p-ISSN: 1907-8056
e-ISSN: 2527-5410
AGROINTEK
Jurnal Teknologi Industri Pertanian
journal homepage: journal.trunojoyo.ac.id/agrointek

Supercritical carbon dioxide extraction as an alternative method to extract natural bioactive compounds

Ananda Regita Olivira[1], Hadi Munarko[1*], Jariyah Jariyah[1], Liza Md Salleh[2]

[1]*Department of Food Technology, Universitas Pembangunan Nasional Veteran Jawa Timur, Surabaya, Indonesia*
[2]*Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Johor, Malaysia*

Article history
Received:
27 September 2023
Revised:
30 August 2024
Accepted:
31 August 2024

Keyword
Bioactive compounds;
Extraction;
Pressure;
SC-CO_2;
Temperature;
Time

ABSTRACT

The study regarding the extraction of bioactive compounds from natural sources such as phenolics, flavonoids, and carotenoids has received significant attention, because these bioactive compounds known to have numerous benefits for human health. Various extraction methods have been used to obtain bioactive compounds. However, the conventional extraction method that is often used has several drawbacks including the use of large amounts of solvent and longer extraction time. Therefore, an alternative extraction method is needed to overcome the limitations of conventional methods. Extraction using supercritical carbon dioxide is an alternative extraction method that is more efficient with a shorter extraction time. This article aims to provide a comprehensive review regarding the extraction of bioactive compounds using supercritical carbon dioxide (SC-CO_2). The use of low critical temperature and pressure during SC-CO_2 extraction prevents the degradation of the bioactive components. In addition, because of its high selectivity, SC-CO_2 extraction can produce a maximum yield with high purity of bioactive components. Therefore, extraction using supercritical carbon dioxide has the opportunity to be used as an alternative method to maximize the extraction process of bioactive compounds.

This work is licensed under a Creative Commons Attribution 4.0 International License.

[*] Corresponding author
Email : hadi.munarko.tp@upnjatim.ac.id
DOI 10.21107/agrointek.v19i2.22473

INTRODUCTION

There has been a growing interest in the past few years in the production of bioactive compounds obtained from natural sources. This is mainly driven by the increased demand for functional foods and other health support items that are made from these natural bioactive compounds. Due to the significant demand, there is currently an increase in the food industry to develop health-enhancing products by utilizing natural bioactive compounds (Sanjeewa et al. 2023). Consequently, numerous studies have been conducted to discover bioactive compounds obtained from different plant materials (Vyavaharkar and Mangaonkar 2016). Various plant materials can be used to identify and characterize bioactive compounds, such as leaves, stems, flowers, fruit (Azmir et al. 2013), and seeds (Sławińska and Olas 2022).

Natural bioactive compounds obtained from natural sources are claimed to possess anti-cancer, anti-degenerative, and anti-inflammatory properties. Fruits, such as grapes and apples, have been recognized for their antioxidant effects. Antioxidants play a crucial role in preventing lipid peroxidation and protecting DNA and proteins from damage caused by oxidative stress. Polyphenols, including flavonoids, tannins, coumarins, and lignans, have been identified for their beneficial impacts on human health. (Shashirekha et al. 2015)

Phenolics are important bioactive compoounds that act as natural antioxidants and have been found in many plants (Tungmunnithum et al. 2018). Plants consist of several phenolic compounds, such as phenolic acids (hydroxycinnamic and hydroxybenzoic acids), coumarins, flavonoids, xanthones, and polyphenols (lignin and tannin) (Do et al. 2014). Natural phenolic compounds and flavonoids are secondary plant metabolites that have aromatic rings containing at least one hydroxyl group (Tungmunnithum et al. 2018).

Extraction is one of the initial steps to obtain beneficial bioactive compounds from plant cells (Borah et al. 2022). According to Sondari et al., (2016) bioactive compounds in plants are found in low concentrations. Hence, it is crucial to use efficient and selective extraction methods to isolate desired bioactive compounds from plant materials. Conventional extraction methods are commonly used to obtained bioactive compounds from different plant sources including maceration, soxhlet extraction, hydrodistillation, and infusion (Marathe et al. 2019). According to Aziz et al. (2016), conventional extraction methods have various drawbacks, including longer extraction times, requiring large amounts of solvent and can cause thermal degradation of targeted compounds due to the continuous use of high temperatures. In addition, solvent residues which can be toxic are difficult to remove from the extract resulting in low product quality and creating large amounts of solvent waste. Therefore, supercritical fluid extraction provides an alternative approach for extraction. Supercritical fluid extraction can overcome the limitations of conventional extraction methods which offers higher efficiency and has a great potential for extracting bioactive compounds (Moslavac et al. 2014).

Over the past 20 years, there has been a significant increase in research on extraction using supercritical fluids. Supercritical fluid extraction has been employed to obtain large amounts of oils, oleoresins, a group of bioactive compounds (alkaloids, terpenes, and phenolics), as well as single compounds (α-humulene, lycopene, and α-tocopherol). (Khaw et al. 2017). Carbon dioxide (CO_2) is the the most common solvent use for supercritical fluid extraction due to its cost-effectiveness, lack of toxicity, and non-flammability. The disadvantage of carbon dioxide is its low polarity, requiring the usage of additional solvents to enhance its polarity (Aziz et al. 2016)

This article will comprehensively examine the extractio of bioactive compounds from several types of plants using supercritical carbon dioxide (SC-CO2), and various factors that can affect the extraction yield, with the aim of providing a comprehensive overview and information based on previous research.

METHODS

The methodology used for the writing of this article is a literature review. The literature reviewed consists of publications from both national and international publications, over the past decade (2013-2023). The literature reviewed was acquired from various database portals including *ResearchGate, ScienceDirect, PubMed, dan Google Scholar*. The keywords used to obtain reference articles are a combination of words

bioactive component, extraction, supercritical fluid extraction, dan supercritical carbondioxide.

