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Review Article

Extraction, bioavailability, and bioefficacy of capsaicinoids

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ABSTRACT

Capsaicinoids are active constituents responsible for the pungent and spicy flavor in chili peppers. During the past few decades, various extraction methods of capsaicinoids from peppers have been developed with high yields. Through biological studies, pharmacological benefits have been reported such as pain relief, antiinflammation, anticancer, cardio-protection, as well as weight loss. In this paper, the extraction methods and bioavailability of capsaicinoids are reviewed and discussed. In addition, the pharmacological effects and their underlying mechanisms are also studied.

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1. Introduction

Peppers are popular around the world, and are often used as food additives to provide a hot and pungent taste. Capsaicinoids are flavor compounds in red chili peppers, mainly composed of capsaicin (C), dihydrocapsaicin (DHC), nordihydrocapsaicin (n-DHC), homocapsaicin (h-C), and homodihydrocapsaicin (h-DHC; Table 1) [1]. Among these, C and DHC contribute to around 80–90% of the total pungency in most chili peppers [2]. Altogether, more than 20 capsaicinoids have been found in different pepper species [3]. Capsaicinoids are biosynthesized in the placenta of the fruits by condensation of vanillylamine and medium chain length fatty acids [4].

Studies on the anticancer and antitumor effects of capsaicinoids have reported that C could induce apoptosis in

cancer cells as well as suppress carcinogenesis in the prostate, skin, breast, colon, lung, and human bladder [5–10]. Oh et al [11] claimed that DHC could induce catalase-mediated autophagy in HCT116 human colon cancer cells. According to Ziglioli et al [12], apoptosis in prostate cancer cells was triggered by C through two pathways: a direct pathway [transient receptor potential vanilloid type 1 (TRPV-1) receptor-independent pathway] and indirect pathway (TRPV-1 receptor-dependent pathway). In the direct pathway, C worked as the coenzyme Q antagonist in controlling electron transport, resulting in an excess amount of reactive oxygen species (ROS), which induced cell damage and apoptosis. In the indirect pathway, C interacted with receptor TRPV-1, leading to the accumulation of Ca^{2+} within cancer cells and finally to the precocious and late elements of apoptosis. The anticancer study of DHC in lung cancer cell lines was

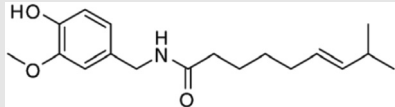
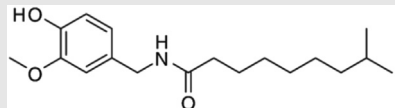
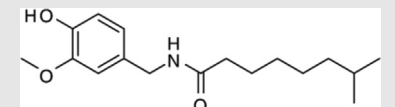
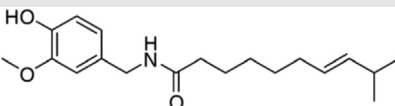
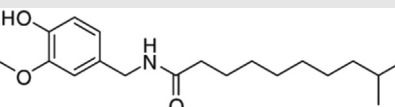
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Table 1 – Chemical structures of capsaicinoids.

Capsaicinoid name	Abbreviation	Molecular formula	Chemical structure
Capsaicin (<i>trans</i> -8-methyl- <i>N</i> -vanillyl-6-nonenamide)	C	C ₁₈ H ₂₇ NO ₃	
Dihydrocapsaicin (8-methyl- <i>N</i> -vanillyl-nonanamide)	DHC	C ₁₈ H ₂₉ NO ₃	
Nordihydrocapsaicin (7-methyl- <i>N</i> -vanillyl-octamide)	n-DHC	C ₁₇ H ₂₇ NO ₃	
Homocapsaicin (<i>trans</i> 9-methyl- <i>N</i> -vanillyl-7-decenamide)	h-C	C ₁₉ H ₂₉ NO ₃	
Homodihydrocapsaicin (9-methyl- <i>N</i> -vanillyl-decamide)	h-DHC	C ₁₉ H ₃₁ NO ₃	

performed by Choi et al [13]. They treated WI38, H1299, H460, and A549 cells with DHC to examine the role of induced autophagy in lung cancer cells. They reported that DHC could reduce the ROS accumulation and induce autophagy in catalase-sensitive cells, which might be involved in cell protection against apoptotic and necrotic cell death.

