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청정생산공정기술 보 문

Effect of Subcritical Water Extraction on the Recovery of Bioactive Compounds from *Allium hookeri* Root using Severity Factors

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Abstract

Plant polyphenols have attracted attention recently because of their abundance in the human diet, high antioxidant effects, and ability to prevent various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases. Therefore, this study demonstrated the extraction of bioactive phenolic compounds from *Allium hoorkei* root (AHR) using subcritical water extraction (scWE) under various temperatures (120 ~ 160 °C) and times (30 ~ 180 min) at a fixed pressure (10 MPa) and AHR to water ratio (1:20, w/w). Furthermore, this study used severity factors, the combined effect of the temperature and time, in order to optimize the conditions for achieving a high yield and efficient recovery of target bioactive phenolic compounds while minimizing the degradation of the extracted products and maintaining a high selectivity. Subcritical water extraction at 160 °C for 30 min (severity of 3.24) produced a relatively high yield (88%) and high number of bioactive compounds including total phenolic contents (31.3 mg GAE/g dry AHR) and rare sugars (D-picose, D-talose, and D-tagatose) compared to Soxhlet extracts obtained from extraction for 8 h with water and 75% ethanol. As a result, the extracts obtained from the green scWE process may have high potential applications in medicines and functional foods because of their high bioactivity and safety.

Keywords : *Allium hookeri* root, Subcritical water, Bioactive compounds, Severity

1. Introduction

Owing to the ever-growing demand for novel natural drugs, the development of new bioactive compounds from unexplored herbal/medicinal plants is becoming increasingly important. As a member of the *Alliaceae* family, *Allium hookeri* root (AHR) has attracted great attention in recent years because of the medicinal and nutritional effects associated with its rich content of nutrients such as sugars, proteins, vitamin C, trace minerals (mainly K), phytosterols, as well as organosulfur and phenolic compounds with potent bioactivity[1-3]. In addition, AHR has been demonstrated to have beneficial effects in forming healthy bones[4], suppressing diseases related to intestinal immune responses[5], and inhibiting melanogenesis[6].

On the other hand, with the awareness of growing environmental pollution originated by the extensive use of volatile and hazardous organic solvents, the exploration of alternative green and sustainable solvents has been priority in both the research community and the chemical industry[7]. In this context, water became a highly favorable candidate due to its non-toxic nature and minimal impact on both human health and the environment[8]. Additionally, water is notable as a universal solvent because it can serve as a not only polar solvent but also self-neutralizing acid-base catalyst through experimental conditions of temperature and pressure variation[9]. For example, in subcritical water region, *i.e.*, water at above its boiling point temperature (100 °C at 0.1 MPa) but below the critical point of water (374 °C, 22.1 MPa) and pressure high enough to maintain the condensed state is used as the extractant, water ionization constant value is three-order of magnitude higher than typical value of normal conditions[10]. Under these conditions, polarity of water decreases with increasing temperature, and consequently its solubility can be tuned to solvate a wide

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range of analysts, including polar, moderately polar, and non-polar compounds. Moreover, as the extraction temperature increases, the extraction rate and solubility increase by decreasing surface tension and viscosity, and thereby increasing the self-diffusivity of water[11]. Based on these advantages, the use of subcritical water extraction (scWE) (or hot compressed water extraction (PHWE)) as a green extraction technique in its application to the extraction of bioactive compounds from natural sources have grown exponentially in a decade[12]. Although extraction yields often increase at high extraction temperatures, so does the risk of degrading the highly thermal-sensitive bioactive biomolecules under harsh conditions. In this respect, extraction temperature and time are critical parameters that affect the efficiency and selectiveness of scWE. Hence, Overend and Chornet[13] initially defined a severity factor (R_0) for modelling combined effect of temperature and time for steam explosion pretreatment based on the assumption that pretreatment affects follow first-order kinetics and obey the Arrhenius equation and expressed as follow:

$$R_0 = t \times \exp\left[\frac{T - T_{\text{ref}}}{14.75}\right] \quad (1)$$

where t is the reaction time (min), T is the reaction temperature (°C), T_{ref} is the reference (base) temperature (100 °C), and 14.75 is the activation energy constant when assuming the overall hydrolysis reaction is first order.

Since that time, numerous reports have demonstrated the effects of severities (the logarithmic values of R_0, Log R_0) on the efficiencies of various pretreatment and fractionation strategies on lignocellulosic feedstocks[14-16]. However, the efficacy of scWE on the yields and recovery of bioactive compounds from herbal/medicinal plants using the severity factors is rarely reported. Therefore, this study attempted to suggest severity factors for achieving high yield and efficient recovery of bioactive molecules (i.e., phenolic compounds, carbohydrates) from the AHR using scWE technique. Besides, Soxhlet extraction of the AHR powder with various organic solvents (water and aqueous ethanol) were performed as control samples to assess the performance of scWE on the AHR.

