# Formation and Characterisation of Stirred Yoghurts Enriched with Avocado Pulp

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# Abstract

Avocado stirred yoghurt has huge market potential with the expanding market of fruit flavoured yoghurt and the increasing health concern of the public. However, the avocado is highly perishable due to its high content of monounsaturated fatty acids sensitive to lipid oxidation as well as its polyphenol compounds sensitive to enzymatic browning. Considering the conventional treatment like heating may not be suitable for use, there is a challenge of preserving the physicochemical properties and microbial stability of avocado added into yoghurt during storage.

This study aimed at investigating the methods of appropriate treatments of avocado for extending the shelf life of avocado-fortified stirred yoghurt and characterising the yoghurt in terms of various properties.

Firstly, to choose suitable treatments, different concentrations (1% and 2%) of citric acid and ascorbic acid, high-pressure processing (HPP) treatment (600 MPa, 5 min) and their combination were used to treat avocado before its incorporation into yoghurt containing 10% avocado pulp. Several parameters, such as pH and colour measurement (CIE L, a*, b* values), were analysed from avocado-fortified yoghurt samples during storage for 4 weeks at 4$^{o}$C. The instrumental measurement of colour change was recorded for 26 days to determine which method of the treatments was more effective in preserving the colour of yoghurt samples. Results showed that the colour change of avocado yoghurt occurred predominantly in the first week which was caused by a decrease of L* value (lightness) and an increase of a* value. The total colour change (ΔE) of avocado yoghurt containing avocado treated with ascorbic acid was below 2 in 26 days. Lower efficiency was found from citric acid where the ΔE value (colour change) was below 4 compared to the untreated sample around 6. Besides, the pH change of avocado stirred yoghurt was more sensitive to citric acid which decreased the pH of avocado yoghurt from around 4.45 to 4.29 and 4.15 at 1% and 2%, respectively. Meanwhile, no significant influence was observed for the HPP treatment on colour stability as well as the pH value of the sample.

The treatments of avocado with ascorbic acid (2%) and HPP (600 MPa/5 min) were further studied about their effects on yoghurt, including microbial quality (lactic acid bacteria and microbial contamination), rheological properties, syneresis, colour and pH. It was found that HPP treatment was the key factor to keep yoghurt syneresis (around 1%) low, minimizing the extra syneresis that could be caused by introduced avocado content. Also, the rheological test illustrated its function to increase and maintain the thickness of avocado stirred yoghurt during storage for 4 weeks. In terms of ascorbic acid, no significant effect on rheological properties was observed by the incorporation of ascorbic acid. Neither of these treatments (HPP or ascorbic acid) influenced the growth of lactic acid bacteria in yoghurt. No yeast and mould or coliform were detected in samples during the whole experiment trials.

The effect of those treatments on sensory properties during the shelf-life of yoghurt (4 weeks) was also evaluated. The results showed that the sensory properties of untreated avocado-fortified yoghurt dropped dramatically in 2 weeks for an overall consumer acceptance score from 7.16 to 2.25. On the other hand, HPP treatment and incorporation of ascorbic acid significantly maintained the overall acceptance. HPP treatment specifically affected the texture profile of the sample by maintaining its thickness. Ascorbic acid significantly ($p < 0.05$) increased the flavour score from 2.72 to 6.89 and the appearance score from 4.79 to 5.55. It was found that the main effect of ascorbic acid to improve the sensory properties was to preserve the green colour and inhibit the development of rancid flavour in the product. Besides, neither of these treatments was observed to significantly influence the sourness of the sample, and the interactive effect was not observed between HPP treatment and ascorbic acid. In conclusion, combining 2% of ascorbic acid and HPP treatment on avocado was found to be most effective in various ways (colour, texture and sensory properties) in extending the shelf life of avocado stirred yoghurt with non-significant influence on pH after 1 week storage.

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# Abbreviations

| | |
|---|---|
| HTST | High Temperature Short Time |
| LDL | Low-Density Lipoproteins |
| LT | Loss Tangent |
| MSNF | Milk Solid Non-Fat |
| NFDM | Non-Fat Dried Milk |
| PCA | Principal Component Analysis |
| PPO | Polyphenol Oxidant |
| QDA | Quantitative Descriptive Analysis |
| REML | Residual Maximum Likelihood |
| SAOR | Small Amplitude Oscillatory Rheology |
| SNF | Soli Non-Fat |
| WPC | Whey Protein Concentrate |

# Chapter 1. Introduction

## 1.1 Background Information

Yoghurt is an important source of protein for people for a long history. As a popular dairy product, yoghurt is traditionally prepared by fermentation milk with specific bacteria, normally *Lactobacillus bulgaricus* and *Streptococcus thermophiles*. In modern life, industrial production provided a big range of yoghurt for consumers. Among those yoghurts, the most popular is fruit-flavoured yoghurt, which combines the health benefits and flavour of yoghurt itself and fruit (Aryana & Olson, 2017). Traditionally, sweet fruit such as strawberries, blueberries, pineapples, etc., are used to provide extra flavour in yoghurt. However, the market of traditional yoghurt is gradually saturated. The eagerness of consumers for diverse flavours has driven the producer to discover more possibilities.

In recent decades, avocado has gradually become popular as healthy fruit. The health benefits of avocado are numerous and increasingly discovered, including reduced coronary heart disease, cataracts, diabetes, benign prostatic hyperplasia, prostate cancer and other cancers, and age-related risk of macular degeneration (Mahmassani, Avendano, Raman, & Johnson, 2018; Schaffer, Whiley, Wolstenholme, & Litz, 2013). Besides, avocado oil is reported to have wound healing and hepatoprotective effects (Schaffer et al., 2013). Some of these benefits appear to be related to pigments in avocado pulp, including chlorophyll, carotenoids and anthocyanins, as well as other unidentified phytochemicals (Schaffer et al., 2013). In addition to the health benefits of avocado oil found in the flesh, avocado and avocado oil are increasingly considered healthy foods due to their high monounsaturated fat content and low saturated fat content. The large consumption of avocados is associated with lowering LDL (harmful/bad cholesterol) and increasing HDL (good cholesterol) (Schaffer et al., 2013).

As a result, the market size of the avocado is expanding globally (Schaffer et al., 2013). Therefore, combining avocado with yoghurt has become a new fruit-flavoured yoghurt trend. Furthermore, the introduction of avocado yoghurt could be helpful for the development of the NZ avocado industry. However, the higher unsaturated fatty acid content of avocado makes it more susceptible to rancidity, and its browning reaction further shortens the shelf life of avocado and related products (Bustos, Mazzobre, & Buera, 2017).

To meet the potential market of avocado flavoured yoghurt, this research was carried out to develop an avocado stirred yoghurt product with a relatively long shelf life. In at least two weeks, the physical properties including colour, rheology, and pH as well as sensory properties could be stable and acceptable for potential consumers. Attempts have been made by some studies investigating the best percentage of avocado yoghurt in terms of palatability (Hettige, Ranadheera, Jayawardena, and Vidanarachchi (2013). Meanwhile, researchers have been investigating chemical and physical processing to increase the stability of avocado pulp. However, the influence of treated avocado on yoghurt needs to be further investigated. Thus, this project reviewed and screened a series of chemical and physical methods to stabilise the avocado pulp, aiming to find the most stable treated avocado pulp utilised in the yoghurt formulation. The avocado flavoured yoghurt prepared were analysed for the determination of colour, pH, rheological properties, microorganisms, and sensory properties during their shelf life.

The objective of this research project was to investigate the effect of chemical and physical methods on the physical, microbial, and sensory stability of avocado flavoured yoghurt.

## 1.2 Overview of Thesis

This thesis consists of seven chapters as follows:

Chapter 1 describes the background information and rationale of developing and characterizing fruit-flavoured stirred yoghurt including the main objective of the research project.

Chapter 2 is a literature review of this project, including the history, ingredients, fermentation mechanism, processing techniques and properties of fruit-flavoured yoghurt. The benefits, drawbacks and relating techniques of incorporating avocado were also introduced. Additionally, previous studies on avocado flavoured stirred yoghurt are also reviewed.

Chapter 3 lists the information about the materials and methods used in this research work.

Chapter 4 describes the use of chemical and physical treating at a different level and their effect on the colour and pH stability of avocado pulp as well as avocado flavoured stirred yoghurt during their shelf life.

Chapter 5 describes the use of screened treating on the colour, pH, rheological properties, and microorganism of avocado flavoured stirred yoghurt during their shelf life.

Chapter 6 describes the use of screened treating on the sensory properties of avocado flavoured stirred yoghurt during their shelf life.

Chapter 7 describes the main findings of this research project, as well as some recommendations for future works.

# Chapter 2. Literature review

## 2.1 History, classification, and health benefits of yoghurt

As one of the main products of animal husbandry, dairy products are an important source of protein for many early human civilizations and play an important role in the development of human society. It is believed that people invented yoghurt as early as the Mesopotamia plains of 5,000 BC, however, yoghurt was first industrially produced by Isaac Carasso in 1919 (Aryana & Olson, 2017). Until today, the yoghurt industry has been relatively mature, and related technologies, standards and industrial chains have been fully developed. Industrially, yoghurt is generally a semi-solid product made by fermenting a standard milk base after heat treatment. According to the Australia New Zealand Food Standards Code, yoghurt means fermented milk where the fermentation has been carried out with lactic acid-producing microorganisms, including *Lactobacillus bulgaricus* and *Streptococcus thermophiles*. Yoghurt products should contain at least $10^6$ cfu/g of microorganisms and their pH should not exceed 4.5. And for yoghurt derived from cow's milk, the minimum protein of 30 g/kg (measured as crude protein) should be met. In addition to the two essential bacteria, adjunct culture can be added to change the flavour of the yoghurt or enhance its health. Common adjunct culture includes *Lactobacillus acidophilus*, *Bifidobacterium spp.*, *Lactobacillus reuteri*, *Lactobacillus casei*, *Lactobacillus rhamnosus GG*, *Lactobacillus gasseri* and *Lactobacillus johnsonii LA1* (Chandan & Kilara, 2013).

Apart from utilizing different probiotics, the producer has fully developed the taste, texture and packaging of the yoghurt to cater to the growing consumer demand for taste, health and convenience. The current type of yoghurt mainly including plain yoghurt, fruit flavoured yoghurt (including fruit-on-the-bottom and blended forms), whipped yoghurt, granola-topped yoghurt, drinkable yoghurt, frozen yoghurt, and Greek yoghurt with varying fat contents (regular, low fat, and non-fat) (Chandan & Kilara, 2013).

Among all kinds of yoghurts, fruit-flavoured yoghurt has won the majority of the market since it perfectly meets consumers growing demand for healthy food (Fernandez & Marette, 2017). Fruits as healthy food in consumers perception may introduce nutrition such as vitamins, minerals, antioxidants, and dietary fibre into yoghurt. Besides, due to the incorporation of fruit, the appearance and taste of the yoghurt are correspondingly enhanced. The abundant species of fruit also grants great potential of diversity to fruit-flavoured yoghurt.

Apart from the health benefits introduced by fruit or other ingredients, yoghurt provides a series of health benefits by itself. Research shows that it helps with several of the most deadly lifestyle diseases in modern society, including diabetes, obesity, cardiovascular and cerebrovascular diseases, and hypertension (Marette, Picard-Deland, & Fernandez, 2017).

## 2.2 Ingredients for yoghurt manufacture

In the commercial production of yoghurt, milk is one of the most important raw materials. Thus, the quality of milk has a great impact on the yoghurt product. Normally, fewer inhibitors are required by yoghurt producers because inhibitors such as antibiotics prevent the growth of lactic acid bacteria and affect the fermentation of yoghurt. Between the two species of microbes necessary for yoghurt, *Streptococcus thermophilus*, is particularly sensitive to antibiotics (Chandan & Kilara, 2013). Additionally, residual disinfectants and sanitizing chemicals may also affect the yield of yoghurt, for instance, chlorine compounds such as hypochlorites and iodophors may slightly inhibit starter cultures at a level of 6–10 mg/l of milk. Therefore, preferably, these compounds should be avoided in plants manufacturing yoghurt and cultured dairy products, and their use on the farm should also be noted (Chandan & Kilara, 2013).

For good quality control, the milk fat and milk solids-not-fat (MSNF) should be standardized in yoghurt-mix composition. Usually, it could be achieved by mixing standard raw materials quantitatively. Commonly used milk base ingredients include whole, partially defatted milk, condensed skim milk, cream, NFDM (Non-Fat Dried Milk), milk solid and WPC (whey protein concentrate) (Chandan & Kilara, 2013).

Besides, for better taste and texture, sweeteners and stabilizers are often added to yoghurt. Most fruit-flavoured yoghurts contain approximately 10–13% sugar equivalent, while flavoured yoghurts (vanilla, lemon, coffee, etc.) contain 8–10% sugar (Weerathilake et al., 2014). For better dispersion of sugar and preventing the growth of the yeasts and moulds, sugar is usually added before the pasteurization of yoghurt-mix. However, before fermentation, the total amount of sugar in yoghurt-mix should not exceed 11% because the excess incorporation of sugar will hinder the growth of the yoghurt culture (Chen et al., 2017). Thus, for fruit-flavoured yoghurt, a certain amount of sugar is introduced by the fruit mix added after fermentation. In recent years, with the popularity of healthy foods, many producers choose to add non-nutritive high-intensity sweeteners, such as aspartame, sucralose, acesulfame-K, neotame and stevia (Chandan & Kilara, 2013).

Stabilizers are often added to the yoghurt to improve consistency and build viscosity. Besides, they are used to decrease whey separation and bind free water, and maintain the gel structure after pumping, mixing and cooling. In general, stabilizers contribute to the shelf-life of the product and provides a reasonable degree of uniformity from batch to batch (Aswal, 2013)

Considering the characteristics of yoghurt, the added stabilizer should be easily dispersed in the yoghurt mix at fermentation temperature and help to form a stable emulsion. Other than that, it should not impart any flavour and has to be able to adapt to low pH values. For whipped yoghurt, it should also be able to maintain the foam structure (Priyanka Aswal, 2012).

Industrially, to sufficiently and uniformly mix the stabilizer with the product, a high-shear-type blender is usually used for stirring. When choosing a specific stabilizer, the following factors need to be considered (Priyanka Aswal, 2012).

1. The type of yoghurt
2. The composition of yoghurt especially the content of fat and total solid
3. Desired texture
4. Desired ingredient labelling
5. Processing equipment
6. The potential masking effect of stabilizer to flavour

Table 2.1 lists stabilizers commonly used in yoghurt and their corresponding amounts.

*Table 2.1 Common stabilizers for yoghurt and yoghurt drinks (Chandan & Kilara, 2013).*

| **Stabilizer** | **Concentration (%)** |
|---|---|
| WPC (34,50 or 80% protein) and /or MPC | 0.7-2.0 |
| Starch, modified(tapioca/corn) | 0.8-2.0 |
| Gelatine (225/250 Bloom) | 0.1-0.5 |
| Agar | 0.25-0.70 |
| Pectin (low methoxy, for *yoghurt*) | 0.08-0.20 |
| Pectin (high methoxy, for *yoghurt* beverages) | 0.30-0.50 |
| Locust-bean gum | 0.3-0.5 |
| Xanthan gum | 0.01-0.05 |

One of the more common stabilizer blends used for stirred yoghurt consists of a combination of modified food starch (0.6-1.5%) and gelatine of 225-250 Bloom (0.25-0.40%). It produces a creamy, firm yoghurt that is resistant to wheying-off and stirs out smooth and free of lumps. If a 'natural' approach is desired, a gelatine-pectin stabilizer or agar-pectin stabilizer can be used. A lot of combinations of stabilizers can be used to meet specific marketing or manufacturing needs, but over stabilization should be noted as it leads to a series of undesired textures such as sandy, chalky or lumpy texture.

## 2.3 Manufacture process for yoghurt

Although the formulation varies with the type of yoghurt produced, the approximate processing is similar. Usually, the first part of yoghurt making is to prepare standard raw ingredients and mix them by calculation. In this step, ingredients such as condensed milk cream, whole milk, skim milk will be pumped into a processing vat. Thereafter, NFDM solid may be added and thoroughly stirred. The second step is to homogenize the product and then pasteurize the mix. Pasteurization, normally heat treatment, helps with the growth of the added culture and denatures the protein in the milk, leading to a better texture of the yoghurt. Finally, the mix is inoculated after cooling to a desirable fermentation temperature. It should be noted that for set yoghurt, fermentation is usually after packaging, while for stirred yoghurt, the product is fermented in a big batch in vat and stirred after fermentation, sometimes with fruit or flavour (Aswal, 2012).

### 2.3.1 Blending

Blending is normally the first stage of yoghurt making, various dry ingredients must be sufficiently dispersed in the liquid phase to ensure uniform yields. Therefore, a certain shear rate and sufficient mixing time must be guaranteed (Chandan & Kilara, 2013). At the same time, it is necessary to minimize the incorporation of air during blending to prevent excess oxygen dissolving which hinders the growth of the anaerobic culture used for fermentation. For better quality control, a composition test is required on the mixed raw ingredients to ensure a consistent amount of fat and solid content (Chandan & Kilara, 2013).

### 2.3.2 Pasteurization and heat treatment

The main role of pasteurization in the production of yoghurt is to kill pathogenic bacteria, reduce the activity of other major microorganisms and inactivating the infant enzymes in milk. The commonly used equipment in industry is a high-temperature–short-time (HTST) heat exchanger. Pasteurization that is applied in yoghurt making processing is generally more intense because additional heat treatment denatures the whey protein in the milk and provide a better growth environment for the fermenting species (Chandan & Kilara, 2013). Besides, the water-binding capacity of whey protein increase during the denaturation process, which helps with the consistency and viscosity of the final product and prevents the syneresis of the product. According to other research, the best whey protein denaturation degree is 80-85%, which usually needs to be achieved by heating at 85°C for 20-30 minutes or at 90°C for 15 minutes (Chandan & Kilara, 2013).

Compared to producing set yoghurt, the heat treatment conditions required for producing stirred yoghurt are relatively mild, as the stability and texture of this type of yoghurt depend mostly on the total solids content of yoghurt as well as the concentration of stabilizer used (Aswal, 2012).

### 2.3.3 Homogenization

Homogenization is necessary for yoghurt making process, it breaks up fat globules in milk, thus leading to the even distribution of the stabilizer in the yoghurt, improving the consistency and viscosity of the final product (Priyanka Aswal, 2012). Studies have shown that applying homogenization after rather than before pasteurization is more conducive to the consistency of the final product. For general yoghurt (MSNF 11-13%), double-stage high pressure of homogenization is typical of approximately 23–28 MPa/6 MPa (Priyanka Aswal, 2012). For yoghurts with higher total solids content, a relatively lower homogenization pressure (usually 6-17 MPa) should be applied (Aswal, 2012). When the modified starch is included in the yoghurt mix, it should be noted whether the starch is resistant to intense agitation. If not, the minimum mean pressure should be applied to protect the structure of starch from vigorous agitation. In this case, a single-stage homogenization pressure of 3-4 MPa is normally employed (Aswal, 2012).

### 2.3.4 Incubation and fermentation

In fresh milk, casein accounts for around 80% of all protein content, it maintains a relatively stable structure in the form of micelles. Since the stability of casein particles is largely attributed to their electrical repulsion, the ionic balance and salt concentration could considerably affect the stabilization of milk (Priyanka Aswal, 2012). During the fermentation of yoghurt, the culture consumes lactose and produces lactic acid, leading to the gradual dissolution of the calcium and phosphorus inside the casein particles, destabilizing the casein micelles. Finally, the casein reaches the isoelectric point at pH 4.6-4.7 and loses electrical repulsive force which results in gel formation (Aswal, 2012).

The physical properties and microstructure of yoghurt are influenced by incubation temperature. The use of high incubation temperature resulted in a decrease in gelation time and $G'$ values at pH 4.6, and whey separation compared with yoghurt gels incubated at low temperature (Lee & Lucey, 2010). This result indicates that gels formed at high temperatures are weak and have a coarse gel network due to extensive rearrangement resulting in the formation of large pores and greater whey separation (Lee & Lucey, 2010). During the formation of yoghurt gels at a low incubation temperature, slow protein aggregation occurs resulting in the formation of many protein-protein bonds. A highly cross-linked and branched protein network that had small pores was observed in micrographs of yoghurt gels incubated at low temperature (Lee & Lucey, 2010). At lower incubation temperatures, there is an increase in the voluminosity of casein particles, which increases the area of the junctions between aggregated casein particles. An increased contact area between casein particles could contribute to the increased stiffness of gels observed at low temperatures (Lee & Lucey, 2010).

Higher viscosity was observed in stirred yoghurts that have been incubated at lower temperatures (e.g. <40°C) compared to gels incubated at high temperature (e.g. >40° C) (Lee & Lucey, 2010). As the incubation temperature increased, there was a decrease in the sensory attributes, such as mouth coating and smoothness of stirred yoghurts (Lee & Lucey, 2010). Recently, a novel two-stage incubation temperature method was proposed. Peng, Horne, and Lucey (2010) reported that if incubation temperature was changed to a new temperature after gelation, the textural properties of yoghurt became similar to those of yoghurts made at that new temperature for the entire fermentation process. It may be possible to use a high incubation temperature for the initial stage of fermentation to facilitate rapid growth of the starter cultures and then slowly reduce the incubation temperature at some stage to achieve better textural properties.

### 2.3.5 Cooling

To maintain a certain pH of the yoghurt product by limiting the further growth of the culture, yoghurt should be cooled immediately after reaching a certain degree of fermentation (Lee & Lucey, 2010). Typically, yoghurt should be cooled when its pH value reaches 4.55-4.65 after fermentation. Cooling at pH 4.7 or higher is likely to result in a loose texture. In addition, excessive cooling allows the bacteria to further growing thus may further decrease the pH, causing undesirable changes in the yoghurt structure (Lee & Lucey, 2010). The specific method of cooling often depends on the type of yoghurt being made. Generally, it is best to lower the temperature of the product to 18-20 °C within one hour to stop further growth of the culture (Panagiotis & Constatnina, 2014).

### 2.3.6 Stirring

Stirring is an important step in making stirred yoghurt. Although the uniformity of stirred yoghurt requires a certain level of agitation, the structure of the yoghurt could also be damaged by intense agitation, leading to a loose and fluid product. A common way is to stir the yogurt after cooling down to around 20°C. Another approach could be applied to ensure the uniformity of the final product where a higher stirring rate is generally employed at the beginning of agitation, while the agitation rate decreases when the temperature drops to about 30 °C. The pH at the time of stirring is also important. Stirring at pH > 4.7 when the protein network is not fully formed in fermentation results in the final product being grainy (Lee & Lucey, 2010).

## 2.4 Quality attributes and characterisation of yoghurt

At different stages of yoghurt production, a series of tests could be carried out for the quality control of the product. The common test timing, test types, and test purposes are listed in the table below (Chandan & Kilara, 2013).

In general, quality testing for yoghurt includes chemical composition testing, physical property testing, microbial quality testing, and sensory testing. For some producers employing raw milk for making yoghurt some bioassays suck as total aerobic plate count and coliform count should be carried out as indicators of contamination of the milk. Besides, the main parameters of chemical testing of raw milk include fat, total solids, protein, lactose, ash, vitamins and minerals. Titratable acidity (TA), added water, foreign materials, antibiotics, sanitisers, aflatoxins, pesticides and other environmental contaminants are also used to indicate the quality of raw milk (Chandan & Kilara, 2013).

