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LIMITED PROTEOLYSIS AS A MEANS TO REDUCE THE ALLERGENICITY OF SEED STORAGE GLOBULINS

(review)

A.M. CHERDIVARĂ

Moldova State University, Republic of Moldova, MD-2009, Chişinău, Mateevich str., 60, e-mail alacherdivara@mail.ru (✉ corresponding author)
ORCID:
Cherdivară A.M. orcid.org/0000-0003-1276-4959
The author declares no conflict of interests
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Abstract

According to SDAP (structural database of allergenic proteins, http://fermi.utmb.edu/), storage 11S and 7S globulins from seeds of peanut, soybean and some other plants are allergens. A β-barrel conjoined with a group of α-helices represents the structural basis of domains of the two-domain 11S and 7S seed storage globulins. During evolution, extended disordered inserts of enhanced susceptibility to proteolytic attack appeared in the amino acid sequences of storage globulins outside the β-barrel-α-helix structural module. Regularities of storage globulin limited proteolysis during seed germination and in vitro are determined by these inserts. In this review, available information on successive reactions of limited proteolysis specific to 11S and 7S globulins from peanut, soybean and some other plants is collected. It was demonstrated that limited proteolysis of 11S globulin from peanut (A. Cherdivară et al., 2017) calls forth destruction of a C-terminal region of α-chains, including the region forming the group of α-helices. Three of the four antigen determinants (IgE epitopes) identified in the peanut subunit Ara h3 (P. Rabjohn et al., 1999) belong to this region. Thus, the limited proteolysis leads to a significant decrease in the allergenicity level of the subunit Ara h3. The presence of IgE epitopes in homologous regions of conserved sequences of α-helices from most other subunits of the peanut 11S globulin, non-identical to Ara h3, is very probable. Thus, the limited proteolysis of not only the Ara h3 subunit, but also the whole hetero-hexamer molecule of peanut 11S globulin can be accompanied by a significant decrease in the level of its allergenicity. Prospects for reducing allergenicity of soybean 11S globulin by limited proteolysis are not so unambiguous. On the one hand, the limited proteolysis of the subunit Gly m G1 leads to the destruction of the C-terminal region of the α-chains (A. Shutov et al., 2012), where both identified IgE epitopes are present (T.A. Beardslee et al., 2000). On the other hand, only one of the IgE epitopes identified in the Gly m G2 subunit (R.M. Helm et al., 2000) can be removed using limited proteolysis of this protein (A. Shutov et al., 1993). The detachment of the α-chain α-helices during limited proteolysis of several other 11S globulins was observed as well. A high degree of conservation of this region in the primary structures of 11S globulins allows suggesting the presence of IgE epitopes, similar to those identified in peanut and soybean 11S globulins, in many other storage proteins of the 11S globulin family. Prospects for the reduction of allergenicity of seed 7S globulins by limited proteolysis are advantageous as well. Limited proteolysis of peanut 7S globulin Ara h1 starts with complete destruction of a disordered N-terminal extension (A. Cherdivară et al., 2016), which contains one third of IgE epitopes identified in the amino acid sequence of this protein (D.S. Shin et al., 1998). Further limited proteolysis calls fourth destruction of another disordered region inside the N-terminal domain that contains an additional IgE epitope identified in the Ara h1 sequence. Summary information considered in the review on the structure of seed storage globulins, as well as on the IgE epitopes identified in their amino acid sequences, evidences the availability of limited proteolysis as a means of considerable reduction of the level of allergenicity, not only of peanut and soybean 11S and 7S globulins, but also of those from other plants whose seeds are used as food either directly or as additives to various food products.

Keywords: seed storage globulins, proteolysis, allergenicity, IgE epitopes, *Arachis hypogaea* L., peanut, *Glycine max* L., soybean

The main part of food plant proteins is seed storage proteins. The overwhelming number of seed storage proteins are grouped in two conserved fami-

lies, the 7S (vicilins) and 11S (legumins) globulins [1]. Their amino acid sequences are inherited from the vicilin- and legumin-like proteins of spore plants [2]. In turn, the latter derived from bacterial oxalate decarboxylase [3]. All these proteins belong to the extensive cupin superfamily, combining dozens of functionally diverse families of proteins, in the structure of which there is a β-barrel from antiparallel β-strands [4].

The tertiary structure of subunits of oligomeric molecules of oxalate decarboxylases and storage globulins is supplemented by a group of α-helices [2]. In the subunits of these proteins, there are two domains, each of which is formed by a structural module of the β-barrel-α-helix. The two-domain structure of oxalate decarboxylases was formed at an early evolutionary stage as a result of duplication of this module which is present in their single-domain bacterial precursor [3].

In the course of evolution, extended hydrophilic variable insertions, taken to the surface of their oligomeric structures, appeared in the amino acid sequences of seed storage globulins, conserved ones on a whole [2, 3]. These insertions determine the sensitivity of native molecules of storage globulins to fast and limited proteolysis, from which their degradation begins in germinating seeds and in vitro [2]. The subsequent massive proteolysis of storage globulins occurs by a sequential mechanism [5], leading to the complete destruction of their molecules [3].

