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CHILI PEPPER AS A NATURAL SOURCE OF CAPSAICIN: DISTRIBUTION, EXTRACTION TECHNOLOGIES, ANALYTICAL METHODS AND FACTORS AFFECTING ITS RECOVERY

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ABSTRACT

Capsaicin is an extremely essential pungent compound present in chili peppers (*Capsicum* spp.). However, the varied concentrations of capsaicin in diverse species of plants, cultivars, tissues, stages of development, growth environments, as well as the diverse methods of extraction have made the process of extraction and quantification of capsaicin quite challenging. This review aimed to provide an integrated overview of chili peppers as sources of capsaicin, with particular emphasis on the factors governing its accumulation, extraction strategies, key extraction parameters, and analytical methods for its determination. The review first examined chili pepper production and diversity in Vietnam, followed by the distribution of capsaicin within fruit and vegetative tissues and the genetic, developmental, and environmental factors influencing its accumulation. It then contrasted the established methods of extraction such as solvent and Soxhlet extraction with the novel methods of extraction such as ultrasonic-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction. Moreover, the influence of solvent and operating conditions on the effectiveness of the extraction process was also considered, together with the chromatography methods such as HPLC, LC-MS and GC-MS, and spectroscopy methods, especially FTIR and NIR for capsaicin and capsaicinoids analysis. The review brings out the issues that should be taken into consideration while choosing appropriate chili materials and extraction process optimization, as well as the ways to ensure accurate capsaicin and capsaicinoid analysis.

Keywords: *Capsicum* spp.; Capsaicin; Capsaicinoids; Chili Peppers; Conventional Extraction; Green Extraction

1 INTRODUCTION

Chili peppers (*Capsicum* spp.) are important fruit crops belonging to the *Solanaceae* family and are cultivated and used worldwide as vegetables, spices, and flavorings. The economic significance of chili peppers is associated with their considerable diversity in color, size, shape, pungency, and chemical composition, as well as their adaptability to diverse agroecological conditions, ranging from humid tropics to deserts and temperate regions [1, 2]. The genus *Capsicum* comprises numerous species, five of which are widely cultivated, including *Capsicum annuum*, *Capsicum chinense*, *Capsicum baccatum*, *Capsicum frutescens*, and *Capsicum pubescens*. *C. annuum* is recognized as the most economically significant species and consists of both hot and non-hot pepper varieties [3]. Chili peppers also provide an important source of income for farmers [1].

The distinctive heat of chili peppers is attributable to capsaicinoids, a group of specialized metabolites characteristic of pungent *Capsicum* species [4, 5]. Among the identified capsaicinoids, capsaicin and dihydrocapsaicin are the predominant compounds, together accounting for the majority of total capsaicinoids in pungent peppers [2, 4-6]. Capsaicin has attracted considerable attention because of its sensory properties and reported biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects [2, 5]. Consequently, chili peppers represent a valuable natural source of capsaicin with potential applications in food, health-related products, and other value-added products.

However, capsaicin content and extraction efficiency are influenced by several factors, including *Capsicum* species and cultivar, tissue type, developmental stage, environmental conditions, and extraction method. Although conventional solvent extraction remains widely used because of its simplicity and accessibility, its limitations, including long extraction times and high solvent consumption, have encouraged the development of more efficient techniques. Reliable analytical methods are also essential for the characterization and quantification of capsaicinoids. Accordingly, this review provides an overview of chili peppers as a natural source of capsaicin, focusing on capsaicinoid distribution and accumulation, conventional and emerging green extraction technologies, analytical methods for capsaicinoid characterization and quantification, and key parameters governing extraction efficiency. Relevant literature was identified through searches of major scientific databases using combinations of keywords related to *Capsicum*, capsaicin, capsaicinoids, extraction, and analytical methods, with a primary focus on publications from 2001 to 2025. Peer-reviewed studies relevant to the scope of the review were included, whereas unrelated studies were excluded.

2 OVERVIEW OF CHILI PEPPER PRODUCTION AND DIVERSITY IN VIETNAM

Chilies belong to the list of economically valuable crops that are cultivated in Vietnam, both for internal consumption and for exportation. Within the group of the most frequently grown chilies, *Capsicum annuum* L. stands out due to its versatility and variety in fruit properties. Chilies are grown throughout northern, central, and southern Vietnam, with the difference in growing conditions and cultivated chilies being the reason behind differences in the results of cultivation. Vietnam is known to be one of the largest chili producer countries in Southeast Asia, which is focused on exportation [1].

Diversity and productivity of cultivated chili peppers in Vietnam have been studied for various production areas. Barchenger *et al.* [1] studied chile pepper entries in Hanoi, Vietnam and Nay Pyi Taw, Myanmar for two growing seasons. The results showed the differences in fruits' properties and yield among studied entries. In Hanoi, the highest-yielding entries varied from year to year; AVPP1324 yielded 15.3 $t.ha^{-1}$ in 2016-2017 season and AVPP1245 yielded 17.4 $t.ha^{-1}$ in 2018-2019 season. This research also showed the necessity of breeding cultivars adapted to the local production area and preferred by farmers and consumers. As one can see from this research, it is important to evaluate varieties' performance in particular production conditions rather than assume that a variety will perform equally well at various locations or years [1].

