

Emerging techniques for the extraction of β -carotene for the valorization of agro-industrial by-products of carrot (*Daucus carota L.*): a review

Técnicas emergentes de extracción de β -caroteno para la valorización de subproductos agroindustriales de la zanahoria (*Daucus carota L.*): una revisión

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Resumen

El objetivo de la presente revisión es reunir, contrastar y analizar algunas de las técnicas convencionales y no convencionales de extracción de β -caroteno a partir de la zanahoria (*Daucus carota L.*), a través de un análisis bibliométrico de estudios e investigaciones recientes, en los que confluyen numerosas técnicas, parámetros y hallazgos. Asimismo, establecer las posibles contribuciones de la extracción con solventes verdes para estos procesos, permitiendo su aplicabilidad a escala industrial para el aprovechamiento de los subproductos agroindustriales de este vegetal. La zanahoria es rica en carotenoides, en especial β -caroteno, fuente de provitamina A, utilizada como colorante natural en la industria de alimentos y farmacéutica. La extracción asistida con microondas (MAE por su sigla en inglés), con enzimas (EAE por su sigla en inglés) y en fluidos supercríticos (SFE por su sigla en inglés), han sido evaluadas en la zanahoria y comparadas con las técnicas convencionales de extracción (CSE por su sigla en inglés), encontrando rendimientos y eficiencias similares e incluso superiores. Sin embargo, la extracción asistida con ultrasonido (UAE por su sigla en inglés), muestra resultados satisfactorios y considerablemente mayores (157,0 mg β -caroteno/100 g base seca). Se requieren estudios posteriores para optimizar las condiciones y los parámetros de extracción, y evaluar las condiciones de conservación de la materia prima y del extracto final que aseguren una mayor estabilidad del producto y, por ende, un rendimiento de extracción superior, al igual que considerar alternativas verdes de extracción para reducir el impacto ambiental.

Palabras clave: extracción; solvente verde; rendimiento; zanahoria; *Daucus carota L.*, β -caroteno; colorante natural.

Abstract

The objective of this review was to collect, contrast and analyze the conventional and non-conventional extraction techniques to obtain β -carotene from carrots (*Daucus carota* L.), by conducting a bibliometric analysis of different recent studies and investigations where different approaches, techniques, and findings converge. Similarly, the contribution of green solvents to the extraction process is established to determine its applicability at an industrial scale. Carrots are rich in carotenoids, and especially in β -carotene, a source of provitamin A used as a natural coloring in the food and pharmaceutical industry. Microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), and Supercritical fluid extraction (SFE) have been evaluated using the carrot and have been compared to conventional solvent extraction techniques (CSE), showing similar and even higher extraction yields and efficiencies. However, ultrasound-assisted extraction (UAE) shows successful and considerably higher results (157.0 mg β -carotene/100 g DB) compared to other non-conventional techniques. Further investigations are required to optimize the extraction conditions and parameters, as well as the evaluation of suitable preservation conditions for both the raw material and the final extract that ensure a higher stability of the end-product, and thus providing a higher extraction yield. Green alternatives should be considered to reduce environmental impact in future extraction processes.

Keywords: carrot; extraction; green solvent; yield; *Daucus carota* L.; β -carotene; natural colorant.

1. Introduction

The growth in demand for food due to the increase in the population worldwide has brought new challenges for the industries of this agricultural and agro-industrial sector. In Colombia, carrot production increased by 30 % between 2007 and 2020, with an average yield of 30.54 % (Ministry of Agriculture, 2017). This generates a problem in the management of residues and by-products remaining from its processes that can reach up to 30 % of the harvest (Clementz *et al.*, 2019). Despite the different uses they currently have, such as biogas production, composting, and animal feed (Martínez; Quintero, 2017), a large part of this volume still does not have any type of useful transformation, causing an economic and environmental problem, since only between 15 % and 20 % have an alternative use (Clementz *et al.*, 2019).

The development of extractive technologies for bioactive compounds from natural sources has allowed the creation of new techniques and processes that establish possible alternatives for taking advantage of components, such as vitamins, starches, and dietary fibers, among others. One of the groups of compounds of high nutritional value and with a wide field of application in different industries are carotenoids. These compounds, belonging to the group of tetraterpenoids, are oil-soluble natural pigments, made up of a hydrocarbon chain of 40 carbon atoms with two terminal rings at the ends, giving them a characteristic yellow-orange-red color (Cheng; Khoo; Kong; Prasad; Galanakis, 2020).

The global consumption of carotenoids grew by 1500 metric tons between 2007 (4193 tons) and 2017 (5693.6 tons) and this figure is expected to reach 622.6 tons by 2022, which is reflected in the global market that had an estimated growth for 2017 of 1.5 trillion dollars; however, other studies forecast a figure of US \$ 2.0 trillion by 2022 (Bogacz-Radomska; Harasym; Piwowar, 2020).

Natural β -carotene belongs to the group of carotenoid compounds that has gained strength in recent years thanks to its nutritional benefits as an antioxidant compound and its effects on health, especially because it reduces the risk of developing some types of cancer (Clementz *et al.*, 2019) and is useful for the prevention of chronic and degenerative diseases; the foregoing, not to mention its applications in the food industry, as a natural coloring, vitamin supplement and active compound for medicines (Martins; Ferreira, 2017). In the market, β -carotene can be found in an oleaginous solution, generally in refined sunflower oil between 0.2-1.0% concentration, whose application is focused on food functional (Underwriters, 2020).

Carrots (*Daucus carota L.*) are known for their high content of carotenoids, especially β -carotene, precursors of vitamin A, fulfilling several relevant functions in the human body (Bogacz; Harasym, 2018). Different solid-liquid extraction techniques, as well as quantification and detection techniques, have been researched and evaluated, in parallel, seeking their optimization, reduction of extraction solvents and replacement of toxic solvents to adjust to the new trends in sustainable, economic, and environment friendly processes (Boukroufa; Boutekedjiret; Chemat, 2017). Unconventional extraction techniques, unlike conventional techniques (CE), are based on the use of high-energy methods that allow reaching the target compound in a plant matrix, providing a series of advantages, such as a reduction in time, energy consumption, temperature, and the amount of extraction solvent, as well as its high extraction yields and its low thermoregulation effects of bioactive compounds (Goula; Ververi; Adamopoulou; Kaderides, 2017). Therefore they have gained great interest in recent years and why they generate great expectations in the scientific and industrial fields.

