Extraction of Phenolic Compounds from Wild Hemp Stem Using Superheated Water

[1,2]Jianhui Zhang, [1,3,4]Xuexue Pan*, [2]Jin Chen, [5]Valery Wilfried Nguemdjo Chimeze
[1]*Zhongshan Polytechnic, Zhongshan, 528400, China.*
[2]*Zhuhai College of Science and Technology, Zhuhai, 519041, China.*
[3]*Faculty of Chemical Technology, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland.*
[4]*Institute of Combustion Problems, Al-Farabi Kazakh National University, Bogenbay Batyr Str. 1721, Almaty 050012, Kazakhstan.*
[5]*Faculty of Sciences, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon.*
panxuexue@zspt.edu.cn*

(Received on 06th May 2024, accepted in revised form 27th November 2024)

Summary: The innovation of this study consists of using superheat water to extract phenols from *Wild hemp stem* leaves. First, the effects of three factors were explored on the yield of phenols, and the results showed that these three factors had a dual impact. Then, based on single-factor experiment results, the most significant yield was achieved by the implementation of the three-factor and three-level Box-Behnken design, and it was 84.78±1.24 mg of gallic acid equivalents under optimal conditions (197 ℃, 43 min, 21 mL/g). In addition, quinic and rosmarinic acids were the main components of phenols by liquid chromatography-mass spectrometry analysis. The outcomes of three distinct extraction procedures—ethanol, hot water, and superheat water—were ultimately evaluated. Both extracts' phenolic yield and antioxidant activity (scavenging capacity, ferric reducing power) by superheat water extraction was higher than by water extraction and ethanol extraction. Therefore, extracting phenols from *Wild hemp stems* by superheating water is feasible.

Keywords: Superheat water, *Wild hemp stem*, Phenolic compounds, Antioxidant activity.

Introduction

Background

Wild hemp stem belongs to Papaveraceae groups and is substantially distributed in the southern, southwestern, and northwestern parts of China [1, 2]. As a kind of Chinese medicinal material, it contains phenols, alkaloids, and some other bioactive compounds, which are highly valued. Its extracts can be used for antioxidants, antiproliferation, antibacterial and insecticides [3]. However, in some parts of China, especially in rural areas, the use of *Wild hemp stem* is still minimal. In some places, the use method is limited to hot water extraction (HWE), which has many disadvantages such as low yield and long time-consuming [4, 5]. Therefore, extracting bioactive compounds from wild hemp stems and making good use of this resource is a subject worthy of study.

Phenols and Their Biological Activities

Phenols, a bioactive compound widely existing in *Wild hemp stem,* have good antioxidant, antibacterial, and antiproliferative activities [6-9]. Among them, antioxidant activity is one of phenols' most essential biological activities. Phenols can scavenge DPPH, ABTS, and other free radicals and have excellent antioxidant activity [10-12].

Conventional Extraction Methods

Conventional phenol extraction methods include Soxhlet extraction, impregnation, heating reflux, etc. [13, 14]. However, these methods have the drawbacks of being onerous, and they usually use organic reagents as extraction solvents, which are costly and pollute the environment. These difficulties prompted the development of superheat water extraction (SWE).

Superheat Water Extraction (SWE)

Superheat water is the liquid state of super hot water at 100-374 ℃, with pressure at 0.1-22.1 MPa [15]. Due to the higher temperature, hydrogen bonds of water molecules begin to break, and the polarity of water is weakened. Therefore, superheat water is similar to an organic solvent, which is why superheat water can efficiently extract natural organic compounds with low polarity [16, 17]. Furthermore, superheat water has lower viscosity, better permeability, and mass transfer characteristics than regular water [18]. Moreover, SWE uses water as the extraction medium, which has the

*To whom all correspondence should be addressed.

advantages of low cost and environmental protection, which makes SWE an up-and-coming extraction method [19].

Previous Research

Previous studies have demonstrated the feasibility and efficiency of SWE in extracting various bioactive compounds. For example, Basile *et al.* extracted the essential oil from rosemary by SWE [20]. The results showed that SWE was a feasible method with a fast extraction rate and good-quality essential oil. Since then, research on the extraction of phenols [19], polysaccharides [21], pectin [22], and protein [23], especially phenols, have also been carried out. Many studies [24-28] showed that extracting phenols by superheating water was efficient with high yield and short time. In addition, superheat water technology can also be applied to the treatment of biomass [29, 30], food waste [31, 32], petrochemical resources [33], and polymers [34]. Therefore, superheat water technology has received more and more attention from scholars.

Objectives of the Study

This study uses superheat water solvent to extract the phenols from *Wild hemp stems*. This study examines the relationship between phenol yield and variables such as liquid-to-solid ratio, extraction temperature, and extraction time. Optimized extraction conditions are investigated by response surface methodology. In addition, the composition of the extract is identified by LC-MS/MS. Finally, the differences in phenol yield and antioxidant capacity of extracts by SWE and traditional extraction methods are compared to determine whether extraction of phenols from *Wild hemp stem* by superheat water was feasible and efficient.

Experimental

Materials and chemicals

The leaves of *Wild hemp stem*, harvested in Sanmenxia, China, were purchased online (taobao.com). The leaves were milled, sieved through 60 meshes, and stored at room temperature. The powder is dried at 105 ℃ for 24 h before extraction. All reagents are analytically pure and purchased from Aladdin Apparatus. Superheat water extractor; UV-2450 (Shimadzu, Japan); 3H16RI Centrifuge (Hunan Herexi Instrument & Equipment Co., Ltd., China); LC-MS/MS (Agilent 1100-API4000). All the chemicals in the analytical grade were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD (Shanghai, China) without further purification.

Superheat water extraction (SWE)

SWE was carried out in the 2 L extractor, as described in Fig 1. The extractor was sealed after filling the material with varying liquid-to-solid ratios (5:1, 10:1, 20:1, 30:1) in milliliters per gram. Different extraction temperatures (from 130 to 250 ℃ at 30 ℃ intervals) and different extraction times (from 15 to 75 min at 15 min intervals) were carried out. The extraction process was finally finished, and the sample was emptied of the extractor when it had cooled to room temperature in ambient circumstances. The extract was filtered and collected.

