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Review

Extraction of Functional Substances from Agricultural Products or By-products by Subcritical Water Treatment

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Water that maintains its liquid state in the temperature range from 100°C to 374°C is called subcritical water, compressed hot water or pressurized hot water. This type of water has unique properties compared to ambient water. One is a low relative dielectric constant and another is a high ion product. Due to these properties, this water can be used to extract functional substances from natural resources. In this article, the application of subcritical water for the extraction of substances from agricultural products or their wastes is reviewed.

Keywords: subcritical water, agro-waste, extraction, functional substance

Introduction

Agricultural products, such as rosemary, grapes, tea leaves, and grain bran, contain bioactive compounds that can function as health-promoting, antioxidative, and radical scavenging substances. Among them, phenolic substances, catechin, epicatechin, anthraquinones, and essential oils are of interest (Deng *et al.*, 2004; Piñeiro *et al.*, 2004; Ibáñez *et al.*, 2003; Shotipruk *et al.*, 2004). Several approaches have recently been used to recover these substances involving their extraction with organic solvents, such as methanol, ethanol and acetone. However, these techniques are time-consuming and require a large amount of organic solvents, which are harmful to human health and cause environmental stress. Thus, an alternative method that is the effective, economical, environmentally friendly, safe and fast, is required to alleviate these drawbacks. The most common techniques, which have recently been discussed, include supercritical fluid extraction (e.g., carbon dioxide), pressurized liquid extraction or accelerated solvent extraction, and subcritical water extraction. Of these techniques, subcritical water extraction using water as the extractant is one of the most interesting methods because water is non-flammable, non-toxic, cheap, and environmentally safe. The subcritical water, also called pressurized hot water, compressed hot water or superheated water, is hot water that maintains its liquid state at temperatures between 100°C and 374°C (the critical temperature and pressure of water are 374°C and 22.4 MPa, respectively) under pressurized conditions. When the temperature of water increases, its physicochemical properties, in particular its relative dielectric constant and ion product, change. The ion product of water is the product of the concentrations of hydrogen and hydroxyl ions. The relative dielectric constant, ε, or polarity of water can significantly decrease with increasing temperature from approximately 80 at 25°C to 27 at 250°C (Fig. 1), which is close to that of methanol (ε = 33) and ethanol (ε = 24) at 25°C (Wagner and Pruß, 2002). Therefore, subcritical water has the ability to recover or dissolve both polar and apolar substances from natural products, such as phenolic, polycyclic aromatic compounds and oils (Deng *et al.*, 2004; García-Marino *et al.*, 2006; Morales-Muños *et al.*, 2002). In addition, the dissociation constant of subcritical water for hydrogen and hydroxyl ions is three orders of magnitude higher than that of ambient water as shown in Fig. 1(a) (Marshall, 1981). Consequently, subcritical water can act as an acidic or basic catalyst during chemical reactions. The potential of subcritical water as a catalyst has been extensively studied: proteins and carbohydrates, the latter including the cellulose and hemicellulose of brans, leaves and grass, could be degraded by subcritical water at 160 to 260°C (Haghighat Khajavi *et al.*, 2006; Rogalinski *et al.*, 2005; Sasaki *et al.*, 2000). In addition, the diffusion coef-

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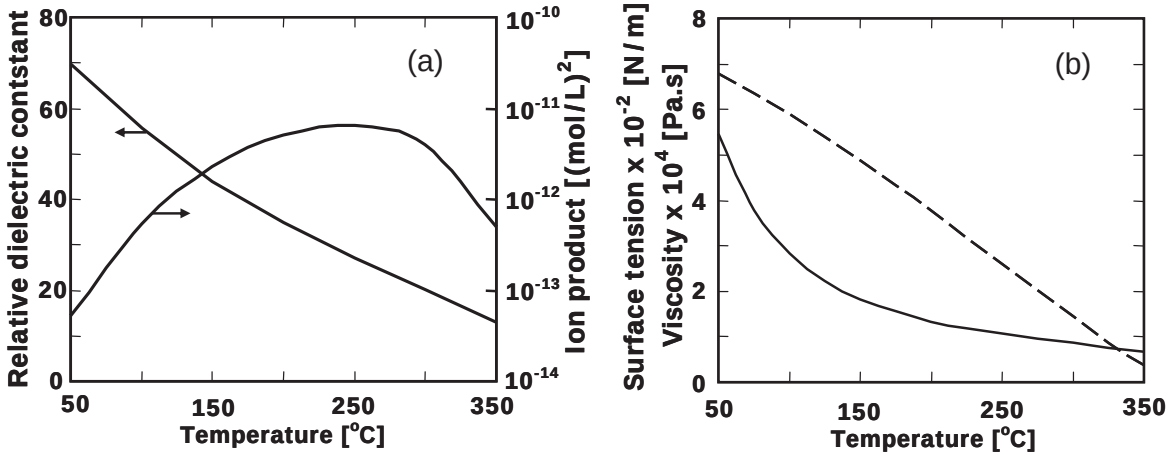

