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Current trends and emerging technologies in biopigment production processes: Industrial food and health applications

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Abstract*—*Recently, driven by the need of finding eco-friendlier and less hazardous pigments than synthetic colorants there is an increasing trend towards their replacement with biopigments. Various types of these biopolymers (such as astaxanthin, canthaxanthin, zeaxanthin, ankaflavin, torularhodin and so on) are produced through the development of proper and efficient bioprocesses. In this review current biocolorants production processes are highlighted. Also, emphasis is given in exploring potential strategies for optimizing biopigments production processes as well as decreasing their total production costs. Therefore, investigation of potential value added biopigment production using various types of agroindustrial by-product streams as well as presentation of efficient extraction, recovery and identification processes and technologies (employing emerging technologies such as supercritical carbon dioxide (SC-CO2)) are carried out. Finally, potential applications of microbial pigments in industrial food and health sector are presented. Biopigments could lead to the production of nutrient supplements and functional food, with improved marketability, displaying various potential health benefits. Use of microbial pigments in food processing and pharmaceutical sector, is an area of promise with large economic potential for several industrial applications.

Keywords*—*biopigments, downstream processes, emerging technologies, functional food, industrial applications

I. INTRODUCTION

Synthetic colorants have been traditionally used in food, cloth, cosmetics, painting, plastics and pharmaceuticals (Lu et al., 2009). The most serious concerns, dealing with synthetic dyes are: environmental toxicity, potential adverse allergenic and intolerance reactions, non-sustainable production processes strongly correlated with their dependence of non-renewable resources; coal tar and petroleum (Amchova, Kotolova, & Ruda-Kucerova, 2015). Currently, research has been focalised in the production of natural originated dyes through bioprocessing design and development, aiming to overturn problems associated with chemical traditional color synthesis. Production of natural colours is of high significance not only for humans, safeguarding their health but also environment and society, promoting sustainable development principles.

Biocolorant is considered any chemical substance obtained either from plants, animals or minerals that is capable of coloring food, drugs, cosmetics or any part of the human body. Natural colors might be extracted from renewable resources of plant origin like seeds, fruits, vegetables and leaves as well as produced via the development of efficient bioprocesses using biocatalysts (like bacteria, yeasts, algae, fungi) as integral part of the whole production process (Heer & Sharma, 2017; Rymbai, Sharma, & Srivastav, 2011). Although a wide range of natural pigments are available, microbial pigments have started to mind the gap, regarding large scale production, due to their: 1) cheaper production 2) easier extraction 3) higher production yields (through proper novel strain improvement) 4) no lack of raw materials owning to climate and seasonal variations and 5) biodegradability.

In that point, it has to be noted that carotenoids (subfamily of isoprenoids also called “terpenoids”) production have witnessed remarkable progress, in response to the great market demands, mainly due to their antioxidant and Vitamin A activity, extensively applied in nutraceuticals, pharmaceuticals, poultry, food and cosmetics. Thinking that the current commercial market value of carotenoids has been estimated $1.5 billion in 2014, with a prospective to grow to $1.8 billion in 2019, with an annual compound growth rate of 2.3% and the fact thatastaxanthin, β-carotene, and lutein together share nearly 60% of total market value (Mata-Gomez, Montanez, Mendez-Zavala, & Aguilar, 2014), is obvious that the production of new generation non-chemically synthesized microbial pigments is of uncontestable interest. Scientific research has divulged that biopigments (Nigam & Luke, 2016) are better alternatives compared to natural colorants of plant and animal origin, not to mention their chemically synthesized counterparts (Mapari et al., 2005). A large spectrum of stable pigments such as zeaxanthin, prodigiosin, astaxanthin, lycopene, β-carotene, pyocyanine blue, can be produced through bioprocessing technology, offering higher biopigment production yields and lower generation of non-desired residues compared to the case of chemically extracted dyes from plants and animal (Heer & Sharma, 2017). Food industry offers a great potential in that area. Each year huge volumes of different types of by-product and waste streams are generated through agro-industrial production processes. Management of these either solid or liquid streams is of high importance for producers, consumers and environment. Production of value added products of high technological, nutritional and economical value such as bio-colorants might address issues related both to their collection, disposal and treatment. Implementation of bioprocessing technology principles, exploiting low cost alternative carbohydrate sources as feedstocks for metabolite production is uncontestable a very challenging prospective. In that point, it has to be highlighted that natural colors apart from their usage as color and flavor modifiers and enhancers in food matrices could be used as nutrients such as vitamin supplements or as functional ingredients in processed food, displaying several promoting health properties. More specifically, biopigments have several functions as antioxidants, anti-inflammatory, antineoplastic, radiation-protective, vasotonic, vasoprotective and chemo as well as hepatoprotective agents(Rymbai et al., 2011).

