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Article

Decomposition of Five Phenolic Compounds in High Temperature Water

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Neste trabalho, a estabilidade de cinco compostos fenólicos (catecol, protocatecualdeído, ácido salviânico A , ácido protocatecuico e ácido ferulaico) em água a alta temperatura foi investigada. Os efeitos de dois fatores principais nos experimentos de estabilidade, como temperatura e tempo, foram investigados. A decomposição de três compostos fenólicos ácidos (ácidos salviânico A, protocatecuico e ferulaico) aumenta com o aumento da temperatura e os ácidos tornam-se menos estáveis com maiores tempos de aquecimento. Os três compostos fenólicos ácidos apresentaram pouca decomposição a 200 °C e a decomposição completa foi observada a 300-350 °C. Os produtos de decomposição do ácido salviânico A, ácido protocatechuico e ácido ferulaico foram caracterizados e quantificados por cromatografia líquida de alta eficiência (HPLC) e posteriormente confirmados por cromatografia líquida de alta eficiência/espectrometria de massas (HPLC/MS). O ácido salviânico A, ácido protocatechuico e ácido ferulaico em água a alta temperatura sofreram, principalmente, reação de descarboxilação, formando 4-etilcatecol, catecol e 2-metoxi-4-vinilfenol, respectivamente. No entanto, catecol e protocatecualdeído permaneceram praticamente estáveis, mesmo a temperaturas até 350 °C. A temperatura e o tempo de reação mostraram pouco efeito na decomposição de catecol e protocatecualdeído. Esses resultados demonstraram que através do ajuste de temperatura ou tempo, a extração de compostos fenólicos pode ser controlada.

In this work, the stability of five phenolic compounds (catechol, protocatechualdehyde, salvianic acid A, protocatechuic acid, and ferulaic acid) under high temperature water was investigated. The effects of two main factors on the stability experiments, such as temperature and time, were investigated. The decomposition of three phenolic acid compounds (salvianic A, protocatechuic, and ferulaic acids) increased with rising temperature and the acids became less stable with longer heating time. The three phenolic acid compounds showed very minor decomposition at 200 °C and their complete decomposition was observed at 300-350 °C. The decomposition products of salvianic acid A, protocatechuic acid, and ferulaic acid were characterized and quantified by high performance liquid chromatography (HPLC) and further confirmed by high performance liquid chromatography/mass spectrometry (HPLC/MS). Salvianic acid A, protocatechuic acid, and ferulaic acid in high temperature water mainly underwent decarboxylation reaction to form 4-ethylcatechol, catechol, and 2-methoxy-4-vinylphenol, respectively. However, catechol and protocatechualdehyde almost remained stable even at temperatures up to 350 °C. Reaction temperature and time showed relatively little effect on the decomposition of catechol and protocatechualdehyde. These results demonstrated that through adjusting the temperature or time, the extraction of phenolic compounds can be controlled.

Keywords: phenolic compounds, high temperature water, HPLC/MS, decomposition

Introduction

Investigations in the area of high temperature water (HTW) as an environmentally benign medium for chemical reactions and extraction are attracting more and more attention in the past three decades.[1-5] This is not only because it is cheap, naturally abundant, and nontoxic, but also because its physicochemical properties can be modified substantially with temperature and pressure. This means that the chemical environment can be modified without changing the composition of solvent.[6-9] So, HTW has been used in a variety of research areas such as in extraction, synthesis, etc.[10-15]

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Recently, in order to achieve reversed-phase separation such as anilines and phenols, HTW in the mobile phase as the sole component in subcritical water chromatograph has also been explored.[16-19] The researchers have also found that this green chromatography technique strives to eliminate the use of organic solvents, so it is promising and interesting. The main advantage of HTW compared to other conventional techniques such as boiling, reflux and sonication is the elimination or minimization of poisonous organic solvents required in the extraction processes, which is consistent with the goals of green chemistry and green engineering.

