Zagazig J. Agric. Res., Vol. 42 No. (3) 2015
http:/www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master

FUNCTIONAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF LENTIL PROTEIN ISOLATE

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ABSTRACT

Lentil protein was isolated from defatted lentil flour by precipitation at its iso-electric point (pI 4.5). The functional properties (solubility, emulsifying and foaming properties) were investigated at a pH range of 2-10 as well as antioxidant activity. The solubility profile of lentil protein isolate indicated that protein solubility was reduced as the pH increased from 2 to 4.5, which corresponding to its isoelectric point, after which subsequent increases in protein solubility occurred progressively. The minimum solubility for lentil protein isolate (22.26%) was recorded at pH 4.5 which corresponds to its isoelectric point (pI). The highest protein solubility (90 %) was observed at pH 10. The maximum emulsifying activity of lentil protein isolate (73%) was obtained at pH 10 of the protein solution. Emulsifying activity decreased with the increase in pH until it reached minimum value (35%) at pH 4.5. Lentil protein isolate recorded 112% foam capacity at pH 10 and this was reduced to 48% at pH 4, where minimum value was observed. DPPH radical scavenging activity at different concentrations (10-40 mg/ml) of lentil protein isolate was measured. The radical scavenging effect was found to increase with increasing concentrations.

Key words: Lentil protein isolate, solubility, emulsifying properties, foaming properties, DPPH.

INTRODUCTION

Legumes play a very important role in human nutrition because they are a very good source of proteins, carbohydrates and dietary fibers. Proteins contribute to the production of a wide variety of food products, mainly through the formulations and stabilization of emulsions. Legumes, which considered as poor man's meat, are generally good sources of nutrients (Tharanathan and Mahadevamma, 2003). They are an important and inexpensive source of protein, dietary fiber and starch for a large part of the world's population, mainly in developing countries (Perla *et al.,* 2003). Lentils are one of the most economic sources of plant proteins and belong to the legume family. The protein content of lentils varies from 22% to 31% (Adsule and Kadam, 1989). Globulins constitute the major protein fraction in lentil. Lentil proteins have an important role in the structure and texture of foods derived from or containing lentils. The functional properties of proteins reflect the inherent properties of proteins, as well as the manner with which they interact with other components of food. There is increased interest in legume proteins, including lentil proteins, as they can be used as good substitute for animal proteins (Alsohaimy *et al.*, 2007). In this regard, the understanding of factors affecting the functional properties of lentil proteins enables better control of these properties, which will facilitate the novel application of these proteins. The functional properties such as solubility, water and oil absorption capacity, gelation, foam and emulsion formation of lentil protein isolate were studied to some extent previously (Hsu *et al.*, 1982). However, there is a lack of fundamental knowledge and supporting data on the interfacial and emulsification properties of lentil proteins. These interfacial and emulsification properties are essential, particularly when the lentil proteins are intended to be used as emulsifiers. Thus, the main objective of this study was to investigate the functional properties of lentil

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protein isolate such as solubility, foaming and emulsifying properties as well as antioxidant activity.

MATERIALS AND METHODS

Plant Materials

Lentil (*Lens culinaris,* Madik) seeds were purchased from local market, Zagazig, Sharkia Governorate, Egypt.

Chemicals

Reagents for electrophoresis were purchased from Bio-Rad laboratories (Richmond, CA, USA). The 1,1-diphyenyl-2-picrylhydrazyl radical (DPPH) was obtained from Sigma-aldrich (St. Louis, Mo, USA). All chemicals used in the experiments were of analytical grade.

Methods

Sample preparations

Lentil seeds were manually cleaned and ground for 3 min using a Moulinex mixer (Type 716, France) at a maximum speed. The ground meal was pass through a 1 mm^2 sieve. The powder was defatted using chloroform: methanol (3:1 *V/V*) for 8 hr., soaking. Solvents were evaporated by air and the dried-defatted meal was stored at 4°C until analysis.

