Lutein: a critical review of its historical context, commercial production and rising alternatives

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Lutein is a molecule that has been studied for a long time. It was indirectly discovered in the human eye in the eighteenth century and has been studied continuously from various angles ever since. It was either found as a pigment in plants, flowers, and fruits, or in the reproductive systems of animals. Over time, different researchers isolated it from these sources and elucidated its structure. However, it was not until the 1950s that the research done on plants started being associated with the findings in the human eye from the 1700s. Thus, interest in identifying its role in the human body began to unfold. At the beginning of the 1990s, people started associating it with eye function and its acquisition. Production methods began to develop, and multiple patents emerged. The topics of the patents varied, ranging from the use of lutein for feed to its use as a supplement and its purification methods. Nowadays, research focuses on both its role in the human body and on sustainable, effective ways to increase its production. This review aimed to recount how lutein has gained importance over time and may also hint at what is to come for lutein production.

Lutein | Lutein history | Marigold flowers | Carotenoid history | Microalgae
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1. Introduction

Lutein is a yellow carotenoid pigment prevalent in animals and acquired through the diet. Photosynthetic organisms produce it, and it is part of their common pigments. It has garnered significant commercial attention due to its industrial and health applications. In this review, the reader will navigate through the first sights of this compound, how its chemical composition and function were elucidated, its initial applications, how its industry grew, the discovery of some of its functions in the human body, its standard production methods, and some alternatives to them. The aim is to reflect on the lutein's history to evaluate how its production and uses can further evolve with current technological advancements, and to explain why this molecule has become relevant.

2. The lutein molecule

To better understand the molecule, some general information is needed. Lutein, (3R,3'R,6'R)-β, ϵ-carotene-3,3'-diol[1, 2], is a pigment with a characteristic yellow color. Its name derives from the name of the *corpus luteum*[3], a name in Latin which means "yellow body" to refer to a yellow mass of the mammalian ovary, produced after ovulation[4]. As a pigment, it belongs to the carotenoids, a class of lipophilic pigments[5] that absorb light at specific wavelengths[6], in particular in the 450-550 nm region[7]. Their coloration ranges from yellow to red[1]. Their backbone is a nitrogen-free 40-carbon polyene chain structure derived from isoprene[1, 5, 8]. They are classified in 2 groups: carotenes and xanthophylls[5]. The name carotene comes from the Latin *carota*, which means carrot[9], while xanthophyll derives from the Greek *xanthós* (yellow) and *phyll* (leaf)[10]. Lutein belongs to the second group.

Structurally, it features a carotenoid base with an ε-ring and a β-ring on each side of the sequence, both of which possess a hydroxyl group at the 3' site [2], as illustrated in Figure 1.

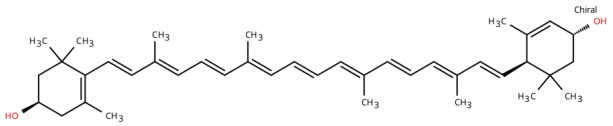


Fig. 1. Lutein structure[11].

Photosynthetic organisms such as plants and algae can synthesize it[2]. Since it is a primary carotenoid[12, 13], it is located alongside the photosynthetic machinery in the thylakoid membranes of the chloroplast[13] in "structural form". However, in flowers and fruits, it exists in an oil form, in which its hydroxyl groups are esterified to fatty acids[2].

The role of lutein in plants may not be directly related to photosynthesis, but it is important regardless. Pogson *et al.* evaluated the *Arabidopsis* mutants *lut1* and *lut2*, impaired in lutein biosynthesis to try to uncover lutein function. Presumably, the mutations affected the hydroxylation and cyclization of the ϵ-ring, respectively, during lutein synthesis. The authors suggested that when lutein was lacking, other carotenoids, such as violaxanthin, could take its place in the light-harvesting complex (LHC)[14]. However, in the *lut2* mutant, which was completely lutein-deprived, it was later confirmed that violaxanthin did not function as well as lutein for efficient chlorophyll triplet quenching. Dall'Osto *et al.* found that *lut2* mutants suffered greater damage from light exposure, including increased reddening and bleaching of older leaves. After multiple tests, they identified that only the presence of lutein enabled the formation of trimers in the LHC, thereby adequately quenching harmful 3Chl*. They also showed other effects of lutein in plants. One such example is the State I-State II transition. When plants receive high

amounts of light, they need to adjust the antenna sizes of photosystems I and II (PSI and PSII). This adjustment is achieved by phosphorylating the light-harvesting complex II (LHC II). When this happens, LHCII detaches from PSII and diffuses to PSI complexes, thereby increasing electron transport and light-harvesting capacity. This phenomenon is called state transition. They found that in the *lut2* mutants, this transition did not occur due to the lack of lutein; they suggested that it was somehow required for the phosphorylation step[15].

Lutein also has important antioxidant properties, which are beneficial not only to plants but also to humans. It may offer a plethora of health benefits. However, unlike other carotenoids (except zeaxanthin), it can cross the blood-brain barrier, allowing access to normally inaccessible tissues such as the brain and retina[16]. In the latter, it has been proven to prevent cataract[12]. Some have suggested it could reduce damage in synapses by mitigating Reactive Oxygen Species (ROS)[16]. Other health benefits include aiding in the development of the infant's brain, reducing the risk of cardiovascular diseases such as atherosclerosis[1], and inhibiting adipogenesis[16, 17]. However, even though there are studies suggesting these possible health benefits, by the time of this research, the authors could not find a specific mechanism or mechanisms which have been proven for any of them. Moreover, in 2006, the US Food and Drug Administration (FDA) reported that based on the information at the time there was no evidence to make any claim regarding the intake of lutein and/or zeaxanthin and the risk of age-related macular degeneration of cataracts[18]. On a recent meta-analysis of randomized controlled trials of lutein supplementation, it was suggested that it could have structural and functional benefits in vision in early stages of AMD, however, those mechanisms ought to be studied further[19] which provides more opportunities for further research.

It is worth noting that humans do not produce lutein[2]; thus, it must be obtained through diet[1, 8]. A study by Seddon in 1994 found that a diet rich in carotenoids, especially those containing lutein and zeaxanthin, such as spinach or collard greens, reduced the risk of age-related macular degeneration (AMD) by 43% in a population aged 55 to 80[20]. According to multiple studies and cohorts, the average intake of lutein from foods is 0.8-2.5 mg/day. However, when evaluating lutein supplementation to obtain health benefits, doses of 8-20 mg per day have been used[21]. In other studies, even 6 mg of lutein per day was sufficient to reduce the risk of AMD[2].

3. Methodology

The earliest hints of the historical origins of lutein were found in the abstract of an article that reported the identification of a yellow spot in the macula in 1782[22]. Afterward, other historical compilations were researched to corroborate this information. The closest was the article titled "Carotenoid research: History and new perspectives for chemistry in biological systems" by George Britton in 2020[23], which provided an overview of carotenoid history. After contacting him, he kindly provided an extensive document on this topic, entitled "Carotenoids: a colourful history," which he also authored alongside Liaaen-Jensen, S., and Pfander, H., in 2017. Other sources also served as guidelines, such as the timeline made by Kemin Industries[24] and the article "The discovery and early discovery of carotene" by Theodore L. Sourkes[25]. The present article, however, focuses mainly on lutein, it provides details of the earlier discoveries and context on how lutein began to be produced, discusses the current source of commercial lutein, and offers some insight into its alternatives.

For historical accuracy, this study aimed at the original sources of information. To reach these sources, the citation cascade was followed backward until the earliest document was reached.

The research used ancient documents recovered from online libraries such as HathiTrust, the Internet Archive, and Gallica (the digital library of the Bibliothèque nationale de France, BnF). Patents were retrieved using the Google Patents research engine. For sources in languages other than English, translations were done using AI language models, including Gemini 3.1 Pro and Perplexity AI.

4. Historical background

After getting some background on the lutein molecule, it becomes clear why it has attracted so much interest. Nevertheless, the journey to reach that knowledge was quite long.

4 1. Early discoveries

The earliest evidence related to lutein found during this research was the observation of a "yellow spot" in the human eye by Francesco Buzzi in 1782. He was developing a method to analyze the functions of the eye in detail, thus designing an "artificial eye" made from a cadaveric eye and wax. He analyzed the eyes of people with jaundice, cataracts, and healthy eyes and found that a lateral point next to the optic nerve was always yellow[26].

Some years later, in 1799, Samuel Thomas Soemmerring claimed no one else had previously found that same spot in the middle of the retina. He narrates how on the 27th of January in 1791, he was examining the eyes of a recently drowned young man through an undisclosed liquid which was used to swell the eyes to observe the retina. While presenting it to his class, he realized the presence of that yellow spot; he corroborated with the other eye that it was something common for both of them and not an anomaly. He also asked the young man's family if he suffered from any disease, and they declined. He then decided to study more healthy eyes to deepen his findings. He was able to describe a way to locate it, but found it very difficult to analyze further as the retina was very fragile. He acknowledged this and left the task of uncovering its properties to future scientists[27].

What was clear was that this yellow spot was a common component of the human eye which would be known as the *macula lutea*, where lutein and zeaxanthin are stored, its location can be seen in Figure 2. An important part of these discoveries were the techniques needed to prepare them. Firstly, at the time, there was no way to analyze the macula without removing the eye, hence both discoveries using cadaveric

samples. Moreover, the eye does not retain its structure naturally due to the loss of fluid which aids in the retention of eye pressure[28], thus both scientists had to figure out how to keep its integrity. Buzzi managed to separate the retina from the eye while retaining its structure using water, while Soemmering managed to analyze the whole eye retaining its integrity while filling it with the undisclosed liquid mentioned in the previous paragraph. Another thing to consider was the technical limitations at the time. One of them was that they had to use candles or daylight as sources of light, or figuring out how to make completely black backgrounds to contrast the image in order to be able to appreciate the macula[29].

Both macular pigments, lutein and zeaxanthin, belong to the xanthophylls, and they are not only common in the human eye but also in plants. The early discoveries of these carotenoid pigments in plants will be discussed in the next section.

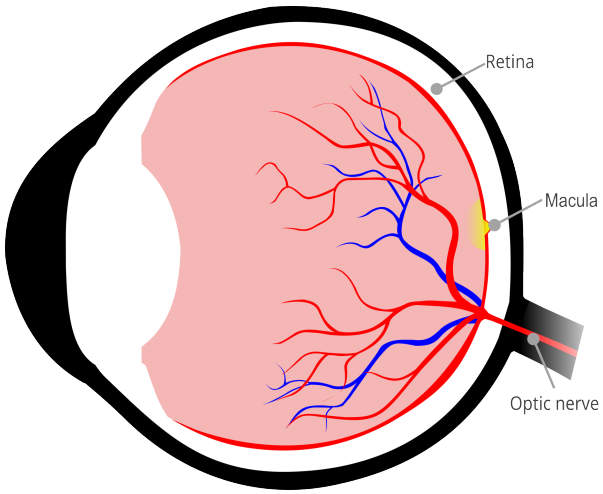


Fig. 2. Graphical representation of the location of the macula lutea in the human eye.

