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Rosa Pilolli, Chiara Nitride, Nathalie Gillard, Anne-Catherine Huet, Christof van Poucke, Marc de Loose, Olivier Tranquet, Colette Larre, Karine Adel-Patient, Hervé Bernard, et al.

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1 **CRITICAL REVIEW ON PROTEOTYPIC PEPTIDE MARKER**  
2 **TRACING FOR SIX ALLERGENIC INGREDIENTS IN INCURRED**  
3 **FOODS BY MASS SPECTROMETRY**

4

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22

## 23        **ABSTRACT**

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

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

40  
41    **Keywords:** food allergens, mass spectrometry, peptide markers, ThRAI, incurred foods.  
42

## 43        **1. Introduction**

44    IgE-mediated food allergies are steadily on the rise representing a huge health concern  
45    worldwide. Although firm prevalence data are lacking, there are extensive investigations  
46    suggesting that food allergies have increased and rates as high as approximately 10 % have  
47    been documented, with main prevalence in industrialized countries, and in children compared  
48    to adults (Scott et al. 2018). There are many manifestations of food allergy with different  
49    severities, and individual investigations rely on specific study populations, specific foods, and  
50    different methodologies, thus impairing the determination of undoubted statistics. Moreover,  
51    further constrains in obtaining solid prevalence data, arise from geographic variations, diet  
52    exposure effects, differences according to age, race, and ethnicity, and myriad other factors.  
53    Like all chronic disease, onset of food allergy is influenced by genetics, environment and  
54    genome-environment interactions including epigenetics effects. Its current management is  
55    based on allergen containing food avoidance. Food labelling legislative provisions are available  
56    in several countries and require a detailed declaration of allergen inclusion in foods, with  
57    number and types of priority allergens differing among countries and depending on specific  
58    dietary habits and allergy prevalence. On the other hand, a precautionary labeling system is  
59    also widespread, representing a safety measure put in place from industries to protect from any  
60    accidental cross-contamination likely to occur in foods expected to be allergen-free and that  
61    can take place at whatever stage along the food chain. All this raised the urgent need to develop  
62    reliable methodologies to trace allergens in foods with the highest confidence. Mass  
63    spectrometry (MS) techniques have been widely exploited in the last ten years to meet this goal  
64    and were specifically applied to food allergen detection, and to the food allergen identification  
65    and characterization, as well. Despite the need for expensive equipment and trained personnel,

66 the chance to provide multiplexing and unequivocal allergen identification accounts for the  
67 overall strength of the MS based approaches compared to previously established methods,  
68 such as immunoassays and DNA based methods. Nonetheless some gaps in the development  
69 of MS-based quantitative methods still need to be addressed. The review paper authored by  
70 Monaci et al., 2018 provides a comprehensive overview of the MS based methods developed  
71 so far for allergen detection and also highlights the need of harmonization in method  
72 development and validation.

73 In this frame, the European project ThRAI (Thresholds and Reference method for Allergen  
74 detection method) titled “Detection and quantification of allergens in foods and minimum  
75 eliciting doses in food allergic individuals”, funded by the European Food Safety Agency will  
76 actively contribute to the advancement in harmonization of MS-based method for food allergen  
77 detection by developing a prototype quantitative reference method for the multiple detection of  
78 food allergens in standardized incurred food matrices (Mills et al. 2019). Five main food  
79 ingredients causing severe IgE-mediated reactions have been prioritized in light of their  
80 occurrence in food recalls (Turner et al., 2015; Worm et al., 2014; Bucchini et al., 2016). Milk,  
81 soybean, tree nuts, egg, and peanuts were selected as responsible for most alerts notified on  
82 the RASFF (Rapid Alert System for Food and Feed) portal for undeclared presence with serious  
83 risk decision. A recent notice from the European Commission (Commission Notice of  
84 13.7.2017) relating to the provision of information on substances or products causing allergies  
85 or intolerances as listed in Annex II of Regulation (EU) No 1169/2011 provides specifications  
86 about how the Annex II of Regulation (EU) No 1169/2011 should be implemented. In particular,  
87 as for milk and egg ingredients the notice clarifies that the legislation should be applied to milk  
88 from all farmed animals and egg from all farmed birds. Given the timing of the ThRAI project,

89 and the urgent demand for harmonization of current analytical methods, the Consortium agreed  
90 to prioritize cow's milk and hen's egg allergens, as first targets, also in light of their main role in  
91 the primary production. Indeed according to recent statistical data (FaoStat, 2017), cow's milk  
92 production represented in 2017 the 97% of total milk production in Europe and the 81% of the  
93 production worldwide. Similarly, in the same year the hen's eggs accounted for the 99% of total  
94 egg production in Europe and for the 82% worldwide. Whether the developed approach would  
95 be applicable to all farmed animals/birds will be specified depending on the final validated  
96 analytical markers. As for tree nuts allergens, two representative ingredients have been  
97 selected, hazelnut and almond, which are widely used in food manufacturing and represent  
98 foods of public health importance in Europe, causing severe reactions (Worm et al., 2014).

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

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

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

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



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

143

## 144 **2. Literature review based marker peptide selection**

145 The analytical workflow displayed in Fig. 1 was designed to provide the comprehensive  
146 evaluation of the current literature. It consists of five main steps aimed at the critical evaluation  
147 of the information gathered from previous works and the proper selection of reported signature  
148 peptides according to specific criteria. We collected all information about the six allergens  
149 selected as targets in the ThRAI project (cow's milk, hen's egg, peanut, soybean, hazelnut,  
150 and almond) (step 1). Afterwards, the list of reported signature peptides have been evaluated  
151 and filtered according to specific features, namely sequence length, matrix similarity with the  
152 ThRAI project, kind of investigation (discovery only or targeted analysis) and occurrence of  
153 amino acid residues prone to endogenous and exogenous modifications (step 2). The third step

154 of the workflow consisted in retrieving all the information available for the relevant allergenic  
155 proteins (relative abundance, post-translational modification sites, protein isoforms and  
156 variants), from different informatics sources detailed in Figure 1. At step 4 peptide specificity  
157 was assessed by sequence alignment with other species searching not only for exact matches  
158 of tryptic peptides (performed for all candidate markers of the six allergenic ingredients) but  
159 also disclosing potential interference from sequence homology with single amino acid  
160 mismatches (performed only on the four plant-derived allergenic ingredients). Finally as last  
161 step of the analytical workflow, for the refined peptide list we collected experimental details  
162 about the MS-based detection, such as the instrumental platform, the precursor ions, and the  
163 selected transitions. Indeed, the peptide ionization efficiency and reproducibility, as well as the  
164 relative intensity of fragmentation pattern might be affected also by instrumental parameters  
165 which need to be assessed case-by-case. For the comprehensive assessment of the allergen  
166 detection literature (step 1 in Fig.1) we exploited the Allergen Peptide Browser (APB) database  
167 available online, and collected all information about the six allergens selected for the ThRAI  
168 project. The gathered information also integrated data fed by other studies based on MS/MS  
169 analysis with either ESI or MALDI ionization, encompassing the most recent publications not  
170 itemized yet on APB. A total of 89 papers were reviewed, published in the last twenty-five years,  
171 which resulted in an exhaustive list of tryptic peptides for milk (total 42 peptides), egg (total 62  
172 peptides), peanut (total 50 peptides) and soybean (total 146 peptides), and a shorter, but still  
173 reasonable list for hazelnut (total 26 peptides) and almond (total 29 peptides) allergens (see  
174 Table S1). In the second step the full list was narrowed down according to specific constraints.  
175 A range of 7-20 amino acids (AA) in length was applied as good compromise between peptide  
176 specificity, ionization yield and reproducibility. About 84% of reported peptides were included

177 in this range. Afterwards, we searched for matrix similarity with the ThRAI project, and marker  
178 peptides that were never validated in either chocolate or highly processed incurred matrices  
179 were rejected. The resulting peptides list is displayed in Fig. 2 and Fig. 3 (and itemized in Table  
180 S2) together with details about the total number of citing papers per each peptide, and the kind  
181 of investigation (targeted vs discovery approaches), marked with different color (orange and  
182 blue, respectively).