The evidence for the presence of varietal diversity can also be found from central Vietnam. The research done by Tran *et al.* [7] in Quang Nam Province examined six different hot pepper varieties of *C. annuum* L., including TN138, TN720, TN185, FAM333, TN738, and TN403, on the basis of their agrobiological features, productivity, and economic benefits. Out of the tested varieties, TN185 and FAM333 showed satisfactory development and gave yields of 45.27 and 43.27 t/ha, correspondingly. These varieties' economic benefits also proved their possibility to cultivate under favorable intensive farming. Replacement of some existing varieties by these prospective ones was recommended by the authors [7].

While these experiments yield helpful data regarding varietal adaptation and productivity levels, such traits are not enough to indicate the suitability of chili peppers for capsicum isolation. Varieties with high productivity may not necessarily contain high capsaicin concentrations, and the studies described above do not establish a direct relationship between yield and capsaicin content. Therefore, selecting promising raw materials for capsaicin extraction requires

consideration of both agronomic performance and the chemical characteristics of the harvested fruits. Further evaluation of capsaicin concentrations in locally cultivated varieties would help identify suitable raw materials and connect Vietnam's chili pepper production diversity with the development of efficient capsaicin recovery processes.

3 CAPSAICIN IN CHILI PEPPER PLANTS

3.1 Chili pepper as a natural source for capsaicin

Capsaicin is naturally found in chili peppers, which are the primary natural source of capsaicinoids. The wide variety of species and cultivars of the genus *Capsicum*, along with variations in cultivation conditions and plant physiology, results in substantial variation in capsaicin content among chili pepper products. Hence, studying chili peppers as a source of capsaicin involves examining their production and the localization of capsaicin in plant organs.

Capsaicinoid biosynthesis exhibits high tissue specificity, with the placenta considered the primary site of synthesis. The placenta is rich in enzymes and genes involved in the phenylpropanoid and branched-chain fatty acid biosynthetic pathways, resulting in capsaicinoid production [3, 6]. Beyond the placenta, capsaicinoids have been detected in other plant tissues, reflecting their uneven distribution throughout the plant [4, 8].

3.2 Distribution of capsaicin within chili's fruit tissues

Capsaicinoids are mainly synthesized and accumulated in the fruit tissues of *Capsicum*, with the placenta and interlocular septa described as the main sites of synthesis. Glands are present in these tissues; however, capsaicinoids primarily accumulate in the vacuoles of placental epidermal cells [2, 9, 10]. Accordingly, the placenta has a higher capsaicinoid content than other fruit tissues [4, 10].

Although the placenta represents the primary site of capsaicinoid accumulation, capsaicinoids can also be detected in the pericarp and seeds [2, 9]. However, their presence in these tissues should not be interpreted directly as evidence of local biosynthesis. In most genotypes, capsaicinoids detected in the pericarp and seeds have been associated with transport, diffusion, or adhesion from adjacent placental tissues [2, 4, 10]. Yet, some highly pungent *Capsicum* species have shown evidence of capsaicinoid biosynthesis in the pericarp, which has been associated with the presence of placenta-like structures [3]. Thus, evidence for capsaicinoid presence in a tissue should be distinguished from evidence demonstrating its capacity for biosynthesis.

Quantitative studies illustrate these differences. In ripe fruits of *Capsicum annuum* cv. Punjab Lal, Pandhair and Sharma [10] reported capsaicin concentrations of 63.96, 7.12, and 5.06 mg/g dry weight in the placenta, pericarp, and seeds, respectively. In another study of *C. annuum* cultivars, Buczkowska *et al.* [9] reported total capsaicinoid concentrations of 262 $mg.kg^{-1}$ in Bronowicka Ostra and 175 $mg.kg^{-1}$ in Orkan, with a reported placental concentration of 401 $mg.kg^{-1}$. Because these studies report different analytical endpoints in distinct materials, the values should not be directly compared without harmonizing the analyte definition and concentration basis.

From a practical perspective, the pericarp still remains important in the recovery of capsaicinoids since it forms a significant part of the total mass of fruits although having low capsaicinoid contents compared to the placenta. Therefore, the selection of fruit tissues for extraction should consider both capsaicinoid concentration and tissue biomass.

3.3 Distribution in chili's vegetative and other tissues

However, even though capsaicinoids are produced and accumulated mainly in the fruits of chillies, capsaicinoids have been detected in non-reproductive tissues, namely leaves and stems [4, 11]. However, capsaicinoids are present at significantly lower amounts in vegetative tissues when compared to fruits, which act as the main source of capsaicinoids in plants [4]. Detection of capsaicinoids in vegetative tissues suggests that capsaicinoids are not limited only to harvested fruits, yet their concentration is variable in different plant tissues.

The distribution of capsaicinoids within vegetative organs is uneven. Estrada *et al.* [4] reported higher capsaicinoid contents in leaves than in stems, as well as greater accumulation in apical tissues than in basal tissues. It is evident from the above results that the amount of capsaicinoids varies not only from one type of plant organ to another but also from one tissue to another in the same organ. Also, it is possible that the composition of the capsaicinoids in the vegetative tissues is quite different from the composition of the fruits. In some vegetative tissues, the amount of dihydrocapsaicin has been detected to be relatively high in comparison with capsaicin [4]. Consequently, the evaluation of capsaicinoids from vegetative tissues should take into account not only their total amounts but also the individual compounds.

From an applied viewpoint, leaves and stems produced during the growth and production of chilies can possibly serve as untapped sources of capsaicinoids and other biological substances. The use of these leaves and stems would help valorize agricultural residues that could be utilized for other purposes. However, a much reduced capsaicinoid content in vegetative parts renders them unsuitable to serve as replacements for the fruits to be used as the source of capsaicin extraction. Their usefulness could, however, be derived from their possible use together with the fruits to form a complementary source of capsaicinoid content as long as it is determined [11].

