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Short Communication

Purification and Characterization of Trypsin Inhibitor from the *Cicer arietinum* L. (Chickpea) Sprouts

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Keywords

- Chickpea sprouts
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Abstract

The proteins fraction of chickpea sprout were extracted and purified by two steps of ion-exchange chromatography and high-pressure liquid chromatography (HPLC) for the first time, the peptides components and molecular weights were determined as11079.57 11440.95, 16619.09, 26016.41, 26032.49, 26016.34 and 28822.02 Da by liquid chromatography and mass spectrometer (LC/MS). The major protein component of this 26032.49 kDa fraction was isolated with trypsin inhibitor activity by HPLC and the partial amino acid sequence was determined as the:

(K)LIEAMVEVEGQLCMDVPSNPGTSAPPFAIVHSSGISLPDRQSATPCSAD-DWRPYLV(-).

ABBREVIATIONS

LC-MS: Liquid Chromatography - Mass Spectrometry; PAAG: Poly Acrylamide Gel electrophoresis; HPLC: High Performance Liquid Chromatography.

INTRODUCTION

Chickpea is a traditional dish and a favorite food in Northwest China for centuries. It is known as a good source of protein, carbohydrates, and another nutrition components [1], and nowadays seeds and sprouts of the chickpea are processed into all kinds of health foods. Furthermore, many pharmacological effects of the chickpea components have been reported including reduction of the risk of diabetes and obesity, [2–4] and colonic cancer [5]. It is also used in the treatment of various diseases including bronchitis, leprosy, skin diseases, blood disorders, and biliousness, [6] diseases of the liver and spleen, and otitis [7] in order to find new uses of chickpea and establishment of effective extraction and separation technology of proteins in Chinese chickpea, as a continues study of polypeptides in Chinese chickpea seeds and spouts, this works we isolated a protein from the check pea sprouts and determined its trypsin inhibitor activity as well as part of the amino acid sequences were characterized by liquid chromatography and mass spectrometry (LC/MS technology) .

MATERIALS AND METHODS

Isolation of total peptides from *Cicer arietinum* sprouts

Sprouts (250 g) were ground, defatted with n-hexane in a Soxhlet apparatus, and dried in air. Peptides from dry sprouts were extracted by cold buffer (1 L) containing Na_2HPO4(10 mM), KCl (100 mM), EDTA (1.5 mM), thiourea (2 mM), -toluenesulfonylfluoride (1 mM), and polyvinylpyrrolidone (1.5%) at pH 7.4 for 2 h at 4°C with constant stirring. The extract was centrifuged at 6,000 rpm for 30 min. The precipitate was discarded. The supernatant was heated for 5 min at 80°C, cooled, and centrifuged again under the aforementioned conditions in order to separate the precipitated high-molecular-weight proteins. The precipitate was discarded. The supernatant was treated with ammonium sulfate to 30% saturation and left overnight at 6°C in order to form a precipitate. The resulting protein precipitate was separated by centrifugation. The supernatant was treated with ammonium sulfate to 50%

saturation. The precipitate that formed overnight was removed by centrifugation. The supernatant was treated with ammonium sulfate to 80% saturation. The resulting precipitate was separated by centrifugation for 15 min at 6,000 rpm. The obtained peptide fractions were dialyzed against distilled water.

Ion-exchange chromatography

The combined peptide fractions obtained after precipitation by 30% and 50% ammonium sulfate were desalted by dialysis and titrated to pH 9 by aqueous ammonia (12 M). The solution was loaded onto a Servacel DEAE-23SN (Hungary) column (2x10 cm) that was equilibrated with 50 mM ammonium acetate solution pH 9, at flow rate 0.5 mL/min. The fraction of peptides that was not bound to the sorbent was titrated to pH 5 by 3 N HCl solution and loaded on a column of CM-TSK-650M (Toyopearl, Japan, 2x15 cm,) that was equilibrated beforehand with ammonium acetate (50 mM, pH 5). Adsorbed cationic peptides were eluted by a linear NaCl gradient (from 0 to 1 M) in ammonium acetate (50 mM, pH 5) at flow rate 0.5 mL/min. Proteins were detected at 280 nm.

