Review

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Posted Date: 29 July 2024

doi: 10.20944/preprints202407.2306.v1

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Review

Extraction Methods for Carotenoids, Xanthophylls and Chlorophylls

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Abstract: The extraction methods for carotenoids, xanthophylls and chlorophylls are essential processes involved in the recovery of bioactive compounds from a wide variety of natural sources, including plants, marine organisms, microorganisms and animals. These bioactive compounds, characterized by their significant functional and structurally active properties, play a crucial role in various industries, such as pharmaceuticals, nutraceuticals and food. Due to their presence in small quantities and conjugated form in nature, the extraction of these compounds can be laborious and often requires meticulous purification steps. Interestingly, the primary and secondary metabolites present in plants contribute significantly to the production of these bioactive compounds, adding to their complexity and importance. Notably, carotenoids, as natural pigments with antioxidant and nutritional properties, are known for offering numerous health benefits and are instrumental in various biological processes, including the photoprotection of plants and the prevention of chronic degenerative diseases. It is fascinating to observe that by-products generated from agricultural activities and food processing serve as abundant sources of these bioactive compounds, aligning with the principles of the circular economy and sustainable resource management. Given the diverse health benefits and multifaceted applications of these natural pigments, there is a growing interest in not only extracting but also utilizing them for industrial, nutritional and pharmacological purposes.

Keywords: extraction methods; conventional methods; non-conventional methods; carotenoids; xanthophylls; chlorophylls; bioactive compounds

1. Introduction

Regardless of origin or type, there are numerous functional and structurally active molecules, called bioactive compounds or biomolecules. These bioactive compounds have been extracted from various sources, in different quantities, and with variable uses in research (Bilal M. and Iqbal H. M., 2020).

González-Peña M. A. et al., 2023, defined bioactive compounds as natural food ingredients derived from plant sources that do not have nutritional value, but which, upon ingestion, will manifest positive or toxic effects on the individual's health. Biomolecules can be derived from natural sources such as plant material, microorganisms, marine organisms, and animals (Jha Avinash Kumar and Nandan Sit, 2022).

They are found in small quantities in nature, in conjugated form, which is a disadvantage for extraction methods because they will be laborious and will require purification steps (Marathe S. J. et al., 2019).

 doi:10.20944/preprints202407.2306.v1

Positive manifestations of biomolecules include modulation of gene expression, induction or inhibition of enzymatic activity, antioxidant activity (Marathe S. J. et al., 2019), immunomodulatory activity, anti-inflammatory (Cerón-García M. C. et al., 2018), anticancer, antidiabetic and also improve digestion and blood circulation (Jha Avinash Kumar and Nandan Sit, 2022). Due to these properties, bioactive compounds are used in the pharmaceutical, nutraceutical, and food industries (Marathe S. J. et al., 2019).

Plants have a biological system made up of primary and secondary metabolites. Primary metabolites are represented by amino acids, proteins, and carbohydrates that have as their main activity the development and maturation of plant tissues. Secondary metabolites are synthesized during the development phase and participate in the adaptation and survival of plants (Jha Avinash Kumar and Nandan Sit, 2022).

According to Ivanović M. et al., 2020, some of the important classes of secondary metabolites are: alkaloids, terpenoids, compounds containing nitrogen, sulfur or phenolic compounds. These classes are also divided into subclasses (Ivanović M. et al., 2020). Carotenoids, with their representative carotene, carotenoid, lutein, β-carotene, β-cryptoxanthin, compounds recognized for their antioxidant activity, are part of the terpenoid class (Jha Avinash Kumar and Nandan Sit, 2022).

Fruits and vegetables are a rich source of bioactive compounds with beneficial functions for the human body, such as carotenoids or phenols, but nevertheless a large quantity can be obtained from plant extracts resulting from food processing or from agricultural waste (Hulkko L. S. et al., 2022). The pulp, peels, leaves, and stems are considered by-products (secondary products or by-products) formed during cultivation or after processing vegetables and fruits in agriculture or in the food industry (Ebrahimi, P. et al., 2023).

A high level of food pigments is contained in these by-products, especially chlorophylls, which are found in almost every green component of the crop, and for this reason, they can be a main source for the recovery of biomolecules, thus addressing the strategy of the circular economy (Ebrahimi, P. et al., 2023). Natural pigments are points of interest for industrial, nutritional, and pharmacological applications because they have various benefits on the health of the body, for example, chlorophyll is involved in the process of recovering damaged tissues, and carotenoids act as precursors in the synthesis of vitamin A (Fatima I. et al., 2023).

Carotenoids are also part of the category of natural pigments. Carotenes and xanthophylls belong to the class of carotenoid pigments and are involved in maintaining the stability of the cell wall structure and in the photoprotection of the plant, having the ability to absorb solar radiation (Fatima I. et al., 2023). According to González-Peña M. A. et al., 2023, recently the interest of researchers for plant pigments has increased due to numerous implications in biological processes. There are studies that prove the importance of these pigments in preventing macular degeneration, chronic degenerative diseases, and the formation of cataracts. Also, carotenoids are also involved in hematopoiesis, cell communication, embryonic development, apoptosis, and have antiproliferative and anti-angiogenic properties (González-Peña M. A. et al., 2023).

2. Lipophilic Bioactive Compounds

2.1. Carotenoids

A healthy diet involves the optimal intake of various necessary vitamins and minerals, and one of the important nutritional components is carotenoids (Cheng S. H. et al., 2020). They are natural colorants with antioxidant and nutritional properties that determine the red, yellow, and orange colors of higher plants (Molina A. K. et al., 2023), green and brown of algae (Fatima I. et al., 2023).

