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## Original Article

# Comparative quality of the forms of decoction pieces evaluated by multidimensional chemical analysis and chemometrics: *Poria cocos*, a pilot study



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## ARTICLE INFO

## Article history:

Received 15 January 2019

Received in revised form

26 February 2019

Accepted 4 March 2019

Available online 21 March 2019

## Keywords:

Chemometric analyses

Forms

Multidimensional chemical evaluation

*Poria cocos* decoction pieces

## ABSTRACT

Many Chinese medicinal materials (CMMs) are parts of plants or fungi that have been processed into different physical forms, termed decoction pieces, that are typically boiled in water for consumption. One CMM may have several decoction pieces forms, e.g., slices, small cubes (dice), or grains. The specifications that have different morphological parameters (shape, size and thickness) for these various decoction pieces have been developed over, in some cases, centuries of practice. Nevertheless, whether and how the form of decoction pieces affects the extraction (decoction) dynamics, and quality stability during storage has not been studied. Here, we investigated *Poria cocos* (PC) as a pilot study; we explore how the form of PC decoction pieces affects its chemistry using multidimensional chemical evaluation such as ultra-performance liquid chromatography-photodiode array-quadrupole time-of-flight mass spectrometry (UHPLC-PDA-QTOF-MS/MS), ultra-performance liquid chromatography-triple quadrupole mass spectrometry (UHPLC-QqQ-MS/MS) and high performance gel permeation chromatography coupled with charged aerosol detector (HPGPC-CAD), combined with analysis of variance (ANOVA), principal component analysis (PCA), factor analysis (FA) and hierarchical cluster analysis (HCA). The results indicated that different specifications had significant differences, and these specifications could be divided into four groups. The comprehensive results of the chemical analyses undertaken here indicate that the highest potentially available quality of PC decoction pieces was in the forms of curl, ultra-small grains and small grains, followed by thin slices. This information not only is conducive to promoting the standardization of the specification/form of PC decoction pieces and maximizing the benefits from its utilization,

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<https://doi.org/10.1016/j.jfda.2019.03.002>

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but also provide a promising strategy for assessing other CMM decoction pieces in different forms.

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## 1. Introduction

Chinese medicinal materials (CMMs) are much appreciated in their long history of preventing and treating human disease [1,2]. Raw CMMs are generally dried parts of plants or fungi, such as roots, rhizomes and sclerotia [3–5]. In order to facilitate their clinical use, raw CMMs are usually processed to be different physical forms, termed decoction pieces. It is the decoction pieces that are used in clinical practice; typically, they are boiled or soaked in water or ethanol to generate decoctions, infusions, or tincture for oral administration [6–8]. During the long-term application of CMMs in different cultures and geographical regions, various forms of decoction pieces have been developed, typically including slices and dice [9]. Even as the same CMM, different forms/specifications of decoction pieces are also clinically prescribed. The specifications for a decoction piece have different morphological parameters, e.g., shape, size and thickness. For example, slices are normally rectangular or square; thin slices will have a thickness of 1–2 mm, while thick slices will have a thickness of 2–4 mm. Dice are cube-like blocks (8–12 mm) [9].

Bioactive chemicals, which comprise either primary or secondary metabolites (e.g., polysaccharides, terpenoids, alkaloids, and flavonoids), are the therapeutic basis of CMMs. Thus, the chemical profile, including both qualitative and quantitative aspects of a CMM, is closely related to its clinical efficacy [10,11]. Accumulating studies have indicated that multiple factors, such as the inherent quality of CMM, extraction solvent, and time of extraction, potentially affect the chemical profile extracted [5,12]. However, no published study has investigated whether and how the form of decoction pieces impacts extraction dynamics in general and the extracted chemical profile in particular. Furthermore, in addition to the extraction, quality stability during storage should also be considered for CMM decoction pieces; this too is unknown. That is, no studies have been done to determine if there is any correlation between the form of CMM decoction pieces and the stability of its quality over time. These issues are significant for producing and processing of CMM decoction pieces and maintaining its quality, thereby ensuring efficacy and safety of CMMs in clinical use. Nevertheless, to the best of our knowledge, no study has addressed these issues, especially those related to the interpretation of their results.

*P. cocos* (PC), derived from the dried sclerotium of the fungus *P. cocos* (Schw.) Wolf, is a widely-used edible CMM called fuling in Chinese and hoelen in Japan [5,13]. Due to its wide range of beneficial physiological activities, unique effects and good nutrition, PC is commonly used not only as a traditional Chinese medicine but also as a functional food and dietary supplement. It is an ingredient in biscuits, bread, cakes, tea, soups

and dishes in China, Korea, India and other southeast Asian and European countries [5,13]. Phytochemical research and our published study indicate that triterpenes and polysaccharides are two major components in PC [5,14], while modern pharmacological research demonstrates that triterpenes and polysaccharides in PC have a broad range of bioactivities including diuretic [15,16], anti-tumor [14,17,18], anti-bacterial [19], anti-inflammatory [20,21], anti-oxidant, cytotoxic [22], anti-emetic [23], and anti-hyperglycemic [24]. Among the triterpenes of PC, pachymic acid (PA), dehydrotumulosic acid (DTUA), tumulosic acid (TUA), dehydrotrametenolic acid (DTRA), poricoic acid A (PAA), poricoic acid B (PAB), polyporenic acid C (PAC), dehydroeburicoic acid (DEA) and eburicoic acid (EA), have been clearly demonstrated to be responsible for the major bioactivities of PC [5,14,15,19,21–24]. These triterpenoid acids and polysaccharides are thus considered as the important bioactive ingredients of PC and are also likely responsible for its taste, so they are usually selected as reference compounds for its quality evaluation [5,13,25]. PC is sold in four forms of decoction pieces: curl, slice, dice, and grain. According to our preliminary market investigation in Hong Kong, mainland China, and Japan, the curl is the major specification of PC decoction pieces used in Hong Kong; slice and dice are mainly used in mainland China; while grain is commonly used in Japan, called Koku in Japanese. Of these, the curl is prepared by firstly cutting raw PC into thin rectangular slices (length 8–10 cm, width 7–9 cm and thickness 1 mm), then softening them on hot bamboo slices and finally rolling them into thin tubes. Grains are obtained by pounding raw CMM and then screening the irregular granular decoction pieces into size categories (1–8 mm diameter).

Qualitative fingerprint profiling, quantitative determination of bioactive components and physical parameter analysis combined with chemometric analysis are a promising strategy for assessing different forms of PC decoction pieces. Ultra-performance liquid chromatography-photodiode array-quadrupole time-of-flight mass spectrometry (UHPLC-PDA-QTOF-MS/MS) has been increasingly used to conduct the qualitative and quantitative characterization in recent years for its rapid analysis, low solvent consumption and good separation performance [5,11,13]. In our published study, untargeted and targeted UHPLC-QTOF-MS/MS was used to explore the differences of secondary metabolites in the three botanical parts of PC [5]. Additionally, multiple reaction monitoring (MRM) mode performed on triple quadrupole mass spectrometry (QqQ-MS/MS), is a powerful quantitative method for dietary and herbal complex matrices by screening of assigned precursor ion-to-product ion pair to achieve high sensitivity and superior specificity [26]. Moreover, the key analytical approaches, UHPLC-PDA-QTOF-MS/MS-based fingerprinting characterization of

triterpenes and UHPLC-QqQ-MS/MS-based quantitative determination of nine triterpenoid acids followed by chemometric analysis such as PCA and HCA, were established to explore the correlation between collection locations and quality of raw PC in our recent study [26]. Therefore, UHPLC coupled with PDA-QTOF-MS/MS is very useful for qualitative fingerprint profiling based on secondary metabolites, while UHPLC coupled with QqQ-MS/MS is a powerful tool for simultaneous quantitative determination of multiple components.

