Zagazig J. Agric. Res., Vol. 46 No. (2) 2019 467-474

Biotechnology Research

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

CHEMICAL EVALUATION AND FUNCTIONAL PROPERTIES OF LUFFA SEEDS PROTEIN

Ibrahim S.A. Mohamed*, A. Osman, K.M. Wahdan and M.Z. Sitohy

Agric. Bioch. Dept., Fac. Agric., Zagazig Univ., Egypt

Received: 24/02/2019 ; Accepted: 02/04/2019

ABSTRACT: In the current study chemical composition of luffa seeds was estimated. An addition, the luffa seed proteins were isolated and the functional properties of these proteins such as solubility, emulsifying properties and foaming properties were evaluated. The proximate composition of luffa seeds was as follows, dry matter 93.53%, crude protein 27.27%, crude fat 26.68%, total carbohydrate 31.25% and ash 8.33% (on dry basis). The pH effect on the solubility of luffa seed protein was presented. The solubility was decreased in the pH range 2-5 and increased gradually from pH 6.0 to pH 10 with a maximum solubility (80%) at pH 8. The solubility for luffa seed protein increased in the alkaline medium. The solubility for luffa seed protein indicates the isoelectric point (less solubility) at pH 5. Emulsifying activity (EA) and emulsion stability (ES) of luffa seed protein is a reflection of the influence of pH on protein solubility. The maximum EA of luffa seed protein (70%) was obtained at pH 8 of the protein solution. The maximum ES of luffa seed protein (77 and 78%, respectively) was obtained at pH 8 and 9. Luffa seed protein recorded 100% foam capacity (FC) at pH 8 and this was reduced to 17% at pH 5, where minimum value was observed. Luffa seed protein had a foam stability of 16%, respectively at pH 7 and increased to 90%, respectively at pH 3. It may be concluded that these values obtained for the luffa seeds protein indicate the potential in its use as a source of vegetable protein in animal and human nutrition.

Key words: Luffa seed protein, proximate analysis, solubility, foaming properties, emulsifying properties.

INTRODUCTION

Luffa sp. belongs to the family of *Cucurbitaceae*. The family is mainly tropical and subtropical consisting of climbing and scrambling herbs with either simple or branched tendrils. The leaves are oval, simple, deeply five to seven lobed- dentate, dark green and frequently hairy. *Luffa* grows well in a well-drained soil that is rich in organic waste. The fruits are smooth and cylindrical shaped with white flesh. The length of the fruit is one to two feet. The young fruit is used as a cooked vegetable although some gardeners grow smooth *Luffa* for the fibrous interior only. The mature fruits are the source of the spongy reticulated material known as the domestic loofah. These loofahs are used for sponges and filters and for stuffing pillows, saddles and slippers. They can also be used for insulation and are attractive sources for packing materials because of their biodegradability. There is an increasing interest in domestic production as studied by **Amoo *et al.* (2008)**.

One mature Luffa sponge will produce at least 30 seeds, where **Oboh and Aluyor (2009)** revealed that in their study. The seeds have laxative properties due to their high oil content. It contains a wide range of secondary metabolites with distinct biological activities. The seeds have been reported to be useful in the treatment of asthma, sinusitis and fever. It is reported to possess antiviral, anti-tumor, antioxidant, anti-inflammatory and immunomodulatory activities, which were investigated by **Nagao *et al.* (1991) and Tannin-Spitz *et al.* (2007)**. *Luffa* seeds contain a high proportion of essential amino acids and has a low atherogenic potential.

* **Corresponding author: Tel. :** +201003743340
E-mail address: i.sayed@agri.zu.edu.eg

Flavonoids (4.53%) were the most concentrated phytochemical in the seed flour as well as saponins, alkaloids and cardiac glycosides were also presented **(Lucy and Abidemi, 2012)**. Seeds are rich sources of minerals but the bioavailability of these minerals is usually low due to the presence of anti-nutrients as studied by **Valencia *et al.* (1999)**.