Carotenoids are found in bulbs, seeds, stems (Butnariu M., 2016), leaves, flowers (Molina A. K. et al., 2023), fruits, and vegetables such as: green leaves, reds, carrots, saffron, papaya, pineapple, sunflower, marigold flower (González-Peña M. A. et al., 2023), red pepper, citrus, peaches, apricots, and mango (Meléndez-Martínez A. J. et al., 2022). These compounds can be synthesized by photosynthetic organisms (Saini R. K. and Keum Y. S., 2018) including photosynthetic bacteria, algae, plants (Molina A. K. et al., 2023), fungi, and some non-photosynthetic bacteria (Saini R. K. and Keum Y. S., 2018).

 doi:10.20944/preprints202407.2306.v1

Animals, except for some species of aphids, cannot synthesize carotenoids, in this way being necessary their intake through food (Saini R. K. and Keum Y. S., 2018). Plants, although they are an important source of pigments, can have a limited production due to geographical factors, seasonal dependence, or variations in colors or shades. Because of these things, microorganisms have been considered a better choice because they can present a higher yield of extraction, cultures represent easily regenerable sources, easily degradable and also, they present antimicrobial properties (Afroz Toma M. et al., 2023).

The pigments resulting from the cultures of microorganisms have attracted the attention of researchers because they can synthesize bioactive compounds under flexible working conditions such as intense light, high temperature, acidic or basic pH, and their greater solubility in aqueous media compared to pigments obtained from plants, and their collection can be continuous and consistent (Afroz Toma M. et al., 2023).

Sources for obtaining microbial pigments can be bacteria, fungi, parasites, microalgae, and basidiomycetes with applicability in fields such as pharmaceutical and food. However, fungi are favorable sources due to the rapid growth and development cell cycle which can also be genetically modified so that the synthesis of pigments is increased (Afroz Toma M. et al., 2023).

Monascus, Cordyceps, Serratia, Penicillium, Aspergillus, Fusarium, Talaromyces are fungi used for large-scale production of carotenoids, lycopene, riboflavin, melanins, quinones, and betalains (Afroz Toma M. et al., 2023). Species of microalgae such as *Dunaliella salina, Porphyridium cruentum, Isochrysis galbana*, and *Haematococcus pluvialis* are intensively used due to their increased synthesis capacity of different types of carotenoids (β-carotene, lycopene, lutein) with biotechnological, industrial, and biorefinery applications (Achour H. Y. et al., 2023).

More than 700 carotenoids have been discovered, of which 40 are included in the diet (González-Peña M. A. et al., 2023). They are composed of eight isoprene units that determine the formation of a chain of 40 carbon atoms, thus they are part of the terpene family (Molina A. K. et al., 2023) and are united through a system of conjugated double bonds (Sousa Clara, 2022).

There are two classes in which these pigments are classified: carotenes comprising hydrocarbon forms without oxygen atoms and xanthophylls which present in their structure at least one oxygen atom forming hydroxy or epoxy groups (Bampidis V. et al., 2019). The most well-known carotenes are β-carotene, α-carotene, lycopene, and torulene (Afroz Toma M. et al., 2023).

α-carotene, β-carotene, γ-carotene, and β-cryptoxanthin are essential for the development and maintenance of healthy vision because they exhibit provitamin A activity, being converted into vitamin A in the body (González-Peña M. A. et al., 2023).

α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin, through their increased antioxidant properties, promote the elimination of reactive oxygen species and free radicals, which contributes to protection against chronic diseases (González-Peña M. A. et al., 2023).

However, their use in the food industry is limited because they are unstable structures predisposed to oxidation in the presence of heat, light, acids, oxidants, and metal ions, they have low solubility in water, availability, and rapid release (González-Peña M. A. et al., 2023).

Table 1. Different extraction methods for carotenoids.

Plant Source	Extraction Method	Solvent	Operating Conditions	Extraction Yield	Source
Solanum lycopersicum (By-product)	Soxhlet	Ethanol	Time of 5h	0.034 mg/g β-carotene 0.703 mg/g lycopene	(Molina A. K. et al., 2023)
Cucumis melo L.	Ultrasound-assisted extraction	Hexane:acetone 80:20	10 minutes, 100% amplitude	124.61 ± 3.82 μg/g	(Molina A. K. et al., 2023)

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Passiflora edulis f. flavicarpa	Immersion	Thermostatic bath Ethanol 90% acidified with 0.03% citric acid Ethanol 90% acidified with 0.03% citric acid	T=29°C, time of 2h, without light, 500 rpm T= 60°C, time of 24h	113.08 ± 8.84 µg β-carotene/100 g 10.34 ± 5.18 µg of β-carotene/100 g	(Molina A. K. et al., 2023)
Cyphomandra betacea	Conventional solvent extraction	n-hexane/petroleum ether 50:50%	Absence of light, time of 48h	0.051g caroten/g	(Molina A. K. et al., 2023)
Pouteria campechiana Kunth	Agitation extraction	n-hexane /dichloromethane 1:1 with a ratio between solvent and sample 15:1 n-hexane /dichloromethane 1:1 with a ratio between solvent and sample 30:1	T=40°C, 30 minutes, 200 rpm, followed by 10 minutes at 6000 rpm T=40°C, 30 minutes, 200 rpm, followed by 10 minutes at 6000 rpm	5.17 ± 0.08 g β-carotene/100g dry matter 3.12 ± 0.01 g β-carotene/100g dry matter	(Molina A. K. et al., 2023)
Daucus carota	Supercritical CO2	Ethanol 15.5%	T=59°C, at a pressure of 349 bar	Extraction yield equal to 86.1%	(Molina A. K. et al., 2023)

2.2. Xanthophylls

According to Petibon F. and Wiesenberg G. L., 2022, xanthophylls are synthesized by adding oxygen to derivatives of tetraterpenes. They are found in the largest quantity in chromoplasts, which through the esterification reaction with fatty acids favor the accumulation of xanthophylls (López-Cruz, R. et al., 2023).

From the perspective of the function they perform, xanthophylls are divided into primary or secondary xanthophylls. The main ones have structural and functional properties at the level of the photosynthetic apparatus of algae, participating in the survival of the organism, and the secondary ones are synthesized in large quantities following exposures to specific environmental stimuli (Zarekarizi A. et al., 2019).

