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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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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.



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

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46 **Nomenclature**

47 ATPE: aqueous two-phase extraction

48 CO₂: carbon dioxide

49 DES: Deep eutectic solvents

50 EAE: enzyme-assisted extraction

51 HSCCC: high-speed countercurrent chromatography

52 IL: ionic liquids

53 ILAE: Ionic liquid-assisted extraction

54 PLE: pressurized liquid extraction

55 PHWE: pressurized hot water extraction

56 MAR: microporous adsorption resin

57 MSPE: magnetic solid-phase extraction

58 SAE: shaker-assisted extraction

59 SFE: supercritical fluid extraction

60 TAPPIR: tunable aqueous polymer-phase impregnated resins

61 TLPE: three-liquid-phase extraction

62 UAE: ultrasound-assisted extraction

63

64 **1. Introduction**

65 Today, there is a current trend in the usage of bioactive compounds for manufacturing
66 new food and pharmaceutical formulations, along with the improvement of the existing
67 foods (Pateiro et al., 2021; Teixeira et al., 2014). The point of utilizing bioactive
68 compounds concerns the production of healthier food options to the customers. The



69 bioactive compounds, generalized as nutraceuticals, are extra-nutritional elements that
70 typically exist in low quantities in several foods, such as fruits, vegetables, fish, seaweeds,
71 herbs, etc. These biologically active compounds are being intensively explored and
72 studied due to their effects on health (Carunchia et al., 2015). Various chemical molecules
73 are classified within the major category of bioactive compounds, including phenolic
74 compounds, carotenoids, terpenes and terpenoids, nitrogen-containing and organosulfur
75 compounds, and alkaloids (Azmir et al., 2013; Castro-Muñoz et al., 2016; Wallace et al.,
76 2020).

77 Among the latter category of compounds, we can find the capsaicin, identified as (N-[(4-
78 hydroxy-3-methoxyphenyl) methyl]-8-methyl-E-6-nonenamide) in IUPAC nomenclature,
79 which is the characteristic ingredient present in chili peppers. In principle, capsaicin is a
80 flavourless, odourless and colourless chemical molecule but displaying a pungent and
81 irritating character when consumed (Al Othman et al., 2011). Capsaicin stands out as the
82 primary compound within the category of capsaicinoids, followed by dihydrocapsaicin,
83 nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin. Capsaicin and
84 dihydrocapsaicin are estimated to be approximately 90% of the total capsaicinoids
85 contained in the chili pepper (Usman et al., 2014); capsaicinoids are alkaloids (see **Figure**
86 **1**) mainly located in the placental tissue. The capsaicin owns a molecular weight of 305.40
87 g mol⁻¹, and it displays great lipophilicity (fat and oil-soluble) and also alcohol-soluble (De
88 Lourdes Reyes-Escogido et al., 2011).

89
90 **Figure 1.** Chemical structures of capsaicin (top) and dihydrocapsaicin (bottom) (Usman
91 et al., 2014).



92 The first approach in capsaicin research has been documented in 1949 (Hippenmeier,
93 1949). Ever since, capsaicin, as the most recognized compound of the chili pepper, has
94 been widely investigated and there is a deep interest in research developments toward
95 its application in various fields (Szolcsányi, 2004). A continuous effort has been devoted
96 to the exploration of capsaicin over the last two decades, as evidenced in **Figure 2**. Apart
97 from the interest of capsaicin as an ingredient and nutraceutical in new food and
98 pharmaceutical formulations, it can also be used as a feedstock for the synthesis of
99 aromas (such as vanillin) via enzymatic treatment (Heuvel et al., 2001). However, the
100 major importance relies on its plenty of biological activities documented by the research
101 community, such as anticancer (Clark & Lee, 2016), mechanosensitive (Drew et al.,
102 2002), antioxidant, anti-iron-binding (Dairam et al., 2008), analgesic (Duarte et al., 2020),
103 anti-inflammatory, antiobesity (Narang et al., 2018), and antimicrobial properties (Molina-
104 Torres et al., 1999), among many others.

105 Such bioactivity can be potentially affected due to the use of unsuitable extraction and
106 purification protocols while R&D centres require pure ingredients (ca. 99.9%) for the
107 development of new food formulations and more importantly for therapeutic assays. In
108 principle, capsaicin may present instability and be less active when downstream
109 extraction techniques use high temperatures (over 100 °C), extreme pH values and longer
110 extraction times (Si et al., 2014). Additionally, capsaicin undergoes many purification
111 stages (such as crystallization and further recrystallization) to industrially obtain a high
112 purity degree compound (Yang, 2010). Therefore, there is a need of finding more cost-
113 effective extraction and purification techniques to preserve the nutritional, functional and
114 biological properties of capsaicin, together with high extraction yields. In this work, we



115 comprehensively review the latest advances in extracting capsaicin from its natural
116 matrix. Apart from analyzing the most relevant insights in the field, great attention is paid
117 to the innovative strategies proposed by the research community, comparing their
118 advantages and disadvantages with conventional extraction techniques. After reviewing
119 the latest research, the perspectives and research gaps are also stated.

120

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

123

124 **2. Traditional extraction techniques for capsaicin**

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



138 proportional to the polarity of the solvent (Lu et al., 2017).

139

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

151 However, UAE has been noted with an enhanced extraction yield when increased the
152 temperature from 30 to 45°C showing a capsaicin recovery of > 95%. In principle, UAE
153 accelerates the swelling and hydration, provoking an enlargement in the pores of the plant
154 cell walls. Thus, an enhanced mass transfer of solutes from the matrix to solvent can be
155 obtained. Also, the authors associated such an enhanced recovery thanks to the
156 disruption of plant cells by microjet after the cavitation bubble collapsed that could
157 potentially promote the rate of solvent penetration into chilli tissue (Toma et al., 2001).

158 Unlike Boonkird's study (Boonkird et al., 2008), Santos et al. (2015) selected
159 dichloromethane in the Soxhlet method to extract the capsaicinoids from Malagueta
160 pepper (*Capsicum frutescens* L.). In preliminary studies, the authors evaluated various



161 solvents with different polarities, as reported in **Table S2**. It was observed that the highest
162 capsaicin yields of Soxhlet were acquired with low polarity solvents, in which
163 dichloromethane showed ca. 92% of total capsaicin recovery. Since the capsaicin is not
164 totally water-soluble, aqueous systems are generally not preferred for the proper
165 extraction of the target capsaicin (Sarma & del Valle, 2020).