Phenolic compounds are bioactive, such as antioxidant activities and antiaging. In order to extract phenolic compounds effectively from plants, some novel extraction techniques have been developed, such as supercritical fluid extraction, ultrasound assisted extraction, and microwave-assisted extraction. Small amounts of catechol occur naturally in fruits and vegetables, along with the enzyme polyphenol oxidase. Salvianic acid A, protocatechuic acid, protocatechualdehyde, and ferulaic acid are found in Chinese medicinal herbs and other plants.[20-23] These components are recognized to protect myocardium from ischemia-induced derangement and platelet from aggregation.[24-27] High temperature water extraction (HTWE) has been applied to the extraction of a host of bioactive components from plants and herbs.[12,16,17] Combined with the characteristics of HTW techniques, investigations into the stability of bioactive components in HTW would be quite meaningful to better assess this method and allow more wide application of this eco-friendly technique. The high temperatures used in these extraction methods may cause the effective components to decompose. Our previous study also showed that decomposition of flavonoids occurred during HTWE of *Radix Scutellariae*.[28] Decomposition of terpene, benzoic acid and its derivatives in HTW has been investigated by Yang's research group.[29,30] Murakami *et al.*[14] used HTWE to recover fluoxetine hydrochloride from both standard solutions and the contents of commercial capsule formulations, and suggest that recoveries were generally incomplete and often produced decomposition by-products during the process.

In the present study, the effects of temperature and time on the stability of five phenolic compounds in HTW were studied. The decomposition experimental temperature ranged from 100 to 350 °C. The single phenolic compound-water mixture was heated in a reactor with heating times ranging from 20 to 60 min to evaluate the kinetic effect on the phenolic compound decomposition. The reaction mixtures were then analyzed by high performance liquid chromatography (HPLC) with a UV detector to determine the analyte stability. If a given analyte was decomposed under certain conditions, the main decomposition products were characterized and quantified by HPLC with a UV detector and further confirmed by HPLC/mass spectrometer (MS).

Experimental

Sample preparation and chemicals

Acetic acid, catechol, 4-ethylcatechol, 2-methoxy-4-vinylphenol were provided by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Salvianic acid A, protocatechuic acid, protocatechualdehyde, and ferulaic acid were acquired from Shanghai Yuanye Biological Technology CO., Ltd. HPLC grade methanol was obtained from Tedia Company. Deionized water (18 MΩ cm) was prepared from Elga Purelab water system (Elga, England).

Salvia miltiorrhiza Bunge were purchased from the local market. They were washed, dried in the air, cut into small pieces, pulverized with a grinder and passed through a 60 mesh sieve. Subcritical water extraction was performed in a 6.1 mL stainless-steel reactor. In a typical extraction, 0.5 g *Salvia miltiorrhiza* Bunge and 4.0 mL water were added into the reactor.

Heating of organic/water mixtures

High pressure cylindrical-shaped 316 stainless steel reactors of 6.1 mL (Haian Company, Jiangsu, China) were used for heating the mixtures of a given phenolic compound and water. The stainless steel reactors used in experiments are inert.

The reaction reactors were rinsed with methanol and allowed to dry prior to each use. The end of reactor was sealed with an end cap. The used solution were prepared in water and stored at 4 °C in a refrigerator. Each 250 μL standard solution was added into each reactor. Then 4.0 mL deionized water was added into the reactor. The loaded reactor was tightly closed and placed into a preheated isothermal furnace. The reaction time was recorded from the time when the reactor was placed in the furnace. After a desired period of reaction time, the reactor was taken out of the furnace and allowed to cool to room temperature. The reactor was opened and the reaction mixture was transferred into a 10.00 mL comparison tube. The reactor was washed with water and the washings were also added to the above comparison tube. The effect of temperature on phenolic compounds in HTW was evaluated in duplicate experiments at temperatures of 100, 150, 200, 250, 300, and 350 °C. The effect of heating time on five phenolic compounds decomposition was determined using 20-60 min.