In the structure of xanthophylls, there are different types of functional groups that include at least one oxygen atom such as: hydroxyl (lutein, zeaxanthin, β-cryptoxanthin), carbonyl (astaxanthin, cantaxanthin, capsanthin) and epoxide (neoxanthin, violaxanthin, fucoxanthin) which contribute to the diversity of these structures (Saini R. K. et al., 2022).

Xanthophylls are not soluble in water, having lipid-like properties (Thomas E. and S. Johnson E. J., 2018). They have a lower predisposition to thermal degradation (Saini R. K. et al., 2022) and are less hydrophilic than carotenes (due to the hydroxyl groups in the chemical structure) (Thomas S. E. and Johnson E. J., 2018). Due to their oxidative properties, they are susceptible to degradation under conditions where they are exposed to light, high temperatures, acids, and longer extraction time (Ahmad N. et al., 2021).

During the sample preparation phase, the extraction yield can be improved by using physical and chemical factors that help destroy the cell wall or other physical barriers (Ahmad N. et al., 2021). From this point of view, the choice of solvent represents a critical point of the extraction process, and due to the oxygen molecule, it is necessary to use polar solvents such as ethanol, acetone, hexane, ethyl acetate or diethyl ether (Ahmad N. et al., 2021).

Xanthophylls such as lutein or zeaxanthin are found in food sources such as corn, green vegetables (Thomas S. E. and Johnson E. J., 2018), parsley, spinach and egg yolk (Nabi B. G. et al.,

 doi:10.20944/preprints202407.2306.v1

2023), β-cryptoxanthin which is found in papaya, peppers, pumpkin (Thomas S. E. and Johnson E. J., 2018), astaxanthin and fucoxanthin are found in green and brown algae (Nabi B. G. et al., 2023), such as Haematococcus pluvialis which is a species of green microalgae, some species of fish (wild salmon), shellfish and certain mushrooms (Thomas S. E. and Johnson E. J., 2018).

Other sources for xanthophylls are Blakeslea trispora (mushrooms), Xanthophyllomyces dendrorhous (yeast), yellow flower petals for non-esterified forms, and from red flower petals for both forms (López-Cruz, R. et al., 2023).

Although they are not part of the category of essential nutrients, xanthophylls have a high bioprotective potential (López-Cruz, R. et al., 2023) in maintaining health or the onset of diseases. Due to their antioxidant activity, they can prevent the onset of cardiovascular diseases or cancer, and by capturing free radicals and acting against ROS, especially singlet oxygen, they prevent lipid peroxidation, oxidative damage to important cellular pathways and DNA damage (Thomas S. E. and Johnson E. J., 2018).

Table 2. Different extraction methods for bioactive compounds.

Pigment	Algae Species	Freshwater/Marine Environment	Extraction Method	Source
β-carotene	Dunaleilla salina	Marine	Supercritical CO2 + supercritical extraction with ethane and ethylene	(Achour H. Y. et al., 2023)
Lutein	Monostroma nitidium	Marine	Extraction with liquefied dimethyl ether	(Fatima I. et al., 2023)
	Chlorella vulgaris	Freshwater	Freeze and thaw extraction	(Kulkarni and Nikolov 2018)
	Scenedesmus almeriensis	Freshwater	Supercritical fluid extraction	(Fatima I. et al., 2023)
	Chlorococum humicula	Freshwater	Extraction with liquefied dimethyl ether	(Babadi et al. 2020)
	Desmodesmus sp	Freshwater	High-pressure extraction	(Fatima I. et al., 2023)
	Scenedesmus sp	Freshwater	DES extraction	(Fan et al. 2022)
Fucoxanthin	Undaria pinnatifida	Marine	Supercritical fluid extraction, Supercritical CO2 extraction	(Fatima I. et al., 2023)
	Chlorococum humicula	Freshwater	Extraction with liquefied dimethyl ether	(Babadi et al. 2020)
	Fucus vesiculosus	Marine	DES extraction	(Obluchinskaya et al. 2021)
	Phaeodactylum tricornutum	Marine	Ultrasound-assisted extraction	(Fatima I. et al., 2023)
	Cylindrotheca closterium	Marine	Microwave-assisted extraction	(Fatima I. et al., 2023)

Chlorophyll a	Chlorococum humicula	Freshwater	Extraction with liquefied dimethyl ether	(Babadi et al. 2020)
	Chlorella vulgaris	Marine	High-pressure extraction	(Fatima I. et al., 2023)
	Chlorella vulgaris	Freshwater	Supercritical CO2 extraction	(Fatima I. et al., 2023)
	Cladophora glomerata, Chlorella rivularis, Ulva flexuosa	Freshwater	Supercritical CO2 extraction	(Fabrowska et al. 2018)
Chlorophyll b	Cladophora glomerata, Ulva flexuosa	Freshwater	Ultrasound-assisted extraction	(Fabrowska et al. 2018)
	Chlorella vulgaris	Freshwater	Supercritical CO2 extraction	(Fatima I. et al., 2023)
	Cladophora glomerata freshwater	Freshwater	Microwave-assisted extraction	(Fabrowska et al. 2018)

2.3. Chlorophylls

Chlorophylls, along with carotenoids, are the most well-known pigments, found in plant leaves where they perform the functions of photoprotection and photosynthesis. Biotic or abiotic stress, UV-B radiation, heat, CO2, and O3 can cause changes in the composition or structure of chlorophylls or carotenoids, thus highlighting the plant's ability to acclimate (Petibon F. and Wiesenberg G. L., 2022).

Chlorophyll (Chl) is the most abundant primary natural pigment in plants, cyanobacteria, and algae (Ferreira et al., 2021). It facilitates the use of sunlight as a source of energy to synthesize glucose molecules and other carbohydrates from water and CO2 (Caesar J. et al., 2018), which will serve as a nutritional source for the entire plant (Ebrahimi, P. et al., 2023). There are five major forms of chlorophyll: a, b, c, d, and e, noting that an f form of chl has also been reported (Singh A. K. et al., 2020).