The previous reserch results which met the criteria were selected by examining full text articles and abstracts. Subsequently, suitable research results are reviewed and compared with other results. Using the specified keywords, resulted in 10 primary papers that cover the process of extracting bioactive compounds using supercritical carbon dioxide. Afterwards, the 10 articles undergo a thorough examination to compile a comprehensive review article

RESULTS AND DISCUSSION

Bioactive Compounds in Plants

Bioactive compounds in plants are defined as secondary metabolites that cause pharmacological effects in humans and animals (Husna et al. 2022). Bioactive compounds can be extracted from various parts of plants such as leaves, stems, roots, seeds, flowers and fruit, using several extraction methods Using several extraction methods, bioactive compounds can be extracted from various parts of plants, such as leaves, stems, roots, seeds, flowers, and fruit (Uwineza and Waśkiewicz 2020). Fruits and vegetables contain a high amount of micronutrients like magnesium, calcium, and potassium, as well as bioactive compounds such as polyphenols, carotenoids, dietary fiber and vitamins (Samtiya et al. 2021). Seeds are considered as a renewable resource that can produce various useful products. The fruit seeds can be used for the extraction of plant oils, which contain many important bioactive compounds and natural antioxidants (Mallek-Ayadi et al. 2018).

Bioactive compounds from plants can be used in the production of various functional foods because of their health benefits. The primary bioactive agents are phenolic compounds which are secondary metabolites from plants. There are two primary categories of phenolic compounds, which are phenolic acids and flavonoids. The first group contains compounds derived from benzoic acid and compounds derived from cinnamic acid. The second group consist of low molecular weight molecules known as flavonoids, namely flavones, flavonols, flavanones, flavan-3-ols, anthocyanidins, isoflavones, coumarins, stilbenes, and lignans. These chemicals are present in several plant components, including leaves, seeds, bark, and flowers. The concentrations of phenolic compounds might be different between bark, leaves, and fruit due to differences in sunlight exposure. Fruit is the part with the highest concentration of polyphenols due to its greater exposure to sunlight. Apart from that, the skin and seeds are also a good source of phenolic acids and flavonoids, especially polymethoxylated flavones and glycosylated flavones, which are mainly found in citrus (Týskiewicz et al. 2018)

A wide variety of secondary metabolites have been found in plants, including glycoalkaloids, antioxidant components, and vitamins. These compounds have been confirmed to have many beneficial effects on human health, such as antioxidant effects, anti-inflammatory and cardioprotective properties, as well as preventing obesity and diabetes (Samtiya et al. 2021). Natural antioxidants derived from plants mostly consist of polyphenols, such as stillene, anthocyanins, flavonoids, lignans, and phenolic acids, in addition to carotenoids (carotene and xanthophyll), vitamin C, and vitamin E. These natural antioxidant especially carotenoids and polyphenols, have a variety of biological properties, such as anti-aging, anti-inflammatory, anti-viral, anti-microbial, and anti-cancer (Xu et al. 2017). In addition, natural antioxidants can regulate blood sugar levels in the body by controlling insulin production through enhancing the function of the pancreas (Oktaviani and Ulilalbab 2020).

Over the past few years, numerous studies has been conducted to extract bioactive compounds from different plant materials. Several bioactive compounds that have been isolated from different plant materials are shown in Error! R eference source not found..

Recently, there has been a high demand in the utilization of by-products such as seeds and skins, as they have the potential to produce a valuable source of bioactive compounds. The use of natural bioactive compounds, especially phenolics derived from fruits and vegetables or their by-products, has experienced a significant surge as a replacement for synthetic compounds (Sharayei et al. 2019). Research by Jokić et al. (2020), showed that orange peel contains significant amounts of bioactive compounds, such as limonoids and phenolics. Hesperidin is the main flavonoid found in every part of orange fruit, including the peel. Hesperidin has the ability as an antioxidant in reducing Reactive Oxygen Species (ROS) in cells and optimizing mitochondrial

enzyme activity (Nandan and Meena 2015). Apart from that, potato skins also reported to have a significant amount of bioactive compounds, especially phenolics consisting of flavonoids, anthocyanins, chlorogenic acid and caffeic acid (Akyol et al. 2016). Various polyphenols such as anthocyanins, gallotannins, ellagitannins, gallagyl esters, hydroxybenzoic acids, hydroxycinnamic acids, and dihydroflavonols have been identified from pomegranate peels which have strong anti-mutagenic, antioxidant, antimicrobial and apoptotic properties. (Akhtar et al. 2015). Coelho et al. (2019)9), have been identifying bioactive components from mango peel extract using High Performance Liquid Chromatography (HPLC). The main bioactive compounds found were flavanols (epicatechin-gallate, epigallocationchin-gallate), flavonols (quercetin-3-O-glucopyranoside and routine), and phenolic acids (gallic acid, o-coumaric acid, and syringic acid).

Various types of fruit also have the potential to be used as sources of various bioactive compounds. Fruit is the pulpy or fleshy part of a plant that has a sweet or sour flavor and can be eaten in its raw form. Fruit is a potential source of carbohydrates, vitamins, and bioactive compounds including phenols and fiber. These substances have biological properties that can help reduce the risk of some diseases (Singh et al. 2017).

Table 1 Various bioactive compounds identified from different commodities

Commodity	Plant Parts	Bioactive Compounds	References
Orange	Peels	Limonoid, hesperidin	(Jokić et al. 2020)
Potato	Peels	Flavonoids, anthocyanins, chlorogenic acid, caffeic acid	(Akyol et al. 2016)
Pomegranate	Peels	Anthocyanins, gallotannins, ellagitannins, gallagyl esters, hydroxybenzoic acid, hydroxycinnamic acid, and dihydroflavonols	(Akhtar et al. 2015)
Mango	Peels	Flavanols, flavonols, phenolic acids	
	Fruit	Polyphenols mangiferin, catechin, quercetin, kaempferol, gallic acid, and benzoic acid	(Coelho et al. 2019)
Bilberry	Fruit	Ellagicpentoside acid, feruloyl hexoside	(Babova et al. 2016)
Dates	Fruit	p-hydroxybenzoic acid, gallic acid, protocatechuic acid, vanillic acid, o-coumaric acid, caffeic acid, syringic acid, ferulic acid, p-coumaric acid, 3-caffeoylquinic acid, and 3- o-caffeoylshikimic acid.	(Hammouda et al. 2013)
Melon	Seeds	Flavonoids (Amentoflavone, luteolin-7-O-glycoside), phenolic acids (gallic acid), tocopherols, and phytosterols (β-sitosterol)	(Mallek-Ayadi et al. 2018)
Apple	Seeds	Phloridzin, dihydrochalcone, hydroxycinnamic acid, flavan-3-ol, flavonol	(Xu et al. 2016)

