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Evaluation of the quality of lotus seed of *Nelumbo nucifera* Gaertn from outer space mutation

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Abstract

Lotus seeds from outer space mutation, named as No. 36 space lotus seed, have been used for 6 years in Jianou county, Fujian province, China and compared to the native counterpart. The proximate composition (ash, moisture, protein, lipid, alcohol extract, 100-seed weight, carbohydrate) and nutritional components (amino acids, vitamins B1, B2, B6, C, E, phospholipids) of No. 36 space lotus seed and native lotus seed embryos were compared. The results indicate that the profiles of proximate composition and nutritional components of No. 36 space lotus seed and native lotus seed embryos were similar; however, most chemical contents were significantly higher ($P < 0.05$) in the former. This result was also confirmed by the HPLC fingerprint. The quality of No. 36 space lotus seed was better than that of native lotus seed and the results support the use of this seed as a food and a herbal medicine product.

Keywords: *Nelumbo nucifera*; Outer space mutation; Lotus seed; Evaluation of the quality

1. Introduction

Nelumbo nucifera Gaertn is a perennial aquatic plant and virtually all parts of it, such as seeds, rhizomes, leaves, flowers and stamens, are consumed worldwide. Besides its popularity as an ornamental flower, it is also used as a herbal medicine, mainly in China, Japan, and possibly India, and it displays strong antipyretic, cooling, astringent, and demulcent properties (Mukherjee, Saha, Das, Pal, & Saha, 1997; Sinha et al., 2000; Qian, 2002).

Three clusters of *N. nucifera* have been identified by clustering analysis according to the random amplified polymorphic DNA (RAPD) method. This result showed that these three clusters corresponded with the three different important organs, namely ornament, seed and rhizome, respectively (Yi, Wang, Wu, & Lin, 2006). However, the reproduction of *N. nucifera* can be sexual (seeds) or asexual (rhizomes) (Dong & Zheng, 2005). In 1994, lotus seed was subjected to space mutation for 14 d and 19 h by the "940703" regainable satellite of scientific research in China, and was given the name space lotus (Xie, Zhang, Yang, & Xu, 2004). During cultivation and selection in Guangchang county, Jiangxi province, China, four strains of space lotus with stable character were identified. The space lotus numbered 36 displayed the best character, e.g. long cultivation

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period, big kernel, high seed set propagation coefficient, and high yield (Tang et al., 2001; Xie et al., 2004). In 2000, this space lotus also showed a better agronomy character in Jianou county Fujian province, China, and its yield has been three times that obtained for the native lotus for the past 6 years (Ge, 2002). Due to its increasing availability and usage, it is necessary to evaluate the quality of space lotus for use as a food or in herbal medicine.

Lotus seed, is widely used as a food in China (by the name of lianzi), and its fruit coat, seed coat and embryo contain alkaloids and flavonoids (Chen, Fan, Wu, Wu, & Mitchell, 2007; Rai, Wahile, Mukherjee, Saha, & Mukherjee, 2006). The embryo of the lotus seed has multiple medicinal effects, e.g. invigoration of the spleen, antidiarrhea, kidney tonic, heart tonic and astringent, and it has been recorded as a traditional Chinese drug in the Chinese Pharmacopoeia (PCCn, 2005a). Since lotus seeds have health-promoting properties, the nutritional substances of its embryo, have been analysed and these include phospholipids, proteins, amino acids, vitamins, and sugars (Xu, 1992; Zheng, Zheng, & Zeng, 2003); however, the secondary metabolic products of the embryo, that have not been investigated, can be identified by residue weight extraction with ethanol or methanol, as reported for other Chinese crude drugs (Ren & Zhou, 2003). According to Chinese medicine theory, all components in crude herbs are responsible for the medicinal effects, while fingerprinting offers integral characterization of a complex system with a quantitative degree of reliability (Liu, Zhou, & Yan, 2007). Hence, in this study, the proximate composition, including alcohol extract, nutritional components and the HPLC fingerprints of methanol extract, from the embryo of the No. 36 lotus seeds, were investigated and compared to native lotus seeds.