According to Roca M. and Pérez-Gálvez A., 2021, there are over 100 structures that form a homogeneous group of chlorophylls. These forms appear as a result of esterification reactions with numerous alcohols, although there are also non-esterified forms. Analyzing the degree of unsaturation of the macrocycle, chlorophylls are classified into: chlorines (chl a and chl b), porphyrins (chl c), or bacteriochlorines (Roca M. and Pérez-Gálvez A., 2021).

Their composition also varies depending on the organism of origin: vascular plants and green algae have Chl a and b, brown algae Chl c, cyanobacteria Chl a, and Rhodophyta which, in addition to Chl a, also have Chl d (Caesar J. et al., 2018). Chl a and Chl b differ by a substitution of a methyl group with a formyl group (Kwartiningsih E. et al., 2021), and this variation in structure results in a variation in colors, Chl a causing the appearance of a blue-green color, and Chl b blue-yellow (Ebrahimi, P. et al., 2023). In plants, Chl a and Chl b are in an approximate ratio of 3:1 (Kwartiningsih E. et al., 2021).

Nettle, spinach, alfalfa, and corn are industrial sources of chlorophyll (Jorge, A. M. et al., 2024), followed by leafy vegetables, green beans, peas, and leaves of Pandanus amaryllifolius (Nabi B. G. et al., 2023).

The beneficial activity of chlorophylls on the body has made them used in the pharmaceutical, food, cosmetic, and health fields (Ferreira et al., 2021) with properties of rapid wound healing, anti-inflammatory (Ebrahimi, P. et al., 2023), anticancer, and antimutagenic (López-Cruz, R. et al., 2023). In addition, different types of chlorophylls can act against reactive oxygen species (ROS) in response, so they are also involved in defense mechanisms, against stress and apoptosis (Roca M. and Pérez-Gálvez A., 2021).

Chlorophylls have a lipophilic character (Murador D. et al., 2021) and because of this they are insoluble in polar solvents, thus resulting in them being liposoluble compounds, like carotenoids (Murador D. et al., 2021). They can change their structure depending on temperature, pH (Murador

D. et al., 2021), solvent, bleaching, ultrasound time, drying temperature, or carrier thus can determine the degradation of chlorophyll (Nabi B. G. et al., 2023). The central Mg ion of the chlorophyll conformation is lost under acidic conditions and is converted into the corresponding pheophytin (Murador D. et al., 2021). For these reasons, to obtain reliable results, it is necessary to standardize appropriate extraction methods, and the solvents most often described in the literature for chlorophylls are: acetone, ethanol, dimethyl sulfoxide (DMSO) (Caesar J. et al., 2018)., methanol (MeOH) and N,N-dimethylformaldehyde (DMF), DMF being the best solvent but due to its toxic nature it is not used (Jorge, A. M. et al., 2024).

Table 3. Different extraction methods for chlorophylls.

Plant Source	Extraction Method	Solvent	Operating Conditions	Extraction Yield	Source
Cynodon spp.	Maceration	Dimethyl sulfoxide (DMSO)	V= 20 mL, 8 evaluations every 12h/12h, T=23-26°C, humidity 40-75%	Chlorophyll a: 316 ± 2.93 µmol·m−2 Chlorophyll b: 66 ± 1.41 µmol·m−2	(Molina A. K. et al., 2023)
		N,N-dimethylformamide	V= 20 mL, 8 evaluations every 12h/12h, T=23-26°C, humidity 40-75%	Chlorophyll a: 297 ± 3.58 µmol·m−2 Chlorophyll b: 85 ± 2.03 µmol·m−2	
		Acetone 80%	V= 20 mL, 8 evaluations every 12h/12h, T=23-26°C, humidity 40-75%	Chlorophyll a: 250 ± 2.65 µmol·m−2 Chlorophyll b: 111 ± 1.50 µmol·m−2	
		Absolute ethanol	V= 20 mL, 8 evaluations every 12h/12h, T=23-26°C, humidity 40-75%	Chlorophyll a: 259 ± 2.84 µmol·m−2 Chlorophyll b: 84 ± 2.25 µmol·m−2	
Brassica napus L.	Maceration	Acetone 80%	Conventional extraction	Chlorophyll a: 0.87 mg·g−1 Chlorophyll b: 0.39 mg·g−1	(Molina A. K. et al., 2023)
	Without maceration	Acetone 80%	Cool room, without light, time of 24h	Chlorophyll a: 0.98 mg·−1 Chlorophyll b: 0.38 mg·g−1	

3. Extraction Methods

Before starting any extraction process, it is essential to first understand the physicochemical properties of the desired compound, with solubility being the most relevant property (Butnariu M., 2016). The diversity of plant materials and the variety of bioactive compounds, even within the same classes, make it impossible to develop a standard extraction technique (Butnariu M., 2016).

The extraction of bioactive compounds from natural sources such as plants and marine organisms is crucial, and extraction methods play a vital role in this process (Molina A. K. et al., 2023). Traditional techniques like maceration and Soxhlet extraction were previously predominant. However, recent innovations have led to the emergence of modern extraction methods that offer superior efficiency and selectivity (Saxena R., 2023). Among these methods are microwave-assisted extraction, ultrasound-assisted extraction, and enzyme-assisted extraction, which have demonstrated potential for improving extraction yield while maintaining compound bioactivity. Selecting the appropriate extraction method is essential for reliable results in phytochemical analysis and pigment extraction from natural sources (López-Cruz, R. et al., 2023).

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Parameters such as solvent, pressure, temperature, time, and cellular matrix influence compound extraction (Azmir J. et al., 2013). Choosing the right solvent, along with the most efficient extraction method, is critical in this process (Singh N. et al., 2024). Solvent selection considers compound polarity (Roca M. and Pérez-Gálvez A., 2021), the need for co-solvents, mass transfer, molecular affinity between solute and solvent, toxicity to organisms, environmental safety, and financial feasibility (Azmir J. et al., 2013).

Carotenoids, except for lutein, are nonpolar molecules similar to xanthophylls but exhibit slightly higher polarity (Pereira A. G. et al., 2021). Polar organic solvents used for carotenoid extraction, regardless of the extraction method, include methyl and ethyl alcohol, acetone, dichloromethane, dimethyl sulfoxide, as well as hexane/acetone/ethyl alcohol, dichloromethane/methanol, and acetone/methanol (Butnariu M., 2016).

