CHAPTER 1: INTRODUCTION

1.1 Background

Red fruit (*Pandanus conoideus* Lam.) is an indigenous plant of Papua Islands that has a special trait of a deep red color appearance. The extracted red fruit oil (RFO) provides multifunctional benefits as a natural dye, functional food, and traditional medicine (Wawo *et al.,* 2019). One of the major compounds found in RFO is carotenoids that exhibit yellow, orange, to red color pigments (Gunawan *et al*., 2020). The abundance of carotenoids in RFO is favorable for the commercial production of natural food colorant. Moreover, current consumers are becoming self-conscious about healthy food ingredients that food and beverage industries have attempted to restrain the use of synthetic and/or natural identical coloring agents and have been proactively seeking naturally-sourced food colorants (Mohamad *et al*., 2019).

All types of carotenoids are known to be lipophilic, moderately heat stable, and likely losing color due to oxidation (Damodaran *et al*., 2008). The majority of food processing methods degrades carotenoids due to the thermal use and oxidative nature of the process itself. Furthermore, converting liquid RFO into powder form could prolong the shelf life, improve the stability of the product, as well as reduce the distribution cost. As such, encapsulation is a promising technique to preserve carotenoids during processing and storage by creating a homogenized oil-in-water (O/W) emulsion of RFO with dissolved coating materials (Indrawati *et al*., 2020). One suitable encapsulation technique for carotenoids is freeze drying. Despite its time-sensitive procedure and high cost, freeze drying is superior for the encapsulation of thermosensitive substances, including carotenoids (Šeregelj *et al*., 2020a). The general principle of freeze drying is utilizing a low-temperature dehydration process by initially freezing the product, and then creating a high vacuum condition to directly convert the ice crystals into vapor through the sublimation phenomenon (Bhatta *et al.*, 2020).

The choice of carrier agent substantially determines the physicochemical properties of the freeze-dried RFO. Maltodextrin is known to be the most widely used encapsulating agent due to its high water solubility, low viscosity even at high concentration, and great encapsulation capability at a low price (Šturm *et al*., 2019). Using proteins as encapsulating agents is also popular since they may protect the core material against oxidation and have high binding properties (Eun *et al.*, 2020). Whey protein isolate (WPI) contains α-lactalbumin and β-lactoglobulin that provide protective film-forming abilities, and good emulsification which is essential for the encapsulation of oil-based products (Šeregelj *et al.*, 2020a). Sodium caseinate is another encapsulating agent derived from milk protein. The exceptional traits of sodium caseinate to stabilize the oil-in-water emulsion and provide a protective barrier have been exploited to encapsulate lipophilic compounds, such as β-carotene (Cornacchia and Roos, 2011). There have been several studies utilizing spray drying for the encapsulation of RFO using maltodextrin (Sarungallo *et al.*, 2019) and sodium caseinate (Yanuwar *et al*., 2007), but the study regarding freeze drying and its influence on the preservation of carotenoid pigments in RFO during storage as a natural food-grade dye is still unknown.

1.2 Objective

The objectives of this study are:

a. to produce red fruit oil (RFO) powder using freeze drying technique while maintaining carotenoid content inside the resulting freeze-dried RFO,
b. to compare different carrier agents and their ratios used in the encapsulation formulation towards the total carotenoid content of freeze-dried RFO,
c. to evaluate carotenoid stability of the freeze-dried RFO during storage, and
d. to identify the correlation between carotenoid pigment concentration and color.

1.3 Scope of the Study

Food colorant has a major function to impart color to the food; nevertheless, this study only focuses on creating carotenoid-rich food colorant from red fruit oil (RFO) using freeze drying technique and finding out which encapsulation formula produces powders with high carotenoid pigment and good carotenoid stability during storage. For the encapsulation process, oil-in-water (O/W) emulsion of RFO will be prepared using different carrier agents, maltodextrin and whey protein isolate, at different ratios. Resulting freeze-dried samples are stored inside the aluminium pouch with moisture absorber at room temperature (25℃) and ambient humidity (±78% RH) storage conditions, then subjected to the analysis of total carotenoid content using spectrophotometry at week 0,1,2,3,4 of storage and color by digital image analysis at week 4 of storage.

1.4 Problem Formulations

According to the project background, the problem formulations are determined as follows:

a. What is the effect of encapsulation process and freeze drying technique towards the total carotenoid content of the RFO powder samples?
b. What is the effect of using different ratios of maltodextrin and whey protein isolate as the carrier agents towards the total carotenoid content of freeze-dried RFO at week 0?
c. What is the effect of storage time towards total carotenoid content and carotenoid stability of freeze-dried RFO?
d. Is there correlation between total carotenoid content and color of freeze-dried RFO?

1.5 Hypothesis

According to the problem formulation, the hypotheses are determined as follows:

<u>Hypothesis 1:</u>

H_0 = Encapsulation process and freeze drying technique do not affect the total carotenoid content of the RFO powder samples.

H_1 = Encapsulation process and freeze drying technique affect the total carotenoid content of the RFO powder samples.

Hypothesis 2:

H_0 = There is no significant difference in total carotenoid content of freeze-dried RFO between ratios of carrier agents.

H_1 = There is significant differences in total carotenoid content of freeze-dried RFO between ratios of carrier agents.

Hypothesis 3:

H_0 = There is no significant difference in total carotenoid content of freeze-dried RFO over storage time.

H_1 = There is significant difference in total carotenoid content of freeze-dried RFO over storage time.

Hypothesis 4:

H_0 = There is no significant correlation between total carotenoid content and color of freeze-dried RFO.

H_1 = There is a significant correlation between total carotenoid content and color of freeze-dried RFO.

1.6 Expected outcome

The expected outcomes of this study are:

a. no significant difference in total carotenoid content between different samples at each processing step throughout the production of freeze-dried RFO,

b. a significant difference in total carotenoid content of freeze-dried RFO between ratios of carrier agents,

c. no significant change in total carotenoid content of freeze-dried RFO over storage, and

d. a correlation between carotenoid pigment concentration and color of the freeze-dried RFO.

1.7 Organization of Thesis

This thesis manuscript consists of six chapters as follows:

Chapter I describes the background of the research project (problem statement, solution proposition, significance), objectives of the research, scope of the study, problem formulation and hypothesis of the research, expected outcome, and the organization of the research.

Chapter II provides the review of literature, explaining red fruit (origin, characteristics, usage, biochemical compositions), food colorant (significance in food and beverage industry, high demand on natural pigment), carotenoids (identity, physical properties, stability), encapsulation by freeze drying technique (principle, advantages, methodology), processing aids used in the encapsulation formulation (maltodextrin, whey protein isolate, sodium caseinate), and research analysis (total carotenoid content using spectrophotometry, color by digital image analysis).

Chapter III explains the research methodology and workflow for this project, consisting of materials and reagents list, coating material formulation, emulsion preparation for encapsulation, freeze drying, total carotenoid content, color, and statistical analysis.

Chapter IV presents the statistical results of this research in the form of tables and figures.

Chapter V provides the interpretation of all collected data and describes the significance of these research findings.

Chapter VI summarizes the overall research work in the form of a conclusion and recommendation for future development of the related study.