In seeds of peanut [6-8], soybean [9-11] and many other cultivated plants used in the dietary intake, e.g. nuts [12-14], almonds [15] and mustard [16], storage 7S and 11S globulins are allergens. This information, as well as some additional data, is included in the SDAP database (Structural Database of Allergenic Proteins) (http://fermi.utmb.edu/) [17]. In some of the storage globulins, 7S globulins of peanut [18] and lentils [19], 11S globulins of peanut [20], soybean [21, 22] and buckwheat [23], antigenic determinants (IgE epitopes) responsible for IgE binding are identified. Many of the IgE epitopes belong to the regions of storage globulins sequences with elevated sensitivity to proteolytic attack, which shows the principal possibility of reducing the allergenicity of seed storage globulins by means of their limited proteolysis.

In this review, to confirm this hypothesis, the experimental data obtained in the study of limited proteolysis of storage globulins of peanut seeds (*Arachis hypogaea* L.), soybean (*Glycine max* L.) and some other plants, have been analyzed.

Limited proteolysis of 11S globulins and IgE epitopes, identified in their sequences. The structure of the α-chains of the subunit Ara h3 (pdb|3c3v) of peanut 11S globulin [24], typical for 11S globulins of soybean seeds [25, 26] and a number of other plants [27, 28], is formed by a β-barrel of antiparallel β-strands BCDEFGHI, connected to the group of α-helices h1, h2, h3, and complemented by β-strands Z, A′-A, E′-F′ and J-J′, as well as by α-helices h0 and h1′. Three hydrophilic disordered regions in the α-chains of 11S globulins are potentially sensitive to limited proteolysis [2]: the loop between the β-strands E and F (E′-F′), the loop between the β-barrel and the α-helices and the C-terminal segment (Fig. 1, Table 1, respectively, the regions a, b and c).

For 11S globulins, the following sequence of limited proteolysis reactions is characteristic [2]. The process begins with the cleavage of the hydrophilic C-terminal region, sensitive to proteolysis (see Fig. 1, Table 1, region c). The further action of proteinases leads to splitting of the loop between the β-barrel and the α-helices (see Fig. 1, Table 1, region b). Depending on the individual features of the structure of 11S globulin and the specificity of proteinase, the loop

between the β-strands E and F can either remain intact or split (see Fig. 1, Table 1, region a).

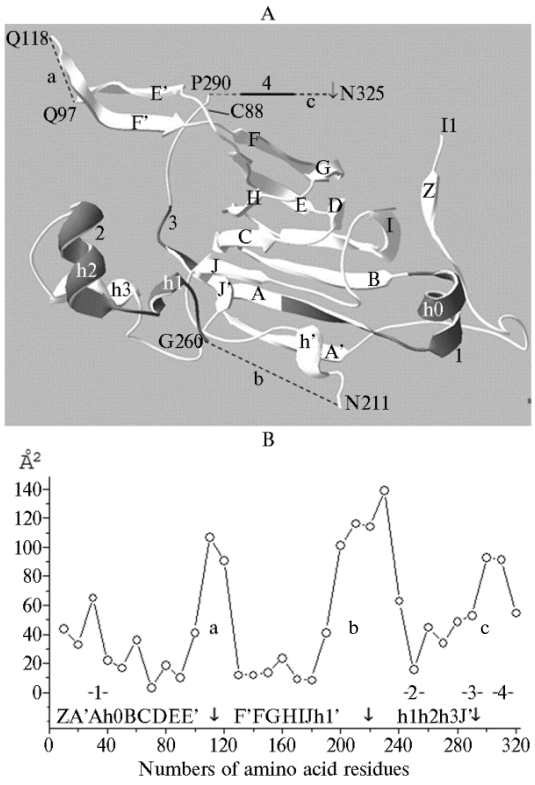

Рис. 1. Третичная структура α-цепи 11S Fig. 1. Tertiary structure of the α-chain of peanut 11S globulin (*Arachis hypogaea*) Ara h3 (pdb|3c3v).

A — the ribbon diagram. Non-ordered regions (a, b, c) in the α-chains of 11S globulins are potentially sensitive to limited proteolysis [2]. The arrow marks the peptide bond N325-G326which is splitted upon maturation of the Ara h3 molecule. The Cys88 residue participates in the formation of a disulfide bond between the α- and β-chains. The dark sections of the diagram correspond to sequences of IgE epitopes 1-4.

B — available to the solvent area of the amino acid residues ASA (accessibility surface area) in the sequence of the α-chain. The reported ASA values [31], expressed in Å^2, correspond to the average values calculated for groups of 10 residues in the amino acid sequence of the α-chain. The graph corresponds to the model quaternary structure of the homohexamer Ara h3 constructed using the pdb|3c3v [31] monomer template. Arrows indicate the location of the cleavage points of the α-chain with limited hydrolysis by trypsin [32]. Figures show the position of IgE epitopes 1-4 in the amino acid sequence Ara h3.

The position of the points of splitting of the α-chains of 11S globulins is determined by the results of N-terminal sequencing of fragments [29, 30] or by the combination of indirect data (specificity of the proteinase, sequence of fragments formation and their molecular weights). The potential sensitivity to the limited proteolysis of disordered regions (see Fig. 1, a, b, c) in the amino acid sequences of 11S globulins is indicated by their relatively high availability to the ASA solvent, shown in the example of Ara h3 (see Fig. 1, B).

