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Review

Lutein from Microalgae: An Industrial Perspective of Its Production, Downstream Processing, and Market

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Abstract: Lutein, a yellow xanthophyll carotenoid, is increasingly recognized for its nutraceutical benefits, particularly in protecting the retina's macula from age-related degeneration. Microalgae are a promising source of lutein, which can be a primary product or a coproduct in biorefineries. Certain microalgae exhibit lutein levels (up to 1.7%) surpassing those of common dietary sources like kale, spinach, and egg yolk (approximately 0.7–0.9%). Predominantly associated with photosystem II's light-harvesting complex, lutein is crucial in photosynthesis and cellular defense. However, being quantitatively minor among cellular constituents, lutein necessitates specialized processing for efficient extraction. Although ubiquitous in microalgae, it is not as easily inducible as β-carotene and astaxanthin in *Dunaliella salina* and *Haematococcus pluvialis*, respectively. Currently, microalgal lutein production predominantly occurs at the bench scale, presenting challenges in scaling up. Factors like culture medium significantly influence biomass and lutein yields in industrial production, while downstream processing requires cost-effective, food-grade solvent extraction techniques. This review delves into contemporary methods and innovative progress in microalgal lutein production, emphasizing industrial-scale processes from biomass cultivation to final product formulation. A conceptual industrial process proposed in this review shows that two 10 m^3 photobioreactors could produce 108 kg dry mass for *Chlorella minutissima*, which can be processed into approximately 616 g of lutein extract, or over 6000 capsules of finished nutraceutical daily. Despite lutein production via microalgae being in nascent stages at large scales, existing research provides a solid foundation for well-informed scale-up endeavors.

Keywords: extraction; *Chlorella*; xanthophyll; optimization; pigments

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1. Introduction

Lutein and its isomers zeaxanthin and meso-zeaxanthin play crucial roles as carotenoid pigments in the human eye's macula and are recommended as essential nutraceuticals for addressing Age-Related Macular Degeneration (ARMD), a leading cause of vision loss in industrialized nations. Oxidative stress seems to be a primary factor responsible for the degeneration of the Retinal Pigmented Epithelium [1]. The European Food Safety Authority (EFSA 2010) advocates a daily intake of 1 mg kg^{-1} of lutein per body weight to replenish macular pigments. Consequently, lutein has gained popularity as a nutritional supplement and an antioxidant, also contributing to the prevention of cardiovascular

disease and atherosclerosis [2]. As a natural colorant (INS 161b), lutein is also used in the food industry [3]. Originally extracted from marigold flowers (e.g., *Tagetes erecta* and *Tagetes patula*), lutein has also become accessible from microalgae, akin to astaxanthin and PUFA-rich oils [4–6].

The global expansion of microalgae production for diverse purposes, encompassing fuel, animal feed, food, and raw materials for nutraceuticals and pharmaceuticals, is evident [7]. In microalgae, lutein (3R,3′R,6′R-β,ε-carotene-3,3′-diol) participates in photosystems as an accessory pigment, absorbing excess light energy in the blue region and quenching it, contributing to photoprotection, and even transferring a minor part to chlorophyll [8,9]. Unlike marigolds, lutein in microalgae is mainly in a free (unesterified) form [10], and it can be produced 3 to 6 times more efficiently, reducing land and water usage, potentially transforming the lutein production industry [11]. Figure 1 illustrates physicochemical characteristics of lutein essential for its extraction, spectrophotometric analysis, and quality control.

Microalgae are known for their ability to accumulate various bioproducts under specific cultivation conditions. Some examples, such as algae from the genera *Chlamydomonas*, *Haematococcus*, *Scenedesmus*, *Arthrospira* (*Spirulina*), and *Chlorella*, can produce high concentrations of lutein [9,12]. However, industrial-scale microalgal production currently lags behind the production of the carotenoids astaxanthin (a potent antioxidant) and β-carotene (an antioxidant and provitamin A) from *Haematococcus* and *Dunaliella*. Other strains can provide lipids for fuel production [13] and fatty acids such as omega 3 [5], as in the case of *Neochloris oleoabundans* and *Schizochytrium* sp., respectively.

Several studies address lutein production from microalgae, but most are limited to the laboratory scale. Factors such as culture medium and large-scale sterilization should be analyzed. Optimizing different conditions is crucial for deciding the stages and conditions for production [14]. The main objective of this review is to comprehensively analyze data on the industrial production of lutein from microalgae. It covers material balances, production, and productivity, as well as the effective extraction and stabilization of lutein from microalgal biomass, providing an assessment of the technological evolution in recent years.