Extraction of protein isolate

A dispersions of 5% (*W/V*) defatted lentil flour in distilled water was adjusted to pH 9 with 0.1N NaOH at room temperature, shaked for 1 hr., and centrifuged for 15min at 2000g. In order to obtain more yields, the extraction and centrifugation procedures were repeated on the residue. The extracts were combined and the pH was adjusted to 4.5 with 1N HCl to precipitate the protein. The proteins were recovered by centrifugation at 2000g for 15min followed by removal of the supernatant by decantation. Crud protein was washed with distilled water, dispersed in distilled water at pH 7.5, dialyzed overnight and lyophilized (Johnson and Brekke, 1983).

SDS-PAGE

SDS–PAGE was performed on a discontinuous buffered system according to the method of Laemmli (1970). Gels (3% and 12%) were prepared from 30% acrylamide and 0.8% N, N-bis methylene acrylamide solution. The final composition of the separation gel was 0.375 mol Tris–HCl (pH 8.8) and 0.1% SDS. Gels were chemically polymerised by addition of 0.025% tetra methyl ethylenediamine (TEMED) and ammonium persulphate. Each 3% gel (stacking gel), contained; 0.125 mol Tris–HCl (pH 6.8) and 0.1% SDS. The electrode buffer (pH 8.3) contained 0.025 mol Tris, 0.192 mol glycine, and 0.1% SDS. Five milligrams of lentil protein were dispersed in 1 ml of 0.03 M Tris buffer (pH 8.0) for 15 min with vortexing every 5 min. The extract was then centrifuged for 10 min at 5000g. An aliquot of the extract (20 µl) was mixed with 20 µl of SDS-sample buffer, heated at 96 °C for 3 min and a 10 µl aliquot from the final mixture was electrophoresed. After running at 10 mA on the stacking gel and 20 mA on the running gel, staining was performed with Coomassie Brilliant Blue R-250 dye (0.2% solution, freshly prepared in 45% methanol and 10% glacial acetic acid and 45% distilled water). Approximate molecular weights of the unknown bands were calculated by relating the distance migrated by these bands to the distances migrated by the bands of standard known molecular weights standard protein MW from 28 to 250 KD.

Functional Properties of Lentil Protein Isolate

pH-solubility profile

One hundred and twenty-five milligrams of lentil sample were dispersed in 25 ml of distilled water and the solution pHs were adjusted to 2-10 using either 0.5 mol/l NaOH or 0.5 mol/l HCl. The slurries were mixed for 1 hr., at 30°C using magnetic bar before centrifuging at 1200g for 20 min at 4°C. The supernatant was filtered and protein content in the supernatant was determined by Kjeldahl method (AOAC, 1996). The solubility was calculated according to the following equation:

$$\text{Solubility (\%)} = \frac{\text{Amount of protein in the supernatant} \times 100}{\text{Amount of protein in the sample}}$$

The solubility profile was obtained by plotting averages of protein solubility (%) against pH.

Emulsifying activity and stability

Emulsifying activity and stability were determined using the method of Neto *et al.* (2001). Five milliliter portions of protein solution (2% *W/V*) were homogenized with 5 ml

corn oil. The emulsions were centrifuged at 1100 g for 5 min. The height of emulsified layer and that of the total contents in the tube were measured.

The emulsifying activity (EA) was calculated as:

$$EA\ (\%) = \frac{\text{Height of emulsified layer in the tube x 100}}{\text{Height of the total contents in the tube}}$$

Emulsion stability was determined by heating the emulsion at 80°C for 30 min before centrifuging at 1100g for 5min:

$$ES\ (\%) = \frac{\text{Height of emulsified layer after heating x 100}}{\text{Height of emulsified layer before heating}}$$

Influence of pH was investigated by preparing protein solutions and creaty similar profiles against pHs ranging from 2 to 10.