Efficiency of extraction varies based on the plant material (fresh, dried, or marine). For fresh material, reducing water content in tissues is necessary, and water-miscible solvents like methanol, ethanol, and acetone are used for drying samples. For dried material, non-water-miscible solvents are employed. In the case of micro and macroalgae, pre-treatment is essential to enhance extraction yield, often using acetone or ethanol:ethanol (Butnariu M., 2016).

3.1. Conventional Methods

Extraction with solvents is a common and widely used method because the solvent acts as a carrier molecule, facilitating the transfer between solution phases (liquid, solid, vapor) based on physicochemical properties such as solubility and volatility (Cheng S.H. et al., 2020). In a study by Arnold C. and colleagues (2014), the effect of temperature on carotenoids and chlorophylls extracted using common solvents (methanol:THF, 1/1, v/v) from plant materials like parsley, dill, cabbage, and spinach was analyzed. Different heat treatments induced varying changes in the concentration of bioactive compounds. For instance, at 121°C, zeaxanthin concentration increased, while lutein decreased in dill samples by 67%, parsley by 73%, and cabbage by 42% (Arnold C. et al., 2014).

For pigment extraction from algae, liquid-liquid extractions, liquid-solid extractions, and Soxhlet extraction methods have been employed. Common solvents used in conventional extraction processes include diethyl ether, benzene, toluene, acetone, ethanol, methanol, cyclohexane, chloroform, petroleum ether, and isopropanol (Fatima I. et al., 2023).

In a study by Heffernan N. et al. (2016), the content of carotenoids and xanthophylls was analyzed using solid-liquid extraction (SLE) and supercritical CO2 (SCO2) from brown macroalgae Fucus serratus and Laminaria digitata. SLE using hexane/acetone (70:30) as the solvent, 24 hours of extraction time, and a temperature of 50°C resulted in higher yield compared to SCO2. Although SCO2 provided greater fucoxanthin purity, SLE exhibited higher purity and concentration for xanthophylls (Heffernan N. et al., 2016).

Soxhlet extraction is a conventional method that uses solvents at their boiling point and low pressure to facilitate interaction between the solvent and the compound. This process increases mass transfer rates. When an organic solvent is combined with high temperature, the solvent's surface tension increases, allowing it to penetrate the matrix solution more easily, thereby solubilizing a variety of dissolved substances. This enhances the efficiency of carotenoid extraction. However, Soxhlet extraction can be costly due to large solvent quantities and long extraction times (Cheng S.H. et al., 2020). Additionally, it generates significant hazardous waste. Efforts have been made to optimize extraction methods and minimize hazardous substances in recent decades (Cunha S. C. and Fernandes J. O., 2018).

Fabrowska J. et al. (2018) compared Soxhlet extraction with ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) for obtaining carotenoids and chlorophylls from marine and freshwater algal biomass. The extraction was performed at 40°C (except for Soxhlet, which depends on solvent boiling point), 60 minutes for MAE with 800W power, and high-purity CO2 for SFE. UAE and MAE were more efficient than Soxhlet and SFE for carotenoid and chlorophyll extraction. A combined ultrasound and microwave-assisted

 doi:10.20944/preprints202407.2306.v1

extraction (UMAE) also showed lower energy consumption compared to Soxhlet, making it a reliable method (Fabrowska J. et al., 2018; Wen L. et al., 2020)

3.2. Modern Extraction Methods

Due to the fact that traditional methods use large quantities of solvents, water, and lengthy processing times, new, more efficient, and environmentally friendly extraction methods have been developed (Kwartiningsih E. et al., 2021). These eco-friendly techniques are known as "green techniques" and utilize recyclable raw materials, less hazardous chemicals, non-toxic and safe solvents, safer chemical synthesis, promote atomic economy, and reduce processing time, thereby minimizing chemical pollution and providing a safer medium for conducting experiments (Chemat F. et al., 2017).

3.2.1. Supercritical/Subcritical Fluid Extraction

It uses CO2 which is an inert gas, it is not toxic, non-flammable, and cheap, whose temperature and pressure have values above the critical point (31.1℃ and 73.8 bar). These supercritical conditions confer increased solubility due to a diffusion coefficient and increased density, cause the appearance of surface tension, low viscosity, and the ability to penetrate the extraction material through small pores in its structure.

If CO2 drops below the temperature of 31.1℃ it will pass into the liquid state called subcritical CO2. This method of supercritical/subcritical CO2 extraction has the advantage of selectively extracting the desired components, but the cost of the equipment is very high because a high-pressure pump is needed to obtain the necessary pressure. For this purpose, dry ice can be used to reach the necessary conditions for supercritical CO2 and in this way the extraction method will be easier and cheaper (Kwartiningsih E. et al., 2021).

The advantages of this extraction method include increased extraction efficiency, safety, simplicity, speed, pre-concentration effects, and reduced environmental hazards. However, a significant drawback is the high equipment cost and the need to modify CO2 for extracting polar molecules (Chemat F. et al., 2017).

In the study conducted by Zarekarizi A. et al., 2019, fucoxanthin was extracted by different methods using solvents (ethanol, acetone, hexane, water, ethyl acetate), the most efficient being ethanol (15.71 mg g−1 dry substance), but lipid co-extraction may result. Supercritical CO2 extraction of fucoxanthin involves the addition of ethanol to increase the polarity of CO2 molecules thus increasing the yield of the process (Zarekarizi A. et al., 2019).

Fucoxanthin is sensitive to high temperatures, low pH, susceptible to thermal degradation, exposure to light induces the degradation of the molecule and changes in color. The fucoxanthin extract from Sargassum binderi was found to be more stable in the dark than in light, and by adding a 1.0% w/v antioxidant agent of ascorbic acid the degradation process was stopped. pH also influences the stability of the molecule, thus the greatest stability was presented at a pH value equal to 9, concluding that fucoxanthin is most stable in the dark, in a basic solution containing ascorbic acid (Zarekarizi A. et al., 2019).