Fig. 1. Effects of temperature on (a) relative dielectric constant and ion product and (b) viscosity (solid line) and surface tension of saturated water (broken line). Data were drawn based on the reference (Marshall, 1981) and using the ChemicaLogic SteamTab Companion software.

ficients of solutes in subcritical water are higher than those in ambient water because of its higher mass transfer properties. The viscosity and surface tension of subcritical water decrease at high temperatures as shown in Fig. 1(b) (Wagner and Pruß, 2002). The low viscosity and surface tension promote the mass transfer properties and penetration into the matrix particles, and consequently lead to enhanced extraction efficiency.

The mechanism of extraction of bioactive substances from agricultural products using subcritical water is similar to that of other extraction methods, which sequentially involve desorption, diffusion and dissolution. For the desorption step, solutes must diffuse from the core of the materials to the surface, transfer from the surface into the stagnant fluid layer and subsequently be distributed or dissolved in the flowing bulk of the extraction fluid in the diffusion step. Finally, the solutes are dissolved in the extraction fluid and eluted out of the extraction cell (Lou *et al.*, 1997). The extraction rate is limited by the slowest of these three steps. For many natural materials, the rate of the initial desorption process will determine the overall extraction rate (Lou *et al.*, 1997; Kronholm *et al.*, 2007).

In addition, a substantial increase in the ion product during subcritical water extraction, in particular, at temperatures between 150 and 250°C, contributes to the hydrolysis into smaller particles and the subsequent shorter time requirement for mass transfer from the core of the matrix particles to the surface.

The subcritical water extraction of functional substances from agricultural products and their wastes is reviewed in this article. We applied the extraction method for the production of useful substances from rice bran. The results are also briefly introduced.

Instruments for subcritical water extraction

Subcritical water extraction can be carried out in three operational modes. Static mode uses a fixed volume of water without any outflow of the subcritical water at elevated temperatures and pressures. Dynamic mode uses water continuously flowing through the sample. The advantage of dynamic mode is that the subcritical water is continuously refreshed during treatment and can subsequently provide a faster recovery, but this mode requires a greater amount of subcritical water than the static mode. Static-dynamic mode is combination of the two previous modes (Lamoolphak *et al.*, 2006; Morales-Muñoz *et al.*, 2002; Lou *et al.*, 1997). A basic extraction system consists of a water reservoir, extraction vessel and extract reservoir. The water is heated to the operating temperature before entering the stainless steel vessel for the subcritical water treatment. The preheating coil and the vessel are generally built into a temperature-controlled oven. After treatment under the desired conditions, the extract is delivered to the cooling system in order to cool the liquid to ambient temperature prior to collecting it in the extract reservoir. The water and extract are delivered and pressurized in the system by a high pressure pump, and a back pressure regulator is fitted at the outlet to maintain the desired pressure in the system. The water reservoir may be connected to a degasser to reduce the amount of dissolved oxygen in the water, which may corrode the line during high temperature operation. Subcritical water treatment may be performed batchwise without the high pressure pump. The main operation unit still consists of the extraction vessel, and the heating and cooling system (Wiboonsirikul *et al.*, 2007a; Lamoolphak *et al.*, 2006; Khuwijitjaru *et al.*, 2004).

In addition, before subcritical water treatment, pretreatment of samples may be required such as sample drying in

air- or freeze-drying. The dried sample should then be ground to obtain a smaller and more homogeneous sample size. Any inert material, such as sea sand, may be used as a supporting material and homogeneously distribute the sample. Sample pretreatment facilitates solute transport to the particle surface.

Factors affecting extraction efficiency

The main factors affecting the extraction efficiency during the subcritical water treatment include treatment temperature, time, and solute characteristics. Other factors, such as the particle size of the samples, the addition of an organic solvent or a surfactant, the geometry of the extraction cell, and the direction of water flow also influence the extraction efficiency, but may not be as significant during subcritical water treatment as the extraction temperature (Kronholm *et al.*, 2007; Lou *et al.*, 1997; Hawthrone *et al.*, 1994).

