

Mini review: Application of supercritical carbon dioxide in extraction of propolis extract

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Article history

Received 28 March 2018

Revised 1 April 2018

Accepted 20 May 2018

Published Online 3 December 2018

Abstract

Propolis is a resinous substance produced by bees functioned to seal holes, exclude draught, protect against contamination and external intruders inside their hives has been substantially studied and reported to have numerous health properties such as antiseptic, antifungal, antibacterial, antiviral, anti-inflammatory and antioxidant characteristics. Propolis cannot be utilized as raw material, due to its complex mixture of compounds. Hence it must be separated by the extraction process. Extraction targets to remove the inert compounds in the propolis sample and preserves the flavonoids and polyphenolic fraction. The most common technique used in propolis extraction is solvent extraction that involves the use of solvents such as ethanol, water, hexane, ethyl-acetate and chloroform. However, this conventional technique has some drawbacks including strong residual flavour, possible adverse reactions, harmful to the environment, low quality of the extract and long process period requirement. Supercritical carbon dioxide (SC-CO₂) is one of the alternative techniques to conventional extraction that was reported to be an excellent method to purify and fractionate bioactive compounds from natural sources. SC-CO₂ extraction was first introduced for analytical application because of the demand to reduce organic solvent utilization in a laboratory environment. It has now become a favourite technique in extraction, fractionation, refinement, and deodorization of natural sample matrices in laboratory scale and industrial scale. Carbon dioxide is an ideal supercritical solvent due to its non-toxic, non-polluting, non-flammable, recoverable and environmentally benign properties. Therefore, this mini review aims to discuss the application of supercritical carbon dioxide extraction specifically on propolis sample starting with a brief introduction on propolis, methods of propolis extraction, the principle of SC-CO₂ extraction, application of SC-CO₂ in propolis extraction, advantages of SC-CO₂ extraction and lastly comparison between SC-CO₂ and conventional extraction techniques.

Keywords: Supercritical carbon dioxide, propolis, extraction

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INTRODUCTION

Propolis is one of the bee products which has gained the attention of researchers and consumers worldwide recently due to its numerous health benefits. It is a resinous substance collected by bees from exudates of surrounding plants, mixed with their saliva and waxes, gathered to their hive to protect against contamination and invaders, to seal holes, and to maintain the temperature inside the hives. This product consists of 50% of resins, 30% of waxes, 10% of essential oil, 5% of pollen and 5% of organic compound and minerals (Almutairi et al., 2014). Propolis has been reported to possess various therapeutic activities, such as antitumoral, antifungal, anticancer, anti-inflammatory, antiviral and antibacterial properties (Abu Mellal et al., 2012). For this reason, it has been widely used in pharmacological, food and beverages industry to enhance human health and prevent diseases such as cancer, heart disease, inflammation and diabetes (Chen et al., 2009).

Propolis has been used for centuries as traditional medicine, especially in Europe and Japan (Biscaia & Ferreira, 2009). Thus, it has become a subject of intense interest in pharmacological and chemical studies for the last 30 years, searching for functional evidence to its diverse advantages. Many studies have proven

therapeutic activities of propolis to be attributed to compounds such as flavonoids, phenolic acids, terpenes and sesquiterpenes (Marcucci et al., 2001, Kumazawa et al., 2004, Nagai et al., 2003, Lu et al., 2005).

Even though it consists vital health functional compounds, propolis cannot be used as raw material because it also contains an inert compound - waxes that comprises up to 30% of its total content (Almutairi et al., 2014). Wax is a complex mixture of organic compounds, predominantly monoesters, formed by moieties of carboxylic acid and alcohol with unbranched carbon chain, followed by hydrocarbons with odd numbers of carbon atom (Custodio et al., 2003). To obtain propolis with a high concentration of functional compounds, it must be purified using extraction process which helps to remove the inert material and preserve the contributing fractions. Solvent extraction with ethanol is the most commonly used method to obtain dewaxed propolis extract with high functional compounds such as flavonoids and polyphenolic content (Pietta, 2000).

Extraction methods are constantly associated with product quality. Extraction techniques that can produce pure extract without solvent residue are considered as good methods (Manirazika et al., 2001). Among well-established conventional extraction methods encompasses hydro-distillation (HD) and organic solvent extraction which includes soxhlet and maceration process (Weinhold et al.,

2008). On the other hand, modern techniques in relation to propolis extraction have been studied by many researchers, for example, ultrasound extraction, microwave-assisted extraction (Pellati et al., 2007), lyophilization, nanofiltration (Tylkowski et al., 2010) and supercritical carbon dioxide extraction (Chen et al., 2009).

Supercritical CO₂ extraction is an extraction technique that employs CO₂ at its supercritical condition. Above its critical temperature of 31°C and critical pressure of 7.38MPa, supercritical CO₂ acts as a solvent to separate targeted compounds from their natural matrixes (Herrero et al., 2010). CO₂ is recognized as a green solvent due to its non-toxic, non-flammable, non-polluting properties and generally acknowledged to be a safe extracting agent by FDA (Food & Drug Administration) and EFSA (European Food Safety Authority) (Herrero et al., 2010). Its easily tuned solvent combined with high diffusivity properties makes supercritical CO₂ a promising method for solute separation. In addition, analyte recovery for SC-CO₂ extraction is very simple and it is able to yield solvent-free analytes as the CO₂ returns into gaseous form at room condition. This allows extraction to be carried out at low temperatures using a non-oxidant medium which is important for food and natural products sample preparation that often involves thermally liable and easily oxidized compound extracts (Herrero et al., 2010).