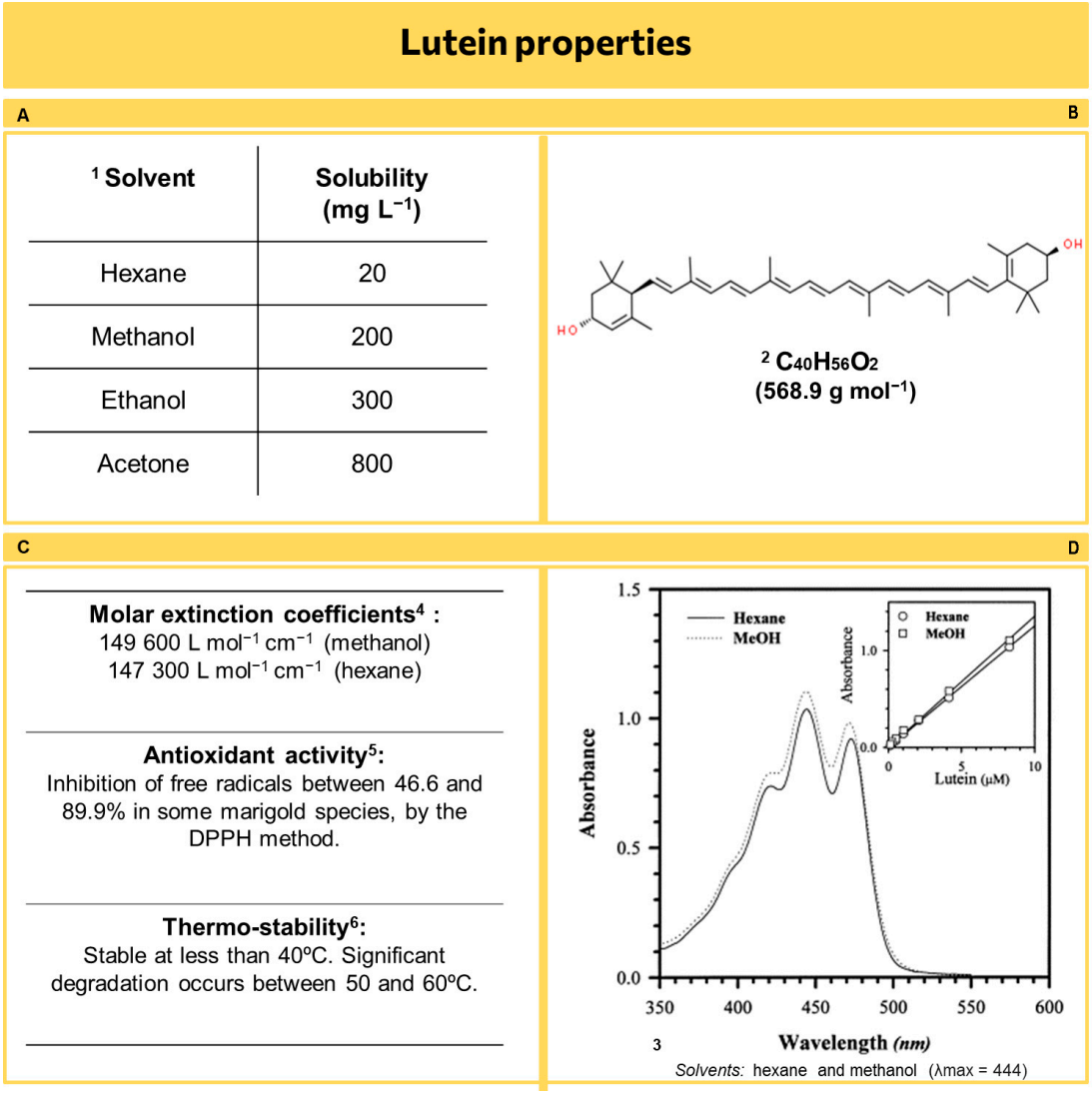

Figure 1. Lutein proprieties. (**A**) Solubility, (**B**) Molecular Structure, (**C**) physicochemical properties, and (**D**) Absorption Spectra. 1 and 4—Craft and Soares [15]. 2—Zang et al. [16] and Sabitha [17]. 3—National Center for Biotechnology Information [18]. 5—Ingkasupart et al. [19]. 6—Manupa et al. [20].

2. Lutein—A Key Molecule in Many Biological Systems: Roles and Bioactivity

Carotenoid biosynthesis in both microalgal and plant cells occurs predominantly in plastids. However, the structural aspects of the metabolic pathways of these pigments may vary depending on the tissue and plastid characteristics. Consequently, carotenoids synthesized through these pathways may specialize into apocarotenoids, serving in cell signaling or acting as structural components in photosynthesis or photoprotection of the thylakoid membrane [21,22].

The distribution of enzymes involved in carotenogenesis varies among plant and microalgal species. For instance, in the genus Arabidopsis, most carotenogenic enzymes are in the chloroplast membrane envelope, with fewer found in the thylakoid membrane. In addition, lutein gene expression can be influenced by abiotic factors such as light, temperature, stress conditions, and carbon sources. In microalgae, chemical inhibitors may also regulate carotenogenesis [22–24]. The carbon molecules and energy required for lutein biosynthesis in microalgae come from the methylerythritol phosphate (MEP) and pentose phosphate pathways during heterotrophic nutrition. In contrast, in the autotrophic mode, these molecules are provided by the Calvin cycle (CBB). While the MEP pathway predominantly facilitates plant carbon flow, the mevalonate pathway is also utilized [23,25].

The precursor metabolite for carotene synthesis in microalgae and plants is isopentenyl diphosphate (IPP), derived primarily from the MEP pathway. IPP is isomerized to dimethylallyl pyrophosphate (DMAPP), catalyzed by IPP isomerase. Subsequently, IPP is condensed with DMAPP to form Geranylgeranyl pyrophosphate (GGPP). The condensation of two GGPP molecules gives rise to a linear, colorless carotene known as phytoene, converted to lycopene. The enzymes lycopene β-cyclase and lycopene ε-cyclase facilitate the addition of β- and ε-rings to the ends of lycopene molecules. The β-carotene is produced by the action of β-cyclase, while hydroxylation of the α- and β-carotenes gives rise to lutein and zeaxanthin, respectively. Zeaxanthin can be converted into violaxanthin via an epoxidase [21,25–27]. Figure 2 summarizes carotene synthesis in microalgae, highlighting the enzymes involved in the process.

Lutein is a crucial component of a light-harvesting protein complex, LHC-II, associated with photosystem II (PSII) in green plants and algae. Xanthophylls, including lutein, play a vital role in microalgal cells, acting as part of the antenna, capturing light for photosystem II reaction centers in the chloroplast. They also provide photoprotection for the thylakoid membrane, mainly against lipid peroxidation of the thylakoid, demonstrating high light tolerance [8,9].

Under high irradiance, xanthophylls can reduce light excitation to the center of the photosystem, thereby reducing oxidative stress. Two groups of xanthophylls present in the microalgal cell are α-carotene derivatives, such as lutein, and β-carotene derivatives, including zeaxanthin, antheraxanthin, and violaxanthin [8,28]. Lutein binds to proteins associated with the light-harvesting complex (LHC), part of photosystem II. The accumulation of the pigment is limited by the amount of LHC present in the chloroplast, linking increased lutein production in microalgae to expanded storage of this pigment [29,30]. Figure 3 illustrates the arrangement of xanthophylls in the microalgal cell.

