



Review

Critical review on proteotypic peptide marker tracing for six allergenic ingredients in incurred foods by mass spectrometry



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ABSTRACT

Peptide marker identification is one of the most important steps in the development of a mass spectrometry (MS) based method for allergen detection, since the robustness and sensitivity of the overall analytical method will strictly depend on the reliability of the proteotypic peptides tracing for each allergen. The European legislation in place issues the mandatory labelling of fourteen allergenic ingredients whenever used in different food formulations. Among these, six allergenic ingredients, namely milk, egg, peanut, soybean, hazelnut and almond, can be prioritized in light of their higher occurrence in food recalls for undeclared presence with serious risk decision.

In this work, we described the results of a comprehensive evaluation of the current literature on MS-based allergen detection aiming at collecting all available information about proteins and peptide markers validated in independent studies for the six allergenic ingredients of interest. The main features of the targeted proteins were commented reviewing all details available about known isoforms and sequence homology particularly in plant-derived allergens. Several critical aspects affecting peptide markers reliability were discussed and according to this evaluation a final short-list of candidate markers was compiled likely to be standardized and implemented in MS methods for allergen analysis.

1. Introduction

IgE-mediated food allergies are steadily on the rise representing a huge health concern worldwide. Although firm prevalence data are lacking, there are extensive investigations suggesting that food allergies have increased and rates as high as approximately 10% have been documented, with main prevalence in industrialized countries, and in children compared to adults (Scott, Sicherer, High, & Sampson, 2018). There are many manifestations of food allergy with different severities, and individual investigations rely on specific study populations, specific foods, and different methodologies, thus impairing the determination of undoubted statistics. Moreover, further constraints in obtaining solid prevalence data, arise from geographic variations, diet exposure effects,

differences according to age, race, and ethnicity, and myriad other factors. Like all chronic disease, onset of food allergy is influenced by genetics, environment and genome-environment interactions including epigenetics effects. Its current management is based on allergen containing food avoidance. Food labelling legislative provisions are available in several countries and require a detailed declaration of allergen inclusion in foods, with number and types of priority allergens differing among countries and depending on specific dietary habits and allergy prevalence. On the other hand, a precautionary labeling system is also widespread, representing a safety measure put in place from industries to protect from any accidental cross-contamination likely to occur in foods expected to be allergen-free and that can take place at whatever stage along the food chain. All this raised the urgent need to develop

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reliable methodologies to trace allergens in foods with the highest confidence. Mass spectrometry (MS) techniques have been widely exploited in the last ten years to meet this goal and were specifically applied to food allergen detection, and to the food allergen identification and characterization, as well. Despite the need for expensive equipment and trained personnel, the chance to provide multiplexing and unequivocal allergen identification accounts for the overall strength of the MS based approaches compared to previously established methods, such as immunoassays and DNA based methods. Nonetheless some gaps in the development of MS-based quantitative methods still need to be addressed. The review paper authored by Monaci, De Angelis, Montemurro, and Pilolli (2018) provides a comprehensive overview of the MS based methods developed so far for allergen detection and also highlights the need of harmonization in method development and validation.

In this frame, the European project ThRAIL (Thresholds and Reference method for Allergen detection method) titled “Detection and quantification of allergens in foods and minimum eliciting doses in food allergic individuals”, funded by the European Food Safety Agency will actively contribute to the advancement in harmonization of MS-based method for food allergen detection by developing a prototype quantitative reference method for the multiple detection of food allergens in standardized incurred food matrices (Mills et al., 2019). Five main food ingredients causing severe IgE-mediated reactions have been prioritized in light of their occurrence in food recalls (Bucchini, Guzzon, Poms, & Senyuva, 2016; Turner et al., 2015; Worm et al., 2014). Milk, soybean, tree nuts, egg, and peanuts were selected as responsible for most alerts notified on the RASFF (Rapid Alert System for Food and Feed) portal for undeclared presence with serious risk decision. A recent notice from the European Commission (Commission Notice of 13.7.2017) relating to the provision of information on substances or products causing allergies or intolerances as listed in Annex II of Regulation (EU) No 1169/2011 provides specifications about how the Annex II of Regulation (EU) No 1169/2011 should be implemented. In particular, as for milk and egg ingredients the notice clarifies that the legislation should be applied to milk from all farmed animals and egg from all farmed birds. Given the timing of the ThRAIL project, and the urgent demand for harmonization of current analytical methods, the Consortium agreed to prioritize cow's milk and hen's egg allergens, as first targets, also in light of their main role in the primary production. Indeed according to recent statistical data (FAOSTAT, 2017), cow's milk production represented in 2017 the 97% of total milk production in Europe and the 81% of the production worldwide. Similarly, in the same year the hen's eggs accounted for the 99% of total egg production in Europe and for the 82% worldwide. Whether the developed approach would be applicable to all farmed animals/birds will be specified depending on the final validated analytical markers. As for tree nuts allergens, two representative ingredients have been selected, hazelnut and almond, which are widely used in food manufacturing and represent foods of public health importance in Europe, causing severe reactions (Worm et al., 2014).

The allergenic ingredients will need to be incorporated into the food before being processed to mimic as closely as possible the manufacturing process. Two model matrices will need to be produced *ad hoc* in a food pilot plant, namely a chocolate bar and a broth powder. These latter are the representative food commodities responsible for recalls, namely baked goods, confectionary products and complex multiphase foods and pose specific challenges in MS based analytical detection. Both matrices were selected because they are extremely complex, with different features, having chocolate high contents of fat and polyphenols whilst broth powder is an extensive processed food (cooking, boiling and drying), enriched with proteins.

One of first tasks scheduled in the ThRAIL project (Objective 1) concerns the identification of protein and peptide markers for the targeted allergenic ingredients. As a fact, markers identification is one of the most important steps in the method development. Different workflows are available to accomplish this objective and they have been

reviewed in a recent paper authored by Downs & Johnson, 2018. All issues besetting such choice including, various ingredients formulations, extraction yield in thermally processed foods, and incomplete genomic and proteomic sequence information, as well as all common issues associated with MS-based protein quantitation were critically discussed. The main point raised by Downs & Johnson was that the target selection cannot be accounted by *in-silico* only approach. Methodological details, such as matrix composition, protein extraction and digestion, should be taken into consideration for marker selection, affecting both the specificity and sensitivity of the final analytical method. The assessment on a model matrix is required firstly for specificity issue because interfering peaks from the matrix background may cause false-positives; in addition the processing degree of the investigated food matrix may also differently affect the target protein/peptide detection resulting in false-negative which can pose safety risk for the allergic consumer. None of these issues can be tackled by the solely *in-silico* based approach, thus the need for an empirical validation of the candidate peptide markers in incurred foods (Monaci et al., 2018).

An alternative approach could rely on the comprehensive assessment of the allergen detection literature, supported by the experimental evidence that if the same peptides for given allergenic proteins have been detected in independent studies and in multiple matrices, they can likely be standardized (Croote & Quake, 2016). In order to promote this approach, an open-source repository of discovery and targeted MS data on allergen detection and quantitation has been developed and made available as public bioinformatic tool (Browner, 2018). The database has been structured around proteins recognized by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) as food allergens.

After a proper screening of the different available options, the partners of ThRAIL consortium agreed on undertaking peptide selection by applying a dual approach with different time scale: (i) critical evaluation of the peptide markers already reported in the existing literature; (ii) validation of the candidate peptide list by discovery analysis on the specific incurred matrices under investigation (chocolate bars and broth powder). Herein, we will describe the results of the first point, namely the comprehensive evaluation of the literature, providing a thorough discussion about the application of general acceptance criteria for harmonization of the markers selection step. The goal of this work is to draw a preliminary list of reliable peptide markers, which will be validated later on by untargeted HR-MS/MS analysis of incurred matrices, representing the bases for the selected reaction monitoring (SRM) based reference method under development within the ThRAIL project.

2. Literature review based marker peptide selection

The analytical workflow displayed in Fig. 1 was designed to provide the comprehensive evaluation of the current literature. It consists of five main steps aimed at the critical evaluation of the information gathered from previous works and the proper selection of reported signature peptides according to specific criteria. We collected all information about the six allergens selected as targets in the ThRAIL project (cow's milk, hen's egg, peanut, soybean, hazelnut, and almond) (step 1). Afterwards, the list of reported signature peptides have been evaluated and filtered according to specific features, namely sequence length, matrix similarity with the ThRAIL project, kind of investigation (discovery only or targeted analysis) and occurrence of amino acid residues prone to endogenous and exogenous modifications (step 2). The third step of the workflow consisted in retrieving all the information available for the relevant allergenic proteins (relative abundance, post-translational modification sites, protein isoforms and variants), from different informatics sources detailed in Fig. 1. At step 4 peptide specificity was assessed by sequence alignment with other species searching not only for exact matches of tryptic peptides (performed for

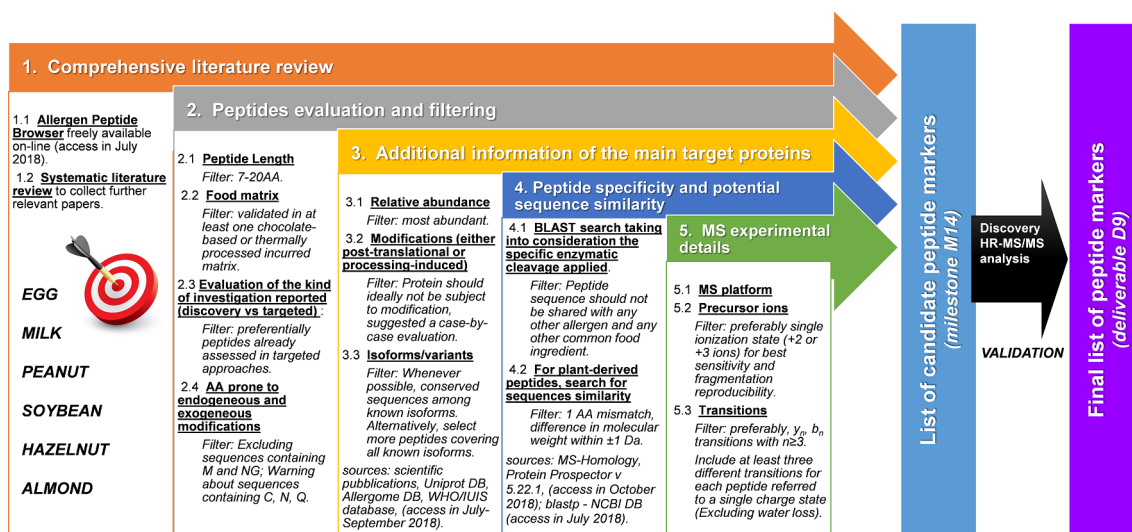


Fig. 1. Analytical workflow describing step-by-step the literature review and the critical evaluation of the proposed peptide markers in order to compile a final list of highly reliable signature peptides to be tested for the ThRAI project.

all candidate markers of the six allergenic ingredients) but also disclosing potential interference from sequence homology with single amino acid mismatches (performed only on the four plant-derived allergenic ingredients). Finally as last step of the analytical workflow, for the refined peptide list we collected experimental details about the MS-based detection, such as the instrumental platform, the precursor ions, and the selected transitions. Indeed, the peptide ionization efficiency and reproducibility, as well as the relative intensity of fragmentation pattern might be affected also by instrumental parameters which need to be assessed case-by-case. For the comprehensive assessment of the allergen detection literature (step 1 in Fig. 1) we exploited the Allergen Peptide Browser (APB) database available online, and collected all information about the six allergens selected for the ThRAI project. The gathered information also integrated data fed by other studies based on MS/MS analysis with either ESI or MALDI ionization, encompassing the most recent publications not itemized yet on APB. A total of 89 papers were reviewed, published in the last twenty-five years, which resulted in an exhaustive list of tryptic peptides for milk (total 42 peptides), egg (total 62 peptides), peanut (total 50 peptides) and soybean (total 146 peptides), and a shorter, but still reasonable list for hazelnut (total 26 peptides) and almond (total 29 peptides) allergens (see Table S1). In the second step the full list was narrowed down according to specific constraints. A range of 7–20 amino acids (AA) in length was applied as good compromise between peptide specificity, ionization yield and reproducibility. About 84% of reported peptides were included in this range. Afterwards, we searched for matrix similarity with the ThRAI project, and marker peptides that were never validated in either chocolate or highly processed incurred matrices were rejected. The resulting peptides list is displayed in Figs. 2 and 3 (and itemized in Table S2) together with details about the total number of citing papers per each peptide, and the kind of investigation (targeted vs discovery approaches), marked with different color (orange and blue, respectively).

The marker selection should comply with specific requirements that value the quantitative information retrieved. Indeed, the allergen quantification depends on the concentration of peptide markers, based on the assumption that the peptide is released completely from the original protein with a specific stoichiometric ratio. Any unpredictable distribution of the released peptide into different precursor ions, for example due to amino acid modification or multiple ionization states, should be traced and susceptible peptides should be excluded, in case of low reproducibility. Concerning the exclusion of signature peptides containing labile amino acids prone to modifications, it deserves to be noted that the number of potential endogenous and exogenous post-

translational modifications is huge, but their occurrence is highly variable; therefore, it is not a realistic goal neither to evaluate nor to exclude them all strictly, rather an empirical validation of the specific case study should be preferable. In this investigation, we focused on the occurrence of specific amino acids of major concern, such as methionine, asparagine, glutamine, and cysteine, excluding only peptide sequences containing methionine residues prone to processing-induced oxidation, and asparagine-glycine motifs prone to deamidation (Li et al., 2005; Li, Schowen, Topp, & Borchardt, 2006). We deemed important for the robustness of the analytical method to avoid M containing peptides as analytical targets, because such residue would be highly prone to oxidation, in thermally processed matrices, in barely predictable proportions. The NG motifs could be subjected to spontaneous deamidation *in-vitro*, however, in case of need the asparagine deamidation might be forced in order to convert all asparagine residues into aspartic acid. As for cysteine residues, most digestion protocols included a reduction/alkylation step (mainly based on dithiothreitol and iodoacetamide), preliminary to actual enzymatic proteolysis, which is specifically designed to break disulfide bridges and block irreversibly the cysteine residues by carbamidomethylation. Common sense is to consider this modification as complete however, in the perspective to avoid source of uncertainty, C containing peptides should be preferentially excluded unless the reliability of the peptide is duly assessed experimentally.

As third step of the analytical workflow reported in Fig. 1, starting from the refined list of peptide markers, we sought for additional information available on the target proteins, which could affect the detection sensitivity and reliability, such as relative abundance, natural post-translational modification (PTM), protein isoforms and variants, gathering data from different sources (either scientific publications or on-line databases, i.e. Uniprot, Allergome, WHO/IUIS).

Peptide specificity was assessed by BLAST search against the most common protein databases disclosing also potential detection interferences in plant-derived proteins (single amino acid mismatches) (step 4). The APB Database queried for this review of peptide markers, already provided information about sequence specificity, retrieved by means of BLAST 2.2.31+ (BLAST DB, Sep 20, 2016). Only hits with 100% identity and no gaps were retained for a given peptide, and the following constraints were applied to exclude hits: (i) bacterial super kingdom; (ii) absent species name or kingdom; (iii) species / common name entry containing: 'synthetic construct' or 'vector'; (iv) title containing 'partial' or 'fragment'. Since it was not clear whether they considered or not the site specific trypsin cleavage, we also double-checked

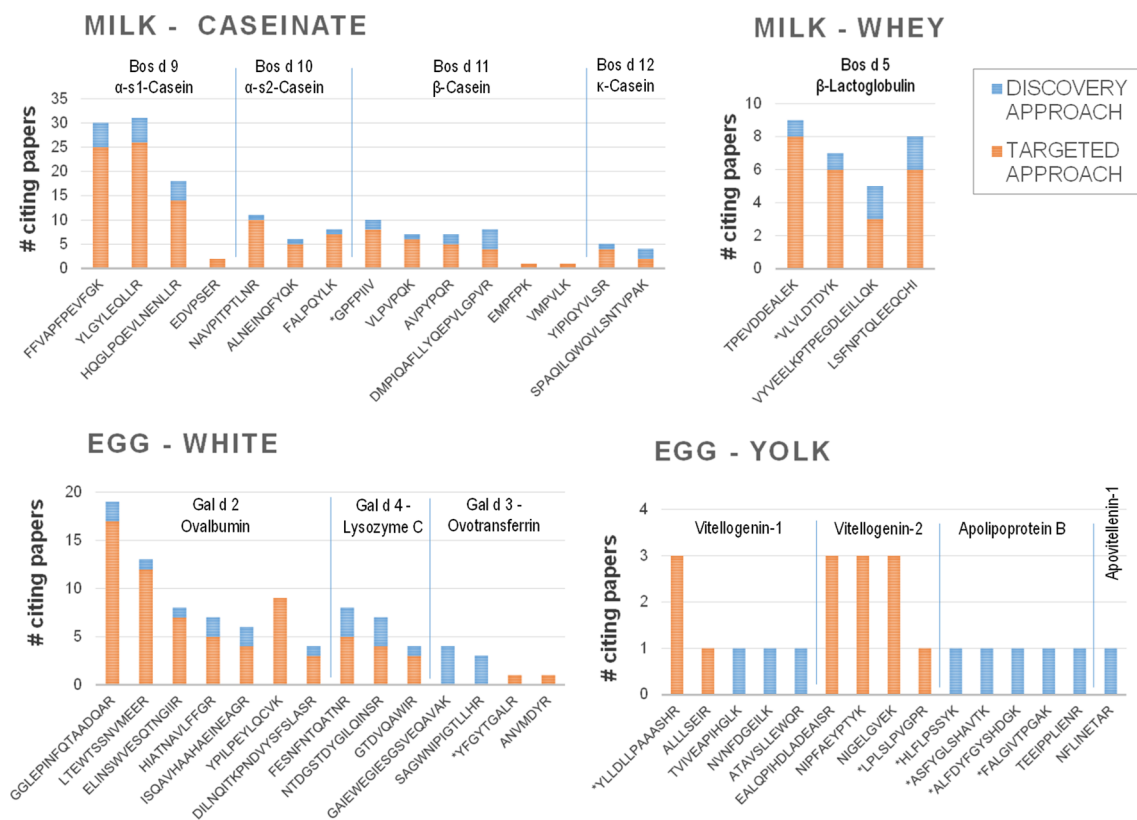


Fig. 2. Citation overview of the peptides markers used for milk and egg allergens, which were validated at least once in hard-to-analyze matrices, highlighting the kind of investigation (targeted approach or discovery only approach) and the presence of amino acids prone to modifications. *peptide without C, M, E, N, or Q amino acid.

the specificity for all the six allergenic ingredients by means of the Protein Prospector tool developed by the University of California San Francisco (<http://prospector.ucsf.edu/prospector/mshome.htm>, accessed on October 24th 2018). In particular, the MS-Homology option searching for 100% identity of tryptic peptides within the main databases, NCBI nr.2013.6.17 and UniProtKB.2017.11.01, was applied. In addition, for plant-derived allergens, protein homology among different taxonomies needs to be considered very carefully. Besides the previously discussed full matches (100% identity), in low-resolution MS detection potential interferences might arise also under particular conditions, from peptides with single amino acid mismatches. Therefore, further BLAST search against non-redundant protein database was carried out (access in July 2018), seeking for single amino acid substitutions, which could result in differences of peptide molecular weight within ± 1 Da (for example, I/L, D/N, E/Q). These kind of substitutions might generate interfering precursor ions isolated and activated at least partially in the set m/z window, and might provide several transitions, which are not distinguishable from the targeted peptides. Finally, as step 5 of the workflow we retrieved experimental details about the MS platform, the isolated ions and the monitored transitions. As a fact, most tryptic peptides feature favorable ionization properties, which provide high ionization efficiency and typically result in either double or triple charged ions due to the occurrence of basic amino acid residues. However, some peptide sequences may present multiple ionization routes resulting in a loss of sensitivity due to the distribution of the total amount of released peptide in different precursor ions, each characterized by a specific fragmentation pattern. Since we use as a calibrant the synthetic natural version of the peptide and use as an anchor the ration measurement to the isotopic labelled version of the peptide, the distribution among different charge state is accounted and thereof can be considered negligible. From the quantitative point of view, by selecting the most intense precursor ions loss of

sensitivity can be limited. Besides the amino acid sequence, several experimental features may affect this ionization behavior, e.g. the electrospray source parameters, the MS platform, the composition of the food matrix, dragging competing species to the ionization step. Therefore it is crucial that the theoretical selection of candidate markers, based either on the *in-silico* approach or on the literature review approach should be confirmed by experimental validation on the specific MS platform and on the main matrices of interest.

In the sections below, we present the results of the analytical workflow reviewing all information collected for the six allergens of interest.

2.1. Proteins and peptide markers reported for cow's milk and hen's egg

The inclusion of milk from domesticated mammalian animals, mainly cattle, buffalo, sheep, goat and camel, boasts a very long tradition in the human diet. According to the FAO data on primary livestock production in 2017, about 96% of the world's fresh milk comes from cows and buffaloes, while the remaining 4.0% is produced by goats, sheep and camels (FAOSTAT, 2017). Noteworthy, milk from all ruminant species contains homologous proteins, which share the same structural, functional, and biological properties (Monaci, Treggoat, van Hengel, & Anklam, 2006). Cow's milk contains about 3 g of proteins per 100 mL and includes at least 25 different proteins classified in two main categories according to their solubility at pH 4.6 and 20 °C (Fox, 2001); the insoluble protein fraction that precipitates are caseins (α 1-casein, α 2-casein, β -casein, κ -casein), while the soluble fraction constitutes the serum characterized by whey proteins (β -lactoglobulin, α -lactalbumin, bovine lactoferrin, bovine serum albumin, and immunoglobulins), corresponding to 80% and 20%, respectively. All the caseins present genetic polymorphisms, which account for the high heterogeneity of such proteins with several identified variants. The

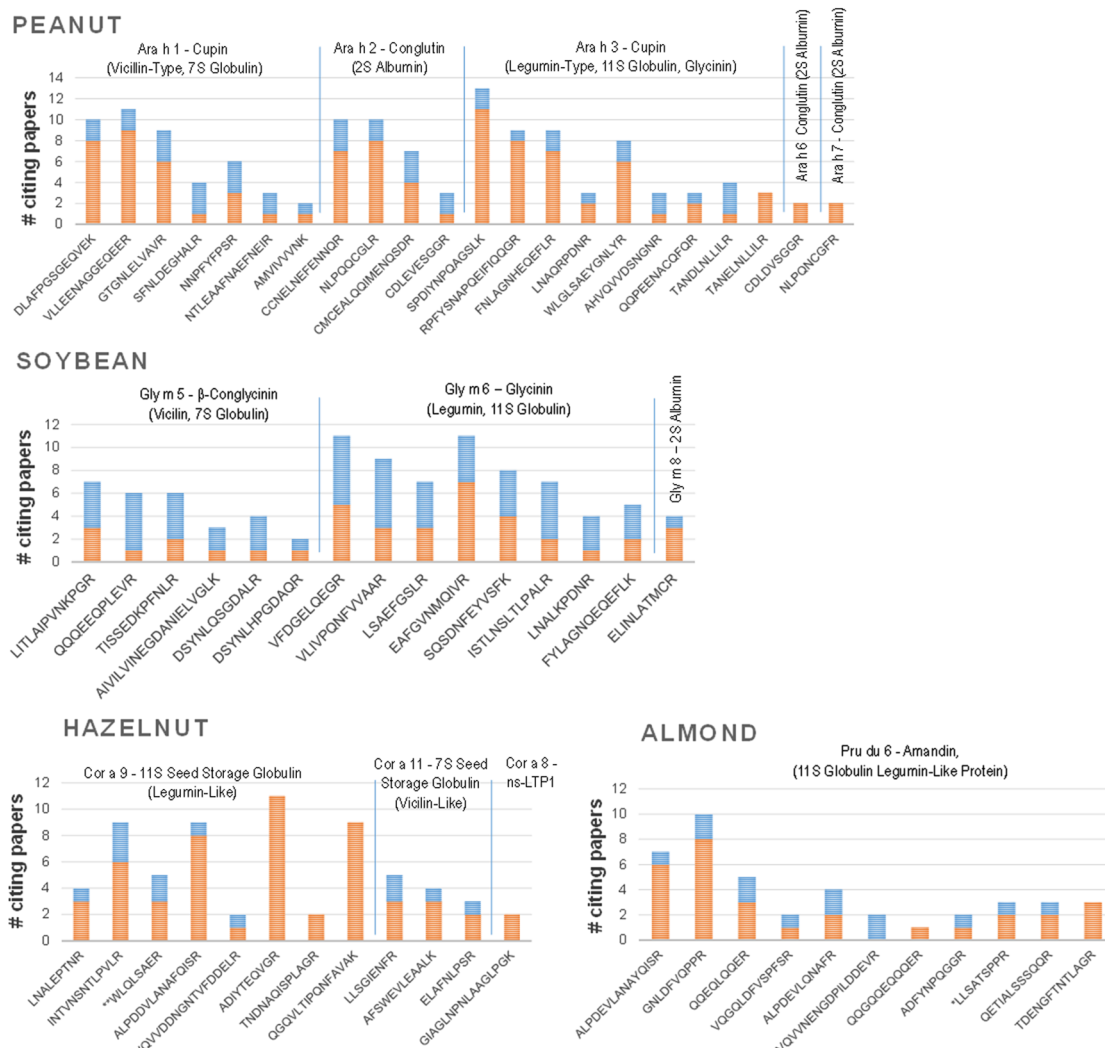


Fig. 3. Citation overview of the peptides markers used for peanut, soybean, hazelnut and almond, which were validated at least once in hard-to-analyze matrices, highlighting the kind of investigation (targeted approach or discovery only approach) and the presence of amino acids prone to modifications. *peptide without C, M, E, N, or Q amino acid.

latter are characterized by single amino acid substitution, by deletions of peptide fragments and by occurrence of post-translational modifications, such as glycosylation, phosphorylation, or partial hydrolysis, which may affect their specific allergenicity (Fox, 2001; Wal, 2001) and peptide utility in LC-MS assays. The main allergenic proteins of whey are the globular proteins β -lactoglobulin and α -lactalbumin, which are not phosphorylated and contain intramolecular disulfide bonds that stabilize their structure.

Besides the direct consumption of raw milk, a wide range of derived food products can be manufactured from either raw milk or its condensed forms, such as cheese, butter products, cream products, powdered formulations; in addition, casein and whey protein fractions can also be used independently as ingredients in several commodities, including cheese, bakery products and glues. Therefore, the design of robust methods of analysis tailored to detect potential milk contaminations requires the selection of specific markers tracking independently the two main milk fractions, caseins and whey.

