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Nitrogen extractability and functional properties of defatted *Erythrina variegata* flour

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Abstract

Defatted *Erythrina variegata* flour was prepared from dehusked seed meal by hexane extraction of residual oil. The resulting flour had 403 g kg^{-1} of protein as compared to 282 g kg^{-1} in the whole seed-defatted meal. Nitrogen extractability of the defatted flour in water was not much influenced by the length of extraction period above 40 min, but an increased extraction was observed at a higher liquid to solid ratio up to a studied limit of 1:60; the optimal ratio was found to be 1:30. The minimum of 26.9% nitrogen was extracted in the pH range 3.0–4.0 and maximum of 94.8% at pH 12. Addition of sodium chloride (0.1 or 0.5 M) broadened the pH range of minimum nitrogen extractability and shifted it toward a lower pH region. At both concentrations of sodium chloride, a marked increase in nitrogen extractability, in the pH range 3.0–7.0, was observed. Precipitation of protein from an extract of pH 10.0 was maximum (85.3%) at pH 4.75. A higher buffer capacity of the flour was observed in the acidic medium (0.195 mmol HCl g^{-1} flour) than in alkaline medium (0.093 mmol NaOH g^{-1}). Water absorption and oil absorption values for the whole *E. variegata* seed flour and the dehusked, defatted flour were 1.81, 1.43 and 1.02, 1.52 kg kg^{-1}, respectively.

Keywords: *Erythrina variegata*; Nitrogen extractability; Protein precipitability; Buffer capacity; Foaming capacity; Foam stability

1. Introduction

Erythrina sp. are leguminous plants which are widely distributed in tropical and subtropical regions (The Wealth of India, 1952). *Erythrina variegata* L. var. orientalis or Indian coral tree is grown throughout India as an ornamental plant. The leaves and tender shoots are eaten as pot-herbs. Rao (1945) reported that the seeds can be eaten after boiling and roasting. The oil yield reported was 11.3% and the residual seed cake had a protein content of 40%. Flowers of *Erythrina* species are consumed in Mesoamerica and tea is made with *Erythrina edulis*. Seeds are eaten in the Andean regions of Columbia and Peru, where they are ground into flour to make a variety of products (Federacion Nacional de Cafeteleros, 1991). *Erythrina* has been used in folk medicine for treatment of insomnia, malarial fever, veneral disease, asthma and tooth-ache and as a narcotic and antihelminthic. The alkaloid Erythroidine was used as a muscle relaxant. Haemoerythrina alkaloids were investigated for their anti-cancer activity (Payne, 1991).

Extensive work has been carried out on nitrogen extractability from various oil seed meals, such as flax or linseed (*Linum usitatissimum*) meal (Madhusudhan & Singh, 1983; Dev, Quansel, & Hansen, 1986; Oomah, Mazza, & Cui, 1994), leguminous seed protein isolates (Pandey & Srivastava, 1991), Bengal gram (*Cicer arietinum*) (Dhawan, Malhotra, Dahiya, & Singh, 1991), pumpkin (*Cucurbita maxima*) seed flour (Lazos, 1992), sunflower (*Helianthus annus*) meal (Pawar, Patil,

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Sakhale, & Agarkar, 2000; Gheyasuddin, Carter, & Matil, 1970), velvet bean (*Mucuna pruriens* var. utilis) (Vadivel & Janardhanan, 2000), rapeseed (*Brassica campestris*) protein isolates (Mahajan & Jha, 1995; Xu & Diosady, 1994), *Hibiscus cannabinus* L. (Kenaf) seed flour (More, Pawar, Deshpande, & Nimkar, 1992), sesame seed (*Sesamum indicum* L.) (Rivas, Jane, & John, 1981) and soy (*Glycine max*) meal (Bass, Singh, & Tanwar, 1997).

There has been little work carried out on *E. variegata*, which is rich in crude fat as well as protein. Hence, an attempt has been made to utilise *E. variegata* as a protein source. The present paper describes the effect of extraction parameters, such as pH, ionic strength, solid:solvent ratio and the time of extraction, on the nitrogen extractability and the buffer capacity of dehusked, defatted *E. variegata* flour.

2. Materials and methods

2.1. Material

Whole pods of *E. variegata* were collected from a nearby garden tree of CFTRI Resource Centre and Indian Institute of Chemical Technology, Hyderabad (India). The seeds were removed from the pods, dried in the shade and stored at 4 °C until the experiments were conducted.