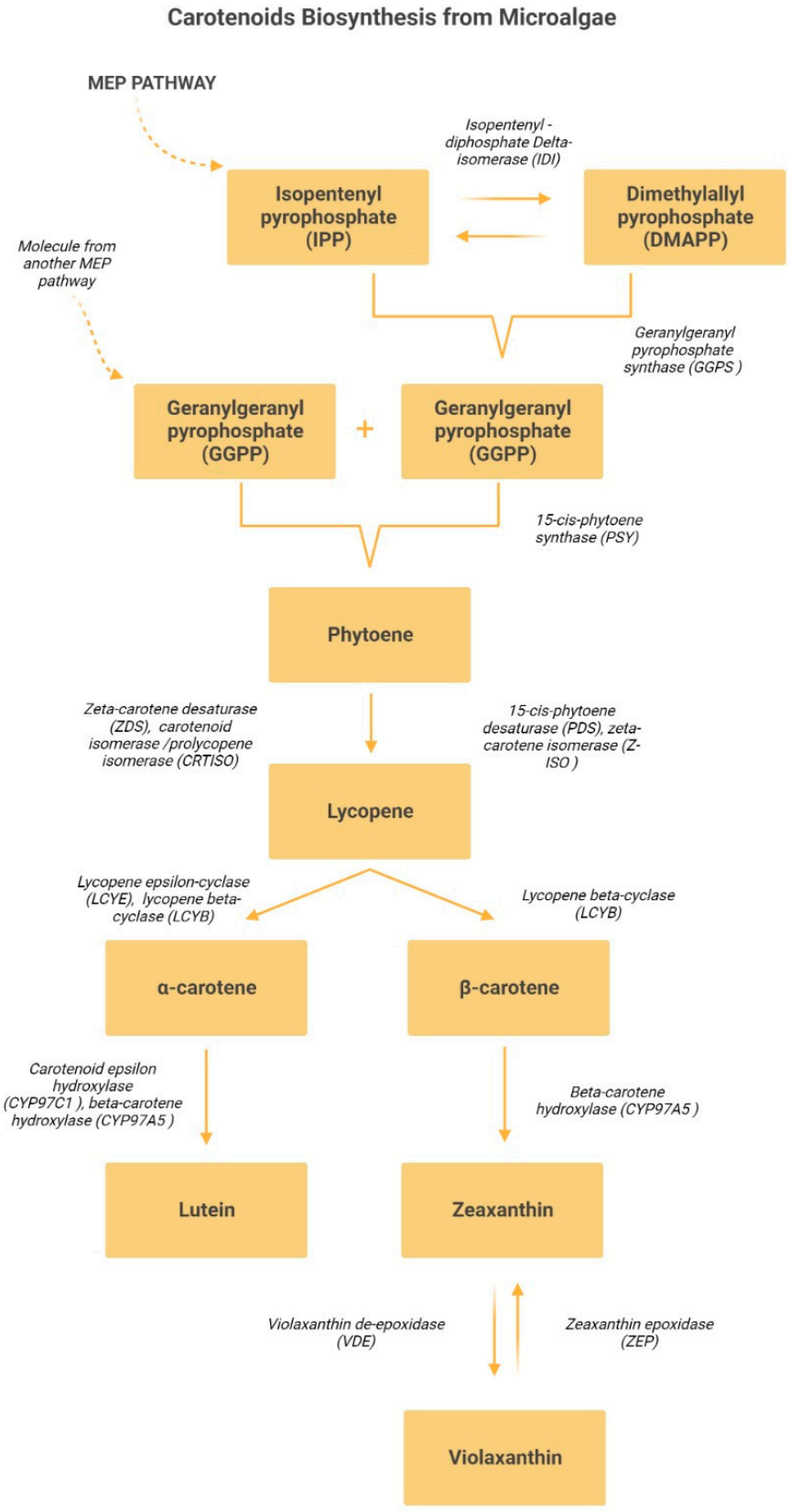

Figure 2. Biosynthesis of lutein in microalgae.

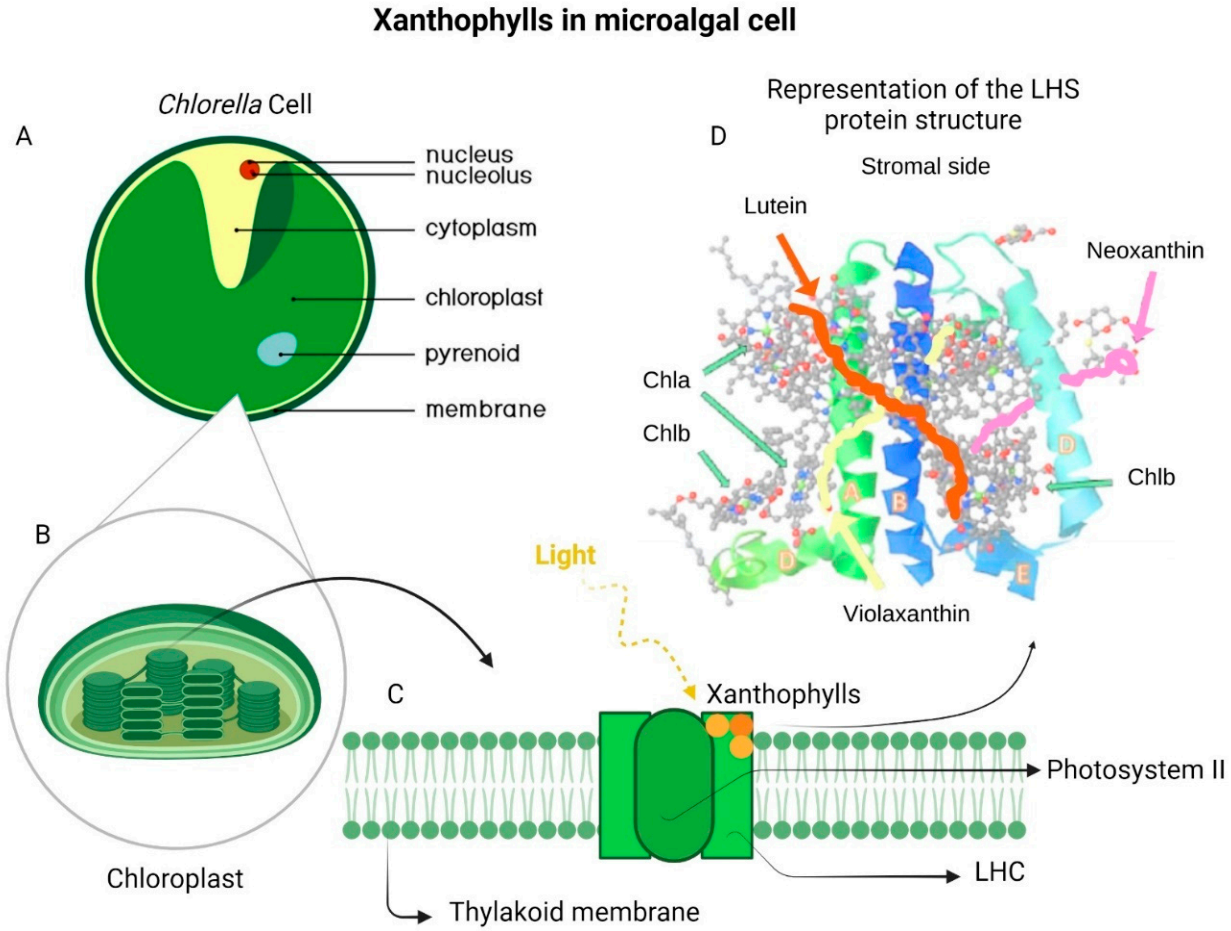

Figure 3. Lutein and other photopigments in microalgal cells. (**A**) *Chlorella*'s cell; (**B**) chloroplast; (**C**) thylakoid membrane; and (**D**) protein structure of the LHS, after Holtzegel [31], based on a structural model of spinach minor light-harvesting complex, obtained by PDB of PDB ID 3PL9, showing the arrangement of some xanthophylls and chlorophylls in the light-collecting complex.

3. Microalgae Cultivation and Lutein Production

3.1. Lutein Sources, Up-and-Coming Microalgae, and Cultivation Enhancement

The presence of lutein, and also zeaxanthin, in human blood and tissues is solely the result of ingesting foods containing these xanthophylls. Corn and eggs are significant sources of lutein and zeaxanthin in human diets, and many other foods, such as fruits, nuts, cereals, and vegetables, can contain fair amounts of these pigments. However, lutein can be found in higher concentrations in green leafy vegetables such as spinach and kale [32], which have lutein contents of 1.1 mg g^{-1} and 1.0 mg g^{-1}, respectively, on a dry basis. In comparison, the lutein content in some microalgae can reach up to 17 mg Lutein g^{-1} dry biomass [33,34].

There is research on industrial microalgae strains capable of high lutein productivity [35,36]. Although some microalgae are already produced for direct consumption (mainly *Spirulina* sp. and *Chlorella* sp.) or for extract valuable fractions (astaxanthin from *Haematococcus* sp. and phycocyanin from *Arthrospira* sp.), there is no consensus on the best strains that could be used to produce lutein-rich biomass. Table 1 presents the microalgae with the highest lutein content in the biomass (mg g^{-1}) and those with the highest lutein productivity (mg L^{-1} d^{-1}). Microalgae from the genus *Chlamydomonas* are commonly used in genetic manipulation [29,37] to improve lutein content, while *Chlorella* is the most studied so far for laboratory and industrial scales [38].