This review paper will lead us to investigate: 1) the current trends and strategies regarding production of biocolors, 2) the potential valorization of agroindustrial residues for the production of natural pigments 3) identify emerging environmentally friendly processes used for biopigment extraction 4) explore the current and future applications of biopigments in industrial food and pharmaceutical sector 5) highlight beneficial health related effects of bioactive natural pigments.

II. NATURAL PIGMENTS PRODUCTION BIOPROCESSES

Designing a bioprocess for colorant production is a very complicated procedure, since many parameters should be considered. Among these the most significant are: 1) the bioreactor design including the configuration, 2) the type of the bioreactor, 3) the raw materials, 4) the microorganism, 5) the type of fermentation, 6) the extraction strategy used for the recovery and 7) the desired actual purity of the biopolymer.

Nowadays, an array of microbial pigments such as carotenoids, melanins, violacein or indigo and others, can be produced via bioprocessing technology, using selected species of microorganisms (bacteria, yeast, fungi and algae) (Heer & Sharma, 2017). For instance, *Blakeslea trispora*, *Xanthophyllomyces dendrorhous*, *Penicillium oxalicum* and *Ashbya gossypii* are capable of efficient production of β-carotene, astaxanthin, Arpink Red and riboflavin, respectively (Heer & Sharma, 2017). Carotenoid production by fungi has been reported by several authors. *Blakeslea trispora* and *Phycomyces blakesleeanus* have been evaluated for their potential carotenoid production not only in laboratory and pilot but also industrial scale(Almeida & Cerdá-Olmedo, 2008). Maximum caretogenic production regarding *Phycomyces blakesleeanus* is achieved under the development of bioprocesses without agitation (Cerda-Olmedo, 2001). *Blakeslea trispora* species are cultivated in large scale fermenters using as fermentation feedstock food grade materials like glucose, corn steep liquor and cheese whey. At the end of the production process, biomass is isolated and transformed into a suitable form for extracting b-carotene using ethyl acetate as solvent. The final product is either b-carotene of high purity or it is formulated as a 30% micronized suspension in vegetable oil (Dufosse, 2006). It has been stated that in that case the pigment produced comply with the specification E 160 aii listed in the directive 95/45/EC. European Food Safety Authority claimed that β-carotene, which now has been commercialized, produced by *Blakeslea trispora* is equivalent to the chemically synthesized alternative, justifying the reasons that make food biocolorants acceptable for use as coloring agent in foodstuffs (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2012). Since 2000, DSM produces β-carotene from *B. trispora* in Europe (Mantzouridou, Naziri, & Tsimidou, 2008). Besides β-carotene also filamentus fungi are capable of generating and other carotenoids like lycopene, γ-carotene, and phytoene as well as ubiquinone, ergosterol and organic acids (Ribeiro, Barreto, & Coelho, 2011). Monascus moulds (*M. pilosus*, *M. purpureus*, and *M. ruber*) are very well known, owning to their ability to produce secondary metabolites of polyketides structures. The final pigment coloration is highly depended on the employed cultivation conditions and strategy followed finding several applications in food processing (Hsu, Hsu, Liang, Kuo, & Pan, 2011).

Traditionally, monascus pigments are produced via solid state fermentation (SSF) (Srianta, Zubaidah, Estiasih, Yamada, & Harijono, 2016). However, solid state fermentation is not suitable for large-scale production due to several upcoming bioprocessing problems such as low productivity of crude pigments, high labor cost, and several control problems. For that reason, it seems that submerged fermentation, is more promising for overcoming problems (such as space, scale-up and process control of solid culture) as well as act beneficial for the production of several pigments and decrease total production costs by minimizing labor costs involved in SSF(Vendruscolo, Tosin, Giachini, Schmidell, & Ninow, 2014). Several microbial pigments such as melanin, monascin, ankaflavin, rubropunctatin, monascorubrin, rubropunctamine, monascorubramine, carotenoids, prodigiosin are produced by various selected microbial strains (Table-1), under different and very specified conditions and strategies.