4 FACTORS AFFECTING CAPSAICIN ACCUMULATION

Capsaicin biosynthesis and accumulation are controlled by interactions between genetic and environmental factors. Differences in capsaicinoid content among *Capsicum* species and cultivars suggest that the genetic background of the plant plays an important role in capsaicin biosynthesis [4-6]. Conversely, differences in pungency within the same species grown under different environmental conditions highlight the influence of the environment on capsaicinoid biosynthesis [4, 12].

4.1 Genetic determinants and developmental dynamics

The genotype of a plant is one of the most important factors determining its capacity for capsaicinoid biosynthesis. Large differences in the level of capsaicinoids have been observed both between species and cultivars of the same species [4-6, 12]. Therefore, the selection of appropriate cultivars is an important initial consideration when chili pepper is intended as a source of capsaicin.

Fruit maturity is another important factor affecting capsaicinoid accumulation. Capsaicinoids begin to accumulate during the early stages of fruit development and generally continue to accumulate until fruit growth ceases, although the timing and level of maximum accumulation may differ among genotypes [2, 4]. Accordingly, capsaicinoid concentrations tend to increase as fruits mature but may decline after reaching a maximum at a particular maturation stage, which varies among varieties [2]. Fruit position on the plant may also influence capsaicinoid concentrations because fruits are exposed to different conditions [4]. Therefore, fruit position and maturation stage should be considered when selecting raw materials for capsaicin extraction.

Developmental measurements also illustrate the importance of harvest timing. For example, a whole-fruit capsaicin concentration of 5.79 $mg.g^{-1}$ dry weight was recorded at 28 days after flowering in *C. annuum* cv. Punjab Lal [10]. This single time-point value should not be generalized as a universal harvest optimum; capsaicin recovery depends on cultivar-specific accumulation patterns and fruit biomass.

4.2 Environmental conditions and abiotic stress factors

Environmental conditions can play an important role in determining capsaicinoid biosynthesis and accumulation. The intensity and quality of light affect both primary and secondary metabolism and can therefore influence the availability of precursors for capsaicinoid biosynthesis [12, 13]. However, the effects of light intensity on capsaicinoid accumulation are not always consistent, as different light conditions may induce different metabolic responses [12]. Temperature also affects capsaicinoid metabolism, although its effects depend on the prevailing growing conditions [5, 12]. These findings indicate that light and temperature should be considered together when evaluating environmental conditions for chili cultivation intended for capsaicin recovery.

Water availability is another important environmental factor. Water stress may stimulate capsaicinoid synthesis as part of the plant's response to unfavorable conditions; nevertheless, severe water stress can adversely affect flowering, fruiting, plant growth, and yield because pepper plants are sensitive to moisture deficits during reproductive stages [5, 14]. Conversely, excessive water availability may also impair plant performance, and the effects of water-related stress on capsaicinoid accumulation can vary with the intensity of the stress [5]. Consequently, an increase in capsaicinoid concentration under stress does not necessarily indicate an improvement in overall capsaicinoid productivity, because changes in compound concentration may coincide with reductions in fruit yield.

Apart from light, temperature, and water availability, soil conditions and mineral nutrition might play an additional role in the biosynthesis of capsaicinoids through altering the metabolic processes of the plant [4-6]. Salinity is another abiotic stress that has been studied in connection with the biosynthesis of capsaicinoids in pungent peppers [8]. However, stress response should not be seen only as a favorable condition for the accumulation of capsaicinoids and metabolism in general. Overall, capsaicinoid accumulation cannot be maximized simply by intensifying environmental stress or optimizing individual cultivation factors independently. Instead, environmental conditions should be managed to support capsaicinoid production while maintaining plant growth and fruit yield.

5 EXTRACTION STRATEGIES FOR CAPSAICIN RECOVERY FROM *CAPSICUM* MATERIALS

Before comparing extraction technologies, it is important to distinguish capsaicin-specific recovery from total capsaicinoid recovery. Capsaicin is the predominant capsaicinoid in many pungent *Capsicum* materials and is therefore a major target of extraction. However, a substantial proportion of the extraction literature reports total capsaicinoids rather than capsaicin alone. Such studies remain relevant for evaluating extraction performance because they provide information on the recovery of the capsaicinoid fraction, but total capsaicinoid recovery should not be interpreted as direct capsaicin recovery. Accordingly, where available, capsaicin-specific results are preferentially considered, while total capsaicinoid data are used as supporting evidence for evaluating extraction technologies. Conventional solvent-based approaches remain widely used because of their simplicity and accessibility, whereas emerging technologies such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) have been developed to improve mass transfer, reduce processing time and solvent consumption, and enhance extract quality [11, 15].

Table 1: Comparison of conventional and emerging extraction technologies for capsaicinoid recovery from *Capsicum* materials

Extraction technology	Relative cost	Advantages	Limitations	Scalability	References
Maceration	Low	Operational simplicity Minimal equipment requirements Flexibility in solvent selection	Prolonged extraction time High solvent consumption Limited extraction selectivity	Compatibility with conventional equipment Straightforward process scale-up	[11, 15-17]
Soxhlet	Low-moderate	Continuous solvent recycling Repeated solvent-sample contact High extraction performance under suitable conditions	Prolonged processing time High energy consumption High solvent demand	Established equipment configuration Limitations in industrial application due to energy and solvent requirements	[11, 17-19]
UAE	Moderate	Enhanced mass transfer Reduced processing time Potential for lower-temperature operation	Specialized equipment requirements Process-dependent extraction performance Scale-dependent ultrasound distribution	Potential for large-scale and continuous processing Requirements for equipment optimization	[17, 20]
MAE	Moderate-high	Rapid internal heating Reduced processing time Potential reduction in energy consumption	Specialized equipment requirements Risk of excessive heating Challenges in achieving uniform energy distribution	Need for scale-up validation Requirements for uniform heating and process control	[11, 18, 21]
SFE	High	Low solvent residue Use of non-toxic and non-flammable CO_2 Ease of CO_2 separation from the extract	High equipment investment High-pressure operating requirements	Feasibility of industrial-scale systems High capital investment and operating requirements	[18, 22, 23]