In terms of cultivation, many advancements have been proposed for microalgae biomass and lutein production: light configuration strategies [39], abiotic stress, and medium optimization [23]. The color of light may affect the lutein content within cells. Li et al. [40] noted that lutein content increased when *Chlorella* sp. AE10 was cultivated under blue light, reaching 9.58 mg g^{-1}, a 63% rise compared to production under white light. The carotenoid protection role can explain this since blue light has higher energy than green and red light. In practical strategy, a two-step culture using white light for eight days, followed by ten days under white and blue lights, increased the lutein content from 1.04 mg g^{-1} to 2.06 mg g^{-1} in *Chlorococcum humicola* [41]. However, light color strategies may be impractical for large-scale lutein production due to the dependence on sunlight [42].

Moreover, using stress factors such as nutrient limitation, as is performed with *Haematococcus*, usually does not work for the accumulation of lutein because the carotenoid is associated with the photosystem and thus can be seen as a primary metabolite, whose productivity is mainly correlated to biomass productivity, not to a secondary biosynthesis phase. In the same work cited before, nitrogen and phosphorus deprivation drastically reduced lutein content to 1.25 mg g^{-1} against 9.58 mg g^{-1} in the regular culture medium [40]. Hence, abiotic stresses, such as high salinities or nutritional deprivation, are associated with decreased lutein content [43,44]. Lutein production can be intensified by optimizing biomass production, and this depends on tailored culture media, discussed in the next section.

Table 1. Microalgae with high lutein content and productivity and important dietary sources of lutein for reference.

Microalgae	Culture Medium	Strategy	Lutein Content, Dry Basis (mg g^{-1})	Productivity (mg L^{-1} day^{-1})	Reference
Asterarcys quadricellulare PUMCC 511	BBM	Optimization	15.5	1.22	[45]
Auxenochlorella protothecoides	Residual	Two stages	4.99	34.13	[46]
Chlamydomonas sp. JSC4	BG11	Two stages	4.24	3.25	[39]
Chlamydomonas reinhardtii	n.d.	Gene manipulation	4.5	n.d.	[38]
Chlorella vulgaris CS-41	BG11	10× Nitrate	10.5	1.3	[44]
Chlorella salina	n.d.	Aeration and light	10.15	5.74	[47]
Chlorella protothecoides UTEX 29	n.d.	Monascus residue	9.11	4.13	[48]
Chlorella sorokiniana FZU60	BG11	Fed-batch N and C	9.57	17.35	[49]
Chlorella zofingiensis CZ-bkt1	n.d.	Mutant	13.1	1.85	[50]
Chlorella minutissima MCC-27	BBM	Optimization	5.58	0.65	[51]
Muriellopsis sp. MCH35	BBM	High-lutein-production strain	4.2	3.81	[52]
Egg yolk (raw)			0.016		[32], recalculated *
Kale (cooked)			1.011		
Spinach (raw)			1.1		
Spinach (cooked)			1.26		

n.d.: data not provided; *: contents in dry basis for comparison, even for raw or cooked sources—recalculated from Perry et al. [32] but correcting for the moisture content of 50% for egg yolk [53], 91.2% for kale [54], and 94 and 87 for raw and cooked spinach [55], respectively.

3.2. Nutritional Requirements and Media Composition

Microalgae cultures are known to have similar requirements for growth as higher plants in terms of mineral nutrients. Consequently, soil water extracts initially formed the basis of culture media. From there, the traditional microalgal media used today were developed a century ago by phycology luminaries such as Arnon, Chu, Bold, and Pringsheim and further improved by many others in the following decades [56]. These media have been successfully used for isolation, adaptation, and maintenance of strains for research but are unsuitable for mass production, being either unbalanced or too expensive.

Many algae are capable of autotrophy and heterotrophy [57]. According to Arnon et al. [58], almost 30 elements are required: macronutrients in a g to 100 mg L^{-1} range and micronutrients in lower concentrations. If water is readily available, algal growth requires adding three main elements for autotrophic cultivation: C, N, and P. The Redfield ratio of 106C:16N:1P has been widely used as a first nutrient limitation [59,60]. Carbon is typically provided as CO_2 through aeration, while N and P are provided as added fertilizers.

Many studies are concerned with comparing traditional media for microalgae growth [61,62] and are not concerned with interactions or concentrations of specific compounds. Such comparisons do not represent the medium's real potential for microalgae growth. Based on Yadav et al. [63], a comparison between four media (BG11, modified BG11, BBM, and M-8) showed that, after 12 days of cultivation of *Chlorella vulgaris*, phosphorus (P) and nitrogen (N) remained in excess. Laboratory culture media are frequently unbalanced regarding the N:P proportion [64].

Culture conditions are linked to the type of carbon source, inorganic or organic. For example, while most microalgae readily assimilate organic carbon, this cannot be achieved in open photobioreactors because of bacterial and fungi competition for carbon sources. However, it is known that the carbon source does not directly affect lutein synthesis; it has an essential role in biomass production [65]. Some works emphasize the importance of organic sources [46,66] for improving microalgae production, stating that, for mass production, just carbon dioxide is insufficient [67]. For instance, *Auxenochlorella protothecoides* was cultivated in two stages and showed a significant increase in biomass production from 0.66 g L^{-1} to 12.65 g L^{-1} after glucose supplementation, with a lutein content of 2.70 mg g^{-1} and productivity of 12.36 mg L^{-1} day^{-1}. Interestingly, this work indicated that a two-stage cultivation with a heterotrophic phase followed by a phototrophic condition resulted in 2.5× more lutein content in the cell than in the mixotrophic condition as the second stage [46]. Two-stage cultivation seems to be a promising strategy for lutein production: using a heterotrophic or mixotrophic stage for high biomass production followed by a phototrophic stage for lutein production [9].

Optimized media become more relevant in large-scale production, as large quantities of each compound are needed, impacting costs. In industry, the residues of nutrients must be treated before water disposal, and limiting compounds can adversely impact production or induce microalgae stress [68,69]. Laboratory (traditional) media are generally unsuitable for mass production. Moreover, the regional water quality must be characterized before nutrient supplementation.

3.2.1. Macro- and Micronutrient Optimization Strategies

The medium composition affects the biomass and lutein production yield and productivity [62]. About 30 chemical elements are required for microalgae cultivation. In traditional media, those elements are combined as anions and cations, resulting in medium recipes mainly containing salts such as cation nitrates, sulfates, or phosphates. Nutrient excess affects the medium's total salinity [70], and even excess micronutrients can be toxic; on the other hand, its limitation can cause microalgae growth limitation [71].