The purpose of this document is to gather and discuss the different findings and developments in the process of extracting β -carotene, from carrot by-products, such as the epidermis or pulp, taking into account the fundamental concepts, parameters, and variables to define its methodology and technique, emphasizing the advantages and potential benefits that they can bring for the valorization of agro-industrial by-products on an industrial scale, considering the great challenges brought by increased production, consumption, and processing of carrots (*Daucus carota L.*) and technological developments towards the consumption of products processed with natural compounds.

2. Methodology

The information collected for the development of this document was consulted in indexed publications, scientific or review articles, and book sections in databases of different scientific communities. The selection criterion was based on a bibliometric analysis establishing a publication time interval from 2015 to the present and selecting studies that present both experimental results and theoretical foundations and methodologies for evaluation, optimization, and analysis of the findings that each one of these exposes. The search was focused only on articles that revolved around the extraction process, using the carrot variety chantenay or (*Daucus carota L.*) as raw material. Once the bibliographic search was carried out, it was found that the percentage of research articles with respect to review articles is 70 % and 30 %, respectively. The keywords or thesauri used as search criteria with the help of the most relevant search operators were “carrot AND β -carotene”, “carrot AND extraction”, “carrot AND ultrasound” and “Green AND extraction”, finding a large influx of research related to these extraction techniques between 2013 and 2017, and specifically in relation to carrots between 2015 and 2017. The sources of information consulted correspond to the Science direct digital databases; Springer link; Scielo and Scopus, as well as sections or chapters of digital books and manuals of equipment or devices related to the mentioned extraction and quantification methods. The initial search yielded a total of 72 scientific articles and publications, which were subsequently refined until a total of 60 documents were obtained. The discard criteria applied to the research articles consisted of a series of requirements where a description of the specified technique and methodology was necessary, as well as providing quantitative results preferably supported by statistical analysis. For review articles and book chapters, it was established that the sources of the theoretical foundations should not exceed the established time range.

3. Topic Development

3.1. Carrot as a natural source of carotenoids

The carrot is a root-type vegetable belonging to the Apiaceae family previously known as Umbelliferae, whose production is specialized in human consumption focused on a biannual planting and harvest (ideal for tropical regions due to its continuous harvest throughout the year) and whose quality is dependent on several factors such as climatic conditions, bark-root relationship, its vascular system and the quality of the root's xylem and phloem (Raees; Prasad, 2015). Its morphology is shown in figure 1, which is composed of the neck and crown (thickest part of the root), the fruit or phloem, the heart or xylem and, finally, the base or tip (the narrowest part). Overall, the carrot can grow from 5 to 20 cm in length (Barzee; El-Mashad; Zhang; Pan, 2019).

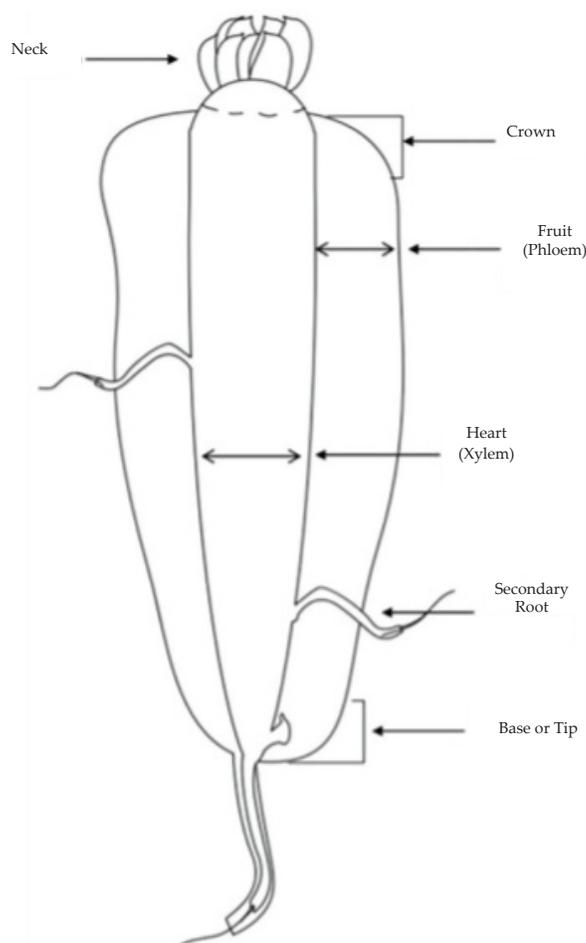


Figure 1. General morphology of carrots
Source: adapted from Barzee *et al.* (2019).

The world consumption of carrots is approximately 37 million tons per year, of which 11 % belongs to the residues of its processing composed, mainly, by the epidermis, tubers, and pieces of fruit attached (de Andrade; Charalampopoulos; Chatzifragkou, 2018). In American and European countries, the generation of waste is a common problem, since between 15-20 % of these are destined for animal consumption and the rest is discarded until decomposition, generating unpleasant odors and the proliferation of insects and pests (Clementz *et al.*, 2019). The main source of carrot by-products is juice production, which creates a large amount of pulp (Barzee *et al.*, 2019). Some figures indicate that up to 50 % of the carrot pulp used for juice production is obtained as waste. This volume is later used as animal feed or even discarded (Tiwari; Upadhyay; Singh; Meena; Arora, 2019), despite being rich in β -carotene content (Purohit; Gogate, 2015). Thanks to the large volume of carrot production and processing and easy access to its low-cost waste, it could be a potential alternative to obtain natural pigments of high economic value and reduce its environmental impact (Mirheli; Dinani, 2018). In Latin America, the market for valuable carrot compounds, such as β -carotene, can reach a sales value of USD 500 per kilogram (Clementz *et al.*, 2019).

Provitamin A (β -carotene) is present in various foods and is also consumed in dietary supplements. As a natural source, it is found in yellow or orange fruits or vegetables, such as melon, mango, pumpkin, and sweet potato; however, carrots are considered one of the best sources of this pigment (Kumari; Rajarani; Bansal; Dahuja; Praveen, 2019). Up to six species of carotenoids have been found in carrots: α -, β -, γ -, ζ -carotene, lycopene, and β -zeacarotene (Figure 2) with an average ratio of 33: 60: 1: 4: 1: 1, respectively, depending on the state and sowing conditions.