2. Material and methods

2.1 Raw materials and chemicals

AHR was purchased from a local market in Gwanak-Gu, Seoul, and thoroughly washed with tap and deionized (DI) water. The washed AHR was dried in a hot air oven at 70 °C for 12 h. The dried AHR was pulverized using an RT-08 grinder (Mill Powder Tech Co., Ltd., Taiwan) and then sieved using a vibratory J-VSS sieve shaker (Jisico Co., Ltd., South Korea) to obtain a fine powder size ranged between 125 and 250 μm. All chemicals and regents used in this study were of analytical or HPLC grade. DI water was prepared using a Milli-Q water-purification system (Millipore, USA).

2.2 Extraction methods

The scWE of AHR powder was performed in custom-built batch reactors, which were fabricated of stainless steel (SUS 316) and had a total inner volume of 23 mL. Approximately 1.0 g of AHR powder and DI water were loaded in the reactor with a solid to water ratio of 1:20 (w/w) and subsequently sonicated using a Powersonic 410 bench-top ultrasonicator (Hwashin Technology Co., South Korea) for 10 min at 25 °C to degas the reactor and thoroughly disperse the particles in the water phase. The reactor was then tightly capped and immersed in the oil bath, which was set to the desired temperature for the experiment. During the scWE, the entire reactor was shaken horizontally. After the scWE, the reactor was removed from the oil bath and immediately immersed in a cold-water bath for quenching. The reaction mixture was separated into liquid extract and solid residue by filtration using Whatman qualitative filter paper No. 1 (110 mm Ø) under vacuum. The liquid extract was then filtered using sterilized Millex-HV syringe filter units (0.45 μm pore size, Merck, Germany) and stored in a freezer at −20 °C until analysis.

Additionally, Soxhlet extractions were carried out for 8 h using DI water and aqueous ethanol (75% Ethanol) for benchmarks to evaluate the performance of scWE on the AHR powder. The ratio of AHR powder to solvent was 1:200 (w/v). After the Soxhlet extraction, each extracted solution was concentrated to a volume of approximately 40 mL using a Büchi Rotavapor R-200 rotary evaporator system (Büchi Labortechnik AG, Switzerland) under vacuum. The concentrated Soxhlet-extracted solution was cooled to room temperature (25 ± 1 °C) and then filtered using sterilized Millex-HV syringe filter units. The filtered Soxhlet extracts were stored in a freezer at –20 °C until analysis.

2.3 Determination of the extraction yield

The solid residue left after scWE or Soxhlet extraction was dried in a vacuum oven at 70 °C for 12 h. Afterwards, the oven dried solid residue was cooled in a desiccator and weighed. Drying, cooling, and weighing were repeated until a constant weight was observed. The yield of solid residue was calculated using the following equation:

$$\textit{Yield of solid residue}\ (\%) = W_{\text{SR}}/W_{\text{feed}} \times 100 \quad (2)$$

where W_{SR} is the dry weight of the solid residue (g), and W_{feed} is the

dry weight of the AHR powder loaded in the extractor (g).

The yield of the liquid extract (%) was calculated by subtracting the solid residue yield from 100%.

2.4 Characterization of the AHR extracts

2.4.1 pH determination

The pH values of the liquid extracts produced from the scWE and Soxhlet extraction on the AHR powder were measured using a portable HI 9024 pH meter (HANNA Instruments, Germany) at room temperature (25 ± 1 °C).

2.4.2 Total phenolic content (TPC) determination

The TPC of the liquid extracts produced from the scWE and Soxhlet extraction on the AHR powder were determined using the Folin-Ciocalteu method as described by Cao et al.[11]. Briefly, an aliquot of the liquid extract (40 μL) was reacted with 200 μL of Folin-Ciocalteu reagent and 1,160 mL of DI water for 5 min at 25 ± 1.0 °C. Subsequently, 20% Na_2CO_3 solution (600 μL) was added to the mixture of the liquid extract, Folin-Ciocalteu reagent, and DI water. After 2 h of incubation at 25 ± 1.0 °C in a dark environment, the absorbance of the mixture at 720 nm was recorded using an Evaluation 201 UV-Vis spectrophotometer (Thermo Scientific Inc., USA) against a blank containing only DI water. The TPC of the extracts was calculated using a standard gallic acid concentration curve ($y = 0.0025x - 0.0018$, $R^2 = 0.9998$, 0 ~ 500 μg mL^{-1}), and the results were expressed as mg of gallic acid equivalent (GAE) per g of dry AHR powder (mg GAE/g dry AHR).