The critical evaluation of data displayed in Fig. 2, pointed out that milk has not been only the most investigated source of food allergens, but also the allergenic food where the highest consensus was achieved in the selection of signature peptides. The milk proteotypic peptides FFV, YLG and HQG (see Table S2 for peptides full sequences) belong to the α S1-casein (Bos d 9), which is considered the most abundant

protein in milk. They have been validated in many independent studies for targeted analysis, including several kind of matrices, such as liquid matrices (wine, ice cream), bakery products (bread, cookie, muffin), high fat and polyphenol matrices (chocolate), acidic formulation (tomato sauce), high protein matrices (meat products) etc. In addition, a few signature peptides were also reported for β -casein (Bos d 11), α S2-casein (Bos d 10), and κ -casein (Bos d 12), in the perspective to monitor two different allergenic proteins tracking for the same ingredient. As for whey proteins, main reliable signature peptides belonged to the β -lactoglobulin (β -LG, MW = 18.3 kDa), which is the most abundant protein in whey. This protein belongs to the lipocalin superfamily and is one of the best characterized lipid-binding proteins. It possesses three disulfide bridges and is present in two main variants with punctual mutations. β -LG occurs naturally as a mixture of monomers and dimers, but the proportion of monomers increases after heating to 70 °C (Monaci et al., 2006). TPE, LVL and LSF were highlighted as the most common signature peptides, already validated into incurred matrices. Noteworthy, the LSF peptide corresponding to the protein C-terminal, despite featuring a cysteine residue, has been selected for its robustness. In addition, some peptide markers were proposed also for α -lactalbumin (VGINYWLAKH, FLDDDLTDDIMCVK, DDQNPHSSNICNISCDK, LDQWLCEK, CEVFR), but none of them was validated in either chocolate-based matrix or thermally processed incurred matrices; therefore, they

Table 1
Summary of the food allergens proteins recorded for the six allergen under investigations. *Acronyms: PTM, post-translational modifications; IUIS, International Union of Immunological Societies. *Information gathered from Uniprot Database (<https://www.uniprot.org/>, Accessed on September 19th 2018).*

Allergenic ingredient	Protein	Allergen Name	MW (kDa)	Number of known PTMs*	Isoforms and Variants	IUIS (YES/NO)	GenBank Protein	UniProt
Milk	α S1-Casein	Bos d 9	23.6	n° 9 Ser phosphorylations	Bos d 9.0101	Yes	NP_851372	P02662
	α S2-Casein	Bos d 10	25.2	n° 13 Ser phosphorylations	Bos d 10.0101	Yes	NP_776953	P02663
	β -Casein	Bos d 11	24	n° 5 Ser phosphorylations	Bos d 11.0101	Yes	XP_005902099	P02666
	κ -Casein	Bos d 12	19	n° 1 N-terminal Gln cyclization to pyroglutamate; n° 3 Disulfide Bonds; n° 3 Ser phosphorylations; n° 1 Thr phosphorylation; n° 6 Thr glycosylations; n° 2 Ser glycosylations	Bos d 12.0101	Yes	NP_776719	P02668
	α -Lactalbumin	Bos d 4	14.2	n° 4 Disulfide Bonds; n° 1 Asn glycosylation	Bos d 4.0101	Yes	AAA30615	P00711
	β -Lactoglobulin	Bos d 5	18.3	n° 3 Disulfide Bonds	Bos d 5.0101	Yes	CAA32835	P02754
	Bovine serum albumin	Bos d 6	66.3	n° 17 Disulfide Bonds; n° 6 Ser phosphorylations; n° 4 Thr phosphorylations; n° 2 N6-succinyllysine; n° 1 Lys methylation	Bos d 6.0101	Yes	AAA51411	P02769
	Immunoglobulins	Bos d 7	160		Bos d 7.0101	Yes		
	Ovalbumin	Gal d 2	44	Initial Met Removal; n° 1 Gly acetylation; n° 2 Ser phosphorylations; n° 1 Disulfide Bond; n° 1 Asn glycosylation	Gal d 2.0101	Yes	CAA23682	P01012
	Ovomucoid	Gal d 1	28	n° 9 Disulfide Bonds; n° 5 Asn glycosylations	Gal d 1.0101	Yes	P01005	P01005
	Ovotransferrin	Gal d 3	78	n° 15 Disulfide Bonds; n° 1 Asn glycosylation	Gal d 3.0101	Yes	CAA26040	P02789
	Lysozyme C	Gal d 4	14	n° 4 Disulfide Bonds	Gal d 4.0101	Yes	CAA23711	P00698
Egg	Serum Albumin	Gal d 5	69	n° 17 Disulfide Bonds; n° 1 Asn glycosylation	Gal d 5.0101	Yes	CAA43098	P19121
	YGP42	Gal d 6	35	n° 3 Asn glycosylations	Gal d 6.0101	Yes	BAA13973	Chain 1628–1912 of the P87498
	Myosin light chain 1f	Gal d 7	22	n° 1 Ala trimethylation	Gal d 7.0101	Yes	K02608.1, K02609.1 and K02610.1	P02604
	alpha-parvalbumin	Gal d 8	11.8	NA	Gal d 8.0101	Yes	CAX32963	C1L370
	Beta-enolase	Gal d 9	50	n° 1 Ser acetylation	Gal d 9.0101	Yes	NP_990450	P07322
	Aldolase	Gal d 10		NA	Gal d 10.0101	Yes		

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Table 1 (continued)

Allergenic ingredient	Protein	Allergen Name	MW (kDa)	Number of known PTMs*	Isoforms and Variants	IUIS (YES/NO)	GenBank Protein	UniProt
Peanut	Vicilin-type, 7S globulin (Cupin)	Ara h 1	64	n° 1 Asn glycosylation	Ara h 1.0101 (clone P41B) Ara h 1 - Clone P17	Yes	AAB00861	P43238
	Conglutin, 2S albumin (Prolamin)	Ara h 2	17	n° 3 Pro hydroxylation; n° 4 Disulfide Bonds	Ara h 2.0101 Ara h 2.0102 Ara h 2.0201 Ara h 2.0202	Yes	AAK96887 see Allergome AAN77576 see Allergome	P43237
	Legumin-type, Glycinin, 11S globulin (Cupin)	Ara h 3	60, 37 (fragment)	NA	Ara h 3.0101 Ara h 3.0201 Ara h 3 genomic clone	Yes	AAC63045 AAD47382 AAM46958	O82580 Q9SQH7
	Profilin	Ara h 5	15	NA	Iso Ara h 3	No	AAT39430	
	Conglutin, 2S albumin (Prolamin)	Ara h 6	15	n° 5 Disulfide Bonds	Ara h 5.0101	Yes	AAD55587	Q9SQJ9
	Conglutin, 2S albumin (Prolamin)	Ara h 7	15	NA	Ara h 6.0101 Ara h 7.0101 Ara h 7.0201	Yes	AAD56337 AAD56719 ABW17159	Q647G9 Q9SQH1 B4XID4
	Pathogenesis-related protein, PR-10, (Bet v 1 like)	Ara h 8	17	NA	Ara h 7.0301 Ara h 8.0101	Yes	AUU21496 AAQ91847	Q647G8 Q6VT83
	Nonspecific lipid-transfer protein type 1 (Prolamin)	Ara h 9	9.8	Disulfide bond	Ara h 8.0201 Ara h 9.0101 Ara h 9.0201	Yes	ABP97433 ABX56711 ABX75045	B0YIU5 B6CEX8 B6CG41
	16 kDa oleosin (Glycosyl transferase GT-C)	Ara h 10	16	NA	Ara h 10.0101 Ara h 10.0102	Yes	AAU21499 AAU21500	Q647G5 Q647G4
	14 kDa oleosin (Glycosyl transferase GT-C)	Ara h 11	14	NA	Ara h 11.0101 Ara h 11.0102	Yes	AAZ20276 AAZ20277	Q45W87 Q45W86
	Defensin (Scorpion toxin-like knottin)	Ara h 12	8 kDa (reducing), 12 kDa (non-reducing), 5.184 kDa (mass)	NA	Ara h 12.0101	Yes		
	Defensin (Scorpion toxin-like knottin)	Ara h 13	8 kDa (reducing), 11 kDa (non-reducing), 5.472 kDa (mass)	NA	Ara h 13.0101 Ara h 13.0201	Yes		
	Oleosin (Glycosyl transferase GT-C)	Ara h 14	17.5	NA	Ara h 14.0101 Ara h 14.0102 Ara h 14.0103	Yes	AAK13449 AAK13450 AAT11925	Q9AXI1 Q9AXI0 Q6J1J8
	Oleosin (Glycosyl transferase GT-C)	Ara h 15	17	NA	Ara h 15.0101	Yes	AAU21501	Q647G3
	non-specific Lipid Transfer Protein 2 (Prolamin)	Ara h 16	8.5 (reducing)	NA	Ara h 16.0101	Yes		
	non-specific Lipid Transfer Protein 1 (Prolamin)	Ara h 17	11 (reducing)	NA	Ara h 17.0101	Yes		

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Table 1 (continued)

Allergenic ingredient	Protein	Allergen Name	MW (kDa)	Number of known PTMs*	Isoforms and Variants	IUIS (YES/NO)	GenBank Protein	UniProt
Hazelnut	Pathogenesis-related protein, PR-10, (Bet v 1 like)	Cor a 1	17	Initial Met Removal	Cor a 1.0101	Yes	CAA50327	Q08407
					Cor a 1.0102	Yes	CAA5032	Q08407
					Cor a 1.0103	Yes	CAA50325	Q08407
					Cor a 1.0104	Yes	CAA50326	Q08407
	Profilin	Cor a 2	14	NA	Cor a 1.0201	Yes	CAA96548	Q39453
					Cor a 1.0301	Yes	CAA96549	Q39454
					Cor a 1.0401	Yes	AAD48405	Q9SWR4
					Cor a 1.0402	Yes	AAG40329	Q9FPK4
					Cor a 1.0403	Yes	AAG40330	Q9FPK3
					Cor a 1.0404	Yes	AAG40331	Q9FPK2
Almond	Non-specific lipid transfer protein type 1 (Prolamin)	Cor a 8	9	n° 4 Disulfide Bonds	Cor a 2.0101	Yes	AAK01235	Q9AXH5
					Cor a 2.0102	Yes	AAK01236	Q9AXH4
					Cor a 8.0101	Yes	AAK28533	Q9ATH2
					Cor a 9.0101	Yes	AAI73404	Q8W1C2
	Legumin-like, 11S globulin (Cupin)	Cor a 9	40	Disulfide bond	-	No	AHA36627	AOA0A0P7E3
					Cor a 11.0101	Yes	AAI86739	Q8S4P9
					Cor a 12.0101	Yes	AAO67349	Q84T21
					Cor a 13.0101	Yes	AAO65960	Q84T91
					Cor a 14.0101	Yes	ACO56333	D0PWG2
					Pru du 3.0101	Yes	ACN11576	C0L0I5
Soybean	Profilin	Gly m 3	14	Initial Met Removal	Pru du 4.0101	Yes	AAI91662	Q8GSL5
					Pru du 4.0102	Yes	AAI91664	Q8GSL5
					Pru du 5.0101	Yes	ABH03379	Q8H2B9
					Pru du 6.0101	Yes	ADN39440	E3SH28
	Pathogenesis-related protein, PR-10, (Bet v 1 like)	Gly m 4	17	NA	Pru du 6.01	No	CAA55009	Q43607
					variant	Yes	ADN39441	E3SH29
					Pru du 6.0201	No	CAA55010	Q43608
					variant	Yes	CAA11756	O65809
					Gly m 3.0101	Yes	CAA11755	O65810
					Gly m 4.0101	Yes	CAA42646	P26987
Soybean	Pathogenesis-related protein, PR-10, (Bet v 1 like)	Gly m 5	63	NA	Gly m 5.0101	Yes	BAA23360	Q22120
					Gly m 5.0201	Yes	BAA74452	Q9FZP9
					Gly m 5.0301	Yes	AAB23463	P25974 (variant F36L)
					n° 1 Asn glycosylation	Yes	AAA33966 BAC78522	V51G F197L
	Legumin, Glycinin, 11S globulin (Cupin)	Gly m 6	54	n° 1 Asn glycosylation	Gly m 5.0302	Yes	AAA33966 BAC78522	P25974 (variant V51G)
					n° 2 Disulfide Bonds;	Yes	BAA00154	P04776
					n° 2 Propeptides	Yes	BAA00154	P04405
					n° 2 Disulfide Bonds;	Yes	CAA33217	P11828
					n° 1 Propeptide	Yes	BAA74953	Q98B11
					n° 2 Disulfide Bonds;	Yes	BAB15802	Q7GC77
Soybean	Seed biotinylated protein 2S albumin (Prolamin)	Gly m 7	76	NA	Gly m 6.0401	Yes	ACS49840	C6K8D1
					Gly m 6.0501	Yes	ACS49840	C6K8D1
					Gly m 7.0101	Yes	ACS49840	C6K8D1
					Gly m 8.0101	Yes	AAB71140	P19594
	Seed biotinylated protein 2S albumin (Prolamin)	Gly m 8	14	Disulfide Bond;	n° 1 Propeptide	Yes	AAB71140	P19594
					n° 1 Propeptide	Yes	AAB71140	P19594
					n° 2 Disulfide Bonds;	Yes	AAB71140	P19594
					n° 2 Disulfide Bonds;	Yes	AAB71140	P19594
					n° 1 Propeptide	Yes	AAB71140	P19594
					n° 1 Propeptide	Yes	AAB71140	P19594

were excluded from the list of candidate markers at the first selection stage. The specificity of all reported milk peptide markers was confirmed, and some of the sequences showed conservation among proteins from other farmed species, still with no specificity issues with other allergenic food ingredients.

Eggs are, together with milk the most common allergenic food affecting European children. Hen's eggs are very common in the human diet as inexpensive source of high quality proteins and they represent a key ingredient in many food products, given its nutritional value and unique functional properties, such as emulsifying, foaming and gelling. Several egg-based formulations have been involved in food manufacturing, such as whole egg, egg white, lysozyme isolate, egg yolk, all containing a different degree of allergenic potential (Benedé, López-Expósito, Molina, & López-Fandiño, 2015). The main egg white allergenic proteins are ovomucoid (Gal d 1), ovalbumin (Gal d 2), lysozyme (Gal d 4) and ovotransferrin (Gal d 3), all listed in Table 1, with specific mention to the known post-translational modifications. Yolk, globally, is significantly less allergenic than albumen, containing two main allergenic proteins, namely Gal d 5 and Gal d 6, both belonging to the livetins fraction. The latter accounts for about 30% of the yolk proteins, however, relative abundance of Gal d 5 and Gal d 6, is hard to define. Gal d 5 is the main α -livetins, whereas Gal d 6 (protein YPG 42) derives from major β -livetins as C-terminal fragment of vitellogenin 1.

Ovomucoid is one of the most important allergen in the egg white, however it was rarely selected as reporter for egg contamination, in MS based methods. Indeed we retrieved information about a single peptide marker (AFNPVCGTDGVTYDNECLCAHK, Montowska & Fornal, 2018) that was never validated in either chocolate-based matrix or thermally processed incurred matrices, thus excluded from the list of candidate markers at the first stage of the literature review. Ovalbumin, as most abundant protein in egg white, is the most common protein used as marker for hen's egg contamination. It is a phosphoglycoprotein with a molecular mass of 45 kDa belonging to the serpin superfamily, with an intra-molecular disulfide bond between C residues in positions 74 and 121. Noteworthy, besides the most cited peptide GGL, which was monitored in several independent investigations, eight of them including hard-to-analyze matrices, all the other peptides LTE, ELI and YPI contain amino acids prone to modifications, methionine, asparagine (NG motif) and cysteine, respectively (Fig. 2). In particular, the peptide LTE was alternatively detected either in its native or in the M-oxidized forms depending on the matrix (De Angelis, Pilolli, & Monaci, 2017; Pilolli, De Angelis et al., 2017; Pilolli, Chaudhari et al., 2017; Pilolli, De Angelis, & Monaci, 2018). The peptide ELI contains the dipeptide NG prone to spontaneous asparagine deamidation. The peptide YPI contains the cysteine residue in position 121 involved in the disulfide bridge. Alternative ovalbumin signature peptides have been also reported, such as HIA and ISQ all displayed in Fig. 2.

Similarly to milk fractions, different egg-derived formulations used in food manufacturing require specific markers to be selected for each part of the allergenic ingredient that could eventually contaminate food commodities. Thus, in addition to egg white markers, it is important to select specific markers of egg lysozyme, industrially applied as protein isolate for its antimicrobial properties, and egg yolk. Only a limited percentage of the collected studies considered this point. As for direct lysozyme detection, we disclosed three main markers, FES, NTD and GTD, which have been validated at least once into incurred baked products, such as cookies and muffins. The egg yolk has been rarely investigated so far, indeed only six studies were collected detecting specific egg yolk proteins, four of them developing a targeted approach (Planque et al., 2016; Planque, Arnould, Dieu et al., 2017; New, Schreiber, Stahl-Zeng, & Liu, 2018; Planque et al., 2019), and two presenting only the identification of potential markers by HR-MS discovery analysis (Gavage et al., 2019; Lee & Kim, 2010). Luckily, most of them already tested a wide diversity of food matrices, with different complexity degree, such as cookie, bread, chocolate, salad dressing, spices, ham (see Table S2 for further details), also taking into

consideration the ingredient inclusion before food processing. Noteworthy, Gavage et al., 2019 proposed a systematic investigation of potential egg markers (selected for both egg white and egg yolk), in raw and processed egg-based commodities, promoting the use of a dual enzyme digestion protocol, which benefits from the use of the lysyl endopeptidase in addition to trypsin to provide the same site-specific cleavage with higher proteolytic yield. Focusing on the four targeted investigations (New et al., 2018; Planque et al., 2016, 2019; Planque, Arnould, Dieu et al., 2017), we disclosed that none of the reported signature peptides traced directly the allergenic protein YGP42, which is the carboxy-terminal portion (284 AAs) cleaved from the primary translation product of vitellogenin-1. Indeed, the peptides YLL and ALL both belong to the amine terminal chain of vitellogenin-1, known as lipovitellin-1. Three additional peptides included in the allergenic protein YGP42 were proposed by Gavage et al., 2019, TVI, NVN, ATA, by mean of the dual enzyme digestion protocol, which could be considered for further assessment in the development of targeted investigations.

The sequence specificity control by BLAST proved that none of the reported peptide markers was common to other allergenic ingredients, however some of them (especially lysozyme and vitellogenin-2 peptides) presented conserved sequence in proteins from bacteria and from other animal species, unlikely to occur in food commodities.

Further non-allergenic proteins were also proposed to trace for egg yolk contamination, such as the vitellogenin-2, the apolipoprotein B and the apovitellenin-1.

2.2. Proteins and peptide markers for legumes: peanut and soybean.

Peanut and soybean are both members of the *Fabaceae* or *Leguminosae* family. Legumes represent 27% of the primary crop production worldwide, highly diffused in the human diet as excellent sources of proteins, water soluble fibers, numerous micronutrients, and phytochemicals (Smykal et al., 2015). Peanut is the best characterized legume in regard to its allergenic content, having sixteen proteins, mainly seed storage proteins, classified as allergens with specific isoforms identified and listed in Table 1. As for soybean, the number of characterized allergens is relatively limited, with eight allergens officially registered by the WHO/IUIS Allergen Nomenclature Subcommittee (Allergen Nomenclature & Allergen Nomenclature, 2018). Legume allergens can be grouped into a restricted number of families and superfamilies, and the most relevant allergens are seed storage proteins belonging to the cupin and prolamin superfamilies. The cupin superfamily share a conserved β -barrel structural core domain, which can be single or double; the seed storage proteins 7S and 11S globulins are both bicupins. The prolamin superfamily includes proteins with conserved cysteine residues at specific positions, sharing a common tridimensional structure with four α -helices stabilized by disulfide bonds. This superfamily includes the 2S albumins and non-specific lipid transfer proteins (ns-LTPs). In addition, minor allergens belong to the profilins and Bet v (*Betula verrucosa*) 1-like superfamilies, which are associated with pollen allergy, the defensins, involved in plant defense against pathogens, and the oleosins, structural proteins of intracellular lipid storage organelles.

Current methods for MS-based peanut detection mainly target Ara h 1 (7S-globulin, vicilin-type), Ara h 2 (2S albumin, conglutin) and Ara h 3 (11S globulin, glycinin) proteins. All peptides reported for these three proteins are highly specific, being conserved only among specific peanut related taxonomies (*Arachis ipaensis* (130454), *Arachis hypogaea* (3818), *Arachis duranensis* (130453)). Ara h 1 is a glycoprotein that makes up 12–16% of the total protein content and forms stable trimers held by non-covalent interactions (Palladino & Breiteneder, 2018). Two isoforms has been identified, encoded by two different clones 41B (Ara h 1.0101) and P17 (Ara h 1 clone P 17), with a sequence identity higher than 90%. Both proteins have an N-terminal 25 amino acid residue signal peptide and a single glycosylation site. The

signal peptide drives the nascent protein to the storage vacuole where both the signal itself and a specific portion of the N-terminal is cleaved-off to yield the mature Ara h 1 found in peanut (Palladino & Breiteneder, 2018). All the Ara h 1 marker peptides reported so far are shared between the two isoforms, and none of them include the glycosylation site (asparagine residue at position 521 of clone 41B and position 516 of clone P17, see Fig. S1 for details). DLA, VLL and GTG are the most frequently selected, already tested in several incurred and hard-to analyze matrices.

Ara h 2 represents the 5.9–9.2% of the total protein content and can be expressed from different genes into two isoforms Ara h 2.01, and Ara h 2.02, the latter differing for the insertion of a 12 amino acid motif at position 75 in comparison to the Ara h 2.01. In addition, the Allergome database further distinguishes the two isoforms in four variants (Ara h 2.0101, Ara h 2.0102, Ara h 2.0201, Ara h 2.0202), which, besides the signal sequence, differ for a single amino acid mismatch (E vs D) close to the C-terminal portion of the full sequence (position 163 in the Ara h 2.0201 sequence). Furthermore, Ara h 2 undergoes proteolytic processing by peanut proteases resulting in the removal of the C-terminal dipeptide RY. Consequently, the Ara h 2 can be found as a mixture of all the four variants as well as their slightly truncated forms. Four intramolecular disulfide bonds hold the tridimensional structure of the Ara h 2 and three proline residues are present in their hydroxylated form (see Fig. S2). Interestingly, none of the markers reported in Fig. 3 for Ara h 2 contain the 4-hydroxyproline residues, however all of them contain cysteine residues directly involved in the formation of the four disulfide bridges. In addition, the CMC also contained two methionine residues, susceptible to oxidation. The first three peptides, namely CCN, NLP and CMC are shared among all the isoforms (see Fig. S2), whereas the peptide CDL includes the position affected by single amino acid substitution, thus both the variants CDLEVESGGR (reported peptide) and CDLDVESGGR (potential alternative sequence) can be found in nature with unpredictable proportions.

Ara h 3 is a glycinin-like protein (11S) with a molecular mass of 60 kDa for the monomer and occurs in peanuts as hexamer of 360 kDa (Palladino & Breiteneder, 2018). The monomer is post-translationally cleaved in 43 kDa acidic and 28 kDa basic subunits, covalently linked by a single disulphide bond. Ara h 3 and Ara h 4, initially considered as different allergenic proteins, have been identified as variants of the same gene, thus they were renamed as isoforms Ara h 3.01 and Ara h 3.02. These are the only two isoforms officially listed by the WHO/IUIS Allergen Nomenclature Subcommittee, whereas further Uniprot entries are classified as Ara h 3 in the Allergome database. A genomic clone encoding Ara h 3 (AF10854) (Viquez, Konan, & Dodo, 2004) was identified as having four exons. The deduced protein (538 AA in length) showed 93% and 91% identity with the Ara h 3 (isoform Ara h 3.01) and Ara h 4 (isoform Ara h 3.02) (Palladino & Breiteneder, 2018), respectively. Furthermore, an additional isoform named *iso-Ara h 3* was reported as sharing only 70–85% of identity with previously cited sequences (Boldt et al., 2005). Several peptide markers tracing for Ara h 3 protein were reported so far (see Fig. 3), however, surprisingly for this particular case, the criterion of sequence sharing among known isoforms has not been valued properly in the current literature (see Fig. S3). Indeed, some of proposed markers were not conserved among protein variants, and according to common sense, they should have been excluded, for their limited representativeness. For example, the peptide SPD, which is cited in 13 investigations, is encrypted only in the Ara h 3.01 isoform (and in the genomic clone). Quite surprising was also the selection of the peptide TANELNLLILR in two very recent papers (Planque, Arnould, Renard et al., 2017; Planque, Arnould, Dieu et al., 2017), which was not encoded by the officially recognized isoforms Ara h 3.01 and Ara h 3.02, but only by the Ara h 3 genomic clone. Noteworthy, the peptide LNA is the only marker fully conserved across the isoforms while, the peptides RPF, QQP are common only to the two main isoforms Ara h 3.01 and Ara h 3.02.

As previously stated, soybean is another edible legume belonging to

the *Fabaceae* family, widely consumed worldwide for its high protein content (approximately 38–40%). It finds also wide applicability as ingredient in meat/poultry products, bakery and pastry products, dairy products, and edible spreads, as well as additive for a variety of pharmaceutical and industrial applications (Verma, Kumar, Das, & Dwivedi, 2013). As such, the number of occurring soy based formulations is very high, ranging from raw to highly processed and/or hydrolyzed commodities and this makes the absolute quantification of soy contamination in complex matrices quite challenging.

The WHO/IUIS recognized eight soybean proteins as official allergens, including hydrophobic protein (Gly m 1), defensin (Gly m 2), soy profilin (Gly m 3), pathogenesis-related protein (Gly m 4), β -conglycinin (Gly m 5, vicilin, 7S globulin), glycinin (Gly m 6, legumin, 11S globulin), seed biotinylated proteins (Gly m 7) and the 2S albumin protein group (Gly m 8). The major storage proteins, β -conglycinin and glycinin, both belonging to the cupin superfamily, represent alone the 70–80% of the total seed globulin fraction (De Angelis, Pilolli, Bavaro et al., 2017). β -Conglycinin is 7S globulin glycoprotein containing 5% of carbohydrate moieties and occur as trimers with molecular masses of approximately 180 kDa. Therefore, the Gly m 5 allergen name refers to the complex of three β -conglycinin subunits that were not characterized at the time the name was assigned (Pomés et al., 2018). The three subunits were first described by Holzhauser et al. (2009) labeled as α (67 kDa), α' (71 kDa) and β (50 kDa), and were assessed to combine at different ratios to form the multimeric complexes. The α and α' subunits are approximately 82% identical, whereas the β -subunit is only 76% identical to the two α subunits. The individual subunits were named as isoallergens Gly m 5.01, Gly m 5.02 and Gly m 5.03 in 2009, with minor variants (e.g. Gly m 5.0301 and Gly m 5.0302 presenting two amino acid mismatches L/F at positions 16 and 198). Interestingly, the *in-silico* simulation of tryptic digestion showed that the isoallergens Gly m 5.01, Gly m 5.02 and Gly m 5.03 do not share any tryptic peptide with minimum length of 7 AA, which could be selected as a common marker for the β -conglycinin subunits (see Fig. S4). This issue makes the absolute quantification of soybean allergen by synthetic peptide analysis quite challenging since the required conversion factors should base on the knowledge of the specific combination of α , α' and β -subunits occurring in the sample under investigation. As expected by *in-silico* prediction, none of the reported peptide markers for Gly m 5 displayed in Fig. 3 were conserved among the three isoallergens (see Fig. S4). Interestingly, an alternative solution, in this case, would be to select unique markers for each subunit in order to disclose the specific combination of α , α' and β -monomers within the trimeric complex. In this frame, the peptide LIT would uniquely trace for Gly m 5.01, the peptides QQQ and DSYNLQ would uniquely trace for Gly m 5.02, and the peptide DSYNLH would uniquely trace for Gly m 5.03 (noteworthy, the latter is conserved between the two variants Gly m 5.0301 and Gly m 5.0302). Attention should be paid to the peptide AIV, even if theoretically unique for Gly m 5.01, since an homologous peptide sequence is expressed in both variants of Gly m 5.03, with a single amino acid substitution L/I at position P19, which could not be discriminated by SRM acquisition. None of the aforementioned peptides contain amino acid residues with natural post-translational modification.