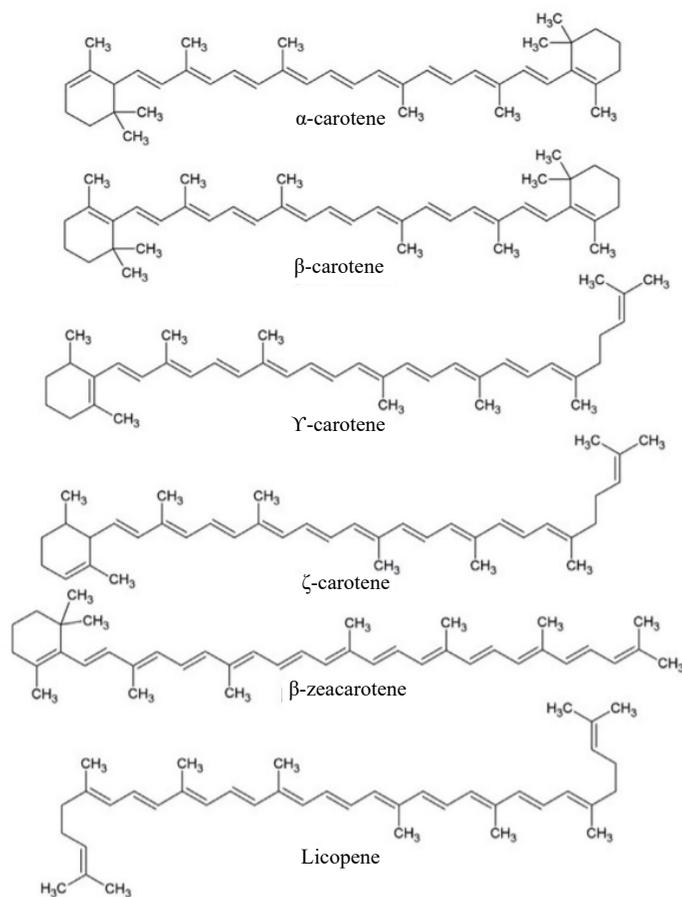


Figure 2. Carotenoids of Carrots
 Source: adapted from Juliana, Indrawati, and Yuliati (2019).

Different studies agree that carrots are the greatest natural source of carotenoids, especially β -carotene, whose values are summarized in Table 1.

Table 1.
Reported values of β -carotene in carrots (*Daucus carota* L.)

Concentration (mg β -carotene / 100 g) dry base	Source
6.5 - 8.8	(da Silveira <i>et al.</i> , 2020)
8.0	(Platel; Srinivasan, 2016)
18.0	(Desobry; Netto; Labuza, 1998)
28.0	(Kumari <i>et al.</i> , 2019)
80.0	(Kyriakopoulou; Papadaki; Krokida, 2015)

Source: own elaboration.

In carrots, this compound exists in crystalline form as a complex adhered to a helical lipoprotein rich in alanine, leucine, glutamic, and aspartic acid, which is believed to have enzymatic functions in its biosynthesis (Desobry *et al.*, 1998). The presence of β -carotene is responsible for the characteristic yellow-orange color of this root (Martins; Ferreira, 2017). Although a wide variety of mineral and synthetic colorants are used in the food industry, thanks to its range of intense colors and its high stability; according to recent studies, some of these are attributed side effects on health from degenerative diseases to attention and hyperactivity problems (Ngamwonglumlert; Devahastin; Chiewchan, 2017). Likewise, solvents such as n-hexane, widely used in conventional carotenoid extraction, are considered reprotoxic and an acute aquatic pollutant (Boukroufa *et al.*, 2017). Therefore, more attention has been paid to natural sources of dyes, such as chlorophylls, anthocyanins, and carotenoids, abundant in nature and whose use in the food industry are approved by the FDA (Ngamwonglumlert *et al.*, 2017). Thanks to the development of new technologies for obtaining pigments from natural sources on a laboratory scale, it has been possible to take these techniques to a larger scale, improving the potential of these trends in healthier consumption and more friendly and sustainable processes. The consumption of fruits and vegetables provides the human body with nutrients, vitamins, fiber, and antioxidant compounds with high nutritional value. These foods are generally processed into juices, purees, or concentrates, leaving peels, skin, and other residues as by-products that contain valuable bioactive compounds that can be used to produce functional foods or active ingredients in the pharmaceutical industry (Sharmin *et al.*, 2016). The search for an approach towards “zero wastes” processes together with the circular economy are topics of current interest, using fruit and vegetable waste, and its subsequent recovery (Ojha; Aznar; O'Donnell; Tiwari, 2020), even allowing a reduction in the costs of natural resources through the reuse of these wastes (Kumari *et al.*, 2019).

3.2. Carotenoids and β -carotene

Carotenoids belong to the family of natural pigments synthesized by different species of plants, algae, and bacteria. In plants, it has been possible to identify around 650 varieties of carotenoids; however, only about 40 belong to fruits and vegetables belonging to the human diet, which represents the largest source of carotenoid consumption (Goula *et al.*, 2017). Carotenoids are compounds of the tetraterpene or tetraterpenoid type, that is, they are made up of a main chain of 40 carbons (C_{40}), made up of eight isoprene units; they are considered natural pigments that vary in the visible range from light yellow, through orange, to deep red. Some of the most common examples in nature (Figure 3) are carotene, lycopene, astaxanthin, zeaxanthin, lutein, bixin, canthaxanthin, violaxanthin, and neoxanthin (Vasconcelos *et al.*, 2020).

