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FLOCCULANTS APPLICATION FOR PRECIPITATION AND SEPARATION OF PROTEINS FROM *LENS CULINARIS* CV. TINA

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Background. Lentil (*Lens culinaris*) is an important crop in many developing countries. Usefulness of protein isolates in the human nutrition and animal feeding have been also studied. Improvement of protein precipitation and fractionation efficiency by using different flocculants was the aim of this study.
Material and methods. The polyelectrolytes Magnafloc LT-22 and Magnafloc LT-25 were tested in the process of coagulation and fractionation of protein from lentil protein extracts. Proteins were extracted from flour with 0.5 M NaCl in the 50 mM Tris-HCl buffer pH 7.5 and 2 mM NaOH at ratio 1:10. Protein were coagulated at different pH (6, 5, 4, 3) using flocculants in three different concentration 0.1, 0.3 and 0.5%. Samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Results. Influence of extraction medium on the yield and quality of protein was visible. In all studied cases proteins were the most effective precipitated with Magnofloc LT-25. Application of different pH condition for coagulation caused fractionation of lentil protein. Gel electrophoresis of protein of all studied samples showed different molecular weight subunit patterns ranging from 8 to 102 kDa.
Conclusions. Using of flocculants as coagulating factors allows obtaining a high concentrated protein isolates, however the yields of flocculation were determined by different extraction systems, concentration of flocculants and pH condition of process.

Key words: flocculants, lentil, Magnafloc, protein precipitation, protein isolates

INTRODUCTION

Lentil (*Lens culinaris*) is an important crop in many developing countries. Lentil proteins have been studied for their composition, nutritional quality and effect of germination on their functional properties [Solanki et al. 1999, Vidal-Valverde et al. 2002,

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Wang and Daun 2006]. Like most legumes, lentil seeds are composed of about two-thirds carbohydrates and 24-30% protein. In addition, lentils are also a good source of certain amino acids, such as lysine and arginine that are important in cereal-based diets [Lee et al. 2007, Longnecker et al. 2002]. Protein preparations from legumes have been produced on an industrial scale for many years. Usefulness of protein isolates in the human nutrition and animal feeding have been also studied [Tanga et al. 2009, Hamilton-Reeves et al. 2008, Papalamproua et al. 2009].

Improvement of protein precipitation and fractionation efficiency by using different flocculants was the aim of this study.

MATERIALS AND METHODS

Lentil (*Lens culinaris*) cv. Tina was used in the present study. Seeds were ground and flour was sieved by 18-mesh screen. The flour was used for the extraction of protein.

Proteins were extracted from flour with 0.5 M NaCl in the 50 mM Tris-HCl buffer pH 7.5 (NaCl-buffer isolate) and 2 mM NaOH (NaOH isolate) at ratio 1:5. The protection of proteins during preparation of samples was assured by adding Protease Inhibitor Cocktail (Sigma, St. Louis). Samples were stirred 30 min at a temperature 4°C and centrifuged (6000x g; 20 min). Supernatants were used in a further analysis.

Protein were coagulated from the protein preparation with flocculants Magnafloc LT-22 and Magnafloc LT-25 in three different concentration 0.1, 0.3 and 0.5% at different pH (6, 5, 4, 3) by decreasing acidity with 2M HCl. In the first step pH of protein isolate was adjusted to value 6. Coagulated protein were removed by centrifugation (6000x g; 20 min), dried in vacuum dryer and used for further analysis. Protein from remaining supernatants was subsequently coagulated in the pH 5, 4 and 3, follow this protocol. Proteins were dissolved in 1mM NaOH and their levels were determined follow Lowry method with bovine albumin as a standard [Lowry et al. 1951].

Samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 12.5% acrylamide [Laemmli 1970]. Samples were treated in denaturing buffer with SDS and β-mercaptoethanol and heated before SDS-PAGE. Gels were stained with 0.2% Coomassie Brilliant Blue R and distained in 50% methanol/10% acetic acid. Electrophorograms were analyzed with Polydoc, Molecular Imaging System, Vilber Lourmat supplied with software PhotoCapt.

