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## Solubility as Influenced by pH and NaCl Concentration and Functional Properties of Lentil Proteins Isolate

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**Abstract:** The functional properties and the effect of pH and NaCl concentration on protein solubility were investigated of proteins isolate of lentil cultivars. The protein content of the seeds was found to be in the range 32.2-35.6%. Lentil protein isolate had 80% protein content. The minimum protein solubility was at pH 5. Lentil protein has good functional characteristics with emulsifying activity of 75.3-62.94% and emulsion stability of 41.4-46.3% and it had low foaming capacity but high stability. The total protein had water absorption capacity of 1.9-2.2 ml H<sub>2</sub>O/g protein, oil absorption capacity of 1.9-2ml oil/g protein and bulk of 1.4 g/ml. Lentil has high protein content with acceptable functional properties, which makes it promising protein source in food application due to its high nitrogen solubility and less fat content; a characteristic generally needed for textured products like meat.

**Key words:** Lentil, protein isolate, functional properties, pH, salt

### Introduction

Plant proteins play significant roles in human nutrition, particularly in developing countries where average protein intake is less than is required. Because of inadequate supplies of food proteins, there has been a constant search for unconventional protein sources, for use as both functional food ingredients and nutritional supplements (Onweluze *et al.*, 1994). Legumes such as lentil contain a high concentration of proteins, carbohydrates and dietary fiber and make an important contribution to human diet in many countries. Lentil (*Lens culinaris* Medic) is a nutritious food legume cultivated for its seeds and mostly eaten as dhal, (Dhal is a seed that's decorticated and split). The primary product is the seed, which has a relatively higher content of protein and calories compared to other legumes. Lentil is a protein calorie crop, its protein content amounting to 22%-35%. Lentil is deficient in the amino acids methionine and cystine; it is an excellent supplement to cereal grain diets because of its good protein/carbohydrate content (Oplinger *et al.*, 1990). Legumes have to be processed prior to consumption due to their content of antinutritional factors such as trypsin inhibitors, phytic acid and galactosides (Vidal-Valverde *et al.*, 2002). Plant protein products are gaining interest as ingredients in food systems throughout many parts of the world; the final success of utilizing plant protein additives depends greatly upon the favorable characteristics that they impart to foods. Therefore, the relationship of protein quality with processing parameters that affect the functional performance of protein products is worthy of extensive investigation. Solubility of protein is a critical functional

attributes required for its use as food ingredient, because solubility greatly influences other properties such as emulsification, gelation and foaming (Wang and Kinsella, 1976). The objectives of this study were to determine the chemical composition of lentil cultivars, the effect of pH and salt concentration on solubility of the proteins isolates and to investigate the functional properties of the proteins isolate.

### Materials and Methods

**Source of lentil cultivars:** Two Sudanese lentil cultivars were obtained from Elhudeba Research Station (Nadi and Selaim). The seeds were cleaned, dehulled and ground to a powder to pass a 0.4mm screen. Unless otherwise stated, all reagents were of reagent grade.

**Preparation of lentil protein isolate:** The flour was defatted and desolventized in an open air. The protein of defatted flour was extracted in an alkaline medium as follows: a sample of defatted flour (100 g) was extracted once with 0.01M phosphate buffer pH 8 containing 10 mM 2-mercaptoethanol (2.ME) at 25°C for 2h using a rotary shaker, then centrifuged at 8000xg for 20 min and the supernatant was acidified to pH 5 with 2N HCl and then centrifuged at 8000xg for 20 min; the precipitate was allowed to dry at room temperature for 5h then milled to pass a 0.4 mm screen. The isolated contained 80% protein.

**Proximate analysis of lentil flour:** The proximate analysis was carried out according to the methods of AOAC (1984) method.

