



Mechanical and thermal processing effects on protein integrity and peptide fingerprint of pea protein isolate

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ABSTRACT

Pea protein is considered to be an emerging alternative for the formulation of numerous food products, including functional foods, because of its purported hypolipidemic, anti-hypertensive and hypoglycaemic activities. The present investigation was designed to evaluate the effects of thermal and mechanical treatments on an industrial pea protein isolate (Pisane[®]) from *Pisum sativum* L. As a preliminary step, the main protein components of *P. sativum* seed were identified, using the canonical proteomic approach, including 2D-separation and mass spectrometry. Most of the main spots were assigned to the major pea storage proteins: legumin, vicilin and convicilin. Differential scanning calorimetry and proteomic techniques were used to investigate the effects of processing on the protein profile and to assess the availability of stable peptides. After prolonged treatments, no proteins were present in their native forms and the protein solubility was greatly decreased; however, some intense spots were still present on 2D-gels. The spot identity was confirmed by HPLC-Chip-MS/MS, showing that the vicilin 30 kDa fragment and legumin acidic subunits were resistant and some specific peptides remained intact. DSC thermograms showed that strong treatments decreased the denaturation enthalpy. The mechanical treatments, instead, did not modify (in a relevant way) either the protein profile or DSC thermograms.

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1. Introduction

Recently, plant protein ingredients are receiving much attention by industry and consumers, thanks to their good environmental sustainability, health-oriented composition, reliable origin, and attractive price (Davis, Sonesson, Baumgartner, & Nemecek, 2010). Although, the main plant protein still remains soy protein, there is a growing interest in the use of other proteins, especially those from pulses, such as pea, chickpea, lentil, common bean and lupin, in food formulation (Boye, Zare, & Pletch, 2010). There are several reasons why pulses are particularly appealing: they provide dietary benefits and are easily available at a low cost, being high in fibre, antioxidants and protein, and low in saturated fat. In addition to providing essential amino acids, pulse proteins also possess functional properties: they have good water-binding capacity (Fernandez-Quintela, Macarulla, Del Barrio, & Martinez, 1997), fat-binding capacity (Kaur & Singh, 2007), and are able to stabilise emulsions, foams and gels (O'Kane, Happe, Vereijken, Gruppen, & van Boekel, 2004). All this may expand their use in a wide variety of food products.

In this context, pea protein may introduce a good opportunity for food innovation. Roquette (Nutralys[®], France), Cosucra Groupe Warcoing (Pisane[®], Belgium), SunOpta Ingredients Group (SunOpta Pea Fibre[®], Canadian), Nutri-Pea (Pro-Flo[™], Canadian), Vital Greens (Phyto-Protein, Australia), and Norben (Propulse[®], USA) are examples of companies focused on innovative pea ingredients.

Actually, pea flours, concentrates and isolates are successfully used in a number of different applications, including the replacement of casein and whey proteins in sports nutrition, weight management products and functional foods, addition to meat, fish, processed foods, soups and sauces to bind fat and/or water, and protein enrichment of baked foods, cereals and snacks (Marinangeli, Kassis, & Jones, 2009). Moreover, pea protein is also considered an emerging alternative for the formulation of gluten-free products (Mariotti, Lucisano, Pagani, & Ng, 2009).

The nutritional quality of pea protein has been evaluated in several studies (Foltz et al., 2008; Leterme, 2002; Mitchell, Lawrence, Hartman, & Curran, 2009). When compared to the FAO reference standard pattern of amino acids (FAO, 1957), pea protein is a good source of essential amino acids, with the exception of a deficiency in the sulphur-containing amino acids, methionine and cysteine. However, pea protein concentrates supplemented with methionine have a protein quality equal to egg protein, fish protein, or casein when included in a mouse diet.

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Recent researches have also highlighted important health benefits, namely hypolipidemic (Kingman et al., 1993; Martins et al., 2004; Rigamonti et al., 2010; Spielmann, Stangl, & Eder, 2008), anti-hypertensive (Humiski & Aluko, 2007; Vermeirssen et al., 2005), and hypoglycaemic (Marinangeli et al., 2009) effects.

The industrial processes used for debittering pea flour or for preparing pea protein concentrates and isolates affect the functional properties of the final products differently. Also, thermal treatments applied to improve protein functionality, namely anti-nutritional factor inactivation and increasing protein digestibility, and mechanical processes (extrusion cooking, high-pressure homogenisation), profoundly affect protein stability. Depending on the thermal stability of proteins and heating conditions, proteins may be either partially or completely dissociated and even denatured and aggregated (Petruccielli & Anon, 1995). Since peptides are considered the bioactive components of the proteins, the objectives of this study were to evaluate the effects of different kinds of thermal and mechanical treatments on the industrial pea protein isolate, Pisane® (Cosucra), and to assess the availability of resistant peptides after these treatments. The focus was on the globulin fraction, which accounts for 65–80% of total pea protein; this fraction is made up of two major components, legumin and vicilin, and a minor one, convicilin. Differential scanning calorimetry and proteomic techniques, such as 2D-electrophoresis and mass spectrometry, were used for the investigation.

A preliminary phase of this study was dedicated to the identification of the main spots in the two-dimensional electrophoretic map of *Pisum sativum* L. seed protein, from which the Pisane® isolate was obtained. This 2D-map allowed a complete overview of the pea protein content, that was the starting point for the future comparison of the treated and untreated protein isolates selected in this study.

2. Materials and methods

2.1. Laboratory procedure for the preparation of pea protein extract (PPE) from the pea seeds

Pea seeds (*P. sativum* cv Attika) were dehulled; the dry cotyledons were milled (60-mesh sieve), and the resulting flour was defatted with *n*-hexane (20 ml/g of flour) for 24 h under stirring. The globulin fraction was extracted as previously described (Ferreira, Ferreira, & Teixeira, 2000) and stored at -80°C . Pea seeds were kindly provided by Cosucra Groupe Warcoing (Belgium).

2.2. Purification of pea globulins from PPE

Pea globulins were purified using a preparative HPLC-DAD HP-1200 series instrument (Agilent Technologies, Santa Clara, CA). An aliquot of 10 ml of the dialyzed PPE was filtered through sterile membrane filters (0.2 μm , Econofilter, Agilent Technologies) and loaded onto a DEAE-Sepharose Fast Flow column (GE Healthcare Bio-Sciences AB, Sweden). Proteins were eluted with a linear salt gradient (0–1 M NaCl) in 100 mM Tris–HCl, pH 8.0. The protein fractions containing vicilins and legumins were pooled and re-injected onto a MonoQ HR 5/5 anion-exchange chromatography column (Amersham Biosciences, Sweden). The samples were eluted with a linear salt gradient (0–1 M NaCl) in 30 mM Tris–HCl, pH 8.0, collected in 0.5 ml fractions and stored at -80°C . Eluted proteins were detected at 280 nm.

The purity of the column fractions was confirmed by MS/MS analysis. Samples were denatured with 6 M urea, reduced with 1 M 1,4-dithiothreitol (DTT) (50 mol of DTT vs. 1 mol of Cys) and alkylated with 1 M iodoacetamide (IAM) (200 mol of IAM vs. 1 mol of Cys). The proteins were then digested with sequencing-grade trypsin (0.5 $\mu\text{g}/\mu\text{l}$, Promega) in the ratio 1:50 w/w enzyme/

protein for 16 h at 37°C and the peptides were analysed using HPLC-Chip-Ion Trap (Agilent Technologies) (Sirtori, Resta, Brambilla, Zacherl, & Arnoldi, 2010).

2.3. 2D-electrophoresis

PPE or desalted globulin fractions were diluted in IEF solubilisation buffer (7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 2% ampholyte, pH 3–10 and pH 4–6); reduced with 65 mM DTT and alkylated with 200 mM IAM (Sirtori et al., 2010). Isoelectric focusing was performed on 7 cm, pH 3–10 non-linear IPG strips (Biorad Laboratories Inc., Hercules, CA); proteins were focused with 20,000 V/h, with a maximum of 2500 V, using the Protean IEF Cell (Biorad). The separation was performed on 13% SDS–PAGE gels, using the Mini Protean 3 Dodeca Cell (Biorad). The gels were stained with Bio-Safe Coomassie (Biorad), scanned with a VersaDoc 3000 (Biorad), and analysed with the PDQuest software (Biorad). All samples were run in duplicate.

In order to increase the spot resolution, proteins were also focused on 7 cm, pH 5–8 IPG strips (Biorad) and the separation in the second dimension was performed on 10% denaturing SDS–PAGE gels.

2.4. Tryptic in-gel digestion for the identification of the spots from 2D-gels

The spots were excised from 2D-gels of PPE and purified proteins. Subsequently, the spots were washed with water, dehydrated with CH_3CN , reduced with 10 mM DTT/0.1 M NH_4HCO_3 (freshly prepared), alkylated with 55 mM IAM/0.1 M NH_4HCO_3 , washed with 0.1 M NH_4HCO_3 , and finally dehydrated with CH_3CN . The gel pieces were rehydrated in 20 μl of trypsin solution (12.5 ng/ μl sequencing-grade trypsin, 50 mM NH_4HCO_3 /5 mM CaCl_2) at 4°C for 50 min and then incubated at 37°C overnight. Gel pieces were sonicated for 10 min and centrifuged briefly. The supernatants were cleaned with a Micro-spin Column (Harvard Apparatus Ltd., Edenbridge, UK) and the peptides were further fragmented and analysed using an HPLC-Chip-Ion Trap (Agilent Technologies) (Sirtori et al., 2010).

2.5. Processing of the industrial pea protein isolate Pisane®

2.5.1. General

The process to obtain the industrial protein isolate, Pisane®, was patented by Cosucra Groupe Warcoing (Belgium). All the information about the product was available at <http://www.cosucra.com>. Both PPE and Pisane® were obtained from the same pea seeds, cv Attika, provided by Cosucra.

Both thermal and mechanical treatments described were performed at the Fraunhofer Institut für Verfahrenstechnik und Verpackung (Freising, Germany).

2.5.2. Thermal treatments

Pisane® was mixed with water in a 1:5 ratio (protein:water). After a 5 min soaking, the slurry was placed in aluminium boxes and heated in an oven at 100°C , 150°C and 200°C , respectively. Samples were taken after 5, 15 and 30 min.

Alternately, Pisane® was dry-heated at 65°C and 100°C . At both temperatures, samples were taken after 30, 120 and 300 min of heating. All the samples were cooled to RT and lyophilised.

Three replicates of each sample were done.

2.5.3. Mechanical process

In order to simulate mechanical stress, Pisane® solution (1:5 ratio, protein:water) were mixed in an Ultraturrax macerator (Janke & Kunkel IKA Labortechnik, Staufen, Germany) for 1 min at 8000, 13,500 and 24,000 rpm, respectively. After treatment, the samples were lyophilised.

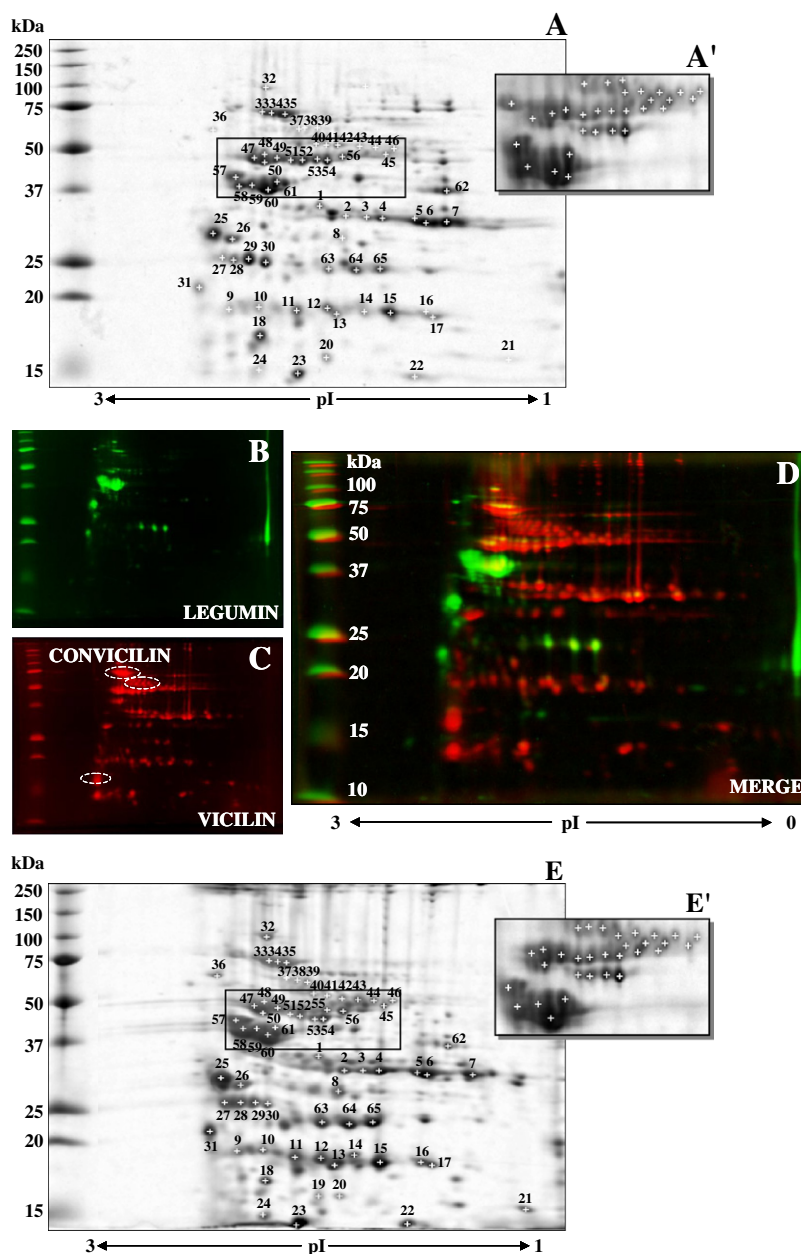


Fig. 1. Proteome reference maps of *Pisum sativum* proteins. The proteins were run in the first dimension on 7 cm, pH 3–10 non-linear IPG strips; the second dimension was performed on 13% denaturing SDS–PAGE gel. Standard marker (Precision Plus Protein) is indicated in kDa on the left. The protein identification and the peptide sequences of each numbered spot are available from authors. The same spot numbers are reported in each map. (A) 2D-map of the reduced total protein extract of Attika mature seeds (PPE). (A') Detailed gel obtained with pH 5–8 IPG strips coupled with 10% denaturing SDS–PAGE gel. (B) 2D-gel of purified 11S legumins from PPE. (C) 2D-gel of both purified 7S vicilins and 7S convicilins, from PPE. (D) Merge obtained overlapping the coloured gels of the purified proteins (red: legumins; green: vicilins and convicilins). (E) 2D-map of the reduced industrial pea protein isolate (Pisane®). (E') Detailed gel obtained with pH 5–8 IPG strips (7 cm) coupled with 10% denaturing SDS–PAGE gel. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Instead, the simulation of the mechanical stress caused by extruders or homogenisers was performed using a high pressure homogeniser (APV-2000, APV, Unna, Germany). Pisane® solutions (1:5 ratio, protein:water) were homogenized at 100, 300 and 1000 bar, respectively. After treatment, the samples were lyophilised. Three replicates of each sample were done.