Luffa seeds are containing high percentage of proteins ranging from 28% to 33% **(Amoo *et al.*, 2008; Salem, 2017)**. Protein is one of the important nutrients required for growth and production in man and livestock. One of the cheap sources of protein for both man and livestock is the grain legumes, many of which had been evaluated to be of good nutritional values when used in formulating diets according to **(Oke *et al.*, 1995)**. The sponge is used in the rural areas of Egypt for washing and scrubbing of household utensils while the seeds are discarded. These properties, such as texture, solubility, water absorption capacity, oil absorption capacity, foaming capacity, gel formation, *etc*. are indices that may be used to a large extent to predict the behavior of the plant protein in food formulations according to **(Dairo *et al.*, 2007)**. In the current study chemical composition of luffa seeds was estimated. Also, the luffa seed proteins were isolated and the functional properties of these proteins such as solubility, emulsifying properties, and foaming properties were evaluated.

MATERIALS AND METHODS

Seed Collection

The seeds of (*Luffa cylindrica*) were kindly collected from a private farm at Sharkia Governorate, Egypt.

Sample preparation

The immature seeds and extraneous materials were removed and the remaining seeds were washed then dried at 50°C. The dried seeds were ground by a Moulinex mixer (Type 716, France) at maximum speed and the meal was ground to pass through a 1 mm^2 sieve. The powder was then defatted using chloroform: methanol (3:1 *V/V*) for 8 hr. The solvent was evaporated by rotary-evaporator and dried-defatted meal stored at 4°C until analysis according the method reported by **Sitohy and Osman (2010)**.

Proximate analyses

Proximate analyses of the luffa seeds, inclusive moisture, ash, total protein, crude fat, and total carbohydrates were estimated in triplicate, as described by **(Horwitz and Latimer, 2000).** Moisture content was evaluated by heating the sample at 105°C for 3 hr., until constant weight; the ash content was evaluated by weighing the incinerated remains acquired at 550°C for 24 hr., The crude protein was obtained by multiplying the nitrogen value by a factor of 6.25 as recommended by **(Dairo *et al.*, 2007)**. The crude fat content was estimated by extraction with petroleum ether in Soxhlet apparatus. Total carbohydrate content was estimated by assessing the absorbance of phenol and concentrated sulphuric acid (H_2SO_4) extracts at 490 nm as followed by **Dubois *et al.* (1956).**

Luffa seed proteins isolation

Dispersions of 5% (*W/V*) defatted luffa seeds flour in distilled water and adjusted to pH 9 with 0.1 N NaOH at room temperature, shaken for 1 hr., and centrifuged for 15min at 2000g. In order to obtain high 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 2000 g for 15min followed by a decantation of the supernatant by decantation. Protein curde was washed with distilled water, dispersed in distilled water at pH 7.5, dialyzed overnight and lyophilized as recommended by **(Johnson and Brekke, 1983)**.

Fourier Transform Infrared (FTIR) Spectroscopy

Luffa seed proteins were prepared with potassium bromide (KBr) pellet method according the study of **Souillac *et al.* (2002)**. Infrared spectra was measured with a FT-IR spectrometer (NICOLET NEXUS 470, DTGS, Thermo Scientific, Waltham, MS, USA) at 25°C. For each spectrum 256 interferograms were collected with a resolution of 4 cm-1 with 64 scans and a 2 cm-1 interval from the 4000 to 400 cm^{-1} region. The system was continuously

purged with dry air. Second derivation spectra were obtained with Savitsky-Golay derivative function soft as followed by **Surewicz and Mantsch (1988)**.