Foaming properties

The foaming capacity and stability were studied according to the method of Conffman and Garcia (1977). 0.1 g of protein isolate was dispersed in 100 ml-distilled water. The resulting solution was whipped vigorously for 2min in a Moulinex mixer (Type 716, France) at the maximum speed. Volumes were recorded before and after whipping. The volume increase as percentage was calculated according to the following equation:

$$\text{Volume}\ (\%) = (V_2\text{-}V_1)/(V_1)\ \text{x}100,$$

Where:

V_2 is the volume of protein solution after whipping and V_1 the volume of protein solution before whipping.

Foam stability was determined as volume of foam that remained after 8 hr., at room temperature and expressed as a percentage of the initial foam volume.

Influence of pH on foam capacity and stability were investigated by preparing protein solutions at various pHs ranging from 2 to 10.

Antioxidants activity (DPPH radical-scavenging activity)

The electron donation ability of the lentil protein isolate was measured by bleaching the purple colored solution of DPPH according to the method of Hanato *et al.* (1988). Different concentrations of lentil protein isolate (10, 20, 30 and 40 mg /ml) were added individualy to 3 mL of 0.1 mM DPPH dissolved in methanol. After incubation period of 30 min at room temperature, the absorbance was determined against a control at 517 nm (Gulcin *et al.*, 2004). Percentage of antioxidant activity of free radical DPPH was calculated as follow:

$$\text{Antioxidant activity (Inhibition)}\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where:

$A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of lentil protein isolate.

RESULTS AND DISCUSSION

SDS-PAGE

The SDS-PAGE of lentil protein isolate demonstrated marked differences in the electrophoretic mobility of its subunits (Fig. 1). It was clear that the molecular weight of protein subunits ranged between 15000 and 116740 daltons.

Functional Properties of Lentil Protein Isolate

pH-solubility profile

Solubility is one of the most important characteristics of proteins because it is not only important by itself, but also their influences on other functional properties. Good solubility of proteins is required in many functional applications, especially for emulsions, foams and gels, because soluble proteins provide a homogenous dispersibility of the molecules in colloidal systems and enhance the interfacial properties (Zayas, 1979). The pH-solubility curve of lentil protein isolate is presented in Fig. 2. The solubility profile of lentil protein isolate indicated that protein solubility decreased as the pH increased from 2 to 4.5, which corresponding to its isoelectric point, Subsequently, as the pH increased the progressive protein solubility increased until pH_{10}. The minimum solubility for lentil protein isolate (22.26%) was recorded at pH 4.5 which corresponds to its isoelectric point (pI). The highest protein solubility (90%) was observed at pH 10. Data are in good agreement with those of Sitohy *et al.* (2001).

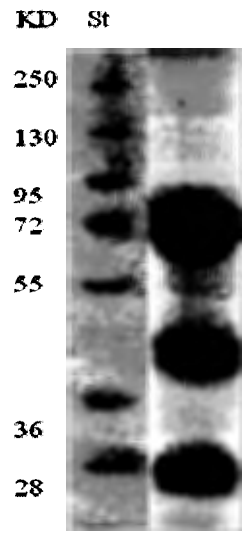

Fig. 1. SDS-PAGE of lentil protein isolates

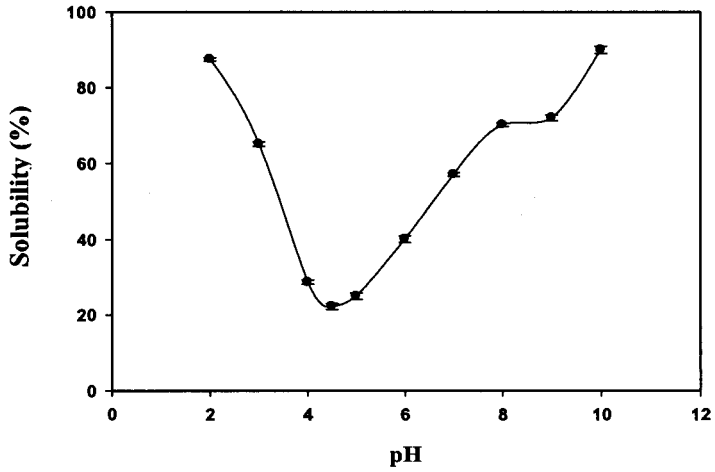

Fig. 2. Protein solubility profile of lentil protein isolates at different pH values

Emulsifying properties

Effect of pH on emulsifying activity and emulsion stability is a reflection of the influence of pH on protein solubility.

