JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by
Faculty of
Biotechnology and
Food Sciences

CAPSICUM EXTRACTS: A REVIEW OF EXTRACTION TECHNIQUES AND FOOD APPLICATIONS

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https://doi.org/10.55251/jmbfs.10015

ARTICLE INFO

Received 29. 3. 2023
Revised 25. 6. 2025
Accepted 30. 7. 2025
Published 1. 10. 2025

Review

OPEN ACCESS

ABSTRACT

For many years, the genus *Capsicum* has been widely utilized in food products due to its hot and spicy flavor, derived from the phytochemical compounds present in most species and varieties. Capsaicinoids, recognized by their aroma, flavor, and pungency effects, have traditionally commanded the spotlight. However, recent researchers have shown a special interest in other compounds present in *Capsicum* fruit matrices, attributing positive health effects to them. Numerous studies have focused on the application of processes and the evaluation of extraction parameters to maximize yield and improve extract composition. This involves reducing solvent consumption, extraction time, environmental impact, and other aspects related to extraction procedures. Furthermore, extraction techniques play an important role in the bioactivity of extracts, prompting several efforts to assess their potential applications in foods. This review presents a comprehensive study of the extraction techniques applied to obtain bioactive compounds from the fruits of the *Capsicum* genus and explores their applications in food products.

Keywords: *Capsicum*, capsaicinoids, extraction parameters, food applications

INTRODUCTION

Capsicum compounds and generalities

Capsicum overview

Capsicum fruits are native to America, and they have dispersed worldwide since the sixteenth century through Spanish and Portuguese traders. Since then, it has become an integral part of the eating habits of many countries **(Gurnani *et al.*, 2016).** The *Capsicum* genus, to which the pepper belongs, is native to tropical and humid areas of Mesoamerica; it belongs to the *Solanaceae* family **(De Aguiar *et al.*, 2016).** It is an annual or perennial herbaceous plant. This genus comprises approximately 22 to 27 wild species. Currently, five domesticated species are recognized: *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, and *Capsicum pubescens*, with more than 3000 varieties. *C. annuum* is the most commercially cultivated species globally **(Gurnani *et al.*, 2016; Yilmaz *et al.*, 2015; Arimboor *et al.*, 2015).** In 2020, global production of bell peppers amounted to 36 million tons, most of which were produced in China, Mexico, Indonesia, and Turkey **(FAOSTAT, 2023).** *Capsicum* fruits are very popular in Mexican food and in Asian cuisines **(Popelka *et al.*, 2017).** After tomatoes, they are the most produced vegetables in the world, and as a spice, they are the most cultivated **(Alabi *et al.*, 2022)**. Cultivated peppers exist in various sizes, shapes, colors, and levels of pungency; they are widely valued for attributes such as flavor and aroma **(Popelka *et al.*, 2017)**. They can be consumed fresh or ground into powders as spices, and their oleoresins are currently used **(De Aguiar *et al.*, 2016)**.

In addition to their consumption within world gastronomy, *Capsicum* fruits play a crucial role as additives in processed foods. They are used as colorants in ketchup, various sauces, dressings, mayonnaise, meat products, beverages, baked goods, snacks, soups, patties, pickles, and canned items, as well as fillings for olives and in baby food, among other applications. *Capsicum* fruits are also employed in pharmaceutical products as well as in the cosmetic and paint industries **(Yilmaz *et al.*, 2015)**. Non-pungent or "sweet" *Capsicum* fruits have also been utilized as coloring and flavoring agents for an extended period, which has reached an approximate annual production of 2.5 million tons **(Alabi *et al.*, 2022; Guijarro-Real *et al.*, 2023)**.

Peppers are excellent sources of phytochemicals such as vitamins A and C, phenolic compounds, flavonoids, carotenoids, and macro- and microelements. Other minor components that significantly influence the taste and aroma of chili peppers include alcohols, terpene aldehydes, ketones, fatty acids, and fatty acid esters. Plants of the *Capsicum* genus can produce alkaloids belonging to the capsaicinoid family, which impart the characteristic pungency of chili peppers **(De Aguiar *et al.*, 2016; Arimboor *et al.*, 2015)**. *Capsicum* is also the only genus known to synthesize capsanthin and capsorubin, red pigments with high economic value **(Gurnani *et al.*, 2016)**.

Capsaicinoids

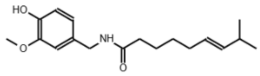
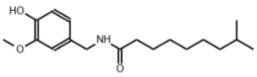
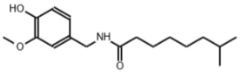
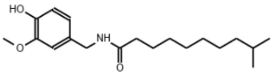
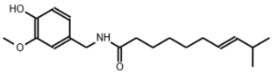
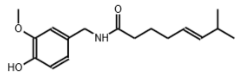
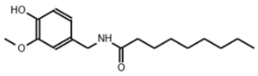
Capsaicinoids possess diverse properties and applications that render them intriguing compounds. Their versatility extends to applications in pharmaceuticals and nutrition, and they have even found use as chemical weapons **(Popelka *et al.*, 2017)**. These compounds exhibit potent pharmacological effects, serving as antimutagenic and antitumor agents, antioxidants, and frequently employed topical analgesics for various clinical pain conditions **(Costa *et al.*, 2022)**. Additionally, capsaicinoids have been explored for their potential in weight reduction and providing gastrointestinal and cardiovascular benefits **(De Aguiar *et al.*, 2016).**

In total, more than 20 capsaicinoids have been discovered in different species of chili peppers. Capsaicinoids are exclusively synthesized in the epidermal cells of the placenta of *Capsicum* fruits through the condensation of vanillylamine and medium-chain fatty acids **(Lu *et al.*, 2017).** They accumulate in vesicles throughout the epidermis **(Costa *et al.*, 2022)** and are excreted to the internal surface of the seeds and the pericarp. High concentrations of capsaicin have been found in the ovary, while the lowest concentrations are in the seeds. Capsaicinoids have evolved in hot peppers as a defense mechanism against predatory mammals; however, this characteristic is a crucial quality attribute of the fruit and one of the most important reasons why hot peppers are consumed **(Popelka *et al.*, 2017)**.

