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A review of capsaicinoid extraction and quantification alternatives and their industrial application

Una revisión sobre las alternativas de extracción y cuantificación de capsaicinoide y su aplicación industrial

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Resumen

Los frutos de chile pertenecientes a la familia *Capsicum annuum* son productos de gran importancia mundial por sus diversas aplicaciones y en específico por el sabor pungente que otorga a los alimentos. Esta pungencia es atribuida a la presencia de compuestos capsaicinoides. Estos compuestos son prometedores en la industria ya que son usados en el sector alimentario, cosmético, farmacéutico, etc. Por esta razón, actualmente se están buscando nuevas alternativas para la obtención de capsaicinoides con el objetivo de conseguir rendimientos más altos a menor tiempos y costos; además, que sean amigables con el medio ambiente. En esta revisión, se busca hacer énfasis en los métodos de extracción y técnicas analíticas para la identificación y cuantificación de los capsaicinoides, así como, las aplicaciones de estos en la industria, además presentando diversas técnicas de extracción y cuantificación, destacando que métodos como ultrasonido y microondas aumentan el rendimiento en un menor tiempo, en comparación a

procesos convencionales. Se comprobó que la HPLC, es un técnica confiable, sensible y rápida para la cuantificación, por lo cual, es la técnica más utilizada y recomendable para la cuantificación de capsaicinoides.

***Palabras clave*:** Capsaicinoides, *Capsicum annuum*, Cromatografía líquida de alta resolución, Extracción asistida por ultrasonido, Microondas.

Abstract

The chili peppers belonging to *Capsicum annuum* family are products of great global importance due to their diverse applications and specifically the spicy flavor they give to food. This pungency is attributed to the presence of capsaicinoid compounds. These compounds are promising in the industry since they are used in the food, cosmetics, pharmaceutical, etc. sectors. For this reason, new alternatives are currently being sought to obtain capsaicinoids with the objective of achieving higher yields at lower costs and times, and that are also environmentally friendly. In this review, we emphasize the extraction and analytical methods for identifying and quantifying capsaicinoids and their applications in industry, also presenting various extraction and quantification techniques, highlighting that methods such as ultrasound and microwaves increase the yield in less time compared to conventional processes. It was proven that HPLC is a reliable, sensitive and fast technique for quantification, which is why it is the most used and recommended technique for the quantification of capsaicinoids.

Key words: Capsaicinoids, *Capsicum annuum*, High-performance liquid chromatography, Microwave-assisted extraction, Ultrasound.

1. Introduction

Chili (*Capsicum* spp.) is an appreciated vegetable in certain countries for its pungency, flavor, and aroma. The spicy sensation of this type of fruit is caused by the presence of capsaicinoids [1]. Capsaicinoids accumulate mainly in the epidermis cells of the placental of chili bell pepper [2]. The amount of these compounds in chili peppers varies due to the influence of factors such as variety (habanero, piquin, tree, cayenne, serrano, jalapeno, etc.), climate, altitude, abiotic stress, and fruit ripening [3-5]. Capsaicinoids have aroused great interest because they exhibit exceptional health benefits and provide flavor and aroma to foods. Capsaicinoids could be used as additives and dyes in the food and pharmaceutical industry [6]. It has been proven that these compounds have an analgesic [7], anti-inflammatory [8], anti-cancerogenic [9], obesity preventative [10], and they improve insulin resistance [11] effects. Due to these properties, obtaining and purifying capsaicinoids from *Capsicum* spp is a topic of interest.

The extraction of capsaicinoids is performed mainly by maceration and agitation [12], using organic solvents such as acetone, hexane, methanol, and ethanol, among others. However, it is often time-consuming, costly, and environmentally unresponsible due to the relatively large volumes of solvents generating waste [13]. Novel extraction methods have been developed to make the process more efficient. Technologies such as microwaves [14], low- and high-power ultrasound [15], and supercritical fluids [16] have been applied to improve the extraction efficiency and yield of these compounds, since various investigations report that when extracting these compounds by conventional

techniques, such as maceration, they use very long extraction periods. For example, [17] reports a higher content of capsaicinoids obtained by maceration, however, they use very long extraction periods of up to 7 days; compared to the aforementioned technologies, they do it in minutes.

