Chapter 4
Production of Valuable Compounds from Leaves by Supercritical CO_2 Extraction

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Abstract Although leaves contain many useful compounds, they are typically considered as a waste. Extraction of leaves with carbon dioxide (CO_2) in its supercritical state (sc-CO_2) allows effective recovery of valuable compounds due to the unique properties of CO_2. Recovered components such as terpenes, phenolics and phytosterols can be isolated and depend on the types of leaves and conditions of the extraction. The extraction kinetics consisted of three steps, that is, extraction of accessible solute from the cells, slower extraction of the solute protected by the cell walls and transition state of these situations. Antioxidants, phenolics and flavonoids can be recovered by sc-CO_2 extraction and antioxidant capacity (AOC), total phenolic content (TPC) and total flavonoid content (TFC) in the extract are shown in this chapter overview. The addition of co-solvent such as ethanol generally increases AOC, TPC and TFC. The temperature and pressure influences AOC, TPC and TFC depends on the contribution of solvent density and vapor pressure of solute. Extraction of plant leaves with sc-CO_2 can provides valuable new chemicals from biomass and can create new sources of biochemicals.

Keywords Leaf · Supercritical carbon dioxide · Extraction · Antioxidant · Phenolics · Flavonoid

4.1 Leaf Extraction with Supercritical CO_2

4.1.1 Background of Supercritical CO_2 Extraction of Leaf

Leaves of many plants are typically treated as biomass waste. On the other hand, the extracted liquid of some tea leaves is popular as a drink that contains desirable compounds such as catechins. The process for recovery of these compounds from

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Z. Fang et al. (eds.), *Production of Biofuels and Chemicals from Sustainable Recycling of Organic Solid Waste*, Biofuels and Biorefineries 11,
https://doi.org/10.1007/978-981-16-6162-4_4

plant leaves by extraction can be used to produce supplements, food additives, fertilizers and so on or to develop a product with added value and a system for efficient use of biomass waste. The appropriate use of leaves contributes to the establishment of a sustainable society.

The solvent for a separation process should be friendly to the human body and environment. CO_2 is an environmentally friendly solvent and safe for humans and different from typical organic solvents, because it can be brought to its supercritical state at temperatures just above room temperature. In particular, it is not necessary to worry about residual solvent in extract where the extract is used for food additives. The solvent properties of supercritical CO_2 (sc-CO_2) will be discussed in this chapter that shows an effective extraction system for obtaining useful components from plant materials including leaf [1].

Extracts from plant leaves contains bioactive compounds such as antioxidants including phenolics and flavonoids. Antioxidants are important component because they prevent neurodegenerative diseases such as cancer and Alzheimer's disease and have protective activity against a multitude of non-communicable human conditions [2, 3].

In this chapter, the specific features of sc-CO_2 for extraction solvent are firstly introduced. After that, extraction of several kinds of leaves is explained from the view point of extraction kinetics, extracted compounds, amount of antioxidants, and compounds such as phenolics and flavonoids in the extract.

4.1.2 Properties of Leaf

The body of vascular plants consists of roots, stems and leaves. The leaf is typically attached to the stem and has a flat shape. The most important function of leaves is photosynthesis. Leaf exchanges water, carbon dioxide, oxygen and water outside of itself and produces organic compounds.

Figure 4.1 shows the cross-section of the tissue of leaf. Each component represents a cell surrounded by cell walls. The major tissue in leaf is the epidermal system, vascular system, and fundamental tissue system other than the former two systems. The epidermal system is the outer layer of the leaf, which is impermeable to water and covered with a cuticle that is the boundary separating the inner cell from the outside. The roles of it are to protect the plant from water loss, regulation of gas exchange and secretion of metabolic compounds. The vascular system consists of veins located in the fundamental tissue system and transport water and nutrition. The fundamental tissue system includes palisade tissue and spongy tissue. The palisade tissue typically exists on the front side of the leaf and consists of one or two vertically elongated cells. The sponge tissue typically exists on the backside of the leaf. The cells in this area are not so tightly packed in an irregular arrangement and there are large intercellular spaces and this is connected outside through the stoma to transport gas to the photosynthetic system.

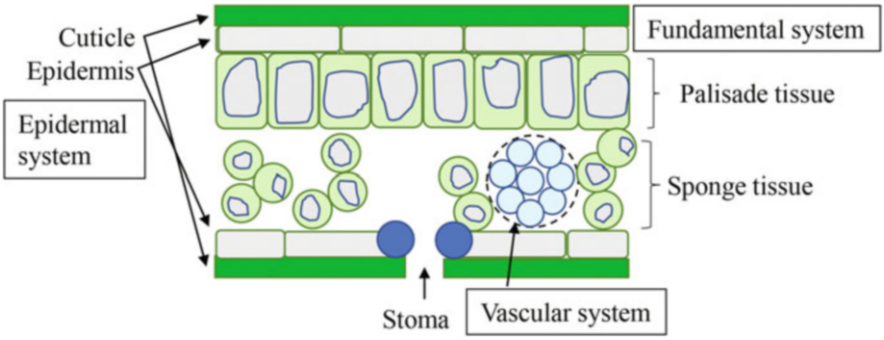

Fig. 4.1 Cross-sectional schematic of leaf tissue

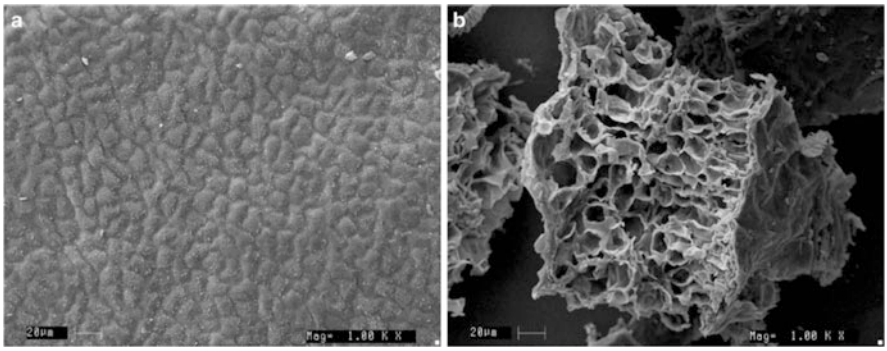

Fig. 4.2 Scanning electron micrographs of the unprocessed tea leaves and sc-CO_2 processed tea leaves. a: Untreated leaves; b: sc-CO_2 processed leaves at 35 MPa, reprinted with permission from [4] Copyright © 2015 Springer Nature

Leaf contains some useful components such as phenolics [2] that have antioxidant properties. Some phenolic compounds exhibit therapeutic benefits including cardio- and neuroprotective effect and health benefits such as preventing many chronic diseases and the integrity of DNA. The antioxidant property and total phenolic content are important to understand the usefulness of extraction of the leaf.

4.1.3 Morphology of Leaf

The solvent power of sc-CO_2 influences the morphology of leaves during extraction. Figure 4.2 shows SEM images of unprocessed tea leaf and tea leaf after extraction of sc-CO_2 at 35 MPa [4]. In the case of the unprocessed leaf, there is an epidermal system on the surface of the leaf. After the extraction with sc-CO_2, the structure of the leaf is cracked and pores in leaf tissue are opened. Sc-CO_2 strongly influences the

structure of leaves such as tissue and cell wall to enhance mass transfer of solvent into the inner side of the leaf, which helps release compounds from the matrix of the leaf.

4.2 Properties of Supercritical CO_2 as an Extraction Solvent

4.2.1 Specific Properties of CO_2

The critical point of CO_2 is 304.1 K and 7.38 MPa as depicted in the P-ρ-T diagram (Fig. 4.3) of CO_2 [5]. Above the critical temperature and critical pressure, CO_2 is in a supercritical state and is thus called supercritical CO_2 (sc-CO_2) fluid. In the supercritical state, the density of CO_2 is continuously varied with temperature and pressure without phase change. In particular, the magnitude of change in density with temperature and pressure is large near the critical point. The high pressure and low-temperature region produces high fluid densities, and the low pressure and high-temperature region gives a low fluid density. Physical properties of CO_2 such as viscosity and diffusivity also greatly change according to the change in the density of CO_2.

The density of sc-CO_2 is between those of a gas phase and a liquid phase. The viscosity and diffusivity of sc-CO_2 are also between those of the gas and liquid phase. The higher density of sc-CO_2 than that of gaseous CO_2 enables sc-CO_2 to dissolve larger amounts of solute than that of the gaseous state. Further, the viscosity

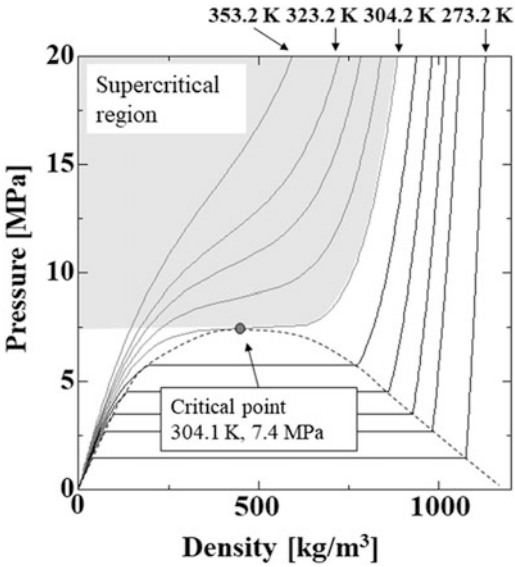

Fig. 4.3 Pressure-density-temperature diagram of CO_2 based on data from Ref. [5]

of sc-CO_2 is lower and the diffusivity of sc-CO_2 is larger than those respective properties for liquid CO_2, which leads to an improvement in the permeation of CO_2 into leaf tissue. In sc-CO_2 extraction of plant materials, bioactive compounds such as phenolic compounds, flavonoids, phytosterol, tocopherols, carotenoids can be separated [1].

