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Astaxanthin: A Comparative Case of Synthetic VS. Natural Production

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Abstract

Astaxanthin, the "king of carotenoids" has been widely used as an animal feed additive for several decades, mainly in the aquaculture industry. Recent studies have led to its emergence as a potent antioxidant available for human consumption. Traditionally it has been chemically synthesized, but the recent market interest has generated interests in producing it naturally via yeast (*Phaffia rhodozyma*) fermentation, or algal (*Haematococcus pluvialis*) induction. This work aims to compare these production processes and their impact on the economical, environmental, and societal scale. We also look at the attempts of increasing production yields by altering various parameters during all three production processes. Ultimately, the decision of sustainable practices in producing carotenoids like astaxanthin involves sacrificing yield/potency for a greener product life cycle.

Background

Astaxanthin (3,3-Vdihydroxy-h,h-carotene-4,4-Vdione) (Fig.1) is a carotenoid that naturally exists in nature and mainly found in marine environments (Lorenz et al. 2000). This pigment adds a reddish-pink color to the flesh of many crustaceans and marine animals, such as shrimp, lobster, crawfish, and salmonids (Johnson et al. 1991). These animals consume microalgae or phytoplankton, which are the primary producers that biosynthesized astaxanthin (Lorenz et al. 2000). Some of the organisms that have been found to produce astaxanthin include microalgae *Chlorella zofingiensis*, *Chlorococcum sp*., *Haematococcus pluvialis*, red yeast *Phaffia rhodozyma*, and the marine *Agrobacterium aurantiacum* (Yuan et al. 2002). Among these, *H. pluvialis* has been found to accumulate the most astaxanthin (Boussiba et al. 1999&2000).

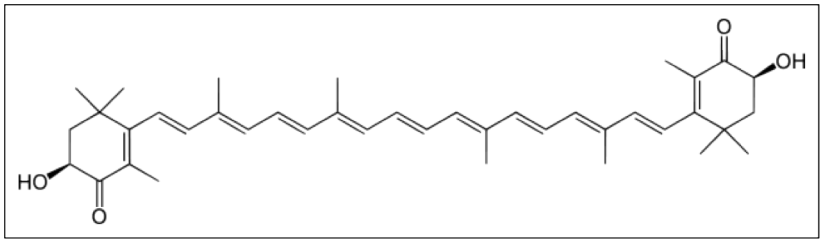

Figure 1. Chemical structure of astaxanthin (3,3-Vdihydroxy-h,h-carotene-4,4-Vdione)

For the past few decades, astaxanthin has reached the commercial market as a food-coloring agent and also a feed additive for the poultry and aquaculture industry (Guerin et al. 2003). The global astaxanthin market is estimated to be around $250 million annually, with an average cost of about $2,500 per kg (Lorenz et al. 2000, BCC). Although it constitutes only about

50-100 ppm of salmon feed, the additive represents around 10-15% of the total cost (Breithaupt 2007).

Recently, there has been a rapidly growing interest for its application for human consumption, acting as a potent antioxidant. The unique structure of astaxanthin allows it to span biological membranes and act as an antioxidant by reducing free radicals, therefor stabilizing them (Hussein et al. 2006, Rao et al. 2010). Studies have suggested that astaxanthin, besides having antioxidant activities, also may provide anticancer, anti-inflammatory, and anti-diabetic activities, among various other health benefits (Higuera-Ciapara et al. 2006). These activities have been compared and astaxanthin was shown to be more potent than all other carotenoids in their Oxygen Radical Absorbance Capacity (ORAC) value (Chew et al. 2004, Palozza et al. 2009).

With these potential health benefits, studies have predicted the potential market value to be over 1.5 billion dollars for 2020, with half for human consumption (Table 1). This rapidly increasing demand for the natural product has generated much interest in its production via algae or yeast, yet the price is still overall higher than that of the synthetic product (Guerin et al. 2003).