To our literature extend, SC-CO₂ technology has been used mainly as a sample preparation technique for the analysis of target compounds from foods and natural product (Engelhardt et al., 1991). It is also used commonly in the process development area, in which target compounds are extracted from different sample matrices. SC-CO₂ has been regarded as a promising tool not only from a laboratory point of view, but also in the industrial scale (i.e., food and natural products industries) (Fiori, 2010). Several papers have been published in the evaluation of the industrial and economical practicality of some SC-CO₂ developed processes, such as brewery spent grain management (Fernandez et al., 2008) and essential oil extraction from anise, fennel and rosemary (Pereira & Meireles, 2007). On the other hand, there are wide studies of SC-CO₂ application in laboratory scale such as extraction of *Lamiaceae* herbs (Babovic et al., 2010), *Strobilanthes crispus* (Pecah Kaca) (Liza et al., 2010), aloe vera (El-Shemy et al., 2010), chia seed oil (Ixtaina et al., 2010), microalgae (Dejoye et al., 2011) and many more.

Vast application and benefits of this assuring SC-CO₂ technique have drawn current researchers to investigate the application of this process in propolis extraction. SC-CO₂ appears to be an ideal solvent to extract bioactive compounds identified in propolis such as flavonoids and phenolic acids including p-coumaric acid, ferulic acid, cinnamic acids, pinobaskin, kaempferol, artemillin C, and others (Lee et al., 2007; Catchpole et al., 2004). A clear positive trend in propolis extraction using SC-CO₂ technique is shown by several successful studies (Chen et al., 2009; Lee et al., 2007; Catchpole et al., 2004).

In recent available literature, the chemical, biological, and nutritional aspects of propolis as a source of functional compounds, as well as technological aspects of the SC-CO₂ extraction have been reviewed. However, to date, there's no reference available for a specific review of the application of SC-CO₂ in propolis extraction. Hence, the intention of this mini review is to summarize the findings related to propolis extraction with an emphasis on using SC-CO₂ technology and related matter.

PROPOLIS

Propolis or sometimes also known as 'bee glue' is defined by the United States Department of Agriculture, USDA as 'a gum that is gathered by bees from various plants. It may vary in colour from light yellow to dark brown. It may cause staining of the comb or frame and may be found in extracted honey' (USDA, 1985). The word propolis was developed from a Greek name in which 'pro' is meant by 'police' and 'polis' is meant by 'city' that describe one of their function is to protect the entrance of beehives against intruders.

The precise composition of raw propolis could vary among different places and source. It is been reported that propolis contains more than 160 constituents and varies significantly because of their

different botanical and geographical sources (Choi et al., 2006). These constituents include polyphenols (flavonoids, phenolic acids and their ester, phenolic aldehydes, alcohol and ketones), amino acids, steroids, coumarins, sesquiterpene quinones and inorganic compounds. Due to this variation in their chemical compositions, it seems to be challenging to identify and standardized propolis profile from different places worldwide (Choi et al., 2006).

The range of pharmacological activities by propolis sample such as antioxidant, antibacterial, antifungal and anti-inflammatory have been attributed to the presence of bioactive compounds. In addition, other pharmacological properties that have also been associated with propolis are immunomodulatory, hematostimulative, hepatoprotective, and cytotoxic activities.

The study by Funakoshi-Tago et al., (2016) found that five types of flavonoids; isoliquiritigenin, chrysin, 3',4'-dihydroxy-4-methoxydalbergione, 4-methoxydalbergion, and ceairon was present in Nepalese propolis. These flavonoids have markedly inhibited inflammatory responses in iNOS (inducible nitric oxide synthase), TNF α (tumour necrosis factor) and CCL2 (monocyte chemoattractant protein-1) mRNA expression. They concluded that propolis's inhibitory effects on inflammatory responses were significantly correlated with the intensities of these five types of flavonoids.

Presence of formononetin, isoliquiritigenin, (3S)-neovestitol, and (3S)-vestitol were suggested to be responsible for antibacterial activity against *Streptococcus mutans*, *Streptococcus sobrinus*, *Staphylococcus aureus* and *Actinomyces naeslundii* in Brazilian propolis (Bueno-Silva et al., 2017). Meanwhile, luteolin and quercetin compound in Brazilian red propolis have been reported to be associated with antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Regueira et al., 2017).

The most commonly known therapeutic properties of propolis was its antioxidant ability which is contributed by the phenolic compounds, particularly phenolic acids and their esters (Socha et al., 2015). Phenolic compounds inhibit the free radical reactions, capturing the superoxide amino radicals and peroxy radicals, reducing transition metal ions and inhibits the formation of reactive oxygen species (Socha et al., 2015). P-coumaric acid and ferulic acid were the dominant phenolic acid found in Poland propolis, whereas, chrysin and galangine were dominant flavonoids found in the same sample. Socha et al., (2015) observed a significant linear correlation between these phenolic compounds with antioxidant activity and reducing power as measured by DPPH (free radical diphenylpicrylhydrazyl) and FRAP (ferric reducing ability of plasma) assay respectively.

