African Journal of Food Science Vol. 6(7), pp. 204-211, 15 April, 2012
Available online at http://www.academicjournals.org/AJFS
DOI: 10.5897/AJFS12.032
ISSN 1996-0794 ©2012 Academic Journals

Full Length Research Paper

Effect of sprouting on the chemical and nutritional qualities and phenolic alkaloid content of lotus (*Nelumbo nucifera Gaertn*.) seeds

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Accepted 20 March, 2012

The chemical and nutritional qualities of lotus seeds sprouted in water at different temperatures (25 and 35°C) and water to seed ratio (1:2.5, 1:3.75, 1:5, 1:6.25 and 1:7.5), and their embryos' phenolic alkaloids content were determined and compared to the non-sprouted lotus seeds (control). Sprouting of the seeds resulted to significant increases in the crude protein and crude lipid and decreases in protein solubility and moisture content ($p<0.05$). Meanwhile, no significant variations are observed on the fiber and ash contents. The sprouted seeds also exhibited significantly lower ($p<0.05$) total phenolics, tannins, and cathechins except phytic acid contents. Furthermore, both of the embryo of sprouted seeds extracted by 80% methanol and water demonstrated significant rise ($p<0.05$) in their total phenolics, flavonoids and phenolic alkaloids (neferine, liensinine and isoliensinine). Results show that sprouted seed at 35°C and water to seed ratio of 1:5.00 (w/v) has higher nutritional qualities of the seeds and higher phenolic alkaloids content of the embryos. Sprouting is a simple method which does not require intensive energy input and also yields chemically unvaried product. The previous results strengthen the use of lotus seed as food and lotus seed flour for the development of new food products and formulation. The embryo, a good herbal medicine, can be used as tea component.

Key words: Lotus seed, sprouting, nutritional, phenolic alkaloids, embryo of lotus seed.

INTRODUCTION

Sprouting as a simple technological method that is used to germinate seeds has been reported to improve the nutritive value of seeds (Amal et al., 2007). At the same time there are indications that sprouting is effective in reducing phytic acid and flatulence causing oligosaccharides (namely stachyose and raffinose), thereby increasing protein digestibility and improving sensory properties (Lintschinger et al., 1997). In case of white kidney beans, faba beans and chickpeas, sprouting improved their protein/amino acid digestibility by decreasing anti-nutritional factors and increasing the true/apparent protein/amino acid digestibility (Rubio et al., 2002). The practice of sprouting of cereal grains has also become popular and it is use in many different foods, for example, breakfast items, salads, soups, casseroles, pasta, and baked products (Lorez, 1980).

Sprouting is affected by different factors such as light intensity which has significant effect on biosynthesis of Ascorbic acid and sprout yield of soybean and chickpea (Bhat et al., 2010). Other factors such as irrigation, water quality, and pH, grain preparation, grain quality and variety, seeding density, temperature and growing duration also affect the yield of sprouts. Sanitation is important to reduce the risk of mould. The length of the sprouting phase, available nutrients and light also has effects.

Legumes are considered to be very important foodstuff, particularly in the developing world. Lentil chickpea (*Cicer arietinum* L.) is an important source of essential amino acids, essential fatty acids and trace minerals which

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(Zia-Ui-Haq et al., 2011). Cowpea (*Vigna Unguiculata (L.)* Walp.), which is widely grown in Pakistan, is a vital nutritional oil source (Zia-Ul-Haq et al., 2010). The lotus seed (*Nelumbo nucifera* Gaertn), which is widely grown in the countries of Australia, China, India and Japan, is a popular health food as it contains rich amounts of health-promoting phytochemicals, proteins and essential minerals (Bhat and Sridhar, 2008). Additionally, the embryo of the lotus seed, commonly known by its Chinese name “Lien zi xin”, has been utilized as a traditional Chinese herbal medicine primarily for the therapy of nervous disorders, insomnia, high fevers with restlessness and cardiovascular diseases, for example, hypertension and arrhythmia, and has also recently been reported to show anti-HIV activity (Yanbin et al., 2008). Liensinine and its analogues, isoliensinine and neferine, are the three main bisbenzylisoquinoline alkaloids components found in the embryo. Liensinine has been reported to slow action potentials in myocardium and slow inward current in canine cardiac Purkinje fibers. Isoliensinine has exhibited a significant inhibitory effect on bleomycin-induced pulmonary fibrosis, probably due to its antioxidant and/or anti-inflammatory activities. Neferine, on the other hand, has been reported to possess a reversal effect of multidrug resistance (MDR) mediated by glutathione (GSH) detoxifcation system in K562/A02 cell line. Neferine also reverses multidrug resistance of human gastric carcinoma SGC7901/VCR cells, which may be associated with the down-regulations of P-gp and MRP expression in SGC701/VCR cells (Yi et al., 2007). The lotus seed’s embryo is rich in phospholipids, proteins, amino acids, vitamins, and sugar (Chen et al., 2007). However, studies on the secondary metabolic products of the seed and the embryo, and the use of sprouting in improving the nutritional qualities of the seed and embryo are lacking.