These spicy properties contribute to their distinctive flavor, making them suitable for culinary preparations. It is important to note that the presence of capsaicinoids in hot peppers is highly variable and strongly depends on the cultivar (genotype), as well as parameters such as ripeness, season, and irrigation **(De Aguiar *et al.*, 2016)**, along with factors like light intensity and temperature, among others **(Rodríguez-Rodríguez *et al.*, 2020)**. Studies conducted in New Mexico found variable levels of capsaicinoids in 13 new landraces of *Capsicum*, with higher levels in fruit grown under field conditions compared to greenhouse conditions **(Rodriguez-Uribe *et al.*, 2014)**.

Capsaicinoids are derivatives of benzylamine, and their structural characteristics determine their properties **(Cunha *et al.*, 2022)**. Structural differences mainly depend on acyl fractions and three elements: the length of the acyl chain (C8-C13), the manner of termination (linear, iso, or anteiso series), and the presence or absence of unsaturation at the carbon atom ω-3 (capsaicin type) or ω-4 (homocapsaicin type I and II) **(Popelka *et al.*, 2017)** (Table 1).

Table 1 Chemical structure of capsaicinoids. Adapted from **Lu *et al.* (2017).**

Capsaicinoid name	Abbreviation	Molecular formula	Chemical structure
Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide)	C	$C_{18}H_{27}NO_3$	
Dihydrocapsaicin (8-methyl-N-vanillyl-nonanamide)	DHC	$C_{18}H_{29}NO_3$	
Nordihydrocapsaicin (7-methyl-N-vanillyl-octamide)	n-DHC	$C_{17}H_{27}NO_3$	
Homodihydrocapsaicin (9-methyl-N-vanillyl-decamide)	h-DHC	$C_{19}H_{31}NO_3$	
Homocapsaicin (trans 9-methyl-N-vanillyl-7-decenamide)	h-C	$C_{19}H_{29}NO$	
Norcapsaicin (7-Methyl-N-vanillyl-5-octenamide)	n-C	$C_{17}H_{25}NO_3$	
Nonivamide N-[(4-Hydroxy-3-methoxyphenyl) methyl]nonanamide	PAVA	$C_{17}H_{27}NO_3$	

Capsaicinoids impart the spicy sensation of hot peppers. Although more than 22 different capsaicinoids have been detected in chili peppers, the most common ones in the fruit are capsaicin and dihydrocapsaicin **(Guijarro-Real *et al.*, 2023)**. **De Aguiar *et al.* (2016)** found that capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide) are the predominant molecules, constituting 90% of the total capsaicinoids in hot peppers. Capsaicin accounts for 69% of the capsaicinoid group; dihydrocapsacinoids for 22%; nordihydrocapsacinoids for 7%; and homocapsaicin and homohydrocapsaicin collectively represent only 1% within the group of capsaicinoids **(Popelka *et al.*, 2017).** Other related capsaicionoids such as norhydrocapsaicin, homodihydroxycapsaicin, norcapsaicin, nornorcapsaicin, and nonivamide, among others, are also present in trace amounts **(Naves *et al.*, 2019; Cunha *et al.*, 2022).**

Capsaicin and dihydrocapsaicin are approximately twice as pungent as nordihydrocapsaicin and homocapsaicin. They are mainly responsible for the spiciness of hot peppers **(Popelka *et al.*, 2017).** On the other hand, the capsaicin content determines the commercial quality of hot peppers due to the greater presence of capsaicinoids **(Alabi *et al.*, 2022).** Therefore, quantifying the pungency of hot peppers is crucial for both consumers and commercial purposes **(De Aguiar *et al.*, 2016).**

The pungency of capsaicinoids and hot pepper preparations can be expressed in Scoville Heat Units (SHU), representing the number of dilutions in water required for a sample to lose its pungency sensation **(Costa *et al.*, 2022).** The human palate can detect pungency in a dilution as high as 1:17,000,000. This measurement indicates the dilution of hot pepper extract at which trained panelists can detect pungency. Currently, the Scoville organoleptic test has been replaced by chromatographic methods, considered more reliable and accurate **(Popelka *et al.*, 2017)**. A universal scale has also been established by the American Species Brand Association (ASTA), based on parts per million (ppm) of capsaicinoids rather than human perception, which can be subjective **(De Aguiar *et al.*, 2016)**. Species and cultivars are the major determinants of pungency, with other factors including the sowing season and environmental conditions of the growing region, such as heat and increased fruit ripeness **(Popelka *et al.*, 2017; De Aguiar *et al.*, 2016)**.

There are variations in the content of capsaicinoids in fruits, from a very low concentration of 0.025% (sweet peppers or peppers), which do not have pungent properties, to concentrations of about 0.25% in hot peppers **(Popelka *et al.* 2017)**. For example, in Brazil, an investigation was carried out on varieties such as Cheiro Verde, Cambuci Vermelha, Cambuci Verde, and Biquinho, which did not show capsaicinoids in their composition. This result was expected since these peppers are popularly known in Brazil as sweet peppers, lacking the typical pungency of the genus **(De Aguiar *et al.*, 2016)**.