2.6. Analysis of the processed pea protein isolate

2.6.1. Differential scanning calorimetry (DSC) analysis

The thermal properties of Pisane® were examined according to a literature procedure (Shand, Ya, Pietrasik, & Wanasundara,

2007). The protein isolate (20% protein concentration, w/w, pH 6.4–6.5) was mixed and dispersed in deionised water, using a vortex. The slurry was weighed into Tzero hermetic pans (TA Instruments, Waters LLC, Waltham, MA). Thermograms were obtained by heating from 40 to 120 °C, at a heating rate of 10 °C/min, using a DSCQ 2000 system (TA Instruments, Waters LLC). A sealed empty pan was used as a reference. The denaturation temperature (T_d), peak transition temperature, and enthalpy of denaturation (ΔH , J/g) were computed from the thermograms, using specific software (Universal Analysis Program, Version 2.5H, TA Instruments, Waters LLC). Data are expressed as mean $\pm$ SD of triplicates.

Table 1

Identification of pea proteins excised from 2D-gels. The peptide sequence matches were determined using the Spectrum Mill software (Agilent Technologies). A detailed table of the peptide sequences that matched the MS spectra obtained from protein spots excised from the 2D gels is available from authors.

No. Spot	Species	Protein identification	NCBI nr accession
1–17, 22–26, 47–54, 55.2, 56.2	<i>Pisum sativum</i>	Vicilin precursor	758248, 137582, 297170
		Vicilin 47 kDa protein	42414627, 42414629
		Vicilin	137581
		Provicilin precursor (Type B)	7339555
		Vicilin 47 kDa precursor	137577
		Vicilin 14 kDa component	137579
		Provicilin (Type A)	1297072
		Vicilin precursor	137584
		Vicilin precursor	137584
		Vicilin precursor	137584
33–35, 37–46, 55, 56	<i>Pisum sativum</i>	Convicilin	7339551, 227928
		Convicilin precursor	117655, 1706205
		Convicilin precursor	1297070
		Convicilin precursor	1297070
		Convicilin	7688242
		Convicilin	7688242
		Convicilin	7688242
		Convicilin	7688242
		Convicilin	7688242
		Convicilin	7688242
19, 20, 25.2, 31, 57–61, 63–65	<i>Pisum sativum</i>	Legumin A2 precursor	126161
		LegA class precursor	4218520
		Legumin (minor small)	2578438
		Legumin B	126169
		Legumin precursor	553082
		Legumin A	126168
		Legumin precursor	282925
		Legumin J precursor	126170
		Legumin A	483449
		N-terminal leg. A1 pre-pro-poly.	388256
27–30	<i>Pisum sativum</i> (garden pea)	Albumin 2 (PA2)	P08688
		Albumin-1 B precursor (PA1 B)	51704208
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
18, 21, 32, 36, 62	<i>Pisum sativum</i>	Albumin 2 (PA2)	P08688
		Albumin-1 B precursor (PA1 B)	51704208
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207
		Albumin-1 A precursor (PA1 A)	51702207

2.6.2. Analysis by 2D-electrophoresis

Treated and untreated Pisane[®] were submitted to 2D-electrophoresis (Section 2.3) for the comparison of the protein profiles. A specific tool of PDQuest software (Biorad) was used to calculate the area of each spot in terms of pixel intensity, permitting comparison between different samples.

2.6.3. MS/MS analysis and database searches

The protein samples were reduced and alkylated, using DTT and IAM, respectively, and then subjected to digestion with trypsin (Promega), as described in Section 2.2. The peptide mixtures obtained were analysed using the HPLC-Chip-Ion Trap (Agilent Technologies). All experiments were performed on an Agilent 1200 LC connected to an Agilent Ion Trap (SL series) mass spectrometer (Agilent Technologies). The ionisation system was an HPLC-Chip Cube, working in nanoflow electrospray positive ion mode. The Ion Trap experimental conditions have been described in detail in a previous paper (Sirtori et al., 2010).

Protein database searches were performed with Spectrum Mill MS Proteomic Workbench (Rev A.03.03). All searches used the NCBI protein database, biweekly updated with trypsin specificity, 1 missed cleavage. The forward database search of the ion trap data was performed using a mass tolerance of ± 2.5 Da for precursor ions and a tolerance of ± 0.7 Da for fragment ions. A reversed database search was also performed. In order to validate the peptide and protein matches, the Spectrum Mill default value for the peptide scores, forward-reversed scores, rank1-rank2 scores, percentage scored peak intensities, and protein scores were considered (Brambilla, Resta, Isak, Zanotti, & Arnoldi, 2009).

3. Results and discussion

3.1. Reference two-dimensional electrophoretic maps of *Pisum sativum* L. seed proteins and Pisane

Since only one 2D-map of pea protein is available in the literature (Bourgeois et al., 2009), a preliminary phase was dedicated to the identification of the main *P. sativum* proteins focused in the 2D maps. This was done on both a total protein extract (PPE) from pea seed prepared in our laboratory and on a commercial protein isolate (Pisane[®]), in order to identify similarities and differences induced by the industrial preparation. Fig. 1 shows the 2D-map of the reduced PPE from *P. sativum* seed (panel A), and the 2D-map of the reduced untreated Pisane[®] (panel E); spot numberings are identical in the two maps.

IPG were run in the presence of 7 M urea; hence, non-covalently-bound oligomers were dissociated in all cases. In total the spots detected by PDQuest Software were 132 for PPE and 125 for Pisane[®]. In both cases, 65 major spots were submitted to MS/MS analysis and 87 polypeptides were identified (Supplementary Table II), corresponding to 30 different protein accessions (Table 1). All accessions corresponded to pea storage proteins (vicilins, convicilins, legumins, and albumins). Some spots (25, 55, and 56) revealed multiple identifications, as previously reported (Bourgeois et al., 2009), and were differently numbered (25.2, 55.2, 56.2). Due to the high sequence homology between legumes, several species had a positive match with the same protein (*Lens culinaris*, *Vicia narbonensis*, *Vicia faba*, *Vicia sativa*).

Numerous identified spots corresponded to 7S globulins (Fig. 1): 32 vicilin spots (Nos. 1–17, 22–26, 47–54, 55.2, and 56.2) were identified on the map, ranging from M_r 50 to 13 kDa and from pI 5.2 to 8.5. A group of spots (Nos. 47–54, 55.2, and 56.2) around 50 kDa corresponded to mature vicilin subunits; instead vicilin spots from M_r 35 to 13 kDa corresponded to different proteolysed forms (Gatehouse, Lycett, Croy, & Boulter, 1982). Often these spot rows are further arranged into clusters of rows of closely spaced M_r values.

Fifteen spots (Nos. 33–35, 37–46, 55, and 56) (Fig. 1) on the upper part of the map from M_r 75 to 48 kDa and from pI 5.5 to 7.5 corresponded to convicilins, with 16 polypeptides identified. These polypeptides corresponded to six different accessions, four of them relative to *P. sativum* (7339551, 227928, 117655, and 1706205), one to *Lens culinaris* (7688242), and one to *Vicia narbonensis* (1297070) (Table 1).

Twelve legumins spots were identified (Nos. 19, 20, 25.2, 31, 57–61, and 63–65), ranging from M_r 20 to 45 kDa and from pI 4.7 to 6.5. They corresponded to 10 sequence accessions (126161, 4218520, 483449, 2578438, 126169, 553082, 126168, 388256, 282925 and 126170) (Table 1) and were divided into acidic and basic forms, resolved at distinct positions in 2D-gels under the denaturing conditions used. In particular, the acidic subunit (~ 40 kDa) had a pI value of 4.6–4.9, and the basic subunit (~ 20 kDa) had a pI value of 6.8–7.1 (Fig. 1).

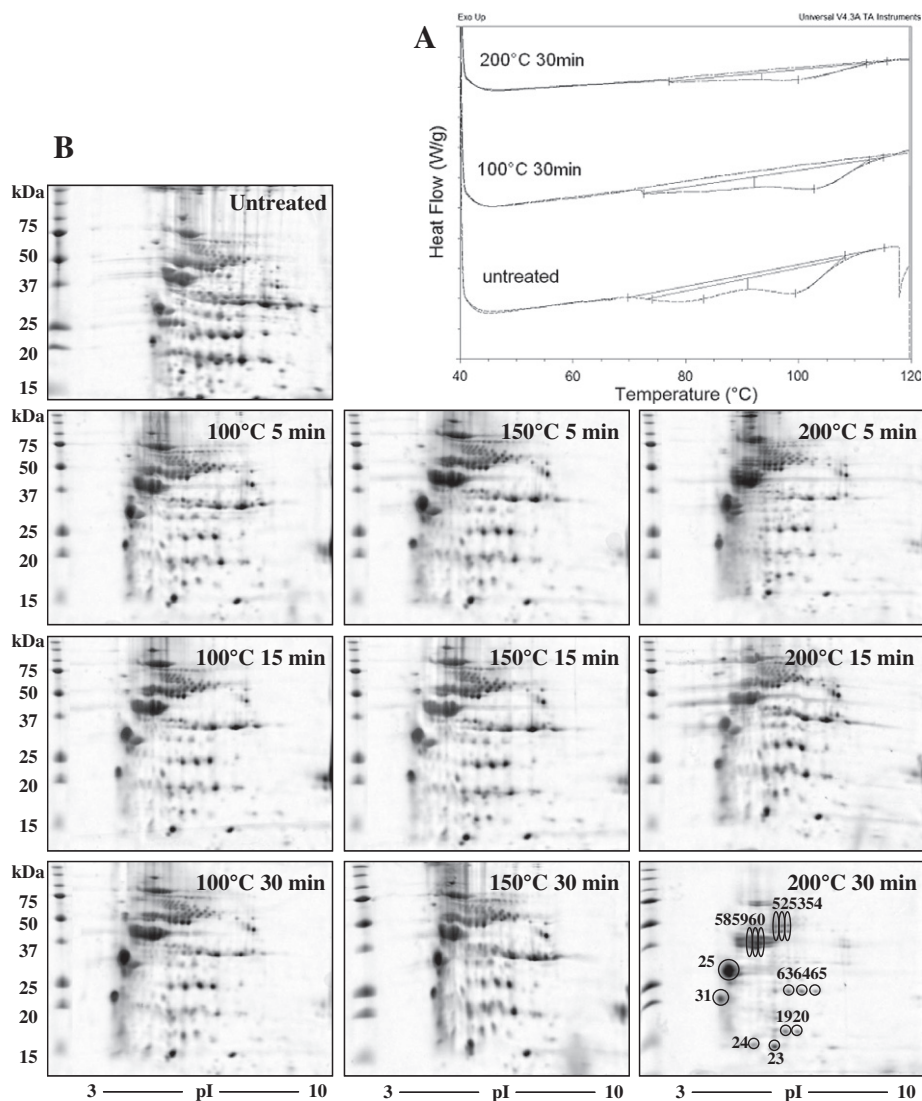


Fig. 2. (A) Exemplary DSC thermograms of the thermally treated (30 min at 100 and 200 °C) and untreated Pisane® (15% solution), at a scanning rate of 10 °C/min at pH 7.0. Denaturation enthalpy and onset point data of protein denaturation are shown in Table 2. (B) Two-dimensional electrophoretic analysis of thermally treated Pisane® at different times of exposure. Untreated Pisane® was used as reference. Isoelectric focusing was performed on pH 3–10 non-linear IPG strips, and the second dimension was done on 13% SDS–PAGE gel. Standard marker (Precision Plus Protein) is indicated in kDa on the left. The 2D-gel of sample treated at 200 °C for 30 min reports the spot numbers corresponding to the 2D-map of pea seeds (Fig. 1).

Four spots (No. 27–30; M_r 25/26 kDa, and pI 5.1 to 5.6) were identified as pea albumin 2 (PA2), the major component of the pea albumin fraction (Fig. 1, Table 1). These four spots corresponded to the same accession (P08688), according to previous data (Bourgeois et al., 2009); in addition, PA1 peptides were also recognised in the same spots, with two different accessions (51704208 and 51702207).

For a further insight, the main storage proteins were separated by chromatographic procedures, starting from PPE. Vicilins, convicilins and legumins, shown in Fig. 1 (panels B and C), were purified by ion-exchange chromatography on a DEAE-Sephacrose column and subsequently by MonoQ column, which is more selective for globulins. Panels B and C show the multi-channel viewers of the purified proteins, obtained with a specific tool of PDQuest Software. Panel D shows the merge obtained by superimposing the coloured gels of the purified proteins. This technical expedient was useful to better correlate the purified proteins with the corresponding spots on the 2D-gel of PPE and Pisane®. This virtual reconstruction corresponds well with panel E, due to the high protein concentrations of both samples.

3.2. Effect of thermal treatments on the protein isolate

With the aim of simulating some industrial processes for producing foods, Pisane® was thermally treated and the effects on the protein quality analysed. Two different kinds of treatment were applied: in the former, Pisane® was suspended in water and heated at temperatures ranging from 100 to 200 °C for different times (humid treatment) whereas, in the latter, Pisane® was heated at 65 °C and 100 °C without solvent (dry treatment).

Differential scanning calorimetry of protein slurries was used to determine the thermal properties of the treated samples in comparison with untreated Pisane®. The thermally-induced transitions of the proteins are plotted in the DSC thermograms shown in Figs. 2A and 3A, and the corresponding denaturation temperatures (T_d) are listed in Table 2.

Two major endothermic peaks were observed in the untreated sample thermogram, corresponding to the vicilin (7S) and legumin (11S) fractions. In fact, these proteins account for the multiple endotherms and variation in denaturation temperature due to their heterogeneity (Sosulski, Hoover, Tyler, Murray, & Arntfield, 1985).

