Purification and ultra-high-performance liquid chromatography tandem mass spectrometry analysis of phenolics extracted from male walnut flowers

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ABSTRACT
In this study, phenolics extracted from male walnut flowers were purified and analyzed by ultra-high-performance liquid chromatography tandem mass spectrometry. After ultrasonic extraction of male walnut flowers with 60% ethanol, AB-8, D3520, NKA-9, D101, and NKA macroporous adsorption resins were compared for purification. The AB-8 macroporous resin gave the best adsorption and desorption performance for 0.7 mg gallic acid equivalents/mL samples (pH 3.0) loaded at a flow rate of 1 mL/min. Elution was carried out with 50% ethanol without pH adjustment. The purity of the phenolics in the eluate (72.5%) was 3.1 times that in the original extract. Analysis by ultra-high-performance liquid chromatography tandem mass spectrometry identified 16 compounds, including phenolic acids, flavonoids, and coumarins in the purified phenolics. The most abundant compounds were chlorogenic acid, kaempferol, and methoxycoumarin. Two compounds were got in male walnut flowers for the first time, and those need yet to be proved by further experiments.

Introduction
Male walnut flowers are one of the main by-products of the growth of walnuts, and their annual production volumes are comparable to those of walnuts. Male walnut flowers are rich in vitamins, minerals, dietary fiber, and multiple bioactive compounds. There is a long history of consumption of male walnut flowers in stir-fries, cold dishes, and soups in China, India, and Greece. The male walnut flowers have a fresh and crisp taste and aromatic flavor. However, the flowers brown soon after harvesting whether they are kept at room temperature or refrigerated, and this affects their quality, appearance, and commercial value.

Phenolics in fruits and vegetables are one of the main causes of browning through oxidative polymerization. Phenolics are classified as flavonoids and non-flavonoids according to the presence or absence of C6–C3–C6 in their backbone. The key to oxidation of phenolics is the B ring in the skeleton (Figure 1a). Phenolics containing catechol, pyrogallol, and hydroquinone groups are more susceptible to oxidation because they are able to form stable radical anions after oxidation and have high electron densities at the adjacent and opposite positions. The aromatic ring in a polyphenol is activated by hydroxyl groups, leading to enhanced reactivity. The main phenolics containing these functional groups are...
gallic acid (GA), catechins, caffeic acid and its esters, and quercetin.\textsuperscript{[5]}  Monophenols, resorcinols, and substituted phenols (especially methoxy derivatives) are not easily oxidized because they cannot produce stable semiquinone radicals.\textsuperscript{[6]}  Phenols also have good antioxidant\textsuperscript{[7]} and anti-aging properties\textsuperscript{[8]} because of the high reactivity of hydroxyl groups and their ability to scavenge free radicals. The catechol structure and the binding of multiple hydroxyl groups with electron donating groups at the fourth position of the aromatic ring could enhance the antioxidant effect of phenols.\textsuperscript{[9]}

Simple phenolics can form different dimers via hydroxyl groups at neighboring or opposite positions. A dimer or polymer produced by oxidative coupling has a lower redox potential than the initial phenolic, and therefore is more easily oxidized. Thus, phenolics that are more easily dimerized are more susceptible to oxidative polymerization\textsuperscript{[10]} and produce large polymers (Figure 1b).

The total phenolics content in male walnut flowers can reach 1350.77 ± 44.58 mg GA equivalents (GAE)/g.\textsuperscript{[11]}  However, the effect of crude extraction is often not good, there are low content of target components, impurities and other shortcomings, need to further purification of crude products. The purity of different compounds can be improved by adsorption-desorption of macroporous resin by virtue of its porous structure, Van der Waals force and intermolecular hydrogen bond,\textsuperscript{[12]} it has many advantages such as high efficiency, energy saving and simple operation\textsuperscript{[13]} making it widely used in the purification of natural active ingredients. In this study, high sensitivity and high resolution UHPLC-MS/MS method was used to analyze the purified polyphenols of walnut male flower,\textsuperscript{[14,15]} and to explore the main phenolic compounds in walnut male flower, which is not only of great significance for the development of antioxidant active components. This knowledge can also be used to prevent the browning during the development and use of male walnut flowers and contribute to the commercialization of male walnut flowers.

