APTEFF, 46, 1-269 (2015) UDC: 633.35:577.112:543.645
DOI: 10.2298/APT1546001B BIBLID: 1450-7188 (2015) 46, 1-18
Review paper

# TECHNO-FUNCTIONAL PROPERTIES OF PEA (*Pisum sativum*) PROTEIN ISOLATES- A REVIEW

*Miroljub B. Barać[1]*, Mirjana B. Pešić[1], Slađana P. Stanojević[1], Aleksandar Ž. Kostić[1] and Slavica B. Čabrilo[2]*

[1] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Beograd-Zemun, Serbia
[2] High Technical School of Vocational Studies, 12000 Požarevac, Nemanjina 2, Serbia

*Due to high nutritive quality, good techno-functional properties and low cost, legume protein products are becoming the most appropriate alternative to protein products of animal origin. In food industries, these products are usually used as techno-functional additives which provide specific characteristics of final food products. Legume proteins are commonly used as flour, concentrates, and isolates. The greatest application on industrial scale has soy proteins, and to a lesser extent, in the past 20 years, pea protein isolates. The modest use of pea protein is partly a result of insufficient information relating to their techno-functional properties. This paper is an overview of techno-functional properties of pea proteins and their isolates. Also, the paper deals with the possible use of limited enzymatic hydrolysis as a method for the improvement of their techno-functional properties.*

**KEY WORDS:** pea protein isolates, techno-functional properties, limited hydrolysis

## INTRODUCTION

For a long time, legumes have been recognized as a valuable and low cost source of high quality protein products such as flour, concentrates and isolates. Nevertheless, the application on an industrial scale has only soybean proteins, whereas other vegetable proteins are less used. Over the last 20 years, especially in Canada and European countries, pea proteins are becoming a viable alternative to soy protein products because of techno-functional and nutritive characteristics (1), which can be as good as those of soybeans. Furthermore, pea seed have a lower content of anti-nutritive components, such as proteinase inhibitors and phytic acid (2) and caused less frequent allergic reactions in humans than soybean (3). In addition, they also contain good quality starch and fibers.

The most promising alternative to soy protein products are pea protein isolates. As in the case of soy protein isolates, techno-functional properties including solubility, emulsifying, foaming and gelling properties of pea isolates are well documented (4-10). In the current literature, opposite results were reported concerning techno-functional properties of pea and soy protein isolates. Some researches (11, 12) obtained better functionality of soy pro-

* Corresponding author: Miroljub B. Barać, University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Beograd-Zemun, Serbia, e-mail: baracm@agrif.bg.ac.rs

UDC: 633.35:577.112:543.645
BIBLID: 1450-7188 (2015) 46, 1-18
Review paper

tein isolates, whereas some other (5, 13, 14) pointed out better properties of pea isolates. Variations in the results among different studies could be due to the differences in the protein purity of the studied samples, method of protein isolation, the specific conditions used for the tests, as well as the different processing conditions (7, 15). Furthermore, significantly different functionalities among pea isolates were observed. Maninder et al. (16) and Barac et al. (6, 17) attributed this to the different ratio of the major proteins, which is in turn influenced by genotype characteristics, environmental conditions, and processing conditions (10, 18-20). To avoid the difference caused by different processing conditions, Barac et al. (15) prepared and compared pea, soybean and adzuki isolates under the same conditions. The results of this investigation showed that techno-functional properties of the isolates prepared from different species depended on several factors such as: choice of species and varieties, preparation conditions, and the pH value at which specific properties were tested.

## STORAGE PEA PROTEINS

Pea seeds contain about 22-23% proteins. The majority of pea proteins are globulins and albumins, which represent about 80% of total seed protein content. Albumins represent 18-25% and globulins 55-65% of total proteins (21). All globulins and some of albumins are storage proteins, which are used as nitrogen sources for the new embryos after seed germination (22).

Major pea storage proteins, legumin, vicilin and convicilin are globulins and represent 65-85% of total proteins (23). According to sedimentation properties these proteins are classified into two fractions, 7S (vicilin, convicilin) and 11S fraction (legumin). Molecular forms of the three major proteins are presented in Figure 1.

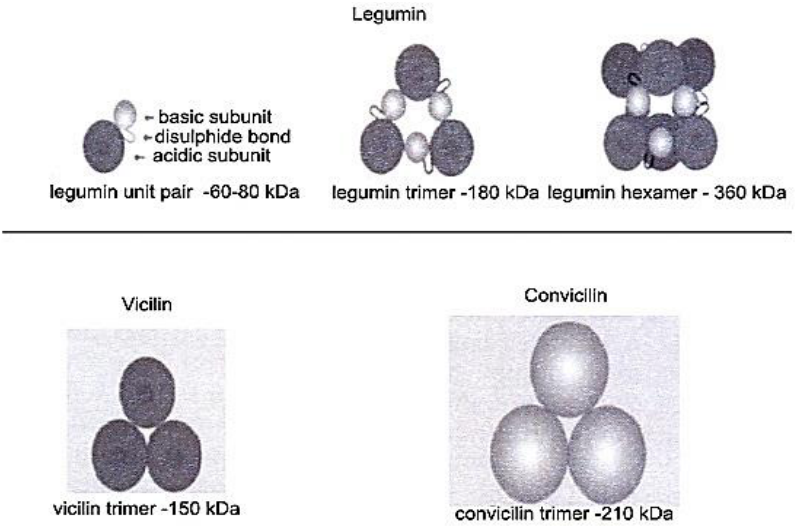


**Figure 1**. Molecular forms of legumin, vicilin and convicilin (22)

## Legumin

Legumin is a protein with compact quaternary structure stabilized via disulphide, electrostatic and hydrophobic interactions. It is a hexamer with a molecular weight (Mw) ~320 to 380 kDa and with beta-sheet-rich structure (24). The mature proteins consist of six subunit pairs that interact non-covalently. Each of these subunit pairs consists of an acidic subunit of ~40 kDa and a basic subunit of ~20 kDa, linked by a single disulphide bond (25). As there are a number of legumin precursors originating from several gene families, different legumin polypeptides have been identified, e.g., 4-5 acidic (α) and 5-6 basic (β) polypeptides. The sizes of these polypeptides range from 38 to 40 kDa for the acidic polypeptides with the isoelectric point (pI) 4.5-5.8, and from 19 to 22 kDa for the basic polypeptides with the pIs of up to 8.8 (26). According to Gueguen et al. (25), more hydrophobic basic polypeptides are placed in the interior of the legumin molecule, whereas acidic polypeptides are oriented towards the outside of the molecule.