The cleavable C-terminal region, encompassing the region of the α-helices h1h2h3 and the β-strand J' (see Table 1), is destroyed to short peptides, which is characteristic not only of Ara h3 [31] but also of other 11S globulins [2]. It should be noted that the N-terminal region of the α-chain

1. Sites of α-chain cleavage (↓) with limited proteolysis of storage 11S globulins of soybean *Glycine max* Gly m G1-G5, peanuts *Arachis hypogaea* Ara h3 and abl14270 (ARAhy), sunflower *Helianthus annuus* aaa33374 (HELan), oat *Avena sativa* aaa32720 (AVEsa), cedar *Pinus sibirica* caa77569 (PINsi) and pumpkin *Cucurbita maxima* pdb|2e9q (CUCma)

Secondary structure A′A-BCD--E--FGHIJ----h1 h2h3J′---- a b c	Subunit	Proteinase
↓ ↓	Gly m G1-G5	Papain [30]
** **↓ * ↓*	Gly m G2	Trypsin [34]
** ↓ **↓ * ↓*	Gly m G2	Trypsin [32]
↓ ↓	Gly m G5	Papain [35]
* ↓ ↓ * ↓**	Ara h3	Papain [31]
↓ ↓ ↓	ARAhy	Trypsin [32]
↓ ↓ ↓	HELan	Papain [36]
↓ ↓ ↓	AVEsa	Papain [37]
↓ ↓	PINsi	Papain [38]
↓ ↓	CUCma	Papain [39]

N o t e. The region h1h2h3J' that is destructed in the limited proteolysis is underlined. The asterisks indicate the position of IgE epitopes identified in the subunits of soybean 11S globulins Gly m G1 [21]and Gly m G2 [22] and peanut Ara h3 [20].

of Ara h3, like all the examined 11S globulins [2], is insensitive to proteolytic attack. Three of the four IgE epitopes identified in the subunit of peanut 11S globulin Ara h3 [20] (see Fig. 1, Table 1) belong to the C-terminal region of the α-chain that degrades by means of hydrolysis with papain [31]. That is, limited proteolysis of the subunit Ara h3 can lead to a significant reduction in its allergenicity.

The hetero-hexamer molecule of peanut 11S globulin contains nine subunits, highly conserved in the C-terminal region of the α-chains where three of the four IgE epitopes Ara h3 are present (see Figs. 1, 2, Table 1). The potential ability to binding of IgE to each of the sequences of other subunits of peanut 11S globulin, homologous to the second and third IgE epitopes Ara h3, can be judged from the results of the definition of PD indexes (property-based peptide similarity index for two sequences) [40]. The method is based on a comparison of the physical and chemical properties of each of the amino acids of the IgE epitope sequence, identified in the protein allergen, with each of the amino acids, which are close in the primary structure of the region of the examined protein's sequence. As the number of differences between the segments of these sequences increases, the value of PD indexes increases from 0 (the sequences are identical) to the limiting value of 10 above which the presence of the corresponding IgE epitope is unlikely [40].

The presence of the second and third IgE epitopes Ara h3 (see Fig. 1, B) in the homologous regions of the sequences of six of the eight subunits of peanut 11S globulin, not identical to Ara h3 [31], is considered very likely (the corresponding PD indexes do not exceed 2.9). Finally, the sequences of the fourth IgE epitope Ara h3 (see Fig. 1, B) and the corresponding putative IgE epitopes in the other two subunits of peanut 11S globulin (aag01363 and abf93402) are identical. That is, the limited proteolysis of not only the subunit Ara h3, but also the whole hetero-hexamer molecule of peanut 11S globulin can be accompanied by a significant decrease in its allergenicity.

Fig. 2. Amino acid sequences of the C-terminal region of the α-chains of 11S globulin of peanut *Arachis hypogaea* (A1 — pdb|3c3v, A2 — abl14270) and soybean *Glycine max* (G1 — pdb|1fxz; G2 — baa00154). The figures on the left correspond to the numeration of amino acid residues in the complete sequences of the subunits. The lower lines show identical amino acid residues (*) and their conserved (:) and semi-conserved (.) substitutions. The arrows correspond to peptide bonds, which are cleavable by trypsin (↓) [29, 32, 34] and papain (↑) [30, 32]. The sequences of IgE epitopes are indicated in bold, and the amino acid residues Arg and Lys of increased accessibility to the solvent (ASA >100 Å) in the model oligomeric structures A1 and G1 are underlined.-

Prospects for reducing the allergenicity of soybean 11S globulin by means of limited proteolysis are not so unambiguous. On the one hand, the limited proteolysis of the subunit Gly m G1 leads to the removal of the region of α-helix h1h2h3-β-strand J', where both identified IgE epitopes are present (see Table 1, Fig. 2). The presence of two corresponding IgE epitopes in homologous sequences of the subunits Gly m G3, Gly m G4, and Gly m G5 is not excluded

(PD indexes from 0 to 5.9). On the other hand, only one of the IgE epitopes identified in soybean 11S globulin Gly m G2 (see Table 1) can be removed by means of its limited proteolysis by papain (see Fig. 2). In this case, the presence of the corresponding IgE epitope in the homologous regions of the sequences of other subunits of soybean 11S globulin is unlikely (the value of PD indexes is close to 10).

