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MINI-REVIEW

Microbial astaxanthin: from bioprocessing to the market recognition

Cassamo U. Mussagy[1] · Anna Kot[2] · Laurent Dufossé[3] · Carmem N. D. P. Gonçalves[4] · Jorge F. B. Pereira[4] · Valeria C. Santos-Ebinuma[5] · Vijaya Raghavan[6] · Adalberto Pessoa Jr.[7]

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Abstract

The attractive biological properties and health benefits of natural astaxanthin (AXT), including its antioxidant and anti-carcinogenic properties, have garnered significant attention from academia and industry seeking natural alternatives to synthetic products. AXT, a red ketocarotenoid, is mainly produced by yeast, microalgae, wild or genetically engineered bacteria. Unfortunately, the large fraction of AXT available in the global market is still obtained using non-environmentally friendly petrochemical-based products. Due to the consumers concerns about synthetic AXT, the market of microbial-AXT is expected to grow exponentially in succeeding years. This review provides a detailed discussion of AXT's bioprocessing technologies and applications as a natural alternative to synthetic counterparts. Additionally, we present, for the first time, a very comprehensive segmentation of the global AXT market and suggest research directions to improve microbial production using sustainable and environmentally friendly practices.

Key points

- *Unlock the power of microorganisms for high value AXT production.*
- *Discover the secrets to cost-effective microbial AXT processing.*
- *Uncover the future opportunities in the AXT market.*

Keywords Astaxanthin · Microbial sources · *Haematococcus pluvialis* · *Phaffia rhodozyma* · Health · Upstream · Downstream · Market

✉ Cassamo U. Mussagy
cassamo.mussagy@pucv.cl

[1] Escuela de Agronomía, Facultad de Ciencias Agronómicas Y de los Alimentos, Pontificia Universidad Católica de Valparaíso, 2260000 Quillota, Chile

[2] Department of Food Biotechnology and Microbiology, Institute of Food Sciences, Warsaw University of Life Sciences, Nowoursynowska 159C, 02-776 Warsaw, Poland

[3] Chemistry and Biotechnology of Natural Products, CHEMBIOPRO, ESIROI Agroalimentaire, Université de La Réunion, 15 Avenue René Cassin, CS 92003, CEDEX 9, 97744 Saint-Denis, France

[4] CIEPQPF, Department of Chemical Engineering, Faculty of Sciences and Technology, University of Coimbra, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, 3030-790 Coimbra, Portugal

[5] Department of Bioprocess Engineering and Biotechnology, School of Pharmaceutical Sciences, São Paulo State University, Araraquara, São Paulo 14800-903, Brazil

[6] Department of Bioresource Engineering, Faculty of Agricultural and Environmental Sciences, McGill University, Montreal, QC, Canada

[7] Department of Pharmaceutical-Biochemical Technology, School of Pharmaceutical Sciences, University of São Paulo, Butantã, São Paulo, Brazil

Introduction

Astaxanthin (AXT) is a ketocarotenoid derived from oxidation of β-carotene, naturally produced by microalgae *Haematococcus pluvialis* and red oleaginous yeast *Phaffia rhodozyma* (Mussagy et al. 2021b; Niizawa et al. 2021). Higher organisms do not possess the capacity to produce AXT de novo, so this outstanding and high-added value carotenoid is usually enriched in humans or other organisms through dietary sources (Yao et al. 2023). As a strong colorant and antioxidant pigment, AXT has several applications in human nutrition and health, aquaculture and pharmaceuticals (Capelli et al. 2019; Lim et al. 2018; Yu & Liu 2020). AXT is a natural pigment that imparts a red-dark colors to microorganisms and protects them from external factors such as oxidative damage and cells protection from UV radiation. Furthermore, recently, this particular pigment has become an important bioindicator for food-quality monitoring (Oslan et al. 2021), and due to the attractive biological properties, several studies have reported the possible applications of AXT as anti-cancer, antioxidant, and anti-inflammatory agent (Park et al. 2018; Penislusshiyan et al. 2020).

To date, the main AXT available on the market is obtained via chemical route, with obvious ecological concerns and human aversion to chemical products. Consumers' concerns created a demand for natural-based AXT generally obtained from the biotechnological route (Mussagy et al. 2021b). Recently, some companies such as Algatech Ltd. from Israel, Astareal Group from the USA and CO_2 GRO Inc. from Canada have explored at industrial scale the production of natural-based AXT using microbial sources (*H. pluvialis* and *P. rhodozyma*) (Grand View Research 2022). However, the industrial production of AXT using yeast, for example, is not economically viable as desirable compared to the synthetic or microalgae counterparts. Some factors, including the high energy requirements and operational costs for the implementation of an AXT-biorefinery and the adequate cultivation conditions for microbial growth, among others, make the production of microbial-based AXT expensive and less competitive with traditional chemical industries (Mussagy et al. 2021a, b, c). In fact, a deep understanding of *upstream* processing (USP) (cell cultivation and AXT biosynthetic route) and downstream processing (DSP) (i.e., extraction, separation, fractionation, purification and polishing units) can allow the effective reduction of manufacturing costs for the production of high commercial value microbial-based AXT. Furthermore, the cost of processing to produce microbial origin AXT and the lack of human trials and approval by food safety regulators in several regions are the main challenges for the nature-based AXT market.

Following these reports, in this review, we aim to provide the readers with a very comprehensive overview to obtain natural AXT from microbial sources. Starting from the basic aspects of structural and chemical forms of AXT, microbial natural sources, and biosynthesis, the analysis of USP and DSP processing technologies includes cultivating conditions, biomass recovery, drying, extraction, purification, and polishing units that must be implemented towards the concepts of circular bioeconomy. Next, we will emphasize the latest research findings of microbial-based AXT health benefits, applications, and the comprehensive segmentation of the global AXT market.

Structural aspects and chemical forms

AXT (3,3′-dihydroxy-β, β′-carotene-4,4′-dione) is an attractive dark-red xanthophyll (carotenoids with carbon, hydrogen and oxygen atoms) with molecular formula of $C_{40}H_{52}O_4$ corresponding to a molecular weight of MW = 596.8 g/mol and CAS no. 472–61-7, naturally synthesized by yeast and microalgae (Mussagy et al. 2021b; Schmidt et al. 2011b) (Fig. 1). The wavelength of maximum absorption (λ_{max}) of AXT ranges from 400 to 650 nm, depending on the solvent in which it is solubilized, as for example: 480 nm in acetone (Mussagy et al. 2022a, b, c). The chemical structure of AXT is constituted by two terminal β-ionone rings linked by a polyene chain, containing two asymmetric carbons located at the 3,3′-position of the β-ionone ring, with a hydroxyl (-OH) and keto (C = O) groups on either end of the molecule (Fig. 1).

The AXT molecule can be found in free form (-OH group not esterified) or in esterified form (which increases the solubility in the cell and makes it more stable to oxidation), viz., when one -OH group reacts with fatty acids (cf., palmitic, oleic, linoleic, stearic acids, among others) to form monoester or when both terminal -OH groups are reacted with fatty acids to obtain di-esters (Brotosudarmo et al. 2020). Furthermore, AXT molecule also reacts with proteins or lipoproteins to form protein-conjugated complexes (Chang & Xiong 2020). The natural AXT optical (enantiomers (3S,3′S and 3R,3′R) and meso (3R,3′S)) and geometric isomers (all-trans, 9-cis, 13-cis and 15-cis) are well defined (Jackson et al. 2008; Schmidt et al., 2011b; Yu & Liu 2020). As for example, the 3S,3′S enantiomers are the principal form of AXT produced by *H. pluvialis*, while 3R,3′R enantiomer is obtained by *P. rhodozyma* yeast (Gong et al. 2020; Storebakken et al. 2004).

