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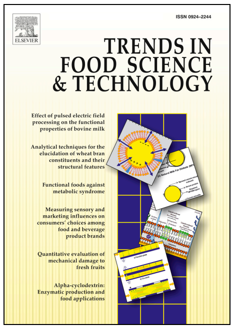

Up-to-date strategies and future trends towards the extraction and purification of Capsaicin: A comprehensive review

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PII: S0924-2244(22)00099-1

DOI: https://doi.org/10.1016/j.tifs.2022.03.014

Reference: TIFS 3751

To appear in: *Trends in Food Science & Technology*

Received Date: 29 July 2021

Revised Date: 11 March 2022

Accepted Date: 12 March 2022

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Abstract

Background: According to the current need of manufacturing healthier products, food companies are seeking specific biomolecules that may offer additional added value (i.e., biological activities) to the new food formulations. Capsaicin, as the pungent ingredient of chili peppers, has become so far one of the target biomolecules explored since the 1950s. There is evidence demonstrating that capsaicin exhibits important biological properties in human health including inhibits acid secretion, stimulates alkali and mucus secretion and particularly gastric mucosal blood flow contributing to the prevention and healing of gastric ulcers, thermoregulation, among many other reported bioactivities.

Scope and Approach: However, one of the main bottlenecks deals with the proper protocol of extraction and purification of this compound since most of the conventional methods based on solvent extraction do not provide efficient yield, along with diminished bioactivity of the compounds. Therefore, this review comprehensively elucidates the current strategies proposed by researchers towards the sustainable extraction and purification of capsaicin from its natural source, and comparison over traditional extraction methods. Particular emphasis has been focused on the innovative extraction techniques and the relevant insights over the last five years.

Key findings and conclusion: A detailed discussion is provided on the advantages and drawbacks of the novel techniques, key interactions with target molecules and their effect on the bioactivity of capsaicin. To finalize, according to the findings of this review, the future trends, perspectives, and research gaps are also given.

Keywords

Capsaicin; innovative extraction techniques; green strategies; nutraceuticals.

Up-to-date strategies and future trends towards the extraction and purification of Capsaicin: A comprehensive review

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Nomenclature

ATPE: aqueous two-phase extraction

CO_2: carbon dioxide

DES: Deep eutectic solvents

EAE: enzyme-assisted extraction

HSCCC: high-speed countercurrent chromatography

IL: ionic liquids

ILAE: Ionic liquid-assisted extraction

PLE: pressurized liquid extraction

PHWE: pressurized hot water extraction

MAR: microporous adsorption resin

MSPE: magnetic solid-phase extraction

SAE: shaker-assisted extraction

SFE: supercritical fluid extraction

TAPPIR: tunable aqueous polymer-phase impregnated resins

TLPE: three-liquid-phase extraction

UAE: ultrasound-assisted extraction

1. Introduction

Today, there is a current trend in the usage of bioactive compounds for manufacturing new food and pharmaceutical formulations, along with the improvement of the existing foods (Pateiro et al., 2021; Teixeira et al., 2014). The point of utilizing bioactive compounds concerns the production of healthier food options to the customers. The bioactive compounds, generalized as nutraceuticals, are extra-nutritional elements that typically exist in low quantities in several foods, such as fruits, vegetables, fish, seaweeds, herbs, etc. These biologically active compounds are being intensively explored and studied due to their effects on health (Carunchia et al., 2015). Various chemical molecules are classified within the major category of bioactive compounds, including phenolic compounds, carotenoids, terpenes and terpenoids, nitrogen-containing and organosulfur compounds, and alkaloids (Azmir et al., 2013; Castro-Muñoz et al., 2016; Wallace et al., 2020).

Among the latter category of compounds, we can find the capsaicin, identified as (N-[(4-hydroxy-3-methoxypheny) methyl]-8-methyl-E-6-nonenamide) in IUPAC nomenclature, which is the characteristic ingredient present in chili peppers. In principle, capsaicin is a flavourless, odourless and colourless chemical molecule but displaying a pungent and irritating character when consumed (Al Othman et al., 2011). Capsaicin stands out as the primary compound within the category of capsaicinoids, followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin. Capsaicin and dihydrocapsaicin are estimated to be approximately 90% of the total capsaicinoids contained in the chili pepper (Usman et al., 2014); capsaicinoids are alkaloids (see **Figure 1**) mainly located in the placental tissue. The capsaicin owns a molecular weight of 305.40 g mol^{-1}, and it displays great lipophilicity (fat and oil-soluble) and also alcohol-soluble (De Lourdes Reyes-Escogido et al., 2011).

Figure 1. Chemical structures of capsaicin (top) and dihydrocapsaicin (bottom) (Usman et al., 2014).

The first approach in capsaicin research has been documented in 1949 (Hippenmeier, 1949). Ever since, capsaicin, as the most recognized compound of the chili pepper, has been widely investigated and there is a deep interest in research developments toward its application in various fields (Szolcsányi, 2004). A continuous effort has been devoted to the exploration of capsaicin over the last two decades, as evidenced in **Figure 2**. Apart from the interest of capsaicin as an ingredient and nutraceutical in new food and pharmaceutical formulations, it can also be used as a feedstock for the synthesis of aromas (such as vanillin) via enzymatic treatment (Heuvel et al., 2001). However, the major importance relies on its plenty of biological activities documented by the research community, such as anticancer (Clark & Lee, 2016), mechanosensitive (Drew et al., 2002), antioxidant, anti-iron-binding (Dairam et al., 2008), analgesic (Duarte et al., 2020), anti-inflammatory, antiobesity (Narang et al., 2018), and antimicrobial properties (Molina-Torres et al., 1999), among many others.

Such bioactivity can be potentially affected due to the use of unsuitable extraction and purification protocols while R&D centres require pure ingredients (ca. 99.9%) for the development of new food formulations and more importantly for therapeutic assays. In principle, capsaicin may present instability and be less active when downstream extraction techniques use high temperatures (over 100 °C), extreme pH values and longer extraction times (Si et al., 2014). Additionally, capsaicin undergoes many purification stages (such as crystallization and further recrystallization) to industrially obtain a high purity degree compound (Yang, 2010). Therefore, there is a need of finding more cost-effective extraction and purification techniques to preserve the nutritional, functional and biological properties of capsaicin, together with high extraction yields. In this work, we comprehensively review the latest advances in extracting capsaicin from its natural matrix. Apart from analyzing the most relevant insights in the field, great attention is paid to the innovative strategies proposed by the research community, comparing their advantages and disadvantages with conventional extraction techniques. After reviewing the latest research, the perspectives and research gaps are also stated.

Figure 2. Documented publications related to the research towards capsaicin over the last two decades (until July 15th, 2021; source: Scopus). Keyword: *Capsaicin.*

2. Traditional extraction techniques for capsaicin

Regardless of the type of purpose (extraction, purification, isolation and concentration), most of the methods towards the separation of bioactive compounds (like capsaicinoids) are classified as traditional and innovative techniques (Sagar et al., 2018). The traditional techniques are those that have been employed for a long time and they are based on solvent extraction combined with heat treatment. The classical methods are considered conventional techniques. Soxhlet extraction, hydro-distillation and maceration are, for instance, some of such traditional extraction techniques (Tsakona et al., 2012; Zhang et al., 2018). To date, hexane-based extraction is likely to be the most explored method to recover capsaicin, however, this solvent is harmful and also produces undesired residues, compromising the final product quality. Alternatively, the extraction has been attempted using other less harmful solvents including methanol, ethanol, acetonitrile, and water, in which organic solvents offer a capsaicinoid recovery ranged from 70 to 92% (see **Table S1, supplementary material**), and interestingly, the extraction time is inversely proportional to the polarity of the solvent (Lu et al., 2017).