Capsaicinoid compounds are also quantified in a variety of ways. The Wilbur Scoville's method has been used to determine the pungency of chili peppers by a panel of trained tasters [18]. However, this test is non-specific and requires a group of people with extensive experience in sensory analysis. Analytical techniques based mainly on chromatographic analysis have been implemented to solve some of the disadvantages of sensory analysis. Chili capsaicinoids are quantified and subsequently related to the pungency of the vegetable [19].

This review aims to integrate the various extraction and quantification methods of capsaicinoids in different chili peppers.

2. Methodology

To prepare the review article, an exhaustive literature review was carried out in order to enrich the manuscript and gain-in depth understanding of the topic. The search for articles was carried out in Scopus and Web of Science, using keywords related to the research topic, such as extractions, quantification, and applications of capsaicinoids. The relevance of the articles was evaluated and whether they were related to the research topic by reading the summary, having a set of 242 articles that were used and that were considered important for the objective of the work. These articles were selected to give a structure based on the introduction, extraction, quantification and applications of capsaicinoids, focusing on extraction and quantification. In conclusion, the review provided relevant information on extraction methods, both traditional and emerging, and the quantification of capsaicinoids; as well as applications in industry.

3. Capsaicinoids

3.1 Location and composition

Capsaicinoids are secondary metabolites of the phenylpropanoid group of alkaloids [20, 21]. They are composed of vanillylamine (4-Hydroxy-3-methoxybenzylamine), acid amides, and fatty acids. The capsaicinoids present in most chili peppers are capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin like shown in figure 1.

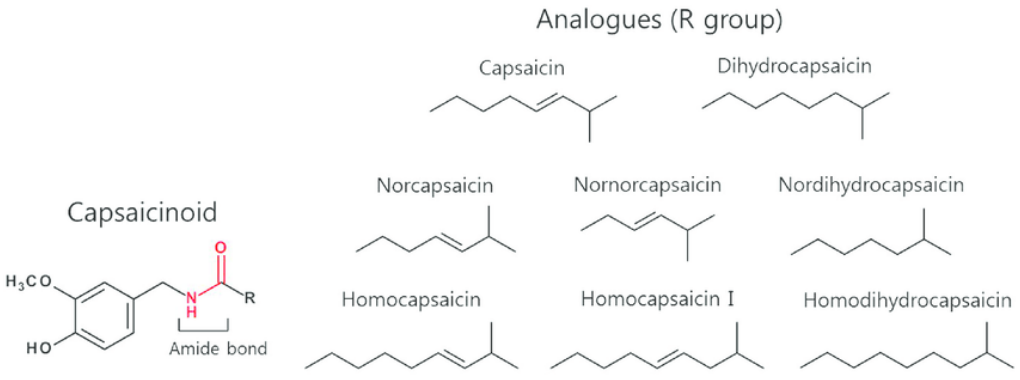


Figure 1. Chemical structure of the main capsaicinoids [22].

It has been reported that capsaicinoids accumulate in vacuoles, mainly in placental cells [23]. However, the location in the fruit is not uniform, and they are not only found in the placental tissue. Capsaicinoids can also be located in the pericarp or seeds, although in smaller quantities than the placenta [24].