2. Materials and methods

2.1. General

The seeds of No. 36 space lotus and native lotus (*N. nucifera*) were provided from the field with GAP (Good Agriculture Practice) by the Department of Jiyang Agricultural Technology Extension in Jianou county, Fujian province, China. The mature lotus seeds, which had a light-purple fruit coat, were harvested in July. After removing the fruit coat, seed coat, spire and embryo root (named plumula nelumbinis), the lotus seeds were dried to constant weight at 70 °C and pulverized to pass through a screen with an aperture of 0.5 mm by using a cyclotech mill (FZ-102, Hangzhou Lantian Instrument Co. Ltd., China). The milled powders were transferred to airtight plastic bags and stored in a desiccator at room temperature (24 °C) prior to proximate and nutritional chemical determination, as well as HPLC fingerprint analysis. All chemicals were analytical grade reagents, obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China), except where stated otherwise.

2.2. Proximate analysis

The ash contents were estimated by heating the powder overnight in a temperature-controlled muffle furnace (SX series, Shanghai Hongji Instrument Co. Ltd., China) at 525 °C (PCCn, 2005b). The moisture content, determined by drying the samples for 5 h in the oven at 100–105 °C (PCCn, 2005c), was expressed as a percentage of the weight of the sample. The crude protein content was calculated by multiplying the nitrogen (N) content by a factor of 6.25, which was determined by the Kjeldahl method (KDN-08A, Shanghai Xinjia Electron Co. Ltd., China) whilst the crude lipids were extracted in a Soxhlet extractor using petroleum ether and the content of the crude lipids was determined gravimetrically after oven-drying the extract at 100 °C overnight. The amylose content was determined by a Chemical Auto-analyzer (SF-IV, Alpkem Co., USA); this method was described by the OI Analytical operating instruction and has been used to determine the amylose content of rice by Zhang, Wang, Ling, and Wu (2006). Briefly, the sample and standard amylose were placed in a paper bag for 3 days for the moisture to equilibrate; 0.05 g of standard amylose was added to a 50 ml measuring flask, followed by the addition of 0.5 ml of 95% ethanol, followed by 4.5 ml of 1 M NaOH with light shaking. The mixture was then placed in a boiling water bath for 10 min, cooled, and distilled water was added to a volume of 50 ml. The sample was treated by the same method as for standard amylose and the residue weight was expressed as percentage by weight of sample.

The carbohydrate contents, e.g. 80% ethanol-soluble saccharide (ESS) and 1.5% HCl-soluble saccharide (HSS), were determined by the colorimetric method (Zheng & Wu, 2004a). Briefly, variable glucose standard solutions were added to 1.0 ml DNS, which was used as a colour-developing reagent, as reported by Zheng and Wu (2004a), followed by the addition of distilled water to a volume of 5 ml; this was then placed in a boiling water bath for 5 min, cooled, and made up to a volume of 25 ml with distilled water. The samples were read at $\lambda = 550$ nm in the ambi-beam spectrophotometer (UV-1600, Beijing Ruili Precision & Scientific Instrument Co. Ltd.). The standard curve was $y = 10.835x - 0.0726$ ($r = 0.9997$). ESS was extracted from the sample by using the procedure described by Zheng and Wu (2004a). In short, 2 g of the sample was extracted by ultrasonic cleaner (KQ2200DE, 80W, Kunshang Ultrasonic Instrument Co. Ltd., China) for 30 min, using 20 ml of 80% ethanol and this extraction was repeated once more; the two samples were filtered and the filtrates were combined and made to a volume of 50 ml with 80% ethanol. ESS content was determined by extraction with 80% ethanol, and the solution was then hydrolyzed to monosaccharide with 1.5% HCl by placing it in a boiling water bath and neutralizing it with 10% NaOH. The HSS content was determined by hydrolyzing 0.2 g of the sample into monosaccharide by adding 35 ml of 1.5% HCl and placing the sample in a boiling water bath

for 10 min, after which it was cooled and neutralized by the addition of 10% NaOH. The amounts of ESS and HSS were expressed as percentage by weight of sample.

