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### Recommended Citation

Yang, J.; Yang, J.; Du, J.; Feng, Y.; Chai, X.; Xiao, M.; Wang, Y.; and Gao, X. (2018) "General survey of Fructus Psoraleae from the different origins and chemical identification of the roasted from raw Fructus Psoraleae," *Journal of Food and Drug Analysis*: Vol. 26 : Iss. 2 , Article 7.

Available at: <https://doi.org/10.1016/j.jfda.2017.10.009>

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## Original Article

# General survey of Fructus Psoraleae from the different origins and chemical identification of the roasted from raw Fructus Psoraleae



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## ARTICLE INFO

## Article history:

Received 19 June 2017

Received in revised form

16 October 2017

Accepted 22 October 2017

Available online 1 December 2017

## Keywords:

Fructus Psoraleae

 $\beta$ -glucosidase

Psoralenoside

Isopsoralenoside

## ABSTRACT

Fructus Psoraleae, a traditional Chinese medicine, is widely used for preventing and treating various diseases such as vitiligo, osteoporosis and psoriasis. Coumarin, such as psoralenoside, isopsoralenoside, psoralen and isopsoralen, are important compounds in Fructus Psoraleae. In our study, ultra performance liquid chromatography coupled with diode array detector was employed for an excellent method validation for simultaneous quantification of psoralenoside, isopsoralenoside, psoralen and isopsoralen, which was further applied in performing general survey of Fructus Psoraleae from the different origins and chemical identification of the roasted from raw Fructus Psoraleae in the light of illuminating the transformed rule of psoralenoside and isopsoralenoside. There is a reciprocal relationship between (iso)psoralenoside and (iso)psoralen, and the total content remains balance in Fructus Psoraleae from the different origins. In addition, we found that (iso)psoralenoside in the powder of the raw Fructus Psoraleae could be easily transformed into (iso)psoralen in methanol aqueous solution, especially above 50% water, rather than the roasted one. Thus, we proposed a hypothesis that transformation between (iso)psoralenoside and (iso)psoralen was hindered by inactivation of  $\beta$ -glucosidase in the process of roasting Fructus Psoraleae, which was further verified by observing transformation of (iso)psoralenoside under the different conditions, such as temperature, pH and  $\beta$ -glucosidase. Therefore, we developed a feasible method to distinguish the roasted from raw Fructus Psoraleae by observing conversion from (iso)psoralenoside to (iso)psoralen in 50% methanol aqueous solution. In summary, these results pave the way for elevating quality standard for Fructus Psoraleae and distinguishing the salt-processed from raw Fructus Psoraleae.

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<https://doi.org/10.1016/j.jfda.2017.10.009>

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## 1. Introduction

Known as Buguzhi in China, *Fructus Psoraleae* (FP), the dried and ripe fruit of *Psoralea corylifolia* L., which belongs to the family Leguminosae, is widely used in Asian country as a traditional medicine. It has been employed for warming kidney and activating yang, promoting inspiration and checking diarrhea, according to Chinese pharmacopoeia (ChP) [1]. And lots of its active components, including monoterpene phenols, coumarins and flavonoids, have been demonstrated to have unique effectiveness against coronary artery disease [2], osteoporosis [3], bacterial infection [4] and vitiligo [5]. As chemical markers for quality control of FP [1], psoralen (P) and isopsoralen (IP) show various biological activities, such as antibacterial, anti-inflammatory [6,7], antitumor [8,9], estrogen-like effect [10,11] and antioxidant activities [12], and so forth.

With the continuously expanded clinical application of FP, toxicity problems, especially the liver toxicity, have been concerned by researchers and doctors. Cheung et al. [13] claimed that FP was recognized as one of the emerging hepatotoxins, in that P and its related chemicals may be responsible for the hepatotoxicity. Kong et al. [14] demonstrated that rats given P and IP (40 mg/kg) showed modest liver injury. P and IP affected hepatic microsomal cytochrome P450 and renal organic ion transport system. Our previous study [15] suggested that a significant increase of AUC and delayed elimination of P and IP were found through conversion of psoralenoside (PO) and isopsoralenoside (IPO) to P and IP by intestinal flora, which possibly worsened the liver toxicity. And according to ChP, only P and IP were the index components in FP. But, in the light of conversion between (I)PO and (I)P, we suggested that PO and IPO should be used as important quality markers for FP, together with the frequently used P and IP.

The ingredients of herbal medicines would be affected significantly by environment, collecting time, storage condition and processing [16,17], as well as FP, which may affect efficacy or even cause toxicity. Therefore, general survey of FP from the different origins should be performed, including the raw and salt-processed one. Furthermore, according to the market surveys, it is challenging to differentiate the raw and salt-processed FP, which have the different functions. Extrinsic feature of ninety-six batches FP was shown in Fig. S1 of supporting information, which resemble each other. So far, the salt-processed FP was only identified by morphological identification, microscopic identification [1,18], leading to erroneous judgment. In clinic, the salt-processed FP is used commonly. Therefore, establishment of a more reliable and simple method to identify the salt-processed FP is imperative.

