Chapter I

Introduction

1.1. Background

Euglena gracilis is a photosynthetic green microalga that lives in freshwater. This microalga has been known for its environmental benefits since it uses CO_2 as a carbon source and light as an energy source. This leads them to be cultured photoautotrophically to perform their cell functions although the influence of this cultivation to the cell composition has not been studied yet (Matsumoto *et al.*, 2008). From the food industry aspect, it has been studied before as an alternative source of protein, fatty acids, vitamins, and minerals and could be easily produced (Matsumoto *et al.*, 2008; Watanabe, Yoshimura, & Shigeoka, 2017). Since it has high nutritional value, the function of *E. gracilis* as nutritional supplements for humans and domestic animals is also being considered.

In addition, *E. gracilis* has the ability to synthesize high levels of antioxidant vitamins such as vitamin C, glutathione, carotenoid, and tocopherols. These antioxidants are essential in human diet which are simultaneously produced by *E. gracilis* cells although the amount is varied by their growth or growth conditions (Watanabe, Yoshimura, & Shigeoka, 2017). These antioxidants and vitamins are also important for the *E. gracilis* to protect itself against Reactive Oxygen Species (ROS) in their living environment (Cazzonelli, 2011; Watanabe, Yoshimura, & Shigeoka, 2017). Although the four antioxidants were correlated to each other, the tocopherols in *E. gracilis* will not be analyzed due to undeveloped methodology to avoid unreliable data gathered in the present research.

In plants, vitamin C has been playing multiple roles in the control of photosynthesis, cell expansion and growth, and transmembrane electron transport additionally to its indispensable role as an antioxidant which also applies in *E. gracilis* (Smirnoff, 2000, 2001; Valpuesta & Botella 2004; Ishikawa *et al.*, 2006; Ishikawa & Shigeoka 2008; Watanabe, Yoshimura, & Shigeoka, 2017). It also constitutes the ascorbic acid-reduced glutathione cycle as a cardinal antioxidative mechanism in *E. gracilis* (Kitaoka *et al.*, 1989; Watanabe, Yoshimura, & Shigeoka, 2017). *E. gracilis* accumulates high concentration of ascorbic acid and excretes 3,000 ng/mg dry weight (DW) of the vitamin into surrounding medium (Shigeoka *et al.*, 1979; Baker *et al.*, 1981; Watanabe, Yoshimura, & Shigeoka, 2017).

Glutathione is an antioxidant in *E. gracilis* which is closely related to ascorbic acid or vitamin C. The glutathione found inside the cells is in reduced form (Watanabe, Yoshimura, & Shigeoka, 2017). The cells have glutathione reductase which keeps the glutathione in reduced form and accelerates the redox process of L-ascorbate that scavenges the peroxides generated in *E. gracilis* (Shigeoka *et al.*, 1987a). However, the total glutathione content in *E. gracilis* is lesser than the other antioxidants. It only presents at millimolar concentration (Shigeoka *et al.*, 1987a).

The carotenoid biosynthetic pathway of *E. gracilis* was discovered recently. Vitamin A or carotenoid is known to be essential for normal cell growth, cell differentiation, immunological functions and vision (Gerster, 1997; Watanabe, Yoshimura, & Shigeoka, 2017). In photosynthesis, carotenoids and chlorophylls both bind peptides in order to form the pigment-protein complexes in the thylakoid membrane (Watanabe, Yoshimura, & Shigeoka, 2017). It was previously reported that the principal carotenoid of *E. gracilis* was antheraxanthin accounting for more than 80% of the carotenoids present, followed by β-carotene at approximately 11% and neoxanthin at 7%, with astaxanthin, astacene, vitamin A, and retinene being undetectable (Kinsky & Goldsmith, 1960; Watanabe, Yoshimura, & Shigeoka, 2017). Although antheraxanthin is the most found in *E. gracilis*, β-carotene is present in most divisions of the reaction center and light-harvesting complexes of photosystem I as well as photosystem II (Takaichi, 2011; Watanabe, Yoshimura, & Shigeoka, 2017).

Antioxidants are essential for humans and plants since they are involved in complex metabolic and signaling mechanisms. Fruits and vegetables are one of the main sources of antioxidants for humans, these antioxidants differ in concentration and identity from each fruit or vegetable (Wilson e*t al.*, 2017). For example, carrots or spinach are sources of β-carotene meanwhile for both oranges and tomatoes, they could be either a source of vitamin C or glutathione since those antioxidants are heavily correlated (Fenech *et al.*, 2018). Accompanied with its ability to produce multiple antioxidants, there is a possibility of *E. gracilis* to be a new source of antioxidant or even could be categorized as superfood because it produces multiple antioxidants if the antioxidants level in the cells are comparable to these food sources.