RESULTS AND DISCUSSION

Protein level in protein isolates or concentrates is conditioned by many factors. One potential limiting factor is protein concentration in raw material, witch is dependent on plant genus, preliminary processing and method of protein isolation and coagulation [Sanchez-Vioque et al. 1999]. Kind of extraction system used for protein isolation from lentil flour significantly influenced on the usefulness polyelectrolytes in a protein coagulation process. Proteins, from prepartions obtained by extraction with NaCl solution, the most effective were coagulated with polyelectrolyte LT-25. It should be noted that

influence of concentration used was not significant. In the alkalic protein solution case the best results were obtained with Magnafloc LT-25 in a concentration 5 mg per ml. Effectiveness of protein coagulation was the lowest in the Magnafloc LT-22 (0.1%) case (Fig. 1).

Based on results concerning NaCl-buffer isolates the most proteins were precipitated in pH 6 with Magnafloc LT-22 (0.1%) and pH 5 with Magnafloc LT-22 (0.5%). In the case of NaOH isolates about 60% of proteins were precipitated with Magnafloc LT-25 in pH 6 (Fig. 2).

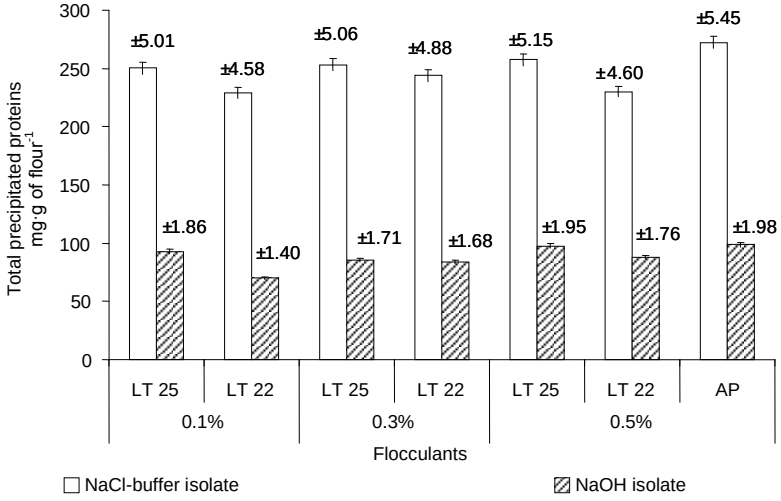

Fig. 1. Influence of polyelectrolites on the protein precipitation process: LT 22 – Magnafloc LT-22, LT 25 – Magnafloc LT-25, AP – acidic precipitation, ±0.00 – standard deviation

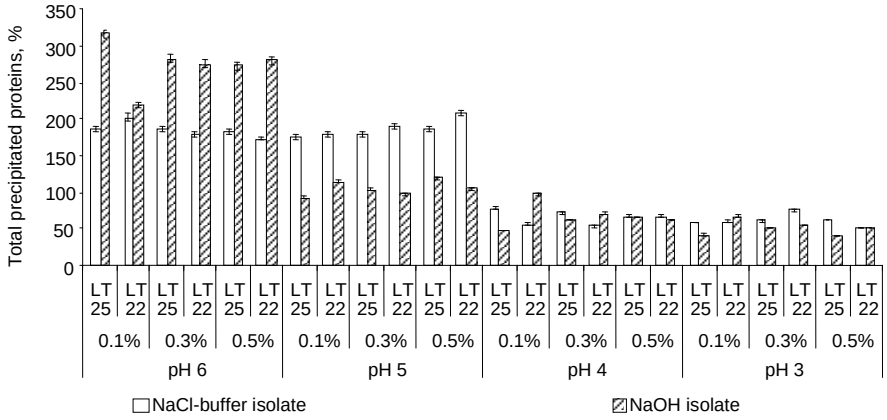

Fig. 2. Precipitation of protein at different pH condition

Co-precipitation factors (flocculants) allowed obtaining slightly lower amounts of precipitated protein than those obtained by acidic precipitation. In the case of solution obtained by isolation with 0.4 M NaCl amounts of precipitated protein were lower from 6 to 16% in comparison to that obtained by acidic precipitation. Similar results have been obtained by Baraniak and co-workers [2004 b]. Authors precipitated pea proteins with polyelectrolytes Magnafloc M-22S and Superfloc A-150. Concentration of protein in the obtained preparations was about 8% lower then in control (precipitation by acidification; Fig. 1). Alamanou and Doxastakis [1995] performed isolation of protein from lupinus seeds using polymers of N-isopropyl acrylamide and methyl bisacrylamide. Yield of extraction was lower up to 50% in the comparison to the isolate obtained by precipitation in isoelectric points. Also methods based on the dialysis and ultrafiltration were less effective, protein loss 9 and 16%, respectively. Similarly to studies performed by Baraniak [1994] we have also observe decreasing of total and true protein level during isolation of protein with polyelectrolyte Magnafloc M-22S.

