Physico Chemical and Functional Properties of Lotus (Nelumbo nucifera) Seed

S. Ashoka and M. L. Revanna

Department of Food Science and Nutrition, College of Agriculture, UAS, GKVK, Bengaluru - 560 065 e-Mail : ashokasonu1995@gmail.com

AUTHORS CONTRIBUTION

S. Ashoka : Designing of experiment, investigation and draft prepration. M. L. REVANNA : Supervision, guidence and manuscript correction.

Corresponding Author : S. Ashoka Department of Food Science and Nutrition, College of Agriculture, UAS, GKVK, Bengaluru The purpose of the current research was to identify the physical, chemical and functional properties of lotus (Nelumbo nucifera) seed. The physical characteristics of the lotus seeds without and with seed coat such as 1000 seeds weight, length and width are ranging from 118.43 to 209.06g, 1.68 to 2.32cm and 1.27 to 1.43cm, respectively. The L*, a* and b* values for lotus seeds to its flour ranging from 83.30 to 88.53, 2.50 to 1.08 and 20.50 to 15.02, respectively. The pH of the lotus flour is 7.43. The water holding capacity, oil holding capacity, emulsifying activity, emulsifying stability, foaming capacity and foaming stability of the lotus seed flour are 4.67ml/g, 2.01ml/g, 60.0%, 81.48%, 12.50% and 60.15%, respectively. Inline, lotus seed flour has a good amount of protein, crude fibre and carbohydrates respectively. Hence, lotus seed flour has a greater potential for exploitation in food and pharmaceuticals.

Abstract

Received : July 2022 Accepted : September 2022

Keywords : Functional properties, Lotus seed, Lotus seed flour, Nutritional composition,

OTUS (Nelumbo nucifera) is an aquatic perennial vegetable belongs to the family Nelumbonaceae. It is also known as water lilly, sacred lotus or Indian lotus which is mainly found throughout the Asian continent (China, India, Sri Lanka, Thailand, Japan and Russia) and in Northern parts of Australia and North America. Almost every part of this plant can be used, with the rhizome (sometimes called root) and seeds being the main consumption parts. Lotus seeds and their processing by-products are widely consumed in Asia (China, Japan, India and other Southeast Asian countries), Americans and Oceania for their high content of physiologically active substances (Mukherjee et al., 2009).

Among all plant parts, the lotus seed has attracted special attention from researchers due to its richness in major food components (lipid, protein, starch, vitamins and minerals) and bioactive compounds. The seed contains 61-62 per cent of carbohydrates, 16-21 per cent of total protein, 2.40 - 3 per cent of crude fat with 5-9 per cent of moisture content (Sathithon &

Nelumbo nucifera

Yan-bin, 2012; Shahzad et al., 2021). One of the dominant components of carbohydrates present in lotus seed is starch. The retrograded starch or resistant starch of lotus seed demonstrated strong prebiotic effects by stimulating the growth of beneficial microbes in the gut (Zeng et al., 2018).

Lotus seeds have been used as a vegetable, functional food and medicine for 7,000 years. These are low caloric, a rich source of multiple nutrients and bioactive constituents, which make them a unique therapeutic food (Arooj et al., 2021). Lotus seeds are consumed in many forms as raw or cooked, ripened or un-ripened and are mostly used in desserts (Chen et al., 2019). These seeds can also be used as roasted/ ground, raw and boiled into syrups (Moro et al., 2013). Lotus seed contains a diverse range of phytochemicals including alkaloids, flavonoids, polysaccharides, essential oils, glycosides, polyphenols, triterpenoids, etc., which have a wide range of pharmaceutical properties (Chen et al., 2012; Huang et al., 2010 and Zhenjia et al., 2010).

Flour and starches are important ingredients in the food industry. As new food products are developed, starches or flours with specific properties are necessary to impart functionally desirable attributes. However, unfortunately, little data has been reported on the physicochemical and functional characteristics of lotus seed flour. Therefore, the study was undertaken to determine the physical, chemical and functional properties of lotus (*Nelumbo nucifera*) seed flour. Results of the present study will help to explore the possibilities of utilizing the lotus seed flour for the development of value-added products as a potential ingredient.

MATERIAL AND METHODS

The study was conducted in the Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bengaluru.