2.4.3 Gas chromatography-mass spectroscopy (GC-MS) analysis

To determine the compositions of chemical species in the liquid extracts obtained from the scWE and Soxhlet extraction, GC-MS analysis of the AHR extracts was performed using an Agilent Technologies 7890A GC equipped with a 30 m × 0.25 mm × 0.25 μm DB-5 fused silica capillary column (J&W Scientific, Folsom, USA) and a mass spectrophotometer 5975 (Agilent Technologies, USA). Ultrahigh purity helium (99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The injector temperature was maintained at 280 °C, and the injection volume was 1.0 μL in the splitless mode. The oven temperature was programmed to hold at 80 °C for 1 min initially, then increase to 220 °C at a rate of 10 °C min^{-1}, from 220 to 310 °C at a rate of 20 °C min^{-1}, and then hold at 310 °C for 6 min. The total run time was 27.5 min. The temperatures of the MS ion source and transfer line were held at 230 °C and 280 °C, respectively. Mass spectra were obtained with a scan range of *m/z* 50 ~ 650 and an electron impact ionization energy of 70 eV. Prior to the GC-MS analysis of the AHR powder, 1.0 mg of the freeze-dried powder sample was derivatized by adding 100 μL of the derivatization reagent BSTFA + 1% TMCS at 70 °C for 4 h. After the derivatization, 15 μg mL^{-1} of methyl heptadecanoate in *n*-heptane was added to each sample as an internal standard. The spectra of the components were identified based on their retention times and comparison of their mass spectra with the database of spectra of known compounds stored in the GC-MS library W9N11. The percent composition of the constituents of the extracts was expressed as the percentage of total peak area.

3. Results and discussion

3.1 Evaluation of scWE performance on the AHR powder using severity factors

To elucidate the effect of the severity of scWE on the efficiency of extraction and recovery of bioactive phenolic compounds from the AHR powder, a wide range of extraction temperatures (120 ~ 160 °C) and residence times (30 ~ 180 min) were performed at a fixed pressure (10 MPa) and AHR powder to water ratio (1:20, w/v). The details of scWE experimental conditions and values of severity (Log R_0) that related to the combined variables of extraction temperature and time are also presented in Table 1. Figure 1 displays that the yields, TPC and pH values of liquid extracts were strongly dependent on the severity of the scWE and fit well with third-order polynomial models with R^2 values of 0.9453, 0.9881, and 0.9846, respectively. The yield of liquid extract increased from 65% to 88% as the severity increased from 1.69 to 3.24. The increased tendency of extraction yield was due to the increased extraction of depolymerized polysaccharides species as the severity increases, and thereby facilitating to enhance solubilization of wider range of organic compounds in the subcritical water reaction environment. In contrast, further increase of the severity beyond this value, the extraction yields gradually decline and resulted in 69% at Log R_0 value of 4.02, which corresponds to the scWE at the highest temperature and longer time (160 °C and 180 min). This was probably due to the decomposition of thermally labile compounds in subcritical water under these conditions, resulting in the production of highly volatile, low-molecular-weight species and/or gaseous products. A similar trend has been observed in previous studies[17,18]. On the other hand, the yields of AHR extracts obtained via 8 h of Soxhlet extraction using water (denoted as H_2O-8), and 75% ethanol (denoted as $EtOH_{75}$-8) were approximately 80% and 24%, respectively, which were 1.1-to 3.6-fold lower than the maximum scWE yield at the severity of 3.24, corresponding to scWE at 160 °C and 30 min. Thus, considering as a promising alternative to high-cost and energy-intensive

Table 1. Experimental conditions related to severity factors of subcritical water extraction (scWE) of the AHR powder

Entry	AHR powder to water ratio (w/w)	Pressure (MPa)	Temperature (°C)	Residence time (min)	Severity factor (Log R_0)
1	1: 20	10	120	10	1.59
2	1: 20	10	120	30	2.07
3	1: 20	10	120	60	2.37
4	1: 20	10	120	90	2.54
5	1: 20	10	120	120	2.67
6	1: 20	10	120	180	2.84
7	1: 20	10	140	10	2.18
8	1: 20	10	140	30	2.65
9	1: 20	10	140	60	2.96
10	1: 20	10	140	90	3.13
11	1: 20	10	140	120	3.26
12	1: 20	10	140	180	3.43
13	1: 20	10	160	10	2.77
14	1: 20	10	160	30	3.24
15	1: 20	10	160	60	3.54
16	1: 20	10	160	90	3.72
17	1: 20	10	160	120	3.85
18	1: 20	10	160	180	4.02