Up to date, several methods including high performance liquid chromatography (HPLC) [19–21], ultra performance liquid chromatography (UPLC) [22], and high performance liquid chromatography–mass spectrometry (HPLC–MS) [23], were employed to determine the quantity of constituents in FP. But, some of the methods mentioned above suffered from limited compounds in FP except for PO and IPO [20,23] and longer analytical time [19–21]. Therefore, it is necessary to establish a simple and effective method to simultaneously

determine the contents of PO, IPO, P and IP, and distinguish the salt-processed from raw FP.

In our study, a UPLC method was developed for the quantification of PO, IPO, P and IP in FP, which was applied to perform the general survey of ninety-six batches FP from the different origins and chemical identification of the salt-processed from raw FP in view of lighting transformed rule of PO and IPO. The aim of this paper was to provide experimental basis for improving quality standard for FP, and distinguish the salt-processed from raw FP, guaranteeing the safety and efficacy of clinic application of FP.

## 2. Materials and methods

### 2.1. Reagents and materials

The methanol was HPLC grade (Sigma Aldrich, St Louis, MO, USA). Formic acid was obtained from Meridian Medical Technologies (Columbia, MD, USA). Water used in the experiment was purified by a Milli-Q water purification system (Millipore, Billerica, USA).  $\beta$ -glucosidase was purchased from Sigma–Aldrich (St Louis, MO, USA). Phosphoric acid, disodium hydrogen phosphate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were bought from DAMAO Chemical Reagent Factory (Tianjin, China). Reference compounds (P and IP) were purchased from National Institute for Food and Drug Control (Beijing, China). PO and IPO with purity above 98% were identified by NMR, LC/MS, IR and UV in our lab.

Ninety-six batches FP, numbered as B-1 to B-96 (the raw and salt-processed FP) were purchased from the different markets of medicinal materials and identified by Professor Tianxiang Li, which were deposited in Tianjin State Key Laboratory of Modern Chinese Medicine. Sample information was summarized in Table S1 of supporting information.

The raw FP (B-5, B-51 and B-96) soaked with 4% salt solution at the ratio of 500 g:250 mL (*m:v*), according to ChP of 2015 edition, were divided into two parts separately. One moiety was dried under the shade at room temperature for 2 days and numbered as SK-5, SK-51 and SK-96, and others were processed by stir-frying at 150 °C for 10 min and numbered SP-5, SP-51 and SP-96, respectively.

### 2.2. Preparation of standard solutions

Four reference compounds were accurately weighted and directly dissolved in methanol to obtain stock solution, separately. Then, the combined standard solution containing 4 standards were prepared for constructing calibration curves. The standard solution was stored at 4 °C for further analysis.

Working standard solutions for calibration curves were obtained by diluting the combined standard solution with 30% methanol (*v:v*, 1:1) to obtain a series of different concentrations of these analytes, whose concentration ranges were 1.20–38.30  $\mu\text{g/mL}$  for PO, 1.29–41.20  $\mu\text{g/mL}$  for IPO, 3.17–101.40  $\mu\text{g/mL}$  for P and 3.13–100.20  $\mu\text{g/mL}$  for IP. All solutions were stored at 4 °C until analysis.

### 2.3. Sample preparation

For general survey of FP, every accurately weighed powder (0.5 g) was transferred into 100 mL volumetric flask. Methanol was added and sonicated for 30 min at 60 °C, then cooled to room temperature. Methanol was added to reach the scale of 100 mL and mixed. The solution was centrifuged at 14,000 rpm for 10 min. The supernatant was diluted with 30% methanol (v:v, 1:1) to obtain the sample solution.

For identifying the salt-processed from raw FP, the powder of samples was deposited in 100 mL volumetric flask. 50% methanol aqueous solution was added and sonicated for 30 min at 60 °C, then cooled to room temperature. 50% methanol aqueous solution was added to reach the scale of 100 mL and mixed. The solution was centrifuged at 14,000 rpm for 10 min. The supernatant was diluted with 30% methanol (v:v, 1:1) and injected immediately into UPLC system for analysis.

For studying transformation between (I)PO and (I)P in water under the different conditions, the testing solution of PO and IPO was prepared at a final concentration of about 50 µg/mL which was subjected to the different impact factors, such as temperatures (30 °C, 50 °C and 70 °C for 12 h), pH (2.0, 4.0, 6.0 and 8.0 for 12 h) and  $\beta$ -glucosidase (37 °C, pH at 5.0 for 19 h in 100 mM sodium acetate buffer). The aliquot of 2 µL solution per hour was directly injected into UPLC system for analysis.