Carotenoids

Carotenoids are tetraterpene pigments that exhibit yellow, orange, red, and purple colors **(Maoka, 2020)**. In higher plants, carotenoids are found in plastids, particularly in chloroplasts of photosynthetic tissues and chromoplasts of flowers and fruits **(Sun *et al.*, 2022)**. Regarding the role of carotenoids in plants, it is well known that they capture light energy during photosynthesis and protect plants from oxidative damage caused by photosensitization **(Maoka, 2020; Sun *et al.*, 2022)**. The content of carotenoids in plant tissues is influenced by various factors, such as the cultivar, ripening stage, variety, the part of the plant used, and growing conditions **(Maoka, 2020; Rodríguez-Rodríguez *et al.*, 2020)**. Changes in carotenoid content can occur not only in live plants but also during post-harvest processing and storage. Plant food processing operations generally break down cells where carotenoids are safely stored, exposing them to oxidative enzymes and other degrading agents. Oxidation contributes significantly to the degradation of carotenoids during processing and storage **(Arimboor *et al.*, 2015; Rodríguez-Rodríguez *et al.*, 2020)**.

In peppers, these different carotenoids are present in the sacrocarps and develop and accumulate quickly as the fruit ripens. Carotenoids are responsible for various colors, ranging from yellow to red, then to green, brown, and orange, as evident in ripe *Capsicum* fruits **(Mohd Hassan *et al.*, 2019; Maoka, 2020).** In peppers, both sweet (bell peppers) and hot varieties, carotenoids are a primary component that dictates the fruit's quality **(Alabi *et al.*, 2022)**. At least 34 unesterified carotenoids have been extracted from peppers. These include α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, violaxanthin, capsanthin, lutein, capsorubin, and other less common ones (neoxanthin, anteraxanthin) **(Mohd Hassan *et al.*, 2019)**. Carotenoids in peppers are of special interest owing both to their provitamin A carotenoid content (β-carotene, α-carotene, and β-cryptoxanthin) and other carotenoids that are important for human eye health (lutein, zeaxanthin) **(Rodríguez-Rodríguez *et al.*, 2020)**.

Pepper carotenoid composition is complex and varies both qualitatively and quantitatively depending on the variety and color. The ketocarotenoids capsanthin, capsorubin, and cryptocapsin impart a bright red color as the fruits ripen, while the yellow-orange color is a result of β-carotene, zeaxanthin, violaxanthin, and β-cryptoxanthin **(Mohd Hassan *et al.*, 2019; Rodríguez-Rodríguez *et al.*, 2020)**. Capsanthin contributes to 30 –70% of the carotenoids in most varieties and cultivars **(Arimboor *et al.*, 2015)**. The structural properties of capsanthin, which include eleven conjugated double bonds, a conjugated keto group, and a cyclopentane ring, neutralize the damaging effects of singlet oxygen. The long chains of double bonds, ending in one or two polar ketones, efficiently absorb green light, giving a red-orange hue. Additionally, the hydroxyl group of the ring structure is esterified with fatty acids in monoesters and diesters, appearing in free form as well **(Arimboor *et al.*, 2015)** (Figure 1).

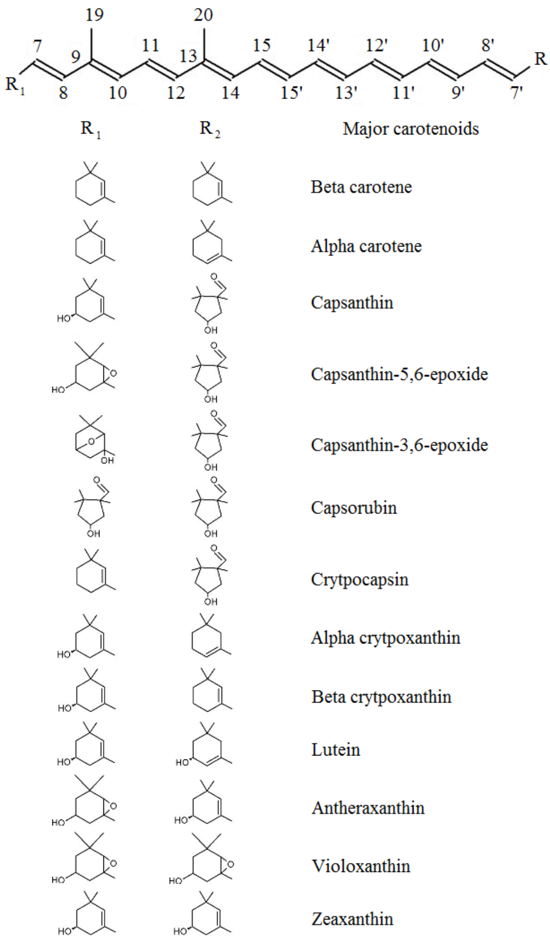


Figure 1 Structure of major carotenoids in red pepper. Mdified from **Arimboor *et al.* (2015).**

In paprika fruits, capsanthin progressively esterifies with fatty acids as ripening advances, incorporating more easily into the membrane structure. Thus, esterified capsanthins can serve as antioxidants in hydrophobic regions, such as the lipophilic globules of chromoplates. The esterified capsanthins of the fully mature fruit represent 70–80% of the total capsanthins **(Arimboor *et al.*, 2015; Maoka, 2020)**. A study conducted by **Rodriguez-Uribe *et al.* (2014)** reported capsanthin levels between 468 and 1007 µg/g of dry weight fruit in fruits grown in the field, while the levels of β-carotene were similarly higher (13 to 22 µg/g of dry weight fruit) in 13 new varieties of peppers in New Mexico.

Most of the xanthophylls in red pepper are produced as esters with C12, C14, and C18 fatty acids, while green pepper extracts are mainly composed of free carotenoids **(Arimboor *et al.*, 2015)**. As chlorophyll pigments such as lutein and neoxanthin disappear, beta-carotene, violaxanthin, capsanthin, and capsorubin increase during ripening **(Sun *et al.*, 2022)**.

Today, food producers pay more attention to colors and additives of natural origin than to synthetics. Carotenoids are commercially exploited as food colorants and feed additives and are used in pharmaceutical, nutraceutical, and cosmetic products **(Sun *et al.*, 2022).** These additives are used to color foods, replacing lost color during processing, enhancing existing color, minimizing batch-to-batch color variations, and restoring color to discolored foods. In addition to coloring, these pigments are essential for plant and animal health, playing a special role in protecting tissues from light and oxygen.