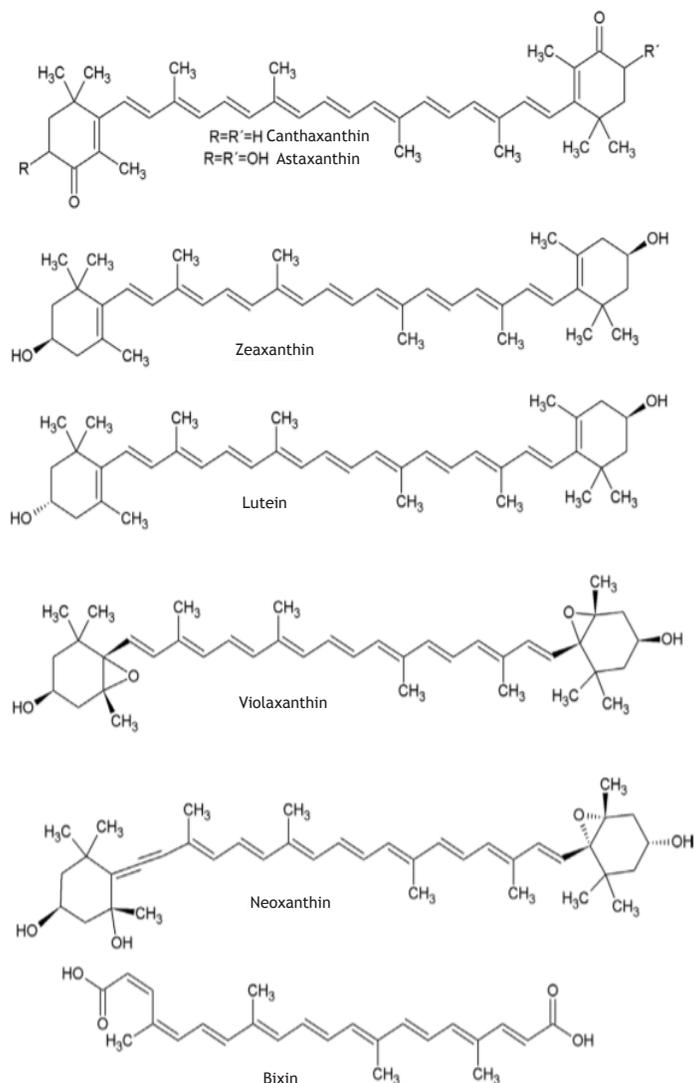


Figure 3. Most common carotenoids in nature
Source: adapted from da Silveira *et al.* (2020).

Carotenoids are synthesized by plants in chromoplasts, following the methylerythrol phosphate (MEP) route starting with the condensation of two C20 GGPP molecules formed from isopentenyl diphosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). In this first step, 15-cis-phytoene catalyzed by the enzyme phytoene synthase (PSY) are obtained; this is then transformed into lycopene through isomerization and desaturation reactions, catalyzed by the enzymes phytoene desaturase (PDS) and de-carotene desaturase (ZDS), respectively. Subsequently, for the formation of the other carotenes and xanthophylls, lycopene goes through reactions of cyclization (formation of α -, β -, γ -carotene), hydroxylation (formation of lutein, astaxanthin, canthaxanthin, and zeaxanthin), and/or ring formation (formation of violaxanthin and neoxanthin) (Vasconcelos *et al.*, 2020). Among some of the most relevant benefits of carotenoids are their antioxidant action and their ability to reduce the risk of degenerative diseases, such as cancer and cardiovascular problems (Miękus; Iqbal; Marszałek; Puchalski; Świergiel, 2019). On the other hand, its numerous applications in the food industry, as a natural colorant, in the pharmaceutical industry as a modulator of the immune system, protector against UV rays and precursor of the visual pigment retinol or vitamin A (Saini; Keum, 2018).

β -carotene is one of the most abundant carotenoids in fruits, plants, and vegetables. It is an apolar molecule of high weight (536.85 g/mol), structurally composed of an unsaturated chain of hydrocarbons whose ends have cyclohexane rings. This pigment represents between 60-80 % of the carotenoids present in carrots (Vasconcelos *et al.*, 2020) (Figure 4).

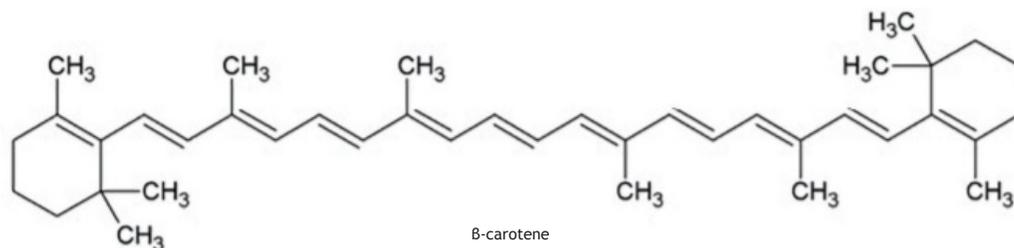


Figure 4. Molecular structure of β -carotene
Source: adapted from Murray and Capelli (2020).

The β -carotene together with the α -carotene and the β -cryptoxanthin are the carotenoids that show the greatest contribution of vitamin A to the human body; however, the latter two represent only 50 % of the activity of this vitamin compared to β -carotene. In the visible spectrum, this pigment absorbs wavelengths between 450 and 460 nm (Murray; Capelli, 2020). Like most carotenoids, β -carotene is used as a coloring agent in various types of foods, beverages, dietary supplements, and even medications. Similarly, its benefits in biological activity and, therefore, in human health, are attributed to its antioxidant effect (Martins; Ferreira, 2017).

3.3. Techniques for extracting β -carotene from carrots (*Daucus carota* L.)

Among the most prominent methods of extracting β -carotene from carrots are conventional solvent extraction (CSE), which includes conventional Soxhlet extraction (SE), and non-conventional extraction methods, consisting of microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE) and Enzyme assisted extraction (EAE) (Kyriakopoulou *et al.*, 2015). Table 2 lists the main advantages and disadvantages of these extraction techniques.

Table 2.

Advantages and disadvantages of carrot β -carotene extraction techniques

Extraction Technique	Advantages	Disadvantages	Source
CSE (Conventional)	High extraction yields. It does not require sophisticated equipment.	Large amounts of solvent. Use of toxic solvents. High energy consumption	(Saini; Keum, 2018)
MAE (Unconventional)	High extraction yields. Allows use of green solvents. Reduction of thermal gradients. Short extraction times.	Losses of volatile compounds. Probable thermal degradation.	(Selvamuthukumar; Shi, 2017)
UAE (Unconventional)	High extraction yields. Low energy consumption. Short extraction times. Allows use of green solvents.	Difficult control of variables. Complex commercial escalation. Requires specific optimization. Probable thermal degradation.	(Li; Fabiano-Tixier; Tomao; Cravotto; Chemat, 2013) (Wen <i>et al.</i> , 2018)
SFE (Unconventional)	Avoids thermal degradation. Low solvent volumes. Simple and Fast	Expensive and sophisticated equipment. Difficult manipulation of the process. High analysis costs	(Miękus <i>et al.</i> , 2019)
EAE (Unconventional)	High extraction yields. Prevents thermal degradation.	Long extraction times. High enzyme costs	(Ngamwonglert <i>et al.</i> , 2017)

Source: own elaboration.

Starting from the fact that carrots are the vegetable source with the highest content of carotenoids, specifically of β -carotene, various researches have focused on optimizing the extraction and quantification conditions and parameters in order to obtain the maximum yield. Considering that, on average, from 50 kg of fresh carrot, 2 g of α - and β -carotene are obtained. Similarly, the physicochemical properties of β -carotene exhibit low resistance to external conditions of light and temperature, which suggests that postharvest conditions should be monitored to avoid losses of this compound (Bogacz; Harasym, 2018).