Table 2 Critical points of some solvents (Khaw et al. 2017)

Solvent	Critical Temperature (°C)	Critical Pressure (Mpa)	Density in Critical Condition (kg.m^{-3})
Carbon dioxide	30.9	7.37	467.6
Nitrogen	-147	3.4	313.3
Ammonia	132.2	11.33	225
n-propane	96.7	4.25	220.5

Mango is a potential source of flavonoids and carotenoids (Coelho et al. 2019). Mango contain several bioactive compounds, including mangiferin, catechin, quercetin, kaempferol, gallic acid, and benzoic acid. These compounds have been associated with the prevention of degenerative diseases such as cancer, cardiovascular disease, and diabetes (Sellamuthu et al. 2013). Babova et al. (2016), showed that bilberries contain a high concentration of polyphenolic and phenolics. Bilberries contain several phenolic compounds such as benzoic acid derivatives, phenylpropanoids, and flavonoids. The most abundant compound from this fraction was ellagicpentoside acid, followed by feruloyl hexosid. Other potential fruit as a source of bioactive compounds is dates. Study by Hammouda et al. (2013), discovered dates as a rich source of polyphenols, including p-hydroxybenzoic acid, gallic acid, protocatechuic acid, vanillic acid, o-coumaric acid, caffeic acid, syringic acid, ferulic acid, p-coumaric acid, 3-caffeoylquinic acid, and 3-o-caffeoylshikimic acid. Proanthocyanidins accounted for about 1.5%, about 95% of total polyphenols.

Seeds are also considered as a potential source of bioactive compounds. Mallek-Ayadi et al. (2018), found that melon seeds contain a significant amount of bioactive compounds, including flavonoids, phenolic acids, tocopherols, and phytosterols. The primary phytosterol found in melon seeds is β-sitosterol. Amentoflavone, luteolin-7-O-glycoside, and gallic acid are the most abundant phenolic compounds found in melon seeds. A study conducted by , showed that various kinds of bioactive compounds are also found in apple seeds. Apple seeds are rich in polyphenols, especially phloridzin. These polyphenols consist mainly of dihydrochalcone; hydroxycinnamic acid; flavan-3-ol which exists in the form of monomers ((+)-catechin and (-)-epicatechin) and oligomers (proanthocyanin B2) or even polymers; and flavonols. Phloridzin has been found to have antioxidant properties that may prevent the process of lipid peroxidation. In addition to its antioxidant activity, phloridzin has been recognized as a potential antidiabetic agent due to its ability to inhibit the absorption of glucose in intestinal and kidneys.

(Jokić et al. 2020)(Akyol et al. 2016)(Akhtar et al. 2015)(Coelho et al. 2019)(Babova et al. 2016)(Hammouda et al. 2013)(Mallek-Ayadi et al. 2018)(Xu et al. 2016)(Khaw et al. 2017)

Supercritical Fluid Extraction (SFE)

Supercritical fluid refers to the condition of a fluid when it surpasses its critical pressure and temperature. Fluids in supercritical conditions exhibit gas-like characteristics while also possessing the ability to dissolve substances like a liquid. This characteristic makes supercritical fluids as a valuable medium for mass transfer due to their similarity to gases in terms of diffusivity and viscosity, while also having a density similar to liquids (Rinawati et al. 2020). Supercritical fluids can be used in several applications of separation technology, such as extracting food and medicines, extracting volatile chemicals from substrates, fractionation, reactive separation, and crystallization. Supercritical fluid extraction (SFE) is the most frequent use of this technology. Supercritical fluid extraction is a method employing a supercritical fluid as a solvent to separate soluble substances from insoluble residues. This extraction method is considerably effective and efficient for extracting natural compounds from plants. (Kwartiningsih et al. 2018).

Supercritical fluid extraction (SFE) is a more modern and eco-friendly method that provides numerous advantages compared to conventional extraction methods. Supercritical fluid extraction is a very promising technology due to the use of environmentally friendly solvents along with its capacity to produce extracts with higher purity (Moncada et al. 2016). The solubility of a substance in a supercritical fluid can be modified by adjusting the temperature and pressure during the process. Another advantages of supercritical fluid are its low viscosity and high solute diffusivity (Goleroudbary and Ghoreishi 2016). Due to their low viscoosity and relatively high diffusity, supercritical fluids possess greater mass transfer capabilities compared to liquids in normal condition. Supercritical fluids can rapidly diffuse through solid materials, resulting in higher extraction rates (da Silva et al. 2015).

Another advantage of SFE is its ability to operate with a smaller sample size compared to conventional extraction methods. Normally, conventional extraction methods require the use of 20-100 grams of material, whereas extraction using SFE only takes 0.5-1.5 grams of sample. It has been reported that from just 1.5 g of fresh plant samples, more than 100 volatile and semi-volatile compounds can be extracted and detected by gas chromatography–mass spectroscopy (GC-MS), of

which more than 80 compounds are present in sufficient quantities for accurate quantification (Ayre et al. 2013).

SFE is carried out by pumping the supercritical fluid through a vessel filled with the sample, and subsequently reducing the solvent pressure to obtain the desired components. SFE instrument usually consists of one or two high pressure pumps for distributing the solvent, ocasionally polar co-solvent such as ethanol is required, a high pressure vessel to contain the sample, a barrier and an extract collection device (Rinawati et al. 2020). Several fluids that can be used as solvents in SFE are carbon dioxide, ammonia, hydrocarbons such as propane and butane. (Escobar et al. 2020), and nitrogen (Khaw et al. 2017)

The extraction process using SFE involves three primary steps. In the initial step, CO_2 is pressurized to around 50 bar and cooled to a temperature below 5°C in order to keep it in a liquid state. The pressure in the system is controlled by a basic regulator or a back pressure regulator. After that, the liquid carbon dioxide (CO_2) is then delivered into the heating zone and exposed to high temperatures until it reaches a supercritical state. Supercritical carbon dioxide is pumped into the extraction vessel, where it quickly spreads throughout the solid matrix and dissolves the desired component. The dissolved component is transferred from the extraction vessel to the separator under lower pressures, allowing the extracted material to separate from the solvent. Then, the supercritical fluid can be put through a cooling process and eventually reused (Geeta et al. 2020).