4 2. First vegetable extracts

In 1821, in the United States, Dr. John Gorham of Harvard University was analyzing a variety of corn called "Indian corn" because it was an important part of public nutrition. There were two varieties of this corn, a yellow one and a white one called Virginian corn. He decided to analyze the yellow one. Firstly, he measured 100 grains of corn, ground them with water, and filtered the resulting mixture. He analyzed the water-soluble fraction on one side, added alcohol to the insoluble fraction, and let it rest for 24 h. He then evaporated the solution and found a yellow, soft, ductile, water-displacing, waxy substance. Not only was it soluble in alcohol, but also in oil of turpentine and sulphuric ether. It represented 3 grains out of the 100 total grains of corn evaluated. He named this substance *zeïne* (later known as zein). He tried to analyze the coloring matter of corn on its own and found that it was soluble in both water and alcohol, and that it turned green with alkali and red with acids[30]. This discovery is of great importance, even though it seems unrelated. Zein in itself is an alcohol-soluble protein, which derives from the processing of corn. It was found to have multiple applications, thus the need for its extraction and purification. However, as Gorham's discoveries show, when extracting zein with alcohols, one must likely extract xanthophylls as well. Thus, to separate them from it, hexane is added, which finally separates xanthophylls from zein[31]. This extract, which at first was only a residue, later became one of the first patented "xanthophyll oils" used as poultry feed to color chicken skin[32]. However, while further in the future the residue gained importance, the opposite happened to zein itself, its high production costs and problematic hygroscopicity made it so that its market dropped dramatically between the 1950s and 1960s. In the 2000s, its interest got renewed due to its biodegradability, however, a lot of information was lost due to the previous information not being preserved properly[33].

Of course, at the point of Dr. Gorham's discoveries, xanthophylls were not called as such just yet. As a matter of fact, that name began to be used 16 years after his research. In 1837, Jöns Jacobs Berzelius published a text elucidating the cause of the yellowing of deciduous trees. He mentioned the observations of someone called Macaire Prinsep, who claimed that in autumn, leaves cease to release oxygen and start absorbing it, thus producing an acid that causes the coloration. At the time, popular belief was that this acid could be neutralized to prevent this color change. He strongly disagreed with these observations, as the yellow leaves could not regain their green color by adding any reagent. Moreover, the red leaves turned green only because green compounds were formed by interaction with alkali. He took it into his own hands to explore what made leaves yellow. Thus, he stored yellow leaves of the lemon tree and the *Pyrus communis* species in a bottle containing 53.3% alcohol for 48 h. The leaves bleached, while the alcohol turned yellow. After removing the alcohol, the leaves turned brown due to exposure to air, but when the alcohol was added again, some yellow could still be extracted. The alcoholic solutions were distilled until he obtained a brownish-yellow liquid with a yellow, greasy substance that contained the yellow coloring matter of the leaves. He managed to saponify it, although he recognized he could not purify it. He mentioned it had bleached completely due to exposure to air and light, and that it may be due to a reaction of the leaf green to the cold weather. He called this pigment "leaf yellow" or xanthophyll[34]. This could be one of the earliest records of actual xanthophyll extraction, or at least one of the first acknowledgments of xanthophylls as a coloring matter. One year later, Berzelius further analyzed leaf green (chlorophyll) to understand its properties. By using different methods, he observed that the leaf yellow appeared in both fresh and dried leaves, as well as in other substances with red/yellow color that did not dissolve in alcohol. This method was highly important because it enabled better separation of chlorophyll from other pigments[35]. Those substances that were not dissolved in alcohol were most likely other carotenoids.

In 1855, P. Harting decided to evaluate whether the chlorophyll in phanerogamic (seed-producing) plants was the same as that in algae, since Ångström had claimed that the chloro-

phyll from plants was different from that of algae because they had a different spectrum. Harting disagreed, claiming there were no differences between the chlorophylls in their pure state, but that the difference relied in the substances that surrounded chlorophyll, which modified their optical properties. Firstly, he decided to use daylight instead of candle or lamp light, as had been used by Ångström and Stokes to analyze the spectrum. He did so using two glass prisms, an achromatic lens, and a white screen inside a black room. The light passed through the glass prism containing the analyzed liquid, and the spectrum was projected onto the screen. He then placed a sheet of paper over the projection to draw the transmitted spectrum. This system could be considered the precursor of the spectrophotometer. However, it is interesting to see the differences in dimensions and the labor required to use it, as well as the dependence on favorable climate conditions. Here, he also noted that the light from a fire was different from that of daylight, the former giving a broader, more defined spectrum and the latter showing greater intensity on the red side. Sadly, the author of this article was unable to retrieve the original images of the spectrum variations, as it would have been interesting to see the comparison. However, Harting did the following: he compared the spectra of the ether extracts of the phanerogamic plant *Syringa vulgaris* and the alga *Spirogyra maxima*. He intended that the extract's intensity and the sample's thickness in the prism be equal. He noticed that in the former, there was less absorption in the blue and yellow spectra. However, he thought the differences were too small to conclude that they were due to chlorophyll, so he isolated it using Berzelius's method and reevaluated the spectrum. He observed that the extract was different in color (more blueish) than the unpurified one, and it behaved differently, as its color did not change upon exposure to light. Through the spectrum analysis, he noticed that the purified chlorophyll absorbed less and shifted slightly in direction. Thus, he concluded that there was no difference in chlorophyll based on the spectrum, but that these differences came from the substances surrounding it[36]. Such substances must have been different types of carotenoids, which are able to absorb at wavelengths that chlorophyll cannot. Their variability must have been due to the different composition of carotenoids of the samples used. This is an indirect implication of the light-absorbing properties of carotenoids and, perhaps also a hint at the blue light absorption of xanthophylls.

4 3. Color theory in the function of yellow pigments in the human eye

At the same time, significant breakthroughs occurred in ophthalmology and color theory, beginning with the development of the ophthalmoscope in 1851 by Hermann von Helmholtz, which enabled the examination of the retina in living eyes, including the "yellow spot" (the *macula lutea*)[37]. The context of this tool was also interesting, as Helmholtz had a military background and when he was released from his service he started teaching to then been offered his position as a physiologist. Since his teaching skills were considered by his peers to be lacking, he opted for practical explanations, hence the use of the ophthalmoscope to explain his class. This development was key, as it allowed to study the eyes without removing them from corpses[38].

At the time, Helmholtz travelled the world to discuss his research, at some point he reached England, where he was not convinced about the quality of the local research, however when he went to Scottland, he was fond of their ways of doing science and engaged in conversation. At the time Thomas Young's research on color science was relevant and he was using it as a baseline for his own research on color theory[38]. He was not the only one using Young's research as a reference, as James Clerk Maxwell, a Scottish scientist was doing as much. He was studying color perception at the University of Edinburgh and during his research he managed to identify common causes of color blindness through his own inventions to view the retina[39]. Thus, Helmholtz discussed his observations with those of James, who, just like he did, managed to observe the yellow spot through a prism only in the blue spectrum[40]. Britton *et al*., in their book "Carotenoids: a colorful history," recognized the importance of these early methods in identifying various phenomena that ultimately enabled accurate identification of this molecule[23]. In one of Max Schultze's lectures, the speaker seemed to imply that discussions arose between Helmholtz and Maxwell. Here, he also mentioned that he had extracted a water-insoluble pigment from the yellow spot in the macula and that it might have blue-light-absorbing properties[41]. Helmholtz's arrival at the same conclusion was confirmed in 1867[42]. At the time, they did not even think that this same substance could be related to those plant pigments; however, these properties later provided better insights into its identity. It is also worth noting that both the spectrum of the plant pigments (section 3.2) and the eye pigments (current section) were being analyzed at the same time. However, there was no mention of one another in either of the research. This is one of many examples of the limitations of research at the time. Some were geographical differences, and others were the over focus of science, scientists were not able to look past their own focus, thus they could not enrich their knowledge with that of other disciplines.

4 4. Uncovering carotenoids and xanthophylls from plant origin

The history of lutein and xanthophylls overall cannot be explained without carotene, as their origins are quite intertwined. The origins of carotene also trace back to the 19th century. In 1831, H. Wackenroder published his own discoveries on the cultivation and use of carrots and carrot juice. His objective was to uncover its components and isolate what he could. He also compared his observations with those of previous analysts of carrots, such as Vauquelin and Hofrath Doebereiner. To obtain carotene, he first precipitated the mixture from the carrot juice with alcohol and nitric acid until it turned white. Then, he applied heat below the boiling point, which allowed the precipitate he called “albumin” to coagulate, clarifying the liquid. He separated the “albumin” and added ether. Then he added “strong alcohol” to

dissolve its fatty part, and he evaporated the ether to obtain ruby-red scales. He found it was not water-soluble but was easily soluble in fatty or essential oils. When dissolved in fat, he observed it bleaching as the fat went rancid, a sign of its reaction to oxygen. Due to its unique behavior compared to other plant pigments, he named it carotene[43]. An interesting characteristic of these early methods of isolation was their empirical quality. Several researchers, like Berzelius or Wackenroder used several acids or solvents to observe what happened with the pigments they extracted. Then they found which of them caused crystallization to isolate them.

The isolation of carotene sparked curiosity about its structure; other scientists took on the task of uncovering its composition. The process of obtaining this formula was quite challenging because fatty acids kept interfering. Then, in 1846, Professor Zeise developed a process to purify it as much as possible. He first added sulfuric acid to the carrot juice to precipitate its coloring components. After washing it, he made a saponification step with potassium hydroxide. Then he performed several washes with water to remove any remaining alkali. He continued with a second round of acid washing followed by increasingly stronger alcohol washings. The key step in the process was the solubilization of the washed matter in carbon disulfide, which he then concentrated by distillation. He continued to remove fat by the continuous mixing with alcohol, to be then filtered and washed with alcohol again. He finally obtained carotene crystals of copper color and luster. They were insoluble in alcohol and slightly soluble in ether and acetone. Through combustion analysis, he elucidated that its elemental composition was C_5H_{10}[44]. Later, he reported it as C_5H_8 in another publication[45]. Some people questioned this formula, though, August Husemann being one of them. He modified the extraction method, using both sulfuric acid and a gallic acid tincture in the precipitation step. He spread the precipitate in the open air, and it formed thin lamellae that were soluble in carbon disulfide and boiling alcohol. He called this thin layer hydrocarotene (proposed formula: $C_{36}H_{30}O_2$). He extracted the carotene by applying carbon disulfide to concentrate it, then washing it with absolute alcohol. He obtained crystals of red-brown color and a velvet luster. He observed its instability in light, noting that the crystals were completely bleached after 3 weeks of exposure to sunlight. This bleaching also changed its physical properties, making it less soluble in carbon disulfide and more soluble in alcohol and ether. He proposed a new formula: $C_{36}H_{24}O_2$[46], and for some time it became quite accepted, although it did face some scrutiny. It is important to point out some context about August Husemann, he was a German born scientist established both in Germany and Switzerland. He was very reputable and multifaceted, as he liked to explore different axis of science, he went from pharmacy to phytochemistry to forensic chemistry. All his carotenoid studies happened while he was studying his doctorate under Professor Bödeker. After he finished he started to explore forensic chemistry, but his career had to halt abruptly due to illness, hence he was not able to respond to criticism right away and afterwards he was not so fond for carotenoids anymore[47].

Finally, in 1867, he published a response to his detractors who claimed that both his carotene and hydrocarotene were, in fact, plant cholesterol, based on their reactions with sulfuric acid, iodine, ferric chloride, and hydrochloric acid, and the shape of their crystals. Husemann previously mentioned that carotene had squared crystals, while the other mentioned that it had rhomboid crystals. Husemann proceeded to disregard their claims and to describe each substance separately to clarify their distinctions. He also claimed that the other authors did not produce a "pure" carotene sample as he did, and he also disregarded the quality of the journal in which they published their observations[48]. In retrospect, the fact that the data were questioned may have reflected the lack of repeatability of Husemann's results; it also shows another aspect of the historical context, namely, the competition to be in the right rather than to pursue further research. Also, this may suggest that discussions about carotenoids were confined to specific circles, leading scientists at the time to ignore related breakthroughs emerging through other routes.