Antioxidation activities of capsaicinoids had been reported both *in vitro* and *in vivo* [14–16]. An *in-vitro* study using serum lipoproteins proved that C and DHC increased the lag time before initiation of low-density lipoprotein oxidation and decreased the oxidation rates, thereby reducing the lipid oxidation [17]. Another study in human umbilical vein endothelial cells demonstrated that C inhibited ROS generation and caspase-3 activation induced by oxidized low-density lipoprotein [18]. *In vivo*, a reduction of oxidative stress in the liver, lung, kidney, and muscle was reported in a mice model after oral administration of capsaicin for 3 days (3 mg/kg body mass/d), showing that C could be an effective antioxidant in lowering oxidative stress even when consumed for a short time [19]. Similar results could be found in the study by Hassan et al [20], who revealed that C could protect the liver against carbon tetrachloride (CCl₄)-induced toxicity in rats by working as an antioxidant to reduce the production of free radicals and suppress the caspase-3 activities.

C has also been used to alleviate pain from neuropathic and musculoskeletal disorders, such as postherpetic neuralgia, diabetic neuropathy, osteoarthritis, and rheumatoid arthritis during topical applications [21]. As was mentioned

previously, after C binds to the TRPV1, intracellular Ca²⁺ content will increase and inflammatory neuropeptides (substance P) will be released [22]. Through this calcium-dependent process, neurons and nerve terminals are damaged and desensitized to further painful stimuli, leading to the analgesic effects or even the degeneration of nociceptive fibers [23]. In brief, C exerts analgesic effect by binding to the vanilloid receptor TRPV-1 and regulating voltage activated calcium channels.

Cardioprotective effects of C in animal models have been reported during topical application, oral administration, and intravenous injections. Studies by Jones et al [24] showed that topical application of C cream could result in significantly reduced infarcts following a 45-minute coronary occlusion, demonstrating a cardioprotective activity in mice. Beneficial cardiovascular effects in rats of metabolic syndrome were also observed by feeding them 0.5–1.0 mg/kg body weight of C together with regular diets [25]. Those beneficial functions include heart rate variability improvement, increased vascular sympathetic drive and increased spontaneous baroreflex sensitivity. In addition, Gross et al [26] also reported that rats receiving C intravenously with doses ranging from 0.1 mg/kg to 1 mg/kg could reduce myocardial infarct sizes via TRPV-1 channel. Therefore, the stimulation of TRPV-1 by C could increase cytosolic calcium and change cholesterol transporters expression, leading to enhanced cholesterol efflux and reduced cholesterol uptake into vascular smooth muscle cells, which lowered the major risk factor in the pathogenesis of atherosclerosis.

Consumption of chili peppers is known to increase energy expenditure. Capsaicinoids as active components in chili peppers has been proven to have thermogenic and antiobesity properties [27–30]. Reinbach et al [27] studied the effects of C on appetite, energy intake, body weight, and heart rate in humans for 6 weeks. Results suggested that C could reduce energy intake as well as help weight loss by relatively suppressing hunger and sustaining satiety. Joo et al [30] fed the rats a high-fat diet with C and performed proteomic analysis to elucidate its molecular action in white adipose tissue. Results revealed that proteins related with lipid metabolism, redox regulations, and signal and energy transduction were significantly altered on the treatment of C, suggesting a possible mechanism of the antiobesity effect of C.

Therefore, beneficial biofunctions of capsaicinoids have been reported with respect to antiinflammation, anticancer, analgesic, cardioprotective, antioxidation, and antiobesity activities, which mainly function through activating the TRPV superfamily of cation-channel receptors [5,31]. However, direct ingestion of C can be lethal at a certain amount. Oral LD50 values of C are 161.2 mg/kg for rats and 118.8 mg/kg for mice. To alleviate its gastric mucosa irritation effects, many encapsulation methods have been employed with enhanced stability, bioaccessibility, and bioavailability [32–34].