In the present study, we used PC as a pilot study to investigate the effects of the decoction pieces forms on the extraction and quality stability using multidimensional chemical evaluation combined with chemometrics. First, from raw PC, we prepared the eight forms of decoction pieces that are commercially available. Second, the qualitative and quantitative differences in chemical profiles of triterpenoid acids and polysaccharides extracted from the different PC decoction pieces were characterized through hyphenated analytical techniques. Of these, UHPLC-PDA-QTOF-MS/MS was used for fingerprinting characterization of triterpenoid acids, while UHPLC-QqQ-MS/MS was applied to quantitative determination of nine bioactive triterpenoid acids. Third, the water-soluble and ethanol-soluble extracts, the drying rate of the decoction pieces, as well as the extraction rate of the polysaccharides were determined. Fourth, quality stability of different forms of PC decoction pieces was assessed by accelerated stability test and long-term stability test. Last, in order to further explore the differences in the forms of PC decoction pieces, the obtained multivariate data were statistically processed by chemometric analyses, including analysis of variance (ANOVA), principal component analysis (PCA), factor analysis (FA) and hierarchical cluster analysis (HCA). The study chemically evaluated the available quality of different forms of PC decoction pieces. The results provide not only the scientific basis for the standardization of producing and processing of PC decoction pieces but also effective approaches for assessing the chemically available quality of other CMM decoction pieces in various forms, with similar benefits.

## 2. Materials and methods

### 2.1. Plant materials

Raw PC was collected from Yunnan, the region famous for its production, and authenticated by Prof. Zhao Zhongzhen from

the School of Chinese Medicine, Hong Kong Baptist University. The collected PC was used to make 24 batches of eight different specifications; that is, three batches for each specification, marked as batch 1, 2 and 3. The specifications/forms were: curls (CUR), thin slices (TNS), thick slices (TKS), dice (DIC), large grains (LAG), medium-sized grains (MSG), small grains (SMG) and ultra-small grains (USG), as specified in Table 1 and Fig. 1. Voucher specimens were deposited in the Bank of China (Hong Kong) Chinese Medicines Centre of Hong Kong Baptist University.

### 2.2. Chemicals and reagents

Chemical markers of glucose, PA, TUA, DTUA, PAA, PAB, PAC, DTRA, EA and DEA, were purchased from Chengdu MUST Biotechnology Co., Ltd. (Chengdu, China), Chengdu PUSH Biotechnology Co., Ltd. (Chengdu, China) and Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). The purity of each standard compound was above 98%, as determined by high performance liquid chromatography (HPLC) analysis. Methanol and acetonitrile were purchased from E. Merck (Darmstadt, Germany). Formic acid with a purity of 96% was of HPLC grade from Tedia Company Inc. (Fairfield, OH, USA). Ethanol for extraction was purchased from Merck (Darmstadt, Germany). Deionized water (>18.2 M Ω cm resistivity) was prepared by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

### 2.3. Qualitative and quantitative characterization of triterpenoid acids

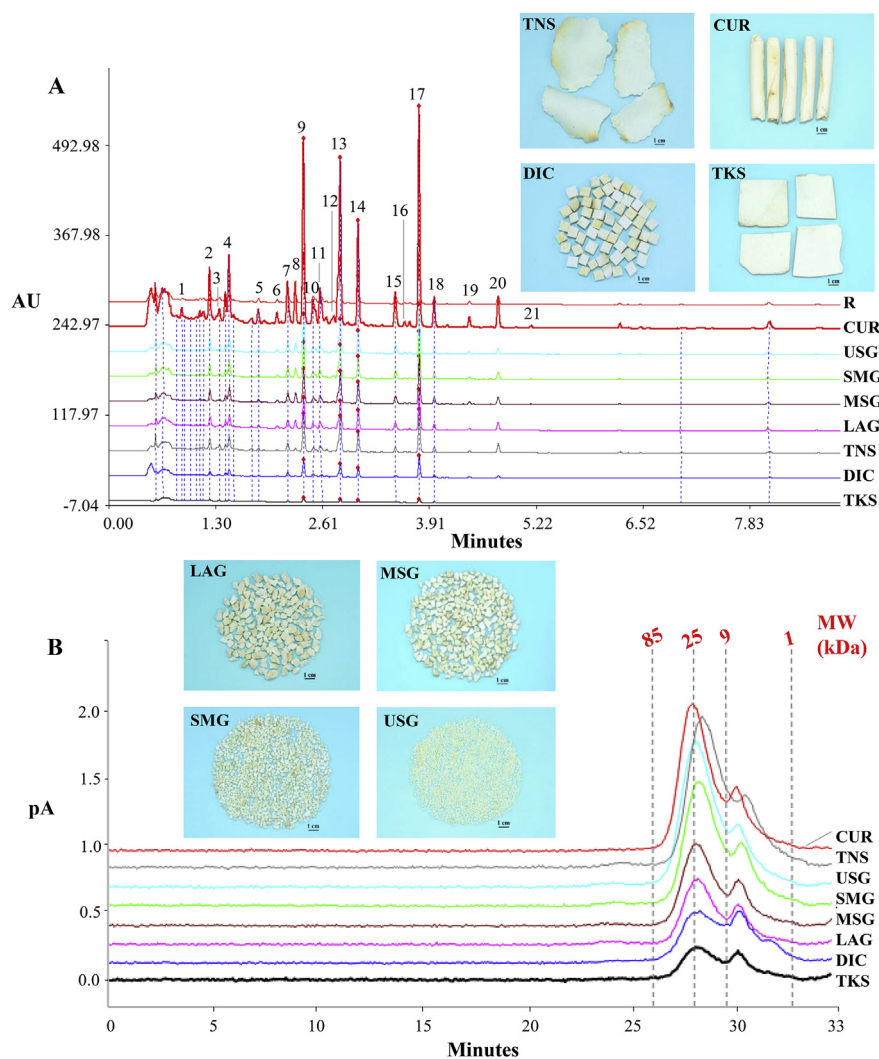
Using protocols described in our recent publication [26], UHPLC-PDA-QTOF-MS/MS was employed for the qualitative fingerprinting analysis and identification of triterpenoid acids, and UHPLC-QqQ-MS/MS was used for quantitative analysis of nine triterpenoid acids, namely PAB, DTUA, TUA, PAA, PAC, EA, PA, DTRA and DEA.

Powdered raw PC was used to optimize the extraction method. The raw PC was dried, ground, and then passed through a 24-mesh sieve. The optimized extraction methods for qualitative and quantitative analysis of this powder was the same as those in our recent study [26]. For the preparation of sample solutions from different decoction pieces specifications of 24 samples, 10.0 g of unground decoction pieces was used and extracted by the optimized extraction methods, keeping the same sample-to-extraction solvent ratio (w/v, g/

**Table 1** – The information on external shape of different specifications of PC decoction pieces.

Specification	Length (cm)	Width (cm)	Thickness (cm)	Weight (g)	Diameter (mm)
LAG	0.68–1.3	0.40–0.70	0.30–0.40	0.13–0.36	6.0–8.0
MSG	0.52–1.1	0.30–0.60	0.20–0.30	0.033–0.13	4.0–6.0
SMG	0.30–0.60	0.20–0.40	0.10–0.20	0.0081–0.043	2.0–4.0
USG	0.10–0.30	0.10–0.20	0.080–0.15	0.0014–0.0082	1.0–2.0
DIC	0.90–1.1	0.80–1.0	0.80–0.90	0.46–1.3	— <sup>a</sup>
TKS	4.0–5.5	3.5–4.8	0.20–0.40	6.0–12	—
CUR	7.0–9.0	—	0.10	4.4–6.0	10–13
TNS	5.5–7.1	2.5–6.5	0.10	2.5–5.1	—

<sup>a</sup> Not measured.