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Table 1: Proximate composition (%) of lentil cultivars

Carbohydrates	Oil	Protein	Ash	Fiber	Moisture	Cultivars
47.040±2.47 <sup>b</sup>	2.100±0.11 <sup>b</sup>	33.390±1.56 <sup>a</sup>	2.700±0.26 <sup>b</sup>	4.130±0.45 <sup>a</sup>	10.370±0.16 <sup>a</sup>	Nadi
51.490±1.39 <sup>a</sup>	1.950±0.32 <sup>b</sup>	32.380±0.31 <sup>a</sup>	3.780±0.68 <sup>a</sup>	2.430±0.16 <sup>b</sup>	7.976±0.84 <sup>c</sup>	Seliam

Values are means ± SD. Means not sharing a common superscript letter in a column are significantly different at p = 0.05 as assessed by Duncan multiple range test.

Table 2: Selected functional properties of proteins isolates of lentil cultivars

ES	EA	BD	FAC	WAS	Cultivars
41.4±1.22	75.3±4.12	1.4±0.32	1.9±0.12	1.9±0.12	Nadi
46.2±3.21	62.9±8.51	1.4±0.02	2.0±0.00	2.0±0.00	Seliam

WAS: water absorption capacity (ml/g); FAC: Fat absorption capacity (ml/g); BD: Bulk density (g/ml); ES: Emulsion stability (%); EA: Emulsifying activity (%).

**Nitrogen solubility:** Nitrogen solubility of the defatted flour was determined by the method of Beuchat *et al.* (1975) at different pH levels and NaCl concentration. The dispersions were shaken for an hour at 25°C and then centrifuged at 3000xg for 30 min. Nitrogen content of the supernatant was determined following micro-Kjeldahl method. Nitrogen solubility was expressed as a percent of nitrogen in the solution to that of the total nitrogen in the sample.

**Water absorption capacity (WAC):** Water absorption capacity was estimated by the method described by Wang and Kinsella (1976). One g of the protein samples was suspended in 10 ml distilled water, stirred for 2 min, shaken for 30 min and then centrifuged at 3700xg for 25 min, the freed water was carefully decanted in a graduated measuring cylinder and the volume recorded. WAC was expressed as ml water retained by one g protein.

**Fat absorption capacity (FAC):** Fat absorption capacity was measured by the method described by Lin *et al.* (1974). FAC expressed as ml oil retained by one g protein.

**Bulk density:** The bulk density was determined according to Wang and Kinselle (1976) method. Ten grams of the sample were placed in 25 ml-graduated cylinder and packed by gently, the volume of the sample was recorded, and the bulk density is expressed as g protein/ml.

**Emulsifying activity (EA) and Emulsion stability (ES):** The procedure described by Volkert and Kelin (1979) was used for both emulsification activity and emulsion stability. Emulsions were prepared with 1g of protein, 50 ml-distilled water at room temperature (~ 25°C) and 50 ml of corn oil. The mixture was emulsified for 30 min. Each emulsified sample was divided equally into 50 ml centrifuge tubes. Content of one tube was directly centrifuged at 3000xg for 30 min while the other centrifuged under the same conditions after heating in a water bath at 80°C for 30 min and cooling to 15°C. The

height of the emulsified layer, as a percentage of the total height of material in the unheated tubes was used to calculate the emulsifying activity and stability using the following formulas:

$$EA (\%) = \frac{\text{height of emulsion}}{\text{height of whole layer}} \times 100$$

$$ES (\%) = \frac{\text{height of emulsion layer after heating}}{\text{height of whole layer}} \times 100$$

**Foaming capacity (FC):** The foaming capacity was determined by the method of Lawhon *et al.* (1972). About 100 ml of distilled water were added to 3g proteins. The mixture was homogenized for 5min in a blender set at high speed at room temperature (~25°C) and then transferred to a 250 ml-measuring cylinder. The volume of foam at 30 second was calculated, and the increase in volume is expressed as a percent foam capacity (FC).

$$FC (\%) = \frac{[\text{volume after whipping} - \text{volume before whipping}]}{\text{volume before whipping}} \times 100$$

**Foam stability (FS):** Foam stability (FS) was determined by measuring the decrease in volume of foam as a function of time up to a period of 120min. The stable foam volumes were recorded at time intervals of 10, 30, 60, and 120min.

$$FS (\%) = \frac{\text{Foam volume after time (t)}}{\text{Initial foam volume}} \times 100$$

**Statistical analysis:** Data represent means of triplicate samples, which were subjected to analysis of variance and means, were separated according to Duncan's multiple range test (Duncan, 1955).