In this review, various extraction methods for major capsaicinoids from fresh pepper fruits and dried samples are reviewed. Bioavailabilities of capsaicinoids are also discussed in terms of absorption, distribution, metabolism, and elimination.

2. Extraction methods

Various extraction methods of capsaicinoids from hot peppers have been developed during the past few decades. When designing an extraction process, the first step is the selection of appropriate solvent that can result in a high yield of desired compound. Among all solvents that have been used for extracting capsaicinoids, methanol, ethanol, acetonitrile, and water are the most common [35]. In addition to the solvent selection procedure, there are many other influencing parameters to be considered in order to achieve high extraction efficiency, such as the temperature, extraction time, volume of solvent, quantity of sample, the repeatability, and reproducibility of the methods. The extraction techniques that have widely been employed by researches include maceration [36], magnetic stirring [37], enzymatic extraction [38], microwave [39] and ultrasound-assisted extraction (UAE) [40], Soxhlet (SOX) [41], supercritical fluid [42], and pressured liquids extraction (PLE) [43]. In this section, common extraction methods for capsaicinoids are reviewed and discussed.

2.1. Enzymatic treatment

Enzymatic processes have been proposed to increase the yield and selectivity during extraction from fruits [44]. In a study conducted by Santamaria et al [38], various commercially available enzymes were used to soften the tissues in C peppers and increase the extraction yield by 7%, with the final recovery of 80% of capsaicinoids. Enzymes used in this research include olivex (mainly pectinase), cellulast (mainly

cellulase), viscozyme L (mainly carbohydrase), and peczyme 5XAL (mainly pectin esterase and arabanase). The treatment took place at 50°C, required 7 hours of agitation in a rotary shaker at 120 rpm, and the ratio of chili powder to water was 1:50. Later, a similar treatment method was adopted by Desikacharya et al [45] using extrazyme (mainly pectinase and multiple carbohydrases) and energex (mainly glucanase), which increased capsaicinoid extraction yield by 32%. In this case, the temperature was controlled at 3°C for 12 hours, and the ratio of chili powder to water was 1:1. Based on the treatment methods stated above, Salgado-Roman et al [46] proposed a noncommercial enzymatic treatment using the enzymatic extracts derived from *Rhizopus nigricans*. After the enzymatic degradations, the chili fruit was dehydrated in a vacuum oven and later got milled. Then powdered samples were extracted in a SOX system with tetrahydrofuran at 60°C. A higher extraction yield above 85% was achieved for capsaicinoids, which demonstrated a more potent cellulose activity of this noncommercial enzymatic extract to soften the cell walls and facilitate the degradation of the cells.

2.2. UAE

The UAE technique is effective due to the phenomenon of cavitation occurring when an ultrasonic wave is passing through the organic solvent, producing energy to enhance the mixing and penetration of solvent into the sample matrix [40]. The application of UAE provides many advantages, such as the reduction of solvents, temperature, and time for extraction, which is very important for the extraction of thermolabile and unstable compounds [47].

Barbero et al have developed a rapid and reproducible UAE method for capsaicinoids from three varieties of peppers in Spain using 25 mL of methanol as solvent at a temperature of 50°C for 10 minutes [35]. The quantitative analysis using high-performance liquid chromatography (HPLC) is listed in Table 2.

2.3. SOX extraction

The SOX process is a traditional method that is widely applied to extract the oil from organic matrix, which is used when the desired compound has limited solubility in a solvent while the impurities are insoluble in this solvent [41]. Bajer et al [48] extracted capsaicinoids from many chili samples using the SOX method with methanol as solvent and an extraction time of 2 hours. The extraction result is listed in Table 2. The same SOX method was used in a study by Liu et al [53], in which extraction of a 1.0 g of *Capsicum annuum* sample was performed with 50-mL methanol for 2 hours. Although SOX is the conventional method for extraction, it has disadvantages such as relative longer extraction time, higher energy consumptions, and lower yields of capsaicinoids compared with other extraction methods, such as UAE, microwave-assisted extraction (MAE), and PLE.

