

A Novel Approach on Propolis Extraction: Supercritical Carbon Dioxide Extraction, Advantages and Disadvantages

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Abstract

Propolis is a unique bee product rich in bioactive compounds. The natural structure of propolis is resinous and waxy, which makes it indigestible for humans. Extraction process is a necessity in order to obtain bioactive compounds of propolis. Ethanol maceration is one of the most employed methods for propolis extraction. However, this method has some shortcomings, such as solvent residue, dark coloration, and a lengthy process. To eliminate these shortcomings, new and environmentally friendly technologies are also employed. Supercritical carbon dioxide extraction is one such method. This method has been increasingly employed in recent years. This review highlights properties, advantages, and disadvantages of supercritical carbon dioxide extraction.

Introduction

Propolis is a bee product produced by bees using various secretions from different plant species, combined with their own secretions and wax. Propolis is mainly produced to strengthen weak points such as holes and cracks in the hive, defend against invaders and maintain a constant temperature in the hive (Devequi-Nunes et al., 2018).

Propolis has various functional properties, especially antioxidant and antimicrobial activities due to the numerous polyphenols in its structure. Propolis' chemical composition is the key factor of determining its biological activity. The quantity and diversity of polyphenols contained in propolis affects its bioactivity and provides different functional properties (Ghisalberti, 1979).

Propolis is not suitable for human consumption in its raw form, making the extraction process essential to benefit from its rich composition. Therefore, it is important that the bioactive components remain undamaged during extraction. Ethanol or methanol solutions are widely used in propolis extraction. According to the existing literature, a 70-80% ethanol solution is reported to dissolve the majority of bioactive components. In their study, Margeretha et al., (2012) extracted propolis with ethanol and water. They reported that the amount of extract obtained from

propolis is associated with its wax content. According to their findings, extraction yields were between 50% and 70% when ethanol was used as a solvent, while the extraction yields were approximately 10% when water was used.

The extraction process for propolis and other natural products typically consists of four steps. Extraction is completed with the stages of entering the solid matrix of the solvent, dissolving the components in solvent, separating the extract from the solid matrices and collection of soluble substances. Factors affecting extraction efficiency are the characteristics of the solvent used, particle size of the raw material, temperature and duration of the process (Zhang et al., 2018). Extraction is a separation technique that involves isolating a desired compound from a matrix. It can be described as the process of removing a soluble substance from an insoluble residue, whether liquid or solid, by using a liquid solvent. Thus, it is a solvation process that relies on mass transfer phenomena (Herrero et al., 2010; Ahmad et al., 2019).

In the context of natural matrices, extraction techniques play a fundamental role in isolating desired compounds. The selection of an appropriate solvent is critical for this process. Ethanol, methanol, ethyl ether, chloroform, and acetonitrile are usually used as solvents. Traditional ethanolic maceration is commonly used for extraction of propolis. But, this method has

several disadvantages, including lengthy processing times, solvent residues, the presence of beeswax in the final product, and the dark coloration of the extracts. This method also offers some advantages such as simplicity, cost-effectiveness, and suitability for small-to medium-scale operations (Machado et al., 2016; Reis et al., 2020; Sun et al., 2022).

According to Brazilian propolis regulations, propolis must contain a maximum of 25% wax (w/w), 8% moisture (w/w), and a minimum of 0.5% flavonoid content (w/w) (Anonymous, 2001). In Turkish propolis regulations, propolis must contain at least 40% balsam (w/w), a maximum of 8% moisture (w/w), a minimum of 10% total phenolics (w/w), 3% total flavonoids (w/w), and a maximum of 50% wax (w/w) (TFL, 2024).

To address the previously mentioned shortcomings, researchers have explored new extraction methods. Consequently, ultrasound-assisted extraction, microwave-assisted extraction, and supercritical carbon dioxide extraction have been utilized for propolis extraction. This review focused on the characteristics of supercritical carbon dioxide extraction, advantages and disadvantages of this method in the context of propolis extraction.