This study focuses on the use of the simple process of sprouting in eliminating or reducing the toxicity of the antinutrients present in lotus seeds. The effect sprouting conditions, namely temperature and water to seed ratio, on the chemical qualities of the seed and the phenolic alkaloids content the seed’s embryo is also examined.

MATERIALS AND METHODS

Dried lotus seeds (*N. nucifera* Gaertn.) were obtained from Hong Xiang Long Company, Xiangtan, Hunan, China. Prior to application of treatments and analyses, the black hard seed coats of the seeds were removed by a sharp knife machine, exposing the brown seed inside.

All chemicals and reagents used were of analytical grade and were obtained from licensed distributors and manufacturers. Folin-Ciocalteu’s reagent and phytate were acquired from Sigma Co., USA; gallic acid equivalents from Tianjin Chemical Reagent Development Center Wing Tai, CN; tannic acid from Sinopharm Chemical Reagent Co., Ltd., CN; cathechins and quercetin from Hefei Hiromi Biotechnology Co., Ltd., CN; and liensinine, isoliensinine and neferine from Tianjin Party Technology Co., Ltd, CN.

Sprouting procedure

The brown lotus seeds were washed with tap water and soaked in hot water for 3 min at 70°C. Afterwards, about 200 g of the brown lotus seeds was soaked in 1000 ml of 80 ppm of peroxyacetic acid for 30 min and then washed with tap water. The seeds were finally rinsed with distilled water until neutral pH.

Different sets of the washed brown lotus seeds were sprouted in distilled water at varying seed to water ratio (1:2.5, 1:3.75, 1:50, 1:6.25, 1:7.5 (w/v) and temperature (25 and 35°C). Another set of seeds was stored in dry container to prevent sprouting and served as the control. After reaching the required sprouting time period (36 h), the embryo was separated from the seed. The seeds were oven-dried at 60°C until their moisture content is reduced to about 10±2%. Similarly, the embryos were oven-dried at 50°C to a moisture content of 5±2%. The dried seeds and embryos were powdered and sieved using 80 mesh screen prior to analyses.

Analysis of the sprouted seeds

Proximate analysis

Moisture content of the seeds (cotyledon portion) was determined by oven drying to a constant mass at 105°C. The crude protein (Humphries, 1986), crude lipid, crude fiber and ash contents were determined as per standard procedures (AOAC, 2000).

Total phenolics: Total phenolics of the seed flours were assayed by adapting the method of Rosset et al. (1982) as outlined in Bhat and Sridhar (2008). Briefly, a known amount of the seed flour was extracted twice with methanol (50%, 5 ml) in a water bath (95°C, 10 min). The pooled extract was made up to 10 ml, and an aliquot of the extract (0.5 ml) was mixed with an equal quantity of distilled water and treated with 5 ml Na_2CO_3 (in 0.1 N NaOH). After 10 min, 0.5 ml Folin-Ciocalteu’s reagent (diluted 1:1 with distilled water) was added and the colour developed was read at 725 nm (Shimadzu, UV-vis 2400). The phenolics determined were expressed as gallic acid equivalents (GAE).

Tannins and catechins: Tannins and catechins were determined according to the method of Brune et al. (1991). Briefly, 1 g of dry seed flour sample was place in a flask containing 50 ml of 50% dimethylformamide in acetate buffer (pH 4.4, DMF acetate). The flask was stoppered and mixed in a shaking machine for 16 h in the dark at room temperature. The extract was then filtered using Whatmann No. 42. One (1) ml aliquot of the extracted sample was placed in a test tube and 4 ml of fresh Ferrous Ammonium Sufate reagent (FAS-reagent) was added. The FAS-reagent was prepared by mixing 89 parts of 50% (w/v) urea-acetate solution (500 g urea in 500 ml 0.1 M acetic buffer, pH 4.4), 10 parts of 1% Arabic solution and 1 part of 5% FAS in 1 M HCl. Absorbance of the sample was determined at 578 and 680 nm against a reagent blank. A food blank was also prepared by replacing FAS in the FAS-reagent with 1 M HCl. Tannic acid and catechins dissolved in 50% DMF acetate are used as standards. The tannins and catechins were expressed as tannic acid and catechin equivalents, respectively.