Treatment temperature Temperature is the major factor affecting the physicochemical properties of water. Consequently, it determines the extraction rate, efficiency, and selectivity of subcritical water treatment (Karásek *et al.*, 2006; Herrero *et al.*, 2005). The two main reasons why subcritical water affects extraction efficiency and selectivity are the solubility and mass transfer effects, and disruption of the surface equilibria.

There are three different points of view regarding subcritical water treatment altering the solubility and mass transfer of solutes from natural products. First, the use of a higher temperature increases the capacity of subcritical water as a solvent to solubilize solutes. The solubility of antioxidants, proteins and carbohydrates from defatted rice bran increased with increasing temperature from 50 to 250°C as shown in Fig. 2 (Wiboonsirikul *et al.*, 2007b; Hata *et al.*, 2008). Antioxidative ability was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) radical-scavenging activity. Second, when the treatment temperature increases, the diffusion rate increases roughly 2 to 10 fold with increase in temperature from 25 to 150°C (Richter *et al.*, 1996). Finally, the concentration gradient between the solution in the extraction cell and the surface of the sample matrix is one of the aspects affecting the extraction efficiency. According to Fick's first law of diffusion, the mass transfer rate, or flux, is higher with a higher concentration gradient. If fresh subcritical water is introduced during a static extraction step, mass transfer is improved, and consequently the extraction rate increases (Morales-Muños *et al.*, 2002).

When the temperature increases during subcritical water treatment, the strong solute-matrix interactions caused by van der Waals forces, hydrogen bonding, and dipole attractions of the solute molecules and active sites on the matrix can be disrupted, and hydrogen bonding is weakened with increasing temperature. The thermal energy can overcome the cohesive (solute-solute) and adhesive (solute-matrix) interactions by decreasing the activation energy required for desorption (Richter *et al.*, 1996).

In addition, as shown in Fig. 1(b), the viscosity and surface tension of subcritical water decrease at higher temperatures, hence promoting better penetration of water into the matrix particles to enhance extraction. An increase in treatment temperature causes a decrease in the surface tension of solutes and the matrix, contributing to better contact of the solutes with the subcritical water, subsequently enhancing extraction efficiency. When the surface tension of the solvent decreases, a solvent cavity is easily formed allowing the solutes to dissolve more quickly in the solvent (Möckel *et al.*, 1987).

Extraction efficiency increases with rising temperature due to promotion of thermal desorption of the solutes and the contribution of the attractive forces of water being closer to those of non-polar substances, leading to a significant increase in the solubility of the apolar substances (Karásek *et al.*, 2006; Hawthorne *et al.*, 1998).

In addition, treatment temperature also influences extraction recovery because chemical reactions, such as hydrolysis and oxidation, substantially increase, and some thermally-labile compounds may be degraded after being liberated from the source matrix (Kronholm *et al.*, 2007). Ibáñez *et al.* (2003) treated rosemary in subcritical water at temperatures

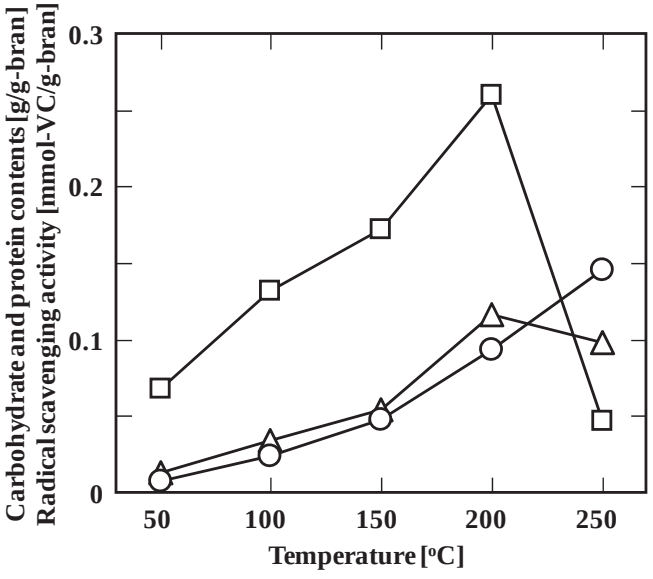

Fig. 2. (□) Carbohydrate and (△) protein content and (○) DPPH radical scavenging activity of the extracts from defatted rice bran by subcritical water treatment for 5 min at different temperatures. The DPPH radical scavenging activity is expressed as mmol-ascorbic acid (abbreviated VC) equivalent per g-bran.

