time in blanching, but lose more nutrients during storage as a result of oxidation. Stability of the product could be enhanced in blanching treatment for 3 min at 95°C under aseptic condition (Pittia *et al.*, 1999). Rani & Bhatia (1986) had studied on preserve and ready to eat product prepared form pear and found out the best preserve preparation on the diced fruits are blanch in hot water at 90°C for 3 minutes (Thompson, 2015) followed by gradual syruping of 40°Brix sugar syrup with 200 ppm of KMS and 0.2% citric acid.

2.4.2 Osmotic solution

Osmotic dehydration is the occurrence of water removal from lower concentration of solute to higher concentration through semi-permeable membrane results in the equilibrium condition in both side of the membrane (Tiwari, 2005). According to Khan (2012), osmotic dehydration involve partial removal of water from plant tissues by immersion in osmotic solutions. It is most preferred method because it can retain vitamin, minerals, colour, flavor and taste compared to other method. Osmotic dehydration also lower the fruits and vegetables water activity and Yadav & Singh (2012) reported that most appropriate osmosis was found at approximately 40 °C, 40 °B of osmotic agent and in near about 132 min. Example of osmotic agents are calcium chloride, ethanol, fructose, invert sugar, lactose, sucrose/sugar and it can be used singly or in combination. The chosen osmotic agents need to have characteristics such as effective, convenient, non-toxic, good taste, reduce water activity of solution and affordable.

As mentioned by Sharma, Pandey & Kumar (2003) moisture of pear could be reduced efficiently by osmosis and they found out change in syrup concentration from 35 to 45° brix resulted in more significant changes in water loss (18.09 to 23.18%). This show parameter are more influenced by syrup concentration than fruit to syrup ratio (Norões *et al*, 2010). Osmotic dehydration and freezing are both important method. In her study revealed the osmotic dehydration of mango was put through (30–50°C), immersion time (60–150 min) and concentration of solution (40–60% w/ w) in sucrose solution. In order to obtain the product similar with unprocessed fruit, water loss was maximum and solid incorporation was minimum (Khan, 2012).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw Materials

Asian pear *(Pyrus pyrifolia)*, Packham pear (*Pyrus communis* L.), sugar and distilled water.

3.1.2 Chemicals and Reagents

Ethanol, 2, 2'-diphenyl-1 picrylhydrazyl (DPPH), Folin Ciocalteu reagent, 2, 6-dichlorophenolindophenol (DCIP), sodium carbonate (Na_2CO_3), ascorbic acid, gallic acid, metaphosphoric acid (HPO_3).

3.1.3 Equipment

Knife, chopping board, beaker, pot, stove, thermometer, conical flask, burette, pipette, conical flask, retort stand, filter funnel, spectrophotometer (Sigma-Aldrich, German), test tube, measuring cylinder, chroma meter (Chroma Meter CR 400, Konica Minolta, Japan) and Brookfield CT3 Texture Analyzer, USA.

3.2 Sample Preparation

Fresh pears were obtained from local market at Pantai Timur, Kelantan. The pears were washed with tap water, peeled and cut into 16 pieces. Each slices of the pears were subjected to different single or combined pre-treatment before freezing. The pear slices were steam blanch at 95°C for 3 minutes. The osmotic dehydration was carried out by immersion of pear slices in osmotic solution at 32°C for 2 hours (Yadav & Singh, 2014). The osmotic solution was prepared with commercial sucrose and distilled water. Syrup concentration was 45° Brix.

Control sample was freshly slices pear which not subjected to any treatment. The second sample was treated with blanching only. Third sample was immersed in osmotic solution. Forth sample was subjected with both blanching and osmotic solution. All the samples were sealed in a sealer bag. Then, the samples were freeze at -21°C for 24 hours. The frozen samples were thawed at the room temperature for around 2 hours. The samples then were used for further analysis.

3.3 Colour Analysis

The colour properties of frozen pear during storage were determined by using chroma-meter. It was determined for day 1, day 2 and day 3. The samples colour value was expressed by the parameters (*L*, a*, b**) measured by colorimeter (Chroma Meter CR 400, Konica Minolta, Japan). L* indicates (whiteness or brightness/darkness), a* (redness/greenness) and b* (yellowness/blueness) (Alhamdan, Hassan, Alkahtani, Abdelkarim, & Younis, 2018).

3.4 Texture profile analysis

The textural properties of the frozen pears were determined by texture profile analysis. Texture profile analysis was performed to all samples by using Brookfiled CT3 Texture Analyzer. TPA of frozen pear was carried out by compressing them with TA4/1000 (cylinder) probe. The studies were conducted at test speed 1.0 mm/s, load cell 5 000g, and trigger load 5g (Raquel & Bruno, 2013). The textural parameters measured were hardness, cohesiveness and chewiness.

3.5 Determination of ascorbic acid content

The vitamin C content was determined by the official AOAC method of dye-titration. 3% (w/v) Metaphosphoric acid (HPO_3) was prepared by dissolving the sticks of HPO_3 in distilled water (Rangnna, 2017). Ascorbic acid standard was prepared by weighing 100mg of L- ascorbic acid and make up to 100 ml with 3% HPO_3 solution (Rangnna, 2017) and dye solution was prepared by dissolving 50 mg of the sodium salt 2.6-dichloroindophenol in approximately 150 ml of hot distilled water containing 42 mg sodium bicarbonate. The dye solution was cooled, filtered and dilute with distilled water to 200 ml.

The standardization of dye was carried out by pipetted out 5 ml of the standard ascorbic acid solution into 100 ml conical flask and 5 ml of the 3% HPO_3 solution was added (Rangnna, 2017). The ascorbic acid solution was titrated against dye solution until it turn into pink colour. Dye factor was calculated using following formula:

$$\text{Dye factor} = \frac{0.5}{\text{Titre (V)}} \qquad \text{Equation 1}$$

The sample was prepared by blend 10-20 g of sample with 3% HPO_3 solution and make up to 100 ml in a volumetric flask with 3% HPO_3. Then, filtered through Whatman No. 1 filter paper. The sample extract were pipetted out around 2-10 ml into conical flask and titrated against dye solution (Rangnna, 2017). End point of the titration will be detected when excess of the unreduced dye give a rose pink colour in acid solution (Tee, Young, Ho, & Mizura, 1988). Ascorbic acid content of the sample in mg per 100 was calculated as follow:

$$= \frac{\text{dye factor} \times V2 \times 10{,}000}{V_1 \times W} \qquad \text{Equation 2}$$

Where: W=weight of sample taken for extraction with HPO_3

V_1 = Volume of sample extract taken for dye titration

V_2 = Volume of dye required (titre)

3.6 Determination of Antioxidant Activity

The antioxidant activity was determined by the DPPH assay, as described by Franco *et al*., (2007). 1 Mm DPPH solution was prepared by dissolving 4 mg of powdered DPPH in 100ml ethanol. The bottle was swirled until the mixture was homogenous. 5 mg of sample extracts were dissolved in ethanol to and different concentrations of pear extracts (Salta *et al,* 2010), ranging from 6.25 to 200 μg/mL, was prepared. Then, each extract concentration (2 mL) was added with 2 ml of DPPH ethanolic solution and test tubes were shaken vigorously (Salta *et al*, 2010). The bleaching of DPPH was measured at 517 nm, after 30 min of reaction, at 25°C (Salta *et al,* 2010). The ability to scavenge the DPPH radical was calculated as follows:

$$\text{DPPH-scavenging effect (\%)} = \frac{\text{(Abs Control - Abs Sample)}}{\text{Abs Control}} \times 100 \qquad \text{Equation 3}$$

where, Abs Control is the absorbance of the control reaction (containing all reagents, except the extract) (Salta *et al.*, 2010).