Supercritical Carbon Dioxide Extraction

Supercritical refers to the state of a substance where it acts as a non-condensing, single-phase fluid, occurring when the substance exceeds its critical temperature and pressure. In this supercritical state, the substance exhibits unique physicochemical properties, such as high density, intermediate diffusivity, and low viscosity and surface tension, combining characteristics of both gases and liquids (Amaral et al., 2017). Supercritical fluid possesses a density similar to that of a liquid, while its viscosity and diffusivity are comparable to those of a gas. Thus, a supercritical fluid can function as a solvent with properties similar to a liquid, and it offers enhanced mass transfer kinetics (Temelli et al., 2012).

Supercritical fluid extraction is a process that utilizes a supercritical fluid to isolate desired compounds from a matrix. This technique has been extensively applied for extraction bioactive substances from natural products (Biscaia & Ferreira, 2009). The method leverages pressure and temperature to enhance the extraction efficiency. Additionally, low viscosity and high diffusivity of supercritical fluids, make the process is notably swift (Yuan et al., 2019).

Supercritical fluid extraction is increasingly recognized as a viable alternative to traditional methods. Solvents in the supercritical phase exhibit unique properties that enhance their ability to extract substances. The high density of these fluids provides them with strong solvating capabilities, while their high diffusivity and low viscosity enable efficient penetration into solid matrices. Choosing the appropriate supercritical fluid is crucial for process development,

with a variety of compounds available as solvents. However, carbon dioxide is often preferred in separation systems for its safety and cost-effectiveness, despite alternatives like ethylene, methane, nitrogen, xenon, and fluorocarbons (Ahmad et al., 2019).

The critical point of carbon dioxide (CO₂) was first identified by Andrews in 1869. Its initial use as a solvent occurred in Russia and the USA during the 1960s. By 1993, 42 distinct oils were commercially extracted using CO₂. Supercritical carbon dioxide is widely favored among supercritical fluids due to its non-toxicity, non-flammability, non-corrosiveness, non-explosiveness, cost-effectiveness, and low critical pressure and temperature (73.8 bar and 31.0°C). CO₂ is easily affordable and readily available in high purity. It is known to minimally alter bioactive compounds, preserving their therapeutic and functional properties. Supercritical carbon dioxide is a preferred alternative to organic solvents as it is, capable of dissolving lipophilic substances, and easily separable from end products. Another advantage is that CO₂ is gaseous at ambient temperature and pressure, simplifying compound recovery and results in solvent-free extracts (Joana Gil-Chávez et al., 2013). Furthermore, CO₂ is eco-friendly and classified as "Generally Recognized As Safe" (GRAS) by both the FDA (U.S. Food and Drug Administration) and EFSA (European Food Safety Authority) (Ahmad et al., 2019). The non-polar nature of CO₂ allows non-polar components to exhibit higher solubility than polar ones of similar molecular weight. Larger molecular size reduces solubility in supercritical fluids. Hence, non-polar solutes with low molecular weight and high vapor pressure are more soluble in supercritical carbon dioxide under low-density conditions, whereas solubilizing larger, slightly polar, and less volatile solutes requires higher densities. This allows for high selectivity by adjusting temperature and pressure, a key advantage of supercritical carbon dioxide extraction technology, often reducing the need for further refining. When the target compound is polar, the polarity of the supercritical solvent can be enhanced by adding a polar cosolvent. The cosolvent interacts with the solute through hydrogen bonding, charge-transfer complex formation, and dipole-dipole interactions, as well as with the solvent, thereby increasing the solvent mixture density and improving solubility. Ethanol, a solvent classified as GRAS, is the preferred cosolvent for food applications. Numerous research groups have employed this gradient method, maintaining a high modifier composition to enable supercritical fluid chromatography separation of polar compounds (Paviani et al., 2010; Ahmad et al., 2019).