Extraction methods

The performance of extraction processes depends on numerous factors, including the solvent, temperature, pressure, extraction time, raw material characteristics, chemical structure of target compounds, and their location within the raw material. However, its significance is contingent on the physical separation mechanism of the extraction method. In this context, it is essential to identify the effects of extraction factors to establish conditions that ensure greater efficiency. Over the years, the extraction of capsaicinoids from pepper has been the subject of study using various extraction methods. These range from traditional approaches such as maceration, percolation, hydrodistillation, and steam distillation to more modern techniques, including ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), and enzyme-assisted extraction (CAE).

Maceration

Extraction by maceration involves the extended contact between a plant matrix and the same solvent within a tank. The extraction method relies on the solubility of an analyte in a liquid-phase solvent or solvent mixture. Various organic solvents may be employed, with their selection based on the polarity of the analyte. Enhanced efficiency in this extraction process is achieved through the application of high temperatures (also called digestion) and periodic stirring. Nevertheless, long extraction times, significant hazardous solvent consumption, and low selectivity in extraction are the primary drawbacks of this method **(Jha and Sit, 2022)**. In the context of capsaicinoid extraction, solvents such as acetic acid, dichloromethane, methanol, ethyl acetate, acetonitrile, acetone, and ethanol, along with their mixtures, are commonly employed for the removal of capsaicin from chili. According to the literature, the extraction of capsaicinoids through maceration with ethanol may require up to 24 hours **(Thongin *et al.*, 2022; Goci *et al.*, 2021)**. Meanwhile, using solvents like acetone, dichloromethane, and ethyl acetate can yield extracts with capsaicin concentrations ranging from 28.8 to 73.97 mg per 10g of chili powder; notably, when ethyl acetate is the solvent of choice, this process can be completed in 1 hour **(Waqas *et al.*, 2022)**. Furthermore, to enhance extraction efficiency, researchers have explored the incorporation of surfactants in the maceration extraction of capsaicinoids. Results from these studies highlight the significant increase in capsaicin extraction, particularly when the nonionic surfactant Tween 80 is used alongside extraction solvents like dichloromethane and acetone **(Waqas *et al.*, 2022)**.

Percolation

Percolation extraction is a traditional method involving the passage of a liquid solvent through a solid sample. Unlike maceration, where there is prolonged interaction between a solvent and a plant matrix, percolation extraction requires consistently adding a fresh solvent to the plant; therefore, percolation extraction prevents solvent saturation and improves the extraction of desired analytes **(Jha and Sit, 2022)**. Also, this technique is suitable for extracting analytes sensitive to heat **(Wang *et al.*, 2020)**. However, its drawbacks include extended extraction times (though shorter than maceration) and the significant consumption of either harmful or non-harmful solvents, along with the associated energy expenses during the concentration process of the extracts **(Rasul, 2019)**. Soxhlet extraction operates under the percolation extraction principle, as it involves the continuous passage of a solvent (typically organic) through the plant matrix. This technique is suitable for extracting compounds that are not sensitive to high temperatures, as the extract remains at the solvent boiling temperature for specific periods, maximizing the risk of analyte degradation **(Belwal *et al.*, 2018)**.

The use of percolation for obtaining bioactives from the Capsicum genus dates back many years, as it is considered an ancient technique. However, due to the development of faster and more environmentally friendly methods in recent times, there is a scarcity of updated literature addressing the study of obtaining Capsicum extracts through this method **(Contreras-Padilla and Yahia, 1998)**. Ethanol percolation (95 –96° purity) at room temperature is commonly used for obtaining Capsicum resin extracts. Additionally, subsequent processes such as evaporation for solvent removal and purification for extract concentration have been reported **(Frial-McBride, 2016)**. Ethanolic extracts of Capsicum pubescens obtained through percolation have shown content of flavonoids, phenolic compounds, tannins, alkaloids, and moderate antibacterial activity against Staphylococcus aureus and Bacillus subtilis **(Carhuancho and Huarcaya, 2018)**. Furthermore, the percolation extraction method is currently employed in the industry for obtaining oleoresin from Capsicum annum **(Apex Botanicals, 2024; Lala Essential Oils, 2024)**. While the extraction conditions are not explicitly mentioned, it is known that the resulting extracts contain capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. Also, the obtaining of capsicum oleoresin through Soxhlet extraction using solvents such as ethanol and acetone was studied. Due to the high polarity of ethanol and the moderate polarity of acetone (resulting from the presence of a carboxyl group and methyl groups), the mixture of both solvents in a 1:1 ratio was the condition that resulted in the highest oleoresin yield (exceeding 15%). However, capsaicinoid-rich extracts were obtained with ethanol. Likewise, Soxhlet extraction also requires subsequent processes of evaporation and drying **(Madhusankha *et al.*, 2023)**.

Hydrodistillation

Hydrodistillation is a traditional extraction method that involves placing the sample with sufficient water (solvent) inside a tank and heating it until the boiling point of the water is reached. The steam dissolves volatile analytes in the sample, which are brought to the surface due to differences in density, and subsequently condensed and captured **(Rasul, 2019)**. This method is widely employed for obtaining essential oils because it is the simplest **(Khan *et al.*, 2023)**. In a study conducted by **Pino *et al.* (2020)**, hydrodistillation was applied to ripe chili peppers to obtain an aromatic distillate that was later encapsulated. The extraction process concluded once 30% of the original mixture was distilled, and among the aromatic distillate characteristics are the presence of over 100 compounds, primarily esters and, to a lesser extent, aldehydes, alcohols, terpenes, ketones, and acids. On the other hand, hydrodistillation has been employed as a subsequent step to percolation extraction for the recovery of essential oils from capsicum oleoresin. After an extraction period of 2 hours, monoterpenes and sesquiterpenes with antioxidant activity were identified in the essential oil **(Madhusankha *et al*., 2023)**. The main disadvantage of this method is the harmful effects it could have on the thermosensitive compounds present in the sample caused by prolonged exposure to temperatures close to 100°C. According to **Melgar-Lalanne *et al.* (2017)**, hydrodistillation can produce hydrolysis of esters, polymerization of aldehydes, or decomposition of other components in capsicum extracts. An alternative to mitigate these effects is the use of subcritical water, since the use of high pressure allows for reducing the time the sample is subjected to high temperatures **(Marcus, 2018)**.