3.7 Determination of Total Phenolic Content

The amount of total phenolics in the frozen pear was determined with the Folin Ciocalteu reagent according to the method of Slinkard & Singleton (1977) using gallic acid as a standard. Samples (1000 µL,) was added with 1.5 mL of Folin-Ciocalteu's reagent and was held for 5 minutes. Then, 2 mL of Na_2CO3_3 (7.5%) will added. The absorbance of all samples was measured at 765 nm using the spectrophotometer after incubating at 30 °C for 2 hours. Calibration curve was constructed for the solvents with gallic acid concentration in range 0.1-100µg/ml. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh weight (Zheng & Wang, 2001) using this equation:

$$T = C \times V/M \qquad \text{Equation 4}$$

Where T= total phenolic content in mg/g of the extract as GAE

C= concentration of galic acid established from calibration curved

V= volume of extract solution in ml

M= weight of the extract in g

3.8 Sensory analysis

Tests were performed on a group of 30 consumers range ages around 22-27 years old. Pear slices sample were placed in a plastic and labeled from A to H. Consumer acceptance was measured as degree of liking (Predieri & Gatti, 2009). As study in Predieri & Gatti, (2009) consumers were asked to taste the samples of pear slices on attributes of colour, hardness, chewiness, sweetness and overall acceptance for each sample to indicate the degree of liking on a seven-point hedonic scale (1 = extremely dislike; 2 = dislike very much; 3= dislike moderately; 4; neither like nor dislike; 5 = like moderately; 6 = like very much; 7 = extremely like) (Lawless & Heymann, 1998).

3.9 Statistical Analysis

All measurements were performed in triplicates and the results were expressed as mean ± SD (standard deviation). Data collected will be subjected to one-way ANOVA for each of the samples and the examined parameters. Statistical analysis was performed using Microsoft Office Excel 2013 (Microsoft Incorporation) and Statistical Package for the Social Science (SPSS) (IBM Corporation) with post-hock Tuckey for all analysis except sensory analysis which use Duncan test. Differences at $p \leq 0.05$ were considered to be significant.

CHAPTER 4

RESULTS & DISCUSSION

4.1 Colour Properties Of Pre-Treated Frozen Pears During Storage

Figure 4.1 show the value for lightness (*L**) of frozen Asian pear and Packham pear treated with different treatments during storage at day 1, day 2 and day 3. The results show both frozen pears exhibit a significant difference at $p \leq 0.05$ with different treatment during storage. For Asian pear, the highest *L** of 62.52 value was recorded in osmotic solution treatment, whereas, lowest value 53.58 was obtained in blanching treatment. For Packham pear treated with osmotic solution also shows the highest *L** value (65.67) and lowest value, 55.12 was found in blanching treatment. Decreasing trend of *L** value throughout the storage from day 1 to day 3 also could be observed on both frozen pears. Lightness of the pear refers to how lightly or darkly the colour is. The higher the *L** value of fresh-cut pear indicated that the brighter the cutting surface. Previous study also reported that *L** values decreased with storage duration (Li *et a*l, 2012).

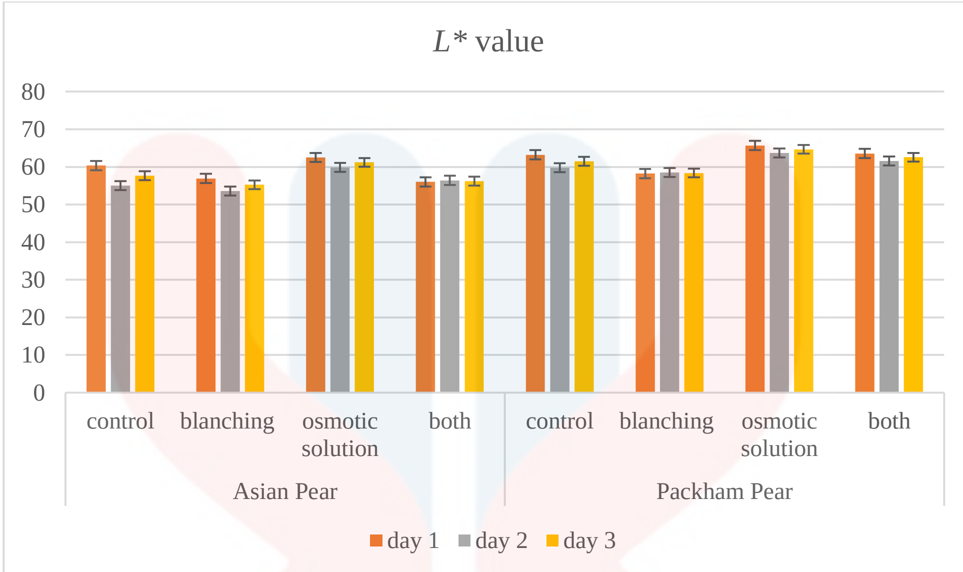

Figure 4.1: Comparison of *L** value for Asian pear and Packham pear during storage. Error bar denotes standard deviation.

Redness (*a**) value of frozen Asian pear and Packham pear with different treatment during storage at day 1, day 2 and day 3 were shown in the figure 4.2. *A** value increase rapidly in control sample for Asian pear at day 1 to day 3 from 2.02 to 4.73. Packham pear also shows the same trend for control samples which the reading increase from 1.06 to 4.18 during storage. The lowest *a** value in Asian pear was recorded when treated in both blanching and osmotic solution at reading of 1.93, 1.83 and 2.32 on day 1, day 2 and 3, respectively. Both blanching and osmotic solution treatment in Packham pear also give lower *a** value at 0.55, 0.59 and 0.89 on day 1, day 2 and day 3, respectively.

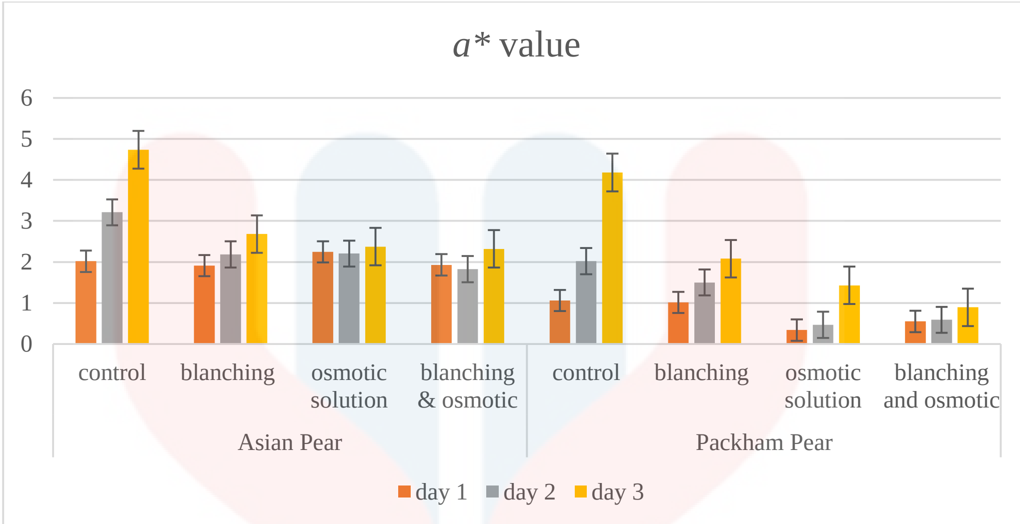

Figure 4.2: Comparison of *a** value for Asian pear and Packham pear during storage. Error bar denotes standard deviation.