## Results and Discussion

**Chemical composition:** The proximate composition of lentil cultivars is illustrated in Table 1. The moisture content of the cultivars was 10.37 and 7.98% for Nadi and Seliam, respectively. The data obtained is similar to

Table 3: Foaming capacity (%) and foam stability (%) of protein isolate of lentil cultivars

Cultivars	Foaming capacity	Foam stability				
		Time (min)				
		0	15	30	60	120
Nadi	22.67 ±4.01 <sup>a</sup>	100.00±0.00 <sup>a</sup>	77.66±6.11 <sup>b</sup>	62.34±8.85 <sup>c</sup>	56.48±6.11 <sup>d</sup>	52.95±0.00 <sup>e</sup>
Seliam	28.33 ±2.14 <sup>b</sup>	100.00±0.00 <sup>a</sup>	75.02±0.00 <sup>b</sup>	68.77±10.8 <sup>c</sup>	62.52±10.8 <sup>d</sup>	56.26±0.00

<sup>a</sup>Values are means ± SD. Means not sharing a common superscript letter in a row are significantly different at  $p=0.05$  as assessed by Duncan multiple range test.

that reported by Muehlbauer *et al.* (1985), but lower than that reported by Adsule *et al.* (1989). The ash content ranged from 2.7-3.8% which is approximately similar to that reported by Adsule *et al.* (1989) and Muehlbauer *et al.* (1985). The protein content ranged from 32.3-33.4% which is consistent to that reported by Adsule *et al.* (1989). The oil content ranged from 1.9-2.1% which is lower than that reported by Duke (1981). The fiber content ranged from 2.43-4.13% which is lower than that found by Duke (1981). These differences may be due to variations in the growing locations of the cultivars.

**Nitrogen solubility:** Fig. 1 shows the nitrogen solubility at different pH levels of lentil protein. The minimum nitrogen solubility of Nadi cultivars was found to be 8% at pH 5 while that of selaim was 9% at the same pH value. The data obtained indicating that the isoelectric point of lentil protein was at pH 5. Hsu *et al.* (1982) found that the lowest solubility of the protein of yellow pea, lentils and faba bean were at pH 4.5- 5.0. On either side of pH 5 the protein solubility started to increase and reached its maximum value at pH 12 (88%). Lentil protein showed good solubility in both acid and alkaline pH regions, which is an important characteristic for food formulation (Idouraine *et al.*, 1991). Parakash and Narasimha Rao (1986) reported similar results. The solubility of lentil protein at different NaCl concentrations is shown in Fig. 2. In dilute NaCl solution (0.2 M), the solubility of lentil protein was lower than that in water. However, protein solubility increased up to 0.6 M (salting-in) and thereafter started to decrease (salting-out). Schut (1976) suggested that NaCl causes a shift in the isoelectric point to a more acidic pH as a result of specific ion binding effects. Since inorganic anions are bound to protein more strongly than inorganic cations due to their smaller hydrated radii, anions are able to attain a closer proximity to the protein molecule and are able to "screen" the charged groups of the protein more effectively than cations. Thus, with the addition of NaCl and selective binding of the chloride anions, the protein would have an excess of negative charges at the pH of original isoelectric point and more acid is, therefore, needed to reach the new isoelectric point. This was observed also by Liu and Hung (1998) who reported that high concentration of NaCl (0.75 M) reduced chickpea protein solubility.

**Water and oil absorption capacity:** As shown in Table 2 lentil protein isolate had a water absorption capacity of 1.9 and 2.0 ml H<sub>2</sub>O/g protein for Nadi and Selaim, respectively. The data obtained within the range of commercial values of protein concentrates (1.9-2.2 ml H<sub>2</sub>O/g protein) as reported by Lin and Zayas (1987). It has been reported that the protein concentrate exhibits poor water-binding capacity compared to that of the isolate; this is likely due to the fact that the protein isolate has great ability to swell, dissociate and unfold exposing additional binding sites, whereas the carbohydrates and other components of the protein concentrate may impair it (Kinsella, 1979). The oil absorption capacity of lentil protein isolate (LPI) was 1.9-2.0 ml oil/g proteins for Nadi and selaim, respectively. LPI showed higher oil absorption capacity than chickpea (Marina, 1986). Kinsella (1979) explained the mechanism of absorption as a physical entrapment of oil; several authors have related the oil absorption capacity to interaction of non-polar side chain of the protein as well as to the conformation features of the proteins. Results obtained in this study suggested that LP1 had both good water and oil absorption capacities.