METHODS OF PROPOLIS EXTRACTION

Currently, there are two categories of extraction technique which are conventional and modern extraction method. Example of modern extraction methods includes supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), and pressurized hot water extraction (PHWE) developed an alternative to conventional extraction method. The conventional extraction method of solid-liquid extraction (SLE) involves solvent application and leaching process which encompasses soxhlet extraction (SE), percolation and maceration extraction (ME) (Socha et al., 2015).

Up until now, several conventional extraction methods such as the ones listed above have been employed in the extraction of bioactive compounds in propolis sample. Conventional extraction methods generally require a huge amount of solvents and it is time-consuming and complicated. Hence, there is a need to develop and establish a greener extraction method with higher efficiency in enriching bioactive compounds from propolis sample. In that regard, a number of researchers had investigated and improved these conventional propolis extraction technique by introducing a new distinct propolis extraction technique.

Machado et al., (2016) had compared Brazilian propolis extract obtained by conventional extraction technique - ethanolic extraction (EtOH) with modern extraction technique - supercritical CO₂

extraction. They found a higher concentration of Artepillin C and p-coumaric acid in propolis extract obtained by supercritical CO₂ extraction at 50°C, 350 bar, and 1% of ethanol addition as co-solvent as compared to conventional ethanolic extraction. Supercritical CO₂ has a higher selectivity for extraction of these target compounds which were particularly related to antioxidant activities (DPPH & ABTS) and antibacterial activities of the propolis extract.

Microwave-assisted extraction (MAE) is one of the modern extraction techniques which can reduce extraction time significantly by allowing rapid and homogenous distribution of energy throughout the solid matrix and solvent (Hamzah & Leo, 2015). A large amount of energy, in the form of heat, is utilized for extraction by microwaves. Moisture in the solid matrix adsorbs microwave energy and creates internal superheating that promotes the solid disruption, thus improving the recovery of bioactive compounds in a short duration (Destandau et al., 2013). Propolis extraction using MAE can be accomplished in a shorter time with less solvent degradation and without severe compound degradation as compared to conventional extraction method such as maceration (Hamzah & Leo, 2015). MAE carried out at 2 × 10 seconds of microwave irradiation exposure to the propolis sample resulted in 73% of extract yield. Meanwhile, maceration extraction had only resulted in 58% of extract yield after 72 hours of extraction period.

On the other hand, ultrasonic extraction had also been mentioned as a fast and efficient extraction method for propolis. Acoustic cavitation by ultrasonic energy inside the sample matrix allowed rapid extraction of bioactive compounds. This technology showed great potential in reducing extraction time and increased extraction yield. Ultrasonic extraction gave a higher percentage of phenolic compounds extracts than MAE. Ultrasonic extraction had resulted in total phenolic of 50% after 30 min extraction period, whilst, MAE had resulted in 40% of total phenolic after 2 × 10s of microwave irradiation exposure to the propolis sample. In addition, this method provided less operating hours and less labour (Trusheva et al., 2007).

Application of membrane concentration process or specifically nanofiltration in propolis extraction has been growing because of several benefits, such as low energy consumption, the absence of phase transition, and operation at low temperature. This technique is based on the principle of solute selective permeation through semi-permeable, inorganic or polymeric membranes. Mechanical pressure is used as the driving force for mass transfer across the membrane for most membrane processes, such as reverse osmosis, nanofiltration, ultrafiltration and microfiltration. Nanofiltration process reported having high efficiency in extraction and concentration of phenolic and flavonoids compounds up to 90% in aqueous and ethanolic propolis solution (Mello et al., 2010). Nanofiltration process had concentrated flavanoids compounds, 71.93 mg/g and phenolic compounds, 105.08 mg/g in ethanolic solution of propolis sample. Meanwhile, the same process had concentrated flavanoids compounds, 96.76 mg/g and phenolic compounds, 104.74 mg/g from an aqueous solution of the propolis sample.

Pressurized liquid extraction (PLE) is becoming an important sample preparation technique in food analysis and its application in the extraction process including propolis extraction process. It requires less solvent, favourable for environmental aspect and allows efficient analytes extraction in an inert and closed environment, thus enabling high-temperature extraction. Parameters such as temperature, pressure, solvent type, extraction time and cell size were stated to affect the performance of propolis extraction significantly. Study by Erdogan et al., (2011) had mentioned that 40°C, 1500 psi, Ethanol:water:hydrochloric acid; (70:25:5, v/v/v) containing 0.1% tert-butylhydroquinone (tBHQ) as solvent, three extraction cycles within 15 min, and a cell size of 11 mL was the most favorable PLE operating conditions for propolis extraction. A recovery rate ranging from 97-99% of polyphenol compounds in propolis which encompasses gallicocatechin (GCT), catechin (CT), epicatechin, gallate (ECTG), caffeic acid (CA), chlorogenic acid (ChA), and myricetin (Myr), can be extracted by PLE (Erdogan et al., 2011).

Table 1 listed details of different extraction methods in propolis extraction encompass the propolis source, solvent, specific method, condition used and their results.

Table 1 List of different extraction methods of propolis extracts.