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

176
177 Even though solvent extraction provides high extraction yields and total capsaicinoid
178 content depending on the method and operating conditions (Martins et al., 2017), most of
179 the traditional techniques demand large amounts of solvents and long extraction times;
180 which certainly raises the overall extraction cost. This is the main drawback that limits
181 their establishment, while overuse of harmful solvents also complicates meeting the
182 environmental and health guidelines. Therefore, according to the principles of "green
183 principles", there is today a strong need in developing cost-effective processes and



184 feedstocks implying the use of less hazardous materials, and minimal production of
185 wastes (Anastas & Eghbali, 2010). Also, a major challenge concerns improving the
186 traditional extraction and purification processes. The following section outlines the up-to-
187 date strategies and innovative protocols towards the sustainable separation of capsaicin.

188

189 **3. Latest strategies for the extraction and purification of capsaicin: A *last-five*** 190 ***years outlook***

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

205

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



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

208

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

215

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

218

219 One of the emerging extraction technologies for the extraction of high-added-value
220 molecules is supercritical fluid extraction (SFE), which utilizes elevated operating
221 pressures and temperatures to reach a critical point, in which the solvent (CO₂) owns the
222 diffusivity properties of a gas and concurrently the solvation power of a liquid (Dias et al.,
223 2021). Afterward, the solvent can be easily removed from the target molecule by
224 depressurization; also, the product displays better stability since there is lower
225 temperature and non-presence of organic solvent (Zougagh et al., 2004). Considering
226 that capsaicin presents good solubility in CO₂ (Knez & Steiner, 1992), SFE is a promising
227 emerging technique for its extraction, e.g., Aguiar et al. (2018) and Aguiar et al. (2020)
228 explored the efficient extraction of capsaicin; they were able to obtain up to 115 mg
229 capsaicinoid/g extract by means of SFE. By performing an economic analysis, they



230 speculated that optimal conditions (at 240 min, 50°C and 15 MPa) could offer an
231 estimated manufacturing cost of about 125.41 USD/kg of extract (Aguiar et al., 2018).
232 Here, Aguiar et al. (2018) provide a more attractive process since offering lower
233 manufacturing costs compared with Rocha-uribe et al. (2014) who estimated
234 approximately 600 USD/kg of extract.

235 Applying SFE combined with high-speed countercurrent chromatography (HSCCC), Yan
236 et al. (2018) claimed exceptionally extraction yield (of about 93%) for both capsaicin and
237 dihydrocapsaicin. This process performed the extraction with aqueous methanol, followed
238 by crystallization via alkali extraction and acid precipitation. As a polishing step, capsaicin
239 and dihydrocapsaicin were subjected to further purification with HSCCC, in which *n*-
240 hexane–ethyl acetate–methanol–water (1.4:0.6:1:1, v/v/v/v) was selected as a solvent.
241 The obtained capsaicin and dihydrocapsaicin presented a purity degree of 98.3 and
242 96.6%, respectively. Hamada et al. (2019) developed an online approach integrated by
243 SFE, dilution line and for the capsaicin extraction and measurement. The concentration
244 of the capsaicin varied from 21 to 60 ng/g in various types of bell peppers. According to
245 the authors' findings, the extraction performance was greatly dependent on the pressure
246 since the pressure increment provokes a higher density of the critical fluid (i.e., CO₂) and
247 thus raises solvating power leading to better extraction yield (Gnayfeed et al., 2001).

248 Unfortunately, the yield could be lowered at low pressure due to the diminished diffusivity
249 decreases the interaction between the fluid and the matrix (Kwon et al., 2011). Therefore,
250 SFE could potentially benefit from other emerging techniques that may promote mass
251 transfer via convection or diffusion (Stoica et al., 2016). For instance, Santos et al. (2015)
252 achieved to increase (up to 77%) the capsaicin yield in SFE by using ultrasound waves,



253 which substantially improved the SFE separation rate. UAE lies on creating small cavities
254 to deliver energy into the product/solvent mixture facilitating the extraction of target
255 analytes. Sricharoen et al. (2017) were able to extract several biomolecules (including
256 capsaicin) embedded into an oleoresin from hot chili peppers. In principle, chili peppers
257 contained between 614-25,976 mg/kg for capsaicin and 609-22,130 mg/kg for
258 dihydrocapsaicin, of which UAE demonstrated a recovery efficiency ranging from 62 to
259 92%.

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

269 Very recently, Martins et al. (2017) screened and compared traditional (such as Soxhlet)
270 and emerging extraction methods, such as UAE and shaker-assisted extraction (SAE),
271 for the recovery of capsaicin from habanero chili. According to the experimentation, the
272 total capsaicin content obtained by Soxhlet (using ethanol) demonstrated that the fruit
273 has 2.2% (ca. 22.0 mg/g) of capsaicin, while UEA yielded 90.7% (between 14.2–19.9
274 mg/g) and SAE 76.1% (between 8.3–16.9 mg/g). In this approach, the highest extraction
275 yield was acquired by the Soxhlet method that was ascribed to high extraction time (225



276 min), which supports Boonkird's idea, together with handled temperature (90°C) and
277 molecule: solvent ratio fostering the solubility increase of capsaicin. Unfortunately,
278 Soxhlet clearly showed a degradation of the capsaicin in the first 30 min of operation,
279 while the other techniques proved their reliability in product stability. Even though SAE
280 and UEA recovered less capsaicin (between 9.3-23.9 % less), they offered a 99% solvent
281 saving and 86% less time in comparison to conventional solvent extraction. By comparing
282 SAE and UEA, the latter extraction technique apparently gave a higher extraction yield
283 thanks to the acoustic cavitation in the solvent due to the ultrasonic waves. In addition,
284 the waves provoked a mechanical effect that results in enhanced penetration of the
285 solvent into the fruits matrix and thus surface contact among the solid-liquid phase (Zhang
286 et al., 2009). As a suggestion, the authors also pointed out that UAE displays several
287 advantages over the other two techniques, however, the usage for the extraction of
288 bioactive compounds must be selected carefully since the formation of cavity bubbles
289 could raise in temperature (over 500 °C) and pressure (up to 550 atm) (Martins et al.,
290 2017), which could indeed speed up the degradation of thermal-sensitive compounds
291 (Schläfer et al., 2002).