Selection and Collection of Sample

The fresh lotus seeds were obtained from the states of Karnataka and Tamil Nadu. The lotus seeds were extracted from the lotus seed heads and used for further processing (Fig. 1).



Fig. 1 : Flowchart of Processing of Lotus seed flour

Physical Properties of Lotus Seeds

Physical characteristics viz., seed length, width was assessed by using a vernier calliper and 100 seed weight was calculated using a digital weighing balance.

The Colour was estimated using a reflecting colorimeter (Chroma meter CR-300). The samples were kept in a colorimeter petri dish and readings were taken in triplicates. The L*, value is a measure of lightness/brightness, ranging from 0 (black) to 100 (white). The a* value is a measure of greenness/ redness, ranging from -60 (green) to +60 (red) and the b* value is a measure of bluishness/yellowness, ranging from -60 (blue) to +60 (yellow).

Bulk Density (Loose and Packed Bulk Density) : The preweighed (W1) bulk density of the flour was determined using the Akpapunam and Markakis (1981) method. A measured amount of flour was deposited in a preweighed (W1) measuring cylinder and both the weight of the cylinder (W2) and the volume of the flour (V1) were recorded.

The loose bulk density (LBD) was calculated as $LBD = W2 - W1 \div V1$.

The flour in the cylinder was gently tapped to remove any air pockets between the flour particles. The sample's new volume (V2) and mass (W3) were recorded and the packed bulk density (PBD) was calculated as PBD = W3 – W1 \div V.

TSS (total soluble solids) were measured with a digital hand refractometer and expressed as degrees Brix (°Brix).

Chemical Properties of Lotus Seed Flour

pH was analyzed using a digital Cole Parmer pH meter

The moisture, protein, fat, crude fibre and ash contents were determined by using standard AOAC, 2005 techniques to determine moisture, protein, fat, crude fibre and ash contents. The differential method was used to calculate carbohydrate content. The energy was also determined using the computing method and all values were expressed as % w/w, based on a dry weight.

CHO (g/100g) = 100-[Protein(g) + Fat(g) + Fibre(g) + Ash(g) + Moisture(g)]

Energy (kcal) = [Protein (g) \times 4] + [Carbohydrate (g) \times 4] + [Fat (g) \times 9]

Functional Properties of Lotus Seed Flour

Water Holding Capacity

Water holding capacity was calculated using a method developed by Gould and others (1989), with minor adjustments. 3 g of the flour sample (dry) was weighed into a centrifuge tube, adding 30ml distilled water and mixed for 30 seconds with a cyclo mixer. The sample was allowed to hydrate at room temperature for 2 hours. This was followed by a 10-minute centrifugation at 2800 rpm. The supernatant was discarded and the hydrated sample was weighed. The findings were expressed as,



Oil Holding Capacity

The oil holding capacity was calculated using the approach of Caprez and others (1986), with minor adjustments. A sample of 3 g dried flour was weighed into a centrifuge tube, combined with 30 mL of corn oil, then mixed for 30 seconds with a cyclo mixer. The sample was allowed to stand at room temperature for 1 hour. This was followed by 10 minutes of centrifugation in a benchtop centrifuge at 2800 rpm. The pellet was weighed after the supernatant was drained. The findings were expressed as,

 $Oil holding = \frac{weight of pellet (g)-weight of dry sample (g)}{capacity (g/g) weight of dry sample (g)}$

Emulsion Activity and Stability

The emulsion activity and stability determined by Yasumatsu *et al.* (1972) were followed and the emulsion (1 g sample, 10 ml distilled water and 10 ml sunflower oil) was prepared in a calibrated centrifuge tube. The emulsion centrifuged at $2000 \times g$ for 5 min. The ratio of the height of the emulsion layer to the total height of the mixture was calculated as emulsion activity in percentage. The emulsion stability was

estimated after heating the emulsion contained in the calibrated centrifuged tube at 80°C for 30 min in a water bath, cooling for 15 min under running tap water and centrifuging at $2000 \times g$ for 15 min. The emulsion stability expressed as a percentage was calculated as the ratio of the height of the emulsified layer to the total height of the mixture.

Dispersibility

Dispersibility was determined by the method described by Kulkarni *et al.* (1991) modified by Akanbi *et al.* (2009). 10g of flour was suspended in a 100 ml measuring cylinder and distilled water was added to reach a volume of 100 ml. The setup was stirred vigorously and allowed to settle for 3 hrs. The volume of settled particles was recorded and subtracted from 100. The difference was reported as percentage dispersibility.