### 2.4. UPLC analysis

Analysis was performed on a Waters ACQUITY UPLC system. The system was controlled by Empower Pro software (Waters). Gradient elution was performed on an ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm) at 60 °C. The mobile phase was composed of 0.1% formic acid aqueous solution (A) and methanol (B). The gradient program applied was as follows: 0–6 min, 10–60% B. The wavelength employed was 246 nm. The flow rate was 0.3 mL/min and injection volume was 2 µL.

### 2.5. Method validation

The analytic method established in this study was validated for linearity, LOD, LOQ, precision (intra- and inter-day), repeatability, stability and recovery. Calibration curves were constructed based on the peak area of analytes (y) versus the corresponding concentrations (x) at six different concentrations in triplicate. The LOD and LOQ were determined by S/N at about 3 and 10 using standard solutions, respectively. The intra- and inter-day precisions were conducted with six replicate injections of the same sample solution performed on the same day and three consecutive days, respectively. To confirm the repeatability, six sample of the same origin were processed and analyzed. The stability of the sample solution stored in UPLC autosampler at 10 °C was investigated by replicate injection of the sample solution at 0, 1, 2, 4, 6, 8, 10 and 12 h. A recovery test was used to further evaluate the accuracy of method. Accurate amount of the mixed standard solution was added to 0.25 g sample powder, whose sample solution was further prepared and analyzed with the method described above.

### 2.6. Data processing

Contents of PO, IPO, P and IP obtained by utilizing different methods of extraction were displayed by GraphPad Prism 5.01 software (Graphpad software Inc., USA). The 'Spider-web' was made by Excel 2016.Lnk software.

## 3. Results and discussion

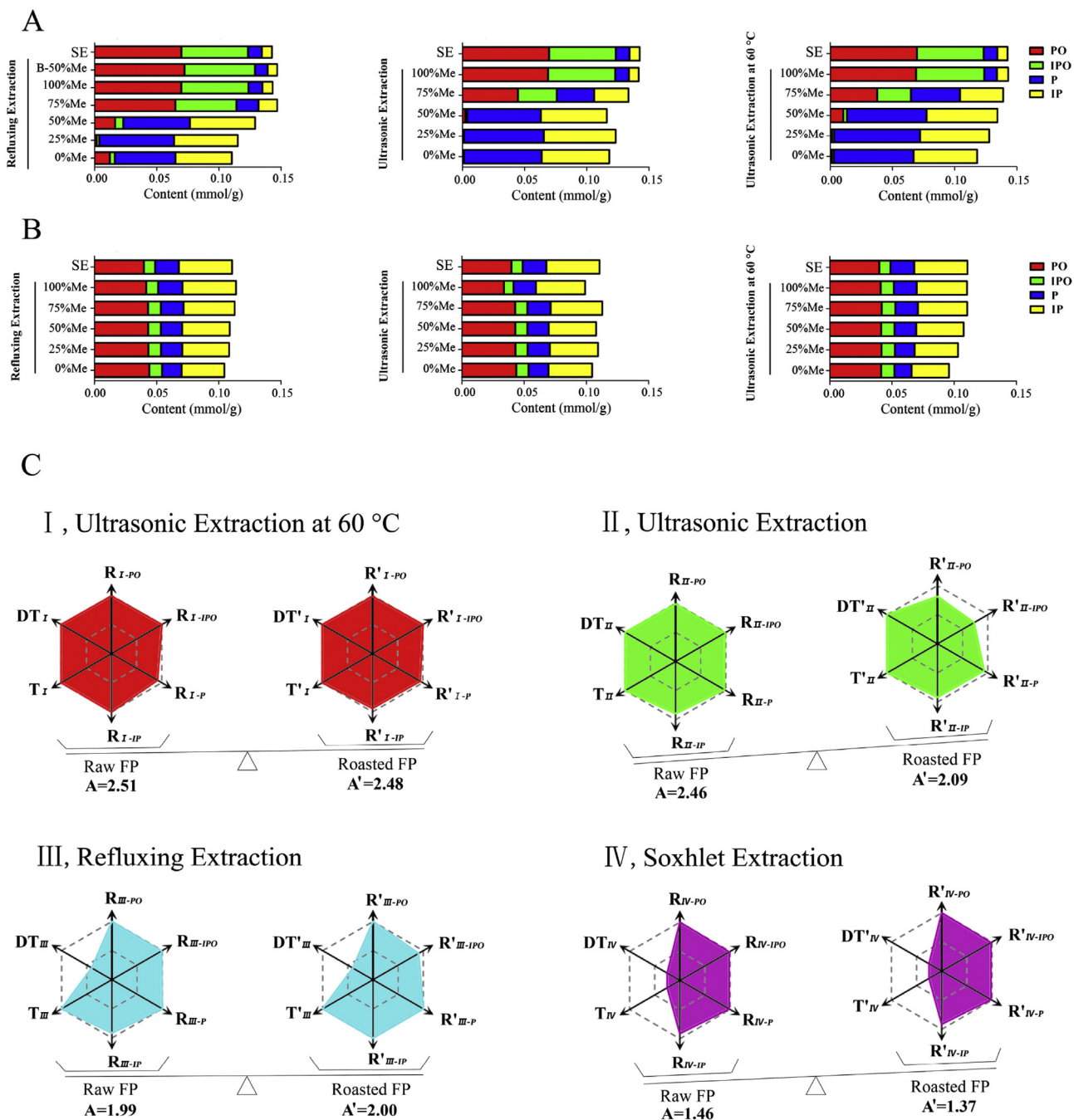
### 3.1. Optimization of extraction conditions