292 A liquid-liquid fractionation technique, such as aqueous two-phase extraction (ATPE),
293 was proposed by Fan et al. (2017) for the extraction and purification of capsaicin from
294 commercial oleoresin. ATPE uses two incompatible phases, such as polymer-salt, ionic
295 liquid (IL)-salt, or alcohol-salt, for efficient extraction. Here, the two incompatible phases
296 take place when one polymer is enriched on the top phase and the salt (or second
297 polymer) is also enriched but at the bottom side. In general, ATPE acts as a promising
298 method for the separation of biologically active compounds since such a technique



299 possesses several advantages in terms of low cost, low equipment requirements, short
300 extraction time, and uses 70-90% water. Based on this, Fan et al. (2017) explored ATPE
301 for obtaining capsaicin and subsequently purified using adsorption process (with ADS-17
302 and AB-8 resin). Initially, the ATPE system (using ethylene oxide-propylene oxide
303 copolymer and K_2HPO_4) exhibited ca. 95% extraction yield, while after the adsorption
304 processes, the integrated technique containing ADS-17 resin showed a capsaicin
305 recovery and purity of 83.7% and 50.3%, respectively, meantime AB-8 resin provided
306 slightly higher recovery rate and high purity product (88.0% and 85.1%, respectively).
307 Comparable outcomes (capsaicin purity of 85%) were also documented by Zhao et al.
308 (2015) who employed D101 and SKP-10-4300 resin, while Cienfuegos et al. (2017)
309 purified capsaicin (> 5-fold) from *Capsicum chinense* via ATPE, which was previously
310 extracted by MAE. At the present work, the overall extraction efficiency of 85 % was
311 reported, in which the active compounds proved \approx 80% antioxidant activity. In a previous
312 study, Dang et al. (2014) achieved to recover over 98% capsanthin from red pepper
313 (*Capsicum annum* L.) via three-liquid-phase extraction (TLPE), without requiring any
314 adsorption or additional purification step.

315 As part of current ideas in process intensification, there is a necessity to developing
316 simple and efficient extraction processes with a fewer number of processing steps, and
317 importantly, they should work at all scales (Fernandez Rivas et al., 2020). Attending such
318 a need, Lu & Cui (2019) integrated two techniques, such as ATPE and (MAR), to develop
319 'tunable aqueous polymer-phase impregnated resins' (TAPPIR), which was subsequently
320 implemented into chromatography. At optimal conditions, the aqueous polymer phase
321 impregnated HZ816 resins containing 18.5% (w/w) PEG6000, 15% (w/w) sodium citrate,



322 and 10% (w/w) [Emim] [OAc] (at pH 6.5) yielded 95% capsaicin extraction, which was
323 ultimately purified by SKP-10-4300 resin in chromatography. Basically, the overall system
324 offered a capsaicin recovery and purity of about 85% and 92%, respectively.

325 Chemists are strongly exploring sustainable alternatives to replace conventional
326 molecular solvents. In this way, ionic liquids (ILs) stand out as a green alternative due to
327 their low toxicity and biodegradability (Welton, 2011). Specific green solvents, such as
328 cholinium (Ch)-based ILs, were explored in ATPE by Santos et al. (2016), who utilized it
329 (together with acetonitrile and water) as an ideal phase to partition capsaicin. The general
330 process followed by Santos et al. (2016) is illustrated in **Figure 4**. It was observed that
331 the acetonitrile phase was preferentially enriched with the capsaicins while the IL
332 captured other metabolites (like phenolic compounds). After the overall extraction
333 process, the extraction efficiency was over 90% with a purification factor of ca. 3.20.
334 Interestingly, it is known that the extraction efficiencies tend to be enhanced by raising
335 the operating temperature when recovering solutes due to the enhanced solubility in liquid
336 phases (Abe et al., 2014; Valencia-Arredondo et al., 2020), nevertheless, Santos et al.
337 (2016) underlined that the extraction efficiency (between 89.7-93.0 %) was not greatly
338 influenced by the temperature, this insight was obtained thanks to the analysis of the
339 effect of the temperature on the capsaicin migration. By calculating thermodynamic
340 parameters (Gibbs energy, enthalpy and entropy), the migration into acetonitrile (using
341 ATPE) was apparently determined as spontaneous (negative Gibbs energy). On the
342 contrary, the analysis suggested that transport of capsaicin into the cholinium (Ch)-based
343 IL behaved as endothermic.

344



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

347
348 Ultimately, the latest research refers to the exploration of new adsorbent materials for the
349 purification and polishing of the pre-concentrated capsaicin. For instance, Lu et al. (2020)
350 focused their research on exploring magnetic solid-phase extraction (MSPE) for the
351 separation of capsaicin from gutter oil (cooking oil recovered from food waste). The
352 authors proposed MSPE as a pre-treatment strategy to directly adsorb the target
353 capsaicin while enriching and separating via an external magnetic field. In this work, a
354 nanocomposite based on graphene oxide (GO)–Fe₃O₄ was synthesized by means of the
355 co-precipitation method. The optimal amount of hybrid adsorbent (ca. 40 mg) offered
356 capsaicin extraction recovery > 80%, which was increased by extending the extraction
357 time (up to 100% for 20 min). It is important to point out that such a nanocomposite also
358 exhibited a similar uptake rate for dihydrocapsaicin and N- vanillylnonanamide, in which
359 its reuse is feasible by several times with no significant loss of performance. Such high
360 recovery efficiencies are credited to the GO due to its large adsorption capacity as a result
361 of its high surface area and two-dimensional structure (Cha-Umpong et al., 2020).

362 Very recently, Genovese et al. (2021a) also assessed nineteen adsorbents (including
363 hydrotalcites, lamellar solids, and phyllosilicates) to pre-concentrate the capsaicin from
364 various cultivars of *Capsicum annuum*. To sum up, hydrotalcite magnesium aluminium
365 azelate and bentonite exhibited the best performing extraction efficiencies, yielding
366 between 73-91% and 68-71%, respectively. Bentonite especially possesses a large
367 surface area and high water uptake properties (Claverie et al., 2018), this latter property



368 was speculated to play an important role since it is quite possible that the transfer of the
369 solvent (water) to the solid phase could have mechanically driven the capsaicin onto the
370 sorbent and thus contributing to the high extraction yield. Regarding hydrotalcite
371 magnesium aluminum azelate, its selectivity towards capsaicin was attributed to Van der
372 Waals and hydrophobic interactions since capsaicinoids present linear 6-8 carbons acyl
373 chains that are able to fit with the linear carbon skeleton of azelaic acid intercalated in the
374 inner structure.