Positive *a** values indicates the red colour in the samples, whereas negative *a** intensify the green colour in the samples (Guiné & Marques, 2013). High value of *a** indicates the sample was subjected to browning. Therefore, untreated samples were subjected to browning faster than pears which undergo pre-treatment. When undergo pre-treatments such as blanching, immerse in osmotic solution and both blanching and osmotic solution, the pears could be preserved longer than without pre-treated pears as no treatment shows rapidly increasing of *a** values throughout the days as shown in figure 4.1.2. Both pre-treatments which were blanching and osmotic solution give better result compared to other treatment as the browning of the samples could be delayed during frozen storage.

Figure 4.3 show the value for yellowness (*b**) of frozen Asian pear and Packham pear treated with different treatments during storage at day 1, day 2 and day 3. Yellowness colour of the sample was shown with the positive value of *b**, whereas negative values of b* indicate the blue colour in the sample. The lowest *b** value in Asian pear was found in non-treated pear with the reading 14.67 on day 1 while the highest value was 20.16 found on non-treated pear on day 3. This show the increasing trend of control sample throughout the storage from 14.67 to 20.17. Other treatment do not show much changes throughout the storage. For Packham pear, lowest *b** value was recorded in blanching treatment, 18.18 and highest value 26.3 was recorded in control pear. Between Asian pear and Packham pear, the highest *b** value (26.3) was found in Packham pear and the lowest *b** value (14.67) was in control Asian pear. Overall, the colour scores presented in *l* a** and *b** value revealed that the colour of frozen pears were significantly affected by treatments as well as storage intervals as the p value $p \leq 0.05$, especially for *a** value as it indicates the browning of the samples.

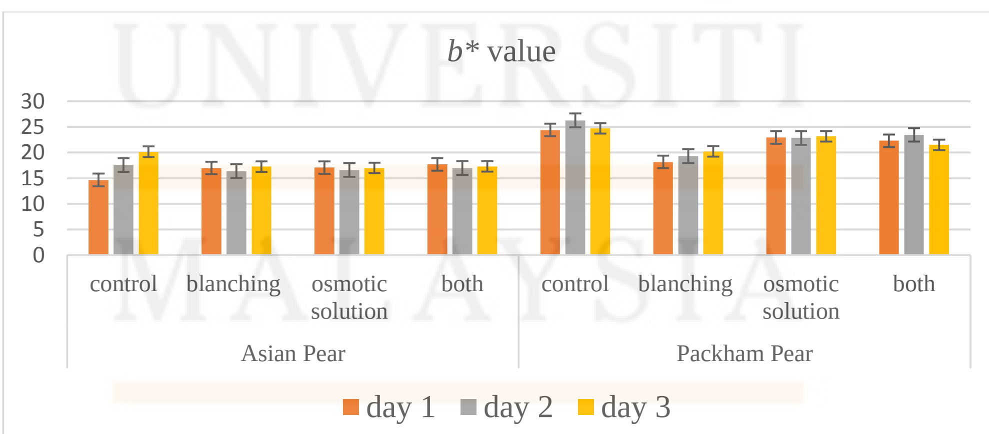

Figure 4.3: Comparison of *b** value for Asian pear and Packham pear during storage. Error bar denotes standard deviation.

4.2 Texture Attributes of Pre-Treated Frozen Pears During Storage

The hardness of the samples were presented in figure 4.4. Hardness often associated with the tensile strength of the sample (Guine & Marques, 2013). The result shows that the highest hardness value was found in control Asian pear at 5071 g while the lowest value (5018 g) was found in blanching treatment. For Packham pear, highest hardness value, 5057 was found in blanching and osmotic treatment while lowest value, 5014 g in control as well as blanching and osmotic solution treatment. Overall, throughout the days, hardness of the samples were decreasing.

The control of the samples show rapidly decreasing from day 1 to day 3. Samples with blanching treatment decrease from day 1 to day 2 but increase on day 3. Pre-treatments in osmotic solution show slowly decreasing of hardness which is 5052, 5038, 5027 for day 1, day 2 and day 3, respectively for Asian pear and for Packham pear 5034, 5027.5 and 5017.5 for day 1, day 2 and day 3. These results are accordance to what was reported by Raquel & Bruno (2013), the hardness of the samples will be reduced upon introduced to freezing due to sublimation of the frozen water lead to very soft product. Plus, textural properties of the fruits were influenced by freezing and freezing method (Alhamdan *et al.*, 2015).

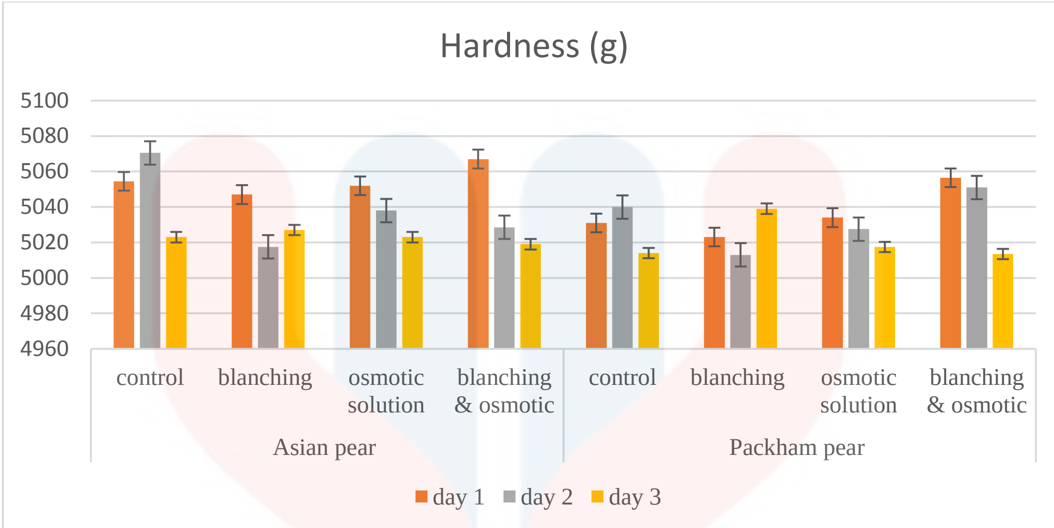

Figure 4.4: Comparison of hardness value of Asian and Packham pear with different treatment during storage. Error bar denotes standard deviation.

Cohesiveness could be regard as the ratio between the work done in the second compression and the work done in the first compression, and reflects the ability of the product to stay in one (Guine & Marques, 2013). According to table 4.2.3 and table 4.2.4, Asian pear shows highest cohesiveness value, 1.11 in control sample where the lowest value, 0.53 was found in blanching treatment. Control for Asian pear has an increasing trend from day 1 to day 3 but in Packham pear, the control sample show decreasing in cohesiveness values. Highest cohesiveness value in Packham pear, 1.37 was recorded in control sample and the lowest value, 0.54 in blanching treatment. This finding indicates that treated samples were less cohesive than control samples.