The isomerization of all-trans natural AXT to cis–trans isomers has been reported in the presence of pure (dichloromethane, dimethyl sulfoxide, acetone, chloroform, acetonitrile or methanol) and mixtures (dichloromethane and methanol) of volatile organic solvents (Yuan and Chen 1999). In this work, 13-cis-AXT was the main cis-isomer

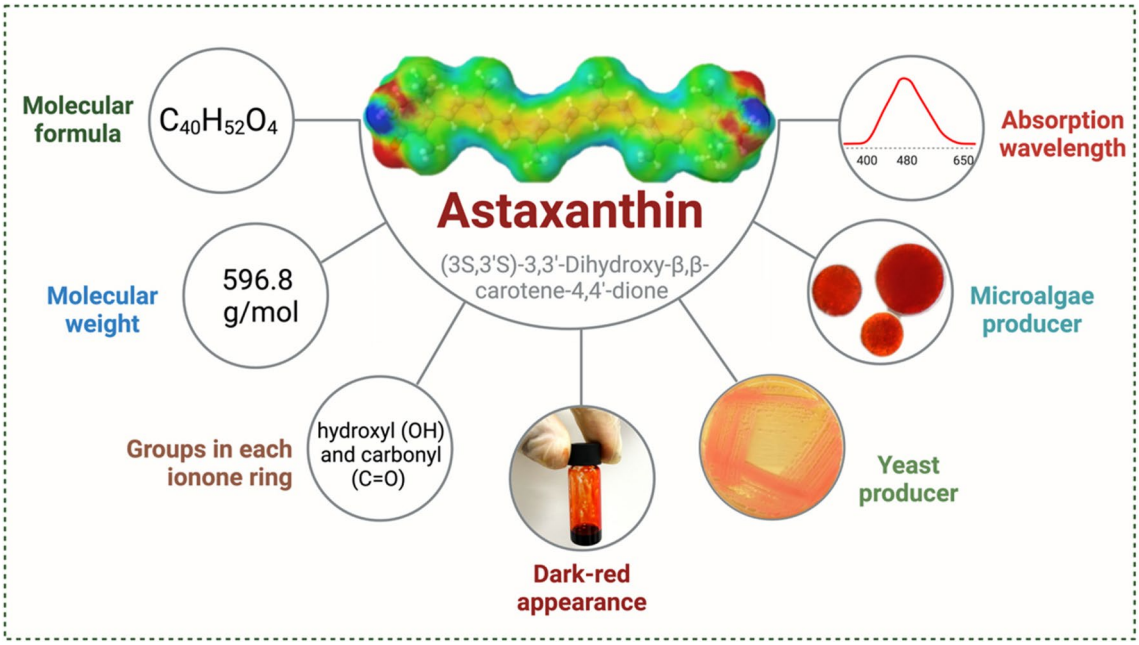

Fig. 1 Tridimensional chemical structure of free astaxanthin, molecular formula and weight, representative groups, absorption wavelength, dark-red visual aspect of the pigment (produced by *P. rhodozyma*), natural yeast (*P. rhodozyma*), and microalgae (*H. pluvialis*) producers

formed from trans-AXT in the presence of dichloromethane or chloroform, which has suggested that high temperatures can promote the isomerization of trans-AXT. The reversible isomerization reaction of cis- to trans-AXT can also occur (Yuan and Chen 2001). As observed, a previous understanding of the microbial AXT isomerization during the downstream processing (DSP) may yield important information towards the stability of the molecule in the presence of solvents during the extraction procedure. To minimize AXT-ester isomerization during the saponification procedure for removing fatty acids in the presence of inorganic solvents, careful evaluation is required prior to implementation. Additionally, special attention should be given to the preparation of AXT standard solutions in the presence of organic solvents and solvent mixtures used in the chromatographic procedures that can lead to the isomerization of the molecule.

Microbial sources and biosynthesis

The most important species used in the industrial production of natural AXT are the green freshwater microalgae *Haematococcus pluvialis* (*H. pluvialis*) and *Phaffia rhodozyma* (*P. rhodozyma*) yeast (Mussagy et al. 2021b; Schmidt et al. 2011b). The advantage to use *P. rhodozyma* is the lack of difficulties in the cultivation, and their growth is possible on cheap substrates compared to plant cultivation (Stachowiak and Szulc 2021). Other fungi used for bioproduction include certain species of the genus *Peniophora* and *Rhodotorula* (Sharma and Ghoshal 2020). Bacteria that synthesize AXT are also known; among them, *Paracoccus carotinifaciens* appears as the most important microbial source (Hayashi et al. 2021). Furthermore, AXT can be found in *Mycobacterium lacticola* and *Brevibacterium* sp. (Yang et al. 2021a, b). The most important microbial hyperproducers of natural AXT are the microalgae *H. pluvialis*. These microorganisms belong to the *Eukaryota* domain, *Viridiplantae* kingdom, *Chlorophyta* division, *Chlorophyceae* class, and *Haematococcaceae* family. *Haematococcus* microalgae are widespread all over the world and can be found in various habitats, viz., from water to rock surfaces (Niizawa et al. 2021). The life cycle of this unicellular algae is quite complex and is divided into two phases: the green motile vegetative and the red non-motile phases. *H. pluvialis* cells in the green motile vegetative phase present a thin cell wall, two flagella, and intensive cell proliferation, while in the red non-motile phase, they can develop a thick cell wall, no flagella, and accumulate high concentrations of AXT (Zhang et al. 2017). Note that in the vegetative stage and under optimal conditions, the *H. pluvialis* cells are green (Masojídek and Torzillo 2008). The green motile cells produce asexual spores which are formed by the sporangia. At this stage, they are usually called zoospores and are mobile mainly due to the appearance of two flagella. Over time, the zoospores are developed to obtain motile cells, and under stressful conditions, the motile cells or zoospores are released from the sporangia, lose their flagella, and are transformed into a red non-motile cell (Zhang et al. 2017). The red non-motile cell

is then transformed into a spherical shape with thick cell wall, their volume increases, and they synthesize and accumulate intracellular red–orange carotenoids including AXT that are located in lipid bodies (Hu et al. 2021).

The precursor of carotenoid biosynthesis in *H. pluvialis* cells is isopentenyl pyrophosphate (IPP) (Gao et al. 2015). This 5-carbon compound is synthesized in the non-mevalonate DOXP pathway. The activity of isopentenyl pyrophosphate isomerase (IPI) results in the isomerization of IPP to dimethylallyl diphosphate (DMAPP). Condensation of IPP and DMAPP molecules leads to the formation of geranyl pyrophosphate — C10 (GPP) (Sun et al. 1998). In the next step, farnesyl pyrophosphate — C15 (FPP) — is formed after attaching the second IPP to GPP by prenyl transferases. FPP is converted to GGPP by attaching another IPP molecule via GGPP synthase. Phytoene synthase (*PSY*) sequentially forms the first non-color carotenoid in the pathway, phytoene, by connecting two GGPP molecules. Two key enzymes take part in the subsequent dehydrogenation reactions — phytoene desaturase (*PDS*) and ζ-carotene desaturase (*ZDS*). *PDS* catalyzes the first two dehydrogenation reactions to form phytofluene and ζ-carotene, while *ZDS* catalyzes two further reactions converting ζ-carotene into neurosporene and lycopene. These enzymes require the activity of a plastid terminal oxidase (*PTOX*) and plastoquinone (PQ) as electron acceptors (Han et al. 2013). The branching point of carotenoid biosynthesis in *H. pluvialis* cells is lycopene cyclization, which is catalyzed by lycopene β-cyclase (*LCY*-b) and lycopene ε-cyclase (*LCY*-e). *LCY*-b catalyzes two β-cyclization reactions at each end of lycopene to form β-carotene, while *LCY*-e catalyzes ε-cyclization of lycopene to ζ-carotene, which is then cyclized by *LCY*-b to form α-carotene (β, ε-carotene), a precursor for the synthesis of lutein. Oxidation of β-carotene in the 4-position by β-carotene ketolase (*BKT*) leads to the formation of echinenone and canthaxanthin. As a result of the hydroxylation of canthaxanthin to adonirubin, AXT is formed in *H. pluvialis* cells. Both reactions are catalyzed by β-carotene hydroxylase (*Crt*R-b) (Han et al. 2013) (Fig. 2).

