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A systematic review on carotenoids: Its health benefits, extraction and fortifications in food

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Abstract
Carotenoids are phytochemicals constituted with a ubiquitous group of isoprenoid pigments with great dietary importance and health benefits. Carotene rich foods like carrot, spinach, sweet potato among several others reduced the incidence of innumerable diseases like cardio-vascular diseases, muscular dystrophy, cancer, diseases related to low immune function and several other degenerative diseases are not synthesized by humans and animals. Carotenoids act as singlet oxygen quenchers and exhibit anti and pro oxidant activity. Recent developments in both conventional and non-conventional methods for the extraction of carotenoids have helped in optimized extraction with highest recovery and methods include Ultra-sound assisted extraction (UAE), pressurized liquid extraction (PLE), Extraction using organic solvents (Green solvents), Supercritical fluid extraction (SFE) and Ionic liquid extraction. The therapeutic and other health benefits of carotenoids lead to fortify them in food and also in the field of feed, cosmetics and pharmaceuticals. Fortification made it convenient for manipulating the carotenoid-profile of the product and thereby to increase the bio-availability of carotenoids Fortification of carotenoid helps to increase its bio availability in various products. This review aims for a systematic approach towards the health benefits, methods of extraction and how they can be used in fortification of various products.

Keywords: Carotenoids, health benefits, extraction, fortification

1. Introduction

Carotenoids are isoprenoid compounds that are lipophilic in nature and are pigmented. They are produced by all photosynthetic organisms and some non-photosynthetic organisms like bacteria and fungi. Animals including humans however cannot produce carotenoids but are taken through diets (Manuel Rodriguez Concepcion *et al.*, 2018) [1]. Carotenoids are richly found in fruits, vegetables and has significant role in phtochemistry, phytobiology and medical fields. Carotenoids possess anti and pro oxidant activity as they interact with singlet oxygen and other wide range of free radicals(R Edge *et al.*, 1997) [2]. Despite being natural pigments, carotene has an important role as the principal precursor of vitamin A. Studies prove that carotenoids are related with enhancement of immune system reduced risk of cardio vascular diseases, muscular degeneration and are anti-cancerous (Debjani Dutta *et al.*, 2005) [3]. Carotenoids are yellow to red isoprenoid phytochemicals which exerts natural antioxidant, anti-cancerous, anti-inflammatory, lipid-lowering, insulin sensitizing properties and are anti-fibrotic (Yoojin lee *et al.*, 2019) [4]. Within the chromoplasts carotenoids exists in association with proteins and other macromolecules covered by rigid cell walls. These act as structural barriers of carotenoid release and hence affects its bioavailability (Ramesh Kumar Saini *et al.*, 2022) [5]. Bioavailability of carotenoids in different food matrices differ due to its intercellular location and intactness of cellular matrices. Blanched vegetables have increased extractability of β- carotene than raw form (Debjani Dutta *et al.*, 2005) [3]. Presence of pectin like compounds which is a primary dietary fibre present in fruits and vegetables affects lipid digestion that inturn influence the carotenoid bioaccessibility. Carotenoids have presence of high contents of antioxidant compounds and thereby minimize the risk of numerous chronic diseases and are anticancerous. Studies proves that this chronic diseases and disorders are due to increased levels of proinflammatory mediators, which carotenoids can regulate by the oxidative stress modulation mechanism (Ramesh Kumar Saini *et al.*, 2022) [5]. Due to the lipophilic nature of carotenoids they are extracted using a mixture of organic solvents after proper sample preparation. For the extraction of carotenoids from foods normally acetone, hexane, ethyl ether or their combination are used considering the stability of different carotenoids with the extracting solvent.

Solvents are selected based on sample matrix and relative polarity with the carotenoid to be extracted (Rachel E Kopec *et al*., 2012) [6]. selection of appropriate solvent or combination of solvents is the crucial factors for efficient extraction of carotenoids. Supercritical carbon dioxide extraction (SCE) is the best known technique for carotenoid extraction. Apart from this Microwave assisted Extraction (MAE) and ultrasonication-assisted extraction (UAE) have also emerged as techniques for carotenoid extraction. Residual toxins from solvents raise critical setbacks and can cause adverse health issues. SCE has less of this residual toxins. Taking into account the harmful effects of toxins recent developments have reported in extraction of carotenoids using organic solvents. It is widely called green solvent extraction, solvents like sunflower oils can be used Aas a replacement for organic solvents (Ramesh Kumar Saini *et al*., 2022) [5].