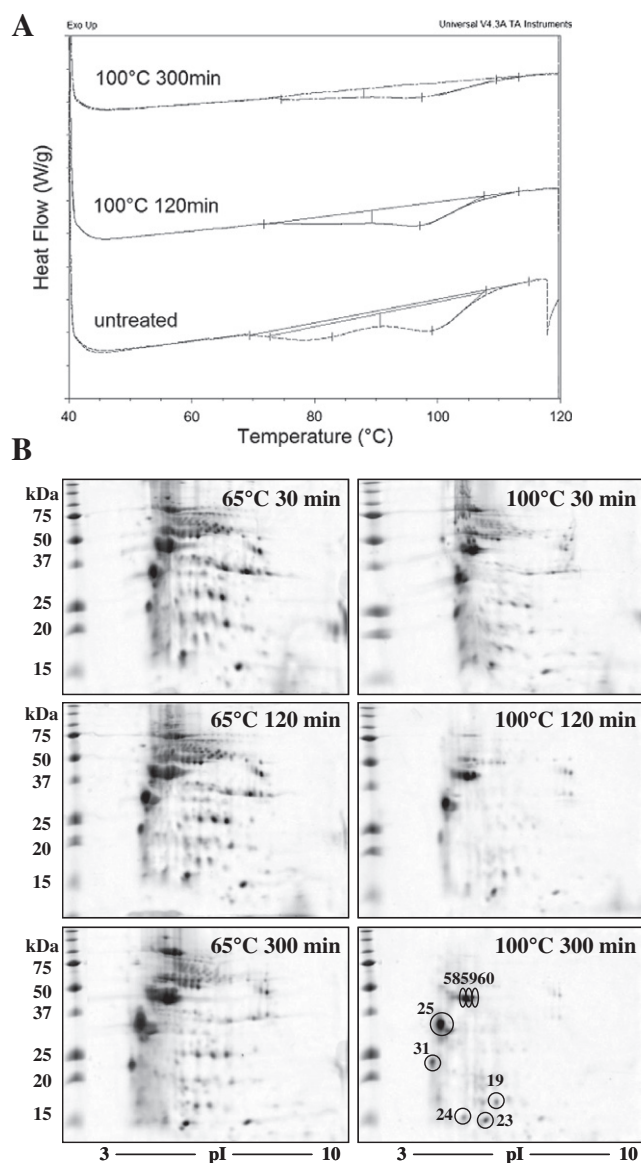


Fig. 3. (A) Exemplary DSC thermograms of the thermally treated (dry treatments at 100 °C for 120 min and 300 min) and untreated Pisane[®], at a scanning rate of 10 °C/min at pH 7.0. Denaturation enthalpy and onset point data of protein denaturation are shown in Table 2. (B) 2D-E analysis of untreated and thermally treated Pisane[®] at different times and temperatures. Isoelectric focusing was performed on pH 3–10 non-linear IPG strips, and the second dimension was done on 13% SDS–PAGE gel. Standard marker (Precision Plus Protein) is indicated in kDa on the left. The 2D-E of sample treated under drastic conditions shows the spot numbers corresponding to the 2D-map of pea seeds (Fig. 1).

The humid heating progressively damaged the native proteins: both peaks were still measurable after short heating times (5 and 15 min) at 100 and 150 °C (data not shown), whereas only the legumin peak could be detected under harsher conditions (30 min at 100 and 200 °C) (Fig. 2A). These thermal transitions were caused by the denaturation of the vicilin at lower temperature (74.7 ± 0.35 °C) and the legumin at higher temperature (99.7 ± 0.70 °C). According to the denaturation behaviour of other legume proteins, such as lupin protein (Sirtori et al., 2010) or soy protein (Shand et al., 2007), the vicilin fraction showed lower heat stability than the legumin fraction.

The heat-induced denaturation of a globular protein depends on its 3D structure and is accompanied by the cleavage of inter- and intra-molecular bonds, followed by intermolecular interaction and aggregation of unfolded molecules (Clark, Kavanagh, & Ross-

Table 2

Denaturation temperature (T_d) and enthalpy of denaturation (ΔH) of untreated and treated samples. T_{d1} and ΔH_1 are referred to vicilin peak; T_{d2} and ΔH_2 are referred to legumin peak. Data are expressed as means ± SD of triplicates. Significance was set at the 5% level ($p < 0.05$, 95% confidence interval) using T-test by GraphPad InStat software version 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

Sample	Onset point of protein denaturation (°C)		Denaturation enthalpy (J/g)	
	T_{d1}	T_{d2}	ΔH_1	ΔH_2
Untreated	74.7 ± 0.35 ^a	99.7 ± 0.70 ^a	4.1 ± 0.35 ^a	6.5 ± 0.50 ^a
<i>Thermal treatments</i>				
100 °C, 30 min	76.3 ± 0.30 ^b	103.9 ± 0.60 ^b	1.6 ± 0.12 ^b	4.2 ± 0.15 ^b
200 °C, 30 min	No peak	100.4 ± 0.35 ^a	No peak	2 ± 1.0 ^c
65 °C, 120 min	75.5 ± 0.31 ^c	98.4 ± 0.51 ^c	2.9 ± 0.25 ^c	3.9 ± 0.95 ^b
65 °C, 300 min	No peak	95.9 ± 0.30 ^d	No peak	3.3 ± 0.30 ^d
100 °C, 120 min	No peak	97.4 ± 0.55 ^e	No peak	3.5 ± 0.60 ^d
100 °C, 300 min	No peak	98.1 ± 0.46 ^c	No peak	3.3 ± 0.35 ^d
<i>Ultraturrax</i>				
8000 rpm	74.5 ± 0.50 ^a	98.5 ± 0.31 ^c	3.6 ± 0.40 ^a	6.3 ± 0.35 ^a
16,000 rpm	74.9 ± 0.65 ^a	96.6 ± 0.55 ^f	3.5 ± 1.50 ^a	6.1 ± 0.55 ^a
24,000 rpm	75.1 ± 0.26 ^a	99.6 ± 0.60 ^a	3.3 ± 1.30 ^a	6 ± 0.65 ^a

^{a–f} Column values followed by the same letter are not significantly different.

Murphy, 2001). The vicilin tertiary structure is mainly bound by weak non-covalent bonds, such as hydrogen bonds, hydrophobic interactions and electrostatic forces. These forces are more labile than covalent bonds, i.e. the disulfide bridges, which form the tertiary protein structure of the legumin. Since covalent bonds require more energy to break, the tertiary structure of a protein containing these bonds shows greater stability to thermal treatments. These phenomena were evident in the dry-heated samples (Fig. 3A), where no vicilin peaks could be observed and also legumin peaks were less evident. The lack of an endothermal transition might indicate partially or completely denaturated proteins (Arntfield & Murray, 1981). These results are in agreement with previous data, where the pea globulin mix (35.7% legumin and 64.3% vicilin) gave thermal transitions between 74 and 95 °C at a 10 °C/min heating rate (Bora, Brekke, & Powers, 1994).

Analysis of the DSC thermogram also enabled the determination of the denaturation enthalpy (ΔH) of the protein samples (Table 2). The ΔH values, calculated from the area under the transition peak, are correlated with the content of secondary structure of the proteins (Koshiyama, Hamano, & Fukushima, 1981). The DSC results showed a decreasing denaturation enthalpy of the treated Pisane[®] with the increased intensity of the thermal treatment. In particular, the ΔH value decreased from 6.5 ± 0.50 (untreated Pisane[®]) to 2 ± 1.0 J/g (200 °C, 30 min) for the legumin fraction, and from 4.1 ± 0.35 to 1.6 ± 0.12 J/g (100 °C, 30 min) for the vicilin fraction. According to our results, it seems that the combination “long time and medium temperature” is preferred to the combination “short time and high temperature”. In fact, the ΔH value was higher for treatment at 100 °C for 300 min (3.3 ± 0.35 J/g) than at 200 °C for 30 min (2 ± 1.0 J/g). The ΔH decreasing trend is in agreement with the lower content of native protein observed.

The extent of protein degradation induced by processing was also analysed by 2D gels (Figs. 2B and 3B). Since the thermal processing can strongly decrease protein solubility, a SDS extraction was performed (Le Gall, Gueguen, Seve, & Quillien, 2005).

Although long times and high temperatures denatured the majority of the proteins, both the basic and acidic legumin subunits were still clearly visible on the 2D-gels, even after a severe treatment (30 min at 200 °C), although their intensities were reduced after 5 and 15 min heatings at 200 °C (Fig. 2B). By contrast, the vicilins appeared to be more labile since, after only a short heat treatment (15 min at 100 °C) the corresponding spots were faint

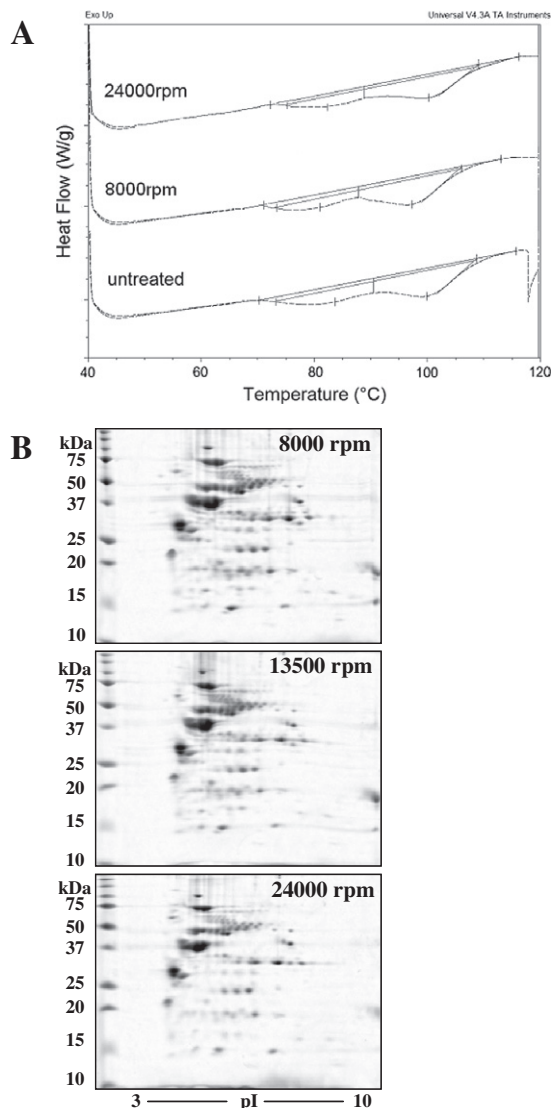


Fig. 4. Exemplary DSC graphs of the mechanically treated samples, at a scanning rate of 10 °C/min at pH 7.0, with the untreated Pisane® used as reference. The samples were treated at 8000 rpm, 13,500 rpm and 24,000 rpm with the Ultraturrax. Denaturation enthalpy and onset point data of protein denaturation are shown in Table 2.

(according to the pixel intensity calculated by PDQuest software). Interestingly, two major spots (Nos. 25 and 31) were visible in all the treatments, with high and constant intensities: spots 25 was identified as both vicilin and legumin, possibly for the simultaneous presence of different polypeptides with similar pI and M_r , that co-migrated in the same area of the 2D-gel; spot 31 was identified as legumin.

The same profile could be observed in the sample treated for 30, 120 and 300 min at 100 °C (Fig. 3B), where only the acidic legumin subunits are present; again spots 25 and 31 showed the highest intensities. These results were in agreement with those of DSC analysis.

3.3. Effect of mechanical stress on pea protein isolate

The mechanical stress was applied using an Ultraturrax macerator (8000, 13,500, and 24,000 rpm) and a homogeniser (100, 300 and 1000 bar), in order to simulate different mixing and extrusion devices. The DSC results (Fig. 4A) did not show any relevant difference in the thermograms of the analysed samples. In all samples

treated either in the Ultraturrax or with the homogeniser (data not shown), the two peaks of vicilins and legumins could be observed and there was not any significant reduction of the onset temperature of the protein denaturation (Table 2). However, a slight decrease of the denaturation peak area was observed, with the ΔH_1 value changing from 4.1 ± 0.35 (untreated) to 3.3 ± 1.30 J/g (24,000 rpm) and the ΔH_2 value decreasing from 6.5 ± 0.50 to 6 ± 0.65 J/g.

Thus, this mechanical treatment did not affect the thermal behaviour of the proteins. These results are in agreement with the protein profiles shown by the gels: neither the Ultraturrax (Fig. 4B) nor the homogeniser (data not shown) produced any obvious differences in the protein profile vs. untreated sample and the spot intensities remained constant.

The modifications induced by mechanical treatments, i.e. disruption of hydrophobic and electrostatic interactions, greatly depend on the pressure applied, duration of treatment and temperature of pressurisation. According to literature data, the legumins tend to form pressure-induced aggregates and the vicilins are denatured, applying pressure higher than 2000 bar (Chapleau & de Lamballerie-Anton, 2003). In fact, high-pressure processing cleaves non-covalent interactions within proteins, leading to structure modification as well as protein aggregation induced by tertiary and secondary structure changes (Masson, 1992). The samples analysed in this work, instead, were treated less severely and no changes in protein profile were observed. Similar results were also obtained for a lupin protein isolate treated in a similar way (Sirtori et al., 2010).

3.4. Peptide fingerprint of the treated samples

In order to identify resistant peptides/proteins which may be potentially released by digestion, even after processing, the untreated and treated samples were digested with trypsin and analysed via mass spectrometry. The peptide sequences identified in treated Pisane® are available from author (only the harshest treatments). In total, 58 stable peptides were identified: 17 belonging to the legumin family, 32 to the vicilin family, and 9 to convilin. Legumins were more resistant to heating for 30 min at 200 °C (11 peptides detected) than heating for 300 min at 100 °C (4 peptides detected). Considering now vicilins and convicilins, although their 2D protein profiles and DSC behaviour are completely modified, some intact peptides were still releasable after industrial processing: more peptides were detected in the sample treated for 300 min at 100 °C (28 peptides) than in the sample treated for 30 min at 200 °C (24 peptides). The mechanical treatment with the Ultraturrax only marginally affected the peptides, since only 16 peptides were no more detected after processing.

Some peptides (9, 14, 19, 27, and 39) were detected only in the treated sample, possibly because the trypsin cleavage sites of these peptides are hidden by the folding in the native proteins and become accessible only after heat-induced protein unfolding.

Some resistant peptides show high identity and homology percentages with allergenic peptides reported in the literature (peanut and lupin proteins). In particular, the peptides 2, 4, 7, 21, 22, 31, 39, 46, 47, 52 and 53 are homologous to the allergen Ara h 1 (NCBI nr 1168391), Ara h 3/Ara h 4 (NCBI nr 21314465), and to the lupin peptides (NCBI nr 85361412, 46451223, 89994190, 169950562, 149208403, 149208401), recognised by the sera of allergic patients (Sirtori, Resta, Arnoldi, Savelkoul, & Wichers, 2011). The peptide alignments between pea and peanut peptides and between pea and lupin peptides are available from authors, together with the corresponding identity and homology % calculated with the Program BLASTp.

Alteration in protein structure by food processing can lead to epitope destruction, modification, masking, or unmasking, thereby

decreasing, increasing, or having no effect on allergenicity. Sanchez and Fremont (2003) have extensively reviewed the effects of food processing on the structural stability of food allergens and concluded that effects of protein–protein interactions (especially aggregation) are unknown. However, the resistance to digestion and processing is thought to be an important characteristic of food allergens (Moreno, 2007).

4. Conclusion

In this work we analysed a commercial pea protein isolate that was thermally and mechanically treated, in order to simulate some of the industrial conditions applied by the food industry for the production of the food products available on the market. The aim was to investigate the final quality of the proteins and to assess the availability of intact peptides after processing.

In fact, technological treatments applied for food production can cause protein unfolding upon denaturation and aggregation phenomena that markedly affect functionality (Alonso, Grant, Dewey, & Marzo, 2000). The final goal of food industries is to optimise the technological conditions for achieving the desired sensory characteristics and safety, as well as inducing the smallest denaturation or insolubilisation, phenomena that could impair nutritive value and protein functionality. This is particularly true in the case of functional foods, whose activity depends on the actual presence of specific proteins/peptides in the final products.