Due to its compact quaternary structure, legumin is a heat-stable protein. Thermal transition point of legumin is above 90$^{o}$C. On the other hand, the quaternary structure of the legumin is more sensitive to pH and salt concentration. Pea legumin is present as a hexamer at the pH 7.0 and high ionic strength (0.1 M), but dissociates at, e.g., the pH 3.35 and 10.0, and, depending on the ionic strength, into a mixture of trimers, dimers, and monomers. Acidic conditions seem to be more drastic than alkaline ones, thus the native legumin is completely dissociated to monomers at the pH 2.4 (25).

As a food protein, legumin is recognized for its sulphur containing amino acid residues. It has been reported to contain approximately two cysteine and three methionine residues per 60-kDa subunit (27).

## Vicilin

Vicilin is a trimeric protein of 150-170 kDa that lacks cysteine residues and hence cannot form disulphide bonds (27). The composition of vacilin subunits varies mostly because of post-translation processing. Mainly, vicilin consists of ~47 kDa, ~50 kDa, ~34 kDa and ~30 kDa subunits (28). Pea vicilin heterogeneity is more complex than the heterogeneity of legumin. Its heterogeneity derives from a combination of factors, including production of vicilin polypeptides from several small gene families encoding different primary sequences, differential proteolytic processing, and differential glycosylation (29). Thermal denaturation temperature of vicilin depends on ionic strength conditions. At low ionic strength conditions ($\mu$ =0.08) the thermal denaturation temperature is 71.7, whereas at higher ($\mu$ =0.5), it is 82.7$^{o}$C (30).

## Convicilin

A third major storage protein, distinct from legumin and vicilin, is convicilin. This protein has a distinctively different amino acid profile and unlike the 7S vicilin, contains very little carbohydrate and has a subunit molecular weight of 71,000 Da. The molecular weight of its native form is 290,000 Da including an N-terminal extension (8). Convicilin is not known to undergo any post-/co-translational modifications other than removal of the signal peptide, and it is not glycosylated. In opposite to vicilin, the residues of sulphur-amino

acids are presented in primary structure of convicilin. However, O'Kane et al. (31) denoted this protein as α-subunits of vicilin. According to these authors, convicilin has an extensive homology with vicilin along the core of its protein, yet is distinguished by the presence of a highly charged, hydrophilic N-terminal extension region consisting of 122 or 166 residues. The homologies of convicilin and vicilin are shown schematically in Figure 2.

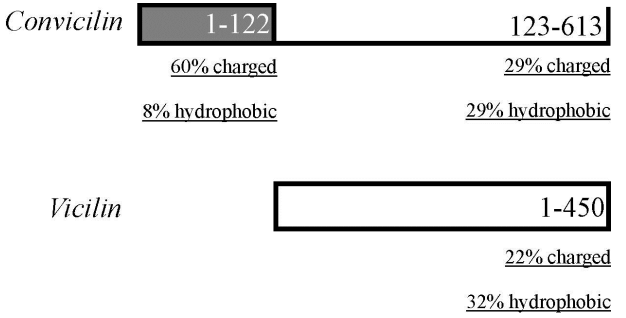


**Figure 2.** Schematic diagram of the highly charged N-terminal extension region (residues 1-122) present in convicilin molecules. The core of convicilin (residues 123-542) is highly homologous to vicilin, as shown by the percentages of charged and hydrophobic residues (40).

Pea protein content and composition vary among genotypes (32, 33). Also, these parameters are influenced by environmental factors (32-34). As a result of genotype and environment-induced variations, the ratio of vicilin to legumin varies and may range from 0.5 to 1.7, with a mean of 1.1 (35). Barac et al (6) investigated protein composition of six different genotypes and showed that the ratio of the sum of vicilin and convicilin to legumin content ranged from 1.30 to 1.78.

The differences in content, composition and structure between vicilin and legumin are exhibited in both nutritional and techno-functional properties. Legumin contains more sulphur containing amino acids than vicilin per unit of protein (27), and its more available fraction from a nutritional point. Furthermore, different techno-functional properties of pure legumin, vicilin and convicilin are well documented (1, 30,36-38).

## PEA PROTEIN PRODUCTS

As a techno-functional ingredient, pea proteins are usually used as flour, concentrates, and isolates. Pea flour is prepared from dehulled and milled seeds. The average composition of pea seeds/flour, concentrate and isolate are given in Table 1.

**Table 1.** The average composition of pea seeds/flour, concentrate and isolate (39)

| Composition (%) | Whole seed/Flour | Concentrate | Isolate |
|---|---|---|---|
| Protein | 25 | 50 | 85 |
| Starch | 50 | 17 | 0 |
| Fat | 5-6 | 4 | <3 |

Commonly, protein concentrates are produced by air-classification of the pea flour (obtained from the milled seeds), which is a dry processing method that blows away the lighter starch granules, thus removing them from the protein. Concentrates have ~50% content of protein. Protein isolates instead undergo a wet processing in which low molecular weight water-soluble components and the salt soluble proteins are extracted from the flour and then the globular proteins are subsequently isolated by a selective precipitation step at the isoelectric point, neutralized and dried (Figure 3). Final protein content of isolates prepared by isoelectric precipitation is approximately about 85%. Protein extraction can be done under alkaline or acidic conditions. The schematic diagram of the most frequently used method based on aqueous alkaline extraction followed by isoelectric precipitation is presented in Figure 3.

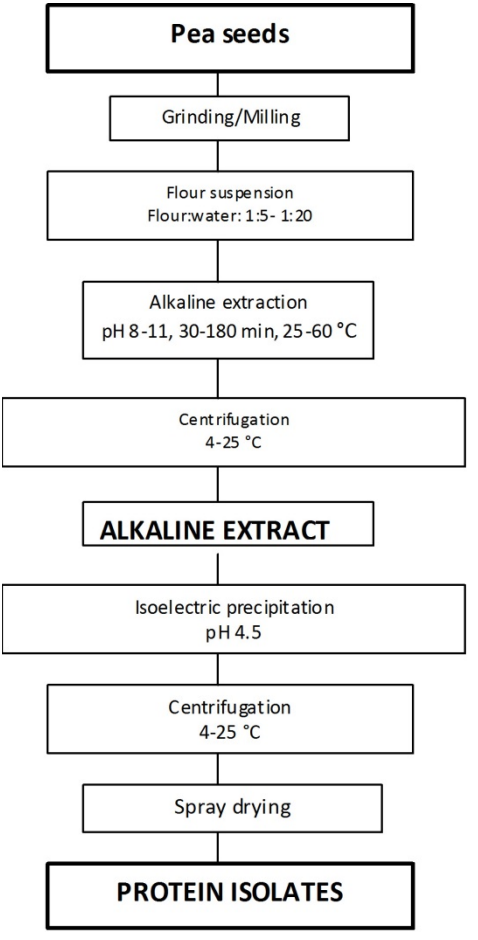


**Figure 3.** Schematic diagram of alkaline extraction and isoelectric precipitation process for production of pea protein isolates (8)

Alternatively, the isoelectric precipitation step can be substituted by ultrafiltration. The use of ultrafiltration increases the yield of isolates and change their composition. Isolates prepared by ultrafiltration contain 90-94% of protein (40). Besides globulins, these products contain other protein fractions and polysaccharides.

