Avenues in Supercritical Carbon Dioxide Extraction and Fractionation of Lipids

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Introduction

Lipids are a group of biomolecules with various types of fatty acids and its derivatives, including monoglycerides, diglycerides, triglycerides, and sterols (Carpenter, 1998). Lipids are mainly consist of carbon, hydrogen and oxygen atoms. Others elements present in the lipids are phosphorus, nitrogen, and sulfur. However, lipids are not soluble in water but soluble in typically hydrocarbons derivatives organic solvents or weakly polar organic solvent including alcohol, ether, benzene and acetone. The term "lipid" is referring to categorize a large number of elements with very different physical and chemical characteristics. Fats and fatty acids (oils) are the main component of lipids present in food and nutritional processes (Carpenter, 1998; Orešič, 2009). Cholesterol and fatty acids and are the most abundant molecules of lipids owing to their important metabolic and nutritional functions (Burlingame et al., 2009; Food and Agriculture Organization of the United Nations (FAO), 2010; Fahy et al., 2009a). Although humans and other mammals utilize numerous biosynthetic pathways to synthesize or break down lipids, some essential lipids must be gained from different source, since some essential lipids cannot be synthesized. Biological lipids originate from two distinct types of biochemical subunits, which are isoprene and ketoacyl groups. Therefore, lipids can be classified into eight categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids, and phenol lipids (Fahy et al., 2009a), as presented in **Fig. 1**.

Lipids have been involved in the evolution of different species, playing an important role in the growth, development, and maintenance of tissues. The requirements of these molecules (mainly fatty acids) change subjected to the physiological state and age of the individual (Valenzuela, 2009; Simopoulos, 2011; Willett, 2012). An example of this importance is the presence of elevated fatty acid concentration in nerve tissues, especially very-long-chained polyunsaturated fatty acids (Valenzuela et al., 2009; Campoy et al., 2012). Among lipids, fatty acids are the crucial relevance in terms of structure and physiology, forming a basic part of phospholipids in cell membranes. It is playing an important role as a primary source of energy, in the formation of cell membranes, synthesis of steroid hormones, structure of vitamin D, synthesis of bile salts, and the composition of bile secretion (Weylandt et al., 2012; Zúñiga et al., 2011; Dawson et al., 2009). Lipids extracted from various food components are also important, since they play a significant role in providing organoleptic characteristics and in fat-soluble vitamins, pigments/dyes, and antioxidants (Valenzuela et al., 2011). Fats and oils are triacylglyceride (TAG) mixtures (i.e., structures formed by linking three similar or different fatty acids to trialcohol glycerol) (Lee et al., 2012). Fats are defined as a mixture of TAGs that is solid or semi solid at room temperature. Conversely, the term "oil" refers to a mixture of triglycerides, which is liquid at room temperature. Generally, TAGs are the main components of fats and oils (over 90%), the substance present in fats and oil are phospholipids, monoacylglycerides, diacylglycerides, sterols, terpenes, fatty alcohols, carotenoids and fat-soluble vitamins (Asensio et al., 2011; Saggini et al., 2011; Fahy et al., 2009b).

Supercritical carbon dioxide ($scCO_2$) has been utilized for the extraction and separation of lipids from various lipid sources. This technology has been viewed as a promising method for extraction, separation, and fractionation to produce refined and deodorized lipids. The present study revises the lipids extraction and fractionation from lipids containing matrices using various extraction technologies. The potential avenue in the application of the $scCO_2$ technology was also reviewed and compared with conventional lipid extraction methods.

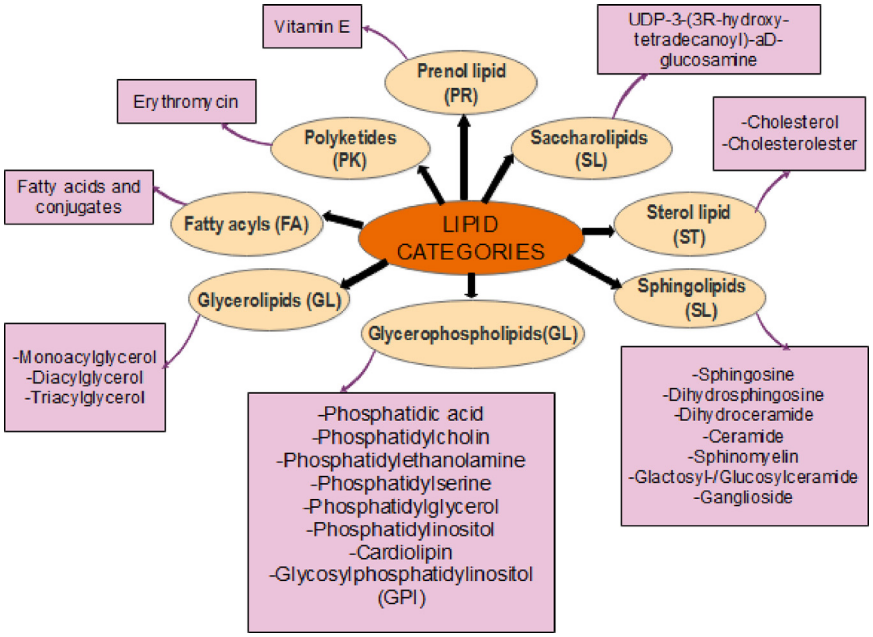

Figure 1 Categories of lipids extracted from lipid containing matrices.

Sources of Lipids

Generally, lipids are organic compounds that are insoluble in water and soluble in nonpolar or weakly polar organic solvents. Lipids can be categorized into two major groups:

i. Fatty acids (saturated and unsaturated)
ii. Glycerides (triglycerides and phosphoglycerides)

The major sources of lipids are food and animals. However, lipids can be extracted from other lipid-containing microbiological substances, such as bacteria and algae (Valenzuela, 2009; Lee et al., 2012). **Fig. 2** shows various lipid sources.