In addition, sc-CO_2 becomes gas by regulating pressure and the extract dissolved in sc-CO_2 is easily precipitated due to the decrease in solvent power by low density in the low-pressure region. The separation of extract from solvent to obtain the desired compounds is simple when sc-CO_2 is used as solvent, because no CO_2 remains in the substrate at atmospheric pressure in contrast to the case in which an organic liquid solvent is used.

4.2.2 Properties of Mixture of CO_2 and Co-solvent

The polarity of CO_2 is low, which means that the solubility of compounds having high polarity is low in CO_2. The addition of high polarity solvent as co-solvent into sc-CO_2 improves the solubility of high polarity compounds in the solvent. The co-solvents generally used are ethanol, methanol or water, because they are popular substances and especially water and ethanol are relatively safe for the human body. The dielectric constants of ethanol, methanol and water at ambient conditions are 25.3, 33.0 and 80.4, respectively [6] and the polarity of co-solvent is in this order. The fraction of co-solvent is usually 5–20 wt%.

The phase behavior of the mixture of CO_2 and co-solvent is important for the extraction process. sc-CO_2 + ethanol mixture is homogeneous above about 12 MPa and below 333 K [7, 8]. sc-CO_2 + methanol mixture is homogeneous above about 8 MPa below 323 K [8]. So the extraction with sc-CO_2 + methanol and sc-CO_2 + ethanol is usually operated under homogeneous conditions. On the other hand, sc-CO_2 + water mixtures are generally in the two-phase region. The vapor-liquid equilibrium for sc-CO_2 + water system from 308 K to 333 K can be estimated with the Peng-Robinson type equation of state [9]. There are CO_2-rich phase and water-rich phase at least less than 30 MPa below 333 K. For example, the mole fraction of CO_2 in CO_2-rich phase and in water-rich phase are 0.96 and 0.03, respectively, at 308 K and 20 MPa. At this condition, the volumetric ratio of the CO_2-rich phase is above 90% in the case of the molar ratio of water/CO_2 being less than 0.3. CO_2 rich phase containing a few % of water would be a major situation of the solvent in the sc-CO_2 + water system.

A mixture of CO_2 and polar co-solvent provides good solvent power for both nonpolar and polar components by the contribution of sc-CO_2 and co-solvent.

4.2.3 Principles of Supercritical CO_2 Extraction System

Figure 4.4 shows the typical CO_2 extraction system in a laboratory scale. In the literature, almost all of the extraction systems are semi-batch system. The principle of a supercritical CO_2 extraction system is as follows.

At first, the leaf is introduced into the extractor. The leaf is generally crushed and sometimes dried before introducing the extractor. CO_2 is supplied with a CO_2 supply pump that can supply CO_2 by cooling the pump head to maintain CO_2 in a liquid phase. The co-solvent that is a liquid is supplied with a high-pressure pump. The feed rate of CO_2 and co-solvent are controlled with the flow rate of each pump. The CO_2 and co-solvent are mixed in line and the mixture is supplied to the preheater as a solvent. The preheater and extractor are in the thermostat that is a water bath or an oven in the case of laboratory scale. In the case of large-scale extraction, preheater and extractor are directly heated with a heater. The solvent is heated in the preheater to the extraction temperature and then flows into the extractor. In the typical extraction, the solvent contact with the leaf ground or whole leaf in the extractor and then penetrates each tissue of a leaf to extract the components in the leaf. Then the extract is moved into the solvent. After that, the solvent flows to the outlet of the extractor.

The solvent dissolving extract is exhausted through a back-pressure regulator. In some cases, a filter is set between the extractor and back-pressure regulator to protect the regulator from dust. The extraction pressure, that is, the system pressure is controlled with a back-pressure regulator. The solvent through the back-pressure regulator is released to the trap to ambient pressure. If the solvent is homogeneous in high pressure, a metering valve can be used instead of back-pressure regulator to control the pressure and flow rate.

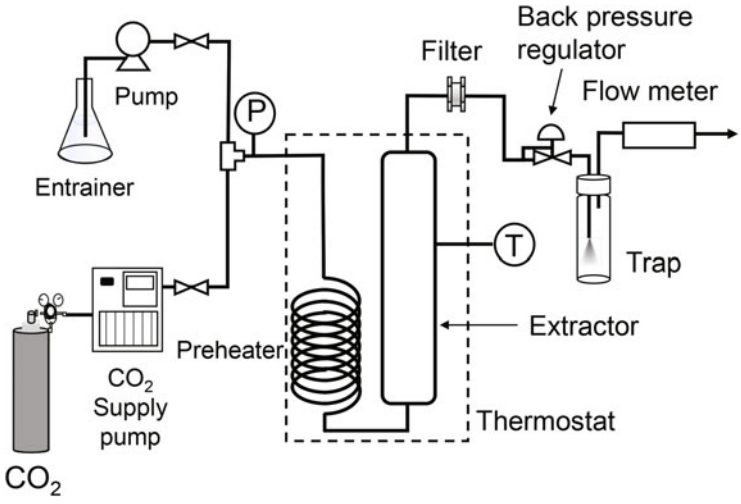

Fig. 4.4 Typical CO_2 extraction system

In the trap, the solvent dissolving extract is separated into CO_2 gas and the precipitated solid or liquid containing extract and co-solvent. The gas is released into the atmosphere after measuring its flow rate. The precipitate in the trap is recovered as the extract. In some cases, the liquid co-solvent is volatilized to obtain the solid extract.

4.3 Extract Obtained by Supercritical CO_2 Extraction of Leaves

4.3.1 Compounds Extracted by Supercritical Extraction of Various Leaves

The extraction of various leaves with sc-CO_2 has been reported (Table 4.1). There are over fifty kinds of leaves extracted with sc-CO_2 and many compounds were recovered. The structures of the main compounds are shown in Fig. 4.5. It was found that the leaves are widely distributed and not limited. The typical co-solvent for leave extraction process in sc-CO_2 is ethanol, methanol and water.

In the extraction of Green Tea (*Camellia sinesis*) leaves with sc-CO_2, triacontanol ((a) in Fig. 4.5) is an important compound that is straight-chain alcohol and improves plant growth by its effect on photosynthesis and plant metabolism [10]. Caffeine ((b) in Fig. 4.5) is also one of the major compounds in tea leaves and should be removed due to its significant effects on the cardiovascular system and gastric acid secretion [11]. Sc-CO_2, sc-CO_2 + water and sc-CO_2 + ethanol solvent extracted caffeine [11–13] from tea leaves. The (−)-epiagllocatechin-3-gallate ((c) in Fig. 4.5) was extracted with sc-CO_2 + ethanol from Green Tea (*Camellia assamica L.*) leaves and it has strong antioxidant and health benefitted potential [4].

In the case of the extraction from Spearmint (*Mentha spicata L.*) leaves, flavonoids such as catechin, epicatechin, rutin, luteolin, myricetin, apigenin and naringenin [14] were extracted with sc-CO_2 + ethanol. Carvone ((d) in Fig. 4.5), Limonene ((f) in Fig. 4.5) and 1,8-cineole ((e) in Fig. 4.5) were extracted with sc-CO_2 [15, 16]. In the extraction of Peppermint with sc-CO_2 + ethanol, *l*-menthol ((g) in Fig. 4.5) and menthone ((h) in Fig. 4.5) were major extracted compounds [17].

Eugenol ((i) in Fig. 4.5) [18, 19] and 1,8-cineole ((e) in Fig. 4.5) [18] were extracted from Tulsi (*Ocimum sanctum*) leaves with sc-CO_2 and these compounds have strong antioxidant potency.

Hempedu bumi (*Andrographis paniculata*) grows widely in the tropical area of southeast Asia and is used for traditional medicine. Andrographolide ((j) in Fig. 4.5) that is one of the main components of its leaves were extracted with sc-CO_2 [20, 21].

Pharmacologically active anthraquinones such as aloe-emodin ((k) in Fig. 4.5) and barbaloin ((l) in Fig. 4.5) were extracted with sc-CO_2 from Aloe (*Aloe vera L.*) leaves [22, 23].