Propolis Source	Solvent	Method	Condition	Results	Authors
Brazil	Water Ethanol	Nanofiltration	NF90 membrane T – 20°C P – 6.0 bar t – 30 min	Phenolic content – 53-94% Flavonoid content – 90 – 99%	Mello et al., (2010)
Italy	Ethanol Ethyl acetate Water HCl Methanol Acetone	Pressurized liquid extraction	T – 20,40,60,80 °C P – 500, 1000, 1500, 2000 psi t – 15, 30, 40, 60, 90, 120 min cell size – 11, 22ml	Optimum condition – 40°C, 1500 psi, 15 min, 11ml cell size	Erdogan et al. (2011)
Bulgaria	Ethanol	Maceration extraction	Propolis: solvent ratio – 1:20 t – 72 hours	Phenolic content – 44% Flavonoid content – 30% Total extract yield – 58%	Trusheva et al. (2007)
Thailand	Ethanol	Ultrasonic extraction	t – 15, 30, 45, 60 min	Optimum inhibitory effects – 15 & 30 min	Sanpa et al. (2012)
Italy	Ethanol Water	Microwave-assisted extraction	Power – 300W Frequency – 2450 MHz EtOH: solvent ratio – 80:20 v/v T – 106°C	Phenolic content – 5-120.8 mg/g Flavonoid content – 2.5 – 168.0 mg/g	Pellati et al., (2013)
Brazil	Ethanol	Supercritical CO ₂ extraction	1% EtOH P – 350 bar T – 50°C CO ₂ flow rate – 6g/min t – 2 hours 30 min	Phenolic content – 157.43 mg/g Flavonoid content – 25.46 mg/g DPPH – 116.49 IC ₅₀ ABTS – 87.60%	Machado et al., (2016)

Hot-pressurized water (HPW) technique is another modern extraction method that has been applied to extract bioactive compounds in propolis sample. This technique is based on the principle of water that has dielectric properties, viscosity, and surface tension that is close to organic solvents while offering more advantages compared to conventional organic solvent extraction. A study by Chen et al., (2007) found seven flavonoids, caffeic acid phenethyl ester and four phenolic acids compounds obtained from HPW extraction were 36% higher than that from hot water extraction at atmospheric pressure. Additionally, the addition of natural surfactant in the process had increased the mentioned compounds extraction to 44% higher as compared to without the addition of natural surfactant. This extract was also reported to have successfully suppressed the growth of leukemia, lung cancer and liver cancer cells (Chen et al., 2007).

PRINCIPLE OF SC-CO₂ EXTRACTION

A supercritical fluid has been applied in the extraction of the natural product since the end of the 1970s. However, for a long period, the applications have been limited only to a few products. Currently, the growth and improvement of supercritical fluid equipment's as well as the processes have begun to pay off and gain much interest from the industries (Herrero et al., 2010). This interest is demonstrated by numerous recent scientific papers regarding the SFE technique. Furthermore, SFE application in industries has been growing tremendously, indicated by patents since the early 1990s (Herrero et al., 2010). Figure 1 illustrated a schematic diagram of the SC-CO₂ extraction process.

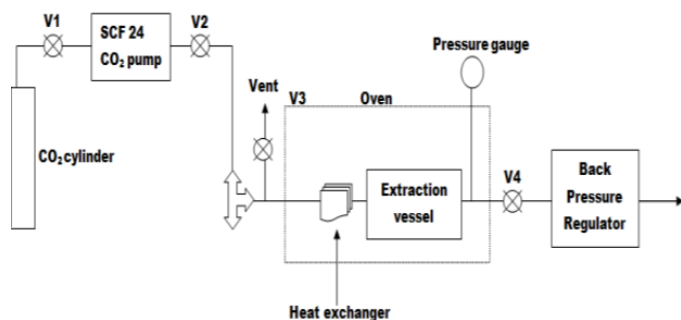


Figure 1 Schematic diagram of SC-CO₂ extraction.

Supercritical fluid extraction is one of the methods used in recovering bioactive compound from natural sources that have shown to be more advantageous and is more environmental friendly compared to conventional extraction methods. SFE technique appears to be a good extraction and fractionation process for industrial application, especially in pharmaceutical and food industries (Lang & Wai, 2001). Each fluid is identified by its critical point, which is described by its critical temperature and critical pressure. This fluid cannot be further liquefied above its critical temperature, despite any pressure applied, however, it may reach the density closer to the liquid state. A substance above its critical temperature and critical pressure is considered as a supercritical fluid. Supercritical fluid is a phase where a distinct gas and liquid phase does not exist (McHugh & Krukoni, 2013). Figure 2 below showed a pressure-temperature diagram of the supercritical fluid.

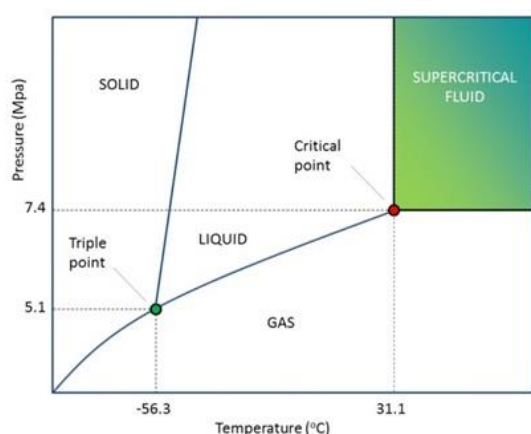


Figure 2 Pressure-temperature diagram.