**Fig. 1** – Typical chromatograms for UHPLC-PDA fingerprint of secondary metabolites (A) and HPGPC-CAD fingerprint of polysaccharides (B) in different specifications of PC decoction pieces. In Figure A, the peaks were identified as poricoic acid E (1), poricoic acid D (2), 26-Hydroxy poricoic acid G (3), 26-Hydroxy poricoic acid G isomer (4), 6,9-Epoxyergosta-7,22-dien-3-ol (5), 16 $\alpha$ -Hydroxytramentenic acid isomer (6), poricoic acid C (7), PAB (8), DTUA (9), TUA (10), PAA (11), poricoic acid HM (12), PAC (13), 3 $\beta$ -Hydroxylanosta-7,9(11),24-trien-21-oic acid isomer (14), 16 $\alpha$ -Acetoxy-3 $\beta$ -hydroxylanosta-7,9(11),24-trien-21-oic acid (15), 3-Oxo-16 $\alpha$ ,25-dihydroxy-Lanosta-7,9(11),24(31)-trien-21-oic acid (16), dehydropachymic acid (17), PA (18), poricoic acid CE (19), DTRA (20), DEA (21); R = the simulated mean chromatogram.

mL). Specifically, PC decoction pieces (10.0 g), accurately weighed, and 100 mL of ethanol were added into a 100-mL bottle with mouth stopper, weighed again, ultrasonically extracted 60 min in an ultrasonic water bath (300 W) at 60 °C, weighed again and made up to original weight with ethanol, then centrifuged at 1800 $\times$ g for 10 min. The extracted solution was filtered by a 0.22- $\mu$ m syringe filter for UHPLC fingerprint and identification analysis by UHPLC-PDA-QTOF-MS/MS. For the quantitative determination of nine triterpenoid acids by UHPLC-QqQ-MS/MS, the sample-to-extraction solvent ratio (w/v, g/mL) was increased to 1:50, and samples were soaked for 30 min at 60 °C before ultra-sonication.

UHPLC was performed on an Agilent 1290 Infinity system (Agilent Technologies, Palo Alto, CA) equipped with an auto-sampler and binary solvent delivery system. The chromatographic separation was performed with a Waters ACQUITY

UPLC<sup>®</sup> BEH-C<sub>18</sub> (2.1 mm  $\times$  100 mm, i.d. 1.7  $\mu$ m) and a VanGuard<sup>™</sup> BEH-C<sub>18</sub> guard column (2.1 mm  $\times$  5 mm, i.d. 1.7  $\mu$ m).

Mass spectrometry (QTOF) was performed on an Agilent 6540 ultra-high definition accurate mass quadrupole time-of-flight spectrometer (Agilent Technologies Inc., Wilmington, DE, USA). The mass spectra were acquired both in the positive and negative mode by scanning from 100 to 1700 in a mass-to-charge ratio ( $m/z$ ). The mass spectrometry analysis was performed under the following optimized conditions: dry gas temperature 300 °C, dry nitrogen gas flow rate 8 L/min, nebulizer pressure 40 psi, Vcap 4500, nozzle voltage 500 V and fragmentor voltage 150 V. The collision energies were set at 15 and 35 eV. The data processing software Agilent MassHunter Workstation and Q-TOF Qualitative Analysis (version B.06.00, Agilent Technologies, Inc. 2012) were used to identify chromatographic peaks.



Mass spectrometry (QqQ) was performed on an Agilent 6460 QqQ/MS system equipped with electrospray ionization (ESI) source. The conditions of the ESI source were as follows: drying gas ( $N_2$ ) flow rate, 8.0 L/min; drying gas temperature, 350 °C; nebulizer pressure, 45 psi; capillary voltage, 3500 V (+) and 4000 V (-); nozzle voltage, 500 V. The analysis was performed using multiple reaction monitoring (MRM) mode, and the detailed MRM conditions for each analyte are given in [Table S1 \(Supplementary data\)](#). Agilent MassHunter Quantitative Analysis Software B.04.00 was used to collect and process QqQ-mass data.

Quantitative comparison of nine triterpenoid acids detected in different specifications of PC decoction pieces was conducted, and the transfer rate (TR) for each analyte was calculated as follows:

$$TR = \frac{CS}{RM} \times 100\% \quad (1)$$

where CS represents the content of each analyte detected in different specifications, and RM represents the content of each analyte in the powdered sample of raw PC. CS and RM were calculated with reference to the dried substance.

#### 2.4. Qualitative and quantitative analysis of polysaccharides

The molecular weight distribution fingerprint of polysaccharides was established by high performance gel permeation chromatography coupled with charged aerosol detector (HPGPC-CAD). For the preparation of polysaccharides, each specification of PC decoction pieces (10.0 g) was accurately weighed, immersed in 70 mL of water for 30 min, and then refluxed for 60 min. The extract solution was immediately filtered, and the dregs were refluxed with 60 mL of water for another 40 min. Next, the two extract filtrates were combined and evaporated in a water bath until dry. The residue was redissolved in 10 mL of water, centrifuged ( $1800 \times g$  for 10 min), and the supernatant (2 mL) was precipitated by adding ethanol to make a final concentration of 80% and left overnight (12 h) at 4 °C. After centrifugation ( $1800 \times g$  for 10 min), the precipitate was collected, washed twice with 80% ethanol, and dried (oven, 40 °C) to remove the residual ethanol; hereafter it was completely redissolved in 10 mL of hot water (60 °C), and a portion of the solution was diluted to the appropriate concentration for quantitative determination of polysaccharides using the reported typical method [27]. Another part of the solution was diluted to the crude herb concentration of 10 mg/mL for HPGPC-CAD analysis.

The polysaccharide extracts of different specifications of 24 samples were qualitatively analyzed using HPGPC performed on a Dionex UltiMate 3000 series UHPLC-PDA system coupled with CAD (Thermo Scientific, Waltham, MA, USA). The separation was achieved by two tandem TSK GMPW<sub>XL</sub> columns (300 mm  $\times$  7.8 mm, i.d. 10  $\mu$ m) kept at 40 °C. Ammonium acetate aqueous solution (20 mM) was used as mobile phase at a flow rate of 0.6 mL/min. The parameters of CAD were set as follows: data collection rate, 2 Hz; filter, 10 s; gain, 100 pA; and nebulizer heater, 60 °C; and gas regulator

mode, analytical. Ultraviolet (UV) detection wavelengths were set at 260 and 280 nm. A 20  $\mu$ L aliquot of the solution was injected for analysis.

Aqueous stock solutions of pullulans (2 mg/mL) with different molecular weights (6, 10, 21.7, 48.8, 113, 210, 366 and 805 kDa) and dextrans (2 mg/mL) with different molecular weights (1, 5, 12, 25, 50, 80, 150, 270, 410 and 670 kDa) were injected into the HPGPC-CAD using the conditions described above for the construction of the molecular weight-retention time calibration curve by plotting logarithm of the molecular weight versus retention time of each standard compound [28].