		Potential for concentrated extracts	Specialized technical requirements		

5.1 Conventional extraction technologies

Conventional extraction largely depends on the interaction between the *Capsicum* material and the selected solvent. Methods such as maceration and hot solid-liquid extraction are commonly used.

5.1.1 Conventional solvent extraction

Solvent selection is crucial since capsaicinoid solubility is determined by physicochemical interactions between the compounds and the solvent. Organic solvents such as ethanol, methanol, acetone, and hexane have been studied for capsaicinoid extraction. Binary solvent systems may provide higher extraction yields than individual solvents because adjusting solvent polarity can enhance interactions between the solvent and capsaicinoids. For example, at the same solid-to-solvent ratio, a 1:1 acetone-water mixture showed a higher capsaicin yield than acetone or 95% ethanol in *Capsicum* samples [16]. This finding illustrates the influence of solvent selection on capsaicin yield under otherwise comparable extraction conditions.

From a practical standpoint, ethanol is particularly suitable for producing extracts intended for food, nutraceutical, and other applications where solvent safety is important. In contrast, solvents such as hexane and other toxic organic solvents can raise concerns regarding residual solvents, occupational exposure, and subsequent solvent removal [15]. Therefore, solvent selection should consider not only extraction efficiency but also the intended application and quality requirements of the final extract.

Conventional solvent extraction is relatively easy to implement and requires limited equipment; however, its major disadvantages include long extraction times, high solvent consumption, and poor selectivity [11]. One study reported that conventional maceration of *C. frutescens* required 15 h, with a maximum reported extraction yield of 79.4% [17]. This result indicates that conventional extraction can achieve substantial yields, although effective extraction may require prolonged processing.

5.2 Soxhlet extraction

Soxhlet extraction technique is a solid-liquid extraction technique that involves the repeated evaporation and condensation of the solvent in the presence of the plant material. The process ensures cycling of solvent, which makes the process of dissolution and diffusion of capsaicinoids from the plant material possible. Cycling of solvent in the presence of the plant material is capable of increasing extraction efficiency in comparison with static solvent extraction techniques [17, 18].

Soxhlet extraction has been applied as a conventional method for capsaicinoid extraction and used as a benchmark for evaluating the performance of other methods [11, 19]. In one example, Boonkird *et al.* [17] extracted capsaicinoids in the form of 92.0% yield from dried *C. frutescens* fruits through Soxhlet extraction using 95% ethanol solvent in a solid/solvent ratio of 1:8 $g.mL^{-1}$ at 78.1 °C after 5 h. It means that the process of Soxhlet extraction could yield high results when applied under appropriate experimental conditions.

However, despite its effectiveness, Soxhlet extraction has several limitations. The process involves lengthy extraction time and usage of solvent in large quantities of solvent leading to high energy and resource use. Hence, though Soxhlet extraction method is relevant in capsaicinoid extraction, fast methods that require lower energy could be more significant [11, 18, 19].

5.3 Green and emerging extraction technologies

In order to address the limitations of conventional methods, emerging techniques have been investigated to improve mass transfer between the plant matrix and the extraction solvent, reduce processing time, and potentially decrease the use of conventional organic solvents [11, 15]. Among the technologies investigated for capsaicinoid recovery, UAE, MAE, and SFE have received particular attention.

5.3.1 Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) makes use of ultrasound waves above the frequency of 20 kHz to enhance the penetrative properties of the solvent inside the plant matrix. The major mechanism involved in UAE is that of acoustic cavitation, which involves the formation and collapse of bubbles in the solvent. This causes shockwaves and microjets which break up the plant cells for extraction of the capsaicinoids into the solvent [17, 20].

A major advantage of UAE is its ability to enhance extraction while reducing processing time and, in some cases, operating temperature compared with conventional methods. The results on the use of UAE on the extraction of capsaicinoids from *C. frutescens* showed that UAE resulted in capsaicinoid recovery of 87.4% when the sample was subjected to 95% ethanol at 1:8 $g.mL^{-1}$ ratio at 45 °C for 3 h [17]. While the result obtained using UAE was relatively low than the 92.0% recovery when Soxhlet was used, UAE had relatively lower extraction time compared to the other method.

Even with these strengths, UAE may need proper process optimization to ensure that the process has a good balance between extraction and resource usage. However, with the less processing time required as well as its ability to run at lower temperatures, UAE is a viable option as compared to other extraction techniques.

5.4 Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) method is a mixture of solvent extraction technique and microwave irradiation technology for increased efficiency of capsaicinoid isolation. This is accomplished by subjecting the samples to microwave heating due to the interaction between the polar molecules and ions in the solvent and biological substance. The increase in temperature and pressure leads to the breaking down of the cell walls and release of the capsaicinoids [11, 18].