To obtain the optimal condition of sample preparation, many factors, including methods of extraction (soxhlet extraction recorded in ChP, refluxing extraction, ultrasonic extraction and ultrasonic extraction at 60 °C), the extraction solvents (0, 25, 50, 75 and 100% methanol in aqueous solution), the extraction time (20, 30 and 40 min), and the material/solvent ratio (1:100, 1:200 and 1:400 g/mL), were optimized by using univariate analysis. The results were shown in Fig. 1.

An interesting phenomenon has happened, when the raw and roasted FP were treated by different extraction solvents (0, 25, 50, 75 and 100% methanol in aqueous solution) using the four different methods of extraction described above except for soxhlet extraction. Noticeably, for the raw FP, compared with the contents of PO, IPO, P and IP obtained by soxhlet extraction, the formal method regulated by ChP, the quantitative results of selected compounds obtained by methanol extraction of the other three methods were consistent with that of soxhlet extraction. But, as methanol in aqueous solution decreased, the content of PO and IPO in the raw FP declined obviously, and P and IP increased remarkably, especially when methanol was below 50% in extraction solvent. Therefore we speculated that the conversion between (I)PO and (I)P was performed by  $\beta$ -glucosidase naturally present in plant [24,25], which was proved by releasing the powder of the raw FP to the boiled 50% methanol to prevent the conversion. In order to further verify this idea, the roasted grain of FP was prepared by keeping the raw FP at 150 °C for 2.5 h in oven. For the roasted FP, the outcomes obtained by methanol extraction using the three methods accorded with that by soxhlet extraction, except for ultrasonic extraction with lower extraction of PO and IPO. Along with the reduction of methanol in aqueous extraction solvent, the conversion between (I)PO and (I)P was not observed. Only the content of P and IP decreased slightly because of the lipophilic property.

Given that high extraction rate and efficiency, the 'Spider-web' mode [26,27] was employed to choose the best method by taking six characteristics into account, including the extraction efficiency of PO, IPO, P and IP, the extraction time, and the dealing time before extraction. The contents of PO, IPO, P and IP in the raw and roasted FP originated from different methods were marked as  $C_{k-m}$  and  $C'_{k-m}$ , and the extraction time was labeled as  $t_k$  and  $t'_k$ , and dealing time before extraction was assigned as  $s_k$  and  $s'_k$ , respectively. The highest content of PO, IPO, P and IP derived from the tested method was marked as  $C_{k-m}(\max)$  and  $C'_{k-m}(\max)$  in the raw or the roasted FP, respectively. The highest value of  $1/t_k$  and  $1/t'_k$  was respectively tagged as  $1/t_k(\max)$  and  $1/t'_k(\max)$ , which was also applied for the





**Fig. 1** – The histograms of PO, IPO, P and IP, and ‘Spider-webs’ obtained by the different methods employed for extracting the raw and roasted FP (SE, the soxhlet extraction method released by Chinese pharmacopoeia; B-50%Me, the boiled 50% methanol aqueous solution; A, the histograms of PO, IPO, P and IP in the raw FP extracted by different methods; B, the histograms of PO, IPO, P and IP in the roasted FP extracted by different methods; C, the ‘Spider-webs’ obtained by the different methods employed for extracting the raw and roasted FP).

dealing time before extraction (DT). The contents of PO, IPO, P and IP divided by their corresponding maximum, which was badged as  $R_{k-m}$  and  $R'_{k-m}$  in the raw and roasted FP, and the inverse of dealing time and preparation time divided by their corresponding maximum, which was tagged as  $T_k$  and  $T'_k$ , and  $DT_k$  and  $DT'_k$ , separately.

