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Research Trends in the Dominating Microalgal Pigments, β-carotene, Astaxanthin, and Phycocyanin Used in Feed, in Foods, and in Health Applications

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Abstract

Three pigments, β-carotene, astaxanthin and phycocyanin are presently well-established microalgal products, produced at large-scale in cultures of microalgae or cyanobacteria and used as natural colours in feed and foods and as nutritional additives. Applied research in these 3 pigments is, however, still developing rapidly; particularly in their effects on human health. This commentary provides a brief overview on the main functional effects of β-carotene, astaxanthin and phycocyanin and presents an analysis of the current trends in research activities in relation to their used in feed, foods and health.

Keywords: Astaxanthin; β-carotene; Phycocyanin; Microalgal culture; Feed; Foods; Health

Introduction

Phototrophic microalgae and cyanobacteria make up a diverse group of organisms. Some species are used in feed or foods or for production of ingredients [1-3]. Their phototrophic mode of living has launched intense interest in microalgal cultivation as these organisms, in principle, need only inorganic nutrients and light in order to grow. However, their need for light also poses a serious challenge. It is inherently difficult to scale up microalgal cultures and at the same time distribute light evenly and maintain adequate light intensities inside the cultures [4]. At culture surfaces will high light intensities typically result in low photosynthetic efficiencies while darker zones with low photosynthetic activities will prevail inside the cultures. Productivity is therefore an unresolved bottleneck in microalgal cultivation and production costs may be in the order of at least 5 €-15 € per kg dry microalgal biomass [3,5].

Only a few microalgal products are presently made at large scale and used in the production of feed and foods or as health promoting nutritional supplements. The most successful microalgal feed and food products belong to the two classes of pigments; carotenoids and phycobiliproteins. Also microalgal oils rich in long-chain polyunsaturated fatty acids have become important ingredients (in infant formula). These oils are however, predominantly produced heterotrophically in cultures of the colourless dinoflagellate *Crypthecodinium cohnii* [6] or in marine protists [7]. Carotenoids and phycobiliproteins function either as light harvesting pigments or used as photoprotecting agents and synthesized mainly by phototrophic species. Carotenoids and phycobiliproteins may provide colour to feed and foods but often their most important roles are as functional health promoting ingredients. All phycobiliproteins, some carotenoids, and also other biologically active molecules [8] are synthesised exclusively by microalgae or cyanobacteria. Still, for only 3 pigments; β-carotene, astaxanthin, and phycocyanin is large scale microalgal cultivations presently a production methods of choice. Table 1 shows world market sizes for β-carotene, astaxanthin, and phycocyanin, and the market shares supplied via microalgal or cyanobacterial cultivation. All 3 pigments are used not only as feed or food colours but also as nutritional supplements. Particularly their health effects have attracted more and more attention during the past years. The main purpose of this commentary is to provide a brief overview of the major functional roles of microalgal and cyanobacterial β-carotene, astaxanthin, and phycocyanin, and analyse current trends in the level of scientific activity and interest in their use in feed, foods and health [9].

Pigment	World market	Publications in WOS
β-carotene	253-280 mio. USD [1,20,78,79]	24,260
β-carotene from *Dunaliella*/ microalgae[1]	8.5-30% produced in microalgae [25,78,80]	678 (2.8%)
Astaxanthin	150-240 mio. USD [1,20,79]	3,090
Astaxanthin from *Haematococcus*/ microalgae[2]	Small fraction produced in microalgae [20,25,45]	882 (28.5%)
Phycocyanin	10-60 mio. USD [1,78,79]	2,317
Phycocyanin from *Spirulina*[3]/ cyanobacteria[3]	Only produced in cyanobacteria	1,519 (65.6%)

Table1: Estimates of world market sizes and fractions covered by microalgal pigments. Number of publications registered by Web of Science (WOS) until January 2016 where topic (title, key words or abstract) includes the pigment name, β-carotene, astaxanthin, or phycocyanin and the pigment name in combination with either the genus name of the main microalgal producer or microalgae or cyanobacteria in general and the percentage of publications on each pigment that also include the name of the main microalgal producer or

microalgae or cyanobacteria in general. Topic search terms included; [1]microalgae, microalga and microalgal, [2]*Spirulina* and *Arthrospira* and [3]cyanobacteria, cyanobacterium and cyanobacterial.