Boonkird et al. (2008) reported a recovery rate of capsaicin as high as 92% using conventional Soxhlet. Using the same solvent (ethanol), the authors also compared Soxhlet extraction (for 5h) with simple maceration (for 15 h) and ultrasound-assisted extraction (UAE) (for 3 h), exhibiting recovery of 79% and 87%, respectively. UAE offered approximately 10% greater capsaicinoid recovery in comparison with maceration but 5% lower recovery than Soxhlet extraction. In these experiments, Soxhlet extraction had the highest extraction yield since it was performed at the highest temperature (~78°C) and having the greatest concentration gradient comparing with all other extractions. The concentration gradient in Soxhlet extraction is due to the basics of the Soxhlet extraction process, where the extraction is done all the time with fresh solvent, unloaded with substances.

However, UAE has been noted with an enhanced extraction yield when increased the temperature from 30 to 45°C showing a capsaicin recovery of > 95%. In principle, UAE accelerates the swelling and hydration, provoking an enlargement in the pores of the plant cell walls. Thus, an enhanced mass transfer of solutes from the matrix to solvent can be obtained. Also, the authors associated such an enhanced recovery thanks to the disruption of plant cells by microjet after the cavitation bubble collapsed that could potentially promote the rate of solvent penetration into chilli tissue (Toma et al., 2001). Unlike Boonkird's study (Boonkird et al., 2008), Santos et al. (2015) selected dichloromethane in the Soxhlet method to extract the capsaicinoids from Malagueta pepper (*Capsicum frutescens* L.). In preliminary studies, the authors evaluated various solvents with different polarities, as reported in **Table S2**. It was observed that the highest capsaicin yields of Soxhlet were acquired with low polarity solvents, in which dichloromethane showed ca. 92% of total capsaicin recovery. Since the capsaicin is not totally water-soluble, aqueous systems are generally not preferred for the proper extraction of the target capsaicin (Sarma & del Valle, 2020).

An important parameter when extracting capsaicin relies on its solubility into the used solvents, in which an estimation of the solubility may guideline for an optimized and efficient separation. Importantly, the solubility of capsaicin depends on both temperature and solvent polarity; e.g. Yan et al. (2012) estimated the solubility of capsaicin in different organic solvents as follows: *n*-hexane ≈cyclohexane < carbon disulfide < butyl ether< isopropyl ether. These findings were corroborated by solute−solvent intermolecular repulsive interactions and values of mixing Gibbs free energy (ΔG). Thanks to Yan's analysis, it was understood that dissolution of capsaicin in solvents comprises a spontaneous process; it means, lower ΔG values correspond to higher solubility values and thus more favourable dissolution.

Even though solvent extraction provides high extraction yields and total capsaicinoid content depending on the method and operating conditions (Martins et al., 2017), most of the traditional techniques demand large amounts of solvents and long extraction times; which certainly raises the overall extraction cost. This is the main drawback that limits their establishment, while overuse of harmful solvents also complicates meeting the environmental and health guidelines. Therefore, according to the principles of "green principles", there is today a strong need in developing cost-effective processes and feedstocks implying the use of less hazardous materials, and minimal production of wastes (Anastas & Eghbali, 2010). Also, a major challenge concerns improving the traditional extraction and purification processes. The following section outlines the up-to-date strategies and innovative protocols towards the sustainable separation of capsaicin.

3. Latest strategies for the extraction and purification of capsaicin: A *last-five years* outlook

The extraction process is a crucial challenging step with a meaningful impact on the production of bioactive compounds, which are generally present in low concentrations in foods and natural sources. As illustrated in **Figure 3**, most of the protocols used for extraction of bioactive compounds from food matrices imply different pre-treatment, extraction, purification and concentration. Of course, the sequence and strategies will depend on the type of target bioactive and food matrix (which usually contains plentiful compounds). In theory, the 'ideal' extraction method should eliminate undesired compounds while displaying high recovery rates in less time (Camara et al., 2021). The modern extraction methods include microwave, ultrasound and high-pressure extraction, supercritical and subcritical fluid extraction, electrotechnologies such as pulsed electric field, high voltage electric discharge and nanosorbent-based extraction techniques, while the purification techniques can be categorized as physical (fractional distillation, chromatographic techniques) and chemical methods (i.e. chemical reactions) (Favela-González et al., 2020).

Figure 3. Usual strategy used for the extraction of bioactive compounds from food

systems. Inspired by Camara et al. (2021).

Today, the research community has provided interesting breakthroughs utilizing the above-mentioned extraction techniques for the purification and polishing of capsaicin. Here, various operating conditions, supplies, and hybrid extraction processes (as the synergistic combination of more than two techniques) have been strategically implemented. **Table 1** enlists the most recent and innovative techniques and protocols used towards capsaicin purification.

Table 1. Ongoing progress on the extraction and purification of capsaicin applying emerging techniques and protocols.

One of the emerging extraction technologies for the extraction of high-added-value molecules is supercritical fluid extraction (SFE), which utilizes elevated operating pressures and temperatures to reach a critical point, in which the solvent (CO_2) owns the diffusivity properties of a gas and concurrently the solvation power of a liquid (Dias et al., 2021). Afterward, the solvent can be easily removed from the target molecule by depressurization; also, the product displays better stability since there is lower temperature and non-presence of organic solvent (Zougagh et al., 2004). Considering that capsaicin presents good solubility in CO_2 (Knez & Steiner, 1992), SFE is a promising emerging technique for its extraction, e.g., Aguiar et al. (2018) and Aguiar et al. (2020) explored the efficient extraction of capsaicin; they were able to obtain up to 115 mg capsaicinoid/g extract by means of SFE. By performing an economic analysis, they speculated that optimal conditions (at 240 min, 50°C and 15 MPa) could offer an estimated manufacturing cost of about 125.41 USD/kg of extract (Aguiar et al., 2018). Here, Aguiar et al. (2018) provide a more attractive process since offering lower manufacturing costs compared with Rocha-uribe et al. (2014) who estimated approximately 600 USD/kg of extract.

Applying SFE combined with high-speed countercurrent chromatography (HSCCC), Yan et al. (2018) claimed exceptionally extraction yield (of about 93%) for both capsaicin and dihydrocapsaicin. This process performed the extraction with aqueous methanol, followed by crystallization via alkali extraction and acid precipitation. As a polishing step, capsaicin and dihydrocapsaicin were subjected to further purification with HSCCC, in which *n*-hexane–ethyl acetate–methanol-water (1.4:0.6:1:1, v/v/v/v) was selected as a solvent. The obtained capsaicin and dihydrocapsaicin presented a purity degree of 98.3 and 96.6%, respectively. Hamada et al. (2019) developed an online approach integrated by SFE, dilution line and for the capsaicin extraction and measurement. The concentration of the capsaicin varied from 21 to 60 ng/g in various types of bell peppers. According to the authors' findings, the extraction performance was greatly dependent on the pressure since the pressure increment provokes a higher density of the critical fluid (i.e., CO_2) and thus raises solvating powder leading to better extraction yield (Gnayfeed et al., 2001).

Unfortunately, the yield could be lowered at low pressure due to the diminished diffusivity decreases the interaction between the fluid and the matrix (Kwon et al., 2011). Therefore, SFE could potentially benefit from other emerging techniques that may promote mass transfer via convection or diffusion (Stoica et al., 2016). For instance, Santos et al. (2015) achieved to increase (up to 77%) the capsaicin yield in SFE by using ultrasound waves, which substantially improved the SFE separation rate. UAE lies on creating small cavities to deliver energy into the product/solvent mixture facilitating the extraction of target analytes. Sricharoen et al. (2017) were able to extract several biomolecules (including capsaicin) embedded into an oleoresin from hot chili peppers. In principle, chili peppers contained between 614-25,976 mg/kg for capsaicin and 609-22,130 mg/kg for dihydrocapsaicin, of which UAE demonstrated a recovery efficiency ranging from 62 to 92%.

