

# Bioactive properties and current trends in the application of pepper extracts in encapsulation technology: A review

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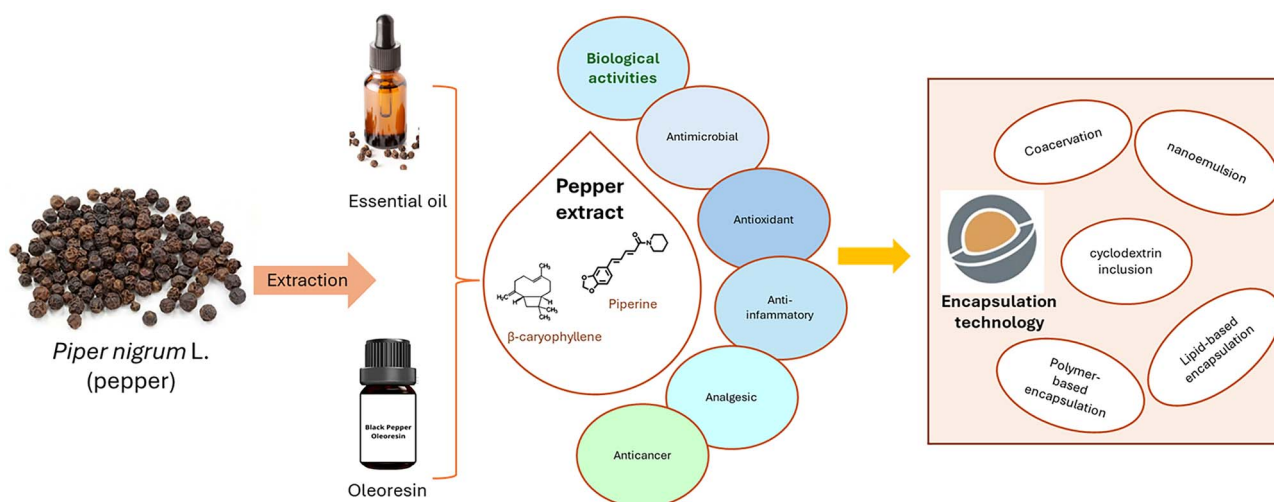
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## Abstract

*Piper nigrum* L. (pepper), a member of the Piperaceae family, is among the most widely used spices worldwide. The essential oil and oleoresin extracted from pepper contain numerous bioactive compounds, such as piperine,  $\beta$ -caryophyllene, sabinene,  $\beta$ -pinene, and limonene. These compounds contribute to the antioxidant, anti-inflammatory, antimicrobial, antifungal, and anticancer activities of pepper extracts. Recent studies have focused on the application of pepper extracts in encapsulation technology. The bioactive compounds in pepper extracts can be encapsulated using various methods such as coacervation, nanoemulsion, polymer-based and lipid-based and hybrid systems encapsulation, and cyclodextrin inclusion, producing micro- or nano-sized particles. This encapsulation enhances the stability and bioactivity of the compounds and allows controlled release during subsequent applications. Additionally, micro- and nano-capsules provide effective protection during food processing as well as oral and gastric digestion, opening promising prospects for their use as food preservatives and functional ingredients.

**Keywords:** *Piper nigrum* L., essential oil, oleoresin, encapsulation, nanoemulsion, coacervation, cyclodextrin

## Graphical abstract



Received: 9 September 2025. Accepted: 8 December 2025

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## Introduction

*Piper nigrum* L. (pepper), often called the “King of Spices” (Nair, 2011), originates from the Malabar region in Southern India (Kapoor et al., 2009). It belongs to the *Piperaceae* family and is widely used for its distinct aroma and spiciness. This spice has a long history of use in both cooking and traditional medicine (Deans, 2001). Black pepper is recognised for its gastrointestinal benefits (Gülçin, 2005), as well as its antioxidant, antimicrobial, anti-inflammatory, and antipyretic properties (Szallasi, 2005; Zhang et al., 2021). Pepper contains many biologically active compounds, such as lignans, alkaloids, flavonoids, amides (Jirovetz et al., 2002), and aromatic compounds, including terpenes and phenolic compounds (Al-Khayri et al., 2022; Lee et al., 2020a). The value of pepper is attributed to its pungency and flavour, which are due to the presence of a naturally occurring alkaloid known as piperine, a main compound in oleoresin, as well as essential oil (EO) (Gorgani et al., 2017a).

In food products, EO and oleoresin of pepper have been frequently studied and applied in food products for their biological properties (Procopio et al., 2022). EO and oleoresin of pepper have high antioxidant activity and can inhibit lipid oxidation in meat products (Bi et al., 2019; Martínez et al., 2006; Mohammadi et al., 2022; Tipsrisukond et al., 1998; Zhang et al., 2016). Besides, pepper extracts have been proven to strongly inhibit food-borne pathogens such as *Escherichia coli* (Chen et al., 2018), *Staphylococcus aureus* (Safari et al., 2023), *Salmonella typhimurium*, and *Listeria monocytogenes* (Tang et al., 2017). Beyond their antimicrobial actions, pepper extracts are also recognised for their therapeutic potential, particularly their antitumor and anticancer properties (Cho et al., 2024; Khongkarat et al., 2024; Zhang et al., 2022). Recent studies have demonstrated that the encapsulation of EOs and oleoresins derived from pepper is an effective strategy for preserving food and functional materials (Bawazeer et al., 2022; Bhatia et al., 2024). The application of bioactive compounds on a micro- or nanometric scale enhances their stability and bioavailability and supports their controlled release (de Souza et al., 2025).

This review aims to provide an overview of the current literature on pepper, including its bioactive compounds, biological properties, and potential applications in encapsulation technology. Additionally, future research directions for pepper extracts are also proposed.

## *Piper nigrum* L. (pepper)

Pepper is believed to have originated from wild species through domestication or selection in Southern India (Ravindran, 2000). Over time, its cultivation spread to other parts of South and Southeast Asia (Ramakrishnan Nair & Dutta Gupta, 2003), particularly in tropical regions (Ravindran, 2000). According to the Food and Agriculture Organisation, most of the world’s black pepper is produced in Asia (Indonesia, India, China, Malaysia, Sri Lanka, and Tajikistan), with Vietnam being the leading producer, growing 288,167 tons in 2021 (Wimalarathna et al., 2024).