However, there is not much study about comparing antioxidants in *E. gracilis* to macro food. Most of the previous studies about the comparison of *E. gracilis* to food sources is comparing the nutrition composition of *Euglena*. One of the studies is comparing nutrition composition of *E. gracilis* such as proteins, fatty acids, micronutrients, and paramylon to *spirulina*, *chlorella*, and yeast (Matsumoto *et al.*, 2008). The comparison of *E. gracilis* nutritional content to other common micro food ingredients is to discover their nutrients function in the cells and its potential as a good food source and supply for the future. In this study, *E. gracilis* sample was cultured photoautotrophically in Cramer-Myers (CM) media and harvested on day 7 of cultivation. It showed a comparable result with yeast and *spirulina* which Matsumoto *et al*. (2008), concluded that *E. gracilis* is a good source of protein and micronutrients. Nonetheless, not all people know of *spirulina* and *chlorella* or the term micro food itself so conducting a research with widely consumed food such as fruit or vegetable is preferred in order to confirm further about the contents in *E. gracilis*.

Based on Matsumoto *et al*. (2008), different analyses were used to analyze each nutrient of *E. gracilis*. Among different analyses used, High Performance Liquid Chromatography (HPLC) is a common method to analyze certain components in both *E. gracilis* and food. It has been used to analyze antioxidant contents in fruits and vegetables (Shui & Leong 2004; Boligon *et al.*, 2012). There are also previous studies about determination of provitamin A, glutathione, ascorbic and content of *E. gracilis* grown under different conditions of media to be analyzed using HPLC (Chen *et al.*, 2008; Kato *et al.*, 2017; Nagy *et al.*, 2016; Rodriguez Zavala *et al.*, 2010; Watanabe, Yoshimura, & Shigeoka, 2017). Aside from the antioxidant analysis using HPLC, the spectrophotometer method is also a common method for determining GSH and total carotenoid (Shigeoka *et al.*, 1987a; Tamaki *et al.*, 2019). Thus, both methods are compatible for this research. The HPLC method will be used to determine the β-carotene and ascorbic acid of the samples and spectrophotometer will be used to determine the GSH activity and total carotenoid from the absorbance.

1.2. Problem Formulation

The initiation of this experiment started from the acknowledgement of *E. gracilis* which are rich in antioxidants so it was seen as a source for future food. Additionally, most of microalga has a simple structure, which also applied on *E. gracilis*, it does not require much land to grow thus making it more preferable as a future food ingredient (Matsumoto *et al.*, 2008; Kumar *et al.*, 2015). Since the experiment that compared the antioxidants of *E. gracilis* towards the macro-food is limited, confirmation regarding the antioxidant contents in the *E. gracilis* is needed. The analysis in this experiment will show whether *E. gracilis* has the potential to be a new source of food grade antioxidants or not.

1.3. Objective

The objective of this research is to compare the antioxidant molecules in *Euglena gracilis* to vegetables or fruits to identify the eligibility of *Euglena gracilis* as a new antioxidant source ingredient.

1.4. Scope of Work

- Culturing *E. gracilis*
- Sample preparation of *Euglena gracilis* (Cell collection and antioxidant extractions)
- Sample preparation of fruits/vegetables that contain the target antioxidants which are β-carotene, ascorbic acid, and glutathione
- Standard Preparation (β-carotene, ascorbic acid, and glutathione)
- High Performance Liquid Chromatography (HPLC) of *E. gracilis* and vegetable/fruit samples
- Total carotenoid and glutathione determination by spectrophotometer
- Antioxidants comparison analysis (specifically β-carotene, total carotenoid, ascorbic acid, and glutathione) by one-way ANOVA and Tukey Post-hoc

1.5. Expected Outcome

This research is expected to know the potential of *E. gracilis* as a new antioxidant source because of their ability to synthesize antioxidants. The comparison to spinach, tomato, yellow bell pepper, kiwi, and carrot represent the large group of fruits and vegetables which usually become the source of antioxidants in the human diet. Hence, if the antioxidants are comparable, *E. gracilis* could be known as a new source of antioxidants and could be incorporated or developed into a food product.