The second important producer of AXT is the *P. rhodozyma* (syn. *Xanthophyllomyces dendrorhous*). These yeasts belong to the domain *Eukaryota*, kingdom *Fungi*, phylum *Basidiomycota*, class *Tremellomycetes*, order *Cystofilobasisiales*, and family *Cystofilobasidiaceae*. *P. rhodozyma* are isolated mainly from the slime fluxes of deciduous trees (Vatankhah and Ramasamy 2021). Vegetative reproduction of anamorphic *P. rhodozyma* cells occurs through budding. The cells are characterized by an ellipsoidal shape and can appear as single cells, in pairs as well as in short chains. The teleomorphic and therefore sexual form of *P. rhodozyma* yeast is *Xanthophyllomyces dendrorhous*. Cells in this form are encapsulated and their reproduction is possible due to the presence of enteroblastic budding. *X. dendrorhous* yeast shows sexual activity through the association and sporulation of two yeast cells (Bellora et al. 2016; Elwan et al. 2019).

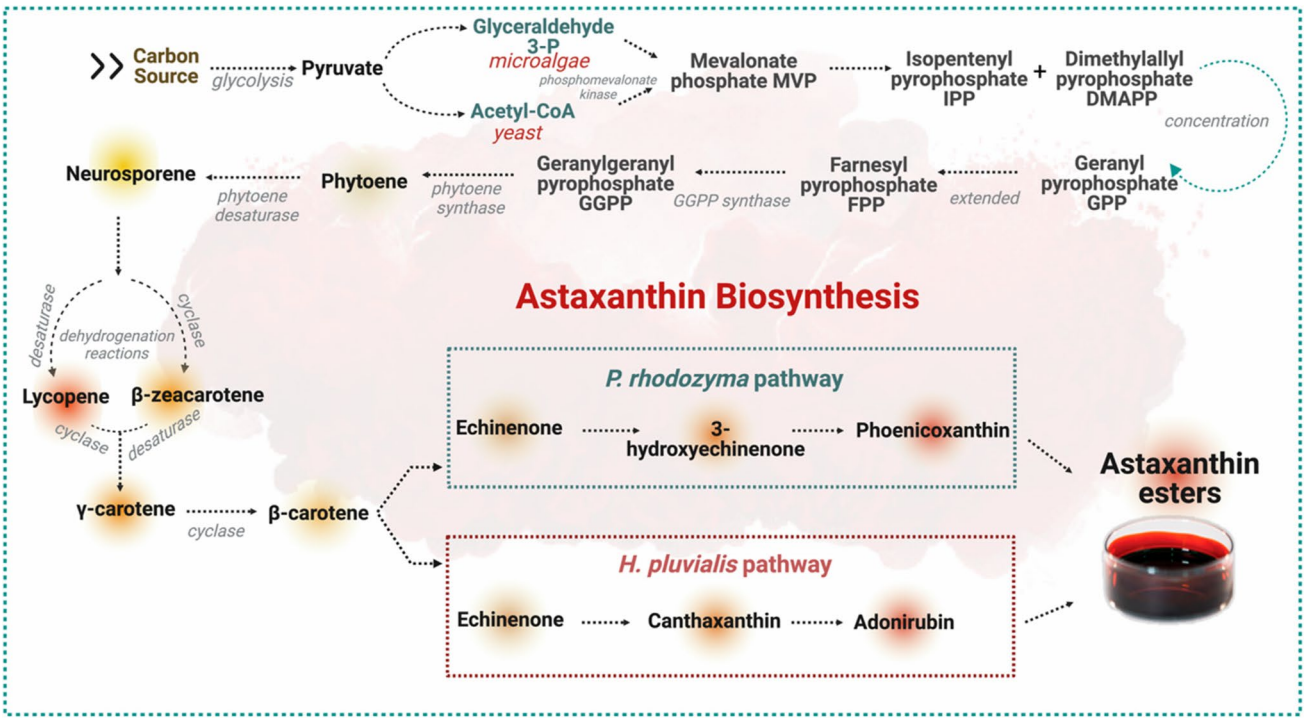

Fig. 2 Biosynthetic pathways of microbial-astaxanthin from microalgae *H. pluvialis* and yeast *P. rhodozyma*

At the initial stage, the biosynthesis of AXT from *P. rhodozyma* yeast cells follows the same route as those of *H. pluvialis*. The immediate precursor of carotenoid biosynthesis is the 5-carbon isopentenyl pyrophosphate unit, which is converted in to a series of reactions towards lycopene and β-carotene. Interestingly, at this stage, the pathway is branched and AXT in *P. rhodozyma* yeast cells can be synthesized in two different ways (Mussagy et al. 2021b). In the first way, called monocyclic pathway, γ-carotene is converted to torulene in a reaction catalyzed by a specific desaturase, and then to 3-hydroxy-3′,4′-didehydro-beta, phi-carotene-4-one from which AXT is formed. In *Phaffia* yeast, this reaction is catalyzed by the ketolase and hydroxylase, which are unified as one enzyme. Moreover, the *crtR* gene encoded cytochrome P450 reductase acts as its helper peptide by providing the electrons necessary for substrate oxidation (Barredo et al. 2017; Elwan et al. 2019). In the second way (bicyclic pathway), cyclase-mediated γ-carotene is converted into β-carotene, which is then converted into echinenone, 3-hydroxyechinoene, phoenicoxanthin and AXT. These reactions are also catalyzed by the ketolase-hydroxylase complex (Mussagy et al. 2021b). The biosynthesis of AXT in *P. rhodozyma* yeast cells depends on many factors, including the type and concentration of the carbon and nitrogen source, the presence of vitamins (including biotin necessary for growth), pH, temperature, and the rate of aeration of the culture.

Upstream and downstream processing to obtain microbial AXT

Upstream processing — cultivation

A two-step process to produce AXT by the microalgae of *H. pluvialis* has been developed based on the organism's life cycle, viz., first, the microalgae is cultivated in a nutrient-rich medium and the stress factors are used to stimulate the biosynthesis of AXT in the second stage. The proliferation stage of microalgae biomass is most often carried out in photobioreactors and then transferred to raceway ponds. In fact, at this stage, the optimization of the main environmental factors such as light intensity, pH, salinity, acetate concentration, nutrients, and specific stressors is required (Li et al. 2020). Hong et al. (2016) revealed that the addition of 50 M iron(II) in the second stage of cultivation increased the production of AXT by 147% due to the production of hydroxyl radicals. In this work, other stress-inducing compounds were also evaluated, cf., succinic acid, sodium hydrosulfide, ZnO NPs, melatonin, and 3-methyladenine. For the industrial production of AXT, *H. pluvialis* strains are known by the following characteristics: high growth rate in the first stage of cultivation (green motile vegetative stage) and high conversion of carotenes into AXT and increase of production titers in the second stage (red non-motile stage). Current research focuses on improving microbial strains using mutagenesis techniques (i.e., UV radiation and chemical agents) (Li et al. 2022) or on improving the production of AXT using low-cost raw materials (e.g., industrial agrifood wastes) as the main carbon and nitrogen sources. For instance, the possibility of using stillage from sweet sorghum (Stoklosa et al. 2019), Jerusalem artichoke powder (Jiang et al. 2017), sugarcane bagasse hydrolysate (Zhuang et al. 2020), mesquite pods and corn steep liquor (Villegas-Méndez et al. 2021), and the paper towels, bones, meat skins, peppers from canteen (Lai et al. 2022) for the production of AXT by *P. rhodozyma* has been investigated. The other approach is focused on genome modifications to improve the biosynthesis of AXT, namely, through plasma mutagenesis under atmospheric and room temperature conditions (Zhuang et al. 2020), NTG mutagenesis, UV radiation (Xie et al. 2014a, b), chemicals (N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)) (Ang et al. 2019), or the regulation of *crtS* and *crtR* gene activity (Torres-Haro et al. 2021). Recently, a strong movement towards the use of phytohormones in AXT biosynthesis by the yeast *P. rhodozyma* has been reported (Nutakor et al. 2022). *P. rhodozyma* was isolated from deciduous tree slime fluxes; thus, the use of phytohormones and plant growth stimulants could be a promising way to increase the efficiency of AXT biosynthesis (Nutakor et al. 2022). For instance, Pan et al. (2020) demonstrated that 6-benzylaminopurine (6-BAP) can be used as a promising stimulator of AXT biosynthesis, increasing the production yields up to 20%. In this work, authors also showed that 6-BAP facilitates the intracellular glucose uptake, promoting the metabolism of glucose. In addition, 6-BAP leads to a high level of ROS, increasing the expression of key genes involved in carotenogenesis, such as HMG-CoA reductase, isopentenyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, and AXT synthase.