## TECHNO-FUNCTIONAL PROPERTIES OF PEA PROTEINS AND THEIR PROTEIN PRODUCTS

In general, techno-functional properties of a protein are affected by numerous factors which can be classified into two groups, intrinsic and extrinsic factors. The intrinsic factors are: amino acid composition and sequence, shape, size, the ratio between hydrophobicity/hydrophilicity, conformation and reactivity. The extrinsic factors which can affect techno-functional properties of pure protein include pH, ionic strength, temperature, conformation, the ratio between hydrophobicity/hydrophilicity, method of extraction. Besides these factors, in the case of protein products, such as flour, concentrate and isolate, several additional factors, including the ratio of major proteins and processing conditions may have crucial effect on their techno-functional properties and consequently on their applicability in food systems.

**Table 2.** Techno-functional properties performed by functional proteins in food systems (41)

| Techno-functional property | Mode of action | Food system |
|---|---|---|
| Solubility | Protein solvation | Beverages |
| Water absorption and binding | Hydrogen bonding of water; Entrapment of water (no drip) | Meat, sausages Breads, cakes |
| Viscosity | Thickening; water binding | Soups, gravies |
| Gelation | Protein matrix formation and setting | Meats, curds, cheese |
| Cohesion-adhesion | Protein act as adhesive material | Meats, sausages, baked goods, pasta |
| Elasticity | Hydrophobic binding in gluten; Disulfide links in gels | Meats, bakery |
| Emulsification | Formation and stabilization of fat emulsions | Sausages, bologna, soups, cakes |
| Fat absorption | Binding of free fat | Meats, sausages, doughnuts |
| Flavor-binding | Adsorption, entrapment, release | Simulated meats, bakery etc. |
| Foaming | Form stable film to entrap gas | Whipped toppings, chiffon desserts, angel cakes |

Techno-functional properties required for a protein product vary due to its specific application in food and food systems (Table 2). In general, a good protein product has to possess multiple functionalities in order to perform well in food systems. The most important techno-functional properties of protein products are solubility, emulsification, foaming, and gelation.

### Solubility of pea protein products

Good solubility of proteins is desired for optimal functionality in food processing applications (6). It is well known that other functional properties such as emulsification,

foaming, and gelation are dependent on the solubility of proteins. Solubility of proteins is variable and is influenced by the number of polar and apolar groups and their arrangement along the molecule (42). Solubility of protein depends on the pH and ionic strengths, whereas processing history of protein products has a great influence on this property (8, 15). Furthermore, the ratio of the major proteins in flour as starting material could affect the solubility of legumes protein product (6, 15, 44)

Major pea proteins are globulins with minimum solubility near the isoelectric point (pI 4.5), high solubility above and moderate below the isoelectric point (6, 11, 15, 46). The maximum value is observed in the pH range of 8-9 (11), whereas less than 20% of proteins are soluble at the pI value. Consequently, native pea proteins and their native products show U-shape of pH-solubility dependence, which is also typical for the other legume proteins (46, 15). However, the variations of solubility of pea protein isolates were observed. It is well known that native as well as thermally-treated proteins from legumes tend to form pH-induced aggregates (47, 48). So, Barac et al. (6, 44) attributed these variations to protein composition of pea isolate and different nature of complexes formed during the processing of the isolate (during isoelectric precipitation) and/or during the solubilization of the isolates at a specific pH.

Thermal treatments reduce the solubility of pea isolates (49). However, thermally treated pea protein products showed similar U-shape dependence (15). The effect of thermal treatment (90$^{o}$C, 3 min) on pea protein isolate solubility is presented in Figure 4.

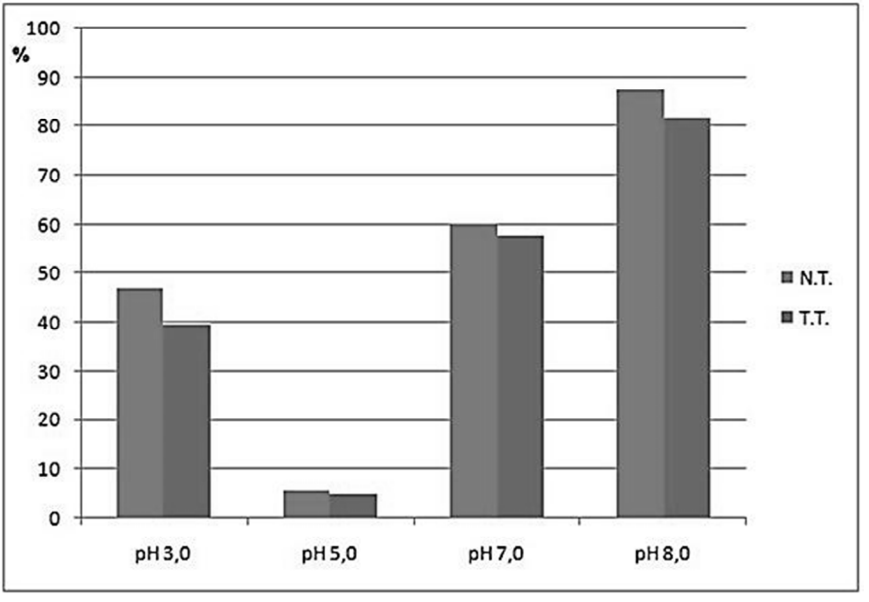


**Figure 4.** The influence of thermal treatment (90$^{o}$C, 3 min) of neutralised suspensions of pea protein isolates on solubility at different pH values (15).
TT - thermally-treated, N.T. - non-treated

## Emulsifying properties

Emulsions are disperse systems of immiscible liquids which are stabilized by emulsifiers – compounds which form interface films and thus prevent the disperse phases from flowing together. Proteins as surface-active and amphiphilic compounds can be used as emulsifying agents on a large scale during the production of food systems. Emulsifying properties of proteins are usually characterized as emulsifying ability or activity and emulsion stability. The emulsion stability is a measure of the stability of the emulsion over a certain time span and emulsion activity is a measurement of how much oil a protein can emulsify per unit protein (7).

