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Evaluation of usefulness of Magnafloc M-22S flocculant in the process of obtaining protein concentrates from peas

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Abstract

The cationic polyelectrolyte, Magnafloc M22S, was tested in the process of obtaining protein preparations from pea var. *Piast*. Additionally, preparations were precipitated from flour subjected to chemical modification with acetic and succinic anhydride. Chemical composition (total protein, true protein, phenol compounds and sugar level), electrophoretic and chromatographic separation of proteins, and functional properties (solubility, water and fat absorption, ability to emulsify and to foam) of the obtained preparations were compared with concentrates precipitated at the isoelectric point of the proteins. The use of polyelectrolyte Magnafloc M-22S in the process of obtaining protein preparations from chemically un-modified pea-flour did not increase the protein content, but it reduced the level of phenol compounds and increased water absorption. Preparations obtained from flour subjected to acetylation and succinylation were characterized by a higher protein content and better fat absorption.

Keywords: Protein concentrate; Pea; Flocculation; Chemical modification

1. Introduction

In an age of increased use of plant products in the diet because they contain many bioactive substances that have a peculiar pro-health action, while simultaneously limiting the consumption of animal proteins and fats, the search for alternative sources of protein arouses much interest. Protein preparations from soybean have been produced on an industrial scale for many years. Work has also been done on seeds of other legumes—bean, pea or lupin. Usefulness of seeds of fodder plants, such as chickpea, pigeonpea or cowpea, has also been studied (Alamanou & Doxastakis, 1995; Bejosano & Corke, 1999; El-Adawy, 2000; Fernández-Quintela, Macarulla, Del Barrio, & Martí, 1997; Porzucek, 1998; Sánchez-Vioque, Clemente, Viogue, Bautista, & Millán, 1999). Properties of the obtained concentrates and isolates often match, or even surpass, those of soybean preparations. In the course of the process of obtaining protein preparations from legume seeds, the content of non-protein substances is reduced (El-Adawy, 2000; Klepacka & Porzucek, 1994; Mahajan & Dua, 1998; Pedrosa, Trisciuzzi, & Ferreira, 1997; Wagner, Sorgentini, & Añón, 2000). Properly modified concentrates and isolates, or hydrolysates obtained from them, are used as nutrients, replacing milk, for allergic people and in diets for people with diseases of the alimentary canal, athletes, convalescents, and elderly people.

The aim of the present study is to define the efficiency of the use of the flocculation process for obtaining protein preparations from peas.

2. Materials and methods

2.1. Material

The material for the study were pea seeds (var. *Piast*). Dry seeds were ground and the flour was the raw material.

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2.2. Obtaining unmodified protein preparations

Proteins were extracted from flour (500 g) with the Tris–HCl buffer, pH = 9.2, at ratio of 1:10 during one hour on a magnetic stirrer at a temperature of 293 °K. After centrifugation (5500×*g*; 15 min), from the obtained supernatant (from the parallel samples), proteins were coagulated in the following ways:

1. acidification to pH = 4.2 using 2M HCl; and
2. by introducing a solution of Magnafloc M-22S flocculant (150 mg/dm^3) and decreasing acidity with 2M HCl to pH = 4.2.

The precipitate was centrifuged (5500×*g*, 15 min), eluted twice with distilled water, frozen and dried in a vacuum drier at the temperature of 298 °K. The dried and ground preparations were stored at a temperature of about 279 °K.

2.3. Obtaining chemically modified protein preparations

Pea flour was modified with acetic anhydride (acetylation) and succinic anhydride (succinylation). Flour was dispersed in Tris–HCl buffer, pH = 7.5 (1:10 w/v) and acylated with acetic or succinic anhydride in amounts of 0.2 g or 0.2 ml per 1 g of flour protein, respectively, added over a period of 60 min. During the acylation process, the pH was maintained at 7.7–8.0 by neuturalization with 1.5 M NaOH.

The obtained extracts were centrifuged (5500×*g*, 15 min), and the concentrates were coagulated from the supernatant with 2M HCl at pH 3.6, and with Magnafloc M-22S flocculant in the amount of 150 mg/dm^3, and then decreasing acidity with 2 M HCl to pH = 3.6.