Depending on its own thermal stability and heating conditions (humid vs. dry heating, short time vs. long-time of exposure), each protein (for example vicilin vs. legumin) may be either partially or completely dissociated and even denaturated and aggregated, as observed in this study. Dissociation could increase the enzyme site accessibility, but unfolded molecules may also aggregate, decreasing their extractability (Petrucelli & Anon, 1995). Protein unfolding and aggregation may also be induced by mixing and shearing that occur during mechanical processing (Burnett et al., 2002).

This paper demonstrates that it is possible to select conditions (mild temperatures, short times, low pressure) that only marginally modify the protein profile. These conditions should be applied in food production. Considering the potential applications of pea protein in functional foods, a major result of our investigation was the demonstration that, even under stronger conditions, some relevant peptides may be still released intact after hydrolysis with digestive enzymes. For example, pea vicilin (NCBI nr 227928, 137582) may be a good candidate for a hypocholesterolemic protein, since it has high identity (from 54% to 56%) with soybean beta-conglycinin alpha prime (NCBI nr 51247837), the main component responsible for the cholesterol-lowering properties of this seed. In addition, it is important to underline that, indeed, some stable peptides, deriving from pea vicilin, which we have identified in this work, show high homologies with soybean beta-conglycinin alpha prime.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.foodchem.2012.02.073](https://doi.org/10.1016/j.foodchem.2012.02.073).

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Supplementary Table I. Protein list for identified spots.

Spot ID	Protein name	Database accession	Species	Experimental MW	Experimental pI	Number of peptides	Score	Coverage %	Mean SPI
1	Vicilin precursor	758248	<i>Pisum sativum</i> L	49515.2	5.33	4	51.26	9	1.31E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	3	38.04	6	1.60E+08
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	2	24.46	3	8.02E+07
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	2	22.89	3	1.92E+08
	Vicilin	42414627	<i>Pisum sativum</i> L	47279.0	5.42	2	22.89	3	1.92E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	2	22.89	3	1.92E+08
2-3	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	2	22.89	10	1.92E+08
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	8	113.57	18	1.15E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	8	113.57	19	1.15E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	7	97.21	15	1.15E+08
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	3	37.11	5	1.53E+08
	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	2	29.65	20	1.56E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	2	24.31	3	1.65E+08
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	2	23.82	10	1.33E+08
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	10	132.46	25	1.96E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	10	132.46	26	1.96E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	9	119.2	24	1.75E+08
4	Vicilin precursor	137582	PEA	52231.7	5.39	5	63.13	9	2.09E+08
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	4	52.89	7	2.46E+08
	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	3	44.03	27	2.43E+08
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	3	36.38	16	1.93E+08
	Provicilin (Type A)	137579	PEA	31540.1	5.57	2	25.72	7	1.51E+08
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	11	142.25	27	2.05E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	11	142.25	28	2.05E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	9	119.51	24	2.18E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	4	46.28	7	2.72E+08
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	3	36.67	16	2.09E+08
	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	3	34.84	27	2.88E+08
5	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	11	143.92	24	3.37E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	11	143.92	26	3.37E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	10	132.58	23	3.49E+08
	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	4	51.4	37	5.11E+08
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	3	38.5	5	5.54E+08
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	2	26.37	14	2.76E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	2	23.47	3	6.48E+08
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	10	140.27	26	1.65E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	10	140.27	28	1.65E+08
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	10	140.27	26	1.65E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	10	140.27	28	1.65E+08

	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	9	126.94	24	1.74E+08
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	5	66.02	41	1.24E+08
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	5	60.83	11	1.86E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	4	44.18	7	2.32E+08
	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	3	43.54	27	2.07E+08
8	Provicilin (Type A)	137579	PEA	31540.1	5.57	5	68.22	22	6.68E+07
	Vicilin precursor	137582	PEA	52231.7	5.39	2	28.17	4	6.36E+07
9	Vicilin precursor	137582	PEA	52231.7	5.39	2	29.37	5	5.40E+07
10	Vicilin precursor	758248	<i>Pisum sativum</i> L	49515.2	5.33	4	53.88	10	4.36E+07
	Vicilin precursor	137582	PEA	52231.7	5.39	3	40.04	7	4.63E+07
11	Vicilin precursor	758248	<i>Pisum sativum</i> L	49515.2	5.33	5	65.83	12	1.47E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	4	53.99	9	1.54E+08
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	4	50.51	9	1.13E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	4	50.51	10	1.13E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	4	50.51	10	1.13E+08
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	3	34.93	6	1.13E+08
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	3	33.94	16	1.36E+08
12	Vicilin precursor	758248	<i>Pisum sativum</i> L	49515.2	5.33	4	54.94	10	4.42E+07
	Vicilin precursor	137582	PEA	52231.7	5.39	3	39.61	7	4.24E+07
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	2	22.47	4	3.14E+07
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	2	22.47	4	3.14E+07
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	2	22.47	4	3.14E+07
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	2	22.47	12	3.14E+07
13	Vicilin precursor	137582	PEA	52231.7	5.39	5	69.65	11	1.26E+08
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	4	47.46	9	8.08E+07
	Vicilin	42414629	<i>Pisum sativum</i> L	46385.1	5.39	4	47.46	10	8.08E+07
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	4	47.46	10	8.08E+07
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	3	35.93	6	1.01E+08
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	3	32.72	16	1.07E+08
14	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	5	68.11	12	1.12E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	46385.1	5.39	5	68.11	13	1.12E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	4	56.44	10	1.38E+08
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	3	41.11	6	1.20E+08
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	3	40.12	16	1.59E+08
	Vicilin precursor	758248	<i>Pisum sativum</i> L	49515.2	5.33	3	37.92	6	1.49E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	2	24.59	3	1.95E+08
15	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	4	58.18	9	2.81E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	46385.1	5.39	4	58.18	10	2.81E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	4	58.18	10	2.61E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	4	54.29	9	2.11E+08

16	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	3	38.16	16	3.22E+08
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	2	24.08	3	2.70E+08
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	3	46.05	7	7.23E+07
	Vicilin	42414629	<i>Pisum sativum</i> L	46385.1	5.39	3	46.05	8	7.23E+07
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	3	46.05	8	6.50E+07
17	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	2	28.56	12	7.60E+07
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	3	38.55	7	7.71E+07
	Vicilin	42414629	<i>Pisum sativum</i> L	46385.1	5.39	3	38.55	8	7.71E+07
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	3	38.55	8	7.71E+07
	Vicilin 47k precursor	7339555	<i>Pisum sativum</i> L	17822.6	9.24	3	37.32	25	5.89E+07
18	Undefined								
19-20	Legumin A2 precursor	126161	PEA	59270.1	6.21	1	14.1	2	4.61E+07
	LegA class precursor	4218520	<i>Pisum sativum</i> L	58789.6	6.16	1	14.1	2	4.61E+07
	Legumin A	483449	<i>Vicia sativa</i>	56565.2	6.63	1	14.1	2	4.61E+07
21	Undefined								
22	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	6	78.36	15	1.83E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	46385.1	5.39	6	78.36	16	1.83E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	6	77.38	16	1.98E+08
	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	4	50.89	37	2.01E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	2	22.01	3	3.08E+08
23	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	6	88.45	15	3.34E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	46385.1	5.39	6	88.45	16	3.34E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	6	88.45	16	3.34E+08
	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	4	59.77	37	3.88E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	2	29.47	3	5.61E+08
24	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	4	54.58	10	3.74E+07
	Vicilin	42414629	<i>Pisum sativum</i> L	46385.1	5.39	4	54.58	11	3.74E+07
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	4	54.58	11	3.74E+07
	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	2	28.87	20	4.68E+07
	Vicilin precursor	137582	PEA	52231.7	5.39	6	76.64	17	2.02E+07
25	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	3	36.97	7	2.71E+07
	Provicilin (Type A)	137579	PEA	31540.1	5.57	2	26.63	7	2.02E+07
	Legumin (minor small)	2578438	<i>Pisum sativum</i> L	64872.7	5.40	1	14.79	4	6.10E+08
25.2	Legumin B	126169	<i>Pisum sativum</i> L	38989.8	5.28	1	14.79	6	6.10E+08
	Vicilin precursor	137582	PEA	52231.7	5.39	8	108.06	20	8.09E+07
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	4	50.8	9	1.06E+08
	Provicilin (Type A)	137579	PEA	31540.1	5.57	2	29.65	7	6.81E+07
	Vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	2	25.7	3	1.15E+08
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	2	25.7	4	1.15E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	2	25.7	4	1.15E+08

27-30	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	2	25.7	13	1.15E+08
	Albumin 2 (PA2)	113570	PEA	26238.5	5.16	1	11.01	6	5.27E+06
	Albumin-1 B precursor (PA1 B)	51704208	PEA	13970.2	6.78	4	33.99	45	3.09E+07
	Albumin-1 A precursor (PA1 A)	51702207	PEA	13912.1	6.68	2	15	26	4.70E+07
	Seed albumin 2	54019705	<i>Pisum sativum</i> L	5748.4	8.03	1	11.01	27	5.27E+06
31	Legumin A2 precursor	126161	PEA	59270.1	6.21	3	35.44	7	2.12E+07
	LegA class precursor	4218520	<i>Pisum sativum</i> L	58789.6	6.16	3	35.44	7	2.12E+07
	Legumin precursor	553082	<i>Pisum sativum</i> L	23414.9	6.75	2	24.83	10	2.33E+07
	Legumin A precursor	126168	PEA	58805.6	6.16	2	17.74	5	1.69E+07
	N-terminal legumin A1 pre-pro-polypeptide	388256	<i>Vicia faba var. minor</i>	56793	6.09	1	17.7	2	2.55E+07
32	Undefined								
33	Convicilin	7339551	<i>Pisum sativum</i> L	72063.4	5.50	11	145.86	20	8.63E+07
	713472A convicilin	227928	<i>Pisum sativum</i> L	71412.7	5.49	9	119.08	16	8.59E+07
	Convicilin precursor	117655	PEA	66990.1	6.29	7	98.05	13	8.37E+07
	Convicilin precursor	1706205	PEA	46396.3	5.32	5	64.82	13	1.27E+08
	Convicilin	7688242	<i>Lens culinaris</i>	60127.9	5.56	3	44.38	6	1.28E+08
34	Convicilin precursor	1297070	<i>Vicia narbonensis</i>	62810.3	6.09	3	38.03	5	1.26E+08
	Convicilin	7339551	<i>Pisum sativum</i> L	72063.4	5.50	11	142.04	22	8.63E+07
	713472A convicilin	227928	<i>Pisum sativum</i> L	71412.7	5.49	9	117.36	18	8.38E+07
	Convicilin precursor	117655	PEA	66990.1	6.29	6	82.12	13	8.97E+07
	Convicilin precursor	1706205	PEA	46396.3	5.32	6	75.7	19	9.15E+07
35	Convicilin	7688242	<i>Lens culinaris</i>	60127.9	5.56	3	38.33	6	9.35E+07
	Convicilin precursor	1297070	<i>Vicia narbonensis</i>	62810.3	6.09	3	35.37	5	1.27E+08
	Convicilin	7339551	<i>Pisum sativum</i> L	72063.4	5.50	13	166.26	25	2.60E+07
	713472A convicilin	227928	<i>Pisum sativum</i> L	71412.7	5.49	11	138.37	22	2.55E+07
	Convicilin precursor	117655	PEA	66990.1	6.29	8	101.37	17	2.53E+07
36	Convicilin precursor	1706205	PEA	46396.3	5.32	8	100.81	24	2.45E+07
	Convicilin	7688242	<i>Lens culinaris</i>	60127.9	5.56	4	48.64	8	2.86E+07
	Convicilin precursor	1297070	<i>Vicia narbonensis</i>	62810.3	6.09	3	34.55	6	2.27E+07
	Undefined								
	713472A convicilin	227928	<i>Pisum sativum</i> L	71412.7	5.49	11	101.33	20	4.92E+07
37-39	Convicilin	7339551	<i>Pisum sativum</i> L	72063.4	5.5	10	98.24	19	4.86E+07
	Convicilin precursor	117655	PEA	66990.1	6.29	6	63.47	11	4.97E+07
	Convicilin precursor	1706205	PEA	46396.3	5.32	6	50.00	16	5.39E+07
	Convicilin	7688242	<i>Lens culinaris</i>	60127.9	5.56	4	35.72	7	4.94E+07
	713472A convicilin	227928	<i>Pisum sativum</i> L	71412.7	5.49	11	101.33	20	4.92E+07
40-42	Convicilin	7339551	<i>Pisum sativum</i> L	72063.4	5.5	4	49.22	8	5.55E+07
	Convicilin precursor	117655	PEA	66990.1	6.29	3	38.18	6	5.74E+07
	Convicilin precursor	1297070	<i>Vicia narbonensis</i>	62810.3	6.09	3	38.18	6	5.74E+07
	Convicilin	7339551	<i>Pisum sativum</i> L	72063.4	5.50	11	145.86	20	7.64E+07
	Convicilin								