Table-1: Biocolors derived from microorganisms

Biopigment	Microorganism	Reference
Melanin	*Streptomyces kathirae* SC-1	(Guo et al., 2014)
Red pigments	*Monascus ruber CCT 3802*	(da Costa & Vendruscolo, 2017)
Astaxanthin, β-carotene, canthaxanthin, neoxanthin, violaxanthin and zeaxanthin	*Nannochloropsis gaditana*	(Millao & Uquiche, 2016)
Torularhodin, torulene, β-carotene and γ-carotene	*Sporobolomyces ruberrimus H110*	(Cardoso et al., 2016)
1-OH-4-keto-carotene and 1-OH-carotene	*Gordonia amicalis* HS-1	(Sowani, Mohite, Damale, Kulkarni, & Zinjarde, 2016)
β-Carotene, astaxanthin, zeaxanthin echinenone, anthaxanthin, phoenicoxanthin, β-cryptoxanthin, asteroidenone, adonixanthiy	*Paracoccus* bacterial strain A-581-1	(Hirasawa & Tsubokura, 2014)
β-Carotene, astaxanthin and lutein	*Scenedesmus* sp.	(Pribyl, Cepak, Kastanek, & Zachleder, 2015)
β-Carotene	*Rhodosporidium toruloides* NCYC 92	(Dias, Sousa, Caldeira, Reis, & Lopes da Silva, 2015)
Yellow pigments (monascin and ankaflavin), orange pigments (rubropunctatin and monascorubrin) and red pigments (rubropunctamine and monascorubramine) pigments	*Monascus anka* GIM 3.592	(Shi et al., 2015)
Monascous pigments	*Monascus purpureus* AS3.531	(Zhang, Li, Dai, Zhang, & Yuan, 2013)
Prodigiosin	*Serratia marcescens, Vibrio psychoerythrus, Streptoverticillium rubrireticuli* and other eubacteria	(Khanafari, Assadi, & Fakhr, 2006)
Violacein and deoxyviolacein mixture	*Escherichia coli*	(Fang et al., 2015)

III. BY-PRODUCTS OF AGROINDUSTRIAL ORIGIN AS RAW MATERIAL FOR BIOCOLORANT PRODUCTION

In recent years, byproducts of agroindustrial origin have been proposed as alternative feedstocks for biocolors production, with the view of reducing total production costs, minimizing environmental pollution related problems and addressing disposal issues (Dimou & Koutelidakis, 2017). Exploitation of costless by-product streams as nutrients for the production of value added products, biopigments, is of high interest, not only for consumers seeking consumption of healthier natural products but also for researchers trying to optimize all parameters affecting the whole bioprocess system. Besides, there is a current trend in food and pharmaceutical industrial sector, targeting production of valuable products with diversified market outputs using costless agroindustrial residues as fermentation feedstocks (Dimou & Koutelidakis, 2016a, 2016b). Several low cost by-product streams and residues of agroindustrial origin have shown their potential in yielding a wide array of biocolors, using different microorganisms. Table-2 presents various low-valued residues of agroindustrial origin that have been used for the production of biopigments.

Carotenoids production using deproteinized hydrolyzed whey (Varzakakou & Roukas, 2009), β-carotene by citrus products (Tinoi, Rakariyatham, & Deming, 2006), rice bran, molasses, sugarcane bagasse (Abdelhafez, Husseiny, Abdel-Aziz Ali, & Sanad, 2016), cabbage, watermelon husk and peach peels (Papaioannou & Liakopoulou-Kyriakides, 2012) and so on, are some interesting studies. Pigments produced by different microorganisms, using agroindustrial by-product streams or residues as nutrient sources of bioconversions are shown in Table-2.

Table-2: Pigment production using different microorganisms and costless by-product and residues streams

Biopigment	Microorganism	Substrate	Reference
b-carotene	*Serratia marcescens*	Rice bran, molasses, sugarcane bagasse	(Abdelhafez et al., 2016)
Carotenoids	*Blakeslea trispora* ATCC 14271, mating type (+), &*Blakeslea trispora* ATCC 14272, mating type (–)	Cabbage, watermelon husk and peach peels	(Papaioannou & Liakopoulou-Kyriakides, 2012)
b-carotene	*Rhodotorula glutinis*	Whey	(Marova et al., 2012)
Carotenoids	*Rhodotorula glutinis*	Chicken feathers	(Taskin & Kurbanoglu, 2011)
Astaxanthin	*Xanthophyllomyces Dendrorhous*	Mustard waste	(Tinoi et al., 2006)
Monascorubramine	*Monascus purpureus*	Rice bran	(Babitha, 2009)
Monascorubramine	*Monascus purpureus*	Sesame oil cake	(Srianta et al., 2016)
Prodigiosin	*Serratia marcescens* MN5	Crude glycerol from biodizel production	(Elkenawy, Yassin, Elhifnawy, & Amin, 2017)

Production of high value added products like biopigments, using costless by-product streams and residues is a field of great interest. More research should be carried out in the field of bioprocess produced by biopigments using costless raw materials.