Carbon Dioxide as a Supercritical Fluid

Carbon dioxide (CO_2) is the most commonly used supercritical fluid for the extraction of natural compounds due to its cost-effectiveness, highly pure, colorless, odorless, non-toxic, non-flammable, and considered as safe. Furthermore, compared to other supercritical solvents (Error! R eference source not found.) CO_2 has a critical point at a comparatively low pressure and close to room temperature. Considering the usage of lower pressure and temperature conditions CO_2 could effectively prevent the thermal and oxidative degradation of bioactive compounds Using lower pressure and temperature conditions, CO2 could prevent bioactive compounds' thermal and oxidative degradation (Babova et al., 2016). Another advantage of using CO_2 is that it is easy to separate from the extract. The decrease in pressure after extraction will cause the transformation of the supercritical fluid into a gaseous state, leading to its separation from the extract. This allows recovery and reuse of the extraction solvent (Farías-Campomanes et al. 2015)

The limitation of CO_2 as a solvent lies in its nonpolar and lipophilic characteristics, CO_2 is considered a weak solvent in extracting compounds with high polarity, such as phenolic compounds (Da Porto and Natolino 2017). However, the addition of polar solvents as co-solvents in small amounts such as water or ethanol (<5% w/w) can enhance the polarity of CO_2 by improving the solubility of more polar substances; in this condition, the system moves in a two-phase subcritical state (Campardelli et al. 2015). Two extraction stages are carried out using a co-solvent, including, removing non-polar compounds with SC-CO_2. The second phase is to extrac(Da Porto and Natolino 2017) polar compounds with SC-CO_2 + co-solvent (Da Porto and Natolino 2017).

Factors Affecting The Extraction Process

The efficiency of the extraction process using supercritical carbon dioxide (SC-CO_2) is influenced by several factors, including particle size, temperature, pressure, solvent flowrate, and solvent volume. The extraction conditions can be modified to enhance the yield of the extract. The SC-CO_2 extraction process is mainly controlled by the pressure and temperature. The temperature and pressure have a significant influence on the solubility of solutes in solvents. These factors impact the density of SC-CO_2 and its ability to dissolve solutes. Increasing the temperature leads to a decrease in the density of SC-CO_2 while increasing the solute pressure. As the pressure of SC-CO_2 decreases towards critical pressure, its density will significantly drop. Consequently, raising the temperature will reduce the solubility of the solute (Masoodi et al. 2019). On the other hand, higher pressure leads to higher extraction yield as it leads to increasing liquid density, hence enhancing the solubility of the solute. The solubility of the solute in the supercritical fluid has a direct impact on the extraction yield (Ara et al. 2015).

Several study have been conducted to observed the influence of these factors in the

extraction yields. Maran and Priya (2015) carried out an extraction process using SC-CO_2 on muskmelon seeds. The research showed that raising the pressure from 30 to 40 Mpa resulted in a higher yield of extracted oil. Under a pressure of 30 Mpa, 18.04% of oil was obtained, while at a pressure of 40 Mpa, only 35.07% of oil was obtained. Another study conducted by Pavlić et al. (2020) on raspberry seeds showed similar results, revealing that an increase in pressure had a positive effect on the amount of extract obtained under pressure conditions of 25 MPa (0.069 kg oil/kg solid) and 30 MPa (0.113 kg oil/kg solid).

Furthermore, the solvent volume and higher flowrate of SC-CO_2 will also enhance the yield. A larger solvent volume can dissolve the desired component more effectively, thus enhancing extraction efficiency (Jokić et al. 2020). According to Wang et al. (2017), the oil extraction from Chinese quince seeds increased from 10.73% to 16.08% when the solvent/solid ratio was adjusted from 5 to 10 mL/g. The outcome lines up with the theory of mass transfer, where the driving force is the difference in concentration between the solid and solvent (S/S) during mass transfer, which increases with greater S/S ratios.

Meanwhile, increasing the flowrate of SC-CO_2 can enhance the yield by increasing the total amount of SC-CO_2 molecules per unit volume and inducing turbulence in the solid particle layer. This leads to an increase in intermolecular interactions between SC-CO_2 and the solute. The increase in the flowrate of SC-CO_2 also reduces the contact time of SC-CO_2 with the sample; as a result, it takes less time for dissolving solute particles (Shrirame et al. 2018). The research conducted by Norodin et al. (2016) on mahogany seeds revealed that the overall yield of oil extract increased as the flowrate of SC-CO_2 increased from 2 ml/min to 4 ml/min, while maintaining a constant pressure of 30 MPa and temperature of 50^{o}C. Priyadarsani et al. (2021), demonstrated similar findings, where the extraction yield of lycopene from grapefruit (*Citrus paradisi*) endocarp increases with the increase in the flowrate of SC-CO_2 from 15 g/min to 45 g/min. This findings are in accordance with (Daraee et al. 2019)(Daraee et al. 2019). They showed that the extraction yield of chlorogenic acid from sunflower seed cernels increased from 37.75% to 52.08% along with the increasing flowrate of SC-CO_2 from 0.6 ml/min to approximately 1.64 ml/min.