HPLC and HPLC/MS analyses

To determine the contents of the five phenolic compounds (catechol, salvianic acid A, protocatechuic acid, protocatechualdehyde, and ferulaic acid), the extracts were analyzed using an UltiMate 3000 LC series made in USA, equipped with a five binary gradient pump, autosampler, and UV detector. The HPLC analysis of the samples was performed with a C-18 Inertsil ODS-3 column (5 μm, 250 mm × 4.6 mm i.d.).

The mobile phase used was 0.5% acetic acid in HPLC grade water (mobile phase A) and methanol (mobile phase B). The gradient was as follows: 90% A for 0-5 min, 30% A for 5-15 min, and 90% A for 15-30 min. The flow rate was set at 1.00 mL min^{-1} and injection volume was 20.00 μL. UV detection was at 254, 260 and 281 nm. Quantification of five phenolic compounds and their decomposition products was obtained by using calibration curves generated using a series of five standard solutions. The phenolic compounds and their respective decomposition products have similar molecular structures, so the response factors were considered the same for all substances.

An Agilent Technologies 6520 Network HPLC System (Santa Clara, CA, USA) coupled with a quadrupole, a hexapole-collision cell and a time-of-flight (TOF) was used to confirm the identity of the decomposition products with a mass hunter data acquisition and processing.

Results and Discussion

Effect of temperature on the stability of phenolic compounds

As shown in Figure 1, catechol and protocatechualdehyde remained stable at temperatures from 100 to 250 °C. Three phenolic acid compounds studied remained stable at temperatures up to 150 °C.

However, ferulaic acid and salvianic acid A showed significant decomposition at 200 °C, but protocatechuic acid still remained stable. Figure 1 also shows the stability trend for the three phenolic acids at 200 °C, with protocatechuic acid being the most stable one followed by salvianic acid A, and ferulaic acid being the least stable one. Ferulaic acid and protocatechuic acid were almost completely decomposed at 250 °C, while salvianic acid A showed obvious decomposition as evidenced in Figure 1. The chromatograms of the water-phenolic acid mixtures obtained after heating at 250-350 °C are compared in Figure 2.

It is also worth noting that the stability of five phenolic compounds in HTW was quite reproducible. For example, duplicate trials of these stability experiments are typically with relative standard deviations of 0.52-6.39%. Therefore, for the times and temperatures investigated, the decomposition values obtained can be used to provide a reliable indication of the stability of five selected phenolic compounds during HTW.

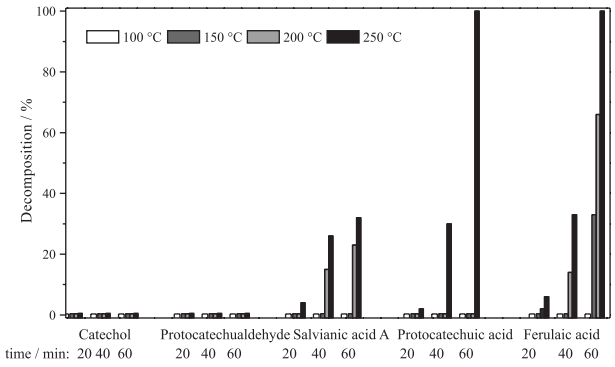

Figure 1. Effects of temperature and heating time on the stability of five phenolic compounds in HTW.

Effect of the heating time on the stability of phenolic compounds

In Figure 1, it is possible to see that at a given temperature, longer heating time caused greater decomposition. For example, 33% of ferulaic acid were decomposed after heating at 250 °C for 40 min as shown in the Figure 2e. However, ferulaic acid decomposition increased to 99% by lengthening the heating time to 60 min at the same temperature. Similar trends were observed for salvianic acid A and protocatechuic acid at 200 and 250 °C as depicted in Figure 1. The stability of catechol and protocatechualdehyde was investigated at 350 °C with much longer heating time as shown in Figure 3. Protocatechualdehyde decomposition at 350 °C was increased to 9.2% by increasing the heating time from 30 to 150 min, while catechol showed the same profile of decomposition at studied temperatures and times.