The fruit of the pepper plant has a complex structure with two main parts: an outer one and an inner one. This directly affects the arrangement of bioactive compounds (Myszka et al., 2019). The outer part of the fruit contains EOs and polyphenols (responsible for the fruit’s colour change from black to green), while the inner part is rich in starch and piperine (Attokaran, 2017). The quality of pepper is primarily determined by its piperine and EO content (Ravindran & Kallapurackal, 2012). The primary components of pepper are starch (41.24%), fibre (30.1%), and protein

(13.25%) (Pradeep et al., 1993). The pepper also contains relatively low amounts of moisture (4.7%) and crude fat (1%–9%) (Gorgani et al., 2017b). Palmitic (16%–30%), oleic (18%–29%), linoleic (25%–35%), and linolenic acid (8%–19%) are the most common fatty acids (Ravindran & Kallapurackal, 2012). Pepper provides potassium (663 mg/100 g), calcium (195 mg/100 g), phosphorus (162.8 mg/100 g), and magnesium (162.8 mg/100 g) (Al-Jasass & Al-Jasser, 2012; Pradeep et al., 1993). Lee et al. (2020b) concluded that the total polyphenol content in pepper was 1,046 mg GAE/100 g, and the total flavonoid content was 705 mg CE/100 g (Lee et al., 2020b).

The EO and oleoresin are responsible for the pepper’s characteristic aroma and pungency. The EO contributes to its distinctive fragrance (Singh et al., 2013), while oleoresin is responsible for the sharp, spicy heat and pungent flavour (Gorgani et al., 2017a). Both EO and oleoresins of pepper have been shown to possess potent antioxidant (Kapoor et al., 2009) and antimicrobial (Morsy & Abd El-Salam, 2017) activities, in addition to anti-inflammatory and anticancer effects. As a result, they hold promise for use as natural preservatives in food products and as functional materials in pharmaceutical applications (Acharya et al., 2024; Jeena et al., 2014; Wang et al., 2025a).

## Extraction methods and bioactive compounds

Several extraction techniques have been used to extract EO and oleoresin from *P. nigrum* L. So far, the volatile compounds of pepper have been extracted using conventional methods (distillation and solvent extraction) and nonconventional methods (including ultrasound, microwave, and enzyme-assisted extraction; supercritical fluid extraction; solid-phase microextraction; solvent-assisted flavour evaporation; and the purge-and-trap technique) (El Asbahani et al., 2015; Chandran et al., 2012; Guan et al., 2007; Jagella & Grosch, 1999; Liu et al., 2007).

Distillation is one of the oldest yet most frequently used methods for extracting EOs from plant materials, including steam distillation and water distillation methods (Bakkali et al., 2008). Plant materials are exposed to boiling water or steam to release the EO within them through evaporation. The process may last 1 to 10 hr, with steam and EOs being condensed and collected in a vessel (Stratakos & Koidis, 2016). The amount of oil produced depends on the length of distillation time, temperature, pressure, and type of plant material (Cannon et al., 2013). Although the extraction of EO by distillation appears to be a straightforward process, it has several drawbacks. For example, the prolonged exposure of EO to boiling water can change the composition of the volatile oils being extracted (Stratakos & Koidis, 2016).

Recently, ultrasound, microwave, and enzyme-assisted extraction of bioactive phytochemicals have attracted researchers’ tremendous interest (El Asbahani et al., 2015; Chandran et al., 2012). Ultrasound-assisted extraction (UAE) is employed to extract bioactive phytoconstituents by using high-frequency sound waves (20–50 kHz) to generate small pores in plant cell walls (Şahin & Şamlı, 2013; Tiwari, 2015). This process induces significant mechanical and thermal effects, which facilitate the release of phytoconstituents from plant materials (Thomas et al., 2015). Ultrasound-assisted extraction offers substantial improvements in efficiency, quality, and sustainability compared to conventional methods. It is proved that UAE achieved higher extraction yields in drastically reduced processing times (Milenković et al., 2025). Furthermore, UAE is critical for preserving thermolabile aromatic

and bioactive compounds that would otherwise be degraded by the high heat of traditional distillation (Mungwari et al., 2025). A comparative analysis of extraction methods for black pepper revealed that UAE yielded the highest total phenolic content, measuring 85.64 mg GAE/g (Milenković et al., 2025). On the other hand, microwave-assisted extraction can enhance yield and efficiency. Under the effect of non-ionising electromagnetic waves with frequencies of 300 MHz–300 GHz, polar molecules such as water inside plant cells are instantly heated by microwaves. This generates evaporation that creates tremendous pressure in the plant cell walls. This pressure pushes and stretches the cell wall and finally destroys it, which facilitates the leaching out of phytochemicals from the broken cells and thus improves the extraction yield (Mandal et al., 2007). Studies comparing extraction methods for black pepper EO consistently show that microwave-assisted hydrodistillation (MHD) provides superior yields compared to traditional methods. For example, Wang et al. (2009) found that MHD produced a 3.8% yield from Chinese black pepper, significantly higher than the 3.1% obtained via conventional hydrodistillation. This finding is supported by Rmili et al. (2014), who also reported that MHD (1.45%) was more efficient, yielding more EO than hydrodistillation (1.24%). Additionally, enzymatic treatment using cellulase, pectinase, hemicellulose, or a complex of enzymes targeting various carbohydrates is necessary to break down the cell wall, boost its permeability, promote oil release, and improve the extraction efficiency of target compounds from the plant matrix (Mungwari et al., 2025). In a study on black pepper, pre-treatment with a multi-enzyme mixture (Lumicellulae) effectively increased the EO yield and significantly improved the recovery of its primary bioactive component. GC–MS analysis demonstrated that the concentration of  $\beta$ -caryophyllene, increased markedly from 15.03% (control) to 25.58% after enzyme application (Chandran et al., 2012).

Extraction with supercritical fluids, such as carbon dioxide, preserves a complete and unaltered flavour of EO (Herrero et al., 2010). It is a suitable method for thermally sensitive compounds, as the extraction is performed at a relatively low temperature (at near ambient critical temperature, around 31 °C) (Majik & Gawas, 2023; Myszka et al., 2019; Stratakos & Koidis, 2016). Additionally, supercritical fluids have lower viscosity and higher diffusivity, allowing them to penetrate porous solid materials more effectively compared to liquid solvents. This results in faster mass transfer and more rapid extraction compared to traditional solvent extraction methods (Otterbach & Wenclawiak, 1999). However, the complexity and high cost of the apparatus limit its use (Herrero et al., 2010).