Phytate analysis

The phytic acid was determined the rapid method by Haug and Lantzsch (1983). Stock solutions were prepared with 1.3 mg/ml phytic acid. Ferric solution was prepared by dissolving 0.2 g $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 100 ml 2 M HCl and the volume was made to 1000 ml with distilled water. 2,2’-Bipyridine solution was prepared by dissolving 1.0 g 2,2’-bipyridine and 1.0 ml thioglycollic acid in distilled water and the volume was made to 100 ml.

Table 1. The effect of sprouting on the proximate composition of lotus seeds (%).

Treatment	Treatment variables		Crude protein	Crude lipid	Crude fiber	Ash	Moisture	Protein solubility
	Temperature	Seed to water ratio (w/v)						
Non-sprouting			20.46^{f}	1.89^{c}	0.12^{a}	4.02^{a}	5.86^{a}	85.9^{a}
		1:1.25	23.20^{bcd}	1.93^{c}	0.14^{a}	3.94^{a}	5.75^{ab}	64.9^{bc}
		1:3.75	22.74^{cde}	1.95^{c}	0.14^{a}	3.84^{a}	5.61^{ab}	65.2^{bc}
	25°C	1:5.00	22.57^{de}	1.96^{c}	0.16^{a}	3.87^{a}	5.52^{abc}	65.7^{bc}
		1:6.25	22.52^{de}	1.98^{c}	0.16^{a}	3.96^{a}	5.41^{bc}	65.9^{bc}
		1:7.50	22.39^{e}	1.99^{bc}	0.16^{a}	3.91^{a}	5.17^{cd}	68.8^{b}
Sprouting								
		1:1.25	24.55^{a}	2.12^{ab}	0.13^{a}	3.97^{a}	4.95^{de}	61.7^{c}
		1:3.75	23.61^{b}	2.12^{ab}	0.15^{a}	3.91^{a}	4.88^{def}	63.0^{bc}
	35°C	1:5.00	23.40^{bc}	2.16^{a}	0.16^{a}	3.99^{a}	4.75^{efg}	63.8^{bc}
		1:6.25	22.46^{de}	2.17^{a}	0.14^{a}	4.02^{a}	4.54^{fg}	68.4^{b}
		1:7.50	22.18^{e}	2.20^{a}	0.14^{a}	4.04^{a}	4.40^{g}	69.0^{b}

Values are means of three determinations (n = 3). Values followed by different superscript letters in a column are significantly ($p < 0.05$) different from each other.

For the analysis of different seed flour, 0.06 g of flour was extracted with 10 ml of 0.2 M HCl at 4°C overnight and 0.5 ml of this extract was pipette out into a test tube fitted with a ground-glass stopper. One (1) ml of ammonium iron (III) sulphate solution was added into it. The test tube was then covered with a stopper and incubated in a boiling water bath for 30 min. After cooling to room temperature, 2 m of (1% v/v) 2',2-bipyridine solution was added. The absorbance was immediately measured at 519 nm against distilled water.

Analysis of sprouted embryos

Extraction method

Heat reflux extraction was done to isolate the three phenolic alkaloids (Liensinine, Isoliensinine and Neferine) from non-sprouted and sprouted embryos. About 0.1 g of the sample was mixed with 10 ml 80% methanol and the suspension was made to boil for 2 h in water bath.

To determine suitability for use as tea component, another set of extract was prepared by adding 10 ml of boiling water to about 0.1 g of the dry embryo placed in a 100 ml conical flask. The mixture was then heated in a water-bath at 90°C for 10 min. After extraction, the obtained extracts were cooled to 25°C, and then filtered through a 0.45 µm filter for analysis (Yao et al., 2006).