In most soybean varieties, the glycinin accounts for over 50% of seed storage proteins representing the predominant protein fraction (De Angelis, Pilolli, Bavaro et al., 2017). Glycinin has a complex hexameric structure with a molecular weight ranging between 320 and 360 kDa. At least five genes encode the monomeric subunits (Nielsen et al., 1989) and the Gly m 6 allergen can be an arrangement of these five proteins, which have individual IgE binding properties (Holzhauser et al., 2009). Each monomer consists of two specific polypeptide chains, one acidic (40 kDa) (A) and one basic (20 kDa) (B), linked together by disulfide bonds. Each monomer can be one of five subunits, namely glycinin G1 ($A_{1a}B_{1b}$), glycinin G2 ($A_{2}B_{1a}$), glycinin G3 ($A_{1b}B_{1a}$), glycinin G4 ($A_{5}A_{4}B_{3}$) and glycinin ($A_{3}B_{4}$) (Wang, Qin, Sun, & Zhao, 2014), being renamed as five isoallergens Gly m 6.0101, Gly m 6.0201, Gly m

6.0301, Gly m 6.0401 and Gly m 6.0501, respectively, in 2009. Similarly to what was already discussed for the quantification of Gly m 5 allergen, the unpredictable composition of the Gly m 6 hexameric complex makes general conversion factors a hard task to achieve. The peptide marker selection carried out so far in current literature completely neglected the different sequence expression in known isoallergens. Indeed, the most frequently used markers, VFD, VLI, and EAF, are expressed uniquely in Gly m 6.0101, whereas the peptide SQS, cited in 8 independent investigations, is conserved across the three isoforms Gly m 6.0101, Gly m 6.0201, Gly m 6.0301, and the peptide IST (cited in 7 independent investigations) is common to the isoforms Gly m 6.0401 and Gly m 6.0501 (see Fig. S5).

2.3. Proteins and peptide markers for tree nuts: hazelnut and almond

Hazelnut and almond allergens were selected in this project as most relevant members of the tree nut category according to the European legislation. Similar to peanut and soybean, the main proteins involved in tree nut allergy belong to cupin (legumins-11S globulin and vicilin-7S globulin) and prolamin (conglutin-2S albumin and ns-LTPs) super-families. Several hazelnut allergens have been identified and are already partly applied in component resolved diagnosis (Geiselhart, Hoffmann-Sommergruber, & Bublin, 2018). However, only partial information have been collected so far about the occurrence of potential allergen isoforms and their complete sequencing, even if their existence is likely, given the high genetic diversity of plant organisms. Even the limited information available for almond allergens, the provided following discussion about the peptide marker selection was driven to the best of the current knowledge for these two allergens, and relevant homologous plant proteins.

WHO/IUIS database included eight hazelnut allergens all listed in Table 1. According to the EuroPrevall population-based survey, including allergic subjects from twelve European cities, the predominance of specific hazelnut allergens is correlated to the geographical area (Pastorello et al., 2002), as well as to the age of the sensitized individuals (Verweij et al., 2012). In the Mediterranean areas, hazelnut allergy is mainly linked to Cor a 8, Cor a 14, Cor a 9, Cor a 11 (Blanc et al., 2015; Schocker et al., 2004). Cor a 8 is a nsLTP type 1, as mature protein, consisting of a unique polypeptide chain of 92 amino acid residues with eight strictly conserved cysteines forming four intramolecular disulfide bridges. Cor a 14 (2S albumin) is a heterodimeric protein with characteristic structural features, including four α -helices and eight cysteine residues engaged in four disulfide bridges (Pfeifer et al., 2015). The precursor protein is post-translationally cleaved into a large and a small subunits linked by disulfide bonds. The N-terminal glutamine shows the cyclization to pyroglutamic acid and the small subunit is variably truncated at the C-terminal, leading to a high micro-heterogeneity (Pfeifer et al., 2015). Cor a 9, as 11S legumin, exhibits some homology with other tree nut allergens, such as cashew Ana o 2, peanut Ara h 3 and the soybean glycinin (Gly m 6). The monomer consists of two polypeptide chains (acidic and basic chains), cleaved post-translationally by site specific endopeptidases, and linked together by a single intermolecular disulfide bond. The WHO/IUIS recorded a single isoform of the Cor a 9 protein corresponding to the Uniprot accession Q8W1C2 and labelled as Cor a 9.0101, first identified in 2002 as IgE-binding protein by 2D-Western blotting, using sera of hazelnut allergic patients (Beyer, Grishina, Bardina, Grishin, & Sampson, 2002). In 2013, a new isoform of the same protein, with a 514 AA full-length sequence, was registered (Uniprot accession A0A0A0P7E3) sharing 96.5% homology (Grishina, Beyer, Bardina, & Sampson, 2013). This isoform has been added to the Allergome database with the general label of Cor a 9. Additionally, at least another 55 kDa protein, with high IgE-reactivity only in the alkaline chain (20.7 kDa), has been identified by Nitride et al. (2013). In this investigation, shotgun proteomics allowed de novo sequencing of six peptides of the basic chain and their assignment to 11S protein isoform sharing high homology with 11S

globulin-like proteins from several plant organisms, including the canonical hazelnut one. Starting from this background, we reviewed the current literature tracing the Cor a 9 selected markers. The peptide WLQ should be excluded by any further discussion since conserved sequence among different tree nut species (hazelnut, pistachio, pecan, and walnut). Besides this, we assessed that all the proposed signature peptides shared 100% identity between the official Cor a 9.0101 (Q8W1C2) sequence and its registered isoform A0A0A0P7E3 (see Fig. S6). However, considering the further potential isoform only partially sequenced by Nitride et al. (2013), three out of the seven reported peptides might be excluded, as non-conserved regions and, noteworthy, this exclusion would involve two of the most cited peptides INT and ALP. Therefore, a wider characterization of most common isoform occurring by natural genetic diversity is urgently needed. In addition, the peptide VQV contains the NG motif, which makes the asparagine residues highly susceptible to deamidation.

Cor a 11 is a 7S globulin like protein, featuring a trimeric structure of MW about 150/190 kDa with subunits of about 50 kDa. The WHO/IUIS lists a single isoform labelled as Cor a 11.0101, corresponding to the Uniprot entry Q8S4P9, which includes a single glycosylation site occurring at the asparagine residue. Three peptide markers have been reported for this protein, namely LLS, AFS, and ELA, and none of them includes amino acid residues prone to modifications.

As for almond, six identified almond allergens, Pru du 3 (ns-LTP), Pru du 4 (profilin), Pru du 5 (60 S ribosomal protein), and Pru du 6 (legumin), are included in the WHO-IUIS allergen list. The specificity in the analytical detection of this allergen is particularly challenging due to both the limited availability of full-length sequences in official database and the high homology of almond proteins (taxonomy *Prunus dulcis*) within the *Prunus* taxonomy genus, mainly *Prunus persica* (peach) (Inman, Groves, McCullough, Quaglia, & Hopley, 2018). For example, the almond allergen Pru du 3.0101 displays 99% amino acid sequence identity to a nsLTP from peach (UniProtKB M5W0S9, not listed in the IUIS) and 57% to two other peach nsLTP isoforms, Pru p 3.0101 and Pru p 3.0102. Two native isoforms have been identified for the almond profilin (Pru du 4), but its relevance is mainly restricted to pollen-associated almond allergy. Pru du 5 has been identified as an 11.4 kDa 60S ribosomal protein involved in protein biosynthesis (Abolhassani & Roux, 2009); nevertheless, the clinical relevance of this allergen is unknown and requires further research. Finally, Pru du 6, a legumin-like protein, also called amandin, is the first and best biophysically characterized allergen in almond to date (Geiselhart et al., 2018). Two cDNA clones encoding almond legumins named prunin-1 (61.0 kDa) and prunin-2 (55.9 kDa) have been isolated, both included in the WHO/IUIS database as Pru du 6.0101 and Pru du 6.0201. By *in-silico* simulation of the enzymatic digestion with trypsin specific cleavage, it was assessed that no tryptic peptide with minimum length of 7 AA presents full identity between the two isoforms Pru du 6.0101 and Pru du 6.0201 (see Fig. S7). In addition, Allergome database also includes two variants of Pru du 6.0101 and Pru du 6.0201, registered with the Uniprot entries Q43607 and Q43608, respectively, which share 99% identity with the relevant official allergen isoform, featuring only few amino acid substitutions (see Fig. S7). In Fig. 3, we presented the signature peptides reported so far for almond detection by SRM analysis all belonging to the Pru du 6 protein, either to the Pru du 6.0101 or to the Pru du 6.0201 isoallergens. Noteworthy, most of the selected peptides highlighted with asterisks presented specificity issues, since the BLAST search against non-redundant protein database showed 100% sequence identity with homologous proteins from *Prunus persica*, *Prunus mume* or *Prunus avium*, (see Figs. S8 and S9). In light of this, we kept in the list reported in Fig. 3 also the two markers QQG both VQV, even if they were not validated in incurred or chocolate based matrices, as only candidates, together with TDE, which feature uniqueness for *Prunus dulcis* species. Interestingly, none of the reported peptides included cysteine residues involved in the disulfide bridges, whereas both the unique peptides VQV and TDE contain the motif NG that make them

Preliminary list of signature peptides selected by the Consortium according to the analytical workflow presented in Fig. 1, which have been monitored in targeted investigations with relevant information on allergens soforms/variants, peptide specificity, and acquisition related technical details (MS platform, precursor ion and selected transitions). The presence of amino acid residues prone to modification was highlighted with a different font (bold and underlined). The transition selected as quantifier ion (when specified) was marked with an asterisk. *Acronyms*: QqQ, triple quadrupole; IT, ion trap; QToF, quadrupole time-of flight; Q-Orbitrap, quadrupole-Orbitrap. LIT, linear ion trap.

[illegible]

(continued on next page)

Table 2 (continued)

Target protein	Peptide Sequence <i>Isoldegens/variants</i>	Specificity (Tax ID)	MS platform	Precursor	Transition 1	Transition 2	Transition 3	References
Bos d 10 – αS2-Casein	NAVPITPLNR <i>Bos d 10.0101</i>	<i>Bos taurus</i> (9913) <i>Bubalus bubalis</i> (89462) <i>Bos motus</i> (72004)	Q-Orbitrap	634.4 (+2)	991.6	771.5	658.4	Y ₅ ⁺
			Dual LIT	634.4 (+2)	934.5	771.5	–	Y ₆ ⁺
			QqQ	634.4 (+2)	552.8	249.1	–	Y ₆ ⁺
			QqQ	634.4 (+2)	991.6	771.5	529.3	Y ₄ ⁺
			QqQ	634.6 (+2)	991.8	771.4	–	Y ₆ ⁺
			QqQ	–	–	–	–	–
			Q-ToF	598.3 (+2)	–	–	–	–
			QqQ	598.3 (+2)	911.5	456.3	285.2	Y ₈ ⁺
			QqQ	598.3 (+2)	911.5	456.3	285.2	Y ₈ ⁺
			QqQ	598.3 (+2)	911.5	701.4	600.3	Y ₅ ⁺
			QqQ	598.3 (+2)	912.0	285.5	–	–
			QqQ	598.3 (+2)	911.5	285.2	–	–
			QqQ	598.3 (+2)	911.5	456.3	–	–
			QqQ	598.4 (+2)	911.6*	456.3	–	–
			QqQ	490.3 (+2)	120.1	648.4	–	–
Bos d 5 - β-Lactoglobulin	TPEVDDEALEK <i>Bos d 5.0101</i>	<i>Bos taurus</i> (9913) <i>Bos motus</i> (72004)	QqQ	490.3 (+2)	120.1	648.4	–	–
			IT	979.3 (+1)	–	–	–	–
			QqQ	490.1 (+2)	761.5	648.4*	–	–
			Orbitrap	979.6 (+1)	–	–	–	–
			QqQ	490.3 (+2)	761.5	648.4	423.3	Y ₃ ⁺
			QqQ	490.2 (+2)	761.5	219.2	–	–
			QqQ	490.3 (+3)	648.4	219.1	–	–
			QqQ	623.3 (+2)	918.4	819.4*	–	–
			Dual LIT	623.3 (+2)	572.8	819.4	–	–
			QqQ	623.3 (+2)	572.8	819.4	918.4	Y ₈ ⁺
			QqQ	623.3 (+2)	–	–	–	–
			QqQ-ToF	623.3 (+2)	1048.2	199.2	918.4	Y ₈ ⁺
			QqQ	623.3 (+2)	572.8	819.4	–	–
			Q-Orbitrap	623.3 (+2)	–	–	–	–
			QqQ	623.3 (+2)	572.8	819.4	–	–
Bos d 5.0101	VLVLDTDYK <i>Bos d 5.0101</i>	<i>Bos taurus</i> (9913) <i>Bubalus bubalis</i> (89462) <i>Capra hircus</i> (9925) <i>Ovis aries musimon</i> (9938) <i>Ovis aries</i> (9940) <i>Rangifer tarandus tarandus</i> (86329) <i>Bos indicus</i> (9915) <i>Bos grunniens</i> (30521) <i>Bos motus</i> (72004) <i>Ovis sp.</i> (9939)	QqQ	533.6 (+2)	–	–	–	–
			QqQ	533.3 (+2)	853.4*	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	641.3	Y ₅ ⁺
			QqQ	533.3 (+2)	853.4	754.4	641.3	Y ₅ ⁺
			QqQ	533.3 (+2)	853.4	754.4	641.3	Y ₅ ⁺
			QqQ	533.3 (+2)	853.4	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	–	–
			QqQ	533.3 (+2)	853.4	754.4	–	–
Bos d 5.0101	VYVPELKPTEGDLEIL- LQK <i>Bos d 5.0101</i>	<i>Bos taurus</i> (9913) <i>Bubalus bubalis</i> (89462) <i>Bos grunniens</i> (30521) <i>Bos motus</i> (72004) <i>Ovis sp.</i> (9939)	QqQ	771.5 (+3)	–	–	–	–
			QqQ	771.8 (+3)	912.0	790.9	627.9	Y ₁₁ ⁺
			QqQ	771.8 (+3)	912.0	790.9	627.9	Y ₁₁ ⁺
			QqQ	858.4 (+2)	928.4	815.3	627.8	Y ₁₀ ⁺
			QqQ	858.4 (+2)	928.4	1254.6	627.8	Y ₁₀ ⁺
			QqQ	858.4 (+2)	1254.6	815.3	627.8	Y ₁₀ ⁺
			QqQ	858.4 (+2)	928.4	627.8	–	–
			QqQ	858.4 (+2)	928.4	627.8	–	–
			QqQ	858.4 (+2)	928.4	627.8	–	–
			QqQ	858.4 (+2)	928.4	627.8	–	–
			QqQ	858.4 (+2)	928.4	627.8	–	–
			QqQ	858.4 (+2)	928.4	627.8	–	–
			QqQ	858.4 (+2)	928.4	627.8	–	–
			QqQ	858.4 (+2)	928.4	627.8	–	–
			QqQ	858.4 (+2)	928.4	627.8	–	–

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Table 2 (continued)

Target protein	Peptide Sequence <i>Isolipogens/variants</i>	Specificity (Tax ID)	MS platform	Precursor	Transition 1	Transition 2	Transition 3	References
Gal d 2 - Ovalbumin	GGLEPINFQTAADQAR Gal d 2.0101	<i>Gallus gallus</i> (9031) <i>Alcaligenes xyloxydans</i> <i>xyloxydans</i> (85698)	QqQ	858.6 (+2)	462.2 ⁺	b ₄ ⁺	685.1	y ₁₁ ⁺⁺
			QqQ	858.4 (+2)	1254.6	y ₁₀ ⁺⁺	627.8	y ₁₀ ⁺⁺
			Q-ToF	—	666.3	y ₁₂ ⁺⁺	—	—
			LIT	844.4 (+2)	—	y ₁₂ ⁺⁺	1331.7	y ₁₂ ⁺⁺
			Orbitrap	844.4 (+2)	666.3	y ₁₂ ⁺⁺	—	—
			Dual LIT	844.4 (+2)	666.3	y ₁₂ ⁺⁺	—	—
			QqQ	844.4 (+2)	1007.5	y ₉ ⁺	1121.5	y ₁₀ ⁺
			Dual LIT /	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1331.7	y ₁₂ ⁺⁺
			Orbitrap	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1121.5	y ₁₀ ⁺
			Dual LIT	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1121.5	y ₁₀ ⁺
			QqQ	844.4 (+2)	—	y ₁₂ ⁺⁺	—	—
			Q-ToF	844.4 (+2)	666.3	y ₁₂ ⁺⁺	—	—
			QqQ	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1331.7	y ₁₂ ⁺⁺
			QqQ	844.4 (+2)	1007.5	y ₉ ⁺	1121.5	y ₁₀ ⁺
Gal d 4 - Lysozyme C	HIAITNAVLFFGR Gal d 2.0101	<i>Gallus gallus</i> (9031) <i>Alcaligenes xyloxydans</i> <i>xyloxydans</i> (85698)	QqQ	—	—	—	—	—
			Q-ToF	673.4 (+2)	1095.6	y ₁₀ ⁺	223.2	a ₂ ⁺
			QqQ	673.4 (+2)	1095.6	y ₁₀ ⁺	223.2	a ₂ ⁺
			Orbitrap	673.4 (+2)	—	y ₅ ⁺	526.3	y ₄ ⁺
			QqQ	449.3 (+3)	639.4 ⁺	y ₅ ⁺	608.3	b ₆ ⁺
			Q-ToF	—	—	—	—	—
			Q-ToF	—	—	—	—	—
			Orbitrap	887.4 (+2)	—	y ₁₁ ⁺	996.5	y ₉ ⁺
			QqQ	887.5 (+2)	1138.6	y ₁₁ ⁺	1067.5	y ₁₀ ⁺
			QqQ	887.5 (+2)	1138.6	y ₁₁ ⁺	1067.5	y ₁₀ ⁺
			QqQ	714.8 (+2)	277.1	b ₂ ⁺	—	—
			Orbitrap	714.8 (+2)	—	y ₁₀ ⁺	804.4	y ₇ ⁺
			QqQ	714.8 (+2)	1152.5	y ₁₀ ⁺	951.5	y ₈ ⁺
			QqQ	714.8 (+2)	589.3	y ₅ ⁺	690.4	y ₆ ⁺
Gal d 4.0101	NTDGSYDYGILQINSR Gal d 4.0101	<i>Gallus gallus</i> (9032) <i>Gallus sonneratii</i> (9033) <i>Gallus gallus</i> (9031) <i>Anas platyrhynchos</i> (8839) <i>Caracus wallachii</i> (9085) <i>Chrysolophus amherstiae</i> (9088) <i>Coturnix japonica</i> (93934) <i>Lophophorus impejanus</i> (9040) <i>Lophura leucomelanos</i> (140445) <i>Meleagris gallopavo</i> (9103)	QqQ	714.8 (+2)	1152.5	y ₁₀ ⁺	951.5	y ₈ ⁺
			Orbitrap	877.4 (+2)	—	y ₄ ⁺	730.4	y ₆ ⁺
			QqQ	585.3 (+3)	489.3	y ₄ ⁺	617.3	y ₅ ⁺
			Dual LIT /	877.4 (+2)	489.3	y ₄ ⁺	617.3	y ₅ ⁺
			Orbitrap	877.4 (+2)	489.3	y ₄ ⁺	730.4	y ₆ ⁺
			Dual LIT	—	—	—	—	—
			QqQ	877.4 (+2)	489.3	y ₄ ⁺	730.4	y ₆ ⁺
			Orbitrap	877.4 (+2)	489.3	y ₄ ⁺	730.4	y ₆ ⁺
			Dual LIT	—	—	—	—	—
			QqQ	877.4 (+2)	489.3	y ₄ ⁺	730.4	y ₆ ⁺
			Orbitrap	877.4 (+2)	489.3	y ₄ ⁺	730.4	y ₆ ⁺
			Dual LIT	—	—	—	—	—
			QqQ	877.4 (+2)	489.3	y ₄ ⁺	730.4	y ₆ ⁺
			Orbitrap	877.4 (+2)	489.3	y ₄ ⁺	730.4	y ₆ ⁺

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Table 2 (continued)

Target protein	Peptide Sequence <i>Isolalergens/variants</i>	Specificity (Tax ID)	MS platform	Precursor	Transition 1	Transition 2	Transition 3	References
Vitellogenin-1	YLIDLIPAAASHR (lipovitellin-1 chain)	<i>Pavo cristatus</i> (9049)	QqQ	480.6 (+3)	709.4	582.3	355.2	Planque et al., 2016; Planque, Arnould, Dieu et al., 2017; New et al., 2018; Planque et al., 2019
		<i>Phasianus colchicus</i>						
		<i>colchicus</i> (9057)						
		<i>Phasianus versicolor</i> (9055)						
		<i>Symaticus soemmerringii</i> (9067)						
		<i>Symaticus reevesii</i> (9066)						
		<i>Tragopan satyra</i> (9070)						
		<i>Tragopan temminckii</i> (9071)						
		<i>Rattus norvegicus</i> (10116)						
		<i>Pavo cristatus</i> (9049)						
		<i>Phasianus colchicus</i> (9054)						
		<i>Phasianus colchicus</i> (9057)						
		<i>Gallus lafayetii</i> (9032)						
		<i>Gallus sonneratii</i> (9033)						
		<i>Alopochen aegyptiaca</i> (30382)						
Vitellogenin-2	NIPFAEYPTYK (lipovitellin-1 chain)	<i>Gallus gallus</i> (9031)	QqQ	671.8 (+2)	1115.5	508.3	558.3	Planque et al., 2016; Planque, Arnould, Dieu et al., 2017; Planque et al., 2019
		<i>Gallus gallus</i> (9031)						
		<i>Larus argentatus</i> (35669)						
		<i>Anas platyrhynchos</i> (8839)						
		<i>Gucullus canorus</i> (55661)						
		<i>Buceros rhinoceros silvestris</i> (175836)						
		<i>Charadrius vociferus</i> (50402)						
		<i>Meryops nubicus</i> (57421)						
		<i>Pelecanus crispus</i> (36300)						
		<i>Mestiornis unicolor</i> (54374)						
		<i>Colinus virginianus</i> (9014)						
		<i>Meleagris gallopavo</i>						

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Table 2 (continued)

Target protein	Peptide Sequence <i>Isotopologues/variants</i>	Specificity (Tax ID)	MS platform	Precursor	Transition 1	Transition 2	Transition 3	References
NIGELGVEK (lipovitellin-1 chain)	(9103) <i>Callipepla squamata</i> (9009)							
	<i>Haliaeetus albicilla</i> (8969)							
	<i>Mesitornis unicolor</i> (54374)							
	<i>Gallus gallus</i> (9031)		QqQ	479.8 (+2)	731.4	674.4	545.3	Planque et al., 2016; Planque, Armoind, Dieu
	<i>Larus argentatus</i> (35669)		QqQ	479.8 (+2)	731.4	674.4	545.3	et al., 2017; New et al., 2018
	<i>Anas platyrhynchos</i> (8839)		QqQ	479.8 (+2)	731.4	228.1	—	
	<i>Nipponia nippon</i> (128390)							
	<i>Calypate anna</i> (9244)							
	<i>Nestor notabilis</i> (176057)							
	<i>Buceros rhinoceros</i> <i>silvestris</i> (175836)							
LPISLPVGPR (lipovitellin-2 chain)	<i>Charadrius vociferus</i> (50402)							
	<i>Pelecanus crispus</i> (36300)							
	<i>Merops nubicus</i> (57421)							
	<i>Apitenodytes forsteri</i> (9233)							
	<i>Paragioenus fasciata</i> <i>monilis</i> (372326)							
	<i>Phalacrocorax carbo</i> (9209)							
	<i>Anrostomus carolinensis</i> (279965)							
	<i>Chlamydotis macqueenii</i> (187382)							
	<i>Opisthocornus hoazin</i> (30419)							
	<i>Gallus gallus</i> (9031)		QqQ	524.8 (+2)	468.3	725.4	—	New et al., 2018
	<i>Hydroprogne caspia</i> (425641)							
	<i>Taeniopygia guttata</i> (59729)							
	<i>Sterna hirundo</i> (108405)							
	<i>Falco sparverius</i> (56350)							
	<i>Larus argentatus</i> (35669)							
	<i>Meleagris gallopavo</i> (9103)							
	<i>Nipponia nippon</i> (128390)							
	<i>Nestor notabilis</i> (176057)							
	<i>Callipepla squamata</i>							

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Table 2 (continued)

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Table 2 (continued)

