Difference of nutritional components between 

Phyllostachys edulis seeds and main grain crops

Phyllostachys edulis tohumları ile temel tahıllardaki besin ögelerinin farkı

Abstract: Objective: Major nutritional components of moso bamboo (Phyllostachys edulis) seeds were compared with main grain crops to study their nutritional and utilization value.

Methods: Older P. edulis seeds were harvested in autumn 2012 and stored at 4°C for 2 years, while fresh seeds were collected in autumn 2014. Starch, protein and fat contents of both old and fresh seeds were determined by ultraviolet spectrophotometer, Kjeldahl method, and acid hydrolysis method, respectively. The amino acid and fatty acid components of the old and fresh seeds were also analyzed with an auto-amino acid analyzer and a gas chromatograph, respectively. In addition, activities of superoxide dismutase, peroxidase and catalase in both old and fresh seeds were measured.

Results: Starch was the main content of P. edulis seeds. Activities of superoxide dismutase, peroxidase and catalase in fresh P. edulis seeds were significantly higher (P<0.05) than those that had been stored at 4°C for two years. Similar decline was also observed in the nutritional content of seeds upon two years of storage.

Conclusion: The seeds are starchy, containing high levels of protein, moderate fat levels and a wide range of amino acids. Notably, P. edulis seeds contain high levels of essential amino acids and polyunsaturated fatty acids and have great value for human nutrition and health. Its protein and essential amino acids contents were higher than that of main grain crops, while the fat content was low. Storage effect of two years on seeds and fresh grain was significant.

Keywords: P. edulis seeds, Starch, Protein, Fat, Antioxidant enzymes
Introduction

Bamboo (Bambusoideae) is by far the largest member of the grass family Poaceae, and is vital to the economy of many countries in the tropics and subtropics. Bamboo has several unique flowering characteristics; many species have 30–60 years, even 120 years in the juvenile phase, and then engage in a suicidal bout of sexual reproduction [1]. More remarkably, the onset of flowering is synchronous among ramets, genets and stands in a range of spatial scales, while only a few species flower annually or periodically [2,3]. Although the flowering cycle of the moso bamboo (Phyllostachys edulis) is elongated, the seed yield after flowering is extremely large. In Chinese folk records, P. edulis seeds were regarded as a crucial food source to allay hunger. Eating P. edulis seeds as food can nourish people and make them feel light and handy [4]. Plump P. edulis seeds with red color smell especially fragrant [5].

Bamboo resources are very rich in China. The bamboo forest area is about 7,200,000 hm², accounting for 6% of China’s forest area [6]. China is not only the country of origin for P. edulis, but also it is the largest P. edulis production, consumption and export country in the world [7].

However, P. edulis seeds are difficult to harvest and store, which restricts seedling culture, development and utilization. They are prone to mildew and decreased viability after harvest [8]. The germination percentage declined by 14% after 180 days of storage at 4°C [9]. Thus, it is vital to choose appropriate storage conditions to protect P. edulis germplasm resources. Most current research is focused on bamboo shoot nutrition and folia bambosae medicinal value [10], but studies on nutritional components of P. edulis seeds have not been reported. It is of important academic significance and practical value to study the nutritional components and antioxidant enzyme activity of P. edulis seeds. This investigation aimed to analyze the nutritional components of P. edulis seeds in order to make full use of germplasm resources and develop utilization of the seeds. The antioxidant enzyme activity of P. edulis seeds was studied to determine causes for the change of seed viability in the storage period and provide a scientific basis for preservation of P. edulis seeds.

Materials and Methods

2.1 Plant material

Older P. edulis seeds were harvested in Guilin City in Guangxi Province in autumn 2012, while fresh seeds were collected in autumn 2014. The older seeds had been stored for two years at 4°C and packed in sealed plastic bags after ventilating and drying. The thousand-grain weight of old seeds was 21.56±0.62 g, while that of fresh seeds was 23.45±0.56 g. The nutritional components and antioxidant enzyme activity of old and fresh seeds were investigated to study the influence of storage. Each group had three replicates.