183 The marker selection should comply with specific requirements that value the quantitative  
184 information retrieved. Indeed, the allergen quantification depends on the concentration of  
185 peptide markers, based on the assumption that the peptide is released completely from the  
186 original protein with a specific stoichiometric ratio. Any unpredictable distribution of the released  
187 peptide into different precursor ions, for example due to amino acid modification or multiple  
188 ionization states, should be traced and susceptible peptides should be excluded, in case of low  
189 reproducibility. Concerning the exclusion of signature peptides containing labile amino acids  
190 prone to modifications, it deserves to be noted that the number of potential endogenous and  
191 exogenous post-translational modifications is huge, but their occurrence is highly variable;  
192 therefore, it is not a realistic goal neither to evaluate nor to exclude them all strictly, rather an  
193 empirical validation of the specific case study should be preferable. In this investigation, we  
194 focused on the occurrence of specific amino acids of major concern, such as methionine,  
195 asparagine, glutamine, and cysteine, excluding only peptide sequences containing methionine  
196 residues prone to processing-induced oxidation, and asparagine-glycine motifs prone to  
197 deamidation (Li et al., 2005; Li et al., 2006). We deemed important for the robustness of the  
198 analytical method to avoid M containing peptides as analytical targets, because such residue  
199 would be highly prone to oxidation, in thermally processed matrices, in barely predictable

200 proportions. The NG motifs could be subjected to spontaneous deamidation *in-vitro*, however,  
201 in case of need the asparagine deamidation might be forced in order to convert all asparagine  
202 residues into aspartic acid. As for cysteine residues, most digestion protocols included a  
203 reduction/alkylation step (mainly based on dithiothreitol and iodoacetamide), preliminary to  
204 actual enzymatic proteolysis, which is specifically designed to break disulfide bridges and block  
205 irreversibly the cysteine residues by carbamidomethylation. Common sense is to consider this  
206 modification as complete however, in the perspective to avoid source of uncertainty, C  
207 containing peptides should be preferentially excluded unless the reliability of the peptide is duly  
208 assessed experimentally.

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

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

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

245 thereof can be considered negligible. From the quantitative point of view, by selecting the most  
246 intense precursor ions loss of sensitivity can be limited. Besides the amino acid sequence,  
247 several experimental features may affect this ionization behavior, e.g. the electrospray source  
248 parameters, the MS platform, the composition of the food matrix, dragging competing species  
249 to the ionization step. Therefore it is crucial that the theoretical selection of candidate markers,  
250 based either on the *in-silico* approach or on the literature review approach should be confirmed  
251 by experimental validation on the specific MS platform and on the main matrices of interest.

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

## 254 ***2.1. Proteins and peptide markers reported for cow's milk and hen's egg***

255 The inclusion of milk from domesticated mammalian animals, mainly cattle, buffalo, sheep, goat  
256 and camel, boasts a very long tradition in the human diet. According to the FAO data on primary  
257 livestock production in 2017, about 96% of the world's fresh milk comes from cows and  
258 buffaloes, while the remaining 4.0% is produced by goats, sheep and camels (FAOStat, 2017).  
259 Noteworthy, milk from all ruminant species contains homologous proteins, which share the  
260 same structural, functional, and biological properties (Monaci et al., 2006). Cow's milk contains  
261 about 3 g of proteins per 100 mL and includes at least 25 different proteins classified in two  
262 main categories according to their solubility at pH 4.6 and 20°C (Fox, 2001); the insoluble  
263 protein fraction that precipitates are caseins ( $\alpha$ S1-casein,  $\alpha$ S2-casein,  $\beta$ -casein,  $\kappa$ -casein),  
264 while the soluble fraction constitutes the serum characterized by whey proteins ( $\beta$ -lactoglobulin,  
265  $\alpha$ -lactalbumin, bovine lactoferrin, bovine serum albumin, and immunoglobulins), corresponding  
266 to 80% and 20%, respectively. All the caseins present genetic polymorphisms, which account

267 for the high heterogeneity of such proteins with several identified variants. The latter are  
268 characterized by single amino acid substitution, by deletions of peptides fragments and by  
269 occurrence of post-translational modifications, such as glycosylation, phosphorylation, or partial  
270 hydrolysis, which may affect their specific allergenicity (Fox, 2001; Wal, 2001) and peptide utility  
271 in LC-MS assays. The main allergenic proteins of whey are the globular proteins  $\beta$ -lactoglobulin  
272 and  $\alpha$ -lactalbumin, which are not phosphorylated and contain intramolecular disulfide bonds  
273 that stabilize their structure.

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

281 The critical evaluation of data displayed in Fig. 2, pointed out that milk has not been only the  
282 most investigated source of food allergens, but also the allergenic food where the highest  
283 consensus was achieved in the selection of signature peptides. The milk proteotypic peptides  
284 FFV, YLG and HQG (see Table S2 for peptides full sequences) belong to the  $\alpha$ S1-casein (Bos  
285 d 9), which is considered the most abundant protein in milk. They have been validated in many  
286 independent studies for targeted analysis, including several kind of matrices, such as liquid  
287 matrices (wine, ice cream), bakery products (bread, cookie, muffin), high fat and polyphenol  
288 matrices (chocolate), acidic formulation (tomato sauce), high protein matrices (meat products)

289 etc. In addition, a few signature peptides were also reported for  $\beta$ -casein (Bos d 11),  $\alpha$ S2-casein  
290 (Bos d 10), and  $\kappa$ -casein (Bos d 12), in the perspective to monitor two different allergenic  
291 proteins tracking for the same ingredient. As for whey proteins, main reliable signature peptides  
292 belonged to the  $\beta$ -lactoglobulin ( $\beta$ -LG, MW=18.3 kDa), which is the most abundant protein in  
293 whey. This protein belongs to the lipocalin superfamily and is one of the best characterized  
294 lipid-binding proteins. It possesses three disulfide bridges and is present in two main variants  
295 with punctual mutations.  $\beta$ -LG occurs naturally as a mixture of monomers and dimers, but the  
296 proportion of monomers increases after heating to 70°C (Monaci et al., 2006). TPE, LVL and  
297 LSF were highlighted as the most common signature peptides, already validated into incurred  
298 matrices. Noteworthy, the LSF peptide corresponding to the protein C-terminal, despite  
299 featuring a cysteine residue, has been selected for its robustness. In addition, some peptide  
300 markers were proposed also for  $\alpha$ -lactalbumin (VGINYWLAHK, FLDDDLTDDIMCVK,  
301 DDQNPSSNICNISC DK, LDQWLCEK, CEVFR), but none of them was validated in either  
302 chocolate-based matrix or thermally processed incurred matrices; therefore, they were  
303 excluded from the list of candidate markers at the first selection stage. The specificity of all  
304 reported milk peptide markers was confirmed, and some of the sequences showed  
305 conservation among proteins from other farmed species, still with no specificity issues with  
306 other allergenic food ingredients.

307 Eggs are, together with milk the most common allergenic foods affecting European children.  
308 Hen's eggs are very common in the human diet as inexpensive source of high quality proteins  
309 and they represent a key ingredient in many food products, given its nutritional value and unique  
310 functional properties, such as emulsifying, foaming and gelling. Several egg-based formulations  
311 have been involved in food manufacturing, such as whole egg, egg white, lysozyme isolate,



312 egg yolk, all containing a different degree of allergenic potential (Benedé et al., 2015). The main  
313 egg white allergenic proteins are ovomucoid (Gal d 1), ovalbumin (Gal d 2), lysozyme (Gal d 4)  
314 and ovotransferrin (Gal d 3), all listed in Table 1, with specific mention to the known post-  
315 translational modifications. Yolk, globally, it is significantly less allergenic than albumen,  
316 containing two main allergenic proteins, namely Gal d 5 and Gal d 6, both belonging to the  
317 livetins fraction. The latter accounts for about 30% of the yolk proteins, however, relative  
318 abundance of Gal d 5 and Gal d 6, is hard to define. Gal d 5 is the main  $\alpha$ -livetin, whereas Gal  
319 d 6 (protein YPG 42) derives from major  $\beta$ -livetins as C-terminal fragment of vitellogenin 1.

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

335 deamidation. The peptide YPI contains the cysteine residue in position 121 involved in the  
336 disulfide bridge. Alternative ovalbumin signature peptides have been also reported, such as  
337 HIA and ISQ all displayed in Fig. 2.