Effect of pH on emulsifying activity of lentil protein isolate was presented in Fig. 3. The maximum emulsifying activity of lentil protein isolate solution (73%) was obtained at pH 10. Emulsifying activity decreased with increasing pH until it reached minimum value (35%) at pH 4.5. Also, the behavior of emulsion stability was pH-dependent (Fig. 4). At pH 4 lentil protein isolate had the minimum emulsion (48%), followed by subsequent increase in emulsion stability as the pH increased. The maximum emulsion stability of lentil protein isolate (82%) was obtained at pH10. Dependence of emulsion activity on pH was expected as it is known that emulsifying activity of soluble proteins depends upon the hydrophilic-lipophilic balance, which is affected by pH. At the oil-water interface, the protein orients lipophilic residues is regard to the oil phase and that of the hydrophilic residues to the aqueous phase, thus reducing surface tension at the interface.

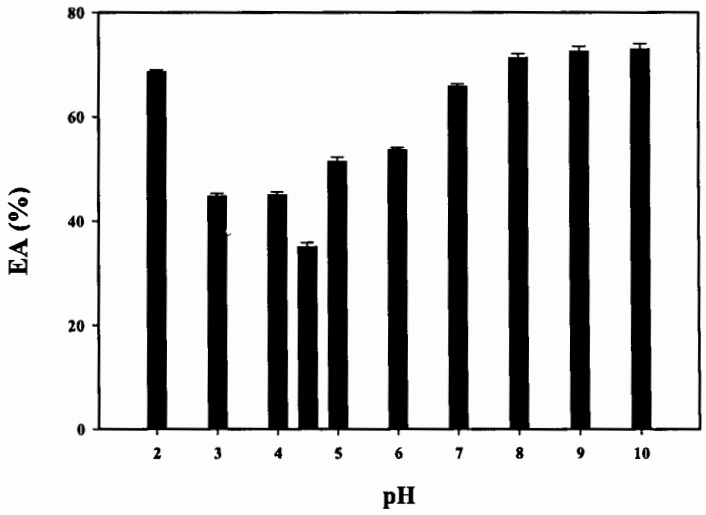

Fig. 3. Emulsifying activity (EA) profile of lentil protein isolate at different pH values

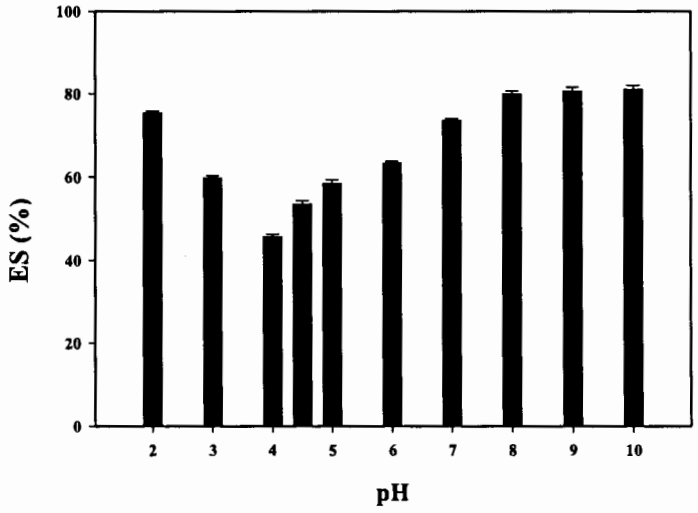

Fig. 4. Emulsifying stability(ES) profile of lentil protein isolate at different pH values

Foaming properties

Effect of pH on foam capacity of lentil protein isolate is presented in Fig. 5. Lentil protein isolate recorded 112% foam capacity at pH 10 and then reduced to 48% at pH 4, where minimum value was observed.