2.4. Supercritical fluid extraction

Supercritical fluids are substances at pressures and temperatures above their critical values, which are strong solvents for nonpolar compounds [55]. After pressure is adjusted to

Table 2 – Quantification of capsaicinoids extracted from peppers through different extraction methods.

Method	Solvent	Conditions	Pepper	C	DHC	n-DHC	h-C	h-DHC	Unit	Reference
UAE	Methanol	Temp: 50°C; Time: 10 min; Pressure: 1 atm (14.696 psi).	Cayenne Bolilla Redondo pepper Bollila Largo pepper	448 ± 28 370 ± 23 275 ± 17	265 ± 15 190 ± 11 122 ± 7	94 ± 6 40 ± 3 25 ± 2	30 ± 1 n.d. n.d.	47 ± 2 20 ± 1 14 ± 1	μmol/kg of fresh pepper	[35]
SOX	Methanol	Time: 2 h; Pressure: 1 atm (14.696 psi).	Trinidad Scorpion Moruga fruit Yellow Bedder fruit Ring of Fire fruit Jamaican Hot Red fruit Yellow Habanero fruit Tabasco fruit Chiltepin fruit Bhut Jolokia spice Trinidad Scorpion Moruga spice Fatalii Red spice	42.88 ± 0.403 2.49 ± 0.09 1.74 ± 0.06 2.08 ± 0.08 0.54 ± 0.02 3.19 ± 0.03 0.29 ± 0.02 8.50 ± 0.07 20.42 ± 0.09	18.09 ± 0.16 2.53 ± 0.09 1.73 ± 0.04 1.17 ± 0.06 0.41 ± 0.02 2.50 ± 0.09 0.22 ± 0.02 5.74 ± 0.05 12.26 ± 0.07	0.42 ± 0.03 0.29 ± 0.02 0.51 ± 0.02 0.20 ± 0.02 0.027 ± 0.01 0.94 ± 0.04 0.07 ± 0.01 0.18 ± 0.02 0.45 ± 0.02	n.d. n.d. n.d. n.d. n.d. n.d. n.d. n.d. n.d.	n.d. n.d. n.d. n.d. n.d. n.d. n.d. n.d. n.d.	g/kg of dried ground sample	[48]
	Ethyl acetate Dichloromethane Ethyl ether Hexane	Time: 6 h; Temp: 25°C	Malagueta pepper (<i>Capsicum frutescens</i> L.)	10.64 ± 0.10 2.16 ± 0.20 2.27 ± 0.30 1.76 ± 0.12 1.88 ± 0.17	3.06 ± 0.09 1.20 ± 0.09 1.22 ± 0.17 0.97 ± 0.07 1.05 ± 0.09	0.10 ± 0.01 0.10 ± 0.01 0.10 ± 0.015 0.08 ± 0.003 0.09 ± 0.015	n.d. n.d. n.d. n.d. n.d.	n.d. 0.04 ± 0.003 0.04 ± 0.006 0.03 ± 0.006 0.03 ± 0.004	g/kg of dried sample	[49]
SFE	Carbon dioxide	Temp: 40°C; Pressure: 25 MPa (3625.94psi)	Dedo de moça pepper	0.88 ± 0.11	0.37 ± 0.04	0.06 ± 0.01	0.04 ± 0.00	0.01 ± 0.00	g/kg of raw material	[50]
	Carbon dioxide	Temp: 40°C; Pressure: 15 MPa (2175.57 psi) Temp: 50°C; Pressure: 15 MPa (2175.57 psi)	Biquinho peppers (<i>C. chinense</i>) Biquinho peppers (<i>C. chinense</i>)	0.30 ± 0.01 0.22 ± 0.001	0.075 ± 0.007 0.042 ± 0.001	n.d. n.d.	n.d. n.d.	n.d. n.d.	g/kg of dried ground sample	[51]

