Subcritical Water for the Extraction of Flavonoids

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1. Introduction

Flavonoids are secondary plant metabolites in various medicinal plants, which occurs in free state (i.e., aglycone) and glycosides. The basic structure of flavonoids consists of a phenolic ring with 2-phenylbenzopyrone (flavone). Their derivatives differ in the substitutions, the number and position of hydroxyl and methoxy groups, and the number of sugar moieties in the molecules (Suntornsuk & Anurukvorakun, 2005). Moreover, flavonoids perform important pharmacological activities, such as antiallergy, anti-inflammatory (Xie, et al., 2012), anti-viral antimicrobial activities (Chirumbolo, 2010), anticancer (Chidambara Murthy et al, 2012; Xie et al., 2012), antimicrobial activities (Al-Oqail et al., 2012) and antioxidant (Haq Ihsan, et al, 2012; Schirrmacher, et al.,2007). However, the traditional extraction method of flavonoids from plant tissues has usually been accomplished using conventional extraction processes such as solid–liquid extraction employing methanol, ethanol, acetonitrile and acetone and also through steam distillation. Such sample preparation procedure is a considerable step. Especially, the methods for the determination of trace analysis in medicinal plants are usually various procedures. These procedures are disadvantaged by the consumption of organic solvents which leads too low to meet the challenges of modern environmental analysis. Whereas, supercritical water extraction (SWE) can play a significant role to overcome the drawbacks. Other common terms of subcritical water are hot water extraction, pressurized (hot) water extraction, high-temperature water extraction, superheated water extraction or hot liquid water extraction. In recent years, numerous papers have been published on the applicability of SWE for the extraction of bioactive compounds from plant. SWE is an environmentally kindly technique for the future based on using water as extraction solvent at temperatures between 100°C and 374°C (critical point of water, 374°C and 22 MPa at high pressure which is high enough to keep the water in the liquid solvent) (Ramos et al.,

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2012). Increasing the temperature at moderate pressure also reduces the surface tension and viscosity of water causes the subcritical water is comparable with organic solvents. Unlike, organic solvents, there is no environmental impact associated with water. The ultimate goal of this article is to explain the fundamental principles of the SWE and its application for the extraction of flavonoids. The main factors affecting the SWE efficiency such as temperatures, pressure, extraction time and flow rate will be discussed

Keywords: Subcritical water extraction, Flavonoids

2. Fundamental principles of SWE

2.1 Physicochemical properties of water

Water is highly polar solvent with a high dielectric constant (ε). Pure water at ambient temperature and pressure has ε = 79 due to the presence of hydrogen boned structure (Teo et al, 2010). Therefore, water is not considered as a suitable solvent for the extraction of non-polar compounds at ambient temperature. However, if the temperature of water is increased, its dielectric constant, viscosity and surface tension will be decreased. The dielectric constants of water will be decreased from 80 to 27 at the temperature of 25°C to 250°C, respectively with the pressure of 50 bar. The dielectric constants falls between those of methanol (ε = 33) and ethanol (ε = 24), the universal solvents for extracting of flavonoids (Teo et al, 2010). Therefore, the dissolving capacity of supercritical water is high, and solubility can be adjusted through changes in temperature and pressure (Yamini & Bahramifar, 2000). Moreover, the relative permittivity of water at room temperature is high (ca. 78.5), but it decreases with increasing temperature, providing non-polar compounds more soluble in water (Yoshii & Miura, 2001). The mass-transfer properties of subcritical water under sufficient pressure are enhanced because diffusion coefficients of solutes are higher than in water at STP, standard temperature and pressure (Kronholm, Hartonen & Riekkola, 2007). The reduced viscosity and the surface tension of subcritical water also allow more desirable mass transfer properties than in water at STP. While beyond a critical point of water, it will be associated in gas phase. Thus, sufficient pressure at a raised temperature could be applied to maintain water render as liquid phase (subcritical water). In summary, subcritical water behaves like organic solvent which is able to dissolve a wide range of medium and low polarity analytes (Ramos et al., 2012; Kronholm et al., 2007).

2.2 Extraction mechanism

The thermodynamic properties of water at both ambient and subcritical conditions are typically described in terms of hydrogen bonding strength and hydrogen bonding structure (Ramos et al., 2012; Kronholm et al., 2007). Hydrogen bonds in water are self associating, in that the strength of one hydrogen bond is governed by the presence of other hydrogen bonds around it. Nevertheless, change to one hydrogen bond affects the whole water volume. Changes in hydrogen bonding strength are reflected in the dielectric constant and heat of vaporization values (Carr et al, 2011). At lower temperatures, hydrogen bonds are stronger and the dielectric constant value is higher. As the temperature of the water is raised, the thermal agitation will be increased and causes the reduction of the strength of each hydrogen bond leading to an amplified reduction of dielectric constant (Caffarena & Grigera, 2004). Thus, the reduction in hydrogen bonding strength within the water molecules and the reduction in water polarity generally lead an increase in flavonoid solubility in the water.