Target protein	Peptide Sequence Isoallergens/variants	Specificity (Tax ID)	MS platform	Precursor	Transition 1	Transition 2	Transition 3	References
Ara h 2 - Conglutinin (2S Albumin)	GTGNLEIVAVR	<i>Arachis hypogaea</i> (3818)	Q/Q	564.4 (+2)	686.6	557.5	Y ₅ ⁺	Heick, Fischer, Kerbach et al., 2011; Heick, Fischer, & Pöpping, 2011; Parker et al., 2015; Gu et al., 2018; Boo et al., 2018; Pilolli et al., 2018
	Ara h 1.0101 (clone P41B)	<i>Arachis duranensis</i> (130453)	Q/Q	564.4 (+2)	686.6	557.5	Y ₅ ⁺	
	Ara h 1 - clone P17	<i>Arachis duranensis</i> (130453)	Q/Q	564.8 (+2)	686.4	557.4	Y ₅ ⁺	
			Q-Q	564.8 (+2)	686.4	557.4	Y ₅ ⁺	
			Q-Orbitrap	564.8 (+2)	—	—	—	
	CCNELNEFFNQVR	<i>Arachis ipaensis</i> (130454)	Q/Q	807.0 (+2)	1050.5	—	—	Careri et al., 2007; Careri et al., 2008; Parker et al., 2015; Pedreschi et al., 2012; Sayers et al., 2016; Sayers et al., 2018; Boo et al., 2018
	Ara h 2.0101,	<i>Arachis hypogaea</i> (3818)	Q/Q	576.2 (+3)	920.3	807.4	Y ₆ ⁺	
	Ara h 2.0201	<i>Arachis hypogaea</i> (3818)	Q/Q	863.8 (+2)	807.4	1050.5	Y ₈ ⁺	
	Ara h 2.0202	<i>Arachis duranensis</i> (130453)	Q/Q	863.9 (+2)	1163.5	1050.5	Y ₈ ⁺	
			Q/Q	863.8 (+2)	1163.5	1050.5	Y ₈ ⁺	
Gly m 5 - β-Conglycinin (Vicilin, 7S Globulin)	NLPQQGGILR	<i>Arachis ipaensis</i> (130454)	Q/Q	543.3 (+2)	858.4	761.4	Y ₆ ⁺	Parker et al., 2015; Pedreschi et al., 2012; Sayers et al., 2016; Sayers et al., 2018;
	Ara h 2.0101,	<i>Arachis hypogaea</i> (3818)	Q/Q	543.3 (+2)	858.4	761.4	Y ₆ ⁺	Vandekerckhove et al., 2017; Planque, Arnould, Dieu et al., 2017; Boo et al., 2018; Planque et al., 2019; Zhang et al., 2019
	Ara h 2.0201	<i>Arachis hypogaea</i> (3818)	Q/Q	543.3 (+2)	858.4	761.4	Y ₆ ⁺	
	Ara h 2.0202	<i>Arachis duranensis</i> (130453)	Q/Q	543.6 (+2)	858.2	429.8*	Y ₇ ⁺	
			Q/Q	543.3 (+2)	858.4	429.7	Y ₇ ⁺	
			Q/Q	543.3 (+2)	858.4	761.4	Y ₆ ⁺	
			Q/Q	543.3 (+2)	858.4	429.7	Y ₇ ⁺	
			Q/Q	543.8 (+2)	858.4	633.3	Y ₅ ⁺	
			Q/Q	543.8 (+2)	858.4	—	—	
			Q/Q	543.8 (+2)	858.4	—	—	
Gly m 5 - β-Conglycinin (Vicilin, 7S Globulin)	LITLAPVNPGR	<i>Glycine max</i> (3847)	Q/Q	464.7 (+3)	767.5*	583.4*	Y ₇ ⁺	Houston et al., 2011; Planque et al., 2016;
	Gly m 5.0101	<i>Glycine soja</i> (3848)	Q/Q	464.6 (+3)	767.5	583.4	Y ₁₁ ⁺	Planque, Arnould, Dieu et al., 2017; Chen, Yang, & Downs, 2019
			Q/Q	464.6 (+3)	767.5	583.4	Y ₁₁ ⁺	
	QQQEEQPLEVR	<i>Glycine max</i> (3847)	Q-Orbitrap	464.63 (+3)	—	—	—	
	Gly m 5.0201	<i>Glycine soja</i> (3848)	Q-Orbitrap	692.34 (+2)	1127.6	870.5	Y ₇ ⁺	Gu et al., 2018; Chen et al., 2019
	DSYNLQSGDALR	<i>Glycine max</i> (3847)	Q-Orbitrap	692.34 (+2)	—	—	—	
	Gly m 5.0201	<i>Glycine soja</i> (3848)	Q/Q	669.8 (+2)	859.5	746.4	Y ₇ ⁺	Gu et al., 2018
	DSYNLHPGDAQR	<i>Glycine max</i> (3847)	Q/Q	458.2 (+3)	643.3	546.3	Y ₅ ⁺	Gu et al., 2018
	Gly m 5.0301,							
	Gly m 5.0302							
Gly m 6 - Glycinin (Legumin, 11S Globulin)	VFDGELQEGR	<i>Glycine max</i> (3847)	Q/Q	575.2 (+2)	903.2	219.2	a ₂ ⁺	Heick, Fischer, & Pöpping, 2011; Heick, Fischer, Kerbach et al., 2011;
	Gly m 6.0101	<i>Glycine max</i> (3847)	Q/Q	575.2 (+2)	903.2	219.2	a ₂ ⁺	Huschek, Bönick, Löwenstein, Sievers, & Rawel, 2016;
			Q/Q	575.3 (+2)	903.2	489.2	Y ₄ ⁺	Planque, Arnould, Dieu et al., 2017; Planque et al., 2019; Chen et al., 2019
			Q/Q	575.3 (+2)	788.4	602.3	Y ₅ ⁺	
			Q/Q	575.3 (+2)	788.4	602.3	Y ₅ ⁺	
	SQSDNFEYVSFK	<i>Glycine max</i> (3847)	Q-Orbitrap	575.28 (+2)	—	—	—	
	Gly m 6.0101,		Q/Q	725.7 (+2)	381.2	1235.4	Y ₁₀ ⁺	Heick, Fischer, Kerbach et al., 2011; Heick, Fischer, & Pöpping, 2011; Pilolli, De Angelis et al., 2017; Gu et al., 2018; Chen et al., 2019
	Gly m 6.0201,		Dual LIT	725.8 (+2)	381.2	1235.4	Y ₁₀ ⁺	
	Gly m 6.0301		Q/Q	725.8 (+2)	772.4	643.3	H1 ⁺	
			Q-Orbitrap	725.83 (+2)	—	—	H ₂ O	
Gly m 6 - Glycinin (Legumin, 11S Globulin)	ISTLSLTPALR	<i>Glycine max</i> (3847)	Q/Q	699.9 (+2)	984.6	870.5	Y ₈ ⁺	Planque et al., 2016; Planque, Arnould, Dieu et al., 2017; Montowska & Fornal, 2019; Chen et al., 2019
	Gly m 6.0401,	<i>Glycine soja</i> (3848)	Q/Q	699.9 (+2)	984.6	870.5	Y ₈ ⁺	
	Gly m 6.0501	<i>Glycine microphylla</i> (45693)	Q-Orbitrap	699.9 (+2)	1097.7	670.4	Y ₆ ⁺	
			Q-Orbitrap	699.92 (+2)	—	—	—	
			Dual LIT	793.9 (+2)	638.8	1163.6	Y ₁₀ ⁺	Pilolli, De Angelis et al., 2017; Hoffmann, Münch, Schwägele, Neustütz, & Jira, 2017
		<i>Glycine max</i> (3847)	Q/Q	793.9 (+2)	638.8	424.2	b ₃ ⁺	

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Table 2 (continued)

Target protein	Peptide Sequence <i>Isoditergens/variants</i>	Specificity (Tax ID)	MS platform	Precursor	Transition 1	Transition 2	Transition 3	References	
Cor a 9 – 11S Seed Storage Globulin (Legumin-Like)	FYLAGNQEQEFLK <i>Gly m 6.0101</i> , <i>Gly m 6.0201</i>	<i>Corylus avellana</i> (13451) <i>Cor a 9.0101</i> (Q8W1C2) <i>Cor a 9 (A0A0A0P7E3)</i>	Q/Q Q/Q Q/Q Q/Q Q/Q Q/Q LIT-Orbitrap Q/Q Q/Q Q/Q Q/Q Q/Q	720.9 (+2)	1013.6	⁺ Y ₉	–	Heick, Fischer, Kerbach et al., 2011; Heick, Fischer, & Pöpping, 2011; Ansari, Stoppacher, & Baumgartner, 2012; Costa, Ansari, Mafra, Oliveira, & Baumgartner, 2014; Planque, Arnould, Dieu et al., 2017; New et al., 2018; Korte, Lepski, & Brockmeyer, 2016	
	720.9 (+2)			1013.6	⁺ Y ₉	–			
	720.9 (+2)			1013.7	⁺ Y ₉	228.2	⁺ b ₂		
	720.9 (+2)			1013.7	⁺ Y ₉	228.2	⁺ b ₂		
	720.9 (+2)			1013.6	⁺ Y ₉	812.5	⁺ Y ₇		
	720.9 (+2)			1013.6	⁺ Y ₉	812.5	⁺ Y ₇		
	720.9 (+2)			1013.6	–	–	–		
	815.5 (+2)			906.6	⁺ Y ₈	–	–		
	815.5 (+2)			906.6	⁺ Y ₈	1019.5	–		
	815.6 (+2)			906.5	⁺ Y ₈	1019.5	–		
	815.6 (+2)			906.5	⁺ Y ₈	185.2	175.2	⁺ Y ₁	
	815.6 (+2)			906.5	⁺ Y ₈	185.2	175.2	⁺ Y ₁	
815.4 (+2)	906.5	⁺ Y ₈	835.4	723.4	⁺ Y ₁₃				
815.4 (+2)	–	–	–	–	–				
815.4 (+2)	906.5	⁺ Y ₈	1019.6	⁺ Y ₆	723.4	⁺ Y ₁₃			
815.4 (+2)	906.5	⁺ Y ₈	1019.6	⁺ Y ₉	–	–			
576.3 (+2)	689.4	⁺ Y ₆	852.5	⁺ Y ₇	–	–	Heick, Fischer, Kerbach et al., 2011; Heick, Fischer, & Pöpping, 2011; Bignardi, Elviri, Penna, Careri, & Mangia, 2010; Bignardi et al., 2013;		
577.0 (+2)	689.0	⁺ Y ₆	567.0	⁺ Y ₇	–	–	Pilolli, De Angelis et al., 2017; Pilolli, Chaudhari et al., 2017; Gu et al., 2018; Pilolli et al., 2018; New et al., 2018; Korte, Lepski, & Brockmeyer, 2016; Planque et al., 2019		
577.0 (+2)	689.0	⁺ Y ₆	567.0	⁺ H ₁	–	–			
576.3 (+2)	689.4	⁺ Y ₆	567.3	⁺ H ₂ O	852.5	⁺ Y ₇			
576.3 (+2)	689.4	⁺ Y ₆	852.4	⁺ [M + 2-	588.3	⁺ Y ₅			
576.3 (+2)	689.4	⁺ Y ₆	852.4	⁺ H ₁	588.3	⁺ Y ₅			
576.3 (+2)	–	–	–	⁺ H ₂ O	–	–			
576.3 (+2)	689.4	⁺ Y ₆	852.4	⁺ [M + 2-	–	–			
576.3 (+2)	–	–	–	⁺ H ₁	–	–			
576.3 (+2)	689.4	⁺ Y ₆	588.3	⁺ H ₂ O	–	–			
807.5 (+2)	1088.6	⁺ Y ₁₀	874.6	⁺ Y ₅	–	–			
807.5 (+2)	1088.6	⁺ Y ₁₀	874.6	⁺ Y ₆	–	–			
807.8 (+2)	874.6	⁺ Y ₈	186.2	⁺ b ₂	314.2	⁺ b ₃	Heick, Fischer, Kerbach et al., 2011; Heick, Fischer, & Pöpping, 2011; Ansari et al., 2012; Costa et al., 2014;		
807.8 (+2)	874.6	⁺ Y ₈	186.2	⁺ b ₂	314.2	⁺ b ₃	Korte & Brockmeyer, 2016;		
807.5 (+2)	1088.6	⁺ Y ₁₀	437.7	⁺ Y ₈	–	–	Pilolli et al., 2018; Pilolli, Chaudhari et al., 2017; Korte, Lepski, & Brockmeyer, 2016;		
807.5 (+2)	1088.6	⁺ Y ₁₀	–	–	–	–	Planque et al., 2019;		
807.5 (+2)	1088.6	⁺ Y ₁₀	987.6	⁺ Y ₉	874.5	⁺ Y ₈	Korte, Oberleitner, & Brockmeyer, 2019		
807.5 (+2)	1088.6	⁺ Y ₁₀	–	–	–	–			
807.5 (+2)	1088.6	⁺ Y ₁₀	874.5	⁺ Y ₈	–	–			

(continued on next page)

Table 2 (continued)

Target protein	Peptide Sequence Isotopologues/variants	Specificity (Tax ID)	MS platform	Precursor	Transition 1	Transition 2	Transition 3	References
Cor a 11 – 7S Seed Storage Globulin (Vicilin-Like)	LLSGIENFR	<i>Corylus avellana</i> (13451)	Q/Q	524.9 (+2)	822.4	565.4	199.3	Ansari et al., 2012; Costa et al., 2014; New et al., 2018
	Cor a 11.0101		Q/Q	524.9 (+2)	822.4	565.4	199.3	
	AFSWEVLEALK	<i>Corylus avellana</i> (13451)	Q/Q	682.7 (+2)	644.4	402.3	191.3	Ansari et al., 2012; Costa et al., 2014; New et al., 2018
	Cor a 11.0101		Q/Q	682.7 (+2)	644.4	402.3	191.3	
Pru du 6 - Amandin, 11S Globulin Legumin- Like Protein	QQQQEQQQER	<i>Prunus dulcis</i> (3755)	LIT	694.0 (+2)	677.0	685.0	-	Bignardi et al., 2010
	Pru du 6.0101							
	TDENGFNTLAGR	<i>Prunus dulcis</i> (3755)	Q/Q	698.3 (+2)	936.5	879.5	732.4	Pilolli, Chaudhari et al., 2017; Korte, Lepski, & Brockmeyer, 2016; Planque et al., 2019
	Pru du 6.0201		LIT-Orbitrap	698.3 (+2)	-	-	-	
			Q/Q	698.3 (+3)	879.5	732.4	-	

susceptible to spontaneous deamidation. In addition, the sequences VQVVNENGDPILQDEVR and ALPDEVLPQNAFR, belonging to the Pru du 6.0201 isoform were not conserved in the alternative allergen variant registered as Q43608 in Uniprot database, both presenting single amino acid substitutions (D/N and N/T, respectively) and the peptide variant VQVVNENGDPILNDEVR is shared with *Prunus persica* (see sequence alignment in Fig. S9).

2.4. List of candidate markers agreed by ThRAI partners

The thorough discussion presented in the previous section allowed the selection of a restricted list of candidate signature peptides that can be fruitfully employed for allergen detection in highly complex and processed foods with a high-confidence on identification accuracy. This list representing the consensus table of the consortium is reported in Table 2 and will be further validated for its reliability in the model matrices selected within the ThRAI project by discovery experiments in untargeted HR-MS/MS analysis mode. As already mentioned, the preliminary marker selection here performed by means of the literature critical evaluation represents only the first step of the dual approach devised for the ThRAI project. The experimental validation of the candidate markers by MS/MS analysis is a mandatory step in method development because several empirical features, such as matrix composition, extraction/digestion conditions, and instrumental parameters might affect both the specificity and sensitivity of the final analytical method. Interferences in the chromatographic analysis from complex food background and information about processing effects on the ingredient itself or finished food products should be traced to avoid false-negative and false-positives. Such information cannot be disclosed merely by any *in-silico* based approach.

Table 2 was populated with all the information about sequence sharing among isoforms/variants per each discussed peptide.

As for the protein homology check among different taxonomies carried out on plant-derived allergens, we concluded that according to the current knowledge of full-length protein sequences, none of the signature peptides selected for plant-derived allergens (Table 2) is affected by homology issues. To the best of the current knowledge, the refined list of peptides provided very high detection specificity. For sake of clarity in Table S3, we reported a short list of peptide markers identified that might experience this kind of interference from other homologous proteins, disclosing both the targeted and the interfering precursor ions, as well as the shared transitions.

As for Pru du 6 peptides, with high sequence identity with homologous proteins from *Prunus persica*, *Prunus mume* and *Prunus avium*, only two peptide markers were kept, including the unique peptide TDE, even if encrypting the NG motif, since it can still be useful for confirmative purposes.

Finally, in Table 2 we also collected information about the reported precursor ions and the transitions monitored. Both the preferential ionization state of the target peptide and its typical fragmentation pattern depend not only on the specific amino acid sequence, but also on instrumental parameters such as the ionization source, the MS platform, and the activation mode; therefore, after proper marker selection, the ionization and fragmentation features need to be assessed on the specific MS platform involved in the study. From a general point of view, we highlighted that most of the selected signature peptides were detected as double charged ions, which indeed are very stable and reproducible both in their ionization yield and in the fragmentation pattern, whereas a heterogeneous scenario was disclosed as for transitions selection. Either two or three transitions per peptide were acquired, in some cases the two monitored fragments are simply two different ionization states of the same transition (y_n^+ and y_n^{++}). This behavior is typical of proline containing peptides that upon collisional induced fragmentation preferentially break the peptide bond at the N-terminal proline site, releasing the same transition as both single and double charged ions (Ma & Johnson, 2012). This transition is usually very sensitive, but the ratio (y_n^+/y_n^{++}) between the two ions is not

fixed, and may change upon different instrumental conditions. Additionally, retrieving the SRM instrumental details for the reported papers we also disclosed that in some investigations quite small fragments have been selected, namely y_1^+ , b_2^+ , a_2^+ , notwithstanding their low selectivity and susceptibility to interference from the background.

In light of the harmonization of detection method, and given the heterogeneous picture retrieved for instrumental set up, we will select at least three transitions for each marker, preferably belonging to the y_n and b_n series. Indeed, y - and b -ion series together would allow read out of the full peptide sequence from the MS/MS spectrum in the two directions starting from the C- and the N-terminus, respectively (Ma & Johnson, 2012); in addition, the two series together would account for most of the MS/MS spectrum ion intensity in both HCD and CID fragmentations (Michalski, Neuhauser, Cox, & Mann, 2012), with very high stability of the y -type transitions. Noteworthy, the monitored fragments should actually correspond to different transitions with a minimum fragment length of 3 amino acids, given the minimum length for peptide markers of 7 amino acids, in order to guarantee the maximum specificity and sequence coverage to the final developed method.

Future development of this work, will be the experimental validation of the reported preliminary list of candidate markers. In particular, discovery high resolution MS/MS analysis will be carried out directly on the two matrices, namely chocolate bar and broth powder as model hard-to-analyze food matrices, produced in a food pilot plant and incurred at the high contamination level. The experimental list of identified peptides belonging to the allergenic ingredients will be compared with the literature based selection either confirming or proposing new options for the SRM set up.

3. Conclusions

In this work we described the results of our comprehensive evaluation of the existing literature on food allergen detection by MS based methods, concerning the selection of peptide markers. We collected all information about the six allergens selected for the ThRAll project, namely cow's milk, hen's egg, peanut, soybean, hazelnut and almond. The selected peptides were critically discussed according to specific considerations and recommended criteria for marker selection that we promote in perspective harmonization of analytical methods development. The in-depth analysis of current knowledge allowed the selection of a restricted list of candidate signature peptides, already validated in several independent analyses, which could be monitored with high confidence. This preliminary list will be validated in the two model matrices selected within the ThRAll project, in a separate investigation for its final confirmation by specific discovery experiments with untargeted HR-MS/MS analysis.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of Competing Interest

The authors declare they have no competing interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2019.108747>.

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Supplementary material of

CRITICAL REVIEW ON PROTEOTYPIC PEPTIDE MARKERS

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TRACING FOR SIX ALLERGENIC INGREDIENTS IN INCURRED

FOODS BY MASS SPECTROMETRY: ~~A CRITICAL REVIEW~~

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25 **Table S1.** Full list of peptide markers belonging to milk, egg, peanut, soybean, hazelnut and almond,
26 reported in current literature of MS-based methods for food allergen detection.

Protein	Peptide Sequence	Total citations	References
Bos d 9 - α -s1-Casein	FFVAPFPEVFGK	32	1. Ansari et al., 2011
			2. Cereda et al., 2010
			3. Heick et al., 2011a
			4. Heick et al., 2011b
			5. Hong et al., 2012
			6. Lamberti et al., 2016
			7. Losito et al., 2013
			8. Monaci et al., 2013
			9. Monaci et al., 2011a
			10. Monaci et al., 2010a
			11. Monaci et al., 2010b
			12. Monaci et al., 2014
			13. Newsome & School, 2013
			14. Parker et al., 2015
			15. Pilolli et al., 2014
			16. Pilolli et al., 2017a
			17. Planque et al., 2016
			18. Tolin et al., 2012a
			19. Weber et al., 2006
			20. Planque et al., 2017a
			21. Planque et al., 2017b
			22. Gu et al., 2018
			23. Boo et al., 2018
			24. Montowska & Fornal, 2018
			25. Groves et al. 2018
			26. Ke et al., 2017
			27. Pilolli et al., 2018
			28. Monaci et al., 2011b
			29. Planque et al., 2019
			30. De Angelis et al., 2017b
			31 Qi et al., 2019
			32. Montowska & Fornal, 2019
	YLGYLEQLLR	34	1. Ansari et al., 2011
			2. Cereda et al., 2010
			3. Heick et al., 2011a
			4. Heick et al., 2011
			5. Hong et al., 2012
			6. Lamberti et al., 2016
			7. Losito et al., 2013
			8. Mattarozzi et al., 2014
			9. Monaci et al., 2013
			10. Monaci et al., 2011a
			11. Monaci et al., 2010a
			12. Monaci et al., 2010b
			13. Monaci et al., 2014
			14. Newsome & School, 2013
			15. Parker et al., 2015
			16. Pilolli et al., 2014
			17. Pilolli et al., 2017a
			18. Planque et al., 2016
			19. Tolin et al., 2012a
			20. Weber et al., 2006
			21. Planque et al., 2017b

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		22. Ji et al., 2017
		23. Gu et al., 2018
		24. Boo et al., 2018
		25. Montowska & Fornal, 2018
		26. New et al., 2018
		27. Groves et al. 2018
		28. Pilolli et al., 2018
		29. Planque et al., 2019
		30. Monaci et al., 2011b
		31. De Angelis et al., 2017b
		32. Qi et al., 2019
		33. Montowska & Fornal, 2019
		34. Pavón-Pérez et al., 2019
HQGLPQEVNLNLLR	18	1. Cereda et al., 2010
		2. Gomaa et al., 2015
		3. Lamberti et al., 2016
		4. Lee & Kim, 2010
		5. Mattarozzi et al., 2014
		6. Monaci et al., 2013
		7. Monaci et al., 2011a°
		8. Monaci et al., 2010a°
		9. Newsome & School, 2013
		10. Parker et al., 2015
		11. Planque et al., 2016
		12. Tolin et al., 2012a
		13. Planque et al., 2017b
		14. Ji et al., 2017
		15. Gu et al., 2018
		16. Montowska & Fornal, 2018
		17. Monaci et al., 2011b
		18. Groves et al. 2018
VPQLEIVPNSAEER	2	1. Cereda et al., 2010
		2. Monaci et al., 2013
EGIHAAQK	1	1. Losito et al., 2013
EPMIGVNVQELAYFYPELFR	4	1. Cereda et al., 2010
		2. Ji et al., 2017
		3. Montowska & Fornal, 2018
		4. De Angelis et al., 2017b
DIGSESTEDQAMEDIK	1	1. Monaci et al., 2013
EDVPSER	2	1. Monaci et al., 2013
		2. New et al., 2018
TTMPLW	1	1. Qi et al., 2019
VNELSK	1	1. Ke et al., 2017
NAVPIPTLNR	12	1. Cereda et al., 2010
		2. Gomaa et al., 2015
		3. Heick et al., 2011a
		4. Heick et al., 2011 .
		5. Monaci et al., 2013
		6. Monaci et al., 2010a
		7. Planque et al., 2016
		8. Planque et al., 2017b
		9. Gu et al., 2018
		10. Planque et al., 2019
		11. Ke et al., 2017
		12. Qi et al., 2019
ALNEINQFYQK	6	1. Cereda et al., 2010
		2. Gomaa et al., 2015
		3. Losito et al., 2013
		4. Lutter et al., 2011

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Bos d 11 - β -Casein	FALPQYLK	9	5. Monaci et al., 2013 6. Gu et al., 2018 1. Cereda et al., 2010 2. Heick et al., 2011a 3. Heick et al., 2011 4. Losito et al., 2013 5. Lutter et al., 2011 6. Monaci et al., 2013 7. Gu et al., 2018 8. Ke et al., 2017 9. Qi et al., 2019
	TVDMESTEVFTK	2	1. Cereda et al., 2010 2. Monaci et al., 2013
	TVYQHQK	1	1. Ke et al., 2017
	FPOYLQYLYQGPIVLNPWDQVK	1	1. Cereda et al., 2010
	ENLCSTFCK	2	1. Monaci et al., 2013 2. Ke et al., 2017
	GPFPPIV	11	1. Ansari et al., 2011 2. Cereda et al., 2010 3. Chen et al., 2015 4. Losito et al., 2013 5. Monaci et al., 2013 6. Monaci et al., 2011a 7. Monaci et al., 2010a 8. Monaci et al., 2011b 9. Tolin et al., 2012a 10. Ke et al., 2017 11. Qi et al., 2019
	VLPVPQK	8	1. Ansari et al., 2011 2. Chen et al., 2015 3. Losito et al., 2013 4. Mattarozzi et al., 2014 5. Monaci et al., 2010a 6. Tolin et al., 2012a 7. Gu et al., 2018 8. Pavón-Pérez et al., 2019
	FQSEEQQTDELQDK	2	1. Cereda et al., 2010 2. Monaci et al., 2013
	AVPYPQR	8	1. Cereda et al., 2010 2. Chen et al., 2015 3. Losito et al., 2013 4. Lutter et al., 2011 5. Mattarozzi et al., 2014 6. Tolin et al., 2012a 7. Gu et al., 2018 8. Qi et al., 2019
	DMPIQAFLLYQEPVLGPVR	8	1. Cereda et al., 2010 2. Chen et al., 2015 3. Monaci et al., 2013 4. Monaci et al., 2010a 5. Tolin et al., 2012a 6. Montowska & Fornal, 2018 7. Hong et al., 2012 8. De Angelis et al., 2017b
	EMPFVK	2	1. Chen et al., 2015 2. Qi et al., 2019
	YMPVLK	1	1. Chen et al., 2015
	YIPIQYVLSR	5	1. Cereda et al., 2010
Bos d 12 - κ -			