Media components are usually of analytical grade at the laboratory scale and are substituted by less expensive, food-grade reagents for large-scale production. Medium optimization involves evaluating and exploring the microalgae's maximum potential for growth and lutein production. Laboratory culture media are often a starting point; Dineshkumar

et al. [51] optimized the BBM medium for newly isolated *Chlorella minutissima* and improved biomass productivity and lutein content from 0.085 g L^{-1} day^{-1} and 2.67 mg g^{-1} to 0.117 g L^{-1} day^{-1} and 5.58 mg g^{-1}, respectively. In another work, isolated *Chlorella vulgaris* DSV77 had a lutein content increase of 1.6 mg g^{-1} to 5.32 mg g^{-1} after medium optimization [72]. Related articles showed that micronutrients had significantly impacted biomass and lutein production for *C. minutissima*, while macronutrients were more relevant for *C. vulgaris*. Both media deviated considerably from the original recipes regarding minor components, again showing that medium optimization is critical in developing a new process.

Medium screening is laborious and time consuming but can avoid waste—using too much of a nutrient that will not be totally consumed. Advancements in automation increase throughput; Radzun et al. [14] simultaneously optimized BM culture media for several newly isolated Chlorophytes, such as *Chlorella* sp. and *Micractinium* sp., using an automated system, improving the growth rate from less than 0.1 h^{-1} to above 0.2 h^{-1}. As a comparison, the automated screening was performed in eighteen units of 96-well plates, which permitted 246 trials during 2 days for each strain analyzed. A conventional experiment (100 mL scale) with 12 trials took almost 10 days for a single strain screening [51].

Finally, the water quality is crucial for scale-up and must be characterized before production. Soil extracts can be an excellent alternative for providing microelements.

3.2.2. Nitrogen Sources

Nitrogen is vital for microalgae growth as it is part of structural compounds: proteins and nucleic acids. Most cultivated microalgae can assimilate N as nitrate (NO_3^{-1}), the central N- source in various synthetic media [9]. Ammonium (NH_4^{+1}) salts and urea ($(NH_2)_2CO$) have been reported as alternative nitrogen sources for cultivation. *Arthrospira platensis* has presented an improved growth rate with both nitrogen sources, with a better biomass production of 1.18 g L^{-1} (in 18 days) when the classical Zarrouk medium was supplemented with KNH_4SO_4 (ammonium potassium sulfate) [73]. *Chlorella minutissima* cultivation showed that a medium supplemented with a cocktail prepared from effluent that contained different concentrations of NH_4^{+1}, NO_3^{-1}, and NO_2^{-1} resulted in 100%, 95.2%, and 100% substrate consumption, respectively. The lutein productivity reached 1.2 mg L^{-1} day^{-1}, 1.4 times higher than the control [74].

Nutritional stress conditions do not lead to lutein accumulation. For instance, the N deprivation showed an 18% loss in lutein content (original content of 4.2 mg g^{-1}) when *Dunaliella tertiolecta* was cultivated with ammonium starvation [75]. Similarly, *Chlorella protothecoides* CS-41 [76] and *Chlorella sorokiniana* FZU60 [77] did not improve lutein production with N deprivation, accumulating approximately 4.33 mg g^{-1} and 9.51 mg g^{-1}, respectively. Moreover, no significant difference was found between these three N sources. Therefore, research supports the hypothesis that industrial processes should focus on maximizing biomass production. However, this is half of the process—after cultivation, microalgae biomass must be harvested and processed.

4. Biomass Downstream Processing Strategies

Typical lutein products are whole algal biomass or concentrated extracts used as nutraceuticals, feed, or color additives. The typical downstream process for these products starts with biomass harvesting, dewatering, cell disruption, and drying for biomass production; for lutein extracts, the process continues with solvent extraction and lutein recovery for purification and stabilization. The residual water content affects pigment extraction if non-polar solvents (e.g., hexane) are used [52]. Moisture is less critical for more polar solvents such as ethanol or acetone. Furthermore, alkaline treatment can be performed before solvent extraction. This treatment is commonly applied to flower processing in the marigold industry [11]. Each stage has different efficiency, energy consumption, and process duration depending on the microalgae type [78].

4.1. Microalgae Harvesting

Harvesting is the first step after microalgae cultivation, aiming to separate the microalgae cells from the liquid cultivation media. Many solid–liquid separation techniques can be applied for microalgae harvesting, including centrifugation, filtration, coagulation and flocculation, flotation, or a combination of more than one technique [79]. The choice of the microalgae harvesting process depends on factors such as scale, cost considerations, and the intended use of the harvested microalgae, requiring attention since this step can account for 20–30% of the total microalgae processing cost [23].

Most processes to recover lutein-rich microalgal biomass use centrifugation. Due to its high separation efficiency, this method is widely applied on all scales, from laboratory to industry. Despite drawbacks such as high energy consumption, extended treatment times, and elevated maintenance and investment costs, it is feasible and efficient for high-value products, as in the case of lutein [23,79,80]. Filtration is another method that presents high efficiency and usually costs less than centrifugation. However, it can take a long time, it requires pressure or vacuum, and membrane fouling or clogging issues can happen, increasing operation and maintenance costs on a large scale [23,79].

Flocculation is an adjuvant technology that aggregates microalgal cells, increasing the effective "particle" size and aiding processes like sedimentation, centrifugation, and filtration. It can be applied on a large scale and with various microalgae species [79,80]. Salt flocculation, an alternative to chemical flocculation, offers high harvesting efficiencies at a lower cost, demonstrating effectiveness. Furthermore, natural coagulants, like jackfruit seed starch, can be used to harvest *Chlorella sorokiniana* for later lutein extraction [81]. Electroflocculation technology provides broad species applicability and low power consumption but requires electrode maintenance, may introduce metal ion contamination, and can lead to temperature and pH changes [23].

Certain microalgae can naturally undergo flocculation in response to environmental stresses like nitrogen levels, pH, and dissolved oxygen variations. This phenomenon is known as auto-flocculation, commonly induced at elevated pH, and is less disruptive to cells than centrifugation. *Desmodesmus* sp., *Chlorococcum* sp., *Scenedesmus* sp., and *Chlorella* sp. are examples of genera that present interesting lutein content and have already been harvested by auto-flocculation [79,80,82]. The biomass concentration must be increased as much as possible, from the cultures' original 1–10 g L^{-1} to the 10–20% by weight that can be applied to the following process, usually a drying step [83,84].

4.2. Drying and Its Influence on Extraction

Drying is essential for biomass production but is optional for lutein extraction—in that case, it depends on the solvent used later in the process. Microalgae biomass is usually freeze-dried at the laboratory scale before the lutein extraction [52,81]. However, freeze-drying is usually too expensive for use in large-scale, commercial recovery of microalgal products [80].

On a large scale, the drying process can streamline industry logistics. In an industry that does not perform lutein extraction immediately after harvesting, drying can extend the biomass shelf life until its processing [85]. Spray drying is the preferred method for large-scale, high-value products, but it may lead to notable degradation of specific algal components, such as pigments. Carotenoids are heat sensitive and highly susceptible to drying temperature and time, e.g., in *Chlorella vulgaris*, using spray drying, 30.8% of total carotenoids were lost [85], presumably from oxidation. The same study proposed using diverse materials such as aquafaba, deactivated baker's yeast, inulin, and maltodextrin in different ratios to encapsulate the microalgae. In most cases, minimal lutein losses were observed.