2. Health Benefits of carotenoids

Carotenoids are lipophilic compounds predominantly found in carrot, apricot, sweet potato, pumpkin and spinach. In a pharmaceutical way of approach they play a very important role as vitamin A precursor. Apart from these, this pigments inhibits a wide variety of cancer and various other chronic diseases (Fazul Nabi *et al*., 2020) [7]. It effectively enhance immune system and are involved in activation of better cell communication (Singh *et al*., 2015) [8].

α-carotene and β-carotene are considered principally as precursor of Vitamin A. Provitamin A activity helps natural carotenoids to form retinol. β-carotene has higher conversion efficacy to retinol than α-carotene (Tang, 2014) [9]. Major carotenoid found in fruits and vegetables is lycopene. Lycopene is known to reduce oxidative stress and minimize the risk of ovarian and prostate cancer(Arain *et al*., 2018) [10]. Studies reported that lycopene reduce the risk of cardiovascular diseases and prevented oxidative damage to the skin barrier (Giovannucci *et al*., 1995, camara *et al*., 2013) [11, 12].

Citrus fruits are rich in β- cryptoxanthin. This carotenoid also has provitamin A activity and has proved to reduce the occurrence of osteoporosis (Yamaguchi, 2018) [13]. β-cryptoxanthin also act as therapeutic agent prominently against bone related issues.

Carotenoids are active compounds that has significant biological effects and potent health benefits. They are potent singular oxygen scavengers and hence act as antioxidants. They help in improving acquired and innate immunity. Carotenoids are anti inflammatory in nature as they block inflammatory process. They possess antimicrobial effect by reducing the growth of pathogenic bacteria. They are anticancerous and are active against breast, cervical and prostate cancers(Milani *et al*., 2017) [14].

3. Chemistry and occurrence of carotenoids

Carotenoids are isoprenoid compounds that cannot be produced by animals but has to taken through diets. They play significant role in animals like modulation of immune system, ornamentations in flaminos and salmon, protection against various cancers and are precursors of vitamin A. They also have photoprotection capability against UV rays hence are used widely in cosmetic products. Carotenoids are broadly classified as two groups; i) xanthophylls ii) carotenes. Xanthophylls contain oxygen as their functional group like lutein and zeaxanthin while carotenes have only hydrocarbon chain without a functional group like β-carotene and lycopene.

Carotenoids differ in their polarity and their alignment in food matrix. Rigid chemical barriers of complex food matrices doesn't allow the mass transfer of carotenoids and hence leads to reduced recovery percentage after extraction. Polar carotenoids can be extracted using solvents like acetone, ethanol, ethyl acetate while non polar solvents like hexane or petroleum ether can be used for extraction of non polar carotenoids. Organic solvents can be used for extraction of carotenoids due to their hydrophobicity. Long time exposure during extraction cannot be followed for carotenoids as they have oxidative property. This also influence its exposure limit to acids, light and heat.

Carotenoids can be extracted from natural sources through the following methods:

- Microwave assisted extraction (MAE)
- Ultra sound assisted extraction(UAE)
- Accelerated Solvent Extraction (ASE) also known as Pressurized Liquid extraction(PLE)
- Pulsed electric field (PEF) assisted extraction
- Supercritical fluid extraction(SFE)
- Enzyme assisted extraction(EAE)

Solvents are used at boiling temperatures and ambient pressure for soxhlet extraction while for ASE and SFE it is maintained at low temperature and high pressure. UAE, PEF AND EAE employs ultrasound waves, high voltage pulses and cellulolytic enzymes respectively for cellular disintegration.

With proper modelling of extraction kinetics, carotenoid availability in micro algae was discussed and downstream processing of carotenoid extract from algae was reviewed (Maki-Arvela *et al*., 2014) [15]. Due to the presence of harmful toxin residues after extraction, thought of using environmentally safe and non-toxic green solvents has gained popularity in recent years. Extraction was coined as green extraction. In this review all this methods are discussed briefly.

Carotenoid extraction from fruits and vegetables are difficult owing to their high moisture content. Carotenoids being hydrophobic makes it difficult for the breakdown of cell wall and other physical barriers. Dehydration methods like conventional thermal dehydration techniques cannot be employed as it cause thermal degradation and isomerization of carotenoids. Thus, food samples with high moisture content are dehydrated using freeze drying or lyophilization. To minimize the degradation of cellular systems a neutralizer like calcium carbonate or sodium carbonate can be added during extraction to neutralize acids released from plant samples. To provide an inert environment during extraction sample tubes are flushed with nitrogen to eliminate oxygen. Care should be given to prevent the samples being exposed to UV light as it cause photodestruction to the cellular systems.

