Food Chemistry 258 (2018) 189–198

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

A new strategy for statistical analysis-based fingerprint establishment: Application to quality assessment of Semen *sojae praeparatum*

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ARTICLE INFO

Keywords:
Semen *sojae praeparatum*
SA
HCA
PCA
Fingerprint
Quality control

ABSTRACT

Semen *sojae praeparatum* with homology of medicine and food is a famous traditional Chinese medicine. A simple and effective quality fingerprint analysis, coupled with chemometrics methods, was developed for quality assessment of Semen *sojae praeparatum*. First, similarity analysis (SA) and hierarchical clusting analysis (HCA) were applied to select the qualitative markers, which obviously influence the quality of Semen *sojae praeparatum*. 21 chemicals were selected and characterized by high resolution ion trap/time-of-flight mass spectrometry (LC-IT-TOF-MS). Subsequently, principal components analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were conducted to select the quantitative markers of Semen *sojae praeparatum* samples from different origins. Moreover, 11 compounds with statistical significance were determined quantitatively, which provided an accurate and informative data for quality evaluation. This study proposes a new strategy for "statistic analysis-based fingerprint establishment", which would be a valuable reference for further study.

1. Introduction

Semen *sojae praeparatum* (Chinese herbal name is "dan dou chi") is a fermented food, native in China, which is extensively consumed as an edible cooking and flavouring product (Chai, Cui, Shan, Yu, & Wen, 2017). Different fermentation processes had an important influence on the discrimination of Semen *sojae praeparatum* and some fermented soybeans (Lee et al., 2017; Jung, Jung, Lee, & Jeon, 2016). Unlike the general soybeans and other fermented products, including tofu in China, natto in Japan, paste doenjiang in Korea, it must be stressed that Semen *sojae praeparatum* is produced by fermentation from *Soybean*, *Sweet Wormwood Herb* and *Mulberry Leaf* (Qu, Fan, Peng, & Mi, 2007). Simultaneously, as the medicine food homology published by the Ministry of Public Health, Semen *sojae praeparatum* is also a famous traditional Chinese medicine that has been widely applied in clinical areas and officially listed in the Chinese Pharmacopoeia (Pharmacopoeia of the People's Republic of China, 2015). In the present investigation, prominent chemical constituents of fermented soybean products, such as isoflavones, have been detected (Handa, Lima, Guelfi, Georgetti, & Ida, 2016; Xu, Du, & Xu, 2015). These obtained components showed extensive pharmacological activities, including prevention of cardiovascular disease and osteoporosis (Lee et al., 2003; Toro-Funes, Bosch-Fuste, Latorre-Moratalla, Veciana-Nogués, & Vidal-Carou, 2015), anticancer activity (Russo et al., 2016;Wu et al., 2017), anti-estrogenic activity (Kurzer & Xu, 1997; Song, Hendrich & Murphy, 1999), protection against oxidative damage and fibrinolytic stress (Li et al., 2012; Shukla et al., 2016).

Semen *sojae praeparatum* classification and quality control are very necessary but difficult due to its various fermentation processes and different origins. However, as far as we know, there have been no previous reports of quality control of Semen *sojae praeparatum* profiles so far. The fingerprint analysis technique, especially chromatographic fingerprinting, combined with mass spectrometry, has been used as an effective and powerful tool for the quality control of traditional Chinese Medicine (TCM) (Sánchez-Salcedo et al., 2016; Han, Wen, Zhou, & Fan, 2015; Rubert, Lacina, Zachariasova, & Hajslova, 2016) and has been internationally acknowledged for the evaluation and quality control of TCM and related products (FDA, 2004).

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https://doi.org/10.1016/j.foodchem.2018.03.067
Received 15 October 2017; Received in revised form 9 February 2018; Accepted 15 March 2018
Available online 16 March 2018

However, the fingerprint analysis method has a series of defects for evaluation of TCM. Generally speaking, in qualitative analysis of fingerprint, the selection of identified objects depends largely upon given compounds or commercially available standards in fingerprint chromatograms (Yin, Xiong, Shang-guan, & Chen, 2017; Xiang, Xu, Zhan, & Zhang, 2016). Moreover, in quantitative analysis of fingerprint, the components regarded as chemical markers are usually chosen for their high contents (Wu, Kao, Ho, & Chang, 2017). Since the selected compounds may not always provide characteristic and representative information, it weakens the significance of the fingerprint approach and hardly reflects the quality of herbal medicines. Although several analytical fingerprint methods have been applied, in combination with chemometrics such as similarity analysis (SA), principal component analysis (PCA) and hierarchical cluster analysis (HCA), the statistical methods were simply conducted to estimate the quality and consistency of medical products along with the qualitative and quantitative analyses. They were not introduced into the authentication of chemical markers in the fingerprints (Zhang, Yang, Zhang, Shi, & Sun, 2017; Yang, Zhang, Jin, Zhang, & Wang, 2016).

In this paper, a purposeful and selective method was developed to evaluate Semen *sojae praeparatum* with chemometrics analysis. A fingerprint of Semen *sojae praeparatum* samples from different areas was established by a high performance liquid chromatography (HPLC) approach. Different statistical methods, including SA and HCA, were initially performed to select qualitative markers in the chemical fingerprint. Then a high resolution ion trap/time-of-flight mass spectrometry (LC-IT-TOF-MS) was explored for characterization of those markers which would have a crucial impact on the quality evaluation of Semen *sojae praeparatum.* Furthermore, PCA and OPLS-DA were used to select the quantitative markers of Semen *sojae praeparatum* from various origins. This study proposed a new strategy for "statistic analysis-based fingerprint establishment", which is a valuable reference for consistent and accurate quality control of Semen *sojae praeparatum* and other fermented soybean products.