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Casein			2. Gomaa et al., 2015
			3. Lutter et al., 2011
			4. Monaci et al., 2010a
			5. Gu et al., 2018 ▲
	▲SPAQILQWQVLSNTVPAK	5	1. Cereda et al., 2010
			2. Monaci et al., 2010a
			3. Gu et al., 2018 ▲
			4. Montowska & Fornal, 2018
			5. Qi et al., 2019
	▲YIPIQYVLSR	1	1. Qi et al., 2019
	▲FFSDK	1	1. Ke et al., 2017
Bos d 4 - α-Lactalbumin	▲VGINYWLAHK	4	1. Ansari et al., 2011
			2. Zhang et al., 2012
			3. Ji et al., 2017
			4. Cucu et al., 2012a
	▲FLDDDLTDDIMCVK	2	1. Ji et al., 2017
			2. Cucu et al., 2012a
	▲DDQNPHSSNICNISCDK	1	1. Ji et al., 2017
	▲LDQWLCEK	1	1. Cucu et al., 2012a
	▲CEVFR	1	1. Ke et al., 2017
Bos d 5 - β-Lactoglobulin	▲TPEVDDEALEK	8	1. Lutter et al., 2011
			2. Monaci et al., 2014
			3. Parker et al., 2015
			4. Yang et al., 2014
			5. Boo et al., 2018
			6. Cucu et al., 2012a
		▲	7. New et al., 2018
			8. Pilolli et al., 2018
	▲VLVLDTDYK	7	1. Figeys et al., 1996
			2. Lutter et al., 2011
			3. Parker et al., 2015
			4. Planque et al., 2016
			5. Planque et al., 2017b
			6. Planque et al., 2019
			7. Cucu et al., 2012a
	▲VYVEELKPTPEGDLEILLQK	5	1. Figeys et al., 1996
			2. Planque et al., 2016
			3. Planque et al., 2017b
			4. Montowska & Fornal, 2018
			5. Cucu et al., 2012a
	▲LSFNPTQLEEQCHI	8	1. Parker et al., 2015
			2. Planque et al., 2016
			3. Planque et al., 2017b
			4. Boo et al., 2018
			5. Montowska & Fornal, 2018
			6. Cucu et al., 2012a
			7. Planque et al., 2019
			8. Ke et al., 2017
	▲DALNENK	1	1. Figeys et al., 1996
	▲LIVTQTMK	1	1. Ji et al., 2017
	▲WENGCAQK	1	1. Cucu et al., 2012a
	▲VAGTWYSLAMAASDISLLDAQSAPLR	3	1. Ji et al., 2017
			2. Montowska & Fornal, 2018
			3. Cucu et al., 2012a
	▲YLLFCMENSAPPEQSLACQCLVR	1	1. Ji et al., 2017
Gal d 2 - Ovalbumin	▲GGLEPINFQTAADQAR	19	1. Azarnia et al., 2013
			2. Mattarozzi et al., 2014
			3. Monaci et al., 2013

Formattato: Tipo di carattere: Non Grassetto, Colore carattere: Automatico

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Formattato: Colore carattere: Automatico

Formattato: Tipo di carattere: Non Grassetto, Colore carattere: Automatico

Formattato: Colore carattere: Automatico

Formattato: Tipo di carattere: Non Grassetto, Colore carattere: Automatico

Formattato: Rientro: Prima riga: 2 car

Formattato: Colore carattere: Automatico

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		4. Monaci et al., 2014
		5. Parker et al., 2015
		6. Pilolli et al., 2014
		7. Pilolli et al., 2017a
		8. Planque et al., 2016
		9. Tolin et al., 2012b
		10. Tolin et al., 2012a
		11. Planque et al., 2017a
		12. Planque et al., 2017b
		13. Boo et al., 2018
		14. Pilolli et al., 2017b
		15. New et al., 2018
		16. Pilolli et al., 2018
	▲	17. De Angelis et al., 2017b
		18. Planque et al., 2019
		19. Cavage et al., 2019
▲ TEWTSSNVMEER	13	1. Azarnia et al., 2013
		2. Monaci et al., 2013
		3. Monaci et al., 2014
		4. Nimata et al., 2017
		5. Pilolli et al., 2014
		6. Planque et al., 2016
		7. Tolin et al., 2012b
		8. Tolin et al., 2012a
		9. Pilolli et al., 2017b
		10. Planque et al., 2017b
		11. Planque et al., 2019
	▲	12. Pilolli et al., 2018
		13. De Angelis et al., 2017b
▲ ELINSWVESQTNGIIR	9	1. Azarnia et al., 2013
		2. Heick et al., 2011a
		3. Heick et al., 2011
		4. Monaci et al., 2013
		5. Parker et al., 2015
		6. Tolin et al., 2012b
	▲	7. New et al., 2018
		8. Tolin et al., 2012a
		9. Pavón-Pérez et al., 2019
▲ HIATNAVLFFGR	7	1. Azarnia et al., 2013
		2. Heick et al., 2011a
		3. Heick et al., 2011
		4. Monaci et al., 2013
		5. Parker et al., 2015
		6. Tolin et al., 2012a
		7. Cavage et al., 2019
▲ ISQAVHAAHAEINEAGR	6	1. Azarnia et al., 2013
		2. Lee & Kim, 2010
		3. Monaci et al., 2013
		4. Planque et al., 2016
		5. Tolin et al., 2012a
		6. Planque et al., 2017b
▲ YPILPEYLQCVK	9	1. Azarnia et al., 2013
		2. Heick et al., 2011a
		3. Heick et al., 2011
		4. Monaci et al., 2013
		5. Monaci et al., 2014
		6. Pilolli et al., 2017a
		7. Boo et al., 2018
		8. Pilolli et al., 2017b

Formattato: Colore carattere: Automatico

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Formattato: Tipo di carattere: Non Grassetto, Colore carattere: Automatico

Formattato: Rientro: Prima riga: 2 car

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Gal d 4 - Lysozyme C	NVLQPSSVDSQTAMVLNVAIV FK	4	9. Pilolli et al., 2018 1. Azarnia et al., 2013 2. Monaci et al., 2013 3. Nimata et al., 2017 4. Tolin et al., 2012a
	EVVGSAAEAGVDAASVSEEF	3	1. Azarnia et al., 2013 2. Monaci et al., 2013 3. Monaci et al., 2014
	DILNQITKPNVDVYSFLASR	4	1. Azarnia et al., 2013 2. Heick et al., 2011a 3. Heick et al., 2011 4. Tolin et al., 2012a
	VTEQESKPVQMMYQIGLFR	3	1. Azarnia et al., 2013 2. Nimata et al., 2017 3. Tolin et al., 2012a
	VASMASEK	2	1. Mattarozzi et al., 2014 2. Tolin et al., 2012a
	ADHPFLFCIK	1	1. Monaci et al., 2013
	FESNFNTQATNR	8	1. Cryar et al., 2013 2. Monaci et al., 2013 3. Parker et al., 2015 4. Pilolli et al., 2014 5. Tolin et al., 2012a 6. Boo et al., 2018 7. Cavage et al., 2019 8. Montowska & Fornal, 2018
	NTDGSTDYGILQINSR	8	1. Lee & Kim, 2010 2. Monaci et al., 2013 3. Parker et al., 2015 4. Pilolli et al., 2014 5. Tolin et al., 2012a 6. Montowska & Fornal, 2018 7. De Angelis et al., 2017b 8. Montowska & Fornal, 2019
	GTDVQAWIR	4	1. Cryar et al., 2013 2. Monaci et al., 2013 3. Boo et al., 2018 4. Montowska & Fornal, 2018
	HGLDNYR	2	1. Cryar et al., 2013 2. Monaci et al., 2013
	GYSLGNWVCAAK	1	1. Monaci et al., 2013
	CELAAMK	1	1. Cryar et al., 2013
	WWCNDGR	1	1. Cryar et al., 2013
	NLCNIPCSALLSSDITASVNCA K	1	1. Montowska & Fornal, 2018
Gal d 3 - Ovotransferrin	GTEFTVNDLQ GK	3	1. D'Ambrosio et al., 2008 2. Lee & Kim, 2010 3. Tolin et al., 2012a
	GAIEWEGIESGSVEQAVAK	5	1. D'Ambrosio et al., 2008 2. Tolin et al., 2012a 3. Cavage et al., 2019 4. Montowska & Fornal, 2018 5. Montowska & Fornal, 2019
	TDERPASYFAVAVAR	3	1. D'Ambrosio et al., 2008 2. Tolin et al., 2012a 3. Montowska & Fornal, 2018
	AQSDFGVDTK	2	1. D'Ambrosio et al., 2008 2. Tolin et al., 2012a
	TCNPSDILQMCSFLEGK	2	1. D'Ambrosio et al., 2008

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Automatico

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			2. Montowska & Fornal, 2018
	ISLTCVQK	1	1. D'Ambrosio et al., 2008
	ECNLAEVPTHAVVVRPEK	2	1. D'Ambrosio et al., 2008
			2. Montowska & Fornal, 2018
	LCQLCQSGGGIPPEK	1	1. D'Ambrosio et al., 2008
	FMMFESQNK	1	1. D'Ambrosio et al., 2008
	WSVWSNGDVECTVVDETK	1	1. D'Ambrosio et al., 2008
	DQLTPSPR	1	1. D'Ambrosio et al., 2008
	SAGWNIPIGTLLHR	3	1. D'Ambrosio et al., 2008
			2. Cavage et al., 2019
			3. Montowska & Fornal, 2018
	SDFHLFGPPGK	1	1. D'Ambrosio et al., 2008
	WCTISSPEEK	1	1. D'Ambrosio et al., 2008
	FFSASCVPGATIEQK	3	1. D'Ambrosio et al., 2008
			2. Montowska & Fornal, 2018
			3. Montowska & Fornal, 2019
	TGTCNFDEYFSEGCAPGSPPNSR	1	1. D'Ambrosio et al., 2008
	FYTVISSLK	2	1. D'Ambrosio et al., 2008
			2. Montowska & Fornal, 2018
	GDVAFIQHSSTEENTGGK	1	1. D'Ambrosio et al., 2008
	NLQMDDFELLCTDGR	1	1. D'Ambrosio et al., 2008
	TSCHTGLGR	1	1. D'Ambrosio et al., 2008
	GEADAVALDGGGLVYTAGVCG	1	1. D'Ambrosio et al., 2008
	LVPVMAER		
	LKPIAAEVYEHTEGSTTSYYA	1	1. D'Ambrosio et al., 2008
	VAVVK		
	YFGYTGALR	1	1. New et al., 2018
	ANVM DYR	1	1. New et al., 2018
	VEDIWSFLSK	1	1. Cavage et al., 2019
Gal d 1 Ovomuroid	AFNPVCGTDGVTYDNECLLC	1	1. Montowska & Fornal, 2018
	AHK		
Gal d 6 Ygp42 (vitellogenin - 1)	VAGNVQAQITPSPR	1	1. Lee & Kim, 2010
	YLLDLLPAAASHR	4	1. Planque et al., 2016
			2. Planque et al., 2017b
			3. Planque et al., 2019
			4. New et al., 2018
	ALLLSEIR	1	1. New et al., 2018
	TVIVEAPIHGLK	1	1. Cavage et al., 2019
	NVNFDEILK	1	1. Cavage et al., 2019
	ATAVSLLEWQR	1	1. Cavage et al., 2019
vitellogenin -2	EALQPIHDLADEAISR	3	1. Planque et al., 2016
			2. Planque et al., 2019
			3. Planque et al., 2017b
	NIPFAEYPTYK	3	1. Planque et al., 2016
			2. Planque et al., 2019
			3. Planque et al., 2017b
	NIGELGVEK	3	1. Planque et al., 2016
			2. Planque et al., 2017b
			2. New et al., 2018
	LPLSLPVGPR	1	1. New et al., 2018
apolipoprotein B (F1NV02)	HLFLPSSYK	1	1. Cavage et al., 2019
	ASFYGLSHAVTK	1	1. Cavage et al., 2019
	ALFDYFGYSHDGK	1	1. Cavage et al., 2019
	FALGIVTPGAK	1	1. Cavage et al., 2019
	TEEIPPLIENR	1	1. Cavage et al., 2019
Apovitellenin-1 (P02659)	NFLINETAR	1	1. Cavage et al., 2019

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Ara h 1 | Cupin
(Vicillin-Type,
7S Globulin)

DLAFPGSGEQVEK	11	- Chassaigne et al., 2007 - Chassaigne et al., 2009 - Heick et al., 2011a - Heick et al., 2011 - Pedreschi et al., 2012 - Sayers et al., 2016 - Sayers et al., 2018 - Shefcheck et al., 2006 - Garber et al., 2016 - New et al., 2018 - Zhang et al., 2019
VLLEENAGGEQEER	11	- Chassaigne et al., 2009 - Pedreschi et al., 2012 - Sayers et al., 2016 - Pilolli et al., 2017a - Sayers et al., 2018 - Shefcheck et al., 2006 - Garber et al., 2016 - Pilolli et al., 2018 - New et al., 2018 - Chassaigne et al., 2007 - Korte et al., 2016a
GTGNLELVAVR	9	- Chassaigne et al., 2009 - Heick et al., 2011a - Heick et al., 2011 - Parker et al., 2015 - Sealey-Voyksner et al., 2016 - Gu et al., 2018 - Boo et al., 2018 - Pilolli et al., 2018 - Garber et al., 2016
SFNLDEGHALR	4	- Chassaigne et al., 2009 - Sealey-Voyksner et al., 2016 - Shefcheck & Musser, 2004 - Gu et al., 2018
NNPFYFPSR	6	- Hebling et al., 2013 - Parker et al., 2015 - Shefcheck & Musser, 2004 - Gu et al., 2018 - Boo et al., 2018 - Garber et al., 2016
NTLEAAFNAEFNEIR	3	- Chassaigne et al., 2009 - Planque et al., 2016 - Shefcheck & Musser, 2004
EGALMLPHFNSK	1	Chassaigne et al., 2009
ISMPVNTPGQFEDFFPASSR	1	Chassaigne et al., 2009
EEEEDEDEEEEGSNR	1	Chassaigne et al., 2009
EGEQEWGTPGSHVR	2	- Chassaigne et al., 2009 - Zhang et al., 2019
AMVIVVVK	2	- Chassaigne et al., 2009 - Pilolli et al., 2018
EHVEELTK	1	Chassaigne et al., 2009
IVQIEAKPNTLVLPK	1	Chassaigne et al., 2009
QFQNLQNHRR	1	Chassaigne et al., 2009
EGEPDLSNNFGK	1	Chassaigne et al., 2009
DQSSYLQGFSSR	2	- Chassaigne et al., 2009 - Zhang et al., 2019
SSENEGVIK	2	- Chassaigne et al., 2009 - Garber et al., 2016

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Ara h 2 - Conglutin (2S Albumin)	CCNELNEFENNQR	10	1. Careri et al., 2007	Formattato	... [172]
			2. Careri et al., 2008a	Formattato	... [174]
			3. Hebling et al., 2013	Formattato	... [175]
			4. Hebling et al., 2012	Formattato	... [176]
			5. Parker et al., 2015	Formattato	... [177]
			6. Pedreschi et al., 2012	Formattato	... [173]
			7. Sayers et al., 2016	Formattato	... [178]
			8. Sayers et al., 2018	Formattato	... [179]
			9. Boo et al., 2018	Formattato	... [181]
			10. Garber et al., 2016	Formattato	... [182]
	NLPQQCGLR	11	1. Hebling et al., 2012	Formattato	... [183]
			2. Parker et al., 2015	Formattato	... [184]
			3. Pedreschi et al., 2012	Formattato	... [185]
			4. Sayers et al., 2016	Formattato	... [180]
			5. Sayers et al., 2018	Formattato	... [186]
			6. Vandekerckhove et al., 2017	Formattato	... [187]
			7. Planque et al., 2017b	Formattato	... [188]
			8. Boo et al., 2018	Formattato	... [189]
			9. Planque et al., 2019	Formattato	... [190]
			10. Garber et al., 2016	Formattato	... [194]
			11. Zhang et al., 2019	Formattato	... [191]
Ara h 3 - Cupin (Legumin-Type, 11S Globulin, Glycinin)	CMCEALQQIMENQSDR	7	1. Apostolovic, et al., 2013	Formattato	... [195]
			2. Careri M., et al. Anal Bioanal Chem 2007	Formattato	... [192]
			3. Careri et al., 2008a	Formattato	... [196]
			4. Hebling et al., 2012	Formattato	... [193]
			5. Parker et al., 2015	Formattato	... [197]
			6. Garber et al., 2016	Formattato	... [198]
			7. Pedreschi et al., 2012	Formattato	... [200]
	CQSGLER	2	1. Apostolovic, et al., 2013	Formattato	... [199]
			2. Hebling et al., 2012	Formattato	... [201]
	CDLEVESGGR	4	1. Hebling et al., 2012	Formattato	... [202]
			2. Pedreschi et al., 2012	Formattato	... [203]
			3. Garber et al., 2016	Formattato	... [204]
			4. Zhang et al., 2019	Formattato	... [205]
	GAGSSQHQR	1	1. Hebling et al., 2012	Formattato	... [206]
	DEDSYGR	1	1. Hebling et al., 2012	Formattato	... [207]
	ANLRPCEQHLMQK	1	1. Hebling et al., 2012	Formattato	... [208]
	QQEQQFK	2	1. Hebling et al., 2012	Formattato	... [209]
			2. Zhang et al., 2019	Formattato	... [210]
	QQWELQGDR	1	1. Hebling et al., 2012	Formattato	... [211]
	DPYSPSPYDR	1	1. Li et al., 2010	Formattato	... [212]
	DPYSPSQDPYSPSQDPDR	1	1. Li et al., 2010	Formattato	... [213]
	DPYSPSQDPYSPSPYDR	1	1. Li et al., 2010	Formattato	... [214]
Ara h 3 - Cupin (Legumin-Type, 11S Globulin, Glycinin)	SPDIYNPQAGSLK	13	1. Bignardi et al., 2010	Formattato	... [215]
			2. Bignardi et al., 2013	Formattato	... [216]
			3. Careri et al., 2007	Formattato	... [217]
			4. Careri et al., 2008b	Formattato	... [218]
			5. Careri et al., 2008a	Formattato	... [219]
			6. Chassaingne et al., 2007	Formattato	... [220]
			7. Parker et al., 2015	Formattato	... [221]
			8. Pedreschi et al., 2012	Formattato	... [222]
			9. Stevenson et al., 2009	Formattato	... [223]
			10. Sayers et al., 2016	Formattato	... [224]
			11. Gu et al., 2018	Formattato	... [225]
			12. Boo et al., 2018	Formattato	... [226]
			13. Garber et al., 2016	Formattato	... [227]
	RPFYSNAPQEIFIQOGR	9	1. Heick et al., 2011a	Formattato	... [228]
			2. Heick et al., 2011	Formattato	... [229]
			3. Planque et al., 2016	Formattato	... [230]

		4. Stevenson et al., 2009
		5. Vandekerckhove et al., 2017
		6. Planque et al., 2017b
		7. Gu et al., 2018
		8. Planque et al., 2019
		9. New et al., 2018
FNLAGNHEQEFLR	10	1. Bignardi et al., 2013
		2. Parker et al., 2015
		3. Planque et al., 2016
		4. Stevenson et al., 2009
		5. Korte & Brockmeyer, 2016b
		6. Planque et al., 2017b
		7. Gu et al., 2018
		8. Boo et al., 2018
		9. Garber et al., 2016
		10. Korte et al., 2019
LNAQRPDNR	3	1. Chassaigne et al., 2009
		2. Pedreschi et al., 2012
		3. Vandekerckhove et al., 2017
WLGLSAEYGNLYR	9	1. Heick et al., 2011a
		2. Heick et al., 2011
		3. Parker et al., 2015
		4. Stevenson et al., 2009
		5. Korte & Brockmeyer, 2016b
		6. New et al., 2018
		7. Garber et al., 2016
		9. Korte et al., 2019
		8. Korte et al., 2016a
SQSENFYVAFK	5	1. Chassaigne et al., 2007
		2. Hebling et al., 2013
		3. Stevenson et al., 2009
		4. Garber et al., 2016
		5. Korte et al., 2016a
AHVQVDSNGNR	3	1. Pedreschi et al., 2012
		2. Sealey-Voyksner et al., 2016
		3. Stevenson et al., 2009
GETESEEEGAIVTVR	2	1. Chassaigne et al., 2009
		2. Stevenson et al., 2009
QQPEENACQFQR	3	1. Sayers et al., 2018
		2. Stevenson et al., 2009
		3. Sayers et al., 2016
TANDLNLILR	5	1. Sealey-Voyksner et al., 2016
		2. Stevenson et al., 2009
		3. Korte & Brockmeyer, 2016b
		5. Korte et al., 2019
		4. Garber et al., 2016
FFVPPSQSPR	1	1. Stevenson et al., 2009
FFVPPFQQSPR	1	1. Stevenson et al., 2009
NALFVPHYNTNAHSIIYALR	1	1. Stevenson et al., 2009
QIVQNLWGENESEEEGAIVTVR	1	1. Stevenson et al., 2009
GYFGLIFGCPSTYEPAQQGR	1	1. Stevenson et al., 2009
ADEEEYDEDEYDEEDR	1	1. Stevenson et al., 2009
VYDEELQEGHVLVPPQNFVAGK	1	1. Stevenson et al., 2009
TANELNLLILR	3	1. Planque et al., 2017a
		2. Planque et al., 2019
		3. Planque et al., 2017b

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Ara h 6 - Conglutin (2S Albumin)	CDLDVSGGR	2	1. Sayers et al., 2016 2. Sayers et al., 2018
	NLPQNCGFR	2	1. Sayers et al., 2016 2. Sayers et al., 2018
Ara h 7 - Conglutin (2S Albumin)	LNALPTNR	5	1. Sealey-Voyksner et al., 2016 2. Korte & Brockmeyer, 2016b 3. Planque et al., 2017b 4. Korte et al., 2016a 5. Korte et al., 2019
	INTVNSNTLPVLR	9	1. Sealey-Voyksner et al., 2016 2. Cucu et al., 2012b 3. Heick et al., 2011a 4. Heick et al., 2011 5. Ansari et al., 2012 6. Costa, et al., 2014 7. Planque et al., 2017b 8. New et al., 2018 9. Korte et al., 2016a
Cor a 9 - 11S Seed Storage Globulin (Legumin-Like)	WLQLSAER	5	1. Sealey-Voyksner et al., 2016 2. Ansari et al., 2012 3. Costa, et al., 2014 4. Gu et al., 2018 5. Cucu et al., 2012b
	ALPDDVLANAFQISR	9	1. Cucu et al., 2012b 2. Heick et al., 2011a 3. Heick et al., 2011 4. Ansari et al., 2012 5. Costa, et al., 2014 6. Pilolli et al., 2017a 7. Pilolli et al., 2018 8. Planque et al., 2019 9. Planque et al., 2017b
	VQVVDNGNTVFDDELRL	3	1. Cucu et al., 2012b 2. Korte & Brockmeyer, 2016b 3. Korte et al., 2019
	QGQQQFGQR	1	1. Cucu et al., 2012b
	ADIYTEQVGR	11	1. Heick et al., 2011a 2. Heick et al., 2011 3. Bignardi et al., 2010 4. Bignardi et al., 2013 5. Pilolli et al., 2017a 6. Planque et al., 2017b 7. Gu et al., 2018 8. Pilolli et al., 2018 9. New et al., 2018 10. Planque et al., 2019 11. Korte et al., 2016a
	QEWER	1	1. Bignardi et al., 2010
	TNDNAQISPLAGR	2	1. Planque et al., 2017b 2. Pilolli et al., 2018
	HFYLAGNPDEHQRL	1	1. Cucu et al., 2012b
	QGQVLTIPQNFVAVK	10	1. Heick et al., 2011a 2. Heick et al., 2011b 3. Ansari et al., 2012 4. Costa, et al., 2014 5. Korte & Brockmeyer, 2016b 6. Pilolli et al., 2018

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			7. Planque et al., 2017b	
		▲	8. Planque et al., 2019	
			9. Korte et al., 2016a	
			10. Korte et al., 2019	
Cor a 11 - 7S Seed Storage Globulin (Vicilin-Like)	LLSGIENFR	5	1. Sealey-Voyksner et al., 2016	
		▲	2. Ansari et al., 2012	
			3. Costa, et al., 2014	
		▲	4. Cucu et al., 2012b	
			5. New et al., 2018	
	GNIVNEFER	1	1. Cucu et al., 2012b	
	HPSQSNQFGR	1	1. Cucu et al., 2012b	
	ALSQHEEGPPR	1	1. Cucu et al., 2012b	
	GSMAGPYNSR	1	1. Cucu et al., 2012b	
	IPAGTPVYMINR	1	1. Cucu et al., 2012b	
	ESFNVEHGDIIIR	1	1. Cucu et al., 2012b	
	NQDQAFFFPQPNK	1	1. Cucu et al., 2012b	
	JWPFGGESSGPINLLHK	1	1. Cucu et al., 2012b	
	LQPVSAAPHGHEAFYGAGGED PESFYR	1	1. Cucu et al., 2012b	
	AFSWEVLEALK	4	1. Ansari et al., 2012	
			2. Costa, et al., 2014	
		▲	3. Cucu et al., 2012b	
			4. New et al., 2018	
	ELAFNLPSR	3	1. Ansari et al., 2012	
			2. Costa, et al., 2014	
			3. Cucu et al., 2012b	
Cor a 14 2S Albumin	QAVMQQQGEMR	1	1. Cucu et al., 2012b	
	QQNLNQQR	1	1. Cucu et al., 2012b	
Cor a 8 - Non- Specific Lipid Transfer Protein 1	GIAGLNPNLAAGLPGK	2	1. Ansari et al., 2012	
			2. Costa, et al., 2014	
Pru du 5 - 60S Acidic Ribosomal Prot. P2	DITELIASGR	1	1. Fasoli et al., 2011	
Pru du 6 - Amandin, 11S Globulin Legumin-Like Protein	ALPDEVLANAYQISR	7	1. Fasoli et al., 2011	
			2. Heick et al., 2011a	
			3. Heick et al., 2011	
			4. Inman et al., 2018	
		▲	5. Planque et al., 2017b	
		▲	6. Planque et al., 2019	
			7. Gu et al., 2018	
	STLNSHNLPILR	3	1. Fasoli et al., 2011	
			2. Inman et al., 2018	
		▲	3. Sealey-Voyksner et al., 2016	
	NGLHLPSYSNAPQLIYVQGR	4	1. Fasoli et al., 2011	
			2. Heick et al., 2011a	
			3. Heick et al., 2011	
		▲	4. Inman et al., 2018	
	GNLDFVQPPR	11	1. Fasoli et al., 2011	
			2. Heick et al., 2011a	
		▲	3. Heick et al., 2011	
			4. Inman et al., 2018	
		▲	5. Planque et al., 2017b	
			6. Gu et al., 2018	
			7. Korte & Brockmeyer, 2016b	
		▲	8. Garber et al., 2016	
			9. Planque et al., 2019	
			10. Korte et al., 2016a	

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β-Conglycinin (Vicilin, 7S Globulin) - Gly m 5	QQEQLQER	5	11. Korte et al., 2019	Formattato	... [387]
			1. Bignardi et al., 2010	Formattato	... [388]
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			3. Fasoli et al., 2011	Formattato	... [391]
			4. Gu et al., 2018	Formattato	... [390]
			5. Garber et al., 2016	Formattato	... [392]
	QQEQEQR	1	1. Garber et al., 2016	Formattato	... [393]
	TEENAFINTLAGR	3	1. Fasoli et al., 2011	Formattato	... [394]
			2. Inman et al., 2018	Formattato	... [395]
			3. Sealey-Voyksner et al., 2016	Formattato	... [396]
	GVLGAVFSGCPETFEESQOS SQQGR	3	1. Fasoli et al., 2011	Formattato	... [398]
			2. Inman et al., 2018	Formattato	... [397]
			3. Heick et al., 2011b	Formattato	... [399]
	VQGQLDFVSPFSR	3	1. Fasoli et al., 2011	Formattato	... [400]
			2. Korte & Brockmeyer, 2016b	Formattato	... [401]
			3. Korte et al., 2019	Formattato	... [403]
	ALPDEVLQNAFR	5	1. Fasoli et al., 2011	Formattato	... [404]
			2. Korte & Brockmeyer, 2016b	Formattato	... [405]
			3. Garber et al., 2016	Formattato	... [402]
			5. Korte et al., 2019	Formattato	... [406]
			4. Korte et al., 2016a	Formattato	... [407]
	ADIFSPR	2	1. Fasoli et al., 2011	Formattato	... [408]
			2. Garber et al., 2016	Formattato	... [409]
	LSQNIQDPSR	2	1. Fasoli et al., 2011	Formattato	... [410]
			2. Garber et al., 2016	Formattato	... [411]
	VQVVNENGDPILNDEV	1	1. Garber et al., 2016	Formattato	... [412]
	VQVVNENGDPILDDEV	2	1. Fasoli et al., 2011	Formattato	... [413]
			2. Garber et al., 2016	Formattato	... [414]
	NGIYSPHWNVNAHSVVYVIR	1	1. Fasoli et al., 2011	Formattato	... [415]
	NLQGQNDNR	1	1. Fasoli et al., 2011	Formattato	... [416]
	FYLAGNPENEFNQGGQSQPR	1	1. Fasoli et al., 2011	Formattato	... [417]
	QQGQQEQQER	1	1. Bignardi et al., 2010	Formattato	... [418]
	QQEEQQSOR	1	1. Garber et al., 2016	Formattato	... [419]
	QQEQQQGQQGRPQQQQQF R	1	1. Fasoli et al., 2011	Formattato	... [420]
	QEGGQGGQQFQGEDQQDR	1	1. Garber et al., 2016	Formattato	... [421]
	FYLAGNPQDEFNPQQQGR	1	1. Fasoli et al., 2011	Formattato	... [422]
	NHLPILR	1	1. Fasoli et al., 2011	Formattato	... [423]
	ADFYNPQGGGR	2	1. Fasoli et al., 2011	Formattato	... [424]
			2. Gu et al., 2018	Formattato	... [425]
	LLSATSPPR	3	1. Fasoli et al., 2011	Formattato	... [426]
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			3. Korte et al., 2016a	Formattato	... [428]
	QETIALSSSQQR	3	1. Fasoli et al., 2011	Formattato	... [429]
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	TDENGFTNTLAGR	3	1. Planque et al., 2017b	Formattato	... [432]
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	QQQQQGEQGR	1	1. Garber et al., 2016	Formattato	... [435]
	LITLAIPVKNKGR	8	1. Agrawal et al., 2008	Formattato	... [436]
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			6. Planque et al., 2017b	Formattato	...
			7. Montowska & Fornal, 2018	Formattato	...
			8. Chen et al., 2019	Formattato	...
	MITLAIPVKNKGR	3	1. Agrawal et al., 2008	Formattato	...