The extraction rate of polysaccharides was also characterized to reveal how the amounts of polysaccharides extracted from the different specifications varied with extraction time. The specific steps were as follows: Each specification of PC decoction pieces (10.0 g) was accurately weighed; 70 mL water was added; the combination was weighed again, then allowed to stand for 30 min, and refluxed for 60 min. Every 15 min during the refluxing, the mixture was weighed again; any weight loss was made up with water; the mixture was well shaken, and then the 15-min extraction solution (1.2 mL) was pipetted and centrifuged ( $3600 \times g$  for 5 min). The supernatant (1 mL) was accurately pipetted and precipitated by 80% ethanol. The polysaccharides were prepared by the method described above, and then were measured by the phenol-sulfuric acid method with glucose as standard [27]. The extraction rate of polysaccharides (ER) in different specifications of PC decoction pieces was calculated based on the dissolution percentage of polysaccharides (DIP), and ER showed how DIP changed with extraction time and was thus expressed as a DIP-extraction time curve.

$$DIP = \frac{C_i}{C_t} \times 100\% \quad (2)$$

where  $C_i$  represents the amount of polysaccharides extracted from the certain specification sample during one 15-min period, and  $C_t$  represents the total amount of polysaccharides extracted from the powdered sample of raw PC during 60 min.

#### 2.5. Drying rate

Each sample from different specifications (10.0 g), not ground, was accurately weighed into a bottle that had been dried to a constant weight, re-weighed, then dried in a constant-temperature oven at 105 °C. The bottle was weighed every 1.5 h, and then put back into the oven until a constant weight was reached. Water loss percentage was plotted against drying time curve to evaluate the drying rate of the different specifications of PC decoction pieces.

#### 2.6. Water-soluble and ethanol-soluble extracts

10.0 g of unground sample from each specification was used to prepare and determine water-soluble and ethanol-soluble extracts by hot dipping and cold soaking method, respectively, using methods described in the Hong Kong Chinese

Materia Medica Standards (Volume I) and Chinese Pharmacopoeia (2015 edition, Volume IV).

For the determination of water-soluble extracts, each specification sample was accurately weighed into a 500-mL round-bottomed flask, added 250 mL of water, tightly sealed with a stopper, weighed together, stood for 1 h, refluxed for 1 h, readjusted to the original weight with water after cooling to room temperature, shaken and filtered through a dry filter. 25 mL of the filtrate were accurately transferred into an evaporating dish that has been previously dried to a constant weight, evaporated to dryness on a water bath, then dried at 105 °C for 3 h, cooled in a desiccator for 30 min, and then weighed immediately and accurately. The water-soluble extracts were calculated with reference to the dried substance.

For the determination of ethanol-soluble extracts, each specification sample was accurately weighed into a 250-mL conical flask, added 250 mL of 70% ethanol, tightly inserted the stopper, weighed together and extracted for 24 h. This mixture was frequently shaken during the first 6 h, then allowed to stand for 18 h, and readjusted to the original weight with 70% ethanol. The extraction solution was rapidly filtered through a dry filter. The other steps are the same as the determination of water-soluble extracts.

### 2.7. Quality stability

Quality stability of different specifications of PC decoction pieces was assessed by accelerated stability test and long-term stability test. In these tests, the key parameters, namely content of polysaccharides and pachymic acid and the fingerprint of triterpenoid acids, were determined by the methods described in Section “Qualitative and quantitative characterization of triterpenoid acids” and “Qualitative and quantitative analysis of polysaccharides”. The conditions of accelerated and long-term stability tests were set by referring to the Part IV of Chinese Pharmacopoeia (2015 edition). Specifically, for accelerated stability test, different specifications of PC decoction pieces were placed in a constant temperature humidity chamber at a temperature of 40 °C ± 2 °C and relative humidity of 75% ± 5% for 6 months. The key parameters were tested in the 0th, 1st, 2nd, 3rd, and 6th months, respectively. For long-term stability test, different specifications of PC decoction pieces were placed in a constant temperature humidity chamber at a temperature of 25 °C ± 2 °C and relative humidity of 60% ± 10% for 9 months. The key parameters were tested in the 0th, 3rd, 6th and 9th months.

### 2.8. Chemometric analyses

PCA, HCA, and FA for dimensionality reduction were applied to further explore the differences between different specifications of PC decoction pieces [5]. PCA was conducted using the software SIMCA-P Version 13.0 (Umetrics, Umeå, Sweden). HCA, one-way ANOVA and FA were performed by IBM SPSS Statistics 21.0 software (IBM, USA). Statistical differences in quantitative determination among the various specifications were assessed by one-way ANOVA followed by Tukey HSD multiple comparisons when the variance was homogeneous; Welch test followed by Dunnett's T3 multiple comparisons

was conducted when the variance was hetero-homogeneous. A p-value no greater than 0.05 was considered as a significant difference.

## 3. Results and discussion

### 3.1. Development of extraction procedure

For quantitatively determining polysaccharides, extraction conditions were optimized to achieve complete extraction of polysaccharides. The polysaccharides in the samples were completely extracted by refluxing with water twice since no polysaccharide was detected by the phenol-concentrated sulfuric acid method in the subsequent third extraction (Table S2, Supplementary data). The sampling amount of the decoction pieces is based on the prescribed dosage described in the Chinese Pharmacopoeia (2015 edition).

### 3.2. Fingerprints of triterpenoid acids

As mentioned in the introduction, triterpenoid acids are a key type of the bioactive compounds of PC, so fingerprinting triterpenoid acids is highly significant for qualitatively comparative study of different forms of PC decoction pieces. The protocol for fingerprinting triterpenoid acids was developed from our recent study [26]. Twenty four batches of unground samples from eight specifications of PC decoction pieces were analyzed by the developed UHPLC-PDA method that is simpler, more economical, and more applicable than UHPLC-MS method for quality evaluation. The similarity between the fingerprint of the tested sample and their simulated mean chromatogram (R) was assessed by Similarity Evaluation Software for Chromatographic Fingerprint of Traditional Chinese Medicine (SES, Version 2004 A). As shown in Fig. 1A, all samples exhibited similar chromatographic profiles, but there were significant differences in peak absorption intensity. In the fingerprints, 21 peaks were detected coexisting in the eight specifications, called as common peaks, and they were identified as triterpenoid acids generated for peak 5 on the basis of accurate molecular mass, generated molecular ions and fragment ions provided by QTOF-MS/MS, and/or by matching this data with corresponding data of known compounds in the reported literature and databases, using the UHPLC-QTOF-MS/MS reported in our recent study [26]. Eight of these peaks, namely PAB (peak 8), DTUA (peak 9), TUA (peak 10), PAA (peak 11), PAC (peak 13), PA (peak 18), DTRA (peak 20) and DEA (peak 21), were unequivocally identified by comparing the mass data of their reference standards. Most common peaks in the curls had the largest peak areas among the eight specifications, while the common peaks had the smallest peak areas in the thick slices and dice (Table S3, Supplementary data), and their similarities to the simulated mean chromatogram were the lowest (Table 2). Thus, it can be seen that the size and thickness of the specification greatly affect the extraction efficiency of triterpenoid acids, and it can be inferred that the smaller or thinner the specifications are, within a certain range, the higher the availability of secondary metabolites (mainly containing triterpenoid acids) may be.