4. Pre-treatments and selection of solvents before extraction

Various physical and chemical pre-treatments are given to food matrix as they have complex mechanical barriers that act as a protective layer thus making the pigment extraction difficult. Pre-treatments helps in release of carotenoids from food samples by facilitating disruption of cellular matrix.

Appropriate technique for pre-treatment is based on cell-wall characteristics and cellular matrix. Disruption of barriers is the first step involved in extraction. It can be by physical means like using methods of drying, osmotic shock, cooking or by chemical means by use of acids and base. It can also be done using biological means with help of enzymes. Microemulsion techniques using surfactants were also used for extraction (Amiri-Rigi & Abbasi, 2016) [16]. Among all the pre treatments, cooking was found to be more efficient as it directly disrupt carotenoid-protein complex and thus helped in highest yield of carotenoids (Castro-Puyana *et al*., 2013) [17]. Extraction method should be properly optimized for higher extraction rates and cost effective production of carotenoids. Cell wall disruption of microalgae *Chlorella* and *Haemotococcus* and thereby extraction of lipids and astaxanthin from them was reviewed by (Kim *et al*., 2016) [18]. Apart from availability of many means of pre treatments for cell wall disruption, huge critical issues like energy consumption, toxic residues and scaling up of compounds and their removal still need to be researched well. Conventional method of carotenoid extraction involves using of organic solvents like hexane, acetone. Methanol, diethyl ether and chloroform or their combinations to provide synergistic effect on extraction process. Appropriate solvent should be selected based on considering moisture content of the food matrix, polarity of carotenoids and complexity of sample matrix. Acetone and hexane are commonly used solvent for the extraction of polar and non-polar carotenoids respectively. A combination of acetone with ethanol or hexane can be used for extraction of polar and non-polar carotenoids simultaneously while acetone and ethanol combination is frequently used for extraction purpose for foods with high moisture content(Amorim-Carrilho *et al*., 2014) [19]. Apart from commonly used solvents like acetone, ethanol and hexane, on the basis of consideration of environmental health and safety issues green solvents and ionic liquids are also used recently for the extraction of carotenoids.

5. Extraction methods of carotenoids

5.1 Soxhlet extraction

It utilizes appropriate solvents and it is the conventional method of extraction that provides highest recovery of carotenoids. Solvents are used at boiling temperature and at low pressures. Soxhlet extraction has higher selectivity for carotenoids under optimized conditions. Since the solvents are used under boiling temperature chances of thermal degradation is higher and can even lead to cis-trans isomerization of carotenoids(Cardenas Toro *et al*., 2015) [20]. ASE can replace time consuming soxhlet extraction in the industrial scale extraction point of view.

5.2 Atmospheric liquid extraction with maceration

Carotenoids are protected by protective rigid cell walls. Cell wall disruption by means of physical, chemical or biological means disrupt cellular structure and thereby helps the organic solvents to enter the cellular matrix for efficient solubilization of intercellular carotenoids thus helping to increase the yield of extraction by 8-10 folds (Michelon *et al*., 2012) [21]. Maceration involves no heat thus minimize thermal degradation and reduces contact time of solvent with sample thus minimizing toxic residue. Extraction of carotenoid from pink shrimp was attempted with maceration using acetone followed by soxhlet extraction. A satisfactory yield of carotenoids was obtained. Authors concluded that highest total yield of targeted carotenoids was obtained by solvent extraction technique while SFE yielded carotenoids with highest purity (Mezomo *et al*., 2011) [22]. Due to the over cost production and presence of extremely harmful residual toxins the use of organic solvents like chloroform, hexane, acetone and ethyl ether are limited in application of food and feed even they provide reasonable yield.