Calculation formulae were as follows.  $k$  represented the methods of ultrasonic extraction at 60 °C (I), ultrasonic

extraction (II), refluxing extraction (III) and soxhlet extraction recorded in ChP (IV);  $m$  represented the compounds of PO, IPO, P and IP.

$$R_{k-m} = C_{k-m}/C_{k-m}(\max) \quad R'_{k-m} = C'_{k-m}/C'_{k-m}(\max)$$

$$T_k = \frac{1}{t_k} / \frac{1}{t_k}(\max) \quad T'_k = \frac{1}{t'_k} / \frac{1}{t'_k}(\max)$$

$$DT_k = \frac{1}{s_k} \bigg/ \frac{1}{s_k} (\max) \quad DT'_k = \frac{1}{s'_k} \bigg/ \frac{1}{s'_k} (\max)$$

Take the method of ultrasonic extraction at 60 °C (I) as example,  $R_{I-PO}$ ,  $R_{I-IPO}$ ,  $R_{I-P}$ ,  $R_{I-IP}$ ,  $T_I$ , and  $DT_I$  were employed to build six dimensions of the ‘Spider-web’ ( $p_i$ ) for the raw FP, and  $R'_{I-PO}$ ,  $R'_{I-IPO}$ ,  $R'_{I-P}$ ,  $R'_{I-IP}$ ,  $T'_I$  and  $DT'_I$  were used to establish six dimensions of the ‘Spider-web’ ( $p'_i$ ) for the roasted FP. The ‘Spider-webs’ of the four methods were shown in Fig. 1. The shaded areas of the ‘Spider-webs’ were established to analyze and determine the most appropriate method, calculating formulae of which were employed for the raw (A) and roasted FP (A') were as follows. The angle between two dimensions was marked as  $\alpha$  and  $\alpha'$ , respectively.

$$A = \frac{1}{2} \times \sin \alpha \left( \sum_{i=1}^{n-1} p_i \times p_{i+1} + p_n \times p_1 \right)$$

$$A' = \frac{1}{2} \times \sin \alpha' \left( \sum_{i=1}^{n-1} p'_i \times p'_{i+1} + p'_n \times p'_1 \right)$$

In general, the extraction method, ultrasonic extraction at 60 °C, did not discriminate against the raw or roasted FP, which showed the balanced shaded area of the ‘Spider-web’ (A, 2.51 and A', 2.48). Moreover, the highest value of the shaded

area demonstrated the perfect extraction rate and efficiency compared with the other methods.

### 3.2. Transformation of psoralenoside and isopsoralenoside under different conditions

As mentioned above, PO and IPO can be transformed into P and IP in the raw FP rather than roasted FP, which suggested that transformation between (I)PO and (I)P may be influenced by the impacts of temperature, pH or  $\beta$ -glucosidase. Therefore, we have systematically performed the effect of temperature, pH, and  $\beta$ -glucosidase on conversion of PO and IPO (Fig. 2). As shown in Fig. 2, the remaining concentration of PO and IPO showed the steady level whether under effect of temperature ranging from 30 °C to 70 °C or pH (2, 4, 6 and 8), which proved that PO and IPO were insensitive to temperature and pH. A slight increase of PO and IPO was detected in the light of evaporation of water in the reaction system.

As illustrated in Fig. 2, exposed to  $\beta$ -glucosidase at 0.84 U/mL, PO was rapidly degraded into P in 6 h, the degradation rate of which was 96.8%. Nevertheless, IPO was found to be more obtuse, which decreased by 67% in 6 h and 94.1% within 18 h. There was a reciprocal transformation between (I)PO and (I)P, which suggested that  $\beta$ -glucosidase held an important role in