Such is the case of what happened in 1868, when Johann Ludwig Wilhelm Thudichum used the term "luteine" to classify a yellow crystallizable substance present in various parts of animals and plants. He said it was observed in the corpora lutea of the ovaries of mammals, in blood serum, in adipose tissue, in butter and a yellow secretion of the mammary gland, in egg yolks, and in various parts of plants, like in seeds (like corn), fruits, roots, leaves, and in the stamens and petals of flowers (like marigold). He also mentioned this substance was not soluble in water, but in alcohol, ether, chloroform and albuminous liquids. Regarding its spectral characteristics it had "great brilliancy" on the red, yellow and green and an absorption in the blue, indigo and violet part of the spectrum. He also pointed out its "affinity to fats and how it was very difficult to separate it from it in the corpora lutea and egg yolks. He mentions that alcohol is effective to extract it from vegetable matter, although in maize it is accompanied by a lot of fat, similar to the egg yolk[49]. This might be the first time ever recorded when lutein was mentioned as such. Although his observations were based on non-purified samples, they were very accurate at the time. Nevertheless, maybe due to the type of publication where it appeared, which was from a medical perspective rather than a botanical one, his results went unnoticed by the botanists focused on carotenoids and xanthophylls. However, there were other factors at the time which influenced this lack of recognition as well: credibility and reputation. Thudichum was a established scientist at the time, he was a German born, English naturalized scientist. He studied under the wing of one of the most respectable German scientists at the time Justus von Liebig, most of the German research regarding carotenoids at the time can be seen published in Liebig's Annalen der Chemie. However, Thudichum had a bad reputation, he was regarded as a solitary scientist, who had no students, assistants or anyone to carry on with his research. His contemporary peers considered his research too far-fetched and obscure, his studies, including the ones regarding lutein were recognized several

years later[50, 51].

4 5. Elucidating the identity of carotenoids

By 1883, there seemed to be some frustration with the lack of success in separating chlorophyll from its accessory pigments, so Borodin approached it in a different manner: a microchemical approach. He shredded, washed, and paper-dried samples of the alga *Spirogyra* and macerated them with alcohol for 24 hours in the dark. He recovered the liquid and examined it under dark-field illumination in a polarizing microscope. He observed bright crystals and classified and characterized them based on their solubility and color. Some were soluble in benzene and had either a red or a violet color. The alcohol soluble substances were yellow in color. These he assumed were xanthophylls and mentioned more than one might have been present[52]. However, by this point, since there were many attempts to isolate the various substances from chlorophyll, people kept giving new names to their apparent discoveries. Tschirch, again recognizing that chlorophyll had not been completely isolated in the past due to its strong association with other dyes and its high lability, decided to group these definitions as synonyms to reduce term ambiguity[53].

Another interesting topic started to arise at the time: the function of plant pigments. Of course, at some point, it was already determined that chlorophyll absorbed certain wavelengths of light. However, it was Engelmann who, through his use of the bacteria method, identified that absorbed light was converted into chemical energy, possibly a precursor to the idea of photosynthesis. He also noted that light was absorbed differently depending on the type and concentration of the plants' pigments[54]. This phenomenon is referenced in early documents discussing the function of carotenoids, making this document one of the earliest pieces of evidence on the topic.

In France, a discussion arose regarding the right carotenoid structure and function. It all started with Arnaud's research in 1885. Albert Arnaud was a French scientist. He showed interest in science early on and self-taught himself how to work in a laboratory making a rudimentary one at home. Through his talents he became the assistant of a very illustrious chemist at the time, Professor Chevreul. Chevreul was an expert in fatty acids and their isolation. At some point he caught interest in the coloring matters of leaves and asked Arnaud to test the methodologies from different authors[55]. He identified the presence of carotenoids in leaves such as spinach, mulberry, peach, maple, and ivy, as well as in carrot roots and pumpkin. The ubiquity of carotenoids was interesting to him, as it suggested that plants commonly possess both carotene and chlorophyll[56]. However, he said that because he did not have the materials at the time, he used Husemann's formula as a reference. Hence, he decided to corroborate it himself; he processed between 500 and 600 kg of carrots to obtain several carotene samples and examine them. The principles of the process were similar, though they were implemented differently. For example, the first step in precipitating the coloring matter from carrot juice was adding lead acetate. The precipitate was dried in a vacuum and then dissolved in carbon disulfide. This difference was key: on this first step, Husemann dried the precipitate in the open air, while Arnaud did so in a vacuum. This was a conscious decision from Arnaud's part, as he wanted to remove the effects of heat, water and oxygen from the equation[55]. The rest of the process was carried out similarly, although Arnoud added a purification step: adding a little carbon sulfide to the carotene obtained and precipitating it with excess alcohol. Afterward, he dissolved it in benzene, evaporated it, washed it with alcohol, and dried it under vacuum. He obtained 3 g of carotene from 100 kg of carrots (nowadays it is known that a raw carrot has in average 7.975 g of β-carotene per 100 kg of carrots[57]). The first thing he observed was that carotene oxidized very quickly in air, both at room and high temperatures. He placed 0.314 g of the sample in an oven at 70°C for 72 h until the weight stopped increasing. He observed that it went from blue to brick red, and the mass increased to get 21% of oxygen. The oven also changed its solubility and its ability to form crystals. He also noticed that after a few months, it lost all color. This discoloration made him realize another challenge in carotene isolation: avoiding oxidation. He observed that if he performed an elementary analysis of the carotene immediately after its preparation, there were no signs of oxygen, or very few. Thus, he decided to analyze it in a way that was difficult to oxidize. He tested mixing it with chloride or bromide with no success; he found that the iodine derivative of carotene was very stable and not easily oxidized. So, to a solution containing excess carotene in dry benzene, he started adding iodine crystals, which formed a benzene-insoluble compound that precipitated. He washed and dried the crystals in a vacuum and performed the elemental analysis again, observing that there was no oxygen present, only carbon, hydrogen, and iodine. This analysis led him to conclude that carotene was an unsaturated hydrocarbon with the formula $C_{26}H_{38}$, and he proposed calling it *carotène*[58]. The same year, he also debunked another of Husemann's discoveries: hydrocarotene. He identified it as vegetable cholesterol, as Husemann's detractors pointed out before; this compound would later be known as phytosterol[59]. He advanced very fast with his carotenoid studies. He still wondered how green leaves could contain carotene yet still look green. So he developed a colorimetric method to associate color with carotenoid concentration. He used a Duboscq colorimeter and performed a proportionality test to determine whether color was directly proportional to concentration. It was done by measuring the color of 15 and 30 g of leaves and their carotene yields, which were 11.57 and 23.45 g, respectively. This way, he obtained approximations of the carotene concentrations in spinach, nettle, and grass[60]. He extended this study to other plants at different stages of their lives and under different culture conditions. He observed that, for example, in nettle and horse chestnut, concentration increased to a maximum during flowering and then decreased continuously until leaf fall. In bean leaves, he also observed that carotenoid concentration decreased in the dark; normal bean leaves had 178.8 mg of carotene, while light-deprived ones had 34 mg.

He mentions how this behavior was similar to that of chlorophyll. He pointed out that its function in the plant must be important because it was strange that a hydrocarbon that absorbed so much oxygen (up to 24% of its weight) remained unaltered in the leaf. This property led him to suggest that carotene might undergo cycles of oxidation and reduction, allowing a stable proportion over time[61]. Arnaud's discoveries suggested yet another function of carotenoids, their antioxidant activity, opening a new line of research regarding carotenoids.

Other scientists were continuing Tschirch's and others' research regarding identifying chlorophyll and separating it from other pigments. Monteverde did so by comparing the absorption spectrum of various living leaves. He did so because, as Tschirch, he was bothered by the heterogeneity of the nomenclature surrounding chlorophyll. He classified the pigments into the yellow ones (carotene and xanthophyll) and the green ones (amorphous chlorophyll and crystallizable chlorophyll)[62]. Following the absorption analysis, Tschirch developed a method for analyzing ultraviolet spectra using quartz prisms, which are transparent to ultraviolet light, on an apparatus he called a quartz spectrograph. He wanted to prove further that, even though plants contain several yellow pigments, they are all different and do not absorb at the same wavelengths. One of them, which he called narrow-sense xanthophyll, had a behavior no other yellow pigment had; it absorbed only in the violet/ultraviolet part of the spectrum. This narrow-sense xanthophyll was extracted from a previously saponified alcoholic solution, which was further extracted with ether. Since only the xanthophyll and phytosterol remained in solution, he removed the latter by continuous crystallization until no further crystallization occurred. The latter solution was placed into a refrigerator, and new crystals formed; he identified them as xanthocarotene (most likely what is known nowadays as a xanthophyll), as they behaved similarly to carotene. What was left was a yellow oil. This oil was resuspended in petroleum ether, and iodine was added, resulting in a green precipitate. After the iodine was removed, the liquid remained yellow. This liquid was identified as narrow-sense xanthophyll, and he found it prevalent in *Corydalis lutea*, *Calendula officinalis*, *Carthamus*, lemon peel, and mace[63]. This ultraviolet absorption distinction was key to differentiating true xanthophylls from other yellow pigments, as modern xanthophylls absorb in the visible spectrum but are transparent to ultraviolet light. In contrast, other compounds, such as flavonoids or oxidation products, do not exhibit this property.

Years later, C.A. Schunk spectroscopically confirmed that the pigment in flowers such as calceolaria, nasturtium (*Tropoelum*), wallflower (*Cheirantlius cheiri*), African marigold (*Tagetes erecta*), daffodil (*Narcissus pseudonarcissus*), and leaves was the same as that of egg yolk. He did so using photography, Iceland spar prism, and quartz lenses with a Welsbach incandescent gas mantle. To extract from the flowers, he first performed an ethanolic extraction and removed interference (ultraviolet-absorbing pigments) via a water extraction and a saponification step, followed by a solution of carbon bisulfide. He did this to preserve the absorption properties of the xanthophylls, which were compromised by other extraction methods. Through this extraction, he identified 3 types of xanthophyll, L, B, and Y, the first one most likely being lutein. He repeated the same extraction method as with flowers for the egg yolks and identified the same L xanthophyll as in the flower species mentioned above. It is worth noting that complete separation of the pigment from the fats was unsuccessful, and that further attempts to saponify it yielded a very low yield of pigment[64].

4 6. Determination of the properties of carotenoids

In 1904, Tschirch wondered whether it was possible to isolate the yellow pigments, as Arnaud had done with carotene, to finally elucidate their chemical formula. He decided to go through a different route by using capillary analysis. This method consisted of him using strips of degreased paper used for the Adams milk analysis method, 5 cm wide, 18 cm long, and 1 mm thick. He immersed these pieces of paper 1 cm deep into a flower or leaf extract, which was positioned in a cylindrical container with a flat bottom. The paper strips remained there for 8 h, were dried, and showed different color zones. The most intense yellow zones were cut and extracted with alcohol. The process was repeated until the yellow zones were pure. Then he used the quartz spectrograph he had previously developed to analyze its spectra. Using this method, he observed several things. Firstly, there were only two types of yellow color in the leaves: xanthocarotene and narrow sense xanthophylls. The first one turns into the second one upon exposure to light, since the yellow pigment readily changes. He also observed that these yellow pigments are normal accessories of chlorophyll, as they were visible in freshly prepared leaf extracts. He also observed that the more processes and attempts to separate xanthocarotene from phytosterol there are, the more it is transformed into narrow-sense xanthophyll. However, if the capillary method were used, xanthocarotene could be recovered easily while maintaining its properties[65].