Functional Properties of Luffa Seed Proteins

Solubility

One hundred and twenty-five milligrams of luffa seed proteins were dispersed in 25 ml of distilled water (0.5%, *W/V*) and the different pH were adjusted to from 2 to 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 1200 g for 20 min at 4°C and the supernatant was filtered to obtain a clear solution. Protein content in the supernatant was determined by Kjeldahl method according a study of **Firestone (1990)**. Triplicate determinations were carried out and the solubility profile was obtained by plotting averages of protein solubility (%) against pH:

$$\text{Solubility (\%)} = \frac{A}{B} \times 100$$

(A) Amount of protein in the supernatant

(B) Amount of protein in the sample

Emulsifying activity and stability

Emulsifying activity and stability were determined using the method of **Neto *et al.* (2001).** Five milliliters portions of luffa seed proteins 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 (%)= Height of emulsified layer in the tube/ height of the total contests in the tube × 100

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

EA (%) = Height of emulsified layer after heating / height of emulsified layer before heating × 100

Influence of pH was investigated by preparing protein solutions of various pHs ranging from 2 to 10.

Foaming properties

The foaming capacity (FC) and stability (FS) for luffa seed proteins were studied according to the method of **Coffmann and Garciaj (1977)**. Two grams from sample (2%, *W/V*) were dispersed in 100 ml-distilled water. The resulting solution was whipped vigorously for 2 min in a Moulinex mixer (Type 716, France) at the maximum speed. Volumes were recorded before and after whipping. The percentage volume increase was calculated according to the following equation:

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

Where:

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

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

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

RESULTS AND DISCUSSION

Proximate Composition

Proximate analyses were executed on luffa seeds and the results of proximate composition are listed in Table 1.

The moisture content for powder was very low (3.5%) which is within the acceptable range for a storage period. Moisture content is a major quality factor in the preservation of some food products and it affects food stability. The relatively low moisture content is an indication that this flour will has high shelf life, especially when properly packaged against external conditions, where this was in accordance with report of **Salem (2017)**. The crude protein content obtained for powder was 27.27%. This suggests that, luffa seeds powder may be useful as a protein supplement in the diet of malnourished people. The high total carbohydrate (31.25%) of the luffa seeds powder may be suggests that, the powder is a good source as an additive to other materials for forming gel in food products. The crude fat content was 26.68% and the ash content was 8.33 %. These results agree with those reported by **Dairo *et al.* (2007) and Salem (2017)**.

Table 1. Proximate composition of *luffa* seeds

Parameters	Concentration [a] (%)
Dry matter	93.53 ±1.5
Crude protein	27.27±0.76
Crude fat	26.68 ±0.34
Total carbohydrates	31.25 ±0.62
Ash	8.33 ±0.13

[a] Values are mean ± standard deviation of triplicate determinations.

Fourier Transform Infrared (FTIR) Spectroscopy

One of the classical methods for structure determination of small molecules is IR. This standing is due to its sensitivity to the chemical composition and architecture of molecules. The high information content in an infrared spectrum carries over also to biological systems. This makes infrared spectroscopy a valuable tool for the investigation of protein structure and interaction with carbohydrate as revealed by **Arrondo *et al.* (1993) and Barth (2007)** of the molecular mechanism of protein reactions as followed by **McClelland *et al.* (2002)** and of protein folding, unfolding and misfolding **(Pozo Ramajo *et al.*, 2005)**. In order to study proteins, the analysis of the secondary structure of protein is often required by FTIR in recent years. FTIR spectroscopy has been proven to be a powerful tool for providing conformational and structural dynamic information of proteins. FTIR spectra of the luffa seed protein was shown in Fig. 1.

The infrared analysis indicated the presence of a sulfate ester with absorptions at 1240 cm^{-1} (S-O vibration) and 723 cm^{-1} (C-O-S vibration) and it was also given evidence of the presence of glycosylation with two typical carbohydrate absorptions at 2925–2855 cm^{-1} and 1459–1399 cm^{-1}. The secondary structure of the protein was commonly based on the amide I band analysis (1700–1600 cm^{-1}). Amide I band peaks identified are more mature and it is the most intense absorption band of the polypeptides. ν(C= O) has a predominant role in amide I, ν (C-N) follows. There is also some in-plane NH bending contribution to amide I. The secondary structure of proteins is reflected by these bands as follows: 1610 ~ 1640 cm^{-1} for the β-sheet; 1640 ~ 1650 cm^{-1} for the random coil; 1650~1658cm^{-1} for the α-helix; 1660 ~ 1700 cm^{-1} for the β-turn. These results agree with those reported by **(Wang *et al.*, 2018)**.