A major advantage of MAE is its ability to substantially reduce extraction time. Under optimal conditions, the extraction of capsaicinoids from freshly macerated Cayenne fruits (*C. frutescens*) was carried out with absolute ethanol in the solvent to sample ratio of 0.5 g/25 mL at 125 °C for 5 min [21]. The experiment showed that the capsaicinoids stability remained unaltered during the studied extraction procedure, showing the possibility of MAE use in extraction with short processing times. This becomes especially significant in comparison with classical extraction methods requiring several hours of processing.

Despite its rapid processing, MAE requires careful control to avoid excessive heating that may compromise capsaicinoid stability. The principal challenge of MAE is to exploit rapid internal heating while maintaining capsaicin and overall capsaicinoid stability. Although the reported study demonstrated extraction within 5 min, the extraction recovery was not specified in the provided data; therefore, its quantitative performance cannot be directly compared with that of Soxhlet extraction or UAE based on recovery percentages alone.

5.4.1 Supercritical fluid extraction (SFE)

SFE uses a fluid maintained above its critical temperature and pressure, where it exhibits gas-like diffusivity and viscosity together with liquid-like density and solvating ability. Supercritical CO_2 has been investigated for capsaicinoid extraction because of its low toxicity, non-flammability, chemical inertness, and ease of separation from the extracted product [18, 22, 23].

Following depressurization, supercritical CO_2 returns to the gaseous state and can be separated from the extract, reducing concerns about residual organic solvents [18, 22]. This feature makes SFE an attractive method for obtaining concentrated extracts with minimal residual solvent.

Reported studies demonstrate that SFE can achieve capsaicinoid extraction within short processing periods. For example, extraction of *C. frutescens* using supercritical CO_2 ($scCO_2$) was conducted at 40 °C for 10 minutes, with a reported capsaicinoid yield of 0.252% (w/w) under the investigated pressure and flow conditions [23]. However, this yield should not be directly compared with percentage recovery values reported for other extraction techniques because yield and recovery represent different response variables.

Although SFE offers several advantages, it requires high-pressure systems, making the process more expensive than conventional solvent extraction. Consequently, SFE involves a trade-off between rapid extraction and low solvent residue on the one hand, and high equipment costs on the other.

The data in Table 5.2 highlight the importance of comparing equivalent response measures. Within one study using dried *C. frutescens* and 95% ethanol at a common 1:8 (g/mL) powder-to-solvent ratio, UAE achieved 87.4% capsaicinoid recovery in 3 h, compared with 79.4% after 15 h of maceration and 92.0% after 5 h of Soxhlet extraction [17]. This controlled comparison illustrates a trade-off between treatment time, temperature, and recovery; it does not establish a universal method ranking. MAE and SFE results should be interpreted separately because sample preparation, extraction conditions, and reported endpoints differ across studies [21, 23].

Overall, the reviewed extraction strategies demonstrate distinct advantages and limitations for the recovery of capsaicin and other capsaicinoids from *Capsicum* materials. Solvent extraction and Soxhlet extraction remain practical because of their ease of use and relatively low equipment requirements, whereas UAE and MAE can enhance mass transfer and

reduce extraction time. SFE offers another approach for obtaining concentrated extracts with minimal residual organic solvents; however, its application is limited by equipment requirements and costs. Reported recovery and yield values should not be interpreted directly as evidence of the superiority of one technique over another, because differences in *Capsicum* species, plant tissue, sample preparation, solvent systems, extraction conditions, and analytical response variables can substantially affect the reported outcomes.

Table 2: Reported performance of selected extraction strategies for capsaicinoid recovery from *Capsicum* materials

Method	*Capsicum* material	Operating conditions	Reported response	Key interpretation	References
Maceration	*C. frutescens*, dried fruit	95% ethanol; 1:8 g.mL^{-1}; 45 ^{o}C; 15 h	79.4% recovery	Low equipment demand; long extraction	[17]
Soxhlet	*C. frutescens*, dried fruit	95% ethanol; 1:8 g.mL^{-1}; 78.1 ^{o}C; 5 h	92.0% recovery	Higher recovery; reflux heating	[17]
UAE	*C. frutescens*, dried fruit	95% ethanol; 1:8 g.mL^{-1}; 45 ^{o}C; 3 h	87.4% recovery	Shorter time than maceration	[17]
MAE	*C. frutescens* (Cayenne), fresh crush fruit	Absolute ethanol; 0.5 g/ 25 mL; 125 ^{o}C; 5 min	Not stated	Rapid treatment; high temperature	[21]
SFE	*C. frutescens*, red pepper fruit	Supercritical CO_2; 40 ^{o}C; 10 min	0.252% (w/w) capsaicinoid yield	Rapid treatment; low residual solvent	[23]

Note: Recovery (%) and yield (% w/w) represent different response measures and should not be directly compared. Recovery refers to the proportion of a target compound recovered relative to a defined reference amount, whereas yield (% w/w) expresses a mass-based proportion.

6 KEY FACTORS AFFECTING CAPSAICIN EXTRACTION

Extraction conditions are interdependent: solvent composition, material moisture, and thermal or mechanical treatment jointly influence capsaicin transfer. The following factors are therefore considered in terms of their interactions and the applicability of published conditions to different *Capsicum* materials.

6.1 Solvent-related factors

The choice of an appropriate solvent plays an important role in capsaicin extraction due to its direct impact on capsaicin solubility and solute transfer. As capsaicin is a hydrophobic compound, solvent selection depends largely on solvent polarity. Methanol, ethanol, acetone, ethyl acetate, and acetonitrile have been widely investigated, whereas water alone generally provides poor extraction performance [21]. The effectiveness of a particular solvent can also depend on the physical condition of the raw material. For example, ethanol and acetonitrile have been found to give good results with fresh chilies, while acetone has been reported to be more efficient for dried chilies [24]. This indicates that, in addition to solvent characteristics, the moisture content and physical state of the chili sample should also be considered.