SFE + US	Carbon dioxide	Ultrasound power: 600 W; Temp: 40°C; Time: 80 min; Pressure: 25 MPa (3625.94psi)	Dedo de moça pepper	0.94 ± 0.09	0.39 ± 0.04	0.06 ± 0.00	0.04 ± 0.00	0.01 ± 0.00	g/kg of raw material	[50]
	Carbon dioxide	Ultrasound power: 360 W; Temp: 40°C; Time: 60 min; Pressure: 15 MPa (2175.57 psi)	Malagueta pepper (<i>Capsicum frutescens</i> L.)	1.93 ± 0.05	1.01 ± 0.03	0.07 ± 0.021	n.d.	0.03 ± 0.003	g/kg of raw material	[49]
PLE	Methanol; ethanol; water	Temp: 200°C; Pressure: 10 atm (146.96 psi)	Long marble pepper	369.8 ± 23.3	190.1 ± 10.9	40.3 ± 2.7	n.d.	19.7 ± 0.9	μmol/kg of fresh pepper	[52]
			Round marble pepper	275.2 ± 17.3	122.5 ± 7.0	25.3 ± 1.7	n.d.	14.5 ± 0.7		
	Methanol	Temp: 100°C; Pressure: 1500 psi	Capsicum annuum samples	0.75	0.34	0.13	n.d.	n.d.	g/kg of dried pepper	[53]
				1.17	0.68	0.14	n.d.	n.d.		
				0.73	0.32	0.064	n.d.	n.d.		
				0.56	0.24	0.047	n.d.	n.d.		
	Water	Temp: 200°C; Pressure: 20 MPa (2900.75 psi)	Trinidad Scorpion	46.45 ± 0.41	15.54 ± 0.16	0.30 ± 0.02	n.d.	n.d.	g/kg of dried ground sample	[48]
			Moruga fruit							
			Yellow Bedder fruit	3.96 ± 0.09	3.10 ± 0.09	0.50 ± 0.03	n.d.	n.d.		
			Ring of Fire fruit	1.86 ± 0.06	1.82 ± 0.04	0.61 ± 0.03	n.d.	n.d.		
			Jamaican Hot Red fruit	2.55 ± 0.08	1.35 ± 0.05	0.26 ± 0.02	n.d.	n.d.		
			Yellow Habanero fruit	0.74 ± 0.03	0.51 ± 0.02	0.02 ± 0.01	n.d.	n.d.		
			Tabasco fruit	3.94 ± 0.04	2.70 ± 0.09	1.07 ± 0.05	n.d.	n.d.		
			Chiltepin fruit	0.31 ± 0.02	0.22 ± 0.02	0.08 ± 0.01	n.d.	n.d.		
			Bhut Jolokia spice	9.13 ± 0.08	4.83 ± 0.08	0.22 ± 0.02	n.d.	n.d.		
			Trinidad Scorpion	20.26 ± 0.21	10.57 ± 0.10	0.52 ± 0.03	n.d.	n.d.		
			Moruga spice							
			Fatalii Red spice	12.40 ± 0.10	3.12 ± 0.09	0.14 ± 0.01	n.d.	n.d.		
MAE	Ethanol	Temp: 125°C; Time: 5 min Pressure: 1 atm (14.696 psi)	Cayenne	451.6 ± 32.8	265.4 ± 18.1	93.8 ± 6.6	29.6 ± 1.7	46.9 ± 2.4	μmol/kg of fresh pepper	[54]
			Long marble pepper	378.8 ± 24.3	185.6 ± 10.3	40.3 ± 2.7	n.d.	18.9 ± 0.8		
			Round marble pepper	265.2 ± 16.8	132.4 ± 8.0	23.2 ± 1.4	n.d.	15.3 ± 0.6		

C = capsaicin; DHC = dihydrocapsaicin; h-C = homocapsaicin; h-DHC = homodihydrocapsaicin; MAE = microwave-assisted extraction; n.d. = not detected; n-DHC = nordihydrocapsaicin; PLE = pressurized liquids extraction; SFE = supercritical fluid extraction; SOX = Soxhlet; UAE = ultrasound-assisted extraction; US = ultrasound.

ambient pressure, the supercritical fluids will return to the gas phase and evaporate without leaving solvent residues. Supercritical fluids extraction (SFE) has been used as an alternative to traditional extraction method during the extraction of bioactive compounds with the advantage of moderate temperatures, reduced energy consumptions, and high purity extracts [50]. Carbon dioxide (CO₂) is frequently used as the supercritical solvent for extraction of capsaicinoids due to its low cost, nontoxicity, nonflammability, inertness, and high extraction capacity [42,51,56].