Figure 1. (a) The flavonoid skeleton. (b) Reaction between two semiquinones or a quinone and a phenolic to generate oligomers.
**Materials and methods**

**Materials and reagents**

Male walnut flowers (Lvling), Hebei Lvling Co., Ltd. Production base: GA standard, Folin–Ciocalteu reagent, and chromatographic grade acetonitrile (Sigma–Aldrich). Macroporous adsorption resins (Solarbio, Inc.). Analytical grade absolute ethanol, anhydrous sodium carbonate, ethyl acetate, methanol, concentrated hydrochloric acid, and sodium hydroxide.

**Instruments**

Agilent-1100 high-performance liquid chromatograph (Agilent, Technologies, Santa Clara, CA, United States). Q Exactive Plus Orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific, Massachusetts, USA). Low temperature centrifuge (Eppendorf, Hamburg, Germany). UV-visible spectrophotometer (UV-1700pc, Meitong Instrument Analysis Co, China, Shanghai).

**Total polyphenol content**

The total polyphenol content (TPC) was determined using the Folin–Ciocalteu colorimetric method. An aliquot (2 mL) of sample solution was mixed with 0.8 mL of Folin–Ciocalteu reagent and then incubated for 3 min. Next, 4 mL of 10% Na₂CO₃ solution was added and the sample was mixed thoroughly before incubation at 50°C for 1 h in the dark. The absorbance was measured at 765 nm. A standard curve was plotted and the regression equation was $y = 0.02147x + 0.00808$, $R^2 = 0.9992$.

**Extraction of crude phenolics**

A sample (2.0 g) of male walnut flowers was added to 50 mL of 60% (v/v) ethanol and ultrasonicated at 60°C for 60 min. The phenolic crude extract was obtained after filtration and concentration, and then stored in a sealed container at −20°C in the dark.

**Purification of phenolics from male walnut flowers**

Screening of macroporous adsorbent resins: After pretreatment, 2.0 g samples of different resins were placed in separate conical flasks, and 50 mL of the crude extract containing 1.0 mg GAE/mL phenolics was added. The flasks were incubated at 25°C with shaking at 120 r/min for 24 h. After this time, the supernatant was discarded, and the phenolics were desorbed from the resin by washing with 50 mL of 60% ethanol for 24 h. The adsorption (A) and desorption(D) rates were calculated as follows:

$$A(\%) = \frac{C_0 - C_1}{C_0} \times 100\%$$

(1)

$$D(\%) = \frac{C_2}{C_0 - C_1} \times 100\%$$

(2)

where $C_0$ is the phenolics concentration before adsorption (mg GAE/mL), $C_1$ is the phenolics concentration after adsorption (mg GAE/mL), and $C_2$ is the phenolics concentration after desorption (mg GAE/mL).

**Static adsorption kinetics**: Samples (2.0 g) of the macroporous adsorption resins were placed in separate conical flasks. Next, 50 mL of 1.0 mg GAE/mL phenolics solution was added and the flasks were incubated at 25°C with shaking at 120 r/min for 12 h. The concentrations of phenolics in the samples were measured at 60 min intervals. The adsorption (Q) and desorption (P) amounts (mg GAE/g) were calculated as follows:

$$Q(\text{mg/g}) = \frac{(C_0 - C_1) \times V}{W}$$

(3)

$$P(\text{mg/g}) = \frac{C_2 \times V}{W}$$

(4)

where $V$ is the volume of the adsorbent and eluent (mL), and $W$ is the resin mass (g).

**Adsorption isotherms**: Aliquots (50 mL) of phenolics extracts with concentrations of 0.31, 0.61, 0.69, 0.92, 1.14, 1.26, 1.74, and 1.96 mg GAE/mL were transferred to separate conical flasks containing 2.0 g of adsorbent resin and incubated at 25°C with shaking at 120 r/min for 6 h. The phenolics
concentration at adsorption equilibrium \((C_e)\) was measured and the adsorption amount was calculated. To further study the adsorption characteristics, the adsorption isotherm was fitted using the Langmuir and Freundlich isotherm models, which are described by Eq. 5 and 6\(^{[19,20]}\)

\[
\ln\left(\frac{Q_e}{Q_e - Q_t}\right) = Kt \tag{5}
\]

\[
\frac{Q}{Q_m} = \frac{bC_e}{1 + bC_e} \tag{6}
\]

where \(Q_m\) is the saturation adsorption amount (mg GAE/g), \(b\) is the adsorption equilibrium constant, \(C_e\) is the equilibrium concentration (mg GAE/mL), \(Q\) is the equilibrium adsorption amount (mg GAE/g), and \(n\) and \(k\) are the adsorption constants.

