Extraction of Phenolic Compounds using Subcritical Hot Water Extraction: A Review

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Abstract. Subcritical hot water extraction (SHWE) is a green technique for extraction of the bioactive compound using water in the subcritical state. This condition occurs when the temperature and pressure are changing between the boiling point and critical point, increasing its solubility on the low and middle polar compounds. It is considered as an environmentally friendly solvent, and many publications have revealed the advantages of using this technique for the extraction of phenolic compounds. The main aim of this review is to provide a brief description of the SHWE application on the extraction of phenolic compounds for the last two years (2018-2019). By optimizing the main parameter of extraction such as extraction time and temperature, the desirable final product could be obtained.

1. Introduction
Extraction is one of the separation techniques which aim to recover bioactive compounds in food materials. High interest is focused on plant-based materials which are proven to be rich in source of phytochemicals such as polyphenols, polysaccharides, essential oil, carotenoids, and sterols [1]. The consumer goods industries use these compounds as ingredients for various purposes [2]. In most cases, the common traditional method of extraction were used to separate and isolate the bioactive compounds, such as extraction using steam, maceration, and Soxhlet extraction [3]. The chemical treatment is then applied to isolate the desired compound in the pure form [4, 5]. However, the separation using conventional methods have several disadvantages. It usually involves the use of large volumes of hazardous solvents, labor and time-consuming, degradation of heat-sensitive components, generate large quantities of waste, and may retain some residual solvents in the product [3, 6]. Thus, over the last few decades, the use of environmental-friendly solvents in food and pharmaceutical industries were considered as the best way for the implementation of the more effective and efficient extraction in the processing of food and less detriment effect to the solutes [1, 4, 7].

Water is known as a ubiquitous solvent because it is cheap, capable of dissolving a variety of different substances. It is considered as the greenest solvent because it is non-toxic, non-flammable, non-corrosive, abundant availability, and nearly negligible environmental impact [5, 8]. Because of tunable properties capability by changing the temperature, many types of research using subcritical hot water extraction (SHWE) for the extraction process. SHWE (Figure 1) is a green extraction technique that gains popularity in recent years for extracting bioactive compounds from natural sources. SHWE utilizes heated water between 100 °C (boiling point) and 374 °C (critical point) under pressurized condition. The extraction using this method is no need to pretreat and dry the raw material, therefore minimize the impact to the environment caused by the extraction process [5].
One of the major classes of phytochemicals that are commonly found in the plant kingdom is phenolic compounds. These compounds comprise several classes, which are produced through the combination reaction of several biochemical pathways, creating different chemical constituents [9,10]. These constituents linked to the plant matrix such as proteins, cell wall, and carbohydrate and the plentiful numbers of these compounds occur naturally, either in their monomeric (sugar-bounded tannin) or polymeric structures (e.g., lignins, brown algae tannins, condensed tannin) [9]. The words “polyphenols” and “plant phenolics” are commonly used and considered by many researchers to have the same meaning. However, the compounds derived from phenylpropanoid with more than one phenolic ring should be categorized as polyphenols. If a compound has only one phenolic ring (e.g., phenolic acids), then it should be considered as a phenolic compound [9]. The compound of phenolic and polyphenol that become the primary interest of the researches (e.g., phenolic acid, flavonoids, lignans, and stilbenes) has been linked with health-improving outcomes, acceptable organoleptic parameters, and proper nutrition quality [11]. This paper aims to provide general information on the fundamental of SHWE and its applications to extract bioactive compounds, mainly focus on phenolic compounds.

![Figure 1. Subcritical hot water extraction design](image)

2. Subcritical Hot Water: Fundamentals

Water at first has not yet received much attention as an analytical extraction solvent because of high polarity. Most organic components are undissolved due to a high dielectric constant at room temperature and atmospheric pressure [12, 13]. However, the perception has changed since the work of Hawthorne et al. [14] reported for the first time on the application of pressurized water as an extraction fluid at elevated temperatures effectively extracting the polar and non-polar analytes. The liquid subcritical water is produced through the combination of proper temperature and pressure (temperature between 100 to 374°C and pressure between 1 to 218 atm) [15]. The efficiency of subcritical water extraction and the physicochemical properties of the samples are not affected by the pressure use in the system [13].

In a subcritical state, the water can penetrate the sample matrix very well due to the improvement of diffusivity of water, continuously decline of viscosity, relative permittivity (ε), and surface tension [13]. The dielectric constant (as a measure of the polarity of the solvent) is a critical parameter in determining solute-solvent interactions. Water is a widespread solvent and naturally abundant with high dielectric constant (ε = 78) at ambient temperature and atmospheric pressure. But, when the water is heated up to 300°C and pressure is increased up to 23 MPa, the dielectric constant value is reduced (ε = 21). This number is comparable to the value of other polar protic and aprotic solvents like ethanol (ε = 24) and acetone (ε = 20.7). Thus, under this condition, it has several properties resembling the organic solvents, which is suitable for extracting low and medium-polarity compounds [12, 14].
Generally, SHWE process can be divided into two modes: dynamic mode and static mode. In order to achieve effective extraction in the dynamic mode, the time of extraction and speed rate of water that flows through the system must be controlled. For instance, the flow rate of water is managed at 1-1.5 ml/min, which generally hikes the extraction until it reaches the equilibrium condition. This condition is preferable for the extraction process because it can consistently extract and enhance the physical mass transfer of the target compounds from the high concentration of samples [16]. Static extraction mode, in contrast to dynamic mode, the sample is extracted by the water in batch conditions. There is no addition of water to the system during the process until it reaches the equilibrium [5]. Based on this reason, the extraction process could run well if the time of extraction, temperatures, and partition-equilibrium constant are taken into consideration to avoid detriment of target compounds [17].