**Table 2 – Similarities between the simulated mean chromatogram (R) and the fingerprint of different specifications of PC decoction pieces (Mean  $\pm$  SD, %, n = 3).**

Specification	TKS	DIC	TNS	LAG	MSG	SMG	USG	CUR
Batch 1	0.664 $\pm$ 0.064	0.732 $\pm$ 0.039	0.925 $\pm$ 0.051	0.968 $\pm$ 0.019	0.939 $\pm$ 0.037	0.944 $\pm$ 0.039	0.953 $\pm$ 0.028	0.954 $\pm$ 0.018
Batch 2	0.752 $\pm$ 0.045	0.769 $\pm$ 0.057	0.941 $\pm$ 0.043	0.903 $\pm$ 0.056	0.950 $\pm$ 0.023	0.961 $\pm$ 0.014	0.947 $\pm$ 0.033	0.932 $\pm$ 0.045
Batch 3	0.535 $\pm$ 0.073	0.801 $\pm$ 0.064	0.932 $\pm$ 0.028	0.936 $\pm$ 0.046	0.951 $\pm$ 0.035	0.943 $\pm$ 0.025	0.963 $\pm$ 0.021	0.904 $\pm$ 0.027

### 3.3. Fingerprints of polysaccharides molecular weight distributions

As mentioned in the introduction, polysaccharides are another important type of the bioactive compounds of PC, so it is significant for qualitative investigation of polysaccharides in different forms of PC decoction pieces. Twenty four batches of unground samples from eight specifications were analyzed and compared, using the established HPGPC-PDA-CAD method, in which UV 260 and 280 nm were selected for monitoring saccharide-conjugated nucleic acids and/or peptides for interference; no absorption was observed at the peaks of polysaccharides under the investigated conditions. The molecular weight distributions of polysaccharides in different specifications of PC decoction pieces were calculated using the constructed molecular weight–retention time calibration curve ( $y = -0.2741x + 9.1402$ ,  $r = 0.9985$ ), and the typical HPGPC fingerprints are shown in Fig. 1B. The chromatograms demonstrated that there is a distinct difference in the molar mass ranges and the peak area of polysaccharides between different specifications. Polysaccharides in curls, ultra-small grains, small grains, medium-sized grains, and large grains possessed the widest molecular weight distribution, ranging from  $1.71 \pm 0.21$  kDa to  $80.16 \pm 5.22$  kDa. Peak areas of polysaccharides in curls, thin slices ( $1.33 \pm 0.09$  kDa– $58.47 \pm 3.58$  kDa), ultra-small grains, and small grains were considerably larger, while the ranges in thick slices ( $2.83 \pm 0.18$  kDa– $54.89 \pm 3.58$  kDa) and dice ( $1.50 \pm 0.09$  kDa– $70.65 \pm 4.61$  kDa) were considerably narrower (Fig. 1B). Hence, it could be seen that the extraction efficiency of polysaccharides was greatly affected by the size and thickness of the specification, and inferences similar to the above UHPLC-PDA fingerprint study could be made.

### 3.4. Transfer rates of nine triterpenoid acids

Nine main bioactive triterpenoid acids, namely PAB, DTUA, TUA, PAA, PAC, EA, PA, DTRA, and DEA, were selected as the quantitative markers, and then they were determined by the MRM mode of UHPLC-QqQ-MS/MS, based on our recent study [26]. As shown from Fig. S1 (Supplementary data), MRM chromatograms demonstrated that the MRM mode provided acceptable chromatographic resolution for the nine analytes. The transfer rate, as expressed by equation (1), shows the percentage between the amount of the analyte extracted from different specifications of PC decoction pieces and the amount of the analyte contained in the raw PC. That is, the transfer rate is the actual extracted percentage for the analyte in PC decoction pieces. The transfer rates of these analytes varied remarkably between different specifications. For example, the transfer rates of all analytes were the lowest in thick slices (i.e., less than  $22.13 \pm 2.10\%$ ) and almost the highest in curls

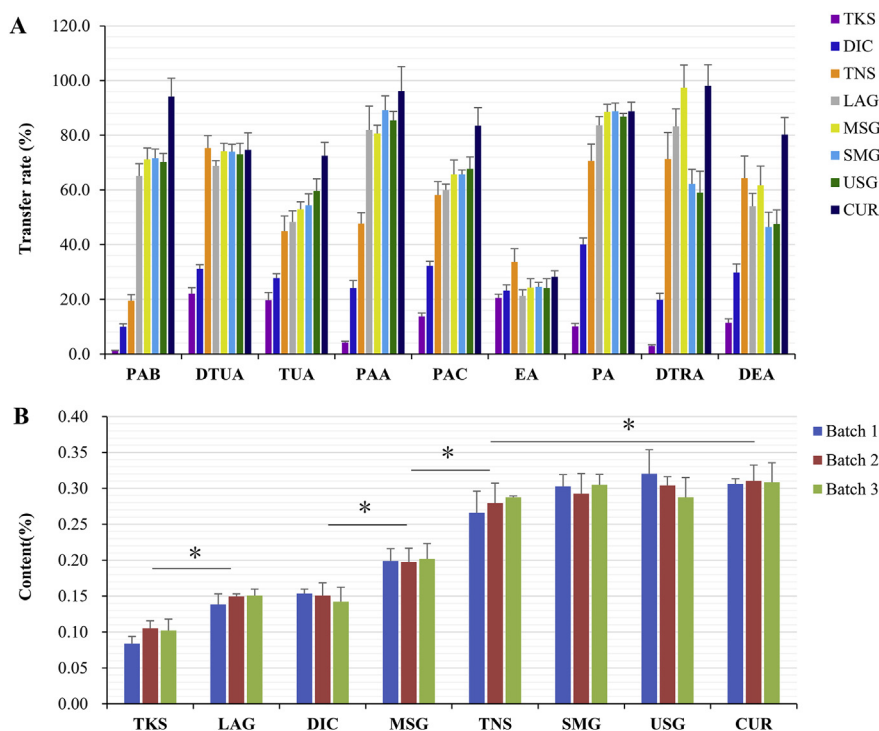
(ranging from  $28.29 \pm 2.17\%$  to  $98.09 \pm 7.75\%$ ). Large grains, medium-sized grains, small grains, ultra-small grains, and curls possessed comparable transfer rates of PAB, DTUA, PAA, EA and PA. The detailed transfer rates of nine analytes in 24 samples from different specifications are exhibited in Fig. 2A and Table S4 (Supplementary data), and the transfer rate of nine analytes as a whole is shown in Fig. S2 (Supplementary data). In order to explicate the differences in transfer rates of nine triterpenoid acids between different specifications, statistical analysis, including one-way ANOVA and the multiple comparisons test, were adopted. The results of multiple comparisons between different specifications are summarized in Table S5 (Supplementary data). In general, it can be seen that the size and thickness of the specifications significantly affect the extraction efficiency for these triterpenoid acids. Thus, it is inferred that the smaller or thinner the specification, within a certain range, the higher the availability of these triterpenoid acids. Among them, the thickness of thin slices and curls were the same, but the transfer rates of PAB, TUA, PAA, PAC, PA, DTRA, and DEA in the two specifications of samples were significantly different. This difference likely results from the different processing method. As mentioned above, the curls undergo the process of heating through the hot bamboo slices during processing, which may bring its bioactive components to the surface where they are more easily extracted; this needs further research.