Bajer et al. (2015), in their work, investigated the application of pressurized hot water extraction (PHWE) and thus compared it with conventional Soxhlet. At this time, the authors applied such processes for capsaicin recovery from several varieties of *Capsicum chinenses* and *Capsicum annuum*. Here, the relative efficiency of PHWE was observed at ca. 110% compared with Soxhlet; for example, PHWE was able to extract capsaicin as high as 12,000 µg/g while Soxhlet exhibited 12,000 µg/g, pointing out that the extraction time of PHWE was significantly shorter. It is worth mentioning that PLE with water presents a great potential when the capsaicin extracts are further processed for pepper sprays, in which solvents, like ethanol, are mostly employed.

Very recently, Martins et al. (2017) screened and compared traditional (such as Soxhlet) and emerging extraction methods, such as UAE and shaker-assisted extraction (SAE), for the recovery of capsaicin from habanero chili. According to the experimentation, the total capsaicin content obtained by Soxhlet (using ethanol) demonstrated that the fruit has 2.2% (ca. 22.0 mg/g) of capsaicin, while UEA yielded 90.7% (between 14.2–19.9 mg/g) and SAE 76.1% (between 8.3–16.9 mg/g). In this approach, the highest extraction yield was acquired by the Soxhlet method that was ascribed to high extraction time (225 min), which supports Boonkird's idea, together with handled temperature (90°C) and molecule: solvent ratio fostering the solubility increase of capsaicin. Unfortunately, Soxhlet clearly showed a degradation of the capsaicin in the first 30 min of operation, while the other techniques proved their reliability in product stability. Even though SAE and UEA recovered less capsaicin (between 9.3-23.9 % less), they offered a 99% solvent saving and 86% less time in comparison to conventional solvent extraction. By comparing SAE and UEA, the latter extraction technique apparently gave a higher extraction yield thanks to the acoustic cavitation in the solvent due to the ultrasonic waves. In addition, the waves provoked a mechanical effect that results in enhanced penetration of the solvent into the fruits matrix and thus surface contact among the solid-liquid phase (Zhang et al., 2009). As a suggestion, the authors also pointed out that UAE displays several advantages over the other two techniques, however, the usage for the extraction of bioactive compounds must be selected carefully since the formation of cavity bubbles could raise in temperature (over 500 °C) and pressure (up to 550 atm) (Martins et al., 2017), which could indeed speed up the degradation of thermal-sensitive compounds (Schläfer et al., 2002).

A liquid-liquid fractionation technique, such as aqueous two-phase extraction (ATPE), was proposed by Fan et al. (2017) for the extraction and purification of capsaicin from commercial oleoresin. ATPE uses two incompatible phases, such as polymer-salt, ionic liquid (IL)-salt, or alcohol-salt, for efficient extraction. Here, the two incompatible phases take place when one polymer is enriched on the top phase and the salt (or second polymer) is also enriched but at the bottom side. In general, ATPE acts as a promising method for the separation of biologically active compounds since such a technique possesses several advantages in terms of low cost, low equipment requirements, short extraction time, and uses 70-90% water. Based on this, Fan et al. (2017) explored ATPE for obtaining capsaicin and subsequently purified using adsorption process (with ADS-17 and AB-8 resin). Initially, the ATPE system (using ethylene oxide-propylene oxide copolymer and K_2HPO_4) exhibited ca. 95% extraction yield, while after the adsorption processes, the integrated technique containing ADS-17 resin showed a capsaicin recovery and purity of 83.7% and 50.3%, respectively, meantime AB-8 resin provided slightly higher recovery rate and high purity product (88.0% and 85.1%, respectively). Comparable outcomes (capsaicin purity of 85%) were also documented by Zhao et al. (2015) who employed D101 and SKP-10-4300 resin, while Cienfuegos et al. (2017) purified capsaicin (> 5-fold) from *Capsicum chinense* via ATPE, which was previously extracted by MAE. At the present work, the overall extraction efficiency of 85 % was reported, in which the active compounds proved ≈80% antioxidant activity. In a previous study, Dang et al. (2014) achieved to recover over 98% capsanthin from red pepper (*Capsicum annum* L.) via three-liquid-phase extraction (TLPE), without requiring any adsorption or additional purification step.

As part of current ideas in process intensification, there is a necessity to developing simple and efficient extraction processes with a fewer number of processing steps, and importantly, they should work at all scales (Fernandez Rivas et al., 2020). Attending such a need, Lu & Cui (2019) integrated two techniques, such as ATPE and (MAR), to develop ‘tunable aqueous polymer-phase impregnated resins’ (TAPPIR), which was subsequently implemented into chromatography. At optimal conditions, the aqueous polymer phase impregnated HZ816 resins containing 18.5% (w/w) PEG6000, 15% (w/w) sodium citrate, and 10% (w/w) [Emim] [OAc] (at pH 6.5) yielded 95% capsaicin extraction, which was ultimately purified by SKP-10-4300 resin in chromatography. Basically, the overall system offered a capsaicin recovery and purity of about 85% and 92%, respectively.

Chemists are strongly exploring sustainable alternatives to replace conventional molecular solvents. In this way, ionic liquids (ILs) stand out as a green alternative due to their low toxicity and biodegradability (Welton, 2011). Specific green solvents, such as cholinium (Ch)-based ILs, were explored in ATPE by Santos et al. (2016), who utilized it (together with acetonitrile and water) as an ideal phase to partition capsaicin. The general process followed by Santos et al. (2016) is illustrated in **Figure 4**. It was observed that the acetonitrile phase was preferentially enriched with the capsaicins while the IL captured other metabolites (like phenolic compounds). After the overall extraction process, the extraction efficiency was over 90% with a purification factor of ca. 3.20. Interestingly, it is known that the extraction efficiencies tend to be enhanced by raising the operating temperature when recovering solutes due to the enhanced solubility in liquid phases (Abe et al., 2014; Valencia-Arredondo et al., 2020), nevertheless, Santos et al. (2016) underlined that the extraction efficiency (between 89.7-93.0 %) was not greatly influenced by the temperature, this insight was obtained thanks to the analysis of the effect of the temperature on the capsaicin migration. By calculating thermodynamic parameters (Gibbs energy, enthalpy and entropy), the migration into acetonitrile (using ATPE) was apparently determined as spontaneous (negative Gibbs energy). On the contrary, the analysis suggested that transport of capsaicin into the cholinium (Ch)-based IL behaved as endothermic.

Figure 4. Integrated system applied for the extraction, purification and polishing of capsaicin (Santos et al., 2016).

Ultimately, the latest research refers to the exploration of new adsorbent materials for the purification and polishing of the pre-concentrated capsaicin. For instance, Lu et al. (2020) focused their research on exploring magnetic solid-phase extraction (MSPE) for the separation of capsaicin from gutter oil (cooking oil recovered from food waste). The authors proposed MSPE as a pre-treatment strategy to directly adsorb the target capsaicin while enriching and separating via an external magnetic field. In this work, a nanocomposite based on graphene oxide (GO)–Fe_3O_4 was synthesized by means of the co-precipitation method. The optimal amount of hybrid adsorbent (ca. 40 mg) offered capsaicin extraction recovery > 80%, which was increased by extending the extraction time (up to 100% for 20 min). It is important to point out that such a nanocomposite also exhibited a similar uptake rate for dihydrocapsaicin and N- vanillylnonanamide, in which its reuse is feasible by several times with no significant loss of performance. Such high recovery efficiencies are credited to the GO due to its large adsorption capacity as a result of its high surface area and two-dimensional structure (Cha-Umpong et al., 2020).

Very recently, Genovese et al. (2021a) also assessed nineteen adsorbents (including hydrotalcites, lamellar solids, and phyllosilicates) to pre-concentrate the capsaicin from various cultivars of *Capsicum annuum*. To sum up, hydrotalcite magnesium aluminium azelate and bentonite exhibited the best performing extraction efficiencies, yielding between 73-91% and 68-71%, respectively. Bentonite especially possesses a large surface area and high water uptake properties (Claverie et al., 2018), this latter property was speculated to play an important role since it is quite possible that the transfer of the solvent (water) to the solid phase could have mechanically driven the capsaicin onto the sorbent and thus contributing to the high extraction yield. Regarding hydrotalcite magnesium aluminum azelate, its selectivity towards capsaicin was attributed to Van der Waals and hydrophobic interactions since capsaicinoids present linear 6-8 carbons acyl chains that are able to fit with the linear carbon skeleton of azelaic acid intercalated in the inner structure.