5.3 Accelerated solvent extraction (ASE)

Accelerated solvent extraction used solvents at higher temperature and pressure. Automated mechanisms are used for optimized control over temperature and pressure under absence of light and oxygen. Larger number of samples can be extracted under precision. Since the method use high pressure it is also called Pressurized liquid extraction (PLE). Higher temperature helps to reduce the viscosity of solvents helping in its better penetration to carotenoid cellular matrix. Higher pressure helps to facilitate intermolecular physical interaction by improving cell permeability. High pressure also leads to denaturation of carotenoid binding protein thus helping in the mass transfer of carotenoids. ASE was used to extract carotenoids of different polarities from lyophilized apricots at 40 °C at 103 bar pressure for 5 min extraction time. High recovery of less polar carotenoids were obtained within short span of extraction time (Zaghdoudi *et al*., 2015) [23]. ASE even utilizes less amount of solvent compared with solvent extraction without much difference in extraction yield. When an attempt was made to extract carotenoid from green vegetative cells and cysts of micro algae using hexane as organic solvent, it was found that extraction temperature was in positive significance with extraction yield. Extraction yield was even higher when solvent used was replaced with ethanol (Jaime *et al*., 2010) [24].

5.4 Ultra sound assisted extraction (UAE)

Ultrasound is an effective technique used in metabolite extraction and microbial inactivation. At a particular pulse rate and suitable power ultrasound technique can create cavitation in liquid media and thus enhance mass transfer of compounds to be extracted. Metabolites can be effectively extracted using this technique under optimized temperature, solvent to sample ratio, power and intensity. For the highest recovery of carotenoids range of ultrasound intensity should be properly optimized else it can lead to accumulation of OH and H radicals. These radicals are usually formed above the optimum intensity range and can interfere with antioxidant compounds like carotenoids (Pingret *et al*., 2013) [25]. UAE was used in extraction of carotenoids from microalgae *Heterochlorella luteoviridis* using 50% ultrasound intensity, 75% ethanol as solvent at 30 °C temperature. Around 80% yield of carotenoids were obtained. There was significant decrease observed in yield when intensity was increased to 100% (Tsiaka *et al*., 2015) [26].

5.5 Microwave assisted extraction (MAE)

MAE is highly economical and rapid technology for the extraction of carotenoids. It is simple and requires a very short extraction time but the yield obtained is comparatively lower to that of soxhlet extraction. Huge variety of samples can be subjected to extraction simultaneously. Samples must subjected to certain pre-treatments before MAE for improving yield of carotenoids. MAE technique provides satisfactory yield when it is properly optimized with respect to power, solvent to sample density and intermittency ratio. MAE involves unavoidable yet minimal thermal degradation and cis-trans isomerization of carotenoids. Thus to control this samples are subjected to intermittent radiation. This has proved to be increasing yields of extracted carotenoids and improve antioxidant activity. To minimize thermal degradation caused by MAE (Hiranvarachat and Devahastin, 2014) [27] attempted an alternative procedure to increase the yield of β-carotene and carotenoids from carrot waste. A lower microwave power (180W) and solvent volume (75 ml) or higher microwave power (300W) and solvent volume (150 ml) was combined with lower intimacy ratio. There was observable changes in β-carotene and total carotenoids content when compared with MAE operated under similar condition in a continuous manner.

5.6 Pulsed Electric Field (PEF) and Moderate Electric Field (MEF) assisted extraction

Pulsed electric field (PEF) is a non- thermal low-energy required method for extraction of carotenoids. It was developed with a view to minimize the effect of thermal destruction to cellular contents. The biological material from which extractant should be taken is kept between two electrodes and electric pulses usually of short span of milliseconds is pulsed at regular time intervals between these electrodes. Through the process of electroporation these pulses makes the cell wall of cellular matrix permeable. The permeability caused is either temporary or permanent. PEF was applied to chlorella vulgaris at different pulse duration of milliseconds and microseconds to check permeabilization and extraction of pigments (Luengo *et al*., 2015) [28]. MEF was used to extract lipid and carotenoids from microalgae using ethanol as solvent. It was found that yield varied with ethanol concentration and electric field strength (Jaeschke *et al*., 2016) [29]. To obtain satisfactory yield appropriate electric field strength must be selected for each food matrix as they may differ in tissue texture.