375

376 **4. Other capsaicinoids and bioactive compounds extracted from chili pepper**

377 Capsaicin and dihydrocapsaicin are the most prominent forms in the chili pepper fruit
378 extracts, accounting for almost 90% of capsaicinoids. Nordihydrocapsaicin (7%),
379 homocapsaicin (1%) and homodihydrocapsaicin (<1%) are always present at very low
380 concentrations when compared to capsaicin and dihydrocapsaicin (De Lourdes Reyes-
381 Escogido et al., 2011). Due to their low concentration, few studies and efforts were made
382 to quantify those capsaicin analogs in chili pepper extracts. For instance, Genovese et al.
383 (2021b) performed solid phase extraction of twenty-two cultivars of chili pepper using
384 solid sorbents. The quantities of extracted capsaicinoids reached 865 mg of capsaicin
385 (76%), 239 mg of dihydrocapsaicin (21%), 17 mg of nordihydrocapsaicin (2%), and 10
386 mg of homocapsaicine (1%) for the most effective sorbent. Liu et al. (2020), for instance,
387 applied solvent extraction of chili pepper seeds. The nordihydrocapsaicin contents ranged
388 from 43 up to 297 $\mu\text{g/g}$ depending on the type of seed and solvent used for extraction,
389 while total capsaicinoids content range was between 1052 – 3692 $\mu\text{g/g}$.

390 A similar group of compounds, named capsinoids, include capsiate, dihydrocapsiate, and



391 nordihydrocapsiate, are also naturally present in chili peppers. Capsinoids have the
392 beneficial properties of capsaicinoids, however, due to their slightly different structure,
393 they do not cause the characteristic of pungency (Hursel & Westerterp-Plantenga, 2010).
394 The research group of Aguiar has worked with different extraction techniques applied to
395 capsinoids recovery from biquinho pepper. They reported the presence of a considerable
396 concentration of capsinoids in extract obtained by SFE at 60 °C and 15 MPa (Aguiar et
397 al., 2014). Capsinoids are less polar than capsaicinoids, due to their ester bond that
398 replaced the amide bond of capsaicinoids, thus supercritical CO₂ was selective for
399 capsinoid compounds – capsiate and dihydrocapsiate. In a subsequent study, the authors
400 pursued the intensification of the extraction process by combining SFE with pressurized
401 liquid extraction (PLE), allowing the production of capsiate-enriched oleoresin from
402 biquinho pepper (Aguiar et al., 2020). An extraction yield of 77% was obtained, together
403 with a reduction 1.39 times of the manufacturing cost. At this point, this finding proves
404 that the smart combination of different techniques makes the process more economically
405 profitable.

406 Chili peppers are also an excellent source of other phytochemicals, such as
407 anthocyanins, vitamins, phenolic acids, flavonoids, and carotenoids. Various traditional
408 and emerging techniques have been employed for the extraction of phytochemicals from
409 chili peppers, including maceration (Luiza et al., 2020), solvent extraction (Bogusz et al.,
410 2018), ultrasound-assisted extraction (Liu et al., 2020), supercritical fluid extraction
411 (Sricharoen et al., 2017), and pressurized liquid extraction (Aguiar et al., 2020). The
412 conventional methods focus in using organic solvents, such as methanol and ethanol, as
413 the extraction vehicle of phytochemicals by the implementation of solvent extraction.



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

416

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

419

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

435

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



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

448
449 **Figure 5.** Process guideline for the efficient extraction and purification of capsaicin from
450 chili peppers considering the current efforts of the research community.

451
452 This process basically implies three fundamental downstream stages such as pre-
453 treatment, extraction and purification, together with polishing. It is worth pointing out that
454 the current efforts have been focused on emerging technologies for the extraction
455 (Sereshti et al., 2019), and novel materials for the selective purification of capsaicin
456 molecules. To finalize, the perspectives and current research gaps for new researchers
457 in the field are given as follows:

- 458 • One of the current research interests deals with the discovery of the biosynthetic
459 pathway of capsaicin and its precursors (Usman et al., 2014). However, such



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

472 • *Pre-treatment and extraction:* To date, maceration stands out as the easy-to-
473 handle and typical methodology for the primary extraction and availability of
474 capsaicin from the placental tissue where they are biosynthesized and
475 accumulated (Santos et al., 2016). Apart from typical maceration for obtaining the
476 capsaicin, enzyme-assisted extraction (EAE) could be an alternative as a way to
477 acquire the bounded capsaicin into the cell wall and thus foster enhanced
478 extraction yield. Until now, EAE has been successfully applied in the recovery of
479 aromas (Galiano et al., 2019) and bioactive compounds (Figoli et al., 2006) from
480 natural products but minimally explored in capsaicin recovery. Importantly, such
481 enzymatic treatment can be combined with other extraction techniques, and it is
482 recognized as an environmentally friendly strategy since water is commonly used



483 as a solvent. Here, different enzymes, such as cellulases, pectinases, beta-
484 glucosidases (Cortés-Ferré et al., 2021), must be suitable to hydrolyze the cell
485 structure releasing the target analyte.

486 • *Polishing of capsaicin as a final stage:* adsorption processes aided with
487 commercial microporous resins, such as SKP-10-4300, ADS-17 and AB-8, are the
488 preferred strategy by the research community. Particular attention should be paid
489 to the new hybrid materials with exceptional adsorption properties, e.g., graphene
490 oxide (GO)-Fe₃O₄ composites have been introduced as an adsorptive material,
491 displaying a capsaicin extraction recovery as high as 98% (Lu et al., 2020). It is
492 worth mentioning that such new hybrid material presented promising reusability
493 since it was used for extracting more than 10 times with unchanged performance.
494 Apart from this, the chemistry should be properly studied to benefit from the large
495 uptake capacities of such new materials. In this regard, further exploration of the
496 physicochemical properties of the sorbents, such as interlayer distances, particle
497 size, the polarity of the inner cavity, surface areas, introduction of selected ions in
498 the crystal lattice, should be performed and extended to possibly tuning for an
499 enhanced extraction. Here, chemical or physical treatments should be adapted
500 emphasizing overall charge, polarity and further interactions, including van der
501 Waals, hydrogen bonding, dipole-dipole forces, and cation-anion interactions
502 (Cartalade & Vernhet, 2006; Galanakis, 2015; Sun & Leung, 2019).