		2. Han et al., 2013	Formattato: Colore carattere: Automatico
QQQEEQPLEVR	7	3. Scholl et al., 2014	Formattato: Colore carattere: Automatico
		1. Agrawal et al., 2008	Formattato ... [438]
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		3. Leitner et al., 2006	Formattato ... [440]
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		6. Cucu et al., 2012	Formattato ... [444]
		7. Chen et al., 2019	Formattato ... [443]
ESYFVDAQPK	4	1. Han et al., 2013	Formattato ... [445]
		2. Leitner et al., 2006	Formattato ... [447]
		3. Monaci et al., 2014	Formattato ... [446]
		4. Scholl et al., 2014	Formattato ... [448]
VPSGTTYVVNPDNNENLR	7	1. Agrawal et al., 2008	Formattato ... [449]
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		3. Leitner et al., 2006	Formattato ... [451]
		4. Scholl et al., 2014	Formattato ... [452]
		5. Montowska & Fornal, 2018	Formattato ... [453]
		6. Cucu et al., 2012c	Formattato ... [454]
TISSEDKPFNLR	6	1. Agrawal et al., 2008	Formattato ... [455]
		2. Han et al., 2013	Formattato ... [457]
		3. Monaci et al., 2014	Formattato ... [456]
		4. Scholl et al., 2014	Formattato ... [458]
		5. Gu et al., 2018	Formattato ... [459]
		6. Cucu et al., 2012c	Formattato ... [460]
DLDFLSIVDMNEGALLPHFN SK	4	1. Agrawal et al., 2008	Formattato ... [461]
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		3. Han et al., 2013	Formattato ... [463]
		4. Montowska & Fornal, 2018	Formattato ... [464]
SQQLQNLRL	3	1. Agrawal et al., 2008	Formattato ... [465]
		2. Han et al., 2013	Formattato ... [466]
		3. Scholl et al., 2014	Formattato ... [467]
SPQLQNLRL	3	1. Agrawal et al., 2008	Formattato ... [468]
		2. Han et al., 2013	Formattato ... [469]
		3. Scholl et al., 2014	Formattato ... [470]
AIVILVINEGDANIELVGLK	3	1. Agrawal et al., 2008	
		2. Gomaa et al., 2015	
		3. Scholl et al., 2014	
AIVVLVINEGEANIELVGIK	3	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Scholl et al., 2014	
QFPFPRPPHQK	3	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Scholl et al., 2014	
NPFLFGSNR	3	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Scholl et al., 2014	
GSEEEQDER	2	1. Agrawal et al., 2008	
		2. Han et al., 2013	
EHPRPHQPHQK	1	1. Agrawal et al., 2008	
NILEASYDTK	4	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Scholl et al., 2014	
		4. Chen et al., 2019	
EQQQEQQQEEQPLEVR	5	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Scholl et al., 2014	
		4. Montowska & Fornal, 2018	
		5. Cucu et al., 2012c	
LQESVIVEISK	4	1. Agrawal et al., 2008	

		2. Han et al., 2013
		3. Scholl et al., 2014
		4. Montowska & Fornal, 2018
EEGQQQGEER	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
EEGQQQGEQR	4	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
		4. Cucu et al., 2012c
QVQELAFPGSAQDVER	3	1. Han et al., 2013
		2. Leitner et al., 2006
		3. Scholl et al., 2014
FFEITPEK	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
ESESESESEL	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
DSYNLQSGDALR	4	1. Agrawal et al., 2008
	-	2. Han et al., 2013
	-	3. Scholl et al., 2014
		4. Gu et al., 2018
DLDVFLSVDMNEGALFLPHF NSK	2	1. Agrawal et al., 2008
		2. Han et al., 2013
DLDFLSSVDINEGALLPHFN SK	2	1. Han et al., 2013
		2. Scholl et al., 2014
DNVISQIPSQVQELAFPGSAK	2	1. Agrawal et al., 2008
	-	2. Han et al., 2013
NFLAGSK	2	1. Agrawal et al., 2008
	-	2. Han et al., 2013
SPQLENLR	2	1. Han et al., 2013
		2. Scholl et al., 2014
AILTLVNDDR	2	1. Han et al., 2013
		2. Scholl et al., 2014
AIVILVINEGDANIELVGK	2	1. Han et al., 2013
		2. Scholl et al., 2014
QEEDDEEEQQR	2	1. Agrawal et al., 2008
		2. Han et al., 2013
ESEEEEEQDEDEEQDK	2	1. Agrawal et al., 2008
		2. Han et al., 2013
SQSESYFVDAQPPQK	2	1. Agrawal et al., 2008
		2. Han et al., 2013
QPEGVIVELSK	2	1. Han et al., 2013
		2. Scholl et al., 2014
NPIYSNNFGK	2	1. Han et al., 2013
		2. Scholl et al., 2014
DPIYSNK	2	1. Agrawal et al., 2008
		2. Han et al., 2013
VPAGTTYVVPNDNDENLR	2	1. Agrawal et al., 2008
		2. Han et al., 2013
FESFFLSSTEAQQSYLQGF	4	1. Agrawal et al., 2008
		2. Han et al., 2013
	-	3. Montowska & Fornal, 2018
	-	4. Cucu et al., 2012c
FESFFLSSTQAQQSYLQGF	2	1. Agrawal et al., 2008
		2. Han et al., 2013
GSEEEDEDEEQDER	2	1. Agrawal et al., 2008
		2. Han et al., 2013
VLFGEEEQ	2	1. Han et al., 2013

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Automatico

Formattato: Tipo di carattere: Non
Grassetto, Colore carattere:
Automatico

Formattato: Colore carattere:
Automatico

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Automatico

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▲NPFHFNSK	2	1.	Agrawal et al., 2008
	-	2.	Scholl et al., 2014
▲DIENLIK	2	1.	Han et al., 2013
	-	2.	Scholl et al., 2014
▲TISSEDEPNLR	2	1.	Han et al., 2013
	-	2.	Scholl et al., 2014
▲QEEEPLEVQR	2	1.	Han et al., 2013
	-	2.	Scholl et al., 2014
▲VQFQSKPNTILLPHHADDFL	1	1.	Han et al., 2013
LFVLSGR			
▲NFLAGEK	1	1.	Scholl et al., 2014
▲ESQSEGESQSR	1	1.	Agrawal et al., 2008
EPQQPGEK	1	1.	Scholl et al., 2014
▲QEEHEQR	2	1.	Han et al., 2013
		2.	Cucu et al., 2012c
▲ESYFVDAQPQOK	1	1.	Scholl et al., 2014
▲PAGTTYLVNPHDHQNLK	1	1.	Han et al., 2013
▲DSYNLHPGDAQR	2	1.	Han et al., 2013
	-	2.	Gu et al., 2018 ▲
▲SSNSFQTLFENQNGR	1	1.	Han et al., 2013
▲EEQEWPR	1	1.	Scholl et al., 2014
▲LFEITPEK	1	1.	Han et al., 2013
▲VLLGEEEEQR	1	1.	Han et al., 2013
EDENNPFYFR	1	1.	Scholl et al., 2014
EDENNPFYLR	1	1.	Han et al., 2013
▲NQYGHVR	1	1.	Scholl et al., 2014
▲QSGDALR	1	1.	Scholl et al., 2014
▲NFLAGSQDNVISQIPSQVQEL	1	1.	Montowska & Fomal, 2018
AFPGSAQAVEK			
▲VFDGELQEGR	12	1.	Agrawal et al., 2008
	▲	2.	Barbosa et al., 2012
	▲	3.	Han et al., 2013
	▲	4.	Heick et al., 2011a
	▲	5.	Heick et al., 2011b
	▲	6.	Huscek et al., 2016
	▲	7.	Leitner et al., 2006
		8.	Scholl et al., 2014
		9.	Planque et al., 2017b
		10.	Planque et al., 2019
		11.	Cucu et al., 2012c
		12.	Chen et al., 2019
▲VLIVPQNEFVVAAR	10	1.	Agrawal et al., 2008
	▲	2.	Gomaa et al., 2015
	▲	3.	Han et al., 2013
	▲	4.	Houston et al., 2011
	▲	5.	Leitner et al., 2006
		6.	Scholl et al., 2014
	▲	7.	Gu et al., 2018 ▲
		8.	Montowska & Fomal, 2018
		9.	Cucu et al., 2012c
		10.	Montowska & Fomal, 2019
▲LSAEFGSLR	7	1.	Agrawal et al., 2008
	▲	2.	Han et al., 2013
		3.	Houston et al., 2011
		4.	Huscek et al., 2016
		5.	Scholl et al., 2014
	▲	6.	Gu et al., 2018 ▲
		7.	Montowska & Fomal, 2018
▲EAFGVNMQIVR	11	1.	Han et al., 2013

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		2. Heick et al., 2011a	
		3. Heick et al., 2011b	
		4. Leitner et al., 2006	
		5. Planque et al., 2016	
	▲	6. Scholl et al., 2014	
	▲	7. Planque et al., 2017a	
		8. Hoffmann et al., 2017	
		9. Planque et al., 2017b	
		10. Planque et al., 2019	
		11. Montowska & Fomal, 2018	
▲ SQSDNFEYVSFK	9	1. Barbosa et al., 2012	
		2. Han et al., 2013	
		3. Heick et al., 2011a	
		4. Heick et al., 2011b	
		5. Pilolli et al., 2017a	
		6. Scholl et al., 2014	
		7. Gu et al., 2018	▲
		8. Montowska & Fomal, 2018	▲
		9. Chen et al., 2019	
▲ GIFGMIYPGCPSTFEPPQQPQ QR	5	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Leitner et al., 2006	
	▲	4. Scholl et al., 2014	
		5. Montowska & Fomal, 2018	
▲ RPSYTNPGQEIIYQQGK	5	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Leitner et al., 2006	
		4. Scholl et al., 2014	
		5. Cucu et al., 2012c	
▲ LSAQFGSLR	5	1. Agrawal et al., 2008	
	▲	2. Han et al., 2013	
		3. Houston et al., 2011	
		4. Scholl et al., 2014	▲
		5. Chen et al., 2019	
▲ HQQEEENEGBSILSGFTLEFL EHAHSVDK	5	1. Agrawal et al., 2008	
		2. Goma et al., 2015	
		3. Han et al., 2013	
		4. Scholl et al., 2014	
	▲	5. Montowska & Fomal, 2018	
▲ ISTLNSLTLPALR	9	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Planque et al., 2016	
		4. Scholl et al., 2014	
		5. Planque et al., 2017b	
		6. Montowska & Fomal, 2018	
		7. Cucu et al., 2012c	▲
		8. Montowska & Fomal, 2019	
		9. Chen et al., 2019	
▲ AIPSEVLASYNLR	5	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Monaci et al., 2014	
		4. Scholl et al., 2014	
		5. Montowska & Fomal, 2018	
▲ NAMFVPHYTLNANSIIYALNG R	3	1. Han et al., 2013	
		2. Leitner et al., 2006	
		3. Montowska & Fomal, 2018	
▲ VESEGLIQTWNSQHPELK	4	1. Agrawal et al., 2008	
		2. Han et al., 2013	
		3. Houston et al., 2011	

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THHNAVTSYLK	3	4. Montowska & Fornal, 2018
▲QQEEENEGSNILSGFAPEFLK	4	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
NGIYSPHWNLNANSVIYVTR	3	1. Han et al., 2013
		2. Leitner et al., 2006
		3. Scholl et al., 2014
		4. Montowska & Fornal, 2018
▲NLQGENEGEDK	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲YQEEQGGHQSQK	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲NGIYSPHWNLNANSVIYVTR	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲MIIAQGK	2	1. Han et al., 2013
		2. Scholl et al., 2014
▲NLQGENEEEDSGAIVTK	4	1. Han et al., 2013
		2. Houston et al., 2011
		3. Leitner et al., 2006
		4. Montowska & Fornal, 2018
▲LNALKPDNR	4	1. Han et al., 2013
		2. Huschek et al., 2016
		3. Scholl et al., 2014
		4. Cucu et al., 2012c
▲SQQLQDSHQK	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲LSAQYGLSR	3	1. Han et al., 2013
		2. Houston et al., 2011
		3. Scholl et al., 2014
▲CAGVTYSK	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲HFLAQSFNTNEDIAEK	5	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
		4. Hoffmann et al., 2017
		5. Montowska & Fornal, 2018
▲HFLAQSFNTNEDTAEK	3	1. Agrawal et al., 2008
		2. Leitner et al., 2006
		3. Scholl et al., 2014
▲EQPQQNECQIQK	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲NGLHLPSSYPYPR	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Houston et al., 2011
▲QFGLSAQYVVLRY	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲QFQLSAQYVVLKY	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲QQEEENEGGSILSGFAPEFLE	4	1. Agrawal et al., 2008

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HAFVDR		2. Han et al., 2013
		3. Scholl et al., 2014
		4. Montowska & Fornal, 2018
▲FYLAGNQEQEFLQYQPQK	3	1. Han et al., 2013
		2. Houston et al., 2011
		3. Scholl et al., 2014
▲WQEQQDEDEDEDEDEDEQI PSHPPR	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Leitner et al., 2006
▲GAIGFAFPGCPETFEKPPQQS SR	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Leitner et al., 2006
▲VVNCQGNVDFGELR	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲VFDGELQEGGVLVIPQNFVA AK	5	1. Han et al., 2013
		2. Monaci et al., 2014
		3. Scholl et al., 2014
		4. Montowska & Fornal, 2018
		5. Cucu et al., 2012c
▲VFYLAGNPDIETPMQQQQ QQK	4	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Leitner et al., 2006
		4. Montowska & Fornal, 2018
▲VFYLAGNPDIETPMQQQQ QQK	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Leitner et al., 2006
▲YEGNWGPLVNPESQQGSPR	4	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
		4. Montowska & Fornal, 2018
▲ALIQVVNCNGER	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
▲QIVTVEGGLSVISPK	4	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Scholl et al., 2014
		4. Montowska & Fornal, 2018
▲FYLAGNQEQEFLK	5	1. Han et al., 2013
		2. Piloli et al., 2017a
		3. Scholl et al., 2014
		4. Hoffmann et al., 2017
		5. Montowska & Fornal, 2018
▲NAMFVPHYNLNANSIIYALNG R	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Montowska & Fornal, 2018
▲IESEGGLIETWNPNNKPFQCA GVALSR	3	1. Agrawal et al., 2008
		2. Han et al., 2013
		3. Montowska & Fornal, 2018
▲THHNAVSSYIK	2	1. Agrawal et al., 2008
		2. Han et al., 2013
▲NNPFSLVPPK	2	1. Han et al., 2013
		2. Scholl et al., 2014
▲YQQQQGGGSQSQK	2	1. Han et al., 2013
		2. Scholl et al., 2014
▲NGLHLPSYSPYQMIIVQVK	2	1. Agrawal et al., 2008
		2. Han et al., 2013
▲KPQEEEDDDDEEQPCVET DK	2	1. Han et al., 2013
		2. Scholl et al., 2014
▲GQHQHQEEEEEGSVLSGFS	2	1. Han et al., 2013

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	K		2. Leitner et al., 2006
	QGQHQQQEEEGSVLSGFS	2	1. Agrawal et al., 2008
	K		2. Han et al., 2013
	LQSPDDER	2	1. Agrawal et al., 2008
			2. Han et al., 2013
	EQDQDQDEDEDEDEDQPR	2	1. Agrawal et al., 2008
			2. Han et al., 2013
	EQPQQNECQIQR	2	1. Agrawal et al., 2008
			2. Han et al., 2013
	WQEQEDEDDEDEEYEQTPS	2	1. Agrawal et al., 2008
	YPPR		2. Han et al., 2013
	VESEGGLIETWNSQHPELQC	2	3. Agrawal et al., 2008
	AGVTVSK		4. Han et al., 2013
	GQLLVVPQNFVVAEQAGEQG	3	1. Agrawal et al., 2008
	FEYIVFK		2. Han et al., 2013
			3. Montowska & Fornal, 2018
	FNECQLNNLNALEPDHR	2	1. Agrawal et al., 2008
			2. Han et al., 2013
	ALVQVVNCNGER	2	1. Han et al., 2013
			2. Scholl et al., 2014
	QSQVSELK	2	1. Han et al., 2013
			2. Scholl et al., 2014
	YQNSGGLVNP	1	1. Agrawal et al., 2008
	IESEGGLIETWNPNNKPFQCA	1	1. Han et al., 2013
	GVALSR		
	NGVEENICTMK	1	1. Agrawal et al., 2008
	NGVEENICTLK	1	1. Agrawal et al., 2008
	AIPSEVLNSYNLGQSQVR	1	1. Han et al., 2013
	NNNPFSLVPPQESQR	2	1. Scholl et al., 2014
			2. Montowska & Fornal, 2018
	FLVPPQESQK	2	1. Scholl et al., 2014
			2. Cucu et al., 2012c
	VFDGELQEGQVLIVPQNFAVA	1	1. Scholl et al., 2014
	AR		
	GQLLVVPQNFVVAEQGGEQG	1	1. Agrawal et al., 2008
	LEYVVK		
	INECQLNNLNALEPDHR	2	1. Han et al., 2013
			2. Montowska & Fornal, 2018
	TNDTPMIGTLAGANSLNALP	1	1. Montowska & Fornal, 2018
	EEVIQHTFNLK		
	TNDRPSIGNLAGANSLNALP	1	1. Montowska & Fornal, 2018
	EEVIQHTFNLK		
	HNIGQTSSPDIYNPQAGSVTT	1	1. Montowska & Fornal, 2018
	ATSLDFPALSWLR		
	HNIGQTSSPDIYNPQAGSITTA	1	1. Montowska & Fornal, 2018
	TSLDFPALSWLK		
	TNDRPSIGNLAGANSLNALP	1	1. Montowska & Fornal, 2018
	EEVIQQTFLNR		
	QNIGQNSSPDIYNPQAGSITT	1	1. Montowska & Fornal, 2018
	ATSLDFPALWLLK		
	QNIGQNSSPDIYNPQAGSITT	1	1. Montowska & Fornal, 2018
	ATSLDFPALQLLK		
Gly m 8 2S Albumin	ELINLATMCR	4	1. Leitner et al., 2006
			2. Planque et al., 2016
			3. Planque et al., 2019
			4. Planque et al., 2017b
	JMENQSEEELEEK	1	1. Leitner et al., 2006
	CCTEMSELR	1	1. Leitner et al., 2006
Kunitz-type trypsin inhibitor KT12	DTVDGWFNIER	1	1. Chen et al., 2019
Lectin	TSNILSDVVDLK	1	1. Chen et al., 2019

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29 **Table S21.** Refined peptide marker list with a sequence length ranging between 7 and 20 amino acids and validated at least once in
30 hard-to-analyze matrices (namely either chocolate-based foods or highly processed incurred matrices). No further exclusion criteria were
31 applied to this list. The amino acids prone to exogenous modifications methionine (M), and asparagine (N) within the NG motif, were
32 underlined. *Official isoforms and variants registered by the WHO/IUIS Allergen Nomenclature Sub-Committee.

Target protein	Peptide Sequence (abbreviation)	Investigated matrices	References
Bos d 9 – α-S1-Casein	FFVAPFPEVFGK (FFV)	Dairy products, Snacks, Wine, Bread, Cookie, Cereal bar, Muffin, Chocolate, Ice cream, Tomato sauce, Poultry meat products (sausages, frankfurters, patés, hot dog), Infant formula, Compote, Ham, Jam, Spices, Mayonnaise	Ansari et al., 2011, Cereda et al., 2010, Heick et al., 2011, Heick et al., 2011b, Hong et al., 2012, Lamberti et al., 2016, Losito et al., 2013, Monaci et al., 2013, Monaci et al., 2011a, Monaci et al., 2010a, Monaci et al., 2010b, Monaci et al., 2014, Newsome & Scholl 2013, Parker et al., 2015, Pilolli et al., 2014, Pilolli et al., 2017a, Planque et al., 2016, Tolin et al., 2012a, Weber, et al., 2006, Planque et al., 2017a, Planque et al., 2017b, Gu et al., 2018, Boo et al., 2018, Montowska & Fornal 2018, Groves et al., 2018, Ke, et al., 2017, Pilolli et al., 2018, Monaci et al 2011b, De Angelis et al., 2017b, Planque et al., 2019, Qi et al., 2019 , Montowska & Fornal, 2019 .
	YLGYLEQLLR (YLG)	Dairy products, Snacks, Wine, Bread, Cookie, Cereal bar, Muffin, Chocolate, Ice cream, Tomato sauce, Poultry meat products (sausages, frankfurters, patés, hot dog), Pies, Waffles, Cookie dough, Salad dressing, Infant formula, Breakfast cereal	Ansari et al., 2011, Cereda et al., 2010, Heick et al., 2011a, Heick et al., 2011b, Hong et al., 2012, Lamberti et al., 2016, Losito et al., 2013, Mattarozzi et al., 2014, Monaci et al., 2013, Monaci et al., 2011a, Monaci et al., 2010a, Monaci et al., 2010b, Monaci et al., 2014, Newsome & Scholl 2013, Parker et al., 2015, Pilolli et al., 2014, Pilolli et al., 2017a, Planque et al., 2016, Tolin et al., 2012a, Weber, et al., 2006, Planque et al., 2017b, Ji et al., 2017, Gu et al., 2018, Boo et al., 2018, Montowska & Fornal 2018, New et al., 2018, Groves et al., 2018, Pilolli et al., 2018, Monaci et al 2011b, De Angelis et al., 2017b, Planque et al., 2019, Qi et al., 2019 , Montowska & Fornal, 2019 , Pavón-Pérez et al., 2019 .
	HQGLPQEVLENLLR (HQG)	Wine, Cookie, Cereal bar, Muffin, Chocolate, Ice cream, Tomato sauce, Poultry meat products (sausages, frankfurters, patés), Pies, Waffles, Compote, Ham, Jam, Spices, Mayonnaise	Cereda et al., 2010, Gomaa & Boye, 2015, Lamberti et al., 2016, Lee & Kim, 2010, Mattarozzi et al., 2014, Monaci et al., 2013, Monaci et al., 2011a, Monaci et al., 2010a, Newsome & Scholl 2013, Parker et al., 2015, Planque et al., 2016, Tolin et al., 2012a, Planque et al., 2017b, Ji et al., 2017, Gu et al., 2018, Montowska & Fornal 2018, Monaci et al 2011b, Groves et al., 2018.
	EDVPSEER (EDV)	Wine, Cookie, Bread, Cookie dough, Salad dressing, Infant formula, Chocolate, Ice cream, Breakfast cereal	Monaci et al., 2013, New et al., 2018.
Bos d 10 – α-S2-Casein	NAVPIPTLNR (NAV)	Wine, Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Infant formula, Compote, Ham, Jam, Spices, Mayonnaise	Cereda et al., 2010, Gomaa & Boye, 2015, Heick et al., 2011a, Heick et al., 2011b, Monaci et al., 2013, Monaci et al., 2010a, Planque et al., 2016, Planque et al., 2017b, Gu et al., 2018, Ke, et al., 2017, Planque et al., 2019, Qi et al., 2019 .
	ALNEINQFYQK (ALN)	Wine, Chocolate, Cookie, Soy-based infant formula, Breakfast cereals, Baby food	Cereda et al., 2010, Gomaa & Boye, 2015, Losito et al., 2013, Lutter et al., 2011, Monaci et al., 2013, Gu et al., 2018.
	FALPQYLK (FAL)	Wine, Bread, Chocolate, Infant formula, Soy-based infant formula, Breakfast cereals, Baby food	Cereda et al., 2010, Heick et al., 2011a, Heick et al., 2011b, Losito et al., 2013, Lutter et al., 2011, Monaci et al., 2013, Gu et al., 2018, Ke, et al.,