In 1907, Richard Willstätter and Walter Mieg determined the molecular formulas of carotene ($C_{40}H_{56}$) and xanthophylls ($C_{40}H_{56}O_2$), which are still used to this day. He performed the extractions using Arnaud's and Kraus's methods. They also described them as mentioned in Table 1. Overall, the solubility and chemical formulas are consistent with current beliefs. As for the melting point, it differs from the information available nowadays, perhaps due to purity issues or mismanagement of the carotenoids resulting from the methods employed for their extraction. For example, carotene was extracted from the leaves by adding 120 L of petroleum ether to 100 kg of nettle leaf meal. It was mixed with an alcoholic potash to remove the chlorophyll, then evaporated under vacuum to 3 L. The carotene was precipitated with absolute alcohol, yielding 3.1 g of carotene crystals. They used the chlorophyll saponification residue to extract the xanthophyll. Then, it was precipitated with petroleum ether, boiled with acetone, and crystallized from wood spirit. In this case, they obtained 12 g of crystals. Another interesting property

Table 1. Properties of carotenoids and xanthophylls according to Willstäter and Mieg, 1907.

Feature	Carotene	Xanthophyll
Formula	$C_{40}H_{56}$	$C_{40}H_{56}O_2$
Appearance	Coppery leaflets	Pleochroic, dark brown-red plates
Transmitted Color	Red	Yellow to orange
Melting Point	167.5–168 °C	172 °C (decomp.)
Petroleum Ether	Considerably soluble	Insoluble
Alcohol	Cold almost insoluble	Cold fairly difficultly; hot fairly easily
Acetone	Very difficult to solubilize	Easily soluble
Carbon Disulfide	Effortlessly soluble	Very difficult to solubilize
Molecular weight (g/mol)	532 (theoretical), real: 521, 523 and 564	564 (theoretical), real: 506, 504, 524
Spectroscopic bands	I. 488-470	I. 480-470
	II. 456-438	II. 453-437
		III. 2 Bands towards the violet in the blue/indigo region

they analyzed was the absorption bands, as the authors noted a shift in the violet region for the xanthophylls, which aligns with their blue-light-absorbing properties. They suggested that both carotene and xanthophylls might act as oxygen carriers in plants[66].

4 7. The study of lutein of animal origin

As the reader might have seen earlier in the text, authors such as Thudichum in 1868 and Schunk in 1903 had already identified similarities between egg yolk pigments and plant pigments in a very clever way, using spectra and, in Thudichum's case, physical properties as well. However, the details of the egg yolk pigment extraction have yet to be given. Although it all begins not with egg yolk but with the *corpus luteum*. As explained at the beginning of this text, the name lutein derives from the *corpus luteum*. This part of the body was first identified by Regnier de Graaf in 1672, in his text "*De mulierum organis generationi inservientibus*" (On the female organs serving reproduction), where he identified that females produced an egg in their testicles (now known as ovaries) and that such an egg produced glandular bodies after coitus, which could be yellow in cows, red in sheep and ashen in others[67]. Those glandular bodies were later formally called *the corpus luteum* in the Encyclopedia Britannica and described as a yellow coagulum that appears in women's fertile years[68].

Studies of the *corpus luteum* continued, trying to explain its function and to distinguish its components. A historical publication by Theodore L. Sourkes in 2009 gives a clue as to what was happening at the time. He credits Adolf Lieben as the first person to identify carotene in animal tissue. Stanislao Canizzaro invited Lieben to join the faculty at the University of Palermo, where he worked for 4 years between 1863 and 1867[25]. Sourkes gives a very well-thought-out summary of Lieben's research, which focused on the pigment in the *corpus luteum*. However, there are other important details in Lieben's actual research that are worth mentioning. Firstly, Lieben suggested that the changes in pigment in the *corpus luteum* might be caused by the differences in nutrition.

Also, at the time, there was discussion about why the *corpus luteum* formed and whether a hemorrhage was needed for it to form. He, alongside his colleague G. Piccolo, took it upon themselves to elucidate this by examining 200 ovaries from cows and sows at different stages of their lives. One of the first things they observed was that hemorrhages in this area were an exception rather than the rule. This observation is quite important, as some authors have attributed the yellow color of the *corpus luteum* to hematoidin, a blood-derived compound rather than a carotenoid. In this research, they acknowledged this belief but decided to further confirm it. To do this, they started by air-drying the ovaries from cows, then treated them with either ether or carbon disulfide. They filtered and evaporated the residue, then observed it under a microscope. They found several things: an amorphous yellow matter, colorless crystals, red crystals that crystallize better when dissolved in ether rather than carbon disulfide, and other crystals that were not found in all samples. After the observation, they took it upon themselves to separate these substances. First, they took several *corpora lutea*; some were yellow, some atrophied and red, and air-dried them as before, then extracted them with ether. That solution (which was yellow) was boiled in a silver capsule for 8 h with caustic potash to saponify the sample. They tried to remove the insoluble fraction by pouring the contents of the capsule into excess water; however, the resulting emulsion remained turbid and did not precipitate after several days. However, they found achroic crystals (which, depending on their position, appeared blue or red) floating on the surface of the liquid and fished them out. After microscopical examination, they identified them as the red crystals observed before. To the turbid liquid, they decided to concentrate it by evaporation and added even more caustic potash; then, a yellow crust formed, which was also recovered. Since this crust was very difficult to wash due to the excess of alkali, they decided to shake it with ether and water; the ethereal solution turned yellow, and they recovered it. Afterward, they evaporated it and digested it with boiling alcohol. After cooling, some colorless crystals were observed and identified as cholesterol. What was left was a yellow, viscous mass, which they were unable to analyze further; however, this mass was most likely lutein, given its extraction method and characteristics, including insolubility in water and solubility in ether, alcohol, and carbon disulfide. They also conducted an interesting experiment with the red crystals, observing that they became discolored when exposed to sunlight. They also observed that discoloration was

not solely due to light, as samples in the dark but exposed to air showed slower discoloration, while the unexposed ones remained almost the same. They concluded that this might be an oxidation process, which hinted at their antioxidant properties. They found that due to their solubility, these crystals could not be either bilirubin or hematoidin. They referenced another author, Holm, who worked in Georg Städeler's laboratory and found the same crystals; however, as they point out, he mistakenly called them hematoidin. The authors decided to call these crystals luteo-hematoidin or emo-lutein[69]. Although they decided to call the crystals this way, in fact, it was more likely another carotenoid with a lower polarity than lutein. Perhaps lycopene or β-carotene, whose solubilities in ether are 100 and 10 times lower than that of lutein.

4 8. The study of the pigments in egg yolk

The name Georg Städeler was quite important, as in his laboratory, they were not only studying the pigment of the *corpus luteum* but also that of egg yolk. According to an article published in 1867, Michel Eugène Chevreul first observed these pigments in 1823[70]. Although the original reference could not be found, some of his discoveries were confirmed by another scientist, Gobley. When he was trying to isolate cholesterol from egg yolk to prove that it was the same one as that of biliary calculi, he observed that in an alcoholic solution at 88 °C, he could obtain cholesterol alongside the coloring matter of egg yolk. However, the study of this pigment was not of his interest[71]. According to Städeler, Chevreul associated the pigment from egg yolk with bilirubin. Städeler proved that assertion to be wrong by showing why they were, in fact, not the same. Firstly, he extracted the pigment and fats from egg yolk with ether. He then saponified it with baking soda and extracted the pigment with ether. This procedure was enough to prove that the two pigments were not the same, as bilirubin mixed with soda was insoluble in ether. He went further by evaporating the ether extract, obtaining a non-saponifiable, golden-yellow fat that separates from cholesterol into a buttery mass. He associated it instead with the yellow mass contained in the fat of the ovaries, wrongfully calling it hematoidin[70].

A year later, Thudichum published his previously referenced research, in which he associated both the egg yolk pigment, the one in the *corpus luteum*, and the pigment in certain plants, fruits, and flowers under the term “luteine”[49]. As mentioned before, these aligned with Schunck’s discoveries in 1903, although with a different name[64].

In 1911 Richard Willstätter and Heinr. H. Escher took on the task of isolating pigments of animal origin, specifically egg yolk, as no one had succeeded before. They referenced the research of Chevreul, Gobley, Städeler, Thudichum, Schunk, and others, including R. Maly and W. Kuhne, both of whom identified it. They compared it with other pigments but were unable to obtain it in crystal form. They called the pigment they isolated "lutein" and claimed it as the first ever pure representative of this pigment from animal origin. They observed that it had the same chemical composition and physical properties as the plant xanthophylls, especially its optical properties. Although they concluded they were not the same, but isomeric. To isolate it, they needed the yolks of 6000 eggs. accounting for a total weight of 110 kg. They found that acetone was a better solvent for isolating the yellow matter from fat, so after a first extraction with ethanol, they performed a second extraction with acetone and filtered the mixture. Afterward, they left it still, which allowed the oil to separate from it. They proceeded to separate it from both lecithin and cholesterol, which involved several steps of crystallization and manipulation of solubility. Finally, they crystallized it by diluting it in boiling ether and then cooling it down. Several crystals formed, and they had to separate the cholesterol further until they obtained 4 g of crude pigment. An important point to note was that they verified there was no carotene formation. They purified it using methanol and found it looked just like the xanthophylls described by Willstätter and Mieg in 1907; not only that, but it also shared the same chemical formula and molecular weight, with only slight differences in its melting point[72].

4 9. Xanthophyll isolation process and disambiguation

In 1913, Willstätter, alongside Arthur Stoll, decided to publish a treatise on chlorophyll isolation, separation, and quantitative determination, which encompassed all of their discoveries on chlorophyll in the last years of their research; they also included carotene and xanthophyll isolation processes. The authors discussed many facts already presented in this text. They also included Tswett's observations from 1911, who, through chromatographic separation, discovered that the xanthophyll pigment was not a single compound but several; he identified them as xanthophylls α, α', α", and β. However, since he did not fully isolate these pigments, they were not accepted by the community at the time. Willstätter and Stoll recognized the possibility of his claims but agreed that further confirmation was needed[73]. Overall, the document served as a set of guidelines for pigment extraction in Europe. Originally in German, this document was translated into English for the public in the United States by Frank Milton Schertz and Albert R. Merz, due to the growing interest in the chlorophyll industry.

In 1931, Richard Kuhn, Alfred Winterstein, and Edgar Lederer disambiguated the terms xanthophyll and lutein. They did so by proving that lutein from plants, egg yolks, and yellow flowers was the same substance. They fractionated the pigments by chromatographic methods and analyzed each fraction individually for melting point and rotation. All horse chestnut, stinging nettle, meadow clover, yellow corn, alfalfa, grass, the yellow flowers *Tagetes grandiflora*, *Helenium autumnale*, *Rudbeckia Neumannii*, and *Helianthus annuus* all shared the same xanthophyll, which melted at 193 °C. They decided that this xanthophyll would be the one referred to as lutein. While xanthophylls would be the group designated for the hydroxyl-containing carotenoids with 40 carbon atoms, at that time, they were lutein, zeaxanthin, violaxanthin, and fucoxanthin. As for the egg yolk, it seemed the problem lay in the difficulty of separating lutein from zeaxan-

thin, which caused a difference in melting point that sparked the identity disagreement. Another interesting finding was that the lutein found in the *Tagetes* flowers was esterified; he called the lutein dipalmitic acid ester helenien[74].