Limited proteolysis of 7S globulins and IgE epitopes identified in the sequence of peanut 7S globulin Ara h1. The N- and C-terminal domains of the subunits of 7S globulins are structurally equivalent to the α- and β-chains of 11S globulins, but differ from the latter in several features [41], i.e. the interdomain linker region in 7S globulins is not split, and their structures lack an interdomain disulfide bond. In the 7S globulins of the known tertiary structure from the seeds of jack bean [42], bean [43], soybean [44], cowpea [45, 46] and pine [47], there is a number of disordered regions, which are potentially sensitive to limited proteolysis [2] (Table 2, Fig. 3).

2. Sites of cleavage (↓) of the N-terminal domain with limited proteolysis of seed storage 7S globulins of *Arachis hypogaea* Ara h1, soybean *Glycine max* Gly m α, Gly m α′, Gly m β, and bean *Phaseolus vulgaris* (PHAvu)

Secondary structure ----------Z-A′ABCDEFGHIJ-h1-h2-h3--J′h4- a b c	Subunit	Proteinase
******↓↓ * * * ↓* ↓	Ara h1	Papain [50]
↓ ↓	Gly m α	In vivo [51]
↓ ↓		C2 [52]
↓ ↓	Gly m α′	Trypsin [32]
↓ ↓		C2 [52]
↓ ↓	Gly m β	In vivo [51]
↓ ↓		CPPh [53]
↓ ↓		Trypsin [54]
↓ ↓	PHAvu	In vivo [55]
↓ ↓		CPPh [53, 56, 57]
↓ ↓		Trypsin [58]
↓ ↓		C2 [52]
↓ ↓		LLP [56, 57]

N o t e. The asterisks indicate the position of IgE epitopes identified in the Ara h1 subunit [18]. C2 and CPPh are endogenous papain-like proteinases from germinating seeds of soybean and bean, respectively.

The variable N-terminal extension of the N-terminal domain (see Table 2, region a), characteristic of 7S globulins of the convicilin type, shows the greatest sensitivity to proteolysis [2]. The region between the α-helix h3 and the β-strand J′ (see Table 2, region b), which is not non-ordered in all 7S globulins, is relatively short; there is no information on its sensitivity to proteolysis. The exception is the peanut 7S globulin Ara h1, in which this region is elongated [48, 49] and cleaved by hydrolysis with papain (see Table 2, see Fig. 3, B). The unregulated region of the interdomain linker is sensitive to proteolysis in all the investigated 7S globulins [2] (see Table 2, region c). Finally, the elongated loop between the β-strands E and F in the C-terminal domain of 7S globulins is known to be sensitive to the limited proteolysis [2].

In the subunit of Ara h1 peanut 7S globulin, exhibiting the greatest allergenicity among the storage seed globulins [59], the presence of 21 IgE epitopes [18] is established. IgE epitopes 1-3 belong to the N-terminal sequence deleted post-translationally [60]. IgE epitopes 4-9 (see Fig. 3, A) localized in the sensitive region of the N-terminal extension (see Fig. 3, B, region a), which is replete with residues corresponding to the substrate specificity of papain [61], are removed at the initial attack by this enzyme [50]. The subsequent action of papain leads to the destruction of the sensitive region between the α-helix h3 and the β-strand J′ (see Table 2, region b), specifically elongated in the Ara h1 sequence, where the IgE epitope 13 is localized (see Fig. 3, A).

It should be noted that there is a relatively high availability for the solvent in the α-helix region h1-h3, which is 3.5 times higher than the ASA for the rest of the N-terminal domain Ara h1. Therefore, it is tempting to try to find

conditions for limited proteolysis that ensure the destruction of this potentially sensitive region of the α-helix, where IgE epitopes 11 and 12 are present (see Fig. 3, A).

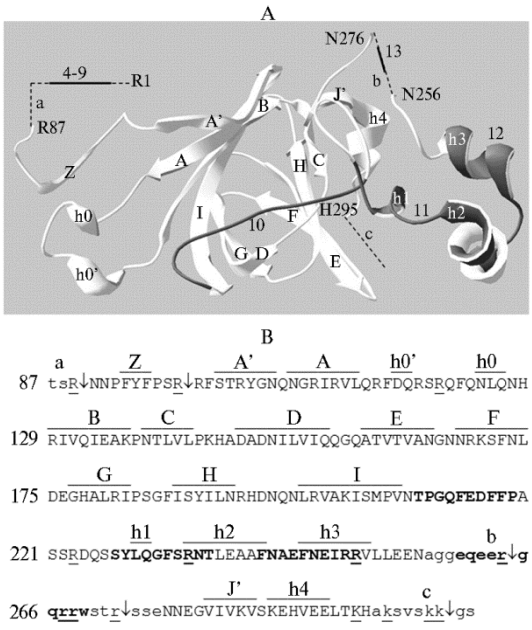

Fig. 3. Structure of the N-terminal domain of peanut 7S globulin Ara h1 (pdb|3smh).

A — the ribbon diagram of a tertiary structure. The disordered regions a, b, and c are shown by dashed lines. The dark sections of the diagram correspond to sequences of IgE epitopes 4-13 in the mature molecule Ara h1 [18].