The alcohol-soluble extract present in the embryos obtained from the lotus seeds was determined according to PCCn protocols (PCCn, 2005d). In short, 100 ml of ethanol were placed in a 250 ml conical flask containing 4 g of the sample and left to stand at room temperature for 1 h. Any losses of ethanol due to the extraction procedure were replaced by the addition of ethanol. The sample was then filtered and 25 ml of the filtrate were placed in an evaporating dish and dried to a constant weight at 105 °C for 3 h, cooled in desiccator for 30 min, and then weighed. The residue weight was expressed as percentage by weight of sample.

The weight of embryos removed from 100 seeds (by a random manner) was calculated by drying the seeds to a constant weight in an oven at 105 °C for 30 min.

2.3. Nutritional component analysis

The contents of amino acids from the embryos obtained from the lotus seeds were determined in a HITACHI 835-50 type Amino Acid Analyzer, using an ion-exchange resin 2619 Column (2.6 × 150 mm) and buffer solution 0.225 ml/min and ninhydrin 0.3 ml/min (Wang, Li, & Liu, 2006; Wu, Li, & Yang, 1990). The amino acid standard was obtained from (Sigma Chemical Co. USA). Before determining the amino acid contents, the protein in sample was hydrolysed to amino acids (Wu et al., 1990). In short, the sample was hydrolysed by 6 N HCl at 110 °C for 24 h under vacuum. The hydrolysed solution was filtered, made up to a volume of 50 ml, by using 0.1 N HCl to rinse the residue, and 2 ml of the solution was then placed in an evaporating dish. This was then dried in a water bath to remove HCl, and further by the addition of 2 ml of distilled water to remove the excess HCl. This procedure was repeated three times and the final solution was then dissolved in 2 ml 0.02 N HCl before assaying. The amino acid content was expressed as percentage by weight of sample.

Vitamins, B1, B2, B6, C, and E from the embryo of the lotus seeds were determined using the methods described in National Standard of the PR China (DNSCn, 2004a, 2004b). The levels of vitamins B1, B2, B6 and E were determined by HPLC (Waters 2695, USA, Waters Co.) with a reversed-phase and UV detector (Waters 2487 UV detector), using internal standard quantitative analysis according to National Standards of the PR China, GB/T 5009.84-2003, GB/T 5009.85-2003, GB/T 5009.154-2003 and GB/T 5009.82-2003, respectively (DNSCn, 2004a). The methods for determining vitamin C were fluorescence spectrophotometry (MPF-4, Japan, Hitachi Co.), OPDA-fluorescence, excitation and emission wavelengths $\lambda =$ 338 nm, 420 nm, respectively, according to National Standards of the PR China GB/T 5009.86-2003 (DNSCn, 2004b). The levels of vitamins were expressed as mg/kg of the sample.

The content of phospholipids was detected by the colorimetric molybdenum blue method (Wang, Wu, Wu, & Wang, 2001). Briefly, phosphonium standard solutions of different concentrations received 0.5 ml 70% perchloric acid, followed by the addition of the coloration reagent (3 M H_2SO_4:H_2O:2.5% $(NH_4)_2MO_4O_{13} \cdot 2H_2O$:10% Vit C = 1:5:1:1) in a shaking water bath at 65 °C for 10 min. After cooling, the optical density at $\lambda = 700$ nm was read using an ambi-beam spectrophotometer (UV-1600, Beijing Ruili Precision & Scientific Instrument Co. Ltd.). The standard curve was $y = 0.1287x - 0.0312$ ($r = 0.999$). Before the above analysis was performed, a 2.5 g sample was extracted twice, with an ultrasonic cleaner (KQ2200DE, 80W, Kunshang Ultrasonic Instrument Co. Ltd., China) for 40 min, using 20 ml of Folch reagent. The filtrates were placed in a 50 ml measuring flask, and then Folch reagent was added to a volume of 50 ml; 2 ml of the sample was used to determine the phospholipids whose amount was expressed as percentage by weight of the sample.