Plant Sources

Plant lipids comprise of fatty acid derivatives, which are triacylglycerides (TAGs), glycerophospholipids (GPLs), terpenes, sterols, carotenoids and waxes (Hatfield et al., 2007). **Table 1** indicates the different methods used for extraction of lipids from various plant matrices. The plant lipids are mainly found as membrane components and source of energy. The major plant lipid compound that are industrially dominant are TAGs and GPLs, which are mainly found in soybean, corn and canola (Tao, 2007). The industrial oleochemistry and hydrocarbon chemistry uses thermal or catalytic reaction technologies for lipid separation methods (Abdelmoez and Mustafa, 2014). Plant lipids are utilized in various industrial and technological applications such as polymers, coatings, printing inks, pharmaceuticals, cosmetics, leather processing, solvents, surfactants, lubricants, hydraulic fluids, pesticide or herbicide adjuvants, glycerol, and biofuels (Tao, 2007). Many plant-lipid-derived products are currently replacing traditional petroleum based products, which are plastics or fuels (Carlsson et al., 2011).

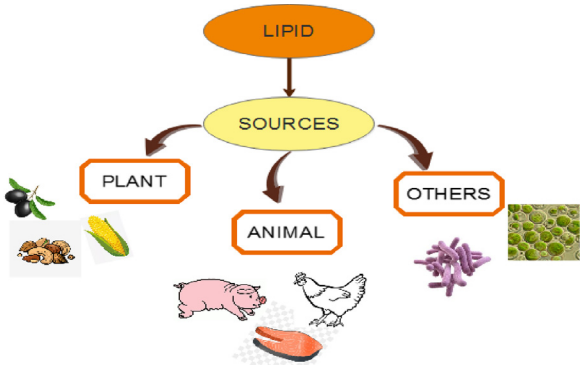

Figure 2 Sources of lipids.

Table 1 Lipids extraction and separation from various plant sources.

Plant	*Scientific names*	*Matrix*	*Technology*	*References*
Cocoa	*Theobroma cacao*	Seed	$scCO_2$	Asep et al., 2008
		Shell	$scCO_2$	Rossi, 1996
Grape	*Vitis vinifera*	Seed	$scCO_2$	Passos et al., 2009; Bravi et al., 2007; Jokić et al., 2016
		Pomace	$scCO_2$	Casas et al., 2010; Louli et al., 2004a
		Skin	$scCO_2$	Marti et al., 2001
Apricot	*Prunus armeniaca*	Kernel seeds	$scCO_2$	Sanal et al., 2004
Tomato	*Solanum lycopersicum*	Skin	$scCO_2$	Chun et al., 2009
		Skin and seeds	$scCO_2$	Vagi et al., 2007
Peach	*Prunus persica*	Pomace	$scCO_2$	Adil et al., 2007
		Seed	$scCO_2$	Mezzomo et al., 2010
		Seed	Soxhlet extraction	Sánchez-Vicente et al., 2009a
Apple	*Malus domestica*	Pomace	$scCO_2$	Adil et al., 2007
Cherry	*Prunus avium*	Seed	$scCO_2$	Bernardo-Gil et al., 2001
Orange	*Citrus sinensis*	Peel	$scCO_2$	Jerković et al., 2015
Olive	*Olea europaea*	Pomace	$scCO_2$	Ibáñez et al., 2000; De Lucas et al., 2003
		Seeds	Microwave	Virot et al., 2007
Rice	*Oryza sativa*	By-products	$scCO_2$	Perretti et al., 2003
Peanuts	*Arachis hypogaea*	By-products	Microwave	Virot et al., 2007
Palm	Arecaceae	Kernel	Solvent extraction	MPOB, 2003
Parsley	*Petroselinum crispum*	Seed	$scCO_2$	Louli et al., 2004b
Canola	*Brassica napus*	Seed	$scCO_2$	Pederssetti et al., 2011
Jojoba	*Simmondsia chinensis*	Seed	$scCO_2$	Salgın, 2007

Animal Sources

Animal lipids primarily consist of triglycerides, phospholipids, and sterols. Each triglyceride molecule comprises three fatty acids, which react with alcohol during transesterification to produce alkyl ester known as biodiesel (Fan and Burton, 2009). Animal fats like suet, tallow, and lard exist in a solid state, which present as saturated fatty acids, compared to plant lipids that contain unsaturated fatty acids (Balat, 2007; Tippayawong et al., 2002). Animal fats are used for lipid extraction in industrial applications, such as pork lard, chicken fat, beef tallow, lamb meat and animal fat mix (Lu et al., 2007). These fats are available readily due meat industry requirements of product handling and control (Feddern et al., 2011). Recently researches aimed at producing high-quality biodiesel from animal fat waste are in the rise (Janaun and Ellis, 2010). Non-human consumption materials due to esthetic and sanitary concern are used as feedstocks for rendering processes. These materials include fatty trimmings, bones, offal and carcasses from slaughterhouses or animals died on farms or in transit (Feddern et al., 2011). **Table 2** presents various processing technologies of animal fats using extraction and separation techniques. Aquatic ecosystems have been known to occupy tow third earth. Aquatic animal lipids are densest form of energy, which yields about 65% more energy compared to proteins or carbohydrates per gram (Parrish, 2013). At present, marine oily fishes such as herring, mackerel, sardine, and salmon and other seafoods are the major dietary source (Ruiz-Lopez et al., 2015).

Table 2 Lipids extraction from animal fats using various processing technologies.