B — the primary structure. Non-ordered regions: a — N-terminal elongation, b — between the α-helix h3 and β-strand J′, c — C-terminal region. The arrows correspond to peptide bonds cleavable by papain [50]. The amino acid residues with increased availability for the solvent (ASA > 100 Å), corresponding to the sub-stratified specificity of papain [61], are underlined. These residues are present in the crystalline structure of the pdb|3smh oligomer, as well as its model (pdb|3s7e as a template) [50] in regions b and c. The sequences of IgE epitopes 10-13 are in bold.

The subunits of peanut 7S globulin in the composition of its heterotrimeric molecule are extremely conserved. It is very likely that IgE epitopes, identified in Ara h1, are also present in other subunits of this protein: the corresponding PD indexes do not exceed 2.5. Limited proteolysis by papain leads to a significant reduction in the allergenicity of the whole hetero-oligomeric molecule of peanut 7S globulin, in connection with the removal of more than a third of the IgE epitopes.

Thus, the reviewed herein data on limited proteolysis of 11S and 7S globulins of peanut, soybean and some other plants suggest that this method is promising for substantially reducing the allergenicity of storage seed globulins.

REFERENCES

1. Dunwell J.M. Structure, function, and evolution of vicilin and legumin seed storage proteins. In: *Biotechnology of biopolymers - from synthesis to patents*. A. Steinbьchel, Y. Doi (eds.). Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2005: 967-997.
2. Shutov A.D., Wilson K.A. Seed storage globulins: their descent from bacterial ancestors and mechanisms of degradation. In: *Globulins: biochemistry, production and role in immunity*. S.D. Milford (ed.). Nova Science Publishers, NY, 2014: 71-104.
3. Rudakova A.S., Cherdivar A.M., Wilson K.A., Shutov A.D. Seed storage globulins: origin and evolution of primary and higher order structures. *Biochemistry (Moscow)*, 2015, 80: 1354-1361 (doi: 10.1134/S000629791510017X).
4. Dunwell J.M., Culham A., Carter C.E., Sosa-Aguirre C.R., Goodenpugh P.W. Evolution of functional diversity in the cupin superfamily. *Trends Biochem. Sci.*, 2001, 26: 740-746 (doi: 10.1016/S0968-0004(01)01981-8).
5. Rupley J.A. Susceptibility to attack by proteolytic enzymes. *Method. Enzymol.*, 1967, 11: 905-917.
6. Hourihane J.O. Peanut allergy — current status and future challenges. *Clin. Exp. Allergy*, 1997, 27: 1240-1246 (doi: 10.1111/j.1365-2222.1997.tb01167.x).
7. Kleber-Janke T., Crameri R., Appenzeller U., Schlaak M., Becker W.M. Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. *Int. Arch. Allergy Imm.*, 1999, 119: 265-274 (doi: 10.1159/000024203).
8. Al-Muhsen S., Clarke A.E., Kagan R.S. Peanut allergy: an overview. *Can. Med. Assoc. J.* (CMAJ), 2003, 168: 1279-1285.
9. Helm R.M., Cockrell G., Connaughton C., Sampson H.A., Bannon G.A., Beilinson V., Living-