### 3.5. Polysaccharides content

Polysaccharides extracted from eight specifications of 24 PC decoction pieces were quantitatively determined by the previous method (Table S6, Supplementary data) [27]. This method was validated for linearity, intra- and inter-day precision, stability, repeatability and recovery (Table S7, Supplementary data). The obtained data were further processed by one-way ANOVA and the multiple comparisons test to explore the dissimilarities between different specifications. As seen from Fig. 2B, the amounts of polysaccharides extracted varied significantly between different specifications. The highest content of polysaccharides was detected in the curls with the average value of 0.308%, followed by ultra-small grains and small grains, while the lowest content was observed in the thick slices and dice.

### 3.6. Water-soluble and ethanol-soluble extracts

According to the records of the Chinese Pharmacopoeia and Hong Kong Chinese Materia Medica Standards, the content of water-soluble and ethanol-soluble extracts is an important indicator for evaluating the quality of traditional Chinese medicine, especially for Chinese medicines whose active ingredients or index components are unclear or low in content.



**Fig. 2** – Transfer rates of nine triterpenoid acids in different specifications of PC decoction pieces (Mean  $\pm$  SD,  $n = 9$ , marked as A) and contents of polysaccharides detected in its different specifications (Mean  $\pm$  SD,  $n = 3$ , marked as B).

Due to low content of triterpenes and polysaccharides, the water-soluble and ethanol-soluble extracts were also selected to assess eight specifications of PC decoction pieces. As shown in Fig. 3A and Table S6 (Supplementary data), the water-soluble and ethanol-soluble extracts from the different specifications varied considerably. The highest yield of water-soluble extracts was detected in the ultra-small grains ( $2.79 \pm 0.16\%$ ), followed by curls ( $2.56 \pm 0.26\%$ ) and thin slices ( $2.05 \pm 0.18\%$ ), while the lowest yield was found in thick slices ( $0.85 \pm 0.13\%$ ). Similar results occurred for the ethanol-soluble extracts.

### 3.7. Drying rate

Moisture is an important condition for mildew growth and deterioration of botanical materials, so drying is a crucial part of ensuring the safety and effectiveness of CMMs. The difficulty of drying affects the processing efficiency of PC decoction pieces. Thus, the drying rates of different specifications of PC decoction pieces were investigated and compared in this study. As seen from Fig. 3B and Table S8 (Supplementary data), ultra-small grains, small grains and curls dried the most quickly, while the thick slices and dice dried the most slowly. The result provides an important reference for finding out the suitable PC decoction pieces specifications that specify those of high available quality.

### 3.8. Extraction rate of polysaccharides

The extraction rate is used to investigate the speed of dissolution of a bioactive ingredient. In other words, the extraction rate of polysaccharides could characterize how the amounts of polysaccharides extracted from different specifications

vary with extraction time, which reflect the extraction efficiency of polysaccharides in different forms of PC decoction pieces. As shown in Fig. 3C and Table S9 (Supplementary data), the extraction rates of polysaccharides markedly varied between different specifications. The curls had the highest extraction rate of polysaccharides ( $1.48 \pm 0.03\%/min$ ), followed by thin slices ( $1.39 \pm 0.05\%/min$ ) and ultra-small grains ( $1.29 \pm 0.06\%/min$ ), while the extraction rates of polysaccharides from thick slices ( $0.44 \pm 0.08\%/min$ ) and dice ( $0.56 \pm 0.09\%/min$ ) were obviously slower. The result also provides an important basis for finding out efficient specifications of PC decoction pieces.

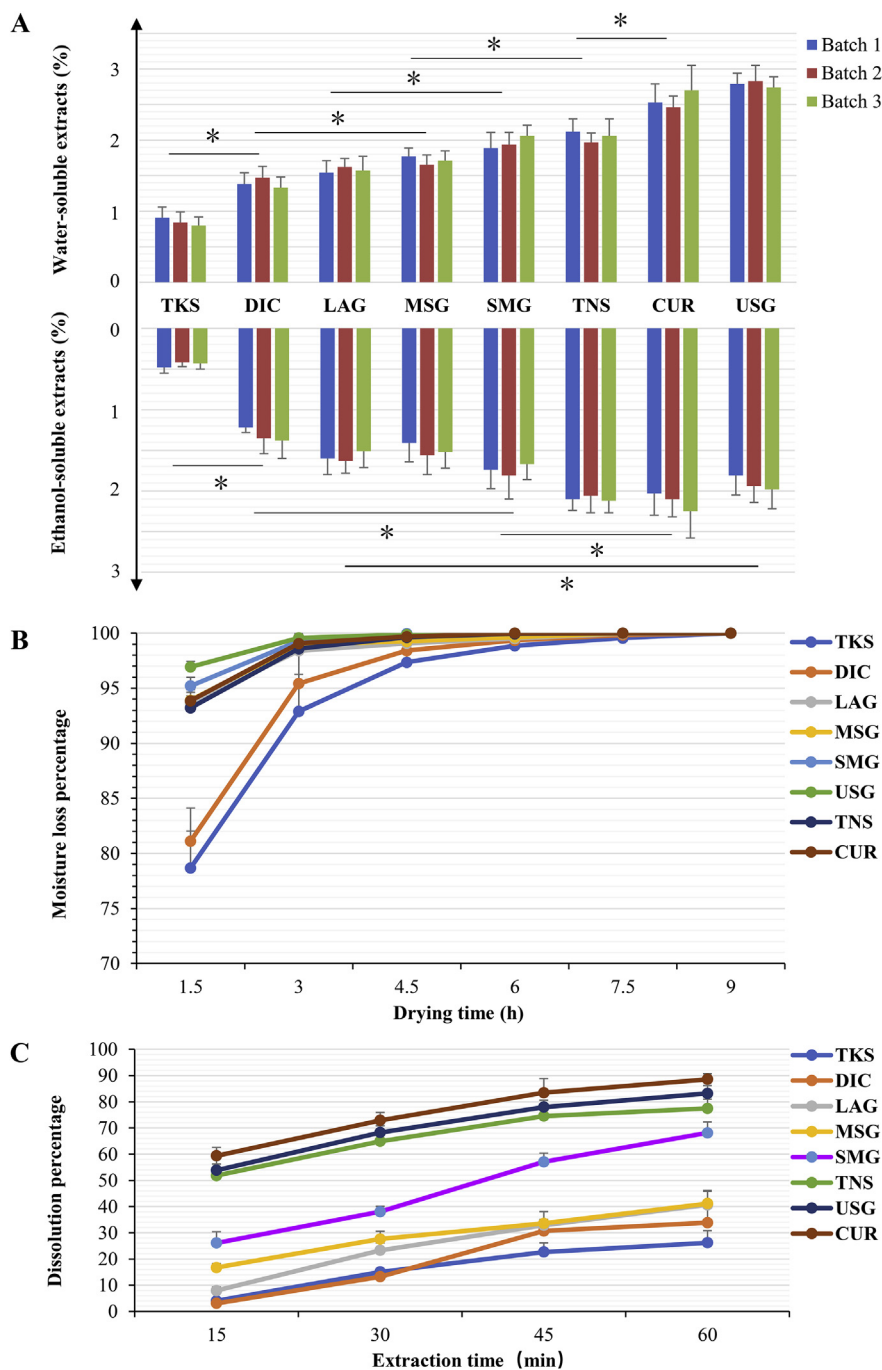
### 3.9. Quality stability

In order to explore whether and how the form of PC decoction pieces impacts the stability of its quality, accelerated stability and long-term stability tests were conducted. The contents of polysaccharides and pachymic acid and the fingerprints of the different decoction pieces forms were determined and compared. As shown in Figs. S3, S4 (Supplementary data) and Tables S10–S12 (Supplementary data), all parameters showed no significant change, which demonstrated that these eight specifications of PC decoction pieces were quite stable in terms of their chemistry; this may be attributed to the stable chemical properties of its main components, polysaccharides and triterpenoids.