Solvent composition can further influence capsaicin recovery. The addition of a limited amount of water may improve solvent penetration into the plant matrix, whereas excessive water can reduce the solubility of hydrophobic capsaicin [21]. Among the commonly investigated solvents, ethanol is particularly relevant to food applications because of its relatively low toxicity and biodegradability. Ethanol also has a good capacity for absorbing microwaves, making it a potential solvent for microwave-assisted extraction [19].

Solvent performance cannot be separated from the physical state of the sample. Solvents that perform well with dried, milled fruits may behave differently in fresh fruits because intrinsic moisture changes the effective extraction medium [21, 24]. Accordingly, optimization studies should report both solvent composition and sample moisture, rather than treating a nominal ethanol or acetone percentage as universally optimal. Selection for food-related applications must additionally consider residual-solvent requirements and downstream removal [15].

Adding water to an organic solvent can promote matrix wetting and solvent penetration, whereas excessive water may lower the solubility of hydrophobic capsaicin [21]. The preferred solvent mixture is therefore matrix-dependent; an optimum reported for one cultivar and preparation method should be confirmed before transfer to another material.

6.2 Process and operating parameters

Several parameters govern the mass transfer of capsaicin from the chili matrix to the extraction medium. Temperature usually enhances diffusion and solubility, thereby promoting capsaicin extraction; however, high temperatures might increase energy costs and cause the degradation of heat-sensitive compounds [21]. Extraction time is another parameter that affects capsaicin extraction efficiency, with longer extraction times generally improving extraction until equilibrium is reached. Once the extraction process reaches equilibrium, further extraction may provide little additional benefit. Temperature and extraction duration should be evaluated together. Heating can increase dissolution and diffusion rates [21], but prolonged thermal exposure also raises energy demand and may change the chemical profile of a complex extract. A useful optimization endpoint is therefore adequate capsaicin recovery at acceptable processing time and temperature, rather than maximization of either condition independently.

The solid-to-solvent ratio, particle size, and matrix state also affect extraction efficiency. An appropriate solid-to-solvent ratio may help maintain the concentration gradient and promote capsaicin diffusion, but excessive solvent consumption is uneconomical and increases the costs of both extraction and solvent removal [21]. A decrease in particle size increases the solvent-accessible surface area, helping to promote solvent penetration into the plant matrix. Mass transfer can also be improved through matrix disruption using methods such as ultrasound or microwaves [19]. In addition, the moisture content of chili materials can alter solvent composition and capsaicin solubility, contributing to differences in extraction performance between fresh and dried samples [24].

In SFE, pressure and temperature jointly influence supercritical CO2 density and extraction selectivity, so neither parameter should be optimized in isolation [22, 25]. UAE and MAE also require reporting equipment-specific operating conditions, including ultrasonic frequency or power and microwave power, respectively, rather than extraction time alone. Across techniques, the analytical endpoint and mass basis must be stated consistently when recovery and yield are compared.

Overall, capsaicin recovery is governed by interactions among raw materials, solvent properties, and processing conditions, rather than by any single parameter. Technology selection should weigh capsaicin-specific recovery against solvent use, process duration, thermal exposure, capital requirements, and downstream purification. Comparisons across studies remain provisional when different materials, pretreatments, or response metrics are used; the clearest evidence comes from controlled comparisons with a common analytical endpoint [17, 21, 23].

7 ANALYTICAL METHODS FOR CAPSAICIN AND CAPSAICINOID DETERMINATION

Reliable and accurate analytical methods are required to detect and quantify capsaicin in chili pepper samples and their extracts. Analytical techniques can be divided into chromatography- and spectroscopy-based methods, depending on whether the objective is compound identification, quantification, or both. These techniques include high-performance liquid chromatography (HPLC), which is commonly used for the accurate separation and quantification of capsaicin, as well as liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS). In contrast, Fourier-transform infrared (FTIR) and near-infrared (NIR) spectroscopy are used when rapid analysis and quantitative prediction are required. Therefore, the choice of technique depends on the required specificity, sensitivity, and analytical purpose.

7.1 High-performance liquid chromatography (HPLC)

HPLC is one of the most frequently applied techniques for the qualitative and quantitative determination of capsaicin and other capsaicinoids in chilies and their extracts. In comparison with sensory tests, which only evaluate the degree of pungency of a sample, HPLC allows the objective quantification of individual compounds following chromatographic separation [26]. This distinction is important because perceived pungency provides an overall sensory response rather than a direct measurement of capsaicin concentration. In HPLC analysis, capsaicin is separated according to its differential interactions with the stationary and mobile phases, producing a characteristic retention time. Qualitative identification is commonly supported by comparing the retention time and detector response of a sample peak with those of an authentic capsaicin standard [27], while quantitative determination is performed using a calibration curve prepared from known concentrations of the capsaicin standard.

An important advantage of HPLC is its ability to separate capsaicin from other capsaicinoids and determine individual compounds rather than providing only an overall measure of pungency [27]. This is particularly relevant when capsaicin is the target compound, as total capsaicinoid content does not necessarily represent the actual concentration of capsaicin. Ultraviolet (UV) detection is commonly employed because capsaicin contains chromophoric groups that absorb UV radiation [26]. However, HPLC-UV identification does not provide the same level of structural confirmation as mass spectrometry-based methods. More selective detectors, such as fluorescence detectors or mass spectrometers,

can provide additional analytical information when structurally similar compounds show closely related chromatographic behavior [28].