IV. BIOPIGMENTS APPLICATIONS IN FOOD PROCESSING AND THEIR BENEFICIAL ROLE IN HEALTH

Up to date the number of biopigment applications in food and pharmaceutical industry are more and more expanded due to the ever-growing consumer acceptance of bioprocessing derived food grade pigments, willing to pay a premium for “all natural based ingredients”.

Biocolorants may have several applications in food and pharmaceutical sector, as colorants, flavor enhancing agents, preservatives, vitamin or nutritional supplements possessing several beneficial health benefits.

4.1 Biopigments as organoleptic enhancers in food production

Several biopigments have been used as food additives for the formation of dairy, fish, meat, juice and other food products, either modifying or enhancing their color and flavor or even sometimes display preservative action.

Monascus species have been identified as potent producers of nontoxic pigments that could be used not only as food colorants and flavor modifiers but also as food preservatives. *Monascus ruber* has been applied for the preparation of flavoured milk by utilizing rice carbohydrate as fermentation feedstock, producing pigment as a secondary metabolite. Solid state fermentation technology using rice broken as fermentation media has led to the production of red, yellow and orange added value pigments (Dufosse, 2006; Vidyalakshmi, Paranthaman, Murugesh, & Singaravadivel, 2009). RMR (also known as Hongqu Hon-Chired koji or anka in Japan and Rotschimmelreis in Europe) meaning the fermented product of steamed rice inoculated with certain Monascus strains has been used as flavoring and coloring agent in a variety of foods (bean curd, preserved dry fish, pork stew, roast duck, roast pork, sausages and so on) (Lin, Wang, Lee, & Su, 2008; Mamucod & Dizon, 2014; Rojsuntornkitti, Jittrepotch, Kongbangkerd, & Kraboun, 2010). RMR due to its bacteriostatic effects, function as a partial substitute for nitrate/nitrite salts in meat and poultry products (including ham and frankfurters) preservation (Bakosova, Mate, Laciakova, & Pipova, 2001a).

Extracted carotenoids from *Rodotorulaglutinis* DFR-PDY has been applied as food coloring additive in puffed products like popcorns, biscuit, ice-creams and fish products (Kot, Błażejak, Kurcz, Gientka, & Kieliszek, 2016). Astaxanthin and other biosynthesized carotenoids have found several applications in fish products leading to the production of perfectly skin colored aquatic animals but also increased consumer acceptability. Recently, astaxanthin produced by *Phafia rhodozyma* and *Xanthophyllomyces dendrorhous* bioconversions have been commercialized. Also, biocarotenoids produced via cyanobacteria and microalgae (such as *Porphyridium*, *Isochrysis*, *Gracillaria*, *Palmaria*, *Arthrospira*, *Haematococcus pluvialis* bioconversions) have been used as color elicitors in cichlid fish, rainbow trout, fish larvae, bivalve mollusks and several gastropods salmon, trout, red sea bream, shrimp, lobster, and fish eggs. Unfortunately, these biopolymers are not yet employed in large scale applications due to their high production costs (Muckherjee, Camellia, Khatoon, & Ruma, 2015; Rao et al., 2010).

Recently, it has been stated that fungus *Penicillium oxalicum* red pigments (Arpink red) could be used for improving organoleptic characteristics of different food commodities such as baby foods, breakfast cereals, pastas, processed cheese, sauces, fruit drinks, and some energy drinks (Neeraj, Neera, & Sayan, 2011). Table 3 presents specific biopigments their applications in food products and their function, either as colour or flavor elicitors.