			2017.
Bos d 11 – β-Casein	GPFPPIV (GPF)	Dairy products, Snacks, Wine, Cookie, Infant formula	Ansari et al., 2011, Cereda et al., 2010, Chen, et al., 2015, Losito et al., 2013, Monaci et al., 2013, Monaci et al., 2011a, Monaci et al., 2010a, Monaci et al 2011b, Tolin et al., 2012a, Ke, at al., 2017, Qi et al., 2019 .
	VLPVPQK (VLP)	Dairy products, Snacks, Wine, Chocolate, Cookie	Ansari et al., 2011, Chen, et al., 2015, Losito et al., 2013, Mattarozzi et al., 2014, Monaci et al., 2010a, Tolin et al., 2012a, Gu et al., 2018, Pavón-Pérez et al., 2019 .
	AVPYPQR (AVP)	Wine, Chocolate, Soy-based infant formula, Breakfast cereals, Baby food, Cookie	Cereda et al., 2010, Chen, et al., 2015, Losito et al., 2013, Lutter et al., 2011, Mattarozzi et al., 2014, Tolin et al., 2012a, Gu et al., 2018, Qi et al., 2019 .
	DMPIQAFLLYQEPVLGPVR (DMP)	Wine, Poultry meat products (sausages, frankfurters, patés), Cookie	Cereda et al., 2010, Chen, et al., 2015, Monaci et al., 2013, Monaci et al., 2010a, Tolin et al., 2012a, Montowska & Fornal 2018, Hong et al., 2012, De Angelis et al., 2017b.
Bos d 12 – κ-Casein	YIPIQYVLSR (YIP)	Wine, Chocolate, Poultry meat products (sausages, frankfurters, patés), Cookie, Soy-based infant formula, Breakfast cereals, Baby food	Cereda et al., 2010, Gomaa & Boye, 2015, Lutter et al., 2011, Monaci et al., 2010a, Gu et al., 2018.
	SPAQILQWQVLSNTVPAK (SPA)	Wine, Chocolate, Poultry meat products (sausages, frankfurters, patés)	Cereda et al., 2010, Monaci et al., 2010a, Gu et al., 2018, Montowska & Fornal 2018, Qi et al., 2019 .
Bos d 5 – β-Lactoglobulin	TPEVDDEALEK (TPE)	Wine, Cereal bar, Muffin, Cookie, Pies, Waffles, Bread, Cookie dough, Salad dressing, Infant formula, Chocolate, Ice cream, Breakfast cereal, Soy-based infant formula, Baby food, Wheat Proteins, Chocolate	Lutter et al., 2011, Monaci et al., 2014, Parker et al., 2015, Yang et al., 2014, Boo et al., 2018, Cucu et al., 2012a, New et al., 2018, Ji et al., 2017, Pilolli et al., 2018.
	VLVLDTDYK (VLV)	Cereal bar, Muffin, Cookie, Chocolate, Ice cream, Tomato sauce, Breakfast cereals, Soy-based infant formula, Baby food, Wheat Proteins, Compote, Ham, Jam, Spices, Mayonnaise	Figeys et al., 1996, Lutter et al., 2011, Parker et al., 2015, Planque et al., 2016, Planque et al., 2017b, Cucu et al., 2012a, Planque et al., 2019.
	VYVEELKPTPEGDLEILLQK (VYV)	Cookie, Chocolate, Ice cream, Tomato sauce, Poultry meat products (sausages, frankfurters, patés), Wheat Proteins	Figeys et al., 1996, Planque et al., 2016, Planque et al., 2017b, Cucu et al., 2012a Montowska & Fornal 2018.
	LSFNPTQLEEQCHI (N-terminal peptide) (LSF)	Cereal bar, Muffin, Cookie, Chocolate, Ice cream, Tomato sauce, Poultry meat products (sausages, frankfurters, patés), Infant formula, Wheat Proteins, Compote, Ham, Jam, Spices, Mayonnaise	Parker et al., 2015, Planque et al., 2016, Planque et al., 2017b, Boo et al., 2018, Montowska & Fornal 2018, Cucu et al., 2012a, Ke, et al., 2017, Planque et al., 2019.
Gal d 2 – Ovalbumin	GGLEPINFQTAADQAR (GGL)	Wine, Cereal bar, Muffin, Cookie, Chocolate, Ice cream, Tomato sauce, Bread, Cookie dough, Salad dressing, Infant formula, Breakfast cereal, Pasta, Compote, Ham, Jam, Spices, Mayonnaise	Azarnia et al., 2013, Mattarozzi et al., 2014, Monaci et al., 2013, Monaci et al., 2014, Parker et al., 2015, Pilolli et al., 2014, Pilolli et al., 2017a, Planque et al., 2016, Tolin et al., 2012b, Tolin et al., 2012a, Planque et al., 2017a, Planque et al., 2017b, Boo et al., 2018, Pilolli et al., 2017b, New et al., 2018, Pilolli et al., 2018, De Angelis et al., 2017b, Planque et al., 2019, Gavage et al., 2019.
	LTEWTSSNVMEER (LTE)	Wine, Cookie, Chocolate, Ice cream, Tomato sauce, Pasta, Compote, Ham, Jam, Spices, Mayonnaise	Azarnia et al., 2013, Monaci et al., 2013, Monaci et al., 2014, Nimata et al., 2017, Pilolli et al., 2014, Planque et al., 2016, Tolin et al., 2012b, Tolin et al., 2012a, Pilolli et al., 2017b, Planque et al., 2017b, Pilolli et al., 2018, De Angelis et al., 2017b, Planque et al., 2019.
	ELINSWVESQTNGIIR (ELI)	Bread, Wine, Cereal bar, Muffin, Cookie, Bread, Cookie dough, Salad dressing, Infant formula, Chocolate, Ice cream, Breakfast cereal, Pasta, Mayonnaise	Azarnia et al., 2013, Heick et al., 2011a, Heick et al., 2011b, Monaci et al., 2013, Parker et al., 2015, Tolin et al., 2012b, New et al., 2018, Tolin et al., 2012a, Pavón-Pérez et al., 2019 .
	HIATNAVLFFGR (HIA)	Bread, Wine, Cereal bar, Muffin, Pasta, Mayonnaise	Azarnia et al., 2013, Heick et al., 2011a, Heick et al., 2011b, Monaci et al., 2013, Parker et al., 2015, Tolin et al., 2012a, Gavage et al., 2019.

	ISQAVHAAHAEINEAGR (ISQ)	Wine, Cookie, Chocolate, Ice cream, Tomato sauce, Mayonnaise, Chicken meat, Pasta	Azarnia et al., 2013, Lee & Kim, 2010, Monaci et al., 2013, Planque et al., 2016, Tolin et al., 2012a, Planque et al., 2017b.
	YPILPEYLQCVK (YPI)	Wine, Bread, Cookie, Pasta	Azarnia et al., 2013, Heick et al., 2011a, Heick et al., 2011b, Monaci et al., 2013, Monaci et al., 2014, Pilolli et al., 2017a, Boo et al., 2018, Pilolli et al., 2017b, Pilolli et al., 2018.
	DILNQITKPNVYSFSLASR (DIL)	Wine, Bread, Pasta	Azarnia et al., 2013, Heick et al., 2011a, Heick et al., 2011b, Tolin et al., 2012a.
Gal d 4 – Lysozyme C	FESNFNTQATNR (FES)	Wine, Cereal bar, Muffin, Cookie, Poultry meat products (sausages, frankfurters, patés), Mayonnaise, Chocolate	Cryar et al., 2013, Monaci et al., 2013, Parker et al., 2015, Pilolli et al., 2014, Tolin et al., 2012a, Boo et al., 2018, Montowska & Fornal 2018, Gavage et al., 2019.
	NTDGSTDYGILQINSR (NTD)	Wine, Cereal bar, Muffin, Poultry meat products (sausages, frankfurters, patés), Cookie, Mayonnaise	Lee & Kim, 2010, Monaci et al., 2013, Parker et al., 2015, Pilolli et al., 2014, Tolin et al., 2012a, Montowska & Fornal 2018, De Angelis et al., 2017b, Montowska & Fornal, 2019 .
	GTDVQAWIR (GTD)	Wine, Cookie, Poultry meat products (sausages, frankfurters, patés)	Cryar et al., 2013, Monaci et al., 2013, Boo et al., 2018, Montowska & Fornal 2018.
Gal d 3 – Qvotransferrin	GAIEWEGIESGSVEQAVAK (GAI)	Wine, Mayonnaise, Chocolate, Poultry meat products (sausages, frankfurters, patés)	D'Ambrosio et al., 2008, Tolin et al., 2012a, Gavage et al., 2019, Montowska & Fornal 2018, Montowska & Fornal, 2019 .
	SAGWNIPIGTLHR (SAG)	Wine, Mayonnaise, Chocolate, Poultry meat products (sausages, frankfurters, patés)	D'Ambrosio et al., 2008, Gavage et al., 2019, Montowska & Fornal 2018.
	YFGYTGALR (YFG)	Cookie, Bread, Cookie dough, Salad dressing, Wine, Infant formula, Chocolate, Ice cream, Breakfast cereal	New et al., 2018.
	ANVMDYR (ANV)	Cookie, Bread, Cookie dough, Salad dressing, Wine, Infant formula, Chocolate, Ice cream, Breakfast cereal	New et al., 2018.
	VEDIWSFLSK (VED)	Mayonnaise, Chocolate	Gavage et al., 2019.
Vitellogenin-1	YLLDLLPAAASHR (lipovitellin-1 chain) (YLL)	Cookie, Chocolate, Ice cream, Tomato sauce, Bread, Cookie dough, Salad dressing, Wine, Infant formula, Breakfast cereal, Compote, Ham, Jam, Spices, Mayonnaise	Planque et al., 2016, Planque et al., 2017b, New et al., 2018, Planque et al., 2019.
	ALLLSEIR (lipovitellin-1 chain) (ALL)	Cookie, Bread, Cookie dough, Salad dressing, Wine, Infant formula, Chocolate, Ice cream, Breakfast cereal	New et al., 2018.
	TVIVEAPIHGLK (YGP42) (TVI)	Mayonnaise, Chocolate	Gavage et al., 2019.
	NVNFEDGEILK (YGP42) (NVN)	Mayonnaise, Chocolate	Gavage et al., 2019.
	ATAVSLLEWQR (YGP42) (ATA)	Mayonnaise, Chocolate	Gavage et al., 2019.
	EALQPIHDLADEAISR (lipovitellin-1 chain) (EAL)	Cookie, Chocolate, Ice cream, Tomato sauce, Compote, Ham, Jam, Spices, Mayonnaise	Planque et al., 2016, Planque et al., 2017b, Planque et al., 2019.
Vitellogenin-2	NIPFAEYPTYK (lipovitellin-1 chain) (NIP)	Cookie, Chocolate, Ice cream, Tomato sauce, Compote, Ham, Jam, Spices, Mayonnaise	Planque et al., 2016, Planque et al., 2017b, Planque et al., 2019.

	NIGELGVEK (lipovitellin-1 chain) (NIG)	Cookie, Chocolate, Ice cream, Tomato sauce, Bread, Cookie dough, Salad dressing, Wine, Infant formula, Breakfast cereal	Planque et al., 2016, Planque et al., 2017b New et al., 2018.
	LPLSLPVGPR (lipovitellin-2 chain) (LPL)	Cookie, Bread, Cookie dough, Salad dressing, Wine, Infant formula, Chocolate, Ice cream, Breakfast cereal	New et al., 2018.
Apolipoprotein B	HLFLPSSYK (HLF)	Mayonnaise, Chocolate	Gavage et al., 2019.
	ASFYGLSHAVTK (ASF)	Mayonnaise, Chocolate	Gavage et al., 2019.
	ALFDYFGYSHDGK (ALF)	Mayonnaise, Chocolate	Gavage et al., 2019.
	FALGIVTPGAK (FAL)	Mayonnaise, Chocolate	Gavage et al., 2019.
	TEEIPLIENR (TEE)	Mayonnaise, Chocolate	Gavage et al., 2019.
	NFLINETAR (NFL)	Mayonnaise, Chocolate	Gavage et al., 2019.
Ara h 1 – Cupin (Vicillin- Type, 7S Globulin)	DLAFPGSGEQVEK (DLA)	Bread, Cookie, Cookie dough, Salad dressing, Wine, Infant formula, Chocolate, Ice cream, Breakfast cereal, Chocolate dessert	Chassaigne et al., 2007, Chassaigne et al., 2009, Heick et al., 2011a, Heick et al., 2011b, Pedreschi et al., 2012, Sayers et al., 2016, Sayers et al., 2018, Shefcheck, et al., 2006, Garber et al., 2016, New et al., 2018, Zhang et al., 2019.
	VLLEENAGGEQEER (VLL)	Cookie, Bread, Cookie dough, Salad dressing, Wine, Infant formula, Chocolate, Ice cream, Breakfast cereal, Chocolate dessert, Multigrain Bread	Chassaigne et al., 2009, Pedreschi et al., 2012, Sayers et al., 2016, Pilolli et al., 2017a, Sayers et al., 2018, Shefcheck, et al., 2006, Garber et al., 2016, Pilolli et al., 2018, New et al., 2018, Chassaigne et al., 2007, Korte et al., 2016a.
	GTGNLELVAVR (GTG)	Bread, Cereal bar, Muffin, Cookie, Chocolate, Cake, Pretzel, Crackers, Crisp, Bars, Cereal flours, Candies, Pasta, Instant Meal, Soups, Puree	Chassaigne et al., 2009, Heick et al., 2011a, Heick et al., 2011b, Parker et al., 2015, Sealey-Voyksner et al., 2016, Gu et al., 2018, Boo et al., 2018, Pilolli et al., 2018, Garber et al., 2016.
	SFNLDEGHALR (SFN)	Chocolate, Muffin, Cookie, Cake, Pretzel, Crackers, Crisp, Bars, Cereal flours, Candies, Pasta, Instant Meal, Soups, Puree, Ice cream	Chassaigne et al., 2009, Sealey-Voyksner et al., 2016, Shefcheck & Musser, 2004, Gu et al., 2018.
	NNPFYFPSR (NNP)	Cereal bar, Muffin, Chocolate, Cookie, Ice cream	Hebling et al., 2013, Parker et al., 2015, Shefcheck & Musser, 2004, Gu et al., 2018, Boo et al., 2018, Garber et al., 2016.
	NTLEAAFNAEFNEIR (NTL)	Cookie, Chocolate, Ice cream, Tomato sauce	Chassaigne et al., 2009, Planque et al., 2016, Shefcheck & Musser, 2004.
	AMVIVVVK (AMV)	Cookie	Chassaigne et al., 2009, Pilolli et al., 2018.
	CCNELNEFENNQR (CCN)	Cereal bar, Muffin, Cookie, Chocolate dessert, Chocolate bar, Chocolate based snacks, Rice crispy based snacks	Careri et al., 2007, Careri et al., 2008a, Hebling et al., 2013, Hebling et al., 2012, Parker et al., 2015, Pedreschi et al., 2012, Sayers et al., 2016, Sayers et al., 2018, Boo et al., 2018, Garber et al., 2016.
Ara h 2 – Conglutin Albumin) (2S	NLPQQGLR (NLP)	Cereal bar, Muffin, Cookie, Chocolate, Ice cream, Tomato sauce, Chocolate dessert, Chili pepper, Compote, Ham, Jam, Spices, Mayonnaise	Hebling et al., 2012, Parker et al., 2015, Pedreschi et al., 2012, Sayers et al., 2016, Sayers et al., 2018, Vandekerckhove et al., 2017, Planque et al., 2017b, Boo et al., 2018, Garber et al., 2016, Planque et al., 2019, Zhang et al., 2019.
	CMCEALQQIMENQSDR (CMC)	Cereal bar, Muffin, Cookie, Cumin, Chocolate based snacks, Rice crispy based snacks	Apostolovic et al., 2013, Careri et al., 2007, Careri et al., 2008a, Hebling et al., 2012, Parker et al., 2015, Garber et al., 2016, Pedreschi et al., 2012.
	CDLEVESGGR (CDL)	Cookie, Cumin	Hebling et al., 2012, Pedreschi et al., 2012, Garber et al., 2016, Zhang et al., 2019.

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Ara h 3 – Cupin (Legumin-Type, Globulin, Glycinin) 11S	SPDIYNPOAGSLK (SPD)	Cereal bar, Muffin, Chocolate, Cookie, Cumin, Chocolate based snacks, Rice crispy based snacks, Breakfast Cereal	Bignardi et al., 2010, Bignardi et al., 2013, Careri et al., 2007, Careri et al., 2008b, Careri et al., 2008a, Chassaigne et al., 2007, Parker et al., 2015, Pedreschi et al., 2012, Stevenson et al., 2009, Sayers et al., 2016, Gu et al., 2018, Boo et al., 2018, Garber et al., 2016.
	RPFYSNAPQEIFIQQGR (RPF)	Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Cookie dough, Salad dressing, Wine, Infant formula, Breakfast cereal, Chili pepper, Compote, Ham, Jam, Spices, Mayonnaise	Heick et al., 2011a, Heick et al., 2011b, Planque et al., 2016, Stevenson et al., 2009, Vandekerckhove et al., 2017, Planque et al., 2017b, Gu et al., 2018, New et al., 2018, Planque et al., 2019.
	FNLAGNHEQEFLR (FNL)	Cereal bar, Muffin, Cookie, Chocolate, Ice cream, Tomato sauce, Cumin, Multigrain Bread, Ice cream	Bignardi et al., 2013, Parker et al., 2015, Planque et al., 2016, Stevenson et al., 2009, Korte and Brockmeyer, 2016b, Planque et al., 2017b, Gu et al., 2018, Boo et al., 2018, Garber et al., 2016, Korte et al., 2019.
	LNAQRPDNR (LNA)	Cookie, Chili pepper	Chassaigne et al., 2009, Pedreschi et al., 2012, Vandekerckhove et al., 2017.
	WLGLSAEYGNLYR (WLG)	Bread, Cereal bar, Muffin, Cookie, Cookie dough, Salad dressing, Wine, Infant formula, Chocolate, Ice cream, Breakfast cereal, Cumin, Multigrain Bread	Heick et al., 2011a, Heick et al., 2011b, Parker et al., 2015, Stevenson et al., 2009, Korte & Brockmeyer, 2016b, New et al., 2018, Garber et al., 2016, Korte et al., 2016a, Korte et al., 2019.
	AHVQVVD ~~SN~~ GNR (AHV)	Cookie, Muffin, Cookie, Cake, Pretzel, Crackers, Crisp, Bars, Cereal flours, Candies, Pasta, Instant Meal, Soups, Puree	Pedreschi et al., 2012, Sealey-Voyksner et al., 2016, Stevenson et al., 2009.
	QQPEENACQFQR (QQP)	Chocolate dessert, Chocolate bar	Sayers et al., 2018, Stevenson et al., 2009, Sayers et al., 2016.
	TANDLNLILR (TAND)	Cumin, Multigrain Bread, Ice cream, Chocolate, Muffin, Cookie, Cake, Pretzel, Crackers, Crisp, Bars, Cereal flours, Candies, Pasta, Instant Meal, Soups, Puree	Sealey-Voyksner et al., 2016, Stevenson et al., 2009, Korte & Brockmeyer, 2016b, Garber et al., 2016, Korte et al., 2019.
	TANELNLLILR (TANE)	Cookie, Chocolate, Ice cream, Tomato sauce, Compote, Ham, Jam, Spices, Mayonnaise	Planque et al., 2017a, Planque et al., 2017b, Planque et al., 2019.
Ara h 6 – Conglutin Albumin) (2S)	CDLDVSGGR (CDL)	Chocolate dessert, Chocolate bar	Sayers et al., 2016, Sayers et al., 2018.
Ara h 7 – Conglutin Albumin) (2S)	NLPQNCGR (NLP)	Chocolate dessert, Chocolate bar	Sayers et al., 2016, Sayers et al., 2018.
Gly m 5 – β-Conglycinin (Vicilin, Globulin) 7S	LITLAI PVNKPGR (LIT)	Cookie, Chocolate, Ice cream, Tomato sauce, Poultry meat products (sausages, frankfurters, patés), Pea, Rice, Wheat proteins	Agrawal et al., 2008, Han et al., 2013, Houston et al., 2011, Planque et al., 2016, Scholl et al., 2014, Planque et al., 2017b, Montowska & Fornal 2018, Chen et al., 2019.
	QQQEEQPLEVR (QQQ)	Chocolate, Meat, Pea, Rice, Wheat proteins	Agrawal et al., 2008, Han et al., 2013, Leitner et al., 2006, Scholl et al., 2014, Gu et al., 2018, Cucu et al., 2012c, Chen et al., 2019.
	TISSEDKPFNLR (TIS)	Chocolate, Cookie, Pea, Rice, Wheat proteins	Agrawal et al., 2008, Han et al., 2013, Monaci et al., 2014, Scholl et al., 2014, Gu et al., 2018, Cucu et al., 2012c.
	AIVILVINEGDANIELVGLK (AIV)	Cookie, Pea, Rice, Wheat proteins	Agrawal et al., 2008, Goma & Boye, 2015, Scholl et al., 2014.
	DSYNLQSGDALR (DSYNLQ)	Chocolate, Pea, Rice, Wheat proteins	Agrawal et al., 2008, Han et al., 2013, Scholl et al., 2014, Gu et al., 2018.
	DSYNLHPGDAQR (DSYNLH)	Chocolate	Han et al., 2013, Gu et al., 2018.
Gly m 6 – Glycinin (Legumin, Globulin) 11S	VFDGELQEGR (VFD)	Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Meat, Pea, Rice, Wheat proteins, Wheat flour, Compote, Ham, Jam, Spices, Mayonnaise	Agrawal et al., 2008, Barbosa et al., 2012, Han et al., 2013, Heick et al., 2011b, Heick et al., 2011a, Huschek et al., 2016, Leitner et al., 2006, Scholl et al., 2014, Planque et al., 2017b, Cucu et al., 2012c, Planque et al., 2019, Chen et al., 2019.

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	VLIVPQN ~~F~~ VVAAR (VLI)	Chocolate, Poultry meat products (sausages, frankfurters, patés), Cookie, Meat, Pea, Rice, Wheat proteins	Agrawal et al., 2008, Gomaa & Boye, 2015, Han et al., 2013, Houston et al., 2011, Leitner et al., 2006, Scholl et al., 2014, Gu et al., 2018, Montowska & Fornal 2018, Cucu et al., 2012c, Montowska & Fornal, 2019 .
	LSAEFGSLR (LSA)	Chocolate, Poultry meat products (sausages, frankfurters, patés), Pea, Rice, Wheat proteins, Wheat flour, Cookie, Bread	Agrawal et al., 2008, Han et al., 2013, Houston et al., 2011, Huschek et al., 2016, Scholl et al., 2014, Gu et al., 2018, Montowska & Fornal 2018.
	EAFGVN ~~M~~ QIVR (EAF)	Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Poultry meat products (sausages, frankfurters, patés), Meat, Pea, Rice, Wheat proteins, Compote, Ham, Jam, Spices, Mayonnaise	Han et al., 2013, Heick et al., 2011a, Heick et al., 2011b, Leitner et al., 2006, Planque et al., 2016, Scholl et al., 2014, Planque et al., 2017a, Hoffmann, et al., 2017, Planque et al., 2017b, Montowska & Fornal 2018, Planque et al., 2019.
	SQSDNFEYVSFK (SQS)	Bread, Cookie, Chocolate, Poultry meat products (sausages, frankfurters, patés), Pea, Rice, Wheat proteins	Barbosa et al., 2012, Han et al., 2013, Heick et al., 2011a, Heick et al., 2011b, Pilolli et al., 2017a, Scholl et al., 2014, Gu et al., 2018, Montowska & Fornal 2018, Chen et al., 2019 .
	ISTLNSLTLPALR (IST)	Cookie, Chocolate, Ice cream, Tomato sauce, Poultry meat products (sausages, frankfurters, patés), Pea, Rice, Wheat proteins	Agrawal et al., 2008, Han et al., 2013, Planque et al., 2016, Scholl et al., 2014, Planque et al., 2017b, Montowska & Fornal 2018, Cucu et al., 2012c, Montowska & Fornal, 2019 , Chen et al., 2019 .
	LNALKPDNR (LNA)	Pea, Rice, Wheat proteins, Wheat flour, Cookie, Bread	Han et al., 2013, Huschek et al., 2016, Scholl et al., 2014, Cucu et al., 2012c.
	FYLAGNQEQEFLK (FYL)	Cookie, Poultry meat products (sausages, frankfurters, patés), Pea, Rice, Wheat proteins, Meat	Han et al., 2013, Pilolli et al., 2017a, Scholl et al., 2014, Hoffmann, et al., 2017, Montowska & Fornal 2018.
Gly m 8 – 2S Albumin	ELINLAT ~~M~~ CR (ELI)	Cookie, Chocolate, Ice cream, Tomato sauce, Meat, Compote, Ham, Jam, Spices, Mayonnaise	Leitner et al., 2006, Planque et al., 2016, Planque et al., 2017b, Planque et al., 2019.
Cor a 9 - 11S Seed Storage Globulin (Legumin-Like)	LNALPTNR (LNA)	Cookie, Chocolate, Ice cream, Tomato sauce, Multigrain Bread, Muffin, Cookie, Cake, Pretziel, Crackers, Crisp, Bars, Cereal flours, Candies, Pasta, Instant Meal, Soups, Puree	Sealey-Voyksner et al., 2016, Korte & Brockmeyer, 2016b, Planque et al., 2017b, Korte et al., 2016a, Korte et al., 2019 , -----
	INTVNSNTLPVLR (INT)	Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Cookie, Cookie dough, Salad dressing, Wine, Infant formula, Breakfast cereal, Multigrain Bread, Muffin, Cake, Pretziel, Crackers, Crisp, Bars, Cereal flours, Candies, Pasta, Instant Meal, Soups, Puree	Sealey-Voyksner et al., 2016, Cucu et al., 2012b, Heick et al., 2011a, Heick et al., 2011b, Ansari et al., 2012, Costa et al., 2014, Planque et al., 2017, New et al., 2018, Korte et al., 2016a.
	WLQLSAER** (WLQ)	Chocolate, Muffin, Cookie, Cake, Pretziel, Crackers, Crisp, Bars, Cereal flours, Candies, Pasta, Instant Meal, Soups, Puree	Sealey-Voyksner et al., 2016, Ansari et al., 2012, Costa et al., 2014, Gu et al., 2018, Cucu et al., 2012b.
	ALPDDVLANAFQISR (ALP)	Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Compote, Ham, Jam, Spices, Mayonnaise	Cucu et al., 2012b, Heick et al., 2011a, Heick et al., 2011b, Ansari et al., 2012, Costa et al., 2014, Pilolli et al., 2017a, Pilolli et al., 2018, Planque et al., 2017, Planque et al., 2019.
	VQVVDN ~~G~~ NTVFDDELRL (VQV)	Multigrain Bread, Ice cream, chocolate	Cucu et al., 2012b, Korte & Brockmeyer, 2016b, Korte et al., 2019 .
	ADIYTEQVGR (ADI)	Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Multigrain Bread, Ice cream, Compote, Ham, Jam, Spices, Mayonnaise	Heick et al., 2011a, Heick et al., 2011b, Bignardi et al., 2010, Bignardi et al., 2013, Pilolli et al., 2017a, Planque et al., 2017, Gu et al., 2018, Pilolli et al., 2018, New et al., 2018, Korte et al., 2016a, Planque et al., 2019.
	TNDNAQISPLAGR (TND)	Cookie, Chocolate, Ice cream, Tomato sauce	Planque et al., 2017, Pilolli et al., 2018.
	QGQVLTIPQNFAVAK (QGQ)	Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Multigrain Bread, Compote, Ham, Jam, Spices, Mayonnaise	Heick et al., 2011a, Heick et al., 2011b, Ansari et al., 2012, Costa et al., 2014, Korte & Brockmeyer, 2016b, Pilolli et al., 2018, Planque et al., 2017, Korte et al., 2016a, Planque et al., 2019, Korte et al., 2019 .
Cor a 11 - 7S Seed Storage Globulin (Vicilin-	LLSGIENFR (LLS)	Cookie, Bread, Dough, Salad dressing, Wine, Infant formula, Chocolate, Ice cream, Breakfast cereal, Muffin, Cake, Pretziel, Crackers, Crisp, Bars, Cereal	Sealey-Voyksner et al., 2016, Ansari et al., 2012, Costa et al., 2014, Cucu et al., 2012b, New et al., 2018.