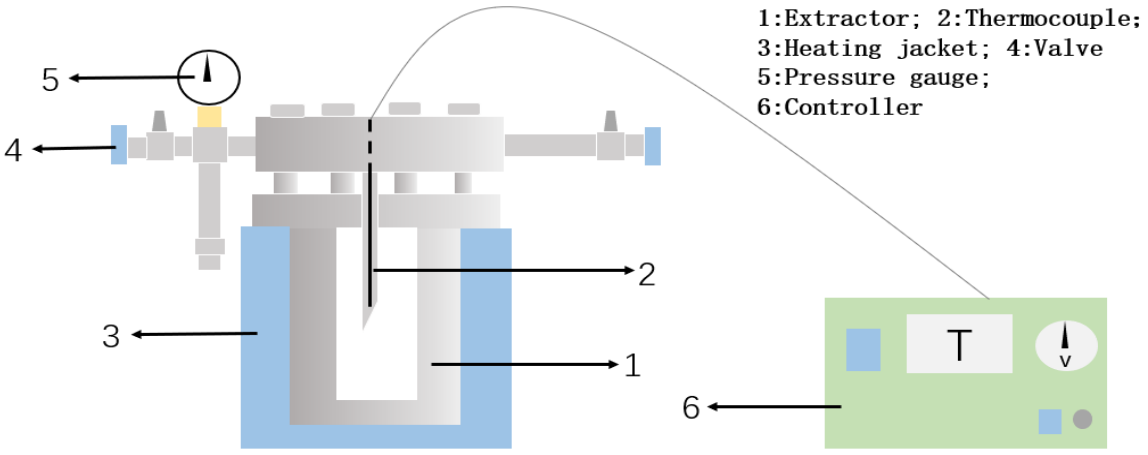

Fig. 1: Schematic diagram of subcritical water extractor.

Table-1: 3 factors and 3 levels of SWE conditions.

Level	X_1 Extraction temperature/℃	X_2 Extraction time/min	X_3 liquid-to-solid ratio/mL·g^{-1}
-1	160	30	10
0	190	45	20
1	220	60	30

Framework for conducting experiments using response surface methodology (RSM)

Data from a three-factor, three-level Box-Behnken experiment are presented in Table-1. The center point was tested repeatedly three times and a total of 15 experiments were performed.

Conventional extraction methods

Ethanol extraction

5 g material and 250 mL ethanol were added to the flask and heated to reflux at 80 ℃ for 3 h. Then, the extract was filtered and collected (experimental conditions have been optimized for obtaining the maximum total phenolic content, extraction temperature (70 ℃, 80 ℃, 90 ℃), extraction duration (1 h, 3 h, 5 h), solid-to-liquid ratio (2 g / 250 mL, 5 g / 250 mL, 10 g / 250 mL).

Hot water extraction (HWE)

5 g material was extracted with 250 mL water at 80 ℃ for 2 h. The extract was filtered and collected (experimental conditions have been optimized for obtaining the maximum total phenolic content, extraction temperature (70 ℃, 80 ℃, 90 ℃), extraction duration (1 h, 2 h, 3 h), solid-to-liquid ratio (2 g / 250 mL, 5 g / 250 mL, 10 g / 250 mL)).

Evaluation of total phenolic content (TPC)

The extracts' yield-to-concentration ratio (TPC) was determined using the Folin-Ciocalteu assay [26].

Fig 2 displays the standard curve that was fitted using the equation y=0.0342x-0.0048.

Identification of phenols by LC-MS/MS

Standards and the sample were treated by centrifugation, ultrasound, and membrane filtration before the test, and the standards were used to identify the phenols in the sample. The MS detection was conducted, correspondingly, in positive and negative ion modes.

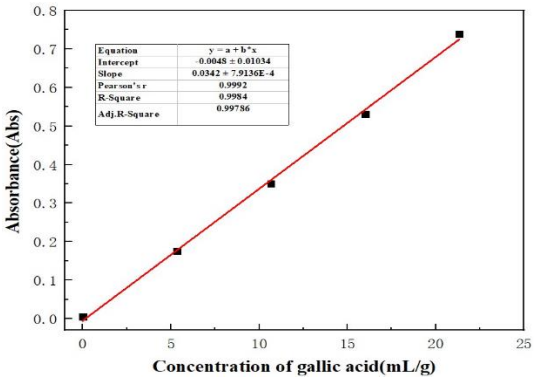

Fig. 2: Standard curve of gallic acid.

Antioxidant activity

The DPPH test, ABTS assay, and ferric reducing power were used to assess antioxidant activity, with some changes, following the methods published in [7, 26].

DPPH free radical-scavenging activity

The calculation method of DPPH free radical-scavenging activity was as follows:

$$\text{Scavenging rate}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \text{ X } 100 \quad (1)$$

ABTS free radical-scavenging activity

The following is the methodology for calculating the free radical-scavenging activity of ABTS based on the absorbance of the sample and the control (which did not contain any extracts) at 734 nm:

$$\text{Scavenging rate}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \text{ X } 100 \quad (2)$$

Ferric reducing power

1 milliliter of sample solution was mixed with 2.5 milliliters of phosphate buffer solution (0.2 mol/L, pH=6.6) and 1% (w/w) $K_3[Fe(CN)_6]$ solution.

Statistical analysis

The standard deviation of all data was less than 6%, and most of the data was less than 5%. The RSM was designed by Design-Expert.V8.0.6.1.

Results and discussion

The factor of extraction temperature on TPC

Fig 3a shows the effect on TPC of varying extraction temperatures (130-250 °C, at 30-degree intervals) with a 45-minute extraction duration and a 20-milliliter-to-gram-weight ratio of liquid/solid. Extraction temperature significantly affects TPC, as seen in Fig 3a. From 130 °C to 250 °C, TPC initially revealed an increase, followed by a decrease. Finally, TPC reached the maximum at 190 °C and the maximum yield was 84.35±2.62 mg GAE/g (190 °C, 45 min, 20 mL/g), 1.6 times more than the lowest yield of 49.97±1.27 mg GAE/g (130 °C, 45 min, 20 mL/g). This indicates that phenols may be more suitable for extraction at higher temperatures, and it may be because as temperature increases, hydrogen bonds of water molecules begin to break. The polarity begins to weaken so that the polarity of superheat water is analogous to that of phenols, which makes it like an organic solvent to extract phenols better. However, as the temperature further increases, some phenols may begin to decompose, causing the phenol production to drop from 190 °C to 250 °C [35, 36]. The efficiency and selectivity of superheat water extraction were primarily affected by temperature. The polarity of water shifted, and mass transfer efficiency improved as temperatures rose because water's viscosity, surface tension, and dielectric constant all dropped. Yan *et al.* [7, 8] and Dinh *et al.* [37] also proved that increasing temperature was beneficial to increase the yield of phenols. Still, a temperature that is too high might cause the product's decomposition, resulting in a reduction in overall productivity.