Table-3: Application of pigments as food quality enhancing agents

Biopigment	Function	Application	Reference
Monascus sppigments such as ankaflavine and monascine (yellow pigments), rubropunctatine and monascorubrine (orange pigments) and rubropunctamine and monascorubramine (purple pigments)	Improve organoleptic characteristics	Bean curd, preserved dry fish, pork stew, roast duck, roast pork, sausages, fish, “Tofu-cheese”, coluring ketchups, rice, wine, brandy and sweets	(Bakosova, Mate, Laciakova, & Pipova, 2001b; Lin et al., 2008; Mamucod & Dizon, 2014; Rojsuntornkitti et al., 2010; Vidyalakshmi et al., 2009)
Carotenoids (such as α-carotene, β- carotene, lutein, lutein 5- 6 epoxide, antheraxanthin, zeaxanthin, violaxanthin, neoxanthin, lycopene, canthaxanthin, astaxanthin)	Skin coloration	Cichlid fish, rainbow trout, fish larvae, bivalve mollusks and several gastropods salmon, trout, red sea bream, shrimp, lobster, and fish eggs	(Muckherjee et al., 2015)
Astaxanthin	Muscle and skin pigmentation effects	Fish originated- by based products	(Parmar & Gupta Phutela, 2015)
Penicillium oxalicum red pigments (Arpink red)	Improve appearance-color	Baby foods, breakfast cereals, pastas, sauces, processed cheese, fruit drinks, and some energy drinks	(Neeraj et al., 2011)

4.2 Biopigments as nutrient supplements or functional ingredients

Bioprocess produced by carotenoids, riboflavin and β-xanthophylles hold a great potential to be used either as nutrient supplements or as functional ingredients for the production of foods of high nutritional value displaying several potential health promoting effects.

It is well known that any carotenoid of cyclic structure, possessing a β-ring has a provitamin A activity. Consequently, such microbial produced by pigments could be used as vitamin supplements or for the formulation of functional foods, serving human health both preventatively regarding the development of hypovitaminosis A as well as therapeutically, regarding its treatment. Hypovitaminosis A is still one of the most significant malnutrition derived problems in underdeveloped and developing countries. Night blindness, xerophthalmia, xerosis of the cornea, corneal ulceration, “keratomalacia” and loss of the eyes or blindness are the most significant clinical manifestations of hypovitaminosis A (Sommer, 2008).So, biocarotenoids hold a great potential to be used as fortifying vitamin “carrier” for the production of nutrient supplements and functional food, preventing and in some cases decreasing the severity of hypovitaminosis-A. Besides biocarotenoids also β-cryproxanthin, a xanthophyll, derived from *Flavobacterium* spp and other microbial strains, could be used for the production of vitamin A functional food and vitamin supplements. It has been reported that β-Cryproxanthin promote osteogenesis and bone disease treatment, stimulating osteoblastic bone formation as well as inhibiting osteoclastic bone resorption (Yamaguchi, 2012). Bio-riboflavin could also be employed to produce B2 biofortified foods such as functional baby foods, specific types of bread, cereal based products or even dairy products as well as vitamin B2 supplements. Unlike carotenoids, riboflavin deficiency persists in both developing and industrialized countries, attributed mainly to malnutrition and unbalanced diets, lacking of dairy and meat products (Ashoori & Saedisomeolia, 2014). Riboflavin deficiency has been associated with impaired vision, reduced growth rate, oxidative stress, anaemia, liver and skin damage (Ashoori & Saedisomeolia, 2014; Z. Shi et al., 2014) as well as dysfunctions in cerebral glucose metabolism. All the above deficiencies become evident with the development of several health symptoms like hyperaemia, sore throat, cheilosis, glossitis and so on (LeBlanc et al., 2013). It should be noted that the production of enriched with vitamins novel functional products might be either the outcome of a complicated external addition of a microbial pigment or the outcome of a physical complicated biochemical process during the fermentation of products. Cappozi et al. (2011) using roseoflavin-resistant *Lactobacillus* strains, produced novel vitamin bio-enriched functional foods (bread and pasta) with enhanced quality characteristics (Capozzi et al., 2011). Another study revealed that roseoflavin-resistant *Lactobacillus* strains were capable of synthesizing riboflavin in soymilk, leading to the production of novel vitamin fortified foods with enhanced consumer appeal, via an economically feasible and adaptable bioprocess (Juarez del Valle, Laiño, Savoy de Giori, & LeBlanc, 2014). Pyo et al. (2007) investigating the potential antioxidant and angiotensin I-converting enzyme inhibitory activity of Monascus-fermented soybean extracts, revealed that the water extract of 1 to 3 kDa molecular mass emerged 6.5 times higher angiotensin I-converting enzyme inhibitory activity (65.3%) than the control (Pyo & Lee, 2007). This evidence highlights the potential usage of Monascus pigments as natural and multifunctional dietary food additives or supplements.