Foaming Capacity

The foaming capacity of the flour was determined by the known method (Coffman & Garcia, 1977). Seed flour (2 g) was dispersed in distilled water (100 mL) and homogenized properly for two minutes using the cyclo mixer. The volumes were recorded before and after homogenization and the per cent increase in the volume was calculated as foaming capacity using the following formula:

Foaming capacity (%) = $100 (V2 - V1 \div V1)$

where,

- V1 = initial volume
- V2 = volume of solution after homogenization

Foaming Stability

The foam was allowed to stand for 8 hours at room temperature and the foam stability (FS) was expressed as the percentage retention of the initial foam volume as:

Foaming stability (%) = $100 (Vt \div V_0)$

where,

 $V_0 = initial$ foam volume and

Vt = foam volume after time (t)

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) for testing the significance of variation using SPSS (Statistical Package for Social Sciences) software 16.0.

RESULTS AND DISCUSSION

Physical Properties of Lotus Seeds

The physical properties of the lotus seed is as summarized in Table 1. Results revealed that the mean scores of 100 seeds weight, length and width of lotus seeds without and with seed coat ranges from 118.43

TABLE 1 Physical properties of lotus seed Parameters Without W seed coat

With

seed coat	seed coat	
118.43 ± 3.41	209.06 ± 4.37	
$1.68 ~\pm~ 0.04$	$2.32 ~\pm~ 0.19$	
$1.27 ~\pm~ 0.01$	$1.43 ~\pm~ 0.05$	
Lotus Seed	Lotus seed flour	
1.80	0.5	
1.80	0.58	
- 15	6.50	
	$seed coat$ 118.43 ± 3.41 1.68 ± 0.04 1.27 ± 0.01 Lotus Seed 1.80 1.80 $-$	

to 209.06g, 1.68 to 2.32cm and 1.27 to 1.43cm, respectively. It's indicating that the whole seed weighs more compared to the seed alone. Whereas the loose bulk density and packed bulk density of lotus seeds to lotus seed flour ranges from 1.8 to 0.5 and 1.8 to 0.58, respectively. Similar results were obtained in the study conducted by Shad *et al.*, 2011 for lotus rhizome flour. Also, the present findings were found

to be comparable to those reported for wheat and sweet potato flour blends (Adeleke and Odedeji, 2010). Bulk density values help to develop suitable nutrient dense products and it mainly depends on the density or particle size of the flour. TSS of the lotus seed flour is 6.50 °Brix.

Colour Analysis of Lotus Seeds and its Flour

The colour analysis of the lotus seeds and its flour results were depicted in Table 2. Results revealed that, L*, a* and b* values for lotus seeds to its flour ranges from 83.30 to 88.53, 2.50 to 1.08 and 20.50 to 15.02, respectively. It shows that there is a significant difference among all the parameters viz., L*, a*, and b*. The L* values of both lotus seeds and its flour indicate white in colour. The value for a* was found to be high in lotus seeds compared to lotus seed flour which means both are red and the values ranges from 2.50 to 1.08. The b* values are also high in lotus seeds when compared to lotus seed flour. However, the b* values of both the lotus seeds and its flour are yellow in colour. It also observed that there is a significant difference among all the parameters viz., L*, a* and b* in both lotus seed as well as lotus seed flour. Similar results were obtained in the study conducted for Thai lotus seed flour by Singthong and Meesit, 2017. The present results are on par with the study conducted by Ham et al. (2017) because they subjected the seeds to different cooking and drying methods.

Chemical and Functional Properties of Lotus Seed Flour

Table 3 depicts the chemical and functional properties of lotus seed flour. Results revealed that pH is 7.43. It

colour unurysis of fotus seeds and its field					
	Samples	L*	a*	b*	
	Lotus seed	83.30 ± 0.88	$2.50~\pm~0.64$	20.50 ± 2.95	
	Lotus seed flour	$88.53 \ \pm \ 0.06$	$1.08~\pm~0.0$	$15.02 \ \pm \ 0.11$	
	$Mean \pm SD$	$85.91 \ \pm \ 2.91$	$1.79~\pm~0.87$	$17.76~\pm~3.53$	
	F value	*	*	*	
	SEM	0.36	0.26	1.2	
	CD @ 5%	1.41	1.01	4.74	

