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PROXIMATE COMPOSITION AND FUNCTIONAL PROPERTIES OF RHIZOMES OF LOTUS (*NELUMBO NUCIFERA*) FROM PUNJAB, PAKISTAN

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Abstract

In the present studies, the proximate composition and functional properties of rhizome flour of lotus cultivated in the Southern Punjab, Pakistan, were determined. The proximate composition (g/100 g flour) was determined as ash (1.10±0.66), the total nitrogen (1.36±0.04) total protein (8.48±0.25), total sugar (19.08±0.01) and free amino acids (0.78±0.035). The lotus rhizome was found to be a poor source of crude oil (2.68±0.04 g/100 g dry weight). Physico-chemical properties of the oil were investigated as acid value (16.66±3.05 mg KOH), saponification value (110.43±1.97 mg KOH) and unsaponifiable matter (0.026±0.11 g/100 g oil). The temperature dependent behaviour of solubility and swelling capacity of the flour showed a linear increase (1.2±0.35-13.84±0.91%) in solubility but an exponential increase in swelling capacity with a gradual increase in the temperature (40-100°C). Water absorption and oil holding capacities were found to be 2.56±0.05 and 2.03±0.25% respectively while least gelation concentration, foaming volume increase, foaming stability, emulsifying capacity and emulsion stability were investigated as 18.0±2.0, 5.23±0.03, 4.97±0.058, 48.93±0.35 and 96.43±0.51% respectively. The present data may provide a guideline for the use of lotus rhizome flour in food formulation.

Introduction

Currently, the whole world especially the developing countries including Pakistan is facing the malnutrition and health problems. Conventional legumes and some dicotyledonous seeds, which have been proved to be a better and economically favourable alternative source of proteins and energy against animal proteins in developing countries (Famurewa & Raji, 2005), played a great role in the management of these problems so far. However, these are still insufficient for complete fulfilment of food and energy requirements of the fast increasing population storm. The increasing demands of developing countries *i.e.* balanced nutrition, food security, medicinal improvements, agricultural development, self-dependence and enhancement of economy, may alternatively be met by the exploration of non-conventional sources.

Lotus, botanically known as *Nelumbo nucifera*, is a rhizomatus aquatic, ornamental, edible and medicinal plant which is grown as a non-conventional vegetable commonly in China, India, Japan and Australia (Qichao & Xingyan, 2005). It is also grown in upper Sindh and lower Punjab regions of Pakistan. All parts of lotus plant are edible in various forms. The rhizomes are used as popular vegetable and exhibit multiple nutritional and medicinal properties (Ono *et al.*, 2006). These are rich source of starch and proteins (Ying & Charls, 1986) and can be eaten in roasted, pickles, dried slices and fried forms.

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Biochemically, the rhizomes are composed of proteins, fats, carbohydrates and minerals and are good source of energy (Sridhar & Bhat, 2007). The rhizomes are used as popular health food. The alkaloid (liensinine) extracted from them is effective to treat arrhythmia (Ling *et al*., 2005), sunstroke, fever, dysentery, diarrhoea, dizziness and stomach problems (Lee *et al*., 2005). The rhizome extracts also posses anti-obesity (Ono, *et al*., 2006) and anti-diabetic properties (Mukherjee *et al*., 1997).

In the light of above facts, it is clear that the lotus plant may be a sole contributor to resolve the malnutrition problems in developing countries. Therefore, this plant must be considered an important candidate deserving to be evaluated. The beneficial uses of plant flours in the pharmaceutical and food industry directly depend on their functional properties (solubility, swelling, water absorption, foaming, gelation and emulsifying capacities (Oshodi & Ekprigin, 1989). The investigations about the functional properties of rhizome flour of lotus may provide an advanced knowledge for its uses in the preparation of different pharmaceutical and food products. A careful survey of literature showed that the work has been done earlier on proximate composition, functional properties, phytochmical analysis, and antioxidant activities of rhizomes of lotus from different areas of the world. However, unfortunately, a little data has been found to be published regarding the characterization of rhizome flour of lotus cultivated in Pakistan. Therefore, in the present study the proximate composition and functional characteristics of rhizome of lotus from the Southern Punjab regions of Pakistan were focussed for determination.