High density provides better compound solubilization by SC-CO₂, whilst low viscosity allows easier solid penetration and enables less friction flow. Solvating strength of SC-CO₂ could be manipulated by adjusting its operating parameters, mainly temperature and pressure (Kalani & Yunus, 2011). Adjusting the critical temperature and critical pressure can alter SC-CO₂ properties and enhance its efficiency to penetrate and subsequently extract targeted bioactive compound from the natural source. In addition, SC-CO₂ is mentioned

to be an excellent solvent to recover the high amount of lipid-soluble compounds (Sahena et al., 2009).

SC-CO₂ extraction parameters comprising extraction temperature, pressure, modifier, flow rate, process time, moisture content, and sample particle size has significant relation with extraction product quantitatively and qualitatively (Zuknik et al., 2012). Temperature as one of the important parameters in the SC-CO₂ process could significantly affect the extraction yield. This had been reported by Favati et al. (1991), with an increase in temperature from 40 °C to 50 °C at 20 MPa, the evening primrose oil yield dropped from 66.1% to 59.6% with a further drop to 31.4% at 60 °C. Meanwhile, they also found that the oil yield increased from 96.8% to 97.5% with increasing temperature from 40 °C to 50 °C and then slightly dropped to 97.2 % at 60 °C. Increase in process temperature will increase the vapour pressure of the solute and reduce the possibility of mass transfer resistance between solute and the sample's matrix to happen.

On the other hand, process pressure could also affect the extraction yield. An increase in process pressure does increase the extraction yield due to an increase in solvent density that enhances the solvating power of SC-CO₂ solvent and risen the intermolecular interaction with the solutes. This phenomenon eases the dissolution of solutes and solvent, thus improving the extraction yield obtained (Machmudah et al., 2006). This has been reported by Tai & Kim (2014) where they found an increase of Gac oil recovery from 72.3% to 91.4% at an increase of pressure from 200 to 400 bar, with constant temperature 323K and flow rate 70 kg-h-1kg-1.

In addition, the SC-CO₂ flow rate has also a strong influence on the extraction yield obtained. The optimum SC-CO₂ flow rate must be sufficient for solvent-solute saturation. Unsaturated SC-CO₂ could exit the extractor at lower flow rate due to mass transfer resistance that limits the amount of solute transported in the solvent. Meanwhile, as an increase of flow rates, the mass transfer resistance will be reduced until the exiting solvent is saturated and thus equilibrium is achieved and the maximum yield can be attained (Kumoro & Hasan, 2007). SC-CO₂ extraction of flaxseed oil had shown that at a lower flow rate of 1L/min required a longer extraction time to reach the maximum extraction yield, whilst the maximum extraction yield had increased at a higher flow rate of 3L/min from 66% to 74% (Bozan & Temelli, 2002).

To summarize advantages of SC-CO₂, i) it can dissolve non-polar to slightly polar compounds, ii) possess high solvating power towards low molecular weight compounds that decreases with bigger molecular weight compounds, iii) high affinity towards medium molecular weight oxygenated organic compounds, iv) low solubility towards water at temperature below 100°C, v) insoluble with protein, polysaccharides, sugars and mineral salts, and vi) is capable of isolating less volatile, higher molecular weight, more polar compounds as the pressure increases.

The main disadvantage of SC-CO₂ is its low polarity, which reduces the efficiency to extract polar compound in the natural sample. However, this drawback can be overcome by applying polar modifier (co-solvent) to alter the SC-CO₂ polarity and help to increase its solvating power towards targeted polar compounds (Herrero et al., 2006). For instance, application of methanol in a small percentage (1-10%) to CO₂ could extend SC-CO₂ extraction range to extract more polar compounds (Herrero et al., 2006). The co-solvent can also reduce the interaction between polar compounds and its matrix, thus improving quality of the extract product.

The design of SC-CO₂ process is highly dependent on the scenario of equilibrium phase which is strongly sensitive to operating parameters and condition changes. Thus, equilibrium phase plays a significant role in the SC-CO₂ extraction process.

One of the vital areas of concern in SC-CO₂ extraction processes is extraction optimization. The adjustment to obtain optimum values for different variables attributing to SC-CO₂ extraction process could significantly increase extraction yield recovery, as well as the concentration of the targeted compound. The main optimization of SC-CO₂ extraction process involves finding its optimum operating parameters. Response surface methodology (RSM) is one of the favoured methods in optimization process of SC-CO₂ extraction.

Simultaneous optimization of different variables could be done by using RSM. Two variables that could be set as response variables are extraction yield and concentration of targeted compounds. RSM requires more experimental points to provides more information on how the extraction is working. Many researchers had studied optimization of SC-CO₂ extraction process of various natural products employing RSM such as extraction of *swietenia mahagoni* seed (Hartati et al., 2016), palm oil mesocarp (Yunus et al., 2015), *jatropha curcas* seed (Azizi & Ismail, 2008), piper betel linn leaves (Aziz et al., 2016) and castor oil (Danlami et al., 2015).