Synthetic Production

The synthetic production still dominates the astaxanthin commercial market today, with BASF and Hoffman-La Roche as the major producers. Chemical synthesis yields different stereoisomers than what is found naturally (3S, 3'S). Many strategies have been developed for this synthesis with the oldest and still most widely used involves a Wittig reaction of two C15-phosphonium salts with a C10-dialdehyde (Fig. 2A)(Widmer 1981). Other methods include the hydroxylation of canthaxanthin (Fig. 2B) (Bernhard et al. 1984), a C10 +C20 +C10 synthesis via dienolether condensation (Rüttimann 1999), and the isomerization of a lutein extracted from marigold to zeaxanthin and then oxidation to astaxanthin (Fig. 2C)(Schloemer and Davis 2001). The synthetic version consists of (3S, 3'S), (3R, 3'S), (3S, 3'R), (3R, 3'R), in a 1:2:2:1 ratio respectively (Fig. 2D)(Higuera-Ciapara et al. 2006). Because farmed fish are fed the synthetic version, one can easily differentiate between wild and farmed fish by analyzing their astaxanthin content (Turujman, 1997).

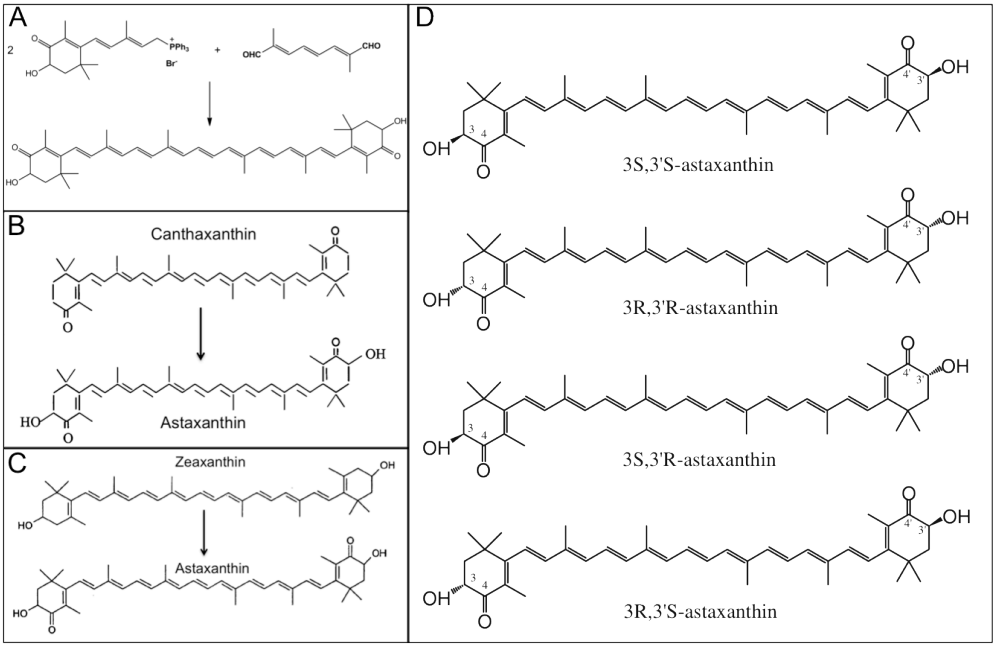

Figure 2. Reactions yielding astaxanthin. (A) Wittig reaction (B) Hydroxylation of canthaxanthin (C) Oxidation of zeaxanthin

Algal Production

Algal production of astaxanthin from *Haematococcus pluvialis* was developed on the industrial scale around the late 1990s (Lorenz et al. 2000, Oleizola 2000). This freshwater unicellular microalga can undergo two cell morphologies, a green vegetative form and a reddening cyst-form. Cyst formation occurs under environmental stress conditions like low nutrients, high light, etc. This stage allows for the rapid accumulation of astaxanthin (Kobayashi et al. 1997). This cellular characteristic allows for an efficient method of production through a two-stage process. Cells are usually grown under optimal conditions and then subjected to stress conditions to induce astaxanthin accumulation (Boussiba et al. 1999).

Industrial systems have utilized nutrient deprivation, high irradiance, and/or high temperature. Yields have hovered around 1-3% astaxanthin harvested from cells in the cysts stage (Oleizola 2003). Harvest is usually achieved by centrifugation, followed by drying and cracking of the cell, releasing astaxanthin (Lorenz et al.2000).