Characterization and quantification of the decomposition products

As discussed above, the three phenolic acid compounds were obviously decomposed at 250 °C. In order to characterize the decomposition products, the heated organic-water mixtures at 250-350 °C were used for characterization of the decomposition products using HPLC. Figure 2b indicates that salvianic acid A was mainly converted to 4-ethylcatechol at 200 and 250 °C. As shown in Figure 2c and 2d, we can easily see the correlation between protocatechuic acid decomposition and catechol formation

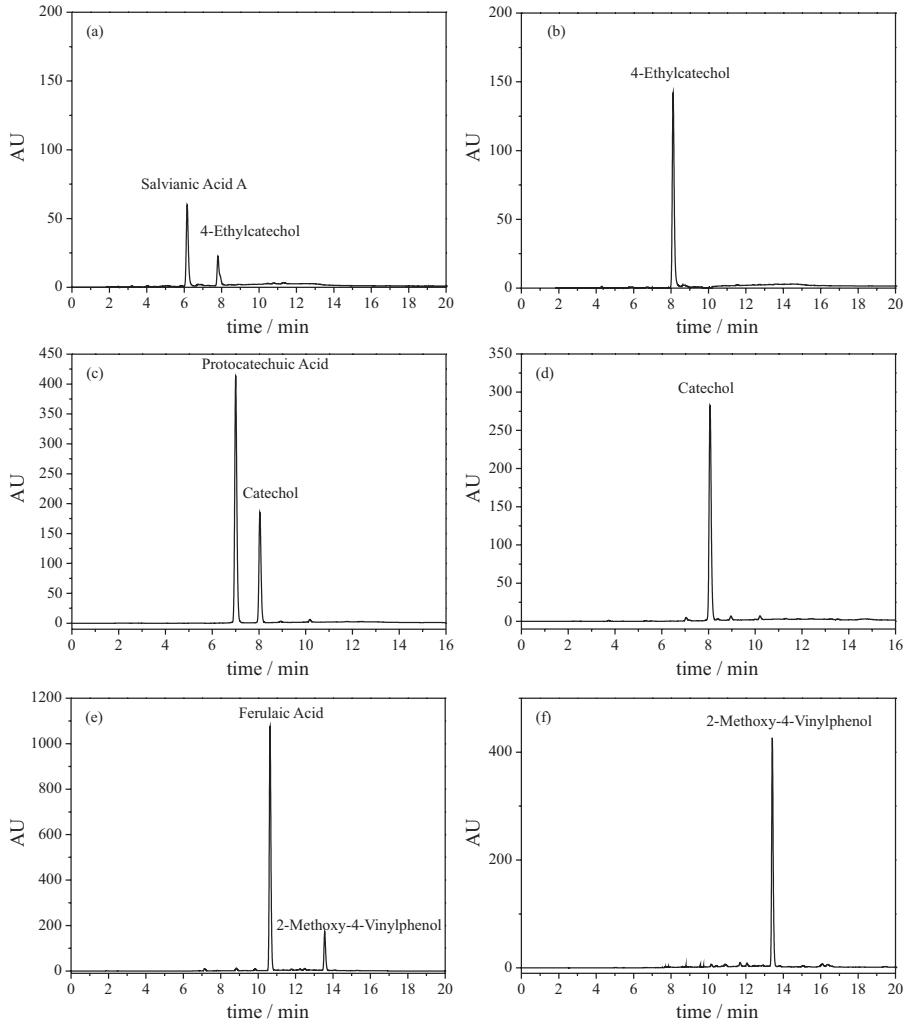

Figure 2. HPLC of water-phenolic acid mixture (a) salvianic acid A: 250 °C, 40 min; (b) salvianic acid A: 350 °C, 40 min; (c) protocatechuic acid: 250 °C, 40 min; (d) protocatechuic acid: 250 °C, 60 min; (e) ferulaic acid: 250 °C, 40 min; (f) ferulaic acid: 300 °C, 40 min.