4.3 Pigments related bioactivities, beneficial health effects and clinical applications

Natural colorants produced via bioprocesses possess a wide range of biological activities including, anticancer, antimicrobial, antimutagenic, potential antiobese effects and in general many health promoting therapeutic functions. It has been indicated that *Monascus* spp bioprocesses, lead to the production of secondary metabolite pigments, which are capable of inhibiting hepatitis C replication by interfering with viral RNA polymerase activity and the mevalonate biosynthesis pathway (Sun et al., 2012). Traditional therapy for chronic hepatitis C virus (HCV) infection treatment is carried out by the usage of interferon (IFN) and the nucleoside analogue ribavirin. Unfortunately, several side effects developed while using alone interferon-α (IFN) or combined with ribavirin (Tanaka et al., 2009). A major player in HCV RNA replication is the 65 kDa HCV NS5B protein, which has RNA dependent RNA polymerase (RdRp) activity. Sun et al. (2012) revealed that Monascus orange pigment (MOP) amino acid derivatives, in which amino acids replaced the reactive oxygen moiety, significantly inhibited HCV replication. This finding indicates that the antiviral activity of the MOP derivatives is an outcome of the mevalonate pathway modulation, since MOP compounds suppressed the mevalonate-induced increase in HCV replication (Sun et al., 2012). Thinking that more than 170 million humans each year all over the world are infected with HCV, strongly associated with liver cirrhosis and hepatocellular carcinoma, the production of natural pigments that offer an alternative strategy for the control of HCV replication is a real "bioproduct" breakthrough of bioprocessing technology. Also it has been documented that *Monascus* spp. fermented products might be beneficial to the antioxidant protection system of the human body against oxidative damage (Vendruscolo et al., 2014). Presently, many clinical trials have evinced that RMR is effective in lowering low-density-lipoprotein–cholesterol levels(Li, Shao, Li, Yang, & Chen, 2010), attributed to its many functional components, such as monacolin K (a total cholesterol decreasing agent), γ-aminobutyric acid (a hypotensive substance), and dimerumic acid (an antioxidant). RMR wine and vinegar products have been strongly associated with health-related functions, such as lowering blood pressure (Chen, Zhu, Zhu, Xie, & Chen, 2011; Zou, Jia, Li, Wang, & Wu, 2013). γ-Aminobutyric acid existing in Monascus-fermented not only prevents hypertension as a hypotensive agent but also is essential for brain metabolism and function in vertebrates (Chiu, Ni, Guu, & Pan, 2006).

Epidemiological and experimental research suggests the antioxidant properties of dietary carotenoids. Carotenoids might prevent the onset of many diseases initiated by free radical damage including age-related macular degeneration, cataracts, arteriosclerosis, multiple sclerosis, bone abnormalities, and cancers. *Haematococcuspluvialis* is considered one of the best natural source of astaxanthin. *Haematococcus* astaxanthin is used as a nutraceutical supplement with no adverse side-effects of its supplementation. Dietary supplements of *H. pluvialis* natural astaxanthin in concentrations ranking from 3.8 to 7.6 mg per day are commercially available in the market, due to potential health benefits (Shah, Liang, Cheng, & Daroch, 2016)

Astaxanthin has a wide range of applications in health sector due to its free radical scavenging capacity. It has been stated that the antioxidant activity astaxanthin is 100 times more effective than α-tocopherol; 65 times more efficacious than vitamin C, 54 times stronger than β-carotene and 10 times higher than β-carotene, canthaxantin, zeaxanthin, and lutein (Lopes et al., 2009). A number of in vitro and in vivo assays using both animal models and humans (Chew et al., 2011; Park, Chyun, Kim, Line, & Chew, 2010) have shown significant effect of carotenoids on immune function. As mentioned before β-cryproxanthin can be produced by selected strains such as *B.lineus* and *Flavobacterium* spp. It has been revealed that this carotenoid pigment could function as health promoting agent, preventing the development of prostate cancer, colon cancer and rheymatoid arthritis (Kim et al., 2012). According to other studies, antioxidant properties of Monascus-fermented soybean extracts were attributed to its content of bioactive mevinolins (r= 0.85) and isoflavone aglycones (r= 0.98), produced from soybean during Monascus-fermentation. Also, it was revealed that the water extract of 1 to 3 kDa molecular mass emerged 6.5 times higher angiotensin I-converting enzyme inhibitory activity (65.3%) than the control, evidencing the great potential of Monascus pigments to be used as natural and multifunctional dietary additives (Pyo & Lee, 2007). These findings are very interesting demonstrating that Monascus pigments function preventatively against the development of several diseases, a bioactivity strongly associated to their antioxidant activity.