Materials and Methods

The rhizomes of lotus were collected directly from the fields of irrigated areas of district Muzaffar Garh, Punjab, Pakistan. The rhizomes were washed with distilled water to remove mud. The rhizomes were crushed in the form of slices with a sharp knife and dried in air under shade. The dried samples were ground with an electric grinder to obtain the flour. The flour was saved in dry and air tight containers for analysis.

Proximate composition: The ash content of lotus rhizome was determined by following the method of Pearson (1980). The total nitrogen content (%N) was determined by Kjeldahl method (Vogal, 1971).The percent crude proteins (%CP) content was calculated as:

$$\%CP = \%N \times 6.25$$

Water soluble and salt [0.5 M $(NH_4)_2SO_4$] soluble protein contents were determined by Biuret method (Plummer, 1979). The sugars and free amino acid contents of rhizomes were extracted and estimated by the methods as mentioned earlier by Shad *et al*., (2009).

Content and Physicochemical properties of oil: Extraction of the oil was done by Soxhlet apparatus using n-hexane as extraction solvent. The acid value and saponification value were determined using the standard method as described in British Pharmacopeia (1973). The unsaponifiable matter was determined by using the method as given in United State Pharmacopeia (1985).

Functional properties

Solubility and swelling capacity: The solubility and swelling capacity (SC) were determined by the method described by Leach *et al*., (1959) with slight modifications. The rhizome flour (1 g) was dispersed in distilled water (50 mL). The resulting slurry was heated at various temperatures (40-90°C) with an interval of 10°C for 30 minutes in a thermally controlled water bath. The mixture was then allowed to cool at room temperature and centrifuged at 2200 × g for 15 minutes. The supernatant was allowed to evaporate at 120°C and the residue thus obtained was subjected to the measurement of the percent solubility of flour in water as:

$$S\ (\%) = (W_1 - W_2 \div W_1) \times 100$$

where W_1 is weight of the sample while W_2 is weight of the residue obtained after centrifugation.

The residue obtained from the centrifugation along with the water it retained was re-weighed. The percent SC was calculated as:

$$SC\ (\%) = (W_3 - W_1 \div W_1) \text{ x } 100$$

where W_1 is weight of the sample while W_3 is weight of the sample along with retained water.

Water absorption capacity (WAC) and oil absorption capacity (OAC): The WAC and OAC of the flour were determined using the methods suggested by Beuchat (1977). Lotus rhizome flour (1 g) was vortexed with distilled water (10 mL) in pre-weighed centrifuge tube for 30 minutes. After standing at room temperature for 30 minutes, the sample was centrifuged for 25 minutes at 3000 × g. The sediments were weighed after complete removal of the supernatant at 40°C and WAC was calculated as:

$$WAC = 100 \times (W_2 - W_1 \div W_0)$$

where W_0 is the weight of the sample, W_1 is the weight of centrifuge tube plus sample and W_2 is the weight of centrifuge tube plus the sediments.

For the determination of OAC, lotus rhizome flour (0.5 g) was homogenized with coconut oil (5 mL) in pre-weighed centrifuge tube. After 30 minutes, the sample was centrifuged for 30 minutes at 4000 × g. The sediments were weighed after the complete removal of supernatant oil by keeping the tube inverted for overnight. The OAC of the flour was calculated as:

$$OAC = 100 \times (W_2 - W_1 \div W_0)$$

where W_0 is the weight of the sample, W_1 is the weight of tube plus sample and W_2 is the weight of the tube plus the sediments.