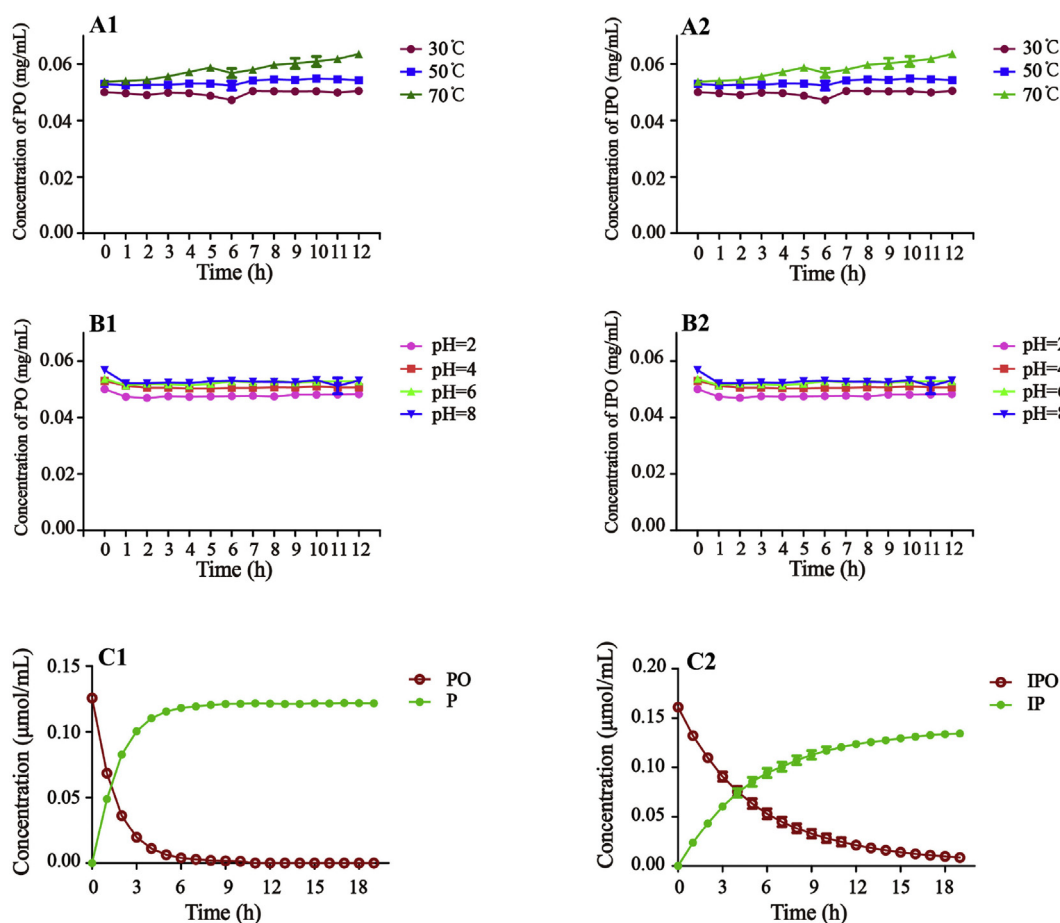


Fig. 2 – The dynamic variations of PO and IPO under different temperature (A1 and A2), pH (B1 and B2) and  $\beta$ -glucosidase at 0.84 U/mL (C1 and C2).

the conversion between (I)PO and (I)P and exerted a significant influence on the content of P and IP in FP.

### 3.3. Method validation

In view of the systematic study above, the method of ultrasonic extraction at 60 °C, which had high rate of extraction and efficiency, was validated methodologically including the linearity, LOD, LOQ, precision, repeatability, stability and accuracy of the interesting compounds, PO, IPO, P and IP. All calibration curves showed good linearity ( $r \geq 0.9999$ ) across the test ranges and the overall LOD and LOQ values were less than 0.08 and 0.25 µg/mL, respectively. The RSDs of the intra- and inter-day precisions, repeatability, and stability were all below 3.0%. The average recoveries of the investigated targets were in the range of 98.30%–101.44% with RSD value below 2.98%. These results demonstrated that the established method was satisfactory for the simultaneous determination of PO, IPO, P and IP in FP.

### 3.4. Quantitative analysis of the samples from the different origins

The established method was applied in outlining the trends on content of the interesting targets of FP from ninety-six origins. Exhibited in Fig. 3A, the total content of P and IP from 0.011 to 0.120 mmol/g was distributed in a scattered form, however, the sum of PO, IPO, P and IP in the range of 0.111–0.170 mmol/g showed in narrow distribution. It could be speculated that the different activity of  $\beta$ -glucosidase contributed to the different degree of transformation between (I)PO and (I)P in the course of cultivation or processing, leading to the different level of P and IP from ninety-six origins.

Specifically, the distribution of total content of P and IP in FP from ninety-six origins was presented in detail in Fig. 3B. The total content of P and IP was divided into the following stages: 0.01–0.024 mmol/g which accounted for 25.00%, 0.024–0.038 mmol/g which made up 31.25%, 0.038–0.052 mmol/g in the proportion of 20.83%, 0.052–0.066 mmol/g which shared 11.46%, 0.066–0.08 mmol/g whose proportion was 8.33%, and 0.08–0.094 mmol/g, 0.094–0.108 mmol/g and 0.108–0.122 mmol/g only accounting for 3.12%, respectively. Released by ChP, the total content of P and IP must be no less than 0.70% (0.038 mmol/g) [1], which qualified only about 43.75% FP. From the performed study, an important clue can be conferred that the great attention

should be paid to the effect of  $\beta$ -glucosidase on the content of P and IP. Many impact factors contributed to the activity of  $\beta$ -glucosidase should be profoundly mined to improve the quality of FP, including growth period, collecting season, processing procedure and storage time.