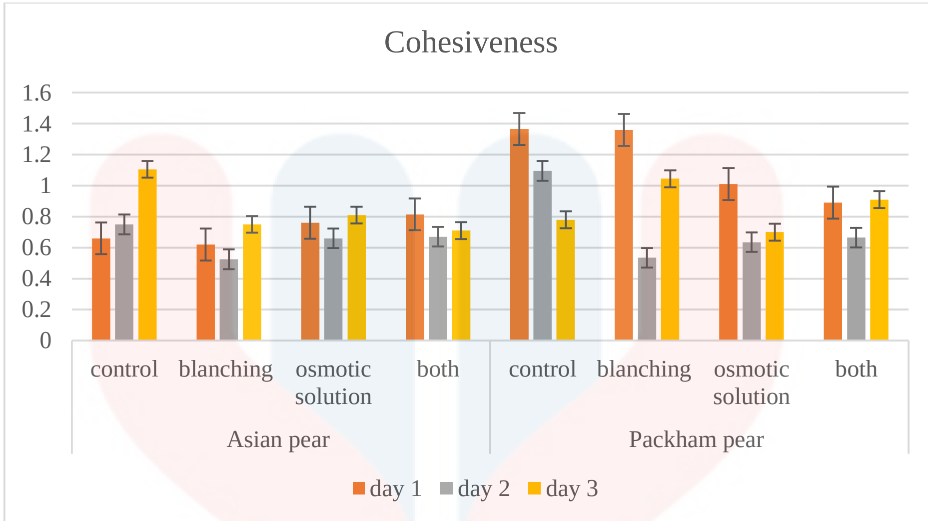

Figure 4.5: Comparison of cohesiveness value of Asian and Packham pear with different treatment during storage. Error bar denotes standard deviation.

Chewiness represents the energy required to disintegrate a solid material in order to swallow it (Guine & Marques, 2013). This textural parameter was directly related to other parameters such as hardness, cohesiveness and springiness. The result in figure 4.6 showed that chewiness was highest for control Asian pear at day 3 with value of 119 mJ while the lowest value of 27.10 mJ found in control sample on day 2. Chewiness value of Asian pear show increasing trend throughout the storage. For Packham pear, highest chewiness value, 90.35 mJ in blanching treatment and lowest chewiness value, 42.00 mJ in blanching and osmotic treatment. All the pre-treatment for Packham pear which is control, osmotic as well as blanching and osmotic treatments show increasing trend but not for blanching treatment. In general, from all the table of hardness, cohesiveness and chewiness of texture of frozen pears, there are no significantly difference between treatments and storage as p≥0.05.

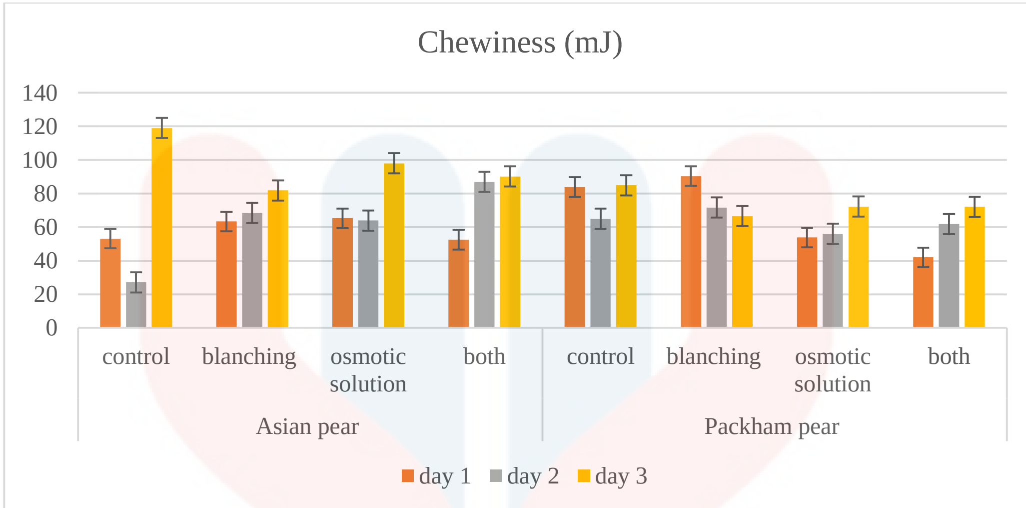

Figure 4.6: Comparison of chewiness value of Asian and Packham pear with different treatment during storage. Error bar denotes standard deviation.

4.3 Ascorbic Acid Content of Pre-Treated Frozen Pears During Storage

From the table 4.3 and 4.4, ascorbic acid content was found in the range of 1.20 to 6.38 mg/100 g in the tested frozen pears. Asian pear treated with blanching has highest ascorbic content at 4.62 mg/100 g while lowest ascorbic content 1.20 mg/100 g was found in control sample. Highest ascorbic acid content (6.38 mg/100 g) was found in treated Packham pear in osmotic solution on day 1 while lowest ascorbic acid content, 1.81 mg/100 g was record in blanching treatment on day 3. From the previous studies conducted by Chen *et al*., 2007; Sanchez *et al*., 2003; Edizer & Gunes 1997; Xie *et al*., 2007, the ascorbic content of pear fruit was in the range of 1.94 to 11 mg/100 g. This show that the current finding on ascorbic acid content of both frozen pears which in range of 1.2 to 6.41 mg/100 g were in line with the previous studies. However, some factors such as genotype difference, harvesting methods, pre-treatments and maturity of pears would affect the ascorbic acid content of the fruits.

Table 4.3: Ascorbic acid content of frozen Asian pear sample (mg/100) with different treatment during storage. (n=12)[1].

Sample	Treatment	Day 1 Mean ± SD	Day 3 Mean ± SD
Asian pear	Control	2.72 ± 0.85	1.20 ± 0.00^{b}
	Blanching	4.62 ± 1.15	2.38 ± 0.24^{ab}
	Osmotic solution	3.69 ± 1.20	2.48 ± 1.91^{ab}
	Blanching & osmotic	3.34 ± 1.11	3.27 ± 1.23^{a}

$^{a-c}$ Different superscript letter in the same column indicate significant differences among treatments (P≤0.05). [1] Data are means ± standard deviation.

Table 4.4: Ascorbic acid content of frozen Packham pear sample (mg/100) with different treatment during storage. (n=12)[1].

Sample	Treatment	Day 1 Mean ± SD	Day 3 Mean ± SD
Packham pear	Control	5.79 ± 1.75	2.17 ± 0.95^{b}
	Blanching	5.48 ± 1.82	1.81 ± 0.48^{b}
	Osmotic solution	6.38 ± 0.96	5.03 ± 1.01^{a}
	Blanching & osmotic	5.29 ± 0.96	5.22 ± 0.75^{a}

$^{a-c}$ Different superscript letter in the same column indicate significant differences among treatments (P≤0.05).

[1] Data are means ± standard deviation.

All pre-treatments subjected prior to freezing show decreasing of ascorbic acid content during storage from day 1 to day 3 for both pears. Blanching cause loss of ascorbic acid content from 4.62 mg/100 g on day 1 to 2.52 mg/100 g on day 3 for Asian pear. Ascorbic acid is a heat sensitive substance as reported by many researchers (Suna *et al*., 2014; Emese & Nagymate, 2008; Bello & Fowoyo, 2014). As stated by Tosun & Yücecan (2008), this losses may be due to leaching and thermal degradation of ascorbic acid to L-dehydro-ascorbic acid and further oxidation might occur. This statement also was supported by Bulut (2015), stating that ascorbic acid content of blanched green bean was reduced about 30%. During frozen storage, blanching significantly protects ascorbic acid in vegetables plus steam blanching provide better protection of ascorbic acid (Bulut, 2015).