2.4. Fingerprint analysis of methanol extract by HPLC

2.4.1. Sample preparation

Sample powder (5.00 g) was extracted with 20 ml of 80% methanol for 30 min by using an ultrasonic cleaner (KQ2200DE, 80W, Kunshang Ultrasonic Instrument Co. Ltd., China). This extraction was repeated once more; the two samples were filtered and the filtrates were combined and made to a volume of 50 ml with 80% methanol. The sample was again filtered, using a film with 0.45 μm pore diameter before analyzing.

2.4.2. HPLC conditions

The fingerprint of 80% methanol extract was analyzed by HPLC (Agilent 1100), which was equipped with a quaternary pump, an on-line solvent vacuum degasser, using an Alltech C18 column (250 × 4.6 mm, ID, 5 μm) under the following conditions: 30 °C column temperature, 10 μl sample size, 0.5 ml/min flow rate, detection at $\lambda = 254$ nm (VWD, UV detector, Agilent), 35 min run time. A binary gradient elution system consisted of acetonitrile (A) and 0.2% HAc hydro-solution (B) and separation was achieved using the following gradient programme: 0–7 min, gradient 3% A; 7–10 min, linear gradient 3–7% A; 10–15 min, linear gradient 7–20% A, 15–35 min, isocratic 20% A. All reagents were of HPLC grade and purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

2.5. Statistical analysis

The data were analysed by one way ANOVA and tests of significant differences were determined by Dunnett's t-test at $P < 0.05$. The results were expressed as means ± S.E.M. to show variations within a group. The evaluation of HPLC fingerprints was performed by software (PCCn, 2004).

3. Results and discussion

3.1. Proximate analysis

The No. 36 space lotus shows a better agronomy character than does the native lotus, and it has been cultivated for 6 years in Jianou county, Fujian province, China. The comparative analyses of proximate composition, between No. 36 space lotus seed and native lotus seed, are shown in Tables 1 and 2. In general, the embryos obtained from the two lotus seeds exhibited similar patterns of proximate composition with a substantial amount of carbohydrate (ranging from 70.1% to 74.1%, DM: dry matter) and extremely low lipid content (ranging from 0.22% to 0.41% DM). A similar pattern of proximate composition of fresh lotus seed has been previously reported (Zheng et al., 2003), and these findings would be comparable if the contents were calculated by using dry matter (DM). Our results, e.g. ash (4.09–4.99% DM), protein (18.7–19.2% DM), lipid, and carbohydrate, were in agreement with those reported previously (Zheng et al., 2003; Zheng & Wu, 2004a). The moistures of the embryos obtained from No. 36 lotus seeds and native lotus seeds were 8.17% and 8.20%, and within the limits set by the Chinese Pharmacopoeia (PCCn, 2005a). The embryos obtained from the dry seeds of lotus are widely available in China and are sold as food or as herbal medicine. Virtually all the contents in the proximate compositions of No. 36 space lotus seed embryos were significantly higher ($P < 0.05$) than those observed in the native lotus seed, except for the contents of moisture and amylose. Notably, amylose is a very important index of rice internal quality (Zhang et al., 2006); however, only a few studies on amylose from lotus, e.g. lotus rhizome, have been reported (Lin et al., 2006). Amylose content (15.6–16.7% DM) was determined in this study to reveal lotus seed internal quality. Eighty percent of ethanol-insoluble saccharide contents (ranging from 63.8% to 66.1% DM), i.e. most polysaccharides, including starch, were tallied with HSS and ESS. The insoluble saccharides were major components in the embryos of the lotus seeds. Regarding the analysis of the crude protein content, a protein conversion factor of 4.38 instead of 6.25 was used for its calculation since the sclerotium of edible mushroom consists mainly of chitin/chitosan (Ude, Ezenwugo, & Agu, 2001; Wong, Cheung, & Wu, 2003). However, it is still not known whether the correct factor for protein content of lotus seed is 4.38 or 6.25 as it contains an alkaloid residue (Chen et al., 2007). Alcohols, (ethanol and methanol) can dissolve many primary and secondary natural products. The alcohol extract from the embryo of the lotus seed can be used as a crude drug and may also be used to estimate the quality of any unclear secondary compounds (PCCn, 2005a). 1000-seed weight has been used to estimate the rice extrinsic quality; however, the weight of lotus seeds was calculated by using the 100-seed method because the seed is bigger than that of rice. 100-seed weight (102.72 g DM) of No. 36 space lotus seed embryo was greater than that of native lotus seed embryo (89.56 g DM). This is one important reason why the former yield was higher than the latter (Ge, 2002).