Gelation properties: The gelation properties of the flour were determined by the method described by Coffman and Garcia (1977). A series of sample suspensions of increasing concentrations *i.e.* 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (m/v) were prepared in distilled water (10 mL). All the suspensions were heated gently for 1 hour in a boiling water bath. The heated suspensions were cooled at 4°C for 2 hours and then inverted one after the other. The least gelation capacity (LGC) was taken as the concentration at which the inverted suspension did not fall or slip.

Bulk Density (Packed Bulk Density and Loose Bulk Density): The procedure of Akpapunam & Markakis (1981) was used to determine the preweighed (W_1) bulk density of the flour. A known weight of the flour was taken into a preweighed (W_1) measuring cylinder and the weight of the cylinder (W_2) as well as the volume of the flour (V_1) was noted. The Loose bulk density (LBD) was expressed as:

$$LBD = W_2 - W_1 \div V_1$$

The flour in the cylinder was tapped gently to eliminate air spaces between the particles of the flour. The new volume ($V_{2)}$ of the sample and mass of the cylinder (W_3) was noted and the packed bulk density (PBD) was expressed as:

$$PBD = W_3 - W_1 \div V_2$$

Foaming capacity: Foaming capacity (FC) of the flour was determined by the known method (Coffman & Garcia, 1977). The rhizome flour (2 g) was dispersed in distilled water (100 mL) and homogenized properly for two minutes in a kitchen blender. The volumes were recorded before and after homogenization and percent increase in the volume was calculated as FC of the flour by using the following formula:

$$FC\ (\%) = 100\ (V_2 - V_1 \div V_1)$$

where, V_1 = initial volume; V_2 = 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 of initial foam volume as:

$$FS\ (\%) = 100\ (V_t \div V_0)$$

where, V_0 = initial foam volume and V_t = foam volume after time (t).

Emulsifying activity: Emulsifying activity (EA) and emulsion stability (ES) were determined by following the method of Neto *et al*. (2001). The rhizome flour (2 g) was dispersed in distilled water (100 mL) and height of solution in the cylinder was measured. After standing for 2 minutes, the solution was homogenized with refined coconut oil (5 mL). The resulting emulsion was centrifuged (1100 × g) for 5 minuets. The height of the emulsified layer was measured and the emulsifying activity was calculated:

$$EA\ (\%) = 100\ (H_2 - H_1 \div H_1)$$

where H_1 is the initial height of unemulsified solution while H_2 is height of emulsion.

Emulsion stability: The emulsion stability (ES) was determined by heating the emulsion at 80°C for 30 minutes followed by centrifugation at 1100 × g for 5 minuets. The emulsion stability was determined by the following formula:

$$ES\ (\%) = 100\ (H_t \div H_2)$$

where H_2 is the height of the emulsified layer before heating while H_t is the height of the emulsified layer after heating.

Results and Discussion

Proximate composition: Table 1 displays the results for the proximate composition of lotus rhizome flour. The ash contents were found to be 1.1±0.66 g/100 g dry weight. The total nitrogen and total protein contents in the lotus rhizomes were investigated as 1.36±0.04 and 8.48±0.25 g/100 g flour respectively. The fractions of water soluble, salt soluble and total salt soluble proteins in lotus rhizomes were found to be 1.23±0.02, 5.73±0.01 and 6.064±0.005 g/100 g flour respectively. The higher value of salt soluble protein fraction was observed as compared to water soluble protein fraction. The ash and total protein content in lotus rhizomes were found to be higher than those reported by Reid, (1977) for lotus rhizome.

The sugars contents were investigated as the total sugar, reducing sugar and non-reducing sugars amountig 19.08±0.01, 0.168±0.01 and 18.87±0.100 g/100 g flour, respectively. The reducing sugar contents were found to be lower while the total sugar content and free amino acids contents (0.78±0.035 g/100 g flour) were found to be higher than those reported by Xueming (1987) for lotus seeds.