The easiest way to separate a supercritical fluid from a solution is by changing the pressure. Since the critical temperature of CO₂ is close to room temperature, it can be separated from the solution by altering the pressure while keeping the temperature constant.

In the supercritical carbon dioxide extraction the cosolvent plays a vital role in improving solubility and efficiency. Short-chain alcohols, such as ethanol and methanol, are commonly preferred due to their effectiveness (Tirado et al., 2018). Other polar cosolvents including acetonitrile, acetone, water, ethyl ether, and dichloromethane are also utilized (Salleh, 2012). Supercritical CO₂, being non-polar, benefits from the addition of cosolvents to enhance polarity and improve extraction efficiency. This approach also allows for operation at lower pressures with reduces the required amount of supercritical solvent, offering economic advantages. Ethanol is particularly advantageous as a cosolvent in supercritical extraction due to its non-toxic nature (Salleh, 2012; Pimentel-Moral et al., 2019).

Previous Studies with Supercritical Carbon Dioxide Extraction

The data indicated that optimal extraction conditions, yielding higher amounts of the target compounds kaempferol and formononetin, as well as the greatest total polyphenols and antioxidant activity, were attained using a 4% cosolvent. The presence of ethanol alongside supercritical CO₂ increased the extraction of total phenolics from red propolis by up to 57%. Additionally, the antioxidant capacity of the extracts improved by 70%. This enhancement is likely due to the increased polarity of the solvent and ethanol's ability to expand the extraction surface area within the natural solid matrix (Souza et al., 2018; Reis et al., 2020).

According to Biscaia and Ferreira (2009), both single-step and two-step procedures were applied for extraction. The single-step process used a fixed pressure and temperature, and required ethanol as a cosolvent. In the two-step process, two different pressure values were used. During the first step, carried out at an average pressure of 100–150 bar, soluble components such as wax and essential oils were separated. In the second step, the pressure was increased to 250–300 bar allowing for the isolation of components responsible for the antioxidant and antimicrobial properties of propolis, such as phenolic acids and flavonoids.

Machado et al., (2015) investigated optimal conditions for the supercritical fluid extraction of Brazilian green propolis. They explored cosolvent ratios of 1-2%, temperatures of 40-50°C, and pressures of 250-350-400 bar. The study was evaluated in terms of total phenolic, total flavonoid, antioxidant capacity, Artepillin C, and p-coumaric acid. The best results were obtained at 50°C, 350 bar, and 1% ethanol. In addition, their findings showed that supercritical extraction reduced total phenolic and total flavonoid values, while increasing the amounts of Artepillin C and p-coumaric acid.

Stahl et al. (1988) extracted raw propolis using supercritical CO₂ at 600 bar and 40°C, separating wax and retaining insoluble flavonoids. Catchpole et al.

(2004) utilized supercritical carbon dioxide both as an antisolvent to precipitate high molecular mass components and as a solvent to extract ethanol and soluble components from ethanolic propolis extracts. Lee et al. (2007) obtained highly pure 3,5-diprenyl-4-hydroxycinnamic acid from Brazilian propolis by extracting with supercritical carbon dioxide modified with ethanol as a cosolvent, followed by column chromatography. Chen et al. (2009) found that a supercritical carbon dioxide extract containing 41.2% (wt) 3,5-diprenyl-4-hydroxycinnamic acid effectively inhibited the growth of human colo-205 cancer cells, despite its relatively low yield compared extraction with ethyl acetate using a Soxhlet apparatus. Paviani et al. (2010) explored the supercritical fluid extraction of a dried ethanolic extract from Brazilian propolis, focusing on component fractionation. They reported greater selectivity at lower solvent densities, highlighting significant differences in the phenolic content between the extracts and raffinates.