**Bulk density:** LPI had bulk density of 1.4g/ml for both Nadi and selaim cultivars. This value is higher than that of cowpea as reported by Ragab *et al.* (2004). Bulk density depends on combined effects of interrelated factors such as intensity of attractive interparticle forces, particle size and number of contact points (Peleg and Bagley, 1983). Higher bulk density is desirable since it helps to reduce the paste thickness which is an important factor in convalescent and child feeding (Padmashree *et al.*, 1987)

**Emulsifying properties:** As shown in Table 2, lentil protein has emulsifying activity of 75.3 and 62.9% for Nadi and Selaim, respectively and emulsion stability of 41.4 and 46.3% for the cultivars, respectively. The data obtained is lower than that obtained by Khalid *et al.* (2003) who reported that the emulsion stability of sesame protein isolate at neutral pH was high. Elizalde *et al.* (1991) reported that the emulsion stability is enhanced by high protein and oil concentrations and these factors are highly interrelated. They also reported that the emulsion stability depends primarily upon the water and oil absorption capacity.

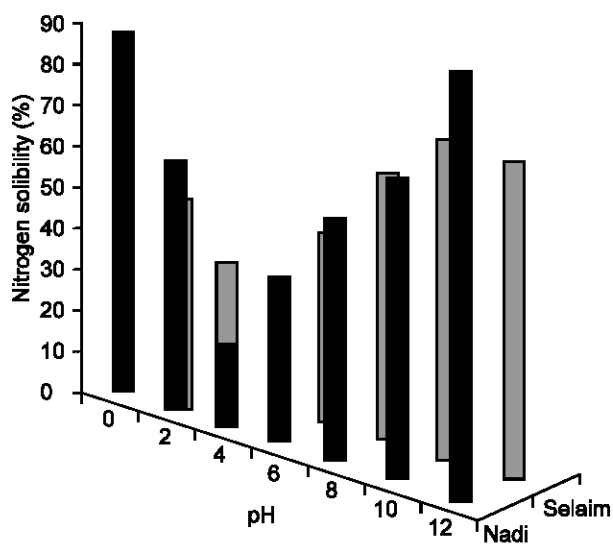


Fig. 1: Effect of pH levels on nitrogen solubility of lentils proteins isolate of two cultivars.

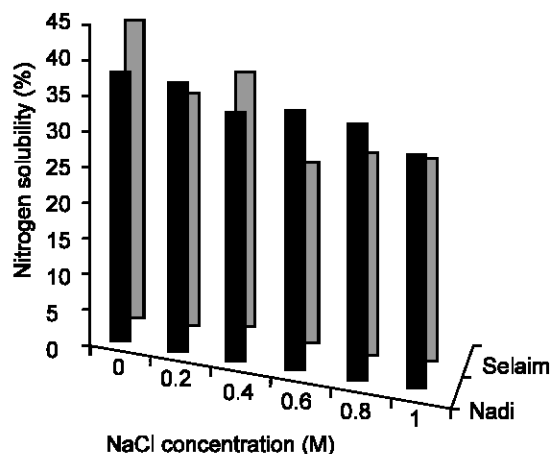


Fig. 2: Effect of NaCl concentration (M) on nitrogen solubility of lentils proteins isolate of two cultivars.

**Foaming properties:** Table 3 shows the foaming capacity and foam stability of protein isolated from lentil cultivars. Lentil protein foam had a lower capacity but highly stable compared to soy protein that studied by Soetrisno and Holnes (1992).

**Conclusion:** Protein isolates from lentil seeds was found to be solubility in acidic and alkaline pHs with good emulsifying and foaming properties. Therefore, it is possible to incorporate it in beverages or soups; it can also be used as animal protein replacer or extender or as a protein supplement to cereal based foods, infant formula and meat products.

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