4 10. The birth of carotenoid applications

As mentioned above, the chlorophyll industry was gaining importance in the United States by the time the book was translated in 1928. The aforementioned importance is evident in the "Foreword" of the document, where Schertz and Merz stated that the United States had been importing 45000 lbs of chlorophyll for the past ten years[73]. Such imports must have come from established dye companies in Europe, specifically in Switzerland, where the first was founded in 1864. Names such as Ciba, Geigy, and Sandoz dominated the market for natural dyes by the beginning of the 20th century, in competition with Germany, the main producer of synthetic dyes. However, due to World War I, German exports were blocked, so Swiss dye exports reached 211 million Swiss francs between 1913 and 1920[75].

The Industrial and Engineering Chemistry journal published several articles on the growth of the chlorophyll industry in the United States, which was promoted by Schertz. Who, before releasing his and Merz's translation of Willstätter & Stoll's treatise in 1928, published an article regarding the commercial applications of chlorophyll and its derivatives. He mentioned that the United States had not yet engaged in chlorophyll production, and that it was being imported at more than $1 USD per pound (translated to 42 USD per kg in 2026). At that time, it was used as a pigment for foods such as cottonseed oil and olive oil, and to color stearin candles, leather, waxes, resins, pomades, ointments, and soaps. They were also evaluating its potential as an anemia treatment[76]. It was Schertz who developed the chlorophyll extraction and purification method, which was then used nationwide in his position at the Bureau of Chemistry and Soils in the U.S. Department of Agriculture. The methodology was based on that of Willstätter and Stoll. In this methodology, they were not only able to benefit from the chlorophyll but also to extract and purify xanthophyll and carotene. Using this process, they obtained 1.2 g of carotene, 3.2 g of xanthophyll, and 40 g of chlorophyll from 6 kg of stinging nettle meal, using 20 liters of petroleum ether, 20 liters of acetone, and 8 liters of methanol[77]. The same year, 1938, William H. Test decided to file for a patent for "vegetable concentrates" intended to retain some potential, mainly provitamin A, as well as vitamins, chlorophyll, carotene, and xanthophylls. He suggested a method for selectively separating all of these substances. Although the description of the discovery was quite broad and unspecific, he was essentially patenting another process for pigment extraction[78]. This fact is a testament to the growing interest in the pigment industry at the time, as well as the increasing value of carotene and xanthophylls.

In the meantime, others were finding uses for xanthophyll extracts; the most prominent one was for poultry pigmentation. The relevance of pigmentation in poultry is determined by its appeal to customers. In some documents from the mid 1950's, it was reported that poultry fed a rich xanthophyll ration exhibited a healthy color of fatty tissue and skin, which was well accepted[79]. To measure these color nuances, several tools had to be developed.

Early on, developments were made in tools for measuring color. In 1886, Joseph Williams Lovibond filed for a patent for the first colorimeter[80], and in 1890, he filed for a variation of it called the tintometer[81]. This invention served as a tool to understand how pigmentation changed in egg yolk, for example. Before 1915, it was believed that the excess of egg laying in hens reduced the amount of pigment in egg yolks. That year, Palmer published his research clarifying that the color of egg yolks, body fat, and serum from hens was due to the hens' diet rather than the hens themselves[82].

This research continued with W.J. Peterson *et al.* publishing a bulletin in 1939 on carotenoid pigments and their metabolism in hens. Here, they further prove the effect of feed pigment (using corn) over yolk pigment, directly determining how much xanthophyll content changed in egg yolks[83]. This research was fundamental as it preceded different uses of corn and its derivatives as feed. An example is a patent filed by Hunter *et al.* in 1941, which describes the use of corn gluten meal or xanthophyll oil as feed to increase the yellow color of poultry skin[32]. This xanthophyll oil is a byproduct of the production of zein, an alcohol-soluble protein from corn, when the water-soluble phase containing the protein is separated from it[31]. This same process was reported by L.C. Swallen and J.B. Gottfried in 1942[84]. These publications by different companies, the patent attributed to Allied Mills Inc., and the article funded by the Corn Products Refining Company show the increasing interest in using xanthophyll extracts as feed.

Thus, chlorophyll-processing companies decided to take advantage of the chlorophyll-producing process to also obtain other high-value products. Such is the case of Valley Vitamins, Incorporated in McAllen, Texas. They started producing chlorophyll because natural vitamin sources were in short supply following the reduction in fishing during World War II. They based their procedures on Tswett's chromatographic techniques because they used activated carbon, one of the company's original products. At the time, carotene and chlorophyll were its biggest sources of revenue, while xanthophylls accounted for only 25% of its income, as they were rarely used as feed. They chose alfalfa meal because it was the most economically feasible raw material, required the least amount of operations, was not seasonal, and grew well in the kind of soil they were working on. It was also what they could afford due to several shortages caused by the war. Apart from Valley Vitamins, two other companies competed by providing the same products: American Chlorophyll, Incorporated, and Midwest Extraction Company. Due to the nature of the extractions and the technology at the time, extraction efficiencies were quite low, 33, 30, and 24% for carotene, xanthophyll, and chlorophyll, respectively[85]. In 1954, American Chlorophyll, Incorporated released a note about the process. By that time, the chlorophyll industry had evolved. In

1952, according to the article, the chlorophyll industry had reached more than $50,000,000 USD in the United States alone. By that time, it was also used in toothpastes, chewing gums, candies, and other medical applications. In this case, they used the techniques developed by Schertz in 1938 for the Department of Agriculture. They rapidly grew; by 1954, they produced pigments in 24 h turns, yielding 400 to 500 lbs of chlorophyll per day at a selling price of $45 USD per pound (equivalent to 1235 USD per kg in 2026)[79].

Interest in xanthophyll production for feed increased, prompting people to diversify their sources. In Kruse's case, he filed a patent for the production of poultry feed using soybean xanthophylls. At the time, the biggest sources of xanthophylls were corn and alfalfa meal. The disadvantages of the first one were that the annual production of corn gluten meal was 20 times lower than the total amount needed to feed the entire poultry population in the United States; at the same time, its xanthophyll concentration was too heterogeneous. This disadvantage was a shared problem with the alfalfa meal, which had another issue: it interfered with the poultry's nutrition due to excess fiber. Soybean oil, on the other hand, seemed to have a more stable xanthophyll content and, at the same time, was more bioavailable due to its emulsifying action[86]. Bioavailability remains one of the main challenges for any supplement to be effective.

Another source was the residue from processing pyrethrum flowers, patented by Hoffman-Taff Inc.[87]. The original process was developed to produce insecticides, as described by Hisao and Kanichi in 1926[88]. This process garnered so much interest that Luis Levy modified it to recover the highest possible amount of byproducts[89]. He founded the company INEXA in 1958. From 1970, they began commercializing lutein extract in Ecuador, and they still do so to this day[90]. This interest indicates how profitable xanthophyll extracts were and still are.

4 11. The production of lutein by marigold flower petals

Although xanthophyll extracts were already being manufactured and marketed for poultry feed, a problem arose: the sources were inadequate. The use of alfalfa meal, for example, had almost no nutritional value for poultry, yet to get a considerable increase in pigmentation of chicken and their eggs, it had to represent almost 10% of the total feed. Seeing this problem, in 1961, George Dewey Lackey Jr. filed for the same patent in multiple countries for a "Procedure for obtaining pigmentation agents for birds and their products" [91–93], which consisted of the use of dry marigold flower (*Tagetes erecta*) petals as feed to increase the yellow pigmentation in poultry skin. He claimed that marigold flowers had a higher xanthophyll content and did not interfere with poultry nutrition. This proposal is particularly relevant in the history of lutein, as marigold flowers are its main source today. Although this knowledge was not new, xanthophyll content from marigold flowers had already been identified in the 19th century[49].

In the 1960s, xanthophyll feed for poultry became more sought after as new products, such as egg noodles and yellow cake mixes, were introduced that required egg yolks with an intense yellow color. Thus, in 1965, Peter M. Grant of Eastman Kodak Co. filed a patent for a treatment of marigold petals to obtain a xanthophyll extract that could be more readily integrated into feed, since the marigold flower meal developed by Lackey had low bioaccessibility. The patent was granted in 1970[94]. Thus, further studies on marigold flowers' xanthophyll content and comparisons to other sources arose. It was found that most of the pigments in marigold flowers were xanthophylls, which were highly esterified with palmitic acid (60.4%), myristic acid (22.6%), stearic acid (14.4%), and lauric acid (2.4%)[95]. Other researchers, just like Grant, were wondering if (like Grant) the addition of xanthophyll extracts instead of the whole meal was effective to increase the pigment content on the chicken skin. They observed that the xanthophylls in the alfalfa and corn meals were highly present in a free, unesterified state. By this time, specific xanthophylls had already been identified as responsible for the yellow pigmentation: lutein, zeaxanthin, and cryptoxanthin. So they compared the xanthophyll extracts from alfalfa and corn meal (adding a pure standard of lutein made from marigold flowers) and compared them to the whole meal, identifying that the extracts had a higher availability than the whole meal, and also identifying the esterified forms of the pigments in the chicken skin[96].

The production of marigold flowers for feed was not limited to the United States. Marigold flower, also known as *flor de cempasúchil*, is a native plant of Mexico and was originally used in traditional medicine. At the end of the 1960s, Mexican business owners in the northeastern part of the country recognized marigolds' potential as a poultry feed ingredient. They began making rudimentary extracts and selling them locally. By 1977, the owners joined forces to create Prodemex (now Industrias Vepinsa, S.A. de C.V.), which produced the pigments industrially[97]. In 1966, the company Industrial Orgánica (IOSA) was also founded[98]. The birth of all these companies established to date is a testament to the profitability of lutein. It was also needed for the development of new scientific discoveries, as most of them provided the sources for scientific research. An example of this occurred in 1972, when using marigold petal meal from Florafil S.A. (formerly Vepinsa), they managed to separate 17 pigments from marigold flowers, identifying that lutein plus zeaxanthin accounted for approximately 92% of the total. For example, in the Mexican variety, lutein accounted for 88% of the pigments, while zeaxanthin accounted for 3.6%[99].

On another axis, helenien, the lutein dipalmitic acid ester identified by Kuhn *et al.* in 1931, was used for a completely different purpose. In 1956, it started being sold in Japan under the brand name of Adaptinol by Bayer Yakuhin. It is still sold to this day and is used as a drug in the treatment of retinitis pigmentosa. It is said to promote the aerobic metabolism of the retina, either expanding the visual field or delaying its narrowing. It is also said to improve vision in the dark[100].

With the popularization of HPLC analyses and the development of model diets containing carotenoids, the industry

grew and grew. However, it reached a plateau due to the limited availability and high cost of high-purity standards for lutein and its diesters. Thus, Julius Tyczkowski and Pat Hamilton decided to develop a purifying method for lutein using marigold flower petal extracts. By that time, Kemin Industries, Inc. and Lynteq, Inc. were already producing them at high concentrations; examples include Kemin's OroGlo and OroGlo 7, which contained 14.7 and 7.2 mg/g of lutein, respectively. They obtained a fairly cheap extract ($70 USD/mg or 172 USD/mg in 2026), which, depending on the source's lutein concentration, yielded better results. For example, their extraction from OroGlo yielded a 99.2% pure lutein product. In contrast, their OroGlo 7 extraction had a 96% pure product[101]. The 90s marked a new era in lutein research, mainly focused on its effects on human health.

4 12. Lutein and its effects on the human body

Several years after the discovery of the yellow spot in the cornea, people started elucidating its functions. Eventually, it was observed that they were different-colored lenses that absorb light in the violet and ultraviolet regions. In the retina, these pigments were found in the *macula lutea*. In 1945, George Wald wanted to explain the absorption of these specific pigments. He found that, effectively, it absorbed blue and violet light, and its absorption spectrum reflected that of a carotenoid, specifically lutein or leaf xanthophyll[102].