4. Other capsaicinoids and bioactive compounds extracted from chili pepper

Capsaicin and dihydrocapsaicin are the most prominent forms in the chili pepper fruit extracts, accounting for almost 90% of capsaicinoids. Nordihydrocapsaicin (7%), homocapsaicin (1%) and homodihydrocapsaicin (<1%) are always present at very low concentrations when compared to capsaicin and dihydrocapsaicin (De Lourdes Reyes-Escogido et al., 2011). Due to their low concentration, few studies and efforts were made to quantify those capsaicin analogs in chili pepper extracts. For instance, Genovese et al. (2021b) performed solid phase extraction of twenty-two cultivars of chili pepper using solid sorbents. The quantities of extracted capsaicinoids reached 865 mg of capsaicin (76%), 239 mg of dihydrocapsaicin (21%), 17 mg of nordihydrocapsaicin (2%), and 10 mg of homocapsaicine (1%) for the most effective sorbent. Liu et al. (2020), for instance, applied solvent extraction of chili pepper seeds. The nordihydrocapsaicin contents ranged from 43 up to 297 μg/g depending on the type of seed and solvent used for extraction, while total capsaicinoids content range was between 1052 – 3692 μg/g.

A similar group of compounds, named capsinoids, include capsiate, dihydrocapsiate, and nordihydrocapsiate, are also naturally present in chili peppers. Capsinoids have the beneficial properties of capsaicinoids, however, due to their slightly different structure, they do not cause the characteristic of pungency (Hursel & Westerterp-Plantenga, 2010). The research group of Aguiar has worked with different extraction techniques applied to capsinoids recovery from biquinho pepper. They reported the presence of a considerable concentration of capsinoids in extract obtained by SFE at 60 °C and 15 MPa (Aguiar et al., 2014). Capsinoids are less polar than capsaicinoids, due to their ester bond that replaced the amide bond of capsaicinoids, thus supercritical CO_2 was selective for capsinoid compounds – capsiate and dihydrocapsiate. In a subsequent study, the authors pursued the intensification of the extraction process by combining SFE with pressurized liquid extraction (PLE), allowing the production of capsiate-enriched oleoresin from biquinho pepper (Aguiar et al., 2020). An extraction yield of 77% was obtained, together with a reduction 1.39 times of the manufacturing cost. At this point, this finding proves that the smart combination of different techniques makes the process more economically profitable.

Chili peppers are also an excellent source of other phytochemicals, such as anthocyanins, vitamins, phenolic acids, flavonoids, and carotenoids. Various traditional and emerging techniques have been employed for the extraction of phytochemicals from chili peppers, including maceration (Luiza et al., 2020), solvent extraction (Bogusz et al., 2018), ultrasound-assisted extraction (Liu et al., 2020), supercritical fluid extraction (Sricharoen et al., 2017), and pressurized liquid extraction (Aguiar et al., 2020). The conventional methods focus in using organic solvents, such as methanol and ethanol, as the extraction vehicle of phytochemicals by the implementation of solvent extraction.

Nevertheless, various innovative technologies for the extraction of bioactive compounds from chili peppers have been also reported (see **Table 2**).

Table 2. Comparison of different extraction techniques used to recover the phytochemicals other than capsaicinoids.

Although the scientific interest focuses on capsaicinoids extraction and purification, most of the research has focused on characterizing the volatiles ones, since sensory characteristics are crucial factors determining the quality of chili peppers and thus affect consumer acceptance. The volatile fraction of chili peppers typically includes low molecular weight compounds and a class of lipophilic secondary metabolites with high vapor pressure (Sosa-Moguel et al., 2017). A few studies have been conducted to identify volatile compounds present in chili peppers varieties, such as Brazilian chili peppers (Bogusz Junior et al., 2015), Shimatogarashi chili peppers (Manikharda et al., 2018) and traditionally pickled Chinese chili peppers (Ye et al., 2020). Their results revealed a complex chemical composition with a total number of compounds, ranging from 127 up to 184, including esters, alcohols, aldehydes, alkanes, ketones, terpenes, ethers, pyrazine, and sulfur compounds. Esters are usually the major group in the volatile compounds profile of chili peppers in terms of amount and varieties, however, in the study reported by Patel et al. (2016), terpenes were found to be predominant volatiles (a total of 45 out of 127 identified compounds) in Peruvian chili pepper samples.

5. Concluding remarks, perspectives and guidelines for the new researchers

This review has elucidated the ongoing progress (over the last five years) on novel and emerging extraction techniques for the cost-effective purification of capsaicin from their natural-containing matrix (i.e., chili peppers). According to the current findings and other researchers' works (Wen et al., 2020), the combination of multiple extraction techniques (either traditional, emerging and novel) will continue to be a common practice for synergistic purification protocols conducting to an enhanced capsaicin recovery efficiency (Wang et al., 2021). In recent years, interesting yields (from 76 to 95%) and purity degrees (up to 98%) have been documented by the research community using hybrid systems (including SFE, PLE, UAE, ATPE, MAE, MARS, TAPPIR, among others, see **Table 1**). After reviewing the current literature, a general guideline process scheme, which is inspired by the efforts of researchers, can be established, as illustrated in **Figure 5**.

Figure 5. Process guideline for the efficient extraction and purification of capsaicin from chili peppers considering the current efforts of the research community.

This process basically implies three fundamental downstream stages such as pre-treatment, extraction and purification, together with polishing. It is worth pointing out that the current efforts have been focused on emerging technologies for the extraction (Sereshti et al., 2019), and novel materials for the selective purification of capsaicin molecules. To finalize, the perspectives and current research gaps for new researchers in the field are given as follows:

- One of the current research interests deals with the discovery of the biosynthetic pathway of capsaicin and its precursors (Usman et al., 2014). However, such unknown knowledge has not been limited to the research community at exploring alternatives for the capsaicin separation and purification towards its exceptional biological and pharmaceutical properties, along with sensorial features to new food formulations. Importantly, since chili pepper fruits are so far the only source used for obtaining such high-added-value molecules, future research will be certainly devoted to new strategies, including techniques, solvent phases, purification supplies, etc., for the cost-effective purification of capsaicin. To date, it is likely that most of the research has been done to directly extract the capsaicinoids from the chili peppers. However, researchers should extend their efforts on considering the wastes produced from artisanal and industrial processing since crop waste materials (seeds, skin, defective fruits) (Castro-Muñoz et al., 2020) are also a potential source of biomolecules and not limited to capsaicin only.

- *Pre-treatment and extraction*: To date, maceration stands out as the easy-to-handle and typical methodology for the primary extraction and availability of capsaicin from the placental tissue where they are biosynthesized and accumulated (Santos et al., 2016). Apart from typical maceration for obtaining the capsaicin, enzyme-assisted extraction (EAE) could be an alternative as a way to acquire the bounded capsaicin into the cell wall and thus foster enhanced extraction yield. Until now, EAE has been successfully applied in the recovery of aromas (Galiano et al., 2019) and bioactive compounds (Figoli et al., 2006) from natural products but minimally explored in capsaicin recovery. Importantly, such enzymatic treatment can be combined with other extraction techniques, and it is recognized as an environmentally friendly strategy since water is commonly used as a solvent. Here, different enzymes, such as cellulases, pectinases, beta-glucosidases (Cortés-Ferré et al., 2021), must be suitable to hydrolyze the cell structure releasing the target analyte.