Apart from that, particle size also affects the extraction yields. Norodin et al. (2016), extracted mahogany seeds with three different particle sizes (0, 25; 0.5; and 0.75 mm). The results showed that the highest yield was obtained from samples with a particle size of 0.5 mm (20.68%), compared with a particle size of 0.25 mm (11.64%), and a particle size of 0.75 mm (18.26%). A smaller particle size will enhance the extraction yield due to the increased mass transfer associated with smaller sample particle size. Nevertheless, a drawback arises when the sample size is too small, as it can lead to a decrease in the extraction rate caused by the channeling effect within the extraction vessel. The channeling effect in the extraction vessel is caused by the sample particles being too fine, which leads to a decrease in the interaction between the sample and the solvent. This occurs because when the particle size is excessively small, the porosity of the sample decreases, causing a deceleration in the penetration of CO_2 into the sample. Consequently, the extraction yields will decreased. The study conducted by Putra et al. (2018) peanut skins showed similar results, where peanut skins with a particle size of 425 μm produced the highest yield (12.33%) compared to larger particle sizes of 500 μm (7.55%) and smaller particle sizes of 355 μm (10.31%).

Application of SC-CO_2 for Bioactive Compounds Extraction

Several studies have been carried out to extract bioactive compounds especially phenolics from various natural sources using different extraction conditions (Tabel 3). Study by Syukriah et al. (2015), shows that to extract phenolic compounds from *Quercus infectoria* galls using optimal extraction conditions at temperature of 40^{o}C, with a pressure of 30 Mpa, and a SC-CO_2 flowrate of 2 ml/min for 2 hours can obtain a phenolic compound content 2 times higher (203.53 mg GAE/g) than soxhlet extraction using methanol solvent (95.86 mg GAE/g) which requires an extraction time of 6 hours. Previous research by (Syukriah and Azizi 2014), showed similar results with extraction conditions at a temperature of 49.6^{o}C, pressure of 26.84 Mpa, and SC-CO_2 flowrate of 2 mL/min for 120 minutes. The total phenolic content from *Quercus infectoria* galls using SC-CO_2 showed higher results (143.75 mg GAE/g) compared to Soxhlet extraction using 70% methanol (112.28 mg GAE/g) which required an extraction time of 6

hours. However, in both studies, the soxhlet extraction method produced a greater amount of extract compared to SC-CO_2 extraction. This demonstrates that while SC-CO_2 extraction may result in a lesser yield, it offers greater selectivity compared to soxhlet extraction. As a result, the desired component can be obtained with a higher level of purity.

In general, phenolics exhibit a polar compound properties. Therefore, some studies applying the use of co-solvent to enhance the polarity of SC-CO_2. Vyavaharkar and Mangaonkar (2016), extracted phenolics from *Buchanania lanzan Spreng* seeds. Optimal extraction conditions using SC-CO_2 were obtained at a temperature of 35^{o}C, pressure 19.61 Mpa, with a CO_2 flowrate of 3 ml/minute, and ethanol as a co-solvent with a concentration of 5.66%. Extraction lasted for 60 minutes. From these optimal conditions, a yield of 20.50% was produced, with a total phenolics content of 52.14 mg GAE/g. Research by Lima et al. (2021) used methanol as a co-solvent to extract phenolic compounds from potato peels. The optimal extraction conditions are at a temperature of 80^{o}C, a pressure of 350 bar, CO_2 flowrate of 18 g/min, and a methanol concentration of 20%. The extraction process was carried out for a duration of 60 minutes. The total phenolic compounds collected under these conditions were 5.24 mg GAE/g. Out of the overall total phenolic content, contains 3.87 mg/g of chlorogenic acid, which accounts for 64% of the total phenolic compounds, and an additional 0.92 mg/g of caffeic acid. The two bioactive compounds mentioned are the primary phenolic compounds found in potato skins, contributing to approximately 80% of the overall phenolic content. Apart from potato skins, chlorogenic acid can also be obtained from sunflower seed kernels, as demonstrated by oleh Daraee et al. (2019). For this study, ethanol was used as a co-solvent at a concentration of 5%. The optimal extraction conditions were obtained using SC-CO_2 at a temperature of 40^{o}C, pressure of 16.9 MPa, CO_2 flowrate of 1.6 ml/min, for a duration of 104.6 minutes. Under these conditions, a yield of 52.08% chlorogenic acid was successfully extracted.

Goyeneche et al. (2020) extracted phenolic compounds from *Beta Vulgaris* L. (beetroot) leaves. The extraction was performed under optimal conditions, with a temperature of 35^{o}C, a pressure of 400 bar, CO_2 flowrate of 0.6 kg/h, and the use of ethanol as a co-solvent. The extraction process was conducted for 30 minutes. Applying these optimal conditions resulted in a total phenolic content of 3370.8 g GAE/ g d.m. Other study conducted by Pimentel-Moral et al. (2019), successfully obtaining bioactive compounds from *Hibiscus sabdariffa* flower petals. Optimal extraction conditions were obtained at a temperature of 50^{o}C, pressure 250 bar, CO_2 flowrate 25g/min. The extraction process was carried out for 90 minutes. Additionally, a co-solvent consisting of 16.7% ethanol was used in this study. This extraction condition can obtain a total phenolics content of 113 ± 1 mg/g extract. This study demonstrates a positive correlation between the concentration of ethanol as a co-solvent and the total phenolic compounds that can be obtained. It shows that, the higher the concentration of ethanol as a co-solvent, the total phenolic compounds that can be obtained will also increase.

On the other hand, numerous studies have been conducted to obtain lycopene. Lycopene is a highly important carotenoid because of its strong antioxidant abilities. Study by Priyadarsani et al. (2021) extracted lycopene from ripe grapefruit endocarp. The optimal conditions for extraction were achieved at a temperature of 70^{o}C, a pressure of 305 bar, and CO_2 flowrate of 35 g/min. The extraction process conducted for a duration of 135 minutes. Under these conditions, up to 93% lycopene was successfully obtained. Haddadin and Haddadin (2015), also succeeded in extracting lycopene from tomato fruit by-products (skin, seeds and inner tissue) using lower pressure at a higher temperature, namely 80^{o}C, with a pressure of 276 bar, CO_2 flowrate 8ml/ min, extraction lasted for 60 minutes. In this condition, 82.50% lycopene was successfully obtained. This result showed a lower lycopene concentration compared to study conducted by Priyadarsani et al. (2021). This can be influenced by the use of lower pressure conditions, increasing pressure will also increase the density of the solvent thereby increasing the solubility of lycopene.

