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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