Suitability of a pure protein and protein isolate as an emulsifier depends on the rate at which proteins diffuse into the interface and on the deformability of its conformation under the influence of interfacial tension (surface denaturation). A protein with ideal qualities as an emulsifier for an oil-in-water emulsion would have a relatively low molecular weight, a balanced amino acid composition in terms of charged, polar and non-polar residues, good water solubility, well-developed surface hydrophobicity, and a relatively stable conformation (42).

Different emulsifying properties of pure solutions of vicilin and legumin are documented. Results of several researchers (30, 36, 37, 50) showed that, in the native form, vicilin had better emulsifying properties than legumin. This could be attributed to the less compact and less rigid native structure of vicilin. Furthermore, due to conformational changes, emulsifying properties of vicilin and legumin are pH-dependent. Namely, the minimum emulsifying activity and stability the major pea proteins showed in the range of pI (4-5). Also, at the pI values their emulsions are extremely unstable. Above and below pI value, emulsifying properties increase due to intensive dissociation, which is more pronounced in the case of legumin (21). Due to this, besides the processing history of the isolate, the vicilin to legumin ratio has significant influence on the emulsifying properties.

Gharlsallaoui et al (51) investigated the emulsifying characteristics of acid-treated pea protein isolates. They showed that acid treated pea proteins adsorb faster on the water-oil interface at the pH 7.0 than at an acidic pH (pH 2.4). But, fast adsorption leads to the formation of more inhomogeneous film structures. In opposite to this, a slower adsorption is regular and slow but it leads to a higher surface viscoelasticity. Due to this, pea-protein-stabilized emulsions are more stable to creaming at acidic pHs, and their particle-size distributions are more homogeneous in these conditions.

Kimura et al. (30) investigated the emulsifying properties of pure 7S and 11S fractions of different legumes at the different pH and ionic strength. These authors showed that 7S fraction of pea had a slightly lower emulsifying ability and stability than 7S fraction of other legumes, whereas no significant differences were observed in the case of 11S globulins.

Several researchers compared the emulsifying properties of pea and soy protein isolates and opposite results were obtained. Earlier work of McWatters and Cherry (52) showed that the emulsifying properties of pea protein are minor compared to soy protein, but it is still able to produce both semi-thick and thick mayonnaise-like emulsions at different pH values. Vose (53) reported that pea isolate had similar or better emulsifying properties

than soy protein isolates. Also, Tömössközi et al. (46) found that pea isolates had quite good emulsifying capacity but low emulsion stability in comparison to soy protein isolate. Aluko et al. (45) and Adebiyi and Aluko (54) showed that pea protein isolate had better emulsifying capacity than soy protein isolate when emulsions were prepared at different concentrations of isolate and at the pH 5.0 and 7.0. The better emulsifying capacity these authors attributed to the higher level of sugar in pea protein isolates than in soy protein isolates. Namely, a higher content of sugar may contribute to the increased protein solubility and emulsifying capacity. To avoid processing induced differences between soy and pea protein isolate, Barac et al. (15) compared these isolates prepared under the same conditions and showed that pea isolates in general had slightly lower emulsifying properties than those of soybean. However, they were quite usable in the food industry. Furthermore, this investigation clearly showed that the comparison of protein isolates from different species, even if they are prepared and used under the same conditions is difficult, as it is related to the selection of genotypes within species.

## Foaming properties

In food systems (such as in baked goods, sweets and desserts), proteins function as foam-forming and foam-stabilizing components. Different proteins have different abilities to form and stabilize foams, and just as in the case of proteins and their different emulsifying properties, this is related to the different physico-chemical properties of the proteins. (6, 15, 55). The ideal foam-forming and foam-stabilizing protein is characterized by a low molecular weight, high surface hydrophobicity, good solubility, a small net charge in terms of the pH of the food, and easy denaturability (42). Foaming properties of proteins are usually characterized as foaming capacity (FC) and foaming stability (FS). FC is measured in volume (%) when whipped, while the volume of the foam over time (normally 0-30 min) gives the protein's FS (8).

Several authors investigated foam properties of pea protein isolates (6, 11, 55-57). According to these investigations, foaming properties of pea isolates are pH- and concentration-dependents. Furthermore, protein level and protein composition of starting seed, processing method used for their production affect foaming properties of pea protein products (7, 8, 20, 57, 47, 55).

Aluko et al. (45) compared foaming properties of soy and pea protein isolates. They showed that pea protein isolates were foaming agent with a more flexible polypeptide conformation at the pH 3.0 and 7.0 when compared to soy protein isolate. Similar observation was reported by Sosulski et al (14), whereas Tömösközi et al. (46) showed poorer foaming ability of pea protein isolate when compared to soy protein isolate. The opposite results of these authors could be attributed to numerous factors including processing conditions and different protein composition of the investigated isolates. To avoid the influence of processing conditions, Barac et al. (15) compared foaming properties of native and thermally treated soy and pea protein isolates prepared under the same conditions. They reported that pea protein isolates had slightly lower foam activity than soy protein isolates in a wide range of pH (3.0-8.0), but foams formed with pea protein isolates at the investigated pHs were more stable.

## Gelling properties

Gel is a dispersed system of at least two components in which dispersant forms a cohesive network. It is characterized by the lack of fluidity and elastic deformability. Globular proteins, such as legume proteins, under specific conditions (after heating and denaturation), can form gel. Usually, this type of gel is characterized as aggregated dispersion (42). Namely, after the denaturation and further heating, the proteins will aggregate and interact with other proteins and form either a gel or a coagulum. Which type will be formed it depends on the conditions such as molecular weight, heating time and protein concentration (58). Gel formation is complicated and affected by the concentration of protein, amount of water, ionic strength, time and temperature, as well as by the pH and interaction with other components in the food system (58). These process conditions can be manipulated for gel formation.

Only a few authors investigated gelling properties of pure pea proteins and pea isolates. Shand et al (59) showed that both globulins and albumins of pea protein isolates contribute to gel formation. Studies on the gelation properties of mixed pea globulins, vicilin and legumin have been reported by Bora et al. (38) and O'Kane et al. (31, 1). It was found by Bora et al. (38) that pea globulin underwent heat-induced gelation, whereas pure legumin did not gel under the same conditions. According to these authors, the relationship between protein (globulin) concentration and log gel hardness was linear. Furthermore, at all protein concentrations studied, as proportion of legumin decreased, the gel hardness increased. In contrast to their findings, O'Kane et al. (1) and O'Kane (39) indicated that both pea vicilin and legumin could form gels. This was probably caused by a difference in pea cultivars since O'Kane et al. (1) indicated that the contribution of legumin to the pea protein gels was cultivar specific and related to its disulphide bonding ability rather than the absolute amount of legumin protein present. Furthermore, these authors showed that the third pea globulin can hinder the gel formation of pea protein isolates when present in sufficient quantity. In large amounts, this protein increases the minimum gelling concentration of purified pea proteins at a near-neutral pH, and causes formation of transparent heat-induced gels. This behaviour was attributed to the repulsive forces on the N-terminal extension region at a near-neutral pH, and was supported by the fact that no difference in the gelation behaviour of vicilin and convicilin fractions was observed at low pH values, where the repulsive charges would have been neutralised.

