CHAPTER I

INTRODUCTION

1.1 Rationale

Haematococcus pluvialis is a microalgae that produces carotenoids and other biologically active compounds. One of the most important carotenoids found in *H. pluvialis* includes beta-carotene, zeaxanthin, lutein, echinenone, canthaxanthin, and astaxanthin. The chemical structures of these carotenoids are shown in Figure 1.1.

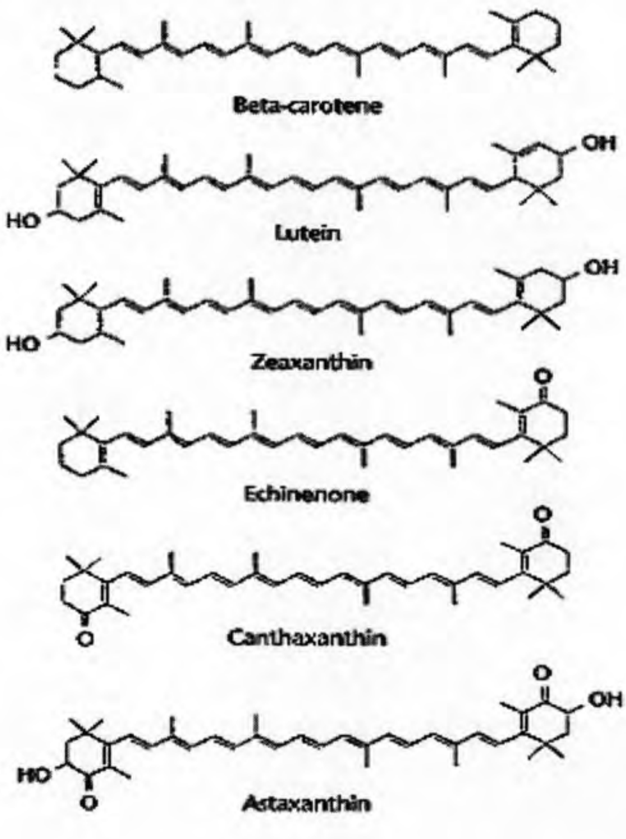

Figure 1.1 The chemical structures of carotenoids in *H. pluvailis*

Of these carotenoids, astaxanthin is found in the largest amount in *H. pluvialis* and is the most powerful biological antioxidant. Astaxanthin is usually added to aquaculture feeds to ensure the proper colorization, growth, and survival of these farmed animals. Moreover, the compound exhibits strong free radical scavenging activity and protects against lipid peroxidation and oxidative damage of LDL-cholesterol, cells and tissues, and cell membranes, and is now approved by FDA for its use as human supplement.

In general, astaxanthin products are highly sensitive to light, oxygen, and heat. So the conventional extraction of astaxanthin content from *Haematococcus pluvialis* with organic solvents, such as acetone or dichloromethane may not be appropriate because these conventional solvent extraction techniques require that the harmful solvents. To separate the solvents, it requires evaporation of the solvent, the process which may cause product degradation. For this reason, new extraction methods that minimize the use of toxic chemicals must be developed.

Supercritical fluid extraction (SFE) is a modern technology with increasing applications in the food and food processing industry. The core of the process consists of utilizing a supercritical fluid whose physicochemical properties are between those of a liquid and a gas. Its temperature and pressure are above the critical values, which lead these solvents to possess special properties such as high diffusivity, low viscosity, and low surface tension, allowing them to better diffuse through natural solid matrix, and thus better extract the natural compounds than the conventional liquid solvents. When the operating pressure and temperature are reduced, the loss of these special characteristics occurs. Thus, the solute can be extracted from the solvent at supercritical condition, and separated when reduce pressure and temperature below the critical condition. The most frequently employed supercritical solvent in food and natural product processing is carbon dioxide (CO_2) due to its low toxicity, good safety, and low critical temperature.

Although supercritical carbon dioxide ($SC\text{-}CO_2$) is nowadays accepted as a new alternative for benign extraction of natural compounds, the selection of the operating conditions for specific application is still an area of active research. For extraction of carotenoids from marine materials, many recent studies have been carried out to investigate the effect of operating conditions and to find optimal conditions for the process (Careri et al., 2001; Sanchez et al., 2005). Studies on extraction of astaxanthin from *Haematococcus pluvialis* have also been reported

(Majewski et al., 2000; Machmudah et al., 2004). It was found in our previous investigation that the total amount of astaxanthin in the extract and its concentration in the extract are influenced by the extraction pressure and temperature. Furthermore, using ethanol as a co-solvent could enhance the amount of astaxanthin in the extract but the antioxidant activity was decreased possibly due to the presence of large amount of other impurities extracted along with the compound (Machmudah et al., 2004). Despite the interesting information obtained, these previous studies investigated the process conditions by merely conducting one-variable-at-a-time experiments. In such cases, no interaction between process variables was assumed, and this can lead to biased results, and thus the true optimality might not be reached. Statistical experimental design has been demonstrated to be a powerful tool for determining the factors effects and their interactions, which allows process optimization to be conducted effectively. The techniques are used widely in various applications including in chemical industry. As an example related to this proposed study, the method has successfully applied in order to investigate the effects of extraction parameters on supercritical fluid extraction of carotenoids in *Spirulina platensis* (Careri et al., 2001).

In this work, the experimental design was used to investigate the effect of operating pressure, temperature, and extraction time on the amount and concentration of astaxanthin as well as antioxidant activity of the extract. In addition, based on the statistical experimental design investigation, the optimal conditions would therefore be proposed. It should be noted here that the effect of co-solvent was not considered in this study since it was shown by the previous study by Machmudah et al. (2004) that addition of co-solvent lowered the antioxidant activity of the extract and that the use of co-solvent was unnecessary at sufficiently high pressure.

1.2 Objectives

1.2.1 To investigate the effects of operating pressure, operating temperature, and extraction time for the SC-CO_2 extraction of astaxanthin from *Haematococcus pluvialis*, using a central composite design.

1.2.2 To model the factor effects with 2^{nd} order response surface equations and to determine the optimal experimental conditions for the SC-CO_2 of astaxanthin from *Haematococcus pluvialis* based on these equations.

1.3 Expected benefits

1.3.1 This investigation provides a new alternative for extraction of high antioxidant substance, astaxanthin, from plant material, *Haematococcus pluvialis* algae.

1.3.2 This investigation provides useful information for scale-up of extraction processes in the industries.

1.4 Working Scope

1.4.1 Using the experimental design to investigate the effect of these extraction variables, pressure in range of 300-500 bar, temperature in range of 40-80 °C, and extraction time in range of 1-4 hours on supercritical carbon dioxide of astaxanthin.

1.4.2 Determination of the best experimental extraction condition for the SC-CO_2 of astaxanthin.

1.4.3 Determination of the astaxanthin antioxidant activity at the best experimental extraction condition.