*Table 2.2. Typical tests analysed for yoghurt products.*

| *Fresh product* | *Test* | *Purpose* |
|---|---|---|
| *Yoghurt after packaging (day of manufacture* | Coliform count | Detect unsanitary processing or packaging conditions |
| | | Indicate post-pasteurization contamination |
| *Yoghurt 24 hours after packaging (D+1)* | pH/titratable acidity | Control acidity |
| | Flavour and texture | Ensure sensory quality |
| | Viscosity | |
| | Preincubation of product in its container at 30°C for 24 hours, followed by yeast and mould count | Predict shelf-life |
| *End of code ( store shelf-life samples at 4 °C* | Sensory | Ensure quality standards are met at end of the code |
| | pH | |
| | Observe spoilage | |

Once the yoghurt is produced, freshly packaged yoghurt needs to be tested immediately for the coliform count. Other quality tests, including pH, viscosity, texture, yeast and mould count, are generally required 24 hours after product packaging is completed (Lee & Lucey, 2010). This is because the microbial environment of the yoghurt straight after fermentation is unstable, and it is necessary to stop the growth of the microorganisms after storage for a certain period at low temperature, leaving the quality of the product stable for testing.

### 2.4.1 Composition and chemical assessment of yoghurt

To ensure a certain level of nutrients in the yoghurt, most of the regulations on yoghurt specify the chemical composition of the yoghurt, some of which is regarding the percentage of solid non-fat (SNF) and/or fat content (Lee & Lucey, 2010). In the United States and the European Union, the SNF and fat content of yoghurt-type fermented milk are between 8.2-8.65% and 2.25-5.0%, respectively. In the Netherlands, the SNF content of yoghurt varies widely (8.2–12.6%) (Panagiotis & Constatnina, 2014). In New Zealand and Australia, there is only regulation on yoghurt made from milk: contain no less than 30 g/kg protein

(Panagiotis & Constatnina, 2014).

Studies have shown that the total solids content between 15.0-16.0% contribute to the best palatability of yoghurt. While fat is another major ingredient that affects the taste of yoghurt. As the consumers' health awareness increases, the market for low-fat products is expanding. However, lower fat levels result in a sharp drop in the sensory quality, especially the rich mouthfeel of the yoghurt. To compensate for the effect of fat loss, a series of thickener and fat replacers have been studied to maintain the texture of low-fat yoghurt. In these studies, protein and starch-based fat substitutes have received widespread attention. However, results show that no current alternatives could ideally replace the functionality of fat in yoghurt. Further research should be done to discover some new methods of fat relacing techniques in yoghurt. Currently, to form the yoghurt structure that meets the basic expectation of consumers, the minimum fat content is 1% (Weerathilake et al., 2014).

*Table 2.3 Typical formulations of Swiss/blended-style yoghurt base (Chandan & Kilara, 2013).*

| **Composition** | **Nonfat yoghurt** | **Low-fat yoghurt** | **Full-fat yoghurt** |
|---|---|---|---|
| Milk fat, % | 0.1-0.4 | 1.0-2.0 | 3.25-3.50 |
| MSNF, % | 10.0-12.0 | 10.0-12.0 | 10.5-11.0 |
| Sugar, % | 0.0-6.0 | 0.0-6.0 | 0.0-6.0 |
| Stabilizer, % | 0.4-2.0 | 0.3-1.8 | 0.3-1.2 |

In addition to the composition, another important chemical factor is acidity, which affects the palatability as well as the shelf life of the product. The acid in the yoghurt is primarily lactic acid, which is produced by the culture from the controlled fermentation process. Lactic acid not only enhances the flavour and taste of the yoghurt but is also essential for the formation of a gel network in yoghurt. However, the acidity produced during storage is generally undesirable because it could lead to whey separation and other texture issues, besides, strong acidity could mask the flavours of yoghurt. Therefore, to provide a product with guaranteed quality, the acidity of the yoghurt should be accurately and effectively controlled. The International Dairy Federation (IDF) suggested that yoghurt should contain at least 0.6% lactic acid (Aswal, 2012; Weerathilake et al.,2014).

The standards and operating procedures for titrating acidity vary among countries. The most common method is to titrate a mixture of 10 g of product and 10 mL of distilled water using 0.1 M NaOH in the presence of phenolphthalein (1 mL) as an indicator. The colour of the mixture turns to pink at the end of titration. The result is expressed as °SH (Sox Henkel) where 1 °SH is equal to 0.0225% lactic acid.

In addition to titratable acidity, pH is another indicator of the acidity of the yoghurt. Although the relationship between pH and titratable acidity is not straightforward in a highly buffered system such as yoghurt, monitoring pH during yoghurt production is a more routine operation.

It is not a common practice to identify other dairy compounds such as protein, lactose and ash in the quality control of yoghurt. However, it is helpful to confirm the protein content when the manufacturer is concerned about the effect of total solids on product quality (Priyanka Aswal, 2012). Besides, although yoghurt contains detectable riboflavin, thiamine, folic acid, pantothenic acid and vitamin A, yoghurt is usually not considered a source of vitamins, so the detection of these nutrients is not conventional except for fortified yoghurt (Panagiotis & Constatnina, 2014).

### 2.4.2 Physical assessment of yoghurt

The study of the physical properties of yoghurt optimizes the production conditions and equipment, help with good quality control. And it could be combined with sensory experience to produce more desirable yoghurt for the consumer.

Yoghurt gel is a heat-induced acidic casein gel composed primarily of non-covalent protein bonds (such as hydrophobic and electrostatic bonds) and covalent thiol disulphide bonds (Panagiotis & Constatnina, 2014). Many factors influence the physical properties of yoghurt, including not only the type and amount of protein in the raw material but also the size and shape of the network they form (Aswal, 2012). From a production perspective, the type of raw materials, the manufacturing method, the heat treatment process, the type of starter, the incubation temperature and the pH are all the factors that influence the texture of yoghurt (Aswal, 2012).

One of the most noteworthy physical properties of yoghurt is the spontaneous syneresis, which refers to the shrinking of gel without external force, which usually causes the whey to exit the protein network of the yoghurt and float on the surface of the yoghurt (Aswal, 2012). This phenomenon generally harms the acceptance of consumers toward yoghurt. In industrial production, gelatine, pectin and starch is commonly used to prevent syneresis. Another method is to increase the total solids content of the yoghurt milk, especially the protein content (Aswal, 2012; Weerathilake et al., 2014).

Spontaneous whey separation may be associated with rearrangement of the gel matrix due to pH change and may also be induced by weaker gel networks (e.g. by vibration or cutting). In the experiment, the whey output in the yoghurt can be measured by high-speed centrifugation, or the whey can be discharged from the stirred yoghurt through a sieve (Aswal, 2012).

Since the yoghurt gel is viscoelastic, viscous and elastic features can be quantified to characterize the rheological properties of the yoghurt. This feature indicates that the material has certain elastic properties of the solid and certain flow characteristics of the ideal (viscous) liquid (Lee & Lucey, 2010). The rheological parameters that can be obtained from deformation physical tests include yield stress, which is defined as the point in time at which the shear stress begins to decrease. A higher yield value indicates a stronger network of yoghurt gels, while a lower yield value indicates that the yoghurt gel is a brittle or short-skin gel. The strength of the protein bond, the number of bonds in the cross-section of each chain, the relaxation time of the network bond, and the orientation of the chain in the matrix contribute to the yield characteristics of the gel (Ozcan, 2013).

The deformation characteristics of food gel are normally related to their functional properties, including shaping, cutting/slicing and feeding characteristics (Priyanka Aswal, 2012). The formation of yoghurt gel is also widely studied, and small amplitude oscillatory rheology (SAOR) has been used to characterize the rheological properties of yoghurt during gel formation (fermentation) without destroying the weak gel network. Small deformations are defined as deformations (strain or dimensional changes) (e.g. $\leqslant$ 1%) that do not disrupt the development of the network structure when stress is applied. The following rheological parameters were determined in the SAOR test: The modulus of elasticity or storage modulus (G') represents a measure of the energy stored in each deformation cycle and represents the nature of the solid. The viscous or loss modulus (G'') represents the amount of energy lost by viscous dissipation per deformation cycle and reflects liquid-like properties. The loss tangent (LT) is defined as the ratio of the loss modulus to the storage modulus (G''/G') and indicates the type of viscoelastic material in the material. Briefly, G' stands for elasticity and G'' stands for viscosity or liquid properties of the gel network (Priyanka Aswal, 2012). A high LT value (i.e., G" & G') means that the material has a liquid behaviour.

The determination of the physical characteristics of yoghurt also differs due to their types. Since the gel structure of the stirred yoghurt has been destroyed during processing, the terms used in the determination of food gel is no longer practical. Therefore, in most studies, researchers used viscometers or rheometers to determine the flow behaviour of stirred yoghurt as their most important physical property (Chandan & Kilara, 2013). The flow behaviour of stirred yoghurt has been modelled from shear rate sweep tests (Lee & Lucey, 2010). The data from flow curves are fitted to the power-law model or Casson equations. The parameters obtained from these flow curve models are useful in comparing samples, but these models are essentially empirical or mathematical. It should be noted that the power-law model does not have a yield stress term while all stirred yoghurts have yield stress unless they have been sheared first and no recovery time allowed to rebuild some structures. Below are the equations for these models (Lee & Lucey, 2010).

Power law model:$\sigma = K(\dot{\gamma})^n$

Herschel-Bulkley model: $\sigma = \sigma_0 + K(\dot{\gamma})^n$

Casson model: $\sigma^{1/2} = \sigma_0^{1/2} + \eta_a(\dot{\gamma})^{1/2}$

Where $\sigma$ is the shear stress, $\sigma_0$ is the yield stress, $\eta_a$ is the apparent viscosity, $\gamma$ is the shear rate, K is the consistency index, $n$ is the flow behaviour index (Lee & Lucey, 2010).

It's worth noting that during stirring, the physical feature of yoghurt changes from a viscoelastic solid to a viscoelastic fluid. However, its viscosity can partially recover with the structure after the shear is stopped. The restoration of structure is called "reproduction" and is a phenomenon that changes with time (Chandan & Kilara, 2013)

However, practically, in the determination of the physical properties of yoghurt, a wide variety of probes, penetration method, penetration depth and temperature are used, so it is almost impossible to compare results between laboratories (Chandan & Kilara, 2013).

### 2.4.3 Microbiological quality control

Microbiological quality control is essential to ensure the safety of the yoghurt and to meet existing local standards and/or regulations. To this end, it is common practice to monitor the quantity of both desirable and pathogenic microorganisms during processing. Besides, determining the level of microbial metabolites in yoghurt is also a common tool for microbiological quality control (Aswal, 2012).

According to Australia New Zealand Standards 2.5.3-3, the number of lactic acid bacteria in yoghurt should be more than $10^6$ cfu/g (*Australia New Zealand food standards code*, 2021). It is worth noting that the number of fermenting bacteria may decrease during the shelf-life, so the initial number of bacteria in yoghurt should be rich enough to ensure a sufficient number till the end of the product's shelf life. Besides, excessive amounts of specific microorganisms, such as *lactobacilli*, in yoghurt may cause an atypical aroma/flavour in the final product. In general, retail yoghurt should not only have a certain number of fermenting bacteria during the shelf life but also a proper ratio of strain for better palatability (Chandan & Kilara, 2013).

### 2.4.4 Sensory Evaluation of yoghurt

Although the determination of the biological, chemical and physical properties of yoghurt provides abundant scientific data on the quality of yoghurt, these data do not directly reflect the consumer's sensory experience with yoghurt. Even for products that are chemically or physically identical or similar, the sensory experiences they offer to consumers could be different. Thus the coordination between the instrumental and sensory characteristics of the product is critical to ensuring its acceptability (Aswal, 2012).

In the sensory evaluation of fermented milk products, non-descriptive hedonic tests based on product texture, appearance and colour, and product aroma and flavour are commonly used. These tests focusing on assessing consumer preferences for products. This kind of assessment is simple, but far from reflecting the actual sensory nature of the product. Another sensory evaluation method for yoghurt is the Karl Ruher 9-point evaluation protocol as shown in the table below. According to this evaluation scheme, the scores assigned to the sample range from 1 (very poor) to 9 (excellent) and are divided into three intervals: I, II and III. Each interval has three sub-categories: upper, middle, and lower, and the samples taken at intervals I, II, and III are each judged to have no objection to consumption and are still commercially acceptable and non-saleable (Chandan & Kilara, 2013).

*Table 2.4. Karl Ruher 9-Point Evaluation Scheme (Tamime, Robinson, & Tamime, 2007b).*

<table>
<tr><th>Point</th><th>Decision</th><th>Quality</th><th></th><th>Class</th><th>Overall judgment</th></tr>
<tr><td>9</td><td>Excellent</td><td>-</td><td rowspan="3">I</td><td>Upper</td><td rowspan="3">No objection</td></tr>
<tr><td>8</td><td>Very good</td><td>Very good</td><td>Medium</td></tr>
<tr><td>7</td><td>Good</td><td>Good</td><td>Lower</td></tr>
<tr><td>6</td><td>Satisfactory</td><td>Satisfactory</td><td rowspan="3">II</td><td>Upper</td><td rowspan="3">Still acceptable in commerce</td></tr>
<tr><td>5</td><td>Mediocre</td><td>Average</td><td>Medium</td></tr>
<tr><td>4</td><td>Sufficient</td><td>Sufficient</td><td>Lower</td></tr>
<tr><td>3</td><td>Imperfect</td><td>Bad</td><td rowspan="3">III</td><td>Upper</td><td rowspan="3">Unsaleable</td></tr>
<tr><td>2</td><td>Bad</td><td>Bad</td><td>Medium</td></tr>
<tr><td>1</td><td>Very bad</td><td>Bad</td><td>Lower</td></tr>
</table>

The American Dairy Science Association has developed another sensory evaluation program for fermented dairy products with a clearer description of the defects. As shown in Figure 2.3. In this model, the evaluation is based on the aroma/flavour, body/texture and appearance/colour defects of the product, and the samples are ranked according to the total score of the sample. In recent years, quantitative descriptive analysis (QDA) has been popular in evaluating the sensory properties of dairy products. This descriptive analysis is based on an oral description of the panellists who have undergone a training course that lasts 75 to 100 hours (Chandan & Kilara, 2013).

| | | | | | | |
|---|---|---|---|---|---|---|
| **Date:** | | **Panelist:** | | | | |
| | | | **Sample No.** | | | |
| **Aroma/Flavor Maximum 10 Points** | **Contestant Score** | → | **1** | **2** | **3** | **Total Grades** |
| | Score | | | | | |
| | Grade | | | | | |
| | Critisism | | | | | |
| No critisism: 10 | Acetaldehyde (coarse) | | | | | |
| | Bitter | | | | | |
| | Cooked | | | | | |
| | Foreign | | | | | |
| | High acid | | | | | |
| | Lacks fine flavor | | | | | |
| | Lacks flavoring | | | | | |
| | Lacks freshness | | | | | |
| | Lacks sweetness | | | | | |
| | Low acid | | | | | |
| Normal range 1–10 | Oxidized | | | | | |
| | Rancid | | | | | |
| | Too high flavoring | | | | | |
| | Too sweet | | | | | |
| | Unnatural flavoring | | | | | |
| | Unclean | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | **Sample No.** | | | |
| **Body/Texture Maximum 5 Points** | **Contestant Score** | → | **1** | **2** | **3** | **Total Grades** |
| | Score | | | | | |
| | Grade | | | | | |
| | Critisism | | | | | |
| No critisism: 5 | Gel-like | | | | | |
| | Grainy | | | | | |
| | Ropy | | | | | |
| Normal range 1–5 | Too firm | | | | | |
| | Weak | | | | | |
| **Appearance Maximum 5 Points** | **Contestant Score** | → | | | | |
| | Score | | | | | |
| | Grade | | | | | |
| | Critisism | | | | | |
| No critisism: 5 | Atypical color | | | | | |
| | Color leaching | | | | | |
| | Excess fruit | | | | | |
| | Wheying off | | | | | |
| Normal range 1–5 | Lumpy | | | | | |
| | Lacks fruit | | | | | |
| | Shrunken | | | | | |
| | Surface growth | | | | | |
| **Total score** | **Total score of each sample** | → | | | | |
| | Total grade per sample | | | | | |
| | | | Final grade | | | |
| | | | Rank | | | |

*Figure 2.1. Sensory evaluation scheme developed by the American Dairy Science Association (ADSA).*

In general, the sensory evaluation of yoghurt is carried out at 4-6°C to assemble the conditions where yoghurt is consumed. And to avoid bias on the product, in the sensory evaluation of yoghurt, no brand-related images appear on the package of the sample, and the display order is specified by a statistical program. The sensory test is followed by statistical analysis where Residual Maximum Likelihood (REML) is widely used. To simplify the data obtained from REML analysis, a multivariance analysis method called principal component analysis (PCA) is normally used, which can reduce the dimension of data by performing covariance analysis between factors (Chandan & Kilara, 2013).

## 2.5 Composition, features, and history of avocado

Avocado is an energy-rich food that can be eaten fresh. The chemical composition of the edible portion of Hass avocado is shown in Table 2.5. The archaeological evidence found that avocado is one of the earliest domesticated crops. It has a long history of use as a food ingredient, such as the famous avocado sauce (from Ahuaca-multi in Nahuatl) and as a spread on tortillas. Both uses are considered to originate from Colombia (Schaffer et al., 2013). According to the early records of Spain, the avocado was a well-known dessert to be eaten with sugar, and it was also prevalent to season avocado with salt and/or pepper (Schaffer et al., 2013; Shepherd & Bender, 2002). Today, avocados are still an important food in American and Caribbean cuisine. The consumption of avocados in Latin America is currently the highest in the world (Popenoe & Zentmyer, 1963; Schaffer et al., 2013).

In Mexico, the hometown of avocado, they are ubiquitous on Mexican plates as a condiment and rich and cool ingredients, along with coriander, onions and tomatoes as part of Salsa Verde (green seasoning). It is also often placed on top of black beans and enchiladas, or with burritos, panuchos and sardines. Throughout the Caribbean, avocados can be sliced and served with soup as a condiment, or as a fruit salad (Schaffer et al., 2013; Shepherd & Bender, 2002).

In Brazil and most of Asia, avocados are sweets, mixed with sugar and condensed milk, and consumed in milkshakes, ice cream and mousse. In Java, the avocado puree is mixed with sweet coffee, while in Indonesia, the avocado chocolate milkshake is popular. Most avocado oil is used in the cosmetics industry because of its high vitamin E content and emollient properties (Bost & Smith, 2013; Schaffer et al., 2013).

*Table 2.5. Hass avocados (Persea Americana) composition of the edible portion (Dreher & Davenport, 2013).*

| Nutrient/phytochemical | Value per 100 g | Nutrient/phytochemical | Value per 100 g |
|---|---|---|---|
| Water (g) | **72.3** | **Riboflavin (mg)** | **0.14** |
| Energy (kcal) | **167** | **Folate, food ($\mu$g)** | **89.0** |
| Energy (kcal) (insoluble fibre adjusted) | **148** | **Choline, total (mg)** | **14.2** |
| Protein (g) | **1.96** | **Betaine (mg)** | **0.7** |
| Total lipid (fat) (g) | **15.4** | **Vitamin B-12 ($\mu$g)** | **0.0** |
| Ash, g | **1.66** | **Vitamin A ($\mu$g RAE)** | **7.0** |
| Carbohydrate, by difference (g) | **8.64** | **Carotene, beta ($\mu$g)** | **63.0** |
| Fibre, total dietary (g) | **6.80** | **Carotene, alpha ($\mu$g)** | **24.0** |
| Sugars, total (g) | **0.30** | **Cryptoxanthin, beta ($\mu$g)** | **27.0** |
| Starch (g) | **0.11** | **Lutein + zeaxanthin ($\mu$g)** | **271** |
| Calcium (mg) | **13.0** | **Vitamin E (alpha-tocopherol), mg** | **1.97** |
| Iron (mg) | **0.61** | **Tocopherol, beta (mg)** | **0.04** |
| Magnesium (mg) | **29.0** | **Tocopherol, gamma (mg)** | **0.32** |
| Phosphorus (mg) | **54.0** | **Tocopherol, delta (mg)** | **0.02** |
| Potassium (mg) | **507** | **Vitamin $K_1$ (phylloquinone) ($\mu$g)** | **21.0** |
| Sodium (mg) | **8.0** | **Fatty acids, total saturated (g)** | **2.13** |
| Zinc (mg) | **0.68** | **16:0 (g)** | **2.08** |
| Copper (mg) | **0.17** | **Fatty acids, total monounsaturated (g)** | **9.80** |
| Manganese (mg) | **0.15** | **18:1 (g)** | **9.07** |
| Selenium (ug) | **0.40** | **Fatty acids, total polyunsaturated (g)** | **1.82** |
| Vitamin C (mg) | **8.80** | **18:2 (g)** | **1.67** |
| Thiamine (mg) | **0.08** | **18:3 (g)** | **0.13** |
| Niacin (mg) | **1.91** | **Cholesterol (mg)** | **0** |
| Pantothenic acid (mg) | **1.46** | **Stigmasterol (mg)** | **2.0** |
| Vitamin B-6 (mg) | **0.29** | **Campesterol (mg)** | **5.0** |

The health benefits of avocado consumption are numerous. Especially for avocado oil, there's growing research found their curing functionality towards coronary heart disease, cataracts, diabetes, benign prostatic hyperplasia, prostate cancer and other cancers, and age-related macular degeneration (Mahmassani et al., 2018; Schaffer et al., 2013). Other properties of the oil include wound healing and hepatoprotective effects (Schaffer et al., 2013). Some of these benefits appear to be related to pigments in the avocado pulp, including chlorophyll, carotenoids and anthocyanins, as well as other unidentified phytochemicals (Schaffer et al., 2013; Shepherd & Bender, 2002). In addition to the health benefits of avocado oil, avocado flesh is increasingly considered as healthy foods due to their high monounsaturated fat content and low saturated fat content. The large consumption of avocados is associated with lowering low-density lipoproteins (LDL) and high-density lipoproteins increasing HDL (Bost & Smith, 2013; Schaffer et al., 2013).

## 2.6 Stability of avocado

Avocados may contain oil as high as 15-30% (depending on the variety). Avocado oil is of high value due to the high concentrations of monounsaturated fatty acids (about 70% of total fatty acids)(Bost & Smith, 2013). Besides, avocados are a rich source of biological activity for antioxidant vitamins, carotenoids, phenols and phytosterols (Schaffer et al., 2013). However, avocados have a relatively high metabolic rate and are therefore highly perishable fruits. When the whole fruit is preserved at optimal temperature and relative humidity, its shelf life is only 3-4 weeks (Schaffer et al., 2013). The shelf life of avocado pulp is even shorter (about 5 days). Due to the higher unsaturated fat content in avocado, which is significantly affected by the oxidation process during storage. The oxidating process not only affects lipids content but also affects the aqueous portion of avocado. The mass degradation promoted by the enzyme-mediated oxidation reaction occurs especially in the aqueous portion, where the phenolic substrate is hydroxylated and then oxidized to form a brown compound (Shepherd & Bender, 2002). This means that controlling enzymatic browning is important in the processing and storage of avocados and related products.

### 2.6.1 PPO

Polyphenol oxidase (PPO), also known as polyphenols or phenolase, is a large class of enzymes found in higher animals, plants, fungi and bacteria (Toledo & Aguirre, 2017a). They are capable of catalysing the oxidation of monophenols and polyphenols in the presence of molecular oxygen, resulting in the formation of o-quinones, which are subsequently non-enzymatically polymerized to form melanin (Martinez, 1995; Nicolas et al., 1994; Toledo & Aguirre, 2017a). In mammals, melanin functions to prevent harmful solar radiation. While in plants and fungi, the activity of PPO enzymes appears to be related to the mechanism of defence against harmful substances, although its function remains unrevealed (Toledo & Aguirre, 2017a).