Table 4.1 Main components extracted from leaves with supercritical CO_2

Name	Primary active compounds that are extracted[a]	Co-sol.[b]	Ref.
Aloe (*Aloe vera L.*)	Aloesin, Aloe-emodin (k), Barbaloin (l)		[22]
	Aloe-emodin (k), Barbaloin (l)		[23]
Annual wormwood (*Artemisia annua L.*)	Artemisinin	E	[64]
Asteraceae (*Tithonia diversifolia*)	Tagitinine C (t)		[33, 34]
Bamboo (*Sasa palmata)*	β-Amyrene, α-Amyrin acetate, Gluconic acid	E, W	[52]
Balu (*Rhododendron anthopogon*)	γ-Terpinene, Limonene (f), β-Caryophyllene		[65]
Bright eyes (*Catharanthus roseus*)	Vindoline, Catharanthine		[66]
Bushy matgrass (*Lippia alba* (Mill.) N. E. Brown)	Carvone (d), β-Guaiene, Thymol		[67]
Cajuput (*Melaleuca cajuputi*)	β-Caryophyllene, Humulene, Eugenin		[68]
Common juniper (*Juniperus communis L.*)	Limonene (f), β-Selinene, α-Terpinyl acetate		[26]
	Limonene (f), α-thoujone		[27]
Congo Bololo (*Vernonia amygdalina* Delile)	Hexadecanoic acid, 9,12-Octadecadienonic acid, α-Linolenic acid		[37]
Cupressaceae (*Juniperus oxycedrus* ssp. *oxycedrus*)	Germacrene D (n), Manoyl oxide (o), 1-*epi*-Cubenol (p)		[28]
Date palm (*Phoenix dactylifera)*	Tetratriacontanol, Tetratriacontanoic acid, Hexadecanoic acid		[69]
Dandelion (*Teraxacum officinale* Ewber et Wiggers)	β-Amyrin, β-Sitosterol		[35]
Five leaved chaste tree (*Vitex negundo L.*)	Benzoic acid, Caryophyllene, Caryophyllene oxide		[70]
Ginkgo (*Ginkgo biloba L.*)	Bilobalide, Ginkgolides	M, E, W	[71]
Grecian foxglove (*Digitalis lanata Ehrh.*)	Digoxin, Acetyldigoxin	M	[72]
Green Tea (*Camellia assamica L.*)	(−)-Epiagllocatechin-3-gallate (c)	E	[4]
Green Tea (*Camellia sinesis*)	Triacontanol (a)		[9]
Green Tea	Caffeine (b)	W	[10]
Hempedu bumi (*Andrographis paniculata*)	Andrographolide (j)		[20, 21]
Herb (*Orthosiphon stamineus*)	Sinensetin, Isosinensetin, Rosmarinic acid	E	[73]
Indian borage (*Coleus aromaticus*)	Carvacrol (q)		[74]
Jambú (*Spilanthes acmella ver oleracea*)	Spilanthol	E, W	[53]
Lamiaceae (*Origanum vulgare L.*)	Carvacrol (q)		[29]
	Carvacrol (q), *trans*-Sabinene hydrate (r)		[30]

(continued)

Table 4.1 (continued)

Name	Primary active compounds that are extracted[a]	Co-sol.[b]	Ref.
Lantana (*Lantana camera*)	Ar-curcumene, α-humulene, α-Zingiberene		[75]
Laurel (*L. nobilis L.* Lauraccae)	1,8-Cineole (e), Linalool, α-Terpinyl acetate		[38]
Lemon balm (*Melissa officinalis L.*)	α-Citral, β-Citral		[76]
Lemon-scented gum (*Corymbia citriodora*)	Citronellal		[77]
Limau purut (*Citrus hystrix*)	Cinnamic acid, *m*-Coumaric acid, Vanillic acid	E	[61]
Lupine (*Lupinus albescens*)	Stigmasterol, Ergosterol		[39]
Maté tea	Caffeine (b)	E	[11]
	Caffeine (b), Theophylline, Theobromine		[12]
Moringa (*Moringa oleifera*)	α-Linoleic acid	E	[40]
Palo Negro (*Leptocarpha rivularis*)	α-Thujone, β-Caryophyllene, Resveratrol	E	[54]
	α-Thujone, β-Caryophyllene, Caryophyllene oxide		[41]
Pandan (*Pandanus amaryllifolius* Roxb.)	2-Acetyl-1-pyrroline (s)		[31, 32]
Pecah Kaca (*Strobilanthes crispus*)	Rutin, Luteolin, Kaempferol	E	[62]
Peppermint	*l*-Menthol (g), Menthone (h)	E	[17]
Pimento (*Pimenta dioica* Merrill.)	Eugenol (i)		[78]
Piper Betle	2,3-Dimethyl-benzoic acid, 9-Octadecenoic acid (Z)-, methyl ester		[79]
Piper klotzschianum	Germacrene D (n), Piper callosidine, 14-oxy-α-Muuroleno	M, E, P	[42]
Physic nut (*Jatropha curcas Linn.*)	Gallic acid	M	[80]
River red gum (*Eucalyptus camaldulensis* Dehn.)	1,8-Cineole (e), Allo-aromadendrene, Globulol		[81]
Rock Samphire (*Crithmum maritimum L.*)	Dillapiole, γ-terpinene, thymol methyl ether		[82]
Rose cactus (*Pereskia bleo*)	Cholest-5-en-3-ol (3beta.)-, Erythritol, 9-Octadecenoic acid		[58]
Rosemary (*Rosmarinus officinails L.*)	Carnosic acid (m), 1,8-Cineole (e), Camphor		[24]
	Carnosic acid (m), Wogonin		[25]
Rose-scented geranium (*Pelargonium graveolens*)	Citronellol, Geraniol, 6,9-Guaiadiene		[83]
Sage (*Salvia officinails L.*)	Carnosol, Fatty acids (C18), Allobetulonlactone-1-en-2-ol		[25]
Seseli bocconi Guss. Subsp. *praecox* Gamisans (Apiaceae)	β-Phellandrene, α-Humulene		[84]

(continued)

Table 4.1 (continued)

Name	Primary active compounds that are extracted[a]	Co-sol.[b]	Ref.
Southern magnolia (*Magnolia grandiflora*)	Parthenolide, Costunolide, Cyclocolorenone		[85]
Spearmint (*Mentha spicata L.*)	Luteolin, Apigenin, Naringenin	E	[14]
	Carvone (d), Pulegone, Limonene (f)		[15]
	Carvone (d), 1,8-Cineole (e), Limonene (f)		[16]
Swamp mallet (*Eucalyptus spathulata*), Coolabah (*Eucalyptus microtheca*)	1,8-Cineole (e), α-Pirene	M	[43]
Sweet cherry (*Prunus avium L.*)	$_{DL}$-Malic acid, α,α-Trehalose, $_{D}$-(−)-Mannitol		[86]
Tasmanian bluegum (*Eucalypus globulus L.*)	Eudesmol, 1,2-Benzenedicarboxylic acid		[87]
Tulsi (*Ocimum sanctum*)	Eugenol (i), 1,8-Cineole (e)		[18]
	Eugenol (i)		[19]
Yerba mate folium (*liex paraguariensis* A. St.-Hil., *Aquifoliaceae*)	Theobromine, Caffeine (b)		[44]

[a]Character in parenthesis is chemical structure in Fig. 4.5
[b]Co-solvent, W: water, M: methanol, E: ethanol

Rosemary (*Rosmarinus officinails L.*) has been recognized as the plant with a high antioxidant capacity. The extraction of rosemary leaves with sc-CO_2 gives carnosic acid ((m) in Fig. 4.5) as the main product [24], and abietane-type diterpenoids including carnosic acid ((m) in Fig. 4.5) and flavonoids including wogonin were also detected in the extract [25].

The extraction of Common juniper (*Juniperus communis L.*) leaves with sc-CO_2 was studied and the main product was limonene ((f) in Fig. 4.5) [26, 27]. Cupressaceae (*Juniperus oxycedrus* ssp. *oxycedrus*) that is a big tree being native to the Mediterranean region, the extraction of the leaves of it with sc-CO_2 provided germacrene D, manoyl oxide and 1-*epi*-cubenol (n, o and p in Fig. 4.5, respectively) [28].

The extraction of Lamiaceae (*Origanum vulgare L.*) leaves with sc-CO_2 was conducted. Lamiaceae is a kind of oregano that is widely used in the flavoring of food products as well as perfume compositions. The components in the extract were slightly different depending on the region harvested [29], and the main components for all samples were carvacrol ((q) in Fig. 4.5) [29, 30] and *trans*-sabinene hydrate ((r) in Fig. 4.5) [30].

Pandan (*Pandanus amaryllifolius* Roxb.) is a tropical plant and a source of natural flavoring. The main component obtained in the extraction of Pandan leaves with sc-CO_2 was 2-acetyl-1-pyrroline ((s) in Fig. 4.5) [31, 32].