2.3 Instrumentation

Most extractions have employed relatively simple homemade instrument. Typically, it consists of a water supply, a pump for transporting the solvent, a heater for heating solvent, a vessel where the extraction occurs. A general instrumentation can be set–up with the following laboratory assembled system as shown in Fig.1.

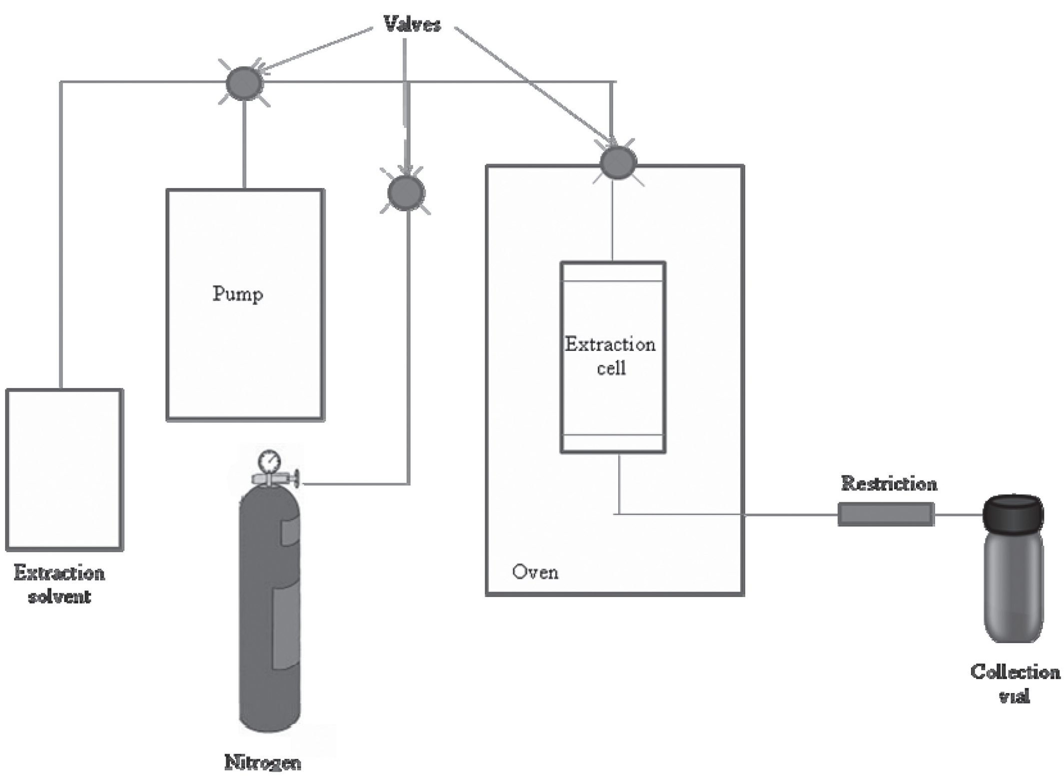
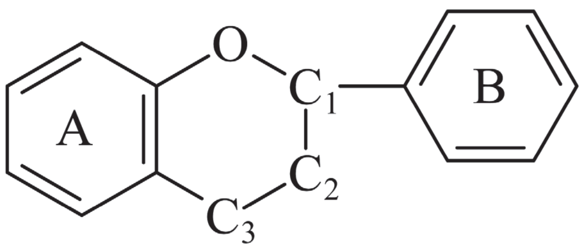


Fig.1 Schematic of the basic set-up for SWE. (Ramos et al., 2012).

2.4 Properties of flavonoids

Flavonoids are plant secondary metabolites, which occur in free states (aglycone) or as glycosides. Their structures are based on 2-phenylbenzopyrone (Fig.2), and differ in their degree of saturation and the position of hydroxyl, methoxyl and sugar residues (Fernandez et al, 1995)