338 Similarly to milk fractions, different egg-derived formulations used in food manufacturing require  
339 specific markers to be selected for each part of the allergenic ingredient that could eventually  
340 contaminate food commodities. Thus, in addition to egg white markers, it is important to select  
341 specific markers of egg lysozyme, industrially applied as protein isolate for its antimicrobial  
342 properties, and egg yolk. Only a limited percentage of the collected studies considered this  
343 point. As for direct lysozyme detection, we disclosed three main markers, FES, NTD and GTD,  
344 which have been validated at least once into incurred baked products, such as cookies and  
345 muffins. The egg yolk has been rarely investigated so far, indeed only six studies were collected  
346 detecting specific egg yolk proteins, four of them developing a targeted approach (Planque et  
347 al., 2016; Planque et al., 2017b; New et al., 2018; Planque et al., 2019), and two presenting  
348 only the identification of potential markers by HR-MS discovery analysis (Lee & Kim, 2010;  
349 Gavage et al., 2019). Luckily, most of them already tested a wide diversity of food matrices,  
350 with different complexity degree, such as cookie, bread, chocolate, salad dressing, spices, ham  
351 (see Table S2 for further details), also taking into consideration the ingredient inclusion before  
352 food processing. Noteworthy, Gavage et al., 2019 proposed a systematic investigation of  
353 potential egg markers (selected for both egg white and egg yolk), in raw and processed egg-  
354 based commodities, promoting the use of a dual enzyme digestion protocol, which benefits  
355 from the use of the lysyl endopeptidase in addition to trypsin to provide the same site-specific  
356 cleavage with higher proteolytic yield. Focusing on the four targeted investigations (Planque et  
357 al., 2016; Planque et al., 2017b; New et al., 2018; Planque et al., 2019), we disclosed that none

358 of the reported signature peptides traced directly the allergenic protein YGP42, which is the  
359 carboxy-terminal portion (284 AAs) cleaved from the primary translation product of vitellogenin-  
360 1. Indeed, the peptides YLL and ALL both belong to the amine terminal chain of vitellogenin-1,  
361 known as lipovitellin-1. Three additional peptides included in the allergenic protein YGP42 were  
362 proposed by Gavage et al., 2019, TVI, NVN, ATA, by mean of the dual enzyme digestion  
363 protocol, which could be considered for further assessment in the development of targeted  
364 investigations.

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

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

## 371 ***2.2. Proteins and peptide markers for legumes: peanut and soybean.***

372 Peanut and soybean are both members of the *Fabaceae* or *Leguminosae* family. Legumes  
373 represent 27% of the primary crop production worldwide, highly diffused in the human diet as  
374 excellent sources of proteins, water soluble fibers, numerous micronutrients, and  
375 phytochemicals (Smýkal et al., 2015). Peanut is the best characterized legume in regard to its  
376 allergenic content, having sixteen proteins, mainly seed storage proteins, classified as allergens  
377 with specific isoforms identified and listed in Table 1. As for soybean, the number of  
378 characterized allergens is relatively limited, with eight allergens officially registered by the  
379 WHO/IUIS Allergen Nomenclature Subcommittee (Allergen Nomenclature, accessed in

380 September 2018). Legume allergens can be grouped into a restricted number of families and  
381 superfamilies, and the most relevant allergens are seed storage proteins belonging to the cupin  
382 and prolamin superfamilies. The cupin superfamily share a conserved  $\beta$ -barrel structural core  
383 domain, which can be single or double; the seed storage proteins 7S and 11S globulins are  
384 both bicupins. The prolamin superfamily includes proteins with conserved cysteine residues at  
385 specific positions, sharing a common tridimensional structure with four  $\alpha$ -helices stabilized by  
386 disulfide bonds. This superfamily includes the 2S albumins and non-specific lipid transfer  
387 proteins (ns-LTPs). In addition, minor allergens belong to the profilins and Bet v (*Betula*  
388 *verrucosa*) 1-like superfamilies, which are associated with pollen allergy, the defensins,  
389 involved in plant defense against pathogens, and the oleosins, structural proteins of intracellular  
390 lipid storage organelles.

391 Current methods for MS-based peanut detection mainly target Ara h 1 (7S-globulin, vicilin-type),  
392 Ara h 2 (2S albumin, conglutin) and Ara h 3 (11S globulin, glycinin) proteins. All peptides  
393 reported for these three proteins are highly specific, being conserved only among specific  
394 peanut related taxonomies (*Arachis ipaensis* (130454), *Arachis hypogaea* (3818), *Arachis*  
395 *duransensis* (130453)). Ara h 1 is a glycoprotein that makes up 12-16% of the total protein  
396 content and forms stable trimers held by non-covalent interactions (Palladino & Breiteneder,  
397 2018). Two isoforms has been identified, encoded by two different clones 41 B (Ara h 1.0101)  
398 and P17 (Ara h 1 clone P 17), with a sequence identity higher than 90%. Both proteins have an  
399 N-terminal 25 amino acid residue signal peptide and a single glycosylation site. The signal  
400 peptide drives the nascent protein to the storage vacuole where both the signal itself and a  
401 specific portion of the N-terminal is cleaved-off to yield the mature Ara h 1 found in peanut  
402 (Palladino & Breiteneder, 2018). All the Ara h 1 marker peptides reported so far are shared

403 between the two isoforms, and none of them include the glycosylation site (asparagine residue  
404 at position 521 of clone 41B and position 516 of clone P17, see Fig. S1 for details). DLA, VLL  
405 and GTG are the most frequently selected, already tested in several incurred and hard-to  
406 analyze matrices.

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

425 Ara h 3 is a glycinin-like protein (11S) with a molecular mass of 60 kDa for the monomer and  
426 occurs in peanuts as hexamer of 360 kDa (Palladino & Breiteneder, 2018). The monomer is  
427 post-translationally cleaved in 43 kDa acidic and 28 kDa basic subunits, covalently linked by a  
428 single disulphide bond. Ara h 3 and Ara h 4, initially considered as different allergenic proteins,  
429 have been identified as variants of the same gene, thus they were renamed as isoforms Ara h  
430 3.01 and Ara h 3.02. These are the only two isoforms officially listed by the WHO/IUIS Allergen  
431 Nomenclature Subcommittee, whereas further Uniprot entries are classified as Ara h 3 in the  
432 Allergome database. A genomic clone encoding Ara h 3 (AF10854) (Viquez et al., 2004) was  
433 identified as having four exons. The deduced protein (538 AA in length) showed 93% and 91%  
434 identity with the Ara h 3 (isoform Ara h 3.01) and Ara h 4 (isoform Ara h 3.02) (Palladino &  
435 Breiteneder, 2018), respectively. Furthermore, an additional isoform named iso-Ara h 3 was  
436 reported as sharing only 70-85% of identity with previously cited sequences (Boldt et al., 2005).  
437 Several peptide markers tracing for Ara h 3 protein were reported so far (see Fig. 3), however,  
438 surprisingly for this particular case, the criterion of sequence sharing among known isoforms  
439 has not been valued properly in the current literature (see Fig. S3). Indeed, some of proposed  
440 markers were not conserved among protein variants, and according to common sense, they  
441 should have been excluded, for their limited representativeness. For example, the peptide SPD,  
442 which is cited in 13 investigations, is encrypted only in the Ara h 3.01 isoform (and in the  
443 genomic clone). Quite surprising was also the selection of the peptide TANELNLLILR in two  
444 very recent papers (Planque et al., 2017a; Planque et al., 2017b), which was not encoded by  
445 the officially recognized isoforms Ara h 3.01 and Ara h 3.02, but only by the Ara h 3 genomic  
446 clone. Noteworthy, the peptide LNA is the only marker fully conserved across the isoforms

447 while, the peptides RPF, QQP are common only to the two main isoforms Ara h 3.01 and Ara  
448 h 3.02.

449 As previously stated, soybean is another edible legume belonging to the *Fabaceae* family,  
450 widely consumed worldwide for its high protein content (approximately 38-40 %). It finds also  
451 wide applicability as ingredient in meat/poultry products, bakery and pastry products, dairy  
452 products, and edible spreads, as well as additive for a variety of pharmaceutical and industrial  
453 applications (Verma et al., 2013). As such, the number of occurring soy based formulations is  
454 very high, ranging from raw to highly processed and/or hydrolyzed commodities and this makes  
455 the absolute quantification of soy contamination in complex matrices quite challenging.