In a study conducted by Georgiopoulou I. et al. (2023), the total content of carotenoids and chlorophylls obtained through supercritical fluid extraction (SFE), SFE with 10% ethanol co-solvent, microwave-assisted extraction (MAE), and solid-liquid extraction (SLE) from Chlorella vulgaris was analyzed. The results revealed that SFE-10% ethanol yielded the highest total carotenoid extract (37.60 mg/gextract), followed by plain SFE (34.61 mg/gextract), MAE (24.88 mg/gextract), and SLE (19.06 mg/gextract). Adding a polar co-solvent to SFE improves the simultaneous extraction of polar carotenoids, including lutein, as well as chlorophylls (Georgiopoulou I. et al., 2023).

3.2.2. Ultrasound-Assisted Extraction (UAE)

The use of acoustic waves for extracting bioactive compounds has led to the emergence of new green technologies that enhance food processing techniques. Ultrasound-assisted extraction (UAE)

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utilizes ultrasonic waves at specific frequencies and amplitudes to enhance extraction mechanisms by creating cavitation bubbles. Once these bubbles surpass their stability threshold, implosion occurs, resulting in the release of high temperatures and pressures. This process favors the disruption of cell walls and the liberation of metabolites. Parameters that can be adjusted to optimize the extraction process include frequency (Hz) (Lefebvre T. et al., 2021) within the range of 20 kHz to 100 MHz (Azmir J. et al., 2013) and amplitude (MPa). Power (W) represents the amplitude over time, while intensity reflects the power exerted over a period. Power significantly influences the selectivity of the extract (Lefebvre T. et al., 2021).

Ultrasound can efficiently extract a multitude of bioactive compounds using both polar and non-polar solvents and accessible equipment (Chemat F. et al., 2017). Additionally, factors such as frequency, pressure, temperature, and sonication time influence the action of ultrasound (Azmir J. et al., 2013). However, Lefebvre T. et al. (2021) studied the impact of temperature on UAE and did not obtain significant results supporting the idea that increased temperature leads to higher extraction yields.

In a study published by Chemat F. et al. (2017), UAE was three times more efficient than the conventional Soxhlet method. When combined with ultrasound, mass transfer from the solid phase to the solvent was enhanced during extraction.

According to research by Minchev I. et al. (2020), the chlorophyll content extracted from Spirulina platensis using UAE at a frequency of 45 kHz was similar to that obtained by sonication using acetone as the solvent and conventional solid-liquid extraction over 24 hours. Optimal conditions for ultrasound-assisted extraction of chlorophyll a with ethanol were found to be at a temperature of 32.59°C and a duration of 4.91 hours at a frequency of 20.52 kHz, resulting in an extract yield of 17.98 mg/g. Chlorophylls are known to be thermosensitive compounds, so increasing temperature and extraction time for better yields could lead to their decomposition (Minchev I. et al., 2020).

3.2.3. Microwave-Assisted Extraction (MAE)

This method is recognized for its extraction efficiency in a short time using high temperatures for homogeneous heating of solutions. The advantages of the method are that it is possible to automate the extraction and the speed of unfolding, but the disadvantage lies in the high cost and the need for cleaning stages. Also, the use of deep eutectic solvents (DES) or natural deep eutectic solvents (NADES) increases the extraction yield compared to conventional solvents (Nakhle L. et al., 2021).

DES are obtained by heating at 80℃ or cold drying two or more compounds without the need for a purification stage, and the reaction conditions are accessible. Due to the physicochemical properties they possess, such as miscibility, viscosity, density, conductivity, and polarity, DES are frequently used for the extraction of various types of solutions, but these are dependent on the constituent components of DES (Cunha S. C. and Fernandes J. O., 2018).

Most have at room temperature a viscosity greater than that of water, facilitating phase separation, low conductivity due to viscosity, and polarity is the most important characteristic of DES.

Carbohydrate derivatives of DES have a higher polarity than short-chain base alcohols (2-propanol, ethanol) or polar aprotic solvents such as dimethyl sulfoxide and dimethylformamide (Cunha S. C. and Fernandes J. O., 2018). NADES have low toxicity compared to conventional solvents and increased efficiency thus reducing extraction costs, environmental impact, and working time, making them optimal for green extraction technologies (Cannavacciuolo C. et al., 2022).

In the article published by Ahamad N. and colleagues in 2021, various extraction methods using ultrasound, microwave, and hot soaking with either 90% ethanol or 90% acetone as solvents were compared. The analysis revealed that for each of the three extraction methods, the highest xanthophyll extract concentration was obtained using 90% ethanol as the solvent. Furthermore, microwave-assisted extraction (MAE) demonstrated superior efficiency with a 6-second irradiation duration (Ahamad N. et al., 2021).

 doi:10.20944/preprints202407.2306.v1

In another study by Pasquet V. et al. in 2011, the performance of ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), and vacuum microwave-assisted extraction (VMAE) was compared to conventional hot and cold soaking methods for chlorophyll extraction from two microalgal species, C. Closterium (CC) and D. Tertiolecta (DT). Hot and cold soaking highlighted the importance of temperature in chlorophyll extraction, emphasizing the need to establish a maximum temperature that does not degrade the extracted compound. MAE at 50W and 56°C for 3-5 minutes yielded the highest extraction efficiency. In contrast, vacuum microwave-assisted extraction (VMAE) performed at 22°C yielded lower efficiency compared to MAE and involved a more labor-intensive process (Pasquet V. et al., 2011).

3.2.4. Enzyme-Assisted Extraction (EAE)

An enzyme-assisted extraction (EAE) process involves the use of enzymes to facilitate the destruction of cell walls and to allow the release of bioactive compounds such as carotenoids, chlorophylls, and xanthophylls. Enzymes can improve the efficiency of the extraction process, can increase selectivity, and can shorten the duration of extraction. They contribute to improving the quality of pigments obtained from natural sources, making this method valuable for extracting bioactive compounds (Marathe S. J. et al., 2019).