### 3.10. Chemometric analyses

In order to further characterize the dissimilarities between different specifications of PC decoction pieces, PCA and HCA





**Fig. 3** – Water-soluble and ethanol-soluble extracts (A), drying rates (B) and extraction rates of polysaccharides (C) from different specifications of PC decoction pieces.

were employed to process the data of the above-mentioned 14 indicators, namely: transfer rates of nine triterpenoid acids, polysaccharides content (POC), water-soluble extracts (WAE), ethanol-soluble extracts (ETE), average extraction rate (AER) and average drying rate (ADR).

PCA, an unsupervised multivariate analysis method, aims to explore the relationship between different samples represented by the multiple indicators/variables by dimensionality reduction [5]. In PCA, several principal components can be used to represent multiple variables if there is a strong

correlation between variables; otherwise, these principal components cannot comprehensively reflect the common characteristics of all variables in the analyzed sample [29]. Therefore, it is necessary to perform a correlation analysis on the data to obtain the correlation matrix of the above 14 variables (Table S13, Supplementary data). The correlation analysis between the above 14 indicators was accomplished by FA. As shown in Table S13, nearly 90% of the correlation coefficients had an absolute value greater than 0.5, and the indicators with extremely significant correlation accounted for

**Table 3 – Rotated component matrix<sup>a</sup> for factor analysis.**

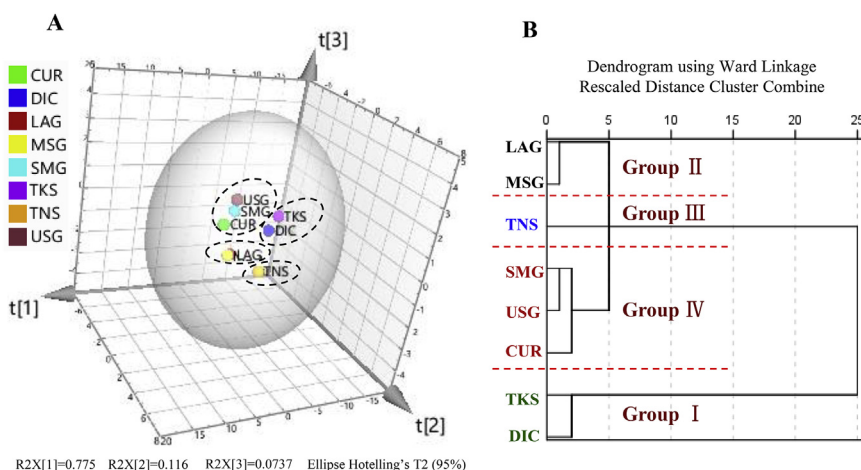
Components	PAB	DTUA	TUA	PAA	PAC	EA	PA	DTRA	DEA	POC	WAE	ETE	ADR	AER
1	0.752	0.790	0.695	0.792	0.775	0.197 <sup>b</sup>	0.830	0.944	0.804	0.301	0.375	0.572	0.335	0.247
2	0.005	0.454	0.356	0.139	0.400	0.954	0.283	0.264	0.525	0.706	0.605	0.714	0.060	0.765
3	0.653	0.318	0.611	0.584	0.488	-0.136	0.425	0.155	0.156	0.618	0.652	0.318	0.916	0.578

Extraction method: Principal Component Analysis.

Rotation method: Varimax with Kaiser Normalization.

<sup>a</sup> Rotation converged in 10 iterations.

<sup>b</sup> Gray font value represents absolute value of loadings less than 0.40.



**Fig. 4 – PCA/3D Scores plot (A) and HCA (B) based on 14 indicators containing POC, WAE, ETE, AER, ADR and the transfer rates of nine triterpenoid acids.**

95.6%, indicating that there was a strong correlation between these 14 variables. Moreover, the cumulative variance contribution rate of the first three principal components reached 96.5%, which demonstrated that the three principal components could explain the information of 96.5% of the raw data. As seen from Table 3, the first principal component possessed a high loading coefficient in ETE and the transfer rates of eight triterpene acids (PAB, DTUA, TUA, PAA, PAC, PA, DTRA, and DEA), indicating their transfer rates and ETE were highly positively correlated with the first principal component; this was not true for EA. Similarly, POC, WAE, ETE, AER, and the transfer rate of EA, DTUA, PAC and DEA were highly positively correlated with the second principal component, and POC, WAE, ADR, AER, and the transfer rates of PAB, TUA, PAA, PAC and PA were highly positively correlated with the third principal component [29]. Therefore, POC, WAE, ETE, AER, ADR, and the transfer rates of nine triterpenoid acids could be considered as characteristic elements to discriminate different specifications of PC decoction pieces. In order to distinctly exhibit the differences and similarities between different specifications, the 3D scores plot of PCA was drawn by SIMCA-P Version 13.0 software based on taking these 14 indicators as variables [5]. The 3D scores plot of PCA indicated that the eight specifications were distinctly divided into four groups (Fig. 4A). Furthermore, the scores plot showed significant differences between groups and great similarities within groups. In the PCA model, the first principal component accounted for 77.5% of the variance, and the fit parameters

indicated that the developed PCA model has good reproducibility ( $R^2X = 0.965$ , close to 1) and good predictability ( $Q^2 = 0.833$ , greater than 0.5).

In addition, HCA was also employed to characterize the dissimilarities among different specifications of PC decoction pieces on the basis of the above 14 indicators. The result showed that the eight specifications could be mainly classified into four groups (Fig. 4B), which nicely confirmed the results of PCA. Therefore, these 14 indicators could be regarded as highly discriminating elements to distinguish different specifications. The chemically available quality of different specifications of PC decoction pieces in order were: Group IV > Group III > Group II > Group I.

#### 4. Conclusion

In this study, we investigated *P. cocos* as a pilot study to evaluate the chemically available quality of different forms of its decoction pieces by combining qualitative and quantitative characterization of triterpenes and polysaccharides, physical parameter analysis, quality stability test and chemometric analyses (such as ANOVA, PCA, FA and HCA). The untargeted/targeted metabolomics demonstrated that not only triterpenes but also polysaccharides in different forms of PC decoction pieces were qualitatively and quantitatively different. The shape, size and thickness of the specification greatly affected the efficiency of extracting bioactive

components from decoction pieces. The comprehensive results chemically demonstrated that the most effective forms among these eight specifications were grains with a screen diameter of 1.0–4.0 mm, curls, and thin slices with a thickness of 1.0 mm. In fact, this appears to be the first study conducted to evaluate the specifications/forms of any CMM decoction pieces using multidimensional chemical analysis combined with chemometrics. This study not only chemically proposed the most effective forms for PC decoction pieces but also provided a promising strategy for evaluating other botanical materials that might appear in different forms, e.g., herbal teas, natural medicines. The research outcomes can be used to promote the efficient utilization of PC, ensure the safety and efficacy of its clinical use, and stimulate its specification/form standardization and industrial development.

### Funding

This work was funded by the Research Grants Council, Hong Kong, China (Project No. 12141516) and the National Natural Science Foundation of the People's Republic of China (Project No. 11475248).

### Conflicts of interest

The authors declare that there is no conflict of interest.

### Acknowledgments

We acknowledge Mr. Alan Ho from the School of Chinese Medicine, Hong Kong Baptist University, for his technical support in the UHPLC-QTOF-MS/MS and UHPLC-QqQ-MS/MS analysis.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jfda.2019.03.002>.