456 The WHO/IUIS recognized eight soybean proteins as official allergens, including hydrophobic  
457 protein (Gly m 1), defensin (Gly m 2), soy profilin (Gly m 3), pathogenesis-related protein (Gly  
458 m 4),  $\beta$ -conglycinin (Gly m 5, vicilin, 7S globulin), glycinin (Gly m 6, legumin, 11S globulin),  
459 seed biotinylated proteins (Gly m 7) and the 2S albumin protein group (Gly m 8). The major  
460 storage proteins,  $\beta$ -conglycinin and glycinin, both belonging to the cupin superfamily, represent  
461 alone the 70–80% of the total seed globulin fraction (De Angelis et al., 2017a).  $\beta$ -Conglycinin  
462 is 7S globulin glycoprotein containing 5% of carbohydrate moieties and occur as trimers with  
463 molecular masses of approximately 180 kDa. Therefore, the Gly m 5 allergen name refers to  
464 the complex of three  $\beta$ -conglycinin subunits that were not characterized at the time the name  
465 was assigned (Pomés et al., 2018). The three subunits were first described by Holzhauser et  
466 al. (2009) labeled as  $\alpha$  (67 kDa),  $\alpha'$  (71 kDa) and  $\beta$  (50 kDa), and were assessed to combine at  
467 different ratios to form the multimeric complexes. The  $\alpha$  and  $\alpha'$  subunits are approximately 82%  
468 identical, whereas the  $\beta$ -subunit is only 76% identical to the two  $\alpha$  subunits. The individual

469 subunits were named as isoallergens Gly m 5.01, Gly m 5.02 and Gly m 5.03 in 2009, with  
470 minor variants (e.g. Gly m 5.0301 and Gly m 5.0302 presenting two amino acid mismatches  
471 L/F at positions 16 and 198). Interestingly, the *in-silico* simulation of tryptic digestion showed  
472 that the isoallergens Gly m 5.01, Gly m 5.02 and Gly m 5.03 do not share any tryptic peptide  
473 with minimum length of 7 AA, which could be selected as a common marker for the  $\beta$ -  
474 conglycinin subunits. This issue makes the absolute quantification of soybean allergen by  
475 synthetic peptide analysis quite challenging since the required conversion factors should base  
476 on the knowledge of the specific combination of  $\alpha$ ,  $\alpha'$  and  $\beta$ -subunits occurring in the sample  
477 under investigation. As expected by *in-silico* prediction, none of the reported peptide markers  
478 for Gly m 5 displayed in Fig. 3 were conserved among the three isoallergens (see Fig. S4).  
479 Interestingly, an alternative solution, in this case, would be to select unique markers for each  
480 subunit in order to disclose the specific combination of  $\alpha$ ,  $\alpha'$  and  $\beta$ -monomers within the trimeric  
481 complex. In this frame, the peptide LIT would uniquely trace for Gly m 5.01, the peptides QQQ  
482 and DSYNLQ would uniquely trace for Gly m 5.02, and the peptide DSYNLH would uniquely  
483 trace for Gly m 5.03 (noteworthy, the latter is conserved between the two variants Gly m 5.0301  
484 and Gly m 5.0302). Attention should be paid to the peptide AIV, even if theoretically unique for  
485 Gly m 5.01, since an homologous peptide sequence is expressed in both variants of Gly m  
486 5.03, with a single amino acid substitution L/I at position P19, which could not be discriminated  
487 by SRM acquisition. None of the aforementioned peptides contain amino acid residues with  
488 natural post-translational modification.

489 In most soybean varieties, the glycinin accounts for over 50% of seed storage proteins  
490 representing the predominant protein fraction (De Angelis et al., 2017a). Glycinin has a complex  
491 hexameric structure with a molecular weight ranging between 320 to 360 kDa. At least five



492 genes encode the monomeric subunits (Nielsen et al., 1989) and the Gly m 6 allergen can be  
493 an arrangement of these five proteins, which have individual IgE binding properties (Holzhauser  
494 et al., 2009). Each monomer consists of two specific polypeptide chains, one acidic (40 kDa)  
495 (A) and one basic (20 kDa) (B), linked together by disulfide bonds. Each monomer can be one  
496 of five subunits, namely glycinin G1 (A<sub>1a</sub>B<sub>1b</sub>), glycinin G2 (A<sub>2</sub>B<sub>1a</sub>), glycinin G3 (A<sub>1b</sub>B<sub>1a</sub>), glycinin  
497 G4 (A<sub>5</sub>A<sub>4</sub>B<sub>3</sub>) and glycinin (A<sub>3</sub>B<sub>4</sub>) (Wang et al., 2014), being renamed as five isoallergens Gly m  
498 6.0101, Gly m 6.0201, Gly m 6.0301, Gly m 6.0401 and Gly m 6.0501, respectively, in 2009.  
499 Similarly to what was already discussed for the quantification of Gly m 5 allergen, the  
500 unpredictable composition of the Gly m 6 hexameric complex makes general conversion factors  
501 a hard task to achieve. The peptide marker selection carried out so far in current literature  
502 completely neglected the different sequence expression in known isoallergens. Indeed, the  
503 most frequently used markers, VFD, VLI, and EAF, are expressed uniquely in Gly m 6.0101,  
504 whereas the peptide SQS, cited in 8 independent investigations, is conserved across the three  
505 isoforms Gly m 6.0101, Gly m 6.0201, Gly m 6.0301, and the peptide IST (cited in 7 independent  
506 investigations) is common to the isoforms Gly m 6.0401 and Gly m 6.0501 (see Fig. S5).

### 507 ***2.3. Proteins and peptide markers for tree nuts: hazelnut and almond***

508 Hazelnut and almond allergens were selected in this project as most relevant members of the  
509 tree nut category according to the European legislation. Similar to peanut and soybean, the  
510 main proteins involved in tree nut allergy belong to cupin (legumins-11S globulin and vicilin-7S  
511 globulin) and prolamin (conglutin-2S albumin and ns-LTPs) superfamilies. Several hazelnut  
512 allergens have been identified and are already partly applied in component resolved diagnosis  
513 (Geiselhart et al., 2018). However, only partial information have been collected so far about the

514 occurrence of potential allergen isoforms and their complete sequencing, even if their existence  
515 is likely, given the high genetic diversity of plant organisms. Even the limited information  
516 available for almond allergens, the provided following discussion about the peptide marker  
517 selection was driven to the best of the current knowledge for these two allergens, and relevant  
518 homologous plant proteins.

519 WHO/IUIS database included eight hazelnut allergens all listed in Table 1. According to the  
520 EuroPrevall population-based survey, including allergic subjects from twelve European cities,  
521 the predominance of specific hazelnut allergens is correlated to the geographical area  
522 (Pastorello et al., 2002), as well as to the age of the sensitized individuals (Verweij et al., 2012).  
523 In the Mediterranean areas, hazelnut allergy is mainly linked to Cor a 8, Cor a 14, Cor a 9, Cor  
524 a 11 (Schocker et al., 2004; Blanc et al., 2015). Cor a 8 is a nsLTP type 1, as mature protein,  
525 consisting of a unique polypeptide chain of 92 amino acid residues with eight strictly conserved  
526 cysteines forming four intramolecular disulfide bridges. Cor a 14 (2S albumin) is a heterodimeric  
527 protein with characteristic structural features, including four  $\alpha$ -helices and eight cysteine  
528 residues engaged in four disulfide bridges (Pfeifer et al., 2015). The precursor protein is post-  
529 translationally cleaved into a large and a small subunits linked by disulfide bonds. The N-  
530 terminal glutamine shows the cyclization to pyroglutamic acid and the small subunit is variably  
531 truncated at the C-terminal, leading to a high micro-heterogeneity (Pfeifer et al., 2015). Cor a  
532 9, as 11S legumin, exhibits some homology with other tree nut allergens, such as cashew Ana  
533 o 2, peanut Ara h 3 and the soybean glycinin (Gly m 6). The monomer consists of two  
534 polypeptide chains (acidic and basic chains), cleaved post-translationally by site specific  
535 endopeptidases, and linked together by a single intermolecular disulfide bond. The WHO/IUIS  
536 recorded a single isoform of the Cor a 9 protein corresponding to the Uniprot accession

537 Q8W1C2 and labelled as Cor a 9.0101, first identified in 2002 as IgE-binding protein by 2D-  
538 Western blotting, using sera of hazelnut allergic patients (Beyer et al., 2002). In 2013, a new  
539 isoform of the same protein, with a 514 AA full-length sequence, was registered (Uniprot  
540 accession A0A0A0P7E3) sharing 96.5% homology (Grishina et al., 2013). This isoform has  
541 been added to the Allergome database with the general label of Cor a 9. Additionally, at least  
542 another 55 kDa protein, with high IgE-reactivity only in the alkaline chain (20.7 kDa), has been  
543 identified by Nitride et al. (2013). In this investigation, shotgun proteomics allowed de novo  
544 sequencing of six peptides of the basic chain and their assignment to 11S protein isoform  
545 sharing high homology with 11S globulin-like proteins from several plant organisms, including  
546 the canonical hazelnut one. Starting from this background, we reviewed the current literature  
547 tracing the Cor a 9 selected markers. The peptide WLQ should be excluded by any further  
548 discussion since conserved sequence among different tree nut species (hazelnut, pistachio,  
549 pecan, and walnut). Besides this, we assessed that all the proposed signature peptides shared  
550 100% identity between the official Cor a 9.0101 (Q8W1C2) sequence and its registered isoform  
551 A0A0A0P7E3 (see Fig. S6). However, considering the further potential isoform only partially  
552 sequenced by Nitride et al. (2013), three out of the seven reported peptides might be excluded,  
553 as non-conserved regions and, noteworthy, this exclusion would involve two of the most cited  
554 peptides INT and ALP. Therefore, a wider characterization of most common isoform occurring  
555 by natural genetic diversity is urgently needed. In addition, the peptide VQV contains the NG  
556 motif, which makes the asparagine residues highly susceptible to deamidation.