In a study, conducted by Monroy et al. (2022), following extraction, the ethanolic and hydroalcoholic extracts were fractionated using supercritical CO₂ as an antisolvent at a constant temperature of 50°C. The process involved four incremental pressures across a series of separators operating at 200, 100, and 80 bar, concluding with atmospheric pressure (1.013 bar). The method was evaluated based on extraction yield, total phenols, total flavonoids, antioxidant activity, and color. The results indicated that pressure impacted both the yield and phenolic compound concentration, with the most effective fractionation occurring in the first and second separators. Notably, all extracts exhibited potent antioxidant activity.

Machado et al. (2016) compared total phenolic compounds and total flavonoids obtained via both ethanolic extraction and supercritical carbon dioxide extraction. Their findings demonstrated that ethanolic extraction is generally more efficient for total phenolic compounds and total flavonoids. On the other hand, supercritical carbon dioxide extraction proved more effective for isolating specific compounds, such as p-coumaric acid and Artepillin C.

Fractionation of ethanolic propolis extracts with supercritical carbon dioxide yielded 11 to 18% returns, with minor differences in Artepillin C composition compared to the original ethanolic extracts. However, the chemical profiles of the four markers were distinctly different from those of the ethanolic extracts. The selectivity of supercritical carbon dioxide was evident in the chemical profile changes of the extracts, which varied with temperature and pressure. This suggests that higher temperatures and pressures than those applied in this study might result in extracts with increased yields and higher marker concentrations, especially Artepillin C (Reis et al., 2020).

Advantages of Supercritical Carbon Dioxide Extraction

Supercritical fluid extraction is regarded as a technological breakthrough, particularly for high-value products, due to its low-temperature operation, efficient solvent usage, recyclability, reduced energy requirements, and enhanced product quality owing to the absence of solvent residue. Versatility of supercritical fluid extraction lies in its selective nature, achieved by precisely controlling temperature and pressure during the extraction process. Consequently, supercritical fluid extraction is increasingly viewed as a viable option for the pharmaceutical, fine chemical, and food industries (Paviani et al., 2013).

Supercritical CO₂ particularly stands out due to its lower critical temperature and pressure (31°C and 74 bar) compared to other supercritical solvents, making it advantageous for extracting thermosensitive compounds (Novak et al., 2014). Moreover, supercritical fluid extraction preserves the chemical integrity of the extracted substances, including their antioxidant capacity, owing to the use of low temperatures (Machado et al., 2019).

The environmentally friendly properties of supercritical CO₂ provide a key incentive for substituting organic solvents. In the event of accidental release, supercritical carbon dioxide poses no environmental hazard due to its non-toxic and safe nature. The use of non-flammable supercritical carbon dioxide as a solvent significantly lowers the risk of explosions reactions, especially those involving highly reactive substances. The superior heat transfer capability of supercritical carbon dioxide ensures effective temperature management, preventing hot spots or runaway reactions in highly exothermic processes (Ahmad et al., 2019; Paviani et al., 2010).

Gas-liquid catalyzed chemical reactions are typically diffusion-controlled. This limitation can be minimized by removing the gas-liquid interface and enhancing diffusivity with supercritical carbon dioxide, thereby increasing reaction rates by reducing mass transfer barriers (Pereda et al., 2005).

The activity and selectivity of porous catalysts are affected by adsorption/desorption and pore transport. In conventional gas or liquid reaction media, one of these factors is typically favorable while the other is not. Traditional media often make it challenging to achieve desired fluid properties such as gas-like transport, liquid-like solvent power, and heat capacity, which are essential for optimal system performance and enhancing the stability of porous catalysts in supercritical reaction media. Supercritical carbon dioxide addresses these challenges by providing adjustable fluid properties, such as diffusivity and viscosity, through changes in pressure or temperature. These properties enhance catalyst activity, product selectivity, and the stability of porous catalysts. Additionally, supercritical carbon dioxide aids in the

penetration of reactants into the porous structure of the catalyst (Zhang et al., 2014).