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Like)		flours, Candies, Pasta, Instant Meal, Soups, Puree	
	AFSWEVLEAALK (AFS)	Cookie, Bread, Dough, Salad dressing, Wine, Infant formula, Chocolate, Ice cream, Breakfast cereal	Ansari et al., 2012, Costa et al., 2014, Cucu et al., 2012b, New et al., 2018.
	ELAFNLPSR (ELA)	Chocolate	Ansari et al., 2012, Costa et al., 2014, Cucu et al., 2012b.
Cor a 8 – nsLTP 1	GIAGLNPNLAAGLPGK (GIA)	Chocolate	Ansari et al., 2012, Costa et al., 2014.
Pru du 6 – Amandin, Globulin Legumin-Like Protein	ALPDEVLANAYQISR** (ALP)	Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Almond milk, Orgeat syrup, Compote, Ham, Jam, Spices, Mayonnaise	Fasoli, et al., 2011, Heick et al., 2011a, Heick et al., 2011b, Inman, et al., 2018, Planque et al., 2017, Gu et al., 2018, Planque et al., 2019.
	GNLDFVQPPR** (GNL)	Bread, Cookie, Chocolate, Ice cream, Tomato sauce, Multigrain Bread, Ice cream, chocolate, Almond milk, Orgeat syrup, Compote, Ham, Jam, Spices, Mayonnaise	Fasoli, et al., 2011, Heick et al., 2011a, Heick et al., 2011b, Inman, et al., 2018, Planque et al., 2017, Gu et al., 2018, Korte & Brockmeyer, 2016b, Garber et al., 2016, Korte et al., 2016a, Planque et al., 2019, Korte et al., 2019 .
	QQEQLQQR** (QQE)	Chocolate, Cumin, Cookie, Almond milk, Orgeat syrup	Bignardi et al., 2010, Bignardi et al., 2013, Fasoli, et al., 2011, Gu et al., 2018, Garber et al., 2016.
	QQGQQEQQQR (QQG)	Cookie	Bignardi et al., 2010.
	QETIALSSSQQR** (QET)	Cookie, Chocolate, Ice cream, Tomato sauce, Almond milk, orgeat syrup	Fasoli, et al., 2011, Planque et al., 2017.
	VQGQLDFVSPFSR** (VQG)	Multigrain Bread, Ice cream, chocolate, Almond milk, Orgeat syrup	Fasoli, et al., 2011, Korte & Brockmeyer, 2016b, Korte et al., 2019 .
	ALPDEVLQNAFR** (ALP)	Cumin, Multigrain Bread, Ice cream, chocolate, Almond milk, Orgeat syrup	Fasoli, et al., 2011, Korte & Brockmeyer, 2016b, Garber et al., 2016, Korte et al., 2016a, Korte et al., 2019 .
	VQVVNE ~~NG~~ DPILDDEV (VQV)	Cumin, Almond milk, Orgeat syrup	Fasoli, et al., 2011, Garber et al., 2016.
	ADFYNPQGGR** (ADF)	Chocolate, Almond milk, Orgeat syrup	Fasoli, et al., 2011, Gu et al., 2018.
	LLSATSPPR** (LLS)	Chocolate, Multigrain Bread, Ice cream, chocolate, Almond milk, Orgeat syrup	Fasoli, et al., 2011, Gu et al., 2018, Korte et al., 2016a.
	TDEN ~~NG~~ FTNTLAGR (TDE)	Cookie, Chocolate, Ice cream, Tomato sauce, Multigrain Bread, Compote, Ham, Jam, Spices, Mayonnaise	Planque et al., 2017, Korte et al., 2016a, Planque et al., 2019.

33

34

35 **Table S2.** Summary of the plant-derived peptide markers collected from the literature, which might experience detection bias due to
 36 similar sequences from homologous proteins. *peptides which present also full matches (100% identity) with other plant species.

Target Allergenic Protein	Peptide Marker		Interfering peptide			
	Sequence	Precursor Ion (z)	Sequence	Precursor Ion (z)	Taxonomy	Shared Transitions
Cor a 9 11S Seed Storage Globulin	LNALEPTNR	514,2802 (+2)	LD <u>A</u> LEPTNR	514,7722 (+2)	Juglans regia Carya illinoensis Eucalyptus grandis	from y ₁ ⁺ to y ₇ ⁺
Pru du 6 Amandin	ALPDEVLANAYQISR*	830,4387 (+2)	ALPDEVIANAYQISR	830,4387 (+2)	Punica granatum	All
	VQVVNENGDPILDDEV	955,9763 (+2)	VQVVNENGDPIL <u>N</u> DEV	955,4843 (+2)	Prunus persica	from y ₁ ⁺ to y ₄ ⁺ from b ₂ ⁺ to b ₁₂ ⁺ from a ₂ ⁺ to a ₁₂ ⁺
	NLQGQDDNR	530.2443 (+2)	NLQGQ <u>N</u> DNR	529.7523 (+2)	Prunus Persica Prunus Avium Prunus Mume	from y ₁ ⁺ to y ₃ ⁺ from b ₂ ⁺ to b ₅ ⁺ from a ₂ ⁺ to a ₅ ⁺
Gly m 5 β-Conglycinin	TISSDKPFNLR*	703,8673 (+2)	TISSQDKPFNLR	703,3753 (+2)	Cajanus cajan	from y ₁ ⁺ to y ₇ ⁺ from b ₂ ⁺ to b ₄ ⁺ from a ₂ ⁺ to a ₄ ⁺
Gly m 6 Glycinin	LSAEFGSLR	490,264 (+2)	LSA <u>Q</u> FGSLR	489,7720 (+2)	Cajanus cajan	from y ₁ ⁺ to y ₅ ⁺ from b ₂ ⁺ to b ₃ ⁺ from a ₂ ⁺ to a ₃ ⁺
	LNALKPDNR	520,796 (+2)	LNALE <u>P</u> DNR	521,2698 (+2)	Lupinus angustifolius Pisum sativum	from y ₁ ⁺ to y ₄ ⁺ from b ₂ ⁺ to b ₄ ⁺ from a ₂ ⁺ to a ₄ ⁺

39 **Figure S1.** Full-length sequence alignment for known isoforms of the allergenic protein Ara h 1
 40 accomplished by means of the Clustal Omega Program provided by Uniprot
 41 (<https://www.uniprot.org/align/>). The signal peptide was highlighted in pink and the asparagine residue
 42 involved in the post-translational glycosylation was highlighted in orange. The tryptic peptide markers
 43 collected from the literature were displayed with red squares.

Ara h 1.0101 (clone P41B) →	P43238	ALL12_ARAHY	1	MRGRVSPFLMLLLGILVLASVSATHAKSSPYQKKTENPCAQRCLQSCQQEPDDLKQKACES	60
Ara h 1.01 (clone P17) →	P43237	ALL11_ARAHY	1	MRGRVSPFLMLLLGILVLASVSATQAKS-PY-RKTENPCAQRCLQSCQQEPDDLKQKACES	58
				*****:*** ** :*****	
P43238	ALL12_ARAHY		61	RCTKLEYDPRCVYDPRGHTGTTNQRSPPGERTRGRQPGDYDDRRQPRREEGGRWGPAFP	120
P43237	ALL11_ARAHY		59	RCTKLEYDPRCVYD----TGATNQRHPGERTRGRQPGDYDDRRQPRREEGGRWGPAEP	114

P43238	ALL12_ARAHY		121	REREREEDWRQPRDWRPFSHQQPKIRPEGREGQEWGTPGSHVREETSNNPFYFPSR	180
P43237	ALL11_ARAHY		115	REREREEDWRQPRDWRPFSHQQPKIRPEGREGQEWGTPGSEVREETSNNPFYFPSR	174

P43238	ALL12_ARAHY		181	RFSTRYGNQNGRIRVLQRFQRSRQFQNLQNHRIVQIEAKPNTLVLPKHADADNILVIQQ	240
P43237	ALL11_ARAHY		175	RFSTRYGNQNGRIRVLQRFQRSRQFQNLQNHRIVQIEARPNTLVLPKHADADNILVIQQ	234

P43238	ALL12_ARAHY		241	GQATVTVANGNNRPSFNLDEGHALRPSGFISYILNRHDMQNLRVAKISMVNTFPGQFED	300
P43237	ALL11_ARAHY		235	GQATVTVANGNNRPSFNLDEGHALRPSGFISYILNRHDMQNLRVAKISMVNTFPGQFED	294

P43238	ALL12_ARAHY		301	FFPASSRDQSSYLQGFSTNLEAAFAFNEIRVLEENAGGEQEESSQRRWSTRSEN	360
P43237	ALL11_ARAHY		295	FFPASSRDQSSYLQGFSTNLEAAFAFNEIRVLEENAGGEQEESSQRRRSTRSS-D	353

P43238	ALL12_ARAHY		361	NEGVIVKVSKEHVEELTKHAKSVSKKGSEEGDITNFINLRGEPDLSNNFGKLFVFKPD	420
P43237	ALL11_ARAHY		354	NEGVIVKVSKEHVQELTKHAKSVSKKGSEE-EDITNFINLRGEPDLSNNFGRLFEVKPD	412

P43238	ALL12_ARAHY		421	KKNPQLQDLDMMLTCVEIKEGALMLPHFNSHAMVIVVNVHSTGNLELVAVRKEQQQRGR	480
P43237	ALL11_ARAHY		413	KKNPQLQDLDMMLTCVEIKEGALMLPHFNSHAMVIVVNVHSTGNLELVAVRKEQQQRGR	472

P43238	ALL12_ARAHY		481	EEE---EDEDEEEGSNREVRRYTARLKEGDVVFIMFAAHFVAIASSELHLLGFGINAEN	537
P43237	ALL11_ARAHY		473	EQEWEEEEEEEGSNREVRRYTARLKEGDVVFIMFAAHFVAIASSELHLLGFGINAEN	532

P43238	ALL12_ARAHY		538	NHRIFLAGDKDNVIDQIEKQAMDLAFFPGSGEQVERLIKQKESHFVSARPQSQSQSPSSP	597
P43237	ALL11_ARAHY		533	NHRIFLAGDKDNVIDQIEKQAMDLAFFPGSGEQVERLIKQKESHFVSARPQSQSQSP----	587

P43238	ALL12_ARAHY		598	EKESPEKEDQEEENQGGKGFLLSILKAFN	626
P43237	ALL11_ARAHY		588	--SPEKEDQEEENQGGKGFLLSILKAFN	614

46 **Figure S2.** Full-length sequence alignment for known isoforms of the allergenic protein Ara h 2
47 accomplished by means of the Clustal Omega Program provided by Uniprot
48 (<https://www.uniprot.org/align/>). The signal peptide was highlighted in pink, the proline residues
49 involved in the post-translational hydroxylation was highlighted in grey, the cysteine residues involved
50 in four disulfide bridges were highlighted in green. The tryptic peptide markers collected from the
51 literature were displayed with red squares.



60 **Figure S4.** Full-length sequence alignment for known isoforms of the allergenic protein Gly m 5
61 accomplished by means of the Clustal Omega Program provided by Uniprot
62 (<https://www.uniprot.org/align/>). The signal peptide was highlighted in pink and the asparagine residue
63 involved in the post-translational glycosylation was highlighted in orange. The tryptic peptide markers
64 collected from the literature were displayed with red squares; in addition, we also marked all the
65 potential trypsin specific cleavage sites, looking for further sequences conserved among isoforms.

Gly m 5.0101	→ O22120 O22120_SOYBN	1	-VEKEECCEEGEIPRPRPQHPEREPOQGEKEEDEQPRPIFFRPQ-PRQEEHEQR	58
Gly m 5.0201	→ Q9F2P9 Q9F2P9_SOYBN	1	VEEEECCEEGQIPRP--RQHPERERQHQGEKEEDEQPRPIFFRPQPHQEEHEQR	58
Gly m 5.0301	→ P25974 GLCB_SOYBN	1	-----	0
Gly m 5.0302	→ Glym5.0302	1	-----	0
O22120 O22120_SOYBN	59	EEQEWPRKEEKRGKSGSEEDDEDEEQDERQFPFPPPHQKEERKQE-----	106	
Q9F2P9 Q9F2P9_SOYBN	59	EEHEWHRKEEKHGGKGSEEEQ-----DEREHPRHPHQKEEKHEWQHQRKHQKQE	111	
P25974 GLCB_SOYBN	1	-----MMRVRFPLL	9	
Glym5.0302	1	-----MMRVRFPLL	9	
O22120 O22120_SOYBN	107	-----EDEDEFQQRSEES---EDSELRLHNNPFLF-GSNHFTLFTFNOYGRIRV	154	
Q9F2P9 Q9F2P9_SOYBN	112	SEEEEDQDEDEEQDKESQESGESQEPREPHNNPFFHF-NSHFFOTLFTFNOYGHVRV	170	
P25974 GLCB_SOYBN	10	-----VLLGTVFLASVCVSLKVRDEDNNPFYFSSNSFOTLFENQVRIRL	55	
Glym5.0302	10	-----VLLGTVFLASVCVSLKVRDEDNNPFYFSSNSFOTLFENQVRIRL	55	
O22120 O22120_SOYBN	155	LQRFNHRSPQLQNLFDYRILEFNSKPNLTLPPHADADYLVILNGTAILSLVNNDDEDS	214	
Q9F2P9 Q9F2P9_SOYBN	171	LQRFNHRSPQLQNLFDYRILEFNSKPNLTLPPHADADYLVILNGTAILTLVNNDDEDS	230	
P25974 GLCB_SOYBN	56	LQRFNHRSPQLENLFDYRIVQFQSKPNTILLPHADADFLFVLSGAILTLVNNDDEDS	115	
Glym5.0302	56	LQRFNHRSPQLENLFDYRIVQFQSKPNTILLPHADADFLFVLSGAILTLVNNDDEDS	115	
O22120 O22120_SOYBN	215	YNLQSGDALVPAGTYYVYNPDNENLILITLAI PVNKPGRFESFFLSSTEAQQSYLQ	274	
Q9F2P9 Q9F2P9_SOYBN	231	YNLQSGDALVPAGTYYVYNPDNENLILITLAI PVNKPGRFESFFLSSTEAQQSYLQ	290	
P25974 GLCB_SOYBN	116	YNLHPGDAQIPAGTYYVYNPDHQNLIILITLAI PVNKPGRYDDFFLSSTEAQQSYLQ	175	
Glym5.0302	116	YNLHPGDAQIPAGTYYVYNPDHQNLIILITLAI PVNKPGRYDDFFLSSTEAQQSYLQ	175	
O22120 O22120_SOYBN	275	FSHNILEASYDTFFEEINNVLFSGEEGQQQGEORLOESVIVEISFQIRALSHRAKSSSR	334	
Q9F2P9 Q9F2P9_SOYBN	291	FSHNILEASYDTFFEEINNVLFSGEEGQQQGEORLOESVIVEISFQIRALSHRAKSSSR	350	
P25974 GLCB_SOYBN	176	FSHNILETSFHFSEFEEINNVLFGEEEQ-----RQEGVIVELSHQIRQLSHRAKSSSR	230	
Glym5.0302	176	FSHNILETSFHFSEFEEINNVLFGEEEQ-----RQEGVIVELSHQIRQLSHRAKSSSR	230	
O22120 O22120_SOYBN	335	FTISSEDRPFNLRSNPDIYSNHLGKFFEITPEHPNQLDLDFLSVDMNEGALLLPHFN	394	
Q9F2P9 Q9F2P9_SOYBN	351	FTISSEDRPFNLRSNPDIYSNHLGKFFEITPEHPNQLDLDFLSVDMNEGALLLPHFN	410	
P25974 GLCB_SOYBN	231	FTISSEDEPFNLRSNPDIYSNHLGKFFEITPEHPNQLDLDFLSVDMNEGALLLPHFN	290	
Glym5.0302	231	FTISSEDEPFNLRSNPDIYSNHLGKFFEITPEHPNQLDLDFLSVDMNEGALLLPHFN	290	
O22120 O22120_SOYBN	395	SHAIVILVINEGDANIELVGLRQQQEQQQEQPLEVRYRAELSEQDIFVIPAGYPVVV	454	
Q9F2P9 Q9F2P9_SOYBN	411	SHAIVILVINEGDANIELVGLRQQQEQQQEQPLEVRYRAELSEQDIFVIPAGYPVVV	470	
P25974 GLCB_SOYBN	291	SHAIVILVINEGDANIELVGLRQQQEQQQEQPLEVRYRAELSEDDVFVIPAAYPFVV	350	
Glym5.0302	291	SHAIVILVINEGDANIELVGLRQQQEQQQEQPLEVRYRAELSEDDVFVIPAAYPFVV	350	
O22120 O22120_SOYBN	455	NATSNLNFFAIGINAENNRNFLAGSDNVISQIPSOVQELAFPGSAQDVERLLHNCES	514	
Q9F2P9 Q9F2P9_SOYBN	471	NATSDLNFFAIGINAENNRNFLAGSDNVISQIPSOVQELAFPGSAQDVERLLHNCES	530	
P25974 GLCB_SOYBN	351	NATSNLNFLAFGINAENNRNFLAGSDNVVRIEIQVQELAFPGSAQDVERLLHNCES	410	
Glym5.0302	351	NATSNLNFLAFGINAENNRNFLAGSDNVVRIEIQVQELAFPGSAQDVERLLHNCES	410	
O22120 O22120_SOYBN	515	YFVDAQPKHHEEGNKGKRGPLSSILAFY	543	
Q9F2P9 Q9F2P9_SOYBN	531	YFVDAQPOOHEEGNKGKRGPLSSILAFY	559	
P25974 GLCB_SOYBN	411	YFVDAQPOOHEEGSKRGKGPFPFSGILGALY	439	
Glym5.0302	411	YFVDAQPOOHEEGSKRGKGPFPFSGILGALY	439	

68 **Figure S5.** Full-length sequence alignment for known isoforms of the allergenic protein Gly m 6
69 accomplished by means of the Clustal Omega Program provided by Uniprot
70 (<https://www.uniprot.org/align/>). The signal peptide was highlighted in pink, the cysteine residues
71 involved in two disulfide bridges were highlighted in green, and two short propeptides were highlighted
72 with a red shadow. The tryptic peptide markers collected from the literature were displayed with red
73 squares; in addition, we also marked all the potential trypsin specific cleavage sites, looking for further
74 sequences conserved among isoforms.



76 **Figure S6.** Full-length sequence alignment for known isoforms of the allergenic protein Cor a 9
 77 accomplished by means of the Clustal Omega Program provided by Uniprot
 78 (<https://www.uniprot.org/align/>). The signal peptide was highlighted in pink. The tryptic peptide markers
 79 collected from the literature were displayed with red squares.

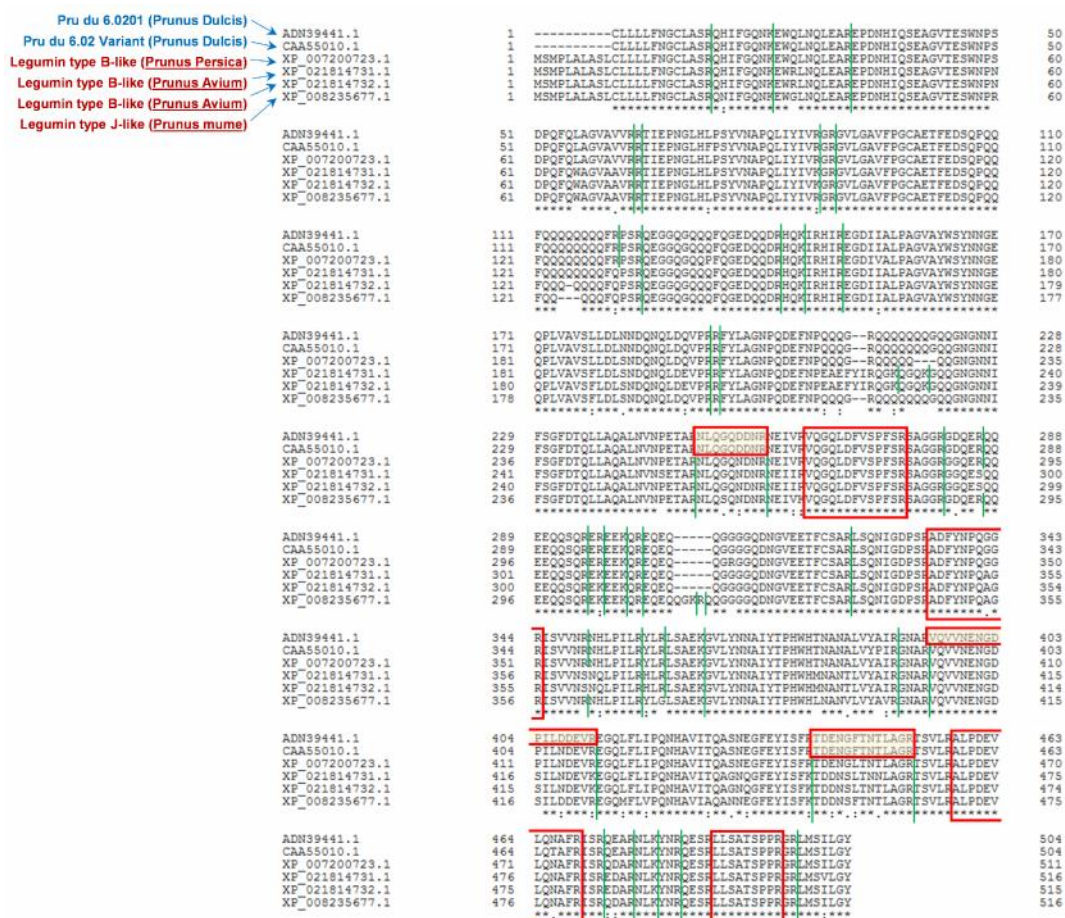
Cor a 9.0101 →	Q8W1C2	Q8W1C2 CORAV	1	MAKLILVFSFSLCLLVLFNGCLGINVGLRRQQQRYFGECNLDELNALEPTNRTEAEACQIE	60
Cor a 9.01 Variant →	A0A0A0P7E3	A0A0A0P7E3_CORAV	1	MAKLILVFSFSLCLLVLFNGCLGIDVGLRRQQQRHFGECNLDELNALEPTNRTEAEAGQIE	60
				*****;*****;*****	
	Q8W1C2	Q8W1C2 CORAV	61	SWDHNDQQFQCAGVAVIRRTIEFNGLLLPQYSNAPELIYIERGRGITGVLFPGCPETFED	120
	A0A0A0P7E3	A0A0A0P7E3_CORAV	61	SWDHNDQQFQCAGVAVIRRTIEFNGLLLPQYSNAPELIYIERGRGITGVLFPGCPETFED	120

	Q8W1C2	Q8W1C2 CORAV	121	PQQSQSQGQRQGGQSQSRSEQDRHQKIRHFREGDIIALPAGVAHWCYNDGDSPVVTVSLL	180
	A0A0A0P7E3	A0A0A0P7E3_CORAV	121	PQQSQSQGQ--GQGQSRSEQDRHQKIRYFQEGDIIALPAGVAHWCYNDGDSPVVAVSLL	178

	Q8W1C2	Q8W1C2 CORAV	181	HTNNYANQLDENPRHFYLAGNPDDQHQRGGQQQFGQRRRQQQSHSGEQGEQEGGEGNNV	240
	A0A0A0P7E3	A0A0A0P7E3_CORAV	179	HTNNYANQLDENPRHFYLAGNPDDQHQRGGQQQFGQRRRQQQSHSGEQGEQEGGEGNNV	238
				*****;*****;*****	
	Q8W1C2	Q8W1C2 CORAV	241	FSGFDAEFLADAFNVDDTARRLQSNQDKRRNIVKVEGRQVVRPERSRQEWERQERQER	300
	A0A0A0P7E3	A0A0A0P7E3_CORAV	239	FSGFDAEFLADAFNVDDTARRLQSNQDKRRNIVKVEGRQVVRPERSRQEWERQERQER	298
				*****;*****;*****	
	Q8W1C2	Q8W1C2 CORAV	301	ESEQERERQRRQGGGRDVGNGFEETICSLRLRENICTRSHADIYTEQVGRINTVNSNTLP	360
	A0A0A0P7E3	A0A0A0P7E3_CORAV	299	ESEQERERQRRQGGGRDVGNGFEETICSLRLMENIGSRSHADIYTEQVGRINTVNSNTLP	358
				*****;*****;*****	
	Q8W1C2	Q8W1C2 CORAV	361	VLRWLQLSAERGDQREGLYVPHWNLNAHSVVYAIRGRAHVQVVDNGNTVFDELFQGG	420
	A0A0A0P7E3	A0A0A0P7E3_CORAV	359	VLRWLQLSAERGDQREGLYVPHWNLNAHSVVYAIRGRAHVQVVDNGNTVFDELFQGG	418
				*****;*****;*****	
	Q8W1C2	Q8W1C2 CORAV	421	VLTIPQNFVAHRAESEGFENVAFHTNDNAQISPLAGRTSAIRALPDDVLANAFQISRRE	480
	A0A0A0P7E3	A0A0A0P7E3_CORAV	419	VLTIPQNFVAHRAESEGFENVAFHTNDNAQISPLAGRTSAIRALPDDVLANAFQISRRE	478
				*****;*****;*****	
	Q8W1C2	Q8W1C2 CORAV	481	ARRLKYNRQETTLVRSSRSSSEKRR-RSESEGRAEA	515
	A0A0A0P7E3	A0A0A0P7E3_CORAV	479	ARRLKYNRQETTLARSSRSSSERMRRRSESEGRAEA	514
				*****;*****;*****	

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81

96 **Figure S9.** Full-length sequence alignment for specificity assessment of the protein Pru du 6.0101 and
 97 its variant, with homologous proteins from *Prunus persica*, *Prunus mume*, and *Prunus avium*
 98 taxonomies, accomplished by means of the Clustal Omega Program provided by Uniprot
 99 (<https://www.uniprot.org/align/>). The tryptic peptide markers collected from the literature were
 100 displayed with red squares; in addition, we also marked all the potential trypsin specific cleavage sites,
 101 looking for *Prunus dulcis* specific tryptic peptides.



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