HPLC analysis and quantification of phenolic alkaloids (Liensinine, Isoliensinine and Neferine)

The diluted extracts were directly injected into an Agilent 110 High Performance Liquid Chromatography unit equipped with Agilent Zorbax Extend Column (150 × 4.6 mm I.D., 5 µm, 120 Å). A linear gradient elution of solvent A (0.1% triethylamine aqueous solution) and solvent B (CH_3CN) was used. The gradient elution was programmed as follows: 0 to 10 min, 30 to 60% B followed by 60% B for 5 min and return to 30% B in 5 min. A flow rate of 0.8 ml/min was employed in all cases. All chromatograms were acquired at 280 nm and each injection volume was 20 µl. Under these conditions, the three phenolic alkaloids were baseline separated. Peak identification was carried out by comparing their retention time with corresponding peak in the standard solution. A comparison of the chromatograms of the three phenolic alkaloids obtained from standard solutions with those contained in the samples was also done (Yanbin et al., 2008).

Total phenolics: Total phenolic contents of the embryo were determined by the Folin–Ciocalteu method by Meda et al. (2005). The diluted extracts (0.1 ml) was mixed with 2.8 ml of deionized water, 2 ml of 2% sodium carbonate (Na_2CO_3), and 0.1 ml of 50% Folin–Ciocalteau reagent. After incubation at room temperature for 30 min, the absorbance of the reaction mixture absorbance was measured at 750 nm against a deionized water blank on a spectrophotometer (Shimadzu, UV-Vis 2400). Gallic acid (GA) was chosen as a standard.

Total flavonoids: The total flavonoid content was determined using the aluminum chloride colorimetric method by Chang et al. (2005). Briefly, the diluted extracts (0.5 ml) was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of deionized water. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm against a deionized water blank on a spectrophotometer (Shimadzu, UV-Vis 2400). Quercetin solution was used as standard.

Statistical analysis

Analysis of variance (ANOVA) followed by Duncan multiple range test (DMRT) was performed on the data obtained using SPSS 11.5 for Windows. The level of significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of sprouting on the proximate composition of lotus seeds

Sprouting increased protein content of lotus seeds to from 20.5 to 24.5% (Table 1). The non - sprouted lotus

seeds had the lowest protein value (16.0%). Statistical comparison of the protein contents of the sprouted and non-sprouted seeds suggests a significant difference at 95% confidence level. The amount of water used in sprouting also affects the protein content of sprouted lotus seeds. There was an observed decreased in the protein content of the sprouted seeds with increasing amount of water, given the same weight of seeds used. Temperature of sprouting also played an important role in the protein content values measured. Sprouting at higher temperature had significantly ($p<0.05$) increase the protein content of the lotus seeds.

Sprouting also increased ($p<0.05$) the crude lipid content of sprouted lotus seeds in comparison with non-sprouted seeds. The crude lipid of sprouted seed samples ranged from 1.9 to 2.2% and was significantly higher at higher temperature and seed to water ratio. Conversely, no significant effect of sprouting was observed on the crude fiber and ash of the lotus seeds.

Inversely, sprouting had a negative effect on the moisture content of sprouted seeds. The moisture content also decreases with increasing seed to water ratio and temperature, with the lowest temperature and seed to water ratio being significantly different than the others. Similarly, sprouting had a negative effect on the protein solubility value of sprouted seeds. Non-sprouted seeds had significantly higher protein solubility of 85.9% ($p<0.05$) compared to sprouted seeds. However, the protein solubility value increased with increasing seed to water ratio for the sprouted seeds, although the increase was significant. No significant effect of temperature on the protein solubility was observed.

The nutritional changes that occur during sprouting are mainly due to the breakdown of complex compounds into a more simple form, transformation into essential constituents, and breakdown of nutritionally constituents. The significant upswing in the protein content of the seeds as germination progressed could be partly explained by the resumption of protein synthetic activity by certain enzymes (for example, proteases) following imbibition (water uptake). The increase in crude lipid of sprouted seeds can be attributed to increase in essential fatty acids due to upsurge in lipase activity. Increased lipolytic activity during germination and sprouting causes hydrolysis of triacylglycerols to glycerol and constituent fatty acids (Chavan and Kadam, 1989). Higher temperature promotes faster sprouting and therefore, significantly higher crude protein and crude lipid are observed at higher temperature. The decrease in crude protein with increasing seed to water ratio can be partially explained by the leakage of solutes due to the hydration of the seeds and the increase in protein solubility. When seeds are hydrated, solutes leak out of them. Leakage is fastest at the start of imbibitions. Solutes that leak include proteins, amino acids, sugars, organic acids, and inorganic ions. As lipids are insoluble in water, they are unaffected by the increase in the amount of water added to the seeds for sprouting. Leakage of organic and inorganic compounds during the soaking of the seeds before sprouting may also be responsible for the slightly reduced ash content. The slight increase in the crude fiber may be due to the synthesis of structural carbohydrates such as cellulose and hemicellulose, a major constituent of cell walls (Chung et al., 1989).