A factor of extraction time on TPC

With an extraction temperature of 190 °C and a liquid-to-solid ratio of 20 mL/g, Fig 3b displays the effect of varying extraction times (15-75 min, at 15 min intervals) on TPC. Fig 3b shows that when the extraction period was increased from 15 to 45 minutes, TPC exhibited an increasing trend at 190 °C. This is mainly because a more extended extraction time aids in the dissolution of phenols. However, when the extraction time exceeded 45 minutes, TPC decreased sharply. The reason is that the long extraction time leads to the decomposition of phenols, especially at high temperatures of 190 °C. Another study of extracting phenols from the lotus seedpod by superheat water [9] confirmed this point. The yield of phenols was much enhanced when the extraction duration was increased to 15 minutes. But as the time was further extended, the phenols gradually decomposed, and the yield decreased. He *et al.* [26] also mentioned that too long extraction time was unsuitable for extracting phenolic compounds, and the concentration was mainly affected by the distribution equilibrium.

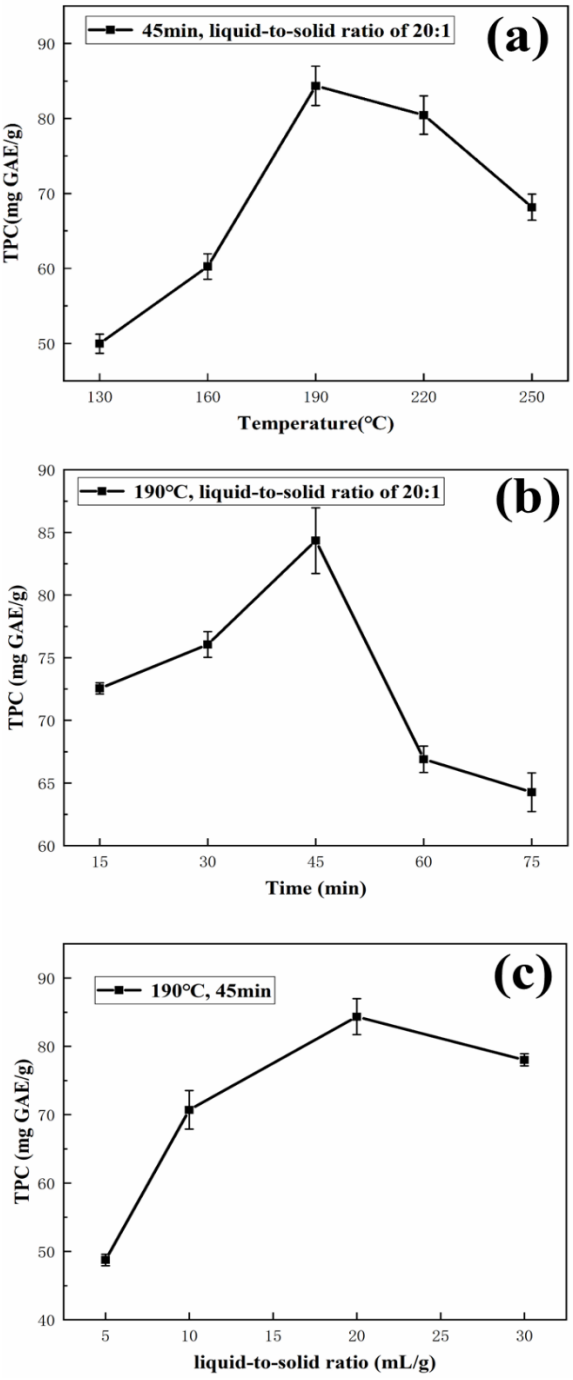

Fig. 3: (a) Factor of extraction temperature on TPC. (b) Factor of extraction time on TPC. (c) Factor of liquid-to-solid ratio on TPC.

Factor of liquid-to-solid ratio on TPC

Fig 3c shows the factor of different liquid-to-solid ratios (5 mL/g, 10 mL/g, 20 mL/g, 30 mL/g) on TPC when extraction temperature and time were 190 ℃ and 45 min, respectively. As the liquid-to-solid ratio increased from 5 mL/g to 20 mL/g, TPC showed an upward trend, but TPC gradually tended to be stable with a further increase in the liquid-to-solid ratio. The optimal liquid-to-solid ratio was 20 mL/g. A similar result was found in [8], and it mentioned that enhancement in liquid-to-solid ratio promoted contact area between the phenols and the water, which was conducive to an augment in yield, but when the liquid-to-solid ratio exceeded a certain threshold, this increase was not evident, and further extractions were not significant. In addition, economic factors also need to be considered [27].

Process optimization and RSM analysis

The following equation was used to fit the data in Table-2 using Design-Expert software and quadratic polynomial regression:

$$Y=84.75 + 6.58 * X_1 - 2.26 * X_2 + 1.60 * X_3 - 0.62 * X_1 X_2 + 0.22 * X_1 X_3 - 0.58 * X_2 X_3 - 13.67 * X_1^2 - 8.89 * X_2^2 - 9.98 * X_3^2$$

where Y is TPC, and X_1, X_2, and X_3 are extraction temperature (℃), extraction time (min) and liquid-to-solid ratio (mL/g), respectively.

The results from Table-3 show that the regression variance model was statistically significant ($P < 0.05$), whereas the absence of an equation fit did not reach statistical significance ($P > 0.05$). R^2, and R_{Adj}^2 were 0.9827 and 0.9515, respectively. This indicated that the model fitted well and was accurate and reliable, which could be used for the theoretical prediction of TPC. Furthermore, there were no**table** impacts of X_1 temperature and X_2 time ($P < 0.05$), but no significant impact of X_3 liquid-to-solid ratio ($P > 0.05$) and no considerable interactions of X_1X_2, X_1X_3, and X_2X_3.

Table-2: Box-Behnken experimental design and results.