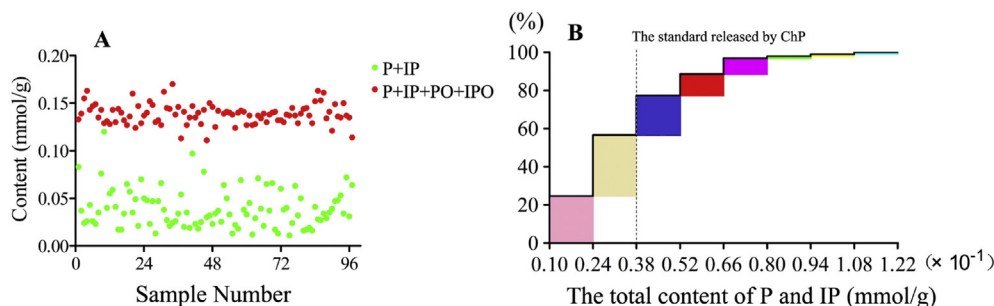
### 3.5. Chemical identification of the roasted from raw FP

Based on the fact that PO and IPO extracted from the raw and soaked FP can be rapidly and obviously transformed into P and IP in 50% methanol aqueous solution or less methanol in solution, which was not applicable to the roasted FP. But, transformation between (I)PO and (I)P was not detected in methanol extract of the raw, soaked or roasted FP. Making the best of this phenomenon originated from the effect of  $\beta$ -glucosidase, in order to prove the feasibility of identifying the roasted from raw and soaked FP, we employed methanol and 50% methanol aqueous solution as the extraction solution to extract the raw FP (B-5, B-51 and B-96), the dried FP under the shade after being soaked with 4% salt solution (SK-5, SK-51 and SK-96) and the salt-processed FP, i.e. the roasted FP after being soaked with 4% salt solution (SP-5, SP-51 and SP-96), respectively. The determined results and representative chromatograms of samples were shown in Table 1 and Fig. 4, respectively. As displayed in Table 1, the content of PO, IPO, P and IP in FP extracted by 50% methanol aqueous solution was tagged as  $C_{PO}$ ,  $C_{IPO}$ ,  $C_P$  and  $C_{IP}$ , whose content of PO, IPO, P and IP extracted by methanol was marked as  $C'_{PO}$ ,  $C'_{IPO}$ ,  $C'_P$  and  $C'_{IP}$ . The ratio of content of PO, IPO, P and IP in FP extracted by 50% methanol aqueous solution to that obtained by methanol was assigned as  $N_{PO}$ ,  $N_{IPO}$ ,  $N_P$  and  $N_{IP}$ , respectively, which was utilized to decide whether FP was roasted or not. Calculating formulae were as follows.

$$N_{PO} = C_{PO}/C'_{PO} \quad N_{IPO} = C_{IPO}/C'_{IPO}$$

$$N_P = C_P/C'_P \quad N_{IP} = C_{IP}/C'_{IP}$$

As illustrated from the result of the studied sample, the roasted FP (SP-5, SP-51 and SP-96) has given  $0.9 \leq N_{PO}$  and  $N_{IPO} \leq 1.1$  or  $N_P$  and  $N_{IP} \leq 1.1$ , and the unroasted FP (B-5, B-51 and B-96, and SK-5, SK-51 and SK-96) has shown  $N_{PO}$  and  $N_{IPO} \leq 0.9$  or  $N_P$  and  $N_{IP} \geq 1.1$ . Therefore, according to the value of  $N_{PO}$ ,  $N_{IPO}$ ,  $N_P$  and  $N_{IP}$ , we can identify the roasted FP (the salt-processed FP) from unroasted FP (the raw FP and the dried FP under the shade after being soaked with 4% salt solution).