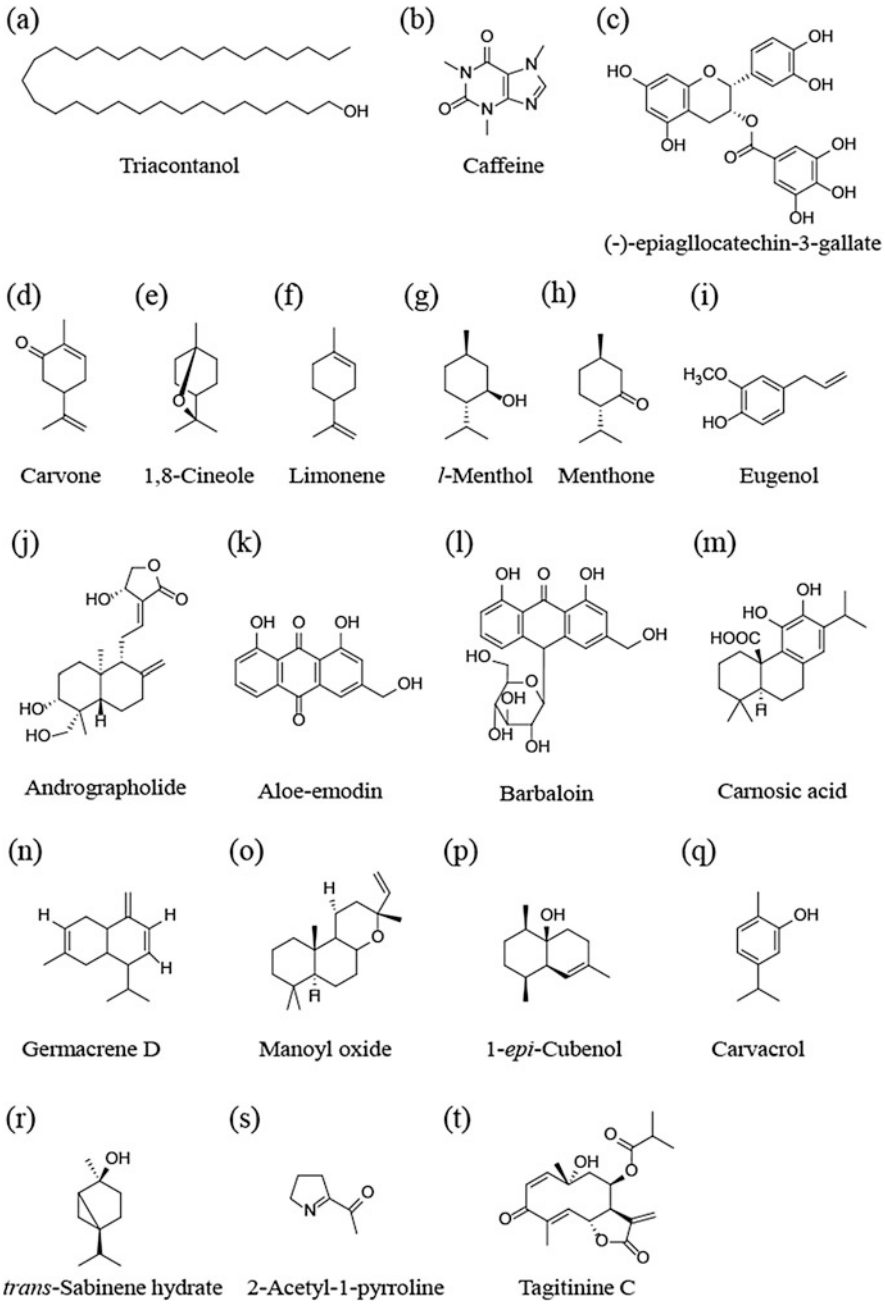

Fig. 4.5 Structure of primary compounds found in leaves obtained with supercritical CO_2

Asteraceae (*Tithonia diversifolia*) is a shrub and its general extract has been used for the treatment of diarrhea, fever and malaria. The extraction of its leaves with sc-CO_2 gave tagitinine C ((t) in Fig. 4.5) as main product that has significant antiproliferative activity [33, 34].

β-Sitosterol was extracted from Dandelion (*Teraxacum officinale* Ewber et Wiggers) leaves [35]. β-Sitosterol is one of phytosterols that are useful components because that provide health benefit to lower cholesterol.

There are many other compounds extracted as shown in Table 4.1. As a whole, terpenes, phenolics and phytosterols were mainly detected as compounds extracted from various leaves with sc-CO_2.

4.4 Kinetics of Supercritical CO_2 Extraction of Leaves

4.4.1 Extraction Model

Extraction of natural products such as leaf includes the extraction of components in the cell and absorbed on the solid in the cell and bound to the cell, which leads to complicated extraction kinetics. The extraction step consists of (1) penetration of solvent into the cellular tissue, (2) dissolution of solute to the solvent, (3) transportation of solute from the cell to the surface of the solid matrix, (4) transportation of solute from the surface of solid to bulk solvent in the laminar film of fluid. The transportation step of (3) is often a rate-limiting step. A typical extraction curve is the properties of extract such as extract yield against the total amount of solvent supplied. The example of the extraction curve is shown in Fig. 4.6 afterward. In the early extraction stage, the solubility of the solute controls the extraction rate because there is a lot of solute in the extraction atmosphere and the extraction proceeds towards saturated concentration. After that, the effect of transportation inner the sample becomes major and the extraction rate decreases.

Several models for the extraction curve have been established. Eqs. (4.1)–(4.6) is one of the basic models [36]. This model is based on the fixed-bed extractor that solvent flows axially with superficial velocity through a bed of milled plant material in a cylindrical extractor.

$$e = qy_r[1 - \exp(-Z)] \tag{4.1}$$

$$e = y_r[q - q_m \exp(z_m - Z)] \tag{4.2}$$

$$e = x_0 - \frac{y_r}{W} \ln\left\{1 + \left[\exp\left(\frac{Wx_0}{y_r}\right) - 1\right] \exp[W(q_m - q)]x_k/x_0\right\} \tag{4.3}$$

$$\frac{z_W}{Z} = \frac{y_r}{Wx_0} \ln \frac{x_0 \exp[W(q - q_m)] - x_k}{x_0 - x_k} \tag{4.4}$$

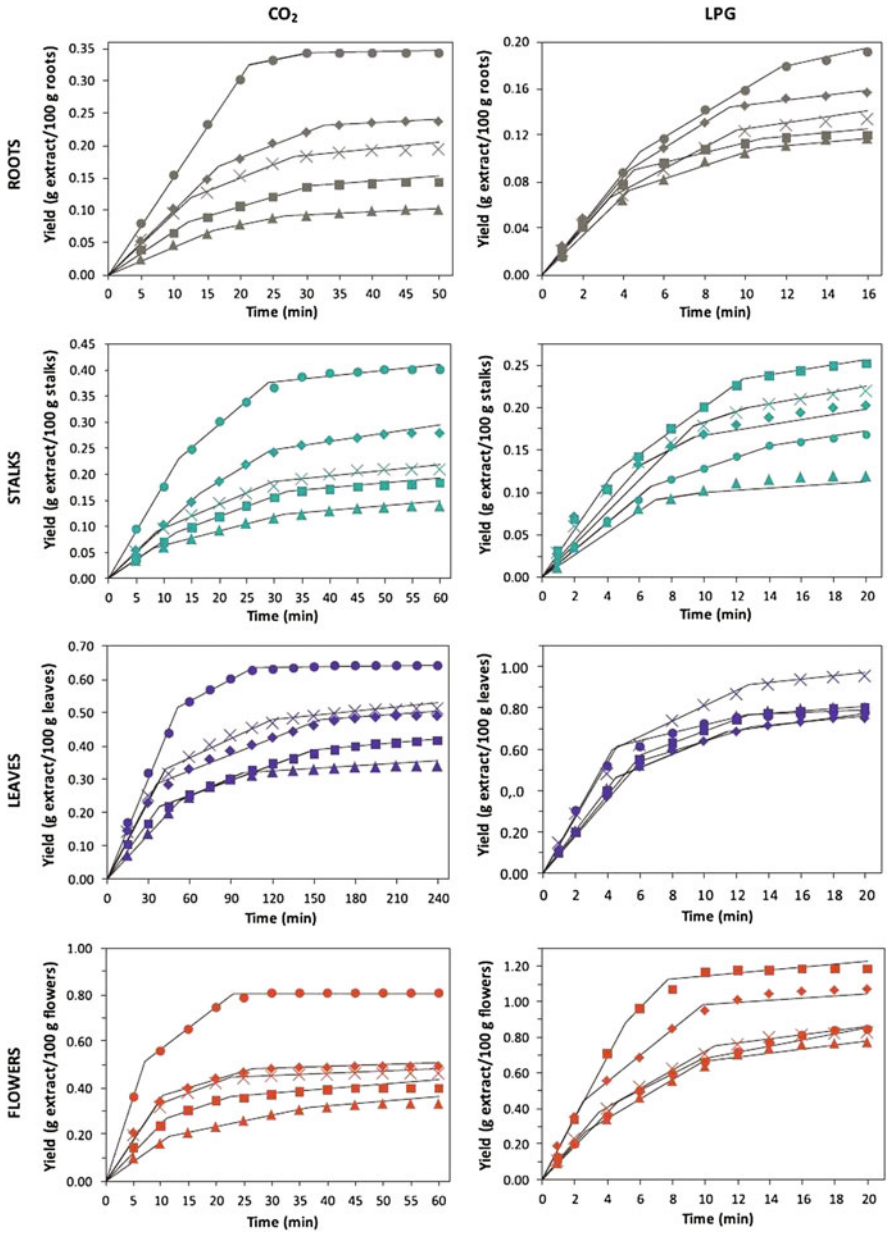

Fig. 4.6 Kinetic yields of extracts obtained from Lupine (*Lupinus albescens*) by sc-CO_2 and LPG (For sc-CO_2, ■: 313 K, 15 MPa, ◆: 313 K, 25 MPa, ▲: 333 K, 15 MPa, ●:333 K, 25 MPa, ×: 323 K, 25 MPa; For LPG, ■: 298 K, 1.5 MPa, ◆: 298 K, 3.5 MPa, ▲: 318 K, 1.5 MPa, ●:318 K, 3.5 MPa, ×: 323 K, 25 MPa; sold line: calculated results, reprinted with permission from [39] Copyright © 2017 Elsevier)