The summarisation of extraction techniques for pepper extracts and their bioactive components is shown in Table 1. Studies suggest that differences in extraction methods can significantly impact the yield and composition of bioactive compounds and subsequently influence the antioxidant and antimicrobial activities of pepper extracts (Ozdemir et al., 2018). The yield of EO and oleoresin from pepper has varied between 1.24% and 5.06%, and 4.27% and 12.73%, respectively (Kurian et al., 2002; Sruthi et al., 2013). Previous studies have shown that the main compounds in pepper EO were  $\beta$ -caryophyllene, limonene, and sabinene (Jeena et al., 2014; G. Singh et al., 2004; Teneva et al., 2016; Wang et al., 2018). Meanwhile, pepper oleoresins have been demonstrated to possess some key compounds such as piperine,  $\beta$ -caryophyllene, sabinene, and limonene (Attokaran, 2017; Zachariah & Parthasarathy, 2008). Piperine, the primary alkaloid found in pepper, constitutes between 2.13% and 5.80%

of its seed composition (Hussain et al., 2017; Kurian et al., 2002; Rmili et al., 2014; Sruthi et al., 2013).

## Biological activities of pepper extracts

### Oleoresin

Volatile oil accounts for approximately 15% to 20% in pepper oleoresin, while piperine accounts for 35% to 55% (Milenković & Stanojević, 2021). The primary alkaloid compound in oleoresin, piperine, is a solid, water-insoluble substance (Badmaev et al., 1999; Kumoro et al., 2009).

Piperine has been shown to effectively inhibit Gram-positive bacteria, including *S. aureus* and *Bacillus subtilis* (Hikal, 2018), and significantly inhibit the growth of Gram-negative bacteria such as *Salmonella spp.* and *Proteus mirabilis* (Alves et al., 2023). Moreover, black pepper oleoresin exhibits analgesic properties, effectively alleviating mild aches and muscle pain (Ashokkumar et al., 2021).

Beyond its antimicrobial and analgesic actions, piperine is recognised for its anti-cancer potential. Both black pepper extract and pure piperine signify anticancer potential, thus proposing black pepper as a potent nutraceutical for preventing the progression of chronic myeloid leukaemia (Banerjee et al., 2021). Besides, piperine has been reported to exhibit anti-cancer activity across various cancer cell lines (Mitra et al., 2022). Recent studies have demonstrated that piperine treatment significantly inhibits tumour growth in several types of cancer, including prostate cancer (Samyikutty et al., 2013; Yakubu et al., 2025), osteosarcoma (Gokul & Vivekkumar, 2023; Zhang et al., 2015), lung cancer (Cho et al., 2024; Lin et al., 2014), melanoma (Khongkarat et al., 2024; Yoo et al., 2019), and breast cancer (Ahmadi et al., 2023; Elimam et al., 2022). Additionally, piperine demonstrates considerable potential as a therapeutic agent for the management of chronic inflammatory conditions, such as arthritis, joint pain, and autoimmune disorders (Pop et al., 2024).

Pepper oleoresin has been highlighted for its efficacy against various pathogens, suggesting its potential as a natural preservative in food applications (Ozdemir et al., 2018). Studies have demonstrated its antimicrobial efficacy against a variety of pathogens, including Gram-positive bacteria such as *S. aureus*, *Bacillus cereus*, *B. subtilis*, and *Enterococcus faecalis* (Martinelli et al., 2017; Morsy & Abd El-Salam, 2017; Singh et al., 2013; Stoyanova et al., 2006), and Gram-negative bacteria such as *E. coli*, *Pseudomonas aeruginosa*, and *S. typhimurium* LT2 (S. Singh et al., 2013; Teixeira et al., 2013).

In addition to antibacterial activity, pepper oleoresin also exhibits strong antifungal effects. It has been reported to inhibit the growth of several fungal species, including *Penicillium viridicatum*, *Aspergillus ochraceus*, *Candida albicans*, *Fusarium graminearum*, *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, and *Fusarium moniliforme* (Martinelli et al., 2017; Morsy & Abd El-Salam, 2017; Singh et al., 2004; Singh et al., 2013). A study by Martinelli et al. (2017) further revealed that black pepper oleoresin extracted with acetone exhibited a minimum inhibitory concentration (MIC) of 1% against *B. cereus*. Interestingly, white pepper oleoresin showed superior antimicrobial activity compared to black pepper oleoresin in the same study (Martinelli et al., 2017).

Recent studies have demonstrated that pepper oleoresin exhibits considerable antioxidant activity, playing a vital role in reducing lipid peroxidation (Wang et al., 2021) and protecting against the degradation of fats in both food and biological systems (Bi et al., 2017). The antioxidant activity is mainly attributed to the phenolic compounds present in black pepper, which have demonstrated effective free radical scavenging properties

**Table 1.** Extraction methods and bioactive compounds in pepper extracts.

Pepper extracts	Extraction methods	Operating parameters	Main compounds	Reference
Oleoresin	Aceton extraction	m(substrate):V(solvent) = 1:20 (wt/vol) temperature: 40 °C time: 3 hr	18 compounds, including main compounds: piperine (33.5%), $\beta$ -piperolein (13.7%), piperamide (3.43%), and guineensine (3.23%)	Singh et al. (2004)
	Ethanol extraction	time: 3 hr	25 compounds, including main compounds: piperine (43.5%), piperanine (5.2%), $\beta$ -piperolein (6.6%), and guineensine (4%)	Singh et al. (2013)
		m(substrate):V(solvent) = 1:5 (wt/vol) time: 3 days	28 compounds, including main compounds: caryophyllene (24.4%), limonene (15.1%), $\alpha$ -pinene (11.1%), and sabinene (11.6%).	Morsy and Abd El-Salam (2017)
	Ethyl acetate extraction	m(substrate):V(solvent) = 1:10 (wt/vol) time: 3 hr	47 compounds, including main compounds: piperine (39%), $\beta$ -caryophyllene (6.7%), piperolein (5.5%), and piperanine (5.1%)	Kapoor et al. (2009)
	n- hexane extraction	time: 3 hr	26 compounds, including the main compounds: piperine (43%) and piperolein B (5%)	Singh et al. (2013)
	Microwave refluxation extraction	time: 120 min, power: 350 W particle size: 0.105 mm m(substrate): V(solvent) = 1:4.8 (wt/vol)	23 compounds, including main compounds: piperine (44.8%), caryophyllene (10%), sabinene (5.9%), and limonene (4.4%).	Abayomi Olusegun et al. (2018)
	High pressure CO <sub>2</sub> extraction	time: 180 min temperature: 28–32 °C, pressure: 6.8–7.2 bar	44 compounds, including main compounds: $\beta$ -caryophyllene (43.9%), allo-aromadendrene (4.4%), limonene (17.1%), 3-carene (11.8), $\alpha$ -phellandrene (4.4%), sabinene (3.1%), and myrcene (3.1%)	Stoyanova et al. (2006)
	C <sub>2</sub> H <sub>2</sub> F <sub>4</sub> -laboratory-extractor	time: 20 min temperature: 19–21 °C, pressure: 5.0 bar	45 compounds, including main compounds: $\beta$ -caryophyllene (56.8%), limonene (18.9%), terpinolene (3.9%) and p-cymene (3.1%)	Stoyanova et al. (2006)
Essential oil	Ultrasound-microwave assisted hydrodistillation	m(substrate):V(solvent) = 1:10 (wt/vol) microwave power: 500 W ultrasonic power: 50 W time: 20 mins	30 compounds, including main compounds: 3-carene (33.2%), limonene (19.2%), $\beta$ -pinene (14%), and caryophyllene (13%).	Wang et al., 2018
	Hydrodistillation	time: 5 hr	49 compounds, including main compounds: $\beta$ -caryophyllene (24.2%), limonene (16.9%), sabinene (13%), $\beta$ -bisabolene (7.7%), and $\alpha$ -copaene (6.3%)	Singh et al. (2004)
		time: 2 hr	20 compounds, including main compounds: caryophyllene (23.3–36%), carene (13–20.4%), limonene (10.2%–16.1%), and $\beta$ -pinene (5.67%–9.42%).	Myszka et al. (2019)
		time: 5 hr	31 compounds, including main compounds: sabinene (21.4%) followed by $\beta$ -caryophyllene (19.2%), limonene (15.6%), $\alpha$ -pinene (11%), and carene (6.9%).	Morsy and Abd El-Salam (2017)
		time: 3 hr	43 compounds, including main compounds: $\beta$ -caryophyllene (18.6%), limonene (15%), sabinene (13.2%), $\beta$ -pinene (9.7%), carene (8.6%), and $\alpha$ -pinene (8%)	Moosavi-Nasab et al. (2016)
		time: 6 hr	40 compounds, including main compounds: $\beta$ -caryophyllene (16%), sabinene (12.6%), limonene (11.9%), and torreyol (9.3%)	Singh et al. (2013)