3.4. Conventional extraction (CSE)

According to Varón *et. al* (2017), a solvent is defined as: “a liquid capable of dissolving, diluting or extracting other substances without causing chemical modification to the substance or itself” (p. 10). Conventional extraction is conducted on a laboratory scale and its main purpose is to evaluate the performance of different solvents under different conditions. Within this, we find three main methods: Soxhlet extraction, maceration, and hydro distillation. The first method consists of a set-up, in which, through the boiling and condensation of a volatile solvent, a direct and continuous contact of this is provided with the sample contained in an extraction chamber that has a siphon, which allows the recovery of liquid solvent. The second, which is maceration, is considered the oldest method and consists of a process of mixing the solid material with a specific solvent and its subsequent separation by means of simple or vacuum filtration, where the main variables to control are the type of solvent and the surface area. The third and last method consists of the use of water for the generation of steam and its use as an extraction agent, where the bioactive compounds or essential oils are recovered by the volatilization of the water in the samples (Selvamuthukumaran; Shi, 2017). Table 3 gathers the main findings in different investigations for the extraction of β -carotene from carrots using conventional extraction methods.

Table 3.

*Conventional extraction (CSE) of β -carotene from carrot (*Daucus carota* L.)*

Extracted β -carotene (mg / 100g) dry base	Parameters	Quantification Technique	Source
31.00	Solid-solvent 1:10 m/v Maceration t: 1 h Cis-Pinene and n-Hexane	UV-VIS/HPLC	(Varón <i>et al.</i> , 2016)
18.10 - 4.90	Solid-solvent 1: 1 m/v Heating and Filtration t: 5 min Ethanol and dichloromethane	UV-VIS	(Hasan; Mohamad; Aldaiek, 2019)
38.05	Solid-solvent 1: 4 m/v Thermal bath (60 °C) t: 240 min Ethanol, methanol and petroleum ether	UV-VIS	(Sharmin <i>et al.</i> , 2016)
65.00	Solid-solvent 1: 5 m/v Stirring (room temperature) 1000 rpm, 20 Wh Hexane	UV-VIS	(Kyriakopoulou <i>et al.</i> , 2015)
69,60	Solid-solvent 1:20 m/v Shaking in incubator (65 °C) t: 270 min Linseed oil	UV-VIS	(Elik; Yanık; Göğüş, 2020)

Source: own elaboration.

From the analyzed studies, it was found that the most relevant variables and parameters in these conventional techniques are solid-solvent ratio, polarity, solvent affinity and toxicity, and stability of the bioactive compound of interest. Of the conventional methods exposed at the beginning, it is worth mentioning that hydro-distillation is not used to extract β -carotene, since its chemical structure is made up of carbon and hydrogen units makes it lipo-soluble, that is, its solubility in polar solvents, such as water, is not sufficient to obtain high extraction yields (Murray; Capelli, 2020). The obtained extraction yield results showed an average value of 37.8 mg β -carotene/100 g dry base. The variability of results of this extraction technique is related to the solubility of the carotenoids, which depends on their physical and chemical properties and, in the same way, on the polarity of the solvent used and the conditions of temperature and pressure during extraction (Varón *et al.*, 2016). On the other hand, the processing or treatment of the samples, affecting the structural integrity of the matrix, produces both a negative effect (loss of carotenoids due to oxidation) and a positive effect (increase in the bioavailability of carotenoids) (Sharmin *et al.*, 2016). When comparing the results of the different researches, a possible increase in the extraction yield of β -carotene is also observed when using oils of vegetable origin, as is the case of the study carried out by Elik *et al.* (2020), who used linseed oil and an extraction temperature and time higher than the other studies (65 °C and 270 min, respectively).

3.5. Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction consists of the use of electromagnetic irradiation at frequencies between 0.3 and 300 GHz (Vinatoru; Mason; Calinescu, 2017), which generates an excitation of the molecules of the medium by the action of rotation of dipoles and conduction of ions, causing heat and mass gradients that manage to break the structures by pressure generation inside the biological matrix and facilitate access to the bioactive compounds of the plant source (Selvamuthukumaran; Shi, 2017). Unlike conventional processes, microwave radiation generates an increase in internal temperature through the volume of the cell or plant matrix where the compound of interest is stored, causing an increase in internal pressure that ends up causing lysis or rupture of the system, thus releasing the target molecule (Vinatoru *et al.*, 2017). The solid-solvent ratio, power, extraction time, and type of solvent are the most evaluated variables in this technique (Table 4). In addition to ultrasound-assisted extraction (UAE), they are considered the most practical methods due to their applicability on an industrial scale, convenience of operation and their good results in extraction performance (Chuyen; Nguyen; Roach; Golding; Parks, 2018).

Table 4.
Carrot (*Daucus carota* L.) β -carotene extraction technique (MAE)

Extracted β -carotene (mg/100 g) dry base	Parameters	Quantification Technique	Source
61.98	Solid-solvent 1: 8.06 m/m Power 165 W t: 9.39 min Linseed oil	HPLC	(Elik <i>et al.</i> , 2020)
112.60 - 110.90	Solid-solvent 1:37.5 and 1:75 m/v Power 180 W - 300 W t: 3.5- and 6.5-min Hexane, acetone and ethanol	UV-VIS/HPLC	(Hiranvarachat; Devahastin, 2014)

Source: own elaboration

The microwave extraction yields (Table 4) show a significant difference between each author. One of the possible causes of this is the use of higher powers in the study of (Hiranvarachat; Devahastin, 2014), who affirm that the extraction's performance obeys a directly proportional behavior of the microwave power and the volume of solvent, given that an increase only in the volume of solvent results in a lower content of β -carotene and carotenoids, since there is a greater dissipation of power. However, considering that in the study by Elik *et al.* (2020) an alternative solvent (linseed oil) was used, and the analysis was conducted with lower microwave power. The results are promising and similar to those that would be obtained through conventional extraction techniques.

3.6. Super Critical Fluid Extraction (SFE)

Unlike the other extraction methods mentioned, supercritical fluid extraction (SFE) is characterized using specific fluids in a supercritical state, in which the fluid behaves like a gas, i.e., it acquires a viscosity, diffusion, and surface tension of the nature of this state, maintaining its properties of dilution and density in the liquid state. This technology mainly uses carbon dioxide CO₂ in a supercritical state, as it does not create toxic waste after extraction; in addition, it avoids the degradation of the bioactive compound and allows it to be obtained in high purity quickly after the process without requiring a subsequent purification treatment or recovery of the extract (de Andrade *et al.*, 2018). The bibliographic review yielded limited results of experimental studies with carrots (*Daucus carota* L.), for this reason, it was necessary to select the study by (Sun; Temelli, 2006). In carrots, some investigations have been carried out (Table 5) in which even recycled co-solvents of a vegetable nature have been used, such as canola oil, to finally obtain this enriched with β -carotene.