Animal	*Scientific names*	*Matrix*	*Technology*	*References*
Cow	*Bos taurus*	Meat	Microwave	Virot et al., 2008a
Lamb	Artiodactyla	Meat	Heat explosion	Fogerty et al., 1990
Porcine	*Sus scrofa domesticus*	Meat	Heat explosion	Fogerty et al., 1990
		Pulmonary artery	$scCO_2$	Fogerty et al., 1990
Chicken	*Gallus domesticus*	Meat	Heat explosion	Fogerty et al., 1990
Fish longtail tuna	*Thunnus tonggol*	Meat	$scCO_2$	Ferdosh et al., 2013
Krill	*Euphausiacea*	Freeze-dried/By-product	$scCO_2$	Li et al., 2014
Hake	*Merluccius merluccius*	Scraps	$scCO_2$	Gil-Ramirez et al., 2019
Fish salmon	*Salmo salar*	Meat offcut	$scCO_2$	Rubio-Rodriguez et al., 2012
Fish orange	*Hoplostethus atlanticus*	Meat offcut	$scCO_2$	Rubio-Rodriguez et al., 2012
African catfish	*Clarias gariepinus*	Meat offcut	$scCO_2$	Sarker et al., 2012
Indian mackerel	*Rastrelliger kanagurta*	Meat offcut	$scCO_2$	Sahena et al., 2010
Black soldier fly	*Hermetia illucens*	Larvae	$scCO_2$	Kim et al., 2019

Other Biological Sources

Lipids can be extracted from other biological resources, including algal organisms and bacteria. Algal are photosynthetic aquatic organisms, which are grouped as macroalgae and microalgae. Microalgae are known as unicellular photosynthetic microorganisms that live in saltwater or freshwater (Ozkurt, 2009). These algae's lipids can be converted into energy, such as biofuel oil and gas. However, due to their high content of water in microalgae, not all biomass can be converted into energy. Thermochemical process is used to produce oil and gas, whereas biochemical process is used to produce ethanol, biodiesel and biohydrogen (Amin, 2009). The lipids of microalgal are categorized into two structural divisions: (1) nonpolar lipids (free fatty acids, waxes, acylglycerols, steryl esters and sterols), (2) polar lipids (phosphoglycerides, glycosylglycerides, and sphingolipids). These have different but important functions in growth and metabolism of microalga (Chen et al., 2018). Some of them, such as phosphoglycerides, sterols, and glycosylglycerides are the main content of cell membrane structure, whereas sphingolipids, inositol lipids, and polyunsaturated fatty acid's oxidative products might represent as key intermediates in cell signaling pathways and may have a role in cellular sensing of any environmental changes (Borowitzka and Moheimani, 2013).

The contents of these microalgal lipids differ dependent of species type, ambient environment and growth conditions. Previous research indicated that the content of lipid in *Chlorella*, *Crypthecodinium*, *Cylindrotheca*, *Isochrysis*, *Nannochloris*, *Nannochloropsis*, *Nitzschia*, *Porphyridium*, *Dunaliella*, *Schizochytrium*, *Neochloris*, *Tetraselmis*, and *Phaeodactylum* ranges from 20% to 50% of the dry biomass (Mata et al., 2010). Vegetable oils, animal oils/fats, and microbial lipids are existing feedstocks for lipids such as TAGs (Wang et al., 2014). Bacteria can overcome inhibition and uses carbohydrate from downstream process to produce TAGs, which is a candidate for bioconversion of lignocellulosic biomass to biodiesel. Recent researches have shown that several type of *Rhodococcus* have demonstrated the ability to collect TAGs (Kosa and Ragauskas, 2012a; Xiong et al., 2012). Examples for the extraction and fractionation of lipids from microbiological sources are presented in **Table 3**.

Lipids Extraction Methods

There are some commonly implemented lipid extraction methods, which are mechanical pressing, microwave heating, steam explosion, and solvent extraction (Kumar et al., 2015; Mubarak et al., 2014; Roy, 2017; Saifuddin et al., 2016; Arsad et al., 2014). This technique is mainly known to be an efficient lipid extraction technique that can increase the yield and quality (Cravotto et al., 2008). Microwave heating is a method based on non-contact heat, where the sample is simultaneously heated, as compared to conductive heating. For instance, MAE was used to efficiently extract lipids in microalgae with solvents (Balasubramanian et al., 2011; Lee et al., 2010b). On the extraction process using conventional solvent, the transfer of mass occurs from inside to outside, meanwhile the heat is transferred from outside to inside (Lee et al., 2010b). In case of microwave-assisted solvent extraction, the mass and heat is transferred from inside extracted material to solvent (Virot et al., 2008b). Generally, microwave-assisted processes have a different mechanism of heat transfer, in which energy absorption occurs via polar molecules. This aids in internal heating and allows selective heating according to the polarity of the components in the system (Flamini et al., 2007).

Polar molecules inside cells can consume energy and heat up, increasing the pressure inside the cell, which then rapidly surpasses the maximum pressure that the cell can endure, leading to cell rupture and discharge of cell compounds, such as lipids, into the extraction solvent (Tigrine-Kordjani et al., 2011; Zhai et al., 2009). The electromagnetic fields produced by microwaves also speed up the process of target dissolution into extraction medium. Proficiency of a substance in absorbing microwaves is correlated to its polarity. For instance, materials with strong polarity have a better potential of absorbing microwave energy. Thus, the polarity of the solvent has a great influence on the efficacy of the lipid extraction method (Flamini et al., 2007; Terigar et al., 2011). The transesterification of vegetable oil to biodiesel has been extended to the use of microwave energy (Leadbeater and Stencel, 2006; Barnard et al., 2007). Moreover, a pilot-scale continuous microwave system has also been used for oil extraction from Chinese tallow tree, soybean, and rice bran (Vian et al., 2008; Virot et al., 2008c).

Soxhlet extraction is a common method that has been implemented for lipid extraction purposes. However, this method is typically time-consuming, requiring around 3–6 h for extraction. One of the disadvantages of using for solvent extraction is time consuming. The extraction of solid samples using solvent is known as solid liquid extraction (also called leaching or lixiviation), is the oldest techniques for solid sample pretreatment (Luque de Castro and García Ayuso, 2000). Conventional Soxhlet extraction

Table 3 Lipids extraction from microbiological sources.