APPLICATION SC-CO₂ IN PROPOLIS EXTRACTION

Supercritical carbon dioxide extraction of propolis yielded an extractant that has been proven to possess better biological activities as compared to conventional organic solvent extraction. Vast researches had been carried out to investigate SC-CO₂ extraction on propolis sample from different origin worldwide. It has been reported that the optimum operating parameters to remove wax and extract rich fat-soluble flavonoids was at a pressure of 600 bar and temperature of 313K (Catchpole et al., 2004). Application of co-solvent, for instance, ethanol and water, in SC-CO₂ extraction of Brazilian and Taiwanese propolis have significantly enhanced water solubility and anti-cancer activity of the product (Wu et al., 2009). A patent had also described SC-CO₂ extraction of propolis sample with the addition of co-solvent yielded different classes of bioactive compounds at increasing extraction pressure (Wu & Luyu, 2000). Propolis extract obtained from SC-CO₂ extraction are sold as commercial products in Japan, specifically marketed for their anticancer properties. In other approaches, a fine particle consisting concentrated bioactive compounds from propolis extract could be produced through SC-CO₂ anti-solvent precipitation technique (Wu et al., 2009). Furthermore, SC-CO₂ extraction technique had also been used in the fractionation of propolis sample mixture, and at the same time in the fractionation of lecithin with triglyceride oil, curcuminoid with triglyceride oil and essential oil with triglyceride oil mixtures (Teberikler et al., 2001).

Several bioactive compounds in propolis, such as flavonoids and cinnamic acid derivatives are categorized as a polar compound, hence they have minimal to zero solubility in SC-CO₂. Therefore, incorporating a modifier or co-solvent in SC-CO₂ extraction could help increase solubility strength between solvent and solute, thus improving the extraction efficiency. CO₂ alone and CO₂ with water were mentioned to be less effective for dissolving flavonoid compounds, whilst CO₂ with ethanol was observed as a more effective solvent (Catchpole et al., 2004). Non-polar flavonoid derivatives, flavone, and 3-hydroxyflavone, were measured to be soluble in CO₂ in range of 10⁻⁵ to 10⁻⁴ mole fraction (Catchpole et al., 2004).

Amount of flavonoids had increased in water-soluble propolis extracted by the SC-CO₂ process as reported by You et al., (2002). Antioxidant compounds from propolis were favourably extracted using SC-CO₂ fractionation technique (Wang et al., 2004). Ethyl-acetate was used as a modifier in SC-CO₂ extraction of Brazilian propolis and has obtained an anti-cancer associated compound, DHCA (Lee et al., 2007). 3,5-diprenyl-4-hydroxycinnamic acid, DHCA, also known as Artepillin C found in Brazilian propolis, exhibited anti-cancer properties by inhibiting effects of renal carcinogenesis through its oxy-radical scavenging characteristic (Ahn et al., 2007). Concurrently, Chen et al., (2009) reported that SC-CO₂ extract from Brazilian propolis contained 95% wt DHCA that can inhibit the growth of colon cancer and human leukemia (HL-60) effectively.

SC-CO₂ extraction steps could be adjusted so that the process temperature and pressure are set in sequential steps. Through this approach, a different fraction of compounds can be extracted from one raw material as the supercritical fluid is at its moderate density which separates highly soluble components such as essential oil. Meanwhile, heavier substances such as antioxidant compounds are separated by the fluid which becomes a higher density compounds towards the end of the process. A mixture of Brazilian propolis extract had been fractionated using this technique and yielded a high concentration of

interest compound in one fraction, with higher selectivity at a low solvent density (Paviani et al., 2010).

Considering the good relevance of SC-CO₂ extraction technology as an alternative method for propolis extraction, in addition to promising and important health compounds present in propolis source, there is a need for more investigation regarding this subject. Recently, demand to establish a good and optimum condition for SC-CO₂ extraction of propolis extraction has increased among researchers (Revercon & De Marco, 2006). However, application of this technique was only slightly explored. Listed in Table 2 are all available literature reported on the extraction of propolis sample using SC-CO₂ technology until 2017, hence, this may be used as a reference for future investigation and improvement.

ADVANTAGES OF SC-CO₂ EXTRACTION

Raventos et al., (2002) had summarized advantages of SC-CO₂ extraction as follow; i) good quality and better purity of recovered product, ii) faster extraction and separation process, iii) extract are free from solvent residue, iv) able to fractionate specifically targeted compounds and v) low in production cost. Recently, direct applications of high-value product in pharmaceutical and food industries have demanded interest in extraction and fractionation of bioactive compounds from plant and animal sources. Awareness of the health, environmental and safety hazards related to the application of organic solvents and possible solvent contamination in food processing's final products among the public had risen. The need to search for excellent extraction techniques to recover natural bioactive health beneficial compounds in various natural material is increasing. The urgent needs for development and improvement of new separation technologies in food processing industries are contributed by the high cost of organic solvents, stricter environmental regulations and new requirement for ultra-pure and high value-added products in this area (Mohamed & Mansoori, 2002). Conventional extraction techniques can cause the destruction of valuable compounds due to the high-temperature application (Chemat & Khan, 2011). Moreover, the usage of organic solvents may also produce an extract with solvent residue, hence reducing the product quality.

