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THE EFFECTS OF AG NANOPARTICLES TO THE PRODUCTION OF ASTAXANTHIN IN ALGAE WITH DIFFERENT pH AND SALINITY GROWTH CONDITIONS

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Abstract

Astaxanthin, a xanthophyll carotenoid which often been refer as "super-antioxidant" compare to other antioxidant like β-carotene and vitamin E. There are two type of astaxanthin in the current market which are the natural form from living organisms and synthetic form synthesized from petrochemical source. However, the mass production in the commercial scale has been a technical challenge. Among all the sources of natural astaxanthin, *Haematococcus pluvialis* is one of the best sources astaxanthin up to 3% of dry weight of biomass has been extracted. This green microalgae is able to produce astaxanthin as a secondary metabolite when encounter any stress conditions and appear as "red" stage of its life cycle. In my research, I was aiming to determine the effect of silver nanoparticle in the production of astaxanthin by *Haematococcus pluvialis* with optimal pH and salinity. The optimal pH and salinity were treated with silver nanoparticles powder at different concentrations to study the effect of silver nanoparticle. There were 5 different pH and salinity (NaCl) to be applied in order to find the optimal pH and salinity after 30 days of cultivation. The silver nanoparticle was used to treat both of the optimal pH and salinity obtained from the previous cultivation. The result showed that highest production of astaxanthin in different pH and salinity were pH 7 and salinity 0.25% which is as obtained in previous research done by Sarada, et.al, 2002. The production of astaxanthin by *H. pluvialis* treated with Ag nanoparticle was found to be highest in 10ppm which is 3.26mg/L at day 30th of extract. Besides, the astaxanthin content decreases as the concentration of Ag nanoparticle increases. Ag nanoparticle seem to have inhibitory effect on microalgae cells in the higher concentration as reflected in cell density. In conclusion, silver nanoparticle have negative effect in promoting the production of astaxanthin in *H. pluvialis* even with optimal pH and salinity.

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List of Abbreviation

%	percentage
µg mL-1	micrograms per millilitre
Ag	Silver
AX	Astaxanthin
BBM	Bold Basal Medium
BG-11	Blue Green Medium
°C	Celcius
$C_{40}H_{52}O_4$	Astaxanthin
C. zofingiensis	*Chlorella zofingiensis*
CO_2	Carbon dioxide
g/mol	grams per mole
H. pluvialis	*Haematococcus pluvialis*
HCl	Hydrochloric acid
KOH	Potassium hydroxide
M	moles per litre
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per litre
min	minute
mL	millilitre
NaCl	Sodium chloride
nm	nanometer
OH	Hydroxyl group
OHM	Optimal *Haematococcus* medium
ROS	Reactive oxygen species
rpm	Revolutions per minute
USFDA	The Food and Drug Administration
% (w/w)	percentage weight per weight

Chapter 1

INTRODUCTION

Astaxanthin, a red pigment carotenoid, belongs to the xanthophyll group existing in various kind of living organisms (Ambati, Moi, Ravi, & Aswathanarayana, 2014; Wu et al., 2015). Fish like salmon actually contains astaxanthin in its meat which causes its color to be red. However, astaxanthin cannot be synthesized by animals and can only be obtained from consumption through diet (Guerin, Huntley, & Olaizola, 2003). Astaxanthin has been well known for its antioxidant effect to living organisms and has been applied in industries such as food, nutraceutical, cosmetics and feed industry (Guerin, Huntley, & Olaizola, 2003). Astaxanthin has been reported as a stronger antioxidant than other carotenoid and vitamins which reduces and stabilizes free radicals produced by our body under stress or unhealthy lifestyle (Liu et al., 2014).