Table 5.
Carrot (*Daucus carota* L.) β -carotene extraction technique (SFE)

Extracted β -carotene (mg / 100g) dry base	Parameters	Quantification Technique	Source
110	Pressure: 55.1 MPa Temperature: 70 °C Medium: SC-CO ₂ (2 L/min) Co-solvent: canola oil	HPLC	(Sun; Temelli, 2006)
21.0	Pressure: 306 bar Temperature: 58.5 °C Medium: SC-CO ₂ (2 L/ min) Co-solvent: ethanol	HPLC	(from Andrade <i>et al.</i> , 2018)

Source: own elaboration.

From Table 5 it can be inferred that a higher extraction performance can be obtained by increasing the operating temperature and pressure, as this has a significant positive effect on the extraction of carotenoids except for lutein (Sun; Temelli, 2006). Likewise, a positive effect of substituting the extraction co-solvent for one of natural origin and a renewable source, such as canola oil, can be evidenced. Nitrous oxide N₂O has also been used as an extraction fluid in a supercritical state, showing a solubility comparable to those found in the use of co-solvents without altering the process in its recovery; however, this fluid has not been investigated in the same way as S-CO₂, as it is considered risky when in contact with natural organic products (de Andrade *et al.*, 2018).

3.7. Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction is not aimed directly at the extraction stage, instead, it uses hydrolytic enzymes to break down the structure or matrix that conceals the bioactive compounds of interest, that is, by focusing on the separation stage of micro- and macro-molecules. The most used enzymes in the extraction of β -carotene from carrots reported in research (Table 6) are cellulase and pectinase. The first is responsible for breaking or hydrolyzing the cellulose present in the main cell wall of plant cells, degrading the 1,4- β -d-glycosidic junctions. The second degrades the pectin present at the cell wall interface (Saini; Keum, 2018).

The main reason that led to the development of this technique for the extraction of carotenoids lies in the fact that these bioactive compounds are heat sensitive and prone to degradation (isomerization) under alkaline conditions. The use of enzymes provides greater stability, since after this treatment, the extraction will not require elaborate techniques to obtain high extraction yields. However, some studies combine this technique with other unconventional ones to obtain the best possible performance.

Table 6.

Carrot (Daucus carota L.) β -carotene extraction technique (EAE)

Extracted β -carotene (mg / 100g) dry base	Parameters	Quantification Technique	Source
42.96	Time: 3 h Temperature: 45 °C Enzymes used: Cellulase (0.8 U/mg) Pectinase (1.15 U/mg) pH Buffer: (7) Ratio: 0.35/0.5 % Source: (Aspergillus Niger)	HPLC	(Tiwari <i>et al.</i> , 2019)
7.0	Time: 120 min Temperature: 40 °C Surfactants used: Tween 20, 60 and 80 Span 20, 40 and 60 Ratio: 1.5/0.4 g	UV-VIS	(Zhao; Zhang; Wu; Ouyang, 2015)

Source: own elaboration.

The main advantage of implementing enzymatic hydrolysis is to decrease the activation energy of the chemical reaction and provide the system with ideal conditions as far as possible to carry out the subsequent extractive process (Ghosh; Biswas, 2016).

3.8. Ultrasound-Assisted Extraction (UAE)

This unconventional extraction technique is based on the use of ultrasonic waves, between the frequencies of 20 kHz and 10 MHz, and is divided into low and high-intensity sonication. The force generated by the extraction process is known as acoustic cavitation, in which microbubbles are generated by the pressure difference located at different points in the extraction medium, which later collapse and generate sources of heat and energy that allow the rupture of the plant matrix, facilitating the entry of the solvent or extraction agent to the compound of interest (Vernès; Vian; Chemat, 2020). Low frequency-ultrasound treatments have also been evaluated in plant matter treatment processes, finding effects that promote greater moisture migration and, therefore, a more effective drying process due to the breakdown of the internal structure of the plant matrix (Wang; Xu; Wei; Zeng, 2018).

The effectiveness of this method depends on the following variables: ultrasonic amplitude, intensity, power and frequency, extraction temperature and time, nature, and physicochemical properties of the solvent and, finally, the nature of the matrix. This technique has shown a very promising future thanks to its flexibility regarding the design of continuous and batch processes on an industrial scale, as long as intensification processes are carried out, to ensure energy consumption that is sufficiently sustainable and friendly to the environment, as well as safe conditions (Tiwari, 2015).

Table 7 brings together the findings and parameters used from the numerous studies found, related to the extraction of β -carotene from carrots published in the last decade.

Table 7.

Carrot (*Daucus carota* L.) β -carotene extraction technique (SFE)

Extracted β -carotene (mg/100g) dry base	Parameters	Quantification Technique	Source
104.5	Solid solvent 1:30 m/v Power and frequency: 165 W/25kHz Temperature: 55 °C t: 60 min Sunflower oil	UV-VIS	(da Silva <i>et al.</i> , 2020)
31.69	Frequency: 20 kHz Temperature: 35 °C t: 20 min Ethanol 55 %	UV-VIS	(Barzee <i>et al.</i> , 2019)
53.88	Solid solvent: 1.5: 100 Power/ frequency: 20 kHz Temperature/time UAE: 50 °C/80 min Temperature/ stirring time: 30 °C/120 min Ethanol	UV-VIS	(Mirheli; Dinani, 2018)
83.32	Equipment: Solid-solvent ultrasonic probe: 0.3: 20 m/v Power: 100 W Temperature/time: 50 °C/50 min Ethane	UV-VIS	(Purohit; Gogate, 2015)
64.66	Equipment: Solid-solvent ultrasonic bath: 0.3: 20 m/v Power/frequency: 180 W/40 kHz Temperature/time: 50 °C/50 min Ethanol	UV-VIS	(Purohit; Gogate, 2015)
46.10	Equipment: ultrasonic probe and Ultra Turrax Solid solvent: 1: 1 m/v Power: 750 W Centrifugation: 20000 rpm/12 min Temperature/ time: room/12 min Linseed oil	HPLC	(Tiwari <i>et al.</i> , 2019)

11.10	Equipment: Solid-solvent ultrasonic probe: 1: 2 m/v Power/frequency: 1000 W/20 kHz Time: 40 min Surfactant solution	UV-VIS	(Zhao <i>et al.</i> , 2015)
157.00	Equipment: Solid-solvent ultrasonic bath: 0.2: 15 m/v Power/frequency: 1000 W/20 kHz Temperature/time: 25 °C/30 min Hexane/acetone/ethanol (50:25:25) v/v	UV-VIS/HPLC	(Lau; Van; Vuong, 2018)

Source: own elaboration.