Functional Properties of Luffa Seed Protein

The functional properties of a protein are: "Those physical and chemical properties, which affect the behavior of proteins in food systems during storage, processing, preparation and consumption, also, these characteristics, are influenced the 'quality' and organoleptic attributes in food" as revealed by **Kinsella (1981)**.

Protein solubility

The solubility of a protein is the most important functional property since the protein needs to be soluble in order to be applicable in food systems. Other functional properties like emulsification, foaming and gelation are dependent on the solubility of proteins, where **Cooper *et al.* (2003)** showed that in their study. The solubility is correlating with pH, therefore pH-solubility of luffa seed proteins is presented in Table 2. The solubility profile of luffa seed proteins indicates that protein solubility reduces as the pH increases from 2 to 5, which corresponding to its isoelectric point (pI), after which subsequent increases in pH increased protein solubility progressively. The minimum solubility for luffa seed proteins (7%) was noticed at pH 5 which corresponds to its pI. The highest protein solubility for luffa seed proteins (80 and 68%, respectively) was observed at pH 8 and 9. These results agree with those reported by **Dairo *et al.* (2007)**.

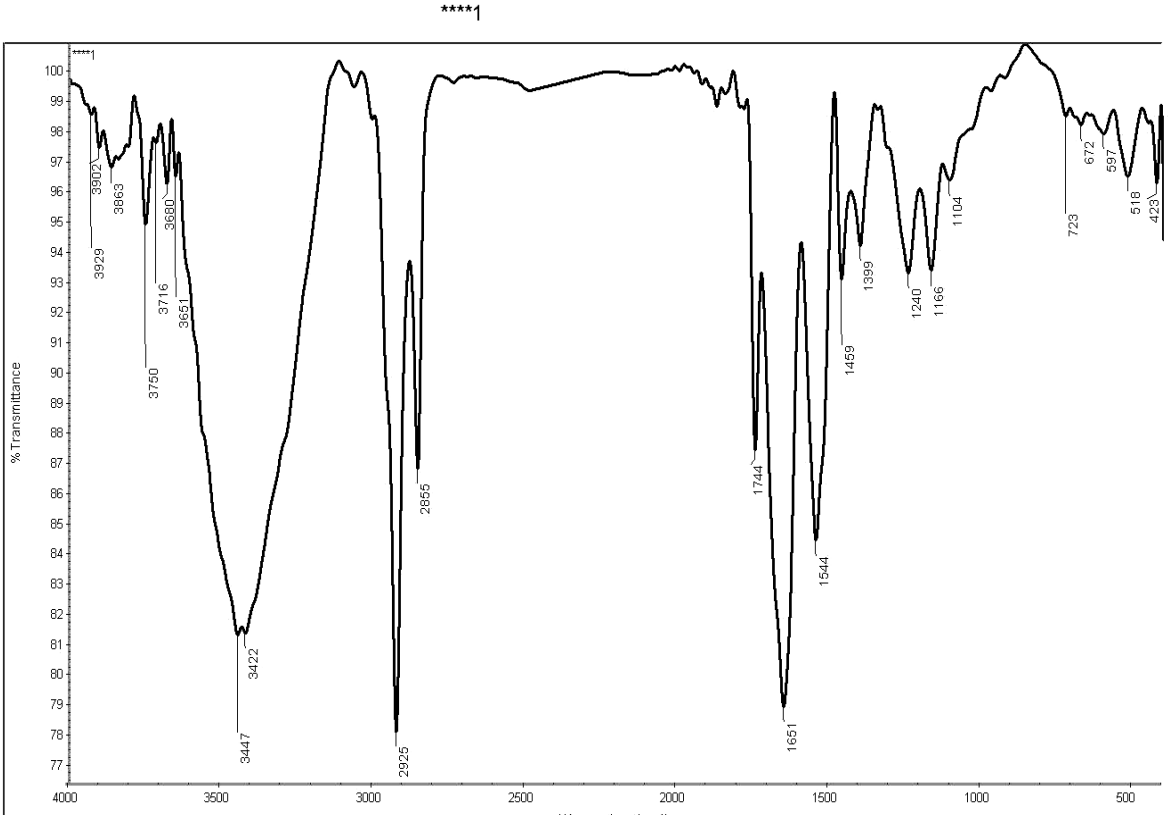

Fig. 1. FTIR spectra of luffa seed protein

Table 2. Protein solubility of luffa seed proteins at different pH

pH	Solubility (%)
2	50 ±2.0
3	45 ±1.5
4	16 ±1.0
5	7 ±0.1
6	16 ±0.8
7	26 ±1.4
8	80 ±3.2
9	68 ±3.1
10	52 ±2.0

Emulsifying Activity and Stability of Protein

Effect of pH on emulsifying activity (EA) and emulsion stability (ES) of luffa seed protein is a reflection of the influence of pH on protein solubility as presented in Table 3. The maximum EA value of luffa seed protein (70%) was obtained at pH 8 of the protein solution. Emulsifying activity was decreased with an increase of pH and recorded minimum value (9%) at pH 5. The maximum ES values of luffa seed protein (77 and 78%, respectively) were obtained at pH 8 and 9. At pH 5 luffa seed protein had the minimum ES of 12%.