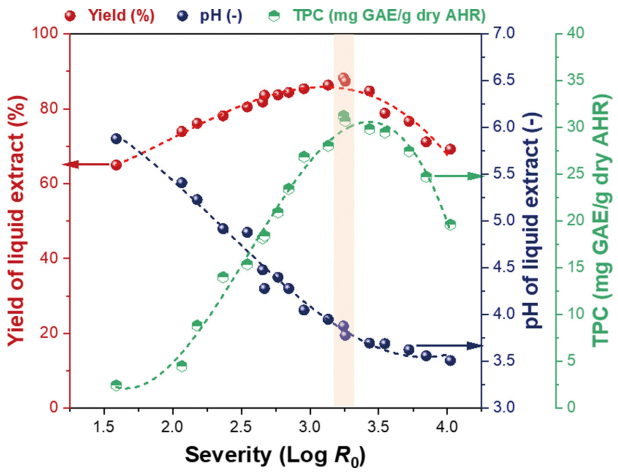

Figure 1. Yield, pH values and total phenolic content (TPC) of the liquid extracts obtained from the subcritical water extraction (scWE) process with respect to the severity of the process.

conventional Soxhlet extraction, scWE is highly efficient for the high-yield recovery of hydrolysates from the AHR with the need of a small amount of water and short extraction time (within 30 min), thereby demonstrating its economic viability and scalability for large-scale production with environmentally friendly aspects.

Furthermore, TPC of the scWE extract significantly increased when increasing the severity from 1.59 to around 3.43 via its fitting curve in Figure 1, and then that decreased accordingly as the severity increased up to 4.02. As the experimental results, an AHR extract produced from the scWE at 160 °C and 30 min (Log R_0 = 3.24) possessed the maximum contents of TPC (approximately 31.3 mg GAE/g dry AHR); this value was 10.1- and 13.0-fold higher than that of the H_2O-8 and $EtOH_{75}$-8 extracts (*i.e.*, 3.1 mg GAE/g dry AHR and 2.4 mg GAE/g AHR, respectively). The high recovery of TPC in the extract achieved by the scWE at the severity ranges 3.42 ~ 3.43 could be due to the high solubility potential of target phenolic compounds in subcritical water through favorable transport properties under the examined conditions; the high diffusivity and low surface tension of subcritical water can penetrate quickly and deeply into the AHR matrix and thereby facilitating to transport solubilized phenolic compounds from the matrix to the solution phase[19,20]. In controversy, the high severity of scWE (Log $R_0 \geq 4.54$, corresponding to the scWE at 160 °C for longer time of ≥ 60 min) resulted in decreased TPC in the extracts; this can be attributed to the degradation of thermolabile phenolic compounds through thermo-oxidation and/or hydrolysis, which agreed with previous works[11,21]. Moreover, Plaza et al.[22] described the formation of new types of bioactive compounds (neoantioxidants) in natural sources during scWE through Maillard, caramelization, and thermo-oxidation; particularly formed at high temperature (200 °C). Nevertheless, the extent of newly formed Maillard reaction (non-enzymatic browning reaction) products highly relies on the nature of chemical species

in the natural source, analysts formed in the reaction system during the scWE, and experimental conditions employed for the scWE. In this respect, pH values have been used as an indicator to quickly monitor the extent of Maillard reaction in the thermal processes, including scWE[20,22,23]. As depicted in Figure 1, the pH values of scWE extracts continuously decreased from 5.9 to 3.6 with increasing the severity of the scWE conditions while these of the Soxhlet H_2O-8 and $EtOH_{75}$-8 extracts were 5.5 and 4.8, respectively. Interestingly, the degree of decreasing trend in the pH values of the scWE extracts with increasing severities (especially from 1.59 to 3.24) was quite similar to the increasing trend of their respective TPC values, which was consistent well with previous reports. For instance, the enhanced extraction of various kinds of acidic compounds (*e.g.*, phenolic acids) at 200 °C was suggested to be responsible for the decrease in pH[22]. This was also attributed to the loss of amino groups as a consequence of the increased rate of Maillard reaction, which was based on the observation of similar behavior in a model glycation system[23]. Similarly, Vhangani et al.[24] reported that the browning index of the Maillard reaction products in their model systems (fructose-lysine and ribose-lysine) coincided with a reduction in the pH and proposed that the decrease in pH during the Maillard reaction was caused by the formation of acetic and formic acids from glucose.