Physichochemical properties of oil: The lotus rhizome was found to be a poor source of crude oil *i.e*. 2.68±0.04 g/100 g dry weight (Table 2). The oil content investigated in the present studies was found to be higher than those reported by Reid (1977) for lotus rhizome. The physichochemical characteristics of the lotus rhizome oil were investigated as acid value (16.66±3.05 mg KOH), saponification value (110.43±1.97 mg KOH) and unsaponiofiable matter (0.026±0.11 g/100 g oil). The acid value was found to be higher while the saponification value and unsaponifiable matter were found to be low as compared to those reported by Bi *et al*., (2006) for lotus plumule oil.

Functional Properties

Solubility: The temperature dependent behaviour of solubility of rhizome flour in water (Fig. 1) showed a linear increase in the percent solubility (1.2±0.35-13.84±0.91%) in response to a gradual increase in the temperature (40-100˚C) after 30 minutes. A comparable temperature dependent behaviour of solubility has already been reported by Yusuf *et al*., (2007) for studies on Jack bean (*Canavalia ensiformis*) starch. The experimental data were analysed statistically using semi-empirical model as:

$$S = S_0 T - S_C \quad (1)$$

where S is the solubility of the lotus rhizome flour, T is temperature, $S_0\,(=0.21)$ is pre-exponential factor and $S_C(=-7.7321)$ is solubility coefficient. The pre-exponential factor indicates the degree of solubility under the negligible temperature, whereas the solubility coefficient indicates the solubility of the flour in response to change in temperature. Thus, using the values of S_o(= 0.21) and S_c(= - 7.7321) from the intercept and the slope of Fig. 1 respectively, the Eq. 1 can be written as:

$$S = 0.210\, T - 7.732 \quad (2)$$

Table 1. Biochemical composition of lotus rhizome (g/100 g flour).

Components	Values*
Total ash	1.1 ± 0.66
Total nitrogen	1.36 ± 0.4
Total protein	8.48 ± 0.25
Water soluble proteins	1.23 ± 0.02
Salt soluble proteins	5.73 ± 0.01
Total salt soluble proteins	6.064 ± 0.005
Reducing sugars	0.168 ± 0.01
Non-reducing sugars	18.87 ± 0.100
Total sugars	19.08 ± 0.01
Free-amino acid	0.78 ± 0.035

*Means ± standard deviation of three replicates

Table 2. Physicochemical properties of oil extracted from lotus rhizome.

Properties	Values*
Crude Fat (g/100 g dry weight)	2.68 ± 0.04
Acid value (mg KOH)	16.66 ± 3.05
Sponification value (mg KOH)	110.43 ± 1.97
Un-sponifiable matter (g/100 g)	0.026 ± 0.11

*Means ± standard deviation of three replicates

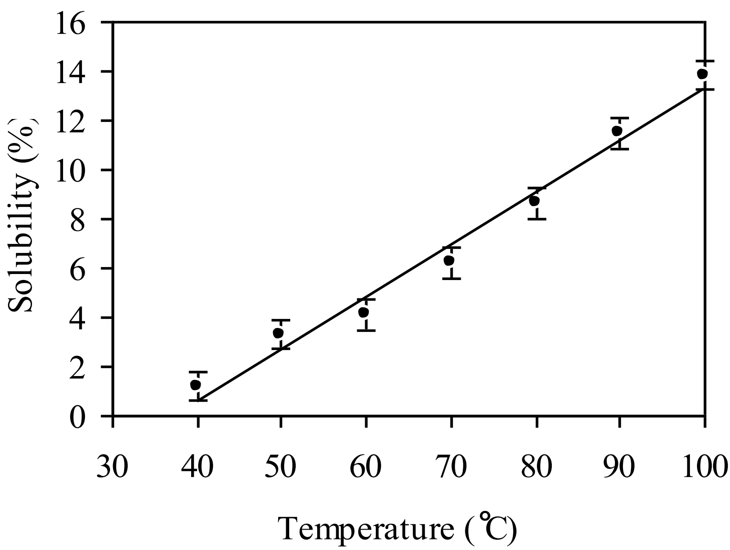

Fig. 1. Temperature dependant patterns of solubility of lotus rhizome flour.