One of the most interesting behaviours of this enzyme in certain plant species is its dual activity. The International Union of Biological and Molecular Biology (IUBMB) has designated its tyrosinase (Tyr) activity (monohydroxylase or phenolase and combined oxidase activity) as EC 1.14.18.1. This activity is responsible for the hydroxylation of the phenol to form o-diphenol, which is not released from the active site and is oxidized in the subsequent reaction to form the final product of the coupling reaction, the ortho-quinone molecule (Toledo & Aguirre, 2017a). Other activities associated with the PPO family are called catechol oxidase or catecholase (EC 1.10.3.1), hereinafter referred to as Cat, which, unlike Tyr, can only catalyse the second step of the Tyr reaction. Cat uses o-diphenol as a substrate and then converts them to orthoquinones. The enzyme is highly variable in terms of species, sequence, substrate selectivity and catalytic ability (Toledo & Aguirre, 2017a). It has been suggested that the enzyme can even be encoded by different genes in different parts of different plants and appears at different times (Toledo & Aguirre, 2017a).

A study identified sequences of 47 PPO species (from animals, plants, fungi, and bacteria) suggesting that the clustering of PPO is nearly impossible because of the large differences of sequences among them (Toledo & Aguirre, 2017a). However, it should be noted that all enzymes belonging to this family share a common feature of high sequence homology at their catalytic sites, thus representing a common evolutionary ancestor. In food science, the activity of this enzyme is useful in products with specific desirable colours, such as tea, coffee and cocoa (Martinez, 1995; Nicolas et al., 1994; Toledo & Aguirre, 2017a). However, the side effect of PPO is the main concern in most cases. As polyphenol oxidase (PPO) activity is involved in the browning reaction in minimally processed fruits and vegetables, phenolic compounds that are present in fruits and vegetables are oxidized by PPO to quinones, which are then polymerized to melanin pigments, resulting in the development of undesirable colours and texture as well as losses of flavour and nutrients (Toledo & Aguirre, 2017a). In addition, consumers also believe that browning is a sign of inferior or not fresh products.

In summary, according to global statistics from the World Food Organization, enzymatic browning reactions can cause damage in certain products, such as avocados. Since 1993, global avocado production has been systematically increasing. Although many techniques have been employed to control PPO in avocados since 1937, there is still an area that remained for researchers because the PPO in avocados is particularly effective (Toledo & Aguirre, 2017a).

### 2.6.2 Chemical methods

Inhibiting the Tyr has attracted great interest because of its role in pigmentation. Due to the increasing health concern of the public in terms of food additives. Therefore, more research focuses on natural inhibitors of Tyr. Chemicals including kojic acid, arbutin, catechin, hydroquinone and sebacic acid are widely studied. The common inhibitor of pigmentation used in avocado pulp is citric acid and salt, after which various additives (ascorbic acid, EDTA and sodium metabisulfite) were combined with physical techniques. However, the study has found that PPO in avocados is more resistant to the inhibitors than other fruits (Almeida & Nogueira, 1995).

Recently, a study has shown that garlic, onion broccoli and brassica vegetable extracts inhibit the browning of mushrooms and avocado slices (Bustos et al., 2017). Besides, inhibition of enzymatic browning of avocado pulp by these extracts was also observed (Bustos et al., 2017). These vegetables also possess a high level of antioxidants, which scavenge free radicals and singlet oxygen, thereby significantly preventing the oxidation of lipid components (Bustos et al., 2017). However, the incorporation of those extracts could be further studied in terms of their effect on the flavour and acceptance of avocado.

### 2.6.3 Heat treatment

As enzymes, PPO is essentially a series of proteins, so the most direct and effective way to inactivate it is to denature it by heat treatment, thereby inhibiting browning in the avocado pulp. This is also one of the earliest methods and has been declared the most effective way to control PPO activity (Golan-Goldhirsh, Whitaker, & Kahn, 1984). After understanding the diversity between different varieties of enzymes, a study evaluated the heat tolerance of PPO in three variants of avocado (*Choquette, Fortuna and Quintal*). The study has shown that regardless of the avocado ripening state and heat treatment temperature (60–80ºC), the enzyme activity in the avocado extract heated for ten minutes dropped to 50% to 60%, suggesting the heat resistance of the enzymes in the avocado matrix (Bustos et al., 2017). Although high-temperature processing has been widely used in many fields, the side effects of heat treatment also draw considerable attention. For instance, heat treatment accelerates the oxidation of fat in the avocado and also brings bitterness and other unpleasant flavours in the avocado product (Bustos et al., 2017). Therefore, this technique is rarely used to preserve avocado pulp.

### 2.6.4 High-pressure processing

Another physical method to inhibit PPO activity in avocado pulp is to apply high-pressure processing, a promising emerging technology that can only affect the hydrophobic interactions of biological structures without affecting their covalent bonds. Therefore, this technique has minimum influence on the nutrition of foods while destabilizing the secondary structure of the protein (enzyme) and inactivating the microorganisms (Woolf et al., 2013). However, the resistance of PPO in Hass avocado to high hydrostatic pressure was also found. Not necessarily correspond to heat resistance, each PPO reacts differently to high pressure. Therefore, it is difficult to extrapolate this result to other varieties of avocados.

In a study aimed at analysing PPO activity in Hass avocado pulp, the researchers applied a four-cycle high pressure (four pressures, 689 MPa every 5 minutes) on guacamole with detected a residual activity of PPO at approximately 15% (Palou et al., 2000). However, in a recent study of Hass avocado slices, the inhibition of PPO (as measured by Cat activity) in avocado treated with high pressure (200 to 600 MPa for 3–6 minutes) was not detected, while oppositely, the activity has increased by approximately 30%. With the help of an electron micrograph, the increased activity was found to be related to the release of PPO from the cell membrane which was destroyed by ultra-high pressure (Woolf et al., 2013). In summary, high-pressure treatment has proven to be an effective method to partially inhibit avocado PPO activity. However, its effectiveness depends on the specific fruit variety. For instance, the PPO in Hass avocado is partially resistant to HPP. Last but not least, the cost of implementing this technology in industrial applications is high.

### 2.6.5 Ultrasound

The application of ultrasound is another label-clean method of inhibiting avocado PPO (Başlar & Ertugay, 2013). High frequencies cause the formation, growth and rupture of small bubbles in the liquid, thereby affecting the activity during enzymatic decomposition. However, since ultrasound ruptures the cell membrane which facilitates substrate contact with their enzymes, this treatment may also increase the activity of PPO in avocados (Başlar & Ertugay, 2013). Therefore, ultrasound is not commonly used to preserve avocado fresh.

### 2.6.6 Modified atmosphere packaging

Modified atmosphere is the practice of modifying the composition of the internal atmosphere of a package to improve the shelf life. Although the application of a modified atmosphere packaging does not inhibit the catalytic activity of PPO, the lack of oxygen in the reaction will inhibit the browning reaction, at least till the system is re-incorporated with oxygen. Many studies have reported a positive effect of the modified atmosphere ($CO_2$, $N_2$ and their combination) on the preservation of the fresh avocado product, primarily from the Hass and Fuertes varieties (Gonzalez, Yahia, & Higuera, 1989; Meir et al., 1997; Sellamuthu, Mafune, Sivakumar, Soundy, & Agriculture, 2013).

### 2.6.7 Combined methods

In addition to the physical or chemical methods described above, the combination method is more common and practical in industry. Almeida and Nogueira (1995). measured the effect of temperature (2 minutes at 70°C) and 4 additives (ascorbic acid, citric acid, EDTA and sodium metabisulfite) on eight fruits and vegetables (avocado, banana, apple, pear, peach, potato, mushroom, eggplant and avocado), and avocado PPO was found to be most resistant to inhibition (Almeida & Nogueira, 1995). Subsequently, Weemaes and Ludikhuyze (1999) combined high pressure and high-temperature treatment and observed that at near-neutral pH, the optimum temperature for inactivating avocado PPO is close to 65°C, and changes in pH (at around 4) cause PPO to be sensitive to high-pressure processing. In 2002, a combination of the packaging in the different atmosphere (air/$N_2$/vacuum), antioxidant (without antioxidant/ascorbic acid/EDTA) and antimicrobial agent (no reagent/sorbic acid) was carried out to determine the kinetics associated with the deterioration of the browning resistance. It was found that in the presence of EDTA, after 3 months, the enzyme can be completely inactivated (Soliva-Fortuny, et al.,2002). Weemaes and Ludikhuyze (1999) proposed using high pressure as a physical reagent combined with five chemical reagents (EDTA, NaCl, benzoic acid, 4-hexylresorcinol and glutathione) at 25 ° C and pH 5.0 to inhibit avocado PPO activity. Based on their results, the authors suggest that there are two types of PPO in the avocado matrix, and one type is resistant to high-pressure treatment, accounting for 10–20% of the total enzyme, and the other is sensitive to high-pressure treatment. In addition, the researchers believe that isozymes may have different isoelectric points (Toledo & Aguirre, 2017a).

## 2.7 Yoghurt fortified with avocado

Avocado and yoghurt are considered healthy foods. With the popularity of fruit-flavoured yoghurt such as yoghurt with strawberry, pineapple, mango etc., it’s natural to improve the taste of yoghurt with avocado.

Although related products have not appeared on the NZ market due to the perishable nature of avocado, relevant research has emerged. Most of the research focused on the effect of the amount of avocado added on the sensory quality of yoghurt. Hettige et al. (2013) used four levels of avocado proportion (0, 5, 10, and 15%) for research. And the sensory test results showed that 10% of avocado incorporated yoghurts were significant ($p < 0.05$) preferred over other groups by the panellists. Citric acid was used in this experiment to prevent enzymatic browning of avocado, and gelatine and pectin to improve the texture of yoghurt.

Meanwhile, similar results were obtained even in experiments without the addition of these additives. According to Gunawardhana and Dilrukshi (2016), among the four yoghurts with different avocado content (8, 10, 12, and 14%), the yoghurt with an avocado content of 8% was favoured by panellists. The study also found that 6% of avocado significantly benefits the probiotics in yoghurt to preserve during the shelf life. However, none of the studies mentioned above researches the sensory properties of avocado yoghurt after a certain period of storage, neither the side effect of anti-browning treatment on the quality of the product.

Additionally, as a considerable amount of avocado pulp was incorporated into yoghurt, new contamination such as *Salmonella* and *Listeria* could be a problem. However, as the studies have shown these bacteria exist only on the peel of avocado and could be effectively diminished by hygiene practice during preparing avocado pulp (Shiferaw & Kibret, 2018). Besides, HPP has been widely studied on its efficiency of inactivating bacterial cells, thus a recent study focused on HPP treatment on microbiology safety in avocado pulp focusing on yeast and mould as their spores could survive HPP treatment (Gamage, 2007).

## 2.8 Literature Review Conclusions

Yoghurt (especially fruit-flavoured yoghurt) and avocado are popular health foods with a variety of health benefits. Its global market size is steadily expanding. Moreover, yoghurt fortified with avocado has also been reported to be beneficial to the skin. According to the research mentioned on avocado yoghurt, their sensory attributes is better than that of ordinary yoghurt, so avocado stirred yoghurt potentially have broad market prospects. However, there are two key challenges in preserving avocado products, the first being PPO which causes browning, and the second of which are unsaturated fatty acids which are susceptible to oxidation. Comparing to other fruits, PPO in avocados exhibits varying degrees of resistance to different treatments, including heat and high hydrostatic pressure inactivation. Although sufficient heat treatment can effectively inhibit the activity of PPO, high temperature promotes the rancidity of unsaturated fat in the avocado and also brings bitterness to the avocado, so the practical use of heat treatment on avocado is limited. Despite the research on the avocado fruit, more relevant experiments are needed to demonstrate the effect of different treatments on the shelf life of avocado yoghurt.

# Chapter 3. Effects of different methods (chemicals and HPP) of avocado treatment on the colour stability of avocado stirred yoghurt

## 3.1 Abstract

Avocado stirred yoghurt has huge market potential with the expanding market of fruit flavoured yoghurt and the increasing health concern of the public. However, the avocado is highly perishable due to its high content of monounsaturated fatty acids and polyphenol oxidants. Considering the conventional treatment like heating is not preferred by consumers, there is a challenge of preserving the biochemical as well as microbial stability of avocado content in yoghurt during storage. Several treatments have been studied to be effective in preserving avocado, including chemical treatment like ascorbic acid, citric acid, and physical methods such as HPP. However, little has been researched about its efficiency in preserving avocado content in stirred yoghurt. In this article, different concentration (1% and 2%) of citric acid and ascorbic acid, HPP treatment (600 MPa, 5 min) and their combination was used to treat the avocado content in avocado stirred yoghurt. The instrumental colour change was recorded during storage for 26 days to determine the effective treatments that preserve the colour of the samples. Results show that the colour change of avocado yoghurt occurs predominantly in the first week which was caused by the decrease of L value and increase of a* value, while the influence of b* value is limited. The total colour difference change (ΔE) of avocado yoghurt with ascorbic acid was lowest below 2 in 26 days. Lower efficiency was found in citric acid where the colour change was below 4 comparing to the untreated sample around 6. Besides, the pH value of avocado stirred yoghurt is more sensitive to citric acid which decreases the pH from around 4.45 to 4.29 and 4.15 at 1% and 2% respectively. Nevertheless, the development of post acidification has gradually decreased the influence of added citric acid and ascorbic acid for 26 days, leaving all samples to pH around 4.2. Meanwhile, no significant influence was observed for HPP treatment on colour stability as well as pH value of the sample.

## 3.2 Introduction

Over these ten years, avocado has become a trending fruit as its world market has increased its size to almost four times larger ("World avocado market," 2020). A crucial reason is that the health benefits of avocado have drawn the attention of the public to growing health concerns.

The shelf life of the product with avocado incorporated is essential, as avocado itself is easily oxidized. The preservation of avocado has long been trouble since the widely used heat treatment brings bitterness and other unpleasant flavours in the final product (Bustos et al., 2017). Thus, other chemical and physical treatments have been raised to minimise oxidation and extent the shelf life of avocado. For chemicals, antioxidants have been focused on such as ascorbic acid, citric acid, EDTA and extract from allium and brassica extracts (Bustos et al., 2017). And the physical methods focusing on preventing the product from exposure to oxygen by packaging or deactivate the polyphenol oxidase (PPO) which is usually done by high-pressure pasteurization.

For avocado yoghurt, the type and application level of those treatments should be filtered to ensure their minimized effect on the flavour of yoghurt. It's also important to choose from non-artificial additives considering the increasing consumer demand worldwide for high quality, minimally processed products that are free from artificial food additives (E Palou et al., 2000). Therefore, the effect of different levels of ascorbic acid, citric acid, HPP methods and their combination on avocado yoghurt were analysed in this article.

An effective approach to analyse the efficiency of treatments is to evaluate their influence on the colour stability of the product as colour is the first and foremost property of avocado that changed over time and is perceived by the consumer as a crucial indicator of freshness. In avocado, browning plays an important role in colour change, and the brown pigments are caused by the polymerization of o-quinones that formed due to PPO and its substrates.

In this study, HPP treatment, different percentages of ascorbic acid and citric acid were used to treat avocado pulp before it was incorporated into avocado yoghurt, and the colour and pH stability was evaluated in 26 days by determining the colour of avocado yoghurt routinely. This experiment is aiming at choosing proper treatments which could potentially develop stabilized avocado yoghurt.

## 3.3 Materials and Methods

### 3.3.1 Materials

Fresh avocado (Hass) incorporated in yoghurt was purchased in a local supermarket. Citric acid and ascorbic acid were supplied by Hawkins Watts Ltd. (New Zealand). The ingredients used in preparing yoghurt included standard whole milk (Anchor Dairy, New Zealand), sugar (Chelsea., New Zealand), trim milk powder (Anchor, New Zealand), pectin (CM020, Herbafood (H&F) Ingredient GmbH, Germany), and starter culture (Lyofast Y 438 B, 10UC, Sacco., Italy). The chemical composition of whole milk and trim milk powder used in this study is chosen in Table 3.1. The base formulation for making stirred yoghurt in this project was based on the literature in Chapter 2 and some preliminary trials (not shown in this thesis).

*Table 3.1. The average quantity of nutrition in the standard whole milk and trim milk powder (Anchor, 2021).*

| | **Average quantity in 100ml standard milk** | **Average quantity in 100g trim milk powder** |
|---|---|---|
| **Energy** | 263 kJ (63 Cal) | 1520 kJ (363 Cal) |
| **Protein** | 3.3 g | 33.0 g |
| **Fat - Total** | 3.4 g | 1.0 g |
| **- Saturated** | 2.3 g | 1.0 g |
| **Carbohydrate** | 4.8 g | 54.0 g |
| **-sugars** | 4.8 g | 54.0 g |
| **Sodium** | 40 mg | 450 mg |
| **Calcium** | 117 mg | 1100 mg |

### 3.3.2 Preparation of avocado pulp

Figure 3.1 below shows the process of avocado pulp making. The avocado was bought from the supermarket and stored at room temperature (20 ± 1°C) till fully ripen in 4 days. Then spoiled or partially spoiled avocado was discarded while chosen avocados were washed with care and peeled and cut in half. The two avocado fresh halves were then placed in vacuum bags (175*200 mm, Contour International, New Zealand) and stored in the fridge at 4 ± 1°C. On the next day, part of the sealed avocado halves was taken to NZ Food Innovation Auckland Ltd (The Food bowl) and processed in vacuum bags by high-pressure processing (HPP) using Uhde 055 HPP machine (Uhde, Germany) at 600MPa, 20 °C for 5 min. The samples were then stored in a freezer at -20 ± 0.1°C until use.

Before its use for the incorporation into yoghurt, the frozen avocados treated with HPP were thawed at 4°C and blended using a hand blender (Living & Co., 250W, New Zealand) at 300 rpm for 10 minutes to make avocado pulp. The avocado pulp was then added and mixed with 1% or 2% w/w of ascorbic acid or citric acid powder in order to investigate their effects against enzymatic browning. The avocado pulps prepared with and without HPP treatment or with and without the addition of ascorbic or citric acids were mixed with yoghurt to make avocado-fortified yoghurt samples containing avocado with different treatments as shown in Table 3.2.

*Table 3.2. The sample codes of avocado yoghurts were prepared by mixing yoghurt with avocado pulp treated with different methods.*

| **Sample code** | **Treatment of avocado** | |
|---|---|---|
| | **HPP treatment** | **Ascorbic or citric acid (1 or 2%)** |
| Untreated | - | - |
| As1 | - | Ascorbic acid 1% |
| As2 | - | Ascorbic acid 2% |
| Ci1 | - | Citric acid 1% |
| Ci2 | - | Citric acid 2% |
| HPP | HPP | - |
| Asi-HPP | HPP | Ascorbic acid 1% |
| As2-HPP | HPP | Ascoribic acid 2% |
| Ci1-HPP | HPP | Citric acid 1% |
| Ci2-HPP | HPP | Citric acid 2% |

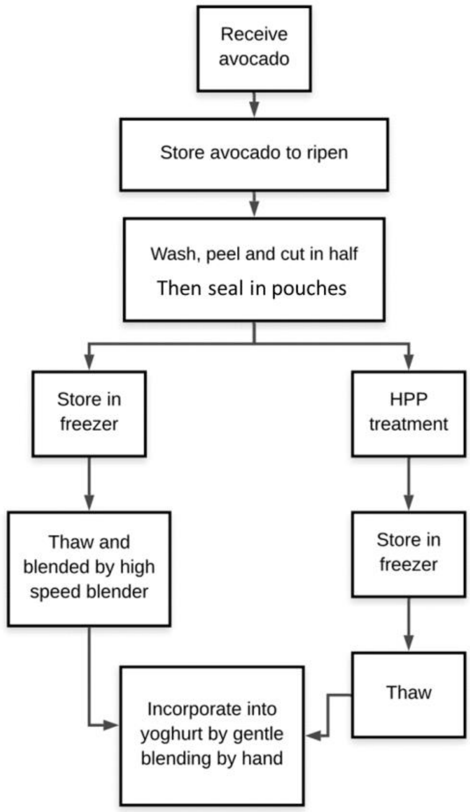


*Figure 3.1 Process of avocado pulp making.*

### 3.3.3 Preparation of avocado stirred yoghurt

Details of the formulation are shown in Table 3.3. The formulation was derived from preliminary trials based on various literatures. First of all, standard whole milk was heated up to 50°C in a 3 L glass jar with a lid. Then trim milk powder was weighted and blended into the milk by Laboratory Mixer (L5M-A, Silverson, USA) for 2 min. The milk was kept at a 50°C water bath for the fine dispersion of milk solid. Then the milk was further heated to 60°C followed by slowly adding a mix of weighted pectin and sugar when agitated by the mixer. After 5 min of agitation, the milk was then heated up to 90°C for pasteurisation for 5 min. The milk was then placed in the cold-water bath. After cooling to 45°C, milk was inoculated by adding weighted freeze-dry culture powder. The milk base was then placed into a water bath at 42°C for 5 hours, followed by a routine pH check every 10 minutes.

When the pH fell to 4.6, a wooden spoon was used to gently break the curd by hand. After agitation for about 10 seconds, the product was cooled down immediately by placing in a cold-water bath until the temperature was decreased to around 20°C. The avocado pulp (10% w/w) was then added, and the yoghurt mix was uniformly agitated by using a wooden spoon for 5 minutes. It was then stored in a fridge at 4°C for a specific time based on the schedule.

*Table 3.3. Formulation of yoghurt base plus avocado pulp.*

| Ingredient | Quantity (g) in 1000 g |
|---|---|
| **Standard milk** | 867.5 |
| **Strim milk powder** | 10 |
| **Pectin** | 2.5 |
| **Sugar** | 20 |
| **starter culture powder** | 0.2 |
| **Avocado pulp** | 100 |

### 3.3.4 pH measurement of yoghurt

The pH of yoghurt samples was measured at 20 ± 1°C in triplicate by using a pH meter (Sartorius, basic pH meter PB-20) after the pH meter was calibrated using pH 4.00 and pH 7.00 buffer solutions (Biolab Ltd., Australia). The pH was also determined during storage at different time intervals (day 0, 2, 5, 12, 19 and 26).

### 3.3.5 Colour measurement of avocado yoghurt

The colour of avocado yoghurt samples was analysed in triplicate during storage (0, 2, 5, 12, 19 and 26 days) by using a colourimeter (Minolta Co. ltd, CR-300, Japan). The sample was firstly agitated to even colour and then a 5 g of sample was placed into the 35 mm diameter Petri dish (Thermo Fisher Scientific, USA). A spatula was used to pack the sample tightly ensuring no air bubbles. The measurement of colour was expressed in CIE L*a*b* colour space to determine a colour change as one of the quality characteristics of yoghurts during cold storage. The colour values of the control sample (day 0) were taken as the reference to calculate a colour change during storage expressed as the total colour difference (ΔE) being calculated by using the equation shown below. In the CIE L*a*b* values, L* represents lightness/darkness. The a* and b* values represent colours from red to green, and yellow to blue, respectively, depending on their values as positive or negative.

$$\Delta \mathrm{E} = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$$

### 3.3.6 Data analysis

The results were statistically analysed using the Minitab (Minitab LLC, version 17, USA). Tukey's multiple comparison test was used to examine differences between the mean values at a significance level of $p < 0.05$. All the measurements (pH and colour) were carried out at least in duplicate for each sample from duplicate experiments.

## 3.4 Results and Discussion

### 3.4.1 pH changes of avocado yoghurts

Initially, the pH changes of yoghurt samples containing avocado pulp treated by different methods were monitored during storage at 4°C for 26 days. The results are shown in Figure 3.2. In general, the pH of all avocado yoghurt samples decreased to around 4.2 during the 26 days, and the effect of HPP on pH was not significant as its pH was similar to the untreated avocado-containing yoghurt sample. The effect of ascorbic acid and citric acid on pH was detected resulting in a decrease in the initial pH compared to the untreated sample, while the acidification caused by citric acid was significantly ($p < 0.05$) stronger than that of ascorbic acid. Initially, the incorporation of citric acid (1% and 2%) decreased the pH of yoghurt from around 4.43 to around 4.18 and 4.22, respectively, while ascorbic acid (1% and 2%) reduced the pH of avocado pulp to 4.37 and 4.28, respectively. On day 5, the significant effects of both concentrations of ascorbic acid no longer existed. However, the sample treated with citric acid (1% and 2%) was 4.23 and 4.19, respectively, compared to the pH of the untreated sample which was around 4.3. Then the gap was further narrowed during the further storage. On day 26, the influence of each chemical was not significant.