ranging from 25 to 200°C. The most active antioxidants from rosemary, including carnosol, rosmanol, carnosic acid, methyl carnosate and some flavonoids, such as cirsimaritin and genkwanin, were recovered in the extracts using subcritical water extraction with DPPH radical scavenging activity as high as that using supercritical carbon dioxide extraction. The selective recovery depended on the extraction temperature. The major components in the extract at low temperature were mostly the polar compounds and their proportion decreased with increasing temperature. The solubility of apolar compounds, such as carnosic acid, increased with increasing temperature. High temperature, however, led to a decrease in the recoveries of the polar and apolar compounds except for carnosic acid.

Several investigations on the effects of treatment temperature on extraction efficiency, selectivity and recoveries of bioactive substances and agricultural by-products have been done as shown in Table 1. Increasing the extraction temperature from 50 to 200°C resulted in higher recoveries of bioactive compounds, including catechins, proanthocyanidin, phenolic substances, organic flavor and fragrance compounds, and essential oils (Wiboonsirikul *et al.*, 2007b; Gracía-Marino *et al.*, 2006; Ozel *et al.*, 2003; Miller and Hawthorne, 2000).

Treatment time Subcritical water treatment can be conducted in static and dynamic modes, and their combination with very short treatment times in comparison with those for conventional solid-liquid extraction using an organic solvent. Subcritical water treatment in static mode may be result in incomplete extraction because of the limited volume of subcritical water. In contrast, treatment in dynamic mode by continuously refreshing the water during an entire course of the extraction has a better extraction recovery, but requires a larger volume of fluid (Morales-Muñoz *et al.*, 2002; Lou *et al.*, 1997). Treatment time is notably influenced by treatment temperature and the nature of the sample matrix and solutes. The treatment time for eugenol and eugenyl acetate from cloves at 250 and 300°C was only 15 min and the required time was 80 min at 125°C to obtain the same 100% recovery (Rovio *et al.*, 1999). Thus, an increase in treatment temperature would shorten the required treatment time for quantitative recovery. Hawthorne *et al.* (1994) revealed that quantitative recoveries of non-polar substances, such as polycyclic aromatic hydrocarbons, could not be attained or that the extraction time would be very long when extraction temperature was low. The partition-equilibrium and solubility of solutes should, however, be considered. Extraction by subcritical water can be carried out from several minutes to hours as listed in Table 1.

Pressure Pressure plays only a minor effect on the extraction recoveries during the subcritical water treatment. However, a specific minimum pressure is required to maintain the water in the liquid state at the treatment temperature. Pressures elevated from 1 to 10 MPa at treatment temperatures ranging from 100 to 300°C are generally used to maintain water in the liquid state during subcritical water treatment (Wagner and Pruß, 2002). Rovio *et al.* (1999) revealed that the pressure only slightly affected the extraction recoveries of eugenol and eugenyl acetate from cloves when it was changed from 2.5 to 17.1 MPa at a certain temperature. The pressure may facilitate extraction from the samples in which the solutes have been trapped in the matrix pores and solvents under atmospheric conditions may not be able to come in contact with them (Richter et al., 1996).

Solutes and sample matrix Extraction efficiency and recovery also depend on the diversity of the intrinsic samples, such as nature of the matrix, porosity, surface to volume ratio, and size or mass, in particular, in the diffusion-limited extraction rate. Extraction efficiency, rate and recovery increase when the samples contain a higher porosity or surface to volume ratio and smaller particle size. Thus, prior to subcritical water treatment, pretreatment of a sample by a technique, such as grinding, sieving, and mixing with an inert solid, is necessary. Pawlowski and Poole (1998) investigated the effect of the mass of a lemon sample on extraction of thiabendazole and carbendazim as pesticide residues by subcritical water and found that the extraction recoveries increased from 67% to 95% when the sample size decreased from 4 to 2 g at an extraction time of 5 min, temperature of 75°C and flow rate of 2 mL/min. Eikani *et al.* (2007a) found that an increase in particle size from 0.25 to 1 mm caused a substantial decrease in extraction recoveries of linalool and an essential oil from coriander at a treatment temperature of 125°C, pressure of 2 MPa, treatment time of 120 min and flow rate of 2 mL/min.