Another carotenoid that can be extracted using SC-CO_2 is fucoxanthin. Fucoxanthin is a carotenoid compound that has significant anticancer, antihypertensive, antiobesity and anti-inflammatory properties. Wakame (*Undaria pinnatifida*) is known as a potential source of fucoxanthin. The optimal extraction conditions to obtain fucoxanthin from wakame are at a

temperature of 40°C, pressure of 40 MPa, with a CO_2 flowrate of 4.0 mL/min, and an extraction time of 180 minutes. This extraction condition was able to obtain a maximum fucoxanthin content of 38.5 ± 2.5 mg/g extract (Quitain et al. 2013).

CONCLUSION

Supercritical carbon dioxide (SC-CO2) has the potential to be used to extract bioactive compounds from various materials of plants and has been proven to be more effective compared to conventional extraction methods. SC-CO2 provides more selective extraction, and can produce purer extracts with a high content of bioactive compounds. Various kinds of research have been carried out to determine suitable extraction conditions. The use of polar solvents as co-solvents needs to be considered because it can expand the range of extraction of polar bioactive compounds.

REFERENCES

Akhtar, S., T. Ismail, D. Fraternale, and P. Sestili. 2015. Pomegranate peel and peel extracts: Chemistry and food features. *Food Chemistry* 174:417–425.

Akyol, H., Y. Riciputi, E. Capanoglu, M. Caboni, and V. Verardo. 2016. Phenolic Compounds in the Potato and Its Byproducts: An Overview. *International Journal of Molecular Sciences* 17(6):835.

Ara, K. M., M. Jowkarderis, and F. Raofie. 2015. Optimization of supercritical fluid extraction of essential oils and fatty acids from flixweed (Descurainia Sophia L.) seed using response surface methodology and central composite design. *Journal of Food Science and Technology* 52(7):4450–4458.

Ayre, A., K. Ghude, P. Mane, M. Nemade, S. Gosavi, A. Pathare, and A. Lad. 2013. *Supercritical Fluid Extraction-A Green Paradigm in the Area of Separation Science*.

Aziz, A. H. A., M. A. C. Yunus, N. H. Arsad, N. Y. Lee, Z. Idham, and A. Q. A. Razak. 2016. Optimization of supercritical carbon dioxide extraction of Piper Betel Linn leaves oil and total phenolic content. *IOP Conference Series: Materials Science and Engineering* 162(1):012031.

Azmir, J., I. S. M. Zaidul, M. M. Rahman, K. M. Sharif, A. Mohamed, F. Sahena, M. H. A. Jahurul, K. Ghafoor, N. A. N. Norulaini, and A. K. M. Omar. 2013. Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering* 117(4):426–436.

Babova, O., A. Occhipinti, A. Capuzzo, and M. E. Maffei. 2016. Extraction of bilberry (Vaccinium myrtillus) antioxidants using supercritical/subcritical CO2 and ethanol as co-solvent. *Journal of Supercritical Fluids* 107:358–363.

Borah, A., S. Selvaraj, S. R. Holla, and S. De. 2022. Extraction and characterization of total phenolic and flavonoid contents from bark of Swietenia macrophylla and their antimicrobial and antioxidant properties. *Arabian Journal of Chemistry* 15(12).

Campardelli, R., L. Baldino, and E. Reverchon. 2015. Supercritical fluids applications in nanomedicine. *The Journal of Supercritical Fluids* 101:193–214.

Coelho, E. M., M. E. A. O. de Souza, L. C. Corrêa, A. C. Viana, L. C. de Azevêdo, and M. dos Santos Lima. 2019. Bioactive Compounds and Antioxidant Activity of Mango Peel Liqueurs (Mangifera indica L.) Produced by Different Methods of Maceration. *Antioxidants* 8(4):102.

Daraee, A., S. M. Ghoreishi, and A. Hedayati. 2019. Supercritical CO2 extraction of chlorogenic acid from sunflower (Helianthus annuus) seed kernels: modeling and optimization by response surface methodology. *Journal of Supercritical Fluids* 144:19–27.

Do, Q. D., A. E. Angkawijaya, P. L. Tran-Nguyen, L. H. Huynh, F. E. Soetaredjo, S. Ismadji, and Y.-H. Ju. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis* 22(3):296–302.

Escobar, E. L. N., T. A. da Silva, C. L. Pirich, M. L. Corazza, and L. Pereira Ramos. 2020, April 23. Supercritical Fluids: A Promising Technique for Biomass Pretreatment and Fractionation. Frontiers Media S.A.

Farías-Campomanes, A. M., M. A. Rostagno, J. J. Coaquira-Quispe, and M. A. A. Meireles. 2015. Supercritical fluid extraction of polyphenols from lees: overall extraction curve, kinetic data and composition of the extracts. *Bioresources and Bioprocessing* 2(1):45.

Geeta, H. P., G. Srinivas, and P. S. Champawat. 2020. Supercritical Fluid Extraction of Bioactive Compounds from Bioresource: A Review. *International Journal of Current Microbiology and Applied Sciences* 9(4):559–566.

Goleroudbary, M. G., and S. M. Ghoreishi. 2016. Response surface optimization of Safranal and Crocin extraction from Crocus sativus L. via supercritical fluid technology. *The Journal of Supercritical Fluids* 108:136–144.

Goyeneche, R., K. Di Scala, C. L. Ramirez, and M. A. Fanovich. 2020. Recovery of bioactive compounds from beetroot leaves by supercritical CO2 extraction as a promising bioresource. *The Journal of Supercritical Fluids* 155:104658.

Haddadin, M. S. Y., and J. S. Haddadin. 2015. Lycopene Extraction from Tomato Pomace with Supercritical Carbon Dioxide: Effect of Pressures, Temperatures and CO2 Flow Rates and Evaluation of Antioxidant Activity and Stability of Lycopene. *Pakistan Journal of Nutrition* 14(12):942–956.

Hammouda, H., J. K. Chérif, M. Trabelsi-Ayadi, A. Baron, and S. Guyot. 2013. Detailed Polyphenol and Tannin Composition and Its Variability in Tunisian Dates (Phoenix dactylifera L.) at Different Maturity Stages. *Journal of Agricultural and Food Chemistry* 61(13):3252–3263.