3.2 Characteristics of chemical compounds in the AHR extracts

To gain a better understanding of the degree of severity of the scWE on the quality and characteristics of bioactive compounds in the AHR extracts, GC-MS analysis was conducted for three scWE extracts obtained at 140 °C and 30 min (Log R_0 = 2.65), and at 160 °C and 30 min and 180 min (Log R_0 = 3.24 and 4.02, respectively). The GC-MS analysis of the two Soxhlet extracts (H_2O-8 and $EtOH_{75}$-8) was also conducted to assess the performance of scWE on the AHR powder. The GC-MS chromatograms, chemical compositions (percent area), and classes of compounds identified in the various extracts are shown in Figures 2 ~ 4, respectively. Notably, all the selected scWE extracts possessed relatively high amounts of monosaccharides and disaccharides isomers (26 ~ 82%) such as β-D-galactofuranose, D-lyxopyranose, D-lyxofuranose, 2-α-mannobiose, D-arabiofuranose, D-frutose, D-lyxofuranose, D-piscofuranose, D-piscopyranose, D-ribose, D-tagatose, D-Talopyranose, D-xylopyranose, glucofuranose, glucopyranose, maltose, and α-L-fucopyranose as compared to the Soxhlet extracts, which are shown in Figures 2, 3 and 4c. The Soxhlet H_2O-8 extract is primarily composed of D-fructofuranose (13.57%) and sucrose (6.27%) while the D-lyxofuranose (3.77%), D-frutofuranose (5.34%), D-galactose (2.19%) and sucrose (1.70%) were found in

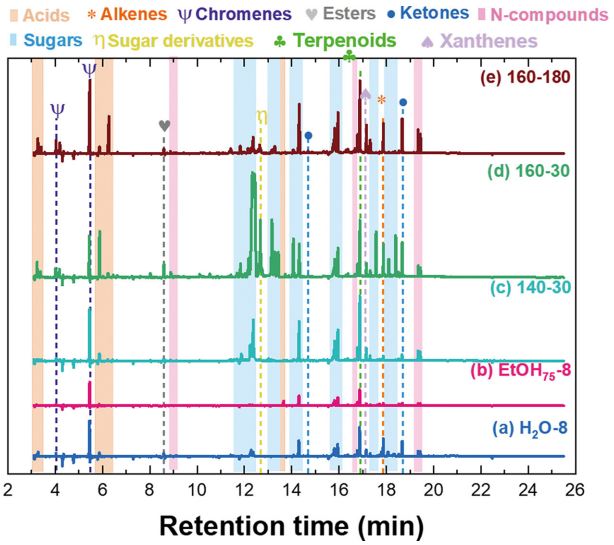

Figure 2. GC-MS chromatograms of selected AHR extracts produced using subcritical water extraction (scWE) and Soxhlet extraction. H_2O-8 is Soxhlext extraction with H_2O for 8 h, $EtOH_{75}$-8 is Soxhlext extraction with 75% EtOH for 8 h, and 140-30, 160-30 and 160-180 are scWE extraction at 140 °C and 30 m, 160 °C and 30 m, and 160 °C and 180 m, respectively.

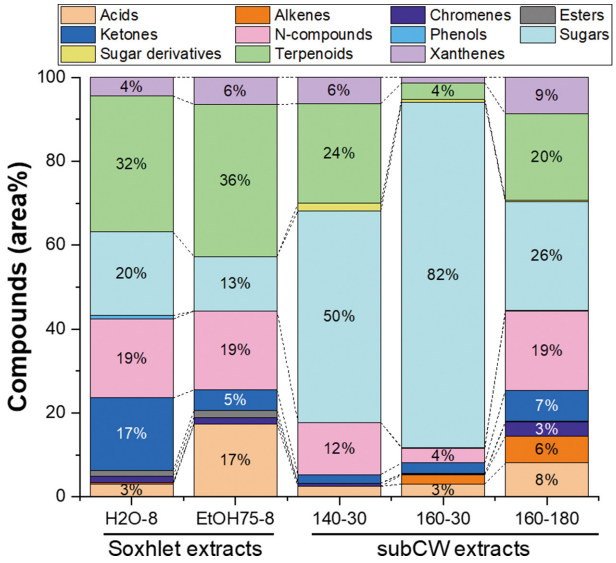

Figure 3. Composition of chemical groups found in selected AHR extracts produced using subcritical water extraction (scWE) and Soxhlet extraction. H_2O-8 is Soxhlext extraction with H_2O for 8 h, $EtOH_{75}$-8 is Soxhlext extraction with 75% EtOH for 8 h, and 140-30, 160-30 and 160-180 are scWE extraction at 140 °C and 30 m, 160 °C and 30 m, and 160 °C and 180 m, respectively.

the Soxhlet $EtOH_{75}$-8 extract, Figure 4c. The scWE extracts also contained sugar derivatives and others molecules including allonic acid, γ-lactone arabinoic acid, arabinonic acid, D-galacturonic acid, D-glucitol, galactofuranoside, γ-lactone xylonic acid, β-DL-arabinofuranoside, 4-hydroxyphenylethanol, 1H-indol-2-carboxylic