(Teixeira et al., 2013). Several experimental investigations have quantified the antioxidant potential of pepper oleoresin using various extraction methods and assay techniques. Dang and Phan (2014) assessed the antioxidant potential of black pepper oleoresin extracted by supercritical fluid extraction under conditions of 250 bar, 45 °C, and 2.5 hr, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and comparing it with ascorbic acid as a standard. The result indicated that the antioxidant activity of the oleoresin was relatively high, approximately 80%–90% of that of ascorbic acid. At a concentration of 80 mg/L, the pepper oleoresin exhibited an inhibition

rate of 87%, while ascorbic acid showed a 92% inhibition rate. In another study, (Aziz et al., 2018) determined the antioxidant activity of the oleoresin extract from pepper retting wastewater. The result showed that the antioxidant activity of white pepper oleoresin in DPPH was  $84.58\% \pm 4.93\%$ , while the ferric reducing antioxidant power assay was  $49.55 \pm 6.11$  mM TE/g. Furthermore, pepper oleoresin extracted by solvent extraction exhibited good antioxidant activity, better than that of butylated hydroxyanisole and butylated hydroxytoluene (BHT), but lower than that of propyl gallate (Kapoor et al., 2009; Singh et al., 2004; Singh et al., 2013). Pepper oleoresin and its primary alkaloid, piperine, serve as highly

**Table 2.** The antimicrobial activity of pepper essential oils.

Microorganism	Name	Antimicrobial activity			Reference
		MIC (mg/ml)	MBC (mg/ml)	IZ (mm)	
Bacteria	<i>Escherichia coli</i>	0.4–12.5	2.1–25	6.18	Nie et al. (2023); Nikolić et al. (2015); Rakmai et al. (2017); Teixeira et al. (2013); Zhao et al. (2024)
	<i>Staphylococcus aureus</i>	1–12.5	2–25	6.8	Nie et al. (2023); Nikolić et al. (2015); Rakmai et al. (2017); Rukayadi et al. (2013); Teneva et al. (2016); Zhao et al. (2024)
	<i>Pseudomonas aeruginosa</i>	0.07–12.8	0.31–25.6	-	Hien and Dao (2022); Nikolić et al. (2015); Rukayadi et al. (2013); Zhao et al. (2024)
	<i>Salmonella typhimurium</i>	0.9–1.25	2.0–5.0	-	Nikolić et al. (2015); Teixeira et al. (2013)
	<i>Proteus vulgaris</i>	>0.6	-	-	Nikolić et al. (2015); Teneva et al. (2016)
	<i>Micrococcus flavus</i>	0.31	0.6	-	Nikolić et al. (2015)
	<i>Streptococcus mutans</i>	0.12	>1.0	-	Rosas-Piñón et al. (2012)
	<i>Listeria monocytogenes</i>	0.31	0.6	-	Nikolić et al. (2015)
	<i>Enterobacter aerogenes</i>	0.5	-	-	Noumedem et al. (2013)
	<i>Porphyromonas gingivalis</i>	1.0	>1.0	-	Rosas-Piñón et al. (2012)
Fungi	<i>Aspergillus ochraceus</i>	2.5	5.0	-	Nikolić et al. (2015)
	<i>Aspergillus niger</i>	4.5–12.8	10–25.6	-	Nikolić et al. (2015); Rukayadi et al. (2013); Zhao et al. (2024)
	<i>Penicillium funiculosum</i>	2.5	5.0	-	Nikolić et al. (2015)
	<i>Penicillium ochrochloron</i>	2.5	5.0	-	Nikolić et al. (2015)
	<i>Penicillium aurantiogriseum</i>	2.5	5.0	-	Nikolić et al. (2015)
	<i>Trichoderma viride</i>	2.5	5.0	-	Nikolić et al. (2015)

Note. MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; IZ = inhibition zone.

effective bioactive compounds. A wealth of evidence supports their wide-ranging pharmacological properties, including strong antimicrobial (both antibacterial and antifungal), antioxidant, anti-inflammatory, analgesic, and notable antineoplastic effects. These findings highlight the potential of both the oleoresin and piperine as compounds of significant interest for future research, with promising prospects for development into novel therapeutic agents within the pharmaceutical industry, as well as functional ingredients or nutraceuticals for use in food applications.