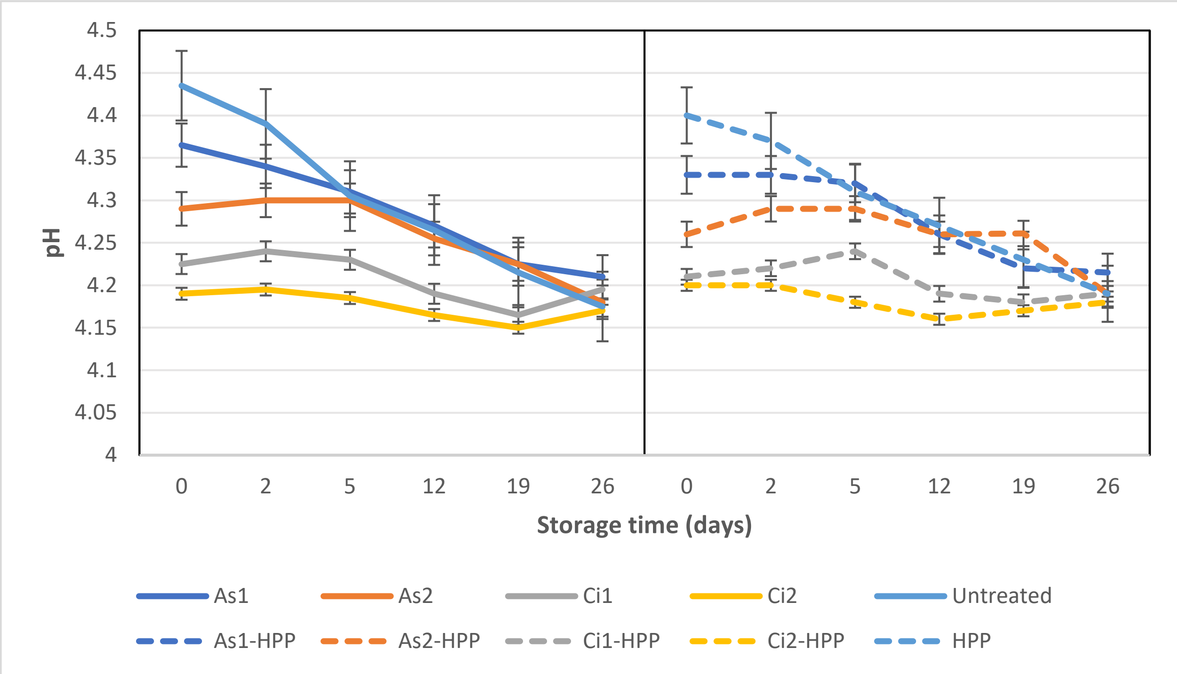


*Figure 3.2 Changes in pH of yoghurt samples, which contained avocado pulp with or without HPP treatment and/or acidified by 1% ascorbic acid (As1) 2% ascorbic acid (As2), 1% citric acid (Ci1) or 2% citric acid (Ci2), during storage at 4°C for 26 days. Untreated means no HPP and no acidification.*

Considering the pH of the sample treated by ascorbic acid was equal to that of the untreated sample after 5 days, the influence of ascorbic acid on yoghurt pH was acceptable. However, for citric acid, the initial pH of yoghurt dropped to around 4.2, which was close to the pH of the untreated sample on day 26. Due to the main changes in the yoghurt during storage is the acidity increases, which will increase the acidity of the product thus decrease the sensory property. Therefore, a low pH at the beginning of storage is not conducive to the quality of the product.

For HPP methods, no effect on pH was detected, thus its influence on the pH of yoghurt was minimum compared to the incorporation of ascorbic acid and citric acid.

### 3.4.2 Effect of ascorbic and citric acids on colour change of avocado yoghurts

Figure 3.3 shows changes in the L*, a*, b* colour values of avocado stirred yoghurt samples, which contained avocado pulp that was not treated with HPP but was treated by adding ascorbic acid or citric acid at two different concentrations of 1% and 2% (As1, As2, Ci1, Ci2) or without any treatment (untreated, UT). The colour of these samples was measured during storage at 4°C for 26 days.

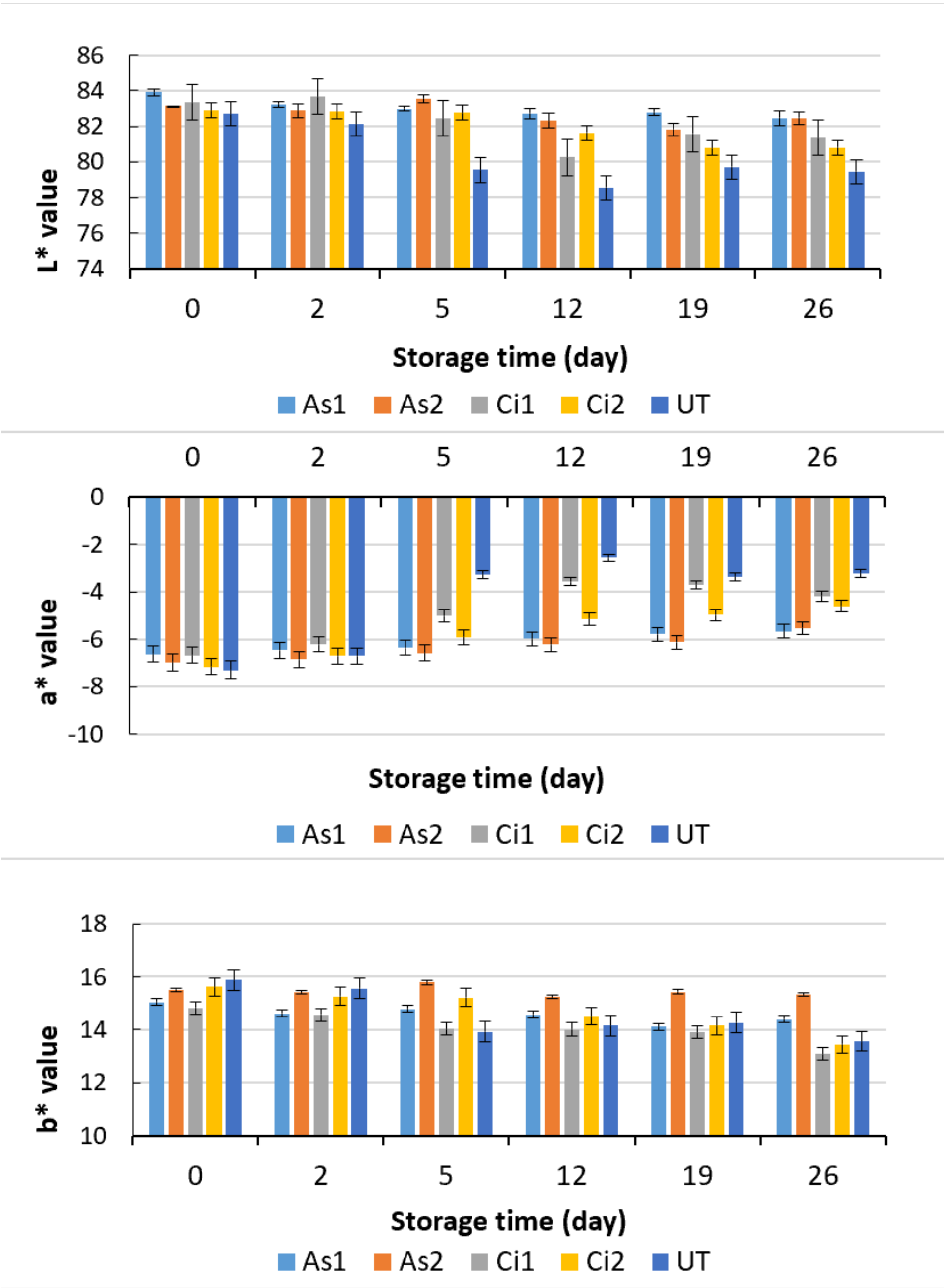


*Figure 3.3. Changes in L*, a* and b* colour values of avocado yoghurt samples, which contained avocado pulp treated with 1% ascorbic acid (As1), 2% ascorbic acid (As2), 1% citric acid (Ci1) and 2% citric acid (Ci2) or without any treatment (untreated, UT), during storage at 4°C for 26 days.*

L* value (lightness/darkness): The L value of all samples showed no significant change in 2 days after preparation, while for the untreated sample (UT), it dropped dramatically on day 5 from around 82.3 to 79.5 and then remained relatively steady during the rest storage period of 26 days. Other treated samples maintained a similar L* value compared to day 0, while on day 12, the L* value of the sample treated by citric acid (Ci1 and Ci2) significantly decreased and then remained stable in the following days on a higher level (80.8) than that of the control sample (UT). Within the total 26 days, the experimental samples with ascorbic acid (As1 and As2) were relatively stable where the difference of L* value compared to day 0 was less than 2. The total change in L* value for samples Ci1 and Ci2 was around 3 but it was 4 for the control sample. In general, the two different concentrations (1% and 2%) of ascorbic and citric acids showed no significant difference in the L* value.

a* value (redness or greenness): As expected, all samples had a negative a* value initially at 0 day which was between -6.5 and - 7.5, indicating the samples had a green hue. The negative a* value however had a significant decrease during storage, which indicates the loss of greenness (less green) which might be related to the degradation of chlorophyll combined with enzymatic browning. Similar to the change of L* value, a* value of all samples remained stable in 2 days, while the control sample (UT) dramatically decreased (less negative a*) on day 5 and then remained steady in the following days at around -3. For the sample treated with citric acid (Ci1 and Ci2), especially for 1% of citric acid (Ci1), the a* value was significantly decreased (less negative) from day 5 and reached around -4. Meanwhile, the a* value of the sample treated with ascorbic acid (As1 and As2) remained at -5.5 on day 26. With the a* value of all samples being decreased in this experiment, the incorporation of ascorbic acid and citric acid significantly delayed this trend, while ascorbic acid was observed to be relatively more efficient. Besides, for ascorbic acid, the concentration of 1% and 2% didn't have a significant difference. On the other hand, for citric acid, the data showed that even though its concentration didn't influence the final a* value on day 26. However, the sample (Ci2) treated with a higher concentration of citric acid sustained a* value for a significantly longer time before decreasing.

The greenness of avocado yoghurt samples would be mainly resulting from the presence of chlorophyll derived from avocado. However, the chlorophyll in avocado is easily degraded due to enzymatic reactions and non-enzymatic reactions influenced by environmental conditions. As a fresh fruit pulp abundant in chlorophyll, the discolouration of green avocado pulp to dark-green, yellow, or even black is primarily due to the degradation of chlorophyll (Heaton & Marangoni, 1996; Strain, 1954). The results of this study were in line with the reports that the incorporation of an antioxidant such as ascorbic acid can prevent the induction of the oxidation of chlorophyll by light, peroxidase and/or lipoxygenase, therefore controlling the degradation of chlorophyll (Heaton & Marangoni, 1996).

b* value (yellowness or blueness): In all samples, the b* value was positive, thus, the avocado yoghurt samples had a yellow hue, too, besides the green hue represented by the negative a* values. In general, the b* value decreased during the experiment but with a smaller difference in comparison with other values. The samples treated by ascorbic acid (As1 and As2) showed no significant change during storage for 26 days. On the other hand, for the samples treated with citric acid (Ci1 and Ci2), the b* values were decreased by about 2, which was similar to that of the untreated sample (UT). It should be noted that the scale of b* value was higher than that of a* value, indicating the avocado yoghurt samples prepared in this study were more yellowish than greenish although they were yellowish-green in colour.

### 3.4.3 Effect of HPP treatment on colour change of avocado yoghurts

Figure 3.4 also shows the changes in L*, a* and b* values of yoghurt samples containing avocado pulp with or without HPP treatment and/or added with 2% ascorbic acid (As2) or 2% citric acid (Ci2). These samples including the control sample containing untreated avocado pulp (UT) were measured for their colour changes during storage at 4°C for 26 days.

L* value (lightness/darkness): The L* value of all samples showed no significant change during the first 2 days of storage. However, the main noticeable change in the L* value was observed from the control sample containing untreated avocado pulp which was significantly decreased on day 5 unlike the other samples treated with ascorbic acid and citric acid had the L* value maintained relatively consistent. Also, the HPP treatment alone had a significant influence on the sample on day 5, where the *L value was significantly higher than the untreated sample. However, this influence decreased over time till the end of the storage trial. For the combination of ascorbic acid or citric acid with HPP (As2-HPP and Ci2-HPP), no noticeable difference due to the application of HPP was observed when compared to the samples treated with ascorbic acid or citric acid only without HPP.

a* value (greenness): The negative a* value was shown to decrease (less negative a* value) in all samples as shown in Figure 3.3. The effect of HPP treatment with or without adding ascorbic or citric acids was not observed on the change of a* value.

b* value (yellowness): For the samples treated with both ascorbic acid and HPP (As2-HPP), the b* value was slightly increased which was opposite to the general trend. However, for other samples, all b* values were decreased at various degrees with no significant effect of HPP treatment.

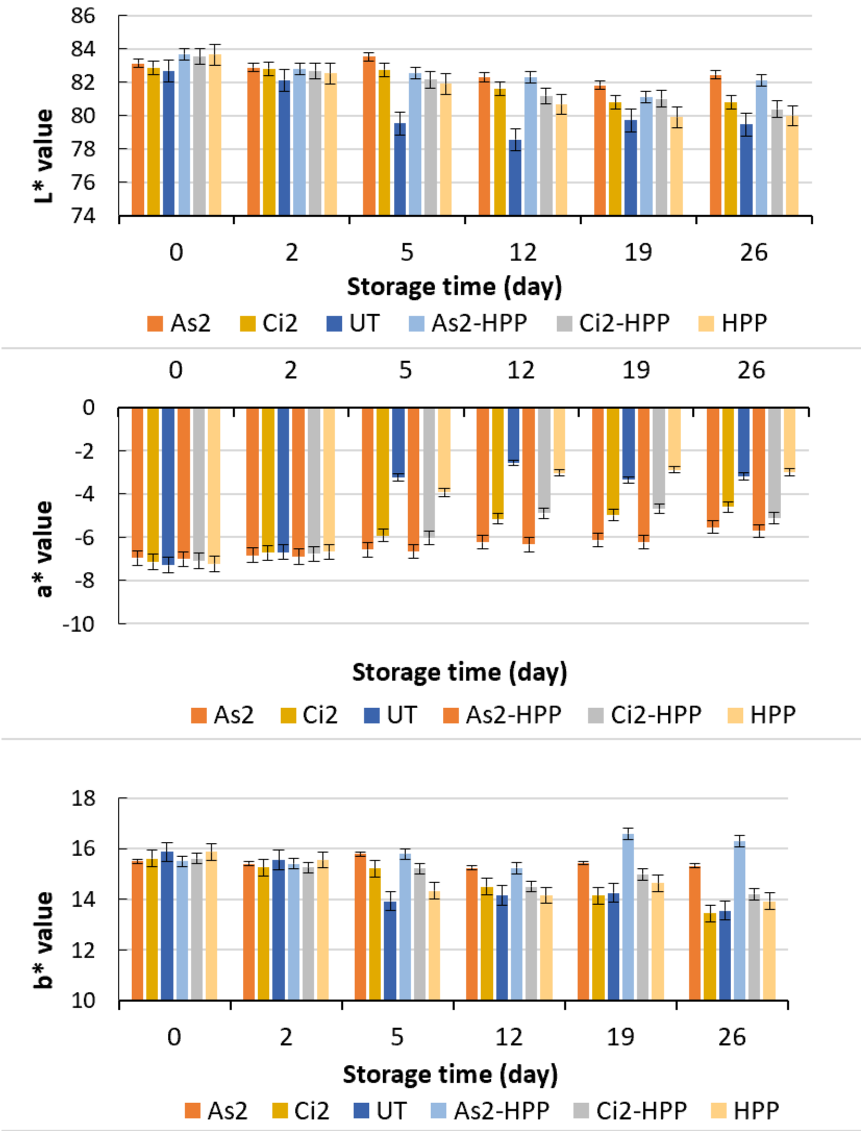


*Figure 3.4 Changes in L*, a* and b* values of yoghurt samples, which contained avocado pulp with or without HPP treatment and/or acidified by 2 % ascorbic acid (As2) or 2% citric acid (Ci2) or untreated (UT), during storage at 4°C for 26 days.*

### 3.4.4 Total colour difference (ΔE)

The ΔE value representing a total colour difference was calculated from all samples based on the CIE L*, a*, b* values shown in the previous sections above. It should be noted that the CIE L*, a*, b* values of each sample measured at 0 days was used as the reference sample in calculating the ΔE value of the sample during storage. In general, the ΔE increased in 12 days dramatically, then stayed relatively stable afterwards (see Figure 3.5). The incorporation of ascorbic acid effectively lowered the ΔE during the experiment. On day 12, the ΔE value of the samples with 1% and 2% of ascorbic acid (As1 and As2) was around 1.1 and 1.05, respectively, which was significantly lower than the ΔE of the untreated sample which was 6.4 on day 12. The ΔE values for the As1 and As2 samples remained lower than 2 during the entire storage period, indicating that the colour change was not significant and its colour change might not be detectable visually. Furthermore, the effect of its concentration in this experiment was not significant. For citric acid, although a significant effect was observed, its efficiency was lower than that of ascorbic acid. On day 12, the ΔE of the samples treated by 1% and 2% of citric acid was around 4.3 and 2.8., respectively, while the difference in the ΔE values between the two samples was narrowed after storage for 26 days as both samples had the ΔE of around 3.9 on day 26.

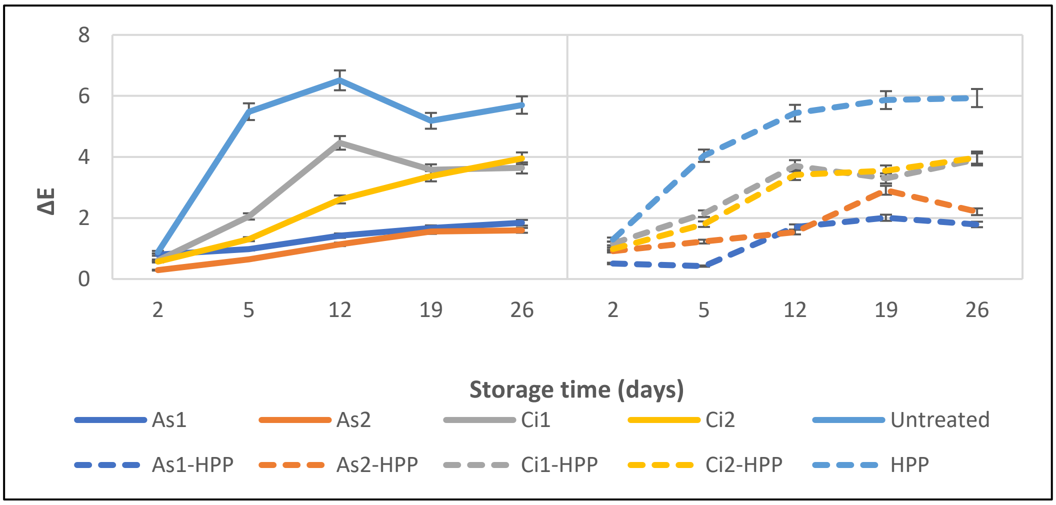


*Figure 3.5 The total colour difference (ΔE) values determined during storage of avocado yoghurt samples containing avocado pulp with or without HPP treatment and acidified by 1% of ascorbic acid (As1) 2 % ascorbic acid (As2), 1% of citric acid (Ci1) or 2% citric acid (Ci2).*

The effectiveness of citric acid could be due to its functionality as an antioxidant that reduces the formation of o-quinones enzymatically formed, thus delaying browning (Soccol, Vandenberghe, Rodrigues, Pandey, & Biotechnology, 2006). The function of ascorbic acid as an antioxidant is similar to citric acid in this case. However, as preservatives, ascorbic acid is more versatile and noted for its complex multi-functional effects. Depending on conditions, ascorbic acid can act as an antioxidant, a pro-oxidant, a metal chelator, a reducing agent or as an oxygen scavenger (Frankel, 1996). Its effect on the PPO and browning of avocado pulp has been widely reported (Elez-Martínez et al., 2002).

Without the combination of antioxidants, the effect of HPP alone on colour change was not very significant although, on day 5, the ΔE of HPP treated sample was 4.0 which was lower than the untreated sample's ΔE value of 5.5. However, no difference was observed between these two samples after storage for 26 days. On day 26, the ΔE of the HPP treated sample was 5.9 and the untreated sample had the ΔE value of 5.8. As for the samples treated with chemicals, no significant effect of HPP was observed. This result agreed with a report on avocado pulp where the HPP methods showed no significant influence on the colour stability during storage (Gamage, 2007).

The HPP treatment on the colour change is limited. In terms of enzymatic browning, it could inactivate extracted PPO, but in avocado fruit, opposite consequences may occur. In a study reported by Ebbage (2011) that aimed at analysing PPO activity in Hass avocado pulp, the extraction of PPO from avocado was attempted which was then followed by the HPP treatment at 600 MPa for 3 minutes. The main results showed the residual activity of the enzyme which was about 50.72%. and its activity was restored 10-15 days after the treatment (Ebbage, 2011). Another study applied a four-cycle high pressure (four times, 689 MPa every 5 minutes) on guacamole which showed a residual activity of PPO at approximately 15% (Palou et al., 2000).

Besides the enzymatic browning, the degradation of chlorophyll is another reason that contributes to the colour change of avocado. The chlorophyll in avocado is easily degraded due to enzymatic and non-enzymatic reactions influenced by various environmental factors. As the fresh avocado pulp is abundant in chlorophyll, the discolouration of green avocado pulp to dark-green, yellow, or even black is primarily due to the degradation of chlorophyll (Heaton & Marangoni, 1996; Strain, 1954). It is reported that the incorporation of an antioxidant, such as ascorbic acid, slows down the induction of oxidative degradation of chlorophyll induced by light, peroxidase and/or lipoxygenase, therefore controlling the degradation of chlorophyll to some extent during storage (Heaton & Marangoni, 1996).

Little has been studied on the effect of HPP on chlorophyll in avocado. Nevertheless, based on the result of this research, the effect of HPP was found to be limited.

## 3.5 Conclusions

The colour change of avocado yoghurt occurred predominantly in the first week of storage. It was mainly caused by a decrease of L* value and an increase of a* value. Ascorbic acid was observed to minimise the total colour change (ΔE less than 2) of avocado yoghurt samples during storage for 26 days compared to the other treatments. Although the effect of citric acid was observed in the experiment, its efficiency was not comparable to ascorbic acid, but it eventually led to keep the colour change below 4 compared to the untreated sample (ΔE at round 6). Besides, the pH value change in avocado stirred yoghurt was more sensitive to citric acid which decreased the pH from around 4.45 to 4.29 and 4.15 at 1% and 2%, respectively. Nevertheless, the development of post acidification leaving all samples to pH around 4.2 was observed at the end of storage for 26 days. On the other hand, the HPP treatment was found to be ineffective in controlling the stability of colour and the pH change of the samples. In conclusion, ascorbic acid was found to be most effective in preserving the colour of avocado yoghurt samples and controlling the pH change during storage, although its concentration was 1% or 2% or whether avocado was treated by HPP or not.