Run	X_1 Temperature/℃	X_2 Time/min	X_3 Liquid-to-solid ratio/mL·g^{-1}	TPC/ mg GAE·g^{-1}
1	220	60	20	64.74
2	160	45	30	53.47
3	190	60	30	65.24
4	160	45	10	52.60
5	160	60	20	55.82
6	190	45	20	84.35
7	190	45	20	83.95
8	190	60	10	61.33
9	190	30	30	71.59
10	220	30	20	69.82
11	220	45	30	70.06
12	220	45	10	68.29
13	190	45	20	85.95
14	190	30	10	65.37
15	160	30	20	58.40

Optimal extraction conditions

Following these ideal parameters, the theoretical yield was 85.78 mg GEA/g, the extraction duration was 42.92 minutes, and the liquid-to-solid ratio was 20.86 mL/g. The temperature of extraction was 197.32 ℃. Considering the convenience of experimental operation, extraction process parameters were slightly adjusted: extraction temperature at 197 °C, extraction time for 43 min, and the liquid-to-solid ratio at 21 mL/g. Three parallel tests were performed, the average yield was 84.78 mg GEA/g, and the relative error was 1.17%. This indicates that optimized conditions obtained by the model are accurate and reliable and have practical value.

Table-3: ANOVA in response surface quadratic model.

Source	Sum of squares	df	Mean square	F-value	p-Value	Significance[1]
Model	1589.47	9	176.61	31.53	0.0007	Significance
X_1	346.11	1	346.11	61.79	0.0005	Significance
X_2	40.73	1	40.73	7.27	0.0430	Significance
X_3	20.38	1	20.38	3.64	0.1147	Not significance
$X_1 X_2$	1.56	1	1.56	0.28	0.6200	Not significance
$X_1 X_3$	0.20	1	0.20	0.036	0.8567	Not significance
$X_2 X_3$	1.33	1	1.33	0.24	0.6462	Not significance
X_1^2	689.60	1	689.60	123.12	0.0001	Significance
X_2^2	291.73	1	291.73	52.09	0.0008	Significance
X_3^2	367.66	1	367.66	65.64	0.0005	Significance
Residual	28.00	5	5.60			
Lack of Fit	25.76	3	8.59	7.67	0.1175	Not significance
Pure Error	2.24	2	1.12			
Cor Total	1617.48	14				
R^2=0.9827	R_{Adj}^2=0.9515					

[1] Significance (P<0.05); Not significance (P>0.05)

Analysis of response surface plots

The response surface plot showed the effects of two independent variables on TPC (the other variable was at 0 levels). It can be found from Fig 4 that the contour plots of the response surface 3D plots were circular, demonstrating that there was no evidence of statistically significant interactions between the independent variables.

It can be found from Figs 4a and 4b that extraction temperature was the most critical factor among the three factors. When other variables were constant, TPC increased significantly with increasing extraction temperature and reached the maximum at 190 ℃. As the temperature was further increased to 220 °C, the extract might be thermally decomposed, causing a decrease in TPC. Literature [38] mentioned that temperature was one of the most essential effects of SWE and had a dual effect. Increasing the extraction temperature properly could increase the yield of bioactive compounds, but increasing the extraction temperature excessively would decrease yield.

It can be found from Fig 4a that extraction time had a dual effect on TPC when extraction temperature was constant. With the extension of extraction time, TPC increased first and then decreased. A study of Essien *et al.* [36] mentioned that the dissolution rate of bioactive compounds was fast at the beginning of extraction. Still, with an extension of extraction time, the extract yield is reduced due to the solubility limit and thermal decomposition.

With a fixed extraction temperature, Fig 4c shows that TPC increased initially and decreased significantly as the liquid-to-solid ratio increased. When weighed against the effects of extraction time and temperature on TPC, there may not be much of an impact here.

LC-MS/MS analysis

Phenolic components in the extract under the optimal condition (197 ℃, 43 min, 21 mL/g) were tested by LC-MS/MS. 8 phenolic components (phenylalanine, vanillic acid, vanillin, rosmarinic acid, gallic acid, quinic acid, ethyl gallate, and ethyl vanillin) were identified by comparing total ion chromatogram (TIC), ion-pairs (precursor ion and product ion) and retention time of extract with those of the standards and the literature [25, 28]. The TIC of extract in positive and negative ion modes are shown in Fig 5. The retention time, optimized ion pairs (precursor ion and production), and molecular weight of 8 phenolic components in the extract are listed in Table-4. Based on the analysis of retention time and peak height of TIC, it may be tentatively deduced that the predominant phenolic constituents in the extract were quinic and rosmarinic acids. In contrast, the extract's content of phenylalanine, vanillic acid, vanillin, gallic acid, ethyl gallate, and ethyl vanillin was relatively low.

Table-4: Phenolic components in the extract and their some information gotten by LC-MS/MS analysis.

Component	Retention time/min	Precursor ion	Product ion	Molecular weight	ESI
Phenylalanine	0.926	165.903	77.019; 103.033	165.19	+
Vanillic acid	3.320	169.000	92.900; 125.000	168.15	+
Vanillin	4.780	152.883	93.074; 125.004	152.15	+
Rosmarinic acid	14.70	361.200	129.000; 185.100	360.31	+
Gallic acid	0.329	169.000	124.900	170.12	-
Quinic acid	0.629	191.000	85.081	192.16	-
Ethyl gallate	5.370	197.000	123.900	198.17	-
Ethyl vanillin	7.180	165.000	107.900	166.18	-