The preparations were centrifuged (5500×*g*, 15 min), eluted twice with distilled water, frozen and dried in a vacuum drier at a temperature of 298 K.

Total protein content (N×6.25) was determined according to the Kjeldahl method in the Kjel-Tec automatic apparatus, while true protein was determined as that insoluble in 10% trichloroacetic acid.

Electrophoresis was carried out in 8% polyacrylamide (PAGE-SDS) in a Bio-Rad Mini-Protean electrophoreser at pH = 8.8, with a constant current of 20 mA.

Separation of the supernatants by gel filtration chromatography, with the Sephadex G-50 columns, was conducted with a phosphate buffer solution (0.1 M, pH = 5.5) and absorbance, at 280 nm, was measured using the UV/Vis Philips spectrophotometer.

Carbohydrate content was determined by the HPLC method, on Tessek Sepharon-SG-NH_2 (4 mm×250 mm) with acetonitrile and water (75/25 v/v) using a Laboratorni Pristroje Praha liquid chromatograph LC-5A with refractometric detector RID-K-102. The samples were deprotenised with 30% trichloroacetic acid, and then 1 cm^3 dissolved acetonitrile.

The degree of protein modification was calculated by determining the amount of free amino groups, using 2,4,6-trinitrobenzensulfonic acid (TNBS) (Achouri, Zhang, & Shiying, 1998).

Solubility of the protein, water absorption, fat absorption, emulsifying activity, efficiency of foaming and foam stability were determined according to the procedures of Rutkowski and Kozłowska (1981).

3. Results and discussion

Total protein content in the obtained preparations ranged between 80 and 88.5% (Table 1). The use of polyelectrolyte Magnafloc M-22S, as an agent for co-precipitating proteins, did not increase its content in the preparations obtained in the present study, and the obtained decrease in the content, amounting to about 8%, was significantly smaller than that obtained by Alamanou and Doxastakis (1995) who precipitated proteins extracted from lupin seeds by a polymer of N-isopropyl acrylamide with methylene bisacrylamide. Protein agglomeration must be preceeded with changes in the secondary structure of the proteins to such a degree that molecules can be bound with each other. The process is most efficient when protein molecules are devoid of electrical charge. Coagulation of colloids under the influence of polyelectrolytes, takes place in two ways (Vasilin-Reimann, Lafuma, & Audebert, 1990): they can neutralize the colloid molecules' charge, or they can form additional inter-molecular bonds (bridging effect). The flocculant used in the study did not increase the amount of precipitated proteins—it displayed a protective effect towards them. On the other hand, the use of polyelectrolyte Magnafloc M-22S in the coagulation of protein (subjected to chemical modification by acetylation and succinylation) caused an increase in the protein level, which shows a possibility of two mechanisms acting simultaneously, especially after modification with succinic anhydride. The use of this anhydride does not give a similar effect when only the acid is used for precipitating the concentrates because, in such cases, the protein level in preparations from modified flour is lower than in preparations obtained from flour without chemical modification. Succinic anhydride increases the charge on the protein molecules, in this way increasing their solubility. On the other hand, acetic anhydride first reacts with hydrophobic amino acids and, in modified proteins, the agglomeration ability should be increased in this way through an increase in hydrophobic actions. Zheng, Matsumura, and Mori (1993) shows with their studies of changes in conformation and surface properties of plant proteins, a significant participation of these bonds in the process of

Table 1
Chemical composition of protein concentrates obtained from modified and unmodified pea flour

Concentrate	Protein			Saccharides				
	Total protein %d.m.	True protein %d.m.	True protein % of total	% of modification	Glucose (%)	Maltose (%)	Stachyose (%)	Inositol (%)
PA	88.5	85.5	96.6	–	0.18	10.2	0.39	0.16
PM	80.4	75.6	94.0	–	0.73	2.46	0.39	0.24
PAS	81.1	75.6	93.3	75	1.31	9.30	0.72	0.25
PAA	88.6	83.3	94.0	89	0.98	4.53	0.97	0.22
PMS	88.6	83.0	93.2	83	1.03	4.83	0.72	0.17
PMA	86.6	77.4	89.5	62	1.87	9.60	1.35	0.42
F	21	18.0	88.5	–	0.01	1.36	0.63	0.73