Santos et al [49] extracted capsaicinoids from the mala-gueta pepper (*Capsicum frutescens* L.) using SFE assisted with ultrasound with CO₂ as the solvent at pressure, temperature, and flow rate of 15 MPa, 40°C, and 1.673×10^{-4} kg/s, respectively. The enhanced SFE rate was achieved when the ultrasound power was applied at 360 W during 60 minutes (Table 2). Later in 2016, Dias et al [50] performed a similar SFE test on dedo de moça pepper with (25 MPa, 40°C, 600W, and 80 min) and without (25 MPa, 40°C) application of ultrasound. The CO₂ flow rate was kept constant at 1.7569×10^{-4} kg/s. Results showed that the global yield of SFE was successfully increased. In summary, the application of ultrasound can increase the SFE yield of capsaicinoids from peppers, which can work as an alternative for traditional extraction techniques that use toxic organic solvents.

2.5. PLE

The operation of PLE is often conducted at a high temperature and pressure, enabling high solubility of compound in the solvent while keeping the solvent below its boiling point, and therefore resulting in a high penetration of the solvent into the sample matrix [43,57]. Many researches have adopted the PLE method in the extraction of capsaicinoid from hot peppers [48,52,53]. Barbero et al developed a PLE method with the extraction solvent of water, methanol, and ethanol; temperature of 200°C and pressure of 100 atm. The result was analyzed using HPLC-mass spectrometry (MS) [52]. According to an experiment conducted by Liu et al [53], three capsaicinoids (C, DHC, and n-DHC) were extracted from dried *C. annuum* samples through the PLE method with methanol as the solvent; temperature at 100°C and pressure at 1500 psi, combined with LC tandem MS as the quantitative analysis method. Pressurized hot water extraction method was also used to extract capsaicinoids from 10 chili samples following the procedures reported by Bajer et al [48]. In this assay, water was selected as the environmentally friendly solvent and heated to 200°C at a pressure of 20 MPa. The quantitative analysis was performed by HPLC-MS. They also compared the extraction efficiency of three capsaicinoids (C, DHC, and n-DHC) through different extraction methods (UAE, MAE, PLE, and SOX) and found that the highest yields were achieved using PLE (Table 3).

2.6. MAE

The technique of MAE is developed through the combination of microwave and traditional solvent extraction, which applies the energy generated through microwave radiation to heat the solvents and increase the kinetic of extraction. MAE has been used for the extraction of capsaicinoids from

Table 3 – Extraction efficiency (%) of capsaicinoids from *Capsicum annuum* sample using different extraction methods [53].

Extraction method	C	DHC	n-DHC
UAE	85.26 ± 1.35	89.46 ± 1.31	86.72 ± 1.31
MAE	86.36 ± 1.12	88.26 ± 1.21	87.46 ± 1.27
PLE	98.31 ± 1.46	97.27 ± 1.13	97.91 ± 1.05
SOX	88.31 ± 1.03	87.32 ± 1.22	1.13

C = capsaicin; DHC = dihydrocapsaicin; MAE = microwave-assisted extraction; n-DHC = nordihydrocapsaicin; PLE = pressurized liquids extraction; SOX = Soxhlet; UAE = ultrasound-assisted extraction.