Most of the previously cited investigations were based on isolates prepared by isoelectric precipitation. Sun and Arntfield (12) showed that processing conditions significantly changed gelling properties of pea protein isolates. These authors investigated the heat-induced gelation properties of salt-extracted pea protein. They showed that the salt-extracted and freeze dried isolates formed gel at much lower concentrations than those prepared by isoelectric precipitation and spray drying. The minimum gelation concentration of salt-extracted pea protein isolate was 5.5%, while that of commercial pea protein isolate was 14.5%. Furthermore, Taherian et al. (10) showed that gelling temperatures of pea isolates prepared by water and KCl extraction and subsequent diafiltration at the pH 6.0 trimmed down to 75.7 ±0.63$^{o}$C and 81.6 ±0.55$^{o}$C, in contrast to that of commercial isolate at above 90$^{o}$C. Similarly, the formation of firm gels, after 1 h of heating at

90$^{o}$C, was associated with membrane processed isolates, whereas commercial isolates did not develop any gel.

Pea protein form weak, heat-induced gels. The gelation of pea protein is temperature-dependent, and primarily influenced by the degree of protein denaturation. If the degree of denaturation is lower, a stronger gel is formed. Protein concentration also plays an important role in gelation properties. Higher concentrations generally produce stronger gels.

However, the gelling point was concentration independent. Heating and cooling rates are minor factors influencing the gelation properties of pea protein. The heating rate influenced the gelling point in the way that higher heating rates resulted in delayed gelling (higher gelling temperatures). Higher heating and cooling rates caused a weakening effect on gel elasticity.

O'Kane et al., (1) and Shand et al. (59) compared gelling properties of pea and soy protein isolates. Both groups of authors concluded that pea protein isolates formed more unstructured gel than soy protein isolates and thus their gelling properties are not that as good as those of soy. For example, Shand et al (59) showed that the optimal conditions for formation of strong heat-induced gels from the pea isolate were 19.6% (w/w) protein content, pH 7.1, 2.0% (w/w) NaCl, and heating at 93°C. The gels prepared with soy protein isolates under the same conditions were stronger and more elastic than those prepared with pea protein isolates. However, Nunes et al. (60), by studying pea protein as a replacer of dairy and egg proteins in a gelled vegetable dessert showed that pea proteins produced good gels that were highly applicable as a food product.

## LIMITED PROTEOLYSIS AS A METHOD FOR IMPROVEMENT OF TECHNO-FUNCTIONAL PROPERTIES OF PEA PROTEIN ISOLATES

Techno-functional properties of pea protein isolates can be improved by chemical, physical and enzymatic treatments. From the standpoint of safety, the most appropriate method for modification of legume protein properties is limited proteolysis (61). Peptides produced by partial proteolysis have smaller molecular size and less compact structure than the original proteins. Such peptides contribute to the improvement of techno-functional properties compared to those of the native proteins (60). To obtain desirable techno-functional properties of pea protein hydrolysates, hydrolysis must be done under strictly controlled conditions to a specified degree of hydrolysis (DH). A limited DH usually improves solubility, as well as emulsifying and foaming capacities, whereas excessive hydrolysis often causes decline in some of these functionalities (62, 63, 44).

Partial enzymatic hydrolysis of plant proteins has been the subject of extensive research by various authors. Most of these studies have been conducted on soy protein products, including soy flour, concentrates and isolates (63-67). Less attention has been paid to pea proteins (44, 69-72). These studies have been conducted on pure proteins and pea isolates and showed that, as well as in the case of soybean proteins, 7S and 11S protein expressed different susceptibility to the enzyme-induced hydrolysis (70,73-75). Proteases preferentially hydrolyze vicilin over legumin (75). This is due to their different structures; the compact structure of legumin makes it difficult protease to act. Braudo et al. (77) compared susceptibility of pea legumin and soy glycinin and concluded that pea 11S

protein was more resistant to proteolysis than soy 11S protein. The differences between these two proteins were attributed to the differences in their primary structures.

In most of the studies reported in the literature, commercial proteases, such as trypsin, alcalase, papain and chymosin have been used for pea protein hydrolysis. In general, these investigations showed that the hydrolysis up to 10% significantly improved solubility, foaming, emulsifying and other properties. For example, the hydrolyzates (characterized with DH of 8%) prepared with trypsin had improved solubility, especially in the range of pH of 4-7 which was 90-98.6% (78). Furthermore, a linear dependence between the degree of hydrolysis and solubility of pea protein hydrolysates were registered. The later work of Huminski and Aluko (71) showed that trypsin isolates with higher DH values (18.28%) had better emulsifying properties than pea protein hydrolyzates obtained with papain, α-chymotrypsin, Alcalase and Flavourzyme. However, most of these studies were focused on the relationship between the action of one or several proteases and techno-functional properties of commercial or laboratory-prepared isolates of one variety. The influence of protein composition in the initial isolate on these properties was less investigated. Barac et al. (44) compared techno-functional properties of pea isolates from two different genotypes and those of modified with two different proteases (Streptomyces griseus protease and papain). They suggested that proper selection of pea variety (besides other factors) could result in the production of enzymatically–modified pea protein isolates with excellent functional properties.

## CONCLUSION

This paper clearly showed that pea protein isolates can be a very useful substituent for soy protein products as techno-functional additives. Pea protein isolates could find application in a wide range of food products, but their proper selection and preparation conditions could be of great importance. Furthermore, the studies reported in the current literature suggest that physico-chemical properties of pea proteins could be extensively improved, and enzymatic hydrolysis is a good tool to achieve this.

### Acknowledgement

The authors acknowledge financial support the Ministry of Education, Science and Technological Development of the Republic of Serbia, TR-31069, 2011-2015.

## REFERENCES

1. O'Kane, F. E.; Vereijken, J. M.; Gruppen, H.: Van Boekel M. A. J. S. Gelation behavior of protein isolates extracted from 5 cultivars of Pisum sativum L. *J. Food Sci.* **2005**, *70*, 132-137.
2. Gwiazda, S.; Rutkowski, A.; Kocoń, J. Some functional properties of pea and soy bean protein preparations. *Nahrung* **1979**, *23*, 681-686.