Flow rate In dynamic mode extraction, extraction efficiency and recovery increased with increasing flow rate due to promotion of mass transfer of the solutes from the sample matrix. Eikani *et al.* (2007b) found that the rate of essential oil extraction increased when the flow rate increased from 2 to 4 mL/min. The increase in flow rate resulted in an increased superficial velocity, and consequently, faster mass transfer. The optimum flow rate is related to the extraction time and desired final extract concentration. A high flow rate is imposed when the extraction is limited by the solute solubility in the extractant, diffusion in the sample matrix and transfer from the sample surface to the extractant. If the extraction recovery does not change when the flow rate is increased, the solute solubility does not affect the extraction efficiency. Consequently, the extraction rate can be increased

Table. 1. Subcritical water extraction or hydrolysis of agricultural products or by-products.

Sample matrix	Solutes	Conditions			Analytical method	Reference
		Temperature [°C]	Time [min]	Pressure [MPa]		
Grape seeds (winery by-products)	catechins, proanthocyanidins	50,100 and150	30	10	HPLC-DAD-MS	Carcía-Marino *et al.* (2006)
Leaves from *Thymbra spicata* L.	essential oils	100, 125, 150 and 175	30	2, 6 and 9	GC (TOF-MS)	Ozel *et al*. (2003)
Rosemary plants	antioxidants	25 to 200	30	4 to 7	LC-MS	Ibáñez *et al*. (2003)
Clove	eugenol and eugenyl acetate	125, 200 and 300	5 to 100	2.5, 4.9, 9.8, 17.2	GC-MS	Rovio *et al*. (1999)
Agricultural commodities	fungicides (thiabendazole and carbendazim)	23 to 75	5 to 10	5	RP-HPLC with UV and fluorescence detector	Pawlowski & Poole (1998)
Cumin (*Cuminum cyminum* L.)	volatile oil	100 to 175	60 to 180	2	GC-FID and GC-MS	Eikani *et al.* (2007b)
Coriander (*Coriandrum sativum* L.)	essential oil (linalool)	100, 125, 150 and 175	20	2	GC-FID and GC-MS	Eikani *et al*. (2007a)
Medicinal plants	berberin, glycyrrhizin and baicalein	80 to 160	40	1 to 2	HPLC with UV detector	Ong and Len (2003)
Ginkgo biloba leaves	terpene trilactones	20 to 140	15	2.5 to 30	GC-FID	Anekpankul *et al*. (2007)
Defatted soybean flakes	isofavones	60 to 100	60 to 180	2 to 4.8	HPLC	Li-Hsun *et al*. (2004)
Acorus tatarinowii Schott	essential oils	125 to 175	5	5	GC-MS	Deng *et al*. (2004)
Deoiled rice bran	protein and amino acids	100 to 220	0 to 30	0.1 to 4	Lowry's assay	Sereewatthanawut *et al*. (2007b)
Eucalyptus leaves	eucalyptus oils	50 to 200	0 to 75	3 to 10	GC-FID and GC-MS	Jiménez-Carmona & Luque de Castro (1999)
Flaxseed meal	lignans, proteins and carbohydrates	130, 160 and 190	180 to 420	5.2	HPLC and Bradford method	Ho *et al*., (2007)
Rosa damascena	volatile compounds	150	30	6	GC-TOF/MS	Özel *et al*. (2006)
Grapes	phenolic compounds	40 and 100	30	4 to 15	HPLC with PDA	Palma *et al*. (2002)
Ginger bagasse	reducing sugars	132 to 188	15	8 to 22	Somogyi method and HPLC	Moreschi *et al*. (2004)
Morinda citrifolia (Yor in Thai)	anthraquinones	150 to 220	0 to 450	4	RP-HPLC with UV detector at 250 nm.	Anekpankul *et al*. (2007)
Origanum vulgare L. (oregano leaves)	antioxidants	25, 50, 100, 150 and 200	30	10	DPPH radicals, HPLC-DAD	Rodríguez-Meizoso *et al*. (2006)
Piper methysticum (kava root)	lactones	50 to 200	20 to 120	6	GC-FID and GC-MS	Kubátová *et al*. (2001)
Defatted rice bran	antioxidants, emulsifiers	50 to 250	5	0.01 to 4	DPPH radicals, HPLC-RI	Wiboonsirikul *et al*. (2007b)
Yeast cells	protein and amino acids	100 to 250	5 to 30	0.01 to 4	Lowry method, Ninhydrin assays	Lamoolphak *et al*. (2006)
Soil	polycyclic aromatic hydrocarbons (PAH)	250 and 300	30 and 60	5	GC-FID, GC-MS	Hawthorne *et al*. (2000)
Savory (*Satureja hortensis*) and peppermint (*Mentha piperita*)	flavor and fragrance compounds	50 to 200	2 to 42	6 to 7	GC	Kubátová *et al*. (2001)

by increasing the extraction temperature.