- *Polishing of capsaicin as a final stage*: adsorption processes aided with commercial microporous resins, such as SKP-10-4300, ADS-17 and AB-8, are the preferred strategy by the research community. Particular attention should be paid to the new hybrid materials with exceptional adsorption properties, e.g., graphene oxide (GO)-Fe_3O_4 composites have been introduced as an adsorptive material, displaying a capsaicin extraction recovery as high as 98% (Lu et al., 2020). It is worth mentioning that such new hybrid material presented promising reusability since it was used for extracting more than 10 times with unchanged performance. Apart from this, the chemistry should be properly studied to benefit from the large uptake capacities of such new materials. In this regard, further exploration of the physicochemical properties of the sorbents, such as interlayer distances, particle size, the polarity of the inner cavity, surface areas, introduction of selected ions in the crystal lattice, should be performed and extended to possibly tunning for an enhanced extraction. Here, chemical or physical treatments should be adapted emphasizing overall charge, polarity and further interactions, including van der Waals, hydrogen bonding, dipole-dipole forces, and cation-anion interactions (Cartalade & Vernhet, 2006; Galanakis, 2015; Sun & Leung, 2019).
- A non-typical practice from research regards the techno-economic feasibility of the applied emerging techniques. It could be interesting if the authors may provide an estimation of the extraction cost of capsaicin using these novel techniques and

related strategies (Aguiar et al., 2018; Aguiar et al., 2020; Rocha-uribe et al., 2014), this opens the possibility to have a clear and realistic overview about the feasibility of such techniques.

- The use of ILs, as non-volatile and tunable solvents, has been done for the capsaicin extraction (Lau et al., 2015), however, the green analogues of ILs, so-called deep eutectic solvents (DESs) or natural deep eutectic solvents (NADES), can potentially be applied. DESs are currently employed in various applications including extraction of biologically active compounds and pharmaceuticals (Faraz et al., 2021), analytical determinations (Zhang et al., 2012), extraction of heavy metals (Haq et al., 2021), among others. Therefore, a new research gap in the near future will refer to the sustainable extraction of capsaicin using DESs. However, particular attention should be devoted to the polarity of the DESs, which is influenced by the type of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) and their molar ratio (Smith et al., 2014).
- In recent years, membrane-based technologies stand out as an alternative for recovery and concentration high-added values compounds from natural products and their by-products (Castro-Muñoz et al., 2020). Such technologies, implemented with ultrafiltration and nanofiltration membranes, could be an alternative for the concentration of capsaicin once they are available in a liquid system. Considering the molecular weight of the capsaicin (~305,41 g/mol), nanofiltration membranes can easily assist the concentration of these compounds depending on the membrane properties (e.g., polymer type, hydrophilicity/hydrophobicity, pore size, structure, morphology, etc.). It is important

to point out that there is no report so far on processing capsaicin extract via membrane techniques.

Acknowledgments

Financial support from Polish National Agency for Academic Exchange (NAWA) under Ulam Programme (Agreement No. PPN/ULM/2020/1/00005/U/00001) is gratefully acknowledged. R. Castro-Muñoz also acknowledges the School of Engineering and Science and the FEMSA-Biotechnology Center at Tecnológico de Monterrey for their support through the Bioprocess (0020209I13) Focus Group.

Conflict of Interest

The author declares no conflict of interest.

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933

Figure 1. Chemical structures of capsaicin (top) and dihydrocapsaicin (bottom) (Usman et al., 2014).

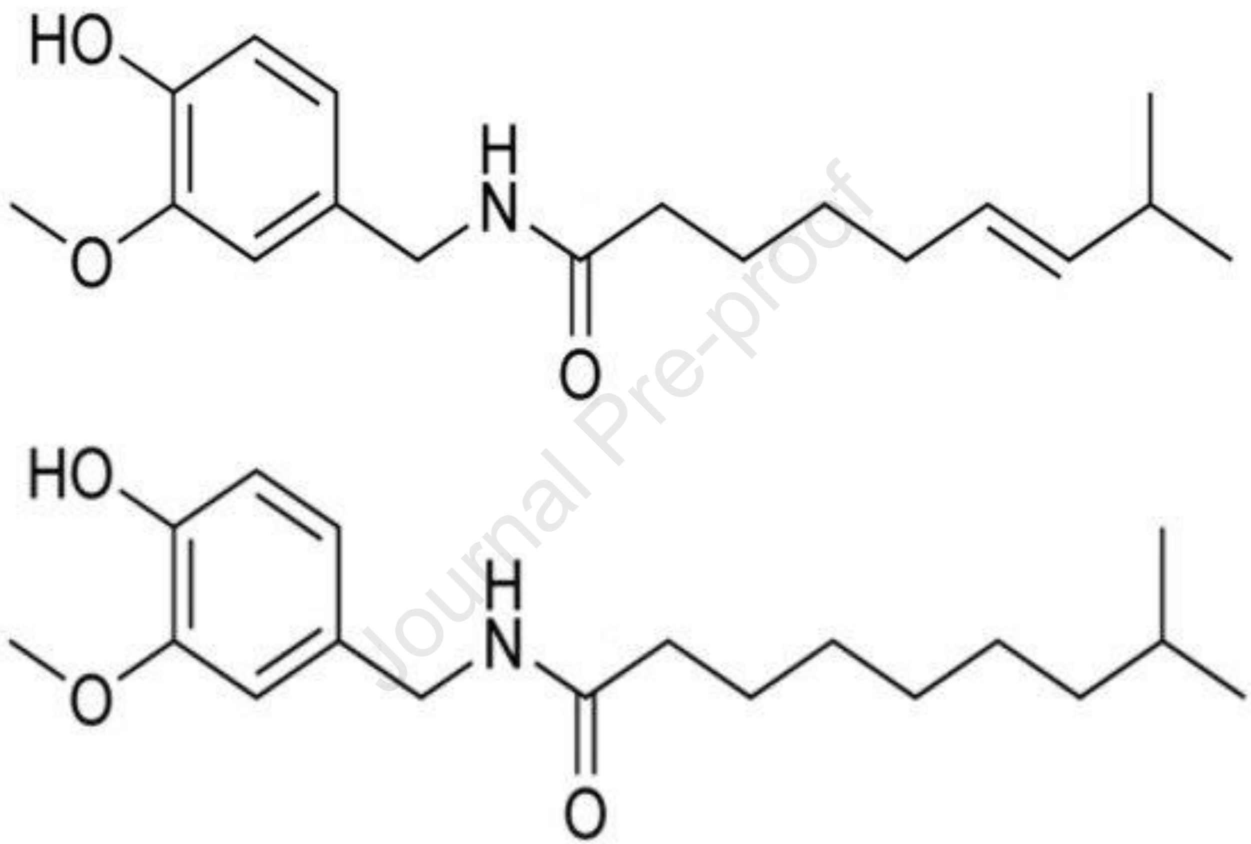

Figure 2. Documented publications related to the research towards capsaicin over the last two decades (until July 15th, 2021; source: Scopus). Keyword: *Capsaicin.*