3. San Ireneo, M.; Ibáñez Sandín, M.D; Fernández-Caldas, E.; Marañón Lizana, F.: Rosales Fletes, M.J., Laso Borrego, M.T. Specific IgE levels to Cicer arietinum (Chick pea) in tolerant and nontolerant children: evaluation of boiled and raw extracts. *Int. Arch. Allergy Imm.* **2000**, *121*, 137-143.
4. Karaca, A.C.; Low N.; Nickerson, M. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Res Int.* **2011**, *44*, 2742-2750.
5. Aluko, R.E.; Mofolasayo, O.A.; Watts, B.M. Emulsifying and foaming properties of commercial yellow pea (*Pisum sativum* L.) seed flours. *J. Agric. Food Chem.* **2009**, *57*, 9793-9800.
6. Barac, M.; Cabrilo, S.; Pesic, M., Stanojevic, S.; Ristic, N. Profile and functional properties of seed proteins from six pea (Pisum sativum) genotypes. *Int. J. Mol. Sci.* **2010**, *11*, 4973-4990.
7. Boye, J.I.; Aksay, S.; Roufik, S.; Ribéreau, S.; Mondor, M.; Farnworth, E.; Rajamohamed, S.H. Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Res. Int.* **2010**a, *43*, 537-546.
8. Boye, J.; Zare, F.; Pletch, A. Pulse proteins: processing, characterization, functional properties and applications in food and feed. *Food Res Int.* **2010**b, *43*, 414-431.
9. Adebiyi, A.P.; Aluko, R.E. Functional properties of protein fractions obtained from commercial yellow field pea (Pisum sativum L.) seed protein isolate. *Food Chem*. **2011**, *128*, 902-908.
10. Taherian, A.R.; Mondor, M.; Labranche, J.; Drolet, H.; Ippersiel, D.; Lamarche, F. Comparative study of functional properties of commercial and membrane processed yellow pea protein isolates. *Food Res. Int.* **2011**, *44*, 2505-2514.
11. Fernandez-Quintela A.; Macarulla, M.T.; Del Barrio, A.S.; Martinez, J.A. Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. *Plant Foods Hum. Nutr.* **1997**, *51*, 331-342.
12. Sun, X.D.; Arntfield, S.D. Gelation properties of salt-extractedpea protein induced by heat treatment. *Food Res. Int.* **2010**, *43*, 509-515.
13. Sumner, A.K.; Nielsen M.A.; Youngs, C.G. Production and evaluation of pea protein isolate. *J Food Sci.* **1981**, *46*, 364-372.
14. Sosulski, F.W.; McCurdy, A.R. Functionality of flours, protein fractions and isolates from field peas and bean. *J. Food Sci.* **1987**, *52*, 1010-1014.
15. Barac, M.B.; Pesic, M.B.; Stanojevic, S.P.; Kostic, A.Z.; Bivolarevic, V. Comparative study of the functional properties of three legume seed isolates: adzuki, pea and soy bean. *J. Food Sci. Technol.* **2014**, in press, DOI 10.1007/s13197-014-1298-6.
16. Maninder, K.; Sandhu, K.S.; Singh N. Comparative study of the functional, thermal and pasting properties of flours from different field pea (*Pisum sativum L.*) and pigeon pea (*Cajanus cajan L.*) cultivars. *Food Chem*. **2007**, *104*, 259-267.
17. Barac, M.; Cabrilo, S.; Pesic, M.; Stanojevic, S.; Pavlicevic, M.; Macej, O.; Ristic, N. Functional properties of pea (*Pisum sativum, L*) protein isolates modified with chymosin. *Int. J. Mol. Sci*. **2011**, *12*, 8372-8387.

18. Periago, M.J.; Vidal, M.L.; Ros, G.; Rincón, F.; Martínez, C.; López, G., Rodrigo, J.; Martínez, I. Influence of enzymatic treatment on the nutritional and functional properties of pea flour. *Food Chem.* **1998**, *63*, 71-78.
19. Fuhrmeister, H.; Meuser, F. Impact of processing on functional properties of protein products from wrinkled peas. *J. Food Eng.* **2003**, *56*, 119-129.
20. Dua S.; Mahajan, A.; Sandal Gagan, M. Physico-chemical properties of defatted pea seed meal proteins with emphasis on salts and pH. *J. Food Sci. Tech.* **2009**, *46*, 251-254.
21. Tsoukala, A.; Papalamprou, E.; Makri, E.; Doxastakis, G.; Braudo, E.E. Adsorption at the air-water interface and emulsification properties of grain legume protein derivatives from pea and broad bean. *Colloids Surf. B* **2006**, *53*, 203-208.
22. Tzitzikas, E.N.; Vincken, J.P.; De Groot, J.; Gruppen, H.; Visser, R.G.F. Genetic variation in pea seed globulin composition. *J. Agric. Food Chem.* **2006**, *54*, 425-433.
23. Owusu- Ansah, Y. J.; McCurdy S.M. Pea Proteins: A review of chemistry, technology of production, and utilization. *Food Rev. Int.* **1991**, *7*, 103-134.
24. Schwenke, K. D.; Henning, T.; Dudek, S.; Dautzenberg, H.; Danilenko, A. N.; Kozhevnikov, G. O.; Braudo, E. E. Limited tryptic hydrolysis of pea legumin: molecular mass and conformational stability of legumin-T. *Int.l J. Biol. Macromol.* **2001**, *28*, 175-182.
25. Gueguen, J.C.M.; Barbot J.; Schaeffer F. Dissociation and aggregation of pea legumin induced by pH and ionic strength. *J. Sci. Food Agric.* **1988**, *53*, 167-182.
26. Heng, L.; Van Koningsveld, G.A.; Gruppen, H.; Van Boekel, M.A.J.S.; Vincken J.P.; Roozen, J.P.; Voragen, A.G.J. Protein-flavour interactions in relation to development of novel protein foods. *Trends Food Sci. Technol.* **2004**, *15*, 217-224.
27. Shewry, P. R.; Napier, J. A.; Tatham, A. S. Seed storage proteins: Structures and biosynthesis. *Plant Cell* **1995**, *7*, 945-956.
28. Griga, M;. Horáček, J.; Klenotičová, H. Protein patterns associated with *Pisum sativum* somatic embryogen*esi*s. *Biol. Plantarum* **2007**, *51*, 201-211.
29. Casey, R.; Domoney, C. The biochemical genetics of pea seed storage proteins. *Kulturpflanze* **1984**, *32*, S99-S108.
30. Kimura, A.; Fukuda, T.; Zhang, M.; Motoyama, S.; Maruyama, N.; Utsumi S. Comparison of physicochemical properties of 7S and 11S globulins from pea, fava bean, cowpea, and French Bean with those of Soybean. French Bean 7S globulin exhibits excellent properties. *J. Agric. Food Chem.* **2008**, *56*, 10273-10279.
31. O'Kane, F.E.; Happe, R.P.; Vereijken, J.M.; Gruppen, H.; Van Boekel, M.A.J.S. Characterization of pea vicilin. 1. Denoting convicilin as the α-subunit of the pisum vicilin family. *J. Agric. Food Chem.* **2004**, *52*, 3141-3148.
32. Guleria, S.; Dua, S.; Chongtham, N. Analysis of variability in different genotypes of pea (*Pisum sativum L.)* on the basis of protein markers. *Legume Res.* **2009**, *32*, 265-269.
33. Wang, N.; Hatcher, D. W.; Warkentin, T. D.; Toews, R. Effect of cultivar and environment on physicochemical and cooking characteristics of field pea (*Pisum sativum*). *Food Chem.* **2010** *118*, 109-115.