During sprouting, dry matter is lost due to the increased metabolic activity of the sprouting seeds. The energy for this metabolic activity is derived from the partial degradation and oxidation of starch. Amylase and maltase activity during sprouting of seeds results in a gradual decrease in starch with a concomitant increase in reducing and non-reducing sugars, which is available to the developing embryo. If no external nutrients are added, only water and oxygen are consumed by sprouting seeds. In general, sprouting of seeds can result to a decrease total dry matter, increase in total protein, a change in amino acid composition of the seeds, a decrease in starch content, increases in sugars, a slight increase in crude fat and crude fiber, and slightly higher amounts of certain vitamins and minerals (Chavan and Kadam, 1989).

Effect of sprouting on nutritional value of lotus seeds

Sprouting of the lotus seeds caused decrease in the concentration of total phenolics (Table 2) from 39.7 mg/g in non-sprouted seeds to as low as 30.9 mg/g. The concentrations of tannins (that is, from 4.9 mg/g to as low as 0.7 mg/g) and catechins (that is, from 5.7 mg/g to as low as 0.8 mg/g) also decrease significantly. However, no statistically significant difference is observed in the phytic acid contents of the sprouted seeds and non-sprouted seeds. It is observed that the concentrations of the total phenolics, tannins and catechins of the sprouted seeds decreases as the ratio of water and temperature of sprouting are increased.

Results show that sprouting helps reduce total phenolic, tannins and catechins contents of the lotus seeds, except phytic acid. It is expected that the total polyphenolic, tannin and catechin concentrations will changed after germination. Khandelwal et al. (2010) reported that the total phenolic and tannin contents were reduced significantly in germinated green gram compared to Bengal gram, red gram and lentil. In germinated kidney bean, the loss of total phenolic and tannin contents can be as high as 96% as shown by Shimelis and Rakshit (2007). However, Duenas et al. (2009) found that germination increased total phenolics content in lupin seeds after 9 days. The same was reported by Chai (2011) in germinated peanut. The observed reduction in tannin content after germination was a result of formation of hydrophobic association of tannins with seed proteins and enzymes. In addition, loss of tannins during germination also may be due to the leaching of tannins

Table 2. The effect of sprouting on various nutritional factors (mg/g) of lotus seeds.

Sprouting		Total phenolics	Tannins	Catechins	Phytic acid
Temperature	Seed to water ratio (w/v)				
Non-sprouting		39.67^{a}	4.93^{a}	5.73^{a}	29.29^{c}
25°C	1:1.25	37.24^{b}	0.93^{b}	1.64^{b}	31.48^{ab}
	1:3.75	35.57^{c}	0.91^{b}	1.06^{c}	31.71^{ab}
	1:5.00	33.97^{def}	0.89^{b}	1.03^{cd}	31.77^{ab}
	1:6.25	33.44^{egh}	0.84^{c}	0.92^{cd}	31.87^{ab}
	1:7.50	32.41^{g}	0.83^{c}	0.88^{cd}	32.71^{ab}
35°C	1:1.25	35.00^{cd}	0.84^{c}	0.96^{cd}	30.57^{bc}
	1:3.75	34.55^{cde}	0.83^{c}	0.96^{cd}	31.18^{abc}
	1:5.00	33.41^{efg}	0.77^{d}	0.89^{cd}	31.85^{ab}
	1:6.25	33.21^{fg}	0.74^{d}	0.85^{cd}	32.02^{ab}
	1:7.50	30.90^{h}	0.75^{d}	0.76^{d}	32.77^{a}

Values are means of three determinations (n = 3). Values followed by different superscript letters in a column are significantly ($p < 0.05$) different from each other.

into the water (Shimelis and Rakshit, 2007) as well as washing during germination and binding of polyphenols with other organic substances such as carbohydrate or protein (Saharan et al., 2002). Apart from that, during the period of soaking prior to germination, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols (Saxena et al., 2003; Khandelwal et al., 2010). The decrease might also be due to break down of protein-tannin complex and release of free tannins into soaking water during sprouting (Megat and Azrina, 2012).