In certain chemical reactions, carbonaceous byproducts can lead to catalyst deactivation through coke formation, which accumulating on both the internal and external surfaces of the catalyst. Supercritical carbon dioxide helps mitigate this issue by removing and transporting these materials due to its high diffusivity, thereby increasing the catalyst's lifetime and facilitating its regeneration. Separating products from traditional solvents is often laborious and energy-intensive. In contrast, within a supercritical carbon dioxide reaction medium, products can be easily separated by merely reducing the CO₂ pressure. The acceleration of reaction rates and simplification of product separation enable the use of smaller continuous reactors compared to traditional ones with equivalent performance. This advantage enhances process safety and reduces the spatial requirements of chemical plants (Baiker, 1999; Reverchon & De Marco, 2006).

Disadvantages of Supercritical Carbon Dioxide Extraction

Supercritical CO₂ extraction is a well-established method for extracting propolis (Banskota et al., 2001). However, its widespread use in propolis processing is limited due to its low yield of flavonoids, high costs, significant energy consumption, and inefficient raw material usage.

Some drawbacks of supercritical fluid extraction compared to traditional liquid solvents for separation processes include the requirement for high pressure, the complexity of recycling measures to lower energy costs, and the significant capital investment in equipment (Sun et al., 2022).

Conclusion

In the context of extraction, bioactive compounds are the most important substances derived from natural resources. Propolis is one of the most valuable bee products, containing numerous bioactive compounds, such as polyphenols, flavonoids, and terpenes. The primary problem with propolis' consumption is its poor digestibility in humans. Consequently, researchers have been exploring effective extraction techniques for years.

Extraction efficiency has traditionally been the primary focus. However, with increasing environmental concerns, new methods have been developed. From this perspective, supercritical carbon dioxide extraction is applied for propolis extraction due to its non-toxic properties for both humans and the environment. Additionally, supercritical carbon dioxide extraction offers many advantages, including cost-effectiveness, reduced energy requirements, recyclability, and the absence of solvent residue. Moreover, it is one of the best methods for obtaining specific compounds.

There are two significant disadvantages of supercritical carbon dioxide extraction. Firstly, due to its

Table 1: Some studies using supercritical carbon dioxide extraction on propolis extraction

Materials from	Cosolvent	Process Conditions	Authors
Italy	-	w: 32 g t: 30 min f: 2 L/min P: room conditions	De Zordi et al., 2014
Brazil	EtOH	w: 5 g t: 118 min f: 1.65 g/min P: 250 bar T: 50°C	Monroy et al., 2017
Brazil	EtOH	w:- t:- f: 1 mL/min P: 20 Mpa T: 54.85°C	Wu et al., 2009
Brazil	EtOH	w:- t:- f: - P: 20-15-10 Mpa T: 60°C	Wang et al., 2003
Indonesia	-	w: 50 g t: 240 min f: 6.59-23.41 g/min P: 150 bar T: 50°C	Fachri et al., 2020
Brazil	EtOH	w: 2.5 g t: 20 min f: P: 200-300-400 bar T: 40-50-60°C	Saito et al., 2021
Commercial Samples	EtOH	w:- t:- f: - P: 250-300 bar T: 59.85°C	Catchpole et al., 2004
Taiwan	EtOH	w: 10 g t:- f: 10 mL/min P: 13.8-27.6 Mpa T: 34.85-59.85°C	C. R. Chen et al., 2009
Türkiye	EtOH	w: 10 g t: 150 min f: 6 g/min P: 150-250-350 bar T: 50°C	Sonverdi et al., 2024
Brazil	EtOH	w: 7.5 g t: 150 min f: 6 g/min P: 350 bar T: 50°C	Dantas Silva et al., 2017

w: weight of propolis; t: time; f: flow rate; P: pressure; T: temperature

non-polar nature, carbon dioxide is not effective for extracting polar compounds. To address this shortcoming, the use of ethanol as a cosolvent is essential. The second disadvantage is high investment costs. However, this disadvantage can be compensated by the production of high-value-added products.

Ethical Statement

There are no ethical issues with the publication of this article.

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Conflict of Interest

The author declare that there is no conflict of interest.

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