Table 2
The carbohydrate content of No. 36 space lotus and native lotus (%, sample DM)[A]

Lotus name	HSS	ESS	EIS	Amylose
No. 36 space	74.1 ± 2.15^{a}	7.95 ± 0.17^{a}	66.1^{a}	15.6 ± 0.47^{a}
Native	70.1 ± 1.18^{b}	6.33 ± 0.22^{b}	63.8^{b}	16.7 ± 0.08^{b}

[A] Mean values (±standard deviation $n = 3$) with different letters (a,b) are significantly different ($P < 0.05$, one way ANOVA), DM: Dry matter. HSS: 1.5% HCl-soluble saccharide. ESS: 80% ethanol-soluble saccharide. EIS: 80% ethanol-insoluble saccharide. EIS% = HSS% − ESS%.

3.2. Nutritional component analysis

The two embryos (No. 36 space lotus seed and native lotus seed) had 17 different amino acids except for tryptophan, which was acid-hydrolysed and destroyed (Zheng et al., 2003) (Table 3). The total amino acid contents (19.4–20.0% DM) were higher than the protein contents (18.7–19.2% DM). This result implies that the free amino acid contents in lotus seed embryos were 0.19–1.29% (MD). Neutral amino acid was higher (8.92–9.92% MD) than acidic amino acid (7.28–7.29% MD) or basic amino acid (3.20–3.39% MD). Glu was the most abundant amino acid (4.98–4.99% DM) and seven different essential amino acids were determined from both lotus seed embryos. The total essential amino acid content was 6.20–6.30% (DM), and the amount of Leu was the highest (1.40–1.41% DM) of the essential amino acids. Vit C (87–106 mg/kg DM) was the principal vitamin detected from both lotus seed embryos and was followed by Vit E (8.7–9.5 mg/kg DM), and Vit B6 (6.0–8.2 mg/kg DM), which implied that lotus seeds may exhibit antioxidant activity (Rai et al., 2006). Phospholipids are reported to enhance many health

Table 1
Proximate composition of No. 36 space lotus and native lotus (%, sample DM)[A]

Lotus name	Ash	Moisture	Protein	Lipid	Alcohol extract	100-Seed weight[B]
No. 36 space	4.99 ± 0.12^{a}	8.20 ± 0.16^{a}	19.2 ± 0.21^{a}	0.41 ± 0.01^{a}	4.32 ± 0.18^{a}	102.72 ± 2.54^{a}
Native	4.09 ± 0.10^{b}	8.17 ± 0.23^{a}	18.7 ± 0.15^{b}	0.22 ± 0.01^{b}	4.18 ± 0.09^{b}	89.56 ± 5.16^{b}

[A] Mean values (±standard deviation $n = 3$) with different letters are significantly different ($P < 0.05$, one way ANOVA), DM: Dry matter.
[B] 100-Seed weight was expressed as 100 seeds weight (g/100 g seed).

Table 3
Amino acid contents of No. 36 space lotus and native lotus (%, sample DM)[a]

Lotus name	Thr[b]	Ser	Glu	Pro	Gly	Ala	Cys	Val[b]
No. 36 space	0.73	1.09	4.99	0.20	0.96	0.91	0.20	1.07
Native	0.76	1.11	4.98	0.25	0.98	0.94	0.22	1.07
	Met[b]	Ile[b]	Leu[b]	Tyr	Phe[b]	Lys[b]	His	Arg
No. 36 space	0.34	0.91	1.40	0.41	0.91	1.18	0.50	1.52
Native	0.37	0.92	1.41	0.56	0.93	1.21	0.50	1.69

	Neutral amino acids	Acidic amino acids	Basic amino acids	Essential amino acids	Total amino acids
No. 36 space	8.92	7.29	3.20	6.20	19.4
Native	9.29	7.28	3.39	6.30	20.0

[a] Mean values of two measurements. DM: Dry matter.
[b] Essential amino acid.

properties (Xu, 1992; Zheng et al., 2003), and their presence in the embryos of the lotus seeds supports their usage in herbal medicine (PCCn, 2005a). The phospholipid content was significantly higher ($P < 0.05$) in No. 36 space lotus seed embryo (0.81% DM) than in the native lotus seeds (0.74% DM) (see Table 4).