2.2 Examination of seed nutritional components

The nutritional components in old and fresh P. edulis seeds were respectively determined and analyzed. Seeds were placed in a Soxhlet extractor and extracted for 7 h using 90% alcohol. Alcohol was volatilized using water bath extraction for 1.5 h. They were then washed three times with distilled water at 80°C. After cooling, the capacity of the 500 mL volume was determined.

Starch content was determined by anthrone–H₂SO₄ colorimetry [11,12].

Protein content was calculated from the nitrogen content which was determined by Kjeldahl method [13,14].

Fat content was analyzed by the acid hydrolysis method [15,16]. Seeds were placed in concentrated
hydrochloric acid and hydrolyzed in a water bath. Then alcohol was added and the mixture was blended. This was extracted by diethyl ether and then recycling solvent, then dried and weighed.

The amino acid component was determined using an auto-amino acid analyzer [17,18]. The fatty acid component was analyzed by gas chromatography [13,19]. Samples were placed in a mixture of light petroleum and benzene. Then 0.4 mol/L KOH-alcohol was added and the mixture was blended. Distilled water was added after 5–10 min and the supernatant extracted and nitrogen was added. The obtained concentrate was used for gas chromatography.

The above methods were in accordance with the National Standard of the People’s Republic of China.

### Table 1: The comparison of the main nutrient contents between *P. edulis* seeds and other crops (%).

| Nutrient content | Old seeds of *P. edulis* | Fresh seeds of *P. edulis* | *O. sativa* | *Z. mays* | *T. aestivum* |
|------------------|-------------------------|---------------------------|------------|-----------|-------------|
| Starch           | 61.97±0.15<sup>a</sup>  | 59.00±0.61<sup>b</sup>   | 74.90      | 69.40     | 70.40       |
| Protein          | 17.00±0.00<sup>a</sup>  | 18.73±0.70<sup>b</sup>   | 8.00       | 9.90      | 11.00       |
| Fat              | 1.80±0.10<sup>a</sup>   | 2.13±0.07<sup>b</sup>    | 1.40       | 4.40      | 1.90        |

Values with different letters differ significantly from each other by ANOVA and Tukey’s test (P<0.05). *The data of *O. sativa*, *Z. mays* and *T. aestivum* were quoted from reference [41].

### 3 Results

#### 3.1 Comparison of main nutrition components between old and fresh *P. edulis* seeds

Carbohydrate was the main component of *P. edulis* seeds (Table 1). The thousand-grain weight of old seeds was 21.56±0.62 g, while that of fresh seeds was 23.45±0.56 g; and the corresponding starch contents were 61.97% and 59.00%, respectively. In addition, the fat content of fresh *P. edulis* seeds was 1.73% higher than that of old seeds. However, the protein content of old and fresh seeds reached 17.00 and 18.73%, respectively. The starch content was significantly higher in old than in fresh seeds. However, the fat and protein contents in *P. edulis* seeds showed different trends to the starch content.

#### 3.2 Comparison of amino acid composition between old and fresh *P. edulis* seeds

Old *P. edulis* seeds had a wide range of amino acids – 33.75% of which were essential amino acids compared to 33.58% in fresh seeds– glutamic acid content was the highest, reaching 19.72 and 20.09% of the total, respectively. The total of essential amino acids in fresh *P. edulis* seeds was 5.90% (Table 2). The total of essential amino acids was notably lower in old than in fresh *P. edulis* seeds. The content of phenylalanine, leucine and valine was relatively high, while that of methionine and tryptophan was correspondingly low.

#### 3.3 Comparison of fatty acid composition between old and fresh *P. edulis* seeds

The fatty acids of *P. edulis* seeds were mainly oleic, linoleic, linolenic, palmitic and stearic acids (Table 3). Unsaturated fatty acid (UFA) content was 1.31 and 1.58% in old and fresh seeds, respectively. The contents of UFA and
total fatty acids were lower in old than in fresh seeds, indicating significant reductions after two years of storage at 4°C. The content of linoleic acid, an essential amino acid for humans, was 0.82% and 1.82% in old and fresh seeds, respectively, while the corresponding content of linolenic acid was 0.03% and 0.05%. The ratio of UFA to saturated fatty acid (SFA) in fresh \( P. edulis \) seeds was up to 2.63.