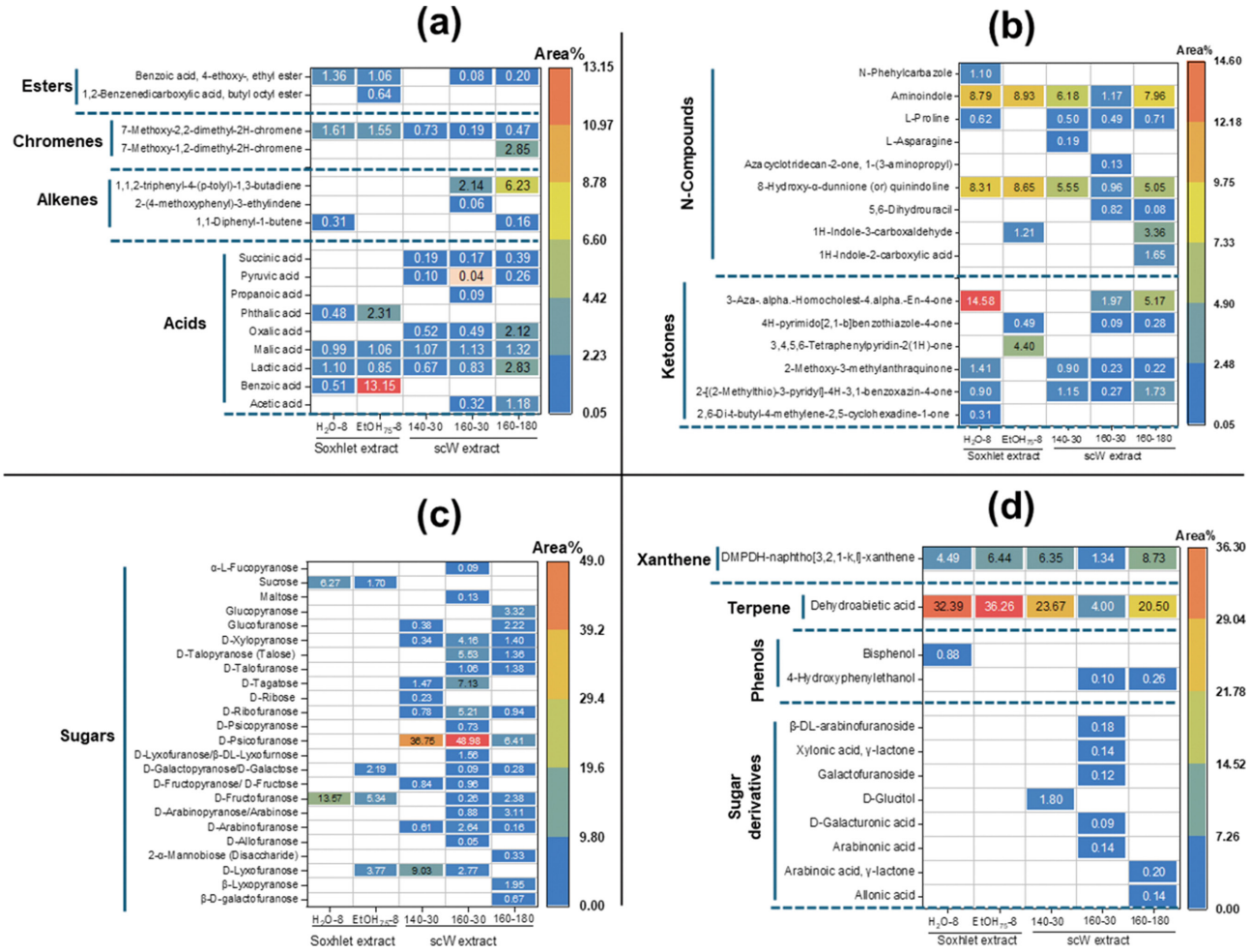

Figure 4. Individual composition of chemicals found in selected AHR extracts produced using subcritical water extraction (scWE) and Soxhlet extraction. H_2O-8 is Soxhlext extraction with H_2O for 8 h, $EtOH_{75}$-8 is Soxhlext extraction with 75% EtOH for 8 h, and 140-30, 160-30 and 160-180 are scWE extraction at 140 °C and 30 m, 160 °C and 30 m, and 160 °C and 180 m, respectively. DMPDH represents 5a,8-Dimethyl-9-phenyl-5a,6-dihydro.