2.2. Preparation of defatted seed flour

The seed coats were removed after overnight soaking and drying of the cotyledons in the shade and powdered using a Sumeet (India) high speed mixer and sieved to pass through a 80 mesh BS sieve (0.18 mm). This flour was extracted with cold hexane at room temperature in a laboratory flask shaker (Toshniwal, India). The solvent was removed using a high-speed centrifuge (Remi, India) and the flour was air-dried and stored in metallised polyester polyethylene laminate pouches at 4 °C until further use.

2.3. Proximate composition of whole seed and defatted flour

Moisture and ash were determined by AOAC (1980) methods. Crude fat was determined by Soxhlet extraction with petroleum ether (b.p. 40–60 °C) for 16 h. Total nitrogen content was estimated by Kjeldahl procedure and crude protein was calculated as $N \times 6.25$ (Pellett & Young, 1980). Crude fibre was estimated by the AOAC (1980) method.

2.4. Nitrogen extractability

The nitrogen extractability of defatted *E. variegata*, as a function of solid:solvent ratio (1:10 to 1:60 w/v), extraction time (10–100 min.), pH (2–12) in water and in 0.1 and 0.5 M NaCl, was determined essentially by the procedure described by Dev et al. (1986).

All extractions were done at ambient temperature (25 °C) with magnetic stirring, using 1 g flour in water or in 0.1 and 0.5 M NaCl solution and pH was adjusted to the desired level using 0.5 M HCl or 0.3 M NaOH. The volumes were kept constant in all the experiments. After each extraction, the suspension was centrifuged (2500*g*) for 30 min. at room temperature and the supernatant was filtered. Nitrogen was determined by the Kjeldahl method using 5 ml of supernatant and nitrogen extractability was expressed as the percent nitrogen extracted from the original defatted flour. All extractions and determinations were performed in triplicate.

2.5. Protein precipitability

Precipitability of protein was done by the method described by Taher, Abbassy, El-Nockroshy, and Shoeb (1981), with minor modifications. Flour (10 g) was dispersed in 300 ml distilled water and the pH of the suspension was adjusted to pH 10.0 using 0.5 M NaOH. After extraction for 40 min with magnetic stirring, the suspension was centrifuged and the supernatant was collected. Aliquots (20 ml) of the supernatant were taken in graduated centrifuge tubes and adjusted to the desired pH, ranging from 3.5–6.0 using 0.5 M HCl. After centrifuging (2500*g* for 30 min), the volume of the supernatant was noted. The yield of protein precipitation was calculated as

$$\frac{V_1N_1 - V_2N_2}{V_1N_1} \times 100,$$

where V_1 and V_2 are the volumes of the aliquots before and after precipitation and N_1 and N_2 are mg nitrogen in 1 ml of V_1 and V_2, respectively.

2.6. Buffer capacity

Two sets of 1.0 g flour were dispersed in 30 ml distilled water. To one set was added a known volume of 0.1 M NaOH and, to the other, 0.2 M HCl and the corresponding changes in pH in both acid and alkaline ranges were noted. The amount of HCl or NaOH added was plotted against pH and the buffer capacity in each range was expressed as the mean value of mmol of HCl or NaOH per gram of flour required to bring about a change in pH of one unit.

2.7. Water absorption

Whole and dehulled, defatted *Erythrina* seed flour, (1.0 g) was dispersed in 10 ml of water, vortexed thoroughly, and centrifuged at 2500*g* for 30 min. The water absorbed by the samples was noted and expressed as kg water absorbed per kg of flour (Johnson, 1970).

2.8. Oil absorption

Whole and dehulled, defatted *Erythrina* seed flour, (1.0 g) was dispersed in 10 ml of refined groundnut oil, vortexed thoroughly, and centrifuged at 2500*g* for 30 min. The oil absorbed by the samples was noted and expressed as kg oil absorbed per kg of flour (Johnson, 1970; Beuchat, 1977).

2.9. Foaming capacity and foam stability

Foaming capacity and foam stability were determined by a modified method of Lin, Humbert, and Sosulski (1974). Two gram of sample were put into a container containing 50 ml of distilled water. The sample was mixed, using a blender, at a speed set for fast blending. The volumes before and after whipping in a 250 ml graduated measuring cylinder were recorded. The percent of volume increase due to whipping was calculated according to the method of Lawhon, Carter, and Matil (1972). The volumes of foam in the standing cylinder, for foam stability at 1 and 2 h after whipping, were recorded.

All the experiments were done in triplicate and the data presented as means of triplicate analyses. In the Figures, mean values were used to plot the curves.

3. Results and discussion

3.1. Proximate composition

The proximate compositions of whole seed and defatted *E. variegata* flour, on an oil-free dry basis, are shown in Table 1. The values are generally comparable with those reported in the literature (The Wealth of India, 1952).