503 • A non-typical practice from research regards the techno-economic feasibility of the
504 applied emerging techniques. It could be interesting if the authors may provide an
505 estimation of the extraction cost of capsaicin using these novel techniques and



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

509 • The use of ILs, as non-volatile and tunable solvents, has been done for the
510 capsaicin extraction (Lau et al., 2015), however, the green analogues of ILs, so-
511 called deep eutectic solvents (DESs) or natural deep eutectic solvents (NADES),
512 can potentially be applied. DESs are currently employed in various applications
513 including extraction of biologically active compounds and pharmaceuticals (Faraz
514 et al., 2021), analytical determinations (Zhang et al., 2012), extraction of heavy
515 metals (Haq et al., 2021), among others. Therefore, a new research gap in the
516 near future will refer to the sustainable extraction of capsaicin using DESs.
517 However, particular attention should be devoted to the polarity of the DESs, which
518 is influenced by the type of hydrogen bond acceptor (HBA) and hydrogen bond
519 donor (HBD) and their molar ratio (Smith et al., 2014).

520 • In recent years, membrane-based technologies stand out as an alternative for
521 recovery and concentration high-added values compounds from natural products
522 and their by-products (Castro-Muñoz et al., 2020). Such technologies,
523 implemented with ultrafiltration and nanofiltration membranes, could be an
524 alternative for the concentration of capsaicin once they are available in a liquid
525 system. Considering the molecular weight of the capsaicin (~305,41 g/mol),
526 nanofiltration membranes can easily assist the concentration of these compounds
527 depending on the membrane properties (e.g., polymer type,
528 hydrophilicity/hydrophobicity, pore size, structure, morphology, etc.). It is important



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

531

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538

539 **Conflict of Interest**

540 The author declares no conflict of interest.

541

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

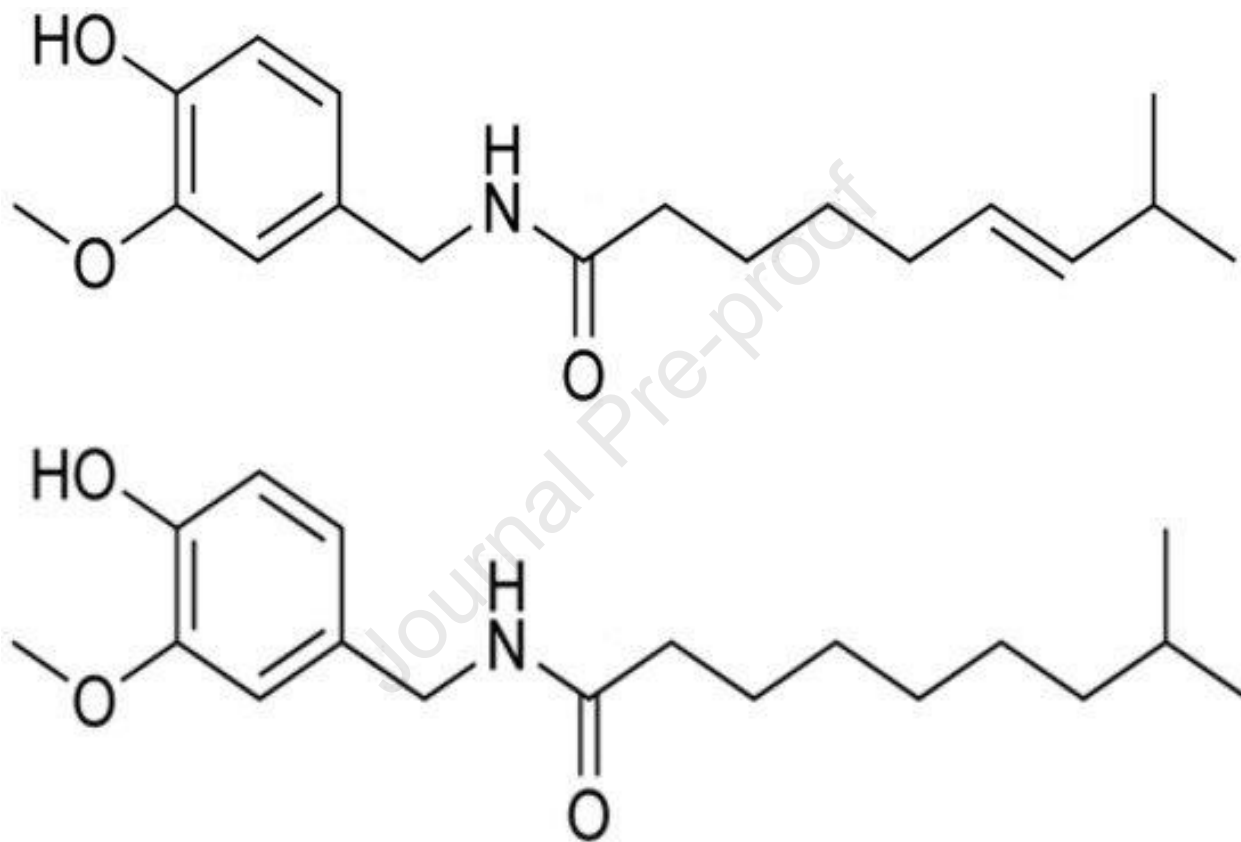


Figure 2. Documented publications related to the research towards capsaicin over the last two decades (until July 15th, 2021; source: Scopus). Keyword: *Capsaicin*.