$$Z = \frac{k_f a_0 \rho}{[\dot{q}(1-\varepsilon)]\rho_s} \tag{4.5}$$

$$W = \frac{k_s a_0}{[\dot{q}(1-\varepsilon)]} \tag{4.6}$$

where a_0: specific interfacial area [m^{-1}], e (=E/N): extract yield [−], E: weight of extract [kg], k_f: solvent-phase mass transfer coefficient [m s^{-1}], k_s: solid-phase mass transfer coefficient [m·s^{-1}], N: weight of initial sample [kg], Q: mass of solvent [kg], q (=Q/N): specific amount of solvent [−], $\dot{q}$: mass flow rate of solvent related to N [s^{-1}], x_0: initial concentration of solute related to solute-free solvent [−], x_k: initial concentration of solute in easily accessible area related to solute-free solvent, y_r: solubility [g-solute/g-solvent], ε: void fraction [−], ρ: density of solvent [kg/m^3], ρ_s: density of sample [kg/m^3].

Equations (4.1)–(4.3) indicate the extraction curve considering firstly extraction of accessible solute from the cell (Eq. 4.1), then slower extraction of the solute protected by the cell walls (Eq. 4.3) that is controlled by diffusion in the solid phase and there is a transition state between these two situations (Eq. 4.2).

The extraction kinetics of leaves with sc-CO_2 is analyzed for various leaves. The profiles of the properties of extract such as extract yield against the amount of solvent supplied or time are useful to analyze the kinetics [11–13, 15–17, 19–21, 23, 25, 29, 31, 33, 34, 37–51].

In the case of extraction of leaves, the complicated structure of leaf including the existence of plant cell provides various barriers against dissolution and transfer of solute, which leads to influences on extraction kinetics. The typical extraction curve is shown in Fig. 4.6 that is the extraction curve for Lupine (*Lupinus albescens*) [39]. Although there are extraction curves of sc-CO_2 and liquid propane in this figure, the extraction curves of sc-CO_2 were only concerned in this chapter. The figures in the left row are sc-CO_2 extraction. The parameter related to the extract, that is, extract yield in this case, generally increases with increasing extraction time. The extraction is classified into three steps [37, 39, 45]. The first step is the constant extraction rate step (CER) corresponding to the firstly extraction of accessible solute from the cell. The second step is the falling extraction rate step (FER) corresponding to the transition state between the first step and third step. The third step is diffusion-controlled (DC) step corresponding to slower extraction of the solute protected by the cell walls that being controlled by diffusion in the solid phase.

Some researchers conducted a detailed analysis for extraction. The theoretically mathematical model is proposed [11, 17, 20]. The mathematical model contains two differential solute mass balances in fluid and solid phase, and local equilibrium adsorption representing the relationship between the fluid and solid.

The mass balance of solute on the fluid phase is.

$$\alpha \frac{\partial C}{\partial t} + U_s \frac{\partial C}{\partial z} = -k_f a_p (1-\alpha)(C - C_{ps}) \tag{4.7}$$

where C: solute concentration in the fluid phase, t: time, z: bed height, C_{ps}: solute concentration in pore at the surface of the particle, α: void fraction, a_p: specific surface area, U_s: superficial velocity, k_f: external mass transfer coefficient.

The mass balance for the solute on the solid phase is.

$$\beta \frac{\partial C_p}{\partial t} = D_e \frac{\partial^2 C_p}{\partial r^2} - (1-\beta)\frac{\partial C_s}{\partial t} \tag{4.8}$$

where C_p: solute concentration in pores within the particle, β: particle porosity, D_e: effective interparticle diffusion coefficient, r: particle radius, C_s: solute concentration in solid phase.

Consequently, the cumulative fractional yield of solute extracted is.

$$F(\theta) = \left[\frac{A}{1-\alpha}\right] \times \left\{ \left[\frac{\exp(a_1\theta) - 1}{a_1}\right] - \left[\frac{\exp(a_2\theta) - 1}{a_2}\right] \right\} \tag{4.9}$$

$$a_1 = \frac{-b + \left(b^2 - 4c\right)^{1/2}}{2} \tag{4.10}$$

$$a_2 = \frac{-b - \left(b^2 - 4c\right)^{1/2}}{2} \tag{4.11}$$

$$b = \frac{\phi}{\beta + (1-\beta)K} + \frac{1}{\alpha} + \frac{\phi(1-\alpha)}{\alpha} \tag{4.12}$$

$$c = \frac{\phi}{\beta + (1-\beta)K\alpha} \tag{4.13}$$

$$A = \frac{(1-\alpha)\phi}{[\beta + (1-\beta)K]\alpha(a_1 - a_2)} \tag{4.14}$$

where $\theta = t/\tau$, $\phi = K_p\, a_p\, \tau$, K: equilibrium adsorption constant.

The most common regression calculation with the above equation and experimental data is performed by mass transfer coefficient k_f and the equilibrium constant K as a fitting parameter.

The simple models are proposed by considering three steps in the extraction [37, 39]. Confortin et al. [39] conducted the extraction of several parts of Lupine (*Lupinus albescens*) at (313–333) K and (15–25) MPa with sc-CO_2 and LPG. The extraction curve of this system is shown as Fig. 4.6. The proposed sprine model is as follows:

$$Yield\ (t) = b_1 t\ (t \leq t_{CER}) \tag{4.15}$$

$$Yield\ (t) = (b_1 + b_2)\ t - b_2\ t_{CER}\ (t_{CER} < t \leq t_{FER}) \tag{4.16}$$

$$Yield\ (t) = (b_1 + b_2 + b_3)\ t - b_2\ t_{CER} - b_3\ t_{FER}\ (t_{FER} \leq t) \tag{4.17}$$

where b_i: adjustable parameters of spline model, t: extraction time variable, t_{CER}: time-span period of CER region, t_{FER}: time-span period of FER region.

Costa et al. [37] analyzed the extraction kinetics about Congo Bololo (*Vernonia amygdalina* Delile) leaves for extract yield. They also proposed a similar simple extraction model (Spline model) considering three extraction steps and claimed that the Spline model presented the best fit to experimental data among other models and was able to characterize constant and decreasing extraction rate periods. When three steps are presented in the extraction curve, the spline model is one of the appropriate models.

Further, a very simple model is shown in Eq. (4.18) and applied for Palo Negro (*Leptocarpha rivularis*) [41].

$$\frac{q}{q_0} = \frac{k_1 t}{1 + k_2 t} \tag{4.18}$$

where k_1: the parameter related to extraction rate at the very beginning of the process [min^{-1}], k_2: the parameter related to maximum extraction yield [min^{-1}], q: amount of extract [$g{\cdot}kg^{-1}$ d. s.], q_0: maximum amount of extracted [$g{\cdot}kg^{-1}$ d. s.], t: time [min].

Other very simple model is proposed in the case of Sage (*Salvia officinails L.*) leaves [46].

$$\text{Extract yield } [\%] = 100\ (1 - \exp\ (a\ t + b)) \tag{4.19}$$

where t: extraction time, a, b: constant.

By using this model, the experimental data are correlated by using two parameters.

4.5 Antioxidant Capacity, Total Phenolic Content and Total Flavonoid Content in the Extract Obtained with Supercritical CO_2 Extraction of Leaves

4.5.1 Typical Experimental Conditions

Table 4.2 shows the experimental condition of sc-CO_2 extraction of leaves for evaluation of antioxidant capacity and total phenolic content in the extract. The extraction conditions of these studies are almost at (313–333) K and (10–30) MPa.