PA—concentrate coagulated by acid from unmodified pea flour; PM—concentrate coagulated by Magnafloc M22S from unmodified pea flour; PAS—concentrate coagulated by acid from succinylated pea flour; PAA—concentrate coagulated by acid from acetylated pea flour; PMS—concentrate coagulated by Magnafloc M 22S from succinylated pea flour; PMA—concentrate coagulated by Magnafloc M22S from acetylated pea flour; and F—pea flour.

agglomeration. The results of the present paper also confirm this assumption, whereas Porzucek (1998) obtained different results.

The differences between protein levels in the concentrates coagulated from non-modified and from chemically modified flour cannot be unequivocally explained by the degree of modification (Table 1). In the case of acid used as the precipitating agent, the degree of modification was similar in the acetylated preparation to the one obtained earlier. (Klepacka & Porzucek, 1994) in peas (var. Ramir) and a little higher than the values obtained by the same authors for the concentrate from pea var. Poa. Studying the use of three different pea varieties (Poa, Ramir and Koral), two different varieties of bean (Jaœ Biczykowy and Wenta) and two varieties of lupin (Hetman and Wat) as raw material in the process of obtaining protein preparations, Klepacka and Porzucek (1994) always obtained a greater degree of modification when they used acetic anhydride. Both its value and difference in relation to the degree of modification after the use of acetic anhydride were dependent on the species and variety of the plant. Similarly, in and earlier study (El-Adawy, 2000), acetic anhydride modified bean proteins to a greater degree than did succinic anhydride when applied at the same concentration. The results of the present study clearly show the influence of the method applied in the coagulation process on the degree of modification. Both the method used in coagulation of the preparations and chemical modification determined their composition, which is proved by the electrophoretic separations of the proteins (Fig. 1). After using polyelectrolyte, the electrophoresis patterns showed protein bands of molecular mass from 88 to 106 kDa and a much greater amount of proteins between 33 and 75 kDa. All the proteins obtained in the study showed molecular masses of 71–73 kDa, which suggests subunits of convicilin, a protein characteristic of pea seeds. The protein is a trimer consisting of three subunits with a mass of 71 kDa (Pasqualini et al., 1991), i.e. a polypeptide with a similar molecular weight is probably a subunit of this fraction. Molecular masses of polypeptides, obtained by electrophoresis separation of protein preparations from peas, prove that the preparations, independent of the method used for precipitating them, are mainly mixtures of subunits consisting of globulins. Only in the case of concentrations precipitated with acid and preparations obtained after chemical modification of pea flour, were there any polypeptides with molecular masses above 91 kDa.

The results of the present study prove that the composition of proteins in concentrates is also dependent on the coagulation method. A polyelectrolyte used for coagulation of proteins from pea prevents precipitation of proteins with molecular weight above 70 kDa. It may be assumed that it causes covalent bond cleavage between protein subunits. Chemical modification of proteins, and especially acetylation, gives the opposite result. Electrophoresis patterns (Fig. 1) prove the presence of proteins with a greater mass (above 100 kDa), independent of the method used for their agglomeration.

Also, chromatograms of supernatants remaining, after separation of preparations, show changing interactions between proteins extracted from pea flour and the agent used for their precipitation (Fig. 2). Prasand (1990), fractionating (on Sephadex gel) the supernatant obtained (after extracting proteins from defatted sunflower seed flour with phosphate buffer in the presence of sodium chloride and coagulating the preparations with acetone) also obtained only one peak of low-molecular weight proteins (<3500 Da), with which most phenol compounds present in the flour were connected. Modification with acetic anhydride leads also to precipitation, along with concentrates of greater molecular mass proteins, whereas the amounts of proteins with smaller molecular mass, left in the supernatant, depend on the method used for coagulating the concentrates.