As illustrated in Table S1 from Supplementary Material, AXT productivity in different strains from two major natural producers (*P. rhodozyma and H. pluvialis*) may differ according to growth conditions. For example, Li et al. (2022) evaluated the production of AXT in a photobioreactor using BG-11 media and *H. pluvialis LUGU (KM115647.1)*, at 25 °C for 8 days, reaching 25.92 mg/L of AXT. Following a similar approach but using a glass column, Zhang et al. (2014) produced 160 mg/L of AXT using *H. pluvialis* strain *SAG 34-1b* in a BG-11 media after 10 days of cultivation at 25 °C. Regarding the production of AXT using *P. rhodozyma* cells, the productivity is lower compared to that obtained with *H. pluvialis* strains. For instance, the cultivation of *P. rhodozyma* cells in 3-L stirred bioreactor with marigold flower medium at 22 °C for 7 days (Bhatt et al. 2013) only

allowed a production of 1.3 mg/L of AXT. Recently, there is some improvements in the production of AXT using *P. rhodozyma*, for example, Jian et al. (2017) cultivated these yeast cells in a medium containing Jerusalem artichoke extract in a 3-L bioreactor (at 22 °C) and after 5 days and half increased the production of AXT up to 108.9 mg/L. Recently, Mussagy et al. (2022a, b, c, d) evaluated the effect of xylose/glucose and light irradiation on the production of natural AXT using *P. rhodozyma* NRRL Y-17268 in a 4-L stirred tank bioreactor, achieving 503.6 $\mu g/g_{DCW}$ of AXT.

Downstream processing

The microbial production of AXT is intracellular, thus requiring an efficient selection of downstream operations, particularly for cell disruption and AXT solubilization (Mesquita et al. 2020). Therefore, after the USP, a series of processing steps are required, including operation units for clarification, cell disruption, purification (if required), as well as adequate polishing steps to obtain the commercial AXT-based products. In recent years, the search for more sustainable, economical, and environmentally friendly alternatives for the extraction and purification of intracellular biomolecules has been of paramount importance, mainly aiming to reduce the current scenario of environmental global warming (Mendes and Mesquita 2019).

Several operations for the recovery of AXT from microbial sources have been investigated; however, the selection of the most adequate operation should be carefully analyzed according to the nature of biomass, the stability of AXT, as well as the purity level of the final AXT-based product. Consequently, through an adequate integration of operation units (a number that depends on the purity degree of AXT), the maximization of the recovery yields and the energetic and economic efficiency of the global manufacturing bioprocess will be guaranteed (Galanakis 2013). In the next subsections, some of these aspects will be briefly described, starting from a description of the processing steps for the biomass recovery and some of the most common approaches for separation, purification and polishing of the AXT. A conceptual schematic representation of the USP and DSP for the production and recovery of AXT from microorganisms is presented in Fig. 3.

Biomass recovery

After the microbial cultivation step, i.e., USP, the AXT remains inside the cells, requiring efficient extraction procedures for its recovery. However, before the recovery stage, the biomass must be separated from the culture medium (i.e., supernatant), which can be done by applying mechanical (gravity sedimentation, flotation, filtration and centrifugation), chemical (coagulation/flocculation), and/or biological (bio-flocculation) operations (Panis and Carreon 2016). Among these procedures, gravity sedimentation is the simplest and economical technique, but depends on physical aspects such as particle size (biomass), cellular concentration, and the most important, a significance difference between the density of cells (biomass) and supernatant (clarified broth) to be industrially applicable (Branyikova et al. 2018). Flocculation under stress condition tends to

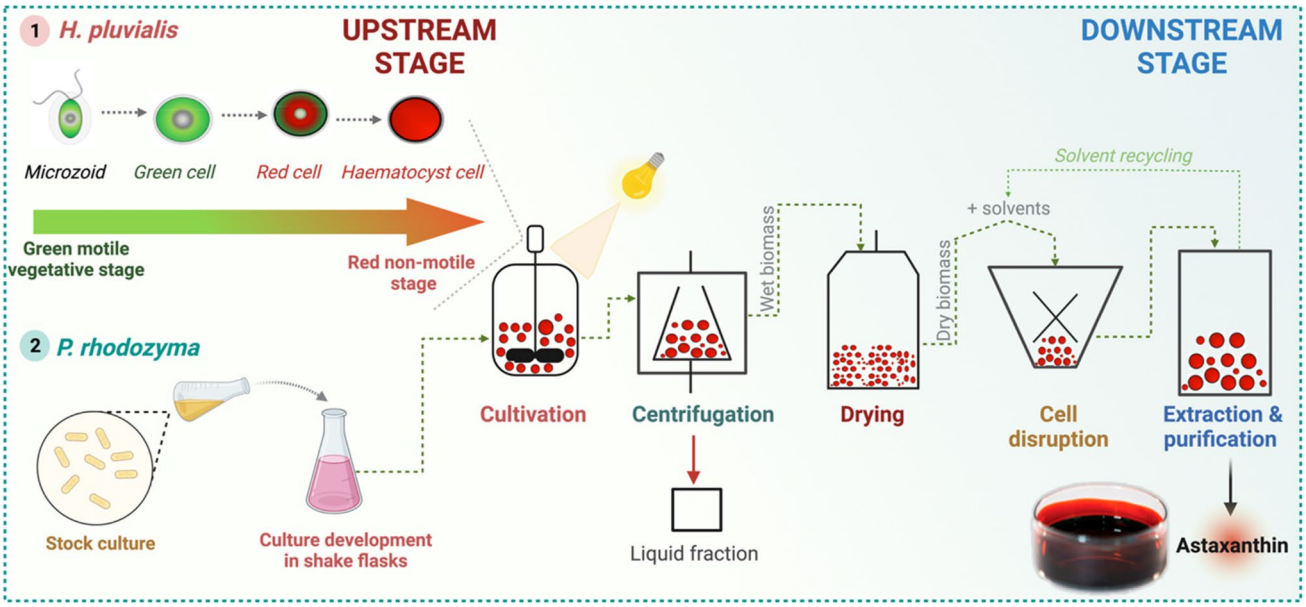

Fig. 3 Representative scheme of upstream and downstream processing to obtain microbial-based astaxanthin

aggregate particles through surface charge neutralization, electrostatic patching, or bridging after addition of flocculants, which depends on pH, cell surface properties, and cell concentration. However, the separation of yeast-based biomass using flocculation approaches can bring processual limitations, particularly related to the contamination of biomass with flocculants, increasing the complexity of the following separation/purification operations (Branyikova et al. 2018; Matter et al. 2019; Rodríguez-Sifuentes et al. 2021; Sanyano et al. 2013). Despite the abovementioned methods, the most common approach for industrial recovery of yeast cells is centrifugation. In fact, centrifugation is the operation unit mainly used in several industries to separate different types of biomasses from the culture media, allowing the recovery of almost 100% of the total cell biomass. Despite the excellent performance, centrifugation operations are highly energetic, contributing to increase the overall cost of the DSP when performed on a large scale (main limitation) (Khoo et al. 2019).

After separating the biomass from the culture medium, the ideal is to perform the recovery of intracellular AXT directly from wet biomass, i.e., the solid fraction recovered after centrifugation. However, due to the hydrophobic nature of the AXT, several extraction operations use nonpolar solvents (with no or low solubility in water), therefore requiring the introduction of a lyophilization (freeze drying) or a drying operation before the following cell disruption and solid–liquid extractions of AXT molecules. In addition, the use of a pre-drying treatment of cellular biomasses can allow efficient removal of the water from biomass and reduce the possible contamination of the yeast biomass. At the industrial scale, spray drying and lyophilization are the most used (Li et al. 2022), but these operations consume high amounts of energy, also increasing the procedural cost. Therefore, an adequate balance between the efficiency on using nonpolar pure solvents for the extraction of AXT and the increase of the costs because of the introduction of the drying pretreatment should be carefully evaluated.