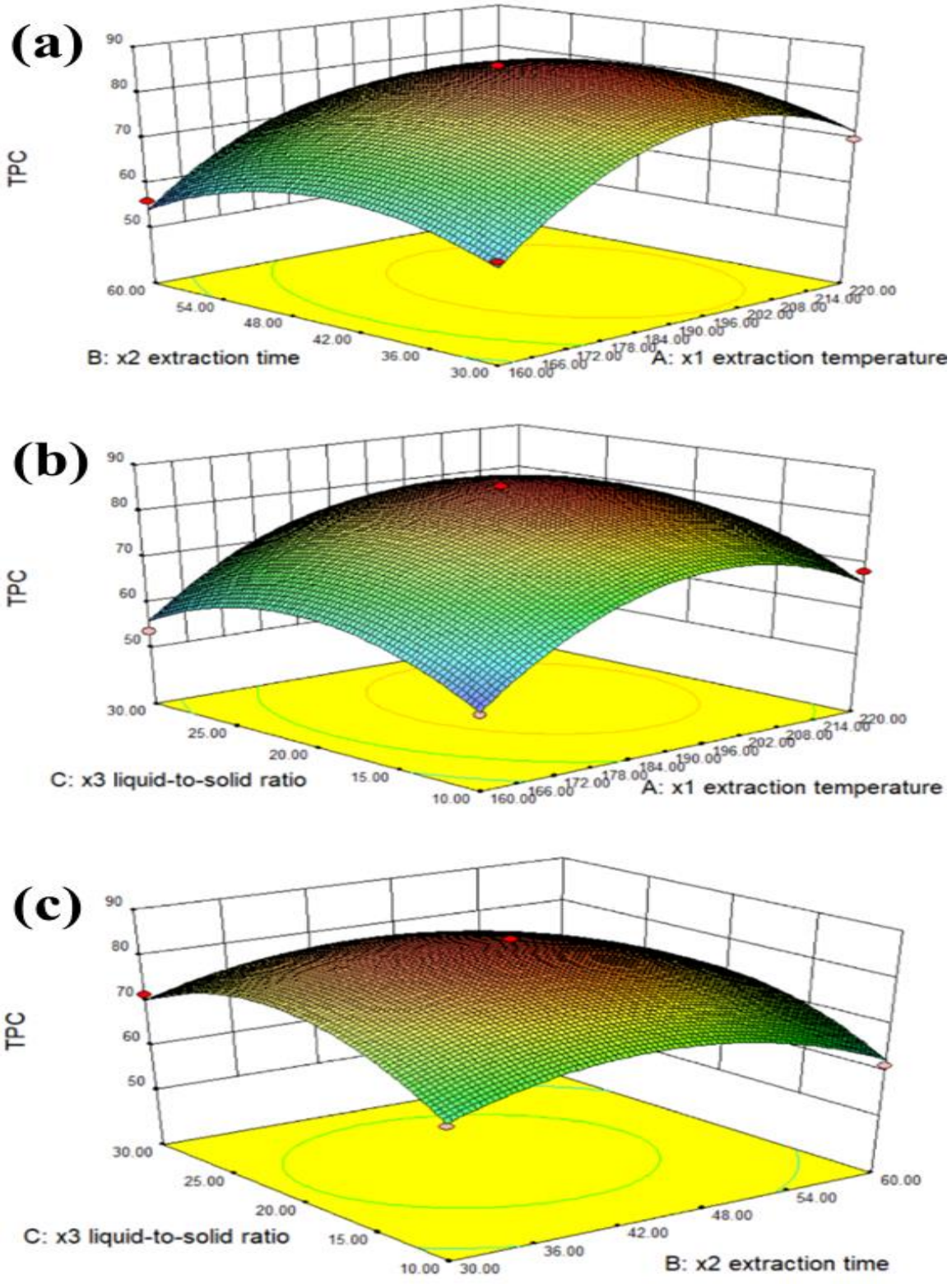

Fig. 4: Response surface 3D plots of the effects of (a) temperature, (b) time, and (c) liquid-to-solid ratio on TPC.

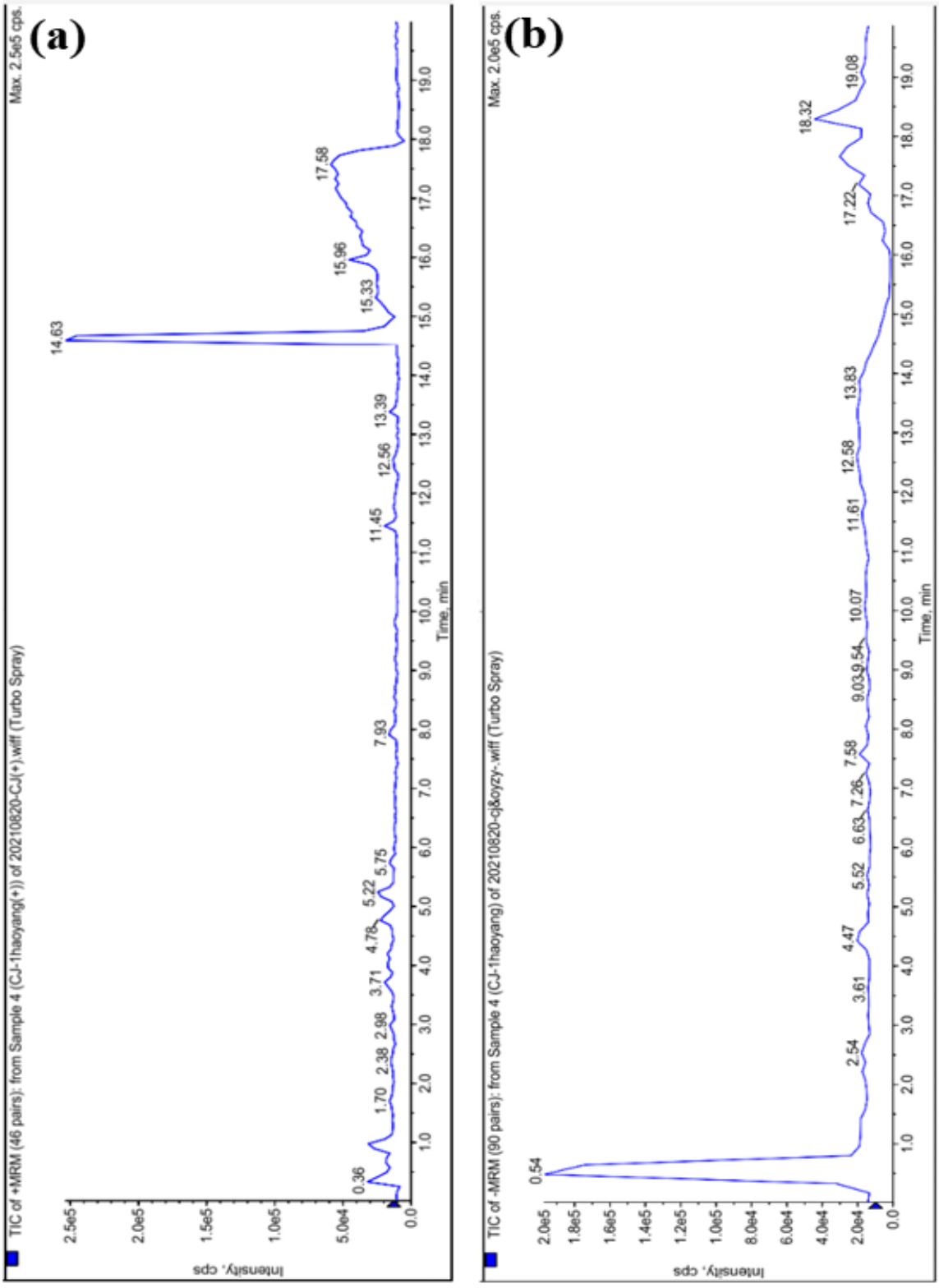

Fig. 5: TIC of +MRM (a) and -MRM (b) from extract under optimal extraction conditions.