5.7 Supercritical fluid extraction (SFE)

Supercritical fluid extraction technique use supercritical carbon-dioxide as extraction solvent. It is an economical replacement for extraction highly pure of heat labile compounds like carotenoids. Supercritical carbon-dioxide rapidly penetrates the cellular matrix of food sample and allows mass flow of carotenoids as they have higher diffusion coefficient and lower viscosity. It penetrates even the complex food matrix thus enhancing the extraction process. Highest recovery of carotenoids from freeze dried powder of marine microalga *S. almeriensis* was obtained at 400 bar pressure and 60 °C temperature. 50% yield was obtained (Macias-sanchez *et al*., 2010) [30]. Extracts of compounds that are obtained after SFE is highly concentrated and carbon-dioxide can be easily separated using depressurizing technique. Thus SFE is considered as a green technique for extraction as it has no residual toxins of solvent. Depressurization can also be used for rapid cell disruption to improve extraction and thereby reducing time and labor. Temperature around 40-60 °C, pressure of 300-400 bar and time between 30-120 min are considered to be optimized extraction parameter for carotenoids. To increase the yield of extraction process co-solvents of entrainers like ethanol can be added as they help in increasing solubility of analytes and sometimes helps in modification of matrix and thereby helping in release of analytes from matrix. Apart from ethanol, acetone, propane, methanol and ethyl acetate can also be used as organic modifiers. SFE can be used for extraction of polar and non polar carotenoids. Levels of pressure, temperature, flow rate and carbon-dioxide density must be changed accordingly with the polarity of compounds. SFE can be coupled with Liquid or gas chromatography for simultaneous extraction and analysis of photo-constituents. SFE is the best employed method for extraction of highly pure carotenoids without the use of toxic solvents. It allows fast and accurate analysis of carotenoids and other thermo-labile metabolites.

5.8 Enzyme-assisted extraction

Enzyme assisted Extraction technique is a promising method for industrial scale extraction of carotenoids. In this methods structural integrity of cell walls of food matrix is disrupted with the help of hydrolytic enzymes (Sowbhagya and Chitra, 2010) [31]. Cellulase and pectin enzymes are generally used respectively for altering the structural integrity of cell wall and pectin substances of of cellular matrix. These enzymes are used as pre-treatment before the extraction with organic solvents. Cellulase breakdown the 1,4-β-d-glycosidic linkage of the primary cell wall and pectin breaks down pectic substances of lamella and primary cell wall. This increases the yield by 6-10 fold. Samples are then subjected to organic solvent extraction using ethyl acetate or any other suitable solvent (Strati *et al*., 2015) [32].

5.9 Extraction using green solvents

All the extraction methods used for industrial scale extraction of carotenoids requires organic solvents obtained from renewable sources. These solvents contains highly toxic residues and are highly volatile and inflammable thereby leading to green house effects and other toxic effects and health hazards to the consumer. Taking this to accounts industries demands a drift to green solvents to replace the organic solvents. Green solvents like cyclopentyl methyl ether (CPME), dimethyl carbonate (DMC), Ethyl acetate(EA), isopropyl alcohol (IPA) and 2-methyltetrahydrfuran (2-Me-THF) are now being replaced for n-hexane, acetone, ethyl ether and various other solvents. Vegetables oils like sunflower oils can also be used as an efficient replacement for organic solvents. The optimization in the application of vegetable oils as solvents in carotenoid extraction must be investigated for its future application at an industrial scale. Owing to the higher viscosity of vegetable oils, diffusion of carotenoids from food matrix is a limiting factor. This can be improved by increasing the diffusivity with the help of ultrasound technique. Carotenoids were extracted using ultra sound assisted technique from pomegranate peels using sunflower oil as green solvent with an extraction time of 30 min at 51.5 °C (Goula *et al*., 2017) [33].

Ionic liquids which are composed of loosely held anions and cations are recently an interest of research. They are successfully used in green extraction of carotenoids. They gain attraction as they have lower volatility and are non-flammable. They are environmental friendly and are widely studied for their utilization for extraction of bioactive compounds including carotenoids. Proper examination of ionic liquids must be examined focusing on their chemical properties, cyclotoxicity and environmental impact.