peppers in many studies [39,54,58]. According to Williams et al [39], the capsaicinoids yield through the MAE method doubled and the extraction time was significantly shortened compared with traditional reflux and shaking flask extraction methods. MAE conditions for the extraction of capsaicinoids from fresh pepper samples were optimized by Barbero et al [54]. In this study, extraction conditions of 125°C extraction temperature, 0.5-g triturated pepper in 25-mL solvent (ethanol), 500 W of power, and 5 minutes' extraction time was found to be optimum. The authors also compared the extraction efficiency of commonly used methods such as magnetic stirring, and confirmed that MAE is a much faster method. Chuichulcherm et al [58] made a comparison of three different extraction techniques (SOX, MAE, and UAE; Table 4). The amount of capsaicinoids derived from SOX, MAE, and UAE methods at each optimum condition were 5.243-mg/g, 5.282-mg/g, and 4.014-mg/g dried chili, with the extraction time of 300 minutes, 20 minutes, and 20 minutes, respectively. The results showed that the MAE method generated highest amount of capsaicinoids with 20-minute extraction time and medium energy consumption, while SOX gave the highest energy consumption with extraction time of 300 minutes. The UAE method had the minimum energy consumption per capsaicinoids and shortest extraction time among three methods.

3. Bioavailability of capsaicinoids

3.1. Absorption and metabolism

The absorption, distribution, metabolism, and elimination of capsaicinoids (mainly C and DHC) have been reported for a long time [59–63]. According to Kawada et al [63], about 85% C and DHC were rapidly absorbed from the stomach and small intestine after administration to male Wistar rats. The absorbance efficiency of 1mM C in the stomach, jejunum, and ileum was 50%, 80%, and 70%, respectively, indicating that absorption of C was higher in the small intestine than in the stomach. They suggested that a small amount of DHC was hydrolyzed to vanillylamine and 8-methylnonanoic acid when passing through the epithelial cells of the jejunum after absorption. The majority of C and DHC were metabolized in the liver after being transported via the hepatic portal vein. In a similar study, Donnerer et al [59] examined the metabolism and absorption of capsaicinoids in the anesthetized male

Table 4 – Energy consumption to capsaicinoids ratio of *Capsicum frutescens* Linn using different extraction methods [58].

Extraction method	Extraction time (min)	Energy consumption (kJ)	Capsaicinoid (mg/g dried chili)	Energy consumption per capsaicinoid (kJ/mg)
UAE	20	102	4.014	25.411
MAE	20	384	5.282	72.700
SOX	300	21600	5.243	4119.779

MAE = microwave-assisted extraction; SOX = Soxhlet; UAE = ultrasound-assisted extraction.

Table 5 – Tissue distribution of orally administered capsaicin in rats at dosage of 30 mg/kg body weight (n = 6) [62].

Time (h)	Serum (μg/mL)	Blood (μg/total blood)	Liver (μg/whole tissue)	Kidney (μg/whole tissue)	Intestine (μg/whole tissue)
1	1.90 ± 0.18	11.11 ± 1.05	24.7 ± 2.1	3.61 ± 0.32	1057.0 ± 157.0
3	1.47 ± 0.09	8.59 ± 0.53	44.7 ± 3.37	5.71 ± 0.33	700.2 ± 42.2
6	0.93 ± 0.10	4.85 ± 0.59	14.8 ± 1.50	6.73 ± 0.45	249.3 ± 24.0
24	0.05 ± 0.01	0.29 ± 0.06	8.71 ± 2.55	3.35 ± 0.45	43.5 ± 3.75
48	0.006 ± 0.001	0.035 ± 0.006	0.60 ± 0.03	0.48 ± 0.09	1.14 ± 0.21
96	0.00	0.00	0.045 ± 0.005	0.00	0.72 ± 0.01
192	0.00	0.00	0.00	0.00	0.00

Sprague–Dawley rats through intragastric administration. They reported that C and DHC were almost completely metabolized in the liver before entering the systematic and general circulation. Recently, Kuzma et al [60] analyzed the intestinal absorption and metabolism of capsaicinoids in male Wistar rats using ex-vivo perfusion of standard *Capsicum* extraction through the proximal jejunum. Results showed that capsaicinoids were fast absorbed in the jejunum and metabolized into C glucuronide and DHC glucuronide by the UDP-glucuronyltransferase enzymes, which were then excreted back into the intestinal lumen. While hepatic metabolism of C and DHC had been illustrated in previous studies, this study reported for the first time the detailed intestinal metabolism of two capsaicinoids.