Enzymes frequently used in the extraction process of biofactors include α-amylase, β-glucosidase, cellulase, β-glucanase, pectinase, xylanase, and other similar enzymes (Marathe S. J. et al., 2019). The interaction between enzyme and substrate is influenced by factors such as the size of the plant material, enzyme concentration, reaction duration, temperature, pH, and solid-liquid ratio. To maximize the interaction between enzyme and substrate, these parameters must be adjusted to optimal values. When considering the optimal pH, the isoelectric point should be avoided, as proteins become insoluble in this area, which can affect the extraction of bioactive substances (Shakoor R. et al., 2023).

Also, at sub-optimal temperatures, enzymes exhibit reduced activity, while higher temperatures can lead to enzyme degradation, both situations reducing the efficiency of extracting bioactive substances. Although a higher concentration of enzyme could improve substrate binding and decomposition, the costs of the process must be appropriately considered (Marathe S. J. et al., 2019).

The enzyme-assisted extraction (EAE) method offers numerous advantages compared to conventional extraction tools. These include:

- Higher selectivity,
- Induced efficiency,
- Ease in extraction,
- Safer and friendlier working conditions,
- Minimum energy and usage requirements,
- Absence or reduced use of harsh substances,
- Superior yield,
- Absence of wasteful protection or deprotection stages,
- Ease in isolation and recovery of the product,
- Possibility of process recycling (Bilal M. and Iqbal H. M., 2020).

In their study, Saeed R. et al. (2022) investigated the effect of pH on extraction yield. They found that at a temperature of 65°C, 150 minutes, 5.5 mL of enzyme, and a pH of 5.5, the highest yield (36.51%) was achieved. In contrast, at a pH of 3.5, a temperature of 45°C, 30 minutes, and 5.5 mL of enzyme, the yield was 13.36%. These results highlight the importance of extraction time, as it facilitates the interaction between the enzyme and the cell wall, leading to increased extraction of bioactive compounds.

Enzymatic extraction methods have been enhanced by combining them with green procedures and technologies, such as ultrasound-assisted enzymatic extraction (UAEE), microwave-assisted

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enzymatic extraction (MAE), enzyme-assisted supercritical fluid extraction (EASCFE), and enzyme-assisted ionic liquid extraction (ILEAE) (Marathe S. J. et al., 2019).

The use of UAE in conjunction with enzymatic extraction (EAE) has been demonstrated by Saeed R. et al. (2022) to be much more efficient, yielding 32.35% compared to 12.15% obtained through maceration. This efficiency is attributed to the combined effect of the enzyme hydrolyzing the cell wall and the porous texture induced by ultrasound (Saeed R. et al., 2022).

3.2.5. Ultrasound-Assisted Enzymatic Extraction (UAEE)

The efficiency of the extraction process can be enhanced by using ultrasound, which generates pressure waves and cavitation phenomena. These exert a mechanical effect that allows the solvent to penetrate deeper into the tissue, thus increasing the contact surface between the solid phase and the liquid phase, leading to improved diffusion into the solvent (Marathe S. J. et al., 2019).

However, ultrasound produces intense heat, which can denature heat-sensitive compounds. Therefore, combining enzyme-assisted extraction (EAE) with ultrasound-assisted extraction (UAE) can help combat this disadvantage (Shakoor R. et al., 2023). Acoustic cavities present hydrophobic surfaces in the extraction liquid, thus increasing the hydrophobic character of the extraction medium. Micro-jets impact the surface, causing exfoliation, erosion, and decomposition of particles, leading to the creation of new surfaces and increased mass transfer (Marathe S. J. et al., 2019).

Thus, it is possible to extract polar fractions in aqueous, hydrophilic extraction media, thus reducing the need to use hydrophobic or strongly polar extraction solvents, generally unwanted. Optimizing extraction parameters is essential for improving extraction yields (Shakoor R. et al., 2023).

One of the essential parameters is the solvent, which is chosen based on the solubility of the desired compound for UAE. However, other factors such as surface tension, vapor pressure, and viscosity should also be considered. When dealing with a sample of high viscosity, it will naturally resist movement caused by ultrasound. Therefore, adjusting the amplitude becomes necessary (Chemat F. et al., 2017).

Another crucial parameter is temperature, which generally leads to better extraction yields. However, operating close to the solvent's boiling point may decrease the process efficiency. The literature suggests that the optimal extraction temperature for UAE typically ranges between 20°C and 70°C, with the most favorable results observed below 30°C (Chemat F. et al., 2017).

According to Marathe S. J. et al., 2019, ultrasound treatment of carrot residues led to the production of β-carotene in proportions of up to 83.32% under optimized operating conditions (50 minutes of ultrasound irradiation, 50°C, 100 W, 60% duty cycle, and solid-solvent ratio of 0.3:20).

Also, the maximum level of lycopene release from tomatoes was obtained following the use of cellulase and pectinase enzymes with a concentration of 3% (w/w), a pH equal to 5.0, a temperature of 50 C, power 10W, and an incubation time of 20 minutes (Marathe S. J. et al., 2019).

3.2.6. Microwave-Assisted Enzymatic Extraction (MAEE)

Through this technique, carotenes and chlorophylls were extracted from marine biomass. The mechanism of action assumes that the source material is subjected to microwave radiation, specifically non-ionizing electromagnetic waves at approximately 2000 MHz, in a controlled environment and for a short period of time. In this way, the movement of polar molecules and the rotation of dipoles are induced so that the solvents are heated. For increased yield, this process is repeated several times with a cooling break to avoid thermal degradation of the sample. In this way, the transfer of bioactive compounds from the matrix to the solvent is favored (Bilal M. and Iqbal H. M., 2020).

The extraction process begins by homogenizing the source material with the solvent. The most commonly used MAE solvents include acetone, acetonitrile, ethanol, methanol, and dichloromethane, each with different polarity indices. According to microwave theory, the radiated waves are composed of two perpendicular oscillating fields, the electric field and the magnetic field. The electric field is responsible for heating the sample. The principle of heating is governed by two phenomena, namely dipole rotation and ionic conduction (Bilal M. and Iqbal H. M., 2020).