6. Fortification

Vitamin A deficiency is a major micronutrient deficiency that effects half of the world population. Carotenoids are essential components for human health and nutrition, mainly due to their antioxidant and pro-vitamin A activity. Carotenoids are essential micronutrient that must be taken in accordance with the daily recommended intake limit. Fortification of carotenoid is very challenging as it is highly unstable in the presence of heat, oxygen, light and pH. Due to their higher sensitivity and rapid chemical degradation addition of carotenoids through fortification is difficult. Biotechnological and genetic engineering based strategies and techniques are widely studied and are used to manipulate carotenoid metabolism. This was successfully implemented in many crops like maize and golden rice and are widely consumed (Enriqueta Alos *et al*., 2016) [34]. Owing to the fact carotenoids loose their biological activity when exposed to light, heat and oxygen, microencapsulation is a promising and proven technique for incorporation of carotenoids in food matrix without losing their biological activity. Practical impossibility of incorporating micronutrients in water-based formulations can be overcome by encapsulating systems. (Gisleania Dourado *et al*., 2021) [35] successfully incorporated carotenoids extracted from carrots in tapioca pancakes with microencapsulation technique. The carotenoid microparticles were produced from carrot juice. Gum arabic and whey protein isolate were used as stabilizers for complex coacervation process. Chocolate bars fortified with nano-encapsulated carotenoids extracted from spirulina to enhance premium nutrition at the composition of chocolate paste : cocoa butter at 27.5:25 and 0.372% of added nanocapsule didn't show any difference in aroma, taste and texture (Nurfitri *et al*., 2019) [36]. (Tarek and Sahar, 2022) [37] extracted carotenoids from carrot pomice using ultrasonication and nanoemulsion carotenoids were incorporated with alginate to produce beads by extrusion technique and the double-encapsulated carotenoids were incorporated to functional orange flavoured stirred yougurt. To alleviate malnutrition and other nutritional- related problems the strategies for the adoption of bio-fortification of carotenoids in staple foods are widely investigated.

7. Conclusion

Carotenoids being essential components of human nutrition are hydrophobic isoprenoid compounds which are found abundantly in carrot, tomato, pumpkin, apricot, sweet potato and spinach. They are natural pigments and principal precursor of vitamin A. Carotenoids are related with enhancement of immune system reduced risk of cardio vascular diseases, muscular degeneration and are anti-cancerous. Carotenoids are yellow to red phytochemicals which exerts natural antioxidant, anti-cancerous, anti-inflammatory, lipid-lowering, insulin sensitizing properties and are anti-fibrotic. Carotenoids are active compounds that has significant biological effects and potent health benefits. They are potent singular oxygen scavengers and hence act as antioxidants. They help in improving acquired and innate immunity. Carotenoids are broadly classified as two groups, xanthophylls and carotenes. Xanthophylls contain oxygen as their functional group like lutein and zeaxanthin while carotenes have only hydrocarbon chain without a functional group like β-carotene and lycopene. Pre-treatments helps in release of carotenoids from food samples by facilitating disruption of cellular matrix and thereby increasing the extraction yield. Appropriate technique for pre-treatment is based on cell-wall characteristics and cellular matrix. Disruption of barriers is the first step involved in extraction. It can be by physical means like using methods of drying, osmotic shock, cooking or by chemical means by use of acids and base. In general, acetone and hexane are solvents used for extraction. The effective method for extraction of carotenoids from various samples with different polarity and nature of matrix has been widely studied. Proper optimization of technique with suitable solvent mixture for each kind of sample must be chosen for higher extraction yield of carotenoids.

Soxhlet extraction technique provided highest yield of carotenoid extraction. Supercritical fluid extraction technique minimized the time of process and amount of solvent. Since the technique used carbon-dioxide as solvent, there was no trace of residual solvent left in final product. Since carotenoids are heat sensitive bio-compounds use of non-thermal methods like Ultra sound assisted extraction, Pulsed electric field extraction and Enzymes assisted extraction can be used for rapid and efficient recovery. Recent innovations in extraction are made through use of green solvents in the extraction process. Further investigations have to be done for its application in large scale industrial use.

Carotenoids are essential micronutrient that must be taken in accordance with the daily recommended intake limit. Fortification of carotenoid is very challenging as it is highly unstable in the presence of heat, oxygen and light. Biotechnological and genetic engineering based strategies and techniques are widely studied and are used to manipulate carotenoid metabolism. Owing to the fact carotenoids loose their biological activity when exposed to light, heat and oxygen, microencapsulation is a promising and proven technique for incorporation of carotenoids in food matrix without losing their biological activity. Still methodological and technological advancements in these field are desirable.

8. Conflict of interest

The authors have declared that there is no conflict of interest.