Many different systems have been developed for the large scale processing of *H. pluvialis*. The first stage of growth is usually facilitated by either closed indoor photo-bioreactors or enclosed outdoor systems. Because of the likelihood of contamination by other microorganisms, the enclosed systems are much more popular, of which also provide a controllable environment where optimal growth conditions can be achieved. The cyst-

induction, “reddening phase” however, can be conducted via open raceway ponds, where nutrient deprivation and cellular stress are actually preferred. Genetic strain manipulation of *H. pluvialis* was also made possible (Steinbrenner and Sandmann 2006), allowing further increase in astaxanthin synthesis. Aside from various strain manipulations, growth media, reactor design, and induction methods (one vs. two stage) have also been looked at to increase yield (Table 2).

Group	Date	Max Astaxanthin Concentration (mg/L)	% Dry Biomass	Accumulation Rate	Variants	Optimal Condition	Growth Method
Lee and Soh	1991		0.43	3.5 mg/g/d	Dilution rate		(2.5L stirred tank fermenter)
Lee and Ding	1995	3.9	0.074	0.1 mg/L/d	Dissolved O2		chemostat culture system
Fa´bregas	1998	49.5			N, Mg2+, Light	High Light + N-deficiency	minireactors (70 ml) circadian dark/light cycle (12h:12h)
Olaizola	2000		2.5	2.2 mg/L/d			25,000-L outdoor photobioreactor Aquasearch Growth Module (AGM)
Sarada	2002	10.7			Stresses	calcium nitrate as nitrogen source, pH 7.0	
Dominguez-Bocanegra	2004		9.8				BAR medium with continuous illumination
Kang	2005	175.7	7.72	6.3 mg/L/d	Induction agent	Continuous 5% CO2	Flasks
Del Rio	2005		0.8	5.6 mg/L/d			1-Step continuous photoautotrophic cultures.
Lopez	2006		2	4.5 mg/L/d		Airlift tubular	Airlift tubular vs bubble column photobioreactors
Garćia-Malea	2006		0.15		Irradiance		
Suh	2006	357	5.79				Double-layered photobioreactor
Alfalo	2007		4	11.5 mg/L/d			
Del Rio	2008		1.1	25 mg/L/d			
Zhang	2009	51.06	2.79	4.3 mg/L/d	Strains		open pond by two-stage growth one-step process
Wu	2010		0.32		UV-B		
Kang	2010	190	4.8	14 mg/L/d			sequential photoautotrophic fed-batch culture
Choi	2011	100		3.3 mg/L/d			Multistage Operation of Airlift Photobioreactor
Yoo	2012	217.78		1.4 mg/L/d			V-shaped bottom design

Table 2. Improvements in astaxanthin yield from H. pluvalis from various studies. Published yields were either in concentration or accumulation rate.

Yeast Production

Primary astaxanthin production from yeast fermentation is mainly with the heterobasidiomycetous yeast, *Phaffia rhodozyma*, also known as *Xanthophyllomyces dendrorhous* (Golubev 1995). One big advantage of this organism is its quick growth and high cell density. This organism produces astaxanthin via the mevalonate pathway (Shmidt et al. 2011). With the metabolic pathway of P. rhodozyma being well known, strain variations have been shown to increase astaxanthin yields. Substrates for the fermentation process have also been analyzed on their economical availability versus product yield (Table 1). Aside from strain and substrate changes, other factors like pH, temperature, nutrients, oxygen content, light and extraction method have also been looked at to increase astaxanthin yield. Unlike the algae, yeast produce the (3R,3’R) version of astaxanthin, which is also contained in the mixture produced by chemical synthesis. The total astaxanthin concentration from yeast is still lower than algae, yet yeast will generate higher biomass and much less heavy metals that are found in the algae process (Xiao et al. 2009).

Sustainability Comparison

Here we consider the economical, environmental, and societal impacts of astaxanthin production via chemical synthesis (Wittig reaction), yeast fermentation (*Phaffia rhodozyma*), and algal induction (*H. pluvialis*). Results are shown in Table 3.

Per kg of Astaxanthin	Economical				Environmental		Societal		
Production Method	Raw Materials Cost ($)	Land Usage (sq. km)	Energy Usage (kWh)	Energy Cost ($)	Waste Water *	Emissions (Air)*	Cost($)	Human Consumption?	ORAC
Chemical Synthesis	40	10	170	26	0	2	2,000	No	33%
Yeast Fermentation	140	20	1,062	160	9	5	2,500	Most	66%
Algal Synthesis	164	25	796	120	2	9	>7,000	Yes	100%

Table 3. Comparison of production methods. Green indicates the best option, yellow medium, and red is the worse. *Values for waste water and emissions are relative and base on the BASF report.