Perhaps this discovery inspired others to research its effects on mammalian eyes. For example, what happened with helenien. In 1957, H. Pfeifer conducted research on whether helenien could increase dark adaptation. This helenien was obtained from petals of *Tagetes patula flore pleno*. He administered it in emulsified and non-emulsified forms because he believed the human body would more easily absorb the emulsified form. By this point, other scientists had already tried to evaluate the same thing. Studnitz, Lovenich, Monje, Cuppers, Wagner, Klaes, Riegel, and Niedermeier concluded that supplementation with Adaptinol and Arovit in both healthy and pathological patients could increase dark adaptation.

In contrast, Wustenberg, Rose, and Schmidt found no effect in healthy patients. Pfeifer evaluated them in 22 patients, 9 healthy and 13 unhealthy. He found that administering the lutein dipalmitic acid ester in emulsified and non-emulsified forms showed no difference in absorption. He also found that, out of the 13 patients with unpaired vision, only 2 with myopia showed slight improvement in this regard. However, one thing to note about these studies is that they assumed the effects of the lutein ester were immediate, as with a regular medicine; they evaluated its effect after 30 minutes of intake[103]. It might not yet be known how lutein moves around the body or how long it takes to settle in the eyes. Another thing to consider is that the substance was already being sold in Japan. At the time of writing this article, no documents from the Japanese literature have been found that could indicate how much they knew about the effects of helenien.

Research continued on the effects of lutein and xanthophylls in mammals. Manilow *et al.* decided to evaluate how xanthophyll levels in the macula and plasma of monkeys decreased following xanthophyll depletion in their diet. They did not see any visual disturbance; the only effect, apart from the axanthochromia (yellowing of the skin), was a change in some physical properties of the retina that did not affect visual performance[104]. This experiment was done more than 20 years later, and the approximation was completely different. For example, the effects were analyzed by comparing animals on different diets over several years rather than several minutes, and this is how they confirmed that xanthophylls must be acquired through diet to settle in the eyes. Kirschfeld in 1982 suggested that macular pigment might play a filtering role in the human eye, based on studies in flies[105].

In 1985, Bone *et al*. finally identified both lutein and zeaxanthin as components of macular pigment[106], thereby corroborating Wald's findings. In the same period of time, lutein was being identified in human tissue and blood by several scientists, like K.W. Miller and C.S. Yang in 1985, or M. Spacewicz-Sapuntzakis, P.E. Bowen, J.W. Kikendal, and M. Burgess in 1987, and David Nierenberg and Sandra Nann in 1992[107], thus, lutein's effect on human health started being studied in depth.

In 1993, the Eye Disease Case-Control Study group found that people with a higher carotenoid intake had a lower risk of Age-related Macular Degeneration (AMD) (Eye Disease Case-Control Study Group, 1993). However, it was in 1994 that Seddon *et al.* first directly linked lutein to that effect[20]. In 1996, Alan Norman Howard *et al.* (The Howard Foundation) filed a patent for "High dosage lutein and zeaxanthin for macula therapy", which consisted of the use of 7.5 mg/day of lutein supplements to replenish pigment content on the macula of people suffering from AMD[108].

In 1994, Frederick Khachik (Catholic University of America) filed a patent for the "Process for isolation, purification and recrystallization of lutein from saponified marigold oleoresin and uses thereof"[109] in order to specifically isolate lutein from marigold flower extracts in order to make standards which could also be used for human consumption. Kemin Industries acquired this patent, thus creating FloraGLO Lutein, the first lutein supplement brand for humans commercially available in the American market in 1994[24]. Further patents focused on developing improved methods for purifying lutein from marigolds to remove pesticide residues and other impurities[110].

4 13. Lutein supplementation: market, challenges and alternatives

As was mentioned before, lutein supplements were developed because of its insufficient intake in a normal diet. The European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA) regard lutein supplements derived from marigold flowers as safe. These supplements also have an Observed Safe Level (OSL) of 20 mg/day[111].

The market size of these supplements was valued at more than 365 million USD in 2024, and it is expected to reach almost $600 million USD by 2032, according to Maximize Market Research (MMR)[112]. Nowadays, the main source for commercial production of lutein is the extraction pro-

cess from marigold flower petals[12]. This process has several drawbacks; it is seasonal and requires large amounts of water and land for irrigation[16, 113]. A significant amount of manual labor is also required[113], mostly for harvesting. Hand harvesting has been proven necessary due to losses of 45-55% in flower yield caused by alternative mechanical methods[114].

Other strategies have been explored to produce lutein, like chemical or biological production[16, 115, 116]. The first one is a complicated strategy. Firstly, because lutein's structure has 3 stereogenic centers in the positions C3, C3', and C6', which means that it can have 8 possible stereoisomers. However, only one of them, (3R, 3'R, 6'R)-Lutein, is found in foods. In early attempts in 1980 by Mayer and Rüttiman, only 1% lutein yield was achieved. After several enhancements, Khachik and Chang achieved an overall 5% yield at laboratory scale by parting from (±)-α-Ionone. The process was so complex that it involved obtaining crystallized ketonitrile from (±)-α-Ionone (28% yield), followed by 4 other reactions (18% yield) to finally obtain the known lutein stereoisomer[117]. If the prices of lutein and its precursor (±)-α-Ionone are brought into the equation (152.26€, 162.00€ per g, respectively, at 90% purity[118, 119]), it is not profitable to produce lutein out of it. Thus, lutein chemical synthesis is far from being industrially feasible nowadays, which leaves biological production as the only possible alternative.

4 14. Biological production of lutein

To be able to understand how lutein is produced biologically, it is necessary to identify how its production mechanism works. The major precursors of carotenogenesis are isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). In the case of microalgae and photosynthetic bacteria, both precursors appear to be synthesized exclusively via the non-mevalonate methylerythritol 4-phosphate (MEP) or the 1-deoxy-d-xylulose-5-phosphate (DOXP) pathway. In the MEP one, two molecules of 2-geranyl diphosphate (GGPP) condense to form phytoene, which becomes phytofluene, ζ-carotene, neurosporene, and lycopene. Here it comes to a branching point where different lycopene cyclases (LCY) act (LCY -β and -ε). The function of the lycopene cyclases is to add -β or -ε rings at both ends of lycopene. Here is when branching occurs: the type of ring added determines the end product. One such example is what happens with β- or α-carotene. The latter is later transformed into lutein by sequential hydroxylation of its rings by two heme-containing cytochrome P450 mono-oxygenases[2, 116, 120]. All the details of this pathway from GGPP to lutein synthesis are shown in Figure 3. However, it is worth noting that it also includes deviations in the route to the production of β-carotene and its derivatives, such as astaxanthin and the phytohormone abscisic acid. Figure 3 indicates whether each step of the pathway is used by plants or by green microalgae and points out the oxygen and metal ions needed for each reaction. The pathway is mostly the same for both higher plants and algae. In a previous study of 22 microalgae from the major classes (Chlorophyta, Rhodophyta, Heterokonta, Haptophyta, and Cryptophyta), researchers found that the framework of carotenoid synthesis pathways was conserved across algal lineages. *Arabidopsis* shares its key enzymes with microalgae as well. However, there are some exceptions[116, 120]. One such example is the astaxanthin branch, which is exclusive to microalgae (Figure 3). According to the resources in the Kyoto Encyclopedia of Genes and Genomes (KEGG), a database for understanding the mechanisms of biological systems, it appears that abscisic acid production is plant specific. However, there is evidence that the enzyme is produced by microalgae, as observed in *Chlorella sorokiniana* in 2017[121].

Microorganisms such as bacteria and yeast have been explored using pathway engineering. It involves integrating biosynthetic pathways or compounds into microbial hosts that do not normally produce them. This strategy has already been explored for the production of common commercial carotenoids such as lycopene, β-carotene, zeaxanthin, and astaxanthin[1]. Some consider this an important green and sustainable production method, which could replace plant extraction techniques[123]. One challenge these strategies face is the limited expression of certain proteins. An example of this is the limited expression of *crtE* in *E. coli* for lutein synthesis. This enzyme is key in the production of lycopene, a direct precursor of lutein. In 2021, Takemura *et al.* engineered an *E. coli* strain capable of producing 11 mg/L of free lutein. The strain needed the introduction of 3 plasmids and the direct integration of genes into its genome However, they were unable to complete the transformation of intermediates such as zeinoxanthin and α-carotene[1].

Another challenge arises from the compartmentalization barriers within the cell that limit enzyme-substrate interactions. This limitation happens, for example, when producing lutein with yeast. Qin *et al*. produced 675.4 mg/L of lutein from *Yarrowia lipolytica,* with a yield of 6.13 mg/g DCW. To obtain this yield, though, they had to implement several strategies. Firstly, the original strain had to be transformed 9 times with several plasmids. They had to develop a way to move the metabolites across the cell membrane to enable the reactions to occur. This relocation was due to the enzyme responsible for converting lycopene into α-carotene, which is in the cytoplasm, whereas lycopene is synthesized in the endoplasmic reticulum. Another important issue is that the strain lacks the proper machinery to protect products as unstable and prone to oxidation as carotenoids. Thus, the need to add exogenous antioxidants during fermentation, such as 2,6-ditert-butyl-4-methylphenol (DHT), was observed in *Yarrowia lipolytica*. Without its addition, lutein production would have been 12.52% of the final amount, likely due to early degradation of both lutein and α-carotene. Even with relatively positive results, the authors did not consider the outcome adequate for industrial application yet[123].