RP-HPLC separation of peptides

Peptides were separated on a DuPont 8800 chromatograph over a 250/8/4 Protein@Peptide C18 column using solution A (0.1% TFA) and solution B (MeCN) at flow rate 1 mL/min, detection at 226 nm, and gradient 0–5 %(0–5 min), 5–60% (5.1–35 min), 60–60% (35.1–40 min), and 60–5% (40.1–45 min) [8].

Electrophoretic analysis of peptides

Electrophoretic analysis of peptides used the Laemmli method [9] in 15% PAAG and 0.1% Na-DDS at pH 8.9. Protein concentration was determined by the Bradford method [10] using trypsin and ovalbumin as standard proteins.

1 ml of protein solution was taken to test-tube and 5 ml Coomassie R250 solution was added. The gained solution thoroughly stirred in room temperature and the color intensity was determined after 30 minutes at 595 nm with UV-2550.

Mass spectrometric analysis of peptides

Mass spectrometric analysis of peptides was carried out on an Agilent Technologies Series 6520B CHIP-Q-TOF LC-MS instrument. The ionization source was ESI+; drying gas flow rate 4 L/min; drying gas temperature 350°C, skimmer cone potential 65 V; fragmentation at 175 V, mass range in MS mode 300–3000 m/z. Positive ionization mode was used. Samples were injected into the mass spectrometer by an Agilent Technologies Series 1200 chromatograph from a Zorbax SB C18 column (75 m 43 mm, 5 m) using mobile phases A (0.1% formic acid) and B (MeCN + 0.1% formic acid) and an Agilent Technologies Series 1260 Cap Pump at flow rate 4 L/min. An Agilent Technologies Series 1260 Nano Pump at flow rate 0.6 L/min was used for the elution. The concentration gradient of solution B (min) was 20%, 3 min; 80%, 30 min; and 20%, 35 min. Solutions were degassed on an Agilent Technologies 1260 degasser. Samples (2 µL) were placed onto the column using an Agilent Technologies Micro WPS instrument.

Determination of trypsin inhibitor activity

Samples of 1 ml containing 50 µg trypsin dissolved in 0.0025 M HCl were mixed with 1 ml containing various amounts of chickpea inhibitor dissolved in 0.0025 M HCl. 1 ml of each mixture added to 1 ml of 1% casein pH7.6 was digested 20 minutes at 37°C, then mixed with 3 ml 5% trichloracetic acid. The precipitate formed is centrifuged after standing 1 hour at 25°C. The optical density of solutions at 280 nm was corrected for blank solutions which are prepared by mixing 1 ml of 1% casein solution with 3 ml of 5% trichloracetic acid. [11].

RESULTS AND DISCUSSION

In the present study, we isolated and purified another trypsin inhibitor from chickpea sprout with molecular weight was 26 kDa by ion-exchange chromatography and Reverse Phase chromatography, and studied its part of the primary amino acid structure. Proteins and polypeptides fraction in the chickpea sprouts were extracted and purified as methods described in our previous publications [8, 12]. The molecular weights of the basic proteins fraction of YZDB-80%Y were determined by SDS PAGE electrophoresis in (15%) under dissociating conditions [9]. It can be seen that the YZDB-80%Y fractions are rich in proteins and their molecular weights concentrated on range 20-27kDa.

This fraction 1 subject to the LC/MS analysis. In order to further identify their molecular weights, the LC/MS analysis picture for proteins fraction 1 from YZDB-80%Y for as follow (Figure 1).

The LC/MS analysis results of this fraction (Figure 2) were indicated that this fraction contains a six proteins with molecular weight 11440.9 Da that appear at 13.21 min, a proteins with molecular weight of 26032.49 Da that appear at 13.55 min, a protein with proteins molecular weight of 26016.34 Da that appear at 13.69 min, a protein molecular weight of 28822.02 Da that appear at 14.24 min and a protein 11079.57 Da, 16619.09 Da that appear at 14.56 min.