Although the profiles of proximate composition and nutritional components of No. 36 space lotus and native lotus embryos were similar, most chemical contents were significantly higher ($P < 0.05$) in the former, especially the contents of phospholipids and alcohol extract. However, external quality is also important and the No. 36 lotus seeds exhibited this property.

3.3. HPLC fingerprint analysis

3.3.1. General

Recently, chromatographic fingerprint methods, such as thin-layer chromatography (TLC), high-performance chromatography (HPLC), gas chromatography (GC), high speed counter current chromatography (HSCCC), mass spectrum (MS), high-performance capillary electrophoresis (HPCE), X-ray, have been used to control the quality of Chinese Crude Drugs (Zheng & Wu, 2004b). Out of all these methods, HPLC is the most popular method and is widely used in fingerprint analysis (Liu et al., 2007) and introduced and accepted by WHO as a strategy for the assessment of herbal medicines (WHO, 1991). It is also required by the Drug Administration Bureau of China to standardize injections made from traditional Chinese medicines (TCM) and their raw materials (DABC, 2000). Hence, the HPLC fingerprint method was used to further compare qualities of No. 36 space lotus seed embryo and its native counterpart.

Table 4
Vitamin (mg/kg, sample DM)[A] and phospholipid (%, sample, DM)[B] contents of No. 36 space lotus and native lotus

Lotus name	B1	B2	B6	C	E	Phospholipids
No. 36 space	2.20	0.12	6.0	106	9.5	0.81 ± 0.04^{a}
Native	5.40	0.16	8.2	87	8.7	0.74 ± 0.11^{b}

[A] Mean values of two measurements. DM: Dry matter.
[B] Mean values (±standard deviation $n = 3$) with different letters are significantly different ($P < 0.05$, one way ANOVA).

3.3.2. Sample preparation and HPLC conditions

The different extracts from native lotus seed embryo were scanned using 70%, 80%, 90%, 100% of methanol, and methanol/chloroform (4/1, v/v) from 190 nm to 600 nm. A strong UV-absorbing was observed at wavelengths from 190 nm to 330 nm. However, wavelengths from 240 nm to 330 nm, namely 240 nm, 254 nm, 280 nm, 300 nm, 320 nm, were used to scan the different extracts, respectively, because methanol has a UV absorption around 200 nm and could interfere with this assay (Liu et al., 2007). Methanol/water (ranging from 5/95 to 95/5, v/v) was used for gradient elution during HPLC analysis. The result showed that a solution of 80% methanol extract, scanned at 254 nm and HPLC fingerprinted, had more peaks with strong absorption at a retention time of less than 5 min. The same result was obtained when the mobile phase was acetonitrile/water. To lengthen retention time with high resolution of peak, different concentrations of acetic acid–water solution, 0.1%, 0.2%, 0.5%, and 1%, instead of water, were used as mobile phase; however, similar fingerprints were obtained when mobile phase was 0.2%, 0.5%, and 1% of acetic acid–water solution. The ion pair reagent, tetra-butyl hydroxyl amine, was also used as a mobile phase instead of acetic acid–water solution; however, although the HPLC fingerprint displayed a high resolution, little peaks and baseline drift were observed (data not shown).

Consequently, an extraction solvent of 80% methanol, with 254 nm detection wavelength, and a binary gradient elution system consisting of acetonitrile (A) and 0.2% HAc-water solution (B) were used to determine the HPLC fingerprint.