### Essential oils

Pepper EO has been reported to exhibit potent antibacterial properties, inhibiting the growth of Gram-negative bacteria such as *E. coli*, *Salmonella spp.*, and *Proteus vulgaris*, as well as Gram-positive bacteria like *S. aureus* (Morsy & Abd El-Salam, 2017; Teneva et al., 2016). Additionally, EO demonstrates antifungal activity in inhibiting *A. niger*, *A. flavus*, *A. ochraceus*, *A. oryzae*, *F. moniliforme*, *F. graminearum*, *Penicillium citrinum*, *P. viridicatum*, *Penicillium madriti*, *P. vulgaris*, and *Curvularia lunata* (Morsy & Abd El-Salam, 2017; Singh et al., 2004; Singh et al., 2013; Zhang et al., 2021). Martinelli et al. (2017) studied the antibacterial and antifungal activities of both black and white pepper EO. The results showed that white pepper EO exhibited stronger antimicrobial activity, inhibiting the growth of *B. cereus* (MIC: 0.25%), *E. coli* (MIC: 1%), and *S. aureus* (MIC: 0.5%). In contrast, black pepper EO showed a higher antifungal effect, particularly against *C. albicans* (MIC: 0.5%). In a recent study, Sultana et al. (2022) conducted a comprehensive analysis of the chemical composition of black pepper EO using GC-MS. Their results revealed that black pepper EO also displayed a substantial inhibition zone against *Trichoderma harzianum*. The

detailed antimicrobial activities of pepper EOs are presented in Table 2.

Pepper is not only a widely used spice but also a source of EO that exhibits notable antioxidant properties. Bag and Chattopadhyay (2015) investigated the synergistic effects of several EOs, including black pepper, on antioxidant activity using the DPPH. The findings from this study emphasised the potential of combining pepper EO with other EO to improve the overall antioxidant performance, suggesting its applicability in the development of functional food products. The antioxidant capacity of pepper EO is closely linked to its chemical composition. Wang et al. (2021) found that black pepper EO is rich in limonene,  $\alpha$ -pinene,  $\beta$ -pinene, and 3-carene, while white pepper EO contains higher levels of caryophyllene. As a result, black pepper EO exhibited significantly higher total and intracellular antioxidant activity than white pepper EO. This difference in activity can be partly explained by the structural characteristics of the compounds. Caryophyllene, a sesquiterpene with a large molecular size and steric hindrance, is less effective as a reactive oxygen species scavenger than small monoterpenes (Mukai et al., 2018). Furthermore, several studies have concluded that pepper EO exhibited more effective and significant antioxidant activity than BHT and butylated hydroxyanisole, but lower than propyl gallate (Singh et al., 2004; Singh et al., 2013; Wang et al., 2021). Zhang et al. (2021) demonstrated that black pepper EO exhibited weaker antioxidant activity than BHT but showed strong antioxidant effects in inhibiting  $\beta$ -carotene discoloration, lipid peroxidation, and oxidation of linoleic acid.

Besides, pepper EO is has significant anti-inflammatory and antinociceptive effects (Jeena et al., 2014; Zhang et al., 2014).

Jeena et al. (2014) demonstrated that black pepper EO effectively reduced inflammation in both acute (carrageenan- and dextran-induced) and chronic (formalin-induced) inflammation models. Moreover, it exhibited antinociceptive effects in the acetic acid-induced writhing test in mice, indicating its potential in pain management. Beyond its anti-inflammatory effects, black pepper EO has also shown promising antitumor and anticancer activities (Majdalawieh & Carr, 2010; Zhang et al., 2022). Notably, nanoparticles formulated with pepper EO significantly inhibited the proliferation, migration, and invasion of MDA-MB-231 cells, a human breast cancer cell line (Zhang et al., 2022). Recent studies have also highlighted the potential of black pepper EO as a sustainable anti-virulence agent against drug-resistant pathogenic bacteria (Chatterjee et al., 2025), providing an alternative strategy in combating antimicrobial resistance. In addition, pepper EO possessed a dual anxiolytic and antidepressant-like effect through the possible involvement of serotonergic transmission (Ghosh et al., 2021). Based on the findings, pepper EO emerges as a highly versatile and potent natural compound with a broad array of biological activities. Its robust antimicrobial properties (including antibacterial and antifungal effects) and antioxidant capabilities position it as a valuable substance for both preservation and health-related applications. Moreover, the evidence underscores its considerable therapeutic potential, demonstrating significant anti-inflammatory, antinociceptive, antitumor, and anti-virulence properties. In addition, its promising anxiolytic and antidepressant-like effects further emphasise its potential as a valuable candidate for advanced development in pharmaceutical and functional food applications.

## Current trends in the application of encapsulation technology

Encapsulation is a technique that involves enclosing a core substance, such as an EO, within a protective matrix or shell. This protective coating, referred to as the wall material, surrounds small droplets of the EO. Functioning at both the micro- and nanoscale levels, this technology is predominantly employed as a strategy for preservation and controlled release (El Asbahani et al., 2015; Charikleia & Constantina, 2015). The primary rationale for encapsulating EOs is to address their intrinsic compositional instabilities. Due to their highly volatile and lipophilic (oil-based) nature, EOs are prone to degradation when exposed to environmental factors such as oxygen, light, moisture, and heat, resulting in diminished stability (Turek & Stintzing, 2013).

Encapsulation of EOs offers significant functional benefits. By isolating the active compounds from pro-oxidant factors, the process enhances chemical stability, preserving the potency of the oils and extending their shelf life (Jamir et al., 2025; Wang et al., 2025b). This isolation also addresses sensory challenges by mitigating the intense aromas or flavours that may be undesirable in food products, thereby improving their overall acceptability (Madhusankha et al., 2025). Beyond preservation, encapsulation is a delivery strategy, enabling the tailored, controlled release of the core material in response to defined triggers like heat, moisture, or pH changes (Bastos et al., 2020a). The functionality of the final capsule is heavily dependent on the chosen wall materials and techniques (Nezamdoost-Sani et al., 2024). A variety of encapsulation techniques are employed, spanning industrial-scale physical processes, such as spray-drying, to physicochemical methods like coacervation and advanced delivery systems, including nanoemulsions (Rezagholidzade-shirvan et al., 2024). The selection of an appropriate methodology is predicated on a careful

consideration of inherent trade-offs. For instance, while spray-drying is widely adopted due to its cost-effectiveness and scalability, the requisite high processing temperatures can induce thermal degradation of labile (heat-sensitive) compounds (Pudžiulytė et al., 2025). Conversely, coacervation, although a more complex and typically higher-cost process, frequently achieves superior encapsulation efficiency and enhanced protection of the core material (Emon et al., 2025). Analogous trade-offs govern the selection of the wall material. Biopolymers are the most frequently utilised class of encapsulants due to their advantageous film-forming properties. These include polysaccharides (maltodextrin, gum arabic, alginate, and chitosan), proteins (whey protein isolate, gelatin, and soy protein), and lipids (waxes, lecithin, and solid fats) (Díaz-Montes, 2023). Each presents distinct advantages and limitations. Polysaccharides, for example, are favoured for their low cost and compatibility with processes like spray-drying (Halahlah et al., 2023). In contrast, proteins such as whey protein or gelatin often provide superior interfacial properties, enhancing emulsification and oxidative stability (Taherian et al., 2011). However, proteins may also exhibit greater sensitivity to environmental parameters, such as pH and thermal stress, when compared to lipids (Dumetz et al., 2008). It is therefore evident that no single method or material possesses universal optimality. The selection of the most efficacious strategy depends upon the unique physicochemical properties of the specific EO and its intended application.