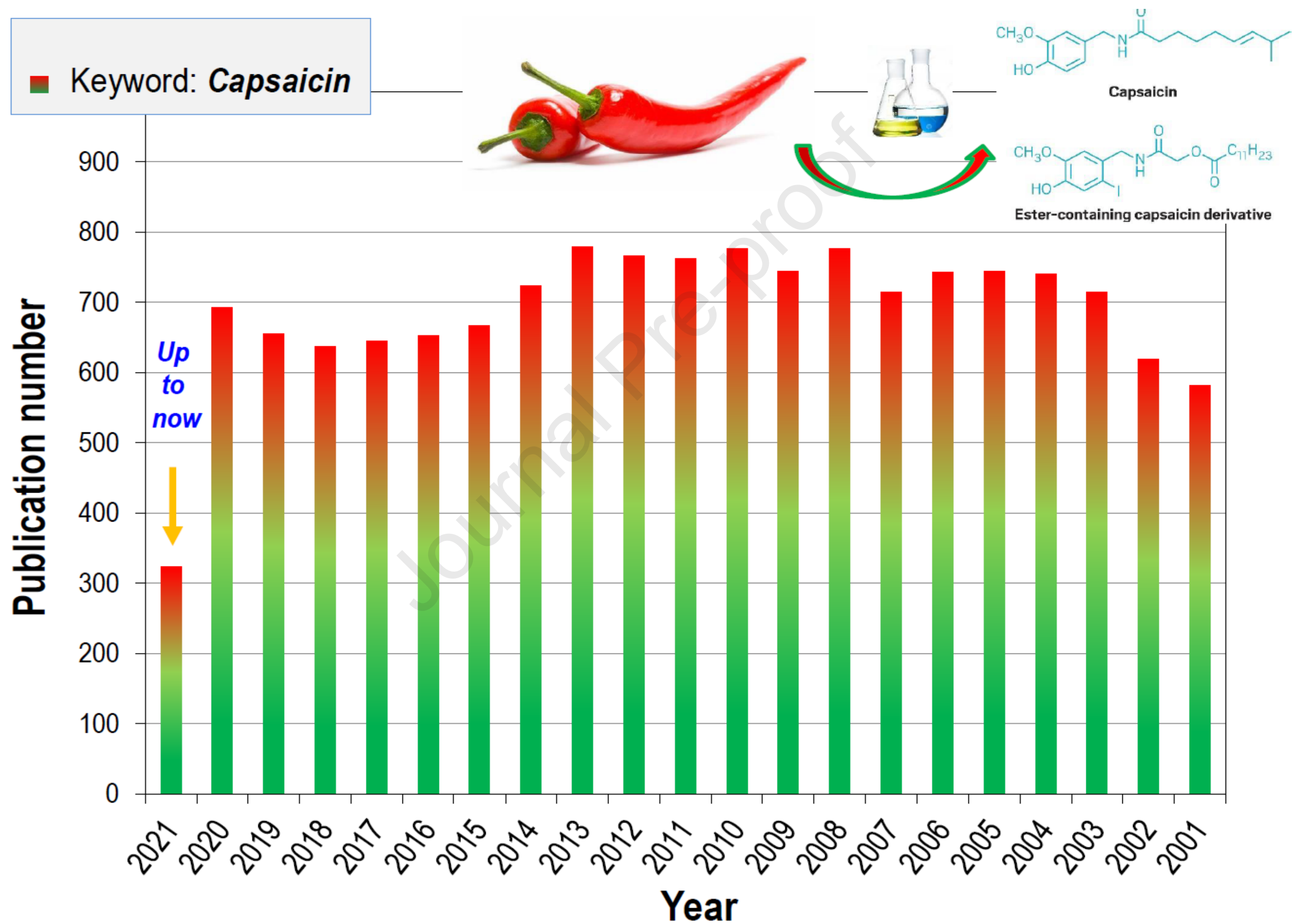

Figure 3. Usual strategies used for the extraction of bioactive compounds from food systems. Inspired by (Camara et al., 2021).

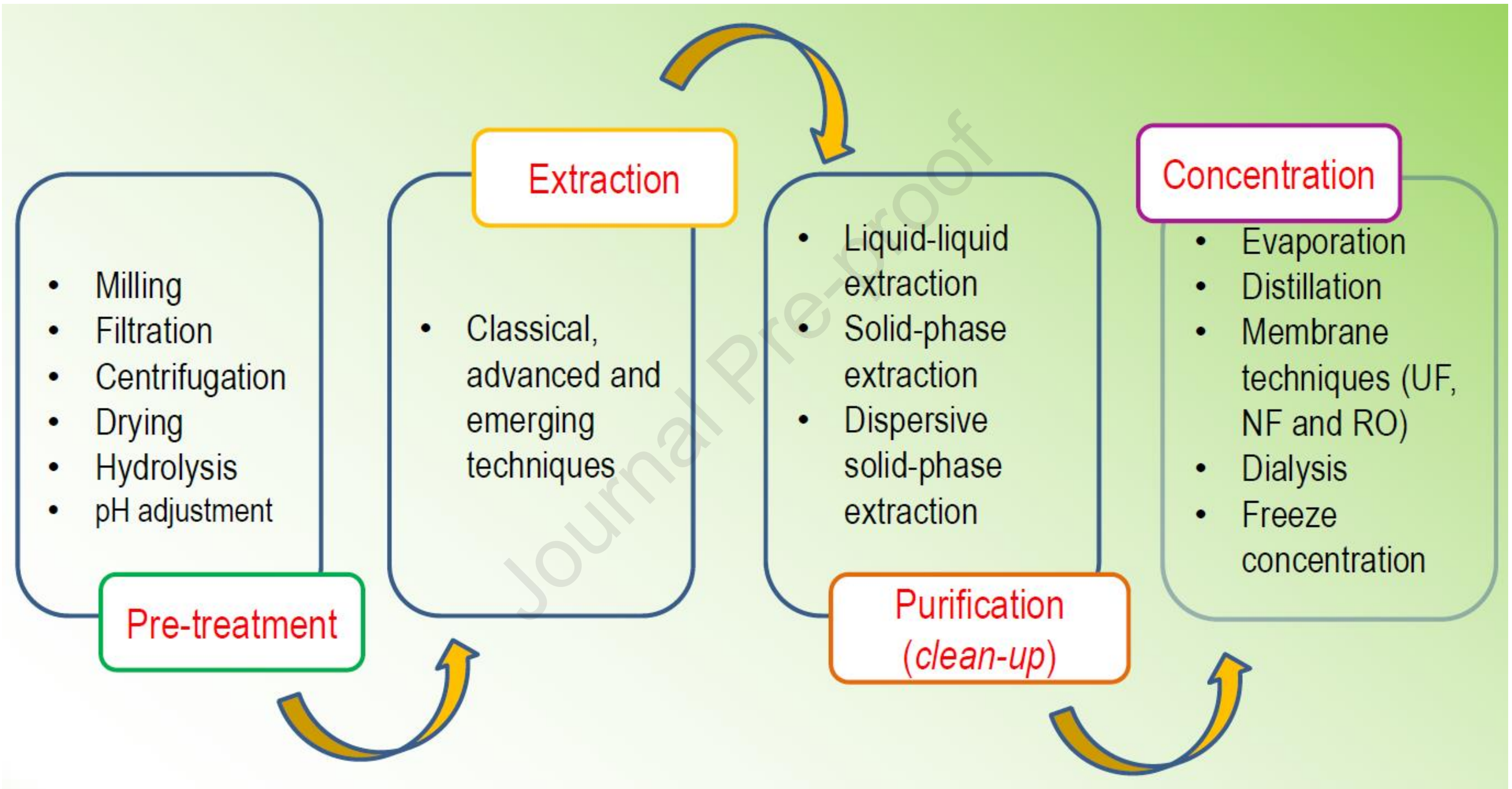

Figure 4. Integrated system applied for the extraction, purification and polishing of capsaicin (Santos et al., 2016).