9. References

1. Amiri-Rigi A, Abbasi S. Microemulsion-based lycopene extraction: effect of surfactants, co-surfactants and pre-treatments. Food chemistry. 2016;197(part A):1002-1007.
2. Amorim-carrilho, Cepeda KT, Fente A, Regal P. Review of methods for analysis of carotenoids. Trends in analytical chemistry. 2014;56:49-73.
3. Arain MA, Mei Z, Hassan F, Saeed M, Alagawany. Lycopene: a natural antioxidant for prevention of heat-induced oxidative stress in poultry. Worlds poultry science Journal. 2018;74(1):89-100.
4. Camara Montana, Maria De Cortez, Virginia Fernandez, Rosa Maria. Lycopene: A review of chemical and biological activity related to beneficial health effects. Studies in natural products chemistry. 2013;40:383-426.
5. Cardenas-Toro, Alcazar Alay FP, Coutinho JP, Godoy HT, Foster-carneiro. Pressurized liquid extraction and low-pressure solvent extraction of carotenoids from pressed palm fiber: Experimental and economical evaluation. Food and bioproducts processing. 2015;94:90-100.

6. Castro-Puyana M, Herrero M, Urreta, Mendiola, Cifuentes JA, Ibanez E *et al*. Optimization of clean extraction methods to isolate acrotenoids from the microalga *Neochloris oleoabundans* and subsequent chemical charecterization using liquid chromatography tandem mass spectroscopy. Analytical and Bioanalytical chemistry. 2013;405(13):4607-4616.
7. Debjani Dutta, Utpal Ray Chaudari, Runu Chakraborty. Structure, health benefits, antioxidants property and processing and storage of carotenoids. African Journal of Biotechnology. 2005;4(13):1510-1520.
8. Enriqueta Alos, Maria Jesus Rodrigo, Lorenzo Zacarias. Manipulation of carotenoid content in plants to improve human health. Carotenoids in nature: biosynthesis, regulation and function. 2016;311-343.
9. Fazul Nabi, Muhammed Arain A, Nasir Rajput, Mahmoud Alagawany, Jamila Soomro, Muhammed Umer, *et al*., Health benefits of carotenoids and potential application in poultry industry: A review. Journal of animal physiology and animal nutrition. 2020;104:1809-1818.
10. Giovannucci E, Ascherio A, Rimm EB, Stampher MJ, Colditz GA, Willet WC. Intake of carotenoids and retino in relation to risk of prostate cancer. Journal of national cancer institute. 1995;87(23):1767-1776.
11. Gisleania Dourado Landim Parente, Bruno Davinci Nunes de Melo, Jaciel Albuquerque de Souza, Marta Maria da Cnceicao, Job Ubbink, Ana Luiza Mattos Braga. Fortification of traditional tapioca b''pancakes'' from the brazilian northeast with microencapsulated carrot carotenoid. LWT-Food science and technology. 2021;152:112301.
12. Goula AM, Ververi M, Adamopoulou A, Kaderides K. Green ultrasoundassisted extraction of carotenoids from pomegranate wastes using vegetable oils. Ultrasonics Sonochemistry. 2017;34:821-830.
13. Hiranvarachat B, Devahastin S. Enhancement of microwave-assisted extraction via intermittent radiation: Extraction of carotenoids from carrot peels. Journal of Food Engineering. 2014;126:17-26.
14. Jaeschke DP, Menegol T, Rech R, Mercali GD, Marczak LDF. Carotenoid and lipid extraction from Heterochlorella luteoviridis using moderate electric field and ethanol. Process Biochemistry. 2016;51(10):1636–1643.
15. Jaime L, Rodríguez-Meizoso I, Cifuentes A, Santoyo S, Suarez S, Ibáñez E, *et al*. Pressurized liquids as an alternative process to antioxidant carotenoids' extraction from Haematococcus pluvialis microalgae. LWT - Food Science and Technology. 2010;43(1):105-112.
16. Kim DY, Vijayan D, Praveenkumar R, Han J, Lee I, Park J. Cell-wall desruption and lipid/astaxanthin extraction from microalgae: Chlorella and haematococcus. Bioresource technology. 2016;199:300-310.
17. Luengo E, Martínez JM, Coustets M, Álvarez I, Teissié J, Rols MP *et al*. A Comparative Study on the Effects of Millisecond- and Microsecond-Pulsed Electric Field Treatments on the Permeabilization and Extraction of Pigments from Chlorella vulgaris. The Journal of Membrane Biology. 2015; 248(5):883-891.