Comparison of SWE and other conventional extraction methods

Table-5 shows the difference in TPC of extracts by SWE and the two conventional methods: HWE and ethanol extraction. Compared with the two traditional extraction methods, the TPC of the extract by SWE was significantly higher. The main reason is that when the superheated water is at high pressure and temperature, hydrogen bonds of water molecules begin to break, and the polarity weakens, dramatically increasing the solubility of lower polar compounds in water [39]. In addition, compared with HWE and ethanol extraction (2 h and 3 h, respectively), SWE had better extraction efficiency (43 min). The reason may be that superheat water exhibits high diffusion, low viscosity, and permeability, making SWE a high mass transfer efficiency [40, 41].

Table-5: TPC of extracts by three extraction methods.

Method	TPC /mg GAE·g^{-1}	Condition
SWE[1]	84.78±1.24	197℃, 43min, 21mL/g
HWE[2]	39.51±0.68	80℃, 2h, 50mL/g
Ethanol extraction[3]	62.71±3.29	80℃, 3h, 50mL/g

Regarding antioxidant activity, extracts by SWE, ethanol extraction, and HWE were dried into a powder. The powder was prepared into the solutions with different concentrations for antioxidant activity measurement. With the increase in concentration in Fig 6, sample solutions had better antioxidant activity. Besides, compared with ethanol extraction and HWE, the sample solutions by SWE had better ferric-reducing power and the ability to scavenge DPPH and ABTS free radicals. Literature [7, 37, 42] confirmed that the antioxidant activity of extract by SWE was much higher than that by standard extraction methods. In addition, literature [35, 43, 44] also mentioned that phenols extracted at high extraction temperatures would have excellent antioxidant activity.

The results of this study provide new ideas for the extraction and application of phenolic compounds. The SWE method applies not only to wild hemp stems and leaves but can also be extended to other plant materials rich in phenolic compounds. Here are some potential application areas:

Food industry: Phenolic compounds, as natural antioxidants, can extend the shelf life of food, prevent oxidation and spoilage, and improve the nutritional value of food.

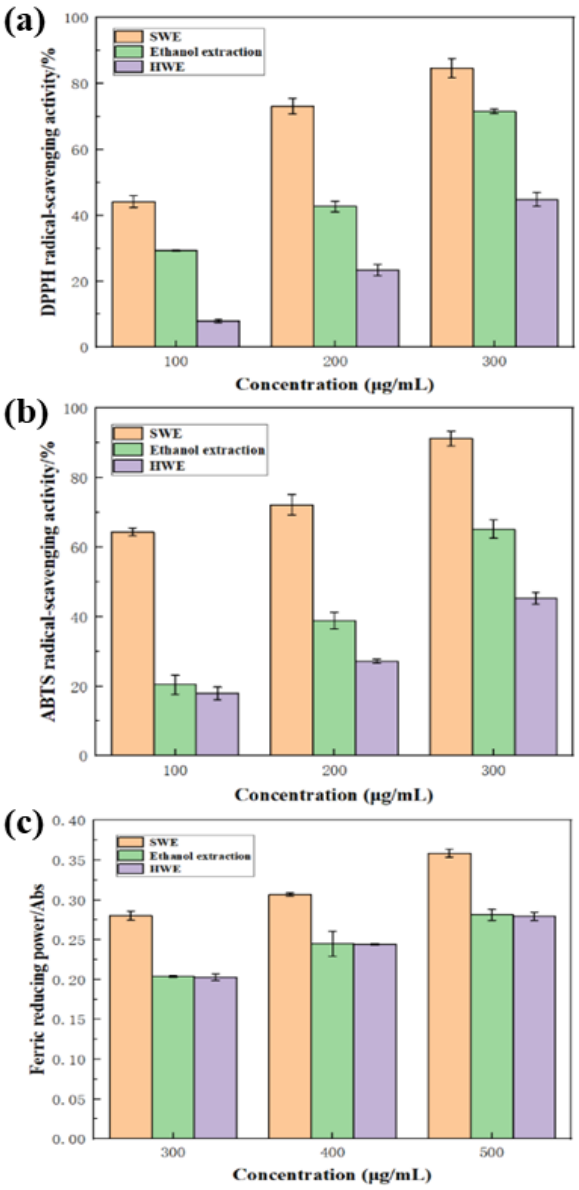

Fig. 6: Antioxidant activity of extracts by three extraction methods: (a) DPPH free radical-scavenging activity; (b) ABTS free radical-scavenging activity; (c) ferric reducing power.

In medicine, phenolic compounds have various biological activities, such as anti-inflammatory, antibacterial, and anticancer, and can be used to develop new drugs and health products.

Cosmetics industry: The antioxidant and anti-aging properties of phenolic compounds make them ideal ingredients in cosmetic formulations, helping to protect the skin from free radical damage.

Conclusions

This study innovatively used the superheat water extraction (SWE) method to extract phenolic compounds from wild hemp stems and leaves and achieved significant results. The key findings are as follows: Extraction efficiency: Under optimized conditions (197 ℃, 43 minutes, liquid-solid ratio of 21 mL/g), the total phenolic content (TPC) extracted by the SWE method reached 84.78 ± 1.24 mg GAE/g, significantly higher than that of hot water extraction (HWE) and ethanol extraction. Main components: Through liquid chromatography-mass spectrometry (LC-MS) analysis, the main phenolic components in the extract were determined to be quinic acid and rosmarinic acid. Antioxidant activity: SWE extract's antioxidant activity (scavenging ability and iron ion reduction ability) is significantly higher than that of HWE and ethanol extract. Influencing factors: Temperature is the most crucial factor affecting the efficiency of phenolic extraction and has a dual effect, while liquid-solid ratio is the least affected factor.

Based on the results of this study, future research can consider the following directions: Expand application scope: Explore the application of SWE method in other plant materials rich in phenolic compounds, and verify its universality in different plants. Process optimization: Further optimize the extraction process parameters to improve the purity and yield of phenolic compounds, and reduce energy consumption and costs. Functional research: Conduct in-depth studies on the relationship between the structure and function of phenolic compounds in extracts, and evaluate their potential applications in fields such as food, medicine, and cosmetics. Mechanism research: Study the mechanism of extraction and degradation of phenolic compounds during the SWE process, revealing the specific effects of temperature, time, and liquid-solid ratio on extraction efficiency. Therefore, this study demonstrates the efficiency and feasibility of the SWE method in extracting phenolic compounds and provides important theoretical basis and technical support for the industrial production and application of phenolic compounds.