The seed was constituted of 70% kernel and 30% hull. As most of the oil is present in the kernel, defatting gives a high protein meal from the kernel. Complete removal of the hulls by soaking and defatting resulted in a considerably high protein content (403 g kg^{-1}) compared with the whole seed (243 g kg^{-1}). The dehusked and defatted flour, which is devoid of hulls, has less crude fibre (30.5 g kg^{-1}) than the whole seed (147.1 g kg^{-1}). The ash contents of the whole seed and dehusked defatted flour are 44.3 and 57.9 g kg^{-1}, respectively, comparable to the reported value of 42 g kg^{-1} (Rao, 1945). Protein content of the *Erythrina* species utilized in Costa Rica, namely *E. peoppigiana*, was reported to the highest at 420 g kg^{-1} (Pezo, Kass, Benavides, Romero, & Chaves, 1990). The oil content of the whole seed is 140 g kg^{-1}, comparable to the reported value of 120 g kg^{-1} (Rao, 1945). The carbohydrate contents (nitrogen-free extract) of whole seed and dehusked, defatted flour were found to be 426 and 509 g kg^{-1}, respectively.

Table 1
Proximate compositions of *Erythrina variegata* flours

Components (g kg^{-1})	Whole seed		Dehusked defatted flour
	Flour	Defatted flour	
Crude protein (N × 6.25)	243 ± 1.5	282 ± 2.0	403 ± 2.3
Crude fat	140.0 ± 1.2	–	–
Fibre	147.1 ± 1.3	171.0 ± 1.4	30.5 ± 0.6
Ash	44.3 ± 0.7	51.5 ± 0.8	57.9 ± 0.9
Carbohydrates (by difference)	426 ± 2.0	496 ± 2.5	509 ± 2.8

All values are expressed on a dry weight basis.

3.2. Influence of extraction parameters on nitrogen extractability

3.2.1. Extraction time and solid-to-liquid ratio

Nitrogen extractability, as a function of time in distilled water at pH 6.08, is shown in Fig. 1(a). The length of the extraction period had very little influence on nitrogen extractability, which remained practically constant with longer extraction times. Nitrogen extraction increased from 43% to 52% up to 35 min, above which it remained constant. A similar trend was reported for linseed flour (Dev et al., 1986). However, variation in flour:solvent ratio had a pronounced effect on nitrogen extraction with a maximum at 1:30, which is illustrated in Fig. 1(b). A further increase in solvent to flour ratio had no effect on nitrogen extractability. Oomah et al. (1994) reported that the soluble protein content of defatted flax seed was maximum at a solid-to-liquid ratio of 1:40 and further increase in solvent-to-flour ratio did not have a significant effect.

3.2.2. pH and ionic strength

The nitrogen extractability from defatted *E. variegata* flour, in distilled water and in NaCl solutions of 0.1 M and 0.5 M in the pH range 2–12, is illustrated in Fig. 1(c). In water, the nitrogen extractability for *E. variegata* defatted flour was 62% at pH 2.0 and increased steadily from pH 6.0 (60%) to pH 12.0, where the extractability was 95%. At alkaline pH (8.0–10.0), nitrogen extractability showed a levelling tendency. In water, nitrogen extractability increased steadily on both sides of the isoelectric range, with a minimum of nitrogenous matter (26.8%) extracted in the isoelectric pH region of 3.0–4.0. Minimum extractability of nitrogenous matter from oil-free linseed meal was reported to occur at pH 3.8–4.6 (Dev et al., 1986; Smith, Johnson, & Beckel, 1946). However, Madhusudhan and Singh (1983) reported a much broader pH range of minimum nitrogen solubility at pH 3.0–6.0 for demucilaged, defatted and dehulled linseed meal. Mahajan and Jha (1995) reported that the isoelectric points (pI) of two protein isolates of rapeseed, RPI-1 and RPI-2, were in the range 5.0–6.0. Lazos