34. Nikolopoulou, D.; Grigorakis, K.; Stasini, M.; Alexis, M. N.; Iliadis, K. Differences in chemical composition of field pea (*Pisum sativum*) cultivars: Effects of cultivation area and year. *Food Chem.* **2007**, *103*, 847-852.
35. Schroeder, H.E: Quantitative studies on the cotyledonary proteins in the genus *Pisum*. *J. Agric. Food Chem.* **1982**, *33*, 623-633.
36. Cserhalmi, Z.; Czukor, B.; Gajzágó-Schuster, I. Emulsifying properties, surface hydrophobicity and thermal denaturation of pea protein fractions. *Acta Aliment. Hung.* **1998**, *27*, 357-363.
37. Rangel, A.; Domont, G.B.; Pedrosa, C.; Ferreira, S.T. Functional properties of purified vicilins from cowpea (*Vigna unguiculata*) and pea (*Pisum sativum*) and cowpea protein isolate. *J. Agric. Food Chem.* **2003**, *51*, 5792-5797.
38. Bora, S.P.; Breeke, J.C.; Powers, R.J. Heat induced gelation of peas (*Pisum sativum*) mixed globulins, vicilin and legumin. *J. Food Sci.* **1994**, *59*(3), 594-596.
39. O'Kane, E.: O'Kane, E. Molecular Characterisation and Heat-Induced Gellation of Pea Vicilin and Legumin.Ph.D. Thessis, Wageningen University, November 2004.
40. Gueguen, J.; Cerletti, P. Protein of some legume seeds: soybean, pea, fababean and lupin. In *Developments in food proteins;* Hudson, B. J. Ed.; Elsevier Applied Science: London, 1994; pp 145-156.
41. Kinsella J.E.: Relationships between structure and functional properties of food proteins. In *Food proteins*; Fox, P.F., Condon, J.J. Eds.; Applied Science Publishers LT: England, 1982; pp 51-78.
42. Belitz, H.D.; Grosch, W.; Schieberle, P. *Food Chemistry*, Springer-Verlag, Berlin, 2009; pp 65- 95.
43. Pesic, M.B.; Vucelic-Radovic, B.V.; Barac, M.B.; Stanojevic, S.P. The influence of genotypic variation in protein composition on emulsifying properties of soy proteins. *J. Am. Oil Chem. Soc.* **2005**, *82*, 667-672.
44. Barac, M.; Cabrilo, S.; Stanojevic, S.; Pesic, M.; Pavlicevic, M.; Zlatkovic, B.; Jankovic M. Functional properties of protein hydrolysates from pea (*Pisum sativum, L.*) seeds. *Int. J. Food Sci. Technol***. 2012**, *47*, 1457-1467.
45. Aluko, R.E.; Mofolasayo, O.A.; Watts, B.M. Emulsifying and foaming properties of commercial yellow pea (*Pisum sativum L.*) seed flours. *J. Agric. Food Chem.* **2009**, 57, 9793-9800.
46. Tömösközi, S.; Lásztity, R.; Haraszi, R.; Baticz O. Isolation and study of the functional properties of pea proteins. *Nahrung* **2001**, *45*, 399-401.
47. Ferreira, R.B.; Franco, E.; Teixeira, A.R. Calcium- and magnesium-dependent aggregation of legume seed storage proteins. *J. Agric. Food Chem*. **1999**, *47*, 3009-3015.
48. Yan, B.; Ren, J.; Zhao, M.; Luo, D.; Gu, L. Effects of limited enzymatic hydrolysis with pepsin and high-pressure homogenization on the functional properties of soybean protein isolate. *LWT- Food Sci. Technol*. **2012**, *46*, 453-459.
49. Habiba, R.A. Changes in anti-nutrients, protein solubility, digestibility, and HCl-extractability of ash and phosphorus in vegetable peas as affected by cooking methods. *Food Chem.* **2002**, *77*, 187-192.
50. Dagorn-Scaviner, C.; Gueguen, J.; Lefebvre J. Emulsifying properties of pea globulins as related to their adsorption behaviours. *J. Food Sci.* **1987**, *52*, 335-341.

51. Gharsallaoui, A.; Cases, E.; Chambin O.; Saurel R. Interfacial and emulsifying characteristics of acid-treated pea protein. *Food Biophys.* **2009**, *4*, 273-280.
52. McWaters, K.H.; Cherry, J.P. Emulsification, foaming and protein stability properties of deffated soybean, peanut, field pea and pecan flours. *J.Food Sci.* **1977**, *42*, 444-461.
53. Vose, J. R. Production and functionality of starches and protein isolates from legume seeds (field peas and horsebeans). *Cereal Chem.* **1980**, *57*, 406-410.
54. Adebiyi, A.P.; Aluko, R.E. Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum Sativum L.*) seed protein isolate. *Food Chem.* **2011**, *128*, 902-908.
55. Fuhrmeister, H.; Meuser, F. Impact of processing on functional properties of protein products from wrinkled peas. *J. Food Eng.* **2003**, *56*, 119-129.
56. Toews, R.; Wang N. Physicochemical and functional properties of protein concentrate from pulses. *Food Res. Int.* **2013**, *52*, 445-451.
57. Pedrosa, C.; Trisciuzzi, C.; Ferreira, S.T. Effects of glycosylation on functional properties of vicilin, the 7S storage globulin from pea (*Pisum sativum*). *J. Agric. Food Chem*. **1997**, *45*, 2025-2030.
58. Raikos, V.; Campbell, L.; Euston, S.R. Rheology and texture of hen's egg protein heat-set gels as affected by ph and the addition of sugar and/or salt. *Food Hydrocolloids* **2007**, *21*, 237-244.
59. Shand, P.J.; Ya, H.; Pietrasik, Z.; Wanasundara, P.K.J.P.D. Physicochemical and textural properties of heat-induced pea protein isolate gels. *Food Chem.* **2007**, *102*, 1119-1130.
60. Nunes, M. C.; Raymundo, A.; Sousa, I. Gelled vegetable desserts containing pea protein, K-carrageenan and starch. *Eur. Food Res. Technol.* **2006**, *222*, 622-628.
61. Barać, M.; Stanojević, S.; Jovanović, S.; Pešić M. Soy protein modification - A review. *APTEFF* **2004**, *35*, 3-17.
62. Adler-Nissen J. Some fundamental aspects of food protein hydrolysis. In *Enzymatic hydrolysis of food proteins;* Adler-Nissen, J. Ed; Elsevier Eplied Science: London, 1986; pp 9-24.
63. Wu, W.; Hettiarachchy, N.; Qi, M. Hydrophobicity, solubility, and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. *J. Am. Oil Chem. Soc.* **1998**, *75*, 845-850.
64. Martinez, K. D.; Carrera Sanchez, C.; Rodriguez Patino, J. M.; Pilosof, A. M. R. Interfacial and foaming properties of soy protein and their hydrolysates. *Food Hydrocolloids* **2009**, *23*, 2149-2157.
65. Chen, L.; Chen, J.; Ren, J.; Zhao, M. Effects of ultrasound pretreatment on the enzymatic hydrolysis of soy protein isolates and on the emulsifying properties of hydrolysates. *J. Agric. Food Chem*. **2011**, *59*, 2600-2609.
66. Hou, Y.; Zhao, X. H. Limited hydrolysis of two soybean protein products with trypsin or neutrase and the impacts on their solubility, gelation and fat absorption capacity. *Biotechnology* **2011**, *10*, 190-196.
67. Jung, S.; Murphy, P. A.; Johnson, L. A. Physicochemical and functional properties of soy protein substrates modified by low levels of protease hydrolysis. *J. Food Sci.* **2005**, *70*, C180-C187.