The primary capsaicinoid, and the most prevalent in most varieties of chili peppers, is capsaicin (C), followed by dihydrocapsaicin (DHC), shown in figure 2. These two compounds account for approximately 90% of the capsaicinoids present in fruits of the genus *Capsicum annuum* and represent 100% of the relative pungency of the fruit. After these, nordihydrocapsaicin (n-DHC), homodihydrocapsaicin (h-DCH), and homocapsaicin (h-C) are found in lower concentrations [25] having a pungency range between 20 to 50% of capsaicin [26]. Nordihydrocapsaicin (7% of capsaicinoids) is an analog of capsaicin that is less irritating and stinging. Homodihydrocapsaicin (2% of capsaicinoids) is highly irritating. Homocapsaicin (3% of capsaicinoids) [27] produces a slight burning in the throat and a slow stinging sensation. However, the latter compounds are not widely described in the literature as capsaicin and dihydrocapsaicin due to their lower presence in chili peppers. In plants, these compounds are present as a defense mechanism against animal attack, such as insects, repelling them and causing an itching sensation [28, 29].

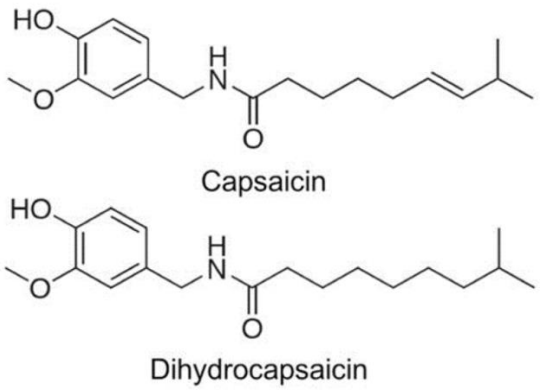


Figure 2. Main capsaicinoid compounds: Capsaicin and dihydrocapsaicin [30].

Capsaicinoids are lipophilic compounds, and although insoluble in water, they are soluble in ethanol, acetone, and oils [31]. Their melting points are between 62 and 65 ºC, and their boiling points occur between 210 and 220 ºC. The study of the functional properties of these compounds has been limited to capsaicin and dihydrocapsaicin, as they are the most common in plants, and because of their anticancer, antimicrobial, anti-inflammatory, analgesic, and gastroprotective properties, among others [32, 33].

4. Applications of Capsaicinoids

Capsaicinoids are of great importance in the food and pharmaceutical industries [6, 34] and some applications are shown in Table 1. Several studies have shown that, in addition to being compounds with high antioxidant power [35], they are also used as topical analgesics for the treatment of diseases that can cause pain, for example, osteoarthritis, postherpetic neuralgia, rheumatoid arthritis, diabetic neuropathy, among others [36-38]. Topical capsaicin formulations are used to control pain and low-concentration creams, patches and lotions have been used for daily use since 1980 [19]. These compounds have been reported to have an antibacterial effect on *Staphylococcus aureus, Salmonella typhimurium, Vibrio cholerae*, *Shigella dysenteriae*, and *E. coli* [6, 39-41]. Therefore, due to its antimicrobial activity, capsaicin has been used as an additive in innovative packaging in biofilms and antifouling coatings [42] to extend the shelf life of minimally processed food products [13, 43]. However, this application is still being investigated. Capsaicin was also found to suppress the growth of malignant cell lines by apoptosis and inhibition of angiogenesis, mainly in human cancers, such as breast, skin, pancreatic [44], stomach, and colon [45, 46].

Table 1. Application of capsaicinoids in the pharmaceutical, food, cosmetics and agriculture industries.

Use	Application	How to use	Administration	Reference
Food	Food flavoring		Chili pepper powder, paprika, sauces	[47]
	Food preservation	Used as the antioxidant and antimicrobial agents in long-term food preservation due to the release behavior of encapsulate capsaicin	Capsaicin microcapsules	[48]
	Functional foods	Capsaicin-enriched noodles to obtain a functional product capable of retaining capsaicin within the noodle, keeping it longer in the matrix and releasing it in the gastrointestinal tract	Chili powder	[49]
	Packaging films	Films applied in the conservation of peppers, delaying the ripening process.	Ethylcellulose-capsaicin biofilms	[50]
Pharmaceutical	Anti-inflammatory activity	It suppresses the production of prostaglandin E2 by inhibiting the enzymatic activity of cyclooxygenase-2 (COX-2). Prostaglandin and COX-2 are proinflammatory mediators in the development of inflammatory diseases.	Topical gels patches	[51]