### 3.4 Comparison of antioxidant enzyme activity between old and fresh \( P. edulis \) seeds

The antioxidant enzyme activity of \( P. edulis \) seeds showed a clear decline after two years of storage at 4°C (Table 4). The SOD activity was significantly higher in fresh seeds (314.48 U·g⁻¹ FW) than in old seeds (259.26 U·g⁻¹ FW). There was a similar situation for POD activity, with fresh seeds having three times the POD activity of old seeds. Additionally, the CAT activity of old seeds was also clearly reduced after long-term storage.

### 4 Discussion

Starch stored in seeds and tubers is globally important as a source of food and has a wide range of industrial applications. Much of this agriculturally produced starch is synthesized in developing seeds, where its biological function is to provide energy for seedling establishment. Starch is a crucial plant nutrient and can also provide energy for the human body [21]. However, it can be converted to fat in the body and excess starch is harmful to human health. The starch content of \( P. edulis \) seeds was significantly lower than that of \( O. sativa \), \( Z. mays \) and \( T. aestivum \). Proteins are the foundation substances of life and are closely connected with all sorts of vital activities, participating in all cells and important parts of the body. There are many kinds of protein with diverse functions in the human body, consisting of 20 different amino acids in various proportions, and are metabolized and reno-
Tables 4:

| Antioxidant enzyme activity | Old seeds of P. edulis | Fresh seeds of P. edulis |
|-----------------------------|-----------------------|-------------------------|
| SOD (U·g⁻¹·FW)             | 259.26±9.47b          | 314.48±2.88a            |
| POD (OD₄₇₀·min⁻¹·g⁻¹·FW)  | 12.07±0.43c           | 49.52±1.75c             |
| CAT (OD₃₄₀·min⁻¹·g⁻¹·FW)  | 8.57±0.40b           | 11.93±0.66c             |

Values with different letters differ significantly from each other by ANOVA and Tukey’s test (P<0.05).

Amino acids are organic compounds comprising azyl and carboxyl groups, and make up the required protein of animal nutrition. They have special physiological functions in antibodies and crucial roles in vital movement. Moreover, amino acids can be oxidized to carbon dioxide (CO₂), water (H₂O) and urea to produce energy [22]. Fat is triacylglycerides consisting of glycerol and fatty acids. The chemical elements in fat are mainly carbon, hydrogen, oxygen, nitrogen and phosphorus. Fat in subcutaneous tissue of animals and plants is part of living organisms and energy storage material, providing animal bodies with essential fatty acids and fat soluble vitamins. It has a trophic function, a metabolic function and an intimate connection with cell recognition, species specificity and histogenic immunity [23]. We found that P. edulis seeds had high levels of protein and moderate fat contents. The protein content of P. edulis seeds was obviously higher than that of O. sativa, Z. mays and T. aestivum while the fat content of P. edulis seeds was only half of that of Z. mays. This phenomenon indicated that P. edulis seeds has high nutritional value, development potential as food and are beneficial to human health.

Amino acids are organic compounds comprising azyl and carboxyl groups, and make up the required protein of animal nutrition. They have special physiological functions in antibodies and crucial roles in vital movement. Moreover, amino acids can be oxidized to carbon dioxide (CO₂), water (H₂O) and urea to produce energy [22]. Fat is triacylglycerides consisting of glycerol and fatty acids. The chemical elements in fat are mainly carbon, hydrogen, oxygen, nitrogen and phosphorus. Fat in subcutaneous tissue of animals and plants is part of living organisms and energy storage material, providing animal bodies with essential fatty acids and fat soluble vitamins. It has a trophic function, a metabolic function and an intimate connection with cell recognition, species specificity and histogenic immunity [23]. We found that P. edulis seeds had high levels of protein and moderate fat contents. The protein content of P. edulis seeds was obviously higher than that of O. sativa, Z. mays and T. aestivum while the fat content of P. edulis seeds was only half of that of Z. mays. This phenomenon indicated that P. edulis seeds has high nutritional value, development potential as food and are beneficial to human health.