The data analysis showed that the solubility of lotus rhizome flour in water is a linear function of temperature (R^2 = 0.9815). The degree of solubility may depend on the type and species of starch and proteins present in the flour. The milling process causes the break down of starch granules which further leads to an improvement in the solubility index. An increase in temperature facilitates the hydrolysis of starch leading to an improved solubility (Hoover & Maunal, 1996).

Swelling capacity: A number of experiments were carried out to investigate the temperature dependent behaviour of SC of the floor. Depending on the process conditions, an exponential increase in the SC was observed with a gradual increase in the temperature (40-100˚C) after 30 minutes time (Fig. 2). A similar temperature dependent behaviour of SC have been reported by Mandala & Bayas (2004) for studies on wheat starch, Ikegwu *et al*., (2010) for starch from achi (*Brachystegia eurycoma*) seeds and Saw *et al*., (2008) for palm kernel cake.

The statistical analysis of the experimental data showed that the SC of lotus rhizome flour is an exponential function of temperature ($R^2 = 0.9821$) following a semi-empirical model:

$$SC = S_{C_0} e^{S_{SC} T} \tag{3}$$

where $S_{C_0} (= 2.0436)$ is pre-exponential factor, $S_{SC} (= 0.0248)$ is swelling sensitivity coefficient and T is temperature. Using the values of $S_{C_0} (= 2.0436)$ and $S_{SC} (= 0.0248)$ from the intercept and the slope of Fig. 2 respectively, the Eq. 3 can be written as:

$$SC = 2.0436\, e^{0.0248T} \tag{4}$$

The increase in the SC may be attributed to the fact that the outer membranes of the starch granules present in the flour are ruptured during the milling process and swell up in the form of a gel by absorbing water at low temperature *i.e.* 40-50˚C. A remarkable increase in swelling power may be observed between 60-90˚C (Hoover & Sosulki, 1996). The swelling pattern of the flour suggests the level of crystalline packing of the starch granules present in the flour. An increase in entropy disturbs the hydrogen bonding in the crystalline regions, resulting in an increase in the swelling power (Billiadaris, 1982).

Water and oil holding capacities: The water and oil holding capacities are essential functional properties of protein which may be defined as the amount of water or oil retained by a known weight of flour under specific conditions. The water holding capacity depends on capillary, pore size and the charges on the protein molecules. This is due to strong correlation of extent of protein hydration with polar constituents along with the hydrophilic interaction through hydrogen bonding. The higher protein content in the flour might be responsible for high hydrogen bonding and high electrostatic repulsion (Altschul, & Wilcke, 1985). The oil holding capacity is also due to enhanced hydrophobic character of proteins in the flours.

Water and oil holding capacities of lotus rhizomes were found to be 2.56±0 and 2.03±0.25% respectively (Table 3). Lotus rhizomes were found to possess less water holding capacity than those reported by Bhat & Sridhar (2008) for lotus seeds. The present results were also low as compared to those reported for jack bean starch (Yusuf *et al*., 2007).

Gelation properties: Least gelation concentration (LGC) is considered as the gelling ability of flour which provide structural matrix for holding water and other water soluble materials like sugars and flavours. The LGC of different flours may vary depending on the relative ratios of different constituent like proteins, carbohydrates and lipids (Sathe *et al*., 1982). It also serves as a good binder or provides consistency in food preparations especially the semi-solid products (Adeyemi & Umer, 1994). The increasing concentration of proteins in the flour facilitates the gelation properties which may be due to the enhanced interaction among the binding forces (Lawal *et al.,* 2004). LGC of lotus rhizomes flour (18.0±2.0%) was found to be higher than those reported by Bhat & Sridhar (2008) for lotus seeds.