Funding

The authors thank the Characteristic Innovation Project of Ordinary Colleges and Universities in Guangdong Province (No. 2022KTSCX339), Special Project in Key Fields of General Universities in Guangdong Province (No. 2022ZDZX3086), 2023 High-level Talents Research Initiative Project (No. KYG2302), Topic 4 of the Second Batch of Social Public Welfare and Basic Research Projects in Zhongshan City in 2023: Basic and Applied Basic Research (No. 2023B2016), Young Innovative Talents Project for Ordinary Universities in Guangdong Province in 2024 (No. 2024KQNCX162) and the 11th Batch of "Zhongshan Talent Plan" Innovation and Entrepreneurship Team Project (No. CXTD2024009).

References

1. G. Benítez, J. Molero-Mesa and M. R. González-Tejero, Wild edible plants of andalusia: traditional uses and potential of eating wild in a highly diverse region, *Plants*, **12**, 1218 (2023).
2. R. group, A. Becker, J. B. Bachelier, L. Carrive, N. Conde e Silva, C. Damerval, C. Del Rio, Y. Deveaux, V. S. Di Stilio and Y. Gong, A cornucopia of diversity—Ranunculales as a model lineage, *J. Exp. Bot.*, **75**, 1800 (2024).
3. T. Alsufyani, N. Al-Otaibi, N. J. Alotaibi, N. H. M'sakni and E. M. Alghamdi, GC Analysis, Anticancer, and Antibacterial Activities of Secondary Bioactive Compounds from Endosymbiotic Bacteria of Pomegranate Aphid and Its Predator and Protector, *Molecules*, **28**, 4255 (2023).
4. A. S. Martinez, O. Lanaridi, K. Stagel, H. Halbwirth, M. Schnürch and K. Bica-Schröder, Extraction techniques for bioactive compounds of cannabis, *Nat. Prod. Rep.*, **40**, 676 (2023).
5. E. E. Golia, J. Bethanis, N. Ntinopoulos, G. -G. Kaffe, A. A. Komnou and C. Vasilou, Investigating the potential of heavy metal accumulation from hemp. The use of industrial hemp (Cannabis Sativa L.) for phytoremediation of heavily and moderated polluted soils, *Sustain. Chem. Pharm.*, **31**, 100961 (2023).
6. K. A. Lee, W. J. Kim, H. J. Kim, K. T. Kim and H. D. Paik, Antibacterial activity of Ginseng (Panax ginseng CA Meyer) stems–leaves extract produced by subcritical water extraction, *Int. J. Food Sci. Technol.*, **48**, 947 (2013).
7. Z. Yan, X. Luo, J. Cong, H. Zhang, H. Ma and Y. Duan, Subcritical water extraction, identification and antiproliferation ability on HepG2 of polyphenols from lotus seed epicarp, *Ind. Crops Prod.*, **129**, 472 (2019).
8. Z. Yan, H. Zhang, C. S. Dzah, J. Zhang, C. Diao, H. Ma and Y. Duan, Subcritical water extraction, identification, antioxidant and antiproliferative activity of polyphenols from lotus seedpod, *Sep.*

Purif. Technol., **236**, 116217 (2020).
9. Z. Ahmadian-Kouchaksaraie, R. Niazmand and M. N. Najafi, Optimization of the subcritical water extraction of phenolic antioxidants from Crocus sativus petals of saffron industry residues: Box-Behnken design and principal component analysis, Innov. *Food Sci. Emerg. Technol.*, **36**, 234 (2016).
10. S. Aytac, O. Gundogdu, Z. Bingol and İ. Gulcin, Synthesis of Schiff bases containing phenol rings and investigation of their antioxidant capacity, anticholinesterase, butyrylcholinesterase, and carbonic anhydrase inhibition properties, *Pharmaceutics*, **15**, 779 (2023).
11. K. Godlewska, P. Pacyga, A. Najda and I. Michalak, Investigation of chemical constituents and antioxidant activity of biologically active plant-derived natural products, *Molecules*, **28**, 5572 (2023).
12. J. Rumpf, R. Burger and M. Schulze, Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins, *Int. J. Biol. Macromol.*, **233**, 123470 (2023).
13. I. Susanti, R. Pratiwi, Y. Rosandi and A. N. Hasanah, Separation methods of phenolic compounds from plant extract as antioxidant agents candidate, *Plants*, **13**, 965 (2024).
14. M. Zhang, J. Zhao, X. Dai and X. Li, Extraction and analysis of chemical compositions of natural products and plants, *Separations*, **10**, 598 (2023).
15. T. Xiang, R. Yang, L. Li, H. Lin and G. Kai, Research progress and application of pectin: A review, *J. Food Sci.*, **89**, 6985 (2024).
16. R. La Tella, D. Donnarumma, F. Rigano, P. Donato, P. Appelblad, P. Dugo, L. Mondello and A. Mondello, Development of a Superheated Water Chromatography Method for the Sustainable Determination of Preservatives in Foods and Cosmetics, *Green Anal. Chem.*, **11**, 100155 (2024).
17. L. Knierim, A. Uhl, A. Schmidt, M. Flemming, T. Höß, J. Treutwein and J. Strube, Pressurized Hot Water Extraction as Green Technology for Natural Products as Key Technology with Regard to Hydrodistillation and Solid–Liquid Extraction, *ACS Omega*, **9**, 31998 (2024).
18. W. Bicheng, Y. Umehara, M. Tanaka, R. Kobayashi, S. Hida, A. Eto and S. Mori, Mechanism of the rapid generation of superheated and saturated steam using a water-containing porous material, *Appl. Therm. Eng.*, **257**, 124172 (2024).
19. E. Rosales-Asensio, E. Segredo-Morales, N. Gómez-Marín, A. Pulido-Alonso and C. Sierra, A Techno-Economic Appraisal of Green Diesel Generation through Hydrothermal Liquefaction, Leveraging Residual Resources from Seaweed and Fishing Sectors, *Water*, **15**, 3061 (2023).
20. A. Basile, M. M. Jiménez-Carmona and A. A. Clifford, Extraction of rosemary by superheated water, *J. Agric. Food Chem.*, **46**, 5205 (1998).
21. Y. Zhang, K. Hawboldt and S. MacQuarrie, Extraction of bioactive compounds from beach-cast brown algae: a review on accelerated solvent extraction and subcritical water extraction, *RSC Sustain.*, **2**, 2069 (2024).
22. X. Wang, Q. Chen and X. Lü, Pectin extracted from apple pomace and citrus peel by subcritical water, *Food Hydrocoll.*, **38**, 129 (2014).
23. W. Lu, X. -W. Chen, J. -M. Wang, X. -Q. Yang and J. -R. Qi, Enzyme-assisted subcritical water extraction and characterization of soy protein from heat-denatured meal, *J. Food Eng.*, **169**. 250 (2016).
24. D. Nkurunziza, P. Pendleton, S. P. Sivagnanam, J. -S. Park and B. S. Chun, Subcritical water enhances hydrolytic conversions of isoflavones and recovery of phenolic antioxidants from soybean byproducts (okara), *J. Ind. Eng. Chem.*, **80**, 696 (2019).
25. X. Liu, Y. Wang, J. Zhang, L. Yan, S. Liu, A. A. Taha, J. Wang and C. Ma, Subcritical water extraction of phenolic antioxidants with improved α-amylase and α-glucosidase inhibitory activities from exocarps of Castanea mollissima Blume, *J. Supercrit Fluids*, **158**, 104747 (2020).
26. L. He, X. Zhang, H. Xu, C. Xu, F. Yuan, Ž. Knez, Z. Novak and Y. Gao, Subcritical water extraction of phenolic compounds from pomegranate (Punica granatum L.) seed residues and investigation into their antioxidant activities with HPLC–ABTS+ assay, *Food Bioprod. Process.*, **90**, 215-223 (2012).
27. S. Essien, B. Young and S. Baroutian, Subcritical water extraction for selective recovery of phenolic bioactives from kānuka leaves, *J. Supercrit Fluids*, **158**, 104721 (2020).
28. D. Pinto, E. F. Vieira, A. F. Peixoto, C. Freire, V. Freitas, P. Costa, C. Delerue-Matos and F. Rodrigues, Optimizing the extraction of phenolic antioxidants from chestnut shells by subcritical water extraction using response surface methodology, *Food Chem.*, **334**, 127521 (2021).
29. S. S. Toor, L. Rosendahl and A. Rudolf, Hydrothermal liquefaction of biomass: a review of subcritical water technologies, *Energy*, **36**, 2328 (2011).