Double pre-treatment which were blanching and osmotic on both Asian pear and Packham pear showed not much changes of ascorbic acid loss on day 1 and day 3. At the end of the storage ascorbic acid content of double pre-treatment were higher that control samples. Favell (1998) also found that in quick-frozen products, level of ascorbic was equal or much better than fresh product. Only treatment on day 3 was found significantly difference at $p \leq 0.05$ for both frozen pears whereas effects of treatments on day 1 was not significantly difference.

4.4 Antioxidant Activity (DPPH) of Pre-Treated Frozen Pears During Storage

The antioxidant activity of pears were determined by using DPPH radical scavenging assay. DPPH Scavenging activity (%) of frozen Asian pear treated with different treatment during storage at concentration of 200µl/ml was shown on the table 4.4.1. Different treatment subjected in Asian pear has significance difference at $p \leq 0.05$ throughout the storage.The highest antioxidant activity was found in pear treated with osmotic solution with the reading of 50.77%, whereas the lowest value 26.41% was observed in control sample. Control sample show decreasing trend during storage but other treatment show increasing value of % of inhibition during storage. The decrease in antioxidant activity might be due to decreasing of phenolic content during storage. The increasing trend of percentage of inhibition in all pre-treated samples might be due to the fruit itself which has many compound present and could retain the antioxidant compound from being scavenge. This trend also observed in apples where free radical scavenging activity increase during storage due to the synthesis of phenolic compounds (Silva, Gomes, Fidalgo, Rodrigues & Almeida, 2010). Therefore, it could be deduce that blanching treatment cause heat stress which induce synthesis and accumulation of high level of phenolic compound (Salveit, n.d.).

Table 4.5: DPPH Scavenging Activity (%) of frozen Asian pear with different treatment during storage at concentration of 200µl/ml. (n=12)[1].

Sample	Treatment	Day 1 Mean ± SD	Day 3 Mean ± SD
Asian pear	Control	49.17 ± 0.95^{b}	26.41 ± 0.00^{d}
	Blanching	42.69 ± 0.19^{c}	55.61 ± 1.86^{c}
	Osmotic solution	50.77 ± 0.19^{a}	67.63 ± 2.37^{a}
	Blanching & osmotic	36.03 ± 0.62^{d}	60.87 ± 0.58^{b}

[a-c] Different superscript letter in the same column indicate significant differences among treatments (P ≤0.05).

[1] Data are means ± standard deviation.

Table 4.6: DPPH Scavenging Activity (%) of frozen Packham pear with different treatment during storage at concentration of 200µl/ml. (n=12)[1].

Sample	Treatment	Day 1 Mean ± SD	Day 3 Mean ± SD
Packham pear	Control	39.81 ± 1.17[c]	29.52 ± 1.22[a]
	Blanching	84.61 ± 0.69[a]	22.49 ± 0.18[b]
	Osmotic solution	44.36 ± 0.48[b]	21.79 ± 0.09[b]
	Blanching & osmotic	18.85 ± 1.17[d]	17.50 ± 0.24[c]

[a-c] Different superscript letter in the same column indicate significant differences among treatments (P≤0.05).

[1] Data are means ± standard deviation.

In addition, Packham pear also has significance difference among treatment performed on the sample during storage as p≤0.05. The highest percentage (%) of inhibition for Packham pear (84.61%) was found in blanching treatment on day 1 and for day 3 (29.52%) in control sample. The lowest reading was obtained when Packham pear treated in both blanching and osmotic solution with the reading of 18.85% on day 1 while 17.50% on day 3 in the same treatment. Therefore, the highest scavenging activity was found in pear treated with blanching while the lowest value was found in blanching and osmotic treatment with regard of the day. DPPH scavenging activity (%) of frozen Packham pear show decreasing trend during storage from day 1 to day 3. This was supported by previous study by stated that owing to period of storage, free radical scavenging activity decrease on average of 42% in first 60 day in storage and remain constant during remaining storage (Silva *et al.*, 2010).

Based on previous studies conducted by Zhang *et al.*, (2006), found that capacities of pear extracts were similar among cultivars ranging from 79.3% to 92.0%. However, in the other paper by Ali (2013), antioxidant activity was found in the range of 27.96 to 46.73% among different cultivars. When comparing pear cultivars with other fruits, pear has low antioxidant activity (9.97-14.07%) (Ozturk *et al.*, 2009). These different findings on percentage of inhibition might be due to the different cultivars of pear used, processing method, harvesting and storage.

4.5 Total Phenolic Content Pre-Treated Frozen Pears During Storage

The data (table 4.7) in respect of total phenolic content of frozen Asian pear with different treatment during storage show significant different at $p \leq 0.05$ for the treatment and storage on day 1 only. The effect of different treatments showed that mean maximum phenol content was in blanching treatment on day 1 (3.79 mg GAE/g) and mean minimum in blanching treatment on day 3 (1.42 mg GAE/g). The interaction between treatments and storage indicated that maximum and minimum phenolic content was in blanching treatment. Throughout the storage studied, total phenolic content was decreasing for all the treatments conducted.

Table 4.7: Total phenolic content of frozen Asian pear with different treatment during storage at concentration of 100µl/ml. (n=12)[1].

Sample	Treatment	Day 1 Mean ± SD	Day 3 Mean ± SD
Asian pear	Control	3.30 ± 0.10[c]	2.51 ± 0.00
	Blanching	3.79 ± 0.00[a]	1.42 ± 0.00
	Osmotic solution	3.60 ± 0.00[b]	2.14 ± 0.00
	Blanching & osmotic	2.51 ± 0.00[d]	2.14 ± 0.00

[a-c] Different superscript letter in the same column indicate significant differences among samples (P≤0.05).

[1] Data are means ± standard deviation.

Table 4.8: Total phenolic content of frozen Packham pear with different treatment during storage at concentration of 100µl/ml. (n=12)[1].

Sample	Treatment	Day 1 Mean ± SD	Day 3 Mean ± SD
Packham pear	Control	14.77 ± 0.00[a]	2.51 ± 0.00[b]
	Blanching	5.25 ± 0.00[d]	4.89 ± 0.00[a]
	Osmotic solution	5.98 ± 0.00[c]	2.14 ± 0.00[c]
	Blanching & osmotic	6.29 ± 0.10[b]	2.45 ± 0.11[b]

[a-c] Different superscript letter in the same column indicate significant differences among treatments (P≤0.05). [1] Data are means ± standard deviation.

Total phenolic content of Packham pear were presented in table 4.8. Total phenolic content show significantly different between treatments and storage for both day 1 and day 3 as $p \leq 0.05$. The highest total phenolic content was found in Packham pear in control sample with the reading of 14.77 mg GAE/g while the lowest mean reading (2.14 77 mg GAE/g) was found in osmotic treatment at day 3. The interaction between treatments and storage indicated that maximum and minimum phenolic content was in control and osmotic treatment, respectively. Total phenolic content of frozen Packham pear also show decreasing trend during storage for all pretreatments as well as Asian pear. Rickman *et al.* (2007) in his reviewed paper, reported that frozen storage of fruits causes a small reduction in phenolic compounds.