3. Subcritical Hot Water Extraction (SHWE) of Phenolic Compounds

The diverse groups of phenolic compounds are characterized by their chemical structures, from low molecular weight phenol to the polymeric phenol. For a long time, the beneficial health of these compounds has been connected to the prevention of degenerative maladies associated with oxidative stress [10]. Its application in food preservation has been described to inhibit several pathogenic microorganisms [18]. Because the bioavailabilities of phenolic compounds are related to their chemical structure, many types of research are focused on the optimization of extraction conditions [19]. From 2018 till present, numerous researchers published the application of SHWE for obtaining the target compounds from diverse sources (Table 1).

| Materials | Bioactive compound (Method of analysis) | Modes | Temperature | Pressure | Time/Flow Rate | References |
|-----------|----------------------------------------|-------|-------------|----------|----------------|------------|
| Moringa (<i>Moringa oleifera</i> Lam.) | Vitamin C, quercetin, and kaempferol (UHPLC-DAD-qTOF-MS) | Dynamic | 50–200 °C | - | 5-60 min 1.0 mL/min | [20] |
| Pacific oyster (<i>Crassostrea gigas</i>Thunberg) | Total phenolic content Total antioxidant activity (ABTS, DPPH, FRAP) Antihypertensive activity | Dynamic | 125–275°C | 2-14 MPa | 5 min | [21] |
| Black tea (<i>Camelia sinensis</i>) | Xanthines, Flavanols (HPLC) | Dynamic | 90–160 °C | 5 MPa | 15-60 min 9-18 mL/min | [22] |
| Green coffee beans (<i>Coffea arabica</i>) | Total Antioxidant activity (DPPH, ABTS,HPLC) | Static | 40–200 °C | 3.45–17.24 MPa | 2–18 min | [23] |
| Walnut (<i>Juglans regia</i> L.) | Total Antioxidant activity (DPPH, ABTS,HPLC) | Static | 36.4–103.6 °C | 1.5-2.34 MPa | 15-28.409 min | [24] |
| Stevia (<i>Stevia rebaudiana</i> Bertoni) | Total phenolic content (Folin–Ciocalteu) | Static | 100-160 °C | 10.34 MPa | 5-10 min | [25] |
| Pomegranate peels (<i>Punica granatum</i> L.) | Composition of Phenolic compounds (HPLC) | Static | 50–100 °C | 10 MPa | - | [26] |
### Materials

| Bioactive compound (Method of analysis) | Modes | Temperature | Pressure | Time/Flow Rate | References |
|----------------------------------------|-------|-------------|----------|----------------|------------|
| Guarana seed (*Paullinia cupana* Kunth.) | Total phenolic content | Static | 40–60 °C | 10 MPa | 10-60 min | [27] |
| | Total antioxidant activity (DPPH, FRAP) | | | | | |
| Onion waste skin (*Allium cepa* L.) | Antioxidant capacity (DPPH) | Static | 170–230 °C | 3.0 MPa | 30 min | [28] |
| | Total phenolic content (FC) | | | | | |
| | Flavonoids (HPLC-DAD) | | | | | |
| | Flavonoid content (ACM) | | | | | |
| Purple coneflower (*Echinacea purpurea* L.) | Total flavonoid content (ACM) | Static | 147 °C | 3.0 MPa | 8.4 min | [29] |
| | Total phenolic content (FC) | | | | | |

ABTS: 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); ACM: aluminium chloride method; DAD: diode-array detector; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FC: Folin-Ciocalteau; FRAP: fluorescence recovery after photobleaching; HPLC: high performance liquid chromatography; qTOF-MS: quadrupole time-of-flight mass spectrometry; UHPLC: ultra-high-performance liquid chromatography

Table 1 describes the researcher’s interest by utilizing SHWE to extract target interest compounds and maximize the process [22-29]. Generally, the optimization was conducted on extraction temperature, time, and flow rate. The temperatures range of 40-275 °C and extraction time range of 5-60 minutes were applied to extract the phenolic contents. The yield of SHWE are comparable to conventional methods (steam distillation, Soxhlet extraction, sono-extraction and heat extraction with alcohol 96% or below) with the resulting tendency of SHWE are higher [13,33]. However, it should be considered that the use of high temperature may exhibit an adverse effect on the phenolic contents. The increasing of solubility of heat-stable phenolic compounds in higher temperature may affect the other heat-sensitive compounds and trigger unwanted chemical reactions such as Maillard reaction and caramelization [30]. Besides, some studies revealed the decrease of phenolic compounds using extraction temperature above 180 °C due to the degradation of some phenolic compounds [31,32]. Therefore, to improve the solubility and effective mass transfer and to prevent the unwanted degradation and chemical reactions, the suitable extraction temperature must be achieved [5].

### 4. Conclusion

Subcritical hot water extraction (SHWE) is a promising green technology that currently attracts researchers and industries for extracting phytochemicals such as phenolic compounds. This technique utilizes water in subcritical conditions, which are safe, organic solvent-free, and environmentally friendly. It assists fastidious separation, resulting high amount of and high purity of target compounds. For industrial application, optimized SHWE conditions could be considered as the solution technique for handling larger sample sizes. Nevertheless, it should be noted that the degradation of phenolic compounds and unwanted chemical reactions may occur during the extraction. Further investigations on the optimization of the extraction process must be continued to achieve all desirable parameters.
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