	Anticancer	Gastric, breast, lung, prostate, colorectal and pancreatic cancer	Investigation work. Prostate cancer stem cells (CSCs) induces apoptosis of cancer cells	[52]
	Analgesic effect	Osteoarthritis, rheumatoid arthritis, post-herpetic neuralgia and diabetic neurophathy	Topical agent Dermal patch	[53]
	Anti-obesity effect	Has been demostrated to inhibit adipogenesis and increase lipid oxidation in adipocytes.	-	[54]
	Antibacterial activity	Evaluation of the antibacterial activity of capsaicin (in vitro) against H. pylori	-	[55]
Industrial	Self-defense spray	Used as raw material for tear gas used for self-defense	-	[56]
Agriculture	Pesticides	Increases plant resistance because it causes an increase in some defense enzyme levels, such as chitinase. Elimination of pests such as insects and mites, especially in organic agriculture.	-	[56]
Cosmetics	Lipsticks	Increased blood flow, due to capsaicin, has the effect of enlarging the lips and manifests itself in slightly swollen, red and toned lips.	-	[57]

	Masks with capsaicin	The use of masks with capsaicin on the scalp generates local irritation and increases blood flow, which leads to the activation of cell renewal, affecting skin regeneration, as well as hair growth.	-	[58]
	Antioxidant	Reduces the intensity of lipid peroxidation processes, delaying the oxidation process of lipids, extending the shelf life of cosmetics	-	[59]

5. Capsaicinoid Compound Extraction Methods

Capsaicinoids, particularly capsaicin, and dihydrocapsaicin, have aroused great interest because of their linked functional properties in relation to health and nutrition. Therefore, the obtaining, purifying, and quantification of these molecules has motivated numerous studies. The most studied methods for extractions of capsaicinoids from plant tissues have been reflux heating [60], enzymatic extraction [61], magnetic stir extraction (MSE) [62], maceration [63], soxhlet extraction [64], ultrasonic-assisted extraction (UAE) [65], supercritical fluid extraction (SFE) [66], liquid-liquid extraction (LLE) [67], solid phase microextraction (SPME) [68], pressurized liquids extraction (PLE) [69] and microwave-assisted extraction (MAE) [70].

It is well known that the yield, quantity, and types of capsaicinoids obtained from the chili pepper plant tissues are influenced by the variety [5], the selected part of the fruit (pericarp, seed, placenta, or septa), the extraction method [71], the extraction method [72], the solvent used [73], the sample preparation (fresh or dried) [73], and the particle size of the crushed material [74] among others. Consequently, the extracts obtained will have different organic compounds, such as pigment, aromatic compounds, capsaicinoids, etc. The type of solvent used is perhaps the most crucial factor in achieving greater efficiency in capsaicinoids extraction [75]. Many solvents have been used for this process, including methanol, ethanol, propanol, ethyl acetate, acetone, n-butyl chloride, dichloromethane, acetonitrile, hexane, and combinations thereof [76, 77].

Table 2 lists the methods commonly used for the extraction of capsaicinoid compounds, the conditions used, and the average yield obtained. It can be observed that ultrasound-assisted extraction (UAE) and microwaves are the techniques that have been most studied for the recovery of capsaicinoids. The UAE has aroused great interest as an extraction method mainly because it is considered environmentally friendly. UAE is characterized by low temperatures, low solvent consumption, and, in general, short extraction periods [78, 79]. In addition, it is known that ultrasonic waves help to break down the cell wall of the sample, improving mass transfer through cavitation and facilitating higher extraction yields. In addition, small sample quantities can be used, and the process is reproducible [80].

Microwave-assisted extraction (MAE) is a technique that by radiating samples with microwaves, the solvent and, consequently, the sample are heated, allowing an accelerated rupture of the cell membrane of the plant tissues and, with this, generating pores and promoting greater mass transfer [62, 81]. Similarly, to the UAE, MAE can be carried out in short processing times (5-10 min) [82]; however, MAE can be used if high pressures and extraction temperatures are required, which is not possible in the UAE.