Drawing from this broader context, the encapsulation of pepper extract serves as a pertinent case study, illustrating the critical need to address the challenges of instability and volatility. Pepper extracts are valued for their strong, warm, and spicy aroma, which is primarily attributed to volatile terpenes such as piperine,  $\beta$ -caryophyllene, limonene, and sabinene. These compounds are highly volatile and prone to degradation during processing and storage. Therefore, encapsulation is especially crucial for preserving the bioactive compounds in pepper extracts. Encapsulating pepper extracts through techniques such as micro- and nanoencapsulation, coacervation, and nanoemulsion (Figueroa-Lopez et al., 2018; Nie et al., 2023) helps preserve their stability and efficacy. This process not only preserves the oil's sensory and antimicrobial properties but also converts it into a convenient powder, ideal for diverse applications, notably in novel food products. Table 3 provides a comprehensive overview of encapsulation technologies and their applications for pepper extracts.

## Complex coacervation

Complex coacervation is a liquid-liquid phase separation process where two polymers with opposing electrical charges are mixed in a solution. This interaction causes the polymers to separate from the solution, forming a dense, polymer-rich liquid phase called the coacervate, which can be used to encapsulate materials (Eghbal & Choudhary, 2018; Tĩmilsena et al., 2019). Bastos et al. (2020a, 2020b, 2020c) conducted a series of studies demonstrating the effectiveness of complex coacervation for encapsulating black pepper EO. They successfully used a gelatin-to-sodium alginate ratio of 6:1 at a pH of 4.0, which resulted in a high encapsulation efficiency of 82.36% (Bastos et al., 2020c). In a separate investigation using lactoferrin and sodium alginate, the researchers produced microcapsules that were highly resistant to the low pH of the stomach, ensuring the EO was released primarily during intestinal digestion (Bastos et al., 2020b). A third study by the same group used  $\beta$ -lactoglobulin and sodium alginate, finding that the resulting capsules had a low release rate during gastric

**Table 3.** The application of pepper extracts by using encapsulation technologies.

Encapsulation methods	Core material	Wall material/emulsifier/carrier	Functions and effects	Reference
Complex Coacervation	Black pepper EO	Lactoferrin and sodium alginate; Cross-linker: transglutaminase	Good protection of active ingredients, stability under oral and gastric digestion conditions.	Bastos et al. (2020b)
	Black pepper EO	Gelatin and gum arabic; Oil phase: soybean or grape seed oil	High cohesiveness and poor flowability of the microcapsules.	Napiórkowska et al. (2023)
Nanoemulsion	Black pepper EO	$\beta$ -lactoglobulin and sodium alginate; Cross-linker: transglutaminase	A low release of black pepper EO during oral and gastric digestion and a higher release in intestinal digestion.	Bastos et al. (2020a)
	Black pepper EO	Tween 80	Inhibiting the growth of <i>E. coli</i> and <i>Salmonella enterica</i> in pork samples; lower aerobic microbial counts in meat samples.	Hien and Dao (2022)
	Black pepper EO	Tween 80	Inhibiting the growth of <i>S. aureus</i> , <i>E. coli</i> .	Nie et al. (2023)
	Black pepper EO	Tween 80, milli Q water	Inhibition of biofilm formation and disruption of the cell membrane in <i>P. aeruginosa</i> ; efficacy in controlling bacterial infections in <i>Penaeus monodon</i> shrimp.	Swathy et al. (2018)
	Turmeric and black pepper extracts	Fractionated coconut oil, tween 80, glycerin, honey, sodium benzoate, sodium alginate/CMC-Na	Nanoemulsions exhibited good stability at 25 and 40 °C for four weeks and after six freeze–thaw cycles. It also significantly reduced the ulcer index in rats with gastric ulcers.	Adlia et al. (2025)
Polymer-based encapsulation	Black pepper EO	Gelatin, bacterial cellulose nanofibrils	Decreasing the lipid oxidation, inhibiting microbial growth, and extending the shelf life of duck meat at 4 °C by 3 days.	Acharya et al. (2024)
	EOs (thyme, cinnamon, black pepper, garlic)	Tween 80, glycerol, water	Increasing stability, antioxidant, and antibacterial activities of nanoemulsions.	Jamir et al. (2025)
	Pepper EO	Gum arabic, maltodextrin	Antimicrobial activity ( <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>E. faecalis</i> ).	Karaaslan et al. (2021)
	EOs (thyme, summer savoury, peppermint, black pepper)	Modified starch, whey protein concentrated, maltodextrin	Improving final weight, total feed intake, feed conversion ratio, body antioxidant status, ileal morphostructure, and intestinal microbial population. Regulating antioxidant and inflammation-related gene expression in ileal tissue of broiler chickens challenged with <i>Salmonella enteritidis</i> .	Moharreri et al. (2022)
	Pepper EO	Octenyl succinic acid starch	High stability, enhanced antioxidant effect, strong antibacterial and anti-inflammatory activities, compared with pure pepper EO.	Wang et al., 2025b
Lipid-based and hybrid systems	Oleoresins (coriander, black pepper, chilli, ginger, turmeric)	Gum arabic, Tween 80, Hi-Cap 100	Retaining more than 85% of the bioactive compounds after 3 months of storage.	Madhusankha et al. (2025)
	Cucurmin, piperine	Lipids (tripalmitin, cholesterol), tween 80	Enhancing antimicrobial, anti-biofilm, and wound-healing effects.	Ramalingam et al. (2025)
	Black pepper oleoresin	Sunflower seed oil, glycerol monostearate, soy lecithin, NaCl nanocrystals	Addressing the flavour discrepancy often associated with reduced salt content, with potential for developing solid food products aimed at salt reduction.	Wang et al. (2025a)
Cyclodextrin inclusion	Black pepper oleoresin	$\beta$ -cyclodextrins	Inhibiting the growth of <i>L. monocytogenes</i> , delaying oxidative reactions and inhibiting the growth of Gram-positive microorganisms in food products	Ozdemir et al. (2018)
	Black pepper EO	HPBCD	Increasing their stability, improving antibacterial activity of black pepper EO fourfold against both <i>S. aureus</i> , <i>E. coli</i> .	Rakmai et al. (2017)
	Black pepper oleoresin	Tween 80, HPBCD	Improving solubility and bioavailability, with potential applications in the food industry.	Kulal et al., 2025a
	Pepper oleoresin	Tween 80, HPBCD	Enhancing bioactivity and demonstrating potential antidepressant effects.	Kulal et al., 2025b

Note. EO = essential oil; HPBCD = hydroxypropyl- $\beta$ -cyclodextrin.

digestion and maintained a high stability of 84.8% and good bioaccessibility (31.16%) after digestion (Bastos et al., 2020a). This work collectively highlights that the choice of polymer combination is critical for controlling release kinetics and enhancing the stability of encapsulated EOs. A further study of Napiórkowska et al. (2023) on complex coacervation for black pepper and juniper EOs found that a 1:1 ratio of gelatin and gum arabic yielded the highest encapsulation efficiency. However, the resulting powders consistently had poor flowability, presenting a key challenge for industrial applications.