2. Materials and methods

2.1. Plant materials and reagents

Semen *sojae praeparatum*, a form of Chinese fermented preparation obtained from the ripe seed of *Glycine* max (L.) Merr. (Fam. Leguminosae), has been widely produced throughout the country. Generally, the primary manufacture of Semen *sojae praeparatum* comprised three steps: (1) Mix the Folium Mori and Herba Artemisiae Annuae filtrate with clean soybean evenly, and then steam thoroughly. (2) Take out the seeds, dry in air briefly, cover with decoated Mori Folium and Artemisiae Annuae Herba, and ferment until yellow hyphae appear completely. (3) Remove the residue, wash clean, ferment again for 15–20 days until an aromatic odour is produced. 12 batches of Semen *sojae praeparatum* samples were collected by labelling their sources (Table 1a). All of them were collected in March 2016 and authenticated by Dr. Luping Qin from The Second Military Medical University (Shanghai, China).

Semen *sojae praeparatum* reference substance: Daidzin (lot number A0315AS, purity > 98%), Glycitin (lot number A0401AS, purity > 98%), Genistin (lot number A0802AS, purity > 98%), Daidzein (lot number A1009AS, purity > 98%), Glycitein (lot number A0609AS, purity > 98%) and Genistein (lot number M1210AS, purity > 98%) were purchased from Dalian Meilun biotechnology CO. LTD. Malonyl daidzin, Malonyl glycitin, Acetyl daidzin, Acetyl glycitin and Malonyl genistin were isolated and purified by our laboratory from Semen *sojae praeparatum* with the HPLC levels of purities over 98%. Acetonitrile (Analytical grade) and acetic acid (HPLC grade) were purchased from Merck Company (Rahway, NJ, USA) and Tedia (Fairfield, OH, USA). Deionized water was prepared by a laboratory water purification system (HITECH Instruments CO., LTD).

2.2. Extraction procedure

The products were first dispensed as powdered samples, and the crude extracts were weighed out, in 100 ml stainless steel extraction tubes. Extraction was conducted using ASE 300 (Dionex, United States). The conditions of the instrument were as follows: extraction temperature, 125 °C; extraction pressure, 1500 psi; static extraction period, 5 min; cycles, 3; nitrogen purge time, 60 s; purge volume, 60% of the extraction cell. 70% ethylalcohol was used as an extracting solvent. After extraction, the collected extract was centrifuged for 10 min at 3000 r/min and then the crude extract was concentrated by evaporating to dryness by rotary evaporator at 60 °C.

The concentrated samples were used by weighing about 50 mg of crude extract from 12 batches of Semen *sojae praeparatum,* then dissolving in 10 ml of acetonitrile–water (50:50, v/v); all of the crude extracts were soluble. The obtained solution was centrifuged for 10 min at 12,000 r/min for all samples. All the solutions were stored in the refrigerator at 4 °C.

2.3. LC-IT-TOF-MS analysis

HPLC-IT-TOF-MS analysis of Semen *sojae praeparatum* crude extract was performed in the Shimadzu LC-IT-TOF-MS system equipped with an auto-sampler, a degasser, a binary pump, a thermostatted column compartment, a model SPD-M20A array detector and an ion trap/time-of-flight mass spectrometer (Shimadzu Corp, Kyoto, Japan). The mass spectrometry detector (MSD) was equipped with an electrospray ionization (ESI) source. The ionization mode was positive and negative alternatingly. The data acquired were processed by Shimadzu LC-solution software (Kyoto, Japan). A reverse phase column (C_{18} analytical column, 4.6 mm × 250 mm, 5 μm, YMC, Japan) was carried out for the analysis. For HPLC fingerprint chromatographic analysis, the mobile phase was composed of (A) water containing 0.2% (v/v) acetic acid and

Table 1a
A summary of tested sample.

Sample no.	Samples	Origins	AMSL (m)	Temperature (°C)
S1	Semen *sojae praeparatum*	Chengdu, Sichuan	503	16.0
S2	Semen *sojae praeparatum*	Baoding, Hebei	1000	13.4
S3	Semen *sojae praeparatum*	Dali, Yunnan	2052	18.0
S4	Semen *sojae praeparatum*	Guangzhou, Guangdong	4.2	21.0
S5	Semen *sojae praeparatum*	Jiangmen, Guangdong	28.2	22.0
S6	Semen *sojae praeparatum*	Jinhua, Zhejiang	64.7	18.2
S7	Semen *sojae praeparatum*	Liuyang, Hunan	66	17.5
S8	Semen *sojae praeparatum*	Shenzhen, Guangdong	40	22.4
S9	Semen *sojae praeparatum*	Jiande, Zhejiang	87.2	17.4
S10	Semen *sojae praeparatum*	Yizhou, Guangxi	160	20.2
S11	Semen *sojae praeparatum*	Yulin, Guangxi	83	16.5
S12	Semen *sojae praeparatum*	Zhejiang	13	22.0