Husna, N. El, E. Noor, F. Fahma, and T. C. Sunarti. 2022. Teknik Ekstraksi dan Nanoenkapsulasi Komponen Bioaktif Buah Malaka: Tinjauan Literatur. *Agrointek : Jurnal Teknologi Industri Pertanian* 16(2):171–185.

Jokić, S., M. Molnar, A. M. Cikoš, M. Jakovljević, S. Šafranko, and I. Jerković. 2020. Separation of selected bioactive compounds from orange peel using the sequence of supercritical CO2 extraction and ultrasound solvent extraction: optimization of limonene and hesperidin content. *Separation Science and Technology (Philadelphia)* 55(15):2799–2811.

Khaw, K. Y., M. O. Parat, P. N. Shaw, and J. R. Falconer. 2017, July 1. Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. MDPI AG.

Kwartiningsih, E., W. B. Sediawan, M. Hidayat, and A. T. Yuliansyah. 2018. Preparation of supercritical fluid extraction using dry ice and exploration of equation of state to predict the operating conditions. Page 020012.

Lima, M. de A., R. Andreou, D. Charalampopoulos, and A. Chatzifragkou. 2021. Supercritical carbon dioxide extraction of phenolic compounds from potato (Solanum tuberosum) peels. *Applied Sciences (Switzerland)* 11(8).

Mallek-Ayadi, S., N. Bahloul, and N. Kechaou. 2018. Chemical composition and bioactive compounds of Cucumis melo L. seeds: Potential source for new trends of plant oils. *Process Safety and Environmental Protection* 113:68–77.

Maran, J. P., and B. Priya. 2015. Supercritical fluid extraction of oil from muskmelon (Cucumis melo) seeds. *Journal of the Taiwan Institute of Chemical Engineers* 47:71–78.

Marathe, S. J., S. B. Jadhav, S. B. Bankar, K. Kumari Dubey, and R. S. Singhal. 2019. Improvements in the extraction of bioactive compounds by enzymes. *Current Opinion in Food Science* 25:62–72.

Masoodi, F., S. Ahmad Rather, and S. Mohd Wani. 2019. *Supercritical Fluid Extraction: A Review Walnut View project Food Hydrocolloids as Encapsulating Agents in Delivery Systems View project*. Page *J. Biol. Chem. Chron*.

Moncada, J., J. A. Tamayo, and C. A. Cardona. 2016. Techno-economic and environmental assessment of essential oil extraction from Oregano (Origanum vulgare) and Rosemary (Rosmarinus officinalis) in Colombia. *Journal of Cleaner Production* 112:172–181.

Moslavac, T., S. Jokić, D. Šubarić, K. Aladić, J. Vukoja, and N. Prce. 2014. Pressing and supercritical CO2 extraction of Camelina sativa oil. *Industrial Crops and Products* 54:122–129.

Nandan, M. P., and V. Meena. 2015. Extraction, Modelling and Purification of Flavonoids from Citrus Medica Peel. *International Journal of Applied Sciences and Biotechnology* 3(4):588–591.

Norodin, N. S. M., L. M. Salleh, Hartati, and N. M. Mustafa. 2016. Supercritical carbon

dioxide (SC-CO2) extraction of essential oil from Swietenia mahagoni seeds. Page *IOP Conference Series: Materials Science and Engineering*. Institute of Physics Publishing.

Oktaviani, I. I., and A. Ulilalbab. 2020. Pengaruh Penambahan Tepung Biji Alpukat (Persea americana Mill) Dalam Pembuatan Roti Tawar Terhadap Kadar Air dan Daya Terima. *Jurnal Teknologi Pangan dan Kesehatan (The Journal of Food Technology and Health)* 2(1):44–52.

Pavlić, B., L. Pezo, B. Marić, L. P. Tukuljac, Z. Zeković, M. B. Solarov, and N. Teslić. 2020. Supercritical Fluid Extraction of Raspberry Seed Oil: Experiments and Modelling. *Journal of Supercritical Fluids* 157.

Pimentel-Moral, S., I. Borrás-Linares, J. Lozano-Sánchez, D. Arráez-Román, A. Martínez-Férez, and A. Segura-Carretero. 2019. Supercritical CO2 extraction of bioactive compounds from Hibiscus sabdariffa. *The Journal of Supercritical Fluids* 147:213–221.

Da Porto, C., and A. Natolino. 2017. Supercritical fluid extraction of polyphenols from grape seed (Vitis vinifera): Study on process variables and kinetics. *Journal of Supercritical Fluids* 130:239–245.

Priyadarsani, S., A. S. Patel, A. Kar, and S. Dash. 2021. Process optimization for the supercritical carbondioxide extraction of lycopene from ripe grapefruit (Citrus paradisi) endocarp. *Scientific Reports* 11(1):10273.

Putra, N. R., D. N. Rizkiyah, A. S. Zaini, M. A. C. Yunus, S. Machmudah, Z. binti Idham, and M. S. Hazwan Ruslan. 2018. Effect of particle size on yield extract and antioxidant activity of peanut skin using modified supercritical carbon dioxide and soxhlet extraction. *Journal of Food Processing and Preservation* 42(8).

Quitain, A. T., T. Kai, M. Sasaki, and M. Goto. 2013. Supercritical Carbon Dioxide Extraction of Fucoxanthin from Undaria pinnatifida. *Journal of Agricultural and Food Chemistry* 61(24):5792–5797.

Rinawati, R., G. Gustami Pangesti, and N. L. Gede Ratna Juliasih. 2020. Review: Green Analytical Chemistry: Pemanfaatan Supercritical Fluid Extraction (SFE) dan Microwave-Assisted Extraction (MAE) Sebagai Metode Ekstraksi Senyawa Diterpena Pada Minyak Biji Kopi Shangrai. *Analit:Analytical and Environmental Chemistry* 5(1):24–33.

Samtiya, M., R. E. Aluko, T. Dhewa, and J. M. Moreno-Rojas. 2021. Potential Health Benefits of Plant Food-Derived Bioactive Components: An Overview. *Foods* 10(4).