TABLE 2Colour analysis of lotus seeds and its flour

Foaming stability (%)

TABLE 3				
Chemical and functional properties				
of lotus seed flour				
Parameters	Values			
pH	7.43			
Water holding capacity (g water/g dry sample)	4.67			
Oil holding capacity (g oil/g dry sample)	2.01			
Emulsifying activity (%)	60.00			
Emulsifying stability (%)	81.48			
Dispersibility (%)	30.50			
Foaming capacity (%)	12.50			

60.15

indicates that lotus seed flour was neutral. The pH of the present study was neutral compared to the study conducted for wheat and sweet potato flour by Adeleke and Odedeji, 2010 and it indicates that wheat and sweet potato are slightly acidic when compared to the lotus seed flour. The water holding capacity and oil holding capacity of the lotus seed flour are 4.67ml/g and 2.01ml/g, respectively. The WHC and OHC results were in line with the study conducted by Kayitesi et al., 2012 for tapioca flour. Whereas, the WHC and OHC of the present study are slightly higher than the study reported for Thai lotus seed flour, tiger nut flour, wheat and sweet potato flour, respectively (Singthong & Meesit, 2017; Oladele & Aina, 2007; Adeleke & Odedeji, 2010 and Muttagi et al., 2017). WAC and OAC values of seed flours indicate the ability of the protein to absorb and retain water or oil and may help to improve binding of the structure, flavour retention and mouthfeel and reduce moisture and fat losses in extended meat products and baked products (Maninder et al., 2007 and Bhat & Sridhar, 2008). The Emulsifying activity and emulsifying stability, dispersibility, foaming capacity and foaming stability of the lotus seed flour are 60.00, 81.48, 30.50, 12.50 and 60.15, respectively. The results of emulsifying activity and emulsifying stability of the present study are in line with the study of (Singthong & Meesit, 2017 and Bhat et al., 2009), respectively. Lotus seed flour can be used as stabilizer in the production of sausage, soup, mayonnaise, salad dressing and bakery products (Singthong and Meesit, 2017). Also, the results of foaming capacity and foaming stability of the lotus seed flour are in line with the study of Singthong and Meesit, 2017 and Oladele & Aina, 2007, respectively. Increased protein concentration has been found to facilitate enhanced protein-protein interactions at the air-water interface and promote the formation of a highly viscoelastic multilayer film that offers resistance to the coalescence of bubbles (Adebowale and Lawal, 2003).

Nutritional Composition of Lotus Seed Flour

The nutritional composition of lotus seed flour is shown in Table 4. The results revealed that the moisture, protein, fat, ash, crude fibre, carbohydrate and energy contents were 4.59 per cent, 19.25g, 1.66g, 5.23g, 4.25g, 65.02g and 352.02 kcal, respectively. The present results were in line with the study conducted by Singthong & Meesit, 2017; Shukla & Chaturved, 2015; Antarkar et al., 2019 and Shahzad et al., 2021. However, the protein and moisture content are more in the study conducted by Jirukkakul and Sengkhamparn, 2018. It might be due to the differences in the cultivar, growing zone and climatic condition of the seeds. The proximate composition of the lotus seed in the present study indicates that the utilization of lotus seed flour will help to develop value-added products and bakery products with high protein and carbohydrates.

TABLE 4

Nutritional composition of lotus seed flour per 100g

Nutrients	Lotus seed flour
Moisture (%)	4.59 ± 0.25
Protein (g)	19.25 ± 0.24
Fat (g)	1.66 ± 0.30
Ash (g)	5.23 ± 0.04
Crude fibre (g)	4.25 ± 0.09
Carbohydrates (g)	65.02 ± 0.15
Energy (Kcal)	352.02 ± 0.44

The results of the present study showed that lotus seed flour is a good source of protein, fibre and carbohydrates. Also, it had better functional properties like water holding capacity, oil holding capacity and emulsion properties. Hence, utilization of lotus seed and its flour will help to develop value added functional and bakery products. Hence, the present results of the study will provide basic information about the lotus seed and its flour.