Carotenoids

Carotenoids are used in animal feed to provide colour to e.g. salmon, chicken, egg yolk, and butter and for colouration and nutritional purposes in foods, as reviewed by Shahidi et al. [10]. Primary carotenoids are integral parts of the photosynthetic apparatus in all photosynthetic organisms. They act as light harvesting pigments or play essential structural or photoprotective roles. Secondary carotenoids have no roles in photosynthesis but may still play protective roles because of their ability to absorb excess light and their antioxidant properties and capabilities to scavenge free radicals. Primary carotenoids make up less than 1% of the biomass in phototrophic microalgae [11] and only the two secondary carotenoids, β-carotene and astaxanthin are produced commercially in large scale microalgal cultures [12].

β-carotene

β-carotene is one of the most widespread pigments in nature. Although it is a primary carotenoid and an essential component of the core complex of photosystems I and II in plants and algae [13,14], some microalgae also accumulate β-carotene as a secondary carotenoid. In the halophilic chlorophyte, *Dunaliella salina* (syn. *D. bardawil* [15]) can β-carotene make up as much as 8% of the biomass [16]. *D. salina* is grown in warm, hypersaline, solar exposed shallow lagoons or ponds where most other organisms do not thrive [17,18]. Between 8.5 and 30% of the β-carotene world market is supplied from *D. salina* cultures (Table 1) and at least 8 companies are marketing *D. salina* β-carotene [19,20]. The fungus *Blakeslea trispora* is an alternative source of natural β-carotene [21]. Synthetic β-carotene made by chemical synthesis contains only the all-trans isomers of β-carotene [22] while natural β-carotene is a mixture of isomers. In *D. salina* can 9-cis β-carotene be the dominating isomer depending on the growth conditions [23,24].

The most important functions of β-carotene in feed and foods are its antioxidant and pro-vitamin A activities, see reviews [1-2] but also cancer prevention, immune response modulations, and hepato-protection have been associated to β-carotene [25]. β-carotene is safe to eat [26] and isomeric differences between natural and synthetic β-carotene have been an important argument to justify the use of natural β-carotene in feed and foods over less costly synthetic β-carotene. Uptake of β-carotene depends on the initial solubilisation of the carotenoid in lipid micelles in the stomach [27]. It is however, not obvious which β-carotene isomer composition is preferable. Natural β-carotene from *D. salina* composed of equal amounts of all-trans and 9-cis isomers seem to be more bioavailable to rats than synthetic all-trans β-carotene [28], probably because all-trans β-carotene is the lesser soluble of the two isomers [16]. The 9-cis β-carotene isomer also acts as precursor for the synthesis of 9-cis retinoic acid [29], which is involved in the regulation of a number of cellular processes [30]. Other studies, however, suggest that all-trans β-carotene is absorbed more efficiently in the human gut than 9-cis β-carotene [31] and has the highest pro-vitamin A activity of all carotenoids [32].

Astaxanthin

Astaxanthin is synthesized only by a number of green microalgae and yeast but is still a widespread pigment in aquatic environments since it is bioaccumulated in crustaceans and certain fish [10]. The richest source of natural astaxanthin is resting spores, haematocysts, of the freshwater microalga *Haematococcus pluvialis* (Chlorophyta) where it can make up to 3% of the biomass [33]. At least 10 companies are marketing natural astaxanthin from *Haematococcus pluvialis* [20,34]. Cultivation takes place in outdoor, closed photobioreactors where contamination organisms are physically excluded [35]. At least one company also grows *H. pluvialis* indoor in mixotrophic cultures illuminated by artificial light [20]. Astaxanthin is found as all-trans and a number of cis isomers, and has in addition two asymmetric carbon atoms that give rise to 3 optical astaxanthin isomers [36]. Synthetic astaxanthin is a mixture of the 3 optical all-trans isomers [37]. *H. pluvialis* synthesise a mixture of all-trans, 9-cis and 13-cis astaxanthin isomers but only one optical isomer [38-40].