This study show that there was variation of total phenolic content among pre-treatments of the pears. Several studies conducted by Spanos & Wrolstad, (1990); Lee *et al.*, (2003) suggest that total phenolic can vary with fruit variety. Prior research reported that total phenolic content was between 29 and 38 mg GAE/100g among pear cultivars (Hussain *et al*, 2013). In another studies total phenolic contents was identified in the pear sample ranged from 0.51± 0.001 mg/g in cultivar “Concordia” cultivar to 1.11 ± 0.013 mg/g in “Patten” cultivar (Liaudanskas *et al*, 2017). Studies conducted by Mrad *et al.*, (2012) suggest that total phenolic contents in pear peel was much higher than its flesh plus polyphenol degraded during drying and rate increased with increasing in temperature. This suggest that why the total phenolic content result was lower value found in current study due to the measured part was pulp not the peel as many researchers found higher total phenolic content in peel rather than pulp.

Furthermore, freezing and long-term frozen storage has an effect on total phenolic content as found out by De Ancos *et al.*, (2000), where the findings on phenolic content of raspberry fruit was slightly affected by freezing. The decreasing trend in phenolic content was because of enzyme polyphenol oxidase (PPO) releases from the fruit's cell wall during storage. The higher the activity of PPO, the higher the chances of polyphenols is oxidized. Thus, degradation of phenolic compounds could be the activity of PPO. This may contribute to the lower total phenolic content during storage.

4.6 Sensory Acceptability of Frozen Pears

Sensory evaluation of frozen Asian pear in term of colour, hardness, chewiness, sweetness and overall acceptance was shown in table 4.6.1. For colour attributes of frozen Asian pear, osmotic solution has highest score among treatment with 5.07 value and the lowest value with the score of 3.97 was in blanching and osmotic treatment. For hardness attributes, the osmotic treatment rated highest again with the mean value of 5.20 ± 1.06, and the lowest was blanching treatment at 3.97±1.13. Panelist mostly rated like moderately the hardness of osmotic treatment over with others pretreatment. Chewiness attributes also has osmotic solution with the highest mean (5.43±0.87) followed by control, both blanching and osmotic treatment and blanching treatment at 5.07±1.02, 5.00±0.87 and 4.13±1.11 respectively. For sweetness, highest mean value (5.97±0.81) recorded in osmotic treatment while the lowest mean (3.20±1.34) recorded in blanching treatment. For overall acceptance, highest mean was 5.80±0.85 in osmotic treatment and the lowest (3.83±1.09) in blanching treatment. According to hedonic scale, osmotic treatment was moderately like by most of the panels and blanching was dislike moderately. Previous literature has mentioned that sweetness could increase acceptability (Simon *et al.*, 1980; Alasalvar *et al.*, 2001). Thus, most panelist rate pear treated in osmotic solution higher than other treatment.

Table 4.9: Sensory acceptability of frozen Asian pear with different treatment (n=120)[1]

Treatment	Colour Mean ± SD	Hardness Mean ± SD	Chewiness Mean ± SD	Sweetness Mean ± SD	Overall acceptance Mean ± SD
Control	4.63 ± 1.16^{ab}	4.63 ± 1.03^{b}	5.07 ± 1.02^{a}	4.17 ± 1.42^{c}	4.90 ± 0.15^{b}
Blanching	4.10 ± 1.40^{b}	3.97 ± 1.13^{c}	4.13 ± 1.11^{b}	3.20 ± 1.35^{d}	3.83 ± 1.09^{c}
Osmotic solution	5.07 ± 1.29^{a}	5.20 ± 1.06^{a}	5.43 ± 0.82^{a}	5.97 ± 0.81^{a}	5.80 ± 0.85^{a}
Blanching & osmotic	3.97 ± 1.43^{b}	4.73 ± 0.87^{ab}	5.00 ± 0.87^{a}	5.00 ± 1.53^{b}	4.60 ± 1.28^{b}

[a-c] Different superscript letter in the same column indicate significant differences among treatments ($P \leq 0.05$).

[1] Data are means ± standard deviation.

Overall, from ANOVA table C.8 in appendices, it was found that the significance value, $p \leq 0.006$ for colour and $p \leq 0.000$ for hardness, chewiness, sweetness and overall acceptance, which was smaller than 0.05. When p-value is less than 0.05, the null hypothesis is rejected. It can be concluded that there was a significant difference in the all attributes of sensory analysis of pretreatment on frozen Asian pear.

The statistical result in table 5.0 showed the sensory attributes of frozen Packham pear with different treatment. For colour attributes, osmotic solution has the highest mean (5.30±1.24) and the lowest mean (3.33±1.61) found in both blanching and osmotic treatment. For hardness attributes, the blanching and osmotic treatment rated highest mean value at 4.20±1.45, and the lowest was control sample at 3.50±1.68. From ANOVA table, the p value was 0.254 which was higher than 0.05. It indicated that the effect of treatments has no significant difference on hardness. The highest mean for chewiness was in both blanching and osmotic treatment at 4.53±1.33 and 3.60±1.67 was the lowest mean found in control sample. For sweetness attributes, highest mean value (5.67±0.99) was recorded in osmotic treatment and the lowest mean (3.43±1.10) was recorded in blanching. Sweetness was the main reason for consumer liking of European pear cultivar (Elkins *et al.*, 2008). Overall acceptance also has high mean value (4.87±1.22) in osmotic and lowest (3.90±1.13) in blanching. The panelist preferred osmotic over other pretreatments because the score for osmotic (4.87) was higher than other treatments.

From ANOVA table C.9 in appendices, colour, chewiness, sweetness and overall acceptance has significance difference because p value was lower than 0.05. However, hardness and have no significance different compared to colour, chewiness, sweetness, and overall acceptance. Between Asian pear and Packham pear, overall acceptance showed consumer choose pear treated with osmotic solution treatment due to the highest score obtain 5.80 for Asian pear and 4.87 for Packham pear.

Table 5.0: Sensory acceptability of frozen Packham pear with different treatment. (n=120)[1].

Treatment	Colour Mean ± SD	Hardness Mean ± SD	Chewiness Mean ± SD	Sweetness Mean ± SD	Overall acceptance Mean ± SD
Control	4.83 ± 1.05^{a}	3.50 ± 1.68	3.60 ± 1.67^{b}	4.67 ± 1.30^{b}	4.27 ± 1.36^{ab}
Blanching	3.63 ± 1.33^{b}	3.67 ± 1.24	3.90 ± 1.18^{ab}	3.43 ± 1.10^{c}	3.90 ± 1.13^{b}
Osmotic solution	5.30 ± 1.24^{a}	3.93 ± 1.34	4.07 ± 1.48^{ab}	5.67 ± 0.99^{a}	4.87 ± 1.22^{a}
Blanching & osmotic	3.33 ± 1.6^{b}	4.20 ± 1.45	4.53 ± 1.33^{a}	4.93 ± 1.34^{b}	4.43 ± 1.31^{ab}

$^{a-c}$ Different superscript letter in the same column indicate significant differences among samples ($P < 0.05$).

[1] Data are means ± standard deviation.

CHAPTER 5

CONCLUSION

5.1 Conclusion

In a conclusion, effects of pre-treatments which is blanching, osmotic and both blanching and osmotic treatments of frozen pears have better result in term of physicochemical, antioxidative and sensory properties compared to untreated frozen pears. Blanching and osmotic treatment could improve the colour properties of frozen pears. However, there are no significant effects observed in textural properties in term of hardness, cohesiveness and chewiness attributes among pre-treated pears and untreated pears. Asian pears introduced with blanching treatment has the highest ascorbic acid and phenolic compound while Packham pear treated with osmotic solution has the highest ascorbic acid. Ascorbic acid content, antioxidant activity and total phenolic content significantly decreased during frozen storage which was rapidly occur in untreated sample compared to pre-treated samples. Osmotic treatments and freezing storage are the best combination for preserving frozen pears which get the highest scores among consumers. Different pear cultivars give different effects on pre-treatment on physicochemical, ascorbic acid content, antioxidant, and total phenolic content and sensory properties. Overall, the frozen pears that had been pre-treated with osmotic solution and blanching tend to retain their quality and can be preserved for a longer time.