### Nanoemulsion

Nanoemulsions are defined as dispersions of at least two immiscible liquids, stabilised by an emulsifying agent, surfactant, and co-surfactant (Acosta, 2009). These systems have droplet sizes ranging from 10 to 100 nm and are kinetically stable (Montes de Oca-Ávalos et al., 2017). Recently, nanoemulsion technology has become a subject of extensive research owing to its wide range of potential applications in diverse fields such as polymer production, the food industry, pharmaceuticals, and agriculture. Moreover, research on nanoemulsions has focused on optimising the preparation processes for nanoemulsions containing EOs, as these formulations demonstrate enhanced bioactivity compared to pure EO (Vinh et al., 2020). Jiménez et al. (2018) successfully formulated oil/water nanoemulsions of black pepper and cinnamon EOs using ultrasound and high-pressure homogenisation, highlighting their potential as effective antimicrobial delivery systems. This was further supported by Swathy et al. (2018), who demonstrated the antibacterial activity of black pepper oil nanoemulsions against *P. aeruginosa*. Hien and Dao (2022) found that black pepper EO nanoemulsion showed stronger antimicrobial activity against *E. coli* and *Salmonella enterica*, with lower MIC values (137 and 273 µg/ml) compared to the free EO (547 µg/ml). The nanoemulsion remained stable for six months, maintaining a droplet size of 18 nm and the content of key components ( $\alpha$ -pinene,  $\beta$ -pinene, D-limonene, and 3-carene) similar to pure EO. Furthermore, the nanoemulsion effectively inhibited bacterial growth on pork, and when used as a preservative in minced beef, seasoning-cured pork, and chicken, significantly reduced aerobic microbial counts compared to control samples (Hien & Dao, 2022). Similarly, (Nie et al., 2023) reported significant improvements in the stability and bioactivity of black pepper EO nanoemulsion. Their study showed that black pepper EO nanoemulsion in Tween 80 and water could inhibit the growth of *S. aureus* and *E. coli*, with the MIC values 12.5 mg/ml and MBC values 25 mg/ml. On the other hand, the nanoemulsion of black pepper EO, dispersed within a gelatin film and bacterial cellulose nanofibrils, effectively slowed down lipid oxidation, inhibited microbial growth, and extended the shelf life of duck meat stored at 4 °C by 3 days (Acharya et al., 2024). Jamir et al. (2025) concluded that nanoemulsions made from a blend of EOs (thyme, cinnamon, black pepper, and garlic) are highly effective against several drug-resistant bacteria. The nanoparticles enhanced EOs' bioactivity, leading to a 16-fold increase in antioxidant activity and a halving of the MIC against several bacterial strains, including *Klebsiella pneumoniae*, *E. coli*, *S. aureus*, and *S. typhimurium*. Based on the reported studies, nanoemulsification is a powerful and highly effective strategy for overcoming the inherent limitations of EOs, such as poor stability and solubility. This technology acts as a bioactivity enhancer, transforming black pepper EO into a more potent and practical antimicrobial and antioxidant agent. The demonstrated success in food applications (pork and duck meat) and the significant efficacy against drug-resistant bacteria position nanoemulsions

as a leading technology for developing natural, effective food preservatives and potential alternatives to conventional antibiotics. However, there is a critical lack of sensory data to confirm that the final products retain the desired flavour and aroma profile, which is essential for food applications.

### Polymer-based encapsulation

Polymer-based encapsulation is a technique in which active compounds are enclosed within a polymeric matrix or shell to protect them from environmental degradation, control their release, and enhance their stability, bioavailability, or functionality (Rojas et al., 2024). The polymers used can be natural (e.g., carbohydrates, proteins) (Chakka & Zhou, 2020; Fathi et al., 2014; Pires et al., 2023) or synthetic (e.g., poly- $\epsilon$ -caprolactone, polylactic acid) (Azevedo et al., 2022; Mendes et al., 2023). Microcapsules of spray-dried black pepper EO, made with 1% pea protein and 39% maltodextrin, exhibited a low surface oil content (~0.8%) and a high encapsulation efficiency (95%), proving to be highly effective carriers for black pepper EO (Can Karaca, 2020). Karaaslan et al. (2021) concluded that gum arabic and maltodextrin as the wall material successfully microencapsulated pepper EO. The results showed that pepper EO powder after spray drying demonstrated an improved antimicrobial activity, inhibiting a broader range of bacteria like *P. aeruginosa* and *E. faecalis*, compared with pure EO. The encapsulation also provided significant protection against oxidation (Karaaslan et al., 2021). Besides, Moharreri et al. (2022) successfully encapsulated the blend of EOs of thyme, summer savoury, peppermint, and black pepper at 4:2:1:1 using a combination of whey protein concentrate, modified starch, and maltodextrin. The resulting microcapsules showed antioxidant and antibacterial properties, which, when used as a feed additive for broiler chickens, improved their growth, feed efficiency, and overall gut health (Moharreri et al., 2022). Recent studies have demonstrated promising advancements in the encapsulation of spice-derived compounds for food applications. Induruwa et al. (2024) concluded that microencapsulation of pepper oleoresin through spray-drying, using gum arabic and modified starch as wall materials, significantly enhances encapsulation efficiency, product solubility, and stability, and prevents the loss of piperine during storage. Further, Wang et al. (2025b) reported that a Pickering emulsion stabilised with octenyl succinic acid starch (OSA starch) effectively encapsulated pepper EO, enhancing its antioxidant, antibacterial, and anti-inflammatory properties while also improving preservation by reducing free fatty acid production. These findings highlight the potential of OSA starch-based emulsions for functional food applications (Wang et al., 2025b). Similarly, Madhusankha et al. (2025) explored the use of carbohydrate-based wall materials for spray-drying oleoresin blends. Gum arabic demonstrated the highest encapsulation efficiency (77.3%) and best retention of pungent compounds. Temperature, salt concentration, and wall materials impacted flavour release, with gum arabic excelling at high temperatures (90 °C) and Hi-Cap showing stable release at lower temperatures and salt concentrations. Besides, a 12-week storage study revealed that encapsulation improved compound stability, with only 10% loss compared to 25% in the unencapsulated control. The GA/Hi-Cap blend provided superior long-term retention of bioactive compounds. Sensory analysis of chicken showed unencapsulated and GA preparations had the highest acceptability and spiciness, while Hi-Cap improved texture and the GA/Hi-Cap blend enhanced aroma (Madhusankha et al., 2025). Polymer-based encapsulation was assessed as the most industrially mature and commercially ready. It uses a cost-effective, scalable process (spray-drying).