Extraction and separation of AXT

Despite the importance of the USP, mainly to increase production yields, the design of DSP is still challenging, as it contributes not only to the reduction of processual costs but also to ensure the maintenance of the outstanding AXT characteristics (Mussagy et al. 2021b). Among the DSP operations, due to the nonpolar nature of AXT, the success of DSP design will depend mainly on cell disruption and extraction operations, specifically, through the selection of the most suitable solvent(s), which will allow both solubilization and maintenance of the biological activities of AXT (Jiang et al. 2017, Mussagy et al. 2021b). The success of an extraction is mainly a result of a suitable selection of the best solvent(s) (Dursun et al. 2020, Jiang et al. 2017). Therefore, the solvent selection must consider, as key parameters, the degree of toxicity to human or animal health and environmental concerns of the solvents used in the extraction (Xie et al. 2014a, b). As widely accepted, a good solvent is one that exhibits a low toxicity degree, has high ecofriendly and biocompatible characters, and guarantees high recovery yields and recyclability (Wan et al. 2014).

On the other hand, AXT extraction is also strongly related and affected with the recovery of other intracellular biomolecules, namely lipids and proteins. Consequently, following the precepts of the biorefinery, the process of integration needs to include adequate operations for the fractionation of all bioproducts from the microbial biomasses. In this case, knowledge of the physical and chemical properties of biomolecules is essential, as some of them are sensitive to light, heat, oxygen, acids, and alkaline bases (Mussagy et al. 2022b).

The extraction of intracellular AXT from different microbial biomasses has been carried out through conventional and non-conventional procedures, including physical, chemical, and biological approaches or even a combination between them to guarantee high recovery yields (Ambati et al. 2014; Mussagy et al. 2021b). Due to the simplicity and high efficiency, the conventional procedures are the most applied, but are less selective and usually more time-consuming (Kim et al. 2016). These conventional SLE and LLE conventional operation use nonpolar (or slightly polar) hazardous volatile organic compounds (VOCs) as extractant agents (e.g., acetone, hexane, and dimethyl sulfoxide) (Mussagy et al. 2019). However, we are currently facing a climate change scenario and, therefore, the use of non-renewable and non-environmentally friendly solvents as extractants is avoided, as these significantly contribute to the worsening of the situation (Karimi 2020; Mussagy et al. 2022a, b, c, d).

To overcome these environmental concerns, the use of non-conventional techniques, such as supercritical fluids extraction (SFE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), and high-pressure homogenization (HPH), has been investigated as sustainable and efficient alternatives for boosting the recovery of natural AXT (Sharayei et al. 2021). These alternatives combined with green biobased solvents (mostly those of a non-polar nature), ionic liquids (aprotic or protic), deep eutectic solvents (DES), and supercritical fluids are of great interest to the industrial and academic community (Sarkarat et al. 2022).

Many studies have already been carried out for the extraction of microbial AXT from *P. rhodozyma* and *H. pluvialis*, but unfortunately, some of them still use non-renewable and non-environmentally VOCs (Desai et al. 2016; Schmidt

et al., 2011a), although some studies have been also evaluating the use of neoteric solvents (Joaquín et al. 2021). As described in Table S2 from Supplementary Material, several approaches and solvents can be employed for the effective extraction of natural AXT (from *H. pluvialis* and *P. rhodozyma*) by evaluating different experimental conditions such as time, temperature, pressure, solvent nature and type of procedure among others. Following this line, Praveenkumar et al. (2015) tested several ILs to extract AXT from *H. pluvialis*, increasing the AXT recovery up to 32.5 pg/cell. In a similar work, Pitacco and co-workers (2022) evaluated three new hydrophobic DES based on oleic acid and thymol (TAO), DL-menthol (MAO), and geraniol (GAO), demonstrating that all are efficient for the recovery of AXT from dry biomass of *H. pluvialis*, that is, extracting more than 60% of the total AXT without any pre-treatment. José et al. (2014) demonstrated in their studies the comparison of the efficiency of $scCO_2$ and CO_2-expanded ethanol (CXE) extractions. Interestingly, these authors revealed that CXE seems to be a strong candidate to compete with $scCO_2$ extraction and is a promising alternative for recovery of AXT from *H. pluvialis* biomass. The CXE was performed at a pressure (7 MPa) lower than the critical pressure of CO_2 (7.38 MPa) and at three different temperatures (30 °C, 45 °C and 60 °C). The results obtained demonstrated that a recovery of 62.57 mg/g AXT content at 45 °C using 50% (w/w) of ethanol in CO_2, while 53.48 mg/g of AXT was obtained at 55 °C using 13% (w/w) of ethanol in CO_2. As noted, these promising results revealed that CXE can be used as an alternative for microbial AXT recovery.

Purification and polishing of AXT

After the extraction of intracellular AXT, to ensure that natural AXT does not contain any impurities, such as other types of carotenoids, proteins, salts, cell debris, antifoams, and traces of solvents, among other contaminants, a subsequent purification step is necessary to achieve the purity levels of the commercial AXT-products, particularly if used for human consumption (Mussagy et al. 2021a). Note that the obtaining high purity natural AXT is of paramount importance to ensuring commercialization and consumer acceptance (Chang et al. 2022).

Several researchers have evaluated different AXT purification techniques, with the aim of finding one that fits both economic and sustainable parameters (Carolina et al. 2021). As with other biomolecules, chromatographic techniques are frequently applied for purification of AXT, such as high-performance liquid chromatographic (HPLC), semipreparative reversed phase column (SP-HPLC), high-speed counter-current chromatography (HSCCC), and low-pressure chromatographic column (LPCC) (J. Yuan & Chen 2000).

However, before the application of chromatographic techniques for the separation and purification of microbial AXT (some also used for AXT quantification), depending on the commercial application, some attention should be paid to the presence of contaminants. In most cases, the extracts containing microbial AXT are subjected to saponification (usually NaOH) to remove esters compounds (Sun et al., 2015a). For example, Fabryova et al. (2020) isolated five AXT monoesters from *H. pluvialis* using high-performance counter-current chromatography (HPCCC) with a mixture of *n*-heptane and acetonitrile (ratio 5:5 v/v) as mobile phase. These authors obtained 33 mg of monoesters from 1 g of microbial extract in the first purification step, while a second purification using SP-HPLC allowed the recovery of 71.7% of AXT with 98% of purity. Sun et al. (2015) evaluated the purification of all-*trans*-AXT from *H. pluvialis* previously saponified with 0.02 M NaOH. In this work, they stated that an LLE procedure was ineffective to separate alkali and chlorophyll from *trans*-AXT due to the presence of other carotenoids and fats, therefore requiring an integration with a high-resolution preparative LPCC to obtain > 80% of high-pure *trans*-AXT. Interestingly, these researchers included a subsequent crystallization operation (at 4 °C for 72 h) and achieved > 96.5% of *trans*-AXT. Another successful example was the study of Du et al. (2016), who obtained a highly pure (99%) AXT from *P. rhodozyma* by applying a HSCCC (850 rpm, at 25 °C) with a biphasic system composed of *n*-hexane:acetone:EtOH:water (at a 1:1:1:1, v/v/v/v) (Table S3 from Supplementary Material).

Usually after purification, a polishing step is required to obtain a product in its commercial form. Polishing is the final operation stage where the commercial product will be formulated and conditioned in the final form (solid or liquid), which will be properly designed to ensure the AXT stabilization without losing its biological activity (Ichihara et al. 2019). Two important polishing methods are frequently used, cf., drying (Gonzalez et al. 2021) and crystallization (Sun et al., 2015b). The drying method, usually a spray drying unit, is used to obtain high amount of pure natural AXT in powder form. This protocol allows for consistent powder quality throughout the drying process, applicable to both heat-sensitive and heat-resistant materials (Ziaee et al. 2019). Another "drying" method is freeze-drying, widely known as lyophilization. This method has been shown to be less aggressive to the polishing bioproducts that are sensitive to heat, as well as to preserving shelf life for a longer when compared to spray drying (Ahmed et al. 2015a). As mentioned above, crystallization is also effective for polishing natural AXT, as it allows to obtain highly pure crystals of AXT. For instance, Sun et al. (2015a) obtained all-trans AXT crystalline powder from *H. Pluvialis* with a purity of 96.5% using cold acetone. Alternatively, since AXT is insoluble in aqueous solutions, a small amount of water can

be used to promote crystallization of AXT. To increase the purity of the crystal, an acetone and distilled water wash can be performed to remove the mother liquor remaining mother on the crystal surface (Sun et al. 2015a).