acid, 5,6-Dihydrouracil, azacyclotridecan-2-one, 1-(3-aminopropyl), L-asparagine, 2-(4-methoxyphenyl)-3-ethylidene, 1,1,2-triphenyl-4-(p-tolyl)-1,3-butadiene, and 7-methoxy-1,2-dimethyl-2H-chromene that were not identified in the H_2O-8 and $EtOH_{75}$-8 extracts, as shown in Figures 4(a, b and d). Indeed, a higher content of ketone functional groups (17%) were observed in the Soxhlet H_2O-8 than that in all selected AHR extracts, Figure 4b. Also, the Soxhlet $EtOH_{75}$-8 extract possessed significantly high proportion of organic acids (17%); particularly benzoic acid (13.2%) and phthalic acid (2.3%), as compared to the Soxhlet H_2O-8 and scWE extracts, Figure 4a. Among these scWE extracts, the scWE extract obtained at 160 °C and 30 min (Log R_0 = 3.24) showed the highest contents of rare sugars including D-picose, D-talose, and D-tagatose, Figure 4c. Nagata et al.[25] reported that D-picose, a C3 epimer of D-fructose, could contribute to achieving a lower body weight and healthy blood lipid and glucose levels in many individuals, especially obese individuals with insulin resistance. On the other hand, the scWE extract produced under harsh conditions at 160 °C and 180 min (Log R_0 = 4.02) contained notably larger contents of various chemical species such as organic acids, alkenes, chromenes, ketones, nitrogen containing compounds, terpenoids, and xanthene than the other two scWE extracts produced under mild and moderate conditions (Log R_0 = 2.65 and 3.24). Considering the combined facts of its lowest extraction yield, TPC, and pH of the scWE extract obtained at 160 °C and 180 min, the diverse small-large molecular structures of chemicals formed in this extract could be due to the consequences of series of sequential and parallel reactions including thermo-oxidation, dehydration, isomerization, and aldol condensation occurred during at the high extraction temperature and prolong time in the system[22-24].

4. Conclusions

This study demonstrates the effect of severity (Log R_0) of the

scWE on the extraction efficiency and selectively recovery of bioactive phenolic compounds from the AHR powder by varying key extraction parameters such as temperature and holding time in the reaction system. Evidently, the intensity of the severity factors strongly influenced the yield and quality of the extracts obtained by using the subcritical water. At an optimal condition (Log R_0 = 3.24, which corresponds to scWE at 160 °C and 30 min), the scWE produced relatively high yield (88%) with high bioactive phenolic compounds (31.3 mg GAE/g dry AHR) and rare sugars (D-picose, D-talose, and D-tagatose) of an AHR extract as compared to the Soxhlet extracts obtained via 8 h extraction with water and aqueous ethanol (75%). Considering its bioactive compounds produced via the green process, the scWE extract has a high potential for its application in pharmaceuticals and functional foods with high bioactivity and safety.