Accession	Protein	Gene	Mass (kDa)	pI	MW (kDa)	pI	MW (kDa)	pI	MW (kDa)
47	713472A convicilin	<i>Pisum sativum</i> L	71412.7	5.49	9	119.08	16	8.52E+07	
	Convicilin precursor	PEA	66990.1	6.29	7	98.05	13	3.73E+07	
	Convicilin precursor	PEA	46396.3	5.32	5	64.82	13	1.27E+08	
	Vicilin precursor	PEA	52231.7	5.39	9	102.88	22	3.38E+07	
	Vicilin precursor	<i>Vicia narbonensis</i>	52405.9	5.52	4	48.65	8	4.46E+07	
	Vicilin 47kD protein	<i>Pisum sativum</i> L	49622.0	5.50	2	22.35	3	4.05E+07	
	Vicilin	<i>Pisum sativum</i> L	46385.1	5.39	2	22.35	4	4.05E+07	
	Provicilin precursor (Type B)	PEA	46385.1	5.39	2	22.35	4	4.05E+07	
48	Vicilin, 14 kDa component	PEA	14039.6	5.28	2	22.35	13	4.05E+07	
	Provicilin (Type A)	PEA	31540.1	5.57	2	21.12	7	4.29E+07	
	Vicilin precursor	PEA	52231.7	5.39	10	135.6	26	6.01E+07	
	Vicilin precursor	<i>Pisum sativum</i> L	49515.2	5.33	9	123.7	23	6.19E+07	
	Vicilin precursor	<i>Vicia narbonensis</i>	52405.9	5.52	4	50	8	7.73E+07	
	Vicilin precursor	<i>Vicia faba</i>	52694.5	5.77	2	29.92	4	8.51E+07	
	Provicilin (Type A)	PEA	31540.1	5.57	2	29.92	7	8.51E+07	
	Vicilin 47kD protein	<i>Pisum sativum</i> L	49622.0	5.50	2	24.32	3	8.59E+07	
49	Vicilin	<i>Pisum sativum</i> L	46385.1	5.39	2	24.32	3	8.59E+07	
	Provicilin precursor (Type B)	PEA	46385.1	5.39	2	24.32	3	8.59E+07	
	Vicilin precursor	<i>Pisum sativum</i> L	49515.2	5.33	12	162.55	30	1.91E+08	
	Vicilin precursor	PEA	52231.7	5.39	11	146.67	26	1.84E+08	
	Vicilin precursor	<i>Vicia narbonensis</i>	52405.9	5.52	6	75.71	12	2.01E+08	
	Vicilin 47kD protein	<i>Pisum sativum</i> L	49622.0	5.50	4	49.33	7	2.52E+08	
	Vicilin	<i>Pisum sativum</i> L	46385.1	5.39	4	49.33	7	2.52E+08	
	Provicilin precursor (Type B)	PEA	46385.1	5.39	4	49.33	8	2.52E+08	
50	Provicilin (Type A)	PEA	31540.1	5.57	2	28.7	7	2.33E+08	
	Vicilin, 14 kDa component	PEA	14039.6	5.28	2	24.77	13	2.82E+08	
	Vicilin 47k precursor	<i>Pisum sativum</i> L	17822.6	9.24	2	24.56	10	2.23E+08	
	Vicilin precursor	<i>Pisum sativum</i> L	49515.2	5.33	8	96.69	22	3.79E+07	
	Vicilin precursor	PEA	52231.7	5.39	7	83.86	18	3.70E+07	
	Vicilin precursor	<i>Vicia narbonensis</i>	52405.9	5.52	3	40.66	6	5.77E+07	
	Vicilin 47kD protein	<i>Pisum sativum</i> L	49622.0	5.50	2	27.83	3	6.43E+07	
	Vicilin	<i>Pisum sativum</i> L	46385.1	5.39	2	27.83	4	6.43E+07	
51-53	Provicilin precursor (Type B)	PEA	46385.1	5.39	2	27.83	4	6.43E+07	
	Vicilin, 14 kDa component	PEA	14039.6	5.28	2	27.83	13	6.43E+07	
	Provicilin (Type A)	PEA	31540.1	5.57	2	26.01	7	5.07E+07	
	Vicilin precursor	<i>Pisum sativum</i> L	49515.2	5.33	16	202.08	43	1.68E+08	
	Vicilin precursor	PEA	52231.7	5.39	14	176.93	35	1.58E+08	
	Vicilin precursor	<i>Vicia narbonensis</i>	52405.9	5.52	6	75.71	12	1.83E+08	
	Vicilin 47kD protein	<i>Pisum sativum</i> L	49622.0	5.50	4	49.33	7	2.16E+08	
	Vicilin	<i>Pisum sativum</i> L	47298.0	5.44	4	49.33	7	2.16E+08	

	Vicilin	42414627	<i>Pisum sativum</i> L	47279.0	5.42	4	49.33	7	2.16E+08
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	4	49.33	8	2.16E+08
	Provicilin (Type A)	137579	PEA	31540.1	5.57	3	38.34	13	1.45E+08
54	Vicilin precursor	758248	<i>Pisum sativum</i> L	49515.2	5.53	12	134.56	32	3.50E+07
	Vicilin precursor	137582	PEA	52231.7	5.39	11	121.73	28	3.42E+07
	Vicilin precursor	1297072	<i>Vicia narbonensis</i>	52405.9	5.52	5	61.76	11	4.31E+07
	vicilin 47kD protein	297170	<i>Pisum sativum</i> L	49622.0	5.50	2	27.83	3	5.19E+07
	Vicilin	42414629	<i>Pisum sativum</i> L	47298.0	5.44	2	27.83	4	5.19E+07
	Vicilin	42414627	<i>Pisum sativum</i> L	47279.0	5.42	2	27.83	4	5.19E+07
	Provicilin precursor (Type B)	137581	PEA	46385.1	5.39	2	27.83	4	5.19E+07
	Vicilin, 14 kDa component	137577	PEA	14039.6	5.28	2	27.83	13	5.19E+07
55	Convicilin precursor	117655	PEA	66990.1	6.29	6	63.47	11	4.94E+07
	Convicilin precursor	1706205	PEA	46396.3	5.32	6	50.00	16	5.37E+07
55.2	Vicilin precursor	758248	<i>Pisum sativum</i> L	49515.2	5.33	16	202.08	43	1.68E+07
	Vicilin precursor	137582	PEA	52231.7	5.39	14	176.93	35	1.58E+07
56	Convicilin precursor	117655	PEA	66990.1	6.29	6	63.47	11	1.21E+07
	Convicilin precursor	1706205	PEA	46396.3	5.32	6	50.00	16	1.38E+07
56.2	Vicilin precursor	758248	<i>Pisum sativum</i> L	49515.2	5.33	16	202.08	43	1.89E+07
	Vicilin precursor	137582	PEA	52231.7	5.39	14	176.93	35	1.76E+07
57	LegA class precursor	4218520	<i>Pisum sativum</i> L	58789.6	6.16	4	47.43	10	1.21E+07
	Legumin A2 precursor	126161	PEA	59270.1	6.21	3	35.23	8	1.38E+07
	Legumin A	483449	<i>Vicia sativa</i>	56565.2	6.63	1	13.78	2	1.89E+07
	Legumin K	282925	garden pea	56276.6	5.64	2	30.79	8	4.56E+07
	Legumin J precursor	126170	PEA	56895.4	5.70	2	29.85	8	3.63E+07
58	Legumin K	282925	garden pea	56276.6	5.64	3	44.83	11	3.04E+07
	Legumin J precursor	126170	PEA	56895.4	5.70	1	18.24	4	3.02E+07
	Legumin A2 precursor	126161	PEA	59270.1	6.21	2	26.58	4	1.53E+08
	LegA class precursor	4218520	<i>Pisum sativum</i> L	58789.6	6.16	1	13.59	2	1.56E+08
	Legumin A	483449	<i>Vicia sativa</i>	56565.2	6.63	1	13.59	2	1.56E+08
59	LegA class precursor	4218520	<i>Pisum sativum</i> L	58789.6	6.16	8	102.05	14	4.02E+08
	Legumin A precursor	126168	PEA	58805.6	6.16	7	88.92	12	3.09E+08
	Legumin A2 precursor	126161	PEA	59270.1	6.21	6	76.46	12	3.05E+07
60	Legumin A2 precursor	126161	PEA	59270.1	6.21	5	66.96	12	3.46E+08
	LegA class precursor	4218520	<i>Pisum sativum</i> L	58789.6	6.16	4	58.75	9	3.55E+08
	Legumin A precursor	126168	PEA	58805.6	6.16	3	44.09	7	8.42E+07
	Legumin A	483449	<i>Vicia sativa</i>	56565.2	6.63	1	14.66	2	7.60E+08
61	Legumin A2 precursor	126161	PEA	59270.1	6.21	3	38.61	8	5.31E+07
	LegA class precursor	4218520	<i>Pisum sativum</i> L	58789.6	6.16	3	38.61	8	5.31E+07
	Legumin A precursor	126168	PEA	58805.6	6.16	2	23.83	5	1.97E+07
	Legumin A	483449	<i>Vicia sativa</i>	56565.2	6.63	1	14.78	2	8.65E+07

62	Undefined								
63	Legumin (minor small)	2578438	<i>Pisum sativum</i> L	64872.7	5.40	1	15.39	3	1.67E+08
	Legumin J precursor	126170	PEA	56895.4	5.70	1	15.39	3	1.67E+08
64	Legumin (minor small)	2578438	<i>Pisum sativum</i> L	64872.7	5.40	1	19.17	3	2.38E+08
65	Legumin (minor small)	2578438	<i>Pisum sativum</i> L	64872.7	5.40	2	34.27	6	6.05E+07

Supplementary Table II. Peptide list for identified spots.

Spot ID	Peptide sequence	MH+	z	Score	SPI (%)	Spectrum intensity
1	(K)NILEASFNNTDYEEIEK(V)	1914.897	2	15.15	78.9	9.68E+07
	(K)AILTVLKPDDR(N)	1240.726	3	13.22	85.4	4.23E+07
	(K)IFENLQNYR(L)	1196.606	2	11.65	74.2	2.66E+08
	(R)NSFNLER(G)	879.432	2	11.24	73.7	1.18E+08
2-3	(R)RQEINEENVIVK(V)	1470.791	3	17.29	87.9	7.00E+07
	(R)AIVIVTVTEGK(G)	1129.683	2	16.93	93.5	1.49E+08
	(K)NQQLQDLDFVNSVDIK(E)	1989.029	3	16.36	90.4	1.18E+08
	(K)NILEAAFNTNYEEIEK(V)	1265.279	2	14.68	77.9	3.13E+07
	(K)GDFELVGQR(N)	1020.511	2	13.29	86.8	1.94E+08
	(K)NILEAAFNTNYEEIEK(V)	1897.918	3	13.01	73.8	1.17E+08
	(R)SDQENPFIFK(S)	1224.59	2	12.8	83.3	1.30E+08
	(R)QEINEENVIVK(V)	1314.69	2	11.2	76.1	9.31E+07
	(R)NSFNLER(G)	879.432	2	11.02	80.3	1.36E+08
4	(K)NQQLQDLDFVNSVDIK(E)	1989.029	3	15.46	86.5	1.40E+08
	(K)GDFELVGQR(N)	1020.511	2	14.43	90.4	3.15E+08
	(R)AIVIVTVTEGK(G)	1129.683	2	14.18	87	1.10E+08
	(K)FFEITPEK(N)	1010.52	2	14.14	94.9	4.04E+08
	(R)RQEINEENVIVK(V)	1470.791	3	13.54	84.9	1.69E+08
	(K)VLLQQEQEPQHR(R)	1633.829	3	11.67	85	3.43E+07
	(R)SDQENPFIFK(S)	1224.59	2	13.11	83.4	1.85E+08
	(R)RQEINEENVIVK(V)	1470.791	2	12.44	78.4	3.84E+07
	(K)IFENLQNYR(L)	1196.606	2	12.06	81.1	1.84E+08
	(R)NSFNLER(G)	879.432	2	11.21	79.9	2.10E+08
	(K)NILEAAFNTNYEEIEK(V)	1265.279	2	11.07	70.6	5.46E+07
	(R)AIVIVTVTEGK(G)	1129.683	2	17.32	97.6	2.11E+08
	(K)NILEAAFNTNYEEIEK(V)	1265.279	2	15.88	84.5	1.07E+08
	(R)RQEINEENVIVK(V)	1470.791	3	14.8	82.2	1.87E+08
	(K)IFENLQNYR(L)	1196.606	2	13.08	80.9	2.05E+08
	(K)NQQLQDLDFVNSVDIK(E)	1989.029	3	12.97	76.3	2.68E+08
5	(K)NILEAAFNTNYEEIEK(V)	1265.279	3	12.67	73	2.68E+08
	(R)SDQENPFIFK(S)	1224.59	2	12.26	82.3	1.52E+08
	(K)FFEITPEK(N)	1010.52	2	11.49	91.7	4.87E+08
	(K)VLLQQEQEPQHR(R)	1633.829	3	11.55	82.9	3.67E+06
	(R)NSFNLER(G)	879.432	2	11.33	84.1	2.71E+08
	(R)EQIEELSK(N)	975.5	2	11.3	85.1	1.40E+08
	(K)GDFELVGQR(N)	1020.511	2	10.38	82.3	1.30E+08
	(R)AIVIVTVTEGK(G)	1129.683	2	16.81	93	3.56E+08
	(K)NQQLQDLDFVNSVDIK(E)	1989.029	3	15.87	87.5	5.90E+08
	(K)NQQLQDLDFVNSVDIK(E)	1326.019	2	15.49	82.2	3.43E+07
	(R)SDQENPFIFK(S)	1224.59	2	15.03	86.8	4.19E+08
	(K)NILEAAFNTNYEEIEK(V)	1265.279	2	15	79.8	1.32E+08
	(K)NILEAAFNTNYEEIEK(V)	1897.918	3	13.35	80.7	2.76E+08
	(R)RQEINEENVIVK(V)	1470.791	3	12.99	78.8	4.34E+08
	(K)SVSSESGPFNLR(S)	1351.649	2	12.12	73.2	4.19E+07
	(K)FFEITPEK(N)	1010.52	2	11.85	93.3	8.92E+08
6	(K)GDFELVGQR(N)	1020.511	2	13.91	87.9	9.01E+07
	(K)LPAGTIAYLANR(D)	1259.711	2	11.34	78.1	9.13E+07
	(R)QEINEENVIVK(V)	1314.69	2	10.69	76	4.94E+08
	(K)KSVSSESGPFNLR(S)	1407.723	3	10.66	76.7	6.55E+07
	(R)RQEINEENVIVK(V)	1470.791	2	10.5	75.8	8.83E+07
	(K)NQQLQDLDFVNSVDIK(E)	1326.019	2	20.46	91.1	2.90E+07
	(K)NQQLQDLDFVNSVDIK(E)	1989.029	3	18.55	94.5	2.76E+08
	(R)TLFLPQYTDADFILVVLGSK(A)	2240.221	3	17.66	88.5	2.26E+07
	(R)AIVIVTVTEGK(G)	1129.683	2	17.26	94.2	1.95E+08
	(K)NILEAAFNTNYEEIEK(V)	1265.279	2	16.35	87.7	5.93E+07
7						