Steam distillation

Steam distillation involves using steam as a solvent to extract the desired analytes (primarily essential oils) from a sample. Steam is produced by boiling water in a separate vessel and is then directed towards the sample, penetrating it. Subsequently, steam dissolves the essential oils, and the resulting mixture is condensed and collected **(Tolulope *et al*., 2019)**. The bioactivity of essential oils obtained through this extraction technique remains unclear, as research findings are conflicting. According to **Rasul (2019)**, the degradation of analytes cannot occur because the sample does not reach the boiling temperature of water. However, **Tolulope *et al.* (2019)** state that, similar to hydrodistillation, steam distillation promotes the chemical modification of heat-sensitive essential oils. Additionally, the polarity of the water used in steam distillation results in low extraction efficiency for capsaicinoids with medium or low polarity **(Melgar-Lalanne *et al*., 2017)**. Nevertheless, this extraction method is widely adopted by industry for the large-scale production of capsicum essential oil.

Currently, there are few studies on the steam distillation of analytes from plant matrices, primarily due to the availability of more efficient techniques. In 1980, **Teranishi *et al.* (1980)** studied steam distillation in both continuous and batch modes for the recovery of volatile compounds from capsicum oleoresin (Capsicum frutescences). Their study concluded that continuous extraction provided a higher yield when conducted at 225°C, and a lower yield was obtained as the temperature decreased (150°C), similar to the Bach process. Moreover, the extract obtained through steam distillation in continuous mode at 225°C showed a content of free acids, mainly acetic acid, and methyl esters of lauric, palmitic, and stearic acids **(Teranishi *et al*., 1980)**. Additionally, **Pino *et al.* (2011)** also used steam distillation to isolate the volatile fraction present in three cultivars of Capsicum chinense Jacq, determining that the volatile fraction is composed of 136 compounds responsible for the flavor of pepper.

Ultrasound-assisted extraction (UAE)

In ultrasound-assisted extraction (UAE), ultrasound energy is used together with a solvent or mixture of solvents to remove the analytes of interest from the matrix. Ultrasound waves. With a frequency exceeding 20 kHz, induce cycles of compression and rarefaction favoring the displacement and detachment of molecules from their original positions. During the rarefaction phase, the negative pressure caused by ultrasound waves overcomes the molecular attraction force, leading to the release of the molecule and the formation of cavitation bubbles. These bubbles grow in size through coalescence and subsequently collapse during the compression phase, generating hot spots and extreme conditions **(Kumar *et al*., 2021). According to Patel *et al*. (2019)**, this technique offers several advantages over conventional methods, including the use of low temperatures, minimal solvent consumption, low energy requirements, short extraction times, the use of small sample quantities, and easy operation, among others. Nevertheless, it also notes that a potential drawback may be the formation of free radicals capable of modifying the structure of the analyte molecule.

Sricharoen *et al.* (2017) extracted phytochemicals, including flavonoids, carotenoids, phenols, carotenoids, flavonoids, capsaicinoids and reducing sugars, exhibiting antioxidant and antidiabetic activity, from capsicum oleoresin using ultrasound-assisted extraction with a water and methanol mixture at 323 K. Moreover, **Herrera-Pool *et al.* (2021)** assessed various solvents such as water, methanol, hexane, ethyl acetate and acetone, both individually and in different combinations. Their study revealed that the extraction of phenolic compounds from habanero pepper (*Capsicum chinense*) leaves achieved the highest yields when employing a methanol and water mixture (1:1) and 313 K through ultrasound assisted extraction.

Microwave Assisted Extraction Method (EAM)

Microwave-Assisted Extraction (MAE) can enhance the content of bioactive compounds in plant extracts while reducing the extraction time and solvent volume compared to thermal reflux, ultrasound-assisted extraction, or traditional enzyme-catalyzed extraction methods **(Chaturvedi, 2018; Akhtar *et al*., 2019)**. This is because microwave electrical oscillations induce repeated rotations of water molecules in the presence of electromagnetic fields, generating heat through molecular friction **(Jimenez *et al.* 2018)**. Heating is caused by intermolecular friction at extremely high speeds in a high frequency alternating electric field environment **(Eskilsson and Björklund, 2000)**. Therefore, microwave-assisted extraction can only be performed in a very short time and at a low frequency **(Akhtar *et al*., 2019)**. Microwave pretreatment of plant material during extraction can improve the recovery of secondary metabolites and aromatic chemicals **(Prado and Rostagno, 2022)**.