Fatty acids are organic molecules with a long aliphatic hydrocarbon chain and a carboxyl group. Fatty acid can be decomposed into CO₂ and H₂O with sufficient oxygen supply, releasing a large amount of energy. Therefore, they are regarded as major sources of energy. Essential fatty acids are necessary for human health and life and cannot be synthesized in the human body and so must come from the food supply. They are all UFAs, belonging to the ω-3 and ω-6 families of polyunsaturated fatty acids (PUFAs). The essential fatty acids are not only essential nutrients, but are also related to human growth, development and health [24] – with relationships to mental development, memory and other physiological functions. Professor Okuyama Harumi compared the effects of linoleic and linoleic acids and found that linolenic acid prolonged the average life of rats by 12% [25]. UFA, especially PUFA content is an important index for evaluating plant nutritional value. The contents of the essential fatty acids oleic and linoleic acids accounted for 23.39% and 46.79%, respectively, of total fatty acids in the fresh seeds of P. edulis, and were clearly higher than levels in old seeds. The contents of linoleic and linolenic acids were both significantly higher than that of Brassica campesiris and Arachis hypogaea [26]. Oleic and linoleic acids not only apply to clinical medicine, but are also widely used in industry. In addition, they can play a crucial role in conquering the blood grease and preventing and treating coronary disease [27]. Yang et al. [28] found that the ratio of edible oil structure should be SFA:monounsaturated fatty acid (MUFA):PUFA of 1:1:1; that of fresh P. edulis seeds was 1:0.85:1.78 (Table 3), indicating that the fatty acids of P. edulis seeds had high value for nutrition development and utilization. The seeds have great potential in developing blended oil that is beneficial to human health. UFAs are one of the main ingredients of cell membranes and can increase cell membrane fluidity and improve the cold resistance of seeds. However, cell senescence and seed deterioration caused by membrane lipid peroxidation occur if seeds are not properly stored. More attention should be given to storage of P. edulis seeds [29].

SOD, POD and CAT synergistically constitute the antioxidant enzyme system [30,31], which plays a protective role by stabilizing the amounts of reactive oxygen species (ROS) in plant cells [32]. SOD is a crucial part of the antioxidant defense system in plants. The major function of SOD is to catalyze the disproportionation of O₂⁻[32]; and the decline of O₂⁻ concentration in plants may prevent O₂⁻ damage to plant cells. Increased O₂⁻ concentrations may also result in increased SOD activity [33]. POD is also an important enzyme that is widely expressed in plant tissues [34] and has many physiological functions, including detoxification [35]. POD can eliminate hydrogen peroxide (H₂O₂), a major substance degraded by SOD, and increased H₂O₂ concentration may result in increased POD activity [36]. CAT is the most general oxidoreductase that converts H₂O₂ to H₂O and O₂ to protect plant cells from damage [37]. The results showed that antioxidant enzyme activities in P. edulis seeds were significantly decreased with prolonged storage, likely because excessive ROS produced in...
metabolic processes actually decreased SOD activity [38]. The ability of scavenging free radicals and peroxides was weakened; and free radicals likely kept accumulating and attacking the membrane lipids, fatty acid chains and proteins, inducing peroxidation and inhibiting antioxidant enzyme activity [39]. Dai [40] indicated that the dehydrogenase and SOD activity of Camellia oleifera seeds was reduced in the aging process. Free radicals and peroxides play a vital role in integrity of the plasma membrane, so their scavenging system function is particularly important in seeds. Research showed that the antioxidant enzyme activity was positively correlated with seed vigor index. Therefore, we deemed that the P. edulis seed vigor index was significantly decreased after two years of storage. Treatment with the gibberellin GA3 may improve the antioxidant enzyme activity and seed vigor and extend seed longevity. The differences in antioxidant enzyme activities between old and fresh seeds were analogous to those of protein and fat.