In certain instances, the solvent extraction of dry biomass has proven to be considerably more effective in recovering intracellular metabolites, such as lutein, than extracting from wet biomass [80]. However, drying is hugely energy consuming, and, by eliminating this step, cost and energy savings can be expected [86]. Studies on lutein extraction

directly from wet biomass showed that the high water content is deleterious for non-polar solvent extraction. To avoid this problem, many solvent mixtures are proposed. Moreover, sequential extraction progressively removed the biomass water content, increasing the extraction yield [86–88]. For example, extraction of dried and wet biomass of *Chlorella vulgaris* showed that wet biomass yielded 2× more lutein (8.5 mg g^{-1}) than dry biomass (3.9 mg g^{-1}). The result was achieved with a single-step extraction with ether/ethanol binary solvent, which was higher than hexane single-solvent extraction [86]. Therefore, a possible industrial option would be using moderately polar solvents, such as ethanol or acetone, in a multistage countercurrent process; that would not be efficient in extracting the non-polar beta-carotene but can be effective for lutein.

4.3. Alkaline Pretreatment

The following unitary process, alkaline treatment, also known as saponification, is applied for two reasons: for lutein transformation of esterified lutein into free lutein [89] and chlorophyll removal from the biomass [90]. In almost all references, KOH is the primary reagent for the saponification process [86,91,92]. For saponification, the alkaline treatment uses a minimum of 10% of KOH for complete lutein conversion, although reported concentrations reached 60% of KOH [93–96]. For chlorophyll removal, this concentration is considerably lower; no more than 4% is related [97–99].

In terms of lutein yield, in *Arthrospira platensis*, a quantity of 115.08 µg g^{-1} of free lutein could be obtained with 4% KOH treatment [91], and *Chlorella sorokiniana* NIOT-2 yielded 14.86 mg g^{-1} of free lutein after 22% KOH treatment [92]. The alkaline treatment for this process was optimized, and the importance of a temperature between 60 °C and 75 °C for maximum yield was highlighted. Despite the reports that include KOH treatment as a step for carotenoid extraction for microalgae [91,100], the practice seems inspired by the marigold industry, and tailored pretreatment should be developed for specific microalgal strains. The lutein esters predominate in marigold flowers, and saponification is necessary for feed or food applications; the ester isomers have an in vivo hydrolysis efficacy of less than 5% [101]. Interestingly, pharmaceuticals and nutraceuticals benefit from having both free and esterified lutein as one has higher antioxidant properties and the second is more stable and remains for a long time in the blood [102,103]. Some microalgae contain mostly free lutein [10], dispensing harsh alkaline treatment. Even so, alkaline treatment can be beneficial in dissolving the cytoplasmic membrane and improving the process speed in the extraction step. The cell membrane is the main barrier for the extraction of small molecules. Therefore, mechanical disruption of the cell wall is usually unnecessary, although tailored pretreatment for each microalgal species, depending on the cell wall composition, can be necessary for fractionation into multiple products [7].

4.4. Extraction, Purification, and Stabilization of Lutein

Organic solvents are commonly used for carotenoid extraction. Ethanol, methanol, hexane, acetone, and diethyl-ether [52,81,104] have been widely used for lutein extraction. As an innovative solvent, THF (tetrahydrofuran) is gaining attention due to its high affinity for lutein [105]. In solvent extraction, the polarity of the solvent plays a significant role. Also, residual intracellular moisture drastically reduces the extractability of non-polar solvents [86]. In terms of affinity, the relative solubility of lutein in ethanol, methanol, acetone, DMSO, and THF is 300 mg L^{-1}, 200 mg L^{-1}, 800 mg L^{-1}, 1000 mg L^{-1}, and 8000 mg L^{-1}, respectively [15]. However, safe solvents must be used in nutraceutical lutein production, making ethanol a good choice [9].

Many innovative processes have improved lutein extraction on the lab scale. Supercritical CO_2 extraction from *Haematococcus pluvialis* yielded 4.03 mg g^{-1} of lutein, and, despite an environmentally friendly solvent, only 52.32% of total lutein could be extracted [106]. In a pressurized liquid extraction with ethanol, the optimized process for *Chlamydomonas* sp. showed a low yield of lutein, only 26% [107]. Ultrasound [108] and microwave-assisted extraction [109] can be highlighted to improve lutein recovery. However, supercritical and

pressurized processes have high capital and operational costs. Ultrasound or microwave processing, popular in laboratory studies, are generally not applicable on a large scale [110].

In conventional extraction (batch mode), multiple steps are necessary for the high-yield recovery of lutein [111]. Each step is followed by centrifugation for solvent removal, and the biomass is subjected to the subsequent extraction. Yadav et al. [111] reported that complete lutein extraction by ethanol from *Chlorella vulgaris* wet biomass was attained in four consecutive steps. In another work, Gong et al. [97] obtained 85% lutein recovery from wet and frozen biomass (−20 and −85, respectively) from *Chlorella vulgaris* UTEX 265. Similarly, the wet biomass of *Scenedesmus obliquus* yielded >95% lutein extracted by methanol after five consecutive extractions [88]. After the last step of extraction, the residual solvent in the lutein must be purged with N_2 gas (99.9% purity) to avoid oxidation [105]. In the case of non-GRAS solvent extraction, the lutein oleoresin is redissolved in acetone or ethanol and purged by nitrogen gas until the residual solvent level drops to GRAS-acceptable levels. These small-scale extractions can be readily scaled up since the mass transfer should occur similarly if the biomass-to-solvent ratio, temperature, and mixing conditions are replicated.

5. Industrial-Scale Microalgae-Based Lutein Production

A conceptual process for industrial lutein production from microalgae, based on average yields from the literature, is presented in Figure 4. As previously reported, optimization for microalgae biomass production is relevant, being perhaps the most critical aspect. Strains to be used should have been explored in research regarding productivity and safe consumption. Consequently, the microalga *Chlorella minutissima* MCC-27, previously optimized for lutein production [51], was considered for this process. The expected biomass productivity for this strain is 0.57 g L^{-1} day^{-1}, with a lutein content of 6.05 mg g^{-1}—within the average range among best-reported strains. A single photobioreactor module of 10 m^3 (i.e., a raceway with dimensions of 10 m length, 3 m width, and 0.3 m depth) could achieve, after 95% efficiency centrifugation [112], a total of 54.15 kg of dry weight biomass after over ten days. Additionally, biomass washing is required to remove residual medium salt (Figure 4—stream 4). This requires a new centrifugation step, resulting in clean, concentrated biomass (stream 7). However, it results in low salt biomass, enhancing purity and reducing equipment corrosion. To streamline production, several photobioreactors could be run in parallel, with daily sequenced harvesting or cold storage of wet biomass, as suggested by [97].