A conventional technique, Soxhlet extraction [73], is commonly used in laboratories; however, compared to the UAE and MAE, it has some disadvantages since large amounts of solvents are used and a significant residue is produced. In addition, long periods of time and large sample amounts are required to carry out the process. Because of these drawbacks, UAE and MAE have been trending in the distinguished methods of obtaining capsaicinoids. Nevertheless, Soxhlet extraction is used as a control to compare the efficiency of new extraction methods.

Table 2 shows, in general, the higher yields of capsaicinoids obtained by extraction with UAE and MAE compared to other procedures.

Also, other less frequently used techniques are listed in Table 2. One of them is enzymatic extraction [83]. The enzymatic treatment of the sample with enzymes generates a minor cell membrane rupture and mass transfer, which, when compared to UAE and MAE, is not as significant. Consequently, it has a lower concentration of the compounds of interest. Another newer technique is solid phase microextraction (SPME) [84]. This solvent-free method is done in a well-defined time, so it does not generate carryovers; however, it is expensive, and small amounts of capsaicinoids are recovered.

Finally, an extraction method should have the following characteristics: short time, high performance, minimally invasive, uses little solvent, does not generate toxic waste, and is environmentally friendly [85].

Table 2. Methods and conditions of extraction of capsaicinoids from different *Capsicum* varieties showing the recovered capsaicinodes and the performance of the process.

Reference	Method	Material	Solvent	Conditions	Recovered Capsaicinoids	Yield
[83]	Enzymatic extraction	Guajillo puya	Ethanol - viscozyme L	50 mL, 1 g, 50 ºC, 7h, shaker 120 rpm	C	0.0029 *
[86]	Magnetic stirring	Cayenne	Ethanol	25 mL, 0.5 g, 25 ºC and 25 min	C DHC n-DHC h-C h-DHC	0.1361** 0.078 ** 0.027** 0.009 ** 0.014 **
	MAE	Cayenne	Ethanol	25 mL, 0.5 g 125 ºC, 5 min and 500W	C DHC n-DHC h-C h-DHC	0.136 ** 0.081 ** 0.027 ** 0.009 ** 0.015 **
[82]	UAE	Cayenne	Methanol	25 mL, 1 g, 50 ºC, 10 min and 360W	C DHC n-DHC h-C h-DHC	0.136 ** 0.081 ** 0.027 ** 0.009 ** 0.015 **
[84]	SPME	Árbol Canica Habanero Jalapeño Piquín Guajillo puya	Destiled water	5 mL, 50 ºC, 8 mg, 30 min, 1200 rpm	C; DHC	1.316; 0.299 * 0.323; 0.705 * 25.46; 4.574 * 0.420; 0.124 * 2.533; 0.393 * 0.512; 0.109 *

	Soxhlet	Árbol Canica Habanero Jalapeño Piquín Guajillo puya	Ethanol	50 mL, 500 mg, 5 h	C; DHC	1.86; 0.749 * 0.709; 0.816 * -; - 0.477; 0.187 * 5.24; 0.70 * 0.782; 0.551 *
[87]	Manual stirring	Cayenne Cayenne rojo Serrano	Ethanol	5 mL, 5 g, 80 ºC, and 4h	C; DHC	4.249 *;4.482 * 0.309 *;0.238 * 0.138 *;0.146 *
[88]	Magnetic stirring	Habanero	Methanol	2 g, 60 ºC and 2 h	C DHC	1.592 * 0.843 *
[89]	UAE	Árbol Canica Habanero Jalapeño Piquín	Ethanol	50 mL, 250 mg, 50 ºC, 25min and 78W	C; DHC	1.244*; 0.823* 1.012 *;1.211* 6.804*;2.736* 0.629 *;0.435* 1.182 *;0.748*
[90]	Supercritical fluid	Biquinho	CO_2	2.5 g, 40 ºC, 20MPa and $2.15x10^{-4}$kg/s	C DHC Capsiato dihidrocapsiato	0.19 * 0.023 * 6.0 * 0.53 *
[91]	Supercritical fluids ultrasonic	Malagueta	CO_2	1 g, 40ºC, 60 min, 360 W, 15 MPa, $1.673x10^{-4}$ kg/s	C DHC n-DHC h-DHC	1.93 * 1.01 * 0.07 * 0.03 *