Emulsions consist of two liquids that are immiscible, where one of the liquids is dispersed in the other in form of small droplets, where **McClements (2015)** was investigated that in his study.

Foaming Properties of Protein

Effect of pH on foam capacity (FC) and foam stability (FS) of luffa seed protein is presented in Table 4. Luffa seed protein recorded 100% FC at pH 8 but this reduced to 17% at pH 5, where minimum value was observed. For a protein to has superior foaming properties, it must possess high solubility in the liquid phase as well as the ability of quickly forming a film around the air bubbles in the food system, where these were studied by **Kinsella (1981)**. Luffa seed protein had a foam stability of 16%, at pH 7 and increased to 90%, at pH 3. Foams consist of a gas phase, a liquid phase and a surfactant (*e.g.* proteins) and whipping or shaking form foams. Foods made up by foams are (*e.g.*) whipped toppings, meringues, ice creams, chiffon desserts and angel cakes as observed by **Castella (2010)**.

Table 3. The percentage of emulsifying activity (EA) and emulsion stability (ES) for luffa seed protein at different pH

pH	EA (%)	ES (%)
2	63 ±2.1	55 ±1.2
3	62 ±1.8	51 ±1.1
4	53 ±1.1	33 ±1.7
5	9 ±0.2	12 ±0.4
6	38 ±0.9	32 ±0.8
7	53 ±1.3	65 ±1.1
8	70 ±1.9	77 ±2.5
9	63 ±2.2	78 ±2.0
10	55 ±1.2	70 ±1.4

Table 4. The percentage of foaming capacity (FC) and foam stability (FS) for luffa seed protein at different pH

pH	FC (%)	FS (%)
2	95 ±2.0	45 ±1.1
3	93 ±2.0	90 ±1.6
4	45 ±1.3	79 ±1.8
5	17 ±0.9	31 ±0.9
6	54 ±1.5	54 ±0.8
7	86 ±1.7	16 ±0.2
8	100 ±2.2	57 ±1.5
9	96 ±1.7	59 ±1.1
10	90 ±2.8	58 ±1.3

Conclusions

It can be concluded that these values obtained for the luffa seed protein may be indicated to the potential in its use as a source of vegetable protein in animal and human nutrition.