Adsorption rate constant: The adsorption rate of a macroporous resin can be evaluated by plotting a graph of \(-\ln(1 - F)\) against time.\(^{[21]}\) The adsorption rate constant \(K\) of each macroporous adsorption resin was calculated using Eq. 7.

\[
\ln Q = \frac{1}{n} \ln C_e + \ln k \tag{7}
\]

where \(Q_t\) is the amount adsorbed on the resin at time \(t\) (mg GAE/g), \(Q_e\) is the amount adsorbed on the resin at equilibrium (mg GAE/g), and \(K\) is the adsorption rate constant. For the graph, \(F\) is equal to \(Q_t / Q_e\).

Effects of different parameters on the adsorption and desorption: Adsorption was carried out with phenolics extracts \((C_0)\) at different pH values (2.0, 3.0, 4.0, 5.0, 7.0, and 8.0) using 1 mol/L NaOH or HCl for pH adjustment. The polyphenol concentration \(C_1\) was recorded after reaching adsorption equilibrium to investigate the relationship between the amount adsorbed and the sample pH. After adsorption, the resin was eluted with ethanol/water mixtures of different volume fractions (20%, 40%, 50%, 60%, 80%, and 100%). The polyphenol concentration after desorption \((C_2)\) was recorded to investigate the relationship between desorption and the eluent.

Dynamic adsorption and elution: Pretreated resin (10 g) was wet loaded onto a column. The crude extract of male walnut flowers (pH 3.0, 0.7 mg GAE/mL) was loaded onto the column at a flow rate of 1 mL/min for dynamic adsorption. Fractions of the effluent were collected and the phenolics concentrations were used to plot a dynamic adsorption curve. After washing the column with water at room temperature to remove any water-soluble substances, the resin was eluted with 50% ethanol. The eluate was collected to determine the concentration of phenolics, and a dynamic elution curve was plotted.

Purification of male walnut flower phenolics: Under the optimum purification conditions, the crude extract of male walnut flower phenolics was purified using the AB-8 macroporous adsorption resin, concentrated under reduced pressure, and lyophilized to obtain a powder. The purity \((P)\) of the powder was calculated as follows:

\[
P(\%) = \frac{C \times V}{m} \times 100\% \tag{8}
\]

where \(C\) is the TPC of the sample solution (mg GAE/mL), \(V\) is the sample volume (mL), and \(m\) is the mass of the powder (mg).

Analysis of phenolic compounds

The purified male walnut flower phenolics were dissolved in 50% methanol and sonicated for 30 min. After centrifugation at 14,000 rpm for 5 min, the supernatant was filtered through a 0.22 \(\mu\)m filter and transferred to a vial for ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). UPLC system: Dionex Ultimate 3000. Column: DiKMA Platsil ODS (250 x 4.6 mm, 5 \(\mu\)m). Mobile phase: acetonitrile (A) and 0.1% formic acid/water (v/v)(B), introduction of formic acid decrease the suppression of ESI signal and provide better efficiency (A and A et al.)\(^{[22]}\). Column temperature: 30°C. Flow rate: 0.5 mL/min. Injection volume:
10 μL. Detection wavelength: 280 nm. Gradient elution: 0–2 min, 8%–10% A; 2–27 min, 10%–30% A; 27–50 min, 30%–90% A; 50–52 min, 90%–100% A; 52–56 min, 100% A; and 56–60 min, 100%–8% A.

Mass spectrometry system: Q Exactive Orbitrap high-resolution mass spectrometer. Scan range: m/z 100–1200. Resolution: 70,000. Sheath gas flow rate: 40 arb. Auxiliary gas flow rate: 15. Spray voltage: 3.20 kV. Capillary temperature: 320°C. Auxiliary gas heater temperature: 350°C.

Data processing
Data are expressed as means ± standard deviations. The data were analyzed using Xcalibur 4.1 and SPSS 22 software. Origin 86 software was used for graphing.

**Results and discussion**

Screening of macroporous adsorption resins
Comparison of different macroporous resins for extraction of walnut phenolics: Resins can be screened using their adsorption and desorption properties. Macroporous adsorbent resins with different polarities, pore sizes, and specific surface areas have different adsorption and desorption capacities for phenolics. In this study, AB-8, D3520, NKA-9, D101, and NKA resins were screened (Figure 2a). The D3250 and AB-8 resins had the best adsorption rates of 64.66% and 64.38%, respectively. The resolutions for the D3250 and AB-8 resins were 89.55% and 85.92%, respectively. Although the D101 resin had a high resolution, it had the lowest adsorption rate of 54.94%, and was not suitable for this application. After considering the adsorption rates and resolutions of the macroporous resins, AB-8 and D3250 were selected for further experiments.