[92]	MAE	Cumari-do-Pará	Ethanol with NaH_2HPO_4	70% v/v, 0.2 g, 125 ºC, 1 min and 250W	C	0.07mg/mL bs
[93]	Soxhlet	Habanero	Ethanol	100 mL, 2 g, 90 ºC, 5 ciclos de 45 min	C	22 *
	Ultrasonic	Habanero	Ethanol	5 mL, 37 ºC, 15, 30, 45 y 60 min and 40 kHz	C	14.2 – 19.9*
	Shaker	Habanero	Ethanol	30 min	C	8.3 – 16.9*
[94]	UAE	Nagaking	Methanol	10 mL, 50 mg, 70 ºC, 5h	C DHC n-DHC	0.00148 * 0.00038 * $1.7x10^{-5}$ *
			Ethanol		C DHC n-DHC	0.00135 * 0.00037 * $1.6x10^{-5}$ *
			Acetonitryl		C DHC n-DHC	0.00127 * 0.00037 * $1.5x10^{-5}$ *
[95]	Stirring	Habanero rojo	Methanol, ethanol, water (6:2:2; v/v)	10 mL, 0.2 g, 10 min	C	15**; 12*
[75]	Manual stirring	*Capsicum annuum* L.	Ethyl acetate-tween 80	30 mL, 1g, 6h	C	7.27 *
[73]	Soxhlet	Chinda pepper	Water	240 mL, 20 g, 24 h	C	0.22 ppm bs
			95% Ethanol			0.18 ppm bs

			Acetone			0.26 ppm bs
			water: acetone			0.48 ppm bs
[79]	UAE	Cayenne	Methanol	4 mL, 0.5 g, 1h	C DHC n-DHC h-C h-DHC	0.268 ** 0.0302 ** 0.0022 ** 0.0064 ** 0.0028 **
	Supercritical liquids	Habanero	CO_2 and ethanol 20% (co-solvents)	40g, 45 ºC, 1450 psi, 60 min	C DHC	37.09 * 10.17 *

(C) Capsaicina, (DHC) Dihidrocapsaicina, (n-DHC) Nordihidrocapsacina, (h-C) Homocapsaicina, (h-DHC) homodihidrocapsaicina
mg/g bs= dry weight*, mg/g bh= fresh weight**

6. Analytical Methods for Capsaicinoid Quantification

Due to consumer demand for food quality and the increased use of capsaicinoids in pharmaceuticals [97], the quantification of capsaicinoids is required to be more precise and accurate. The most commonly used methods to determine the presence of capsaicinoids are organoleptic, spectrophotometric, and chromatographic analyses [98]. The conventional way to establish the degree of hotness of chili-based products has been an organoleptic test resulting in the Scoville scale, (shown in Table 3) where the degree of pungency is measured as Scoville Heat Units (SHU) [99, 100]. However, the analysis is non-specific, and an organoleptic scale must be used, depending on the sensitivity of the tasters [101]. Thus, it is being replaced by instrumental techniques [102]. The analytical techniques for quantifying capsaicinoids are more reliable, accurate, low-cost, and environmentally responsible [68].

Table 3. Pungency of Capsicum varieties in Scoville heat units [103].