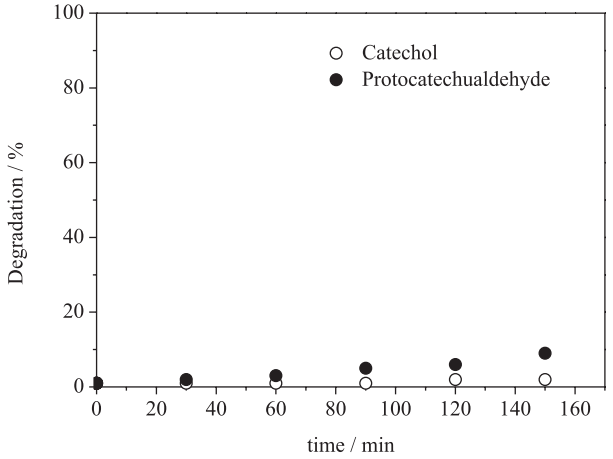

Figure 3. Effect of heating time on the decomposition of catechol and protocatechualdehyde in HTW at 350 °C.

by comparing the chromatograms obtained at 40 and 60 min. The higher the decomposition of protocatechuic acid was, the greater the yield of catechol. Figure 2e and 2f revealed that 2-methoxy-4-vinylphenol is the main decomposition product of ferulaic acid at high temperatures.

The identification of the decomposition products was identified by comparing their ultraviolet absorption with standard compounds and further studied by HPLC/MS analysis. When the ionization mode was negative, the electrospray ionization mass spectrometry (ESI/MS) spectra of the decomposition product are given in Supplementary Information (Figure S1-S3, in the Supplementary Information (SI) section). Figure S1 shows that the molecular weight was mainly 137.0 g mol^{-1}, which is in agreement with the molecular weight of 4-ethylcatechol. Figure S2 shows that the molecular weight was 149.1 g mol^{-1}, which is in agreement with the molecular weight of 2-methoxy-4-vinylphenol. Figure S3 shows that the molecular weight was 109.0 g mol^{-1}, which is in agreement with the molecular weight of catechol. Based on both retention time and the MS spectra of the decomposition products, the HPLC/MS analyses further confirmed the decomposition products

determined by HPLC as discussed above. As already known, catechol and protocatechualdehyde are much more stable in HTW. Therefore, the identification of catechol and protocatechualdehyde decomposition products were performed after heating at 350 °C. Efforts were also made to characterize the decomposition products of catechol and protocatechualdehyde by MS. However, the results obtained were not conclusive. Further characterization of the decomposition products formed in these experiments was not undertaken.

Based on the results in Figure 4, it is possible to note that the decomposition reaction of salvianic acid A started with the decarboxylation and dehydration reaction. The main decomposition product of protocatechuic acid and ferulaic acid is the result of the loss of carbon dioxide. In other words, the carboxyl group in each acid was replaced by the hydrogen originally attached to the carboxyl group. This has been reported that *ortho*-substituted benzoic acids undergo decarboxylation in HTW.[31] The transition state of a water molecule bridging the hydroxyl and amino groups was created. Lindquist and Yang[30] has also found

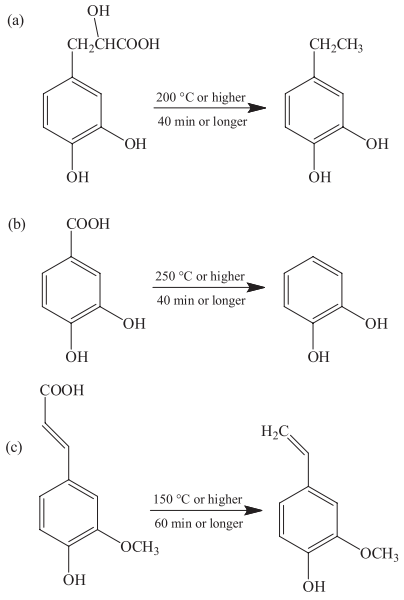

Figure 4. Decomposition reactions of three phenolic acid compounds: (a) salvianic acid A; (b) protocatechuic acid; and (c) ferulaic acid.