Aquaculture is the largest market for astaxanthin. It is the most important pigment in the flesh of salmonids, the skin of sea bream and ornamental fish, and in crustacean shells, reviewed by [10]. The aquaculture market is dominated by synthetic astaxanthin with the salmon industry as the largest consumer [33]. Salmonids do not discriminate between isomeric differences between natural and synthetic astaxanthin [41]. Astaxanthin is also used as food additive, and no health related problems seem associated to the intake astaxanthin [42,43]. Numerous health effects have been linked to astaxanthin, see reviews [44-47], including positive effects in eyes, skin and muscles, the heart, the immune system, the liver, and to metabolism, cognitive functions, and sperm quality. Astaxanthin may be used against e.g. inflammation, cancer, neurogenerative diseases and diabetes. Astaxanthin exhibits higher antioxidant activity than other carotenoids [48] and the 9-cis and 13-cis isomers have higher *in-vitro* antioxidant activities than all-trans astaxanthin [49]. Astaxanthin is a particular efficient antioxidant when dissolved in phospo-lipid bilayer membranes [50] and able to scavenge electrons or radicals on the membrane surfaces as well as in the interior of the membrane, interact synergistically with β-carotene, other non-polar carotenoids, and α-tocopherol (Vitamin E) in the membrane, and with water soluble ascorbic acid (Vitamin C) at the membrane surface [47,51,52]. While apolar carotenoids like β-carotene dissolve deep inside phospo-lipid bilayer membranes oriented in parallel to the membrane surface [53,54], astaxanthin dissolves perpendicular to the membrane surfaces, spans the phospo-lipid bilayer, and exposes its end-positioned polar keto- and hydroxyl-groups on both sides of the membrane [51].

Phycobiliproteins

Phycobiliproteins are light harvesting pigments found only in cyanobacteria, red algae, and cryptophytes. Phycobiliproteins can be used in feed and foods to provide colour and for health purposes. Phycobiliproteins are multichain proteins and it is covalently bound prosthetic phycobilin groups that provide colour to the phycobiliproteins [55,56]. The 3 common phycobiliproteins are red coloured phycoerythrin with phycoerythrobilin chromophores, and blue coloured phycocyanin and allophycocyanin with phycocyanobilin chromophores. Macroalgae (Rhodophyta) are the main source of phycoerythrin, used mainly as a fluorophore [57] while cyanobacterial cultures are the major source for allophycocyanin (also used mainly as fluorophore) and phycocyanin.

Phycocyanin

Phycocyanin is the phycobiliprotein that has attracted most attention for use in feed, foods, and health probably because it is the most readily available phycobiliprotein. Phycocyanin cannot be made synthetically but is synthesised in cultures of *Arthrospira platensis* (syn. *Spirulina platensis* [15]) and possibly other cyanobacteria and cannot be made synthetically. Phycocyanin can make up more than 15% of the biomass in *A. platensis* [58]. This cyanobacterium tolerates pH values up to pH 10.5 [59] and is grown photoautotrophically in outdoor, open ponds or raceways in tropical and subtropical regions [2,60,61]. Phycocyanin can actually be produced more efficiently in heterotrophic cultures of the unicellular rhodophyte, *Galdieria sulphuraria* [62,63] though this organism has no history for use in feed of foods. *A. platensis* cells are, in contrast, already used as feed, food and in health food products. *A. platensis* is believed to stimulate the immune defence system and possess antioxidant, anti-inflammatory, anti-viral, anti-cancer, and cholesterol-lowering effects because of their high contents of phycocyanin and other biologically active molecules [64,65].

Purified phycocyanin is quite a novel food ingredient in most parts of the world. Phycocyanin from *Arthrospira* extracts was approved for use in candy, chewing gum and other types for confection in the US in 2013 and 2014 by the US Food and Drug Administration [66]. In EU have 'Guidance notes for the use of colouring foodstuffs' since 2013 provided novel opportunities for the use of phycocyanin rich Arthrospira extracts as a so-called colouring food [67]. Phycocyanin itself is not yet on the list of approved food additives in the EU [68]. The nutraceutical value of phycocyanin is a second reason for its use in foods. The phycocyanobilin groups provide antioxidant and radical scavenging activities to phycocyanin [69-73]. The list of potential health effects related to phycocyanin includes anti-inflammatory effects, anti-platelet aggregation, anti-cancerogenic effects, prevention of cholesterol-induced artherosclerosis, kainic acid-induced neural damage, kidney stone formation, thioacetamide-induced hepatic encephalopathy, and reduced cardiotoxicity of doxorubicin, see reviews [74,75]. It may be that it is actually a second compound, phycocyanorubin that is the true antioxidant species *in vivo* [76]. Phycocyanorubin is produced from phycocyanobilin *in vivo* by biliverdin reductase and is similar to bilirubin, a natural antioxidant in plasma that also inhibits formation of superoxide radicals by NADPH oxidase.