Polyphenolics and tannins, which are usually present in the testa layer of seeds, have been recognised as toxicant factors. These are known to inhibit several hydrolytic enzymes, such as trypsin, chymotrypsin, amylases, cellulases and β-galactosidase. In addition they bind with proteins and form tannin-protein complexes, thus making protein unavailable. Tannins have also been found to adversely affect the nutritive value of black beans by decreasing the proteolytic enzymes' digestibility (Aw and Swanson, 1985). However, epidemiological studies have shown that polyphenolic compounds possess rich antioxidant properties and are effective in, reducing cardio-cerebrovascular diseases and cancer mortality, so drinks such as green tea that contain large amounts of these compounds might be good for the health of some people and elimination of phenolic compounds will entirely rest on the consumer's needs (Hertog et al., 1997). Zia-Ul-Haq et al. (2007) found that extracts from *Ferula assafoetida* resin, *Grewia asiatica* leaves, *Ipomoea hederacea* seeds, *Lepidium sativum* seeds, *Nigella sativa* seeds and *Terminalia chebula* have potent activity against certain microorganisms. The antibacterial action of the extracts may be due to the presence of tannins. Detrimental effects of polyphenolics and tannins on the availability of minerals and vitamins have also been reported (Salunkhed et al., 1984). The decrease in the total phenolic and tannin contents of lotus seeds due to sprouting can be due to the break down of the tannin-protein complexes, which also partially explain the increase in protein during sprouting.

Phytic acid is primarily present in the seed coats and germ of plant seeds. It forms insoluble or nearly insoluble compounds with minerals including Ca, Fe, Mg and Zn. Diets high in phytic acid and poor in these minerals produces mineral deficiency symptoms in experimental animals (Gontzea and Sutzescu, 1958). Recently, phytic acid has been recognized for a variety of possible benefits to human health, and not only considered as an antinutrient (Reddy et al., 1986). Phytic acid serves as an antioxidant, an anti-inflammatory selective inhibitor, an energy store, and a regulator of vesicular via binding to various proteins (Talamond et al., 2000). The slight increase in the phytic acid may be due to decortication of the seeds.

The effect of sprouting on the phenolic alkaloids, total phenolics and flavonoid contents of the embryo of lotus seeds

The effect of sprouting on the phenolic alkaloids, total phenolic and flavonoid content of the embryo of sprouted lotus seed extracted by 80% methanol is presented in Table 3. Significant increase in the total phenolic content of lotus seed's embryo is observed due to sprouting Increasing the amount of water added to the seeds for sprouting and the temperature also resulted in increasing total phenolics of the embryo. The same effect and trend can be observed on the flavonoid contents of the embryo of the lotus seeds due to sprouting. The percentage

Table 3. The effect of sprouting on the phenolic alkaloids, total phenolic and flavonoid contents of the embryo of sprouted lotus seed extracted by 80% methanol (mg/g).

Sprouting		Total phenolics	Flavonoids	Phenolic alkaloids		
Temperature	Seed to water ratio (w/v)			Neferine	Isoliensinine	Liensinine
Non-sprouting		42.49^{d}	43.77^{f}	14.03^{e}	5.54^{d}	1.00^{d}
25°C	1:1.25	47.98^{c}	53.94^{e}	17.27^{d}	9.72^{c}	1.24^{cd}
	1:3.75	48.32^{c}	54.29^{de}	18.22^{c}	9.77^{c}	1.40^{c}
	1:5.00	49.74^{bc}	54.99^{de}	18.66^{c}	10.15^{c}	1.52^{c}
	1:6.25	49.68^{bc}	55.28^{de}	17.33^{d}	10.27^{c}	1.93^{b}
	1:7.50	50.83^{abc}	56.20^{d}	17.20^{d}	10.78^{c}	2.13^{b}
35°C	1:1.25	52.40^{abc}	59.08^{c}	18.34^{c}	10.37^{c}	1.96^{b}
	1:3.75	52.05^{abc}	60.91^{bc}	18.90^{bc}	10.77^{bc}	2.03^{b}
	1:5.00	53.01^{ab}	61.97^{b}	20.58^{a}	11.49^{ab}	2.17^{b}
	1:6.25	54.68^{a}	62.33^{b}	19.50^{b}	12.03^{a}	3.00^{a}
	1:7.50	55.03^{a}	68.79^{a}	18.98^{bc}	12.35^{a}	3.15^{a}

Values are means of three determinations (n = 3). Values followed by different superscript letters in a column are significantly ($p < 0.05$) different from each other.