Comparison of the adsorption kinetics of the AB-8 and D3250 resins: Adsorption kinetics are important to consider when selecting equipment and designing processes. With both the AB-8 and D3250 resins, the amount of male walnut flower phenolics adsorbed increased over time and then leveled off after 3 h as the resins reached saturation (Figure 2b). The K values of the AB-8 and D3250 resins were 0.92 and 0.75, respectively. Phenolics contain multiple hydroxyl groups, and some phenolic derivatives contain sugar groups and are polar. The AB-8 resin is slightly more polar than the D3250 resin, and therefore has a higher K value. Taking into considering the adsorption rate, adsorption effect, and time, the AB-8 macroporous adsorption resin was more suitable than the D3250 resin for the separation and purification of phenolics from male walnut flowers.

Adsorption isotherm with the AB-8 resin: An adsorption isotherm describes the relationship between the equilibrium concentration of an adsorbate and the equilibrium adsorption amount of an adsorbent at a certain temperature. The adsorption isotherm for male walnut flower phenolics on the AB-8 macroporous resin was measured at 25°C. The adsorption isotherm showed the adsorption of phenolics on the resin initially increased with increases in the phenolics concentrations (Figure 2c). Above 1.74 mg GAE/mL, the adsorption decreased as the adsorbent reached saturation.

Langmuir and Freundlich models are often used to describe adsorption isotherms because of their relative simplicity and reasonable accuracy. The Langmuir model assumes that there are no interactions between adsorbates and that the adsorption occurs only in a localized monomolecular layer on the adsorbent. The Freundlich model is an empirical model for non-ideal adsorption on heterogeneous surfaces and where many different types of interaction sites act simultaneously giving both monolayer and multilayer adsorption. The adsorption equations of the AB-8 resin were obtained by plotting 1/Q against 1/C_e (Eq. 5) and lnQ against lnC_e (Eq. 6) (Table 1). The correlation coefficient of the Langmuir model was higher than that of Freundlich model, and in the Freundlich model, 1/n was less than 1, which indicated that the adsorption was mainly monolayer adsorption. This meant that the adsorption increased with increases in the concentration of the adsorbate, but did not exceed the saturation adsorption capacity of the
The $1/n$ was 0.565, which indicated that the adsorbent could adsorb solutes relatively easily. Therefore, the AB-8 macroporous resin had a good adsorption effect for phenolics from male walnut flowers.

Effect of sample properties on the adsorption capacity

Effect of sample pH: The pH of a sample solution plays an important role in the resin adsorption process because it can influence solute ionization, which affects the adsorption characteristics of the solute on the resin.\[28\] The adsorption capacity of the resin decreased with increases in pH (Figure 3a), which was consistent with the results of.\[29\] The decrease in the adsorption rate could be explained by ionization of phenolic hydroxyl groups at high pH.\[30\] A pH of 3.0 was favorable for the adsorption of male walnut flower phenolics by the resin.

Table 1. Adsorption isotherm equations.

| Adsorption isotherm equation | Adsorption equation | parameter | Correlation coefficient $R^2$ |
|-----------------------------|---------------------|-----------|-------------------------------|
| Langmuir                    | $Q = \frac{102.04C_e}{(1 + 3.4796C_e)}$ | $Q_m = 29.3255; b = 3.4796$ | 0.9748                        |
| Freundlich                  | $Q = 27.6520C_e^{0.5648}$      | $n = 1.77; k = 27.6520$      | 0.9665                        |
**Figure 3.** Factors affecting adsorption and desorption. (A) Effect of sample pH on the adsorption performance. (B) Influence of ethanol concentration on the desorption. (C) Dynamic adsorption curve. (D) Dynamic desorption curve.

Effect of ethanol concentration: The concentration of ethanol affected desorption of phenolics from the AB-8 resin. Desorption increased with increases in the ethanol concentration from 20%–50% (Figure 3b). This might be caused by enhancement of breaking of hydrogen bonds. When the ethanol concentration was higher than 50%, the desorption of phenolics decreased. At this point, the polarity difference between the desorbent and the phenolics increased, and the phenolics could not be fully desorbed. It is also possible that some impurities were desorbed by the concentrated ethanol, resulting in a decrease in desorption. Considering the cost and desorption rate, 50% ethanol was selected as the optimum concentration for elution.