	(R)RQEINEENVIVK(V)	1470.791	3	14.76	78.4	1.19E+08
	(R)SDQENPFIFK(S)	1224.59	2	13.93	89.7	1.65E+08
	(K)LPAGTIAYLANRDDNEDLR(V)	2117.062	3	13.33	85.6	4.22E+07
	(K)GDFELVGQR(N)	1020.511	2	12.38	86.2	3.15E+08
	(K)FFEITPEK(N)	1010.52	2	10.7	89.3	3.34E+08
	(K)IFENLQNYR(L)	1196.606	2	10.61	77.7	1.90E+08
	(R)NSFNLER(G)	879.432	2	10.49	71.6	1.97E+08
8	(K)DLTFPGSAQEVD(R)	1434.686	2	16.81	94.4	1.02E+08
	(R)AIVIVTVNEGK(G)	1129.683	2	14.63	92.1	5.27E+07
	(K)GDFELVGQR(N)	1020.511	2	13.54	88.7	7.46E+07
	(K)LTPGDVFPAGHPVAVR(A)	1845.038	3	12.33	90.4	7.57E+07
	(K)QSYFANAQPQQR(E)	1437.687	2	10.91	81	2.89E+07
9	(K)NILEASFNNTDYEEIEK(V)	1914.897	2	16.68	80.3	2.81E+07
	(R)NSFNLER(G)	879.432	2	12.69	81.2	8.00E+07
10	(K)NILEASFNNTDYEEIEK(V)	1914.897	2	16.86	80.4	5.34E+07
	(K)AILTVLKPDDR(N)	1240.726	3	13.84	91	3.55E+07
	(R)SDPQNPFIFK(S)	1192.6	2	11.83	87.6	1.50E+07
	(R)NSFNLER(G)	879.432	2	11.35	82.9	7.05E+07
11	(K)NILEASFNNTDYEEIEK(V)	1914.897	2	18.04	91	4.59E+07
	(R)SDPQNPFIFK(S)	1192.6	2	12.9	92.7	1.28E+08
	(R)NSFNLER(G)	879.432	2	12.2	81.2	2.30E+08
	(K)AILTVLKPDDR(N)	1240.726	3	11.84	90.7	1.21E+08
	(K)IFENLQNYR(L)	1196.606	2	10.85	80	2.11E+08
12	(K)NILEASFNNTDYEEIEK(V)	1914.897	2	15.89	78.3	1.72E+07
	(K)AILTVLKPDDR(N)	1240.726	3	15.33	90.8	4.98E+07
	(K)IFENLQNYR(L)	1196.606	2	12.38	71.9	5.51E+07
	(R)SDPQNPFIFK(S)	1192.6	2	11.34	88.6	5.48E+07
	(R)SDQENPFIFK(S)	1224.59	2	10.09	76.7	7.70E+06
13	(K)NILEAAFNTNYEEIEK(V)	1265.279	2	14.74	86.1	3.70E+06
	(K)AILTVLKPDDR(N)	1240.726	3	14.39	93.9	8.21E+07
	(R)SDPQNPFIFK(S)	1192.6	2	13.35	89.9	1.63E+08
	(K)IFENLQNYR(L)	1196.606	2	11.21	74.2	1.48E+08
	(R)SDQENPFIFK(S)	1224.59	2	10.89	77.2	2.19E+07
	(R)NSFNLER(G)	879.432	2	10.62	75.2	1.49E+08
14	(K)NILEAAFNTNYEEIEK(V)	1265.279	2	16.32	88.2	3.27E+07
	(R)SDQENPFIFK(S)	1224.59	2	15.53	89.3	1.48E+08
	(K)AILTVLKPDDR(N)	1240.726	3	13.33	93.4	9.80E+06
	(K)IFENLQNYR(L)	1196.606	2	12.34	81.6	2.05E+08
	(R)NSFNLER(G)	879.432	2	12.25	83	2.38E+08
	(K)VLLQQEQEPQHR(R)	1633.829	3	11.67	85	3.43E+07
15	(K)NILEAAFNTNYEEIEK(V)	1265.279	2	20.02	95.6	7.63E+07
	(K)IFENLQNYR(L)	1196.606	2	14.08	86.6	4.25E+08
	(K)NILEAAFNTNYEEIEK(V)	1897.918	3	12.77	82.2	3.61E+08
	(R)NSFNLER(G)	879.432	2	12.41	82.9	2.75E+08
	(R)SDPQNPFIFK(S)	1192.6	2	11.9	92.9	1.14E+08
	(R)SDQENPFIFK(S)	1224.59	2	11.67	85.3	2.66E+08
16	(K)NILEAAFNTNYEEIEK(V)	1265.279	2	17.49	88	3.21E+07
	(R)SDQENPFIFK(S)	1224.59	2	15.04	91.3	8.36E+07
	(K)NILEAAFNTNYEEIEK(V)	1897.918	3	13.59	77.3	1.01E+08
	(K)IFENLQNYR(L)	1196.606	2	13.52	79.3	1.13E+08
	(R)SDQENPFIFK(S)	1224.59	2	10.9	70.9	3.10E+07
17	(K)IFENLQNYR(L)	1196.606	2	13.47	78.8	7.32E+07
	(R)SDQENPFIFK(S)	1224.59	2	12.88	82.2	8.24E+07
	(K)NILEAAFNTNYEEIEK(V)	1897.918	3	12.2	75	7.57E+07
	(R)TLFLPQYTDADFILVVLSGK(A)	2240.221	3	10.97	74.2	2.11E+07
19-20	(R)DFLEDAFNVNR(H)	1339.628	2	14.1	81.7	4.61E+07
	(K)QEENEGNNIFSGFK(R)	1740.782	2	10.61	72.3	1.50E+06
	(R)FYLAGNHEQEFLR(Y)	1623.791	3	10.22	79.8	1.81E+06
22	(K)NQQLQDLDFVNSVDIK(E)	1989.029	3	17.45	88.1	6.06E+07

	(R)RQEINEENVIVK(V)	1470.791	3	14.26	81	8.34E+07
	(R)AIVIVTVTEGK(G)	1129.683	2	13.21	84.5	1.58E+08
	(K)SVSSESGPFNL(R)	1351.649	2	11.43	77.2	1.78E+08
	(K)GDFELVGQR(N)	1020.511	2	11.23	82.4	1.16E+08
	(K)FFEITPEK(N)	1010.52	2	10.78	90.6	5.18E+08
	(K)NQQLQDLDFVNSVDIK(E)	1326.019	2	10.75	70.3	9.88E+06
23	(K)NQQLQDLDFVNSVDIK(E)	1989.029	3	18.85	95.5	3.62E+08
	(K)NQQLQDLDFVNSVDIK(E)	1326.019	2	18.11	88.6	2.79E+07
	(R)AIVIVTVTEGK(G)	1129.683	2	16.99	95.8	2.85E+08
	(K)GDFELVGQR(N)	1020.511	2	15.12	92.8	5.67E+08
	(K)FFEITPEK(N)	1010.52	2	14.35	94.2	7.23E+08
	(R)RQEINEENVIVK(V)	1470.791	3	11.69	71.4	5.59E+07
	(K)SVSSESGPFNL(R)	1351.649	2	11.45	77	2.56E+08
24	(K)NQQLQDLDFVNSVDIK(E)	1989.029	3	15.93	88.1	4.53E+07
	(R)AIVIVTVTEGK(G)	1129.683	2	13.29	86.3	2.42E+07
	(K)GDFELVGQR(N)	1020.511	2	12.94	87.5	4.12E+07
	(R)QEINEENVIVK(V)	1314.69	2	12.42	81.2	2.25E+07
25	(K)NPQLQDLDFVNSVEIK(E)	1972.039	3	15.49	88.7	8.72E+06
	(K)ELAFPGSAQEVDR(I)	1418.691	2	13.91	86.7	4.59E+07
	(R)AIVIVTVNEGK(G)	1129.683	2	13.87	87.2	2.09E+07
	(K)GDFELVGQR(N)	1020.511	2	12.76	87	1.96E+07
	(K)ASSNLDLLGFGINAENNQR(N)	2033.005	3	10.31	72.1	1.02E+07
	(K)SVSSESEPFNL(R)	1351.649	2	10.3	74.2	1.57E+07
25.2	(K)VEDGLHISPELQEEEEQSHSQR(K)	2689.271	4	14.79	76.5	6.10E+08
26	(K)NPQLQDLDFVNSVEIK(E)	1972.039	3	18.43	93.5	5.96E+07
	(R)AIVIVTVNEGK(G)	1129.683	2	15.74	93.3	4.62E+07
	(K)GDFELVGQR(N)	1020.511	2	13.91	87.9	9.01E+07
	(K)ELAFPGSAQEVDR(I)	1418.691	2	12.98	91	1.50E+08
	(R)GPIYSNEFGK(F)	1111.542	2	12.83	87	1.10E+08
	(K)SVSSESEPFNL(R)	1351.649	2	12.12	73.2	4.19E+07
	(K)FFEITPEK(N)	1010.52	2	11.79	90.2	1.41E+08
27-30	(K)YVLLDYAPGTSNDK(V)	1555.764	2	11.01	75.5	5.27E+06
	(A)ASCNGVCSPFEMPPCGSSACR(C)	2330.909	3	12.43	84	7.79E+06
	(N)DEHPNLCESDADCR(K)	1717.655	3	7.88	66.3	7.67E+07
	(V)LFATLGMFLTKNVGAASCNG(V)	2072.031	3	7.12	73.6	1.72E+07
	(A)SVKLASLMVLFATLGMFLTK(N)	2170.238	3	6.56	74.7	2.18E+07
31	(R)DFLEDAFNVNR(H)	1339.628	2	17.7	89.1	4.31E+07
	(K)QEENEGNNIFSGFK(R)	1740.782	2	10.61	72.3	1.51E+07
	(K)GGLSIISPPEK(Q)	1097.62	2	7.13	88.8	1.87E+07
	(R)DFLEDAFNVNR(H)	1339.628	3	7	62.6	7.96E+06
33	(R)AIVVLLVNEGK(G)	1154.715	2	17.5	92.7	6.30E+07
	(R)NFLSGSDDNVISQIENPVK(E)	2076.025	3	17.25	84	6.09E+07
	(R)SDLFENLQNYR(L)	1398.665	2	14.94	84.1	1.13E+08
	(K)NILEASYNTR(Y)	1152.59	2	14.45	88.8	1.28E+08
	(K)VLLLEEQEK(D)	987.536	2	13.16	94.8	1.04E+08
	(K)ELTFPGSVQEINR(L)	1489.765	2	12.33	81.2	4.84E+07
	(K)GNLELLGLK(N)	956.578	2	12.11	82.4	5.29E+06
	(K)AILTVLSPNDR(N)	1198.679	2	11.94	93.9	1.28E+08
	(K)LFEITPEK(N)	976.535	2	11.15	85.5	5.47E+07
	(R)NFLSGSDDNVISQIENPVK(E)	2076.025	2	11.02	71.3	1.49E+07
	(K)SLPSEFEPINLR(S)	1401.738	2	10.7	84.5	1.13E+08
	(R)LVDLVIPVNGPGK(F)	1320.789	2	10.33	83.3	7.89E+07
34	(R)NFLSGSDDNVISQIENPVK(E)	2076.025	3	19.29	90.4	6.45E+07
	(R)NFLSGSDDNVISQIENPVK(E)	2076.025	2	17.03	84.2	1.12E+07
	(K)LPAGTTSYLVNQDDEEDLR(L)	2136.01	3	14.09	81.5	6.22E+07
	(K)NILEASYNTR(Y)	1152.59	2	13.81	83.5	1.10E+08
	(K)AILTVLSPNDR(N)	1198.679	2	13.72	89.9	3.00E+08
	(R)AIVVLLVNEGK(G)	1154.715	2	13.37	79.1	3.64E+07
	(R)LVDLVIPVNGPGK(F)	1320.789	2	12.42	87.9	6.44E+07

35	(K)ELTFPGSVQEINR(L)	1489.765	2	12.05	76.8	5.80E+07
	(K)NILEASYNTK(Y)	1152.59	2	11.6	82.7	2.96E+07
	(R)SDLFENLQNYR(L)	1398.665	2	11.24	85.5	5.87E+07
	(K)SLPSEFEPINLR(S)	1401.738	2	11.22	86.1	1.04E+08
	(R)SDLFENLQNYR(L)	1398.665	3	10.66	74.4	1.75E+07
	(K)LPAGTTSYLVNQDDEEDLR(L)	2136.01	3	16.28	85.7	1.18E+07
	(R)NFLSGSDDNVISQIENPVK(E)	2076.025	3	14.53	75.6	2.63E+07
	(K)NILEASYNTR(Y)	1152.59	2	14.37	85.8	3.10E+07
	(R)AIVVLLVNEGK(G)	1154.715	2	14.09	89.8	4.61E+07
	(K)ELTFPGSVQEINR(L)	1489.765	2	13.52	86.3	2.55E+07
37-39	(K)AILTVLSPNDR(N)	1198.679	2	13.17	92.4	2.91E+07
	(R)LVDLVIPVNGPGK(F)	1320.789	2	13	81.7	2.59E+07
	(K)SLPSEFEPINLR(S)	1401.738	2	12.12	81.7	1.59E+07
	(K)FEAFDLAK(N)	940.478	2	11.88	91.9	3.63E+07
	(K)GNLELLGLK(D)	956.578	2	11.19	84.7	2.76E+07
	(R)SDLFENLQNYR(L)	1398.665	2	10.76	75.1	1.42E+07
	(K)VLLEEQEK(E)	987.536	2	10.73	85.6	2.28E+07
	(K)FLTLFENENGHIR(L)	1589.808	3	10.62	71.4	2.49E+07
	(R)NFLSGSDDNVISQIENPVK(E)	2076.025	3	15.55	82.4	4.47E+07
	(K)GNLELLGLK(D)	956.577	2	12.2	82.9	6.73E+07
40-42	(K)ELTFPGSVQEINR(L)	1489.765	2	11.04	77.9	4.60E+07
	(K)AILTVLSPNDR(N)	1198.679	2	10.43	89.7	9.03E+07
	(R)SDLFENLQNYR(L)	1398.665	2	9.67	66.9	3.45E+07
	(R)AIVVLLVNEGK(G)	1154.714	2	9.45	85.1	2.87E+07
	(R)LVDLVIPVNGPGK(F)	1320.789	2	8.25	64.7	3.07E+07
	(R)WEREEDDEEQVDEEWR(G)	2063.858	3	8.01	79.3	2.09E+07
	(R)SDLFENLQNYR(L)	1398.665	2	7.82	74.1	3.77E+07
	(K)FEAFDLAK(N)	940.477	2	7.47	98	7.89E+07
	(K)VLLEEQEKQQR(R)	1625.849	3	7.45	96.7	6.78E+07
	(K)NILEASYNTK(Y)	1152.59	2	6.68	76.6	3.96E+07
43-46	(R)NSYNLER(G)	895.427	2	6.17	76.3	7.37E+07
	(R)NFLSGSDDNVISQIENPVK(E)	2076.024	3	13.17	72.3	4.64E+07
	(K)ELTFPGSVQEINR(L)	1489.765	2	11.04	77.9	4.60E+07
	(R)SDLFENLQNYR(L)	1398.665	2	9.67	66.9	3.45E+07
	(K)AILTVLSPNDR(N)	1198.679	2	9.5	100	8.00E+07
	(R)AIVVLLVNEGK(G)	1154.714	2	9.29	90.6	3.61E+07
	(R)LVDLVIPVNGPGK(F)	1320.789	2	8.25	64.7	3.07E+07
	(K)VLLEEQEKQQR(R)	1625.849	3	7.45	96.7	6.78E+07
	(K)GNLELLGLK(D)	956.577	2	6.77	82.7	5.82E+07
	(K)NILEASYNTK(Y)	1152.59	2	6.68	76.6	3.96E+07
47	(K)ELTFPGSVQEINR(L)	1489.765	2	11.04	77.9	4.60E+07
	(K)AILTVLSPNDR(N)	1198.679	2	10.43	89.7	9.03E+07
	(R)SDLFENLQNYR(L)	1398.665	2	9.67	66.9	3.45E+07
	(R)AIVVLLVNEGK(G)	1154.715	2	9.29	90.6	3.61E+07
	(R)LVDLVIPVNGPGK(F)	1320.789	2	8.25	64.7	3.07E+07
	(R)NFLSGSDDNVISQIENPVK(E)	2076.024	3	8.24	86.5	4.18E+07
	(R)WEREEDDEEQVDEEWR(G)	2063.858	3	8.01	79.3	2.09E+07
	(K)FEAFDLAK(N)	940.477	2	7.47	98	7.89E+07
	(K)VLLEEQEKQQR(R)	1625.849	3	7.45	96.7	6.78E+07
	(K)NILEASYNTK(Y)	1152.59	2	6.68	76.6	3.96E+07
47	(K)GNLELLGLK(D)	956.577	2	6.21	88.9	5.43E+07
	(R)NSYNLER(G)	895.427	2	6.17	76.3	7.37E+07
	(K)AILTVLKPDDR(N)	1240.727	3	14.88	93.2	6.08E+07
	(K)NILEASFNTDYEEIEK(V)	1914.897	2	12.78	74.3	2.02E+07
	(K)ELAFPGSAQEVDR(I)	1418.692	2	11.42	83.2	3.66E+07
	(K)FFELTPEK(N)	1010.52	2	11.37	93.9	2.94E+07
	(K)GDFELVGQR(N)	1020.511	2	10.98	80.6	5.16E+07
	(K)NPQLQDLDFVNSVEIK(E)	1972.039	3	10.75	78.9	9.13E+06
	(R)SDPQNPFIFK(S)	1192.6	2	10.29	85.4	2.36E+07