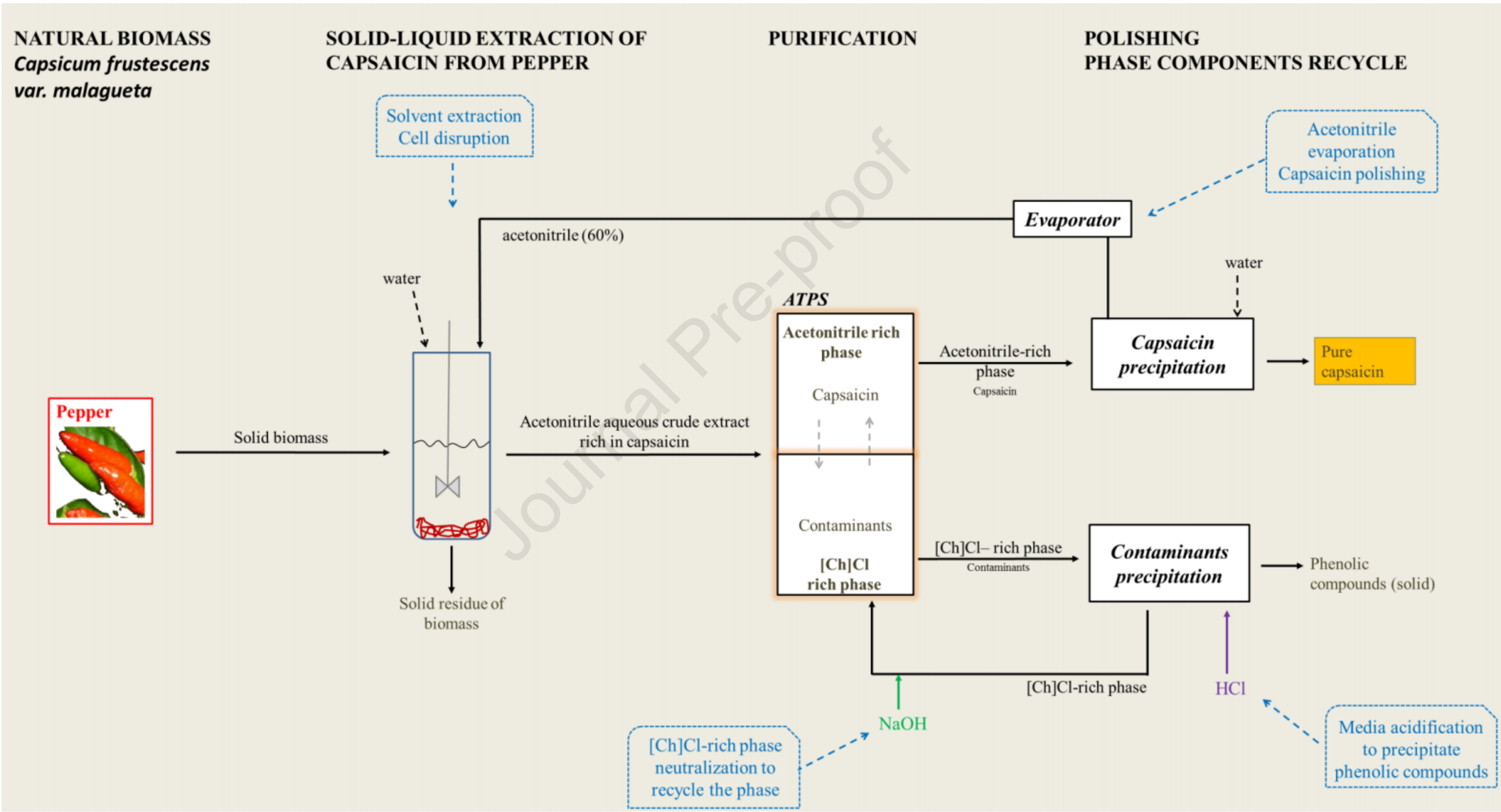

Figure 5. Process guideline for the efficient extraction and purification of capsaicin from chili peppers considering the current efforts of the research community.

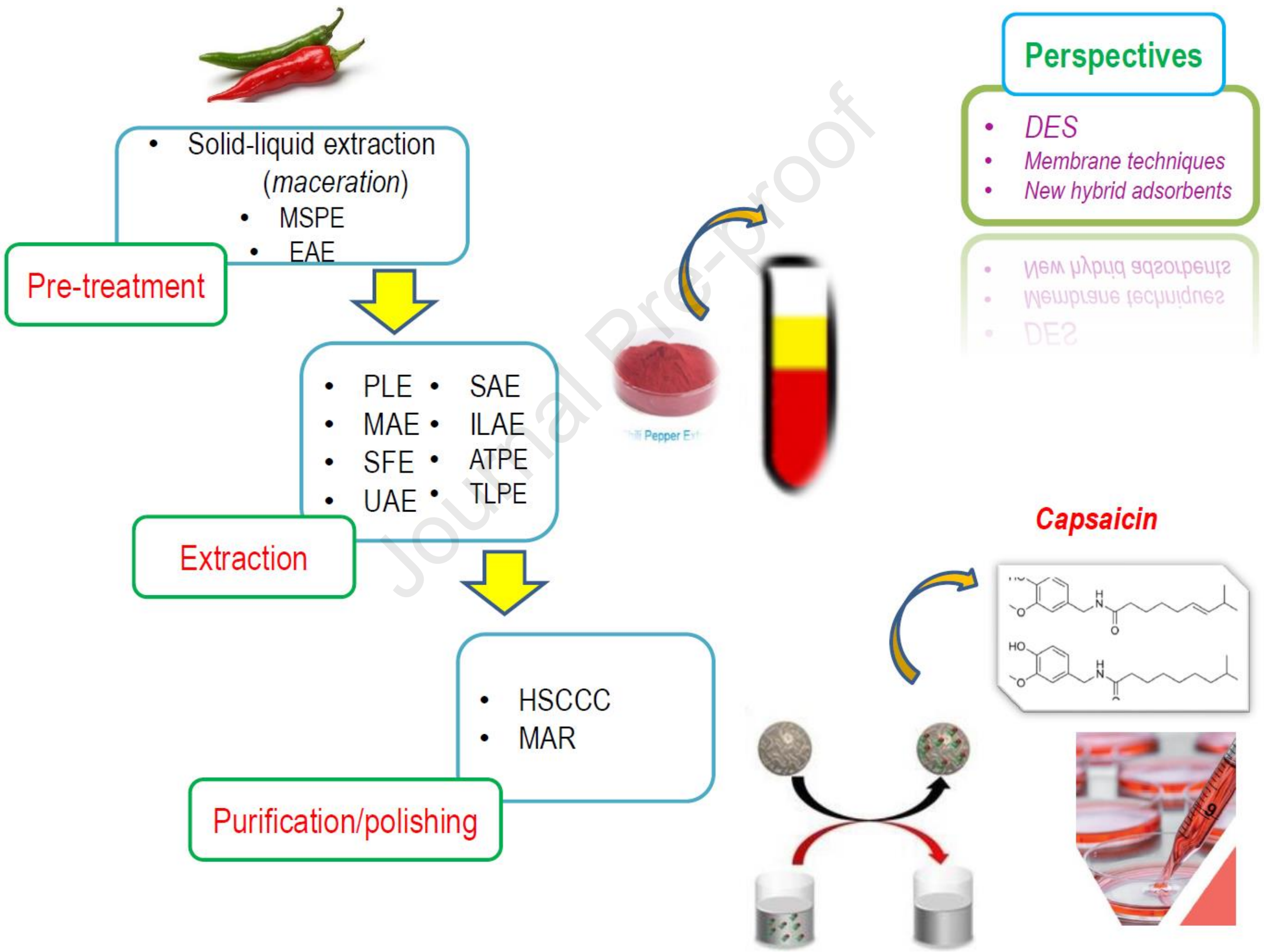

Table 1. Ongoing progress on the extraction and purification of capsaicin applying emerging techniques and protocols.