REFERENCES

Amoo, L., A. Emenike and V. Akpambang (2008). Chemical Composition and Nutritive Significance of *Luffa aegyptica* and *Castena* sp. Seeds. Trends in Appl. Sci. Res., 3:298-302.

Arrondo, J.L.R., A. Muga, J. Castresana and F.M. Goñi (1993). Quantitative studies of the structure of proteins in solution by Fourier-transform infrared spectroscopy. Progress in Biophysics and Molec. Biol., 59:23-56.

Barth, A. (2007). Infrared spectroscopy of proteins. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1767: 1073-1101.

Castella, K. (2010). A World of Cake: 150 Recipes for Sweet Traditions from Cultures Near and Far-Honey Cakes to Flat Cakes, Fritters to Chiffons, Meringues to Mooncakes, Tartes to Tortes, Fruit Cakes to Spice Cakes. Storey Publishing.

Coffmann, C. and V. Garciaj (1977). Functional properties and amino acid content of a protein isolate from mung bean flour. Int. J. Food Sci. and Technol., 12:473-484.

Cooper, S., V. Vaclavik and E. Christian (2003). Essentials of Food Science. Springer: New York.

Dairo, F., P. Aye and T. Oluwasola (2007). Some functional properties of loofah gourd (*Luffa cylindrica* L., MJ Roem) seed. J. Food Agric. and Environ., 5: 97.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.T. Rebers and F. Smith (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Firestone, D. (1990). Official methods of analysis of the association of official analytical chemists. Arlington, USA.

Horwitz, W. and G. Latimer (2000). Official Methods of Analysis of AOAC International, Gaithersburg MA, USA. Association of Official Analytical Chemist.

Johnson, E.A. and C. Brekke (1983). Functional properties of acylated pea protein isolates. J. Food Sci., 48: 722-725.

Kinsella, J.E. (1981). Functional properties of proteins: possible relationships between structure and function in foams. Food Chem., 7:273-288.

Lucy, O.F. and O.B. Abidemi (2012). Food value and phytochemical composition of *Luffa cylindrica* seed flour. Ame. J. Biochem., 2:98-103.

McClelland, J., R. Jones, S. Bajic, J. Chalmers and P. Griffiths (2002). Handbook of Vibrational Spectroscopy. John Wiley and Sons, Ltd.

McClements, D.J. (2015). Food emulsions: principles, practices, and techniques. CRC press.

Nagao, T., R. Tanaka, Y. Iwase, H. Hanazono and H. Okabe (1991). Studies on the constituents of *Luffa acutangula* Roxb. I. Structures of acutosides AG, oleanane-type triterpene saponins isolated from the herb. Chem. and Pharmaceutical Bulletin, 39:599-606.

Neto, V.Q., N. Narain, J. Silva and P. Bora (2001). Functional properties of raw and heat processed cashew nut (*Anacardium occidentale*, L.) kernel protein isolates. Molecular Nutr. and Food Res., 45: 258-262.

Oboh. I. and E. Aluyor (2009). *Luffa cylindrica*-an emerging cash crop. Afr. J. Agric. Res., 4: 684-688.

Oke, D., O. Tewe and B. Fetuga (1995). The nutrient composition of some cowpea varieties. Nigerian J. Anim. Prod., 22 : 32-36.

Pozo Ramajo, A., S.A. Petty, A. Starzyk, S.M. Decatur and M. Volk (2005). The α-helix folds more rapidly at the C-terminus than at the N-terminus. J. Ame. Chem. Soc., 127:13784-13785.

Salem, R.H. (2017). Functional characterization of luffa (*Luffa cylindrica*) seeds powder and their utilization to improve stabilized emulsions. Sci., 7: 613-625.

Sitohy, M. and A. Osman (2010). Antimicrobial activity of native and esterified legume proteins against Gram-negative and Gram-positive bacteria. Food Chem., 120 : 66-73.