Scoville heat unit (SHU)	Category	Type
>80,000 SHU	Very highly pungent	Habanero 100,000 to 300,000
25,000–70,000	Highly pungent	Chile piquín 30,000-50,000
3,000-25,000	Moderately pungent	Serrano 5,000 to 15,000
700-3,000	Midly pungent	Anaheim 500 to 1,000
0-700	Nonpungent	Pimiento 0

Currently, the preferred analytical process for the determination of capsaicinoids is high-performance liquid chromatography (HPLC) [104]. Due to its theoretical foundations, equipment, and analytical process conditions, this technique is very reliable, precise, and accurate [97]. It is characterized by its high sensitivity and selectivity, in addition to the fact that it does not generate degradation or damage to the analytes contained in the sample [105]. However, using considerable amounts of high-purity organic solvents during the analytical process makes this technique environmentally insensitive and carries relatively high processing costs.

Another way to quantify capsaicinoids is by gas chromatography (GC). However, GC is not widely used in the analysis of these compounds since the sample is subjected to high temperatures to be volatilized, and generally, the analysis results in the destruction of the sample [106]. Thus, GC is an unattractive analysis for capsaicinoid evaluation. However, some research has proven the effectiveness of this technique in determining capsaicin and has considered that it has certain advantages over HPLC [107]; for example, it has greater selectivity (having longer columns), more economical processes, and less waste to the environment.

Table 4 details the different analytical techniques and conditions used for the quantification of capsaicinoids. The quantification of capsaicinoids can also be performed by spectrophotometry [75] ; however, due to its low specificity and reliability, it is not widely used [88]. This may be because during extraction, not only capsaicin is recovered, but also components can be present that interfere with the analytical process, resulting in errors in the analysis. Likewise, some studies have been carried out with nuclear magnetic resonance spectrophotometry (NMR) [108, 109];

however, few studies have used this technique to quantify these molecules. According to [102], NMR has some advantages compared to GC-MS or other analytical techniques; for example, it does not require internal standards similar to the analyte of interest, use of small amounts of sample, analytical speed and non-destructive with the sample. On the other hand, Bora et al. [109] mention that with NMR, more reliable data is obtained from the authentication of the structure of the analytes. According to Woodman et al. [104], The NMR does not need to perform calibration curves since the integration of the signals is proportional to the amount of compound, and the run time can be from 1 to 2 minutes. Unfortunately, this method cannot provide information on the amounts of the different capsaicinoids found in the matrix. In addition to the above, NMR is expensive equipment, and most research centers, academic institutions, or private laboratories cannot access this type of instrument. It is worth mentioning that liquid and gas chromatography are methods that allow the determination of validation parameters, contrary to NMR.

Finally, new quantification technologies such as direct real-time analysis method and time-of-flight mass spectrometry (DART-TOF-MS) are being employed, which are useful for rapid detection, but, contrary to HPLC can underestimate the content of capsaicinoids in the matrix by matrix effects, for example, concentration or affinity of other compounds [110].

Table 4. Methods, Conditions, and Analytical Sensitivity in the Determination of Capsaicinoids.

Method	Conditions	LOD C; DHC	LOQ C; DHC	References
HPLC	Acetic acid at 1% en acetonitrile, C-18 de 150 x 3.9 mm, detector UV 280 nm	-	-	[111]
	Water - acetonitrile 50:50, C-18 150 x 4.6 mm, detector UV at 222 nm, 20ºC	0.09; 0.10*	0.30; 0.36*	[112]
	Methanol – DI water (50-70% v/v), C-18, diode array detector at 280nm, RT	0.5**	1.0**	[88]
	Acetonitrile gradient: formic acid at 0.2%, Ascentis Express RP-Amide 100 x 2.1 mm, detector UV at 254 and 280 nm, 40 ºC	0.09; 0.10*	0.30; 0.36*	[113]
	Acetonitrile, methanol and water (5:3:2), C-18 250 mm x 4.6, detector UV at 229 nm	0.22**	0.34**	[93]
	Acetonitrile at 60% (v/v) in 0.5% (v/v) formic acid 60% isocratic flow, C-18 250 x 4.6 mm, detector UV at 280 nm	-	-	[94]
	Acetonitrile-water (65:35), BDS Hypersil C18 (150 × 4,6 mm), detector UV-1575, 25 ºC at 222 nm	-	-	[104]