Melanin, another biopigment, is also associated with several health beneficial effects, displaying self-protective functions such as blocking UV radiation, free radical adsorption, toxic iron chelation, scavenging of phenolic compounds and buffering against environmental stress. Production of melanin by newly isolated strains such as *Streptomyces kathirae* SC-1 culture might lead to higher production yields, moving in the frame of optimizing bioprocess(Guo et al., 2014). Optimization of melanin production processes is of high academic interest and more research should be carried out, aiming to produce melanin enriched food products with increased UV radiation blocking and antioxidant activity.

Bio-violacein, an indole derived purple biopigment, exhibit a wide range of antimicrobial effects, towards gram positive bacterial strains (for instance *Staphyloccocus aureus*), bacteriovorus, protozoans and metazoans (Choi, Yoon, Lee, & Mitchell, 2015). Despite the wide range of academic reports demonstrating the biological activities of violacein, it was only after 2010 that the immunomodulatory effect of violacein verified. In a very recent study the effective violacein gastroprotective activity toward NSAID-induced gastric lesions was proved (at a single oral dose of 40 mg/kg). This activity was partially associated with PGE2 and IL-10 level increase, while on the other hand levels of pro-inflammatory cytokines (IL-1β, IL-6 and especially TNF-α) were decreased by violacein. Another finding of this very interesting study is that violacein restores to normal levels constitutive nitric oxide synthase (cNOS), implying NO in gastric protection (Antonisamy et al., 2014). The potential therapeutic application of bio-violacein to cancer chemoprevention has been the focus of current research. Masueli et al. 2016 revealed that violacein produced by *Janthinbacterium lividum* could become a useful tool for the treatment of head and neck cancer, inhibiting cancer cell growth both invitro and in-vivo (Masuelli et al., 2016). More research should take place regarding the therapeutic action of violacein produced by different microorganisms towards different types of cancer.

Table.4: List of pigments of natural origin related bioactivities and/or beneficial health functions

Biopigment	Color	Activities	Reference
Monascus derivatives	yellow pigments (monascin and ankaflavin), orange pigments (monascorubin and rubropunctatin), red pigments (monascropunctamine and rubropunctamine)	Antimicrobial, tumour suppressive, immunosuppressive, hypolipidaemic, anti-HCV, anti-hypertension	(Chen et al., 2011; Chiu, Ni, Guu, & Pan, 2006; L. Li, Shao, Li, Yang, & Chen, 2010; Sun et al., 2012; Vendruscolo et al., 2014; Zou et al., 2013)
Astaxanthin	Pink-Red	Antioxidant activity	(Pérez-López et al., 2014) (Cyanotech, 2015)
β-cryproxanthin	Yellow-Orange	Preventive action against the development of prostate cancer, colon cancer and	(Kim et al., 2012)

		rheymatoid arthritis	
Canthaxanthin	Orange	Antioxidant, Anticancer	(Dufosse, 2006)
Menalin	Black	Self protective activities (blocking UV radiation, free radical adsorption, toxic iron chelation, scavenging of phenolic compounds and buffering against environmental stress)	(Guo et al., 2014)
Violacein	Purple	Antibacterial effects, antioxidant, detoxify ROS, potential anticancer, curing malaria and leishmaniasis in humans	(Lopes et al., 2009; Subramaniam, Ravi, & Sivasubramanian, 2014; Vynne, Mansson, & Gram, 2012)
Prodigiosin	Red	Antibacterial, anticancer, potential apoptotic and immunostimulation properties, DNA cleavage	(Lapenda, Silva, Vicalvi, Sena, & Nascimento, 2015; Maheswarappa, Kavitha, Vijayarani, & Kumanan, 2013)
Riboflavin	Yellow	Anticancer, antioxidant	(Thakur, Tomar, & Sacchinandan De, 2016)
Torularhodin	Orange-Red	Antioxidant, Antimicrobial	(Ungureanu & Ferdes, 2012)

V. EMERGING EXTRACTION PROCESSES, PURIFICATION METHODOLOGIES AND TECHNOLOGIES FOR BIOPIGMENT RECOVERY

Recovery of biopigments presupposes the development of proper and efficient downstream processes. Following the extraction of biocolors (depending the samples properties) development of effective processes, leading to the production of natural colors of high purity is a key-step facilitating their commercialization. Besides, biopigments are natural polymers of high sensitivity when released from cells. Thus, efficient development of extraction and purification steps are of great challenge aiming to maintain and ensure: 1) the integrity and the quality of the final produced natural pigment 2) minimize undesirable losses 3) optimize recovery process 4) decrease purification costs.