The primary unaddressed challenge is the impact of the high temperature on the EO's most volatile compounds.

### Lipid-based encapsulation

Lipid-based encapsulation techniques have shown considerable promise in enhancing both the functional attributes of food products and the therapeutic effectiveness of bioactive compounds. Wang et al. (2025a) demonstrated that a system combining black pepper oleoresin with lecithin allowed for a substantial reduction in salt content, up to 66.77%, without compromising perceived saltiness. This improvement was linked to an increased release of sodium ions and the formation of stable oil-in-water emulsions featuring smaller droplet sizes (Wang et al., 2025a). Similarly, (Ramalingam et al., 2025) found that emulsomes loaded with both curcumin and piperine exhibited superior antimicrobial, anti-biofilm, and wound-healing properties compared to curcumin alone. These formulations effectively suppressed the growth of *S. aureus* and *P. aeruginosa*, disrupted bacterial membranes, and enhanced fibroblast cell migration in vitro (Ramalingam et al., 2025). These studies highlight the effectiveness of lipid-based delivery systems in improving bioavailability and functional outcomes in food and medical applications. Notably, the lipid-based encapsulation method shows the most novel, high-value functionality. Its critical, unaddressed flaw is the lack of data on oxidative stability (rancidity). Furthermore, its therapeutic claims are based only on in vitro data, and its sensory impact on food is unknown.

### Cyclodextrin inclusion

Cyclodextrin inclusion is a molecular encapsulation process where a guest molecule is spontaneously encapsulated within the hydrophobic cavity of a cyclodextrin molecule (Paiva-Santos et al., 2022). The driving force for this host-guest interaction is the natural tendency of the hydrophobic molecule to escape the surrounding aqueous environment (Liu & Guo, 2002). This complexation effectively protects the guest molecule from degradation, while simultaneously increasing its solubility and bioavailability (Geue et al., 2023; Liu & Guo, 2002; Paiva-Santos et al., 2022). Cyclodextrin inclusion is a highly effective method for encapsulating black pepper EO. Rakmai et al. (2017) found that encapsulating black pepper EO with hydroxypropyl- $\beta$ -cyclodextrin (HPBCD) improved its stability, aqueous solubility, and functional properties as an additive. Additionally, the antibacterial activity of black pepper EO was enhanced fourfold against both *S. aureus* and *E. coli* (Rakmai et al., 2017). A study by (Ozdemir et al., 2018) compared two methods for encapsulating black pepper EO with  $\beta$ -cyclodextrin: freeze-drying and kneading. Results showed that the kneading method was superior, achieving a higher entrapment efficiency and offering better antimicrobial protection against *L. monocytogenes*. While both methods effectively preserved the pepper EO's antioxidant properties, the kneading technique was identified as a more promising approach for food applications (Ozdemir et al., 2018). Recently, Kulal et al. (2025a, 2025b) published two studies on the nano-encapsulation of pepper oleoresins using HPBCD. The first paper focused on physicochemical properties, reporting high encapsulation efficiency (>85%), small particle size (<250 nm), and controlled release in the intestinal phase, which led to a 40%–50% increase in bioavailability (Kulal et al., 2025a). The second study investigated biological activities, finding that while encapsulation reduced antioxidant potential, it significantly enhanced the stability and bioavailability of the oleoresins. Molecular docking suggested that these encapsulated compounds have potential as natural antidepressants due to their

strong interactions with depression-related proteins (Kulal et al., 2025b). Cyclodextrin inclusion is an effective encapsulation technique that enhances the stability, solubility, and bioavailability of bioactive compounds like pepper EO, though its application is limited by reduced antioxidant potential and low payload capacity.

### Conclusion and future perspectives

Black pepper is rich in bioactive compounds, such as piperine and  $\beta$ -caryophyllene, known for their digestive, anti-inflammatory, antioxidant, and anticancer properties. However, these compounds are highly susceptible to degradation from heat, light, and acidic conditions, leading to reduced efficacy during processing and gastrointestinal digestion. To address these challenges, recent research has focused on encapsulating black pepper extracts. Various encapsulation techniques, including nanoemulsion, coacervation, polymer- and lipid-based encapsulation, and cyclodextrin inclusion, have been explored. These methods utilise diverse wall materials like carbohydrates, proteins, and polymers, effectively protecting the bioactive compounds, enhancing their stability, bioavailability, and controlled release in both food and pharmaceutical applications.

Despite significant advancements in encapsulation techniques, several challenges persist, particularly in their application to food products. The sensitivity of bioactive compounds, such as those encapsulated from pepper extracts, to various operational parameters (temperature, humidity, and processing conditions) should be carefully considered. These factors can adversely affect the stability, bioavailability, and overall efficacy of the encapsulated compounds. Furthermore, consumer acceptability remains a crucial issue, as the sensory properties of food products, such as taste, aroma, and texture, can be altered by the encapsulation process. Additionally, enhancing the flowability of encapsulated powders is vital for their widespread application in food products, as poor flowability can lead to issues such as uneven distribution and clumping during processing. Addressing these challenges is essential for ensuring the successful integration of encapsulated bioactive compounds into diverse food formulations.

### Data availability

No data was used for the research described in the article.

### Author contributions

Thi Thu Hang Vo (Conceptualisation, Data curation [equal], Methodology, Visualisation, Writing—original draft [lead], Writing—review & editing [equal]), Eva Samková (Methodology, Supervision, Writing—review & editing [equal]), František Lorenc (Data curation, Writing—review & editing [equal]), Thi Van Anh Nguyen (Data curation, Methodology [equal]), Tereza Janů (Data curation [equal]), Ithannan Suttikhana (Data curation [equal]), Eliška Míková (Data curation [equal]), and Jan Bedrníček (Methodology, Supervision, Writing—review & editing [equal])

### Funding

None declared.

## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This work is supported by the Grant Agency of South Bohemia University (GAJU 023/2025/Z).

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