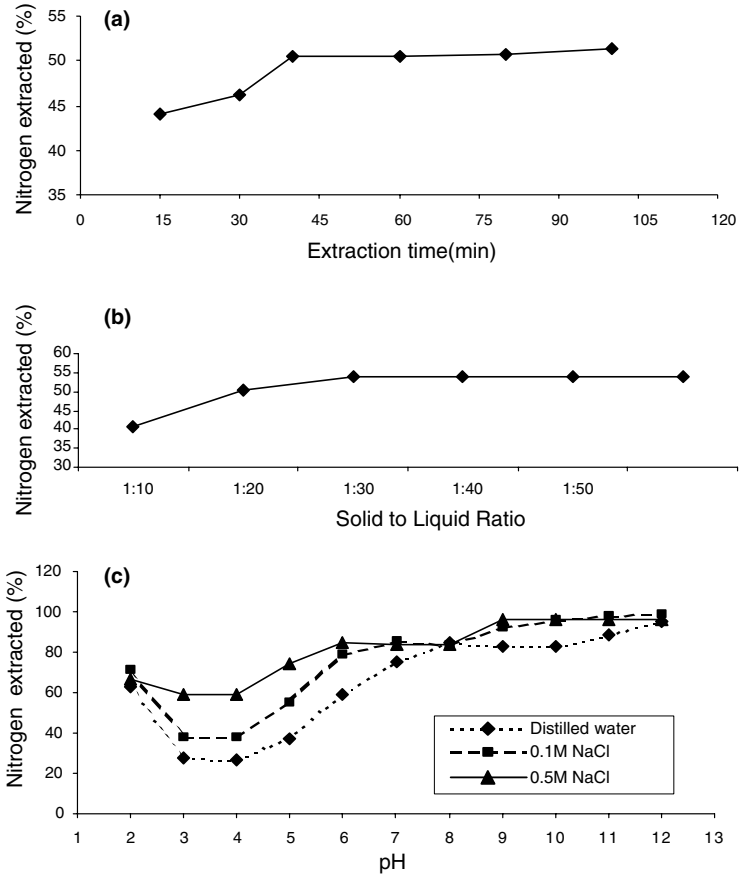

Fig. 1. Effect of time, solid to liquid ratio and pH and sodium chloride on nitrogen extractability of defatted *Erythrina variegata* flour: (a) influence of extraction time on nitrogen extractability; (b) effect of solid:liquid ratio on nitrogen extractability; (c) effect of pH and NaCl concentration on nitrogen extractability of defatted *Erythrina* seed flour.

(1992) reported minimum nitrogen solubility for pumpkin seed flour at pH 3.0–7.0 and a maximum extraction of more than 90% at pH > 9.0. Bass et al. (1997) reported that five commercial samples of defatted soy flour exhibited minimum nitrogen solubilities at pH 4.0 and maximum solubilities at pH 9.8 and above. Sosulski and Bakal (1969) have reported nitrogen extractabilities of 91–96% from whole seed defatted meals of rape seed, flax seed and sunflower. The differences are apparently due to varying degrees of protein denaturation during the preparation of defatted meals. Extraction of protein at alkaline pH >10 will have adverse effects on protein functinality. Hence, the preparation of protein isolates from defatted *E. variegata*, extraction could possibly be done at PH 10.0 at room temperature, as reported in the case of linseed meal (Dev et al., 1986).

NaCl extraction narrowed the isoelectric range of *E. variegata* proteins, as shown in Fig. 1(c) at both concentrations (0.1 and 0.5 M) of NaCl. The percent nitrogen extracted was high at all the acidic pH values. At pH 8.0, extraction with water or with NaCl solutions showed the same nitrogen extractability (82%). However, increased solubility was observed at alkaline pH with 0.1 and 0.5 M NaCl. Lazos (1992) reported that, even at minimum solubility, the extractability of pumpkin seed proteins could be improved by increasing the sodium chloride concentration. Oomah et al. (1994) reported higher protein solubility of defatted flax seed at an ionic strength of 0.8 M and pH above 8.0.

The results were similar to reported results for other protein-rich materials, such as oilseed meals and legumes. The broad pattern of nitrogen extractability, at varying pH and ionic strength, is comparable with those reported previously for other defatted oil seed meals, such as sunflower and sesame (Gheyasuddin et al., 1970; Rivas et al., 1981). Dev et al. (1986) reported that a solid:solvent ratio of 1:40 and addition of sodium chloride (0.1–1.0 M) markedly increased nitrogen extractability in at pH 4.0 for linseed flour, which exhibited an isoelectric range of 4.0–4.6. Our studies with *E. variegata* revealed that a solid:solvent ratio of 1:30 and addition of 0.1 or 0.5 M sodium chloride increased nitrogen extractability in the pH range 4.0–8.0. Nitrogen extractability was increased by the addition of sodium chloride, even at the isoelectric point (pI 4.75–5.0).

3.3. Protein precipitation

The maximum precipitation of protein occurred in the pH range 4.75–5.0; the peak was at pH 4.75 (Fig. 2). About 85% of the extracted protein could be precipitated at this pH. The percentage of protein precipitated decreased on both sides of the isoelectric region. Smith et al. (1946) observed a maximum precipitation of dispersed linseed protein at pH 5.1, and about 21% of the nitrogenous matter remained soluble, which is in agreement with our results where 15% of the nitrogenous matter remained soluble at pH 4.75.

