CHAPTER 1
INTRODUCTION

1.1 Background

Astaxanthin, one of natural carotenoid, is classified as xanthophylls. Basic structure of astaxanthin consists of eight isoprenoid units with two six-carbon rings each harboring oxygen molecule at the polar end. It is of commercial value pigment generally found in flesh, carapace, plumage of birds and marine animals such as lobsters, crab, shrimp, trout, and salmon (Johnson *et al.*, 1980; Johnson, 2003). Typically, microorganisms and plants can exclusively synthesize astaxanthin *de novo* (Roy *et al.*, 2008) whereas animals are unable to synthesize astaxanthin. Therefore, wild animals acquires their characteristics color through their feed (Johnson *et al.*, 1997). Further, farmed animals to whom natural color sources are unavailable, astaxanthin has to be added to their feed to improve their flesh coloration to pinkish-red relevant to consumers' acceptance as well as animal's health (Breithaupt, 2007; Johnson, 2003; Lorenz and Cysewski, 2000). Currently, FDA and EU have approved usage of astaxanthin as a supplement in animal feed (Breithaupt, 2007; Dufossé, 2006). In addition, antioxidant activity of astaxanthin is 10 and 100-500 folds stronger than that of β-carotene and α-tocopherol (Vitamin E), respectively (Naquib, 2000; Miki, 1991; Shimidzu *et al.*, 1996).

Astaxanthin may be obtained either chemically or biologically and costs approximately 2,500 $US per kilogram with an annual worldwide market estimated at 200 $US million, providing that more than 95% available in the market at present was chemically synthesized (Lorenz and Cysewski, 2000). Further, utilization of chemically synthesized astaxanthin must comply with strict regulation as well as customer acceptance when chemical supplement was applied in food (Tangeras and Slinde, 1994). *Xanthophyllomyces dendrorhous* has been identified as the best biological source of astaxanthin because it can be grown in fermentor to very high cell densities (more than 50 g/L dry weigh cell) (Johnson, 2003). Additionally, astaxanthin production by *X. dendrorhous* were identified as both growth-associated (Johnson and Lewis, 1979) and sometimes non growth-associated (Johnson, 2003). In addition, *X. dendrorhous* (formerly *Phaffia rhodozyma*), also commonly known as red yeast, is capable of

accumulating astaxanthin as a major carotenoid approximately 80 % (Andrewes *et al.*, 1976).

Even though production of astaxanthin using *X. dendrorhous* represents the most promising source, wild type strain generally accumulates astaxanthin to a rather low level, approximately 200-400 $\mu g/g_{yeast}$ (Johnson, 2003). Attempts have been made to seek effective and inexpensive cultivation growth medium for *X. dendrorhous* cultivation. There are reports on the use of inexpensive carbon sources for production of astaxanthin by *X. dendrorhous* including cane molasses (Haard, 1988), sugar cane juice (Fontana *et al.*, 1996), grape juice (Meyer and du Preez, 1994), hydrolyzed peat (Martin *et al.*, 1993), and raw coconut milk (Domínguez-Bocanegra and Torres-Muñoz, 2004). In addition, the best nitrogen source capable of promoting high astaxanthin production was yeast extract; however, high price hinders its use in large quantities. Pineapple juice concentrate contains high sugar concentrations including sucrose, fructose, and glucose which are superb carbon sources for cultivating microorganisms. In addition, pineapple, a highly acid fruit, contains 2.95 g/L acid of which 2.18 g/L is citric acid and 0.29 g/L is malic acid (Abdullah and Mat, 2008). In addition, it has been demonstrated that citrate as well as phosphate (Flores-Cotera *et al.*, 2001) could enhance astaxanthin production by *P. rhodozyma*. Moreover, pineapple juice concentrate is rich in protein (0.4-0.7 % w/w) as well as several trace elements such as iron, potassium and phosphorous. Therefore, pineapple juice may potentially be employed to cultivate *X. dendrorhous* and, at the same time, yielding high level of astaxanthin production.

1.2 Objectives

1. Investigate important factors significantly influencing growth and astaxanthin production.
2. To establish effective and inexpensive pineapple juice base growth medium for *X. dendrorhous* cultivation and astaxanthin production.

1.3 Research Outline

1. Characterization of pineapple juice concentrate.
2. Screening of chemical inducers influencing growth by *X. dendrorhous* using Fractional Factorial Design.

3. Optimization of biomass production by *X. dendrorhous* using Doehlert experimental design.
4. Screening of chemical inducers influencing astaxanthin production by *X. dendrorhous* using Fractional Factorial Design.
5. Optimization of astaxanthin production by *X. dendrorhous* using Doehlert design.
6. Investigating growth and astaxanthin production characteristics in 2 liters.

1.4 Expected Output

Add value to agricultural by products particularly pineapple juice concentrate and, at the same time, obtain effective and inexpensive growth medium for cultivating *X. dendrorhous*.