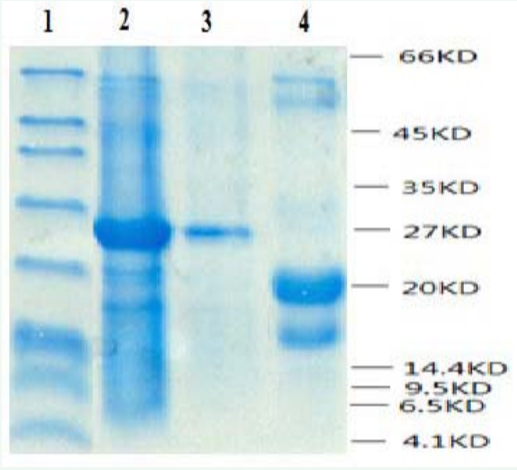

Figure 1 SDS-PAAG gel electrophoresis in 15% PAAG: line 1, marker proteins (parathyroid hormone 1-34, 4.1 kDa; aprotinin, 6.5 kDa; parathyroid hormone 1-84, 9.5 kDa; lysozyme, 14.4 kDa; trypsin inhibitor, 20.0 kDa; triosophosphate isomerase, 27.0 kDa; pepsin, 35 kDa;ovalbumin, 45.0 kDa; BSA, 66 kDa); line 2,YZDB-80% Y; line 3, YZDB-80% fraction 1 after HPLC; line 4, YZDB-80% fraction 2 after HPLC.

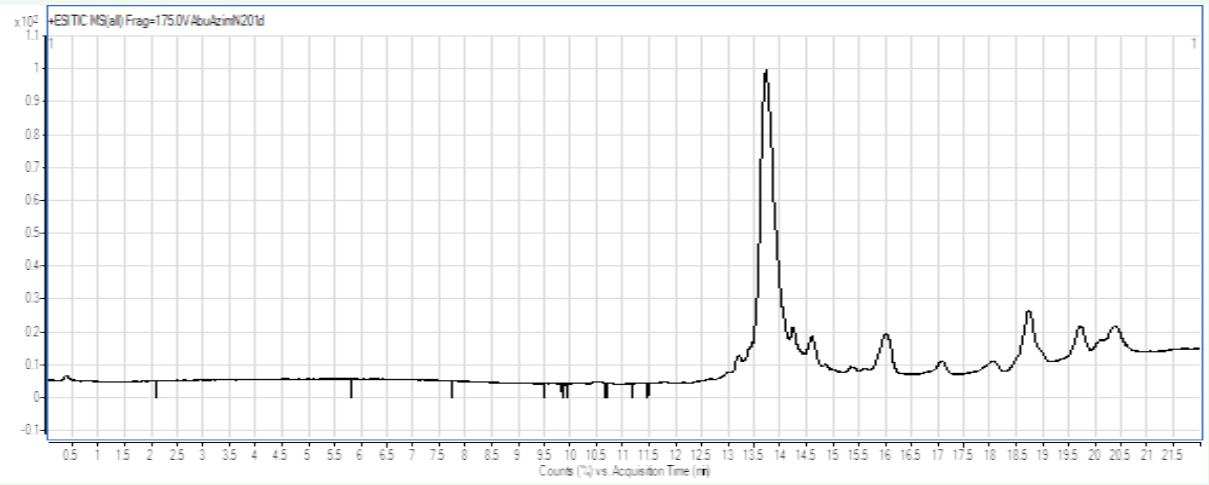

Figure 2 LC chart for protein fraction 1.