48	(R)GPIYSNEFGK(F)	1111.542	2	10.27	85.3	3.87E+07
	(R)AIVIVTVNEGK(G)	1142.679	2	10.14	84.8	3.41E+07
	(K)NPQLQDLDFVNSVEIK(E)	1972.039	3	18.69	92	1.30E+07
	(R)AIVIVTVNEGK(G)	1142.679	2	16.87	93.9	4.14E+07
	(K)NILEASFNTDYEEIEK(V)	1914.897	2	15.98	80.3	3.02E+07
	(K)AILTVLKPDDR(N)	1240.727	3	13.89	93.5	9.47E+07
	(K)GDFELVGQR(N)	1020.511	2	13.05	82.7	8.50E+07
49	(R)NFLAGDEDNVISQIQRPVK(E)	2143.115	3	11.9	85.7	3.88E+07
	(K)SVSSESEPFNL(R)	1351.649	2	11.79	74.1	4.05E+07
	(R)GPIYSNEFGK(F)	1111.542	2	11.32	80.4	1.07E+08
	(R)NSFNLER(G)	879.433	2	11.27	78.8	4.36E+07
	(R)SDPQNPFIFK(S)	1192.6	2	10.84	89	4.36E+07
	(K)NPQLQDLDFVNSVEIK(E)	1972.039	3	18.34	91.9	3.05E+08
	(K)NILEASFNTDYEEIEK(V)	1914.897	2	18.22	88.1	8.48E+07
50	(K)AILTVLKPDDR(N)	1240.727	3	15.88	93.5	2.68E+08
	(R)AIVIVTVNEGK(G)	1142.679	2	15.32	88.1	1.89E+08
	(K)GDFELVGQR(N)	1020.511	2	13.38	85.5	2.77E+08
	(K)IFENLQNYR(L)	1196.606	2	12.84	84.4	2.47E+08
	(K)ELAFPGSAQEVD(R)	1418.692	2	12.68	83.9	9.99E+07
	(R)GPIYSNEFGK(F)	1111.542	2	11.89	87.3	1.97E+08
	(R)NSFNLER(G)	879.433	2	11.72	81.4	1.99E+08
51-53	(K)FFEITPEK(N)	1010.52	2	11.39	95.2	2.87E+08
	(K)SVSSESEPFNL(R)	1351.649	2	10.66	74.6	7.54E+07
	(R)GQIEELSK(N)	903.479	2	10.23	88.9	1.19E+08
	(K)GDFELVGQR(N)	1020.511	2	14.76	90.6	5.57E+07
	(K)FFEITPEK(N)	1010.52	2	13.07	85.4	7.29E+07
	(K)AILTVLKPDDR(N)	1240.727	3	12.83	91	4.46E+07
	(K)NPQLQDLDFVNSVEIK(E)	1972.039	3	11.87	74	2.31E+07
54	(K)ASSNLDLLGFGINAENNQR(N)	2033.005	3	11.6	71.4	2.38E+07
	(R)AIVIVTVNEGK(G)	1142.678	2	11.25	82.9	4.57E+07
	(R)QSQEENVIVK(L)	1301.67	2	10.92	80	2.45E+07
	(K)QSHFADAQPQQR(E)	1412.667	3	10.39	79.3	1.33E+07
	(K)NPQLQDLDFVNSVEIK(E)	1972.039	3	18.34	91.9	3.05E+08
	(K)NILEASFNTDYEEIEK(V)	1914.897	2	18.22	88.1	8.48E+07
	(K)AILTVLKPDDR(N)	1240.726	3	15.88	93.5	2.68E+08
55	(R)AIVIVTVNEGK(G)	1142.678	2	15.32	88.1	1.89E+08
	(K)IFENLQNYR(L)	1196.606	2	12.84	84.4	2.47E+08
	(K)ELAFPGSAQEVD(R)	1418.691	2	12.68	83.9	9.99E+07
	(R)GPIYSNEFGK(F)	1111.542	2	11.89	87.3	1.97E+08
	(K)ASSNLDLLGFGINAENNQR(N)	2033.005	3	11.56	67.2	1.29E+08
	(K)SVSSESEPFNL(R)	1351.649	2	10.66	74.6	7.54E+07
	(R)GQIEELSK(N)	903.478	2	10.23	88.9	1.19E+08
54	(K)LPAGTIAYLVNR(D)	1287.742	2	9.86	75.5	1.33E+07
	(K)FQTLFENENGHIR(L)	1604.782	3	9.27	71.3	3.16E+08
	(K)GDFELVGQR(N)	1020.511	2	8.95	66	1.05E+08
	(K)GDFELVGQR(N)	1020.511	2	14.76	90.6	5.57E+07
	(K)FFEITPEK(N)	1010.519	2	13.07	85.4	7.29E+07
	(K)AILTVLKPDDR(N)	1240.726	3	12.83	91	4.46E+07
	(K)NPQLQDLDFVNSVEIK(E)	1972.039	3	11.87	74	2.31E+07
55	(K)ASSNLDLLGFGINAENNQR(N)	2033.005	3	11.6	71.4	2.38E+07
	(K)ELAFPGSAQEVD(R)	1418.691	2	11.58	61.8	3.72E+07
	(R)AIVIVTVNEGK(G)	1142.678	2	11.25	82.9	4.57E+07
	(R)QSQEENVIVK(L)	1301.67	2	10.92	80	2.45E+07
	(K)QSHFADAQPQQR(E)	1412.667	3	10.39	79.3	1.33E+07
	(R)SDPQNPFIFK(S)	1192.6	2	9.86	90.8	3.58E+07
	(K)SVSSESEPFNL(R)	1351.649	2	9.52	78.7	1.22E+07
55	(R)NFLSGSDDNVISQIENPVK(E)	2076.024	3	15.55	82.4	4.47E+07
	(K)GNLELLGLK(D)	956.577	2	12.2	82.9	6.73E+07
	(K)ELTFPGSVQEINR(L)	1489.765	2	11.04	77.9	4.60E+07

	(K)AILTVLSPNDR(N)	1198.679	2	10.43	89.7	9.03E+07
	(R)SDLFENLQNYR(L)	1398.665	2	9.67	66.9	3.45E+07
	(R)AIVVLLVNEGK(G)	1154.714	2	9.45	85.1	2.87E+07
	(R)LVDLVIPVNGPGK(F)	1320.789	2	8.25	64.7	3.07E+07
	(R)SDLFENLQNYR(L)	1398.665	2	7.82	74.1	3.77E+07
	(K)VLLLEEQEKEPQQR(R)	1625.849	3	7.45	96.7	6.78E+07
	(K)NILEASYNTK(Y)	1152.59	2	6.68	76.6	3.96E+07
	(R)NSYNLER(G)	895.427	2	6.17	76.3	7.37E+07
55.2	(K)NILEASFNTDYEEIEK(V)	1914.897	2	18.22	88.1	8.48E+07
	(K)AILTVLKPDDR(N)	1240.726	3	15.88	93.5	2.68E+08
	(K)GDFELVGQR(N)	1020.511	2	13.38	85.5	2.77E+08
	(K)IFENLQNYR(L)	1196.606	2	12.84	84.4	2.47E+08
	(K)ELAFPGSAQEVD(R)	1418.691	2	12.68	83.9	9.99E+07
	(K)NPQLQDLDFVNSVEIK(E)	1972.039	3	12.46	72.7	1.32E+08
	(R)NSFNLER(G)	879.432	2	11.72	81.4	1.99E+08
	(K)ASSNLDLLGFGINAENNQR(N)	2033.005	3	11.56	67.2	1.29E+08
	(K)FFEITPEK(N)	1010.519	2	11.39	95.2	2.87E+08
	(K)SVSSESEPFNLR(S)	1351.649	2	10.66	74.6	7.54E+07
	(R)GQIEELSK(N)	903.478	2	10.23	88.9	1.19E+08
	(K)LPAGTIAYLVNR(D)	1287.742	2	9.86	75.5	1.33E+07
	(K)FQTLFENENGHIR(L)	1604.782	3	9.27	71.3	3.16E+08
	(K)QSHFADAQPQQR(E)	1412.667	3	8.84	82.4	8.26E+07
56	(R)NFLSGSDDNVISQIENPVK(E)	2076.024	3	15.55	82.4	4.47E+07
	(K)GNLELLGLK(D)	956.577	2	12.2	82.9	6.73E+07
	(K)ELTFPGSVQEINR(L)	1489.765	2	11.04	77.9	4.60E+07
	(K)AILTVLSPNDR(N)	1198.679	2	10.43	89.7	9.03E+07
	(R)SDLFENLQNYR(L)	1398.665	2	9.67	66.9	3.45E+07
	(R)AIVVLLVNEGK(G)	1154.714	2	9.45	85.1	2.87E+07
56.2	(R)NFLSGSDDNVISQIENPVK(E)	2076.024	3	15.55	82.4	4.47E+07
	(K)ELTFPGSVQEINR(L)	1489.765	2	11.04	77.9	4.60E+07
	(R)SDLFENLQNYR(L)	1398.665	2	9.67	66.9	3.45E+07
	(K)AILTVLSPNDR(N)	1198.679	2	9.5	100	8.00E+07
	(R)LVDLVIPVNGPGK(F)	1320.789	2	8.25	64.7	3.07E+07
	(R)NFLSGSDDNVISQIENPVK(E)	2076.024	3	8.24	86.5	4.18E+07
	(R)AIVVLLVNEGK(G)	1154.714	2	8.14	78.1	3.59E+07
	(R)WEREEDDEEVDEEWR(G)	2063.858	3	8.01	79.3	2.09E+07
	(R)SDLFENLQNYR(L)	1398.665	2	7.82	74.1	3.77E+07
	(K)VLLLEEQEKEPQQR(R)	1625.849	3	7.45	96.7	6.78E+07
	(K)GNLELLGLK(D)	956.577	2	6.77	82.7	5.82E+07
	(K)NILEASYNTK(Y)	1152.59	2	6.68	76.6	3.96E+07
	(R)SDLFENLQNYR(L)	1398.665	2	6.38	64.7	3.84E+07
	(K)GNLELLGLK(D)	956.577	2	6.21	88.9	5.43E+07
57	(R)DFLEDAFNVNR(H)	1339.628	2	13.78	83.5	1.89E+07
	(R)EQPEQNECQLER(L)	1559.676	2	12.2	86.2	7.13E+06
	(K)QEENEGNNIFSGFK(R)	1740.782	2	11.25	72.5	1.00E+07
	(R)IESEGGLIETWNPNNK(Q)	1800.876	3	10.2	74	1.25E+07
	(R)VFYLGGNPETEFPETQEEQQGR(H)	2555.169	3	15.68	80.9	4.30E+07
	(R)VESEAGLTETWNPNNPELK(C)	2151.035	3	15.11	87.9	4.82E+07
58	(R)VFYLGGNPETEFPETQEEQQGR(H)	2555.169	3	18.24	87.3	3.02E+07
	(R)VESEAGLTETWNPNNPELK(C)	2151.035	3	16.52	87.5	4.00E+07
	(K)GVLGLSLPGCPETYEPR(S)	1977.27	3	10.07	91.7	2.11E+07
	(R)DFLEDAFNVNR(H)	1339.628	2	13.59	81.9	2.79E+08
	(R)FYLAGNHEQEFLR(Y)	1623.791	3	12.99	89.3	1.43E+08
59	(R)LDALPDNRIESEGGLIETWNPNNK(Q)	2824.375	3	16.98	91.4	9.85E+06
	(R)IESEGGLIETWNPNNK(Q)	1800.876	2	15.34	86	9.30E+07
	(K)GGLSIISPPEK(Q)	1098.58	2	13.7	88.5	3.26E+08
	(K)QEENEGNNIFSGFKR(D)	1740.782	2	13.17	80.2	2.51E+07
	(R)DFLEDAFNVNR(H)	1339.628	2	13.13	79.1	7.34E+08
	(R)EQPEQNECQLER(L)	1559.676	2	12.29	73.3	1.14E+08

	(R)FYLAGNHEQEFLR(Y)	1623.791	3	10.71	85.7	1.40E+08
	(R)LDALEPDNR(I)	1042.516	2	10.19	84.2	1.51E+09
60	(R)LDALEPDNRIESEGGIETWNPNNK(Q)	2824.375	3	16.98	91.4	9.85E+06
	(R)IESEGGLIETWNPNNK(Q)	1800.876	2	15.34	86	9.30E+07
	(R)DFLEDAFNVNR(H)	1339.628	2	14.66	85.9	8.03E+08
	(R)EQPEQNECQLER(L)	1559.676	2	13.56	83.5	1.49E+07
	(K)QEENEGNNIFSGFK(R)	1740.782	2	11.77	83.2	1.50E+08
	(R)FYLAGNHEQEFLR(Y)	1623.791	3	11.63	88.1	2.20E+08
61	(R)DFLEDAFNVNR(H)	1339.628	2	14.78	84.6	1.04E+08
	(K)QEENEGNNIFSGFK(R)	1740.782	2	11.96	71.1	1.98E+07
	(R)IESEGGLIETWNPNNK(Q)	1800.876	2	11.87	77.9	1.97E+07
63	(R)VESEAGLTETWNPNHPELK(C)	2151.035	3	15.39	87.1	1.67E+08
64	(R)VFYLGGNPEIEFPETQQK(Q)	2096.034	3	19.17	92.5	3.35E+08
65	(R)VFYLGGNPEIEFPETQQK(Q)	2096.034	3	22.21	93.5	9.17E+07
	(R)VESEAGLTETWNPNHPELK(C)	2151.035	3	12.06	76.2	7.59E+07
	(R)VFYLGGNPEIEFPETQQK(Q)	2096.034	2	10.25	72.9	6.74E+06

Supplementary Table III. Peptide sequences obtained after tryptic digestion of the samples thermally treated at 200 °C for 30 min and 100 °C for 300 min, and mechanically treated with Ultraturrax at 24,000 rpm. Untreated (UN) Pisane[®] was used as reference. The protein identification with the corresponding NCBI^{nr}, the pI, the MH⁺ value, and the start aminoacid position are reported.