2-phenylbenzopyrane

Fig.2 Skeleton structure of flavonoids

The polarity of flavonoid is of wide range. In general, they can be extracted from medicinal herbs with methanol or ethanol. The solubilities of flavonoids are strongly anion-dependent. H-bonding interaction is the most dominant interaction for a large variety of ionic liquids (ILs) to determine the solubility of flavonoids, and the anionic part has greater effect on the overall H-bonding capability of the IL. (Guo et al., 2007). As which SWE, offers the possibility to perform under an inert atmosphere and to protect active compounds from light. Flavonoids are very sensitive to these two factors. Flavonoids such as catechin and epicatechin react easily at high temperature when they are in contact with the air. However, when higher temperatures are applied under nitrogen atmosphere, there were no degradations, since the degradation process for phenolic compounds as flavonoids is an oxidative process requiring the presence of oxygen. Light can catalyze the reaction transforming the compound from the active to inactive form. Moreover, the short extraction times may reduce the adverse effect of enzyme activity (Palma, Piñeiro & Barroso, 2001). In order to optimize the extraction of flavonoids and its conjugates from natural products using subcritical water as solvent, it is important to measure their physicochemical properties, such as solubility of the quercetin compounds in water at different temperatures for process design purposes. Sriniva et al., (2010) reported the aqueous solubility of anhydrous quercetin varied from 0.00215 g/L at 25°C to 0.665 g/L at 140°C. For SWE of grape seeds, the results were shown that catechin increased by 30% and epicatechin increased by 44% from 50 to 100°C extraction temperature, catechin increased by 32% and epicatechin increased by 99% over the recovery from the 50°C extraction (Palma et al., 2001).

3. Factor affecting the extraction efficiency in SWE

The main factors affecting SWE are temperature and dynamic mode (pressure, extraction time and flow rate).

3.1 Temperature

Temperature is the main factor affecting the extraction capability and selectivity. The applied temperature is usually above the normal boiling point of the water. The higher temperatures have also changed the properties of water. This will enhance the solubility of less polar compounds. Polyphenols, flavonoids and antioxidant activity were achieved using subcritical water extraction compared to the conventional methods, the optimum temperature was at 140°C (Aliakbarian, Fathi et al., 2012). The maximum yields of flavanones including hesperidin and narirutin from *Citrus unshicu* were achieved by SEW at the temperature of 160°C (Cheigh et al., 2012). However, degradation of compounds could be occurred with increased temperature. The extraction of carotenoids could be achieved with ethanol at 80°C for 2 hr. While that of flavonoid could be effectively carried out with SEW at 120°C for 10 min, since the degradation of carotenoids occurred at a high temperature (Prommuak et al, 2008).

3.2 Dynamic mode

Dynamic modes for SWE are pressure, duration and flow rate. For SWE, the water is driven through a narrow sample cell at high pressure which generally enhances the extraction. Nevertheless, prolonged heated may cause compound degradation. Thus, optimization of extraction time is extremely significant. The effect of extraction time was shown in SWE of onion skin which was SEW were run between 5 and 30 min at temperatures of 165°C and 170°C. The total amount of quercetin decreased with increasing extraction time, especially when the duration exceeded 15 min. The results obtained from the SEW obtained at 165°C and 170°C for 15 min were similar (Ko et al., 2011) compared with the 3–24 hr. conventional quercetin extraction (Kang et al., 1998b). Besides, the flow rate of about 1 mL/min is typically applied in dynamic mode for the SWE. A higher flow rate could generally improve extraction potential because the total volume of water is increased. However, the extraction time and flow rate in SWE require the optimization to complete the extraction.

4. Application of SWE on flavonoid extraction

As subcritical water acts in a similar way to hydrophobic organic solvents (subcritical water), it has been demonstrated to be an effective, environmentally friendly solvent for the extraction of flavonoids. A number of SWE have been applied successfully on flavonoid extraction as summarized in Table 1.

5. Future trend

Future trend for SWE, an environmentally friendly extraction technique with little disposal issue, is towards scaled –up operation to handle large volume of sample for industrial-scale. Factor affecting the extraction efficiency are usually monitored (Teo et al.,2010). However, further studies are to evaluate the commercial applicability of SWE of flavonoids to elucidate the relationship between the SWE efficiency and SWE mechanism based on the chemical structure of flavonoids. The potential application is an attractive option for the extraction of active components from medicinal plants which could be lead compounds for drug discovery purposes. In particular for Thailand which has been rich in biodiversity, SWE could be applied to the extraction of

Table 1 Conditions of SWE for the extraction of flavonoids.

Analyte	Temperature (°C)	Pressure	Flow-rate (mL/min)	Extraction time (min)	Reference
Total flavonoids from winery waste	140	11.6 MPa	1	100	Aliakbarian et al., 2012
Total flavonoids from silk waste	120	0.47-4.47 bar	-	10	Prommuak et al., 2008
Quercetin from onion skin	165	90-131 bar	1	15	Ko, et al., 2011
Flavones hesperidin and narirutin from *Citrus unshiu*	160	100±atm	1	10	Cheigh et al., 2012
Phenolic compound from *Momordica charantia*	200	10MPa	2	320	Budrat & Shotipruk, 2009
Flavonoids from Knotwood of aspen	150	220 bar	-	35	Hartonen et al., 2007
Total phenolic compounds from oregano	200	-	-	30	Rodríguez et al., 2006
Total flavonoids	200	1500 psi	-	15	Kumar et al., 2011
Phenolic compounds from potato peel	180	6 MPa	-	60	Singh & Saldaña, 2011
Phenolic compounds from pomegranate (*Punica granatum L.*)	220	6MPa	-	30	He et al., 2012

Reference

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