68. Molina Ortiz, S.; Cristina A.N.M. Analysis of products, mechanisms of reaction, and some functional properties of soy protein hydrolysates. *J. Am. Oil Chem. Soc.* **2000**, *77*, 1293-1301.
69. Periago, M.J.; Vidal, M.L.; Ros, G.; Rincón, F.; Martínez, C.; López, G.; Rodrigo, J.; Martínez I. Influence of enzymatic treatment on the nutritional and functional properties of pea flour. *Food Chem.* **1998**, *63*, 71-78.
70. Le Gall, M., Gueguen, J.; Seve, B.; Quillien, L. Effects of Grinding and thermal treatments on hydrolysis susceptibility of pea proteins (*Pisum sativum L.*). *J. Agri. Food Chem.* **2005**, *53*, 3057-3064.
71. Humiski, L. M.; Aluko, R. E. Physicochemical and bitterness properties of enzymatic pea protein hydrolysates. *J. Food Sci.* **2007**, *72*, S605-S611.
72. Pownall, T. L.; Udenigwe, C. C.; Aluko, R. E. Amino acid composition and antioxidant properties of pea seed (*Pisum sativum L.*) enzymatic protein hydrolysate fractions. *J. Agri. Food Chem.* **2010**, *58*, 4712-4718.
73. Spencer, D.; Higgins, T.J.V.; Preer M.; Dove, H.; Coombe J.B. Monitoring the fate of dietary proteins in rumen fluid using gel electrophoresis. *Br. J. Nutr.* **1988**, *60*, 241-247.
74. Crévieu, I.; Carré, B.; Chagneau, A.M.; Quillien, L.; Guéguen, J.; Bérot, S. Identification of resistant pea (*Pisum stivum L.*) proteins in the digestive tract of chickens. *J. Agric. Food Chem.* **1997**, *45*, 1295-1300.
75. Gabriel, I.; Quillien, L.; Cassecuelle, F.; Marget, P.H.; Juin, H.; Lessire, M.; Sève, B.; Duc, G.; Burstin, J. Variation in seed protein digestion of different pea (*Pisum sativum L.*) genotypes by cecectomized broiler chickens: 2. Relation between in vivo protein digestibility and pea seed characteristics, and identification of resistant pea polypeptides. *Livest. Sci.* **2008**, *113*, 262-273.
76. Govindaraju, K.; Srinivas, H. Controlled enzymatic hydrolysis of glycinin: usceptibility of acidic and basic subunits to proteolytic enzymes. *LWT – Food Sci. Technol.* **2007**, *40*, 1056-1065.
77. Braudo, E.E.; Danilenko, A.N.; Guslyannikov, P.V.; Kozhevnikov, G.O.; Artykova, G.P.; Lapteva, N.A.; Vaintraub, I.A.; Sironi, E.; Duranti, M. Comparative effects of limited tryptic hydrolysis on physicochemical and structural features of seed 11S globulins, *Int. J. Biol. Macromol.* **2006**, *39*, 174-178.
78. Soral-Smietena, M.; Swigon, A.; Amarowicz, R.; Sijtsma, J. The solubility of trypsin pea hydrolysates. *Nahrung* **1998**, *42*, 217-218.

APTEFF, 46, 1-269 (2015) UDC: 633.35:577.112:543.645
DOI: 10.2298/APT1546001B BIBLID: 1450-7188 (2015) 46, 1-18
Review paper

# ТЕХНО-ФУНКЦИОНАЛНЕ ОСОБИНЕ ИЗОЛАТА ПРОТЕИНА ГРАШКА

*Мирољуб Б. Бараћ[1], Мирјана Б. Пешић[1], Слађана П. Станојевић[1], Александар Ж. Костић[1], Славица Б. Чабрило[2]*

[1] Универзитет у Београду, Пољопривредни факултет, Немањина 6, 11080 Београд-Земун, Србија
[2] Виша техничка школа струковних студија, Немањина 2, 12000 Пожаревац, Србија

Захваљујући високој нутритивној вредности, добрим техно-функционалним карактеристикама и ниској цени, протеини легуминоза постају најприхватљивија алтернатива за протеинске производе анималног порекла. У индустрији хране ови производи најчешће се користе као техно-функционални адитиви којима се обезбеђује нека од карактеристика финалног производа. Протеини легуминоза најчешће се користе као протеинска брашна, концентрати и изолати. У индустријским размерама највећу примену имају протеини соје и у знатно мањој мери, у последњих 20 година, протеински изолати грашка. Ређа употреба протеина грашка делом је последица још увек недовољно информација о њиховим техно-функционалним карактеристикама. Овај рад представља преглед техно-функционалних карактеристика протеина грашка и његових изолата. Такође, у овом раду разматра се и делимична протеолиза као метод за побољшање техно-функционалних карактеристика протеина грашка.

**Кључне речи:** протеински изолати грашка, техно-функционалне карактеристике, ограничена протеолиза

Received: 9 February 2015.
Accepted: 15 July 2015.