Supercritical carbon dioxide (SC-CO_2) extraction is an emerging green technology suitable for the development of lipophilic molecules extraction processes, lately diffused in industrial and lab-scale applications, replacing traditional extraction technologies (using organic solvents like acetone, chloroform, dichloromethane, hexane, cyclohexane, methanol) (Mäki-Arvela, Hachemi, & Murzin, 2014). This current trend is mainly attributed to various SC-CO_2 processing advantages; low critical temperature of carbon dioxide, moderate operation temperatures of SC-CO_2, shorter extraction times; limitation of toxic organic solvents usage; retainment of the sample in an oxygen-free and light-free environment; prevention of microbial pigments degradation(Guedes, Amaro, & Malcata, 2011; Reyes, Mendiola, Ibanez, & del Valle, 2014). Supercritical CO_2 extraction has been used for efficient recovery of high quality carotenoids. Macías-Sánchez et al. (2009) succeeded in obtaining 1.2% biocarotenoids yield employing supercritical CO_2 and 5% ethanol as co-solvent at conditions: 60°C and 400 bar (Marcias-Sanchez, Serrano, Rodriguez, & Martinez de la Ossa, 2009). Pan et al. (2012) used environmentally friendly SC- CO_2 techniques to obtain astaxanthin from *Haematococcus pluvialis*. According to that study maximum astaxanthin yield achieved was 73.9% (10.92 mg/g dry *H. pluvialis* powder) after eight cycle of extraction cycles, under optimal operation conditions (*H. pluvialis* weight, 6.5 g; CO_2-flow rate, 6.0 NL/min; extraction time, 20 min; extraction pressure, 4500 psi; volume of ethanol modifier added, 9.23 mL/g; extraction temperature, 50°C; modifier composition, 99.5%) (Pan, Wang, Chen, & Chang, 2012). Apart from extraction also development of purification processes is of high significance regarding the final quality and purity of the recovered biopigment. Traditional purification processes like adsorption column chromatography, differential extraction, differential crystallization and countercurrent extraction could be used for the recovery of biopigments (Pennachi et al., 2015). Liquid chromatography coupled to mass spectrometry (LC-MS) has been broadly used for the purification and identification of carotenoids comparing the mass spectra with databases (Stafsnes et al., 2010). Lycopene production by *Yarrowia lipolytica* has been revealed by HPLC analyses, using as mobile phase various compositions of water, methanol, acetonitrile and ethyl acetate (Matthaus, Ketelhot, Gatter, & Barth, 2014). Gharibzahedi et al. (2012) analysed biopigment canthaxanthin using an UV-HPLC method. Separation took place in a Lichrospher 100 RP-18 silica column. Acetonitrile and methanol (80:20, v/v) at a flow rate of 2 mL/min used as the isocratic mobile phase (Gharibzahedi, Razavi, Mousavi, & Moayedi, 2012). A high speed countercurrent chromatography technique has been effectively applied for the separation and purification of canthaxanthin from the microalga *Chlorella zofingiensis*, leading to the production of a final bioproduct of 98.7% purity from 150 mg of crude extracts (H. B. Li, Fan, & Chen, 2006).

VI. CONCLUSIONS

Pigments produced through the implementation of bioprocesses considering microorganisms as an integral part of the whole upstream and downstream process is probably the most efficient and environmentally-friendly strategy for the formation of naturally originated colorants. On the other hand, traditional synthetic colors for food applications is under severe censuring due to their high pollution and their possible adverse health effects. Thus, optimization of upstream and downstream processes is of high priority for biocolorants production. Costless agroindustrial by-product streams could be used as nutrient sources for the production of biocolorants decreasing total production costs and concurrently addressing several environmental issues regarding their dicposal. Development of efficient downstream processes, regarding extraction and purification is a field of high significance regarding efficiency of the whole production process, final purity and quality of the bioprocessed produced by natural colorant. Taken into consideration the wide range of market outputs of natural pigments in food and pharmaceutical sector accompanied with their possible beneficial health effects, optimization of biocolorant production processes would possible facilitate their commercial production becoming the years to come the “next generation leaders in color market, partially or even in some cases totally substituting synthetic colorants.Also, efficient production of biocolorants could maximize their industrial applications and lead to the production of various novel industrial food products with several beneficial health effects

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