# Chapter 4. Effects of HPP and ascorbic acid on the stability and properties of avocado stirred yoghurt

## 4.1 Abstract

With the increase of public health concerns and pursuit for expanding sensory experience, there's a huge market potential for fruit flavoured yoghurt. Avocado, a globally emerging fruit, has drawn attention as new fruit to be incorporated into yoghurt. However, avocado is highly perishable and sensitive to deterioration, thus it is important to preserve its sensory properties when its incorporation into food products. It was shown in Chapter 3 that the addition of ascorbic acid with and without HPP treatment was effective in preserving the colour of avocado yoghurt, especially against enzymatic browning. Therefore, this study focused on their effects on some other properties of avocado yoghurt samples containing 10% avocado pulp, including viscosity, syneresis, colour, pH and microbial quality (e.g. lactic acid bacteria and contaminating microbes). In the experiments, HPP treatment (600 MPa/5 min), 2% of ascorbic acid and their combination were used to treat avocado. The results showed that the incorporation of 2% ascorbic acid was effective in preventing the colour change of avocado yoghurt, maintain the total colour difference (ΔE) value below 2 during the shelf-life of yoghurt at 4$^{o}$C for 4 weeks. On the other hand, HPP treatment alone was not effective in preventing avocado yoghurt from its colour change, however, HPP treatment was found to be the key factor to significantly reduce syneresis (around 1%) in avocado yoghurt samples by minimizing some extra syneresis caused by the incorporation of avocado pulp. Also, the rheology test showed that the HPP treatment increased and maintained the thickness of avocado stirred yoghurt during storage for 4 weeks. However, no significant effect on rheological properties was observed by the incorporation of ascorbic acid. Neither of these treatments and incorporation of avocado was shown to have no impact on the growth of lactic acid bacteria in avocado yoghurt. Also, no yeast and moulds or coliform were detected in avocado yoghurt samples during their shelf life which can be 4 weeks at 4$^{o}$C.

## 4.2 Introduction

Avocado stirred yoghurt has huge market potential with the expanding market of fruit flavoured yoghurt and the increasing health concern of the public. However, avocado is highly perishable due to its high content of monounsaturated fatty acids and polyphenols which are sensitive to oxidation and enzymatic degradation (Schaffer et al., 2013). Considering the conventional unit operation like heat treatment to inactivate undesirable enzymes and destroy microorganisms is sometimes not suitable to use in some food formulations and processing conditions. Therefore, there is a challenge of preserving the sensory properties and microbial stability of avocado-fortified yoghurt during storage when the fresh avocado pulp is incorporated as fruit into yoghurt during storage.

The sensory properties of yoghurt, including viscosity, texture, flavour and colour, are crucial as indicators of yoghurt quality. The characterisation of yoghurt quality typically includes microbial quality (including lactic acid bacteria and contaminating microbes), rheological properties and acidity (pH) (Chandan & Kilara, 2013). For avocado stirred yoghurt, the contaminating microbes mainly include coliform potentially introduced by milk base and yeast and mould from the avocado pulp. Therefore, it is important to determine the total yeast and mould count as well as the total number of lactic acid bacteria as a measure of microbial quality and safety.

High-pressure processing (HPP), which is known as a nonthermal food preservation technique that inactivates harmful microorganisms and enzymes, has been reported to make the avocado fruit into pulp in a different way when HPP is applied to avocado fruit. As high pressure (e.g. 600 MPa, 5 min) destroys the fibre in avocado, little shearing is thus required when processing the avocado fruit into pulp (Zhang et al., 2015). Compared to blending by high shear blender, the cell wall structure is partially maintained when treated by HPP (Zhang et al., 2015). However, this impact as well as its changes during storage on the avocado yoghurt has not been investigated and understood clearly. Besides, HPP effectively inactivates the enzyme, especially polyphenol oxidase (PPO) present tin avocado, resulting in a delay of enzymatic browning during the degradation of avocado. Ascorbic acid as a chemical agent is well known for its multi-functional roles. Depending on conditions, ascorbic acid can act as an antioxidant, a pro-oxidant, a metal chelator, a reducing agent or as an oxygen scavenger (Frankel, 1996).

Although some studies have been reported as described above, the effects of ascorbic acid addition and HPP treatment of avocado on the physicochemical and microbial properties of avocado-fortified yoghurt have not been investigated. Therefore, the objective of this study was to evaluate the effects of HPP and ascorbic acid on the physical, chemical, and microbial properties of avocado stirred yoghurt within its shelf-life during storage for 4 weeks at 4$^{o}$C.

## 4.3 Material and Methods

### 4.3.1 Preparation of avocado stirred yoghurt

Yoghurt samples containing avocado pulp with or without HPP treatment (600 MPa/5 min) and/or 2% ascorbic acid addition were prepared by using the method as described in Sections 3.3.2 and 3.3.3. The avocado yoghurt samples prepared after mixing with 10% w/w of differently treated avocado pulps were designated by sample codes as U, H, A and HA as shown in Table 4.1, including another yoghurt sample, called the plain yoghurt (P) without containing avocado pulp prepared under the same conditions.

*Table 4.1. The sample codes of avocado yoghurts prepared by mixing yoghurt without or with avocado pulp (10% w/w) treated by different methods.*

| Sample code | Description |
|---|---|
| **P** | Plain yoghurt without containing avocado pulp |
| **U** | Yoghurt containing untreated avocado pulp |
| **H** | Yoghurt containing avocado pulp treated by HPP |
| **A** | Yoghurt containing avocado pulp treated by 2% ascorbic acid |
| **HA** | Yoghurt containing avocado pulp treated by both HPP and 2% ascorbic acid. |

### 4.3.2 Measurement of syneresis by centrifugation

Syneresis of yoghurt samples was measured according to the method of Amal, Eman, and Nahla (2016) with some modifications. Briefly, a 40 g of yoghurt sample was weighed and transferred into a 50 mL centrifuge test tube (Thermo Fisher Scientific, New Zealand). Then, the yoghurt samples in the centrifuge tubes were centrifuged at 4°C for 10 min at 237 x g in a Sigma 6-16KS centrifuge (Sigma-Aldrich Inc., Germany). After centrifugation, the supernatant was taken out by pipette and weighed. The difference in weight between the residue and supernatant was then calculated as syneresis (%) using the equation below.

$$\text{Syneresis (\%)} = \text{weight of supernatant (g)} \times 100 / \text{weight of sample (g)}$$

### 4.3.3 Confocal laser scanning microscopy

The microstructures of avocado pulp and avocado-fortified yoghurt samples were observed using confocal laser scanning microscopy (CLSM) (Zeiss LSM 980 with Airyscan 2, Germany). Samples were placed in a cavity slide and added with 50 µL of 0.2 g/L Nile Red and Fast Green before a coverslip covered the sample. Imaging was captured by the Leica DM6000B SP5 confocal laser scanning microscope system with LAS AF software (version 2.7.3.9723, Leica Microsystems CMS GmbH, Germany). Images were acquired with an HCX PL APO CS 40x oil (N.A.1.25) using the super-resolution mode. Nile Red and Fast Green were sequentially imaged from excitation at 488 nm (argon laser) and 633 nm (HeNe 633 laser), respectively. Emission collection was conducted at 498-569 nm and 643-787 nm, respectively.

### 4.3.4 Microbiological quality analysis of yoghurt

Microbiological testing was conducted to determine the microbial stability of yogurt samples during their shelf-life for 4 weeks at 4°C. Lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) count, total coliform count, and yeast & mould count was measured. The microorganisms were enumerated by using different media and methods mentioned below. After the incubation period, the number of colonies was counted or estimated on two serial plates with 25 to 250 colonies. All tests were analysed in triplicate.

For the lactic acid bacteria count, a 10 g of sample was taken out after gentle agitation and dispersed into 90 g of 0.1% peptone water for 1 min. Then 1 ml of the sample was poured on the Lactic Acid Bacteria Count Plate (3M™ Petri film™, USA) which was later placed into the incubator at 37°C for 24 h.

A similar procedure was applied in the coliform count and yeast and mould count in which the samples were incubated on coliform count plate (3M™ Petri film™, USA) and yeast and mould count plate (3M™ Petri film™, USA), respectively. The incubation time for coliform count plate was 24 h at 37°C, and the yeast and mould count plates were incubated aerobically at 25°C for 3–5 days, the developed colonies were evaluated and counted.

### 4.3.5 Rheology test

The rheological properties of samples were analysed by using a rheometer (AR550 advanced rheometer, TA instruments Ltd., USA) equipped with a cone and plate geometry. Th rotating cone geometry was 20 mm in diameter and the cone angle was 2°. The instrument was controlled by the Rheology Advantage Instrument Control AR software (version 5.8.2, TA instruments Ltd., USA) and the data was collected and analysed by the Rheology Advantage Data Analysis software (version 5.7.0, TA Instruments Ltd., USA). The measurement of flow properties of yoghurt samples was carried out at 20 ± 1°C. For each test, around 1 ml of sample was loaded onto the Peltier plate by a spoon, then the rheometer with the filled sample was initially temperature equilibrated for 2 minutes. The sample was then sheared with a programmed continuous sequence in which the shear rate was increased linearly from 0 to 300.0 $s^{-1}$ over 5 minutes. Shear stress-share rate data were collected continuously at 10-second intervals throughout the test. Each sample from the replicated experiments was analysed in triplicate.

To better describe the rheological properties of the samples, the obtained viscosity-shear rate data were fitted to the Williamson model by using Rheology Advantage Data Analysis software. The model was described by the equation below, where a is described as zero-rate viscosity, b as consistency and c as rate index.

$$Viscosity = \frac{a}{1 + (b * shear\ rate)^c}$$

### 4.3.6 Data analysis

A storage day × HPP × ascorbic acid factorial arrangement of treatments in a completely randomized design with repeated measures was utilized. All experiments for the sample preparation and analysis were carried out at least in duplicate. The results are reported as average and standard deviation. Differences among treatments were evaluated by Tukey's multiple comparison test at a significance level of $p < 0.05$. All analyses were performed using Minitab (Minitab LLC, version 18, USA).

## 4.4 Result and Discussion

### 4.4.1 Changes in pH of yoghurt

The pH change of yoghurt samples is shown in Figure 4.1. All samples showed a similar trend with decreasing gradually from day 0 to day 28. For the yoghurt samples (U and H) containing untreated avocado or HPP-treated avocado without ascorbic acid addition, their initial pH values were around 4.61 and 4.60, respectively. On the other hand, the other two samples added with ascorbic acid (HA and A) had the pH of 4.51 and 4.48, respectively, at day 0. The slightly lower pH observed in the latter two samples would be due to ascorbic acid, known as a weak acid. However, the plain yoghurt (P) without added avocado pulp also had a slightly low pH of 4.53 compared to the samples (H and U).

The change of pH was dramatic in the first 7 days. For the sample without ascorbic acid (U), its pH dropped from 4.61 to around 4.46, while the pH of sample HA dropped from 4.51 to 4.39. For the sample added with ascorbic acid (A), its pH was decreased from 4.48 to 4.40. This trend was in agreement with the study reported by Campos et al. (2017) where the pH of plain stirred yoghurt decreased dramatically in the first week. The pH decline during storage observed in this study could be related to 'post acidification which is an undesirable phenomenon caused by excessive acids produced by the lactic acid bacteria during storage under the cooling temperature (Tamime, Robinson, & Tamime, 2007a).

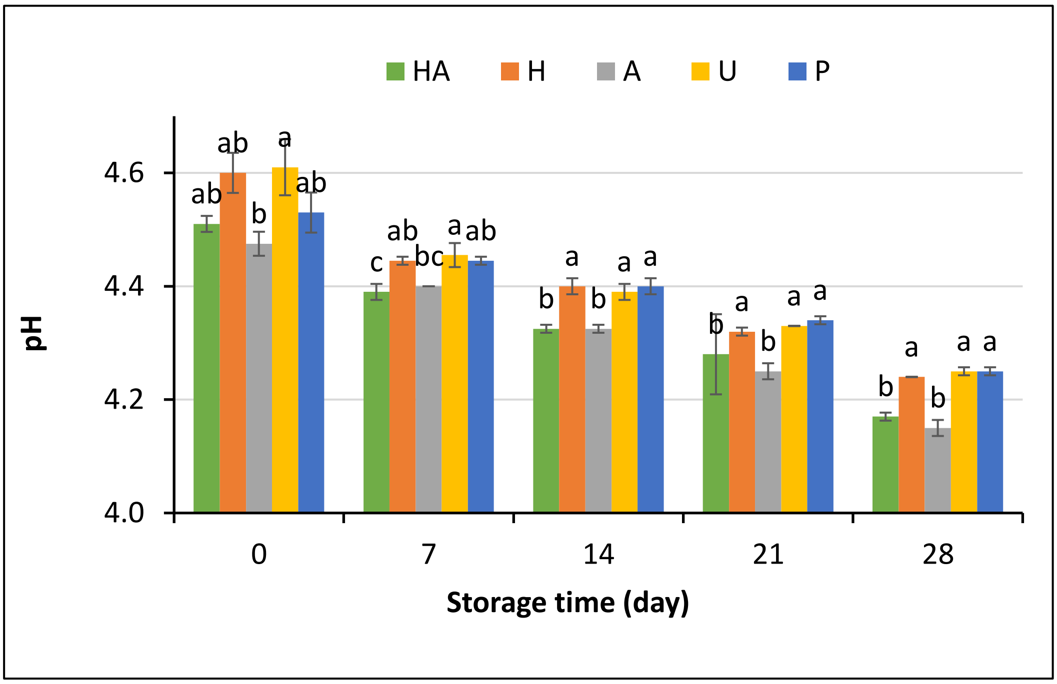


*Figure 4.1 Changes in pH of the plain yoghurt without containing avocado (P) and the yoghurt samples containing avocado that was untreated (U) or treated with HPP (H), 2% of ascorbic acid (A) and a combination of HPP and ascorbic acid treatment (HA). Yoghurt samples were vacuum packed in pouches and stored at 4°C for 28 days. Letters a, b and c in the graph indicating a significant difference ($p < 0.05$) between samples at a given storage time.*

As already mentioned above, the plain yoghurt sample (P) without avocado had a lower pH (4.53), while the pH of samples containing untreated avocado (U) or HPP-treated avocado (H) was 4.61 and 4.60, respectively. This slightly higher pH of the latter two samples compared to the plain yoghurt could be due to the incorporation of 10% avocado pulp, which pH was around 6.65. On the other hand, the sample with ascorbic acid had a significantly lower pH, which was affected by the acidity of ascorbic acid. Therefore, the effect of ascorbic acid on the pH of yoghurt as well as a series of comprehensive changes caused by decreased pH are not to be neglected during the shelf life of yoghurt.

According to the factorial analysis shown in Figure 4.2, the influence of HPP on pH was not significant, while ascorbic acid and time, especially the first week, showed a significant influence in decreasing the pH of samples. No interactive effect was observed, indicating the effect of ascorbic acid and storage time was independent as expected.

In general, the pH of yoghurt dropped over time gradually and was significantly decreased by the treatment of avocado by adding ascorbic acid, while the HPP treatment alone had no effect. Given the other properties of yoghurt, such as yoghurt structure and flavour, which are strongly related to the pH, the incorporation of ascorbic acid would impact them indirectly by influencing pH.

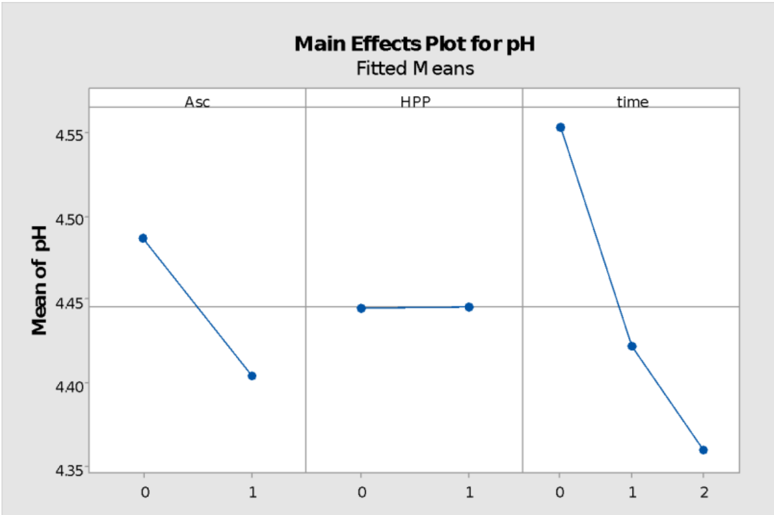


*Figure 4.2 Factorial analysis of pH with factors including Asc (level 0 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level 0 represent no HPP treatment and level 1 represents HPP treatment at 600 MPa for 5 min) and time (level 0, 1, 2 represents storage time at week 0, week 1 and week 2, respectively).*

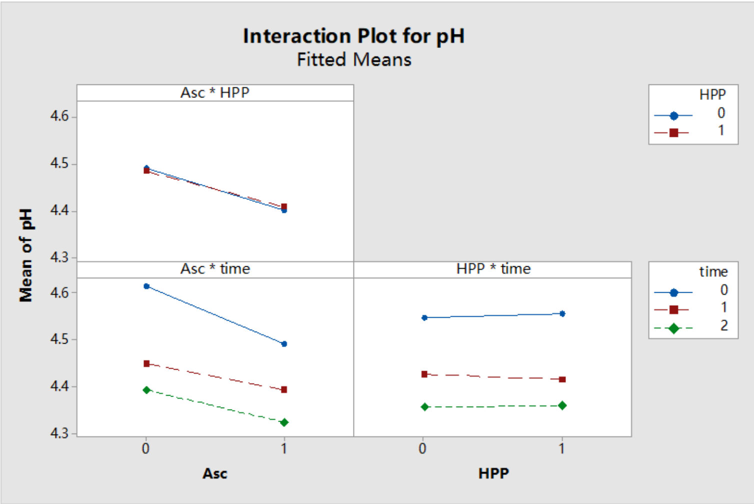


*Figure 4.3 Factorial analysis of pH with interacted factors, including Asc (level 0 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level 0 represent no HPP treatment and level 1 represents HPP treatment at 600 MPa for 5 min) and time (level 0, 1, 2 represents storage time at week 0, week 1 and week 2, respectively).*

### 4.4.2 Measurement of syneresis

The results of syneresis of yoghurt samples measured during storage for 4 weeks are shown in Figure 4.4. All samples had a minimum syneresis rate (< 1%) at day 0, which indicated the incorporation of avocado had a small effect on the syneresis of yoghurt immediately after preparation. However, noticeable differences and changes in syneresis between and within some samples were observed after 1 week. During the entire storage period of 28 days, the syneresis of samples with HPP treatment (H and HA) remained relatively constant being lower than 2%, while the syneresis of the samples without HPP treatment (A and U) gradually increased. For the sample treated with ascorbic acid (A), the syneresis increased to 7.0% at 21 days, which was the highest among all the samples. For the sample containing avocado without any treatment (U), its syneresis gradually increased to around 4% on day 14 and remained steady afterwards. The plain yoghurt without containing avocado (P) had a relatively stable against syneresis being lower than average 1% during the first 3 weeks of storage but its syneresis increased more than 3% after 4 weeks. Although it is not sure why this plain yoghurt had a sudden increase in syneresis after 4 weeks, the formulation and processing of this regular yoghurt sample with no added avocado had a reasonable result of syneresis in the normal shelf-life of yoghurt, i.e. 2-3 weeks at 4°C (Rani, Unnikrishnan, Dharaiya, & Singh, 2012).

The increased syneresis observed from the two samples (U and A) might be related to the incorporation of avocado puree without HPP treatment. This will be discussed later with the analysis of rheological properties. Meanwhile, the syneresis of sample A containing avocado treated with ascorbic acid only was lower than that of sample U containing untreated avocado, while it continuously increased and surpassed sample O (3.82%) at day 21, reaching the highest point (7.0%) at day 21. One possibility is that the syneresis of sample A in the period of the first 2 weeks was mainly caused by the degradation of avocado, while the lower pH might have contributed more syneresis after 2 weeks.

The factorial analyses of syneresis shown in Figure 4.5 which illustrate the influence of each factor and their interaction in the first two weeks. It's shown in Figure 4.5 that the syneresis was significantly decreased by the incorporation of ascorbic acid and HPP treatment. Besides, time was a significant factor in increasing the syneresis. As the post-acidification occurs over time during storage, the increased acidity may contribute to the increase of syneresis (Rani et al., 2012).

As shown in the interactive factor plot in Figure 4.6, time was not significant with the presence of HPP treatment. Besides, the effect of ascorbic acid was less significant with the presence of HPP. This suggested that the HPP treatment was the dominant factor reducing the syneresis among these samples, regardless of with or without the addition of ascorbic acid.

In conclusion, in the shelf-life of yoghurt (2-3 weeks) or in a longer time, HPP treatment was found to be the key factor for the avocado yoghurt to remain low syneresis.

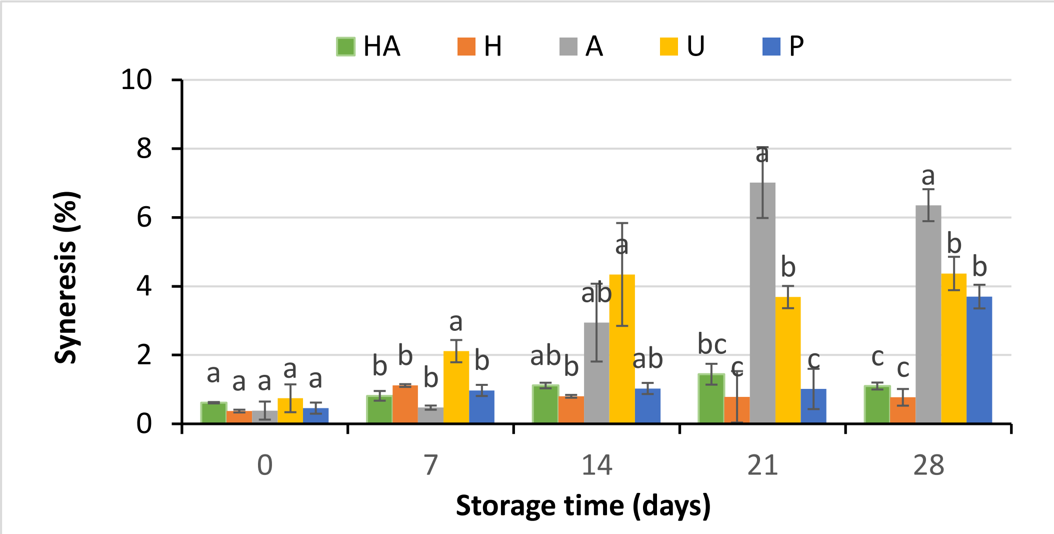


*Figure 4.4 Changes in syneresis of plain yoghurt without containing avocado (P) and yoghurts containing avocado treated by different methods (U, H, A and HA). U: untreated avocado, A: 2% ascorbic acid, H: HPP treatment, HA: a combination of HPP and 2% ascorbic acid. Yoghurt samples were vacuum packed in pouches and stored at 4°C for 28 days. Letters a, b, and c in the graph indicating a significant difference ($p < 0.05$) between samples at a given storage time.*

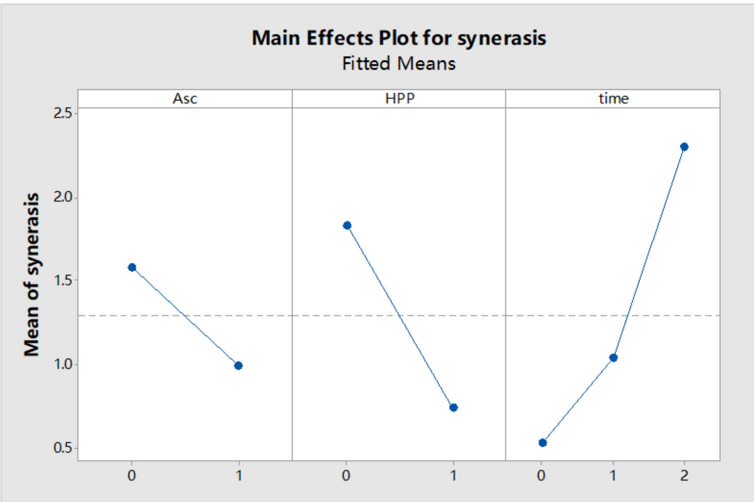


*Figure 4.5 Factorial analysis of syneresis with factors including Asc (level 0 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level 0 represents no HPP treatment and level 1 represent the incorporation and time (level 0, 1, 2 represents storage time at week 0, week 1 and week 2, respectively).*

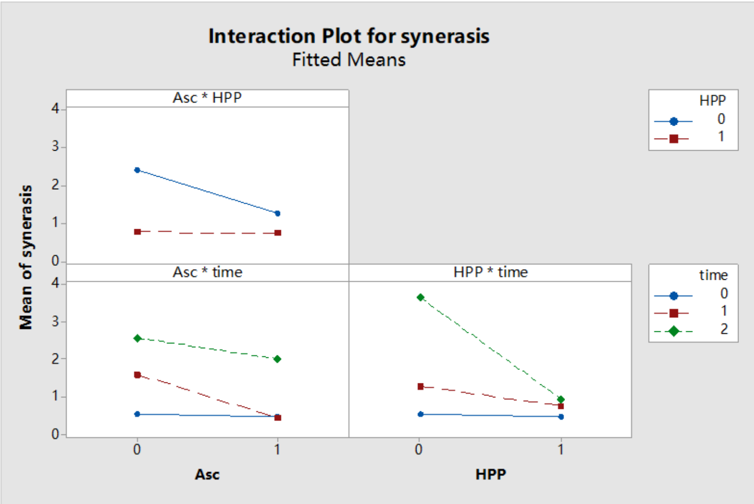


*Figure 4.6 Factorial analysis of syneresis with interacted factors including Asc (level 0 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level 0 represents no HPP treatment and level 1 represent the HPP treatment) and time (level 0, 1, 2 represents storage time at week 0, week 1 and week 2, respectively).*

### 4.4.3 Analysis of colour change

Figure 4.7 shows the visual colour change of avocado stirred yogurt samples during storage at days 0, 7 and 28. There was a colour change of samples (H and U) on day 7 which turned from greenish to yellowish. Besides, the top layer of samples (HA and A) on day 7 turned yellow, indicating the browning started from the surface layer which had direct contact with the air (oxygen). On day 28, all samples appeared to be more yellow than greenish and no visual difference was observed among samples.

