CHAPTER 1

INTRODUCTION

1.1. Background

The fruit *Persea americana*, also known as Avocado, emerged from Central America although is nowadays cultivated and harvested from around the world (Cowan & Wolstenholme, 2016). In Indonesia, there are two varieties of avocado, namely; *ijo panjang* (*Porsea gratissima Gaertn*) and *ijo bundar* (*Persea americana Mill*), where *ijo bundar* is also known as *alpukat mentega* (Anova & Kamsina, 2013). Avocado is known for being nutritious due to its immense amounts of desirable un- and mono-unsaturated fatty acids, antioxidants and vitamins. Based on a study by Salazar-López et al. (2020), an average avocado fruit can be divided into approximately 73% pulp, 16% seed, and 11% peel by total weight. Surprisingly, that high antioxidant content and phenolic compounds mainly originates from the avocado seed rather than the pulp (Bahru et al., 2019). Dabas et al. (2013) reviewed the antioxidant values of avocado seed from various measuring methods. They concluded that the antioxidant values were 55-155 times bigger than what were in the pulp. They also found out that ascorbic acid and phenolic compounds were responsible for the antioxidant activity in the seed, while D-mannoheptulose for the pulp. While in another study, procyanidins were found to be the major phenolic compounds possessing antioxidant activity in the seed (Wang et al., 2010). In addition, other beneficial health effects of avocado seed extract including anticancer, anti-inflammatory, hypocholesterolemic, etc. have also been reported (Dabas et al., 2013).

Despite still being nutritionally useful, the avocado seed (AS) is usually discarded during the industrial process of avocado products. Its high content of bioactive compounds are still rather unknown to most industries and the seeds are usually considered a hindrance to the processing point of view, and thus contributes to food waste (Saavedra et al., 2017). Around 1.3 billion tons of food waste is generated globally every year (FAO, 2011), where 21% is contributed by the central and southeast asian countries (FAO, 2019). Indonesia as part of southeast asia, generated 30 million tons of waste per year in the year 2020 where 41% of that is from food waste and 60% from that 41% is from fruit and vegetables. (Ministry of environment and forestry, 2007; Ministry of environment and forestry, 2020). Therefore, it was suggested to convert the food waste to something valuable (e.g. food additive in a form of powder). However, the phenolic compounds possessing antioxidant capability are prone to damage from environmental factors (e.g. UV light, moisture, oxidation, chemical reaction) (Sun-Waterhouse et al., 2011; Salazar-López et al., 2020), as well as exhibiting astringent and bitter undesired flavor (Calderón-Oliver et al., 2017). Therefore, encapsulation techniques are needed to protect the phenolic compounds and increase its value (Desai & Park, 2005; Gaonkar et al., 2014).

Encapsulation is defined as a process in which small sized food ingredients (active ingredients) are enclosed in a layer of coating or in a matrix for storage, protection, or controlled release (Gaonkar et al., 2014). Freeze drying and coacervation are two of the encapsulation techniques that are commonly used in food industries. Freeze drying allows the transformation of liquid products into solid forms for storage, preservation, and easy handling (Fang & Bhandari, 2012). Meanwhile, coacervation encapsulates the active ingredients with a coacervate phase that is subsequently formed upon the phase separation of one or many hydrocolloids from the starting solution within the same reaction media (Gouin, 2004). In a complex coacervation, oppositely charged polymers will be used to form a coacervate phase that will encapsulate the active ingredients that have been either emulsified or in suspension (Xiao et al., 2013). Other than to protect the food ingredients (active ingredients) from oxidative stress, encapsulation techniques also protect against damage from environmental factors (e.g. water, heat, light, etc.), increasing shelf-life, off-taste masking, preventing undesirable interaction between the active ingredients and other food components, and allowing controlled delivery or release of the active ingredients (Gaonkar et al., 2014). Emulsification of active ingredients has been studied to further protect the active ingredients from environmental stress and for better control of releasing the active ingredients (Zhu et al., 2019).