Table 7 shows a large number of studies associated with the extraction of β -carotene from carrots, where each one presents a different alternative for this technique, using different parameters and evaluating different variables. The average extraction yield from the studies is 69.03 mg β -carotene/100g dry base. The extraction conditions and parameters have a specific effect on the effectiveness of this technique. Among some of the most important effects to highlight, according to the results gathered in Table 7 are:

Solvent: the viscosity and surface tension of the solvent are inversely proportional to the effectiveness of the cavitation phenomenon (Vernès *et al.*, 2020). In the values of Table 7, it can be observed that the substitution of solvents for vegetable oils does not show a significant effect on the degradation of the extract, since the ultrasonic degradation of β -carotene is typically slow compared to other more volatile aromatic solvents.

Extraction temperature: it causes a variation in the properties of the solvent such as its viscosity, improving solubility and promoting the phenomenon of cavitation and, on the other hand, facilitates diffusion and mass transfer (Vernès *et al.*, 2020). However, it is observed in the review's results that a temperature higher than 50 °C can decrease the extraction performance, which may be related to the thermo-sensitivity of β -carotene that can generate isomerization or degradation of the molecule (Juliana *et al.*, 2019).

Extraction time: it is directly proportional to the extraction performance, however, this behavior occurs up to a certain point, where the yield reaches a constant and maximum value for extraction under certain conditions (Purohit; Gogate, 2015). On the other hand, caution should be exercised, as prolonged exposure of β -carotene to high temperatures can have a negative effect on pigment stability.

Ultrasonic frequency: the common ranges of ultrasonic frequency used in carotenoid extraction vary between 20 and 40 kHz, since a significant increase in frequency outside this range makes it difficult for microbubbles to form so that cavitation in the medium may occur (Vernès *et al.*, 2020). On the other hand, in the study by (Purohit; Gogate, 2015), a direct relationship was found between the extraction performance and the ultrasonic frequency, as an effect of the energy dissipation of the ultrasonic waves in the medium. However, a considered increase in frequency has an inhibitory effect on the cavitation process by reducing the formation of microbubbles in the medium (Wen *et al.*, 2018).

Type of equipment: the ultrasonic bath type equipment allows a uniform distribution of the ultrasonic waves; however, it operates at low ultrasonic powers. While in ultrasonic probe-type equipment, the probe is in direct contact with the medium and operates at higher powers, however, there is a greater probability of energy loss due to diffusion into the medium (Tiwari, 2015). In the analyzed studies, a higher average performance can be observed for the tests carried out in ultrasonic probe-type equipment than in those with ultrasonic baths.

3.9. Green extraction as an alternative

A large part of the β -carotene extraction experiments, from natural sources reported by different authors, show the use of solvents from non-renewable sources, such as hexane, ethanol, benzene, chloroform, and acetone. Despite this, nowadays they are considered highly flammable, toxic, and harmful to health and the environment. Likewise, new needs and trends on an industrial scale seek to reduce their consumption and replace them with alternative solvents from renewable and natural sources (Saini; Keum, 2018). Recent studies have evaluated and compared the effect of the extraction performance through a partial or total replacement of conventional solvents with other friendlier ones. Table 8 shows some of these examples where the compared conventional solvent and the result in the final extraction performance are also evidenced.

Table 8.
Comparison of extraction yields with green solvents

Solvent	Extraction Technique	Yield	Compared conventional solvent	Source
Pinene	CSE	17.3 % higher	n-Hexane	(Varón <i>et al.</i> , 2016)
Limonene	CSE	16.7 % higher	n-Hexane	(Aissou; ChematDjenni; Yara-Varón; Fabiano-Tixier; Chemat, 2017)
Limonene	UAE/CSE	Similar	n-Hexane	(Boukroufa <i>et al.</i> , 2017)

Source: own elaboration.

Among some of the solvents considered green, used for the extraction of bioactive compounds, it is worth highlighting ethyl acetate, isopropyl alcohol, and cyclopentyl ethyl ether, as solvents with less environmental impact. On the other hand, natural solvents stand out, such as pinene, limonene and canola, sunflower, and olive vegetable oils (Saini; Keum, 2018). The use of vegetable oils in carotenoid extractions has yielded satisfactory results with high retention percentages, reducing their degradation, especially when they are in a refined and unnatural state. Despite the replacement of conventional solvents with vegetable oils, the diffusivity of carotenoids continues to be one of the greatest risks and inconveniences that are presented by viscous effects during extraction; on the other hand, there are not enough studies that support a considerable reduction in costs at optimal extraction conditions (Saini; Keum, 2018). There is considerable potential thanks to the strengthening of laws and the demand by today's society to migrate to more sustainable processes that use renewable sources, which is in line with the figures of the global market of chemical compounds estimated at 3000 trillion dollars for 2025, of which growth in the participation of renewable compounds is estimated from 3 % to 15 % compared to 2009 (Varón *et al.*, 2017). Although there is a reduction in the environmental impact by using environmentally friendly solvents, some of the unconventional techniques require considerable replicability to reach a reliable quantitative analysis, requiring a higher energy consumption in the operation of the equipment, which can to some extent increase costs and the net environmental impact (Armenta; Garrigues; Esteve-Turrillas; de la Guardia, 2019).

Thus, the main objective that is sought when selecting a solvent considered green is to meet the following requirements: it must not emit volatile organic compounds, low toxicity to humans, limited environmental impact, must come from renewable sources, high power as a solvent, easy to recover and easily adjustable to existing processes (Armenta; Garrigues; de la Guardia, 2015).

4. Discussion of results

In the first instance, there has been a growth in the annual production of carrots (*Daucus carota L.*) in Colombia within the last decade, because of the growth in demand due to the increase in its population. For 2017 the annual figure of carrot production in the country was 306,746 tons, with participation led by the departments of Cundinamarca (30 %), Boyacá (26 %), Antioquia (26 %), and Nariño (14 %) (Ministry of Agriculture, 2017). This indicates a parallel growth of agro-industrial waste in cultivation, harvesting and processing. In Colombia, approximately 22 % of food is lost per year or 6.22 million tons (Martínez; Quintero, 2017). The main cause of the generation of agro-industrial by-products in Colombia has to do with their aesthetics or imperfections and with the wrong handling or storage conditions (Palacio, 2020). The supply centers generate a large amount of food waste, being 50 % of the total waste in the case of vegetables (Gonzalez; Gómez; Abad, 2017).