Dynamic adsorption and elution of male walnut flower phenolics by AB-8

Dynamic adsorption curve: When the sample loading volume of the male walnut flower phenolics extract reached 30 mL, phenolics were detected in the effluent (Figure 3c). After this, the phenolics content in the effluent gradually increased until the sample loading volume was approximately 400 mL. At this point, adsorption on the resin basically reached equilibrium, which indicated that the resin had reached saturation. Many adsorption/desorption cycles for sample loading and multiple columns in tandem could be used to improve the adsorption on the macroporous resin.

Dynamic elution curve: The phenolics adsorbed on AB-8 were easily eluted under dynamic elution conditions (Figure 3d). The desorption peaks were relatively concentrated, with symmetrical peak shapes and no obvious trailing. When the eluent volume reached 10 mL, the phenolics began to elute, and the content of phenolics first increased and then decreased as the eluent volume increased. The
concentration of phenolics peaked at 5.0 mg GAE/mL with 30 mL of eluent, and all phenolics were eluted with 100 mL of eluent. The eluate collected between 10 and 100 mL was combined, concentrated, and lyophilized to obtain the purified male walnut flower phenolics. The phenolics contents of the crude and purified eluates of male walnut flower were measured. The purities before and after purification were 23.2% and 72.5%, respectively. The purity after purification was more than three times that before purification, which showed that purification by the AB-8 macroporous resin enriched male walnut flower phenolics.

**UHPLC-MS/MS**

The positive and negative total ion chromatograms (Figure 4a and 4b) obtained using UHPLC-MS/MS showed that there were 16 main compounds in the male walnut flower phenolics (Table 2). The phenolic acid content in the male walnut flowers was high, and most of the phenolics had an easily oxidized o-hydroxy structure. The contents of chlorogenic acid, kaempferol, and 5,8-dihydroxy-4-oxo-1,2,3,4-tetrahydro-1-naphthalenyl 6-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranoside were relatively high at 27.78%, 19.59%, and 15.54%, respectively. This is the first report to get 5,8-dihydroxy-4-oxo-1,2,3,4-tetrahydro-1-naphthalenyl 6-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranoside (peak 9) and (Z)-3-(4-hydroxyphenyl)-N-[4-[[[(E)-3-(4-hydroxyphenyl)prop-2-enoyl]amino]butyl]prop-2-enamide (peak 14) in male walnut flowers. The relative content of peak 14 was 5.05%. The excimer peak of peak 9 was m/z 507 [M-H]⁻, which gave a molecular weight of 508. Its secondary mass
Table 2. Mass spectrometry data for the main compounds in the male walnut flowers.

| Peak | Identification | Retention time (min) | Molecular formula | Measured mass (m/z) | Fragment ions MS² (m/z) | References |
|------|----------------|----------------------|-------------------|---------------------|------------------------|------------|
| 1    | Epigallocatechin | 15.28                | C₁₅H₁₄O₇          | 305.07              | 179, 165, 125          |            |
| 2    | Chlorogenic acid | 20.20                | C₁₆H₁₈O₉          | 353.09              | 191, 173, 135          | [32]       |
| 3    | Cryptochlorogenic acid | 20.92 | C₁₆H₁₈O₉          | 353.09              | 191, 179, 135          | [33]       |
| 4    | 7-Methoxycoumarin | 22.62                | C₁₀H₈O₃           | 177.05              | 149, 121, 105          | -          |
| 5    | 1-Caffeoylquinic acid | 23.09 | C₁₆H₁₈O₉          | 353.09              | 191, 179, 135          | [34]       |
| 6    | Isoquercitrin    | 24.45                | C₁₂H₁₀O₆          | 465.10              | 303                    | [35]       |
| 7    | Coumarin         | 24.74                | C₈H₆O₂     | 147.04              | 119, 91                | [36]       |
| 8    | Kaempferol       | 26.62                | C₁₅H₁₀O₆          | 287.05              | 153                    | [37,38]    |
| 9    | 5,8-Dihydroxy-4-oxo-1,2,3,4-tetrahydro-1-naphthalenyl 6-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranoside | 30.66 | C₂₃H₂₄O₁₃ | 507.12 | 331, 271, 211, 169, 125, 107 | - |
| 10   | Ellagic acid     | 30.68                | C₁₄H₆O₈           | 300.99              | 284, 257, 245          | [39]       |
| 11   | Quercitrin       | 33.23                | C₁₂H₁₀O₁₁         | 447.60              | 301, 283               | [40]       |
| 12   | 3,5-Dicaffeoylquinic acid | 34.01 | C₂₅H₂₄O₁₂         | 515.12              | 353, 191, 179          | [41]       |
| 13   | afzelin          | 35.96                | C₂₁H₂₀O₁₀         | 431.10              | 285                    | [42]       |
| 14   | (2)-3-(4-hydroxyphenyl)-N-4-[[(E)-3-(4-hydroxypheny]prop-2-enoyl] amino]butyl]prop-2-enamide | 37.25 | C₂₂H₂₄N₂O₄      | 381.18              | 219, 147, 119, 91      | -          |
| 15   | Ferulic acid     | 39.47                | C₁₀H₁₀O₄          | 195.06              | 177, 164, 149          | [M. and 43]|
| 16   | Ethyl caffeate   | 41.44                | C₁₁H₁₂O₄          | 207.07              | 179, 135, 134          | [44]       |