In the encapsulation technique, a coating agent is used to encapsulate the active ingredients. There are different types of coating agents or wall materials including polysaccharides (e.g. alginate, pectin), lipids (e.g. mono- and diglycerides), and proteins (e.g. gelatin), in which their structure and characteristics will contribute different physicochemical properties to the encapsulated active ingredients (Šeregelj et al., 2020). In this study, gelatin (type B), low methoxyl pectin, and sodium alginate were used as the coating agent. Gelatin (GE) has an isoelectric point (pI) of around 8-9 and 4-5 for type A and B respectively, and will have a positive charge when exposed to a pH below the pI (Gómez-Guillén et al., 2011). Low methoxyl pectin (LMP) has a negative charge due to the presence of ionised carboxylic group with pK_a values of around 3.6 when exposed in a mild acidic pH (Xiao et al., 2013). On the other hand, sodium alginate (SA) has a negative charge at pH of 3-7 due to the presence of ionised carboxyl groups with pKa value of around 3.5 (Harnsilawat et al., 2006). Thus, the protein would be able to form complex coacervation with the individual polysaccharide given that the pH is below their respective pI and pK_a. Nevertheless, complex coacervation is affected by pH and polymer ratio (Eghbal & Choudhary, 2018; Ye, 2008).

However, this study is still seen as a novel research due to the lack of understanding of the effect of complex coacervation process on antioxidant activity and literature in the field of complex coacervation of avocado seed. On that account, the focus of this study was to perform a preliminary study to determine the viability of complex coacervation to preserve the antioxidant activity of avocado seed (AS) by investigating the effect of freeze-drying, the presence of avocado seed powder (ASP), and encapsulation process, as well as type and ratio of the encapsulating agents to the TAC of the sample. The purpose of analysing the TAC is due to the presence of wide diversities of the antioxidant compounds that are present within the product as well as for studying the antioxidant properties of the product (Csepregi et al., 2016). The TAC was measured using DPPH assay and was expressed as percentage of antioxidant activity (AA%) through calculation based on the absorbance obtained.

1.2. Research Objective

The objective of this study is to reduce food waste and perform a preliminary study to determine the viability of complex coacervation to preserve the antioxidant activity of avocado seed

(AS). Other objectives include observing the effect of the freeze-drying, the presence of ASP (core), and encapsulation process (W/O/W emulsion and complex coacervation), as well as type (GE, LMP, SA, GE-LMP, and GE-SA) and ratio (1:0:0, 0:1:0, 0:0:1, 4:1:0, 1:1:0, 1:4:0, 4:0:1, 1:0:1, 1:0:4) of the encapsulating agents to the TAC of the sample.

1.3. Problem Formulations

- What is the effect of freeze-drying to the TAC of the sample ?
- What is the effect of the presence of ASP (core) to the TAC of the sample ?
- What is the effect of different encapsulation processes (W/O/W emulsion and complex coacervation) to the TAC of the sample ?
- What is the effect of the type and ratio of the encapsulating agents used to the TAC of the sample ?

1.4. Research Hypothesis

- Null hypothesis (H_0): There are no significant differences between the samples at any stage of this steps (step 1, step 2, step 3, step 4, step 5, step 6)
- Alternative hypothesis (H_1): There are significant differences between the samples at any steps of this study (step 1, step 2, step 3, step 4, step 5, step 6)

1.5. Expected Outcome

- The freeze-dried powder sample is expected to have higher TAC than the liquid state sample
- The samples that contain ASP (core) is expected to have higher TAC than the samples that does not contain the core
- Sample with complex coacervation is expected to have higher TAC than samples with W/O/W emulsion
- One type of sample is expected to have highest TAC among other type of samples

1.6. Scope of the Research

This research involves several stages of complex coacervation process (i.e. dissolution, emulsion, gelation, freeze drying), extraction method of samples, and TAC analysis via DPPH assay of the samples. However, there were several things that were excluded in this research including; comparison of TAC between the AS and ASP, comparison of TAC of AS from different avocado cultivars, identification and classification of chemical composition in ASP and wall materials, the safety and toxicity of any samples containing the ASP, the physical characteristic of the freeze-dried samples, the controlled release properties of the sample, in depth analysis of the interaction between the core and wall materials, application of sample to a food product, and etc.