Additionally, these studies clearly show that quality and levels of precipitated proteins were depended on the kind of flocculants used. Protein agglomeration should be preceded by changes in the secondary structure of protein, which facilitate forming of new bonds. This process is the most effective when protein compound are not charged. Colloids coagulation with polyelectrolytes is mainly cause by neutralization of compounds charges and/or by formation additional, intermolecular bounds (bridging effect) [Vasilin-Reimann et al. 1990]. In the conditions different than pI, proteins are charged and can be neutralized by oppositely charged flocculants compounds. At the time, those interactions disappear and aggregation of compounds ensues. Fluctuations of pH condition significantly influence on the properties of flocculants. They influence on the dissociation of functional groups of polyelectrolyte and total charge of colloidal compounds. Additionally, charge density of flocculant is changing [Vasilin-Reimann et al. 1990]. Therefore, colloids aggregation takes place more effectively in low acidic conditions when polyelectrolytes can easily absorb on the proteins. Finally, flocculation efficiency depends on polyelectrolyte concentration. Too high concentrations of coagulants cause formation of additional layer on the protein surface, that protects colloid against precipitation [Scheutjens and Feler 1982]. Concentrations of flocculants, used in this work, did not influence negatively on the amounts of precipitated protein. Better extraction yields were obtained with anionic polyelectrolyte Magnafloc LT-25 can suggest that colloids particles were in the majority positively charged. These observations confirm results obtained by Baraniak et al. [2004 b], pea proteins were more effectively precipitated with anionic polyelectrolyte Superfloc A-150. It should be noted that coagulation conditions significantly influence chemical composition and functional properties of protein preparations [Femandez-Qurntela 1997]. Electrophoretic profiles of protein precipitate with anionic and cationic flocculants (Magnafloc LT-22 and Magnafloc LT-25) show that amounts and molecular masses of protein were closely bound with coagulation conditions (pH) and protein extraction procedure.

Irrespectively of the concentration and kind of flocculants in the electrophoretic profiles of proteins precipitated from NaOH preparations only one main band was visible, corresponding to protein with molecular mass about 44 kDa (Fig. 3). Probably, it is vivcilin, trimeric protein of lentil seeds that is composed of subunits with molecular mass about 48 kDa and belongs to globulin fraction [López-Torrejón et al. 2003]. Similar results obtained Baraniak et al. [2004 a] during precipitation of proteins from pea