The instrumental colour measurement of yoghurt samples was also conducted by measuring the CIE L*, a* and b* values of samples (data not shown). These numerical values were then used to calculate the total colour change in samples during storage which was expressed as the ΔE values shown in Figure 4.8. On day 28, no significant difference was observed among all samples, indicating that the ΔE values of all samples were similar after storage for 4 weeks. However, the rate of colour change during the storage period of 4 weeks between the samples was significantly different. The ΔE of the yoghurt sample with untreated avocado puree (U) reached sharply up to 5.9 in the first week and then remained steady afterwards.

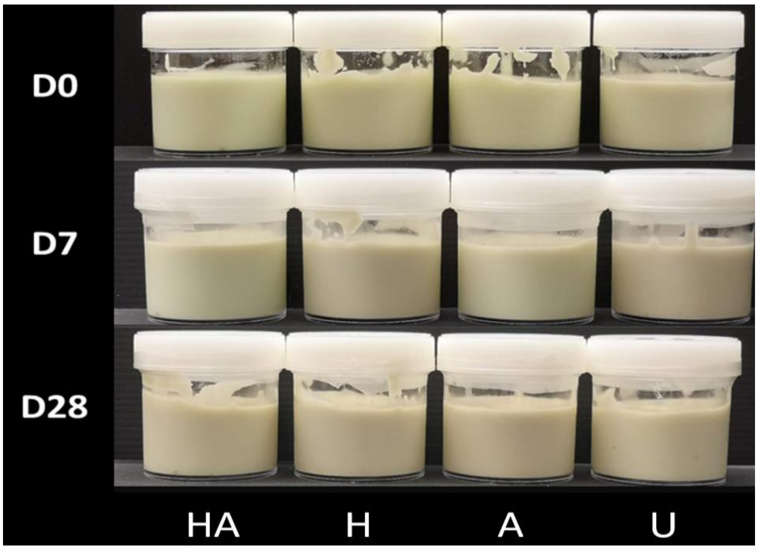


*Figure 4.7 Colour change of yoghurt samples containing avocado that was untreated (U) or treated with HPP (H), 2% of ascorbic acid (A) and a combination of HPP and ascorbic acid (HA) during storage at day 0, 7 and 28.*

The HPP-treated avocado-fortified yoghurt sample (H) had a similar pattern with the ΔE of 4.4 at day 7 and then remained relatively steady without much further change until the end of storage time (4 weeks). In the case of the other two samples containing avocado treated with ascorbic acid (A) alone or a combination of HPP and ascorbic acid (HA), their ΔE value increase was gradual but more slowly and evenly during storage for 4 weeks although the ΔE values of all samples appeared to be similar at the end of storage after 4 weeks as mentioned above. This could be due to the effect of ascorbic acid as an antioxidant and reducing agent to inhibit or retard not only the enzymatic browning of avocado but also the degradation of chlorophyll as a green pigment.

The chlorophyll in avocado is easily degraded due to enzymatic reactions and non-enzymatic reactions influenced by various environmental conditions. As the fresh avocado pulp is abundant in chlorophyll, the discolouration of green avocado pulp to dark-green, yellow, or even black is primarily due to the degradation of chlorophyll (Heaton & Marangoni, 1996; Strain, 1954). It is reported that the incorporation of an antioxidant, such as ascorbic acid, prevents the induction of oxidation of chlorophyll by light, peroxidase and/or lipoxygenase, therefore controlling the degradation of chlorophyll (Heaton & Marangoni, 1996).

Another main reason for the colour change is the enzymatic browning caused by the enzyme called PPO (polyphenol oxidase) in avocado. In avocado fruits, the PPO has been reported to be anchored to cell membranes, which is a latent form of the enzyme, as opposed to its postulated active form when the plant cell membranes are broken, which would then be exposed (Mayer and Harel, 1979). This means the access of the enzyme PPO to its substrates (polyphenols) for enzymatic browning is thus initiated and promoted when the avocado cell tissue is damaged or broken (Toledo & Aguirre, 2017b).

The sample treated with both HPP (600 MPa for 5 min) and ascorbic acid (HA) showed a non-significant change in one week with its ΔE value of around 1.1. On the other hand, the sample (H) containing avocado treated with HPP only without adding ascorbic acid had a significantly higher ΔE value of 4.4 after one week. Although it has been reported that the PPO in avocado could be partially inactivated by HPP treatment, this effect in controlling the colour change of avocado was not significant given by the conditions used in this study. This implies that the degree of enzyme inactivation was not enough to delay the enzymatic browning reaction. Similar results were also reported by Gamage (2007) that the use of HPP treatment (600 MPa, 3 min) had no significant effect in maintaining the colour stability of avocado products (slices, pulp and halves).

In the shelf-life of yoghurt, namely 14 days, the samples with ascorbic acid (HA and A) had a low value of ΔE (<2), while for the other samples containing untreated avocado (U) or HPP-treated avocado (H), their ΔE values after 14 days were 5.6 and 4.5, respectively. This indicates that the incorporation of 2% of ascorbic acid was effective in keeping the colour of avocado yoghurt with 10% of avocado pulp. However, the instrumental data should be combined with the sensory test to further investigate the practical effect of ascorbic acid. The results of the sensory evaluation are discussed in Chapter 5.

The factorial analysis focused on the change of colour on day 7 and day 14 is shown in Figure 4.9. Time was not a significant factor during this period (2 weeks), and it was the same for the HPP treatment. On the other hand, the addition of ascorbic acid was a significant factor. In terms of the interactive factor analysis, Figure 4.10 illustrates that the addition of ascorbic acid further minimised and offset the effect of HPP treatment, suggesting the overlapped functionality of each treatment, such as their similar function in terms of preventing the enzymatic browning of avocado. Meanwhile, as expected, the effect of ascorbic acid was affected by the time factor, indicating that ascorbic acid as an antioxidant was consumed over time.

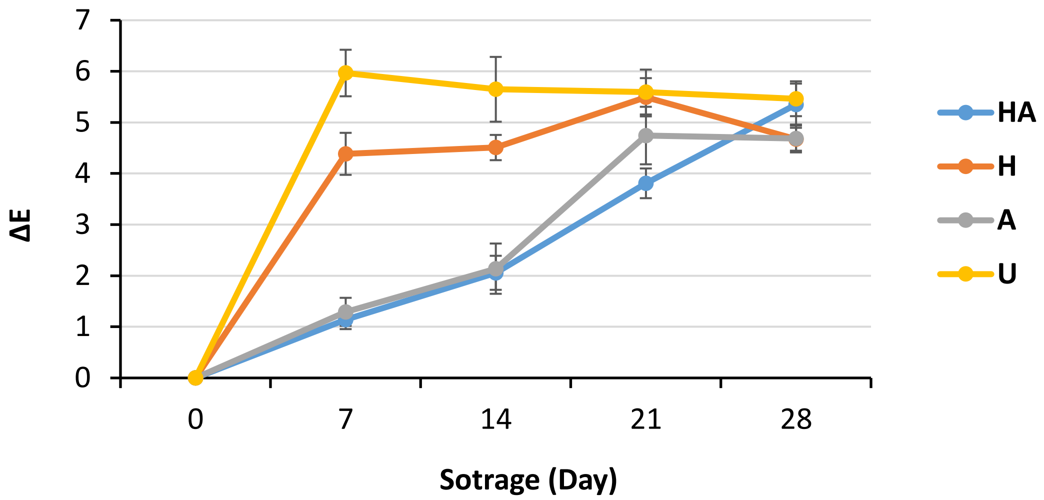


*Figure 4.8 Total colour change (ΔE) values of yoghurts during storage. Yoghurt containing avocado puree that was untreated (U) or treated with HPP (H), 2% of ascorbic acid (A) and a combination of HPP and ascorbic acid (HA). Yoghurt samples were vacuum packed in pouches and stored at 4°C for 28 days.*

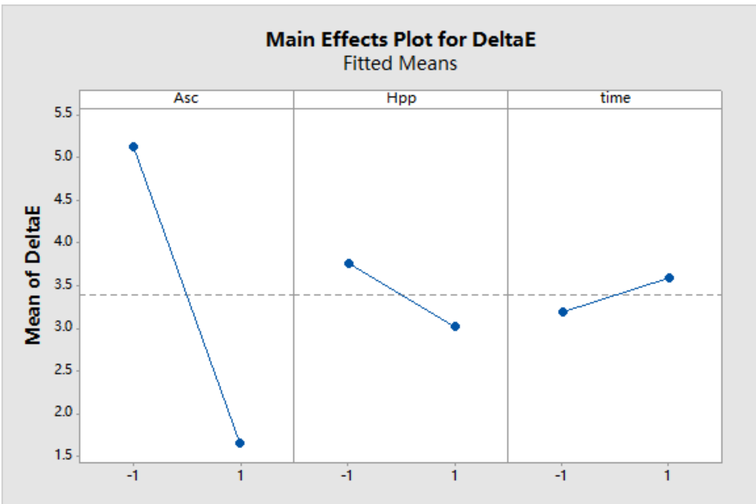


*Figure 4.9 Factorial analysis of ΔE with factors including Asc (level -1 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level -1 represent no HPP treatment and level 1 represents HPP treatment at 600 MPa for 5 min) and time (level -1 represents storage time at week 0 and level 1 at week 1)*

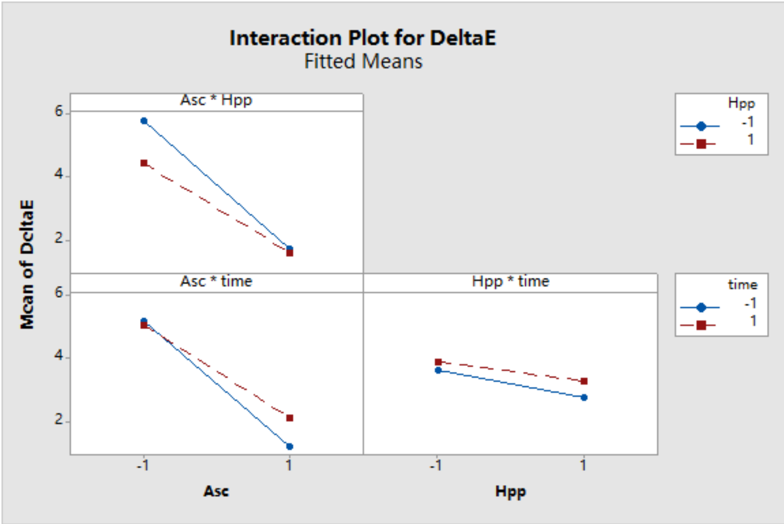


*Figure 4.10 Factorial analysis of ΔE with interacted factors, including Asc (level -1 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level -1 represent no HPP treatment and level 1 represents HPP treatment a 600 MPa for 5 min) and time (level -1 represents storage time at week 0 and level 1 represents at week 1).*

### 4.4.4 Rheological properties of yoghurt

#### *4.4.4.1 Characterization of the flow curve*

In this research, a representative flow curve for all avocado stirred yoghurt samples under a dynamic shearing sequence is shown in Figure 4.11. Although quantitative differences were observed among samples, they shared similar features as the curve shown in Figure 4.11 which was obtained from the yoghurt sample HA on day 7. All avocado yoghurt samples showed a shear-thinning (thixotropic) nature as the viscosity decreased with the shear rate.

The Williamson model was used to fit the flow curve in this research. The model is generally with the standard error between 10 and 12 while the standard error of another commonly used model such as the Herschel-Bulkley model is over 20. This implies the Williamson model can better fit the experimental data, so it is more suitable for describing and analyzing the rheological characteristics of the sample. Referring to the Williamson model, three parameters including zero-shear rate viscosity (a), consistency (b), and rate index (c) were used to compare the rheological characteristics among samples.

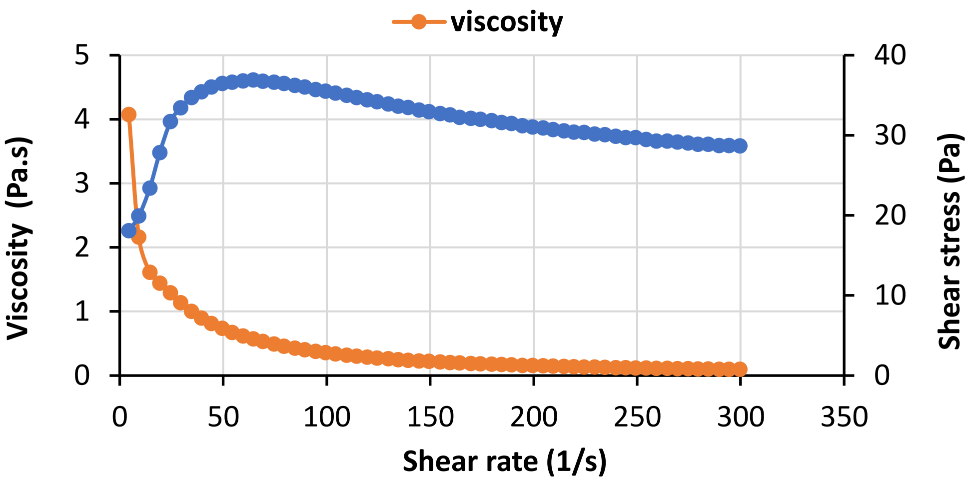


*Figure 4.11 A flow curve (viscosity) of avocado stirred yoghurt.*

#### *4.4.4.2 Analysis of a: zero-shear rate viscosity*

In the Williamson model, the term 'zero-shear rate viscosity' which is denoted as a value refers to the theoretical viscosity of the sample without shear rate. As shown in Figure 4.12, except for the two yoghurt samples, that is, plain yoghurt without avocado (P) and HPP treated avocado fortified yoghurt (H), a value (zero-shear rate viscosity) of the other samples (HA, A and U) was decreased over time during storage, which might be related to the increased acidity or/and degradation of avocado content.

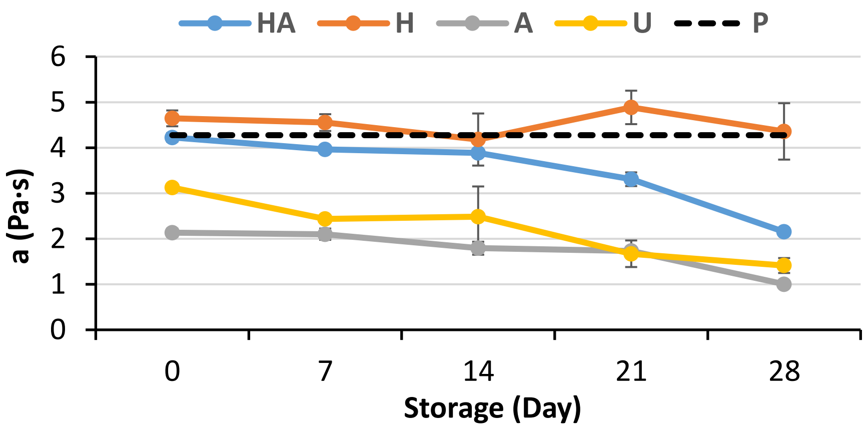


*Figure 4.12 Change of parameter a representing zero-shear rate viscosity of yoghurt samples during storage for 4 weeks at 4°C. Plain yoghurt without avocado (P), yoghurt containing untreated avocado (U) and yoghurt samples containing avocado treated with HPP (H), 2% ascorbic acid (A) or a combination of HPP and ascorbic acid (HA).*

On day 0, the plain yoghurt without avocado (P) had ‘a’ value zero-shear rate viscosity of 4.3, while its ‘a’ value was decreased significantly to 3.1 when the yoghurt was added and mixed with untreated avocado (U). In the case of the yoghurt added with ascorbic acid-treated avocado puree (A), the a value was further decreased to 2.1. However, this was not observed for the samples containing avocado treated with HPP (H and HA), as the ‘a’ value of sample H and sample HA was 4.6 and 4.2, respectively. One possible reason for a difference in the a value (zero-shear rate viscosity) between samples might be related to the different structures of avocado pulp processed by blending and HPP treatment. Researches have shown that the HPP treatment could increase the plant cell membrane permeability and cell disruption, facilitating the occurrence of chemical reactions (De Roeck et al., 2009). During HPP treatment, enzymes, substrates and cations are liberated from various compartments and contact each other. Therefore, pectin methylesterase catalyses the demethoxylation of pectin, resulting in the formation of carboxylated pectin with the release of methanol. At the same time, the low methoxy pectin can form a gel network with divalent cations, such as calcium ions in yoghurt, resulting in increased hardness (De Roeck et al., 2009).

Except for the chemical conversions of pectin, HPP treatment influences the plant tissues differently compared to the blending. As HPP treatment employs isotopic homogeneity of mechanical stress, pressurization is accompanied by a simultaneous decrease in volume. The avocado tissues suffer structural modifications favouring a more compact form (Zhang et al., 2015). As the pressure increases up to 600MPa, the cell suffers deformation and loss of integrity. Although blending also deforms the cell by high shear stress, the cell wall of avocado is predominantly broken. However, a study reported by Woolf et al. (2013) has shown the preservation of cell wall structure where HPP treatment at 600 MPa for 6 min was applied on avocado slices (see Figure 4.13 below). Thus, the preservation of cell wall structure may contribute to the high value of ‘a’ as it indicates higher zero-rate viscosity. Nevertheless, further research is required in terms of the preservation of cell walls and rheological properties.

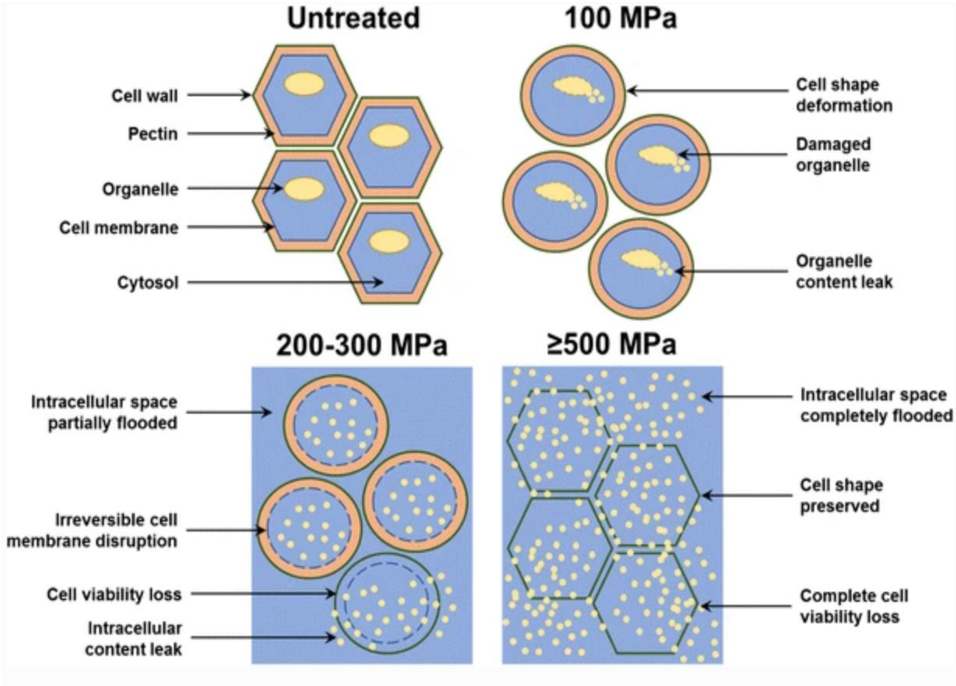


*Figure 4.13 Influence of different levels of HPP treatment on the cell structure of plant (Serment-Moreno et al., 2017).*

#### *4.4.4.3 Analysis of b: consistency*

In the Williamson model, the term 'consistency' which is denoted as a 'b' value refers to how fast the viscosity decreases by the increase of shear rate. According to the model, a bigger parameter of b value indicates the viscosity drops faster with increasing shear rate. Similar to 'a' value, except for sample H, a decreasing trend of the b value was observed during the whole experiment (see Figure 4.14). In contrast, HPP treatment favoured the b value of sample (H) stay around the value of P (0.051) while it deviated a value to a higher degree. For sample HA the b value on day 0 was 0.059 and for sample H 0.064. This indicates that without HPP treatment, the incorporation of avocado pulp had little influence on the b value of stirred yoghurt. However, the HPP treatment significantly increased the b value which indicates stronger shear thinning.

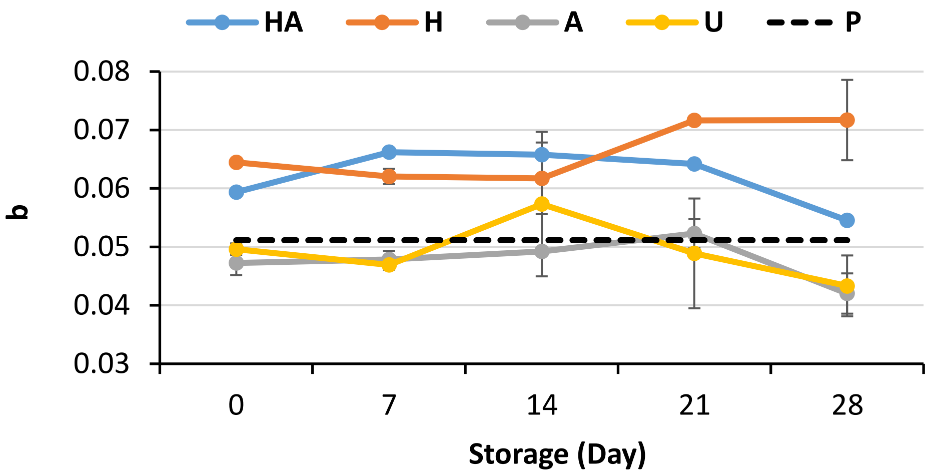


*Figure 4.14 Change of parameter b representing consistency of yoghurt samples during storage for 4 weeks at 4°C. Plain yoghurt without avocado (P), yoghurt containing untreated avocado (U) and yoghurt samples containing avocado treated with HPP (H), 2% ascorbic acid (A) or a combination of HPP and ascorbic acid (HA).*

Some potential reason was explained above, including the presence of cell wall structure and the form of low methoxyl (LM) pectin gel. Another possible reason for the higher thinning properties of avocado yoghurt with HPP treatment may be due to the coalescence of oil droplets in the avocado treated by HPP. As reported by Woolf et al. (2013), after HPP treatment of avocado halves at 600 MPa for 5 min, coalescence of the parenchyma oil droplets into much larger droplets was observed. The coalescence may be a result of the denaturation of protein as a stabilizer in avocado content. The CLSM images shown in Figure 4.15 indicate that in the blended avocado pulp (i.e., avocado puree), the oil droplets (red) were surrounded by protein (green) which functions as an emulsifier and stabilizer that prevents the coalescence of oil droplets. However, the difference was not observed when the avocado pulp was incorporated with yoghurt. On the other hand, in the avocado pulp treated by HPP, the layer of protein adsorbed on the oil droplet surface could not be seen clearly. The oil droplets with increased sizes in HPP-treated avocado might have changed the rheological properties of yoghurt samples. As reported by Pal (1996), in an oil in water emulsion, with a constant total volume fraction of the dispersed phase, when the fine droplets are replaced by coarse droplets, the resulting emulsion exhibits higher viscosity at low shear stresses. At high stresses, the viscosity increases as the proportion of fine droplets increases, indicating lower shear thinning of fine droplets emulsion, in this case, the blended avocado pulp.