Table 4.2 Conditions used to obtain antioxidants and phenolics from leaves with supercritical CO_2

Name	Temp. Press.	Co-sol.[a]	AOC[b]	TPC[b]	Ref.
Aloe (*Aloe vera*)	305–323 K, 35–45 MPa	M	+		[59]
Bamboo (*Sasa palmata*)	323–483 K, 10–25 MPa	E, W	+	+	[52]
Congo Bololo (*Vernonia amyg-dalina* Delile)	313–333 K, 20–25 MPa		+		[37]
Firespike (*Odontonema strictum*)	328–338 K, 20–25 MPa	E	+		[63]
Green Tea (*Camellia sinensis L.*)	313–333 K, 10–20 MPa	E	+		[55]
Hempedu bumi (*Andrographis Paniculata*)	333 K, 30 MPa	M	+	+	[56]
Jambú (*Spilanthes acmella ver oleracea*)	323 K, 25 MPa	E, W	+	+	[53]
Lemon balm (*Melissa officinalis L.*), Rosemary (*Rosmarinus officinails L.*), Spanish lavender (*Lavandula stoechas* ssp.), Thyme (*Thymus serpyllum*)	313–333 K, 20–30 MPa		+		[76]
Lemon-scented gum (*Corymbia citriodora*)	309–343 K, 5.18–10 MPa		+		[77]
Limau purut (*Citrus hystrix*)	313–333 K, 10–36.3 MPa	E	+	+	[61]
Moringa (*Moringa oleifera*)	313–333 K, 10–20 MPa	E		+	[40]
Palo Negro (*Leptocarpha rivu-laris*)	313–333 K 10-20 MPa	E	+		[54]
	313–333 K, 9–15 MPa		+	+	[41]
Rose cactus (*Pereskia bleo*)	313–333 K, 25–45 MPa	E	+		[58]
Rosemary (*Rosmarinus officinails L.*)	313 K, 15–30 MPa		+		[24]
Rosemary (*Rosemarinus officinails L.*), Sage (*Salvia officinails L.*)	313–373 K, 30 MPa		+		[25]
Spearmint (*Mentha spicata*)	313–333 K, 10–30 MPa		+		[60]
Strawberry (*Fagaria ananassa*)	308–333 K, 10–30 MPa	E, W, Ac	+	+	[50, 57]
Tasmanian bluegum (*Eucalypus globulus L.*)	313–353 K, 10–35 MPa		+	+	[87]

[a]Co-solvent, W: water, M: methanol, E: ethanol, Ac: acetone
[b]Antioxidant capacity (AOC) and total phenolic content (TPC) +: Evaluated

This is because of easy operation under relatively milder conditions in supercritical states and considering the thermal stability of natural compounds. The extraction temperature in a few studies is over 333 K and it is a rare case. The range for optimization of temperature and pressure is conducted only 20 K and 20 MPa. Further, co-solvent such as methanol, ethanol and water are sometimes used.

4.5.2 Antioxidant Capacity

Leaves contain antioxidants and the extraction of antioxidants with sc-CO_2 have been widely studied. Table 4.2 summarizes the studies of antioxidant and total phenolic content in the extract obtained with sc-CO_2 extraction of the leaf. One of the major methods for antioxidant capacity is DPPH radical-scavenging assay relative to 2,6-di-*tert*-butyl-*p*-cresol (Butylated hydroxytoluene). "Antioxidant capacity (AOC)" in this chapter means the radical eliminating activity defined in each literature and is not standardized value. It is difficult to organize the condition of extraction and the magnitude of AOC in the extract obtained with sc-CO_2 at this time. In this section, the introduction of some examples of trends are introduced.

The effect of co-solvent for antioxidant capacity were studied [50, 52–57]. In the extraction of Strawberry (*Fagaria ananassa*) leaf with sc-CO_2 + co-solvent at 308 K, 20 MPa and 0.1 of co-solvent/CO_2 molar ratio, the magnitude of AOC was in the order of ethanol > acetone > water > without co-solvent [50]. The solvent probably enhances the extraction of antioxidant compounds in the deep tissue of the leaf. Both nonpolar and polar components were probably present in the extract as an antioxidant because amphiphilic solvents such as ethanol and acetone were effective. In the extraction of Green Tea (*Camellia sinensis L.*) [55], AOC increased with increasing the flow rate of ethanol as co-solvent under constant temperature, pressure and CO_2 flow rate. The addition of polar solvents enhanced the change in the structure of the cellar matrix via intra-crystalline and osmotic swelling and break analyte-matrix bindings. The extraction of Rose cactus (*Pereskia bleo*) with sc-CO_2 + ethanol, AOC increased with increasing the ratio of ethanol to water [58]. The increase in the amount of co-solvent significantly increased AOC. On the other hand, in the case of extraction of Aloe (*Aloe vera*) with sc-CO_2, the effect of co-solvent on AOC was small [59].

In some cases, there is the optimal concentration of co-solvent. The extraction of Bamboo (*Sasa palmata*) leaves with a ternary system of sc-CO_2, ethanol and water were conducted and 25:75 (mol:mol) ethanol-water composition gave the highest AOC at 323 K and 25 MPa [52]. The phenolics contained in Bamboo leaves probably consisted of both ethanol-soluble and water-soluble compounds. Similar results were obtained in the extraction of Jambú (*Spilanthes acmella ver oleracea*) leaves [53]. In the extraction of Strawberry (*Fagaria ananassa*) leaves with sc-CO_2 + water, AOC once increased increasing in water/CO_2 molar ratio and then decreased as shown in Fig. 4.7 [57]. The maximal AOC was 2.48 μmol-BHT/g-sample. The existence of water that is a polar solvent enhanced the dissolution of

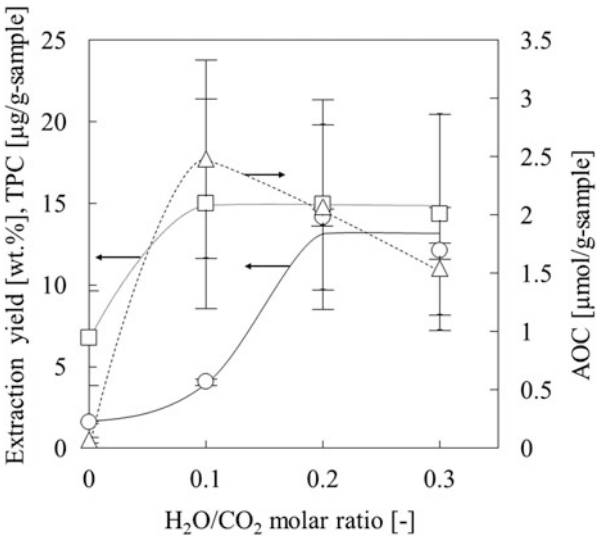

Fig. 4.7 Extraction yield, AOC and TPC at 308 K, 20 MPa and 7.58×10^{-4} mol/s of CO_2 flow rate for 120 min (○: extraction yield, Δ: AOC, □: TPC) of the extract in the extraction of Strawberry (*Fagaria ananassa*) leaves, reprinted with permission from [57] Copyright © 2021 The Society of Chemical Engineers, Japan

antioxidants in the solvent. On the other hand, the phase behavior of sc-CO_2 + water is two-phase regions and the ratio of the volume of CO_2 rich phase decreased with increasing water/CO_2 ratio, which leads to the suppression of the contact between leaves and solvent in CO_2 rich phase as main fluid. The optimal water/CO_2 ratio was probably determined by the balance of positive and negative effects.

The effect of temperature and pressure on AOC was evaluated. The extraction of Strawberry (*Fagaria ananassa*) leaves with sc-CO_2 + water at (308–333) K and (10–30) MPa and 0.1 molar ratio of water/CO_2 was examined [57]. The results were correlated with temperature and pressure, and the response surface against temperature and pressure were constructed as shown in Fig. 4.8. The typical method of response surface analysis is as follows. At first, the experimental conditions such as temperature, pressure and amount of co-solvent are converted to the simple parameter. The parameter is typically between −1 to 1 in the experimental range. After that, the experimental results are correlated with a quadratic polynomial by the determination of the parameters in the equation. AOC was high in higher temperature and lower pressure region. The maximal AOC was 5.65 μmol-BHT/g-sample at 333 K and 10 MPa. The density of solvent decreased with increasing temperature in each pressure. For example, the density of CO_2 decreases from 866 kg/m^3 to 724 kg/m^3 at 20 MPa when temperature increases from 308 to 333 K [5]. This decrease in density is a factor that decreases the amount of solute dissolving in a solvent. On the other hand, the solubility of compounds generally increases with increasing temperature due to an increase in the vapor pressure of compounds. The effect of change in solubility with temperature was probably the main factor in the extraction of antioxidants from strawberry leaves. The extraction of Aloe (*Aloe vera*) with sc-CO_2 + methanol was examined and AOC was the highest at 305 K, 45 MPa and 24% methanol [59]. The relatively lower temperature and higher pressure region