stone D., Nielsen N.C., Burks A.W. A soybean G2 glycinin allergen. 1. Identification and characterization. *Int. Arch. Allergy Imm.*, 2000, 123: 205-212 (doi: 10.1159/000024445).
10. Krishnan H.B., Kim W.S., Jang S., Kerley M.S. All three subunits of soybean beta-conglycinin are potential food allergens. *J. Agr. Food Chem.*, 2009, 57: 938-943 (doi: 10.1021/jf802451g).
11. Wang T., Qin G.X., Sun Z.W., Zhao Y. Advances of research on glycinin and β-conglycinin: a review of two major soybean allergenic proteins. *Crit. Rev. Food Sci.*, 2014, 54: 850-862 (doi: 10.1080/10408398.2011.613534).
12. Beyer K., Grishina G., Bardina L., Grishin A., Sampson H.A. Identification of an 11S globulin as a major hazelnut food allergen in hazelnut-induced systemic reactions. *J. Allergy Clin. Immunol.*, 2002, 110: 517-523 (doi: 10.1067/mai.2002.127434).
13. Wang F., Robotham J.M., Teuber S.S., Sathe S.K., Roux K.H. Ana o 2, a major cashew (*Anacardium occidentale* L.) nut allergen of the legumin family. *Int. Arch. Allergy Imm.*, 2003, 132: 27-39 (doi: 10.1159/000073262).
14. Sharma G.M., Irsigler A., Dhanarajan P., Ayuso R., Bardina L., Sampson H.A., Roux K.H., Sathe S.K. Cloning and characterization of an 11S legumin, Car i 4, a major allergen in pecan. *J. Agr. Food Chem.*, 2011, 59: 9542-9552 (doi: 10.1021/jf2017447).
15. Willison L.N., Tripathi P., Sharma G., Teuber S.S., Sathe S.K., Roux K.H. Cloning, expression and patient IgE reactivity of recombinant Pru du 6, an 11S globulin from almond. *Int. Arch. Allergy Imm.*, 2011, 156: 267-281 (doi: 10.1159/000323887)
16. Palomares O., Cuesta-Herranz J., Vereda A., Sirvent S., Villalba M., Rodrigues R. Isolation and identification of an 11S globulin as a new major allergen in mustard seeds. *Annals of Allergy, Asthma and Immunology*, 2005, 94: 586-592 (doi: 10.1016/S1081-1206(10)61138-6).
17. Ivanciuk O., Schein C.H., Braun W. SDAP: database and computational tools for allergenic proteins. *Nucleic Acids Res.*, 2003, 31: 359-362 (doi: 10.1093/nar/gkg010).
18. Shin D.S., Compadre C.M., Maleki S.J., Kopper R.A., Sampson H., Huang S.K., Burks A.W., Bannon G.A. Biochemical and structural analysis of the IgE binding sites on Ara h 1, an abundant and highly allergenic peanut protein. *J. Biol. Chem.*, 1998, 273: 13753-13759 (doi: 10.1074/jbc.273.22.13753).
19. Vereda A., Andreae D.A., Lin J., Shreffler W.G., Ibanez M.D., Cuesta-Herranz J., Bardina L., Sampson H.A. Identification of IgE sequential epitopes of lentil (Len c 1) by means of peptide microarray immunoassay. *J. Allergy Clin. Immunol.*, 2010, 126: 596-601 (doi: 10.1016/j.jaci.2010.06.023).
20. Rabjohn P., Helm E.M., Stanley J.S., West C.M., Sampson H.A., Burks A.W., Bannon G.A. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J. Clin. Invest.*, 1999, 103: 535-542 (doi: 10.1172/JCI5349).
21. Beardslee T.A., Zeece M.G., Sarath G., Markwell J.P. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *Int. Arch. Allergy Imm.*, 2000, 123: 299-307 (doi: 10.1159/000053642).
22. Helm R.M., Cockrell G., Connaughton C., Sampson H.A., Bannon G.A., BeilinsonV., Nielsen N.C., Burks A.W. A soybean G2 glycinin allergen. 2. Epitope mapping and three-dimensional modeling. *Int. Arch. Allergy Imm.*, 2000, 123: 213-219 (doi: 10.1159/000024446).
23. Yoshioka H., Ohmoto T., Urisu A., Mine Y., Adachi T. Expression and epitope analysis of the major allergenic protein Fag e 1 from buckwheat. *J. Plant Physiol.*, 2004, 161: 761-767 (doi: 10.1016/j.jplph.2004.01.010).
24. Jin T., Guo F., Chen Y.W., Howard A., Zhang Y.Z. Crystal structure of Ara h 3, a major allergen in peanut. *Mol. Immunol.*, 2009, 46: 1796-1804 (doi: 10.1016/j.molimm.2009.01.023).
25. Adachi M., Takenaka Y., Gidamis A.B., Mikami B., Utsumi S. Crystal structure of soybean proglycinin A1aB1b homotrimer. *J. Mol. Biol.*, 2001, 305: 291-305 (doi: 10.1006/jmbi.2000.4310).
26. Adachi M., Kanamori J., Masuda T., Yagasaki K., Kitamura K., Mikami B., Ursumi S. Crystal structure of soybean 11S globulin: glycinin A3B4 homohexamer. *PNAS USA*, 2003, 100: 7395-7400 (doi: 10.1073/pnas.0832158100).
27. Tandang-Silvas M.R., Carrazco-Pena L., Barba de la Rosa A.P., Osuna-Castro J.A., Utsumi S., Mikami B., Maruyama N. Expression, purification and preliminary crystallization of amaranth 11S proglobulin seed storage protein from *Amaranthus hypochondriacus* L. *Acta Crystallogr. F*, 2010, 66: 919-922 (doi: 10.1107/S1744309110021032).
28. Tandang-Silvas M.R.G., Fukuda T., Fukuda C., Prak K., Cabanos C., Kimura A., Itoh T., Mikami B., Utsumi S., Maruyama N. Conservation and divergence on plant seed 11S globulins based on crystal structures. *Biochim. Biophys. Acta*, 2010, 1804: 1432-1442 (doi: 10.1016/j.bbapap.2010.02.016).
29. Shutov A.D., Kakhovskaya I.A., Bastrygina A.S., Bulmaga V.P., Horstmann C., Müntz K. Limited proteolysis of β-conglycinin and glycinin, the 7S and 11S storage globulins from soybean (Glycine max (L.) Merr.): structural and evolutionary implications. *Eur. J. Biochem.*, 1996, 241: 221-228 (doi: 10.1111/j.1432-1033.1996.0221t.x).
30. Shutov A., Rudakova A., Rudakov S., Kakhovskaya I., Schallau A., Maruyama N., Wilson K. Limited proteolysis regulates massive degradation of glycinin, storage 11S globulin from soybean seeds: an