Health benefits of microbial AXT

Recently, several works including Aneesh et al. (2022), Cao et al. (2021), Villaró et al. (2021), and Patil et al. (2022) have extensively reviewed the health benefits of AXT. The health benefits of dietary AXT varied from the common antioxidant, anti-cancer, and anti-inflammatory activities to the recent reported anti-diabetes, wound healing, hepatoprotection, osteoprotection, and neuroprotective potential (Fig. 4). The antioxidant potential of AXT is well documented. Liu et al. (2020) have reported the protective effect of AXT on brain oxidative damage in a D-galactose-induced rat model of aging and revealed that AXT improve the activities of some enzymes, cf., catalase, glutathione peroxidase, and superoxide dismutase, by 26, 53, and 30%, respectively. Following the same line, Park et al. (2010) reported the protective effect of AXT against DNA damage induced by oxidative stress in rodent models.

The anti-cancer potential of AXT is well established, viz., AXT displays anti-cancer effects by inducing apoptosis in DMH-induced rat colon carcinogenesis by modulating the expressions of nuclear factor-κB, cyclooxygenase-2, MMPs-2/9, Akt, and ERK-2 (Nagendraprabhu & Sudhandiran 2011). Ko et al. (2016) also revealed the efficiency of AXT to decrease the expression of protein Rad51, improving the mitomycin C–induced cytotoxic effect on non-small cell lung cancer cells. Recently, Sowmya et al. (2017) evaluated the effect of natural AXT (from shrimp) on molecular events in MCF-7 cells (human breast cancer cell line). In this work, the authors revealed that consumption of AXT from shrimp helps in combating cancer chemoprevention. Furthermore, Chen et al. (2017) also indicated that AXT has potent in vivo and in vitro preventing effects on melanoma tumor growth.

It was previous reported (Park et al. 2018) that AXT treatment improved the anti-inflammatory effect in phthalic anhydride-induced atopic dermatitis in vivo and in vitro models, proving the efficiency of this carotenoid for atopic dermatitis by inhibition of nuclear factor-κB signaling. AXT treatment demonstrates protective effect against acute lung injury in wild-type C57BL/6 J mice, due to the ability to inhibit the inflammatory response and down-regulate nuclear factor-κB P65 expression (Bi et al. 2017). The beneficial effect of AXT against hyperglycemia, oxidative stress, and insulin-deficiency induced by diabetes mellitus in rats was studied by Penislusshiyan et al. (2020). The authors revealed that the administration of AXT in rats with diabetes mellitus, resulted in oxidative stress reduction, insulin secretion restoration, and beta cell rejuvenation.

AXT can be considered as "multi-target-carotenoid," with outstanding neuroprotective effects, as for example, AXT has been able to inhibit the L-glutamate cytotoxicity via multiple signaling pathways and reduce the reactive oxygen species (ROS) production, due to the neuroprotective effects (Park et al. 2018). AXT also exerted hepatoprotective effects by blocking TGFβ1-signaling, hindering the activation of Smad3 pathway in hepatic stellate cells isolated from C57BL/6 J mouse liver (Yang et al. 2015). El-Baz et al. (2019) investigated the effect of *H. pluvialis* AXT on osteoporosis in D-galactose-treated rats. In this work, the authors concluded that oral treatment with microbial-based AXT

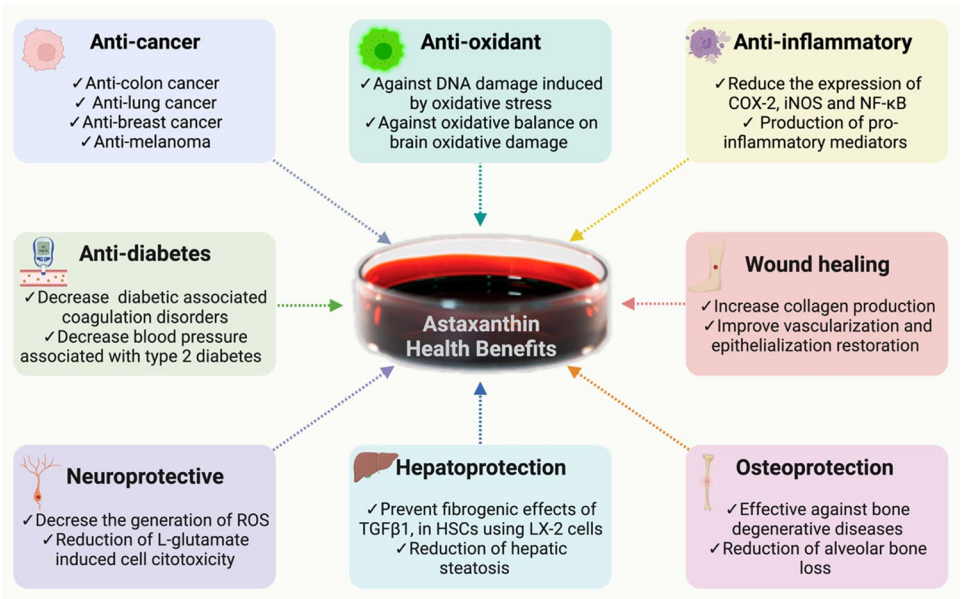

Fig. 4 Health benefits of microbial astaxanthin

ameliorated bone loss through the downstream regulation of serum osteoprotegerin in concurrence with the up-regulation of serum nuclear factor-κβ ligand.

Most of the AXT health benefits reviewed so far are related to anti-oxidant, anti-cancer, anti-inflammatory, among others (Fig. 4). However, some pioneering studies have proposed the use of AXT in alternative and disruptive areas, for example, as additives agents for the production of some biomaterials. Envisioning the application of microbial-based AXT (from *H. pluvialis*) as medical cosmetology agents (wound healing), Chou et al. (2016) revealed the potential of AXT to enhance growth factor secretions and improve collagen production in human dermal fibroblasts. Following the same line, Meephansan et al. (2017) investigated the effect of topical AXT on cutaneous wound healing in mice. The authors observed that AXT-treated wounds in female mice's showed contraction by day 3 and complete wound closure by day 9 of treatment, revealing the effective action of AXT for accelerating wound healing.

As previously observed, the recent research findings of AXT health benefits are directly related to the synthetic AXT and there a few works that uses microbial-based AXT. Another drawback is linked to the lack of human clinical studies related to the AXT intake. Synthetic AXT have demonstrated the effectiveness as supplementary food additives; however, several studies must be further investigated for the relationship between the microbial-based AXT intake and human health effects.