Effect of pH on foam stability of lentil protein isolates is presented in Fig. 6. Lentil protein isolate had a low foam stability of 52% at pH 9, and then increased to 96% at pH 4. Protein adsorption and viscoelasticity at an air/water interface is maximum near or at isoelectric pH because a protein is not strongly repelled. In addition, the protein possesses low net charge near isoelectric pH, which may contribute to the formation of stable molecular layers in the air/water interface, a development that improves foam stability. This observation was quite similar to results reported by Buckingham (1970).

Antioxidant activity

DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of proteins and peptides. *In vitro* antioxidant studies on the lentil protein isolate; the extent of DPPH radical scavenging at different concentrations (10-40 mg/ml) of lentil protein isolates was measured. The radical scavenging effect was found to increase with increasing concentrations (Fig. 7).

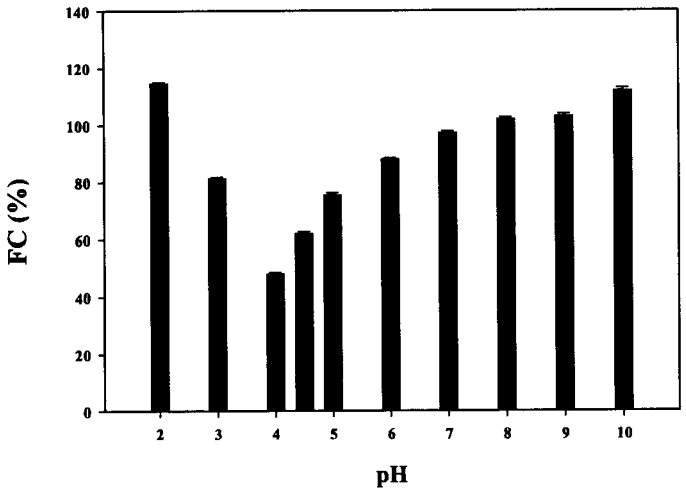

Fig. 5. Foaming capacity (FC) profile of lentil protein isolate at different pH values

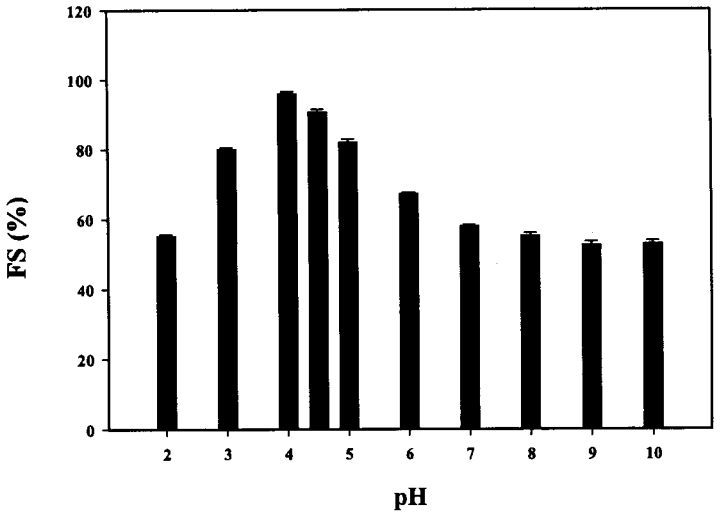

Fig. 6. Foaming stability (FS) profile of lentil protein isolate at different pH values