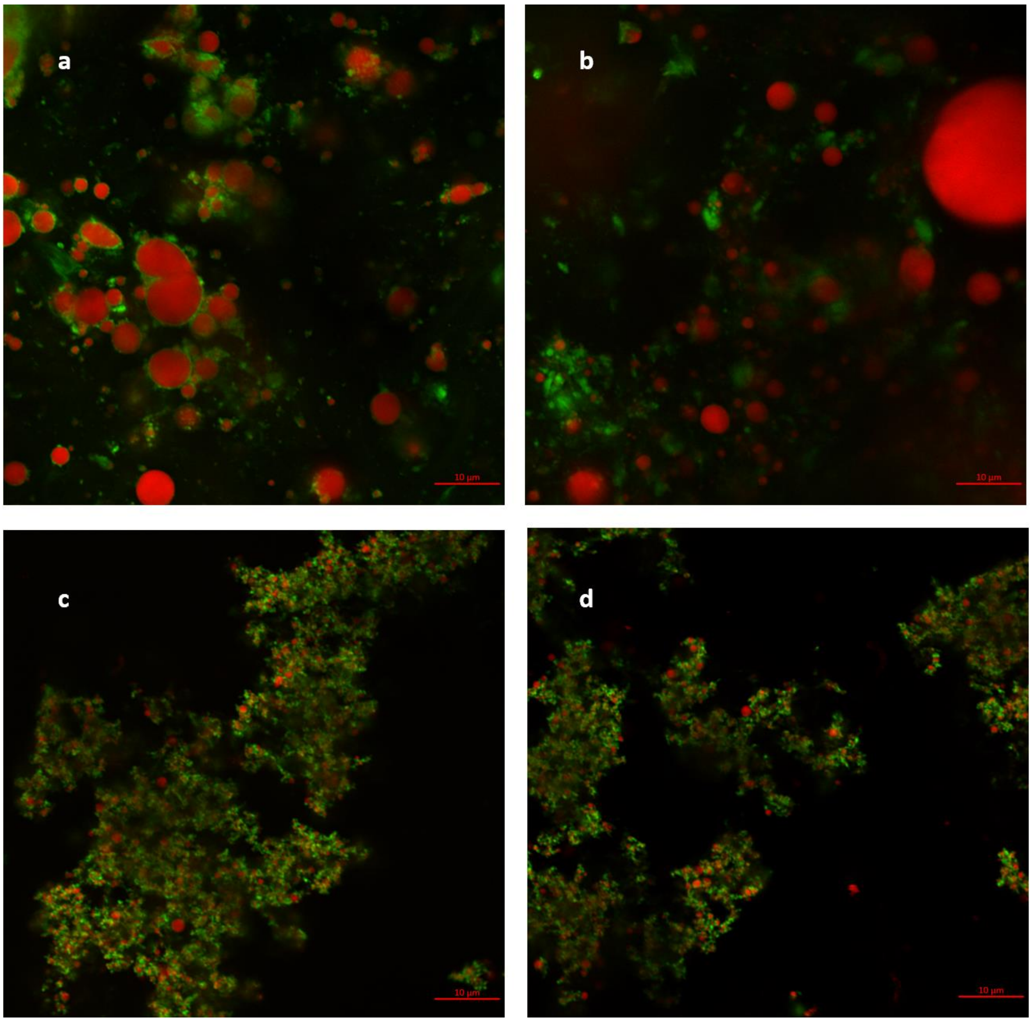


*Figure 4.15 The microstructure of blended avocado (a), avocado treated by HPP at 600 MPa for 5 min (b), yoghurt with blended avocado (c) and yoghurt with HPP-treated avocado (d) as observed by CLSM. Nile Red-stained fat appears red and fast green FCF-stained protein appears green.*

#### *4.4.4.4 Analysis of c: rate index*

According to the Williamson model, a bigger parameter of c (rate index) primarily indicates the lower level of viscosity at a high shear rate (>150 $s^{-1}$). As shown in Figure 4.16, the incorporation of avocado with or without HPP treatment decreased the c level of the sample. For the reference sample (P) which was the plain yoghurt with no avocado, the c value at day 0 was 1.26 while samples HA and U were 1.22 and samples H and A were 1.21 and 1.15, respectively. The c value in the Williamson model mainly indicates the viscosity of the sample at a high shear rate (>150 $s^{-1}$). The lower c value of the avocado sample suggests the incorporation of avocado increased the viscosity of the sample under a high shear rate.

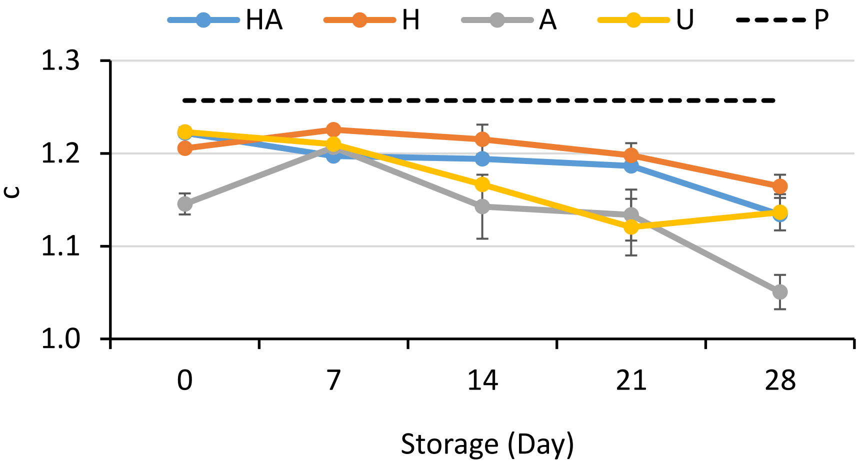


*Figure 4.16 Change of parameter b representing rate index of yoghurt samples during storage for 4 weeks at 4°C. Plain yoghurt without avocado (P), yoghurt containing untreated avocado (U) and yoghurt samples containing avocado treated with HPP (H), 2% ascorbic acid (A) or a combination of HPP and ascorbic acid (HA).*

### 4.4.5 Microbial quality of avocado yoghurt

#### *4.4.5.1 Lactic acid bacteria (LAB)*

In this study, yoghurts were prepared from milk by fermentation with a starter culture containing lactic acid bacteria (*Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus*). Figure 4.17 shows the change of a total lactic acid bacteria count in yoghurt samples during storage for 4 weeks. In all yoghurt samples, the plate count of LAB was high up to around 9.5 log CFU $g^{-1}$ after preparation (day 0) and remained stable during the first two weeks then dropped significantly on day 21 to around 9 logs CFU $g^{-1}$. After storage for 4 weeks, on day 28, the LAB count for all samples decreased to around 8.2 logs CFU $g^{-1}$. Despite the decline of LAB count, the total number of lactic acid bacteria has greatly exceeded the demanded number of 6 log CFU $g^{-1}$, which indicates all yoghurt samples had excellent microbial quality during the storage.

Meanwhile, the results showed that the influence of both HPP treatment and ascorbic acid was not significant. Comparing the two samples between U (yoghurt with untreated avocado pulp) and P (plain yoghurt without any incorporation of avocado), it could be referred that the incorporation of avocado had no adverse effect on the number of lactic acid bacteria in yoghurt. The decrease in the number of lactic acid bacteria after 3 weeks could be due to the limited growing space and the exhausted lactose (Priyanka Aswal, 2012).

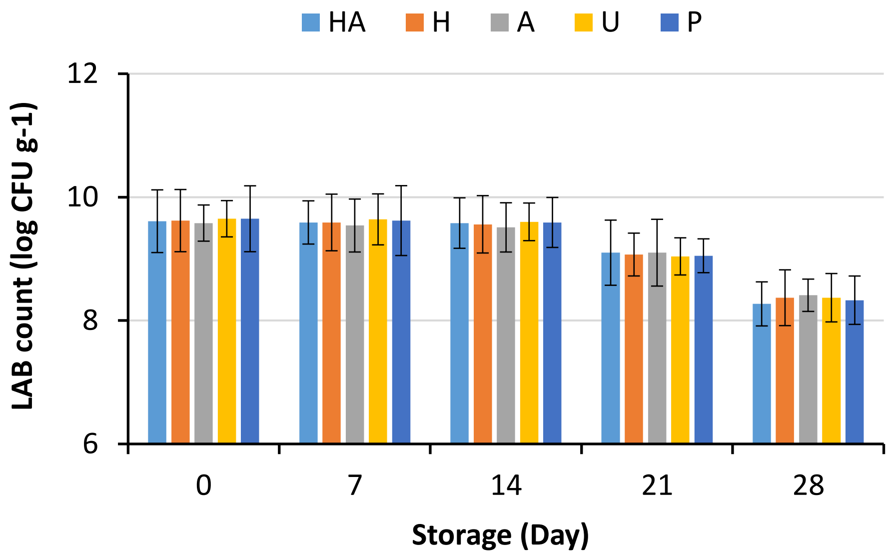


*Figure 4.17 Lactic acid bacteria (LAB) count in avocado yoghurt samples. HA stands for yoghurt with avocado treated by HPP (600 MPa, 5 min) and ascorbic acid; H for HPP treatment only; A for ascorbic acid only; U for untreated avocado-containing sample; and P for plain yoghurt without any avocado.*

### *4.4.5.2 Microbial contamination*

Yoghurt samples were analysed for total coliform and total yeast and mould counts during storage for 4 weeks at 4°C. The results shown in Table 4.2 indicate that no coliform was detected from all yoghurt samples containing avocado without any treatment or with different treatments. In this research, the pH of yoghurt started from around 4.5-4.6 and gradually decreased to around 4.2 after storage of 4 weeks. The processing method used in making yoghurt also contributed to eliminating the coliform as the milk was pasteurized at

90°C for 5 min before fermenting. Thus, it was expected that no growth of coliform could be observed during the experiment.

Similar to the results of coliform, the growth of yeast and mould was not detected in this experiment for any sample during 4 weeks of storage. In an experiment on avocado pulp, the pulp acidified by citric acid to pH 4.4 was shown to have no growth of yeast and mould (Gamage, 2007). Considering the pH in avocado yoghurt (4.2-4.5) in this experiment, the results were within expectation. Yeast and mould can be the main concern in the shelf life of avocado products, especially in avocado yoghurt as no pasteurization or sanitization was employed after the incorporation of avocado. In addition, the avocado was not heat-treated although HPP was used untreated avocado was also used and added into yoghurt (U). Nevertheless, besides the acidic environment in the yoghurt sample, there are other conditions contributing to the absence of yeast and mould, including proper experimental practice (e.g., cleaning, maintenance and hygiene), the small experimental scale of production and a relatively small content of avocado (10%) added into yoghurt.

Yeast and mould are reported as the main factor in the spoilage of fresh-cut fruits and vegetables and it is usually detected by consumers when yeast counts reach levels above 5 log (CFU/g) (Aguilo-Aguayo, Oms-Oliu, Martin-Belloso, & Soliva-Fortuny, 2014). However, most yeast and mould are on the surface or peel of avocado. Recent research suggests a mean yeast and mould count of 3.50, 2.95 log10 CFU/g from the avocado surface and avocado peel (Shiferaw & Kibret, 2018). Thus, proper experimental practices, such as washing the avocado surface and hygienic practices during cutting and peeling could effectively decrease the contamination of yeast and mould. Another reason is the limited amount of avocado used in this experiment so that there is a lower chance of the incorporation of contaminated avocado fruit as well as cross-contamination. An experiment by Shiferaw and Kibret (2018) showed that in a total of avocado fruits, half 38 (47.5%) of the samples were found to be contaminated by yeast and mould counts while the remaining 42 (52.5%) of the fruit samples were free from yeast and mould counts.

In conclusion, the results showed that all samples of avocado yoghurts were safe to consume during 4 weeks of storage. However, in mass production where there is more chance of cross-contamination and improper practice, the contamination of yeast and mould is still nonnegligible. Besides, the effect of HPP treatment and the incorporation of ascorbic acid on the microbial safety of the sample were not illustrated in this experiment. Their effects on the microbial safety of avocado yoghurt on a larger scale of production need to be determined.

*Table 4.1 Growth of coliform, yeast and mould (expressed as log CFU $g^{-1}$) in yoghurts containing avocado with different treatments. H for HPP treatment only, A for ascorbic acid only, U for untreated, and HA for both HPP and ascorbic acid treatment.*

| *Days* | *HA* | *H* | *A* | *U* |
|---|---|---|---|---|
| ***Coliform*** | | | | |
| *0* | n.d. | n.d. | n.d. | n.d. |
| *7* | n.d. | n.d. | n.d. | n.d. |
| *14* | n.d. | n.d. | n.d. | n.d. |
| *21* | n.d. | n.d. | n.d. | n.d. |
| *28* | n.d. | n.d. | n.d. | n.d. |
| ***Yeast and mould*** | | | | |
| *0* | n.d. | n.d. | n.d. | n.d. |
| *7* | n.d. | n.d. | n.d. | n.d. |
| *14* | n.d. | n.d. | n.d. | n.d. |
| *21* | n.d. | n.d. | n.d. | n.d. |
| *28* | n.d. | n.d. | n.d. | n.d. |

## 4.5 Conclusions

The incorporation of 2% of ascorbic acid was effective in controlling the colour change or degradation of avocado stirred yoghurt, keeping the ΔE below 2 in the shelf-life of the product. On the other hand, no significant influence of HPP treatment was observed on the colour stability in this experiment. However, HPP treatment was found to be the key factor to keep the syneresis of yoghurt low, minimizing the extra syneresis caused by the introduced avocado content. Also, the rheological test illustrated its effect on increasing and maintaining the thickness of avocado stirred yoghurt during storage for 4 weeks. No significant effect on rheological properties was observed by the incorporation of ascorbic acid into avocado pulp. Neither of these treatments had an influence on the growth of lactic acid bacteria in yoghurt. No yeast and mould or coliform were detected in all samples during storage of 4 weeks.

# Chapter 5. Sensory evaluation of avocado-fortified yoghurts: Effects of HPP treatment and ascorbic acid on avocado pulp

## 5.1 Abstract

In recent years, avocado has become a trending fruit and its world market has increased its size to almost four times larger due to its numerous health benefits. However, avocado undergoes deterioration readily after ripening, including enzymatic browning, thus its use in various food applications is relatively limited. Various physicochemical methods have been explored to preserve the quality of avocado against enzymatic browning. A commonly used physical method is high-pressure processing (HPP) treatment, which has minimum influence on the nutrition of foods while inactivating polyphenol oxidase (PPO), an enzyme causing enzymatic browning, and destroying microorganisms. Except for physical methods, chemicals, especially ascorbic acid, is also widely used. However, the effects of these treatments applied on avocado on the sensory properties of yoghurts fortified with avocado pulp treated with HPP and ascorbic acid have not been studied. Therefore, the objective of this study was to evaluate the effects of HPP and ascorbic acid treatments of avocado on the sensory properties of avocado stirred yoghurt during its shelf-life. The sensory properties of yoghurt added with untreated avocado were observed to drop dramatically in 2 weeks during storage at 4°C from an overall consumer acceptance score from 8.0 to 2.6. While HPP treatment and incorporation of ascorbic acid were shown to significantly maintain the overall acceptance. HPP treatment specifically affected the texture profile of samples by maintaining the thickness of the samples. Avocado treatment with ascorbic acid significantly ($p<0.05$) increased the flavour score of avocado yoghurt from 3.1 to 7.0 and the colour appearance from 5.4 to 6.1. It is believed that the main mechanism of ascorbic acid to improve the sensory properties was due to its function as an antioxidant and reducing agent to prevent enzymatic browning and preserve the original green colour and also inhibit the development of rancid flavour in the product. Besides, neither of these treatments was observed to significantly influence the sourness of the sample, and the interactive effect was not observed between HPP treatment and ascorbic acid. The results of the sensory evaluation showed that the combination of HPP treatment and ascorbic acid

addition effectively maintained the sensory properties (e.g., texture, flavour, sourness and appearance) of avocado stirred yoghurt.

## 5.1 Introduction

With the increase of public health concerns and pursuit for expanding sensory experience, there's a huge market potential for fruit flavoured yoghurt. Avocado, a globally emerging fruit, has drawn attention as a new fruit to be incorporated into yoghurt (Fernandez & Marette, 2017). However, the perishable nature of avocado including physicochemical changes (e.g., colour degradation, enzymatic browning and lipid oxidation) has brought challenges of preserving its sensory properties within the shelf-life. Due to the shortcomings of heat treatment in processing avocado, non-thermal treatments are employed to extend the shelf-stability of avocado products. A commonly used physical treatment is HPP treatment, which has minimum influence on the nutrition of foods while inactivating enzymes and destroying microorganisms (Hendrickx, Ludikhuyze, Van den Broeck, Weemaes, & Technology, 1998). In addition, some chemicals, especially antioxidants such as ascorbic acid and citric acid, are widely used. Research has found the effectiveness of ascorbic acid in inhibiting the PPO activity and preventing the colour change of avocado puree in up to 4 months (C. et al., 2002). However, the effect of these treatments on sensory properties during the shelf-life has not been investigated.

In a study reported by Hettige et al. (2013) involving the incorporation of untreated avocado into yoghurt at four different levels (0, 5, 10 and 15%), the results of the sensory evaluation showed that 10% avocado yoghurt was preferred most by panellists. Citric acid was used in their experiment to prevent enzymatic browning of avocado, and gelatine and pectin to improve the texture of yoghurt. Meanwhile, similar results were obtained even without the addition of these additives. According to Gunawardhana and Dilrukshi (2016), among the four yoghurts containing different amounts of untreated avocado (8, 10, 12 and 14%), the yoghurt with an avocado content of 8% was favoured by panellists. However, these studies have mainly focused on the determination of an optimum amount of avocado content for incorporation. Little work was done on the sensory properties of avocado-fortified yoghurt after storage. Therefore, the objective of this study was to evaluate the effects of different treatments of avocado with HPP and/or ascorbic acid on the sensory properties of avocado stirred yoghurt during its shelf-life.

## 5.2 Materials and Methods

### 5.2.1 Preparation of avocado stirred yoghurt

Four types of avocado stirred yoghurts containing avocado pulp (10%) with different treatments (untreated, 2% ascorbic acid addition, HPP treatment (600 MPa/5 min), and both HPP treatment and ascorbic acid addition) for sensory evaluation were made 3 times before the sensory test. Namely 14, 7 and 0 days before the sensory test. The sample were placed in sealed plastic bottle (200 ml) and stored in refrigerator at 4°C. The formulation and method used for making yoghurt samples are described in Section 3.3.

### 5.2.2 Sensory analysis

Sensory analysis was carried out in the sensory laboratory in Food Technology, Massey University (Auckland, New Zealand) using 34 untrained panellists with multiple ethnic backgrounds aging from 20 to 38. The panellists were asked to evaluate the yoghurt samples for basic sensory properties, including appearance, colour, texture, flavour, and overall acceptability according to the nine-point hedonic scale. Besides, the rancid flavour was evaluated by a five-point scale. The list of questions (sensory questionnaires) used in the sensory tests can be found in Appendix. The Massey University Human Ethics Committee approved this sensory test: Southern A, Application 4000022967.

### 5.2.3 Statistical analyses

A storage week (0, 1 and 2) × HPP × ascorbic acid factorial arrangement of treatments in a completely randomized design with repeated measures was utilized. Differences among treatments were evaluated by Tukey's multiple comparison test at a significance level of $p < 0.05$. All the results of the sensory evaluation were statistically analysed using Minitab (Minitab LLC, version 18, USA).

## 5.3 Result and Discussion

### 5.3.1 Overall acceptance

The results of sensory evaluation of avocado yoghurt samples, in terms of 5 different sensory attributes, such as texture, flavour, appearance, sourness and overall acceptance, are shown as a spider plot in Figure 5.1. The overall acceptance scored relatively high, whereas it dropped to a different extent for each sample in the following days. For the untreated avocado-fortified yoghurt sample (U), the score of overall acceptance reduced dramatically from 7.16 to 2.25, indicating a severe decline of quality. A similar trend was observed for the sample containing avocado treated with HPP, where the score of its overall acceptance dropped from 7.74 to 3.33 after storage for 2 weeks for the sample treated by ascorbic acid, a slight decrease by time was observed. In two weeks, the overall acceptance of sample A dropped from 6.76 to 5.31. For sample HA, the score decreased from 7.13 to 5.38 which indicates that even with different treatments, the sensory properties of avocado yoghurt were unstable within the shelf life.