Applications and global market of microbial AXT

AXT is a carotenoid exploited on a large scale due to the attractive biological properties, used in the formulation of several products for animal and human consumption or skin care in developed or emerging countries (Mussagy et al. 2019). The growing preferences of consumers for healthy products are some of the main factors for the growth of the natural carotenoid's applications compared to the synthetic counterpart, creating a new business opportunity for natural AXT (Mussagy et al. 2021b). This red pigment has an important antioxidant power, which makes it a particularly interesting active ingredient in foods, nutraceuticals, cosmeceuticals, and pharmaceuticals (Mezquita et al. 2020). For all applications, regulation is a prerequisite and for feed and food, most started with the publication on February 5, 2020, of a text entitled *Safety of astaxanthin for its use as a novel food in food supplements* (EFSA 2020). Previously, in 2014 (European Commission), the Panel on Nutrition, Novel Foods, and Food Allergens (NDA Panel from the European Commission) assessed the safety of the novel AXT-rich ingredient derived from microalgae *H. pluvialis* in the context of an application submitted under Regulation (EC) No. 258/1997. In a new assessment, the panel derived a new acceptable daily intake (ADI) of 0.2 mg AXT/kg bw (bodyweight). In the pioneering company Cyanotech (USA), the ingredient BioAstin® Hawaiian AXT is offered in various formats, including algal biomass and cold water–dispersible powder or oil extracts. At Vitafoods Europe, AstaReal (Sweden) showcased its AstaGummy Boost, whose natural AXT content provides antioxidant benefits in a vegan, soft gel capsule gummy form. If the use of natural AXT as feed ingredient for fish (e.g., salmon) and shrimp was the initial market in the years 2003–2004 with products from *P. rhodozyma* such as Ecotone™ from ADM Archer Daniels Midland (Chicago, IL, USA), or Aquasta™ at UK based Tate and Lyle (Selby, England) in collaboration with Igene Biotechnology, Inc (Columbia, Maryland, USA), now feed market is developing for pets. This drives the market up to new volumes, and company BGG world (Yunnan Province, China) recently announced it is doubling its manufacturing capacity of AstaZine Natural AXT extracted from the microalgae *H. pluvialis*. The use of AXT in processed food is not allowed in many parts of the world; as a result, the chemically synthesized AXT is estimated to reach only 55% of the world market in 2024 because of the legal restrictions introduced due to the differences between natural and synthetic AXT.

In 2021, the global natural AXT market was valued at USD 647.1 million, and it is estimated to reach USD 4.75 billion in 2028, recording a Compound Annual Growth Rate (CAGR) of 8.3% (from 2021 to 2030), 14% (from 2020 to 2030), 16.8% (from 2021 to 2028), and 7.6% (from 2022 to 2028), according to the Markets and Markets (2020), Future Market Insights (2022), Grand View Research (2022), and PR News (2022) reports, respectively (Fig. 5). The segmentation of the AXT global market is divided into 4 different types: (i) by source: natural or synthetic; (ii) by product: dried biomass, oil, soft gel and liquid; (iii) by application: cosmetics, pharmaceuticals, antioxidant nutraceuticals and animal feed coloring agents; and (iv) by region: North America, Europe, Central and South America, Asia Pacific, and Middle East and Africa (PR News 2022) (Fig. 5). The natural AXT market is the fastest-growing segment source type in the last years according to the Globe News Wire (2022); the high growth rate of this natural-based can be attributed to the several benefits offered by AXT in these sources compared to the chemical. As for example, in Asia (i.e., China), Europe, and North America, the natural AXT production increased 40% from 2011 to 2016 (from 43,279 to 73,717 kg), with Europe and North America being the largest markets for natural AXT (PR News 2022). Some reports reveal that several nutraceutical companies around the world used approximately 54.8% of natural AXT from

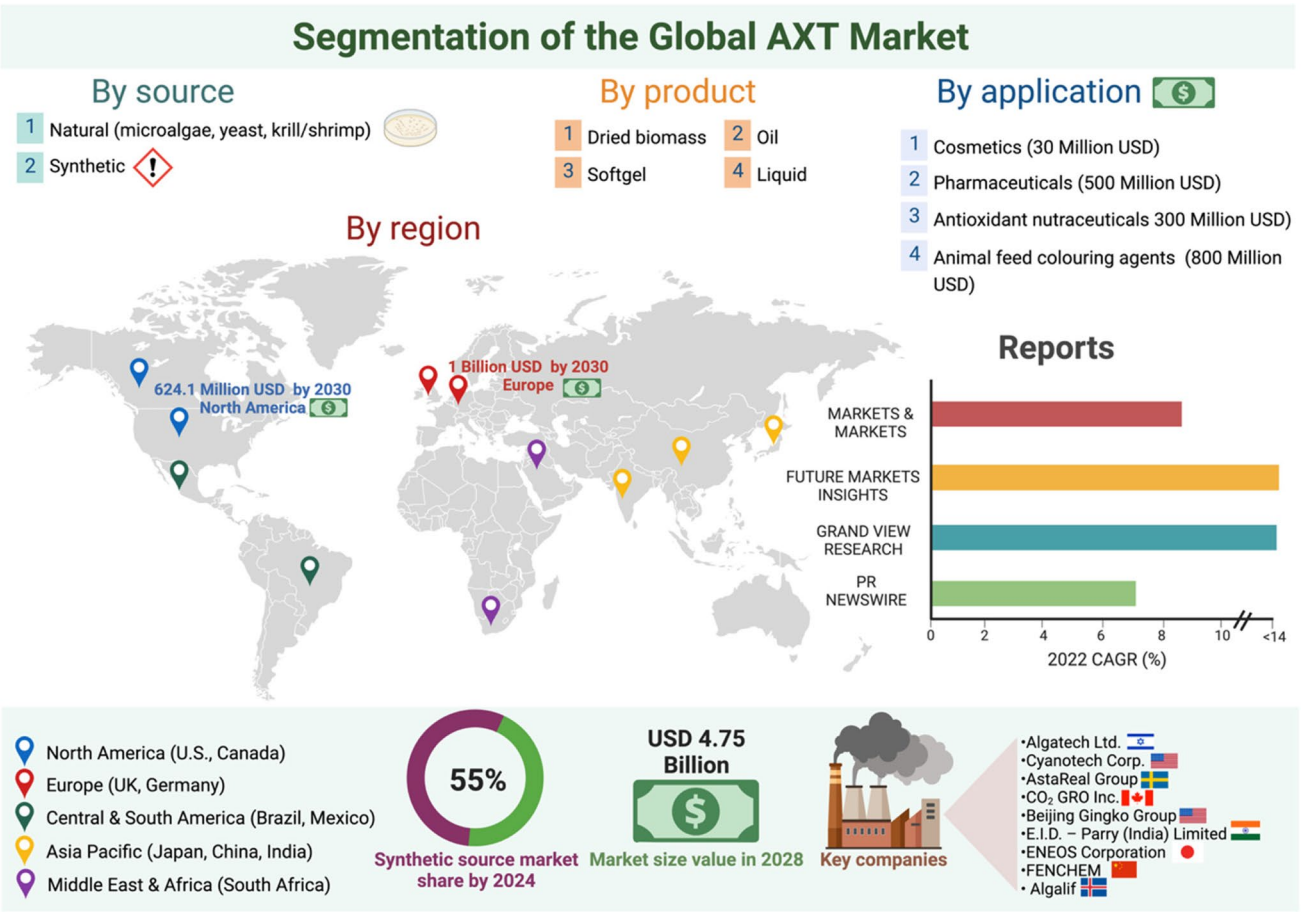

Fig. 5 Segmentation of the global astaxanthin market by source, product, application and geographic region

the microalgae *H. pluvialis* available in the market in 2017, and by 2024, the production of AXT is expected to reach 190 metric tons (PR News 2022). The market value of AXT varies depending on the type of product and its purity. For instance, synthetic AXT can cost between 1200 and 1500 USD/kg, whereas natural AXT from *H. pluvialis* can reach prices of about 6620 to 13,240 USD/kg in certain cases (Shah et al. 2016). Some commercial products including 10% AXT oil in nutraceutical market cost approximately 1200 to 1500 USD/kg, which equates to a conservative estimate of 150,000 USD/ton of algae grown (Pond Tech 2022).

As previously mentioned, the global AXT market can be divided by taking into account the source, the type of product, the final application, and geographic region (Fig. 5). Concerning the source, it is widely known that the synthetic AXT has almost 20 and 50 times lower neutralization of free radicals and reactive oxygen species capacity, respectively, than natural ones and, so far, has not been approved for human consumption (Mussagy et al. 2021b; Schmidt et al. 2011b). However, it is widely known that farmers use synthetic AXT to improve the color of salmons, and as we consume this fish in our diet, we end up indirectly absorbing the synthetic AXT. Note that these biological issues opened new possibilities for the natural AXT, which dominated the market in 2020 and is projected to witness the CAGR of 16.8% from 2021 to 2028 (Grand View Research 2022).