Modifiers and additives Organic and inorganic solvents and some surfactants, such as sodium dodecyl sulfate (SDS) and Triton X-100, were used to enhance the solute recovery or solubility in water and also to increase the interactions between the water and solutes. The concentration of the surfactant added to subcritical water is one of the parameters determining the optimum conditions besides treatment temperature and time. The addition of other solvents or surfactants changes both the physical properties of the water and its critical temperature and pressure (Kronholm *et al.*, 2007). Ong and Len (2003) investigated the effect of the addition of ethanol (0 to 30%) into subcritical water on the extraction of berberine from a medicinal plant and found that the berberine content in the extracts increased with increasing amount of ethanol at treatment temperatures of 95 and 140°C for 40 min. Modification of subcritical water with ethanol from 0 to 20% or urea at 28% (w/w) at 100°C and 5 MPa increased the solubility of atrazine, a type of pesticide found in food commodities, from 500 to 6,000 mg/L (Curren and King, 2001). Choi *et al.* (2003) applied subcritical water containing Triton X-100 at various concentrations above its critical micelle concentration to extract the pharmacologically active ingredients (ginsenosides) from the root of ginseng. The treatment conditions were from 50 to 120°C for 10 min at 10 MPa. The extraction recoveries of the ginsenosides using the subcritical water containing Triton X-100 significantly increased in comparison with using only subcritical water, most noticeably at the lower treatment temperature. Ong *et al.* (2006) explained that the presence of a surfactant, such as Triton X-100, promotes the solubility of solutes of the sample matrix in subcritical water by disrupting the solute-matrix interaction and subsequently increasing extraction efficiency. Fernández-Pérez and Luque de Castro (2000) revealed that the presence of 0.05 mol/L SDS in subcritical water increased the extraction recoveries of polycyclic aromatic hydrocarbons (PAHs) in soil from 30 to 73% using static mode extraction at a temperature, pressure and time of 150°C, 5 MPa, and 15 min, respectively.

Subcritical water treatment of defatted rice bran

Rice is an agricultural staple in many countries including Japan. After the rice grain has been dried, its hull is eliminated to produce brown rice. This process is the first step in polishing and is called husking. The outer brown layer is removed from the brown rice kernel by an abrasive milling machine to yield white rice. The separated brown layer is designated rice bran. Because the rice bran contains lipids at a content of 10 to 23% as well as protein, fiber, ash and moisture, the lipid is extracted with hexane to produce rice bran oil. Defatted rice bran is a by-product of the process, that is, agricultural waste. It still contains, however, significant levels of proteins, carbohydrates and phenolic substances.

We tried to extract functional substances from defatted rice brans using subcritical water treatment. As shown in Fig. 2, the extracts from defatted rice bran contained proteins, carbohydrates and phenolic compounds, which would be responsible for the DPPH radical scavenging ability. The extracts at 100 to 200°C possessed both emulsifying and emulsion-stabilizing activities, while the extracts at 200 to 250°C exhibited antioxidative ability (Wiboonsirikul *et al.*, 2007b). Figure 3 shows the oxidation processes of linoleic acid contained in the extract prepared at 260°C and lyophilized, then added at different weight ratios. Oxidation was retarded more effectively at a higher weight ratio.

The conditions for obtaining an extract with a high phenolic content and radical scavenging ability, relating to the antioxidative activity of the extract, were optimized using the response surface methodology (Wiboonsirikul *et al.*, 2007c). Figure 4 shows the response surface for the DPPH radical scavenging ability for the ratio of bran to water and treatment time. Within the tested regions, an extract with the higher ability was obtained at a higher ratio of bran to water and for a shorter treatment time.