In economical, the raw materials cost, energy cost, and land usage was compared. Only to production process was taking into consideration, while capital costs were excluded for simplicity. For the algal production, the most widely used two-stage process was used. Raw materials list was gathered from (Orosa et al. 2005) and estimated costs were extrapolated from (Li et al. 2011). Energy costs were based on an average U.S. cost of $0.15/kWh and cover electricity and steam used from the initial growth, harvest, extraction, to the final purification steps. Land usage was based on a working site in China from (Li et al. 2011). For the yeast production, the media list and energy costs were extrapolated from (Zheng et al. 2006). Land usage was calculated from the Xiamen Yongxingsheng Biological Science & Technology Co. located in China. For the chemical synthesis materials and energy costs for a Wittig reaction was extrapolated from (Ernst et al. 2002). Land usage was based on the BASF facility in Germany. Although BASF does not solely produce astaxanthin, we also put into consideration that companies using algae or yeast may also have the option to produce other products. Results for raw materials cost showed that chemical synthesis was the cheapest at $40/kg of astaxanthin, followed by yeast at $140/kg and algae at $164/kg. Land usage was about the same for yeast and algae, with chemical synthesis requiring about less than half the space. The most energy intensive was the yeast process, with algae being 20% less, and synthetic requiring only $1/10^{th}$ of the yeast.

For environmental we can look at the BASF report that was presented by Baker and Saling (2003). They took a cradle-to-grave approach in analyzing these astaxanthin production processes including raw materials, total energy usage, and potential toxicity. The results of this study showed that the chemically produced astaxanthin faired the better than both yeast fermentation and algal induction methods for most of the tested criteria. Yeast fermentation, because of its relatively low yield and great dependence on both energy and precursor agricultural products (usually sugar/molasses) was the worse for most criteria. The algal strategy seem to be better than that of yeast but still cannot compete with synthetic simply due to the

yield difference. Chemical synthesis consumed the least amount of energy, with algal requiring 5 times more, and yeast requiring 6 times more. Greenhouse gas emissions were lowest again for chemical synthesis, with yeast having 3 times more and algal having 6 times more.

For the societal view we consider the market cost, ORAC value, and whether or not it is availiable for human consumption. The market cost per kg of astaxanthin is around $2,000, $2,500, and $7,000 for chemical synthesis, yeast fermentation, and algal induction respectively (Li et al. 2011). The ORAC values for algal-made astaxanthin is about 3 times more than that of the synthetic product and about a third more than the yeast-made version (Naguib 1998). This difference is attributed to the various astaxanthin stereoisomers created by each method, also affecting the stability of astaxanthin. The natural product is esterified, which gives it more stability in preventing oxidation (Schmidt et al. 2011).

Only the algal and yeast-made astaxanthin are approved for human consumption, while the synthetic form is used predominantly for animal feed. This may be due to an early scare reported by Newsome in 1986, in which the concerns of using cancer-causing petrochemicals for astaxanthin synthesis were reported.

While the synthetic product seems to be the most economical and sustainable, it still cannot fill the market demand as a human supplement. Although the algal method was the worse for raw materials cost, land usage, air emissions, and market cost, it has the highest reported ORAC value out of the three. Even though as seen in Table 2, many advances have been made in attempts to increase the astaxanthin yield from algae, the results were shown at a laboratory scale and not proven on a full size industrial scale. Associated costs also are unknown for the different method variations. The market for synthetic astaxanthin seems to already be stable as an animal feed supplement, so few people are needing to further advance this process to make the final product resemble the natural product. Many questions then arise concerning our society and how we value sustainability versus having the best available product.

Questions to consider:

-Are the sustainability costs worth the production of another antioxidant? Why not just consume more of what's available?

-Should chemical synthesis be re-evaluated for human consumption?

-How much lower can the price drop for algal synthesis?

-Should algal and yeast production methods be coupled to other products (biomass)?

-Would you buy this antioxidant? Or fish that is pale in color?

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