Microorganisms	*Types*	*Technology*	*References*
Chlorella vulgaris	Microalgae	Industrial ionic liquids (ILs)	Kosa and Ragauskas, 2012a
Chlorella vulgaris	Microalgae	Soxhlet extraction	Aguoru and Okibe, 2015a
Chlorella sp	Microalgae	Sonication (PSSE)	Prabakaran and Ravindran, 2011a
Nannochloropsis sp	Green algae	Soxhlet (CSE)	Balasubramanian et al., 2013
Schizochytrium sp.	Microalgae	Bligh-Dyer (CSE)	Byreddy et al., 2015a
Botryococcus sp	Microalgae	Microwave (PSSE)	Lee et al., 2010a
Tetraselmis sp.	Green algae	$scCO_2$	Yamaguchi et al., 1986

uphold as one of the main pertinent techniques for lipids extraction. During this process, the sample is positioned in a thimble holder and, during operation, is filled with a condensed fresh solvent (Eisert and Pawliszyn, 1997; Hageman et al., 1996). Once the liquid attains an overflow level, all the contents in the thimble holder is aspirated by a siphon and discharged into distillation flask that contains the extracted analytes. This sequenced will be repeated until the extraction completes. In the steam explosion method, the raw material is entered into a steam explosion tank and mixed with saturated steam supplied from a steam boiler (Ciechanska et al., 2009). In this tank, lipid extraction occurs when the steam (solvent) penetrates the small pores of the raw material (solute) and causes them to explode, causing lipids (extract), hot water, and a residue mixture to diffuse out into a cyclone (Kumar et al., 2015; Mubarak et al., 2014). This cyclone separates solids from liquids, where all the residues are removed while the lipids and hot water mixture are entered into an oil and water separator. Lipids generally discharge as a final product, whereas the hot water goes to a counter-current-type heat exchanger where the recycled hot water is cooled down by cool water pumped from a chiller. Meanwhile, the recycled hot water discharging from the heat exchanger is pumped into a steam boiler to be heated until the steam is saturated for the next steam explosion lipid extraction process. **Table 4** shows the various technologies used in extraction of lipids.

Lipids Extraction and Fractionation Using $scCO_2$ Technology

There have been increasing concerns regarding environmental pollution, human health, and hazards associated with the utilization of organic solvents in food byproduct processing. The strict environmental regulations regarding the usage of organic solvents that are toxic and the requirement of solvent-free and ultrapure food products by food industries, food scientists are looking for a cleaner technology in place of the conventional food processing technologies. Although the conventional solvent extraction procedure are vastly utilized in extraction and fractionation of lipids, this traditional technique has a number of limitations (Sarker et al., 2012; Sahena et al., 2010; Yamaguchi et al., 1986):

- It produces potential toxic emissions.
- It produces flammable and hazardous organic solvent residue.
- It requires highly pure organic solvents.
- It is laborious and time-consuming.

Table 4 Existing lipids extraction methods.

Method	*Lipid sources*	*Operating parameters*	*Lipids yield (%)*	*References*
Microwave integrated extraction	Peanuts	30 g of dried and ground samples, 300 mL of hexane,32 min	46.1–47.3	Virot et al., 2008b
Microwave integrated extraction	Olives	30 g of dried and ground samples, 300 mL of hexane, 32 min	39.1–40.3	Virot et al., 2008b
Microwave integrated extraction	*Botryococcus* sp.	2450 MHz, 100 °C, 5 min, normal pressure	28.5	Lee et al., 2010c
Soxhlet extraction	Peanuts	30 g of dried and ground samples, 300 mL of solvent, 8 h	47.3	Virot et al., 2008b
Soxhlet extraction	Olives	30 g of dried and ground samples, 300 mL of solvent, 8 h	40.3	Virot et al., 2008b
Soxhlet extraction	*Chlorella vulgaris*	205 mL of chloroform methanol 2:1(v/v),3	20–32	Aguoru and Okibe, 2015b
Steam explosion	*P. tricornutum*	100 g microalgal, 120–150 °C, 2–4.7 bar and 5 min.	27–29	Lorente et al., 2015
Steam explosion	Camellia seeds	300 g camellia seeds, 120–240 °C, 1.6–2.3 MPa and 30–120 sec.	8–12.4	Zhang et al., 2019
Steam explosion	*Dunaliella tertiolecta*	4 kg microalgae, 150 °C, 4.7 bar and 5 min.	11.4–26.6	Lorente et al., 2015
Steam explosion	*Nannochloropsis gaditana*	100 g microalgal, 120–150 °C, 2–4.7 bar and 5 min.	11–18	Lorente et al., 2015
Steam explosion	*Nannochloropsis gaditana*	4 kg microalgae, 150 °C, 4.7 bar and 5 min.	22.3	Lorente et al., 2018
Steam explosion	*Chlorella sorokiniana*	4 kg microalgae, 150 °C, 4.7 bar and 5 min.	11.8	Lorente et al., 2018
Steam explosion	*Chlorella sorokiniana*	100 g microalgal, 120–150 °C, 2–4.7 bar and 5 min.	14–19	Lorente et al., 2018
Bligh-Dyer (CSE)	*Schizochytrium* sp	Methanol-chloroform, 25 °C, normal pressure	22.1	Byreddy et al., 2015b
Sonication	*Chlorella* sp	50 kHz, 15 min, normal pressure	25.5	Prabakaran and Ravindran, 2011b
Liquefied gas	*M. aeruginosa*	Dimethyl ether, 25 °C, 0.51 Mpa	40.1	Kanda and Li, 2011
Industrial ionic liquids (ILs)	*Chlorella vulgaris*	383∼393 K,1 h	60–75.4	Kosa and Ragauskas, 2012b

Above all, lipid extraction and fractionation with conventional techniques are not environmentally friendly and generate a large volume of organic contaminants. Therefore, a new and clean technology is urgently required for the separation and extraction of food products. In recent years, $scCO_2$ has attracted potential interest as a promising technology to replace conventional mechanical and solvent extraction methods (Kim et al., 2019; Montanes and Tallon, 2018). This technology has been widely utilized to extract and fractionate lipids from various lipid sources in food and pharmaceutical industries.