557 Cor a 11 is a 7S globulin like protein, featuring a trimeric structure of MW about 150/190kDa  
558 with subunits of about 50kDa. The WHO/IUIS lists a single isoform labelled as Cor a 11.0101,  
559 corresponding to the Uniprot entry Q8S4P9, which includes a single glycosylation site occurring

560 at the asparagine residue. Three peptide markers have been reported for this protein, namely  
561 LLS, AFS, and ELA, and none of them includes amino acid residues prone to modifications.

562 As for almond, six identified almond allergens, Pru du 3 (ns-LTP), Pru du 4 (profilin), Pru du 5  
563 (60 S ribosomal protein), and Pru du 6 (legumin), are included in the WHO-IUIS allergen list.  
564 The specificity in the analytical detection of this allergen is particularly challenging due to both  
565 a limited availability of full-length sequences in official database and due to the high homology  
566 of almond proteins (taxonomy *Prunus dulcis*) within the *Prunus* taxonomy genus, mainly *Prunus*  
567 *persica* (peach) (Inman et al., 2018). For example, the almond allergen Pru du 3.0101 displays  
568 99% amino acid sequence identity to a nsLTP from peach (UniProtKB M5W0S9, not listed in  
569 the IUIS) and 57% to two other peach nsLTP isoforms, Pru p 3.0101 and Pru p 3.0102. Two  
570 native isoforms have been identified for the almond profilin (Pru du 4), but its relevance is mainly  
571 restricted to pollen-associated almond allergy. Pru du 5 has been identified as an 11.4 kDa 60S  
572 ribosomal protein involved in protein biosynthesis (Abolhassani & Roux, 2009); nevertheless,  
573 the clinical relevance of this allergen is unknown and requires further research. Finally, Pru du  
574 6, a legumin-like protein, also called amandin, is the first and best biophysically characterized  
575 allergen in almond to date (Geiselhart et al., 2018). Two cDNA clones encoding almond  
576 legumins named prunin-1 (61.0 kDa) and prunin-2 (55.9 kDa) have been isolated, both included  
577 in the WHO/IUIS database as Pru du 6.0101 and Pru du 6.0201. By *in-silico* simulation of the  
578 enzymatic digestion with trypsin specific cleavage, it was assessed that no tryptic peptide with  
579 minimum length of 7 AA presents full identity between the two isoforms Pru du 6.0101 and Pru  
580 du 6.0201 (see Fig. S7). In addition, Allergome database also includes two variants of Pru du  
581 6.0101 and Pru du 6.0201, registered with the Uniprot entries Q43607 and Q43608,  
582 respectively, which share 99% identity with the relevant official allergen isoform, featuring only

583 few amino acid substitutions (see Fig. S7). In Fig. 3, we presented the signature peptides  
584 reported so far for almond detection by SRM analysis all belonging to the Pru du 6 protein,  
585 either to the Pru du 6.0101 or to the Pru du 6.0201 isoallergens. Noteworthy, most of the  
586 selected peptides highlighted with asterisks presented specificity issues, since the BLAST  
587 search against non-redundant protein database showed 100% sequence identity with  
588 homologous proteins from *Prunus persica*, *Prunus mume* or *Prunus avium*, (see Fig. S8 and  
589 S9). In light of this, we kept in the list reported in Fig. 3 also the two markers QQG both VQV,  
590 even if they were not validated in incurred or chocolate based matrices, as only candidates,  
591 together with TDE, which feature uniqueness for *Prunus dulcis* species. Interestingly, none of  
592 the reported peptides included cysteine residues involved in the disulfide bridges, whereas both  
593 the unique peptides VQV and TDE contain the motif NG that make them susceptible to  
594 spontaneous deamidation. In addition, the sequences VQVVENGGPILDDEVR and  
595 ALPDEVLQNAFR, belonging to the Pru du 6.0201 isoform were not conserved in the alternative  
596 allergen variant registered as Q43608 in Uniprot database, both presenting single amino acid  
597 substitutions (D/N and N/T, respectively) and the peptide variant VQVVENGGPILNDEVR is  
598 shared with *Prunus persica* (see sequence alignment in Fig. S9).

#### 599 **2.4. List of candidate markers agreed by ThRAI partners**

600 The thorough discussion presented in the previous section allowed the selection of a restricted  
601 list of candidate signature peptides that can be fruitfully employed for allergen detection in  
602 highly complex and processed foods with a high-confidence on identification accuracy. This list  
603 representing the consensus table of the consortium is reported in Table 2 and will be further  
604 validated for its reliability in the model matrices selected within the ThRAI project by discovery

605 experiments by untargeted HR-MS/MS analysis. As already mentioned, the preliminary marker  
606 selection here performed by means of the critical evaluation of current literature represents only  
607 the first step of the dual approach devised for the ThRAI project. The experimental validation  
608 of the candidate markers by MS/MS analysis is a mandatory step in method development  
609 because several empirical features, such as matrix composition, extraction/digestion  
610 conditions, and instrumental parameters might affect both the specificity and sensitivity of the  
611 final analytical method. Interferences in the chromatographic analysis from complex food  
612 background and information about processing effects on the ingredient itself or finished food  
613 products should be traced to avoid false-negative and false-positives. Such information cannot  
614 be disclosed merely by any *in-silico* based approach.

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

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

625 As for Pru du 6 peptides, with high sequence identity with homologous proteins from *Prunus*  
626 *persica*, *Prunus mume* and *Prunus avium*, only two peptide markers were kept, including the

627 unique peptide TDE, even if encrypting the NG motif, since it can still be useful for confirmative  
628 purposes.

629 Finally, in Table 2 we also collected information about the reported precursor ions and the  
630 transitions monitored. Both the preferential ionization state of the target peptide and its typical  
631 fragmentation pattern depend not only on the specific amino acid sequence, but also on  
632 instrumental parameters such as the ionization source, the MS platform, and the activation  
633 mode; therefore, after proper marker selection, the ionization and fragmentation features need  
634 to be assessed on the specific MS platform involved in the study. From a general point of view,  
635 we highlighted that most of the selected signature peptides were detected as double charged  
636 ions, which indeed are very stable and reproducible both in their ionization yield and in the  
637 fragmentation pattern, whereas an heterogeneous scenario was disclosed as for transitions  
638 selection. Either two or three transitions per peptide were acquired, in some cases the two  
639 monitored fragments are simply two different ionization states of the same transition ( $y_n^+$  and  
640  $y_n^{++}$ ). This behavior is typical of proline containing peptides that upon collisional induced  
641 fragmentation preferentially break the peptide bond at the N-terminal proline site, releasing the  
642 same transition as both single and double charged ions (Ma & Johnson, 2012). This transition  
643 is usually very sensitive, but the ratio ( $y_n^+/y_n^{++}$ ) between the two ions is not fixed, and may  
644 change upon different instrumental conditions. Additionally, retrieving the SRM instrumental  
645 details for the reported papers we also disclosed that in some investigations quite small  
646 fragments have been selected, namely  $y_1^+$ ,  $b_2^+$ ,  $a_2^+$ , notwithstanding their low selectivity and  
647 susceptibility to interference from the background.

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

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

### 666 **3. Conclusions**

667 In this work we described the results of our comprehensive evaluation of the existing literature  
668 on food allergen detection by MS based methods, concerning the selection of peptide markers.  
669 We collected all information about the six allergens selected for the ThRAII project, namely



670 cow's milk, hen's egg, peanut, soybean, hazelnut and almond. The selected peptides were  
671 critically discussed according to specific considerations and recommended criteria for marker  
672 selection that we promote in perspective harmonization of analytical methods development.  
673 The in-depth analysis of current knowledge allowed the selection of a restricted list of candidate  
674 signature peptides, already validated in several independent analyses, which could be  
675 monitored with high confidence. This preliminary list will be validated in the two model matrices  
676 selected within the ThRAI project, in a separate investigation for its final confirmation by specific  
677 discovery experiments with untargeted HR-MS/MS analysis.