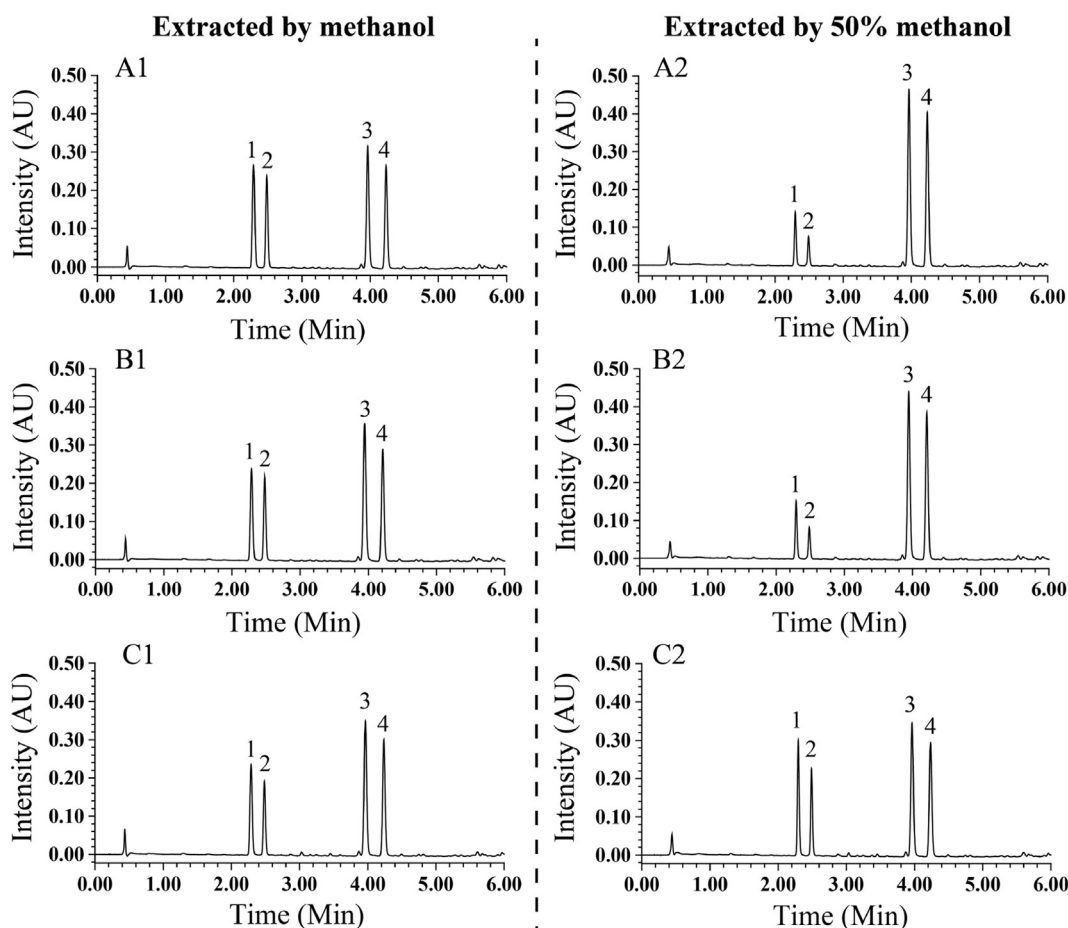


**Fig. 3** – The scattering diagram (A) and accumulative histogram (B) of interesting compounds of FP from the different origins.

**Table 1** – The contents of PO, IPO, P and IP in FP extracted by 50% methanol aqueous solution and methanol (mmol/g) and the value of  $N_{PO}$ ,  $N_{IPO}$ ,  $N_P$  and  $N_{IP}$ .

Sample	$C_{PO}$	$C_{IPO}$	$C_P$	$C_{IP}$	$C'_{PO}$	$C'_{IPO}$	$C'_P$	$C'_{IP}$	$^a N_{PO}$	$^a N_{IPO}$	$^a N_P$	$^a N_{IP}$
B-5	0.0194	0.0077	0.0485	0.0483	0.0524	0.0418	0.0207	0.0188	0.370	0.184	2.343	2.569
SK-5	0.0087	0.0034	0.0597	0.0530	0.0472	0.0379	0.0258	0.0225	0.184	0.090	2.314	2.356
SP-5	0.0464	0.0316	0.0226	0.0242	0.0478	0.0326	0.0246	0.0265	0.971	0.969	0.919	0.913
B-51	0.0147	0.0046	0.0523	0.0468	0.0563	0.0417	0.0156	0.0139	0.261	0.110	3.353	3.367
SK-51	0.0096	0.0039	0.0585	0.0492	0.0500	0.0374	0.0208	0.0179	0.192	0.104	2.813	2.749
SP-51	0.0511	0.0340	0.0185	0.0193	0.0525	0.0351	0.0199	0.0209	0.973	0.969	0.930	0.923
B-96	0.0139	0.0068	0.0524	0.0459	0.0326	0.0244	0.0352	0.0298	0.426	0.279	1.489	1.540
SK-96	0.0165	0.0085	0.0496	0.0437	0.0299	0.0229	0.0410	0.0334	0.552	0.371	1.210	1.308
SP-96	0.0291	0.0197	0.0387	0.0333	0.0289	0.0196	0.0398	0.0345	1.007	1.005	0.972	0.965

<sup>a</sup>  $N_{PO} = C_{PO}/C'_{PO}$   $N_{IPO} = C_{IPO}/C'_{IPO}$   $N_P = C_P/C'_P$   $N_{IP} = C_{IP}/C'_{IP}$ .

**Fig. 4** – The representative chromatograms of samples B-5 (A1 and A2), SK-5 (B1 and B2) and SP-5 (C1 and C2) extracted by methanol and 50% methanol aqueous solution, respectively (1, PO; 2, IPO; 3, P; 4, IP).

#### 4. Conclusion

In this study, a rapid and effective method based on UPLC has been developed for the quantification of PO, IPO, P and IP in FP, which was successfully applied in illustrating the important role of  $\beta$ -glucosidase in transformation between (I)PO and (I)P, surveying the quality of FP from the different origins, and identifying the roasted from unroasted FP. The result could

pave the way for improving the quality and promoting the quality standard of FP.

#### Acknowledgments

This work was supported by the National Science and Technology Major Projects for “Major New Drugs Innovation and



Development” (grant number 2015ZX09J15102-004-004), the Tianjin Support Plans for the Top of the Notch Youth Talents, the National Natural Science Foundation of China (81403060) and National basic scientific and technological project (2014FY1111100).

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jfda.2017.10.009>.

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