CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. In this study, the main effect of pressure (X_1), the main effect of extraction time (X_3), and the interaction effect between temperature and pressure (X_1X_2) were significant to the astaxanthin yields.
2. The following approximate model was proposed for predicting the response surface of astaxanthin yields.

   $$Y = 10.107 - 0.199X_1 + 1.032X_2 + 0.972X_3 - 0.458X_1^2 - 0.393X_2^2 + 0.127X_3^2 + 0.651X_1X_2 + 0.0288X_1X_3 - 0.451X_2X_3$$

   From the model, the optimal condition for the astaxanthin yields is at 90 °C, 640 bar, and 2.9 hours. At this operating point, the amount of astaxanthin extract is estimated to be 22.66 mg/g (or 82.40 %wt.) and the antioxidant activity in terms of IC_{50} is estimated to be 1.83 mg/l. This condition was the extrapolated result and therefore would not be accurately predicted. Thus the optimal condition was proposed within the range of this experiment to be at the temperature of 70 °C, the pressure of 500 bar, and the extraction time of 4 hours, which yielded the amount of astaxanthin extract of 23.04 mg/g dry algae (or 83.78 %wt.) and IC_{50} 2.45 mg/l.
3. In this study, only the interaction effect between operating temperature and operating pressure was a significant factor affecting the antioxidant activity of the *H. pluvialis* SC-CO_2 extract.
4. The following approximate model was proposed for predicting the response surface of extract antioxidant activity ($1/IC_{50}$).

   $$Y = 0.413 + 0.0209X_1 - 0.0116X_2 + 0.0164X_3 + 0.04392X_1^2 + 0.04042X_2^2 - 0.0576X_3^2 - 0.0585X_1X_2 - 0.0313X_1X_3 + 0.0310X_2X_3$$

   From the response surface equation, the least favorable condition for extraction that yielded the extract with the lowest antioxidant activity is at 50 °C, 354 bar, and 2.68 hours. This point represents inappropriate condition for extract antioxidant activity. At this point, the extract antioxidant activity in term of IC_{50} was equal to 2.18 mg/l.

5.2 Recommendations

1. Face-centered central composite design was used in this study to obtain the response surface. However, the optimal point determined from the response equation was outside the experimental domain, particularly, that of the operating pressure. It is recommended that experiments at higher temperature be conducted. This however was not possible due to the constraint in the pressure of the equipment employed in this study. Alternatively, a rotatable design, which consists of a 2^k factorial (coded to the usual ± 1 notation) augmented by 2k axial points (±α, 0, 0, …, 0), (0, ±α, 0, …, 0), (0, 0, ±α, …, 0), …, (0, 0, 0, …, ±α) and n_o center points (0, 0, …, 0), where k is the number of factor and the α equals to $(2^k)^{1/4}$, can be used to extrapolate or predict the results out of the experimental range, it is thus recommended for the future study.
2. This study concerned with the SC-CO_2 extraction of astaxanthin without addition of co-solvent as it was previously found that EtOH co-solvent lowers the antioxidant activity of the extracts. However, there are other natural non-polar substances such as vegetable oil that can dissolve non-polar product as astaxanthin and may not affects to the antioxidant property. In the future study, these co-solvents might be tested for SC-CO_2 extraction of astaxanthin and the antioxidant activity of the extract.
3. Detailed analysis of astaxanthin content with high performance liquid chromatography is recommended. This not only provides a more quantitative analysis of astaxanthin, it allows the qualification and quantification of the other carotenoids compounds present in the extract.
4. Because of high potential of antioxidant activity of astaxanthin for application in fields of medicine and cosmeceuticals, purification of astaxanthin in the *H. pluvialis* extract is recommended to enhance the value of the product.