A novel method for extracting protein from defatted rice bran has been developed (Tsuno *et al.*, 2007). Even after extraction, the bran still contains a significant amount of protein, *ca.* 18% (w/w). This by-product from the production of the rice bran oil and protein was also treated with subcriti-

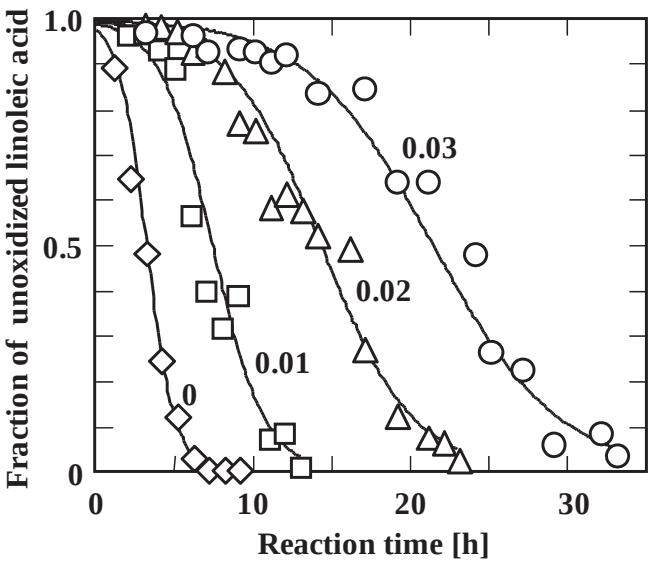

Fig. 3. Oxidation processes at 65°C and 0% relative humidity of linoleic acid mixed with the extract prepared at 260°C (Wiboonsirikul *et al.*, 2007a). The weight ratio of the extract to linoleic acid was 0 to 0.03 as indicated in the figure.

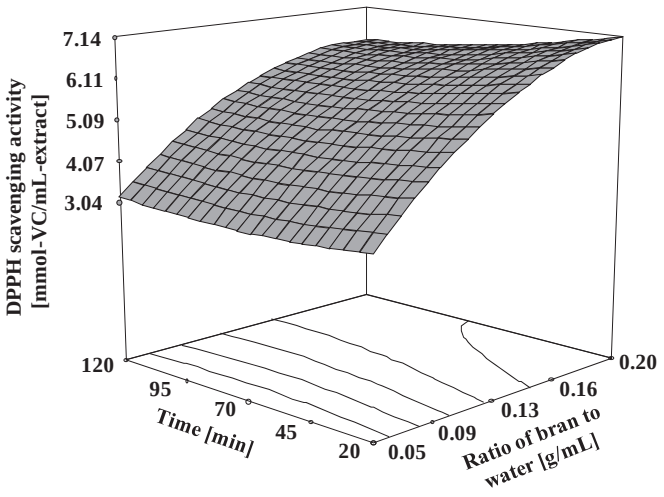

Fig. 4. Response surface for dependence of the DPPH radical scavenging activity on the ratio of bran to water (Wiboonsirikul *et al.*, 2007c). The treatment temperature was fixed at 250°C.

cal water at different temperatures. The extracts contained protein, saccharide and phenolic compounds, and had radical scavenging and emulsifying abilities (Wiboonsirikul *et al.*, 2008).

Defatted black rice bran was also treated with subcritical water, and the extracts also contained proteins and carbohydrates and had radical scavenging and antioxidative abilities (Wiboonsirikul *et al.*, 2007a)

Post-treatment after subcritical water extraction

After subcritical water extraction, the extract is usually a dilute aqueous solution and may be subjected to solute loss by re-adsorption into the sample matrix. The extract cannot be practically used without post-treatment. The matrix residues, which are suspended in the extract, need to be removed by techniques such as filtration and centrifugation. After clarification, the aqueous extract requires concentration or extraction before further application of separation methods. Excess water in the extract may be removed by a rotary evaporator under vacuum before other subsequent extractions to enrich the desired solute concentration (Ong *et al.*, 2006). Another method for enriching the desired solutes from the dilute extract is their partitioning into a small amount of immiscible organic solvent (liquid-liquid extraction) such as ethyl acetate and tetrahydrofuran (Lang and Wai, 2003). However, the general intent of subcritical water extraction is to avoid the use of organic solvents. Alternative solvent-free or minimal solvent extraction methods have been attractive, including solid-phase extraction, solid-phase microextraction and microporous membrane liquid-liquid extraction. Each of these extraction methods can be either separate from or on-line coupled with the subcritical water apparatus (Li-Hsun *et al.*, 2004; Kuosmanen *et al.*, 2003; Palma *et al.*, 2002).