Biomass drying is not used in this conceptual process; as discussed, direct extraction from wet biomass may reduce energy consumption. Sequential extraction can effectively overcome reduced extraction efficiency induced by the residual water. After five sequential methanol extractions with overall > 95% recovery (stream 10 represents the biomass return for re-extraction), the lutein solution in stream 12 proceeds to an evaporator where the solvent is recovered (stream 13), and 99% of the lutein remains in the extract [88], yielding 616.2 g of lutein extract. This first lutein extract must be purged with N_2 to eliminate residual solvent. The lutein extract is then formulated by dissolving it into 28.3 L of vegetal oil for a final concentration of 20 mg mL^{-1} lutein adequate for long-term storage or encapsulation into soft gel beads [105].

To avoid unnecessary costs and steps due to methanol removal after extraction [88], a suggested pressurized extraction by ethanol with 86.2% lutein yield [105] could be more feasible due to the safety and GRAS classification. The final concentration of lutein oil can be adjusted for the desired final product. Typical lutein products contain 30 capsules with 25 mg of lutein + zeaxanthin. Based on that, the proposed industrial concept for lutein production could produce over 6000 capsules daily (24,600 capsules every four days).

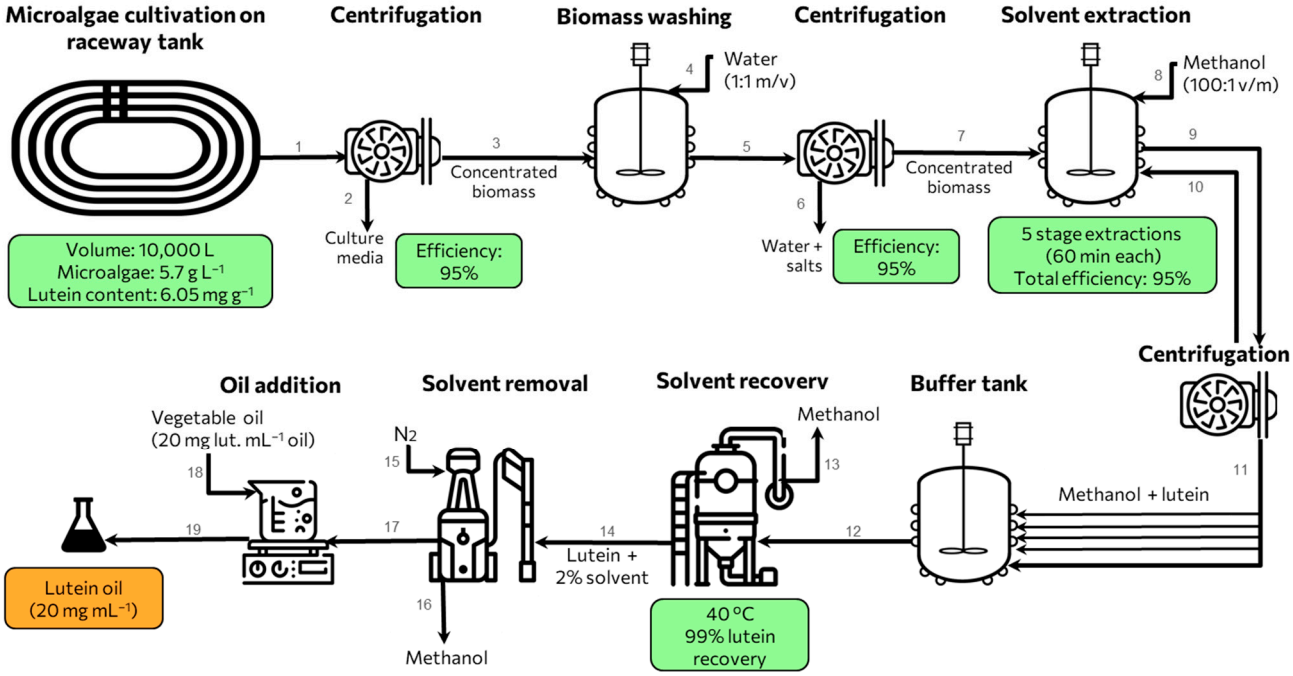

Figure 4. Conceptual industrial process for lutein production from microalgae. Process yields are selected from the literature and discussed in the text. 1 to 3—Biomass recovery; 4 to 7—Residual medium's salts removal and biomass recovery; 8 to 10—Solvent addition and biomass separation for sequential extraction stages; 11—Solvent recovered from five extraction stages; 12 to 14—Solvent removal and lutein recovery; 15 to 17—Residual solvent removal from lutein extract; 18 and 19—Lutein extract stabilized in vegetable oil.

6. Advancements in Microalgae-Based Lutein and Market Prospects

In the modern landscape of lutein production, marigold flowers (*Tagetes* sp.) remain the predominant raw material. However, the high growth rate of microalgae, the ability to control culture conditions, and the ease of harvesting corroborate the advantages of producing lutein using photosynthetic microorganisms [12,23]. A notable distinction arises in lutein production rates between marigold flowers and microalgae. While marigold flowers yield a pigment production rate of 120 kg per hectare, microalgae can achieve 350–750 kg per hectare [11].

GRAS microalgae products (generally recognized as safe by the American Food and Drug Administration) are developed and marketed with high added value. For instance, *Chlorella* biomass, primarily produced by companies in Taiwan and Germany, is sold as a nutraceutical for food supplementation. Production levels range from 130 to 450 tons annually, with an average price of 44 USD kg^{-1}. *Haematococcus*-derived astaxanthin is marketed at an average price of 2500 to 7000 USD kg^{-1}, predominantly produced in the United States and Japan, with around 300 tons per year produced worldwide. β-carotenes are extracted from the microalga *Dunaliella*, with global production reaching 1200 tons per year, mainly in Australia and Israel, and sold for an average of 300 to 1500 USD kg^{-1} [113]. On the other hand, some GRAS microalgae are recognized as lutein producers, i.e., *Haematococcus*, *Dunaliella*, *Scenedesmus*, and *Chlorella*; however, these studies have been conducted on a small scale [11]. The European Union approved the lutein pigment obtained from Tagetes (E161b) as a colorant in food and for medical and pharmaceutical industries with a daily 1 mg kg^{-1} body weight [27].

The lutein market was approximately USD 135 million in 2015. The Compound Annual Growth Rate (CAGR) is expected to be 6.1% during 2020–2027, reaching USD 369 million in 2024 and USD 491.4 million in 2029 [114,115], and countries such as India, China, and Japan hold around 60% of the market; companies Biomed Ingredients, India

Glycols Limited, Prakruti Products, and Bio-gen Extracts Private Limited are the major players, and the leading applications are beverages and health supplements [9].

Knowledge and innovation are strictly related; therefore, patents and articles were analyzed to determine the status of innovations in producing lutein from microalgae. The search was performed for patents using the Derwent Innovations database, while the Scopus database was used for articles. One hundred and eight patents and one hundred and twelve articles (review and experimental) were retrieved. Figure 5A illustrates the evolution of applied papers and articles, which follows an increasing trend with some natural fluctuations. It was observed that, since 2010, there has been a surge in technological growth mainly in genetic manipulation, production conditions, and extraction of lutein from microalgae due to the commercial interest in this pigment. The steady growth over the last few years has made lutein extraction an emerging technology developed jointly between companies, research centers, and universities (Figure 5B). According to WIPO, China has the most patents applied, followed by France and India, which corroborates that the primary market is focused in Asia and Europe (Figure 5C).