In conclusion, P. edulis had starchy seeds, containing higher levels of protein than that of main grain crops, moderate fat levels and a wide range of amino acids. Notably, P. edulis seeds contained high levels of essential amino acids and PUFAs and have great nutritional and health value. Seeds of P. edulis should be considered for food additives or health foods by comparison with main grain crops, thus increasing their utilization. The results confirmed the records of edible P. edulis seeds in Chinese ancient classics and indicated that they should be considered as a green nutritional food. However, high levels of UFAs could easily lead to deterioration of stored seeds and more attention should be paid to seed preservation. The P. edulis seeds did not maintain vigor with storage at 4°C and this storage method, widely used in China, is not appropriate. The comprehensive utilization and processing technology of P. edulis seeds needs to be associated with seed production and economic benefits. Two years of storage treatment significantly affected nutritional components and antioxidant enzyme activity of P. edulis seeds. The nutritional content was significantly higher in fresh than in old P. edulis seeds. The contents of protein, fat, amino acids and fatty acids declined by 1.73%, 0.33%, 1.6% and 0.32% after storage, respectively. The significant reduction of antioxidant enzyme activity indicated that the P. edulis seed vitality and germination percentage declined after two years of storage at 4°C. The results showed that old P. edulis seeds were not suitable for establishing seedlings. Appropriate storage of P. edulis seeds should be further studied to lay the foundation for the conservation of germplasm resources and realization of P. edulis genetic breeding.

Acknowledgements: This project was supported by the National Science and Technology Pillar Program during the Twelfth Five-year Plan Period (No. 2012BAD23B0503), Fundamental Research Funds of ICBR (No.1632015009) and “948” of the State Forestry Project (No. 2012–4–49), International Centre for Bamboo and Rattan, China. Wei Ge and Tao Hu contributed equally to this work. Jian Gao and Zhenghua Peng are both the corresponding authors. The authors hope to express their appreciation to the reviewers for this manuscript.

Conflict of interest: None declared.

5 References

[1] Janzen DH. Why bamboos wait so long to flower? Ann Rev Ecol Syst 1976; 7:347–91.
[2] Nelson BW. Natural forest disturbance and change in the Brazilian Amazon. Remote Sensing Reviews 1994; 10:105–25.
[3] Franklin DC. Synchrony and asynchrony: observations and hypotheses for the flowering wave in a long-lived semelparous bamboo. J Biogeography 2004; 31(5):773–86.
[4] Li SZ. Compendium of Materia Medica. 1590; Chinese Classics Publishing House, Beijing.
[5] Li F, Hu M, Li M, Xu X, Zhao LJ, et al. Into Porcelain Pillow. 978; People’s Literature Publishing House, Beijing.
[6] Leng HN, Zheng KL, Li GD, Gui RY. Aluminum stress with seed germination and seedling growth in Phyllostachys edulis. Journal of Zhejiang Forestry College 2010; 27(6):851–7.
[7] Xu JM, Zhao RJ, Fei BH. Research on properties and utilization of bamboo in China. Wood Process Mach 2007; 3:39–42.
[8] Cai CJ, Peng ZH, Gao J, Wang HX, Liu F. Seed germination characteristics of Phyllostachys edulis. Chinese Agricultural Science Bulletin 2008; 24(12):163–7.
[9] Li N, Jin QY, Peng HZ, Hua XQ, Wang KH. Effects of storage treatments on seed vigor of Phyllostachys edulis. Journal of Bamboo Research 2009; 28(2):29–33.
[10] Zhou WW, Zhou RA, He QJ, Ma YG, Wang B, et al. Content analyses of nutrients in bamboo shoots of Phyllostachys edulis. Journal of Zhejiang Agricultural Sciences 2012; 10:1391–3.
[11] Chen YQ. Biochemistry experiment method and technology. 2002; Science Press, Beijing.
[12] National Standard of the People’s Republic of China (GB/T 5009.9–2003). Determination of starch in food. 2003; China Standard Press, Beijing.
[13] Song ZJ, Ji CG. Modern analysis instrument and test method. 1995; Northwest University Press, Xi’an.
[14] National Standard of the People’s Republic of China (GB 5009.5–2010). Determination of protein in food. 2010; China Standard Press, Beijing.
[15] Lisa KK, Christine MG, Julie KS, George CF. Amino acid, carbohydrate, and fat composition of soybean meals prepared at 55 Commercial U.S. Soybean Processing Plants. Journal of Agricultural and Food Chemistry 2005; 53(6):2146–50.
[16] National Standard of the People’s Republic of China (GB/T 5009.6–2003). Determination of fat in food. 2003; China Standard Press, Beijing.

[17] Zhou CF, Yao XH, Lin P, Wang KL, Chang J, et al. Constituent changes associated with seeds development of *Camellia oleifera* Abel. Chinese Journal of Oil Crop Sciences 2013; 35(6):680–5.