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## 688 **Ethical approval**

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

691 **Competing interests**

692 The Authors declare they have no competing interest.

693 **FIGURE AND TABLE CAPTIONS**

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

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

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

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

709 **Table 2.** Preliminary list of signature peptides selected by the Consortium according to the  
710 analytical workflow presented in Figure 1, which have been monitored in targeted investigations  
711 with relevant information on allergens isoforms/variants, peptide specificity, and acquisition  
712 related technical details (MS platform, precursor ion and selected transitions). The presence of  
713 amino acid residues prone to modification was highlighted with a different font (bold and  
714 underlined). The transition selected as quantifier ion (when specified) was marked with an

715 asterisk. *Acronyms:* QqQ, triple quadrupole; IT, ion trap; QToF, quadrupole time-of flight; Q-

716 Orbitrap, quadrupole-Orbitrap, LIT, linear ion trap.

717

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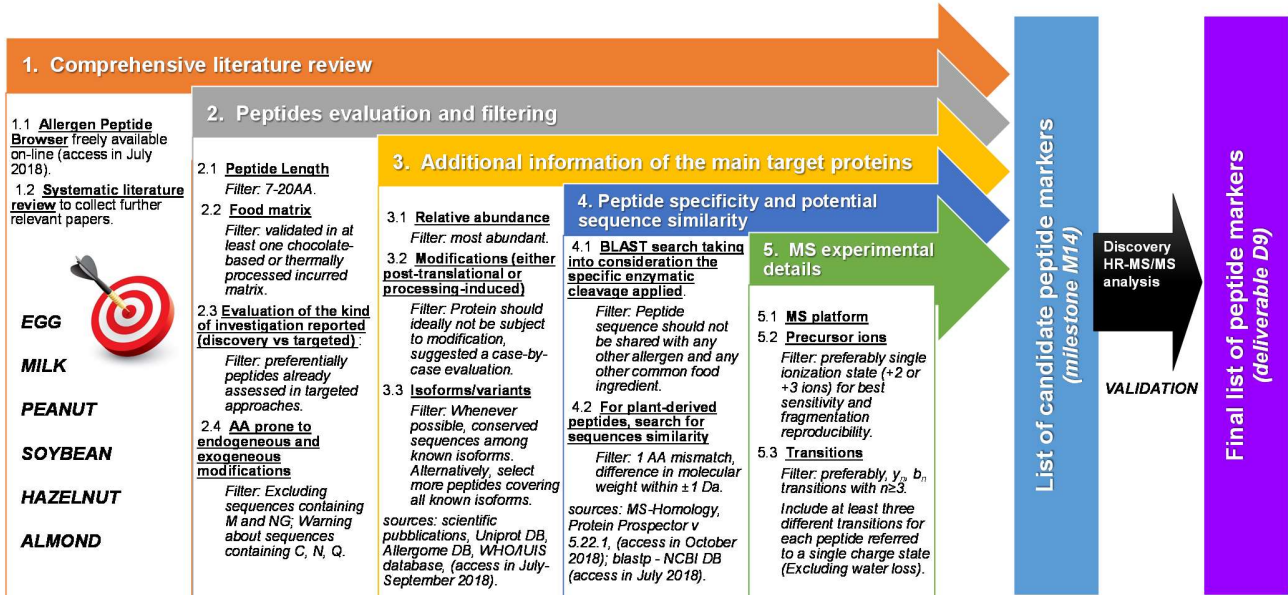
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1083 **FIGURES AND TABLES**

1084 **Figure 1 (color online version only)**

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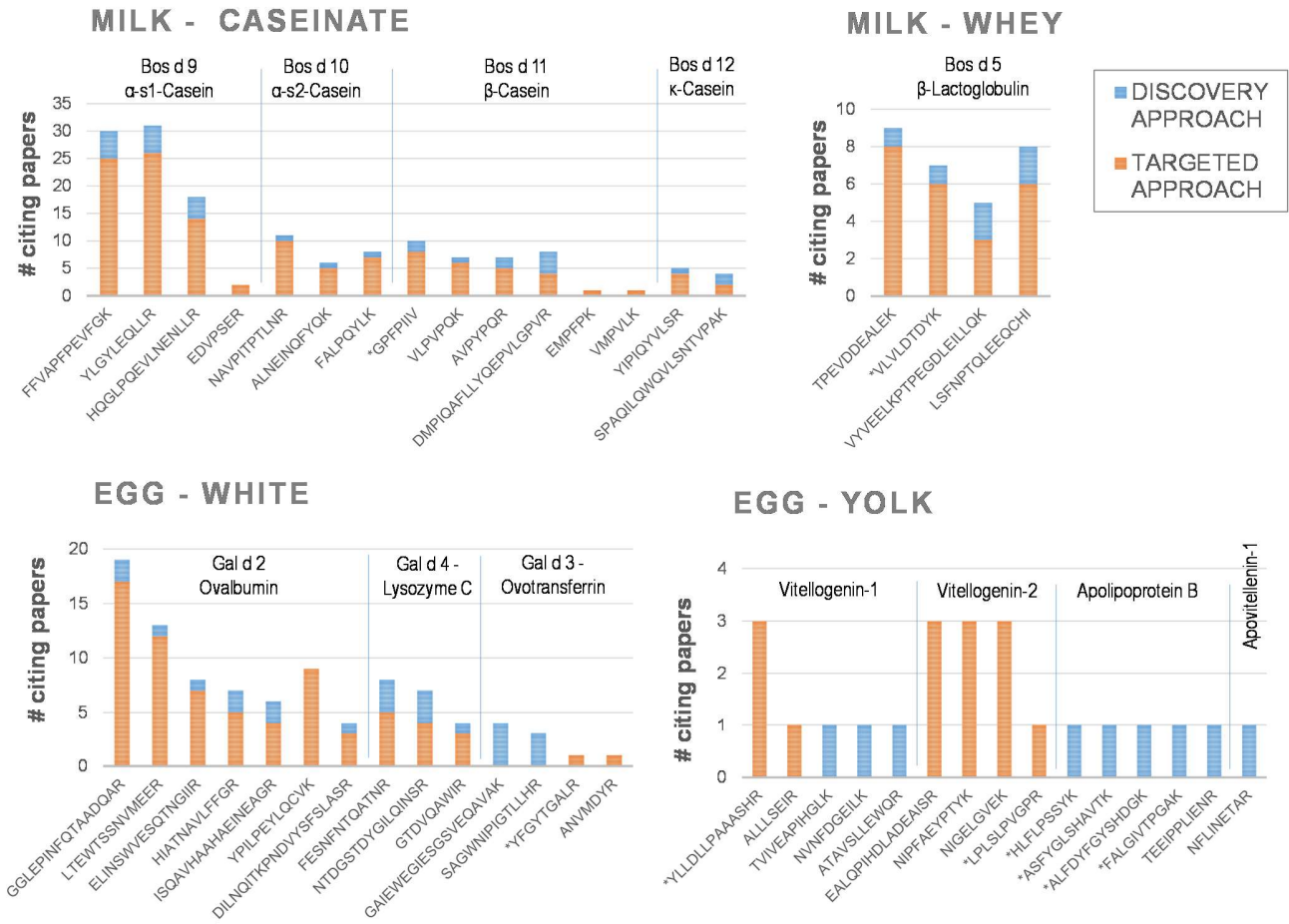
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1088 **Figure 2 (color online version only)**

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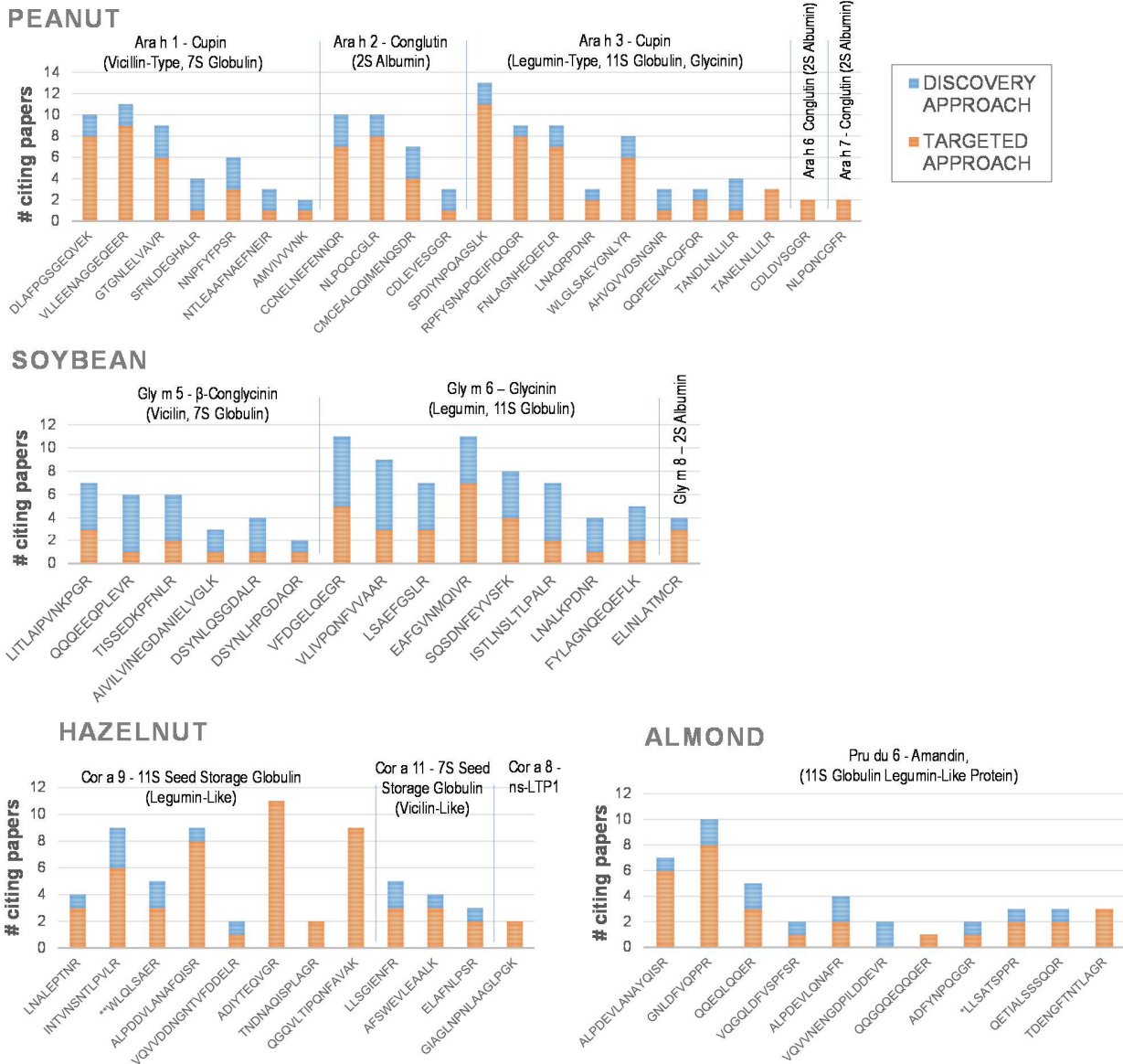
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1092 **Figure 3**