The reliability of HPLC determination depends strongly on the analytical conditions, including column selection, mobile-phase composition, flow rate, detection wavelength, and calibration range. These conditions must be optimized to achieve sufficient resolution and accurate quantitative results. Analytical performance may also be assessed using parameters such as linearity, precision, accuracy, sensitivity, and reproducibility. These validation characteristics are important for ensuring that the reported capsaicin concentrations are reliable and suitable for comparisons among samples or experimental conditions.

In capsaicin extraction research, HPLC therefore serves not only as an analytical tool but also as a means of evaluating extraction performance and selecting suitable processing conditions. By quantifying capsaicin separately from other capsaicinoids, it helps distinguish improvements in capsaicin recovery from changes in total capsaicinoid content. These capabilities support the use of HPLC as a widely used method for the identification and quantification of capsaicin in chili-derived samples [26].

7.2 Mass spectrometry-based methods (LC-MS and GC-MS)

Mass spectrometry-based techniques provide additional molecular information and greater selectivity than conventional HPLC-UV detection. In liquid chromatography–mass spectrometry (LC-MS), compounds separated by liquid chromatography are ionized and detected according to their mass-to-charge ratios, facilitating the identification and discrimination of structurally related capsaicinoids [28]. Combining chromatographic retention behavior with mass spectral information increases confidence in compound identification and enables the detection of both major and minor capsaicinoids [27]. This additional information is particularly valuable when chili extracts contain multiple compounds with similar chromatographic behavior, as retention time alone may not be sufficient to establish compound identity.

LC-MS is therefore useful when analytical objectives extend beyond routine capsaicin quantification to the characterization of more complex capsaicinoid profiles. Its molecular selectivity can support the investigation of individual compounds in chili-derived samples and provide complementary evidence for their identification. However, LC-MS generally requires more expensive instrumentation and greater technical expertise than HPLC-UV [29]. Consequently, its use should be guided by the analytical requirements, particularly when the additional selectivity and molecular information justify the greater instrumental complexity.

Gas chromatography–mass spectrometry (GC-MS) has also been applied to the qualitative and quantitative determination of capsaicinoids. However, their relatively low volatility may require additional sample preparation, particularly derivatization, before GC-MS analysis. In situ derivatization can simplify analysis and improve analyte recovery [30]. Using direct in situ silylation with hexamethyldisilazane (HMDS), Blaško *et al.* [30] demonstrated the GC-MS determination of capsaicin and dihydrocapsaicin without separate extraction and purification steps. Their method enabled sample treatment within 30 min and achieved good recovery and agreement with the standard AOAC method. This approach illustrates how appropriate sample preparation can address analytical limitations and simplify the determination of capsaicinoids.

Overall, LC-MS and GC-MS are valuable when high selectivity and structural confirmation are required. LC-MS is generally more suitable for analyzing non-volatile capsaicinoids directly, whereas GC-MS can be used with appropriate sample preparation. In capsaicin research, these techniques can complement HPLC by providing additional evidence for compound identity and supporting more detailed chemical characterization. The choice of method depends on the required identification level, sensitivity, quantitative performance, sample complexity, and available instrumentation.

7.3 Spectroscopic and rapid analytical methods (FTIR and NIR)

Spectroscopic techniques, particularly FTIR and NIR spectroscopy, have been investigated as rapid, non-destructive methods for the qualitative analysis and quantitative prediction of capsaicin content in chili peppers and their products. Unlike chromatography, these methods do not physically separate capsaicin from other components but rely on spectral data related to the chemical composition of the sample. However, overlapping spectral regions in complex chili matrices can make specific compound identification and quantification difficult, making chemometric analysis important for quantitative determination.

The use of NIR spectroscopy for quality screening is particularly appealing because it requires little sample preparation, does not require organic solvents, and allows non-destructive sample measurement. Quantitative NIR models typically correlate spectral data with capsaicin concentrations determined using reference methods such as HPLC, followed by multivariate analysis, including partial least squares regression (PLSR), to predict capsaicin content in unknown samples [31]. NIR hyperspectral imaging has also been studied for predicting capsaicin and dihydrocapsaicin

concentrations and distinguishing between pungent and non-pungent peppers [32]. Hand-held NIR spectrometers have also been investigated for the rapid determination of capsaicin content and the detection of adulteration in pepper powder [33].

The main advantages of FTIR and NIR include rapid analysis, non-destructive measurement, limited sample preparation, and reduced solvent consumption. However, their quantitative performance depends strongly on the quality and representativeness of the calibration dataset, spectral preprocessing, and the selected chemometric model. Consequently, chromatographic methods such as HPLC and LC-MS remain important reference techniques for establishing and validating spectroscopic prediction models. FTIR and NIR are therefore particularly suitable for rapid screening, quality assessment, and process monitoring, whereas chromatographic methods remain more appropriate for the specific identification and quantitative determination of capsaicin.

Evidence for infrared prediction is method- and matrix-specific. Domínguez-Martínez *et al.* [34] used mid-FTIR with multivariate calibration to predict capsaicin and other chemical attributes in serrano chili and reported calibration coefficients of determination above 0.998 for their optimized models. By contrast, Johnson *et al.* [35] found that fresh-fruit NIR spectra were not suitable for predicting capsaicin and dihydrocapsaicin in their Habanero samples; mid-infrared spectra from dried material provided only modest cross-validated prediction of the capsaicin-to-dihydrocapsaicin ratio (R2cv = 0.45). Other NIR studies have explored capsaicinoid prediction, hyperspectral imaging, and screening of pepper powder [31-33]. These different results caution against assuming that a strong calibration fit guarantees predictive accuracy for new materials.