Scientific Activities on β-carotene, Astaxanthin and Phycocyanin

The scientific interests in microalgal β-carotene, astaxanthin, and phycocyanin in feed, foods and health applications have increased sharply the past decades. The number of scientific papers and the number of citations to these papers recorded by Web of Science [9] can be used as indicators of the developments in scientific activities [77] related to these pigments. In February 2016 were more than 24,000 publications on β-carotene and 2,300-3,000 publications on phycocyanin and astaxanthin, respectively, registered by WOS (Table 1) [78-81]. Publications on β-carotene has been released annually since the 1930's, for the two other pigments since the 1950's. Less than 3% of the publications on β-carotene associate this pigment to either *Dunaliella* or microalgae in their title, key words or abstract (in WOS denoted the topic). Much higher proportions of the publications on astaxanthin or phycocyanin associate these pigments to either *Haematococcus* /microalgae or *Spirulina*/cyanobacteria, reflecting the much narrower range of organisms in which these pigments are present (Table 1).

Scientific interests on microalgal β-carotene, astaxanthin, and phycocyanin in feed, foods, and health began much later. Only since the early 1990's are publications linking these pigments to feed or food released annually, while publications associating these pigments to health have been released regularly since approximately Year 2000. Since then have the interests in all 3 pigments developed rapidly. Figure 1 shows the total number of publications published each year in which the 3 pigment names are mentioned in combination with either the genus name of the main producer (*Dunaliella, Haematococcus, or Spirulina/Arthrospira*) or with microalgae or cyanobacteria in general. The total number of citations these publications have received each year is also shown in Figure 1. Lastly are also the annual number of publications linking the 3 pigments to feed, food, or health, and their annual number of citations shown in Figure 1.

The specific rates by which the annual numbers of publications and their citations have increased can be estimated by fitting a first order exponential equation to the data points in Figure 1.

$$n = e^{k\cdot(t-t0)} \qquad (1)$$

where n is annual number of publications or citations, t is time measured in years, t0 represents the first year publications on a given topic started to appear on a yearly basis, and k is the specific rate constant for the annual growth in numbers of publications or citations. The total numbers of annual publications registered by WOS have increased by 3.2% per year from 1975–2015. The annual numbers of publications on microalgal β-carotene, astaxanthin, or phycocyanin are growing at much faster at almost similar specific rates of 11%-13% per year (Figure 1). Also the annual numbers of publications on the 3 microalgal pigments in association to feed, food, and health are growing at comparable specific rates. In all cases are the highest rates of growth seen in the publication numbers associating the microalgal pigments to health.

The annual numbers of citations to the publications on microalgal β-carotene, astaxanthin, or phycocyanin have increased by 21%-23% per year (Figure 1). The annual numbers of publications linking these pigments to feed or food have experienced only slightly higher specific rates of growth of 22%-28% per year. By far the highest specific rates of growth (38%-40%) are seen in the numbers of annual citations received by the publications linking the 3 pigments to health.

The large specific rates of growth in publications and their citations indicate that applied research in microalgal pigments is an expanding research topic in absolute as well as in relative terms, and reflect how health related aspects of microalgal pigments have become a particularly 'hot' research topic in recent years. A substantial number of pigments and prospective microalgal feed and food products have been identified and characterised [8]. Much research is, however, still centred on the only 3 pigments, β-carotene, astaxanthin, and phycocyanin that successfully have been taken into large-scale production. The scientific interests in their use in feed, foods and health have never been greater than now. Strong interests in health effects also apply to algal pigments not yet produced by microalgal cultivation. One example is the anti-obesity potential of fucoxanthin from seaweed or diatoms [18].