Regarding the type of product, the dried biomass has dominated the segment market in 2020 (more than 25% of revenue share) and is expected to control this market for the next 8 years (Grand View Research 2022). The requirements of less downstream procedures, such as extraction, purification, and polishing units, are the key factors for the growing market demand of dried microbial biomass in the animal feed and other products that use the whole biomass for formulations. According to the Grand View Research (2022), soft gels used in nutraceuticals formulations represent the second AXT segment with the fastest progress (17.4%) and this number is expected to increase during the forecast period (2021–2028). Recently, some companies, viz., NextFerm and Soft Gel Technologies, Inc. have worked in collaboration to produce new soft gel based AXT

products (Astaferm®) derived from *P. rhodozyma* (Nextferm 2022).

The aquaculture and animal feed industry represent the major market segment of AXT (used as coloring agent to improve the quality of seafood), with revenue sharing of 47% in 2020, and is projected to be higher in the next years (Grand View Research 2022). Besides that, in the last year, the AstaReal Group (company from Sweden) launched the new microalgae-based ingredient for pet foods (NOVASTA® AXT) with several beneficial properties for improving egg production in poultries and enhanced the performance in horses and dog health (Astareal 2022). Because of COVID-19 outbreak, the cosmeceutical segment is estimated to grow due to the significant increase in demand for healthy natural products to improve the immune system. The term "cosmeceutical" comes from the integration of "cosmetic" and "pharmaceutical" and therefore refers to the treatments that are generally found in pharmacies. For example, they can be both post-dermatological products, prescribed by doctors, and concentrated anti-aging treatments. Numerous products were launched this last decade, such as Oskia London's City Life I-Zone Balm which is designed for the delicate lip and eye areas to help defend against the damaging effects of indoor and outdoor pollution. The oil-based system comprises emollients and occlusive plant fats: avocado oil, sunflower oil, shea butter, modified hydrogenated vegetable oil, and AXT as one of the active ingredients. Many researchers have successfully developed and evaluated formulations to increase the skin penetration of AXT (Ponto et al. 2021) and to target its antioxidant and anti-inflammatory potential to the epidermis and dermis. The good image of biotech ingredients such as microbial AXT among consumers allows the market to develop rapidly. Enhancing skin health by oral administration of natural compounds and minerals may have strong implications to the dermal microbiome, and this is an emerging research trend. Another aspect of this market is represented by nutricosmetics (Meléndez-Martínez 2022). Very briefly, nutricosmetics refers to all food supplements with a beauty-related action, "beauty coming from inside." This neologism, which is a contraction of the words nutrients and cosmetics, was born in the 1980s in the cosmetics industry. Due to the powerful antioxidant properties, AXT has the ability to penetrate all parts of the body and could be a strong player in nutricosmetics and nutraceuticals (Mehariya et al. 2021).

The dominance of North American region (AXT market share of over 36% in 2020) is attributed to the presence of well-established nutraceutical market and the growing awareness about the use of natural products. This region is anticipated to reach 624.1 million USD by 2030 (Future Market Insights 2022). However, some countries from Europe (Germany and the UK) are estimated to witness the fastest total growth rate (1 billion USD in 2030) (Future Market Insights 2022). The Asia Pacific region (Japan, China, and India) is also considered an attractive potential market of natural AXT due to the considerable number of consumers. Some key companies are crucial to the rapid development of the natural AXT market, including Cyanotech Corp. (USA); Algatech Ltd. (Israel); Astareal Group (USA); CO_2 GRO Inc. (Canada); E.I.D — Parry (India); ENEOS Corporation (Japan); FENCHEM (China); and Algalif (Iceland) (Grand View Research 2022).

A summary of the key aspects regarding the application and marketing of natural AXT is depicted in Fig. 5. With this diagram, we intend not only to differentiate the AXT market segments but also to give a special attention to the AXT CAGR and important key companies. The lack of studies regarding the natural AXT in human formulations and the insufficient technological transfer-of-knowledge from the universities with the key companies are the main drawbacks regarding the application of natural AXT in human diet. However, this is changing as some food technologists are working far in advance of the regulations (Stachowiak and Szulc 2021) and many studies were published in recent years about stabilization of AXT in food processes, through micro- and nanoencapsulations (Bassijeh et al. 2020) and formulation with edible oils, gums, or hydrocolloids (Yang et al. 2021a, b). A lot has to still be done to demonstrate the advantages of using AXT as a food ingredient (Mezquita et al. 2020), as it was earlier demonstrated for the other well-known carotenoid, such as β-carotene. For pharmaceuticals and drugs (Patil et al. 2022), private key companies and the public academic sector are investing a lot of funds to systematically investigate AXT affinity targets, perturbed signaling pathways, and related disease applications (Sun et al. 2020). Hundreds of clinical trials with AXT have been performed or are in progress and can be found in clinical trial registries of many countries, viz., (i) a double-blind, placebo-controlled clinical trial of the insulin sensitization, anti-inflammatory, and antioxidant activities of AXT in insulin-resistant subjects and (ii) effects of AXT on oxidative stress and lipid profile in overweight and obese adults. So, many physiological, metabolic, and health targets are under evaluation, and time will tell us the pathologies, the diseases for which AXT has true and proven beneficial effects (Lin et al. 2021).

Concluding remarks and perspectives

The microbial pigments global market is looking to increase the number of new products with improved effectiveness to the human health. With the increase of this market, viz., for natural microbial AXT, the development of more

biocompatible and sustainable technologies is crucial not only to further boost biomass production and consequently AXT production titers, but also in reducing processing costs. Thus, although the consumption of synthetic AXT still dominates the market, consumer demand for microbial AXT has been growing, providing a market opportunity for natural AXT. *Haematococcus*-AXT has been approved in several regions for some applications (i.e., human consumption); however, the *Phaffia*-based has not been yet effectively implemented in the market due to some regulations and lack of studies. This raises the following questions: *Can microbial AXT really compete with synthetic AXT? Is the biotechnological production of Phaffia-based* AXT *viable for the commercial application? Can Phaffia-based AXT ensure the safety for the consumers?* It is obvious that the microalgae based AXT will dominate the market in the near future. The research findings are very encouraging, and the consumers perception of synthetic-based products in food and preference for natural products may lead to a significant expansion of microalgae-based AXT. Some companies that understand the importance of natural AXT in the market are looking into several processes in order to reduce the production costs and make the price of microalgae based AXT competitive with the synthetic ones in the near future. So far, we believe that there has not been an adequate answer to understand whether *Phaffia*-based AXT is viable or safe for human application. The only company in the world that produces AXT (Astaferm®) from *P. rhodozyma* available in the market is NextFerm from Israel. However, we must highlight that Israel has no specific regulations for some products available in the market for human consumptions. In other countries, even with the GRAS status (Generally Recognized as Safe) (GRAS) of *Phaffia*, the Food and Drug Administration (FDA) only approved the use of AXT from *P. rhodozyma* as feed additives. The main problems with implementing AXT from *P. rhodozyma* in the food market are the lack of approval by regulatory agencies (such as the FDA in the USA, Health and Safety Executive (HSE) in Britain, or the European Food Safety Authority (EFSA) in Europe) and the absence of human trials. We suspect that other factors such as expensive upstream and downstream technologies, will need to be carefully optimized, to accomplish similar market value of microalgae or synthetic-based AXT to remain competitive.

Thus, with this comprehensive review, we hope to encourage the academia and industry authors that works with microbial-based AXT, to perform more studies regarding the bioavailability and bio accessibility, toxicological assessment of natural AXT (mainly from yeasts) to provide the scientific information on AXT safety for human consumption. It is important to highlight that working together (academia and industry), we can achieve our goals more rapidly towards the implementation of these natural outstanding molecules for human consumption with low cost in the market as an alternative to the synthetic ones.

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Author contribution CUM: conceptualization, literature review, figures, writing — original draft preparation, writing — review and editing, visualization. AK: Literature review, writing — review and editing. LD: Literature review, writing — review and editing. CNDPG: literature review, writing — review and editing. JFBP: writing — review and editing. VCSE: writing — review and editing. VR: writing — review and editing. APJ: writing — review and editing.

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Data Availability Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate This article does not contain any studies involving human or animal participants conducted by any of the authors.

Conflict of interest The authors declare no competing interests.

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