The spectrum showed characteristic fragments at m/z 331, 271, 211, 169, 125, and 107. The fragment at m/z 331 was for β-glucogallin and that at m/z 169 was for gallic acid. The fragments at m/z 125 and 107 are characteristic fragments of gallic acid, with that at m/z 125 obtained by loss of one molecule of CO₂ from gallic acid. This is followed by loss of one molecule of water to produce the fragment at m/z 107. The fragment at m/z 211 can be obtained from m/z 331 after loss of C₆H₄O₄, and peak 9 is presumed to be a GA-like substance. The excimer peak of peak 14 is m/z 381 [M + H]^+ with characteristic fragments in the secondary mass spectrum at m/z 219, 147, 119 and 91. The parent ion lost one molecule of glucose to give m/z 219 [M + H-glu]^+, and fragment m/z 147 lost one molecule of CO to give coumaric acid m/z 119. This was followed by loss of CO to give m/z 91, and peak 14 was presumed to be a coumarin-like substance.

Conclusion

Among five macroporous resins investigated for purification of phenolics from male walnut flower phenolics, AB-8 was the best resin. The static adsorption and desorption rates with this resin were 64.38% and 85.92%, respectively. Adsorption equilibrium was reached within 3 h. The purity of the phenolics after processing with the resin was 72.5%. The main compounds in the phenolics extract were chlorogenic acid, ellagic acid, ethyl caffeate, kaempferol, and coumarin. Chlorogenic acid had the highest content. Two previously unreported phenolics were identified. The results suggest that male walnut flowers are a rich source of bioactive phenolics. Browning of the flowers could be solved by reducing the contents of easily oxidized phenolics, adding antioxidants to reduce quinones to polyphenols, and isolating samples from oxygen to avoid contact with phenolics. These steps could be used to maintain the original color and quality of male walnut flowers.
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Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Chenchen Yu: Conceptualization, Investigation, Methodology, Resources, Writing - original draft. Shengyun Li: Investigation, Formal analysis. Xuemei Zhang: Provided experimental materials. Aijin Ma: Thesis design. Zhixiang Cao: Investigation, Software. Guohui Qi: Provided experimental tasks. Suping Guo: Provided experimental materials. Yiling Tian: Conceptualization, Writing - review & editing, Supervision.