For solid-phase extraction, the aqueous extract can be passed through a cartridge or adsorbent resin, such as alkyl- or aryl-bonded silica (C18-trap), carbon black, polymeric absorbent (Amberlite XAD) resin, and the anion exchange resin, SAX, and the desired solutes can be eluted from the cartridge or resin by a small volume of solvent. After concentration, the solutes can be analyzed by an appropriate analytical method, such as LC-MS.

Solid-phase microextraction is an extraction method using no solvent and no complicated apparatus, and less time-consuming than the liquid-liquid extraction. It can be used to concentrate both volatile and non-volatile compounds, such as an essential oil, in both liquid and gaseous samples for analysis by GC, GC-MS or HPLC. Deng *et al.* determined the essential oils from a traditional Chinese medicine, *Acorus Tatarinowii Schott.* using subcritical water combined with a solid-phase microextraction using poly(dimethylsiloxane)-divinylbenzene and further analysis by GC-MS. This method provided a good repeatability and recovery (Deng *et al.*, 2004).

Microporous membrane liquid-liquid extraction is a new technique introduced to replace solid phase extraction, which is subject to poor reproducibility because more sample matrix is extracted and, consequently, the trapping column may become blocked (Kuosmanen *et al.*, 2003). Microporous membrane liquid-liquid extraction is used for extracting, cleaning and concentrating aqueous extracts after subcritical water treatment of various biological and environmental samples (Kuosmanen *et al.*, 2003; Hyötyläinen *et al.*, 2001; Shen *et al.*, 1998). The solutes concentrated by this extraction method can be transferred on-line to a GC or HPLC for quantitative and qualitative analyses.

Comparison between subcritical water extraction and conventional solid-liquid extraction

The efficiency of subcritical water extraction has been commonly compared to that of conventional methods, such as solid-liquid extraction, using organic solvents. The efficiency and selectivity of subcritical water extraction mainly depend upon extraction temperature, as already mentioned.

Jiménez-Carmona and Luque de Castro (1999) isolated the essential oils from eucalyptus leaves using two methods: subcritical water at 150°C and 5 MPa for 20 min and hydrodistillation for 3 h. The recovery of the essential oils by subcritical water extraction was significantly higher and faster than that by the hydrodistillation. The energy cost of subcritical water extraction was estimated to be one twentieth that of the hydrodistillation. The results agreed with those from Gámiz-Gracia and Luque de Castro (2000), who compared

continuous subcritical water with dichloromethane extraction and hydrodistillation for the extraction of essential oils from medicinal plants.

When subcritical water extraction was compared with supercritical CO_2 and liquid CO_2 extraction at various treatment temperatures and pressures for the extraction of cedar wood oils, extraction rates were the highest for the supercritical CO_2 extraction at 100°C and 41 MPa. The highest ratio of cedrol to cedrene was obtained using liquid CO_2 at 25°C and 10 MPa. However, subcritical water extraction at any condition provided a lower recovery than the liquid CO_2 extraction. Subcritical water extraction at 200°C and 3.4 MPa. gave the relatively highest recovery with the off-odor of oil, whereas lower temperatures provided a higher quality of oil but with very low extraction rates (Eller and Taylor, 2004).

García-Marino *et al.* (2006) investigated the recoveries of catechins and proganthocyanidins from winery by-products using subcritical water extraction at 50 to 150°C for 30 min in comparison to 3 extractions of methanol/water (75/25) for 15 min each at atmospheric pressure. The total polyphenols obtained by subcritical water extraction at 150°C provided better recoveries of the flavanol dimers and trimers than those by the methanol/water extraction. In addition, the extracts by subcritical water extraction had higher antioxidant activites than those by the conventional extraction method.

Sereewatthanawut *et al.* (2007) compared the recovery of protein and amino acids by subcritical water extraction at various temperatures and times with those by the alkaline method at 30°C for 45 min. The protein content in the extract by subcritical water extraction at 160°C for 20 min or longer and at 220°C for more than 5 min was higher than that by alkaline extraction.

The recoveries of highly polar substances at a relatively high treatment temperature may be reduced due to a decrease in the dielectric constant of water and also the induced degradation of the solutes. In addition, a high recovery of non-polar substances, such as long chain alkanes, may not be obtained by subcritical water extraction because the relative permittivity of water is not low enough to extract non-polar substances (Kronholm *et al.*, 2007; Hageman *et al.*, 1996). Subcritical water extraction a suitable method for polar and apolar substances with a high extraction efficiency, rate and recovery in comparison with common solid-liquid extraction.

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