Table 4. The effect of sprouting on the phenolic alkaloids, total phenolic and flavonoid contents of the embryo of sprouted lotus seed extracted by water (mg/g).

Sprouting		Total phenolics	Flavonoids	Phenolic alkaloid		
Temperature	Seed to water ratio (w/v)			Neferine	Isoliensinine	Liensinine
Non-sprouting		33.40^{f}	44.79^{f}	2.86^{c}	0.95^{d}	0.26^{d}
25°C	1:1.25	36.84^{e}	50.58^{e}	4.21^{b}	2.35^{c}	0.29^{cd}
	1:3.75	39.60^{d}	52.25^{d}	4.49^{ab}	2.36^{bc}	0.30^{cd}
	1:5.00	40.28^{cd}	52.40^{d}	4.54^{ab}	2.43^{abc}	0.31^{cd}
	1:6.25	40.88^{cd}	52.71^{cd}	4.60^{ab}	2.66^{ab}	0.34^{bcd}
	1:7.50	41.59^{c}	53.27^{cd}	4.79^{ab}	2.69^{a}	0.36^{bc}
35°C	1:1.25	43.75^{b}	54.32^{c}	4.39^{ab}	2.46^{abc}	0.30^{cd}
	1:3.75	45.38^{ab}	57.13^{b}	4.50^{ab}	2.48^{abc}	0.31^{cd}
	1:5.00	45.92^{a}	57.16^{b}	4.91^{a}	2.55^{abc}	0.37^{bc}
	1:6.25	46.06^{a}	58.24^{ab}	4.81^{ab}	2.68^{a}	0.42^{b}
	1:7.50	46.12^{a}	59.83^{a}	4.75^{ab}	2.69^{a}	0.50^{a}

Values are means of three determinations (n = 3). Values followed by different superscript letters in a column are significantly ($p < 0.05$) different from each other.

contents of the individual alkaloids in the methanol crude extract of the embryo of the seeds ranged from 14.0 to 20.6 for neferine, 5.54 to12.35 for isoliensinine and 1.0 to 3.2 for liensinine.

The effect of sprouting on the phenolic alkaloids, total phenolic and flavonoid content of embryo of sprouted lotus seed extracted by water is presented in Table 4. The same effect and trend on the total phenolics and flavonoids contents of methanol-extracted embryo of sprouted lotus seeds is observed with water-extracted lotus seeds. The percentage contents of the individual alkaloids in the embryo of the seeds range from 2.9 to 4.91 for neferine, 0.9 to 2.7 for isoliensinine and 0.3 to 0.5 for liensinine.

Sprouting leads to an increase in the alkaloids content of the embryo of the lotus seeds. There is also an observed general trend of increase in the alkaloids content of the embryo of sprouted lotus seeds with increasing amount of water added to seed for sprouting, with the exception of neferine at 25 and 35°C temperatures for the methanol extract and at 35°C for the water extract. Neferine has a molecular structure which is more easily dissolved in water compared with the others and may have leaked out during soaking.

The results showed that sprouting can be used to improve the phenolic alkaloids, total phenolic and flavonoid contents of the embryo of lotus seeds. Water can also be used in the extraction of phytochemicals in the embryo of lotus seeds, although less effective than methanol. The major phytochemicals present in the seed embryo are phenolic alkaloids, liensinine, isoliensinine and neferine, which are officially used as the diagnostic constitutes for the quality control of the seed embryo.

Conclusion

Sprouting is mainly a catabolic process which supplies important nutrients to the growing plant through hydrolysis of reserve nutrients. Therefore, induction nutrient of seeds and phenolic alkaloids of its embryo is expected. The result of this study shows that the sprouted lotus seeds constitute a rich source of nutrients, can be successfully utilized as an important source of protein and lipid. At a temperature of 35°C, sprouting is faster and there is higher percentage of sprouting.

Low seed to water ratio is not suitable for used because the seeds will ferment. The temperature and seed to water ratio of 35°C and 1:5.00 w/v for 36 h is recommended to achieve increased protein and fat percentages and high phenolic alkaloids content of its embryo.

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