Naturally, astaxanthin has drawn attraction from various industries to research on the large scale production of this substance. Microalgae like *Haematococcus pluvialis,* salmon, trout, shrimp and yeast are natural source of astaxanthin (Ambati, Moi, Ravi, & Aswathanarayana, 2014). Among them, green algae *H. pluvialis* is the most promising source of astaxanthin which has been shown in many studies (Guerin, Huntley, & Olaizola, 2003). Wu et al. (2015) had reported that as much as 3.8% of astaxanthin accumulation on dry weight basis in a few strain of *H. pluvialis* (Ambati, Moi, Ravi, & Aswathanarayana, 2014). Another study also found that *H. pluvialis* produce or accumulate more than 30g/kg dry biomass of astaxanthin, which is suspected to be the highest production level in nature (Guerin, Huntley, & Olaizola, 2003). Nevertheless, the extraction of astaxanthin from *H. pluvialis* has been proven to be safe and approved by USFDA as dietary supplement in 1999 (Wu et al., 2015).

Currently, astaxanthin is mainly produced synthetically instead of by extraction from natural sources due to its poor production from crustacean by-product, mircroalgae and transgenic plants (Liu et al., 2014). Globally, astaxanthin is mainly produced chemically from petrochemical sources in a free form while natural astaxanthin is in the form of di-esterified or mono-esterified with fatty acids (Shah, Liang, Cheng, & Daroch, 2016). However, synthetic astaxanthin has not been approved for human consumption due to safety concerns and natural source is the only way for the consumption of astaxanthin by human (Guerin, Huntley, & Olaizola, 2003).

The fact that *H. pluvialis* is able to enhance its production of natural astaxanthin under stress conditions has opened up a pathway to large scale production and commercialization of natural astaxanthin (Shah, Liang, Cheng, & Daroch, 2016). One of the major problem of producing astaxanthin by this microalgae was the slow cell growth that hinder the mass production of commercially available astaxanthin (Shah et al., 2016). On the other hand, pH seem to exert the greatest effect on the morphological change and formation of carotenoids as the solubility of CO_2 influenced the metabolism (Nagaraj et al, 2012). Besides, salinity is another factor that has proven to have induced the formation of astaxanthin in both *H. pluvialis and C. zofingiensis* (Liu et al., 2014).

Therefore, the aim of this study is to investigate the effect of metal nanoparticle as a stress in the production of astaxanthin. Usually, metal in low concentration can stimulate the growth of microalgae but overdose will be detrimental or even lethal to them in culture media (Miazek, Iwanek, Remacle, Richel, & Goffin, 2015). Research has shown metal stress can be a method to stimulate production of astaxanthin as a protective response to induced oxidative stress by metal (Miazek, et al, 2015). The objective of this proposal is to investigate the combination effects of Ag nanoparticle and different pH and salinity growth condition to enhance the production of *H. pluvialis.*

Chapter 2

Literature Review

2.1 STRUCTURE OF ASTAXANTHIN

Astaxanthin has two hydroxyl group (-OH) that is different from other members of the family of xanthophyll that enables it to esterify fatty acids which can be found joined on both of the terminal polyene ring (Wu et al., 2015). Astaxanthin has a chemical formula of $C_{40}H_{52}O_4$ with 596.86g/mol of molar mass and a symmetric molecule that can exist in *cis* and *trans* isomers (Liu et al., 2014). *Trans* isomers were found to be more thermodynamically stable and considering the molecule has two chiral centers in C3 and C3' position it can be presented in three configuration: two enantiomers (3R, 3'R and 3S, 3'S) and a meso form (3R, 3'S) (Higuera-Ciapara et al., 2006). Among the isomers, 3S, 3'S has found to be the most abundant natural form of astaxanthin which can be biosynthesized by green microalgae species (Liu et al., 2014).