FTIR and NIR may therefore complement chromatographic assays for rapid sorting, quality screening, and process monitoring, provided that calibration and independent validation demonstrate fitness for purpose. Quantitative studies should state reference measurements, the validation design, and prediction errors, not only calibration R2. HPLC and LC-MS remain more suitable for specific identification and quantification of individual capsaicinoids, especially when selectivity and confirmatory information are required.

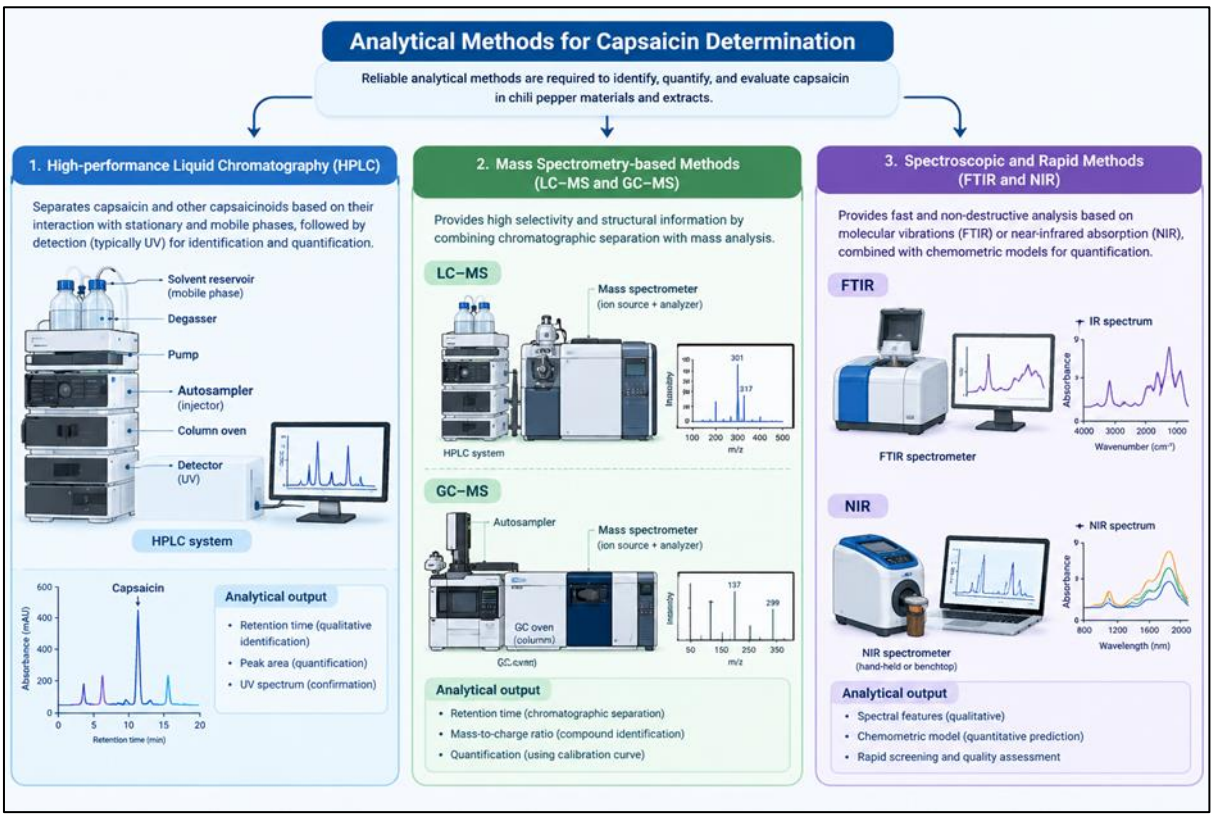

Figure 1: Analytical techniques for the characterization and determination of capsaicin and capsaicinoids

8 CONCLUSION

Capsicum fruit is one of the important natural sources of capsaicin, offering scope for enhancement of its utilization. This paper presents a review of the existing literature on the use of Capsicum as a capsaicin source, linking characteristics of the raw material, technology of capsaicin recovery, and methods of its analysis to give a holistic view

of capsaicin utilization, especially in Vietnam. Much has been done regarding extraction technology and analysis, but more remains to be done before these results can be applied successfully in practice. In future, research on capsaicin can focus on assessing the viability of local chili cultivars and less-exploited plant sources, setting similar standards for assessing capsaicin extraction efficiency, and finding the right processes that will strike a balance between product quality, resource usage, and sustainability issues. Future research efforts need to pay particular attention to the scalability of extraction processes, the adoption of safer and sustainable solvents and analytical techniques for evaluating product quality, among others. Future research on capsaicin should not only focus on extraction efficiency but also process feasibility and application needs. Combination of all of these factors will help to make up the difference between the laboratory level study and implementation. Finally, all these together in an integrated way may lead towards the valorization of the materials derived from the chili and can act as a basis for future capsaicin based products.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

Author contributions

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- Phuong Thi Hai Le: literature collection, proofreading
- Quang Minh Tran: literature collection, proofreading
- Duyen Thi My Nguyen: manuscript writing (review and editing)
- Anh Ngoc Nguyen: manuscript writing (review and editing), revision
- Phuc Nguyen Thien Le: manuscript structuring, revision
- Phu Hong Le: conceptualization, critical revision, administrative support

All authors read and approved the final manuscript

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