Haiyan *et al.* (2013) investigated the extraction of capsaicin from chili powder using microwave-assisted techniques, employing solid-liquid ratio, extraction time, and extraction temperature as optimization parameters. They determined that the influence of the three factors on the extraction yield of capsaicin from chili using the microwave method decreases in the following order: temperature > solid-liquid ratio > time. The optimal extraction conditions were found to be at 120˚C with a solid-liquid ratio of 25 mg/mL for 90 minutes, resulting in a capsaicin extraction yield of 3.85%. In another study, **Adegoke (2023)** employed extraction criteria, including both fixed parameters (microwave power and particle size) and variable ones (heating time and solvent volume), to obtain aqueous extracts from black pepper (*Piper nigrum*). Optimal conditions were achieved with 87.28 minutes of heating and 364.26 mL of solvent volume, reaching a desirability value of 0.624 while keeping microwave power and particle size constant. This approach resulted in an efficient and environmentally friendly extraction process, leading to increased bioavailability of functional compounds.

ltrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) have been implemented and analyzed. Initially, various solvents were individually examined, determining that the optimal combination for UAE was 42% methanol and 58% ethyl acetate, while for MAE, 100% methanol was used. Subsequently, a Box-Behnken experimental design was conducted with four variables for both UAE and MAE (time, temperature, pH, and the "mass sample: solvent volume ratio"); the latter proved to be the most influential variable in UAE, while for MAE, no variable showed greater influence than the others. The results demonstrate the potential of both techniques as highly suitable methods for extracting capsinoids from peppers, antioxidant compounds very similar to capsaicinoids but less irritating, non-spicy, and more flavorful **(Vasquez-Espinosa *et al*., 2019)**.

Supercritical fluid extraction

Among the various methods employed for processing pepper, a particular focus is directed towards supercritical fluid technologies. These include supercritical fluid extraction (SFE) utilizing pure solvents as well as CO_2 with modifiers, along with ultrasound-assisted SFE. Processes based on supercritical fluids offer advantages compared to traditional extraction techniques, such as reduced solvent usage, shorter extraction times, enhanced specificity, and scalability **(De Aguiar *et al*., 2022)**. Supercritical extraction is carried out in a pressurized extraction cell using a solvent at a temperature and pressure above the critical point (critical Temperature-Tc and critical pressure-Pc). A supercritical fluid has the favorable extraction characteristics of liquids (high density) and gases (high diffusivity). The most widely used solvent is carbon dioxide (CO_2) due to its critical conditions (Tc = 31 °C and Pc = 7.3 MPa) that prevent the degradation of heat-sensitive compounds, as well as being economical, non-toxic, non-flammable, and inert **(Brunner, 2013)**. Sometimes, modifiers (solvents with opposite polarity, such as water, ethanol, and methanol) and their combination with ultrasound waves (SFE+US) are used to increase the extraction efficiency.

The primary factors impacting the efficiency of supercritical fluid extraction (SFE) include temperature, pressure, particle size, and moisture content of the feed material, as well as the flow rate of CO2 and the solvent-to-feed ratio **(Dias *et al*., 2021)**. Nevertheless, the majority of studies concentrate their efforts on investigating the effects of temperature and pressure. Typically, temperatures within the range of 40 to 70 ºC are employed in SFE. The lower limit is dictated by its close proximity to the critical temperature of CO_2, while the upper limit affects solvent density. Elevated temperatures result in lower solvent density, leading to reduced extraction yield and increased energy consumption. In terms of pressure, most research explores the range of 100 to 500 bar. Pressure has an opposing impact on CO_2 density compared to temperature; increased pressure results in higher CO_2 density, consequently achieving a greater solvation capacity. Pressures exceeding this range incur high energy costs, while pressures below approach the critical CO_2 pressure **(De Aguiar *et al*., 2022)**.

Dos Santos *et al.* (2013) studied the SFE+US extraction of oleoresin from malagueta pepper (*Capsicum frutescens* L.). The dry and ground raw material with two particle sizes (1.68-1.18 and 0.342-0.177 mm) was subjected to extraction with supercritical carbon dioxide at 15 MPa and 40 °C at a mass flow of 0.5 kg/h for 8 h. The ultrasound probe, installed inside the column, emitted 360-watt waves during the extraction period. The authors concluded that the decrease in particle size increased the yield from 5.14 to 9.10%. Likewise, the ultrasound waves produced changes in the morphology of the plant tissue, favoring the extraction performance, which was more evident when the particle size was larger.

Similarly, **Grande-Villanueva *et al.* (2015)** assessed the extraction of oleoresin using supercritical carbon dioxide from jalapeño pepper (*Capsicum annuum*). The extraction was carried out at 40 and 60 °C with pressures of 15 to 35 MPa for 240 min. The highest yield of extracts was 5.1% and was obtained at 40 °C/30 MPa and 60 °C/20 MPa, concluding that the increase in pressure leads to a rise in the overall yield. Likewise, the authors determined a maximum capsaicin content of

12.1 µg/g of extract (60 °C/15 MPa) and 617 µg/g of lyophilized jalapeno pepper. Other compounds, such as phenolics, were also extracted.

In a recent study, the combination of supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) demonstrated enhanced efficiency in extracting active compounds. **De Aguiar *et al.* (2020)** conducted an economic analysis of this SFE+PLE method for obtaining capsiate-rich oleoresin and phenolic-rich extract from biquino pepper. SFE extraction, using carbon dioxide at 50 °C and 15 MPa with a S/F ratio of 116.6, yielded 3.9 g of oleoresin and 40.04 mg of capsiate per 100g of hawthorn pepper over a 2-hour process. Subsequently, the extraction residue underwent PLE using ethanol and water (75% ethanol) as a solvent at 10 MPa and 65 °C. The PLE process resulted in a 43.7% extract yield, with total phenolics and rutin content from the residue measuring 11.7 mg GAE/g and 130 µg/g, respectively. Finally, manufacturing costs for SFE and PLE extracts were calculated at US$5.291/kg and US$25.39/kg, respectively.

Kostrzewa *et al.* (2020) determined the effect of pressure, temperature, and time on the supercritical carbon dioxide extraction of sweet paprika (*Capsicum annuum L.*) using a response surface methodology. The results revealed that the operational parameters (pressure, temperature, and extraction time) significantly influenced the extraction yield and carotenoid content in the extract. The optimal extraction conditions to achieve a high carotenoid content in the extract were determined to be 323.15 K, 45 MPa, and 56 minutes, resulting in a yield of 8.5% and a carotenoid recovery of 84%.