Extraction of Lipids by $scCO_2$

In the $scCO_2$ technology, lipids are extracted using CO_2 as a solvent at the supercritical state. This technology is viewed as the most efficient method to extract lipids from various lipid sources owing to its distinct properties, such as wide availability, low critical temperature (31.1 °C), moderate pressure (7.4 MPa), nontoxicity, and environmental friendliness (Sapkale et al., 2010). Besides, the lipids extraction method using $scCO_2$ leads to rapid chemical reactions that are difficult or even impossible to achieve with conventional solvent extraction methods, as it is a fast extraction method, which requires 10–60 min to complete an extraction cycle (Sapkale et al., 2010). Sapkale et al. (Pourmortazavi and Hajimirsadeghi, 2007) also mentioned that lipids extracted using $scCO_2$ can be separated from the analyte by simply releasing pressure, leaving almost no trace and yielding a pure residue.

Lipid extraction from different sources, both plants and animals, using $scCO_2$ was first implemented in the early 1980s (Md Zaidul et al., 2006). The critical low temperature of CO_2 (31.1 °C) makes it ideal for food products that are thermally labile. Other solvents such as ethane and propane have been used for the extraction of natural compounds using supercritical fluids method, because these solvents have a great solvation power to enable higher solubility for lipid components as compared to $SC{-}CO_2$. However, the main drawback of propane and ethane is both solvents are highly flammable and expensive. **Table 5** summarizes studies on lipid extraction from various lipid sources using $scCO_2$. Shi et al. (Shi et al., 2010) extracted nut oils (pistachio, almond, pecan, hazelnut, walnut and peanut), seed oils (apricot, grape, borage, rosehip, cherry, sesame, evening primrose, pumpkin, flax, sea buckthorn, etc.), cereal oils (rice bran, oat, wheat germ and amaranth), and fruit and vegetable oils (buriti, olive husk, carrot, tomato and cloudberry) using $SC{-}CO_2$. Another study reported that the main advantage of the $SC{-}CO_2$ extraction method to extract and fractionate oils to maintains the unique flavor and aroma, which are volatile and often lost with traditional solvent extraction processing (Akanda et al., 2012a). Specialty oils contain high percentage of bioactive compounds, such as squalene, tocopherols, tocotrienols, polyunsaturated fatty acids, phytosterols, and carotenoids, which have positive effects on human health thanks to public awareness. $SC{-}CO_2$ extraction is known to be the best extraction method for isolation of bioactive lipid components in the literature (Shi et al., 2010).

Linoleic acid, as an example of essential fatty acids, is necessary for human metabolism but cannot be synthesized inside the human body. Thus, this essential fatty acid must be supplied externally through diet. Essential fatty acids are the major component of membrane structure and for the development and optimal function of the nervous system and brain (Obeid et al., 2018). Hormone-like substances, such as eicosanoids, formed by essential fatty acids are important for regulation of blood pressure and viscosity, vasoconstriction, and inflammatory and immune responses (Sánchez-Vicente et al., 2009b). Fatty acid deficiency has also been proven to be associated with several human diseases (Obeid et al., 2018). $scCO_2$ is proven to be an effective technology to extract and separate various lipids from plant matrixes. Since this technology does not require multiple processing steps and chemicals for refining the extracted lipids, therefore there is a little change to contaminate with impurities. $scCO_2$ technique is widely used in palm oil industries to extract palm kernel oil (PKO). The $scCO_2$-extracted PKO are higher triglycerides and minor components, such as phytosterols, squalene, and carotenes compared to commercially extracted palm oil using hexane (Lau et al., 2006).

$scCO_2$ Assisted Lipids Fractionation

Besides, extraction and refining of palm oil using $scCO_2$, simultaneous fractionation is also possible. Further fractionation of crude lipid extracts is important for refining or obtaining fractions rich in specific bioactive substances. In process of refining, it is known that $SC{-}CO_2$-extracted oils do not requires refining as compared to hexane solvent extracted oils. Furthermore, many of the bioactive components are removed in conventional refining method. However, phospholipids and chlorophyll are extracted to a very limited extent or not extracted by $scCO_2$. As the efficiency of removing bioactive substances, such as phytosterols, tocopherols, and tocotrienols, by conventional refining is questionable. The $scCO_2$-extracted oils can be used with minimal refining that is achieved by fractionation step followed by extraction. Fractionation of lipid mixtures is viable through three different approaches. First, the fractional extraction is the collection of fractions over a period of time in which the extraction temperature and/or pressure may be changed at certain intervals over time. Thus, allows the fraction from different compositions to be isolated separately. In addition, fraction contains high level of free fatty acid will be collected first because of it's high solubility and followed by triglycerides. Phospholipid fractions are collected after triglyceride fractions. In addition, ethanol is added as a co-solvent in order to separate fraction rich in phospholipids (Temelli, 2009).

The second procedure is fractional separation, where few separators are utilized in a series. The pressure and temperature of extraction can be set to achieve the highest possible CO_2 density to maximize the extraction of the solutes. The separator conditions are adjusted accordingly to reduce the CO_2 density. Thus, fractions of high, medium and low molecular weight correspond to low, medium and high volatile compounds, respectively, which can be collected using separator in a sequence configuration (Dunford and Temelli, 1995). Third, liquid feed mixtures can be separated using a fractionation column. Generally, heaters is

Table 5 Extraction and fractionations of lipids using $scCO_2$.