18. Macías-Sánchez MD, Fernandez-Sevilla JM, Fernández FGA, García MCC, Grima EM. Supercritical fluid extraction of carotenoids from Scenedesmus almeriensis. Food Chemistry. 2010;123(3):928-935.
19. Maki-Arvela, Hachemi P, Murzin D. Comparitive study of the extraction methods for recovery of carotenoids from algae: extraction and kinetics and effects of different extraction parameters. Journal of chemical technology and biotechnology. 2014;89(11):1607-1626.
20. Manuel Rodriguez Concepcion, Javier Avalos, Luisa Bonet M, Albert Boronat, Lourdez Gomez, Damaso Hornero-Mendez *et al.* A global perspective on carotenoids: Metabolism, Biotechnology and benefits for nutrition and health. Progress in Lipid research. 2018;70:62-93.
21. Mezzomo N, Martínez J, Maraschin M, Ferreira SRS. Pink shrimp (P.brasiliensis and P. paulensis) residue: Supercritical fluid extraction of carotenoid fraction. The Journal of Supercritical Fluids. 2013;74,:22-33.
22. Michelon M, Borba T de M. de, Rafael R da S, Burkert CAV, Burkert JF de M. Extraction of carotenoids from Phaffia rhodozyma: A comparison between different techniques of cell disruption. Food Science and Biotechnology. 2012;21(1):1-8.
23. Milani A, Basirnejad M, Shabazi S. Bolhassani, Carotenoids: Biochemistry, pharmacology and treatment. British journal of pharmacology. 2017;174(11):1290-1324.
24. Nurfitri Ekantari, Siti Ari Budhiyanti, Wahdan Fitriya, Asep Bayu Hamdan, Ciacia Riaty. Stability of chocolate bars fortified with nanocapsules carotenoid of spirulina platensis. IOP Conference series: earth and environmental science. 2019;370(1):012079.
25. Pingret D, Fabiano-Tixier AS, Chemat F. Degradation during application of ultrasound in food processing: A review. Food Control. 2013;31(2):593-606.
26. Edge R, McGarvey DJ, Truscott TG. The carotenoids as antioxidant- A review. Journal of photochemistry and photobiology. 1997;41(3):189-200.
27. Rachel Kopec E, Jessica Copperstone L, Morgan Cichon J, Steven Schwarts J. Edn 1, Blackwell publishing, West Sussex. 2012; I:105-149.
28. Ramesh Kumar Saini, Arina Ranjit, Kavita Sharma, Parchuri Prasad, Xiomin Shang, Karekar Gowda *et al*. Bioactive compounds of citrus fruits: A review of composition and health benefits of carotenoids, flavanoids, limonoids and terpenes. Antioxidants. 2022;11:239.
29. Ramesh Kumar Saini, Parchuri Prasad, Veeresh Lokesh, Xiomin Shang, Juhyun Shin, Young-soo-Keum *et al*. Carotenoids: Dietary sources, extraction, encapsulation, bioavailability and health benefits- A review pf recent advancements. Antioxidants. 2022;11:795.
30. Singh A, Ahmed S, Ahmed A. Green extraction methods and environmental applications pf carotenoids. A review. RSC Advances. 2015;5(77):62358-62393.
31. Sowbhagya, H. B., & Chitra, V. N. Enzyme-assisted extraction of flavorings and colorants from plant materials. Critical Reviews in Food Science and Nutrition.2010;50(2):146–161.
32. Strati IF, Gogou E, Oreopoulou V. Enzyme and high pressure assisted extraction of carotenoids from tomato waste. Food and Bioproducts Processing. 2015;94:668-674.
33. Tang G. Vitamin A Value of plant food provitamin A-Evaluated by stable isotope technologies. International

journal of vitamin and nutrition research. 2014;84(1)25-29.
34. Tarek Nour Soliman, Sahar Nasser A. Charecterization of carotenoids double-encapsulated and incorporate in functional stirred yogurt. Frontiers in sustainable food systems. 2022;6:979252.
35. Tsiaka T, Zoumpoulakis P, Sinanoglou VJ, Makris C, Heropoulos GA, Calokerinos AC. Response surface methodology toward the optimization of high-energy carotenoid extraction from Aristeus antennatus shrimp. Analytica Chimica Acta. 2015;877:100-110
36. Yamaguchi M. β-Cryptoxanthin and bone metabolism: The preventive role in osteoporosis. Journal of health science. 2008;54(4):356-369.
37. Zaghdoudi K, Pontvianne S, Framboisier X, Achard M, Kudaibergenova R, Ayadi-Trabelsi M, *et al*. Accelerated solvent extraction of carotenoids from: Tunisian Kaki (*Diospyros kaki* L.), peach (Prunus persica L.) and apricot (*Prunus armeniaca* L.). Food Chemistry. 2015;184:131-139.