References

[1] Soumya R. P. Sandeep S. R. Rubeka I. Vasudha S. Payel G. A review on nutritional, bioactive, toxicological properties and preservation of edible flowers. Future Foods. 2021, 4,100078, https://doi.org/10.1016/j.fuofo.2021.100078
[2] Osmont, M. N. L.; Caggia, M.; Polard, E.; Riou, C.; Balusson, F.; Oger, E., et al. Utilisation du PMSI pour la Détection d’Effets Indésirables Médicamenteux. [Use of the PMSI for the Detection of Adverse Drug Reactions]. Thérapie.2013, 68(4), 285–295. DOI: 10.2515/therapie/2013042.
[3] De Paepe, D.; Coudijzer, K.; Noten, B.; Valkenborg, D.; Servaes, K.; De Loose, M.; Diels, L.; Voorspoels, S.; Van Droogenbroeck, B., et al. A Comparative Study between spiral-filter Press and Belt Press Implemented in A Cloudy Apple Juice Production Process. Food Chem. 2015, 173, 986–996. DOI: 10.1016/j.foodchem.2014.10.019.
[4] Ma, L.; Waterhouse, A. L. Flavanols React Preferentially with Quinones through an Electron Transfer Reaction, Stimulating Rather than Preventing Wine Browning. Anal. Chim. Acta. 2018, 1039, 162–171. DOI: 10.1016/j.aca.2018.07.013.
[5] Li, H.; Guo, A.; Wang, H., et al. Mechanisms of Oxidative Browning of Wine. Food Chem. 2007, 108(1), 1–13. DOI: 10.1016/j.foodchem.2007.10.065.
[6] Azahara, L.; Manuel, M.; Merida, J.; Medina, M., et al. Yeast-induced Inhibition of (+)-catechin and (-)-epicatechin Degradation in Model Solutions. J. Agric. Food. Chem. 2002, 50(6), 1631–1635. DOI: 10.1021/jf0109930.
[7] Fiore, M.; Messina, M. P.; Petrella, C.; D’Angelo, A.; Greco, A.; Ralli, M.; Ferraguti, G.; Tarani, L.; Vitali, M.; Ceccanti, M., et al. Antioxidant Properties of Plant Polyphenols in the Counteraction of alcohol-abuse Induced Damage: Impact on the Mediterranean Diet. J. Funct. Foods 2020, 71, 104012. DOI: 10.1016/j.jff.2020.104012.
[8] Russo, G. L.; Spagnuolo, C.; Russo, M.; Tedesco, I.; Moccia, S.; Cervellera, C., et al. Mechanisms of Aging and Potential Role of Selected Polyphenols in Extending Healthspan. Biochem. Pharmacol. 2020, 173, 113719. DOI: 10.1016/j.bcp.2019.113719.
[9] Xie, P.; Huang, L.; Zhang, C.-H.; Zhang, Y.-L., et al. Phenolic Compositions, and Antioxidant Performance of Olive Leaf and Fruit (Olea Europaea L.) Extracts and Their structure-activity Relationships. J. Funct. Foods 2015, 16, 460–471. DOI: 10.1016/j.jff.2015.05.005.
[10] Mcdonald, P. D.; Hamilton, G. A. "On the Mechanism of Phenolic Oxidative Coupling Reactions. Ferricyanide Oxidation of 2,3,4-trihydroxybenzophenone, an Example of a Radical Aromatic Substitution Mechanism. J. Am. Chem. Soc. 1973, 95(23), 7752–7758. DOI: 10.1021/cent.197404142.
[11] Zhang, Y.; Kan, H.; Chen, S.-X.; Thakur, K.; Wang, S.; Zhang, J.-G.; Shang, Y.-F.; Wei, Z.-J., et al. Comparison of Phenolic Compounds Extracted from Diaphagma Juglandis Fructus, Walnut Pellicle, and Flowers of Juglans Regia Using Methanol, Ultrasonic Wave, and Enzyme assisted-extraction. Food Chem. 2020, 321, 126672.10.1016/j.foodchem.2020.126672. DOI: 10.1016/j.foodchem.2020.126672.
[12] Xiong, N.; Yu, R.; Chen, T.; Xue, Y.-P.; Liu, Z.-Q.; Zheng, Y.-G., et al. Separation and Purification of l-methionine from E.coli Fermentation Broth by Macroporous Resin Chromatography. J. Chromatogr. B 2019, 1110-1111, 108–115. DOI: 10.1016/j.jchromb.2019.02.016.
[33] Hong, C.; Chang, C.; Zhang, H.; Jin, Q.; Wu, G.; Wang, X., et al. Identification and Characterization of Polyphenols in Different Varieties of Camellia Oleifera Seed Cakes by UPLC-QTOF-MS. Food Res. Int. 2019, 126, 108614. DOI: 10.1016/j.foodres.2019.108614.

[34] Weisz, G. M.; Kammerer, D. R.; Carle, R., et al. Identification and Quantification of Phenolic Compounds from Sunflower (Helianthus Annuus L.) Kernels and Shells by HPLC-DAD/ESI-MS N. Food Chem. 2008, 115(2), 758–764. DOI: 10.1016/j.foodchem.2008.12.074.

[35] Carazzone, C.; Mascherpa, D.; Gazzani, G.; Papetti, A., et al. Identification of Phenolic Constituents in Red Chichory Salads (Cichorium Intybus) by high-performance Liquid Chromatography with Diode Array Detection and Electrospray Ionisation Tandem Mass Spectrometry. Food Chem. 2013, 138(2–3), 1062–1071. DOI: 10.1016/j.foodchem.2012.11.060.