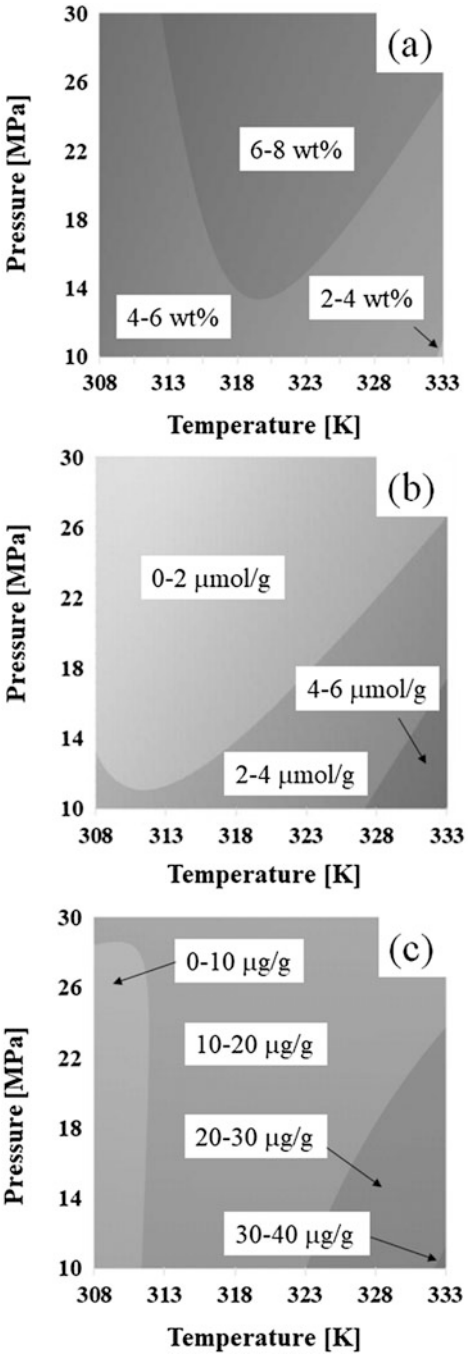

Fig. 4.8 Response surface analysis of (**a**) extraction yield, (**b**) AOC and (**c**) TPC of the extract in the extraction of Strawberry (*Fagaria ananassa*) leaves at 0.1 of H_2O/CO_2 molar ratio and 7.58×10^{-4} mol/s of CO_2 flow rate for 120 min

was preferred to obtain high AOC. The author of this paper claimed that higher pressure contributed to the diffusion of polar components and a heat-sensitive property of polar antioxidants suppressed AOC at high-temperature regions. In the case of the extraction of *leptocarpha rivularis* leaves with sc-CO_2, AOC was high in higher pressure and lower temperature region [41]. The high density of solvent probably promoted the extraction of antioxidants.

In some cases, AOC took a maximal value against temperature or pressure. Maran et al. [55] conducted the extraction of Green Tea (*Camellia sinensis L.*) leaves with sc-CO_2 + ethanol at (313–333) K and (10–20) MPa. AOC once increased and then decreased with increasing temperature and increased with increasing pressure. The high solvent density at high pressure decreased the distance between the molecules and thereby strengthening interactions between the fluid and matrix, which leads to the acceleration of mass transfer rate and diffusion of the solvent into the system to improve the extraction of solute. The increase in temperature increased the solute vapor pressure and contributed to damage the particle cell walls increasing mass transfer of solute whereas decomposition of antioxidants during the extraction would occur. A similar trend is reported in the case of extraction of Spearmint (*Mentha spicata*) with sc-CO_2 [60]. AOC tended to higher in high-pressure region and once increased and then decreased with increasing temperature. In the case of extraction of Strawberry (*Fagaria ananassa*) leaf with sc-CO_2 + ethanol, AOC had a maximal value at 20 MPa and was independent of pressure at 308 K [50]. The balance of vapor pressure and solvent density with temperature and pressure determined the extraction of antioxidants. The extraction of Rose cactus (*Pereskia bleo*) was conducted with sc-CO_2 + ethanol [58] and AOC was relatively high in high-pressure region and the low and high-temperature region. In the extraction of Bamboo (*Sasa palmata*) leaves with sc-CO_2 + ethanol or water, the effect of temperature and pressure on AOC depended on the kind of co-solvent [52]. In sc-CO_2 + ethanol, AOC decreased with increasing temperature at 20 MPa, and once increased and then decreased at 323 K. In sc-CO_2 + water, AOC increased with increasing temperature at 20 MPa and increased and became almost constant with increasing pressure at 323 K. Extraction of Limau purut (*Citrus hystrix*) leaves with sc-CO_2 + ethanol was examined [61] and the optimal condition was 323 K and 31.4 MPa estimated by response surface analysis.

4.5.3 Total Phenolic Content

Total phenolic content (TPC) in the extract was evaluated in several studies. TPC is typically measured by the Folin-Ciocalteu method and the content of phenolics is evaluated relative to gallic acid as the standard. The referral of "TPC" in this chapter means that the TPC values were determined according to procedures in each literature reference.

The addition of a polar co-solvent increases TPC by enhancing the extraction of phenolics. In the extraction of Strawberry (*Fagaria ananassa*) leaves with sc-CO_2 at

308 K and 20 MPa, TPC with sc-CO_2 + ethanol, acetone and water were higher than that with sc-CO_2 [50, 57]. These results indicate that the polar solvent was effective for the extraction of phenolic compounds. In the extraction of Bamboo (*Sasa palmata*) leaves with sc-CO_2+ co-solvent, a mixture of 25:75 (mol) ethanol-water co-solvent gave the highest TPC in the whole composition of water-ethanol mixtures [52]. The phenolic compounds consisted of both ethanol and water-soluble compounds.

The trend of the effect of temperature and pressure on TPC resembled that on AOC. In the case of TPC in the extraction of Strawberry (*Fagaria ananassa*) leaves with sc-CO_2 + water at (308–333) K and (10–30) MPa, TPC tended to increase with increasing temperature and decreasing pressure as shown in Fig. 4.8, and the maximal TPC was 31.0 μg-gallic acid/g-sample at 333 K and 10 MPa [57]. In the extraction of Bamboo (*Sasa palmata*) leaves, TPC increased with decreasing temperature and increasing pressure in sc-CO_2 + ethanol while TPC increased with increasing temperature and pressure in sc-CO_2 + water [52].

In some cases, there were optimal conditions. The extraction of Green Tea (*Camellia sinensis L.*) leaves was conducted with sc-CO_2 + ethanol at (313–333) K and (10–20) MPa, and TPC had an optimal value against temperature and increased with increasing pressure [55]. In the extraction of Strawberry (*Fagaria ananassa*) with sc-CO_2 + ethanol at (308–333) K and (10–30) MPa, TPC had maximal value for the temperature at 20 MPa and pressure at 308 K [50].

4.5.4 Total Flavonoid Content

Flavonoids are contained in plants, belong to a class of plant secondary metabolites and contain polyphenolic structure [3]. The flavonoid in the extract from leaves are analyzed. The total flavonoid content (TFC) is measured typically aluminum chloride method and also evaluated as the sum of individual compounds in some cases. In the extraction of leaves with sc-CO_2, the contents of flavonoids in the extract were reported [14, 25, 51, 54, 56, 62, 63].

TFC was measured for Green Tea (*Camellia sinensis L.*) leaves [55], Hempedu bumi (*Andrographis paniculata*) leaves [56] and Palo Negro (*Leptocarpha rivularis*) leaves [54]. In the extraction of Green Tea (*Camellia sinensis L.*) leaves with sc-CO_2 + ethanol at (313–333) K and (10–20) MPa, the estimated maximal of TFC by response surface analysis was 194.60 mg of quercetin equivalents per 100 ml of extract at 323 K and 18.8 MPa [55]. In the extraction of Hempedu bumi (*Andrographis paniculata*) leaves with sc-CO_2, TFC was 179.81 mg per g of extract at 333 K and 30 MPa [56].

The compounds classified as a flavonoid was detected in the extract. The structures of some flavonoids detected are shown in Fig. 4.9. In the extract of Pecah Kaca (*Strobilanthes crispus*) leaves with sc-CO_2 + ethanol at 333 K and 20 MPa, rutin (a), luteolin (b), epicatechin (mixture of c, d), catechin (mixture of e, f), kampferol (g), myricetin (h), naringenin (i) and apigenin (j) were contained [62]. In the extraction of