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Table 4. Conventional, advanced and integrative extraction methods of bioactive compounds.

	Extraction Method	Advantages	Disadvantages	Source
Conventional	Soxhlet	Favors the kinetic process by applicability at high temperatures, simplicity	Low yield and long extraction time	Soquetta et al. 2018, Jha A. K. and Sit N., 2022
	Maceration	Allows modulation of selectivity by solvent selection, has low costs	Thermal destruction of some samples or compounds	Soquetta et al. 2018
	Hydrodistillation	Automatic separation of bioactive compounds based on physicochemical properties	Volatile compounds can evaporate if temperature rises too much	Jha A. K. and Sit N., 2022
	Infusion	Short time and will consume a latent heat of vaporization smaller than that of water	Use of large amounts of solvent	Jha A. K. and Sit N., 2022
	Digestive	The yield of the extraction process can be increased by heating	Long extraction time	Nadar S. S. et al., 2018, Umair Muhammad et al., 2021
	Exhaustive extraction in series	Higher yield of extract from the matrix of interest, especially in the case of substances that are found in small concentrations or are difficult to extract	Degradation of compounds after prolonged exposure to high temperatures	Nadar et al. 2018
Advanced	Supercritical fluid extraction	Increases the recovery process of bioactive compounds from natural sources	High cost	Saini și Keum 2018
	Microwave-assisted extraction	The amount of solvents used and extraction time are reduced, high yields in a short time	High temperatures	Tsiaka et al. 2018, Umair Muhammad et al., 2021
	Pressurized liquid extraction	Allows obtaining higher yields and can be more efficient than traditional methods	High temperatures, low flow rate and high costs	Bilal M. and Iqbal H. M., 2020
	Pulsed electric fields	Has the potential to improve the yield and quality of extracts from natural materials, and its combination with other extraction techniques can offer additional advantages	Conductivity and enzyme use are necessary	Jha A. K. and Sit N., 2022
	High hydrostatic pressure-assisted extraction	It is an ecological method and does not cause denaturations or major damage	Can induce structural changes at the level of	Jha A. K. and Sit N., 2022

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			sensitive molecules	
	Enzyme-assisted extraction	Improving the yield of extraction and the pharmacological properties of the extract	Additional steps in wet conditions	Nadar S. S. et al., 2018
	High voltage electric discharge extraction	Increase in extraction yield and increase in extract purity	Can cause oxidation of samples by producing free radicals	Jha A. K. and Sit N., 2022
	Ultrasound-microwave-assisted extraction	Efficient and rapid extraction of bioactive compounds from plant materials	Decreases extraction yield if the alkyl chain of the compound increases	López-Cruz, R. et al., 2023
Integrative	Enzyme-assisted, ultrasound and microwave extraction	Reduces extraction time and increases process yield	High costs	Nadar S. S. et al., 2018, Umair Muhammad et al., 2021, López-Cruz, R. et al., 2023
	Supercritical carbon dioxide combined with pressure swing technique	Beneficial for efficient extraction of bioactive compounds from various natural sources	High costs	Nakhle, L., Amat, A. M., Karim, M., and Atieh, E. 2021
	Supercritical fluid extraction – Pressurized liquid extraction (SFE-PLE)	The extraction time can be 2 to 2.5 times faster resulting in a better yield	High costs	Lefebvre T. et al., 2021
	Pulsed electric field and high voltage techniques	Antioxidants can be extracted from mango peel	High cost	Jha A. K. and Sit N., 2022
	Supercritical fluid extraction Assisted by Ultrasound (SFE-UAE)	Significant improvements in yield and extraction kinetics	High cost	Roca M. and Pérez-Gálvez A., 2021
	Extraction with the help of ultrasound, pulsed electric field and high hydrostatic pressure	Increases extraction efficiency and protects heat-sensitive compounds and does not use toxic solvents	High costs	Jha A. K. and Sit N., 2022
	High Hydrostatic Pressure-Extraction by Shaking (HHPE-AE)	Improves the permeability of plant cells and the diffusion of bioactive compounds, thus facilitating extraction efficiency	Expensive equipment	Jha A. K. and Sit N., 2022
	Ultrasound-assisted extraction	Efficiency in extracting bioactive compounds,	High costs	López-Cruz, R. et al., 2023

– Pressurized liquid extraction	especially pigments, from plant and marine raw materials		
High Hydrostatic Pressure - Ultrasound Extraction (HHPE-UE)	Obtains high-quality extracts, having a positive impact on yield and antioxidant activity	Semi-continuous or discontinuous operation	Jha A. K. and Sit N., 2022
Supercritical carbon dioxide extraction (SCCO2) - Subcritical water extraction (SWE)	Does not require filtration and does not produce hazardous waste because it uses Co2 which is recyclable and non-toxic and increases the yield and purity of the extraction process	High technical complexity	Saini R. K. and Keum Y. S., 2018

4. Conclusions

In the food industry, a significant amount of biodegradable organic secondary waste is produced, which can be utilized to reduce biological waste and recover important bioactive compounds. The extraction of carotenoids, chlorophylls, and xanthophylls depends on the type of solvent, the selected method, working conditions (temperature, pH, light), and the solute components.

Choosing the right solvent is critical for achieving satisfactory results because its efficiency determines increased extraction yield, reduced working time, and minimized interference between compounds. Additionally, understanding the material's morphology from which the extraction will be performed is essential.

When using organic solvents such as DES and NADES, they offer the additional advantage of being non-toxic, thus reducing environmental impact. Enzyme-assisted extraction (EAE) involves analyzing catalytic properties, optimal operating conditions, enzyme synergy, and mode of action. Unconventional methods have proven to be more efficient due to reduced extraction time, even though similar extraction yields were obtained. Furthermore, unconventional methods can be combined to enhance extraction potential.

Given the variability in physicochemical properties of the extraction material, differential characteristics among compounds (even within the same class), and their interaction with the solvent, it is challenging to optimize a universally applicable extraction method.

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