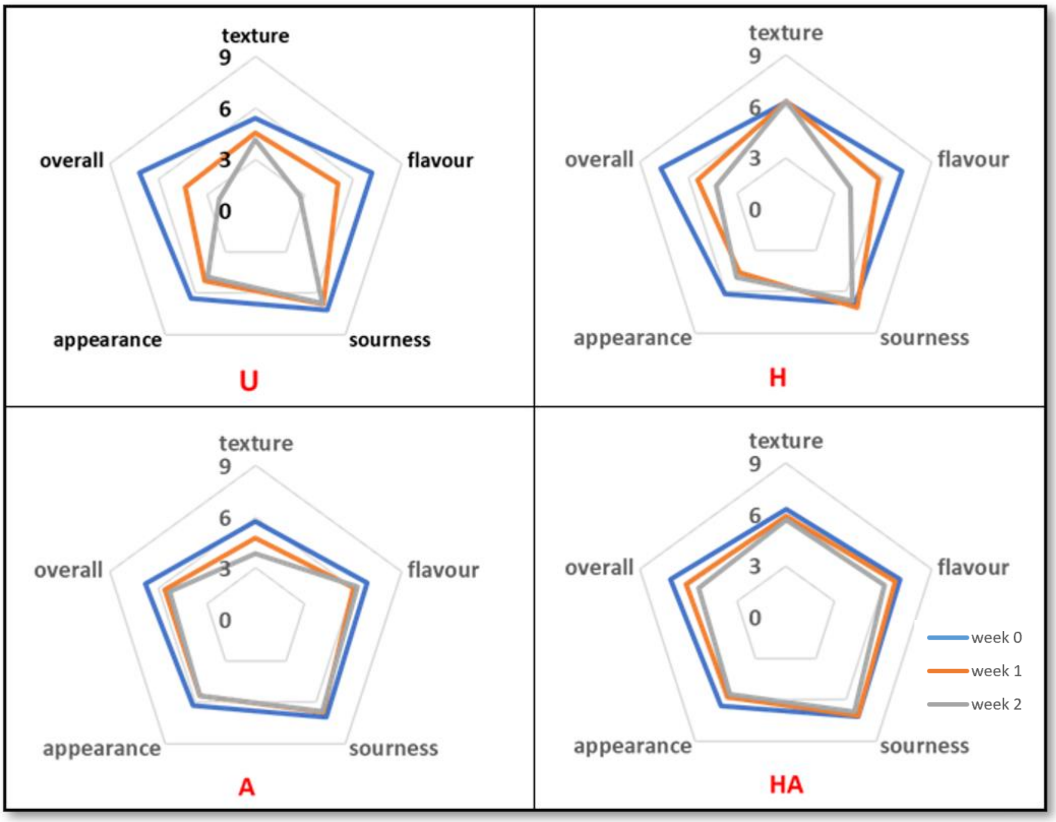


*Figure 5.1 Spider plot for sensory attribute scores of avocado yoghurt samples containing avocado with different treatments during storage (0, 1 and 2 weeks). HA stands for yoghurt with avocado treated by HPP (600 MPa, 5 min) and 2% ascorbic acid; H for HPP treatment only; A for ascorbic acid only; U for untreated avocado.*

The factorial analysis shown in Figures 5.2 and 5.3 illustrates the most significant factor on the overall acceptance was the storage time. The overall acceptance decreased drastically with the increasing storage time of avocado yoghurt, while the other two factors (HPP and ascorbic acid) slightly but positively affected this figure. The decrease in sensory quality during storage was very likely to be related to the avocado content. As for plain commercial yoghurt, its quality change within its shelf life is usually caused by post-acidification (Tamime et al., 2007a). This is due to a phenomenon caused by the excess acidity created by the lactic acid bacteria which could survive at low temperatures (4$^{o}$C) (Tamime et al., 2007a). Typically, post- acidification in yoghurt leads to increased sourness and change of protein structure in yoghurt, thus causing a series of changes in sensory properties. However, there is a limit of growth of lactic acid bacteria, granting a lower limit of pH during storage. Thus, the quality change of commercial yoghurt within its shelf-life is generally under control. In this experiment, the starter culture used is commercially manufactured, therefore it's less likely that the sharp decrease of sensory quality was related to the yoghurt content of the sample.


*Figure 5.2 Factorial analysis of overall acceptance with interactive factors including Asc (level -1 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level -1 represents no HPP treatment and level 1 represents the HPP treatment at 600 MPa for 5 min) and time (level -1 represents storage time at week 0 and level 2 represents storage time at week 2).*

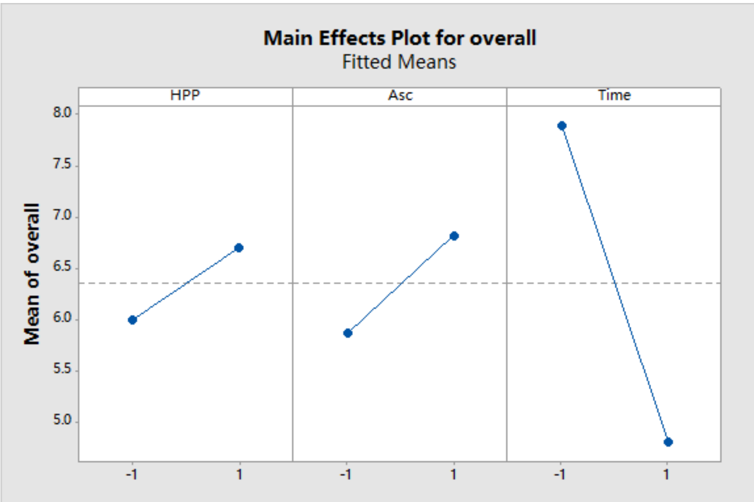


*Figure 5.3 Factorial analysis of overall acceptance with factors including Asc (level -1 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level -1 represents no HPP treatment and level 1 represents the HPP treatment at 600 MPa for 5 min) and time (level -1 represents storage time at week 0 and level 2 represents storage time at week 2).*

### 5.3.2 Texture

As shown in Figure 5.1, panellists showed an intermediate likeness for the texture of all samples. On day 0, the texture score for each sample fell in the range of 5.4-6.3, indicating that the yoghurt formula has room for improvement. Nevertheless, the sample treated by HPP was significantly higher than others on week 0, as the score for sample H and HA was 6.27 and 6.31, respectively, compared to that of sample U (5.42) and sample A (5.75). Besides, the various trends of the score were observed in each sample during storage. For samples without HPP treatment, the score of texture dropped by increasing storage time. Specifically, for the untreated avocado fortified sample, the score of texture dropped from 5.42 to 4.13 in two weeks, while for the sample treated by ascorbic acid from 5.75 to 3.88. Meanwhile, no significant change of texture was observed for samples H and HA, indicating the effectiveness of HPP treatment in terms of maintaining the texture of avocado yoghurt. This effect was also supported by the factorial analysis in Figure 5.4.

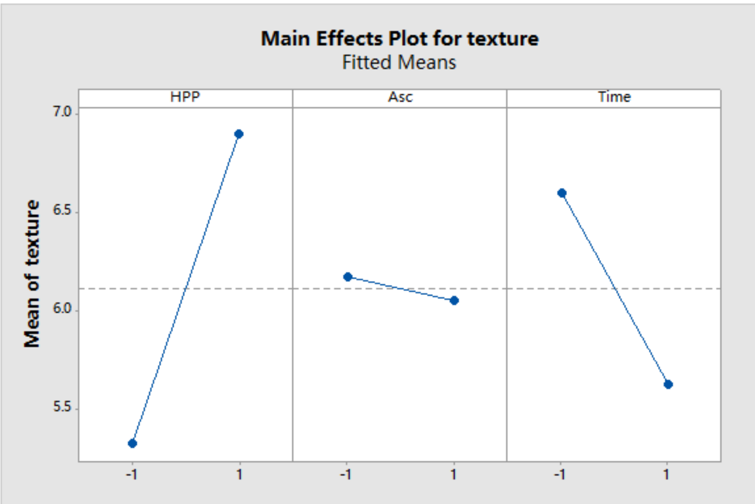


*Figure 5.4 Factorial analysis of texture with factors including Asc (level -1 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level -1 represents no HPP treatment and level 1 represents the HPP treatment at 600 MPa for 5 min) and time (level -1 represents storage time at week 0 and level 2 represents storage time at week 2).*

Given the instrumental results of texture measurement shown in Chapter 4, it could be referred that the decreased texture score was related to the loss of thickness. Participants in the sensory experiment were informed in advance that the sample was a stirred yoghurt, so they had certain expectations about the viscosity of the product. If the viscosity of the sample was out of the range of the normal product, the participants would give a lower score.

A possible reason for texture change could be due to the different tissue of avocados after being treated by blending and HPP. As HPP treatment employs isotopic homogeneity of mechanical stress, pressurization is accompanied by a simultaneous decrease in volume (Zhang et al., 2015). The avocado tissues suffer structural modifications favouring a more compact form (Zhang et al., 2015). As the pressure increases up to 600 MPa, the cell suffers deformation and loss of integrity. However, the cell wall is predominantly preserved during HPP. In contrast, blending breaks most of the cell wall. Research has shown the preservation of cell wall structure when HPP treatment for 6 mins at 600 MPa was applied on avocado slices (Woolf et al., 2013). Thus, the maintenance of cell wall structure may contribute to the high value of texture score. Nevertheless, further research is required in terms of the presence of cell walls and rheological properties.

Besides the difference of avocado tissues, another possible reason may be related to the forming of the gel network caused by the carboxylated pectin which is generated during HPP treatment (De Roeck et al., 2009). As De Roeck et al. (2009) found that the HPP treatment could increase the cell membrane permeability and cell disruption, facilitating the occurrence of chemical reactions. During HPP treatment, enzymes, substrates, and cations are liberated from various compartments and reach with each other. Therefore, pectin methylesterase catalyses the demethoxylation of pectin, resulting in the formation of carboxylated pectin with the release of methanol. At the same time, the low methoxy pectin can form a gel network with divalent cations such as calcium ions in yoghurt, resulting in increased hardness (De Roeck et al., 2009).

### 5.3.3 Flavour

The change of flavour showed the same trend as overall acceptance. On day 0, the score of the flavour of all samples was at around 7, and no significant difference was observed. However, the flavour of the untreated sample and HPP sample dropped dramatically with increasing storage time. As the flavour of untreated sample and HPP sample at week 2 scored 2.72 and 4.00 respectively, indicating that after 2 weeks storage, the majority of participants in sensory evaluation disliked this product very much. Meanwhile, for samples treated with ascorbic acid, a moderate decrease of flavour was observed. For sample A, the score of flavour dropped to 6.29 while for sample HA 6.09. Combining with the factorial analysis in Figure 5.5, it could be referred that the ascorbic acid effectively sustains the deterioration of flavour in the avocado yoghurt.

The deterioration of avocado yoghurt may result from the degradation of avocado rather than the flavour change of yoghurt content. As reported, yoghurt flavour could be stable in a long range of time without microbial contamination (Salvador & Fiszman, 2004). The deterioration of avocado is comprehensive, including a serious biochemical change. The main change relating to flavour change is the development of rancid flavour caused by the oxidation of unsaturated fatty acids. As shown in Figure 5.6, the score of flavour may be highly related to the development of rancid flavour in the avocado yoghurt as the score of flavour decreases by the increased level of rancid flavour. Considering the incorporation of ascorbic acid also prevented the development of rancid flavour, it could be referred that the main function of ascorbic acid to preserve the flavour is due to the prevention of the oxidation of unsaturated free fatty acid in avocado. This result was in agreement with researches of ascorbic acid in terms of its ability to prevent the formation of primary and secondary lipid oxidation compounds that was corroborated by a longer shelf-life time (Aubourg, Pérez-Alonso, Gallardo, & Technology, 2004; Pourashouri et al., 2009; Rostamzad, Shabanpour, Kashaninejad, & Shabani, 2010).

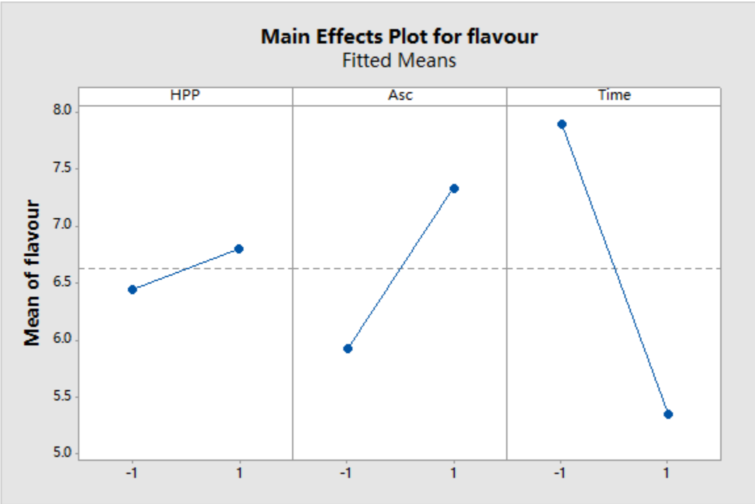


*Figure 5.5 Factorial analysis of flavour with factors including Asc (level -1 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level -1 represents no HPP treatment and level 1 represents the HPP treatment at 600 MPa for 5 min) and time (level -1 represents storage time at week 0 and level 2 represents storage time at week 2).*

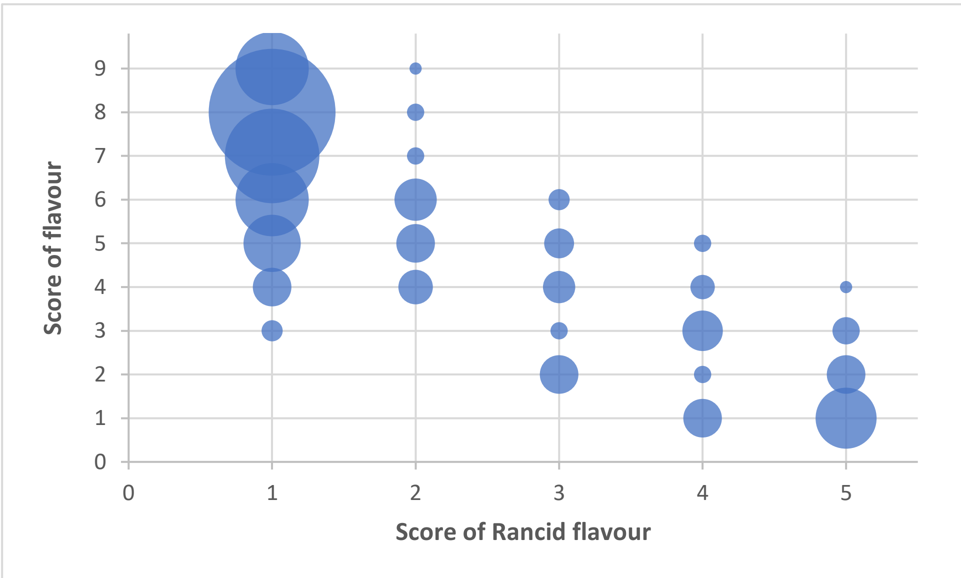


*Figure 5.6 Relation between the score of "rancid flavour" and "Flavour". The size of the bubble illustrates the number of panellists. The rancid flavour is from 1 (undetectable rancid flavour) to 5 (strong rancid flavour).*

### 5.3.4 Sourness

The sourness score of samples was relatively high and stable, and no significant difference was observed among samples on day 0, suggesting a great performance of selected start culture and minimum side effects of ascorbic acid. As the storage time increased, the score was declined slightly for each sample, indicating the effectiveness of post acidification in yoghurt. According to the factorial analysis of each treatment in Figure 5.7, it could be referred that the avocado content and its degradation had no significant influence on the sourness of the product. Besides, the effect of ascorbic acid on sourness was not observed in the sensory evaluation.

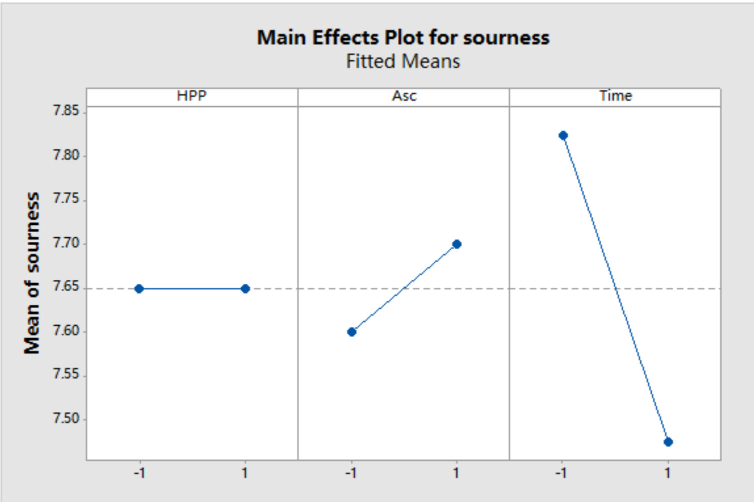


*Figure 5.7 Factorial analysis of sourness with factors including Asc (level -1 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level -1 represents no HPP treatment and level 1 represents the HPP treatment at 600 MPa for 5 min) and time (level -1 represents storage time at week 0 and level 2 represents storage time at week 2).*

### 5.3.5 Appearance

The appearance score of each sample was high for the sample on week 0, which was around 6.2 without a significant difference. However, after 1-week storage, the score of untreated samples dropped from 6.37 to 4.79, while for sample H, the score dropped from 6.13 to 4.96. However, no significant changes were observed for those samples between 1 week and 2 weeks storage, which was also indicated by the factorial analysis in Figure 5.8. This result was in agreement with the instrumental data shown in Chapter 4 where the ΔE value changes mainly occurred in the first week. Meanwhile, no significant change was observed for sample A and sample HA. Combining with the factorial analysis, it could be referred that the incorporation of ascorbic acid effectively preserved the appearance of the sample.

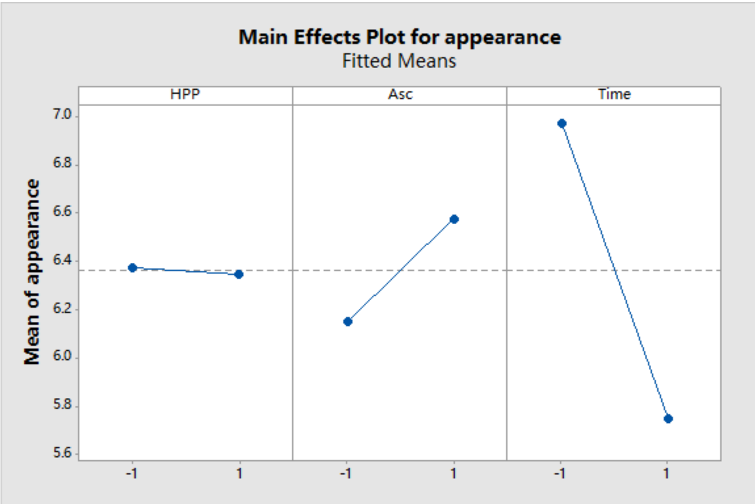


*Figure 5.8 Factorial analysis of appearance with factors including Asc (level -1 represents no ascorbic acid and level 1 represents the incorporation of 2% of ascorbic acid), HPP (level -1 represents no HPP treatment and level 1 represents the HPP treatment at 600 MPa for 5 min) and time (level -1 represents storage time at week 0 and level 2 represents storage time at week 2).*

## 5.4 Conclusions

The sensory properties of untreated avocado-fortified yoghurt dropped dramatically in 2 weeks from an overall acceptance from 7.16 to 2.25. Meanwhile the score for sample treated by both HPP treatment and ascorbic acid dropped from 7.13 to 5.38. HPP treatment specifically affected the texture profile of the sample by maintaining the thickness of the samples. Ascorbic acid significantly ($p < 0.05$) increased the flavour score from 2.72 to 6.29 and the appearance from 4.79 to 5.55. It was found that the main pathway of ascorbic acid to improve the sensory properties was to preserve the green colour and inhibit the development of rancid flavour in the product. Besides, neither of these treatments were observed to significantly influence the sourness of the sample, and the interactive effect was not observed between HPP treatment and ascorbic acid. In conclusion, the combination of HPP treatment and ascorbic acid effectively maintain the sensory properties (texture, flavour, sourness and appearance) of avocado stirred yoghurt.

# Chapter 6. Overall Conclusions and Recommendations

## 6.1 Conclusions

The aim of this study was to investigate appropriate treatments of avocado for extending the shelf life of avocado stirred yoghurt and characterise the yoghurt in terms of various properties.

The colour change of avocado stirred yoghurt occurred predominantly in the first week, and it was mainly caused by the decrease of L* value (lightness) as well as the decrease of a* value indicating the decrease of greenness.

The incorporation of 2% of ascorbic acid was effective in preventing the colour change of avocado stirred yoghurt, keeping the ΔE (total colour change) value below 2.0 during the normal shelf-life of the yoghurt product which is for 2 weeks.

A limited effect of citric acid on colour change was observed, while its efficiency was not comparable to ascorbic acid and eventually kept the colour change (ΔE) below 4.0 compared to the untreated sample's ΔE value of around 6.0.

The pH value of avocado stirred yoghurt was more sensitive to citric acid which decreased the pH from around 4.45 to 4.29 and 4.15 at 1% and 2%, respectively.

No significant influence of HPP treatment (600 MPa, 5 min) was observed on the colour stability in this study, while HPP treatment was found to be the key factor to keep syneresis of yoghurt low, minimizing the extra syneresis caused by introduced avocado content.

The rheological test illustrated HPP treatment increased and maintained the thickness of avocado stirred yoghurt by treating avocado before adding it into yoghurt.

No significant change in the rheological properties of avocado stirred yoghurt was observed by the incorporation of 2% of ascorbic acid.

Neither HPP treatment nor the ascorbic acid had an influence on the growth of lactic acid bacteria in yoghurt.

No yeast and moulds or coliform were detected in yoghurt samples during storage for 4 weeks for the whole experiment.

The sensory properties of untreated avocado-fortified yoghurt dropped dramatically in 2 weeks for an overall acceptance score from 7.16 to 2.25.

While HPP treatment and incorporation of ascorbic acid maintained the overall acceptance.

The sensory evaluation showed that HPP treatment specifically affected the texture profile of the avocado stirred yoghurt by maintaining the thickness of the samples.

Ascorbic acid significantly ($p < 0.05$) increased the flavour score of 2 weeks old avocado stirred yoghurt from 2.72 to 6.29 and the appearance from 4.79 to 5.55.

It was found that ascorbic acid was able to improve the sensory properties by preserving the green colour and inhibiting the development of rancid flavour in the product. However, the combination of ascorbic acid and HPP treatment was most effective in terms of maintaining the sensory properties (e.g. texture, flavour, sourness and appearance) of avocado stirred yoghurt.

## 6.2 Recommendations for further research

Various conclusions could be drawn based on the results obtained in this study as mentioned above. However, several questions arose, and these can only be answered by further investigations.

- In this study, the chemical agent chosen to treat avocado for preventing its deterioration was ascorbic acid which is used as an antioxidant. Although the effect of ascorbic acid was proved on extending the shelf-life of avocado stirred-yoghurt, there can be other types of antioxidant or reducing agents that can be explored for their effectiveness on the inhibition of enzymatic browning from avocado and the maintenance of avocado yoghurt quality during storage.
- The HPP treatment used in this study was 600 MPa for 5 min. However, the properties of avocado treated by HPP methods could vary when treated under different pressures and processing times. Thus, the effect of using different levels

of HPP treatment deserves further study to elucidate the full mechanism of HPP treatment.

- With avocado treated by the HPP method, the rheological properties of avocado stirred yoghurt were significantly different from the control group containing untreated avocado as the latter gradually lost its thickness during storage. Therefore, it would be interesting to investigate the use of some thickeners that maintain the structure of yoghurt with untreated avocado. In this study, microbial contamination was not detected due to the maintenance of hygiene and the limited scale of the experiment.
- The avocados used in this study were of a similar level of ripeness. However, the avocados from different ripening stages have a significantly different flavour and texture. Therefore, it could be interesting to investigate the influence of the ripeness level of avocado on the properties and stability of avocado stirred yoghurt.
- The scope of this study was limited to the avocado stirred yoghurt, but the types of yoghurt are numerous, such as set yoghurt and drinking yoghurt. It could be interesting to develop other types of yoghurt mixed with avocado fruit, including drinking yoghurt.

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# Appendix

1. Overall, How much do you like the sample?

| Like extremely | Like very much | Like moderately | Like slightly | Neither like or dislike | Dislike slightly | Dislike moderately | Dislike very much | Dislike extremely |
|---|---|---|---|---|---|---|---|---|
| ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

2. How much do you like the Flavour of the sample?

| Like extremely | Like very much | Like moderately | Like slightly | Neither like or dislike | Dislike slightly | Dislike moderately | Dislike very much | Dislike extremely |
|---|---|---|---|---|---|---|---|---|
| ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

3. How much do you like the Texture of the sample?

| Like extremely | Like very much | Like moderately | Like slightly | Neither like or dislike | Dislike slightly | Dislike moderately | Dislike very much | Dislike extremely |
|---|---|---|---|---|---|---|---|---|
| ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

4. How much do you like the the Sourness of the sample?

| Like extremely | Like very much | Like moderately | Like slightly | Neither like or dislike | Dislike slightly | Dislike moderately | Dislike very much | Dislike extremely |
|---|---|---|---|---|---|---|---|---|
| ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

5. How much do you like the the Appearance of the sample?

| Like extremely | Like very much | Like moderately | Like slightly | Neither like or dislike | Dislike slightly | Dislike moderately | Dislike very much | Dislike extremely |
|---|---|---|---|---|---|---|---|---|
| ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

6. What do you think of the Rancid Flavour of the sample?

| Not detected | Noticeable | Moderate | Strong | Very Strong |
|---|---|---|---|---|
| ○ | ○ | ○ | ○ | ○ |