30. P. C. Torres-Mayanga, D. Lachos-Perez, A. Mudhoo, S. Kumar, A. B. Brown, M. Tyufekchiev, G. Dragone, S. I. Mussatto, M. A. Rostagno and M. Timko, Production of biofuel precursors and value-added chemicals from hydrolysates resulting from hydrothermal processing of biomass: A review, *Biomass Bioenerg.*, **130**, 105397 (2019).
31. H. Su, D. Hantoko, M. Yan, Y. Cai, E. Kanchanatip, J. Liu, X. Zhou and S. Zhang, Evaluation of catalytic subcritical water gasification of food waste for hydrogen production: Effect of process conditions and different types of catalyst loading, *Int. J. Hydrogen Energy*, **44**, 21451 (2019).
32. E. Ö. Kanmaz, Humic acid formation during subcritical water extraction of food by-products using accelerated solvent extractor, *Food Bioprod. Process.*, **115**, 118 (2019).
33. Y. Sun, S. Kang, S. Wang, L. He, W. Guo, Q. Li and S. Deng, Subcritical water extraction of Huadian oil shale at 300 C, *Energ. Fuel.*, **332**, 106 (2019).
34. J. A. Okolie, S. Nanda, A. K. Dalai, F. Berruti and J. A. Kozinski, A review on subcritical and supercritical water gasification of biogenic, polymeric and petroleum wastes to hydrogen-rich synthesis gas, *Renew. Sust. Energ. Rev.*, **119**, 109546 (2020).
35. J. Zhang, C. Wen, H. Zhang, Y. Duan and H. Ma, Recent advances in the extraction of bioactive compounds with subcritical water: A review, *Trends Food Sci. Technol.*, **95**, 183 (2020).
36. S.O. Essien, B. Young and S. Baroutian, Recent advances in subcritical water and supercritical carbon dioxide extraction of bioactive compounds from plant materials, *Trends Food Sci. Technol.*, **97**, 156 (2020).
37. T. V. Dinh, P. S. Saravana, H. C. Woo and B. S. Chun, Ionic liquid-assisted subcritical water enhances the extraction of phenolics from brown seaweed and its antioxidant activity, *Sep. Purif. Technol.*, **196**, 287 (2018).
38. P. Mottahedin, A. Haghighi Asl and M. Khajenoori, Extraction of curcumin and essential oil from Curcuma longa L. by subcritical water via response surface methodology, *J. Food Process. Preserv.*, **41**, e13095 (2017).
39. M. Munir, H. Kheirkhah, S. Baroutian, S. Y. Quek and B. R. Young, Subcritical water extraction of bioactive compounds from waste onion skin, *J. Clean. Prod.*, **183**, 487 (2018).
40. I. Okajima and T. Sako, Energy conversion of biomass with supercritical and subcritical water using large-scale plants, *J. Biosci. Bioeng.*, **117**, 1 (2014).
41. Ž. Knez, M.K. Hrnčič, M. Čolnik and M. Škerget, Chemicals and value added compounds from biomass using sub-and supercritical water, *J. Supercrit. Fluids*, **133**, 591 (2018).
42. L. G. G. Rodrigues, S. Mazzutti, L. Vitali, G. A. Micke and S. R. S. Ferreira, Recovery of bioactive phenolic compounds from papaya seeds agroindustrial residue using subcritical water extraction, *Biocatal. Agric. Biotechnol.*, **22**, 101367 (2019).
43. S. Erşan, Ö. G. Üstündağ, R. Carle and R. M. Schweiggert, Subcritical water extraction of phenolic and antioxidant constituents from pistachio (Pistacia vera L.) hulls, *Food Chem.*, **253**, 46 (2018).
44. Y. Zhang, Y. Zhang, A.A. Taha, Y. Ying, X. Li, X. Chen and C. Ma, Subcritical water extraction of bioactive components from ginseng roots (Panax ginseng CA Mey), *Ind. Crops Prod.*, **117**, 118 (2018).