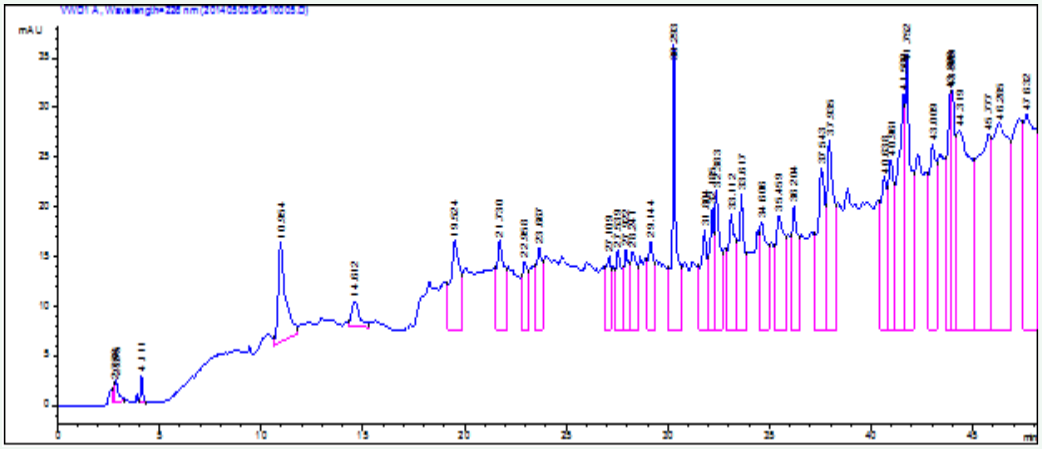

Figure 3 HPLC separation of chickpea sprout YZDB-80%Y protein fraction.

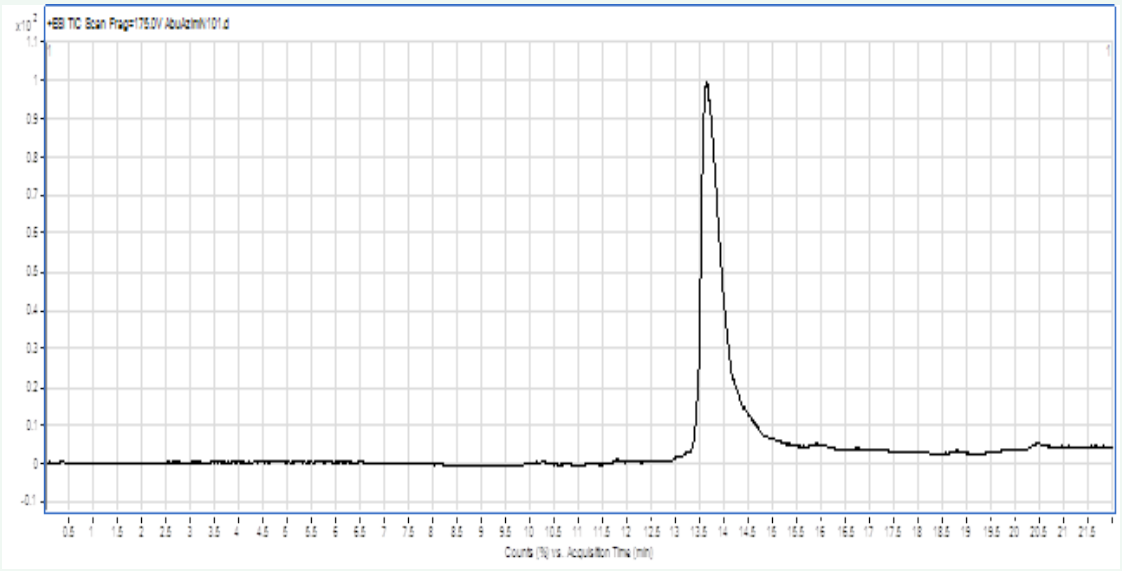

Figure 4 LC Chart for purified protein.