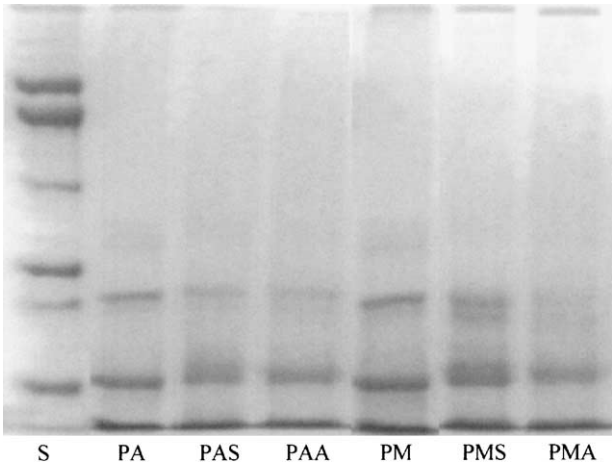

Fig. 1. SDS–PAGE analysis of protein concentrates obtained from modified and unmodified pea flour. S—standards (242, 170, 116, 76, 53 kDa); PA—concentrate coagulated by acid from unmodified pea flour; PAS—concentrate coagulated by acid from succinylated pea flour; PAA—concentrate coagulated by acid from acetylated pea flour; PM—concentrate coagulated by Magnafloc M22S from unmodified pea flour; PMS—concentrate coagulated by Magnafloc M 22S from succinylated pea flour; PMA—concentrate coagulated by Magnafloc M22S from acetylated pea flour.

These changes may be caused by a differentiated dissociation of fraction 11S into subunits with exposure of hydrophobic surfaces of β-conformation of polypeptide chains that may polymerize or react with non-protein ingredients. As well as the method used in the process of precipitating preparations, the conditions of extraction also have an effect as proved by Sánchez-Vioque et al. (1999). These authors obtained a different picture of the fraction, which—in their opinion—points to partial disintegration of fraction 11S, depending on the conditions.

Using the flocculant in the process of precipitation affected the levels of sugars in the concentrates, particularly that of maltose, its concentration being about four times lower than in the preparation precipitated with acid (Table 1). Mostly, glucose, stachyose and inositol were found in the preparation flocculated with Magnafloc M-22S after acetylation of the proteins. The levels of almost all the determined carbohydrates (except maltose in the preparations precipitated with acid and inositol in the preparation flocculated with Magnafloc M-22S after succinylation) were higher after chemical modification of the proteins (from pea flour) than in preparations obtained without modification. Stachyose is the main α-galactoside in the legume seeds that—along with raffinose and verbascose—is responsible for the flatulence effect after consumption of legumes. The amount of this tetrasaccharide is reduced through heat treatment of the seeds or subjecting them to fermentative processes (Duszkiewicz-Reinhard, 1994). A decrease of oligosaccharide level from the raffinose family, in the process of obtaining protein concentrates, allows enriching of food with these preparations without causing a significant increase in the level of flatulance factors.

Chemical modification of concentrates conducted in the present study resulted in preparations with a higher level of stachyose, independent of the applied method of