Lipids sources	Type of lipids sources	Parameters				Yield (wt.%)	References
		Pressure (MPa)	Temperature (^oC)	Time (min)	Modifier		
Chlorella vulgaris	Microalgae	60	60	180	Ethanol	75.2	Aguoru and Okibe, 2015a
P. valderianum	Microalgae	35	40	90	–	30.5	Chatterjee and Bhattacharjee, 2014
Nannochloropsis oculata	Microalgae	40	60	15–120	–	100	Crampon et al., 2013
N. oculata	Microalgae	25–75	50	210–240	Ethanol	40	Obeid et al., 2018
S. obliquus	Microalgae	30–80	50–80	540	–	77.96	Lorenzen et al., 2017
S. obtusiusculus	Microalgae	30–80	50–80	540	–	44.04	Lorenzen et al., 2017
Tetraselmis sp	Microalgae	24.5–39.2	40–80	180–240	Methanol	11.7	Yamaguchi et al., 1986
Thunnus tonggol	Longtail tuna fish	20–40	40–65	360–420	Ethanol	35.6	Ferdosh et al., 2013
Tetraselmis	Microalgae	15	40	720	Water	10.88	Li et al., 2014
Merluccius	Hake	25	40	180	Water	25	Gil-Ramirez et al., 2019
Salmo salar	Salmon fish	25	40	–	–	51	Rubio-Rodriguez et al., 2012
Hake	Fish	25	40	–	–	18	Rubio-Rodriguez et al., 2012
Jumbo squid	Marine animal	25	40	–	–	17	Rubio-Rodriguez et al., 2012
Clarias gariepinus	African catfish	10–40	35–80	60–240	–	67	Sarker et al., 2012
Rastrelliger kanagurta	Indian mackerel fish	20–35	45–75	360	Ethanol	53.2	Sahena et al., 2010
Hermetia illucens	Black soldier fly	15–35	35	120–360	–	5	Kim et al., 2019
Simmondsia chinensis	Plant	25–45	70–90	120	Ethanol	44	Salgın, 2007
Brassica napus	Plant	20–25	40–60	480	–	19.49	Pederssetti et al., 2011
Petroselinum crispum	Plant	10–15	35–45	50	–	30–35	Louli et al., 2004b
Oryza sativa	Rice	35–70	40–80	120–240	–	24.68	Perretti et al., 2003
Malus domestica	Pomace	20–60	40–60	10–40	Ethanol	7.72	Adil et al., 2007
Prunus avium	Seed	18–22	40	60–150	–	40.84	Bernardo-Gil et al., 2001
Citrus sinensis	Peel	10	40	180	–	–	Jerković et al., 2015
Olea europaea	Pomace	10–20	40–60	180	Ethanol	–	Ibáñez et al., 2000
Olea europaea	Pomace						De Lucas et al., 2003
Solanum lycopersicum	Tomato	20–40	40–100	180	–	30.27	Chun et al., 2009
Prunus persica	Peach	30–46	40–80	180	–	15.05	Vagi et al., 2007
Prunus persica	Peach	20–60	40–60	10–40	Ethanol	38.24	Adil et al., 2007
Theobroma cacao	Cocoa	10–30	30–50	150	Ethanol	24	Mezzomo et al., 2010
Theobroma cacao	Cocoa	35	60	600–4200	Ethanol	40.22	Asep et al., 2008
Vitis vinifera	Grape	35	60	600–4200	–	36.84	Rossi, 1996
Vitis vinifera	Grape	16–20	40	240	–	43.5	Passos et al., 2009
Vitis vinifera	Grape	25–30	40–80	120–270	–	44.5	Bravi et al., 2007
Vitis vinifera	Grape	15.68–44.14	25	90	–	37.06	Jokić et al., 2016
Vitis vinifera	Grape	10–40	35–55	180	Ethanol	60	Casas et al., 2010
Vitis vinifera	Grape	10–25	45	180	Methanol	41	Louli et al., 2004a
Prunus armeniaca	Peach	15	40	30	Ethanol	–	Marti et al., 2001
Theobroma cacao	Cocoa	30.4–50.7	40–60	90	–	75	Sanal et al., 2004
Prunus persica	Peach	10	30–50	360	Ethanol	53	Sánchez-Vicente et al., 2009b
Peanut	Plant	15–35	25–55	300	–	24.1	Osseo et al., 2004
C. vulgaris	Microalgae	25–75	50	210–240	Ethanol	20	Obeid et al., 2018

used for packed column in order to obtain thermal gradient throughout the column height, which forms an internal reflux that can enhance the separation efficiency and possibility to produce a reflux through an external reflux pump (Dunford and Temelli, 1997). During extraction at room temperature, the first fraction is in the form of solid and followed by semiliquid faction, and lastly liquid fraction. The solid appearance of the first fraction is solid and it is such because of the high-saturated fatty acids (mainly C16:0) content. Besides, the solid fraction appeared first due to it's high solubility under $scCO_2$ condition. The liquid fractions contain unsaturated fatty acids, which are mostly C18:1 triglycerides. The concentration of palm oil saturated and short-chain fatty acids decreases as the extraction time increases. Conversely, the concentration of unsaturated and heavier fatty acids increases proportional to the extraction time, after most of the shorter-chain fatty acids are removed. Thus, making these longer chains accessible to the $scCO_2$ extraction of low-vapor-pressure oils, which is not possible using distillation technique. These oils cannot achieve good fractionation by distillation due to the existence of impurities in equal volatility as main components (Dunford and Temelli, 1997). Therefore, investigation of $scCO_2$ fractionation was studied to detect chemical composition role in production of oil fractions using different carbon lengths and saturations (Temelli, 2009).