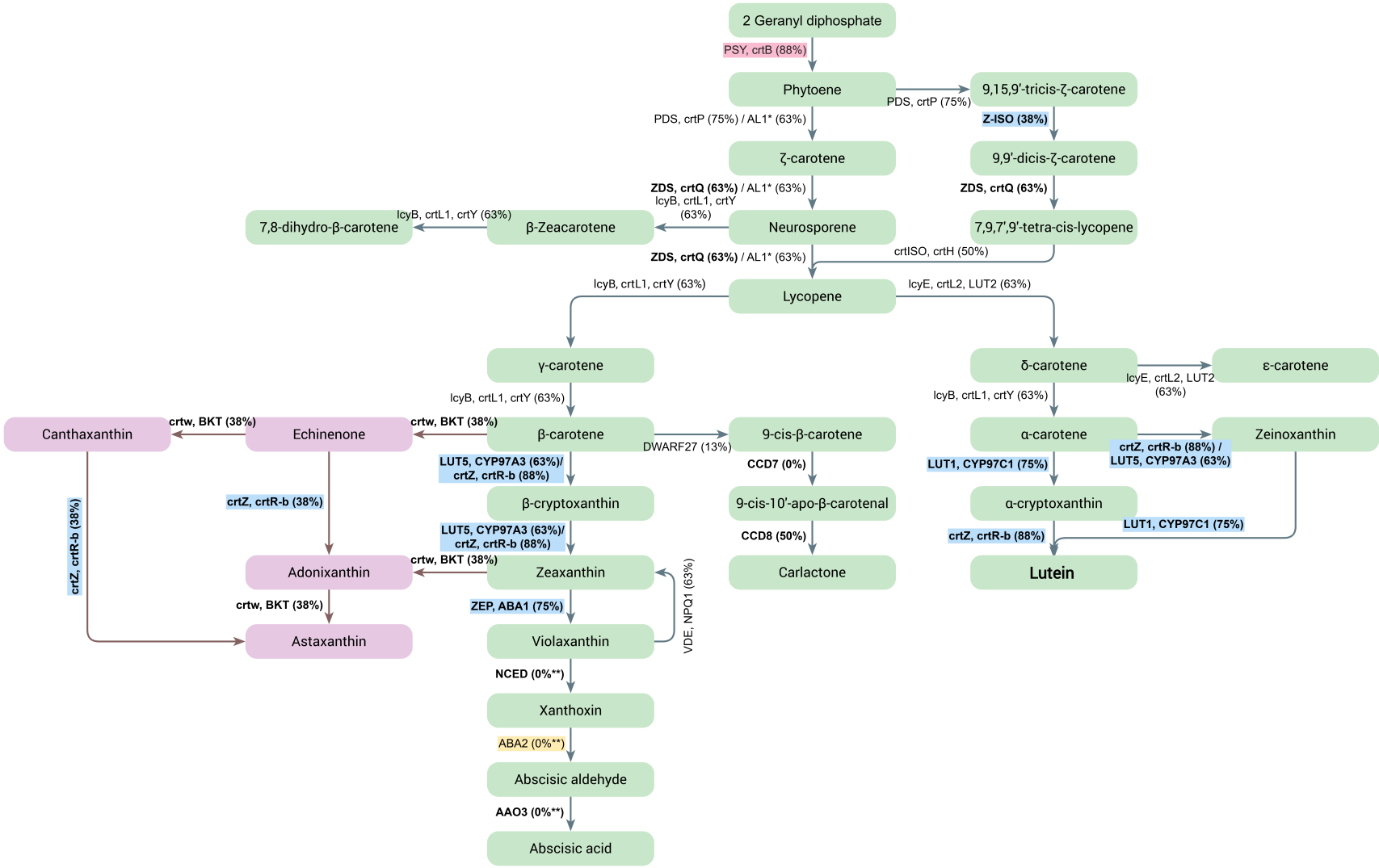


Fig. 3. Lutein synthesis pathway adapted from the KEGG database (map00906)[122]. The green colored boxes are the steps shared by both plants (represented by the *Poaceae* family) and green microalgae (*Chlamydomonadaceae*, *Volvocaceae*, *Selenastraceae*, *Coccomyxaceae*, *Chlorellaceae*, *Bathycocaceae* and *Mamiellaceae*), while the red ones are exclusive for green microalgae. The percentages indicate the proportion of microalgae which share the enzymes. The enzymes in bold signal the need of oxygen for the reaction. The ones highlighted in pink use manganese as a metal ion; blue ones, iron and yellow ones, molybdenum. Abbreviations: PSY/crtB, 15-cis-phytoene synthase; PDS/crtP, 15-cis-phytoene desaturase; Z-ISO, ζ-carotene isomerase; ZDS/crtQ, ζ-carotene desaturase; crtISO/crtH, prolycopene isomerase; AL1, phytoene desaturase; lcyB/crtL1/crtY, lycopene β-cyclase; lcyE/crtL2/LUT2, lycopene ε-cyclase; crtZ/crtR-b, β-carotene 3-hydroxylase; LUT5/CYP97A3, β-ring hydroxylase; LUT1/CYP97C1, carotenoid ε-hydroxylase; DWARF27, β-carotene isomerase; CCD7, 9-cis-β-carotene 9',10'-cleaving dioxygenase; CCD8, carlactone synthase; ZEP/ABA1, zeaxanthin epoxidase; VDE/NPQ1, violaxanthin de-epoxidase; NCED, 9-cis-epoxycarotenoid dioxygenase; ABA2, xanthoxin dehydrogenase; AAO3, abscisic-aldehyde oxidase; crtw/BKT, β-carotene/zeaxanthin 4-ketolase.

Microalgae are other potential competitors for the biological production of lutein. They contain a plethora of useful bioactive components, applied in the healthcare, nutraceutical, and cosmetic industries[124]. These can go from proteins, lipids, and phycocolloids to vitamins, minerals, and pigments[12, 125]. Some of the most common genera for industrial applications are *Arthrospira*, *Chlorella*, *Dunaliella*, and *Haematococcus*[126]. However, they can have high production costs (minimum 5-10 €/kg), which stem from infrastructure, materials, and downstream processes[127]. The use of microalgae for lutein production offers several advantages over other microorganisms, as lutein is a primary metabolite for many species, meaning it has a direct relationship with growth. Additionally, its use implies a potential for higher productivity, lower labor intensity, and reduced space requirements compared to land crops[11, 16, 17, 116, 128].

Lutein content in microalgae oscillates from 1 to 10 mg/g DCW; however, concentrations up to 12 mg/g have been reported in the literature[116, 129]. To compare them, the data between a regular marigold flower production[113] and an outdoor continuous culture of high lutein producing strain *Scenedesmus almeriensis* in a 4000 L tubular system[130] were put on similar terms, it is expected that marigold produces between 87.6 and 262.8 kg of lutein·ha^{-1}·yr^{-1}, while the outdoor microalgae culture had a productivity of about 1000 kg of lutein·ha^{-1}·yr^{-1}. It is important to know that the microalgae culture was done in a smaller scale, however it is done with real production data in a system with high scalability.

This comparison is only based on the amount of lutein they produced. However, their production methods also differ, starting with their end products. The product from marigold flower processing is lutein oleoresin (lutein in ester form), while the one from microalgae is a carotenoid extract (in free form). Both processes can be summarized in Figure 4. As can be observed, in terms of number of processes, the microalgae one still has the advantage. However, marigold flower production is still the preferred method for lutein production. Lin *et al.*, 2015 clearly explain where the problem lies, it is because harvesting, dewatering and homogenization have higher energy costs due to microalgae's increased moisture content and higher energy needs for cell disruption.

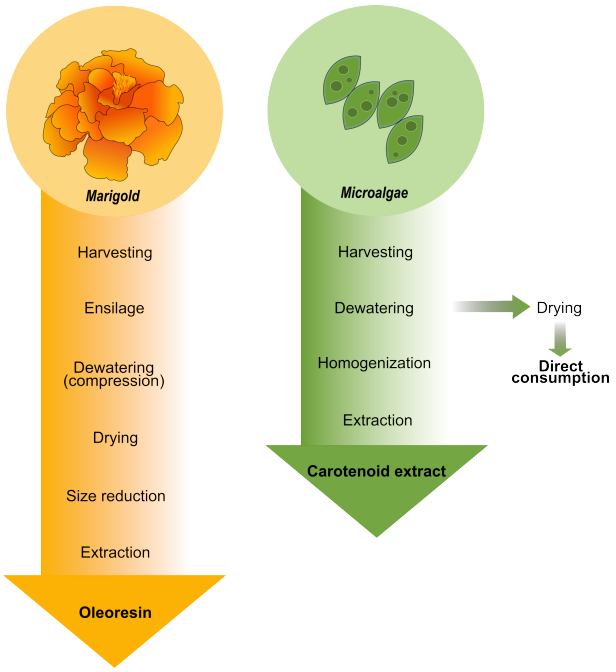


Fig. 4. Comparison between the lutein production processes from marigold flowers and microalgae[113, 130].

4 15. Microalgae lutein production history

Lutein production by microalgae is not new, in fact, pigments were being studied in microalgae since the 1800s. During the early 20th century, there was doubt about whether the pigments in plants, such as chlorophylls and carotenoids, were the same as those of green algae. Willstätter and Page confirmed this in 1914 for chlorophyll, while Kylin confirmed it in 1927 for carotenoids. Moreover, in 1939, Carter, Heilbron, and Lithgoe confirmed that lutein and β-carotene were ubiquitous among green algae. By 1945, it was also known that the Chlorophyceae class of algae could grow without light, preserving their pigments at least qualitatively[131].

By the 60's, as xanthophyll production was increasing rapidly, several companies sought the best way to produce them. They found an alternative in microalgae, although they faced the disadvantage that autotrophic pigment production, which was the common method of cultivation at the time, yielded too low biomass and pigment yields and required very extensive production times. Thus, they decided to produce them under heterotrophic conditions. The oldest patent in the United States was issued to Henry R. Kathrein, who patented the production of carotenoids using microalgae. According to the patent, those algae had to belong to the division *Chlorophyta*, preferably *Chlorococum* or *Chlorella*, using any sugar as a carbon source and various nitrogen sources like casein or peptone, the proportion of carbohydrate was between 0.5 to 10% by weight of the nutrient solution (preferably between 2 to 3%). In comparison, the protein could have gone from 0.1 to 5% (preferably between 0.4 to 2.4%). Kathrein reported that the maximum growth and carotenoid yield were achieved between 6 and 11 days of cultivation[132]. This procedure was of particular interest because, although they reported that light was not needed during cultivation, they still claimed that the addition of light would improve yield, which would, in fact, not make it a strict heterotrophic culture but a mixotrophic one by today's standards. A similar patent, specifically using *Chlorella pyrenoidosa*, was granted in 1966; key differences included the addition of Vitamin B1, methanol or ethanol, ethylenediaminetetraacetic acid, and fumaric acid to the medium, as well as a spray-drying process. They also recognized the nutritional value of the microalgae as a whole[133].

It was later in the 2010s that the true potential of microalgae as xanthophyll sources unfolded. As for poultry feed, in 2013 whole *Chlorella* strains were fed to egg laying hens in a proportion of 1 and 2%, this led to an increase of 46 and 119% respectively of the carotenoid content in the egg yolks of the eggs they produced. More than 90% of these

carotenoids were lutein and zeaxanthin[134]. The same year, it was also found that the intake of whole spray dried *Chlorella* strains increased lutein and zeaxanthin content in egg yolks by 3.82 and 4.22-fold, respectively[135]. Moreover, in 2013, it was discovered that the consumption of 9 g/day of whole *Chlorella vulgaris* could cause a 4-fold increase in lutein in human erythrocytes[136]. A year later, a study with samples provided by *Chlorella* Industry Co., with 3 of its employees participating in it, concluded that the daily supplementation of 6 mg of *Chlorella* to pregnant women from gestational week until delivery increased the lutein, zeaxanthin and β-carotene contents of the breast milk by 2.6-fold, 2.7-fold and 1.7-fold, respectively[137]. Recently, in 2021 in a study funded by Buggypower in Portugal, it was discovered that a single dose of 6 g of *Chlorella* containing 7.08 mg of lutein, increased lutein content in the blood of 11 healthy male subjects[138].

These discoveries further demonstrate the potential of microalgae as whole foods, potentially increasing lutein content without the need for costly purification steps compared to the conventional marigold flower process. It is also a testament to lutein's relevance throughout recent times and to the evolution of methods to improve its production.

As shown throughout this review, the history of lutein did not follow a single path; rather, several lines of research converged to produce our current understanding of the molecule. Figure 5 illustrates and summarizes these facts in a timeline organized into four categories: the ones related to the discoveries on plants and animals, which involve its identification and isolation; the human one, which shows the anatomic processes that were being identified involving lutein at the same time as the research on animal and plant tissue; a category for its industrial and health applications and finally one for the microalgae related lutein research. The timeline also highlights important processes and permits a more complete view of what was happening at a certain moment in time. It is interesting for example to observe that even though marigold flower production of lutein is the golden standard nowadays, it was patented after a microalgal production method which is just recently growing as an alternative.

5. Final remarks and conclusions

Lutein remains a relevant molecule to this day. A Web of Science search conducted in September 2026 identified more than 400 experimental articles using 'lutein' as a keyword, mainly in the categories of "Food Science and Technology" (32.62%), "Nutrition Dietetics" (12.86%), "Chemistry Applied" (9.29%), "Plant Sciences" (8.57%), "Biochemistry Molecular Biology" (8.33%), over the last five years, between 505 and 558 articles have been published annually, suggesting that research interest in lutein has remained relatively stable in recent years.