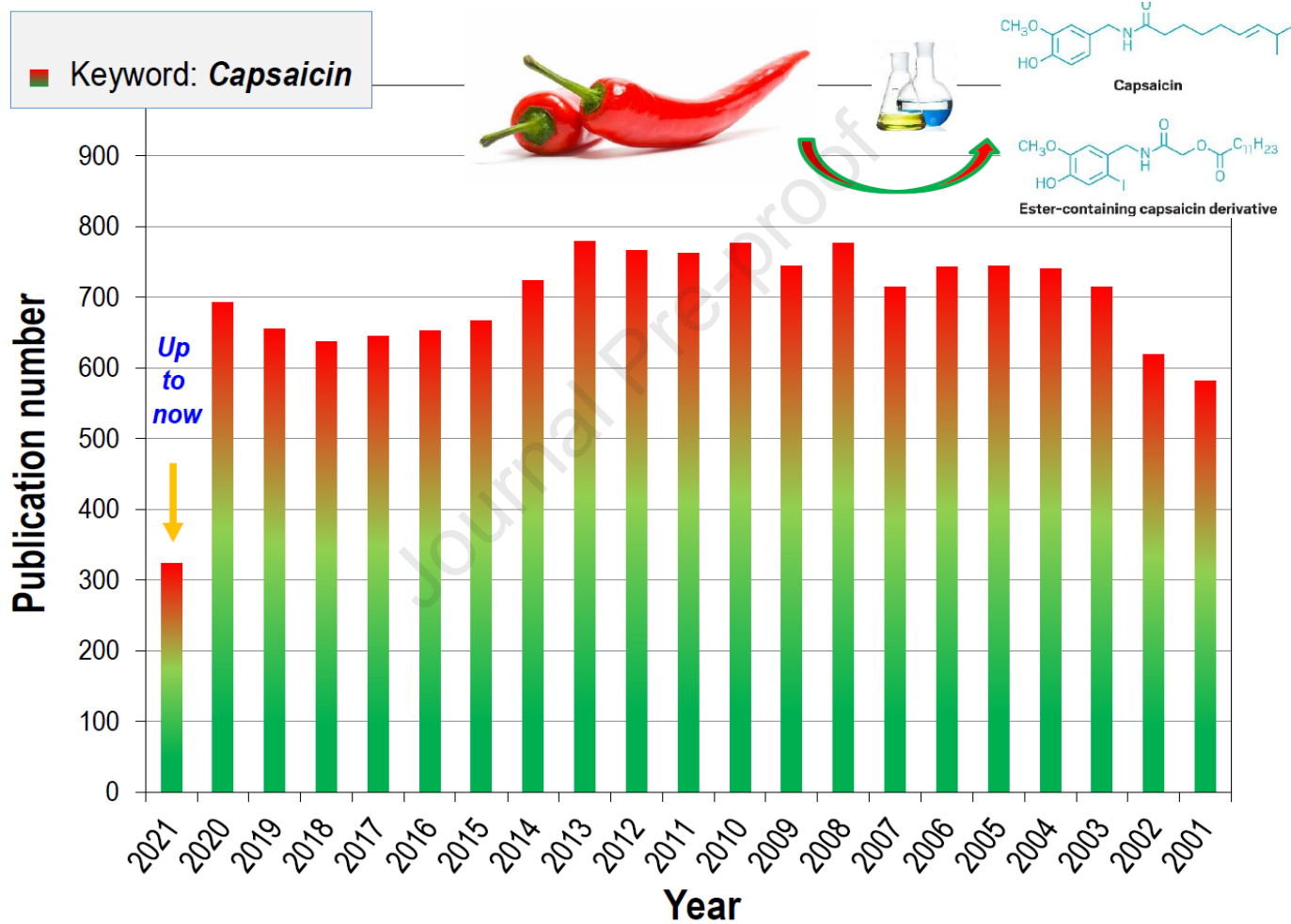


Figure 3. Usual strategies used for the extraction of bioactive compounds from food systems. Inspired by (Camara et al., 2021).

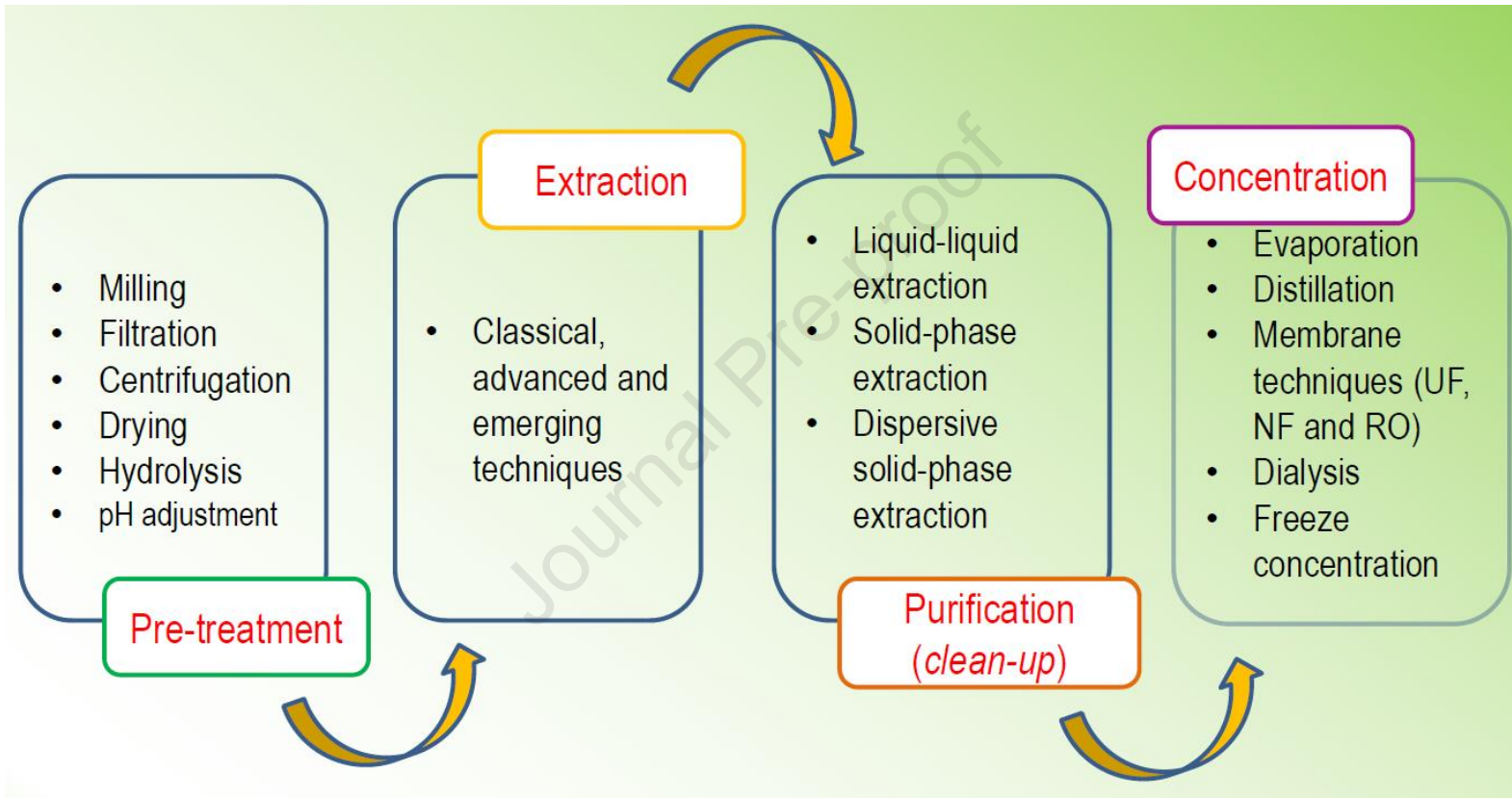


Figure 4. Integrated system applied for the extraction, purification and polishing of capsaicin (Santos et al., 2016).