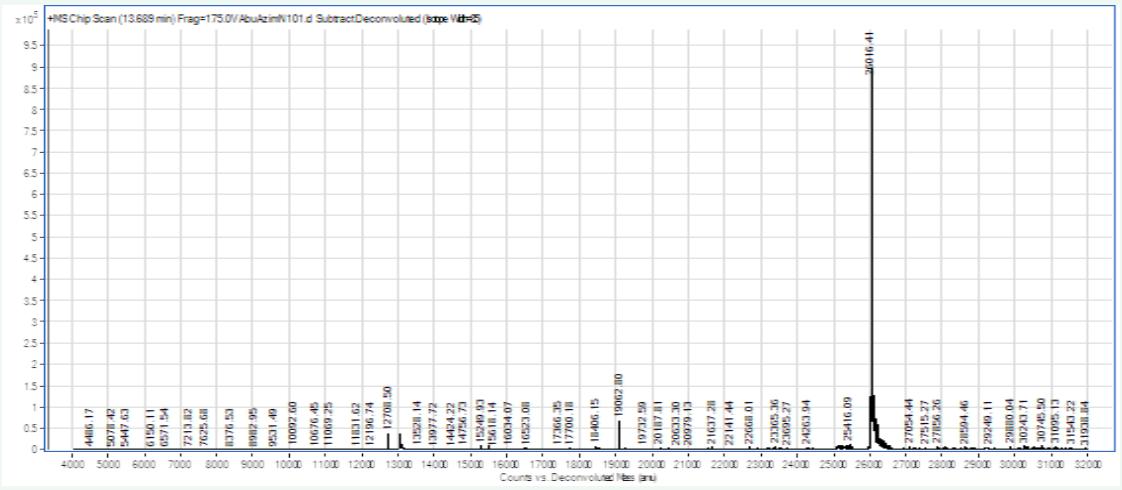

Figure 5 LC/MS Chart for individual protein.

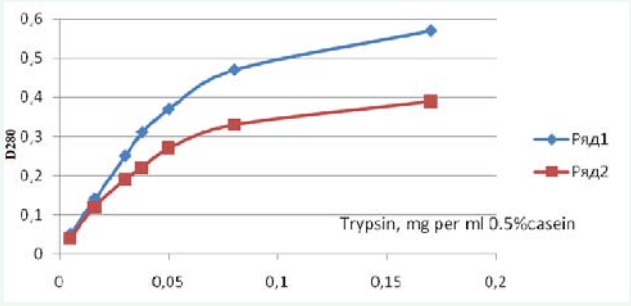

Figure 6 Digestion of casein by trypsin Optical density at 280 nm plotted vs. milligrams of trypsin (1-digestion of casein by trypsin, 2–digestion of casein by trypsin with equivalent amount of chickpea trypsin inhibitor).

Abbreviations: LC-MS: Liquid Chromatography - Mass Spectrometry; PAAG: Poly Acrylamide Gel electrophoresis; HPLC: High Performance Liquid Chromatography

From analysis SDS-PAAG and LC/MS data, we easy find out that this fraction contain a major peak with molecular weight about the 26 - 27 KDa, therefore in order to further identification this major peak, we purified this fraction with high performance reverse phase liquid chromatography (HPLC). The data was shown at (Figure 3). The fraction that was collected by HPLC at 30.29 min and further subject to analysis with LC/MS, the LC/MS spectrum of this protein indicated that this a absolute individual protein which appear at 13.56 min in LC chromatography, the result shown (Figure 4) that it is molecular weight was 26032.49 Da in a good accordance with the SDS-PAAG electrophoresis data.

LC/MS Results Shown (Figure 5) Filtered by Validation Category: the partial amino acid sequences of this individual protein as:

(K)LIEAMVEVEGQLCMDVPSNPGTSAPPFAIVHSSGISLPDRQ-SATPCSADDWRPYLV (-)

Protein inhibitors in legumes are one of the most promising weapons that confer resistances against insects by inhibiting proteases present in the gut of insect larvae. Protein inhibitors are widely distributed in nature and have been isolated from many sources including plants.

Trypsin inhibitors were first found in soybean and isolated by Kunitz [13,14]. The molecular weights of inhibitor II and III were 23 and 24 kDa. Both of them were thermo stable and shown to be serine inhibitors, which is controversial with the result of Yao et al. [15]. A 33 kDa trypsin inhibitor, sporamin A, was purified from sweet potato by trypsin affinity column and SDS PAGE, which had scavenging activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH), could capture hydroxylradical [16], and also had glutathione peroxidase-like activity [11].

A 20 kDa and 30 kDa trypsin inhibitors were purified to homogeneity from chickpea seeds by ammonium sulfate precipitation and chromatography with Sephadex G-100 and DEAE cellulose ion-exchange column [17, 18].

In the present study, we isolated and purified another trypsin inhibitor from chickpea sprout with molecular weight was 26 kDa by ion-exchange chromatography and Reverse Phase chromatography, and studied its part of the primary amino acid structure.