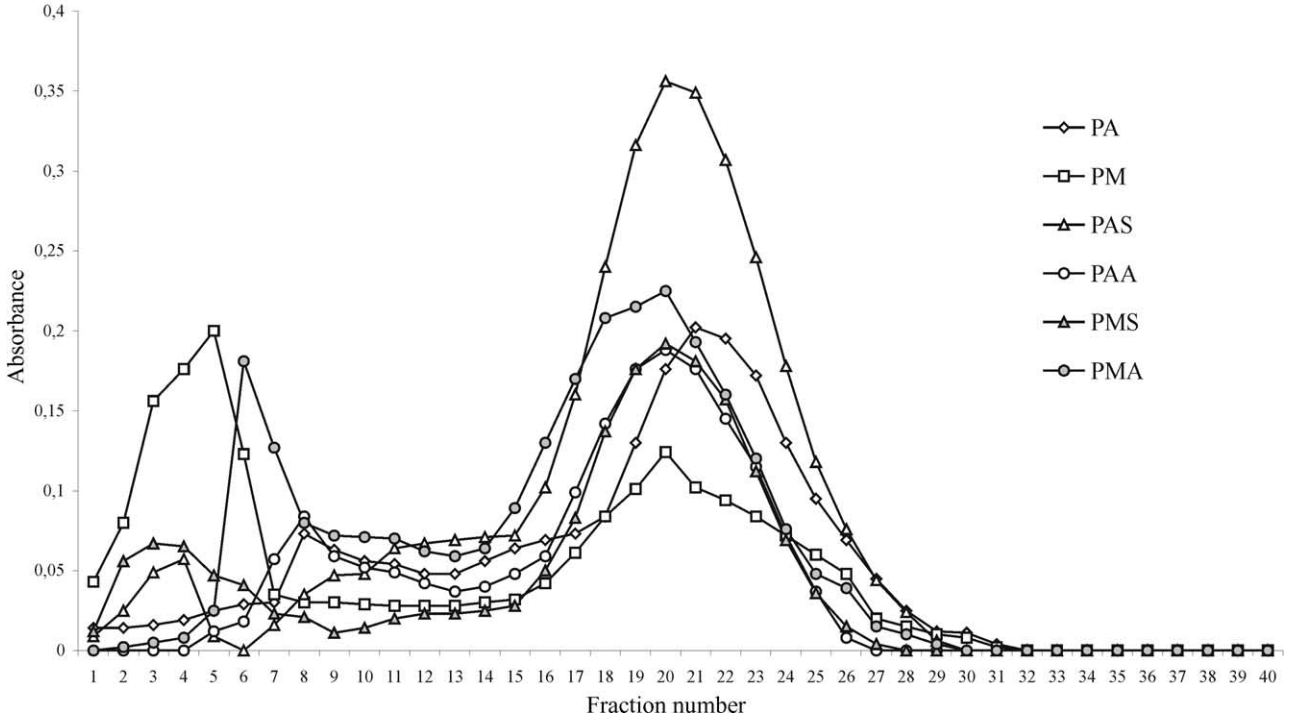

Fig. 2. Sephadez G-50 elution patterns of filtrates after protein concentrates.

precipitation. A smaller increase was obtained in succinylated preparations, and a greater one after acetylation. Porzucek (1998) obtained lower stachyose and raffinose contents both after modification with succinic anhydride and acetic anhydride, with higher results obtained for preparations with succinylated proteins. Changes in protein conformation may weaken the bonds occurring in protein-saccharide complexes and change their affinity for the applied solvent system—the quoted author used 80% methanol, and in the present study carbohydrates were isolated with water. The process is intensified by the presence of polyacrylamide, which is proven by the highest stachyose content obtained in the present study in the concentrate coagulated with Magnafloc M-22S polyelectrolyte from acetylated pea proteins.