3.3.3. Repeatability

According to the methods in 2.4.1 and 2.4.2, HPLC fingerprints of native lotus seed embryo were performed five

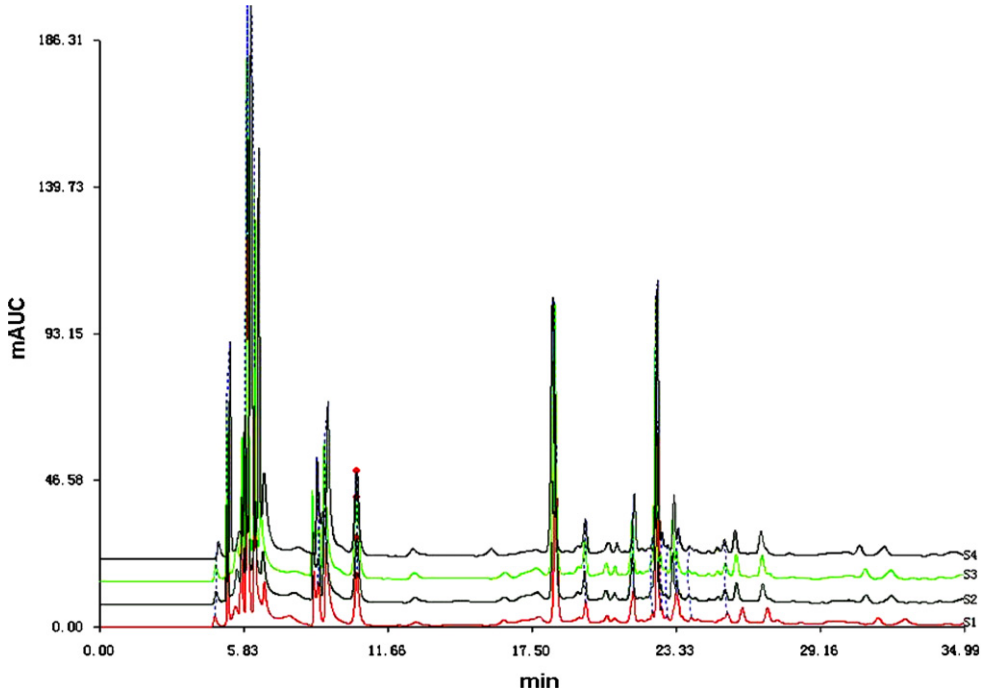

Fig. 1. HPLC fingerprints of 80% methanol extracts stood at room temperature for 0 h (S1), 12 h (S2), 24 h (S3), and 48 h (S4) before determination by HPLC.

times and 11 peaks were determined from the sample. The R.S.D. (relative standard deviation) values of retention time and peak height (means absorbability, eV) were less than 2.5%. This result implies that the methods of extraction and determination were suitable for HPLC fingerprint analysis and displayed feasibility and rationality.

3.3.4. Stability

The solution of 80% methanol extraction from native lotus seed embryo was prepared by using the method in 2.4.1. and stored at room temperature for 0 h, 12 h, 24 h and 48 h before HPLC was performed. The result showed that the extract was stable for 12 h after preparing. In the case of 12 h, a similar HPLC pattern to that observed at the beginning of the experiment was obtained. Both HPLC patterns were evaluated by software ($r = 0.985$) (PCCn, 2004). However, comparing with 0 h, the correlations of 24 h and 48 h were 0.935 and 0.779, respectively (Fig. 1).

3.3.5. Precision

The precision of injection was evaluated by repeated injection of the sample solution (five times). The R.S.D. values of retention time and peak height (means absorbability, eV) were less than 2.5% and the result (Table 5) showed that HPLC gave high precision.

3.3.6. Determination of HPLC fingerprint on lotus seeds

According to the software analysis of HPLC fingerprint semblance (PCCn, 2004), the two HPLC fingerprints had 0.98 correlation (Fig. 2). This result implies that the two

Table 5
The mean values of five (injection time) determinations ±S.D. and R.S.D. values of retention time and peak height (means absorbability, eV) in HPLC fingerprints of native lotus seed removed embryo for equipment precision