Finally, **Soldan *et al.* (2021)** investigated the potential of supercritical fluid extraction (SFE) in obtaining oleoresin from the industrial waste of *Capsicum annuum pepper* (Jalapeno). SFE experiments were conducted using CO2 under different conditions, including varying temperatures (40 and 60 °C), pressures (200 and 250 bar), and with or without the addition of ethanol as a cosolvent. In comparison, Soxhlet extractions using ethanol, acetone, and n-hexane were performed. The inclusion of ethanol as a cosolvent and temperature fluctuations played a significant role in increasing the total mass of extracted oleoresin, while pressure showed no substantial effect. SFE resulted in total mass yields ranging from 9.38% to 10.08%, whereas Soxhlet extraction exhibited a broader range of yields (8.45% to 15.5% w/w). Despite the presence of bioactive compounds such as phenolics, flavonoids, fatty acids, and carotenoids in the extracts, they did not demonstrate significant antioxidant activity.

Pressurized liquid extraction

The extraction with pressurized liquid (PLE), also known as the accelerated extraction with solvent (Accelerated Solvent Extraction, ASE), is an extraction technique friendly to the environment that arises to overcome the inconvenience of the loss of thermosensitive compounds by lengthy procedures at high temperatures. PLE consists of the rapid passage of a solvent in a liquid state at temperatures above its boiling point through a solid matrix **(Mustafa *et al.*, 2011; Bubalo *et al.*, 2018).**

The utilization of pressurized liquids for extracting active compounds from the Capsicum genus is relatively limited. In 2016, **Barbero *et al.* (2006)** investigated the PLE extraction with water, ethanol, and methanol (0–20% in water) at 10 MPa and temperatures of 50–200 °C of capsaicin and dihydrocapsaicin from fresh cayenne pepper, long marble peepers, and round marble peepers, without peduncles or seeds, liquefied. In PLE extracts, the capsaicinoids nordihydrocapsaicin, capsaicin, dihydrocapsaicin, isomers of dihydrocapsaicin, and homodihydrocapsaicin were identified. Likewise, it was determined that no degradation of capsaicinoids occurred in the temperature range studied for up to 30 min. In this sense, the study determined that extraction at 200 °C allowed the most significant recovery of capsaicinoids. Additionally, the solvent that provided the highest extraction yield was pure methanol.

On the other hand, **Kang *et al.* (2016)** evaluated the recovery of lutein by PLE from lyophilized and ground paprika leaves; the effect of temperature, static time, and ethanol concentration was studied.

Enzyme-assisted extraction

According to **Ovando-Chacón *et al.* (2005)**, cellulolytic enzymes are produced by a great variety of aerobic, anaerobic, mesophilic, and thermophilic bacteria and fungi, which are characterized by their ability to hydrolyze the β-1,4 glycosidic bond that exists between glucose molecules, forming cellulose. According to the site of action, this enzyme complex can be endoglucanases (EnG), exoglucanases (ExG), or β-glucosidases (BG). However, in any case, glucose is the product of the hydrolysis of cellulose by the action of cellulolytic enzymes **(Moore, 2003)**.

Gamarra-Mendoza *et al.* (2020) investigated the cellulolytic enzyme-assisted extraction of capsaicinoids from native chili peppers (*Capsicum baccatum*). The process involved pre-hydrolysis of dried and ground native chili pepper through the addition of cellulolytic enzymes in an aqueous medium at ratios of 1:15 and 1:20, carried out at 35 °C with stirring for up to 4 hours. Subsequently, the hydrolyzed chili pepper was combined with a solvent (hexane-75% and ethanol-25%) for extraction at 40 °C with stirring for 3 hours. Results revealed a 21% higher efficiency in cellulolytic enzyme-assisted hydrolysis compared to the treatment without enzymes, as measured by the content of reducing sugars (39 mg/mL after 4 hours of hydrolysis). The highest oleoresin yield (11.4%) was achieved with a 1:20 ratio of native chili pepper to cellulolytic enzymes, coupled with stirring at 190 rpm for 4 hours. The identified capsaicinoids included norhydrocapsaicin, capsaicin, and dihydrocapsaicin. The study concludes that cellulolytic enzymes, by breaking down cellulose, hemicellulose, and other plant tissue compounds, enhance mass transfer rates through increased tissue porosity. This, in turn, reduces extraction time and boosts overall yield.

Enzyme-Assisted Extraction (EAE) enhances the acquisition of bioactive compounds found in fruits and seeds. In a study conducted by **Cortes-Ferre *et al.* (2022)**, the conditions for cellulase-assisted extraction of capsaicinoids and phenolic compounds from Habanero CPS (*Capsicum chinense*) were examined. EAE was carried out with variations in temperature (T1 = 30°C, T2 = 45°C, and T3 = 60°C), enzyme concentrations (E1 = 2500 IU/L and E2 = 250 IU/L), and extraction times (0-150 minutes). The results revealed that the highest content of phenolic compounds (337.96 mg GAE/L) was achieved at 30°C, with an enzyme concentration of 2500 IU/L and an extraction time of 150 minutes. Regarding the maximum content of CAP (310.23 µg/ml), it was attained at 45°C with an enzyme concentration of 250 IU/L over 150 minutes, while for DHC (167.72 µg/ml), the optimal conditions were 60°C, 2500 IU/L, and a 120-minute extraction period.

Food application of *Capsicum* extracts

For food applications, *Capsicum* extracts and raw consumption, compared to other food products, have been lower and are still frequently ignored in research related to food intake **(Gajewska *et al.*, 2019).** The main two consumed peppers are *Piper* and *Capsicum* **(Sá-Mendes *et al.*, 2019)**, as their production increased by 25% between 2006 and 2016, being of great agricultural and economic importance **(Baenas *et al.*, 2019)**. Furthermore, due to the bioactive compounds presented in the matrix, it is possible to apply raw pepper as well as the extract to the food industry, improving functional and sensory quality, antioxidant and antimicrobial properties, shelf life, and bringing health benefits **(Rezazadeh *et al.*, 2022; Procopio *et al.*, 2022)**. However, there are still few works related to the characterization and identification of this raw material or plant extract, with multiple potential uses **(Sá-Mendes and Andrade-Gonçalves, 2020; Baenas *et al.*, 2019)**.