REFERENCES

- ADEBOWALE, K. O. AND LAWAL, O. S., 2003, Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrates. *Food chemistry*, 83 (2): 237 - 246.
- ADELEKE, R. O. AND ODEDEJI, J. O., 2010, Functional properties of wheat and sweet potato flour blends. *Pakistan Journal of Nutrition*, **9** (6) : 535 538.
- AKANBI, T. O., NAZAMID, S. AND ADEBOWALE, A. A., 2009, Functional and pasting properties of a tropical breadfruit (*Artocarpus altilis*) starch from Ile-Ife, Osunstate. *Nigeria. Int. Food Res. J.*, 16: 151 - 157.
- AKPAPUNAM, M. A. AND P. MARKAKIS., 1981, Physicochemical and nutritional aspects of cowpea flour. *Journal of Food Science*, **46**: 972 - 973.
- ANTARKAR, S., GABEL, S., TIWARI, S., MAHAJAN, S. AND AZMI, R. M., 2019, Evaluation of nutritional and functional properties of partially substituted whole wheat flour with taro root and lotus seed flour (Composite flour). *Pharma Innovation*, 8: 125 - 128.
- AROOJ, M., IMRAN, S., INAM UR RAHEEM, M., RAJOKA, M. S. R., SAMEEN, A., SIDDIQUE, R., SAHAR, A., TARIQ, S., RIAZ, A., HUSSAIN, A. AND SIDDEEG, A., 2021, Lotus seeds (*Nelumbinis semen*) as an emerging therapeutic seed : A comprehensive review. *Food Science & Nutrition*, 9 (7) : 3971 - 3987.
- BHAT, R. AND SRIDHAR, K. R., 2008, Nutritional quality evaluation of electron beam-irradiated lotus (*Nelumbo nucifera*) seeds. *Food Chemistry*, **107** (1) : 174 - 184.
- BHAT, R., SRIDHAR, K. R., KARIM, A. A., YOUNG, C. C. AND ARUN, A. B., 2009, Influence of γ-radiation on the nutritional and functional qualities of lotus seed flour. *Journal of agricultural and food chemistry*, **57** (20): 9524 - 9531.

- CAPREZ, A., ARRIGONI, E. V. A., AMADÒ, R. AND NEUKOM, H., 1986, Influence of different types of thermal treatment on the chemical composition and physical properties of wheat bran. *Journal of Cereal Science*, **4** (3) : 233 - 239.
- CHEN, G. L., FAN, M. X., WU, J. L., LI, N. AND GUO, M. Q., 2019, Antioxidant and anti-inflammatory properties of flavonoids from lotus plumule. *Food chemistry*, 277 : 706 - 712.
- CHEN, S., FANG, L., XI, H., GUAN, L., FANG, J., LIU, Y., WU, B. AND LI, S., 2012, Simultaneous qualitative assessment and quantitative analysis of flavonoids in various tissues of lotus (*Nelumbo nucifera*) using high performance liquid chromatography coupled with triple quad mass spectrometry. *Analytica Chimica Acta*, **724** : 127 - 135.
- COFFMANN, C. W. AND GARCIAJ, V. V., 1977, Functional properties and amino acid content of a protein isolate from mung bean flour. *International Journal of Food Science & Technology*, **12** (5) : 473 - 484.
- GOULD, J. M., JASBERG, B. K. AND COTE, G. L., 1989, Structure-function relationships of alkaline-peroxide treated lignocellulose from wheat straw. *Cereal Chem.*, 66: 213 - 217.
- HUANG, B., BAN, X., HE, J., TONG, J., TIAN, J. AND WANG, Y., 2010, Comparative analysis of essential oil components and antioxidant activity of extracts of *Nelumbo nucifera* from various areas of China. *Journal of Agricultural* and Food Chemistry, **58** (1) : 441 - 448.
- HAM, Y. K., HWANG, K. E., SONG, D. H., KIM, Y. J., SHIN, D. J., KIM, K. I., LEE, H. J., KIM, N. R. AND KIM, C. J., 2017, Lotus (*Nelumbo nucifera*) rhizome as an antioxidant dietary fiber in cooked sausage : Effects on physicochemical and sensory characteristics. *Korean journal for food science of animal resources*, 37 (2) : 219.
- JIRUKKAKUL, N. AND SENGKHAMPARN, N., 2018, Physicochemical properties and potential of lotus seed flour as wheat flour substitute in noodles. *Songklanakarin Journal of Science & Technology*, **40** (6).