	Acidic water (0,03 M o-phosphoric acid) and methanol, H_3PO_4/H_2O: CH_3OH 40:60 (v/v), Luna Omega C18 (250 mm × 4,6 mm), 25 ºC at 228 and 282 nm	0.018 mg/L	0.062 mg/L	[110]
	Acetonitrile and formic acid, C-18 150 X 4.60mm, 25ºC	-	-	[79]
	Water with formic acid at 0.1% (60:40), C-18, diode array detector at 280 nm	-	-	[96]
CG	AT1701 (30 m x 0.25 mm), 160 ºC – 250 ºC with a rate of 20ºC/min, selective thermionic detector at 290ºC, helium at 160ºC.	-	-	[114]
CG-SM	ZB-5M (30 m x 0.32 mm), 40 ºC – 300 ºC with a rate of 10 ºC/min, 5973N Selective Mass Detector. The ionization potential of SM was at 70eV, 350µA ionization current, transfer temperatures 230 and 280 ºC, scan mode from 50 to 550 m/z.	0.014; 0.022**	0.069; 0.108**	[89]
	HP-5MS (30 m x 0.25 mm), 90 ºC – 290 ºC with rate of 5 ºC/min. The ionization potential of SM was 70 eV, scan mode from 50 to 550 m/z.	0.25; 0.30*	0.80; 0.90*	[115]
	HP-5ms (30 m x 250 µm x 0,25 µm), 40 ºC – 300 ºC with rate of 10 ºC/min holding it for 5 min. The ionization potential of MS was 70eV	-	-	[102]
UV-Vis spectrophotometry	λ 280 nm	-	-	[116]

	λ 286 nm	-	-	[95]
	λ 280 nm	-	-	[75]
NMR spectrophotometry	Non-deuterated acetonitrile. Advance III NMR spectrophotometer operated at 500.13 MHz (for ^{1}H). The experimental conditions were: 16 transients obtained at 298 ºK, an acquisition time of 1.586 s and a recycling delay of 20 s. The spectral width was 10,330 Hz (∼20 ppm).	-	-	[104]
	Ultrapure water and methanol (HPLC) (1:1), 25 ºC.	639**	2633**	[102]
	Deuterated chloroform. Advance III NMR spectrophotometer operated at 500 MHz connected to a 5 mm probe at 295 ºK, zg30 conventional pulse program, acquisition mode was DQD with a delay of 6.50 μs, 8 scans, acquisition time 2.94 μs, spectral width of 11029.4 Hz	4.4**	14.8**	[109]
DART-TOF-MS	Needle potential 3000 V, grid potential 350 V, fragmenter potential 175 V, skimmer potential 65 V, autosample speed 1 mm/s, ionization gas temperature 350 ºC.	-	-	[110]

Limit of detection (LOD); Quantification limit (LOQ) μg/g* y μg/mL**
(C) Capsaicin; (DHC) Dihidrocapsaicin

7. Conclusions

Due to the great diversity of applications of capsaicinoids and the need to obtain them fast and environmentally friendly, it is necessary to develop new technologies for their extraction, purification, and quantification. In this review, various techniques of extraction and quantification of capsaicinoids are presented. Methods such as ultrasound and microwave increase the yield in a shorter extraction time than conventional processes such as maceration. In addition, these new extraction technologies have been proven to consume smaller amounts of solvents and require less energy, resulting in less expensive and more environmentally responsible processes.

As for analytical methods, HPLC was found to be reliable, accurate, sensitive, fast procedure that does not damage the sample. Therefore, it is the most recommended technique for quantifying capsaicinoids. However, determining capsaicinoids by other methods such as GC is possible, as it is a reliable, inexpensive technique and produces less waste than HPLC. Likewise, NMR quantification could prove to be reliable, with short periods of analysis and non-destructive with the sample; however, it still requires more research to improve confidence in the results.

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