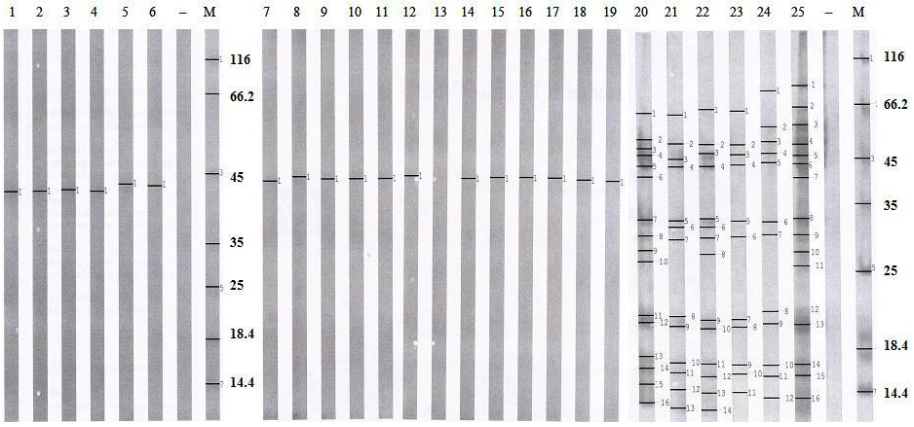

Fig. 3. Electrophoretic profiles of protein precipitated from NaOH extracts. Lines 1-6 Precipitation at pH 3: 0.1% Magnafloc LT-22 and Magnafloc LT-25, 0.3% Magnafloc LT-22 and Magnafloc LT-25, 0.5% Magnafloc LT-22 and Magnafloc LT-25, respectively. Lines 7-12 Precipitation at pH 4: 0.1% Magnafloc LT-22 and Magnafloc LT--25, 0.3% Magnafloc LT-22 and Magnafloc LT-25, 0.5% Magnafloc LT-22 and Magnafloc LT-25, respectively. Lines 14-19 Precipitation at pH 5: 0.1% Magnafloc LT-22 and Magnafloc LT-25, 0.3% Magnafloc LT-22 and Magnafloc LT-25, 0.5% Magnafloc LT-22 and Magnafloc LT-25, respectively. Lines 20-25 Precipitation at pH 6: 0.1% Magnafloc LT-22 and Magnafloc LT-25, 0.3% Magnafloc LT-22 and Magnafloc LT-25, 0.5% Magnafloc LT-22 and Magnafloc LT-25, respectively, M – molecular mass markers [kDa]

protein isolates. Authors obtained protein with molecular mass 78 kDa, main subunit of pea convicilin. It should be noted that only during precipitation in pH 6 more protein fractions have been isolated. In the first fraction were proteins with molecular masses from 13 to 18 kDa, in the second about 20 kDa, in the third one from 27 to 32 kDa. Additionally, all precipitates possessed protein with molecular range from 40 to 50 kDa and single bnd corresponding to protein with molecular mass about 65 kDa. It is noteworthy that during coagulation with high concentrated flocculants additional band was detected (molecular mass about 80-85 kDa; Fig. 3).

Contrary to NaOH extracts, where only at pH 6 precipitated different fraction of protein, in the case of NaCl-buffer preparations wide spectrum of lentil proteins has been coagulated. At all studied pH condition were present protein with molecular masses 45-55 kDa, 27-35 kDa, 21 kDa, 16-18 kDa and 14 kDa. Proteins with molecular masses about 45 and 55 kDa belong probably to vicillin fraction or other protein without disulfide bridges. We can also speculate that proteins with molecular masses 20-25 kDa and 33-38 kDa constitute the alkalic and acidic subunits of legumins. It should be noted that at pH 4 and 5 were found additional band with molecular masses 13, 17, 25-27 and 35 kDa (Fig. 4).

Based on results of these studies, amount and kind of precipitated protein is conditioned by many factors that can influence on the process condition. Between them the most important seems to be a kind and concentration of flocculant and pH of precipitation. This thesis confirms studies performed by Baraniak et al. [2004 a], where also