- KAYITESI, L., VIJAYALAKSHMI, K. G., NATH, K. G. AND SURENDRA, H. S., 2012, Effect of processing on physicochemical and functional properties of tapioca. *Mysore J. Agric. Sci.*, **46** (2) : 265 - 273.
- KULKARNI, K. D., KULKARNI, D. N. AND INGLE, U. M., 1991,Sorghum malt-based weaning formulations:Preparation, functional properties and nutritive value.*Food Nutr. Bull.*, 13 (4): 322 327.
- MANINDER, K., SANDHU, K. S. AND SINGH, N., 2007, Comparative study of the functional, thermal and pasting properties of flours from different field pea (*Pisum sativum* L.) and pigeon pea (*Cajanus cajan* L.) cultivars. *Food chemistry*, **104** (1): 259 - 267.
- MORO, C. F., YONEKURA, M., KOUZUMA, Y., AGRAWAL, G. K. AND RAKWAL, R., 2013, Lotus – A source of food and medicine : current status and future perspectives in context of the seed proteomics. *International Journal* of Life Sciences, 7 (1) : 1 - 5.
- MUKHERJEE, P. K., MUKHERJEE, D., MAJI, A. K., RAI, S. AND HEINRICH, M., 2009, The sacred lotus (*Nelumbo nucifera*) – phytochemical and therapeutic profile. *Journal of Pharmacy and Pharmacology*, 61 (4): 407 - 422.
- MUTTAGI, G. C., USHA, R. AND MAHADEVU, P., 2017, Functional properties of traditional rice varieties of Karnataka. *Mysore J. Agric. Sci.*, **51** (4) : 896 - 899.
- OLADELE, A. K. AND AINA, J. O., 2007, Chemical composition and functional properties of flour produced from two varieties of tigernut (*Cyperus esculentus*). *African Journal of Biotechnology*, **6** (21).
- SATHITHON, P. AND YAN-BIN, X., 2012, Effect of sprouting on the chemical and nutritional qualities and phenolic alkaloid content of lotus (*Nelumbo nucifera* Gaertn.) seeds, *African Journal of Food Science*, **6** (7) : 204 -211.
- SHAD, M. A., NAWAZ, H., HUSSAIN, M. AND YOUSUF, B., 2011, Proximate composition and functional properties of rhizomes of lotus (*Nelumbo nucifera*) from Punjab, Pakistan. *Pak. J. Bot*, **43** (2) : 895 - 904.

- SHAHZAD, M. A., AHMAD, N., ISMAIL, T., MANZOOR, M. F., ISMAIL, A., AHMED, N. AND AKHTAR, S., 2021, Nutritional composition and quality characterization of lotus (*Nelumbo nucifera* Gaertn.) seed flour supplemented cookies. Journal of Food Measurement and Characterization, 15 (1): 181 - 188.
- SHUKLA, K. AND CHATURVEDI, N. J. I. H., 2015, Investigation on preliminary phytochemical and proximate analysis of *Nelumbo nucifera* Gaertn seeds. *Ijppr Human*, 4 : 35 - 43.
- SINGTHONG, J. AND MEESIT, U., 2017, Characteristic and functional properties of Thai lotus seed (Nelumbo nucifera) flours. International Food Research Journal, 24 (4): 1414.
- YASUMATSU, K., SAWADA, K., MARITAKA, S., TODA, J., WADA, T. AND ISHI, K., 1972, Whipping and emulsifying properties of soy bean products. *Agri. Biol. Chem.*, 36 : 719 - 727.
- ZENG, H., ZHENG, Y., LIN, Y., HUANG, C., LIN, S., ZHENG, B. AND ZHANG, Y., 2018, Effect of fractionated lotus seed resistant starch on proliferation of Bifidobacterium longum and Lactobacillus delbrueckii subsp. bulgaricus and its structural changes following fermentation. *Food chemistry*, 268 : 134 - 142.
- ZHENJIA, Z., MINGLIN, W., DAIJIE, W., WENJUAN, D., XIAO, W. AND CHENGCHAO, Z., 2010, Preparative separation of alkaloids from Nelumbo nucifera leaves by conventional and pH-zone-refining counter-current chromatography. *Journal of Chromatography B*, **878** (19) :1647 - 1651.