[36] Faqueti, L. G.; Sandjo, L. P.; Biavatti, M. W., et al. Simultaneous Identification and Quantification of Polyphenol-based Formulas to Replace Sulfur Dioxide for Storage of Sparkling White Wine. Food Control 2016, 60, 606–614. DOI: 10.1016/j.foodcont.2015.09.005.

[37] Cai, Y.; Wu, L.; Lin, X.; Hu, X.; Wang, L., et al. Phenolic Profiles and Screening of Potential α-glucosidase Inhibitors from Polygonum Aviculare L. Leaves Using ultra-filtration Combined with HPLC-ESI-qTOF-MS and Molecular Docking Analysis. Ind. Crops Prod. 2020, 154, 112673. DOI: 10.1016/j.indcrop.2020.112673.

[38] Fracassetti, D.; Gabrielli, M.; Costa, C.; Tomás-Barberán, F. A.; Tirelli, A., et al. Characterization and Suitability of polyphenols-based Formulas to Replace Sulfur Dioxide for Storage of Sparkling White Wine. Food Control 2016, 60, 606–614. DOI: 10.1016/j.foodcont.2015.09.005.

[39] Pavlović, A. V.; Papetti, A.; Zagorac, D. Ć. D.; Gašić, U. M.; Mišić, D. M.; Tešić, Ž. L.; Natić, M. M., et al. Phenolics Composition of Leaf Extracts of Raspberry and Blackberry Cultivars Grown in Serbia. Ind. Crops Prod. 2016, 87, 304–314. DOI: 10.1016/j.indcrop.2016.04.052.

[40] Min, Z.; Jun, X.; Qian, D.; Guo, J.; Jiang, S.; Shang, E.-X.; Duan, J.-A., et al. Identification of Astilbin Metabolites Produced by Human Intestinal Bacteria Using UPLC-Q-TOF/MS. Biomed. Chromatogr.: BMC.2014, 28(7), 1024–1029. DOI: 10.1002/bmc.3111.

[41] Xia, J. X.; Zhao, B. B.; Zan, J.-F.; Wang, P.; Chen, L.-L., et al. Simultaneous Determination of Phenolic Acids and Flavonoids in Artemisiae Argyi Folium by HPLC-MS/MS and Discovery of Antioxidant Ingredients Based on Relevance Analysis. J. Pharm. Biomed. Anal. 2019, 175, 112734. DOI: 10.1016/j.jpba.2019.06.031.

[42] Neugart, S.; Rohn, S.; Schreiner, M., et al. Identification of Complex, Naturally Occurring Flavonoid Glycosides in Vicia Faba and Pismum Sativum Leaves by HPLC-DAD-ESI-MS N and the Genotypic Effect on Their Flavonoid Profile. Food Res. Int. 2015, 76(1), 114–121. DOI: 10.1016/j.foodres.2015.02.021.

[43] Antonia, M.; Hornedo-Ortega, R.; Cerezo, A. B.; Troncoso, A. M.; García-Parrilla, M. C., et al. Determination of Nonanthocyanin Phenolic Compounds Using High-Resolution Mass Spectrometry (UHPLC-Orbitrap-MS/MS) and Impact of Storage Conditions in a Beverage Made from Strawberry by Fermentation. J. Agric. Food Chem. 2016, 64(6), 1367–1376. DOI: 10.1021/acs.jafc.5b05617.

[44] Clifford, M. N.; Johnston, K. L.; Knight, S.; Kuhnert, N., et al. Hierarchical Scheme for LC-MS N Identification of Chlorogenic Acids. J. Agric. Food Chem. 2003, 51(10), 2900–2911. DOI: 10.1021/jf026187q.

[45] Sunil Kumar, P. C. V. B.; Kumar, B.; Bajpai, V.; Singh, A.; Srivastava, M.; Mishra, D. K.; Kumar, B. Rapid Qualitative and Quantitative Analysis of Bioactive Compounds from Phyllanthus Amarus Using LC/MS/MS Techniques. Ind. Crops Prod. 2015, 69, 143–152. DOI: 10.1016/j.indcrop.2015.02.012.

[46] Abu-Reidah, I. M.; Ali-Shayeh, M. S.; Jamous, R. M.; Arráez-Román, D.; Segura-Carretero, A., et al. HPLC-DAD -ESI-MS/MS Screening of Bioactive Components from Rhus Coriaria L. (Sumac) Fruits. Food Chem. 2015, 166, 179–191. DOI: 10.1016/j.foodchem.2014.06.011.