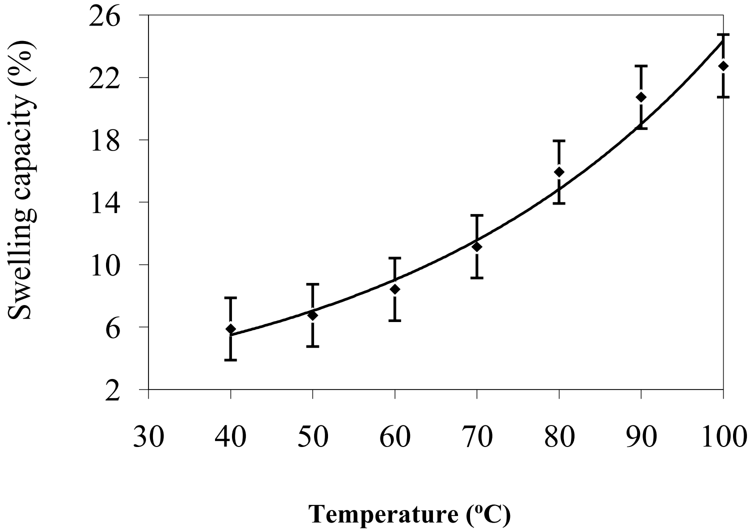

Fig. 2. Temperature dependent behaviour of swelling capacity of lotus rhizome flour.

Table 3. Functional properties of lotus rhizome flour.

Properties	Values*
Water holding capacity (%)	2.56 ± 0.05
Oil holding capacity (%)	2.03 ± 0.25
Least gelation concentration (%)	18.0 ± 2.0
Foaming volume increase (%)	5.23 ± 0.03
Foaming stability (%)	4.97 ± 0.058
Emulsifying capacity (%)	48.93 ± 0.35
Emulsifying stability (%)	96.43 ± 0.51
Loose bulk density (g/mL)	0.59 ± 0.035
Packed bulk density(g/mL)	0.803 ± 0.153

*Means ± standard deviation of three replicates

Foaming properties: Foaming properties are much important in the maintenance of the texture and structure of different food products (ice creams and bakery products) during and after processing. The foamability of the flour depends on the presence of the flexible protein molecules which may decrease the surface tension of water (Sathe *et al*., 1982). The results revealed that foaming volume increase and foaming stability of lotus rhizomes were found to be 5.23±0.03 and 4.97±0.058% respectively. These values of FC and FS were found to be low as compared to those reported by other workers for different flours (Adebowale *et al*., 2005; Mortuza *et al*., 2009; Ogundele & Oshodi, 2010). The low foamability of lotus rhizome flour indicates the presence of highly ordered globular protein molecules which increase the surface tension.

Emulsifying properties: Emulsion capacity determines the maximum amount of oil that can be emulsified by protein dispersion. On the other hand, emulsion stability determines the ability of an emulsion with a specific composition to remain unchanged. The emulsifying capacity and emulsion stability of the flour were found to be 48.93±0.35% and 96.43±0.51% respectively. Emulsion capacity of lotus rhizome flour was found to be lower while emulsion stability was found to be higher than those reported by Shridhar & Bhat (2007) for lotus seeds.

Bulk density: The loose bulk density and packed bulk density of flour were found to be 0.59±0.035 and 0.803±0.153 g/mL respectively. These values were found to be comparable to those reported for wheat blends (Amajeet *et al*., 1993). The low values of bulk densities make the flour suitable for high nutrient density formulation of foods.

Conclusion

In conclusion, the flour of lotus rhizome is a good source of dietary proteins and sugars. It possesses appreciable functional properties which give it an advantage of being used in bakery and food products. Good SC, WAC and OAC make it useful to be used as a thickener in liquid and semiliquid foods *i.e.* gravies soups and bakery products. Low LGC value suggests that the flour of lotus rhizome has greater gel forming ability and may be used as an additive to other gel forming materials in food industry. It also possesses good foaming capacity and foaming stability and, therefore, can be used in formulation of food products. Higher values of bulk density provide packing advantage and make its use in the preparation of high nutrient density weaning food.

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