Sanjeewa, K. K. A., K. H. I. N. M. Herath, Y.-S. Kim, Y.-J. Jeon, and S.-K. Kim. 2023. Enzyme-assisted extraction of bioactive compounds from seaweeds and microalgae. *TrAC Trends in Analytical Chemistry* 167:117266.

Sellamuthu, P. S., G. I. Denoya, D. Sivakumar, G. A. Polenta, and P. Soundy. 2013. Comparison of the Contents of Bioactive Compounds and Quality Parameters in Selected Mango Cultivars. *Journal of Food Quality* 36(6):394–402.

Sharayei, P., E. Azarpazhooh, S. Zomorodi, and H. S. Ramaswamy. 2019. Ultrasound assisted extraction of bioactive compounds from pomegranate (Punica granatum L.) peel. *LWT* 101:342–350.

Shashirekha, M. N., S. E. Mallikarjuna, and S. Rajarathnam. 2015. Status of Bioactive Compounds in Foods, with Focus on Fruits and Vegetables. *Critical Reviews in Food Science and Nutrition* 55(10):1324–1339.

Shrirame, B. S., S. R. Geed, A. Raj, S. Prasad, M. K. Rai, A. K. Singh, R. S. Singh, and B. N. Rai. 2018. Optimization of Supercritical Extraction of Coriander (Coriandrum sativum L.) Seed and Characterization of Essential Ingredients. *Journal of Essential Oil Bearing Plants* 21(2):330–344.

da Silva, R. P. F. F., T. A. P. Rocha-Santos, and A. C. Duarte. 2015. Supercritical Fluid Extraction of Bioactive Compounds. *Trends in Analytical Chemistry* 76:40–51.

Singh, B., J. P. Singh, K. Shevkani, N. Singh, and A. Kaur. 2017. Bioactive constituents in pulses and their health benefits. *Journal of Food Science and Technology* 54(4):858–870.

Sławińska, N., and B. Olas. 2022. Selected Seeds as Sources of Bioactive Compounds with Diverse Biological Activities. *Nutrients* 15(187):1–42.

Syukriah, N., and M. Azizi. 2014. Effect of different extraction techniques on total phenolic content and antioxidant activity of Quercus infectoria galls. *International Food Research Journal* 21(3):1075–1079.

Syukriah, N., M. Azizi, and F. Adibah. 2015. Total Phenolic Content and Antioxidant Activity of Quercus infectoria Galls Using Supercritical CO 2 Extraction Technique and Its Comparison with Soxhlet Extraction. *Pertanika J. Sci. & Technol* 23(2):287–295.

Tungmunnithum, D., A. Thongboonyou, A. Pholboon, and A. Yangsabai. 2018. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines* 5(3):93.

Týskiewicz, K., M. Konkol, and E. Rój. 2018. The Application of Supercritical Fluid Extraction in Phenolic Compounds Isolation from Natural Plant Materials. *Molecules* 23(2625):1–27.

Uwineza, P. A., and A. Waśkiewicz. 2020. Recent Advances in Supercritical Fluid Extraction of Natural Bioactive Compounds from Natural Plant Materials. *Molecules* 25(17):3847.

Vyavaharkar, R. Y., and S. S. Mangaonkar. 2016. Extraction of Flavonoids From Buchania lanzan Spreng. Seeds By Supercritical Fluid Extraction and Determination of Their Antioxidant Activity. *International Journal of Pharmacy and Pharmaceutical Sciences* 8(1):353–358.

Wang, L., M. Wu, H. M. Liu, Y. X. Ma, X. De Wang, G. Y. Qin, and Y. Yang. 2017. Subcritical fluid extraction of Chinese quince seed: Optimization and product characterization. *Molecules* 22(4).

Xu, D.-P., Y. Li, X. Meng, T. Zhou, Y. Zhou, J. Zheng, J.-J. Zhang, and H.-B. Li. 2017. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *International Journal of Molecular Sciences* 18(1):96.

Xu, Y., M. Fan, J. Ran, T. Zhang, H. Sun, M. Dong, Z. Zhang, and H. Zheng. 2016. Variation in phenolic compounds and antioxidant activity in apple seeds of seven cultivars. *Saudi Journal of Biological Sciences* 23(3):379–388.

Table 3 Implementation of SC-CO_2 extraction

Comodities	Plant Parts	Extraction Conditions					Targeted Compound	Results	References
		Temp	Pressure	CO_2 Flowrate	Co-Solvent	Time			
Quercus infectoria	Galls	40°C	30 MPa	2 ml/min	-	120 min	Phenolic	203,53 mg GAE/g	(Syukriah et al. 2015)
Quercus infectoria	Galls	49,6°C	26,84MPa	2 ml/min	-	120 min	Phenolic	143.75 mg GAE/g	(Syukriah and Azizi 2014)
Buchanania lanzan Spreng.	Seeds	35°C	19,61 MPa	3 ml/min	Ethanol 5,66%	60 min	Phenolic	52.14 mg GAE/g	(Vyavaharkar and Mangaonkar 2016)
Potato	Seeds	80°C	300 bar	18 g/min	Methanol 20%	60 min	Phenolic	5,24 mg GAE/g	(Lima et al. 2021)
Sunflower	Seed Cernels	40°C	16,9 MPa	1,6 ml/min	Ethanol 5%	104,6 min	Chlorogenic Acid	52,08 %	(Daraee et al. 2019)
Grapefruit	Endocarp	70°C	305 bar	35 g/min	-	135 min	Lycopene	93%	(Priyadarsani et al. 2021)
Tomato	skins, seeds, and inner tissue	80°C	376 bar	8 ml/min	-	60 min	Lycopene	82,50%.	(Haddadin and Haddadin 2015)
Wakame	Thallus	40°C	40 MPa	4 ml/min	-	180 min	Fucoxanthin	80%	(Quitain et al. 2013)
Beetroot	Leaves	35°C	400 bar	0.6 kg/h,	Ethanol	30 min	Phenolic	3370.8 g GAE/ g	(Goyeneche et al. 2020)
Roselle	Petals	50°C	250	25g/min	Ethanol 16,7%	90 min	Phenolic	113 ± 1 mg/g	(Pimentel-Moral et al. 2019)