For instance, palm kernel oil (PKO) fractionation through $scCO_2$ extraction has been studied using $scCO_2$ as a solvent using pressure in the range of 20.7–48.3 MPa and at a temperature of 40–80 °C (Nik Norulaini et al., 2004). Based on the study, at lower pressures (i.e., 20.7 and 27.6 MPa), the PKO solubility in $scCO_2$ decreases with temperature, whereas at higher pressures (i.e., 34.5, 41.4, and 48.3 MPa), the solubility increases with temperature. The authors found abundance of short-chain triglycerides in the earlier fractions, whereas the later fractions contained longer-chain triglycerides and unsaturated triglycerides in abundance. Another report indicated the short-chain fatty acids in PKO are easily soluble in $scCO_2$ (Nik Norulaini et al., 2004). The $scCO_2$ is known to be a convenient solvent for the fractionation of PKO, reducing short- and medium-chain (C8—C14), but increases long-chain (C18:0–C18:2) fatty acids amount in $scCO_2$ extracted PKO. Many researchers have attempted fractionation of fatty acid triglycerides according to carbon number using $scCO_2$ using a range of temperature (40–80 °C) and pressure up to 50 MPa (Nik Norulaini et al., 2009). In a study, Zaidul et al. (Zaidul et al., 2007) produced superior quality refined and bleached PKO using $scCO_2$ with respect to the physiochemical properties, such as the fatty acid content, slip melting point, cloud point, iodine value, acid value, saponification value and solid fat content.

Factors Affecting $scCO_2$ Lipids Extraction and Fractionation

There are a few factors that affect $scCO_2$ lipid extraction and fractionation, which are the temperature, pressure, CO_2 flow rate, solubility, particle size and humidity, as presented in **Fig. 3**.

Temperature is one of the most important factors affecting $scCO_2$ lipid extraction and fractionation (Saheena et al., 2009). Any increase in temperature decreases the density of $scCO_2$ but increases the solvation power. Besides, at higher temperatures, lower pressures are required, and vice versa, for $scCO_2$ lipid extraction and fractionation (Khaw et al., 2017). Zhang et al. (Zhang et al., 2019) reported that shorter extraction time and a lower pressure are required at higher temperatures during the extraction of edible oils from sunflower seeds because of the changes of solvent density, which is more effective than solute vapor pressure and volatility of some aromatic compounds present in the sunflower oil. Increasing the pressure at a constant critical temperature in $scCO_2$ helps achieve the highest fluid density. Generally, the density of CO_2 increases with pressure because the intermolecular distances between CO_2 decrease, thus enhancing the interaction between the molecules of *solute* and *solvent* (Sauceau et al., 2004), resulting in achieving the maximum solubility of solute in $scCO_2$ and, thereby, increasing the $scCO_2$ lipid extraction and fractionation yield (Norodin et al., 2017). Dabrowski et al. (Dabrowski et al., 2018) extracted lipids from canola flakes and canola and sunflower seeds using $scCO_2$. In their study, they reported that the yield of lipid extraction increases with pressure and temperature, wherein the $scCO_2$ pressure plays a major role in obtaining higher lipid yields (Dabrowski et al., 2018). In addition, Yeddes et al. (Yeddes et al., 2012) extracted palm kernels PKO using $scCO_2$ with pressure swing and continuous extraction methods. They revealed that the extraction yield was doubled in pressure swing techniques compared to the continuous extraction method.

There are two major effects related to cosolvent addition. The first is its contribution to the enhancement of the solute and solvent molecule physical interactions. The interaction is highly dependent to the solute's nature, as it can contribute toward chemical interactions (hydrogen bonds) and enhance the solute solubility. The second effect is the mixed solvent's high critical temperature compared to the pure solvent (Frolov and Kiselev, 2014). In addition, a small increase in modifier or polar co-solvents (i.e., alcohols like ethanol or methanol) can modify solvation characteristics of $scCO_2$ by increasing the polar and

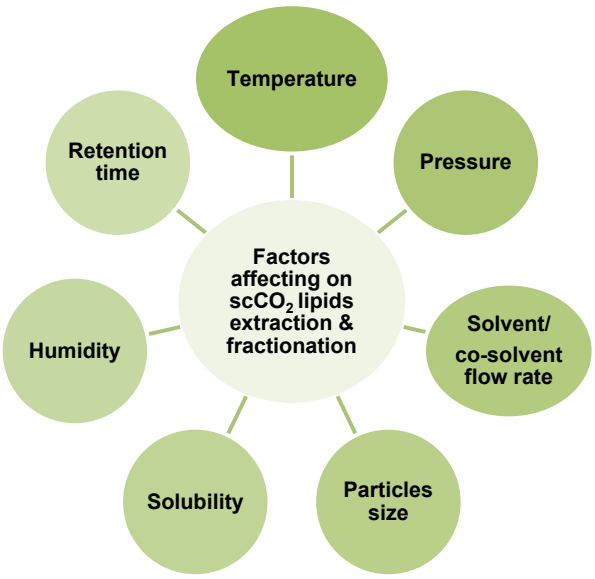

Figure 3 Factors affecting $scCO_2$ lipids extraction and fractionation.