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**Table 1**

Allergenic ingredient	Protein	Allergen Name	MW (kDa)	Number of known PTMs*	Isoforms and Variants	IUIS (YES/NO)	GenBank Protein	UniProt
Milk	αS1-Casein	Bos d 9	23.6	n° 9 Ser phosphorylationsphosphorylations	Bos d 9.0101	Yes	NP_851372	P02662
	αS2-Casein	Bos d 10	25.2	n° 13 Ser phosphorylationsphosphorylations	Bos d 10.0101	Yes	NP_776953	P02663
	β-Casein	Bos d 11	24	n° 5 Ser phosphorylationsphosphorylations	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 phosphorylationsphosphorylations 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		
Egg	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
	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		
Peanut	Vicilin-type, 7S globulin (Cupin)	Ara h 1	64	n° 1 Asn glycosylation	Ara h 1.0101 (clone P41B)	Yes	AAB00861	P43238
					Ara h 1 - Clone P17	No	AAA60336	P43237
	Conglutin, 2S albumin (Prolamin)	Ara h 2	17	n° 3 Pro hydroxylation n° 4 Disulfide Bonds	Ara h 2.0101	Yes	AAK96887	
					Ara h 2.0102	No	see <i>Allergome</i>	
					Ara h 2.0201	Yes	AAN77576	
					Ara h 2.0202	No	see <i>Allergome</i>	
	Legumin-type, Glycinin, 11S globulin (Cupin)	Ara h 3	60, 37 (fragment)	NA	Ara h 3.0101	Yes	AAC63045	O82580
					Ara h 3.0201	Yes	AAD47382	Q9SQH7
					Ara h 3 genomic clone	No	AAM46958	
					Iso Ara h 3	No	AAT39430	
	Profilin	Ara h 5	15	NA	Ara h 5.0101	Yes	AAD55587	Q9SQI9
	Conglutin, 2S albumin (Prolamin)	Ara h 6	15	n° 5 Disulfide Bonds	Ara h 6.0101	Yes	AAD56337	Q647G9
	Conglutin, 2S albumin (Prolamin)	Ara h 7	15	NA	Ara h 7.0101	Yes	AAD56719	Q9SQH1
					Ara h 7.0201	Yes	ABW17159	B4XID4
					Ara h 7.0301	Yes	AAU21496	Q647G8
	Pathogenesis-related protein, PR-10, (Bet v 1 like)	Ara h 8	17	NA	Ara h 8.0101	Yes	AAQ91847	Q6VT83
					Ara h 8.0201	Yes	ABP97433	BOYIU5
	Nonspecific lipid-transfer protein type 1 (Prolamin)	Ara h 9	9.8	Disulfide bond	Ara h 9.0101	Yes	ABX56711	B6CEX8
					Ara h 9.0201	Yes	ABX75045	B6CG41
	16 kDa oleosin (Glycosyl transferase GT-C)	Ara h 10	16	NA	Ara h 10.0101	Yes	AAU21499	Q647G5
Ara h 10.0102					Yes	AAU21500	Q647G4	
14 kDa oleosin (Glycosyl transferase GT-C)	Ara h 11	14	NA	Ara h 11.0101	Yes	AAZ20276	Q45W87	
				Ara h 11.0102	Yes	AAZ20277	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	Yes			
				Ara h 13.0201	Yes			
Oleosin (Glycosyl transferase GT-C)	Ara h 14	17.5	NA	Ara h 14.0101	Yes	AAK13449	Q9AXI1	
				Ara h 14.0102	Yes	AAK13450	Q9AXI0	
				Ara h 14.0103	Yes	AAT11925	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			
Hazelnut		Cor a 1	17	Initial Met Removal	Cor a 1.0101	Yes	CAA50327	Q08407

	Pathogenesis-related protein, PR-10, (Bet v 1 like)				Cor a 1.0102	Yes	CAA5032	Q08407				
					Cor a 1.0103	Yes	CAA50325	Q08407				
					Cor a 1.0104	Yes	CAA50326	Q08407				
					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				
					Profilin	Cor a 2	14	NA	Cor a 2.0101	Yes	AAK01235	Q9AXH5
									Cor a 2.0102	Yes	AAK01236	Q9AXH4
					Non-specific lipid transfer protein type 1 (Prolamin)	Cor a 8	9	n° 4 Disulfide Bonds	Cor a 8.0101	Yes	AAK28533	Q9ATH2
					Legumin-like, 11S globulin (Cupin)	Cor a 9	40	Disulfide bond	Cor a 9.0101	Yes	AAL73404	Q8W1C2
					Vicilin-like, 7S globulin (Cupin)	Cor a 11	48	NA	-	No	AHA36627	A0A0A0P7E3
Cor a 11.0101	Yes	AAL86739	Q8S4P9									
Oleosin	Cor a 12	17	NA	Cor a 12.0101	Yes	AAO67349	Q84T21					
Oleosin	Cor a 13	14-16	NA	Cor a 13.0101	Yes	AAO65960	Q84T91					
2S albumin (Prolamin)	Cor a 14	10	NA	Cor a 14.0101	Yes	ACO56333	D0PWG2					
Almond	Non-specific lipid transfer protein 1 (Prolamin)	Pru du 3	9	Disulfide bond	Pru du 3.0101	Yes	ACN11576	C0L015				
	Profilin	Pru du 4	14	NA	Pru du 4.0101	Yes	AAL91662	Q8GSL5				
					Pru du 4.0102	Yes	AAL91664	Q8GSL5				
	60s acidic ribosomal prot. P2	Pru du 5	10	NA	Pru du 5.0101	Yes	ABH03379	Q8H2B9				
	Legumin-like, Amandin, 11S globulin (Cupin)	Pru du 6	ca. 360	Disulfide bond	Pru du 6.0101	Yes	ADN39440	E3SH28				
					Pru du 6.01 variant	No	CAA55009	Q43607				
					Pru du 6.0201	Yes	ADN39441	E3SH29				
					Pru du 6.02 variant	No	CAA55010	Q43608				
	Soybean	Profilin	Gly m 3	14	Initial Met Removal	Gly m 3.0101	Yes	CAA11756	O65809			
						Gly m 3.0102	Yes	CAA11755	O65810			
Pathogenesis-related protein, PR-10, (Bet v 1 like)		Gly m 4	17	NA	Gly m 4.0101	Yes	CAA42646	P26987				
					Vicilin, β-conglycinin, 7S globulin (Cupin)	Gly m 5	63	NA	Gly m 5.0101	Yes	BAA23360	Q22120
							65		Gly m 5.0201	Yes	BAA74452	Q9FZP9
							48		n° 1 Asn glycosylation	Gly m 5.0301	Yes	AAB23463
48		n° 1 Asn glycosylation	Gly m 5.0302	Yes		P25974 (variant V51G)						
Legumin, Glycinin, 11S globulin (Cupin)		Gly m 6	54	n° 2 Disulfide Bonds n° 2 Propeptides	Gly m 6.0101	Yes	AAA33966 BAC78522	P04776				
			52	n° 2 Disulfide Bonds n° 1 Propeptide	Gly m 6.0201	Yes	BAA00154	P04405				
			52	n° 2 Disulfide Bonds n° 1 Propeptide	Gly m 6.0301	Yes	CAA33217	P11828				