5.2 Recommendation

Future studies should be conducted on the nutritional value of the pears and determine whether different treatment does deterioration of nutritional quality over long period of storage as the present study could not performed for a longer period of time. In order to further commercialize the frozen pears, shelf life studies of the frozen pears under different storage condition on microbial and quality retention is very crucial.

Moreover, further investigation on the other enzymes besides PPO which might involve in polymerization reaction could help us to better understand antioxidant compound loss and color changes during frozen storage. In addition, different assays may be perform for further characterization of antioxidant activity such as oxygen absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP) and ABTS radical cation scavenging assay.

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APPENDIX A

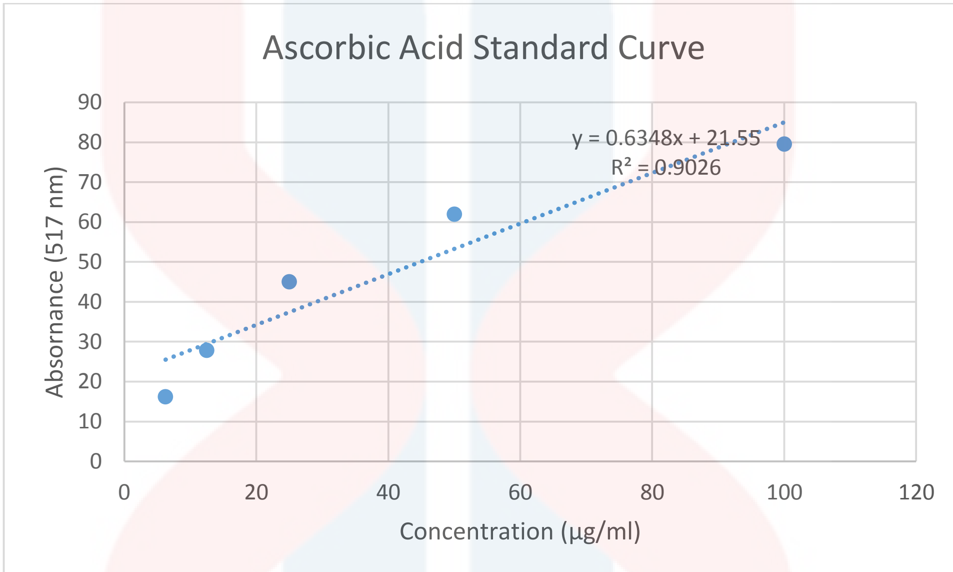

Figure A.1: Ascorbic Acid Standard Curve with concentration of 6.25, 12.5, 25, 50 and 100 µg/ml)

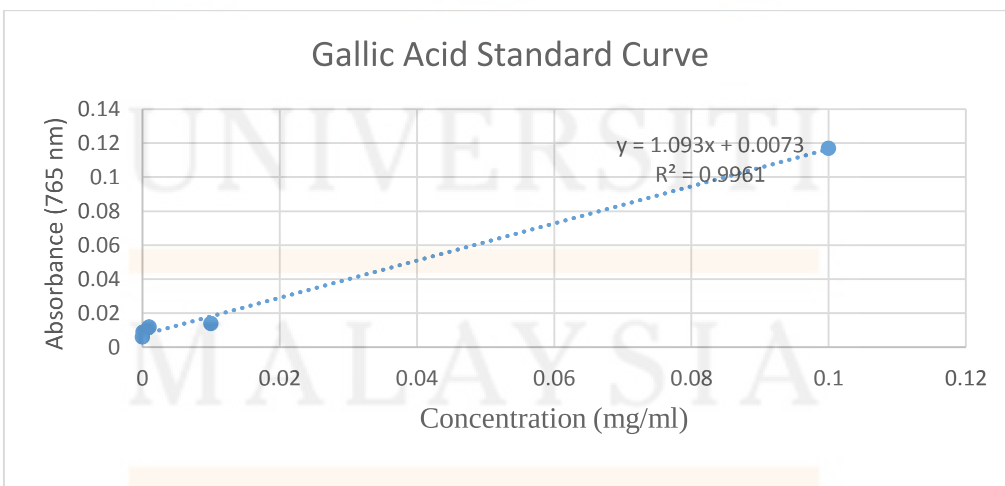

Figure A.2: Standard calibration curve of absorbance against concentration with gallic acid at concentration of 0.01, 0.1, 1, 50, 100 µg/ml.

APPENDIX B

SENSORY EVALUATION TEST OF EFFECTS OF PRE-TREATMENTS ON FROZEN PEAR

Age: ___ years Panel No:

Race: Malay / Chinese / Indian / Others Sample No:

Gender: Male / Female Date:

Directions: Please circle your degree of likeness based on the characteristics below.
Do rinse your mouth before and after testing each sample.

1. Colour

1 2 3 4 5 6 7

Extremely dislike Neither like nor dislike Extremely
like

2. Hardness

1 2 3 4 5 6 7

Extremely dislike Neither like nor dislike Extremely
like

3. Chewiness

1 2 3 4 5 6 7

Extremely dislike Neither like nor dislike Extremely
like

4. Sweetness

1 2 3 4 5 6 7

Extremely dislike Neither like nor dislike Extremely
like

5. Overall acceptance

1 2 3 4 5 6 7

Extremely dislike Neither like nor dislike Extremely

Figure B.1: Sensory Evaluation Form

APPENDIX C

Table C.1: ANOVA table of colour for Asian Pear

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
l value of pear A on day 1	Between Groups	82.315	3	27.438	15.295	.001
	Within Groups	14.352	8	1.794		
	Total	96.667	11			
l value on day 2	Between Groups	66.263	3	22.088	22.537	.000
	Within Groups	7.840	8	.980		
	Total	74.104	11			
l value on day 3	Between Groups	44.849	3	14.950	21.461	.000
	Within Groups	5.573	8	.697		
	Total	50.421	11			
a value on day 1	Between Groups	.210	3	.070	7.184	.012
	Within Groups	.078	8	.010		
	Total	.288	11			
a value on day 2	Between Groups	3.188	3	1.063	35.070	.000
	Within Groups	.242	8	.030		
	Total	3.430	11			
a value on day 3	Between Groups	11.877	3	3.959	413.844	.000
	Within Groups	.077	8	.010		
	Total	11.954	11			
b value on day 1	Between Groups	14.625	3	4.875	18.681	.001
	Within Groups	2.088	8	.261		
	Total	16.713	11			
b value on day 2	Between Groups	2.420	3	.807	3.726	.061
	Within Groups	1.732	8	.216		
	Total	4.152	11			
b value on day 3	Between Groups	20.106	3	6.702	19.422	.000
	Within Groups	2.761	8	.345		
	Total	22.867	11			