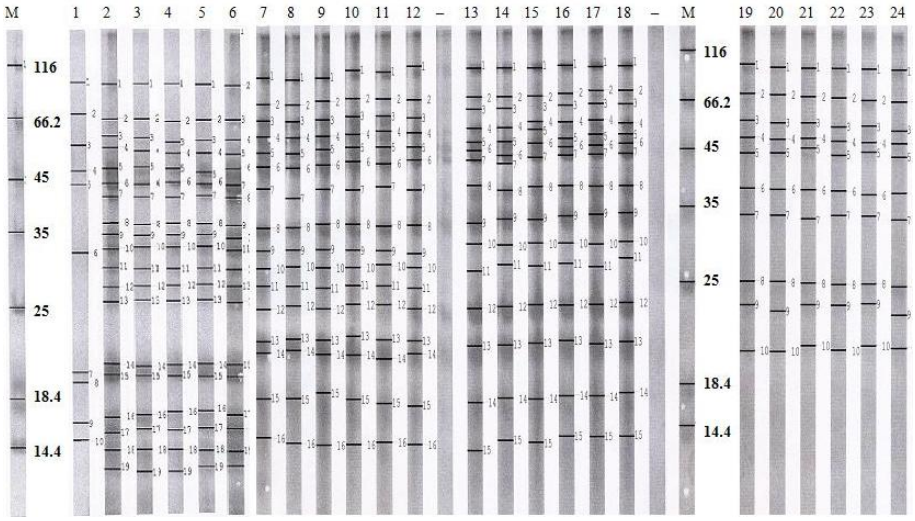

Fig. 4. Electrophoretic profiles of protein precipitated from NaCl-buffer extracts. Lines 1-6 Precipitation at pH 6: 0.1% Magnafloc LT-22 and Magnafloc LT-25, 0.3% Magnafloc LT-22 and Magnafloc LT-25, 0.5% Magnafloc LT-22 and Magnafloc LT-25, respectively. Lines 7-12 Precipitation at pH 5: 0.1% Magnafloc LT-22 and Magnafloc LT--25, 0.3% Magnafloc LT-22 and Magnafloc LT-25, 0.5% Magnafloc LT-22 and Magnafloc LT-25, respectively. Lines 13-18 Precipitation at pH 4: 0.1% Magnafloc LT-22 and Magnafloc LT-25, 0.3% Magnafloc LT-22 and Magnafloc LT-25, 0.5% Magnafloc LT-22 and Magnafloc LT-25, respectively. Lines 19-24 Precipitation at pH 3: 0.1% Magnafloc LT-22 and Magnafloc LT-25, 0.3% Magnafloc LT-22 and Magnafloc LT-25, 0.5% Magnafloc LT-22 and Magnafloc LT-25, respectively, M – molecular mass markers [kDa]

flocculants played key role in the precipitation of protein from pea protein prepartions. It is noteworthy that protein subunits interactions are modified during preparation of protein isolates. Electrophoretic studies of cowpea flour and its protein isolates performed by Sanchez-Vioque and co-workers [1999] clearly confirmed these relationships.

Using of flocculants as coagulating factors allows obtaining a high concentrated protein isolates, however the yields of flocculation were determined by different extraction systems, concentration of flocculants and pH condition of process. Bases on electrophoretic studies is visible that precipitation of proteins with flocculants can be a good tool for protein fractionation although still needs further studies concerning selection of better conditions for protein isolation and precipitation.

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ZASTOSOWANIE FLOKULANTÓW DO WYTRĄCANIA I FRAKCJONOWANIA BIAŁEK Z *LENS CULINARIS* CV. TINA

Wprowadzenie. Soczewica jadalna (*Lens culinaris*) odgrywa istotną rolę w rolnictwie wielu rozwijających się państw. Użyteczność izolatów i koncentratów białkowych w żywieniu ludzi i zwierząt jest przedmiotem wielu badań naukowych. Celem badań było udoskonalenie metod precypitacji i frakcjonowania białek z użyciem flokulantów.
Materiały i metody. Polielektrolity Magnafloc LT-22 i Magnafloc LT-25 zostały użyte w procesie koagulacji i frakcjonowania białek z ekstraktów otrzymanych z nasion soczewicy. Białka izolowano z mąki z zastosowaniem 0,5 M NaCl w 50 mM buforze Tris-HCl pH 7,5 oraz 2 mM NaOH w stosunku 1:10. Precypitację białek przeprowadzono przy różnych wartościach pH (6, 5, 4, 3), używając flokulantów w trzech różnych stężeniach 0,1, 0,3 i 0,5%. Otrzymane frakcje białkowe zostały przeanalizowane z zastosowaniem elektroforezy poliakrylamidowej w obecności SDS (SDS-PAGE).
Wyniki. Bardzo widoczny był wpływ medium ekstrakcyjnego na wydajność izolacji białek. We wszystkich wariantach doświadczenia białka były koagulowane najefektywniej z zastosowaniem flokulantu Magnofloc LT-25. Precypitacja w różnych wartościach pH pozwoliła na rozfrakcjonownie białek soczewicy. Analiza elektroforetyczna otrzymanych frakcji białkowych wykazała obecność protein o masach molekularnych w zakresie od 8 do 102 kDa.
Podsumowanie. Użycie flokulantów jako czynników koagulujących białka pozwoliło na otrzymanie skoncentrowanych izolatów białkowych, jakkolwiek wydajność precypitacji była zdeterminowana wieloma czynnikami, do których należały rodzaj i stężenie użytych flokulantów, pH prowadzonego procesu koagulacji oraz rodzaj układu ekstrakcyjnego użytego do izolacji białek.

Słowa kluczowe: flokulanty, soczewica, Magnofloc, precypitacja białek, izolaty białkowe

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