SC-CO₂ has several vital properties which are regarded as a promising alternative technology to current conventional solvent extraction methods. For example, SC-CO₂ have lower viscosity and higher diffusivity as compare to liquid solvents, hence they can penetrate porous solid material better, resulting in faster extraction due to much faster mass transfer. In addition to comparable and better recoveries in SC-CO₂ extraction, the process time could also be reduced to only a few minutes as compared to hours and days in conventional liquid-solid extraction. The continuous flow of SC-CO₂ through the sample in this extraction process provides complete or quantitative extraction. Solvation power of SC-CO₂ can also be manipulated by adjusting their pressure and temperature which leads to a remarkably high selectivity. This advantage of tunable solvation power is specifically beneficial to the extraction of complex samples, such as plant material. For example, selective extraction of vindoline compounds from among other 100 alkaloid components from *Catharanthus roseus* leaves (Song et al., 1992). Moreover, the extracted solutes dissolved in SC-CO₂ can be easily recovered by the depressurization process, which has replaced the time-consuming sample concentration process. The latter process often results in loss of targeted volatile compounds.

SC-CO₂ extraction method also requires only a little sample consumption, as small as 0.5 – 1.5 gram, as compared to conventional methods which need a bigger amount of sample up to 20 – 100 grams. It has been reported that more than 100 volatile and semi-volatile compounds could be extracted from only 1.5 gram of fresh plant sample as detected by GCMS, in which each of the components was sufficient for accurate quantifications (Lee & Markides, 1990). Furthermore, there is no application of environmentally harmful solvent in SC-CO₂ extraction process while tens to hundreds of millilitres of organic solvents may be used in the conventional extraction process.

Table 2 Summary of studies on the application of SC-CO₂ in propolis extraction. Where, T – temperature; P – Pressure; FR – Flow rate; SC – co-solvent concentration; t – time

Propolis source	Pre-treatment	Solvent	SC-CO ₂ condition	Results	Authors
Brazil	No	Ethyl-acetate	T – 40,50,60°C P - 13891 – 27681 kPa FR – 4L/min SC – 2,4,6 %	SCCO ₂ extract at 50°C & 4% SC resulted in DHCA conc. 13.9%	Lee et al., (2007)
New Zealand	Yes *Ethanol *Water	Ethanol	T – 333K P – 250 – 300bar FR ratio – 0.05 SC – 10%	SCCO ₂ extract resulted in flavonoid content – 20-35%	Catchpole et al., (2004)
Brazil	Yes *Ethyl-acetate	Ethyl acetate Ethanol	T – 308 – 328K P – 10 – 20MPa FR – 10.8 – 32.6 g/min	SCCO ₂ extract at 20MPa & 328K resulted in DHCA conc. 300 mg/g Soxhlet ext – 204 mg/g	Chen et al., (2009)
Brazil	No	Ethanol	T – 30,40,50°C P – 100 – 250bar FR – 3, 5 g/min SC – 2,5,7 %	SCCO ₂ extract – total yield 24.8% Soxhlet extract – 73%	Biscaia & Ferreira, (2009)
Italian	No	No	T – 35,40,45°C P – 130,200,270bar FR – 2L/min t – 2.5,4,5.5h	SCCO ₂ extract at 317bar, 45°C, 6.5hrs, 2L/min CO ₂ FR resulted in total yield 14.3%	De Zordi et al., (2014)
Brazil	Yes	No	T – 328K P – 20MPa FR – 10,15,20 L/min	SCCO ₂ extract concentration 25-250mg/ml – inhibit human colon & breast cancer	Wu et al., (2009)
Brazil	No	Ethyl-acetate	T – 308 – 333K P – 13.8 – 27.6MPa FR – 10L/min SC – 0 – 6 %	SCCO ₂ extract at 20.7MPa, 323K, 6wt% ethyl acetate resulted in DHCA conc. 41.2 wt% Soxhlet extract resulted in DHCA conc. 91.8 wt%	Chen et al., (2007)
Brazil	Yes *Ethanol	Ethanol	T – 20,35,50°C P – 150,200,250bar FR – 1g/min SC – 0,5,10,15%	SCCO ₂ extract without solvent – total yield 7.3% SCCO ₂ extract with solvent – total yield 51% Soxhlet extract – total yield 39.5%	Paviani et al., (2012)
Brazil	Yes *Ethanol	No	T – 60°C P – 5,10,15,20 MPa	SCCO ₂ extract – DPPH activity (27 – 93%), O ₂ scavenging activity (73%), OH scavenging activity (65%)	Wang et al., (2004)

Brazil	No	Ethanol	T – 40-50°C P – 250,350,400bar FR – 6 g/min SC – 1-2%	SCCO ₂ extract at 50°C, 300 bar, 1% EtOH – Artepillin C conc (8.98g/100g) & p-coumaric acid (0.4g/100g)	Machado et al., (2015)
Brazil	Yes *Ethanol	No	T – 60°C P – 150,200,250,300bar FR – 1g/min	SCCO ₂ extract at 60°C, 150-350 bar resulted in a total yield of 3.82 – 13.07%	Paviani et al., (2010)
Brazil	Yes *Ethanol	No	T – 60°C P – 5,10,15,20 MPa	SCCO ₂ extract – suppress lipid peroxidation & increase in antioxidant enzyme activity	Wang et al., (2006)
Brazil	No	Ethanol Water	T – 50°C P – 250 bar FR – 1.65 g/min SC – 0.79 g/min	SCCO ₂ extract resulted in a total yield of 53.5% Soxhlet extract resulted in a total yield of 44.7%	Monroy et al., (2017)

In recent decades, SC-CO₂ extraction coupled with the chromatographic method has been introduced, which is useful in extraction and direct quantification of highly volatile compounds, which also helps to reduce the process time needed (Mendiola et al., 2005).