### REFERENCES

- [1] Li P, Qi LW, Liu EH, Zhou JL, Wen XD. Analysis of Chinese herbal medicines with holistic approaches and integrated evaluation models. *Trends Anal Chem* 2008;27:66–77.
- [2] Liu Y, Li L, Xiao YQ, Yao JQ, Li PY, Chen L, et al. Rapid identification of the quality decoction pieces by partial least squares-based pattern recognition: grade classification of the decoction pieces of *Saposhnikovia divaricate*. *Biomed Chromatogr* 2016;30:1240–7.
- [3] Bai KC, Sheu F. A novel protein from edible fungi *Cordyceps militaris* that induces apoptosis. *J Food Drug Anal* 2018;26:21–30.
- [4] Zhao ZZ, Liang ZT, Guo P. Macroscopic identification of Chinese medicinal materials: traditional experiences and modern understanding. *J Ethnopharmacol* 2011;134:556–64.
- [5] Zhu LX, Xu J, Zhang SJ, Wang RJ, Huang Q, Chen HB, et al. Qualitatively and quantitatively comparing secondary metabolites in three medicinal parts derived from *Poria cocos* (Schw.) Wolf using UHPLC-QTOF-MS/MS-based chemical profiling. *J Pharm Biomed Anal* 2018;150:278–86.
- [6] Chan K. Chinese medicinal materials and their interface with western medical concepts. *J Ethnopharmacol* 2005;96:1–18.
- [7] Wu X, Wang SP, Lu JR, Jing Y, Li MX, Cao JL, et al. Seeing the unseen of Chinese herbal medicine processing (Paozhi): advances in new perspectives. *Chin Med* 2018;13:4–16.
- [8] Guo P, Brand E, Zhao ZZ. Chinese medicinal processing: a characteristic aspect of the ethnopharmacology of Traditional Chinese Medicine. In: Heinrich M, Jäger AK, editors. *Ethnopharmacology*. 1st ed. Hong Kong: John Wiley & Sons, Ltd; 2015. p. 303–16.
- [9] State Pharmacopoeia Commission. General principles for the processing. In: *Chinese Pharmacopoeia*. 4. Beijing: China Medical Science Press; 2015. p. 31.
- [10] Tesoriere L, Butera D, Gentile C, Livrea MA. Bioactive components of Caper (*Capparis spinosa* L.) from Sicily and antioxidant effects in a red meat simulated gastric digestion. *J Agric Food Chem* 2007;55:8465–71.
- [11] Zhong WF, Tong WS, Zhou SS, Yip KM, Li SL, Zhao ZZ, et al. Qualitative and quantitative characterization of secondary metabolites and carbohydrates in Bai-Hu-Tang using ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry and ultraperformance liquid chromatography coupled with photodiode array detector. *J Food Drug Anal* 2017;25:946–59.
- [12] Yi T, Chen QL, He XC, So SW, Lo YL, Fan LL, et al. Chemical quantification and antioxidant assay of four active components in *Ficus hirta* root using UPLC-PAD-MS fingerprinting combined with cluster analysis. *Chem Cent J* 2013;7:115–23.
- [13] Xia B, Zhou Y, Tan HS, Ding LS, Xu HX. Advanced ultraperformance liquid chromatography-photodiode array-quadrupole time-of-flight mass spectrometric methods for simultaneous screening and quantification of triterpenoids in *Poria cocos*. *Food Chem* 2014;152:237–44.
- [14] Ke RD, Lin SF, Chen Y, Ji CR, Shu QG. Analysis of chemical composition of polysaccharides from *Poria cocos* Wolf and its anti-tumor activity by NMR spectroscopy. *Carbohydr Polym* 2010;80:31–4.
- [15] Zhao YY, Feng YL, Du X, Xi ZH, Cheng XL, Wei F. Diuretic activity of the ethanol and aqueous extracts of the surface layer of *Poria cocos* in rat. *J Ethnopharmacol* 2012;144:775–8.
- [16] Feng YL, Lei P, Tian T, Yin L, Chen DQ, Chen H, et al. Diuretic activity of some fractions of the epidermis of *Poria cocos*. *J Ethnopharmacol* 2013;150:1114–8.
- [17] Chen XY, Xu XJ, Zhang LN, Zeng FB. Chain conformation and anti-tumor activities of phosphorylated (1→3)- $\beta$ -D-glucan from *Poria cocos*. *Carbohydr Polym* 2009;78:581–7.
- [18] Huang QL, Jin Y, Zhang LN, Peter CKC, John FK. Structure, molecular size and antitumor activities of polysaccharides from *Poria cocos* mycelia produced in fermenter. *Carbohydr Polym* 2007;70:324–33.
- [19] Wang YZ, Zhang J, Zhao YL, Li T, Shen T, Li JQ, et al. Mycology, cultivation, traditional uses, phytochemistry and pharmacology of *Wolfiporia cocos* (Schwein.) Ryvarden et Gilb.: a review. *J Ethnopharmacol* 2013;147:265–76.
- [20] Lee HC, Cheng WY, Huang BETG, Hsu YH, Huang SY. Anti-inflammatory and hypoglycemic efficacy of *Poria cocos* and *Dioscorea opposita* in prediabetes mellitus rats. *RSC Adv* 2014;4:55649–57.
- [21] Deng JS, Huang SS, Lin TH, Lee MM, Kuo CC, Sung PJ, et al. Analgesic and anti-inflammatory bioactivities of eburicoic acid and dehydroeburicoic acid isolated from *Antrodia*

- camphorata on the inflammatory mediator expression in mice. *J Agric Food Chem* 2013;61:5064–71.
- [22] Zhou L, Zhang Y, Gapter LA, Ling H, Agarwal R, Ng KY. Cytotoxic and anti-oxidant activities of lanostane-type triterpenes isolated from *Poria cocos*. *Chem Pharm Bull* 2008;56:1459–62.
- [23] Tai T, Akita Y, Kinoshita K, Koyama K, Takahashi K, Watanabe K. Anti-emetic principles of *Poria cocos*. *Planta Med* 1995;61:527–30.
- [24] Li TH, Hou CC, Chang CL, Yang WC. Anti-hyperglycemic properties of crude extract and triterpenes from *Poria cocos*. *Evid Based Complement Alternat Med* 2011;128402–9.
- [25] Jia XJ, Ma LS, Li P, Chen MW, He CW. Prospects of *Poria cocos* polysaccharides: isolation process, structural features and bioactivities. *Trends Food Sci Technol* 2016;54:52–62.
- [26] Zhu LX, Xu J, Wang RJ, Li HX, Tan YZ, Chen HB, et al. Correlation between quality and geographical origins of *Poria cocos* revealed by qualitative fingerprint profiling and quantitative determination of triterpenoid acids. *Molecules* 2018;23:2200–16.
- [27] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem* 1956;28:350–6.
- [28] Zhou SS, Xu J, Tsang CK, Yip KM, Yeung WP, Zhao ZZ, et al. Comprehensive quality evaluation and comparison of *Angelica sinensis* radix and *Angelica acutiloba* radix by integrated metabolomics and glycomics. *J Food Drug Anal* 2018;26:1122–37.
- [29] Yang XL, Meng J, Chen QF, Wang SM. Study on quality control standard of charred ginger based on principal component analysis and correlation analysis. *J Guangzhou Univ Tradit Chin Med* 2017;34(3):413–7 [In Chinese, English abstract].