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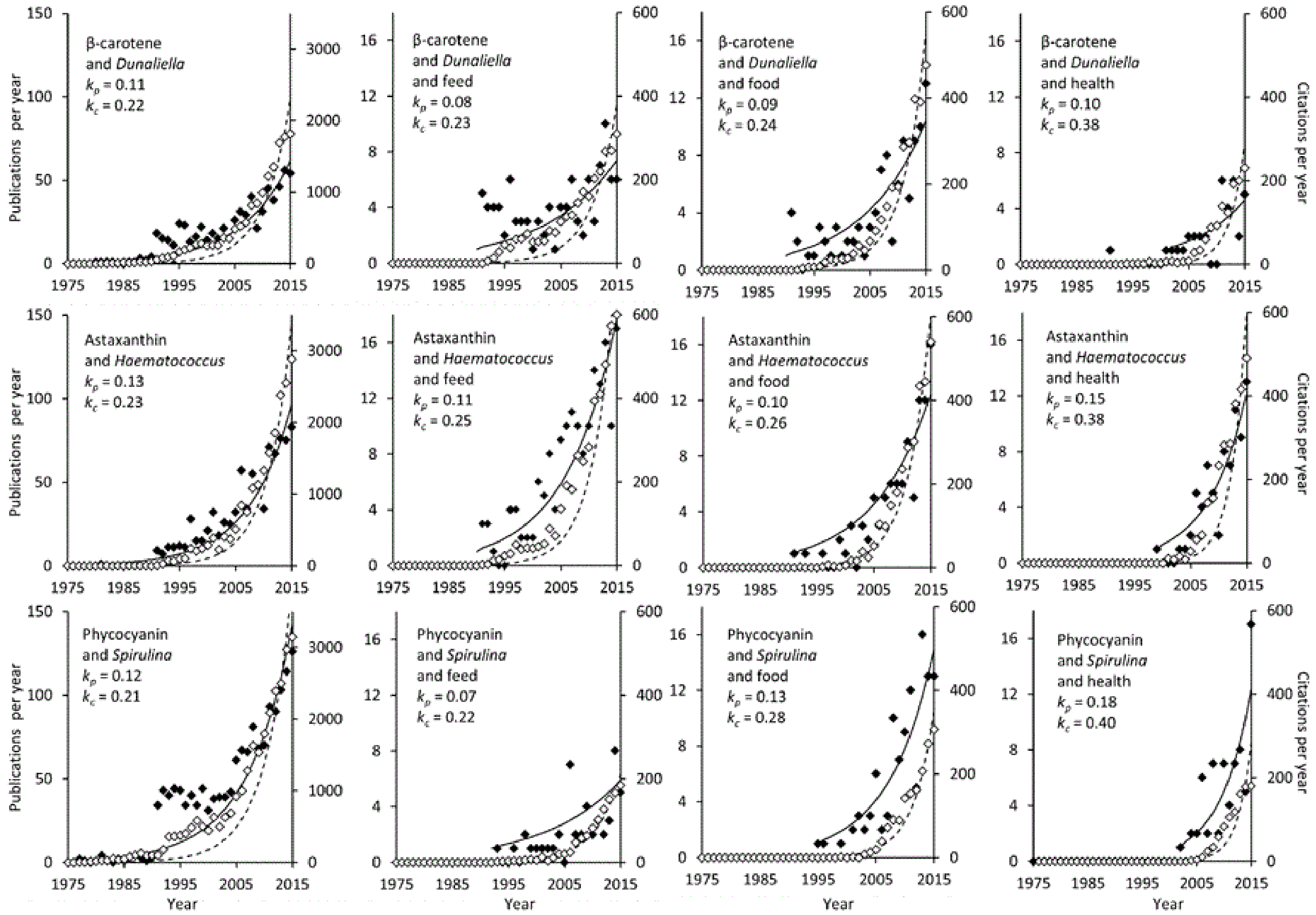

Figure 1: Annual number of publications (◆) registered by Web of Science until January 2016 where topic (title, key words or abstract) includes pigment name, β-carotene, astaxanthin, or phycocyanin in combination with either the genus name of the main microalgal producer or microalgae or cyanobacteria in general (search term *Spirulina* was combined with the synonymous name, *Arthrospira*), and in combination with either feed, food, or health, and annual numbers of times these publications have been cited (◊). Curves represent best fits of Equation 1 to the annual numbers of publications or citations, k_p and k_c are specific rates of growth in annual numbers of publications or citations, respectively.

New developments in the use of microalgal pigments in feed and foods can therefore be expected to relate largely to their potential health benefits.

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