# CHAPTER 5
# CONCLUSION

## 5.1 Conclusions

The main objective of this study lies in utilization of pineapple juice concentrate, an agricultural by-product derived from canned pineapple industry, as a base growth medium to cultivate and simultaneously produce astaxanthin by the red yeast *X. dendrorhous*. Pineapple juice was characterized and revealed that sucrose, glucose, and fructose were present at the concentration of 23.58, 39.14, and 36.86 g/L, respectively. In addition, acetic acid, citric acid, propionic acid concentrations of 21.70, 2751.30, and 51.00 mM were found to be the main acid in pineapple juice concentrate together with several amino acids; therefore, it is possible to be employed as base culture medium to cultivate *X. dendrorhous*. Results on cultivation of *X. dendrorhous* in pineapple juice containing 10 g/L total sugar without nitrogen source supplementation was satisfactory in comparison with that of YM medium. Biomass and astaxanthin concentration and astaxanthin content obtained when cultivated *X. dendrorhous* in pineapple juice were 4.25 g/L, 0.91 mg/L, and 241.47 $\mu g/g_{yeast}$, respectively, while biomass and astaxanthin production and astaxanthin content with YM medium were 4.2 g/L, 1.04 mg/L, and 278.13 $\mu g/g_{yeast}$ (Table 5.1), respectively. Since YM medium was rather expensive, moreover, cultivation of *X. dendrorhous* in pineapple juice concentrate was fairly reasonable, therefore, pineapple juice could be a excellent substrate for cultivating *X. dendrorhous* when appropriately optimized.

Medium components and operating parameters, primarily carbon source (sucrose and glucose), nitrogen source ($KNO_3$), and oxygen vector (*n*-hexadecane) were considered statistically significant ($p<0.05$) on growth and astaxanthin production by *X. dendrorhous*. Results obtained using fractional factorial design further showed that although glucose was considered insignificant on growth but played important role on astaxanthin production. Additionally, optimization using Doehlert design indicated that sucrose, $KNO_3$, and *n*-hexadecane at the concentration of 40.2, 1.23 g/L and 8.2 % (v/v), respectively, yielded high biomass production of 13.24 g/L (Table 5.1) which is 3.2 fold increase in comparison with that of YM medium.

The production of astaxanthin could be usually improved by supplementation of suitable enhancers to the cultivation medium. However, adverse effect on growth of *X.*

*dendrorhous* when some chemical inducers were supplemented into the growth medium has been reported. Therefore, chemical inducers capable of enhancing astaxanthin accumulation while posed insignificant effect on growth were carefully selected of which pyruvate, Tween20, metal ion ($ZnCl_2$), and ethanol were adopted. Screening experiment accomplished using FFD indicated that pyruvate and Tween20 played statistically significant roles ($p<0.05$) on astaxanthin accumulation. However, results indicated that curvature was found to be statistically insignificant suggesting optimum may lie outside the chosen range of variables investigated ($p>0.05$); therefore, path of steepest ascent was adopted to expedite the decision making process of where the future optimization may be performed. Results showed that optimization should be focused at the concentrations of pyruvate and Tween20 of 50 mM and 0.17%, respectively. Response surface methodology using Doehlert design was again adopted in order to optimize the suitable level of enhancers capable of promoting high astaxanthin production. It was found that optimum concentrations of both pyruvate and Tween20 were 49.78 mM and 0.145 %, respectively, which were in good agreement with those indicated by the path of steepest ascent. Additionally, under optimized conditions established astaxanthin concentration of 1177.30 $\mu g/g_{yeast}$ (Table 5.1) which is 4.2 fold higher than that of YM medium could be achieved.

Cultivation of *X. dendrorhous* in batch using 2-Liter fermentor under optimized conditions resulted in astaxanthin production of 906.57 $\mu g/g_{\ yeast}$ or 13.57 mg/L and 17.43 g/L biomass concentration (Table 5.1). Biomass concentration achieved was higher than those obtained with optimization (1.3 fold) using Doehlert design as well as validation study in shake flask (1.35 fold) which may due to the fact that oxygen provide in fermentor were in excess; thus, better aeration than that in shake flask. Additionally, astaxanthin production achieved was slightly lower than that obtained with flask culture; however, volumetric astaxanthin concentration obtained was comparable.

In summary, pineapple juice concentrate supplemented with appropriate carbon and nitrogen sources together with carefully selected chemical inducers could efficiently and satisfactorily promote both growth and astaxanthin production by the red yeast *X. dendrorhous*. Therefore, since pineapple juice concentrate contained high sugar concentration as well as amino acid together with some organic acids, it is feasible to

employ pineapple juice concentrate as an economical base culture medium for mass cultivation of *X. dendrorhous* at the industrial scale.

## 5.2 Recommendation

Since pH of the culture medium during cultivation of *X. dendrorhous* has been reported to critically influence both biomass and astaxanthin production; the fermentation might be conducted in two stages: the biomass production stage and the astaxanthin production stage to increase biomass and astaxanthin production, where pH of each could be maintained precisely at their coressponding optima.

In addition, substrate inhibition typically takes place during *X. dendrorhous* with high sugar content leading to lower growth as well as astaxanthin biosynthesis. Fed-batch fermentation where substrates, namely, carbon and nitrogen source, pineapple juice supplemented with sucrose and $KNO_3$ in this study, in combination with chemical enhancer, i.e., pyruvate, $ZnCl_2$, ethanol and Tween20, may be periodically and slowly introduced into the culture medium which may help improving growth and astaxanthin production and may also reduce time of cultivation in comparison with normal batch fermentation.

**Table 5.1** Summary of experimental work in this study

| Fermentation type | Biomass concentration (g/L) | Astaxanthin content ($\mu g/g_{yeast}$) | Astaxanthin concentration (mg/L) |
|---|---|---|---|
| YM medium in Shake flask culture | 4.20 (day 2) | 278.13 (day 6) | 1.05 (day 6) |
| Pineapple juice base medium in Shake flask culture | 4.25 (day 2) | 241.47 (day 6) | 0.91 (day 6) |
| Optimization of biomass production in Shake flask culture | 13.24 (day 8) | - | - |
| Optimization of astaxanthin production in Shake flask culture | - | 1170.30 (day 9) | - |
| Batch cultivation in 2 liter fermentor | 17.43 (day 7) | 906.57 (day 10) | 13.57 (day 10) |

* Texts in parentheses indicate the day at which results were obtained.