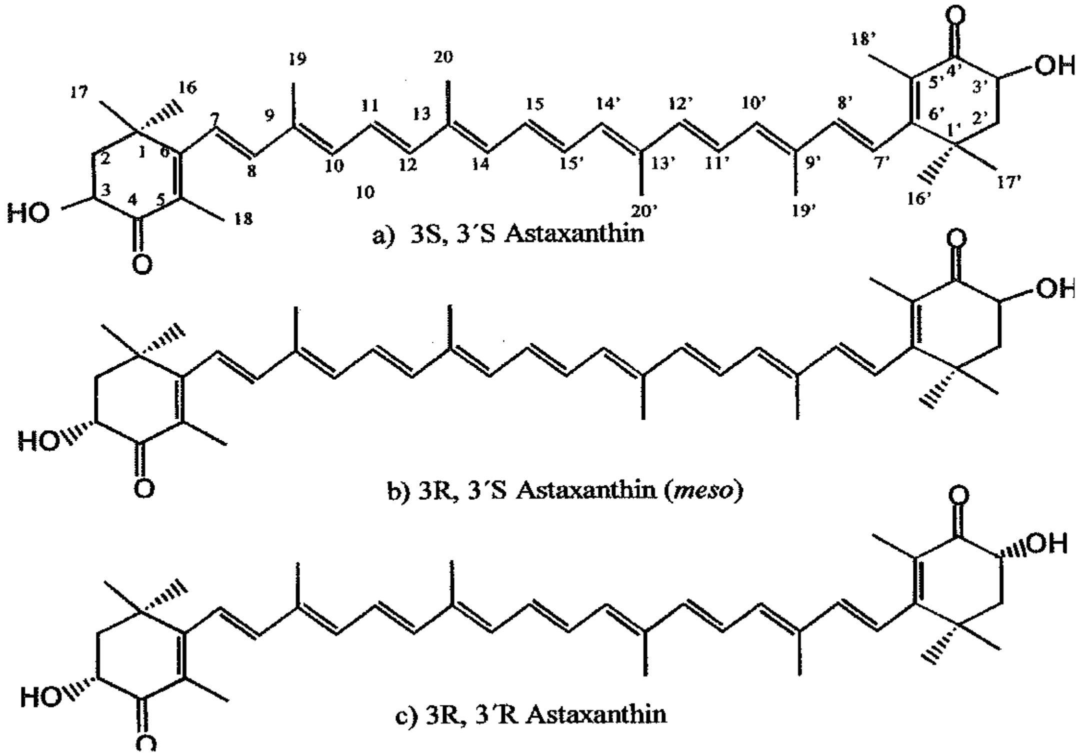

Figure 2.1 Astaxanthin of different isomers

Naturally astaxanthin mostly exists in esteried form as it is usually associated with fatty acids on one or both of its hydroxyl groups like palmitic, oleic and stearic acids. Synthetic astaxanthin maly found in free form inwhere its mixture of isomers is in a ratio of 1:2:1 for (3*S*, 3′*S*), (3*R*, 3′*S*), and (3*R*, 3′*R*) respectively (Higuera-Ciapara et al., 2006)

2.1.1 SOURCE OF ASTAXANTHIN

Astaxanthin(AX) is a carotenoid and belongs to the family of xanthophylls which can be commercially synthesized by microalgae *H. pluvialis* and yeast *Phaffia rhodozyma* in a deep-red form of phytonutrient (Wu et al., 2015). Astaxanthin is widely used as feed additive in the global market especially in aquaculture industry (Higuera-Ciapara et al., 2006) and mostly synthetic form from petrochemical sources (Wu et al., 2015). Natural form of astaxanthin can be extracted from various organisms, for instance, trout, krill, algae, salmon, yeast, crustacean and crayfish. Despite several organisms being sources of astaxanthin, natural form of astaxanthin only contribute to the small portion of astaxanthin in market due to the cheaper price of synthetic astaxanthin. And the technical challenge of mass production of natural astaxanthin (Shah, M. M. R., Liang, Y., Cheng, J. J., & Daroch, M., 2016). Among all the sources, microorganisms has been an ideal source of natural astanxanthin as shown in the *Table1* while *Haematococcus pluvialis* can be considered the best source in the industry (Ambati, Moi, Ravi, & Aswathanarayana, 2014).

Astaxanthin can be extracted from crustacean heads, tails and shells. However, these organisms only generate a minute amount and difficult to digest due to the presence of ash and chitin present (Liu et al., 2014). It is worth mentioning that salmon can contain a maximum of 26-38mg/kg astaxanthin of flesh in sockeye species (Ambati et al., 2014). On average, to consume 3.6mg of astaxanthin, one needs to consume 165 grams of salmon per day and the supplement of astaxanthin reported to be beneficial to health according to (Ambati et al., 2014), Guerin, Huntley, & Olaizola 2003).