Table C.2: ANOVA table of colour for Packham pear

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
l value of on day 1	Between Groups	89.090	3	29.697	10.441	.004
	Within Groups	22.754	8	2.844		
	Total	111.844	11			
l value on day 2	Between Groups	45.280	3	15.093	7.903	.009
	Within Groups	15.278	8	1.910		
	Total	60.558	11			
l value on day 3	Between Groups	41.830	3	13.943	11.523	.003
	Within Groups	9.680	8	1.210		
	Total	51.510	11			
a value on day 1	Between Groups	1.120	3	.373	5.699	.022
	Within Groups	.524	8	.065		
	Total	1.643	11			
a value on day 2	Between Groups	4.979	3	1.660	98.310	.000
	Within Groups	.135	8	.017		
	Total	5.114	11			
a value on day 3	Between Groups	18.670	3	6.223	100.769	.000
	Within Groups	.494	8	.062		
	Total	19.164	11			
b value on day 1	Between Groups	64.417	3	21.472	13.528	.002
	Within Groups	12.698	8	1.587		
	Total	77.115	11			
b value on day 2	Between Groups	73.692	3	24.564	18.404	.001
	Within Groups	10.678	8	1.335		
	Total	84.370	11			
b value on day 3	Between Groups	35.282	3	11.761	25.510	.000
	Within Groups	3.688	8	.461		
	Total	38.971	11			

Table C.3: ANOVA table of texture for Asian pear

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
hardness value on day 1	Between Groups	434.375	3	144.792	1.940	.265
	Within Groups	298.500	4	74.625		
	Total	732.875	7			
hardness value on day 2	Between Groups	3130.375	3	1043.458	4.640	.086
	Within Groups	899.500	4	224.875		
	Total	4029.875	7			
hardness value on day 3	Between Groups	64.000	3	21.333	.057	.980
	Within Groups	1486.000	4	371.500		
	Total	1550.000	7			
cohesiveness value on day 1	Between Groups	.048	3	.016	.935	.502
	Within Groups	.069	4	.017		
	Total	.117	7			
cohesiveness value on day 2	Between Groups	.052	3	.017	.289	.832
	Within Groups	.241	4	.060		
	Total	.293	7			
cohesiveness value on day 3	Between Groups	.192	3	.064	1.112	.443
	Within Groups	.230	4	.058		
	Total	.423	7			
chewiness value on day 1	Between Groups	266.305	3	88.768	1.353	.376
	Within Groups	262.430	4	65.608		
	Total	528.735	7			
chewiness value on day 2	Between Groups	3770.170	3	1256.723	.407	.757
	Within Groups	12347.390	4	3086.848		
	Total	16117.560	7			
chewiness value on day 3	Between Groups	1519.170	3	506.390	.270	.845
	Within Groups	7506.790	4	1876.698		
	Total	9025.960	7			

Table C.4: ANOVA table of texture for Packham pear

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
hardness value on day 1	Between Groups	1236.375	3	412.125	.775	.566
	Within Groups	2126.500	4	531.625		
	Total	3362.875	7			
hardness value on day 2	Between Groups	1606.375	3	535.458	1.219	.411
	Within Groups	1756.500	4	439.125		
	Total	3362.875	7			
hardness value on day 3	Between Groups	883.000	3	294.333	2.251	.225
	Within Groups	523.000	4	130.750		
	Total	1406.000	7			
cohesiveness value on day 1	Between Groups	.355	3	.118	.340	.799
	Within Groups	1.391	4	.348		
	Total	1.746	7			
cohesiveness value on day 2	Between Groups	.369	3	.123	.257	.853
	Within Groups	1.914	4	.479		
	Total	2.283	7			
cohesiveness value on day 3	Between Groups	.137	3	.046	.715	.592
	Within Groups	.256	4	.064		
	Total	.394	7			
chewiness value on day 1	Between Groups	3254.770	3	1084.923	.709	.595
	Within Groups	6124.030	4	1531.008		
	Total	9378.800	7			
chewiness value on day 2	Between Groups	256.774	3	85.591	.046	.985
	Within Groups	7426.725	4	1856.681		
	Total	7683.499	7			
chewiness value on day 3	Between Groups	364.144	3	121.381	.199	.892
	Within Groups	2436.015	4	609.004		
	Total	2800.159	7			

Table C.5: ANOVA table of ascorbic acid content for Asian and Packham pear

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D1_AA_A	Between Groups	5.662	3	1.887	1.600	.264
	Within Groups	9.437	8	1.180		
	Total	15.100	11			
D3_AA_A	Between Groups	6.556	3	2.185	5.403	.025
	Within Groups	3.236	8	.405		
	Total	9.792	11			
D1_AA_B	Between Groups	2.046	3	.682	.332	.803
	Within Groups	16.454	8	2.057		
	Total	18.501	11			
D3_AA_B	Between Groups	29.580	3	9.860	14.627	.001
	Within Groups	5.393	8	.674		
	Total	34.973	11			

Table C.6: ANOVA table for DPPH for both Asian and Packham pear.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DPPH_A_D1	Between Groups	408.262	3	136.087	402.526	.000
	Within Groups	2.705	8	.338		
	Total	410.967	11			
DPPH_A_D3	Between Groups	2967.447	3	989.149	425.685	.000
	Within Groups	18.589	8	2.324		
	Total	2986.036	11			
DPPH_B_D1	Between Groups	6798.155	3	2266.052	2627.078	.000
	Within Groups	6.901	8	.863		
	Total	6805.056	11			
DPPH_B_D3	Between Groups	223.313	3	74.438	186.955	.000
	Within Groups	3.185	8	.398		
	Total	226.498	11			

Table C.7: ANOVA table for TPC on both frozen pears

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TPC_A_D1	Between Groups	2.863	3	.954	353.407	.000
	Within Groups	.022	8	.003		
	Total	2.884	11			
TPC_A_D3	Between Groups	1.874	3	.625	.	.
	Within Groups	.000	8	.000		
	Total	1.874	11			
TPC_B_D1	Between Groups	181.137	3	60.379	22362.546	.000
	Within Groups	.022	8	.003		
	Total	181.158	11			
TPC_B_D3	Between Groups	14.574	3	4.858	1614.825	.000
	Within Groups	.024	8	.003		
	Total	14.598	11			

Table C.8: ANOVA table for sensory acceptability of Asian pear

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
colour	Between Groups	23.092	3	7.697	4.409	.006
	Within Groups	202.500	116	1.746		
	Total	225.592	119			
hardness	Between Groups	23.267	3	7.756	7.338	.000
	Within Groups	122.600	116	1.057		
	Total	145.867	119			
chewiness	Between Groups	27.292	3	9.097	9.890	.000
	Within Groups	106.700	116	.920		
	Total	133.992	119			
sweetness	Between Groups	125.233	3	41.744	24.465	.000
	Within Groups	197.933	116	1.706		
	Total	323.167	119			
overall_acceptance	Between Groups	59.500	3	19.833	18.725	.000
	Within Groups	122.867	116	1.059		
	Total	182.367	119			

Table C.9: ANOVA table for sensory acceptability of Packham pear

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
colour	Between Groups	79.825	3	26.608	15.272	.000
	Within Groups	202.100	116	1.742		
	Total	281.925	119			
hardness	Between Groups	8.492	3	2.831	1.375	.254
	Within Groups	238.833	116	2.059		
	Total	247.325	119			
chewiness	Between Groups	13.692	3	4.564	2.232	.088
	Within Groups	237.233	116	2.045		
	Total	250.925	119			
sweetness	Between Groups	77.758	3	25.919	18.270	.000
	Within Groups	164.567	116	1.419		
	Total	242.325	119			
overall_acceptance	Between Groups	14.467	3	4.822	3.050	.031
	Within Groups	183.400	116	1.581		
	Total	197.867	119			