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References

1. Tong, T., Wang, Y.-N., Zhang, C.-M., and Kang, S.-G., "*In Vitro* and *In Vivo* Antihypertensive and Antioxidant Activities of Fermented Roots of *Allium hookeri*," *Chin. Herb. Med.*, **13**(4), 541-548 (2021).
2. Myint, A. A., Aregay, M. G., Kang, M., Kim, B.-S., Lee, Y.-W., and Kim, J., "Comprehensive Study on the Formation Mechanism of the Highly Bioactive Compounds from *Allium hookeri* Root using Subcritical Water and Their Antioxidant and Anticancer Effects," *J. Supercrit. Fluids*, **157**, 104709 (2020).
3. Yang, M. H., Kim, N.-H., Heo, J.-D., Rho, J.-R., Ock, K. J., Shin, E.-C., and Jeong, E. J., "Comparative Evaluation of Sulfur Compounds Contents and Antiobesity Properties of *Allium hookeri* Prepared by Different Drying Methods," *Evid. Complement. Alternat. Med.*, **2017**, 2436927 (2017).
4. Park, H., Jeong, J., Hyun, H., Kim, J., Kim, H., Oh, H. I., Choi, J. Y., Hwang, H. S., Oh, D. B., Kim, J. I., and Kim, H. H., "Effects of a Hot-Water Extract of *Allium hookeri* Roots on Bone Formation in Human Osteoblast-Like MG-63 Cells *In Vitro* and in Rats *In Vivo*," *Planta. Med.*, **82**, 1410-1415 (2016).
5. Nan, L., Nam, H. H., Choo, B. K., Park, J. C., Kim, D. G., Lee, J. H., and Moon, K. H., "An Ethanolic Extract of *Allium hookeri* Root Alleviates Reflux Esophagitis and Modulates BF-κB Signaling," *Evid. Complement. Alternat. Med.*, **2018**, 1834681 (2018).
6. Moon, H.-R. and Yun, J.-M., "Inhibitory Effect of *Allium hookeri* Root Extract on Melanogenesis in Murine B16F10 Melanoma Cells," *J. Korean Sci. Food Sci. Nutr.*, **52**(7), 758-764 (2023).
7. Clarke, C. J., Tu, W.-C., Levers, O., Bröhl, A., and Hallett, J. P., "Green and Sustainable Solvents in Chemical Processes," *Chem. Rev.*, **118**, 747-800 (2018).
8. Castro-Puyana, M., Marina, M. L., and Plaza, M., "Water as a Green Extraction Solvent: Principles and Reasons for Its Use," *Curr. Opin. Green Sustain. Chem.*, **5**, 31-36 (2017).
9. Plaza, M. and Marina, M. L., "Pressurized Hot Water Extraction of Bioactives," *Trends Anal. Chem.*, **116**, 236-247 (2019).
10. Bandura, A. V. and Lvov, S. N., "The Ionization Constant of Water over Wide Ranges of Temperature and Density," *J. Phys. Chem. Ref. Data*, **35**(1), 15-30 (2006).
11. Cao, R., Myint, A. A., and Kim, J., "High-yield Recovery of Highly Bioactive Compounds from Red Ginseng Marc using Subcritical Water Extraction," *J. Ind. Eng. Chem.*, **109**, 547-558 (2022).
12. Plaza, M. and Marina, M. L., "Pressurized Hot Water Extraction of Bioactives," *Trends Anal. Chem.*, **166**, 117201 (2023).
13. Overend, R. P. and Chornet, E., "Fractionation of Lignocellulosics by Stream-Aqueous Pretreatments," *Phil. Trans. R. Soc. Lond. A*, **321**, 523-536 (1987).
14. Lachos-Perez, D., Tompsett, G. A., Guerra, P., Timko, M. T., Rostagno, M. A., Martínez, J., and Forster-Carneiro, T., "Sugars and Char Formation on Subcritical Water Hydrolysis of Sugarcane Straw," *Bioresour. Technol.*, **247**, 1069-1077 (2017).
15. Kim, D. S., Myint, A. A., Lee, H. W., Yoon, J., and Lee, Y.-W., "Evaluation of Hot Compressed Water Pretreatment and Enzymatic Saccharification of Tulip Tree Sawdust using Severity Factors," *Bioresour. Technol.*, **144**, 460-466 (2013).
16. Torres-Mayanga, P. C., Azambuja, S. P. H., Tyufekchiev, M., Tompsett, G. A., Timko, M. T., Goldbeck, R., Rostagno, M. A., and Forster-Carneiro, T., "Subcritical Water Hydrolysis of Brewer's Spent Grains: Selective Production of Hemicellulosic Sugars (C-5 Sugars)," *J. Supercrit. Fluids*, **145**, 19-30 (2019).
17. Petersson, E. V., Liu, J., Sjöberg, P. J. R., Danielsson, R., and Turner, C., "Pressurized Hot Water Extraction of Anthocyanins from Red Onion: A Study on Extraction and Degradation Rates," *Anal. Chim. Acta*, **663**, 27-32 (2010).
18. Khuwijitjaru, P., Plernjit, J., Suaylam, B., Samuhaseneetoo, S., Pongsawatmanit, R., and Adachi, S., "Degradation Kinetics of Some Phenolic Compounds in Subcritical Water and Radical Scavenging Activity of Their Degradation Products," *Can. J. Chem. Eng.*, **92**, 810-815 (2014).
19. Uematsu, U. and Frank, E. U., "Static Dielectric Constant of Water and Steam," *J. Phys. Chem. Ref. Data*, **9**, 1291-1306 (1980).
20. Plaza, M. and Turner, C., "Pressurized Hot Water Extraction of

Bioactives," *Trends Anal. Chem.*, **71**, 39-54 (2015).
21. Munir, M. T., Kheirkhah, H., and Baroutian, S., Quek, S. Y., and Young, B. R., "Subcritical Water Extraction of Bioactive Compounds from Waste Onion Skin," *J. Clean. Prod.*, **183**, 487-497 (2018).
22. Plaza, M., Amigo-Benavent, M., del Castillo, M. D., Ibáñez, E., and Herrero, M., "Facts about the Formation of New antioxidants in Natural Samples after Subcritical Water Extraction," *Food Res. Int.*, **43**, 2341-2348 (2010).
23. Plaza, M., Amigo-Benavent, M., del Castillo, M. D., Ibáñez, E., and Herrero, M., "Neoformation of Antioxidants in Glycation Model Systems Treated under Subcritical Water Extraction Conditions," *Food Res. Int.*, **43**, 1123-1129 (2010).
24. Vhangani, L. N. and Van Wyk, J., "Antioxidant Activity of Maillard Reaction Products (MRPs) Derived from Fructose-Lysine and Ribose-Lysine Model Systems," *Food Chem.*, **137**, 92-98 (2013).
25. Nagata, Y., Kanasaki, A., Tamaru, S., and Tanaka, K., "D-psicose, an Epimer of D-fructose, Favorably Alters Lipid Metabolism in Sprague-Dawley Rats," *J. Agric. Food Chem.*, **63**, 3168-3176 (2015).