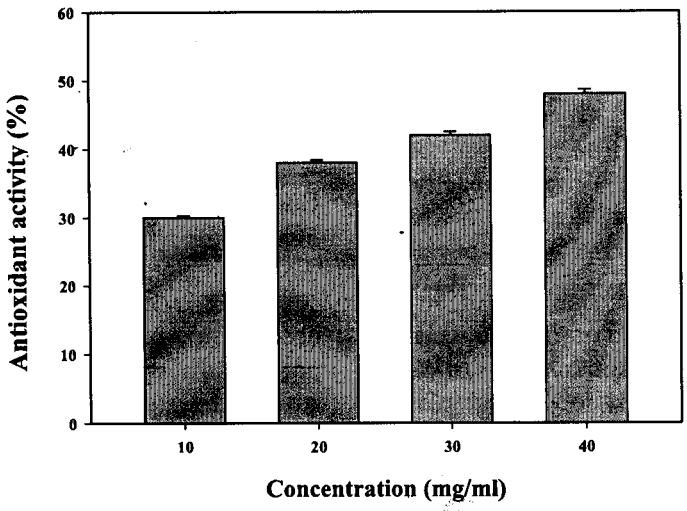

Fig. 7. DPPH radical-scavenging activity of lentil protein isolate at different concentrations (10-40 mg/ml)

At a concentration of 10, 20, 30 and 40 mg/ml the lentil protein isolate scavenged 30, 38, 42 and 49%, respectively. In the literature, Aydemir (2008) studied the antioxidant activity of lentil protein from lentile cultivars grown in Turkey (*Lens culinaris, Madik*) and (*Lens esculenta*) and found that the free radical scavenging activity varied between 110 ad 185 mmol trolox/kg (*Kalkas* and *Ali Dayl*).

Conclusion

The solubility profile of lentil protein isolate indicated that protein solubility was reduced as the pH increased from 2 to 4.5, which corresponding to its isoelectric point, after which subsequent increases in protein solubility occurred progressively. The minimum solubility for lentil protein isolate was recorded at pH 4.5 which corresponds to its isoelectric point (pI). The highest protein solubility was observed at pH 10. The maximum emulsifying activity of lentil protein isolate was obtained at pH 10 of the protein solution. Emulsifying activity decreased with the increase in pH until it reached minimum value at pH 4.5. Lentil protein isolate recorded high foam capacity at pH 10 and this was reduced at pH 4, where minimum value was observed. DPPH radical scavenging activity at different concentrations of lentil protein isolate was measured. The radical scavenging effect was found to increase with increasing concentrations.

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الخصائص الوظيفية والتضاد للأكسدة لمفصول بروتين العدس

شهاب الدين محمد أنور – محمود زكي سطوحي – رجب عبدالفتاح المصري – سيد سليمان السعدني

قسم الكيمياء الحيوية – كلية الزراعة – جامعة الزقازيق - مصر

تم فصل بروتين العدس من مطحون العدس منزوع الدهن بالترسيب عند نقطة التعادل الكهربي للبروتين (٤,٥)، ثم تم فحص الخصائص الوظيفية (الإذابة – الاستحلاب – الخاصية الرغوية) للبروتين المفصول وذلك عند درجات pH من ٢-١٠ وكذلك دراسة نشاطه كمضاد للأكسدة، وقد تناقصت الذوبانية مع ازدياد درجات pH من ٢ حتى ٤,٥ (٢٢,٢٦%) مما يتوافق مع نقطة التعادل الكهربي للبروتين المفصول، وبازدياد درجات pH حتى ١٠ ازدادت معها الذوبانية (٩٠%)، كما ظهر أن أعلى نشاط استحلابي لمحلول البروتين المفصول (٧٣%) عند درجة pH ١٠ ثم تناقصت القدرة على الاستحلاب بانخفاض درجات pH حتى وصلت لأقل قيمة (٣٥%) عند درجة pH ٤,٥، كما حقق البروتين المفصول ١١٢% قدرة على إحداث رغوة عند pH ١٠ ثم تناقصت إلى أقل قيمة ملحوظة (٤٨%) عند pH ٤، وقد أظهر البروتين المفصول قدرته كمضاد أكسدة حيث زادت القدرة على تجميع الشقوق الحرة مع ازدياد التركيز المستخدم من محلول البروتين من ١٠ إلى ٤٠ ملليجرام/ملل.

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