The use of protein preparations in the food industry is, to a large degree, determined by their functional properties, which, in turn, are conditioned by the species of the material and the process by which they are obtained. The course of the curves showing the correlation between protein solubility and the pH of the solution is always similar; it reaches its minimum near the isoelectric point of the proteins, and then solubility increases, as the pH increases. On the other hand, the values obtained for solubility are dependent on the protein content in the preparation, the method used to obtain it and the modifying processes that were applied. Different degrees of solubility are not only shown by proteins in isolates obtained from different plants (Bejosano & Corke, 1999). It is generally considered that factors accelerating protein agglomeration, by increasing hydrophobic areas on their surface, effect a decrease in their solubility. By determining the surface hydrophobicity and water solubility of soybean protein isolates obtained on a laboratory scale under different conditions and commercial isolates subjected to modifications, Wagner et al. (2000) found different correlations. After presenting the solubility of isolates obtained on a laboratory scale as a function of surface hydrophobicity it turned out that the greater the value of enthalpy, the greater was the solubility. The authors suggest that the changing conditions at different stages of obtaining the preparations (extraction, centrifuging, precipitation, elution, temperature, time, presence of sulfates (IV), calcium or urea ions, or the method of drying) differentiate the dissociation of the 7S and 11S globulin fractions, as well as their recombination. The results of the present study also show that the method of obtaining isolates differentiates the protein solubility, which is confirmed by the studies by Alamanou and Doxastakis (1995). Even greater changes in solubility are introduced by chemical or enzymatic modification of proteins of seeds or preparations obtained from their flour (Mahajn & Dua, 1998) In the present study, independent of the factor used (acetic anhydride or succinic anhydride), a decrease of protein solubility occurred in acidic medium, and an increase by over 20% in alkaline medium (Fig. 3). The values of solubility obtained for alkaline medium are in some cases higher than those obtained by Sheen (1991) when he analyzed fraction I, isolated from alfalfa, tobacco, soybean and beet, in which protein content reached more than 97%. A comparison of solubility of protein in isolates obtained without modification and after application clearly suggests that this value is more dependent on the type of modification than on the factor used in the process of precipitation. The obtained correlations also suggest that, independent of the applied method, proteins of preparations do not show a great heterogeneity. Flocculants are absorbed on colloidal molecules of protein and the formed complex may decrease protein solubility by blocking hydrophilic groups (which is proven by higher results obtained in acidic medium). Introduction of chemical factors changes the mutual interactions between the coagulating factor and proteins extracted from pea flour by blocking active groups of amino acids. The obtained differences in water absorption prove that there is a change in protein conformation both under the influence of factors precipitating the preparations and the modifying ones. Contrary to results obtained in the study, where application of flocculant increased water absorption of the preparation, the use of a polymer of N-isopropyl acrylamide with methylene bisacrylamide (Alamanou & Doxastakis, 1995) with seed extracts of lupin decreased this value six times more than with concentrates obtained by means of dialysis, and four times more than with concentrates precipitated isoelectrically. The highest values of water absorption obtained in the present study (after application of the Magnafloc M22S flocculant) were not improved by modification of the flour with acetic anhydride, which gave good results after application of acid (Table 2). Acetylation and succinylation of protein isolates obtained from bean also increased the water absorption, which is documented by earlier studies (El-Adawy, 2000). Generally, the absorption values obtained in the present study are higher than those obtained by Lampart-Szczapa (1993), when she analyzed protein preparations obtained by means of different methods from lupin seeds (var. Topaz), and higher than the values obtained by Alamonou and Doxastakis (1995) for the preparation from lupin precipitated isoelectrically. Hence it follows that preparations obtained from pea seeds are a better addition to meat foods than those obtained from lupin, as a good ability to absorb water is very important, because it determines juiciness and rheological properties. From this point of view, fat absorption is also important. In the analyzed preparations, the activity was lower than that obtained with preparations from lupin by the authors of both quoted papers; however, they tested preparations obtained