CO₂ used in this extraction process can also be recycled or reused, hence minimizing the waste production, particularly in the large-scale SC-CO₂ extraction process. SC-CO₂ extraction can be applied in different scales of production, starting from the analytical scale which requires only less than a gram of sample, to preparative scales which needs several hundred grams of sample, and lastly to large industrial scale which can use tonnes of the sample to operate (Sahena et al., 2009). The SC-CO₂ extraction process can also provide important information regarding the extraction process and mechanisms which provide vital information for scientists to quantitatively assess, evaluate and optimize the efficiency of whole extraction process (Sahena et al., 2009).

SC-CO₂ VS CONVENTIONAL EXTRACTION

In food and natural product area, comparison on the performance of SC-CO₂ extraction and conventional extraction techniques were widely studied by many researchers. SC-CO₂ extraction has been particularly evaluated in comparison with solid-liquid extraction, hydrodistillation, soxhlet extraction, solvent extraction and ultrasound assisted extraction. SC-CO₂ extraction has been reported to have faster extraction time and higher recovery as compared to hydrodistillation for extraction of essential oil of *V.officinalis*. However, contrarily hydrodistillation method yielded a better number of volatile compounds extracted as compare to SC-CO₂ extraction method which may due to loss of several volatile compounds during depressurization step in the process (Safaralie et al., 2008).

Additionally, SC-CO₂ extraction had produced more enriched phenolic compounds as compared to solid-liquid extraction in the extraction of grape pomace (Pinelo et al., 2007). However, the composition of the extracts reported was not similar. SC-CO₂ extraction yielded simpler phenolics while solid-liquid extraction resulted in the higher amount of proanthocyanidins content. Moreover, SC-CO₂ extract demonstrated higher antiradical and antimicrobial activity compared to that in conventional techniques extract (Liu et al., 2009).

Furthermore, SC-CO₂ extraction resulted in similar yield as compared to hot hexane extraction (Wang et al., 2007). SC-CO₂ extraction method can reach a 97 – 100% recoveries for a wide range of food products extract, for instance, puff-dried product, potato chips and seeds. There was no significant difference in fatty acids composition of beef sample extract yielded from SC-CO₂ compared to that of conventional extraction method as per the nutritional labelling and education act (NLEA) procedure (King et al., 1996). Comparable results of fatty acid contents in beef and bakery samples extract were reportedly obtained from acid hydrolysis solvent extraction and SC-CO₂ extraction method. However, SC-CO₂ have recovered significantly higher concentration of lipids and fatty acids in seaweed sample as compared to chloroform and methanol soxhlet extraction (Eller & King, 1998).

Satisfactory results were recorded for fat content and fatty acid profile in dairy product extracted with SC-CO₂, which recovery was between 83.1% and 96.4%. Similarly, the extraction yield of some bakery products using SC-CO₂ was similar to that of the conventional extraction method (Wu et al., 2009). To conclude, various researches mentioned above had demonstrated SC-CO₂ extraction could be a better alternative or replacement for conventional extraction methods for a large variety of natural samples (Wu et al., 2009). Although the method has slight drawback with cost-effectiveness for low volume products, a various study has been carried out to overcome the mentioned disadvantages.

Notably, SC-CO₂ extraction of propolis sample has also been compared to several other extraction techniques such as Soxhlet extraction, maceration, and ultrasound extraction. Lee et al., (2007) has reported that the SC-CO₂ extraction method resulted in better

recovery at 40% wt. as compared to soxhlet ethyl-acetate extraction only at 16.9% wt. The report also mentioned that the SC-CO₂ method has produced the extract purity twice as high the compared to Soxhlet extraction methods. SC-CO₂ extraction was compared to Soxhlet extraction and maceration process in a study by Biscaia & Ferreira (2009) which has recorded the highest yield for SC-CO₂ extraction at 24.8% w/w, while for Soxhlet extraction, at 73% w/w. De Zordi et al., (2014) stated that extraction pressure and time affect the extraction of lipophilic components from propolis sample most significantly, while temperature has less effect. They obtained the highest yield of 14.3% using SC-CO₂ extraction at 317 bar, 45°C and 6.5 h of experimental time (with a gas flow of 2 L/min at room conditions of T and P). There was a different composition of flavones and phenolic components observed between both SC-CO₂ and conventional extraction products. The study suggested that SC-CO₂ extraction process can be utilized as pre-treatment of crude propolis for further ethanolic extraction and as a method to yield new kind of lipophilic-enriched propolis extract.

CONCLUSION

In this mini review, the interest in the application of SC-CO₂ extraction for propolis sample has been discussed. Apparently, researches on SC-CO₂ extraction of propolis is growing enormously around the globe. The aim of this review is to summarize all available literature on the application of this advanced, green and promising technique to extract propolis sample which is known to contain various beneficial health compounds as well as to discuss related matters including principle and advantages of SC-CO₂ extraction. Information presented in this short review may provide current information and insightful details regarding SC-CO₂ propolis extraction for future development and improvement.

ACKNOWLEDGEMENT

Authors thank Research University Grant (Q.J130000.2509.16H87 & Q.J130000.3009.01M10) from Universiti Teknologi Malaysia for supporting this work.

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