The principal applications in the food industry for the matrix, as well as the extract, are sauces, soups, meat products, confectionery, sweets, salad dressing, sausages, bakery products, and alcoholic beverages **(Procopio *et al.*, 2022; Baenas *et al.*, 2019; Yilmaz *et al.*, 2015)**. Additionally, the oil extract, with its high nutritional value due to the presence of linoleic acid and polyunsaturated fatty acids, is used in food processing **(Koncsek *et al.*, 2017)**.

The challenge associated with using capsaicin lies in its strong pungency, taste, and odor, coupled with its low solubility in water and susceptibility to environmental factors like light, leading to a notable decrease during thermal processes **(Rezazadeh *et al.*, 2021)**. Employing encapsulation emerges as an effective approach to mitigate the degradation of bioactive compounds, providing protection against adverse environmental conditions. Moreover, encapsulated compounds exhibit reduced interaction with food components, making them more readily usable in food applications **(Rezazadeh *et al.*, 2022)**.

Rezazadeh *et al.* (2022), evaluated electrospin zein fibers loaded with different concentrations of capsaicin (0.1%–0.4%) in terms of encapsulation efficiency, morphology, and antimicrobial and antioxidant activity. The capsaicin-loaded zein fibers exhibited antioxidant activity in the range of 60%–68%. Additionally, the nanofibers demonstrated higher antimicrobial activity against *Staphylococcus aureus* than against *Pseudomonas aeruginosa* and *Escherichia coli*.

The antioxidant and antimicrobial properties of polyphenol extracts from peppers are attributed to the main phenolic compounds found in peppers, including vanillic, caffeic, ferulic, p-coumaric, and p-hydroxybenzoic acids. These compounds act as natural additives, benefiting both the food industry and human health, as they can have adverse effects on microorganisms, including intestinal bacteria **(Sá-Mendes and Andrade-Gonçalves, 2020)**. It is believed that *Capsicum* peppers, among the possible functional and technological applications, can be used as a natural antioxidant ingredient due to their important barrier properties for ready-to-eat products **(Sá-Mendes and Andrade-Gonçalves, 2020)** to enhance physical, chemical, enzymatic, and microbial stability **(Solomando *et al.*, 2020; Zehiroglu and Sarikaya, 2019)**.

Gurnani *et al.* (2016) assessed the antimicrobial and antioxidant potential of *Capsicum frutescens* L. seeds and characterized the chemical constituents of the crude extracts. They identified pharmacologically active compounds, revealing remarkable antimicrobial activity along with moderate antioxidant activity in the low-polar extracts of red chili seeds. The findings of the present study justify the traditional use of red pepper as a food preservative in hot climates and indicate the tremendous nutraceutical potential of red pepper. Subsequent studies could focus on bioactivity-guided isolation from these crude extracts, potentially providing fractions or constituents with high antimicrobial and antioxidant capabilities. Such substances could serve as substitutes for synthetics with equal efficacy.

In dairy products, the application of cayenne and green pepper ethanol extracts in bull milk during the manufacture of Egyptian Kareish cheese showed antimicrobial activity against the natural microbiota, coliforms, molds, and *S. aureus* **(Wahba *et al.*, 2010)**. The extract contributes to food safety by acting as a control for foodborne pathogens, preventing spoilage, and avoiding the use of synthetic preservatives such as nitrite, sodium benzoate, or sodium metabisulfite, which have occasionally been associated with allergic reactions and the potential formation of nitrosamines **(Baenas *et al*., 2019)**.

The coloring capacity of the carotenoid pigments makes *Capsicum* oleoresins frequently used in the food industry as a natural additive. However, they are sensitive to heat and light conditions, which promote isomerization and oxidation reactions, resulting in the loss of carotenoid activity **(Procopio *et al*., 2022; Jimenez-Escobar *et al*., 2020)**. **Procopio *et al*. (2022)** indicate that carotenoids were more stable to chemical degradation in nanoemulsions of paprika oleoresin. In addition to microparticles, recent studies indicate a tendency to use these extracts directly as antimicrobial and antioxidant agents in films and food products.

CONCLUSIONS

The compounds present in the *Capsicum* genus, especially capsaicinoids and carotenoids, are elements of great significance in both gastronomy and various industries. With a wide diversity of species in the *Capsicum* genus, each possesses unique characteristics reflected in their culinary and commercial applications. Capsaicinoids, responsible for the distinctive spiciness of peppers, have garnered increasing interest due to their pharmacological and nutritional properties. These include antioxidant, antimutagenic, and analgesic effects, making them compounds of great value for human health. On the other hand, carotenoids, which give peppers their various colors, play a fundamental role in both fruit quality and health promotion. The study and utilization of these natural compounds are areas of ongoing research aimed at optimizing their extraction, identification, and application in a wide range of food, pharmaceutical, and cosmetic products.

Methods for extracting capsaicinoids from peppers have been studied for years, ranging from traditional approaches like maceration and percolation to more modern techniques such as ultrasound-assisted extraction and supercritical fluid extraction. The choice of an appropriate method depends on factors such as the type of compound to be extracted and the desired characteristics of the final product. Despite the challenges associated with the strong pungency, taste, and odor of capsaicin, Capsicum extracts find application in a wide variety of food products, including sauces, soups, meat products, confectionery, salad dressings, sausages, bakery products, and alcoholic beverages. This is due to their beneficial properties for health and their ability to enhance the sensory and functional quality of foods.

Acknowledgments: The authors thank Fundacion Universitaria Agraria de Colombia for providing the financial supports (PE180005) to carry out this work.

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