the decarboxylation of benzoic acid and its derivatives in HTW. Their experimental results agree well with the above computational reaction model. Based on these results, we concluded that salvianic acid A, protocatechuic acid and ferulaic acid have the carboxyl groups, which facilitate the thermal decarboxylation of the carboxyl group for each phenolic acid compound in HTW. However, catechol and protocatechualdehyde may lack activating groups, which may account for its exceptional stability.

In order to better indicate the decomposition of the three phenolic acid compounds in HTW, the percent yield (%) of the decomposition products was quantified by HPLC analysis. The comparison of the percent decomposition of each phenolic acid compound and the percent yield of each degradant is shown in Table 1.

The results of Table 1 are obtained from single measurement. From the data, it can be seen that the yield of the degradant for each phenolic acid is increased with decomposition of the corresponding phenolic acid compound. However, the percent decomposition for each phenolic acid compound is higher than the percent yield of degradant. From Figures 2-4, it can be seen that there exists other small decomposition products, which might be caused by the decomposition of the degradants.

High temperature water extraction of *Salvia miltiorrhiza* Bunge

HTWE of *Salvia miltiorrhiza* Bunge was conducted at only 100 and 150 °C because higher temperatures caused significant decomposition of five phenolic compounds as discussed above. *Salvia miltiorrhiza* Bunge is a perennial plant, which contains phenolic compounds, such as salvianic acid A, protocatechuic acid, and protocatechualdehyde.

Table 2 lists the phenolic compounds concentrations found in water extracts at two different temperatures at 40 min. The results of Table 2 are obtained from single measurement. The phenolic compound concentrations found in water extracts at 150 °C are generally equal to those at 100 °C due to the relative stability of phenolic compounds at higher temperature. The phenolic compound

Table 1. Comparison of the decomposition of three phenolic acid compounds and the yields of their decomposition products after heating at 250-350 °C for 40 min

Reactant	Decomposition / %	Degradant	Yield / %
Salvianic acid A, 250 °C	31	4-ethylcatechol	26
Salvianic acid A, 350 °C	97	4-ethylcatechol	81
Protocatechuic acid, 250 °C	35	catechol	27
Protocatechuic acid, 300 °C	99	catechol	93
Ferulaic acid, 250 °C	33	2-methoxy-4-vinylphenol	15
Ferulaic acid, 300 °C	98	2-methoxy-4-vinylphenol	78

concentration range from trace amount for catechol and ferulaic acid to 9.53 mg^{-1} g for salvianic acid A. In order to evaluate the accuracy of proposed method for determination of phenolic compounds in plant material, a recovery test had been performed.

Table 2. Phenolic compound concentrations of *Salvia miltiorrhiza* Bunge found in HTWE at 40 min and the recovery at 100 °C

	100 °C	150 °C	Recovery / %
	Concentration / (mg g^{-1} *Salvia miltiorrhiza* Bunge)		
Salvianic acid A	9.53	9.46	91.8
Protocatechuic acid	0.054	0.059	93.4
Protocatechualdehyde	0.46	0.41	90.8
Ferulaic acid	trace	trace	94.7
Catechol	trace	trace	98.7

Conclusions

The stability of five phenolic compounds in HTW was studied and the decomposition products were identified. The results indicated that salvianic acid A, protocatechuic acid, and ferulaic acid with the carboxyl groups undergo decarboxylation to form 4-ethylcatechol, catechol and 2-methoxy-4-vinylphenol, respectively. However, catechol and protocatechualdehyde without the carboxyl groups were relatively stable and need more severe situations to decompose. These results led to the development of a HTW extraction method for extracting the studied phenolic compounds from herbs or plants. The application of HTWE is promising and should be further studied.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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