Peak no.	Retention time (min)		Peak height (eV)	
	Mean ± S.D. ($n = 5$)	R.S.D. (%)	Mean ± S.D. ($n = 5$)	R.S.D. (%)
1	5.42 ± 0.00	0.06	28.4 ± 0.21	0.74
2	5.63 ± 0.00	0.06	70.4 ± 0.09	0.12
3	5.89 ± 0.00	0.04	120 ± 0.26	0.22
4	6.13 ± 0.01	0.12	13.6 ± 0.17	1.23
5	8.29 ± 0.01	0.06	23.1 ± 0.45	1.94
6	9.86 ± 0.02	0.24	12.7 ± 0.05	0.43
7	19.48 ± 0.01	0.06	15.6 ± 0.19	1.25
8	21.52 ± 0.00	0.02	21.9 ± 0.31	1.41
9	22.48 ± 0.00	0.02	93.9 ± 0.46	0.49
10	23.14 ± 0.00	0.02	11.2 ± 0.28	2.47
11	23.33 ± 0.00	0.02	13.5 ± 0.27	1.98

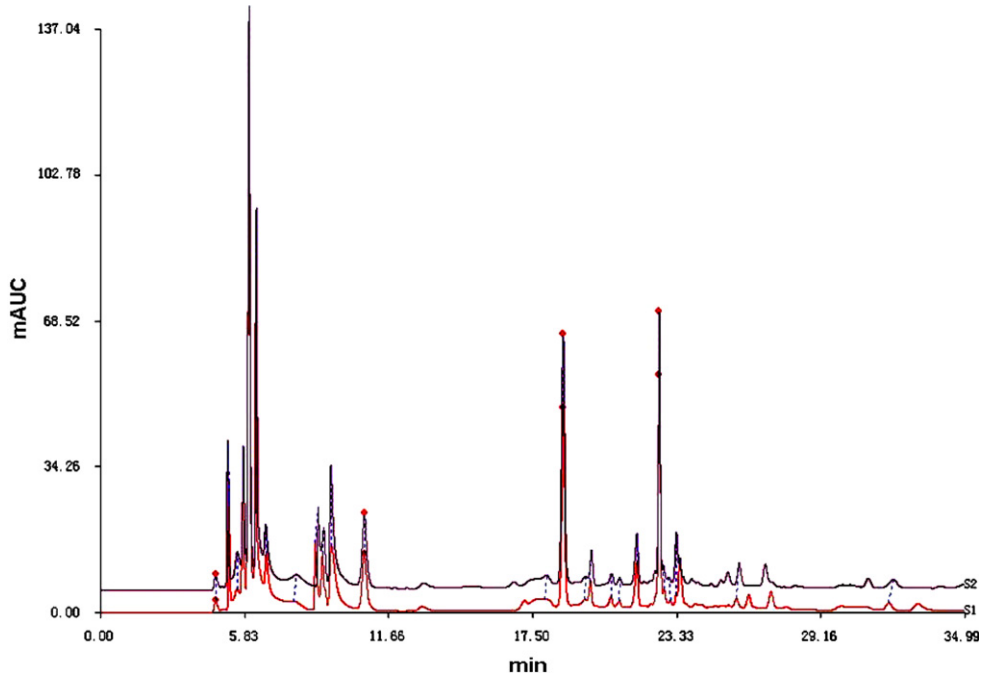

Fig. 2. HPLC fingerprints of 80% methanol extracts between No. 36 space lotus seed removed embryo (S1) and native lotus seed removed embryo (S2). (0.981 correlation).

80% methanol extracts contain similar components which further suggested that both lotus seeds had near internal quality.

4. Conclusion

We have previously investigated the DNA fingerprinting of lotus seeds and the HPLC fingerprinting of *Pseudostellaria heterophylla* (Miq.) Pax ex Pax et Hoffm. The analysis method, consisting of carbohydrate, amino acid, ash, moisture, protein, lipid, of the mushroom, used to estimate the quality of lotus seed embryos showed applicability, availability and reliability (Liao, Wu, Chen, & Guo, 2006; Wong et al., 2003; Yi et al., 2006; Zheng & Wu, 2004a, 2004b). Lotus seeds not only have an important food and medical value, but also propagate well. However, longtime cultivation of lotus seeds can degrade seed quality, leading to a decrease in seed yield. By using space mutation, the quality and yield of lotus seeds have been improved. Further investigations on the chemical analysis of lotus seed embryo are underway.

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