in vitro model. *J. Plant Physiol.*, 2012, 169: 1227-1233 (doi: 10.1016/j.jplph.2012.06.004).
31. Cherdivară A., Rudakova A., Rudakov S., Şutov A. Alergenul Ara h3, globulina de rezervă din seminţele de arahide. 1. Proteoliza limitată cu papaină [Allergen Ara h3, storage 11S globulin from peanut seeds. 1. Papain limited proteolysis]. *Studia Universitatis Moldaviae, seria Ştiinţe reale şi ale naturii*, 2017, 1(101): 37-40 (in Romanian).
32. Cherdivar A., Rudakova A., Rudakov S., Şutov A. Alergenul Ara h3, globulina de rezervă din seminţele de arahide. 2. Proteoliza limitată cu tripsină [Allergen Ara h3, storage 11S globulin from peanut seeds. 2. Typsin limited proteolysis]. *Studia Universitatis Moldaviae, seria Ştiinţe reale şi ale naturii*, 2017, 1(101): 41-45 (in Romanian).
33. Cherdivar A., Rudakova A., Şutov A. Globulinele de rezervă 7S din seminţe ca alergeni. Imunoreactivitatea oncrucişat ontre globulinele de rezervă 7S şi 11S [Storage 11S globulins as allergens. Cross-immunoreactivity between storage 7S and 11S globulins]. *Studia Universitatis Moldaviae, seria Ştiinţe reale şi ale naturii*, 2016, 1(91): 56-60 (in Romanian).
34. Shutov A.D., Senyuk V.I., Kakhovskaya I.A., Pineda J. High molecular mass products of hydrolysis of soybean glycinin by trypsin. *Biochemistry (Moscow)*, 1993, 58: 174-182.
35. Rudakova A., Rudakov S., Kakhovskaya I., Wilson K., Yagasaki K., Utsumi S., Shutov A. Limited proteolysis controls massive degradation of glycinin, storage 11S globulin from soybean seeds. Materialele simpozionului naţional «*Agrobiodiversitatea vegetală on Republica Moldova: evaluatea, conservarea şi utilizarea*» [*Vegetable agrobiodiversity in the Republic of Moldova: evaluation, conservation and use.* Materials of national symposium]. Chişinău, 2008: 396-402.
36. Makaeva E., Lapteva N., Rudakova A., Rudakov S., Kakhovskaya I., SHutov A. 11S globulin podsolnechnika: kinetika ogranichennogo i kooperativnogo proteoliza papainom. [Makaeva E., Lapteva N., Rudakova A., Rudakov S., Kakhovskaya I., Shutov A. Sunflower seed 11S globulin: kinetics of papain limited and co-operative proteolyses]. *Studia Universitatis Moldaviae, seria Ştiinţe reale şi ale naturii*, 2009, 1(21): 24-28 (in Russ.).
37. Shutov A.D., Rudakova A.S., Klimova N.V., Lapteva N.A., Makaeva E.F., Wilson K. Limited proteolysis of oat 11S globulin by papain. *Studia Universitatis Moldaviae, seria tiin e reale i ale naturii*, 2014, 1(71): 85-90.
38. Lapteva N., Makaeva E., Rudakov S., Rudakova A., Kakhovskaya I., Shutov A. Smeshannyi tip proteoliza 11S globulina kedra papainom. [Lapteva N., Makaeva E., Rudakov S., Kakhovskaya I., Shutov A. Mixed-type papain proteolysis of cedar 11S globulin]. *Studia Universitatis Moldaviae, Ştiinţe reale şi ale naturii*, 2010, 6(36): 9-13 (in Russ.).
39. Rudakova A.S., Rudakov S.V., Kakhovskaya I.A., Shutov A.D. 11S storage globulin from pumpkin seeds: regularities of proteolysis by papain. *Biochemistry (Moscow)*, 2014, 79: 820-825 (doi: 10.1134/S0006297914080100).
40. Ivanciuk O., Midoro-Horiuti T., Schein C.H., Xie L., Hillman G.R., Goldblum R.M., Braun W. The property distance index PD predicts peptides that cross-react with IgE antibodies. *Mol. Immunol.*, 2009, 46: 873-883 (doi: 10.1016/j.molimm.2008.09.004).
41. Lawrence M.C. Structural relationships of 7S and 11S globulins. In: *Seed proteins.* P.R. Shewry, R. Casey (eds.). Kluwer Academic Publishers, Dordrecht, 1999: 517-541.
42. Ko T.P., Ng J.D., McPherson A. The three-dimensional structure of canavalin from jack bean (*Canavalia ensiformis*). *Plant Physiol.*, 1993, 101: 729-744 (doi: 10.1104/pp.101.3.729).
43. Lawrence M.C., Izard T., Beuchat M., Blagrove R.J., Colman P.M. Structure of phaseolin in 2.2 E resolution: implications for a common vicilin/legumin structure and the genetic engineering of seed storage proteins. *J. Mol. Biol.*, 1994, 238: 748-776 (doi: 10.1006/jmbi.1994.1333).
44. Maruyama N., Adachi M., Takahashi K., Yagasaki A., Kohno M., Takenaka Y., Okuda E., Nakagawa S., Mikami B., Utsumi S. Crystal structures of recombinant and native soybean β-conglycinin β-homotrimers. *Eur. J. Biochem.*, 2001, 268: 3595-3604 (doi: 10.1046/j.1432-1327.2001.02268.x).
45. Itoh T., Garcia R.N., Adachi M., Maruyama Y., Tecson-Mendoza E.M., Mikami B., Utsumi S. Structure of 8S alpha globulin, the major seed storage protein of mung bean. *Acta Crystallogr. D*, 2006, 62: 824-832 (doi: 10.1107/S090744490601804x).
46. Fukuda T., Maruyama N., Salleh M.R., Mikami B., Utsumi S. Characterization and crystallography of recombinant 7S globulins of Adzuki bean and structure-function relationships with 7S globulins of various crops. *J. Agr. Food Chem.*, 2008, 56: 4145-4153 (doi: 10.1021/jf072667b).
47. Jin T., Wang Y., Chen Y.W., Fu T.J., Kothary M.H., McHugh T.H., Zhang Y. Crystal structure of Korean pine (*Pinus koraiensis*) 7S seed storage protein with copper ligands. *J. Agr. Food Chem.*, 2014, 62: 222-228 (doi: 10.1021/jf4039887).
48. Chruszcz M., Maleki S.J., Majorek K.A., Demas M., Bublin M., Solberg R., Hurlburt B.K., Ruan S., Mattisohn C.P., Breiteneder H., Minor W. Structural and immunologic characterization of Ara h 1, a major peanut allergen. *J. Biol. Chem.*, 2011, 286: 39318-39327 (doi: 10.1074/jbc.M111.270132).
49. Cabanos C., Urabe H., Tandang-Silvas M.R., Utsumi S., Mikami B., Maruyama N. Crystal structure of the major peanut allergen Ara h 1. *Mol. Immunol.*, 2011, 49: 115-123 (doi:

10.1016/j.molimm.2011.08.004).
50. Cherdivar A., Rudakova A., Rudakov S., Shutov A. Proteoliza limitată a alergenului Ara h1, globulina de rezervă 7S din seminţele de arahide [Limited proteolysis of the allergen Ara h1, storage 7S globulin from peanut seeds]. *Studia Universitatis Moldaviae, seria Ştiinţe reale şi ale naturii*, 2016, 6(96): 10-15 (in Romanian).
51. Kawai M., Susuki S., Asano M., Miwa T., Shibai H. Characterization of 30-kDa fragments derived from beta-conglycinin degradation process during germination and seedling growth of soybean. *Bioscience, Biothechnology and Biochemistry*, 1997, 61: 794-799 (doi: 10.1271/bbb.61.794).
52. Seo S., Tan-Wilson A., Wilson K.A. Protease C2, a cysteine endopeptidase involved in the continuing mobilization of soybean β-conglycinin seed proteins. *Biochim. Biophys. Acta*, 2001, 1545: 192-206 (doi: 10.1016/S0167-4838(00)00277-6).
53. Zakharov A., Carchilan M., Stepurina T., Rotari V., Wilson K., Vaintraub I. A comparative study of the role of the major proteinases of germinated common bean (*Phaseolus vulgaris* L.) and soybean [*Glycine max* (L.) Merrill] seeds in the degradation of their storage proteins. *J. Exp. Bot.*, 2004, 55: 2241-2249 (doi: 10.1093/jxb/erh247).
54. Shutov A.D., Rudakova A.S., Rudakov S.V., Kakhovskaya I.A., Schallau A.A., Wilson K.A., Maruyama N. Degradation of β-conglycinin β-homotrimer by papain: independent occurrence of limited and extensive proteolyses. *Bioscience, Biothechnology and Biochemistry*, 2013, 77: 2082-2086 (doi: 10.1271/bbb.130440).
55. Senyuk V., Rotari V., Becker C., Zakharov A., Horstmann C., Müntz K., Vaintraub I. Does an asparaginyl-specific cysteine endopeptidase trigger phaseolin degradation in cotyledons of kidney bean? *Eur. J. Biochem.*, 1998, 258: 546-558 (doi: 10.1046/j.1432-1327.1998.2580546.x).
56. Rotari V.I., Senyuk V.I., Jivotovskaya A.V., Horstmann C., Vaintraub I.A. Proteinase A-like enzyme from germinated kidney bean seeds. Its action on phaseolin and vicilin. *Physiol. Plantarum*, 1997, 100: 171-177 (doi: 10.1111/j.1399-3054.1997.tb03469.x).
57. Rudakova A., Rudakov S., Lapteva N., Morari D., Stepurina T., Rotari V., Kakhovskaya I., Wilson K., Fukuda T., Utsumi S., Shutov A. In vivo and in vitro limited proteolysis of phaseolin: facts, suggestions and problems. *Studia Universitatis Moldaviae, seria Ştiinţe reale şi ale naturii*, 2007, 1(01): 101-111.
58. Jivotovskaya A.V. Senyuk V.I., Rotari V.I., Horstmann C., Vaintraub I.A. Proteolysis of phaseolin in relation to its structure. *J. Agr. Food Chem.*, 1996, 44: 3768-3772 (doi: 10.1021/jf960129l).
59. Pele M. Peanut allergens. *Romanian Biothechnological Letters*, 2010, 15: 5204-5212.
60. Wichers H.J., De Beyer T., Savelkoul F.J., van Amerongen A. The major peanut allergen Ara h1 and its cleaved-off N-terminal peptide; possible implications for peanut allergen detection. *J. Agr. Food Chem.*, 2004, 52: 4903-4907 (doi: 10.1021/jf049697o).
61. Schechter I., Berger A. On the size of the active site in proteases. I. Papain. *Biochem. Bioph. Res. Co.*, 1967, 27(2): 157-162 (doi: 10.1016/S0006-291X(67)80055-X).