The results at (Figure 6) shown inhibition activity of chickpea 26 kDa protein from sprouts to trypsin digestion of casein.

CONCLUSION

In the present study, we isolated and purified another trypsin inhibitor from chickpea sprout with molecular weight was 26 kDa by ion-exchange chromatography and Reverse Phase chromatography, and studied its part of the primary amino acid structure. The partial amino acid sequences of this individual protein as:

(K)LIEAMVEVEGQLCMDVPSNPGTSAPPFAIVHSSGISLPDRQ-SATPCSADDWRPYLV (-).

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REFERENCES

1. Duke JA. Handbook of Legumes of World Economic Importance; Plenum. Press: New York, USA. 1981; 52–57.
2. Panlasigui LN, Panlilio LM, Madrid JC. Glycaemic response in normal subjects to five different legumes commonly used in the Philippines. Int J Food Sci Nutr. 1995; 46: 155–160.
3. Mansour EH. Biological and chemical evaluation of chickpea seed proteins as affected by germination and a-amylase treatment. Plant Food Hum Nutr. 1996; 49: 271–282.
4. Venkateswaran S, Pari L, Saravanan G. Effect of phaseolus vulgaris on circulatory antioxidants and lipids in rats with streptozotocin-induced diabetes. J Med Food. 2002; 5: 97–103.
5. Hangen L, Bennink MR. Consumption of black beans and navy beans (Phaseolus vulgaris) reduced azoximethane-induced colon cancer in rats. Nutr Cancer. 2002; 44: 60–65.
6. Sastry CST, Kavathekar KY. Plants for Reclamation of Wastelands. Council of Scientific and Industrial Research. 1990; 684.
7. Warner PKW, Nambiar VPK, Remankutty C. Indian Medicinal Plants: Orient Longman. Chennai, India. 1995; 773–774.
8. Yili A, Maksimov VV, Ziyavitdinov JF, Ibragimov FA, Beresneva YuV, Salikhov ShI, et al. Comparative investigation of the polipeptide composition of Cicer arietinum sprouts. Chem Nat Comp. 2014; 50: 900-903.
9. Yili A, Ma QL, Lv QY, Gao YH, Zhao B, Veshkurova ON, et al. Antioxidant peptides from Cicer arietinum of Xinjang, China. Chem Nat Comp. 2012; 48: 643-645.
10. Laemmli EK. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (London). 1970; 227: 680.
11. Kunitz M. Science. 1945; 101: 668.
12. Kunitz M. Crystalline soybean trypsin inhibitor. II. General properties. J Gen physiol. 1947; 30: 291-310.
13. Yao PL, Hwang MJ, Chen YM, Yeh KW. Site-directed mutagenesis evidence for a negatively charged trypsin inhibitory loop in sweet potato sporamin. FEBBs Letters. 2001; 496: 134-138.
14. Hou WC, Chen YC, Chen HJ, Lin YH, Yang LL, Lee MH. Antioxidant activity of trypsin inhibitor, a 33 kDa root storage protein of sweet potato (Ipomoea batatas L. Lam cv. Tainong 57). J Agric Food Chem. 2001; 49: 2978-2981.
15. Hou WC, Chen HJ, Han CH, Lin CY, Lin YH. Glutathione peroxidase-like activity of 3 3 kDa trypsin inhibitor from roots of sweet potato (Ipomoea batatas [L.] Lam 'Tainong 57'). Plant Sci. 2004; 166: 1541-1546.
16. Meera Nair, Sardul Singh Sandhu, Babbar A. Purification of trypsin inhibitor from seeds of Cicer arietinum L. and its insecticidal potential against Helicoverpa armigera (Hübner). Theor Exp Plant Physiol. 2013; 25: 137-148.
17. Kansal R, Kumar M, Kuhar K, Gupta RN, Subrahmanyam B, Koundal KR, et al. Purification and characterization of trypsin inhibitor from seeds of Cicer arietinum L. and its efficacy against Helicoverpa armigera. Brazilian J plant physiol. 2008; 20: 313-322.
18. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72: 248.

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