high-molecular-weight substances solubility. It can negate the advantages of residue-free extraction and fractionation as these co-solvents can become a residue in the product (Das and Panda, 2015). However, co-solvent addition can increase the solubility and selectivity of the solute (Manjare and Dhingra, 2019). The solubility of the solute in $scCO_2$ extraction depends on the selection of operating pressure and temperature, where an increase in temperature and pressure increases the density, dissolving power and solubility, thus increase the lipids yield (Zhang et al., 2019). Mezzomo and Ferreira (Mezzomo and Ferreira, 2016) reported that solubility gets affected by increasing the pressure at a constant temperature or decreasing the temperature at a constant pressure, whereas the extraction and fractionation time can be reduced with an increase of the flow rate and adding chemical additives or modifiers. As CO_2-based fluids are nonpolar solvents, the addition of low levels of modifiers able to significantly increase the solubility, particularly of polar compounds (Manjare and Dhingra, 2019). For instance, squalene is highly soluble (0.189 mg/g CO_2) at low $scCO_2$ pressure density (10 MPa) (the higher the pressure, the higher the solubility), owing to its nonpolar characteristic and small molecular size, whereas sterols have a value of 0.132 mg/g CO_2, even though their initial concentration is four times higher compared to squalene (4349 mg/kg and 1117 mg/kg, respectively) (Lozano-Grande et al., 2018). Furthermore, tocopherol and carotene differ greatly in terms of solubility in $scCO_2$ (Akanda et al., 2012b). Besides, $scCO_2$ lipid extraction and fractionation also have the potential to produce various fractions of value-added oils from a single raw material, such as enriched carotene, carotenoids, and vitamin E, from fresh palm-pressed mesocarp fibers (Akanda et al., 2012b). Meanwhile, the solubility of carotenoids lies in the range from 1.31×10^{-4} to 1.58×10^{-3} g/kg at the pressure range of 14–30 MPa (Wei et al., 2005). Vitamin E is more soluble than carotene in $scCO_2$ at 10 and 20 MPa, where the vitamin E with concentration of 3650 mg/kg was extracted in the first fraction, meanwhile low carotene content of 3942–5498 mg/kg in the second fraction at approximately 40%–90% of triglyceride (Othman et al., 2010).

$scCO_2$ extraction and fractionation dependent of the solubility of nonpolar and low-volatility solvent of CO_2 with a low affinity for polar substances (Beckman, 2004). Hence, the solubility of different substances in $scCO_2$ decreases with the increase in the number of polar functional groups (i.e., hydroxyl, carboxyl, amino, and nitro) (de Azevedo et al., 2008). Tyskiewicz et al. (Tyskiewicz et al., 2018) observed that the extraction of alkaloid and phenolic compounds using $scCO_2$ is potentially influenced with the inclusion of cosolvent, such as ethanol. Besides, another study found that lipids yielded from cupuacu was obtained with sorter retention time and addition of co-solvent. This was due to the increases in solvent density and, modification of both physical and chemical intermolecular interaction force than occurs between the solute and solvent (Van Osch et al., 2017). Khaw et al. (Khaw et al., 2017) mentioned that the flow rate of CO_2-based fluids influences lipid extraction using $scCO_2$ since the oil solubility in CO_2 is rapidly saturated with oil at lower flow rate. The increase of molecular weight, the decrease the solvent density and solubility of solute with the increase the temperature, hence it led to the increase of solute vapor pressure (Das and Panda, 2015). Al-Hamimi et al. (Al-Hamimi et al., 2016) reported that the retention time for the extraction and fractionation of lipids may decrease with increasing solvent/cosolvent flow rate owing to the increased density around the solute molecule, increasing physical interactions and formation of hydrogen bonds.

The particle size of the solute also influences lipid extraction and fractionation using $scCO_2$. This is because smaller size particles have larger surface areas and, therefore, enhance solute and solvent extraction, hence increasing the extraction yield (Aris et al., 2018). Tchabo et al. (Tchabo et al., 2018) obtained a higher lipid yield (about 39%) from nutmeg using $scCO_2$ extraction at 60 °C and 41.4 MPa with particle sizes of $\leq$0.5 mm. However, the lipids yield was gained about 29% at particles size of $\leq$2 mm. Smaller particle sizes increases the contact between the solvent surface, which enhances the diffusion of the solvent through the sample and facilitates the oil extraction from the inner part of the intact cells (Pan et al., 2013). Asep et al. (Asep et al., 2016) reported higher lipid yield in $scCO_2$ system with smaller particle size, due to the increase of surface area with the reduction in particle size. Thus, resulted in an increase in the percentage of broken cells compared to undamaged intact cells with mass transfer coefficients in the fluid phase.

Humidity has an effect toward supercritical extraction and fractionation process, because it affect the overall mechanical performance of $scCO_2$ by plugging the restrictor, which requires samples drying prior to extraction and fractionation (Aris et al., 2018). In expansion valves, humidity generally poses a problem (Rezzoug et al., 2005). The formation of ice at the expansion valve generally restricts the flow in the small extractors (Mezzomo and Ferreira, 2016). However, moisture enhances the solubility of the solute during $scCO_2$ extraction and fractionation (Wei et al., 2005). In contrast, Gustinelli et al. (Gustinelli et al., 2018) stated that the presence of moisture could have a negative influence toward extraction and fractionation process, depending on the compound to be extracted. De Azevedo et al. (de Azevedo et al., 2008) obtained a high yield during the extraction of green coffee beans from seeds using $scCO_2$ with a low moisture content (3%–11%) and short extraction time. High moisture of 12% and above has minor effect toward the ability to extract oils from the seeds. Therefore, drying is discontinued to maintain the intergirity of chemical component and to avoid degradation of volatile compounds in samples (Nofer et al., 2018).

Conclusion

In this study, we described the extraction and fractionation of lipids using $scCO_2$. We showed that $scCO_2$ is a promising alternative technology for the extraction and separation of lipids from various lipid sources and that this technology offers some distinct characteristics, such as low viscosity and high diffusivity. CO_2-based fluids can penetrate into porous solid matrices more effectively than liquid-based solvents. The potential advantages of utilizing $scCO_2$ in the extraction and fractionation of lipids are as follows: (i) it

does not require toxic solvents, (ii) it has a fast extraction rate, (iii) it has a good separation rate, (iv) it is highly selective, and (v) it does not require further refining and purification of lipids. Although a number of factors potentially influence the yield during $scCO_2$ extraction and fractionation of lipids, the $scCO_2$ pressure and temperature play the most prominent role in the extraction and fractionation of lipids.

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