Lutein is a molecule found in many living things, from plants to mammals, although it is produced only by photosynthetic organisms. Due to its properties, it has found commercial use in agriculture, health, and the cosmetic industries. The lutein market is still growing today across several axes. Although its main source is marigold flowers, several other sources have attracted interest, such as microalgae. The main goal of this article was to understand why and how lutein became such a relevant molecule, from its isolation and identification to its first use and production methods.

Lutein was indirectly identified in humans and mammals between the 17th and 18th centuries. The 1800s and the beginning of the 1900s represented the century for classification and identification. Xanthophylls, chlorophylls, and carotenes were distinguished over time, given redundant and ambiguous names; their chemical formulas were determined, and their physicochemical characteristics were described. During the 20th century, lutein was fully identified and isolated, and it was also linked to the human body and its functions; thus, research began to uncover it. It was also during this century that xanthophyll and lutein started being commercially available, starting as a "xanthophyll oil", a byproduct of chlorophyll production used to increase the marketability of poultry products (like eggs and chicken skin). Lutein then became a product in its own right, as both a high-purity standard and a supplement.

Marigold flowers became the gold standard for lutein production compared to other sources, such as corn and alfalfa, because lutein was almost their only carotenoid, making downstream processing simpler. Lutein, however, does not need to be pure to be supplemented; the intake of whole *Chlorella* is an effective way to increase lutein concentration in both poultry and humans. That was the case for an increase in the carotenoid profile of eggs from microalgae-fed hens and an increase in lutein in the bloodstream and in breastmilk of humans.

Even though lutein has been researched extensively, some of its possible applications are still under investigation; however, the existence of lutein-producing companies with decades of production experience shows how profitable it is. With the growing need for sustainable industries, marigold-derived lutein may need to be replaced by production processes that do not compete with agriculture, such as microalgal production. They could also pose an advantage as a supplement that requires minimal downstream processing. However, in this regard, microalgae production should evolve to microalgal production should evolve to achieve higher productivity and yields while reducing energy costs. This optimization of microalgal production of lutein is just one of the many lines of current lutein research. With lutein remaining relevant through the years it is no surprise it is still an ongoing history, and perhaps this article could serve as an inspiration on the new chapters to be written of such history.

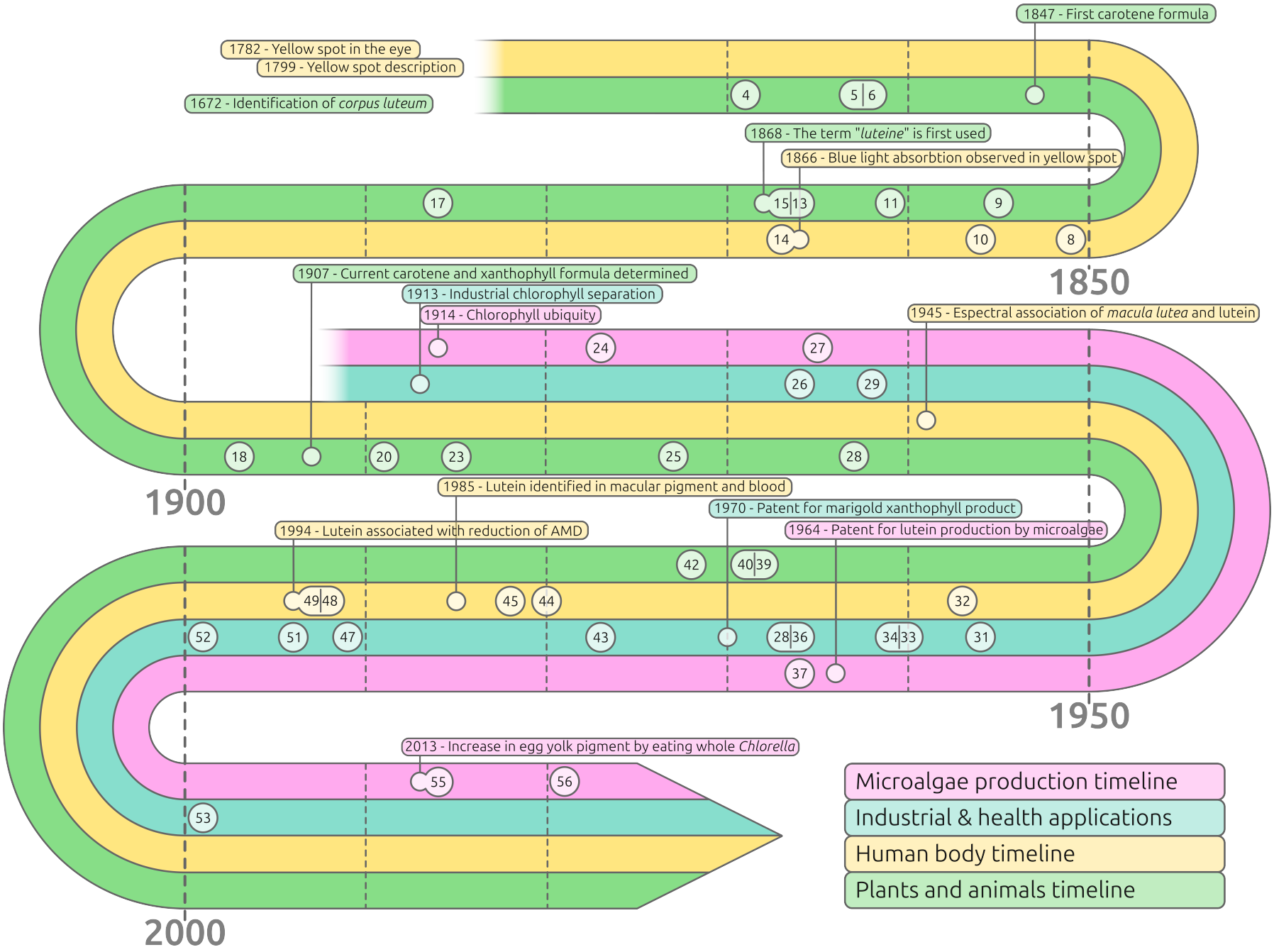


Fig. 5. Lutein history timeline. The events on the timeline are listed in Table 2.

Table 2. List of events for lutein history timeline.

Number	Year	Event
1	1672	De Graaf identifies the *corpus luteum*
2	1782	Buzzi identifies yellow spot in the human eye
3	1799	Soemmering described its prevalence and location
4	1831	Gorham obtains yellow pigment from corn
		Wackenroder obtains pigment from carrots
5	1837	Berzelius isolates coloring matter of leaves and calls it xanthophyll
6	1838	Berzelius develops method to separate chlorophyll from other pigments
7	1847	Zeise proposes elemental composition for carotene (C_5H_8)
8	1851	Helmholtz develops ophtalmoscope, seeing the "yellow spot" in living eyes
9	1855	Harting discovers differences in pigments from plants and algae depend on accessory pigments rather than chlorophyll
10	1856	Maxwell manages to observe the yellow spot through a prism
11	1861	Husemann proposes new carotene formula $C_{36}H_{24}O_2$
12	1866	Schultze describes the blue light absorbing properties of the yellow spot
13	1866	Piccolo and Lieben isolate carotenoids from the *corpus luteum*
14	1867	Helmholtz confirms the blue light absorbing properties of the yellow spot
15	1867	Städeler associates the pigment from egg yolks with the one from the *corpus luteum*
16	1868	Thudichum describes "luteine", a yellow substancefound in animals and plants
17	1886	Arnaud proposes new carotene formula $C_{26}H_{38}$
18	1903	Schunk spectroscopically confirms that the pigments of some flowers and egg yolk were the same
19	1907	Willstätter and Mieg determine formula for carotene ($C_{40}H_{56}$) and xanthophylls ($C_{40}H_{56}O_2$)
20	1911	Willstätter and Escher purify pigment of egg yolk and call it lutein.
21	1913	Willstätter and Stoll publish a treatise on chlorophyll separation, the basis for its industrial production
22	1914	Willstätter and Page confirm that algae and plants have the same chlorophyll
23	1915	Palmer publishes how the color of egg yolks, body fat and serum from hens depended on their diet
24	1927	Kylin confirms the carotenoids in plants are the same as those in algae
25	1931	Kuhn distinguishes lutein from xanthophyll. He also discovers helenien
26	1938	Schertz publishes modified chlorophyll separation method
		Test files patent for the "Production of vegetable concentrates"
27	1939	Carter *et al.* confirmed ubiquity of lutein in green algae
28	1941	Peterson found the effect of feed pigment in xanthophyll concentration of eggs
29	1942	Hunter patents xanthophyll oil from corn
30	1945	Wald associated the absortion of the *macula lutea* with that of lutein
31	1956	Adaptinol (helenien) starts being sold in Japan by Bayer Yakuhin
32	1957	Pfeifer studied the effect of helenien on darkness adaptation
33	1960	Kruse and Cravens patent "Poultry treatment for skin pigmentation" with soybean oil
34	1961	Lackey Jr. files patent in multiple countries for a "Procedure for obtaining pigmentation agents for birds and their products" using marigold flower petals
35	1964	Kathrein patents the "Production of carotenoids by the cultivation of microalgae" in heterotrophic culture
36	1966	Industrial Orgánica is founded in Mexico
37	1966	Hoffman-La Roche Inc.patents "Process for the preparation of lutein" from *Chlorella pyrenoidosa* in heterotrophic culture
38	1967	Prebluda patents "Treatment of pyrethrum plant waste materials and utilization in feed"
39	1968	Alam *et al.* identify pigments in marigold flowers were mostly esterified xanthophylls
40	1969	Livingston *et al.*, identify that xanthophyll extracts have more availability than whole foods
41	1970	Grant patents "Treatment of marigold petal meal to obtain xanthophyll product"
42	1972	Quackenbush and Miller identified that 92% of the total pigments of marigold flowers are lutein and zeaxanthin
43	1977	Industrias Vepinsa is born in Mexico
44	1980	Manilow *et al.*, show the effect on diet on xanthophyll concentration in the eyes of monkeys
45	1982	Kirschfield suggests the protective function of macular pigment
46	1985	Bone, *et al*. identify lutein and zeaxanthin as the carotenoids in macular pigment
		Lutein was identified in the human blood
47	1991	Tyczkowski and Hamilton develop method to obtain a 99.2% pure lutein product from marigold flower extract
48	1992	Nierenberg and Nann identify lutein in skin and other tissues
49	1993	Eye Disease Case-Control Study Group found that a high carotenoid intake associates with a reduction of AMD
50	1994	Seddon, *et al*. associate lutein with the reduction of age-related macular degeneration (AMD)
51	1994	Khachik patents the "Process for isolation, purification and recrystalization of lutein from saponified marigold oleoresin and uses thereof"
52	1999	The Howard Foundation patents a "High dosage lutein and zeaxanthin for macula therapy"
53	2001	Levy patents "Trans-xanthophyll ester concentrates of enhanced purity and methods on making same"
54	2013	Kotrbácek *et al.* increase pigment in egg yolks by feeding whole *Chlorella*
		Englmaierová *et al.* increase pigment in egg yolks by feeding spray-dried *Chlorella*
55	2014	Nagayama *et al.* increased breast milk lutein content by feeding women with *Chlorella*
56	2021	Serra *et al.* increased lutein content in blood with a single dose of *Chlorella*

Authors contributions

CRediT: **Diego López-Salas:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing; **Cristóbal Camarena-Bernard:** Conceptualization, Project administration, Supervision, Validation, Writing – review & editing; **Victor Pozzobon:** Conceptualization, Data curation, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

Cristóbal Camarena-Bernard and Victor Pozzobon would like to underline that this manuscript represents a sizable amount of work, first and foremost led by Diego López-Salas.

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