This creates a significant problem regarding the management, handling, and disposal of these by-products of an environmental nature, due to soil contamination by solid waste, and of economic nature for the industries that lose this raw material, since they do not have the capacity to recover it. Agro-industrial waste not only represents a loss of the raw material itself, but also involves losses in the use of land, fertilizers, water, energy, and labor. In addition, they create a large environmental problem due to their decomposition in the soils, emitting harmful greenhouse gases (Sagar; Pareek; Sharma; Yahia; Lobo, 2018).

B-carotene is a carotenoid compound present in carrots (*Daucus carota L.*) with high commercial value and shows growth in the global market due to its numerous applications in the pharmaceutical, food, and cosmetic industries, in the same way, due to its benefits in health. B-carotene plays a very important role in the prevention of diseases of the immune system and other diseases, such as breast, prostate, colorectal and lung cancer; likewise, for the treatment of osteoporosis, cardiovascular disorders, visual health, and skin sensitivity problems (Singh; Ahmad; Ahmad, 2015).

The review of the development of techniques for the extraction of β -carotene from carrots allows to establish a wide horizon of possibilities and potential alternatives for the use of this natural source and its industrial by-products. In the first instance, the physicochemical characteristics of the carrot and its volumes of availability in the form of an industrial by-product. In the present review, it was possible to show that there are different extraction techniques applied to obtain β -carotene from carrots, demonstrating the emergence of extraction technologies to apply in the food, pharmaceutical, chemical, and cosmetic industries (Tsiaka; Sinanoglou; Zoumpoulakis, 2017). Each of these techniques has advantages and disadvantages, and in the same way, different extraction yields, finding numerous investigations in ultrasound-assisted extraction (UAE). This technique, apparently, is the most effective for this raw material, finding a maximum extraction yield reported in the study conducted by (Lau *et al.*, 2018), with an extraction yield value of 157 mg β -carotene/100 g dry base. Recent studies that used analytical techniques to extract b-carotene from carrot (*Daucus carota L.*) reported in the literature, yielded a concentration value of 7.75 mg β -carotene / 100 g carrot dry base (Owolade *et al.*, 2017), indicating a considerable extraction performance for the unconventional technique assisted with ultrasonic waves.

A contrast analysis performed with the Least Significant Difference (LSD) model for an unbalanced design, for a value of $\alpha = 0.5$ through a Student's t-type model, allowed establishing that in the extraction yields for the selected conventional and non-conventional techniques there is no significant difference between any of the treatments or techniques, that is, this analysis suggests that statistically the extraction technique

compared, either between conventional and non-conventional methods or between non-conventional methods, does not have a significant effect on the extraction yield. However, this finding does not determine the final effectiveness of the method, since the other variables, such as equipment, type of solvent, energy consumption, solid-solvent ratio, temperature, and extraction time, among others, must be considered to establish the real difference between each of the techniques.

For ultrasound-assisted extraction (UAE), it is correct to affirm that a series of results are observed in the findings of the different researches, whose main reason is the type of ultrasonic equipment used, since, generally, the ultrasonic probe can generate ultrasonic intensities up to 100 times greater than those installed in ultrasonic baths (Rutkowska; Namieśnik; Konieczka, 2017). Similarly, the operating conditions and selected parameters were different. Ultrasonic frequency is a relevant parameter that can improve extraction performance by allowing the formation of microchannels within the plant matrix, causing a change in the microstructure that makes contact with β -carotene more accessible (Nowacka; Wedzik, 2016).

The possibility of using vegetable oils in this technique makes this process ecologically friendlier and satisfies the needs that allow its application on an industrial scale (da Silva *et al.*, 2020). For ultrasound-assisted extraction (UAE), it can be seen that temperatures below 40 °C decrease the extraction performance. Despite this, much higher temperatures can generate degradation by isomerization of the molecule as a consequence of its thermo sensitivity, affecting, in the same way, the extraction efficiency. The uniqueness of each technique also leads to the need to control and establish specific parameters, with the main purpose of seeking their optimal values that allow maximizing extraction yields within controllable, safe, and energetically sustainable operating ranges.

The efficiency and effectiveness do not only depend on the extraction conditions, but on the contrary, the postharvest and the recovery and product generation stage's conditions affect the stability of the final β -carotene extract obtained after extraction, depending on its handling and storage conditions, such as pH, temperature, and storage time (Juliana *et al.*, 2019).

Another relevant aspect found is the detection and quantification techniques of β -carotene, where UV-VIS spectrophotometry seems to be preferred due to its flexibility for different extraction solvents and its ease determining the total content of carotenoids in a sample. However, HPLC high-performance liquid chromatography is also a widely used method to specifically detect the types of carotenoids present in samples and their concentration, thanks to its attachable ultraviolet detector (Sarungallo; Hariyadi; Andarwulan; Purnomo; Wada, 2015).

Given the difficulties and limitations that are still evident in the studied techniques, it is necessary to carry out more in-depth research, which allows for more standardized processes and, therefore, to put the biorefinery concept into practice (Moreno; Ballesteros; Negro, 2020). The foregoing to reach a broader level of applicability in which it is possible to merge all the characteristic aspects of this trend, such as the circular economy, use of green solvents and reduction of energy consumption.

5. Conclusions

The growing demand in the consumption of carrots (*Daucus carota* L.) is constantly increasing in line with population growth in Colombia, being a great challenge for the handling and use of by-products after processing. Various authors agree with the great potential of this as a natural source of carotenoids, especially β -carotene, whose obtaining is possible through different non-conventional extraction techniques, such as microwave-assisted extraction (MAE), supercritical fluids extraction (SFE), enzyme-assisted extraction (EAE), and, especially, ultrasound-assisted extraction (UAE). This last technique promises to be simple, inexpensive, fast, and safe. In addition, it allows the use of natural solvents of renewable origin, which meets the new eco-

friendly needs of industrial processes and creates a promising horizon for the use of highly beneficial natural β -carotene for health in the food, pharmaceutical, and chemical industries. The future success of this technique in the industry will depend on research focused on optimizing both the extractive conditions and parameters and the postharvest and post-extraction stability controls, to ensure maximum yield and efficiency of the extract of this valuable compound.

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