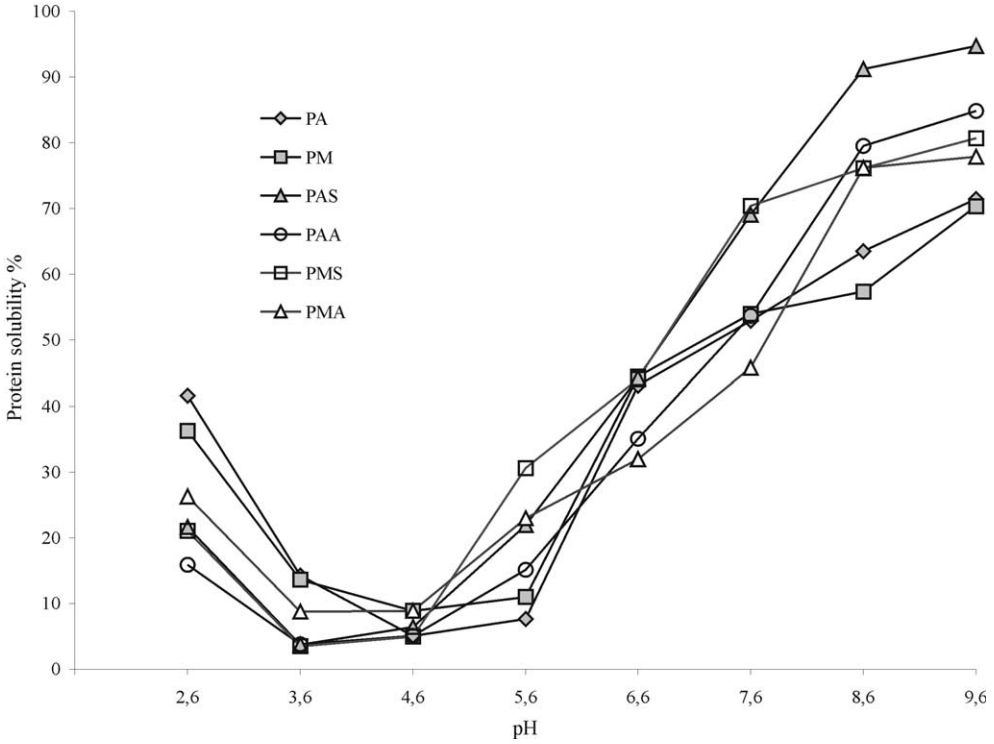

Fig. 3. Protein solubility of protein concentrates obtained from modified and unmodified pea flour. See Fig. 1 for abbreviations.

from defatted flour. In the presented studies flour was not defatted and the preparations already contained fat in their composition. The fat absorption was decidedly increased for all the preparations by chemical modification, especially when it was conducted with acetic anhydride (Table 2). Acetylation also favourably influenced fat absorption in isolates obtained from chick-pea seed (Liu & Hung, 1998) and from bean (El-Adawy, 2000).

The lipid emulsifying ability did not differ significantly from the values that are characteristic of various protein preparations: soybean isolates, milk products and dried egg products, that were studied by Jêdrzejczyk, Swiderski, Cholewińska, and Waszkiewicz-Robak (1993); or lupin preparations analyzed by Lampart-Szczapa (1993). The lipid emulsifying ability is based on hydrophilic-lipophilic balance of molecules and is smaller for proteins whose surface has a lipophobic character. An increase in hydrophobicity of proteins causes an increase in the lipid emulsifying activity. It is also considered that this value is greater if there are proteins with medium size molecules in the emulsion. This observation is confirmed by the lower emulsifying abilities of the preparations obtained in the presence of polyelectrolyte noted in the present study (Table 2).

Another property of proteins that determines the lipid emulsifying ability is their hydrophobicity; the greater it is, the greater are the emulsifying abilities. In the present study, when polyelectrolyte is applied for precipitating the preparations, additional protein-flocculant complexes are formed, which are characterized by a lower hydrophobicity. Chemical modification of proteins, especially with succinic anhydride, caused, complexes to be formed with a higher hydrophobicity, which is proven by the increased emulsifying ability. Pedrosa et al. (1997) also obtained a considerable increase in the emulsifying ability of vicilin, isolated from pea seeds

Table 2
Functional properties of protein concentrates obtained from modified and unmodified pea flour (see Table 1 for abbreviations)

Concentrate	Water absorption (%)	Fat absoption (%)	Foam expansion (%)	Foam stability (%)	Emulsifying ability (%)
PA	248	58	4	0	55.5
PM	310	52	4	0.1	45.0
PAS	238	57	14	0	48.0
PAA	260	72	12	0	51.1
PMS	258	68	0	0	50.4
PMA	286	71	0	0	46.0

and modified by glycosylation with lactose, galactose, and galacturonic acid.

Foam expansion depends on the magnitude of protein molecules in the opposite way, providing that the protein is strongly dispersed. Zhu and Damadoran (1996), in a study of conformational changes of proteins and functional properties of isolates from whey, suggest that the magnitude of protein molecules is not so much responsible for good foam expansion and foam stability, as is the ratio of monomers to polymers.Stability of the obtained foam was low and completely reduced by the modification process (Table 2). Those low values prove that protein isolates do not lower of the gas-liquid interfacial tension and they do not form viscoelastic films surrounding the bubbles, and hence they cannot be components of pulverized desserts.

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