No.	Peptide sequence	pI	MH ⁺ (Da)	Start aa position	UN	200°C 30 min	100°C 300 min	Ultraturrax 24,000 rpm
	Legumin A2 precursor [NCBI ^{nr} 126161]							
1	(R)DFLEDAFNVNR(H)	4.03	1339.628	219	•	•	•	•
2	(R)FYLAGNHEQEFLR(Y)	5.40	1623.792	182	•	•	•	•
3	(K)GGLSIISPPEK(Q)	6.00	1097.621	252	•	•		
4	(K)QEENEGNNIFSGFK(R)	4.25	1740.78	203	•			•
	Legumin A precursor [NCBI ^{nr} 600108, 126168]							
5	(R)LAGTSSVINDLPLDVVAATFNLQR(N)	4.21	2514.357	467	•	•	•	
6	(K)NAMFVPHYNLNANSIIYALK(G)	8.50	2293.18	389	•			•
7	(K)QEENEGNNIFSGFK(R)	4.25	1740.78	202	•			•
	Legumin type B precursor [NCBI ^{nr} 126164]							
8	(R)ATPADVLANAFGLR(Q)	5.88	1415.765	445	•	•		
	Legumin J precursor [NCBI ^{nr} 126170]							
9	(K)CAGVSLIRR(T)	10.35	1031.578	66		•		•
10	(R)TIDPNGLHLPSPSPQLIFIIQGK(G)	6.41	2719.482	75	•			•
11	(R)VFYLGGNPETEFPETQEEQQGR(H)	3.98	2555.169	184	•	•		
	Legumin K [NCBI ^{nr} 126171, 282925]							
12	(R)AAVSHVQQVLR(A)	9.80	1207.691	300	•			•
13	(R)ATPAEVLANAFGLR(Q)	6.05	1429.78	311	•	•		•
14	(K)CAGVSLIRR(T)	10.35	1031.578	66		•		•
15	(R)TIDPNGLHLPSPSPQLIFIIQGK(G)	6.41	2719.482	75	•			•
16	(R)VFYLGGNPETEFPETQEEQQGR(H)	3.98	2555.169	184	•	•		
	Legumin (minor small) [NCBI ^{nr} 2578438]							
17	(R)VFYLGGNPEIEFPETQQK(K)	4.25	2096.034	181	•	•	•	•
	Vicilin 47kDa protein [NCBI ^{nr} 297170]							
18	(R)AIVIVTVTEGKGDFELVGQR(N)	4.68	2131.176	303	•	•	•	
19	(K)FFEITPEK(N)	4.53	1010.52	266			•	•
20	(K)IFENLQNYR(L)	6.00	1196.606	62	•			•

No.	Peptide sequence	pI	MH ⁺ (Da)	Start aa position	UN	200°C 30 min	100°C 300 min	Ultraturrax 24,000 rpm
21	(R)NFLAGEEDNVISQVERPVK(E)	4.41	2144.099	388	•		•	•
22	(K)NILEAAFNTNYEEIEK(V)	4.09	1897.918	176	•			
23	(K)NQQLQDLDFVNSVDIK(E)	3.93	1989.029	274	•	•	•	•
24	(K)QSYFANAQPLQR(E)	8.75	1422.713	426	•	•	•	•
25	(K)QSYFANAQPLQRE(-)	6.22	1551.756	426	•	•	•	•
	Vicilin 14kDa component [NCBIInr 137577]							
26	(K)NQQLQDLDFVNSVDLK(E)	3.93	1989.029	66	•	•	•	•
27	(K)FFELTPEK(N)	4.53	1010.52	58			•	•
	713472A Convicilin [NCBIInr 227928]							
28	(K)LFEITPEKK(Y)	6.14	1104.631	422	•			
29	(K)AILTVLSPNDR(N)	5.88	1198.68	271	•	•	•	•
30	(R)AIVVLLVNEGK(G)	6.05	1154.715	460	•	•		•
31	(R)NFLSGSDDNVISQIENPVK(E)	4.03	2076.025	539	•		•	•
32	(K)NILEASYNTK(Y)	6.00	1180.596	345	•	•	•	•
33	(R)SDLFENLQNYR(L)	4.37	1398.665	231	•	•		•
34	(K)SLPSEFEPINLR(S)	4.53	1401.738	398	•	•		•
	Convicilin [NCBIInr 7339551]							
35	(K)ELTFPGSVQEINR(L)	4.53	1489.765	564	•		•	•
	Convicilin precursor [NCBIInr 1706205]							
36	(K)NILEASYNTR(Y)	6.00	1180.596	345	•	•	•	•
	Provicolin (Type A) [NCBIInr 137579]							
37	(R)ASSNLDLLGFGINAENNQR(N)	6.05	2032.021	189	•	•	•	•
38	(R)AIVIVTVNEGK(G)	6.05	1142.679	119	•	•	•	•
39	(K)DLTFPGSAQEVDRLLENQK(Q)	4.32	2160.094	227			•	
40	(K)NPQLQDLDFVNYVEIK(E)	4.56	2176.165	89	•	•		
41	(K)LTPGDVFPVAGHPVAVR(A)	6.74	1845.039	171	•	•	•	
42	(K)QSYFANAQPQQR(E)	8.75	1437.688	246	•	•	•	•
43	(K)GDFELVGQR(N)	4.37	1020.511	130	•	•		•
	Provicolin precursor (Type B) [NCBIInr 137581]							
44	(R)AIVIVTVTEGKGDFELVGQR(N)	4.68	2131.176	294	•	•	•	
45	(R)AIVIVTVTEGK(G)	6.05	1129.683	294	•	•	•	•
46	(R)NFLAGEEDNVISQVERPVK(E)	4.41	2144.099	379	•		•	•
47	(K)NILEAAFNTNYEEIEK(V)	4.09	1897.918	167	•			
48	(K)IFENLQNYR(L)	6.00	1196.606	53	•			•
49	(R)QEINEENVIVK(V)	4.25	1314.68	203	•		•	•
50	(R)SKIFENLQNYR(L)	8.31	1411.733	51	•			
	Vicilin precursor [NCBIInr 137582]							
51	(K)ASSNLDLLGFGINAENNQR(N)	4.37	2033.005	377	•	•	•	•

No.	Peptide sequence	pI	MH ⁺ (Da)	Start aa position	UN	200°C 30 min	100°C 300 min	Ultraturrax 24,000 rpm
52	(K)ELAFPGSAQEVDRI	4.14	1418.692	415	●	●	●	●
53	(R)NFLAGDEDNVISQIRPVK(E)	4.56	2143.115	396	●		●	●
54	(R)SRGPIYSNEFGK(F)	8.31	1354.676	258	●	●	●	
55	(K)SVSSESEPFNLR(S)	4.53	1351.649	246	●	●		●
56	(R)GPIYSNEFGK(F)	6.00	1111.542	260	●			●
57	(R)QQSQEENVIVK(L)	4.53	1301.66	216	●		●	
	Vicilin [NCBI nr 42414627]							
58	(K)QSYFANAQPLQRE(-)	6.22	1551.756	403	●	●	●	●

Supplementary Table IV. Alignments between pea peptides identified in treated samples and allergenic peptides reported in literature, belonging to peanut (*Arachis hypogaea*) and lupin (*Lupinus albus*, *Lupinus angustifolius*). Both identity and homology % (calculated with BLASTp Program) of each peptide are reported; the NCBI nr of each peptide is also indicated.

No.	Pea peptide [<i>Pisum sativum</i>]	Peanut peptide [<i>Arachis hypogaea</i>]	Lupin peptide [<i>Lupinus albus</i> , <i>Lupinus angustifolius</i>]	Alignment	Identity (%)	Homology (%)
2	FYLAGNHEQEFLR [126161]	FNLAGHEQEFLR (a) [21314465]	FYLSGNQEQEFLQ [85361412]	pea FYLAGNHEQEFLR	85	85
				arah3/4 F LAG HEQEFLR		
				pea FNLAG-HEQEFLR		
4-7	QEENEGNNIFSGFK [126161; 126168]	AGQEENEGNNIFSG(F) (a) [21314465]	GQGQQQEGGNVLSGF(D) [85361412]	pea FYLAGNHEQEFLR	84	92
				arah3/4 FYLAGNHEQEFLR		
				pea FYL GN+EQEFL+		
21	NFLAGEEDNVISQVERPVK [297170]	IFLAGDKDNVID (b) [1168391]	NFLAGSEDNVIR (c) [46451223; 89994190]	lupin FYLSGNQEQEFLQ	93	93
				pea QEENEGNNIFSGF		
				arah3/4 QEENEG NIFSGF		
21	NFLAGEEDNVISQVERPVK [297170]	IFLAGDKDNVIDQIEK (b) [1168391]	NFLAGSEDNVISQLDR (c) [169950562; 149208403; 149208401]	pea QEENEGNNIFSGF	58	72
				arah3/4 QEENEGNNIFSGF		
				pea Q Q+ EG N+ SGF		
22	NILEAAFNTNYEEIEK [297170]	NTLEAAFNAEFNEIR (a,b) [1168391]	NTLEATFNTR (c) [169950562; 149208403; 149208401]	lupin QGQQQEGGNVLSGF	67	75
				pea NFLAGEEDNVIS		
				arah1 FLAG+ DNVI		
22	NILEAAFNTNYEEIEK [297170]	NTLEAAFNAEFNEIR (a,b) [1168391]	NTLEATFNTR (c) [169950562; 149208403; 149208401]	pea IFLAGDKDNVID	83	83
				arah1 NFLAGEEDNVIS		
				pea NFLAG EDNVI		
22	NILEAAFNTNYEEIEK [297170]	NTLEAAFNAEFNEIR (a,b) [1168391]	NTLEATFNTR (c) [169950562; 149208403; 149208401]	lupin NFLAGSEDNVIR	63	75
				pea NFLAGEEDNVISQVER		
				arah1 FLAG+ DNVI Q+E		
22	NILEAAFNTNYEEIEK [297170]	NTLEAAFNAEFNEIR (a,b) [1168391]	NTLEATFNTR (c) [169950562; 149208403; 149208401]	pea IFLAGDKDNVIDQIEK	82	88
				arah1 NFLAGEEDNVISQVER		
				pea NFLAG EDNVIQ +R		
22	NILEAAFNTNYEEIEK [297170]	NTLEAAFNAEFNEIR (a,b) [1168391]	NTLEATFNTR (c) [169950562; 149208403; 149208401]	lupin NFLAGSEDNVISQLDR	56	63
				pea NILEAAFNTNYEEIEK		
				arah1 N LEAAFN + EI		
22	NILEAAFNTNYEEIEK [297170]	NTLEAAFNAEFNEIR (a,b) [1168391]	NTLEATFNTR (c) [169950562; 149208403; 149208401]	pea NTLEAAFNAEFNEIR	44	44
				arah1 NILEAAFNTNYEEIEK		
				pea N LEA FNT		
22	NILEAAFNTNYEEIEK [297170]	NTLEAAFNAEFNEIR (a,b) [1168391]	NTLEATFNTR (c) [169950562; 149208403; 149208401]	lupin NTLEATFNTR	44	44
				arah1 NILEAAFNTNYEEIEK		
				pea N LEA FNT		

No.	Pea peptide [<i>Pisum sativum</i>]	Peanut peptide [<i>Arachis hypogaea</i>]	Lupin peptide [<i>Lupinus albus</i> , <i>Lupinus angustifolius</i>]	Alignment	Identity (%)	Homology (%)
31	NFLSGSDDNVISQIENPVK [227928]	IFLAGDKDNVID (b) [1168391]	NFLAGSEDNVIR (c) [46451223; 89994190]	pea FLSGSDDNVISQIEN	67	67
				arah1 FL G DNVI QIE		
				pea IFLAGDKDNVID		
		IFLAGDKDNVIDQIEK (b) [1168391]	NFLAGSEDNVISQLDR (c) [169950562; 149208403; 149208401]	pea FLSGSDDNVISQIEN	53	60
				arah1 FL GS+DNVI		
				lupin NFLAGSEDNVIR		
39	DLTFPGSAQEVDR [137579]	DLAFPGSGEQVEK (a,b) [1168391]	ELTFPGSAEDIER (c) [46451223; 89994190]	pea FLSGSDDNVISQIEN	67	67
				arah1 FL G DNVI QIE		
				pea IFLAGDKDNVIDQIEK		
				pea FLSGSDDNVISQIEN	67	87
				arah1 FL GS+DNVISQ++		
				lupin NFLAGSEDNVISQLDR		
46	NFLAGEEDNVISQVERPVK [137581]	IFLAGDKDNVID (b) [1168391]	NFLAGSEDNVIR (c) [46451223; 89994190]	pea DLTFPGSAQEVDR	59	84
				arah1 DL FPGS ++V+		
				pea DLAFPGSGEQVEK		
				pea DLTFPGSAQEVDR	62	100
				arah1 +LTFPGSA++++R		
				lupin ELTFPGSAEDIER		
47	NILEAAFNTNYEEIEK [137581]	NTLEAAFNAE (a,b) [1168391]	NTLEATFNTR (c) [46451223; 89994190]	pea NFLAGEEDNVISQVER	50	56
				arah1 FLAG+ DNVI		
				pea IFLAGDKDNVID		
				pea NFLAGEEDNVISQVER	63	63
				arah1 NFLAG EDNVI		
				lupin NFLAGSEDNVIR		
47	NILEAAFNTNYEEIEK [137581]	NTLEAAFNAE (a,b) [1168391]	NTLEATFNTR (c) [46451223; 89994190]	pea NILEAAFNTNYEEIEK	44	44
				arah1 N LEAAFN		
				pea NTLEAAFNAE		
				pea NILEAAFNTNYEEIEK	44	44
				arah1 N LEA FNT		
				lupin NTLEATFNTR		

No.	Pea peptide [<i>Pisum sativum</i>]	Peanut peptide [<i>Arachis hypogaea</i>]	Lupin peptide [<i>Lupinus albus</i> , <i>Lupinus angustifolius</i>]	Alignment	Identity (%)	Homology (%)
52	ELAFPGSAQEVD [137582]	DLAFPGSGEQVEK (a,b) [1168391]	ELTFPGSAEDIER (c) [46451223; 89994190]	pea ELAFPGSAQEVD +LAFPGS ++V+	54	85
				arah1 DLAFPGSGEQVEK		
				pea ELAFPGSAQEVD EL FPGFA++++R	62	93
			ELTFPGSIEDVER (c) [169950562; 149208403; 149208401]	lupin ELTFPGSAEDIER		
				pea ELAFPGSAQEVD EL FPGS ++V+R	62	85
				arah1 ELTFPGSIEDVER		
53	NFLAGDEDNVISQIR [137582]	IFLAGDKDNVID (b) [1168391]	NFLAGSEDNVIR (c) [46451223; 89994190]	pea ELAFPGSAQEVD EL FPGS ++V+R	62	85
				arah1 ELTFPGSIEDVER		
				pea ELAFPGSAQEVD EL FPGS ++V+R	62	85
				lupin ELTFPGSIEDVER		
53	NFLAGDEDNVISQIR [137582]	IFLAGDKDNVID (b) [1168391]	NFLAGSEDNVIR (c) [46451223; 89994190]	pea FLAGDEDNVISQIR FLAGD DNVI	60	60
				arah1 IFLAGDKDNVID		
				pea FLAGDEDNVISQIR FLAG EDNVI	60	60
				lupin NFLAGSEDNVIR		

The no. of peptides are referred to Table 3.

Identity and homology % are referred to the alignments between pea vs. Ara h 1 or Ara h 3/4 and pea vs. lupin.

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