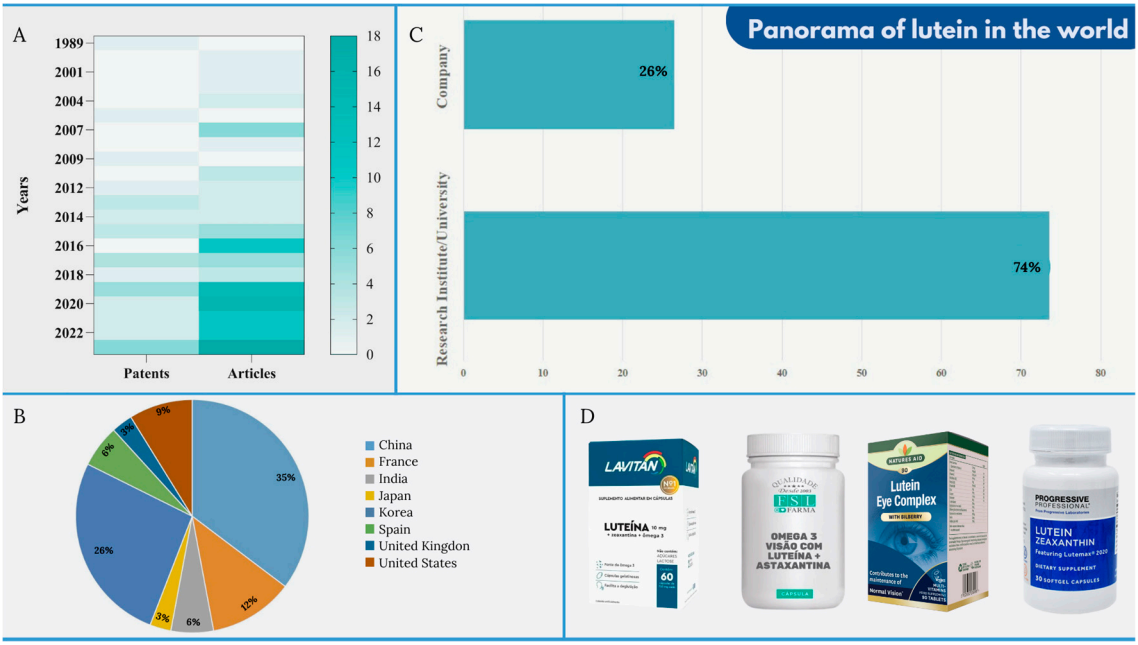

Figure 5. Lutein patents and research landscape. (**A**) Evolution of applied patents and articles over the years; (**B**) number of applied patents by countries; (**C**) comparison between applied patents by industries and academic/research. (**D**) Some lutein products examples on market.

7. Future Perspectives

Microalgal biomass is a rich source of pigments and other valuable bioproducts such as proteins, carbohydrates, essential vitamins, and fatty acids. Large-scale microalgae production is now a reality for selected strains that produce nutritional biomass (mainly *Arthrospira* and *Chlorella*) and carotenoid products (mainly Astaxanthin from *Haematococcus* sp. and β-carotene from *Dunaliella*). Lutein from microalgae has the potential to be the next big product in the microalgae market. Although the existing processes for microalgal products, especially carotenoids, can be adapted for lutein production, targeted process development can be more efficient. Based on the prior discussion, the following aspects of technological development seem particularly promising.

Strain selection—The lutein content varies considerably among different microalgal species. Algal strains with high lutein content, above 1% of the dry weight, and high productivities—above 5 mg L^{-1} d^{-1}—have already been studied and can be used in large-scale processes. Novel strains can be obtained through random mutagenesis or genetic manipulation. However, there is much potential to be explored in nature, and the

targeted isolation of strains—e.g., extremophiles that can grow with less microorganism contamination or strains that grow well in agroindustrial effluents—is a promising pathway for developing new strains, intensifying the process.

Culture media development—Research has shown that biomass titers are the most important factor for high lutein productivity after selecting suitable strains. Automation and artificial-intelligence-backed analysis can join classical statistical analysis of experiments for culture media optimization, leading to the rational use of fertilizers. Agroindustrial effluents are also a rich source of water and nutrients for microalgae production and must be evaluated whenever possible.

Culture media recovery—Not all nutrients are absorbed in microalgae cultivation, even if their concentrations are optimized. Some excess concentration may be necessary to facilitate transport and maintain high productivity. However, these nutrients can be partially recycled after biomass recovery, and their composition can be amended for a new production cycle. Recycling may bring back to the photobioreactors an excess of unwanted substances and microorganisms, and therefore must be optimized for each case, but can lead to resource optimization.

Minimal pretreatment and green solvents—As previously discussed, many processes at the laboratory scale borrowed from marigold processing the idea of alkaline treatment to facilitate lutein extraction. However, diversely from plant sources, microalgal biomass is already a suspension of small particles with a large specific surface area, facilitating solvent extraction. In addition, selected strains may have a predominance of unesterified lutein. Therefore, the minimal processing of lutein may prove effective and have a low cost, even if it means longer processing times for efficient extraction. As for the solvents, research shows that using moderate-polarity solvents such as ethanol can both dehydrate biomass and extract lutein but probably require more solvent than processes with previous drying. To compare wet and dry biomass processing and solvent recovery, process economics must be evaluated.

Biorefinery approaches—Downstream processing technologies developed for microalgae in general, such as harvesting, dewatering, and drying, can adapted for lutein production processes. Inverting this idea, existing processes can benefit from lutein extraction in a biorefinery approach. For example, biomass produced for protein production can be partially extracted with solvents for lutein production, and the dried meal can be further processed into proteinaceous meals or bioenergy. A complete economic evaluation must be performed based on preliminary data for this integrated process. It can increase the feasibility of otherwise uneconomical processes such as algal biodiesel production.

8. Conclusions

Lutein is gaining importance as a nutraceutical and pharmaceutical for ocular diseases and as an antioxidant. Lutein is poised to join other microalgal carotenoids on the market, such as β-carotene from *Dunaliella* sp. and astaxanthin from *Haematococcus* sp. However, although lutein production seems feasible and has higher productivity than land plant sources, there are challenges, and, thus, there is intensive ongoing research into its production.

Highly productive strains such as *Asterarcys quadricellulare* and many *Chlorella* strains were already isolated and can be used to develop efficient industrial processes. However, unlike secondary carotenoids, lutein production does not increase under stress conditions, although it can be modulated by the light quality used. Increasing biomass production is the primary pathway for process intensification and requires culture media optimization. On the lutein recovery side, existing processes (harvesting—drying—extraction—concentration) can be used, but research shows that drying could be skipped if moderately polar solvents are used.

Based on the patent and research landscape analysis, it is clear that lutein production from microalgae is still innovative and growing. Because of the many options, including the integration into biorefineries, lutein production requires a techno-economic evaluation that can be based on the already profuse research available.

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