The hepatic metabolism of C was described in a work by Chanda et al [64], in which the biotransformation of C in rat, dog, and human hepatic microsomes and S9 fractions was examined. Five primary metabolites were detected after incubations. Major side chain-hydroxylated metabolites of C included 16-hydroxycapsaicin and 17-hydroxycapsaicin. 16,17-Dehydrocapsaicin was produced by oxidation of C or dehydration of the hydroxylated metabolites. Vanillylamine was generated by hydrolysis of the amide bond of C, part of which was further metabolized to form vanillin. Metabolism of capsaicinoids by cytochrome p450 enzymes was also reported by Reilly et al [65,66]. 5,5'-dicapsaicin was identified as a novel metabolite of C, indicating that P450 enzymes were also capable of oxidizing C to produce free radical intermediates.

The in-vitro and in-vivo metabolism of DHC in rats was studied by Kawada et al [63]. Forty-eight hours after the oral administration of DHC in male Wistar rats at a dose of 20 mg/kg body weight, the unchanged DHC (8.7% of the total dose) and its metabolites were detected in urine, which included vanillylamine (4.7%), vanillin (4.6%), vanillyl alcohol (37.6%), and vanillic acid (19.2%). In addition, 10% of unchanged DHC was also identified in feces. The in-vitro study was performed using cell-free extracts of rat liver, which contained DHC-hydrolyzing enzymes to transform DHC to vanillylamine and

8-methylnonanoic acid. The vanillylamine was further transformed to vanillin in situ.

3.2. Tissue distribution and elimination

The study of in-vivo tissue distribution and subsequent elimination after oral administration of capsaicin to Wistar male albino rats (30 mg/kg of body weight) were carried out by Suresh et al [62]. During each time interval at 1 hour, 3 hours, 6 hours, 1 day, 2 days, 4 days, and 8 days following the oral gavage of C, six rats were sacrificed and serum were separated from blood samples for HPLC analysis. Liver, kidney, and intestine were excised for distribution study. Urine and fecal samples were collected for elimination study. According to the tissue distribution result in Table 5, the highest concentration was shown at 1 hour in blood and the intestine, 3 hours in the liver, and 6 hours in the kidney. The total concentration of 24.4% of administered C was seen after 1 hour, which was reduced to 1.24% in 24 hours and 0.057% in 48 hours. After 96 hours, no C was detected in all tissues.

The elimination result of orally administered C is shown in Table 6. Within 4 days, the amount of C excreted in feces and urine was 6.34% and 0.095%, respectively. Therefore, about 94% of C was absorbed through oral administration. After 5 days, no C was detected in urine and feces. This result was consistent with a previous bioavailability study [63], which

Table 6 – Elimination of orally administered capsaicin in rats at a dosage of 30 mg/kg body weight (n = 6) [62].

D	Feces	Urine
1	174.0 ± 11.3	4.05 ± 0.45
2	99.8 ± 5.03	0.225 ± 0.035
3	11.3 ± 1.25	0
4	0.375 ± 0.032	0
5	0	0
Total	285.5 (6.34% of administered dose)	4.275 (0.095% of administered dose)

proved that 85% of capsaicinoids were quickly absorbed from the gastrointestinal tract following oral administration.

4. Summary

In this review, common extraction methods for capsaicinoids were examined, including enzymatic pretreatment, UAE, SOX, PLE, SFE, and MAE. Recently, combined methods have been developed to further enhance the extraction yields of capsaicinoids and reduce energy consumption, such as SFE assisted by ultrasound, and multiple-stage extraction methods, etc.

Bioavailability of capsaicinoids were reviewed and explained. After oral administration, C and DHC are absorbed in the gastrointestinal tract and almost completely metabolized in the liver. Biological studies have shown that capsaicinoids has antiinflammation, anticancer, antioxidation, pain relief, cardioprotective, and antiobesity effect. Therefore, these nutraceuticals can be further developed into multi-functional foods with great potential in the food industry. However, how to reduce the extreme pungency and enhance the bioavailability of capsaicinoids should be a major focus in future work.

Conflicts of interest

The authors declare no conflicts of interest.

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