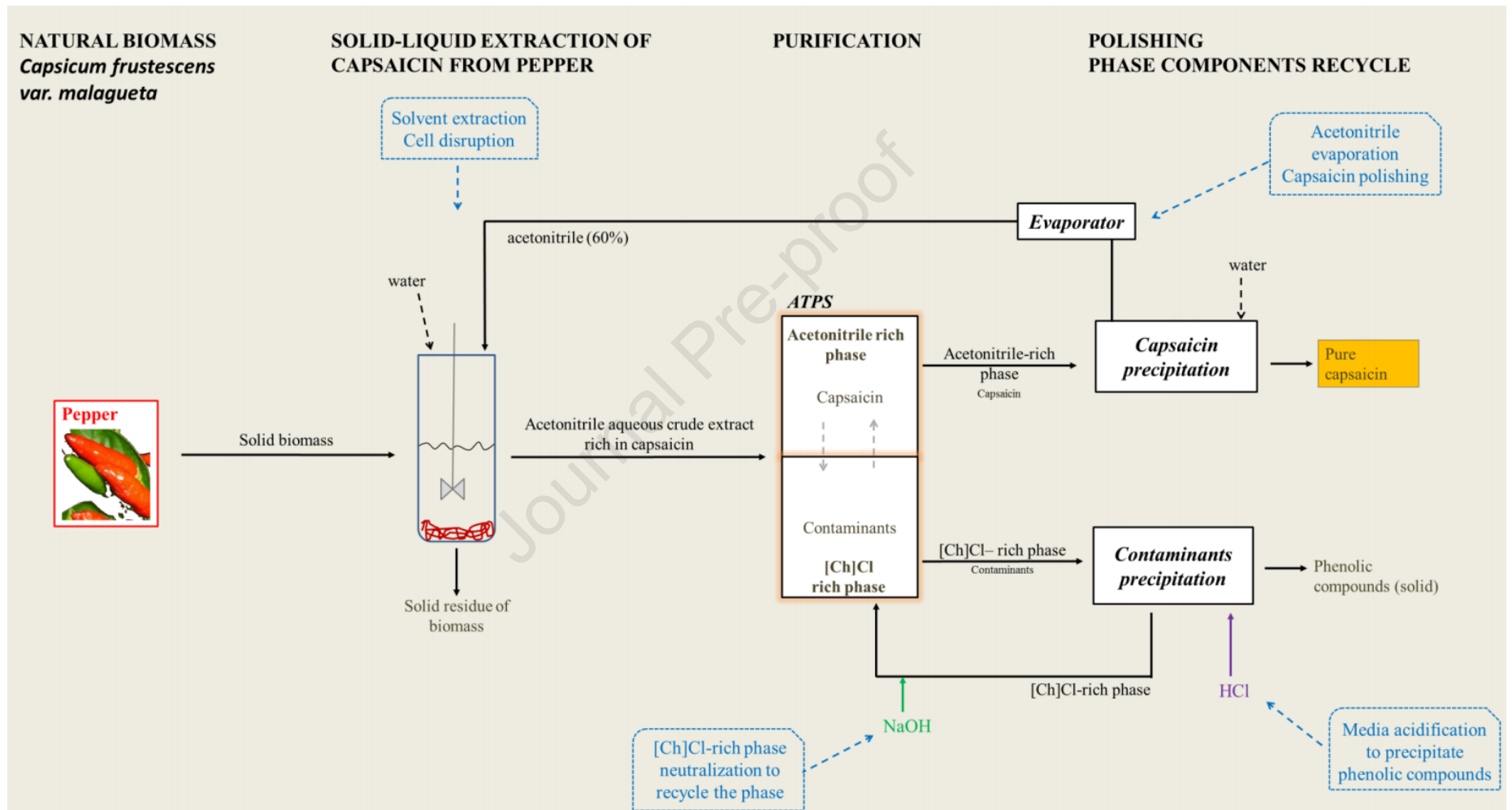


Figure 5. Process guideline for the efficient extraction and purification of capsaicin from chili peppers considering the current efforts of the research community.