Technique/ technology	***Source***	***Operating conditions***	***Yield/ purity/ capsaicin recovery***	***Reference***
SFE	Malagueta pepper (*Capsicum frutescens* L.)	40 ◦C, 15 MPa, acetonitrile, acetic acid, water	115mg/g extract	(Aguiar et al., 2018)
SFE	Malagueta pepper (*Capsicum frutescens* L.)	40◦C, 15 MPa	Yield: 76.1%	(Santos et al., 2015)
SFE+UAE	Malagueta pepper (*Capsicum frutescens* L.)	SFE: 40◦C, 15MPa, 150 min UAE: 360 W, 60 min	Yield: 79%	(Santos et al., 2015)
SFE	dedo de moça pepper (*Capsicum baccatum* L. var. *pendulum*)	60◦C, 25 MPa, 120 min	Yield: 88%	(Dias et al., 2016)
SFE	Habanero chili	10 MPa, 35◦C, 90.2 kg/h CO_2 flow rate	Yield: 92%	(Rocha-uribe et al., 2014)
UAE	Habanero chili	60min, 37◦C, hydroethanolic solution, 40 KHz	19mg/g extract	(Martins et al., 2017)
SAE	Habanero chili	Hydroethanolic solution	16.9mg/g extract	(Martins et al., 2017)
UAE	Red Jalapeno pepper (Capsicum annuum L.)	40% amplitude, 40◦C, 400W and 24 kHz, 15 min, olive oil	-	(Civan & Kumcuoglu, 2019)
UAE	Red hot chili pepper powder (*Capsicum annuum* L.)	28.5/31.5 kHz, 20min, olive oil	169.9 mg/kg pepper	(Paduano et al., 2014)
ATPE+MAR	Capsicum oleoresin	Buffer pH: 2.74, sample: 0.35 g capsicum oleoresin, polymer concentration: 20 % (w/w) ethanol, 22.3 % (w/w) potassium carbonate.	Purity: 85%	(Zhao et al., 2015)
PLE	*Capsicum chinenses*, *Capsicum annuum*	20 MPa, 200 ◦C and 10 + 20 min of static extraction time	Capsaicin concentration: 20, 264 µg/g	(Bajer et al., 2015)
TLPE	Red pepper (*Capsicum annum* L.)	22% (w/w) acetone 20% (w/w) K_2HPO_4 10% (w/w) *n*-hexane, 25 ◦C	Recovery: 98.15%	(Dang et al., 2014)
MAE	Red hot chili pepper powder (*Capsicum annuum* L.)	500 W, 60s, olive oil	164.7 mg/kg pepper	(Paduano et al., 2014)
ILAE	Green *Capsicum annuum* Bird's eye chilli (Thailand)	IL: chili ratio (5: 1), 50 ◦C, 1 h, IL's: 1-ethyl-3-methylimidazolium acetate, 1-ethyl-3-methylimidazolium	-	(Lau et al., 2015)

		hydrogen sulfate		
SFE	Green, yellow and red bell pepper	Modifier: 5% methanol Total flow rate: 5 mL/min	60.33 ng/g, (Green) 31.79 ng/g, (Yellow) 35.38 ng/g, (Red)	(Hamada et al., 2019)
ATPE+MAR	Capsicum oleoresin	Buffer pH: 4.35, sample: 0.24 g capsicum oleoresin, polymer concentration: 16.3% UCON 50-HB-5100, 10% K_2HPO_4, 1% ethanol.	Yield：95.5% Purity：85.1%	(Fan et al., 2017)
MAE+ATPE	Cumari-do-Para (*Capsicum chinense* var.)	20 wt.% of ethanolic extract, 25 wt.% of NaH_2PO_4	Extraction efficiency: 85.6%	(Cienfuegos et al., 2017)
MAE	*Capsicum frutescens* Linn.	90, 320, 360, 600 W ethanol, 5-20 min	5.2 mg/g dried chili	(Chuichulcherm et al., 2013)
UAE	*Capsicum frutescens* Linn.	40 kHz, 600 W ethanol, 5-30 min	4.0 mg/g dried chili	(Chuichulcherm et al., 2013)
UAE	Dietary supplements sold as fat burners	65% methanol	Recovery: 76-89 %	(Werner et al., 2021)
TAPPIR + Chromatography	Capsicum oleoresin	18.5% (w/w) PEG6000, 15% (w/w) sodium citrate, and 10% (w/w) [Emim] [OAc] at pH 6.5 SKP-10-4300 resin	Yield: 95.8 % Recovery: 85% Purity: 92%	(Lu & Cui, 2019)
SFE+HSCCC	Capsici fructus	SFE: 33MPa, 41 ∘C, co-solvent volume 75 mL. HSCCC: n-hexane–ethyl acetate–methanol–water (1.4:0.6:1:1, v/v/v/v), 25 ∘C, flow rate 2 mL/ min	Capsaicin extraction yield:93.1% Dihydrocapsaicin yield:93.4% Capsaicin purity :98.3% Dihydrocapsaicin purity:96.6%	(Yan et al., 2018)
ATPE	*Capsicum frutescens* var. malagueta	30 wt.% acetonitrile, 35 wt.% of [Ch]Cl, 35 wt.% of water, at 318 (±1) K	Extraction efficiency: 90%	(Santos et al., 2016)
SFE	Paprika *(Capsicum annuum L.)*	Temperature 35-75°C, pressure 100-500 bar, extraction time 60-180 min, particle size 0.25-1.25 mm.	Capsaicin content: 2.10%	(Shah et al., 2020)
UAE	Hot pepper (*Capsicum annuum* L.)	2 h, methanol, 50°C, sample-solvent ratio 1:8	Capsaicin concentration: 627 µg/g	(Marincaş et al., 2018)
UAE	Hot pepper (*Capsicum annuum* L.)	Water-methanol (20:80%v/v), 50°C, 20 min, 35 kHz and 640 W	Recovery efficiency: 62-92%	(Sricharoen et al., 2017)

SAE	Hot pepper (*Capsicum annuum* L.)	2 h, sample-solvent ratio 1:8	Capsaicin concentration: 662 µg/g	(Marincaş et al., 2018)
MSPE+ Adsorption	Gutter oil	MSPE: 1 mL dichloromethane, 3 mL, 2% NaOH aqueous solution, 1500 rpm Adsorbent: GO-Fe_3O_4	Recovery: >83%	(Lu et al., 2020)
Adsorption	*Capsicum annuum*	Adsorbents: Hydrotalcite magnesium aluminium azelate, and bentonite	Extraction yields:70-92%	(Genovese et al., 2021a)

PLE: pressurized liquid extraction; MAE: microwave assisted extraction; SFE: supercritical fluid extraction; UAE: ultrasound-assisted extraction;

SAE: shaker-assisted extraction; ILAE: Ionic liquid-assisted extraction; ATPE: aqueous two-phase extraction; MAR: microporous adsorption resin;

TLPE: three-liquid-phase extraction; HSCCC: high-speed countercurrent chromatography, MSPE: magnetic solid phase extraction

Table 2. Comparison of different extraction techniques used to recover the phytochemicals other than capsaicinoids.

Extraction method	***Operating conditions:***	***Extracted compound:***	***Reference:***
Solvent extraction	Methanol 3 h, room temperature	Phenolic compounds	(Bogusz et al., 2018)
	Methanol 24 h, 25 ºC	Phenolic compounds, flavonoids, Ascorbic acid	(Hamed et al., 2019)
	80% methanol 24 h, 25 ± 2 oC	Carotenoids, phenolics and flavonoids	(Ayob et al., 2021)
UAE	ethanol 80%, methanol 80% and acetone 80% 40 min, 40 ºC 40 kHz	Phenolic compounds	(Liu et al., 2020)
Enzyme-Assisted extraction	• Cellulase, viscozyme L, pectinase 1 h, 60 ºC • Solvent Extraction: ethanol, 30 min	Phenolic compounds, flavonoids, carotenoids	(Nath et al., 2016)
Enzymatic maceration	• Pectinex AR, Celluclast and combined 18 h, 50 ºC • Solvent Extraction: Water, 18 h, 50 ºC	Carotenoids	(Luiza et al., 2020)
PLE	65 ºC, 10 MPa, ethanol and water, 60 min	Capsiate, phenolic compounds	(Aguiar et al., 2020)
SFE + PLE	• SFE: 50 ºC, 15 MPa, 120 min • PLE: 65 ºC, 10 MPa, ethanol and water, 60 min	Capsiate, phenolic compounds	(Aguiar et al., 2020)
SFE	50 ºC, 15 MPa, 120 min	Capsiate, phenolic compounds	(Aguiar et al., 2020)
	40 ºC, 25 MPa, 80 min Ultrasonic power 600 W	Phenolic compounds	(Dias et al., 2016)

Highlights

A last 5 years comprehensive review on the current strategies towards capsaicin extraction is given.

Innovative techniques for the purification of capsaicin are highlighted.

The guidelines to the new scientists for the effective extraction are stated.

The current research gaps and perspectives in the field are outlined.