3.4. Buffer capacity

A 1:30 (solid:solvent) dispersion of defatted *E. variegata* flour in water had a pH of 6.08. Adding HCl or NaOH brought about changes in the pH of the dispersion (Fig. 3). At alkaline pH, within the range of pH 6.08–10.0, an average of 0.093 mmol of NaOH per gram of flour was required to change the pH by one unit. To effect a change of one pH unit at acidic pH, within the range 6.08–4.0, 0.195 mmol of HCl per gram of flour was required. This indicates a markedly higher buffer capacity of the defatted flour in acidic medium than in alkaline medium. Similar observations were made by Rutkowski (1975) for rapeseed meal. During extraction and preparation of protein isolates from *E. variegata*, if

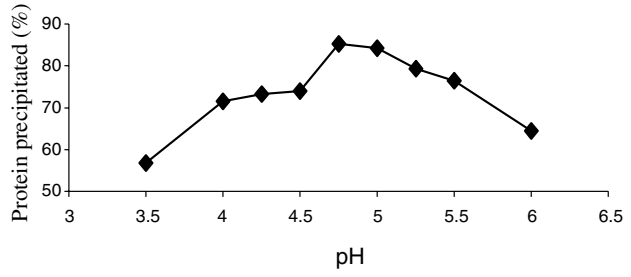

Fig. 2. Precipitability of protein from an alkaline extract in isoelectric pH region.

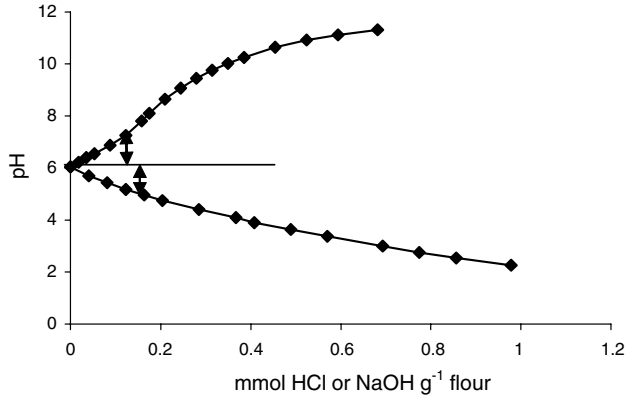

Fig. 3. Buffer capacity of defatted *Erythrina variegata* (white) flour: (a) 0.195 mmol HCl/g flour/unit pH; (b) 0.093 mmol NaOH/g flour/unit pH.

Table 2
Functional properties of *Erythrina variegata* flours

Functional properties	Percentage	
	Whole	Defatted
Water absorption	300 ± 0.50	225 ± 0.40
Oil absorption	102 ± 0.40	152 ± 0.60
Foaming capacity (volume increase)	16 ± 0.50	20 ± 0.50
Foam stability (2 h later)	6 ± 0.05	8 ± 0.05

pH 10 is selected for extraction, 0.350 mmol of NaOH per gram of flour would be required to bring the pH from 6.08 to pH 10.

3.5. Water absorption

Erythrina whole seed flour exhibited a water absorption of 1.81 kg kg^{-1}, whereas the dehulled, defatted seed flour absorbed 1.43 kg kg^{-1}flour. The higher water absorption capacity of whole seed may be due to its husk content (300 g kg^{-1} seed). Mahajan and Jha (1995) reported a water absorption value for rape seed of 2.25 kg kg^{-1}. Bass et al. (1997) reported that five commercial samples of defatted soy flour exhibited water absorption values of 1.70–2.07 kg kg^{-1} of sample.

3.6. Oil absorption

The oil absorption value for whole *Erythrina* seed flour was 1.02 kg kg^{-1}, whereas the value for dehusked, defatted flour was 1.52 kg kg^{-1}. As the whole seed flour contained natural oil, externally added oil was not absorbed very much as compared to the dehusked, defatted flour. Mahajan and Jha (1995) reported an oil absorption value for rapeseed of 150%. Bass et al. (1997) reported that five commercial samples of defatted soy flour exhibited oil absorption values of 110–120 ml per 100 g of sample.

3.7. Foaming capacity and foam stability

The foaming capacity and foam stability of whole and defatted *E. variegata* flours are presented in Table 2. The values were comparable with legume flours.

The present investigation gave results that were in agreement with reported values for other oil seed and legume meals. *E. variegata* seeds were rich in crude fat as well as protein, and hence could be used as a protein and energy source. Value-added products, such as protein isolates, protein-rich meals, and protein concentrates could be prepared from *E. variegata*.

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