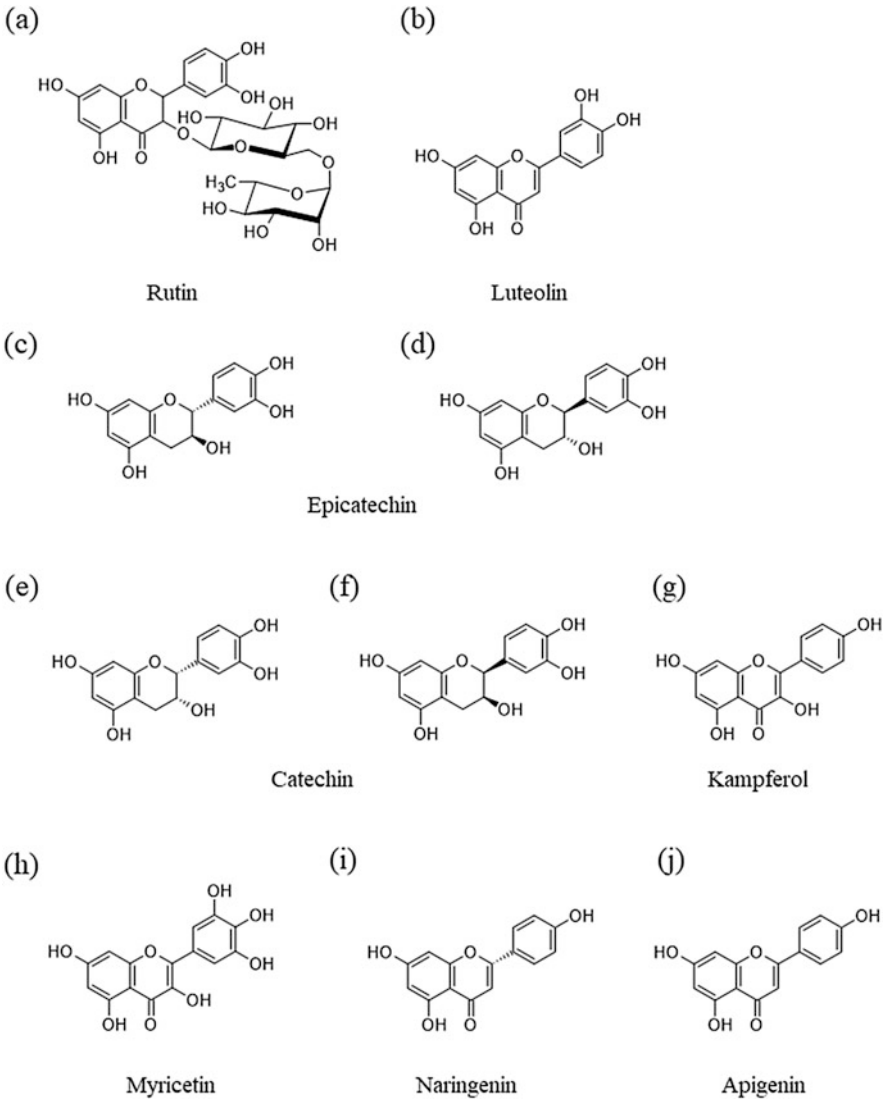

Fig. 4.9 Structure of flavonoids extracted with supercritical CO_2 extraction of leaves from Pecah Kaca (Strobilanthes crispus) [62] and Spearmint (Andrographis paniculata) [14]

Spearmint (*Andrographis paniculata*) leaves with sc-CO_2 + ethanol at 333 K and 30 MPa, the amount of luteolin (b), apigenin (j), naringenin (i), myricetin (h), epicatechin (c,d), ruin (a), cactechin (e,f) was large in this order [14]. The extract from the extraction of Rosemary (*Rosmarinus officinails L.*) leaves with sc-CO_2 at 373 K and 30 MPa, wogonin, genkwanin, oroxylin A, biochanin A, acacctin and 5,7-dihydroxy-6-methoxyflavone were detected in the extract [25].

4.5.5 *Effect of Temperature and Pressure on Kinetics of Supercritical CO_2 Extraction*

The effect of temperature and pressure on sc-CO_2 extraction was discussed throughout this chapter. Here, the contribution of temperature and pressure are summarized. Fig. 4.10 shows the effect of vapor pressure of solute and solvent density with temperature and pressure on sc-CO_2 extraction of compounds from leaves.

In low temperature and high-pressure regions, the density of CO_2 is high and the solubility of compounds becomes high. In this case, there are a lot of solvent molecules around the leaf tissue, which probably enhances the transportation of solute from the leaf tissue to solvent by a high saturated concentration of solute in the solvent. On the other hand, low-temperature conditions make the vapor pressure of the solute low and so vaporization of solute from the leaf is suppressed.

In high temperature and low-pressure region, high temperature leads to the high vapor pressure of solute and the solute easily vaporizes into the solvent. The solvent density in high temperature and low-pressure region is low, which leads to the decrease of the number of solvent molecules, which makes the solubility of the solute low. The amount of solute such as antioxidants, phenolics and flavonoids at each condition is determined by considering above factors [50] and the contribution of these factors depends on the kind of leaves. So it is important to evaluate the effect of solvent properties manipulated with temperature and pressure on the extraction kinetics.

4.5.6 *Comparison of Supercritical CO_2 Extraction and Conventional Extraction*

The comparison of AOC and TPC obtained by sc-CO_2 extraction and that by conventional extraction such as Soxhlet and hydrodistillation were sometimes

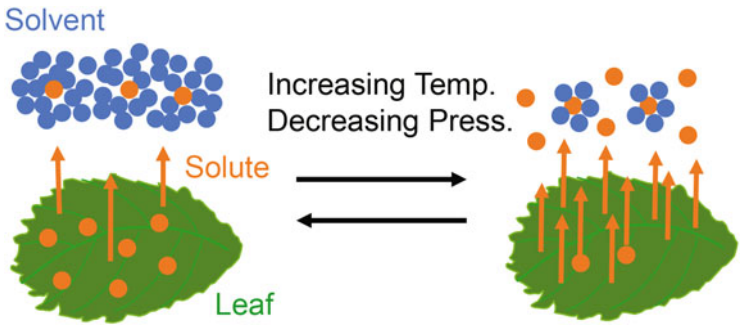

Fig. 4.10 Effect of vapor pressure of solute and solvent density with temperature and pressure on sc-CO_2 extraction of compounds from leaves

examined. In the extraction of Delile (*Vemonia amygdalina*) leaves, AOC and TPC were larger in the order of ethanol > dichloromethane > sc-CO_2 > hexane, which indicates that high polarity of solvent was advantageous of the extraction of antioxidants [37]. In the extraction of Palo Negro (*Leptocar pharivularis*), AOC obtained with hydrodistillation was lower than that with sc-CO_2 extraction because high temperature distillation probably enhanced decomposition of antioxidants. AOC and TPC obtained from the distillation of ethanol-water mixture was larger than sc-CO_2 [41]. In the extraction of Bamboo (*Sasa palmata*) leaves, the ethanol -water solvent (molar ratio 25:75) gave higher AOC than that obtained with sc-CO_2 + ethanol -water solvent (molar ratio 25:75), and TPC for those condition was similar [52]. In the extraction of Jambú (*Spilanthes acmella ver oleracea*), TPC was in the order of hydrodistillation > Soxhlet ethanolic distillation (ethanol-water mixture) > sc-CO_2 extraction [53].

These results indicate that the quantitative comparison was difficult because the extraction system between conventional solvent and sc-CO_2 were different, high polarity of solvent is effective for extraction of antioxidants and phenolics as well as sc-CO_2. In the view of comparison of sc-CO_2 extraction system with conventional extraction one, the advantage of sc-CO_2 system is reducing the amount of polar solvent required for conventional extraction and easier separation of extract from solvent, and disadvantage would be the system being complicated and difficult to make it larger.

4.6 Conclusions and Future Outlook

Extraction of useful components such as antioxidants from leaves with supercritical carbon dioxide (sc-CO_2) is a promising technology for effective usage of biomass. The properties of CO_2 in its supercritical state give it high permeability and provide high solubility of solutes compared with CO_2 in the gas or liquid state. The addition of a polar co-solvent improves extraction of compounds by promoting molecular interactions that are attractive for polar compounds in leaves. Extraction of leaves is typically conducted with a semi-batch system.

Considering the extraction studies of over fifty different types of leaves with sc-CO_2, recovered components such as terpenes, phenolics and phytosterols depend vary widely with the type of leaves. There are three steps in the kinetics of extraction with sc-CO_2 due to the rigid structure of the cell in the leaf. The first step is the constant extraction rate step corresponding to the firstly extraction of accessible solute from the cell. The second step is the falling extraction rate step corresponding to the transition state between the first step and third step. The third step is the diffusion-controlled step corresponding to slower extraction of the solute protected by the cell walls that are controlled by diffusion in the solid phase. Several extraction models were proposed for the extraction curve and some models expressed the extraction curve including the above three steps well.

The total antioxidants (AOC), total phenolics (TPC), total flavonoid content (TFC) in the extract were evaluated. The addition of polar co-solvent such as ethanol and water increased AOC and TPC due to the improvement of solubility of polar compounds in most cases. The effect of temperature and pressure differed according to the kind of leaves. The contribution of both solvent density that becomes large in low temperature and high-pressure region and vapor pressure of solute that becomes large in high-temperature region probably governed the dependence of AOC, TPC and TFC on temperature and pressure.

In the future, generalization of the extract process will be important by extending the extraction conditions, especially to milder conditions. In this case, extraction kinetics in liquid CO_2 just below the critical temperature and critical pressure becomes interesting due to the mingling of different phases. Co-solvents are necessary for the extraction of polar components to compensate for low solubility of CO_2 at low pressures. Water is an inexpensive and environmentally friendly solvent and will become major co-solvent for application in extraction systems. Analysis of extraction kinetics of multiphase systems for sc-CO_2 + water will be necessary.

The extension of the present extraction system to different kinds of leaves is also expected. The recovery of useful components from leave mixtures as a biomass waste is one of the directions of development of this technology. The key factor is finding high added value and highly biological activity compounds from the extraction. It is expected that there will be great progress by making detailed evaluations of compounds obtained from sc-CO_2 extraction of leaves.

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