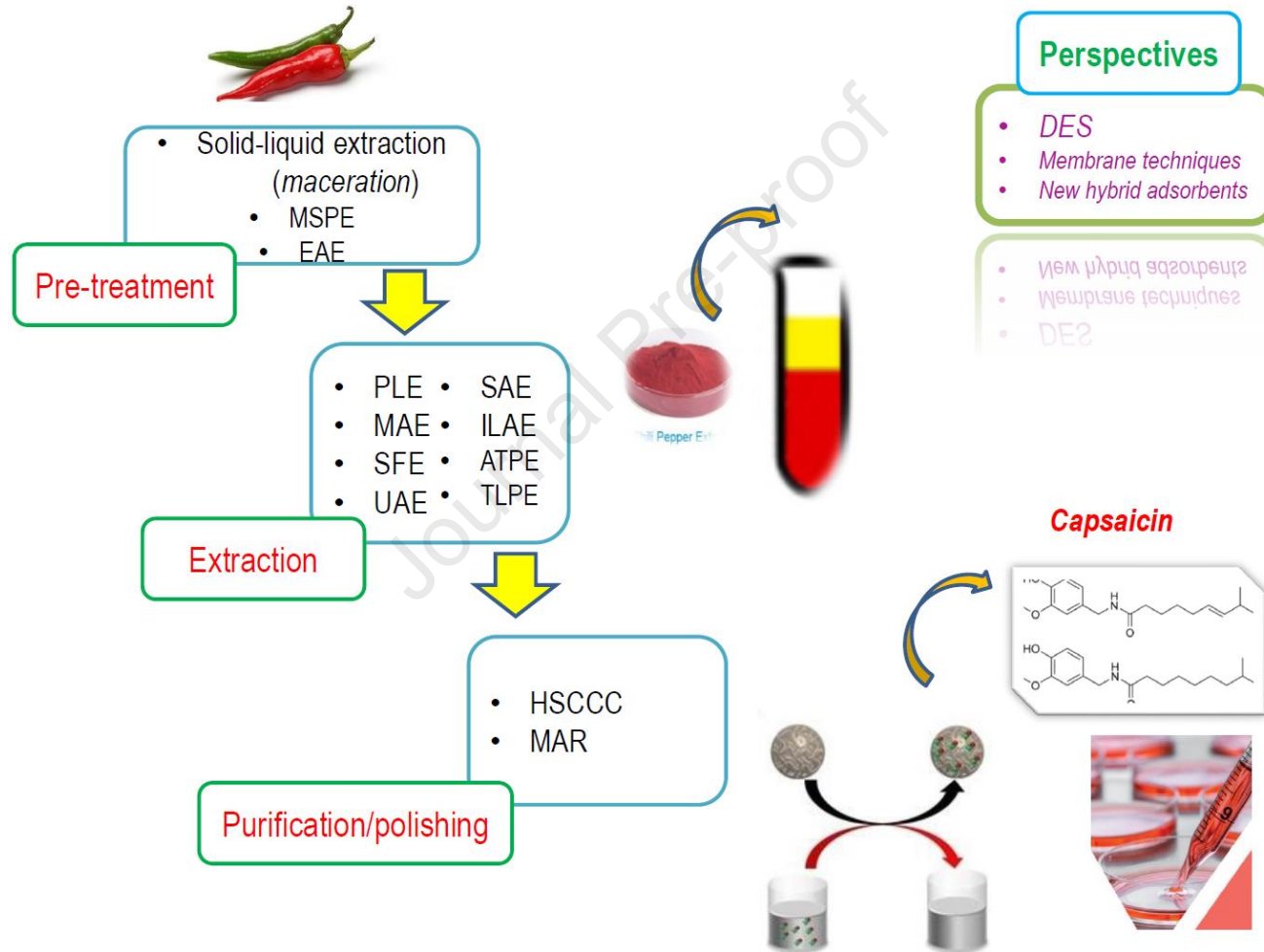


Table 1. Ongoing progress on the extraction and purification of capsaicin applying emerging techniques and protocols.

Technique/ technology	Source	Operating conditions	Yield/ purity/ capsaicin recovery	Reference
SFE	Malagueta pepper (<i>Capsicum frutescens</i> L.)	40 °C, 15 MPa, acetonitrile, acetic acid, water	115mg/g extract	(Aguiar et al., 2018)
SFE	Malagueta pepper (<i>Capsicum frutescens</i> L.)	40°C, 15 MPa	Yield: 76.1%	(Santos et al., 2015)
SFE+UAE	Malagueta pepper (<i>Capsicum frutescens</i> L.)	SFE: 40°C, 15MPa, 150 min UAE: 360 W, 60 min	Yield: 79%	(Santos et al., 2015)
SFE	dedo de moça pepper (<i>Capsicum baccatum</i> L. var. <i>pendulum</i>)	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 (<i>Capsicum annuum</i> L.)	40% amplitude, 40°C, 400W and 24 kHz, 15 min, olive oil	-	(Civan & Kumcuoglu, 2019)
UAE	Red hot chili pepper powder (<i>Capsicum annuum</i> 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	<i>Capsicum chinenses</i> , <i>Capsicum annuum</i>	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 (<i>Capsicum annum</i> L.)	22% (w/w) acetone 20% (w/w) K ₂ HPO ₄ 10% (w/w) <i>n</i> - hexane, 25 °C	Recovery: 98.15%	(Dang et al., 2014)
MAE	Red hot chili pepper powder (<i>Capsicum annuum</i> L.)	500 W, 60s, olive oil	164.7 mg/kg pepper	(Paduano et al., 2014)
ILAE	Green <i>Capsicum annum</i> 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 ₂ HPO ₄ , 1% ethanol.	Yield : 95.5% Purity : 85.1%	(Fan et al., 2017)
MAE+ATPE	Cumari-do-Para (<i>Capsicum chinense</i> var.)	20 wt.% of ethanolic extract, 25 wt.% of NaH ₂ PO ₄	Extraction efficiency: 85.6%	(Cienfuegos et al., 2017)
MAE	<i>Capsicum frutescens</i> Linn.	90, 320, 360, 600 W ethanol, 5-20 min	5.2 mg/g dried chili	(Chuichulcherm et al., 2013)
UAE	<i>Capsicum frutescens</i> 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	<i>Capsicum frutescens</i> 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 (<i>Capsicum annum</i> 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 (<i>Capsicum annum</i> L.)	2 h, methanol, 50°C, sample-solvent ratio 1:8	Capsaicin concentration: 627 µg/g	(Marinçaş et al., 2018)
UAE	Hot pepper (<i>Capsicum annum</i> 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 (<i>Capsicum annuum</i> 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 ₃ O ₄	Recovery: >83%	(Lu et al., 2020)
Adsorption	<i>Capsicum annuum</i>	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

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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 °C	Phenolic compounds, flavonoids, Ascorbic acid	(Hamed et al., 2019)
	80% methanol 24 h, 25 ± 2 °C	Carotenoids, phenolics and flavonoids	(Ayob et al., 2021)
UAE	ethanol 80%, methanol 80% and acetone 80% 40 min, 40 °C 40 kHz	Phenolic compounds	(Liu et al., 2020)
Enzyme-Assisted extraction	• Cellulase, viscozyme L, pectinase 1 h, 60 °C • Solvent Extraction: ethanol, 30 min	Phenolic compounds, flavonoids, carotenoids	(Nath et al., 2016)
Enzymatic maceration	• Pectinex AR, Celluclast and combined 18 h, 50 °C • Solvent Extraction: Water, 18 h, 50 °C	Carotenoids	(Luiza et al., 2020)
PLE	65 °C, 10 MPa, ethanol and water, 60 min	Capsiate, phenolic compounds	(Aguiar et al., 2020)
SFE + PLE	• SFE: 50 °C, 15 MPa, 120 min • PLE: 65 °C, 10 MPa, ethanol and water, 60 min	Capsiate, phenolic compounds	(Aguiar et al., 2020)
SFE	50 °C, 15 MPa, 120 min	Capsiate, phenolic compounds	(Aguiar et al., 2020)
	40 °C, 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.