[18] National Standard of the People’s Republic of China (GB/T 5009.6–2003). Determination of fat in food. 2003; China Standard Press, Beijing.

[19] National Standard of the People’s Republic of China (GB/T 5009.124–2003). Determination of amino acids in food. 2003; China Standard Press, Beijing.

[20] Zhang HY, Jiang YN, He ZY, Ma M. Cadmium accumulation and oxidative burst in garlic (*Allium sativum*). Journal of Plant Physiology 2005; 162:977–84.

[21] Tetlow IJ. Starch biosynthesis in developing seeds. Seed Science Research 2011; 1(21): 5–32.

[22] Shewry PR, Halford NG. Cereal seed storage proteins: structures, properties and role in grain utilization. Journal of Experimental Botany 2002; 53(370):947–58.

[23] Bhattacharjee A, Ghosh SK, Neogi K, Aich A, Willard B, et al. Deposition of stearate-oleate rich seed fat in Mangifera indica is mediated by a FatA Type Acyl-ACP Thioesterase. Phytochemistry 2011; 72(2–3):166–77.

[24] Osburn L. Hemp seed: the most nutritionally complete food source in the world. Hemp Line Journal 1992; 1(1):14–5.

[25] Baidu baike. Flax meal. baike.baidu.com/view/2700933.htm (Last accessed: February 2016)

[26] Zhu YZ, Wang JQ. Determining fatty acids component of main oil crops by Gas Chromatograph. Acta Agriculture Zhejiangensis 1995; 7(4):326–8.

[27] Li SJ, Shu ZM, Wei LZ, Liang ZS, Fu LL. Protein and fattiness evaluation of Salvia miltiorrhiza seed. Acta Bot Boreal Occident Sin 2008; 28(9):1899–903.

[28] Yang HP, Yang WM, Wang HF. Study on new source of health blend oil. China Oils and Fats 2008; 28(3):11–3.

[29] Pan RZ. Plant Physiology. 1995; Higher Education Press, Beijing.

[30] Cakmak KB, Horst WJ. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of Soybean (Glycine max). Physiologia Plantarum 1991; 83:463–8.

[31] Scandalios JG. Oxygen stress and superoxide dismutase. Plant Physiology 1993; 101:7–12.

[32] Yurekli F, Porgali ZB. The effects of excessive exposure to copper in bean plants. Acta Biologica Cracoviensia Series Botanica 2006; 48:7–13.

[33] Zheng YP, Yu HN, Liu P. The effect of aluminum on antioxidant enzyme activities of seven species of compositae plants. Guizhou Agr Sci 2009; 37:30–3.

[34] Zhang FQ, Wang YS, Lou ZP, Dong JD. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedling. Chemosphere 2007; 67:44–50.

[35] Zeng LS, Liao M, Chen CL, Huang CY. Effects of lead contamination on soil microbial activity and rice physiological indices in soil-Pb-rice (*Oryza sativa* L.) system. Chemosphere 2006; 65:56–7.

[36] Chu L, Yu XL, Li Y, Liu DY. Physiological metabolism and antioxidase activity of Parathelypteris glanduligera under Cu stress. Biol 2008; 25(4):51–4.

[37] Ruley AT, Sharma NC, Sahi SV. Antioxidant defense in a lead accumulating plant, Sesbania Drummondii. Plant Physiology and Biochemistry 2004; 42:899–906.

[38] Aravind P, Prasad MNV. Zinc alleviates cadmium induced oxidative stress in Ceratophyllum demersum L.: a free floating freshwater macrophyte. Plant Physiol Biochem 2003; 41:391–7.

[39] Tang ZI, Song M. Physiological and biochemical analysis of artificially aged Chinese Cabbage. Acta Horticulturae Sinica 1999; 26(5):319–22.

[40] Dai RC. A quantitative study on the injury of membrane during accelerated aging in the seeds of Oltea Camellia (*Camellia oleifera* Abel). Journal of Fujian Normal University 2001; 17(4):84–7.

[41] Seed Work Book Writing Group. Seed Work Book. 1979; Shanghai Science and Technology Publishing House, Shanghai.