Souillac, P.O., C.R. Middaugh, Rytting JH (2002) Investigation of protein/carbohydrate interactions in the dried state. 2. Diffuse reflectance FTIR studies. Int. J. Pharmaceutics, 235: 207-218.

Surewicz, W.K. and H.H. Mantsch (1988). New insight into protein secondary structure from resolution-enhanced infrared spectra. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molec. Enzymol., 952:115-130.

Tannin-Spitz, T., M. Bergman and S. Grossman (2007). Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. Biochem. and Biophysical Res. Communications, 364: 181-186.

Valencia, U.S., S. Ann-Sofie, J.R. Silvia (1999). Processing of quinoa (*Chenopodium quinoa*, Willd): effects on *in vitro* iron availability and phytate hydrolysis. Int. J. Food Sci. and Nutr., 50: 203-211.

Wang, Z., A. Tu, D. Tang and Y. Shan (2018). Effectively preparing soluble ovomucin with high antiviral activity from egg white. Int. J. Biol. Macromolecules, 118: 504-510.

التـركيب الكيميـائي والخصـائـص الـوظيفيـة لبـروتيـن بـذور اللـوف

إبراهيم سيد احمد محمد – على عثمان – خالد محمد وهدان – محمود زكى سطوحى

قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة الزقازيق – مصر

في الدراسة الحالية تم تقدير التركيب الكيميائي لبذور اللوف وتم فصل بروتينات بذور اللوف وتقييم خصائصها الوظيفية (الذوبان، خصائص الاستحلاب وخصائص تكوين الرغوة)، وأظهرت نتائج التحليل التقريبى للبذور أن المادة الجافة ٩٣,٥٣%، البروتين الخام ٢٧,٢٧%، الدهن الخام ٢٦,٦٨%، الكربوهيدرات الكلية ٣١,٢٥%، والرماد ٨,٣٣ (على أساس الوزن الجاف)، تم تقييم تأثير الأس الهيدروجيني على قابلية ذوبان بروتين بذور اللوف وأظهرت النتائج انخفاض الذوبانية فى نطاق الرقم الهيدروجيني ٢-٥ وزيادة تدريجية بداية من الرقم الهيدروجيني ٦ إلى الرقم الهيدروجيني ١٠ مع الحد الأقصى للذوبان (٨٠%) عند الرقم الهيدروجيني ٨، ازدادت قابلية الذوبان لبروتين بذور اللوف في الوسط القلوي، كما سجلت نقطة التعادل الكهربى لهذا البروتين عند الرقم الهيدروجينى ٥ إن نشاط الاستحلاب وثبات المستحلب لبروتين بذور اللوف هو انعكاس لتأثير الرقم الهيدروجيني على قابلية الذوبان للبروتين، سجلت النتائج أقصى نشاط للمستحلب لبروتين بذور اللوف (٧٠%) عند الرقم الهيدروجيني ٨ لمحلول البروتين، تم تسجيل الحد الأقصى من ثبات المستحلب لبروتين بذور اللوف (٧٧ و ٧٨% على التوالي) عند الرقم الهيدروجيني ٨ و ٩، وسجلت بروتينات بذور اللوف نسبة ١٠٠% من الرغوة عند الرقم الهيدروجيني ٨ وهذا انخفض إلى ١٧% عند الرقم الهيدروجيني ٥، لذا يمكن الاستنتاج أن هذه القيم التي تم الحصول عليها لبذور اللوف والبروتين تشير إلى إمكانية استخدامه كمصدر للبروتين النباتي في تغذية الحيوان والإنسان.

المحكمـــون:

١- أ.د. أحمـد محمــد أبو العينين أستاذ الكيمياء الحيوية المتفرغ – كلية الزراعة – جامعة القاهرة.

٢- أ.د. رجب عبدالفتاح المصـري أستاذ الكيمياء الحيوية المتفرغ – كلية الزراعة – جامعة الزقازيق.