			61	Disulfide bond	Gly m 6.0401	Yes	BAA74953	Q9SB11
			56	n° 2 Disulfide Bonds	Gly m 6.0501	Yes	BAB15802	Q7GC77
	Seed biotinylated protein	Gly m 7	76	NA	Gly m 7.0101	Yes	ACS49840	C6K8D1
	2S albumin (Prolamin)	Gly m 8	14	Disulfide Bond n° 1 Propeptide	Gly m 8.0101	Yes	AAB71140	P19594



**Table 2**

Target protein	Peptide Sequence <i>Isoallergens/variants</i>	Specificity (Tax ID)	MS platform	Precursor	Transition 1		Transition 2		Transition 3		References			
<b>Bos d 9 – α-S1-Casein</b>	FFVAPFPEVFGK <i>Bos d 9.0101</i>	<i>Bos taurus</i> (9913) <i>Bos mutus</i> (72004) <i>Bubalus bubalis</i> (89462)	QqQ	693.3 (+2)	920.8	y <sub>8</sub> <sup>+</sup>	676.6	y <sub>6</sub> <sup>+</sup>	267.3	a <sub>2</sub> <sup>+</sup>	Ansari et al., 2011			
			QqQ	692.9 (+2)	920.3	y <sub>8</sub> <sup>+</sup>	991.3	y <sub>9</sub> <sup>+</sup>	-	-	Heick et al., 2011a			
			QqQ	692.9 (+2)	920.3	y <sub>8</sub> <sup>+</sup>	991.3	y <sub>9</sub> <sup>+</sup>	-	-	Heick et al., 2011b			
			IT	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	1090.6	y <sub>10</sub> <sup>+</sup>	Lamberti et al., 2016			
			IT	1384.6 (+1)	-	-	-	-	-	-	-	Losito et al., 2013		
			Orbitrap	692.9 (+2)	-	-	-	-	-	-	-	Monaci et al., 2013		
			Orbitrap	692.9 (+2)	-	-	-	-	-	-	-	Monaci et al., 2011		
			Q-ToF	692.6 (+2)	-	-	-	-	-	-	-	Monaci et al., 2010a		
			Q-ToF	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	676.4	y <sub>6</sub> <sup>+</sup>	295.2	b <sub>2</sub> <sup>+</sup>	Monaci et al., 2010b			
			Dual LIT	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	-	-	Monaci et al., 2014			
			QqQ	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	1090.6	y <sub>10</sub> <sup>+</sup>	Newsome & Scholl, 2013			
			QqQ	692.9 (+2)	920.5*	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	1090.6	y <sub>10</sub> <sup>+</sup>	Parker et al., 2015			
			Dual LIT / Orbitrap	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	-	-	Pilolli et al., 2014			
			Dual LIT	692.9 (+2)	920.5*	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	545.8	y <sub>10</sub> <sup>++</sup>	Pilolli et al., 2017a			
			QqQ	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	676.4	y <sub>6</sub> <sup>+</sup>	Planque et al., 2016			
			QqQ	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	676.4	y <sub>6</sub> <sup>+</sup>	Planque et al., 2017a			
			QqQ	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	676.4	y <sub>6</sub> <sup>+</sup>	Planque et al., 2017b			
			QqQ	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	676.4	y <sub>6</sub> <sup>+</sup>	Gu et al., 2018			
			QqQ	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	1090.6	y <sub>10</sub> <sup>+</sup>	Boo et al., 2018			
			QqQ	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	-	-	-	-	-	Groves et al., 2018		
			QqQ	692.9 (+2)	295.1*	b <sub>2</sub> <sup>+</sup>	394.2	b <sub>3</sub> <sup>+</sup>	-	-	-	Ke et al., 2017		
			Q-Orbitrap	692.9 (+2)	-	-	-	-	-	-	-	Pilolli et al., 2018		
			Dual LIT	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	1090.6	y <sub>10</sub> <sup>+</sup>	De Angelis et al., 2017b			
			QqQ	692.9 (+2)	920.5	y <sub>8</sub> <sup>+</sup>	991.5	y <sub>9</sub> <sup>+</sup>	-	-	Planque et al., 2019			
			QqQ	692.9 (+2)	460.8*	y <sub>8</sub> <sup>++</sup>	496.3	y <sub>9</sub> <sup>++</sup>	-	-	Qi et al., 2019			
			QqQ	692.8 (+2)	1090.6	y <sub>10</sub> <sup>+</sup>	676.4	y <sub>6</sub> <sup>+</sup>	450.3	y <sub>4</sub> <sup>+</sup>	Montowska & Fornal, 2019			
				YLGYLEQLLR <i>Bos d 9.0101</i>	<i>Bos taurus</i> (9913) <i>Capra hircus</i> (9925) <i>Ovis aries</i> (9940) <i>Bubalus bubalis</i> (89462) <i>Bos motus</i> (72004)	QqQ	634.8 (+2)	991.7	y <sub>8</sub> <sup>+</sup>	771.8	y <sub>6</sub> <sup>+</sup>	249.4	a <sub>2</sub> <sup>+</sup>	Ansari et al., 2011
						QqQ	634.3 (+2)	991.3	y <sub>8</sub> <sup>+</sup>	249.2	a <sub>2</sub> <sup>+</sup>	-	-	Heick et al., 2011a
						QqQ	634.3 (+2)	991.3	y <sub>8</sub> <sup>+</sup>	249.2	a <sub>2</sub> <sup>+</sup>	-	-	Heick et al., 2011b
						IT	634.4 (+2)	991.6	y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	658.4	y <sub>5</sub> <sup>+</sup>	Lamberti et al., 2016
IT	1267.6 (+1)	-				-	-	-	-	-	-	Losito et al., 2013		
LIT	634.8 (+2)	991.8				y <sub>8</sub> <sup>+</sup>	771.4	y <sub>6</sub> <sup>+</sup>	-	-	Mattarozzi et al., 2014			
Orbitrap	634.4 (+2)	-				-	-	-	-	-	-	Monaci et al., 2013		
Orbitrap	634.4 (+2)	-				-	-	-	-	-	-	Monaci et al., 2011		
Q-ToF	634.2 (+2)	-				-	-	-	-	-	-	Monaci et al., 2010a		
Q-ToF	634.3 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	249.2	a <sub>2</sub> <sup>+</sup>	Monaci et al., 2010b			
Dual LIT	634.4 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	-	-	Monaci et al., 2014			
QqQ	634.4 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	-	-	Newsome & Scholl, 2013			
QqQ	634.4 (+2)	991.6*				y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	658.4	y <sub>5</sub> <sup>+</sup>	Parker et al., 2015			
Dual LIT / Orbitrap	634.4 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	-	-	Pilolli et al., 2014			
Dual LIT	634.4 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	658.4	y <sub>5</sub> <sup>+</sup>	Pilolli et al., 2017a			
QqQ	634.4 (+2)	934.5				y <sub>7</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	658.4	y <sub>5</sub> <sup>+</sup>	Planque et al., 2016			
QqQ	634.4 (+2)	934.5				y <sub>7</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	658.4	y <sub>5</sub> <sup>+</sup>	Planque et al., 2017b			
QqQ-ToF	634.6 (+2)	991.3				y <sub>8</sub> <sup>+</sup>	249.2	a <sub>2</sub> <sup>+</sup>	-	-	Ji et al., 2017			
QqQ	634.4 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	658.4	y <sub>5</sub> <sup>+</sup>	Gu et al., 2018			
QqQ	634.4 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	658.4	y <sub>5</sub> <sup>+</sup>	Boo et al., 2018			
QqQ	634.4 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	249.2	a <sub>2</sub> <sup>+</sup>	-	-	New et al., 2018			
QqQ	634.4 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	-	-	-	-	-	Groves et al., 2018		
Q-Orbitrap	634.4 (+2)	-				-	-	-	-	-	-	Pilolli et al., 2018		
Dual LIT	634.4 (+2)	991.6				y <sub>8</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	658.4	y <sub>5</sub> <sup>+</sup>	De Angelis et al., 2017b			
QqQ	634.4 (+2)	934.5				y <sub>7</sub> <sup>+</sup>	771.5	y <sub>6</sub> <sup>+</sup>	-	-	Planque et al., 2019			
QqQ	634.4 (+2)	552.8				y <sub>9</sub> <sup>++</sup>	249.1	b <sub>4</sub> <sup>++</sup>	-	-	Qi et al., 2019			

			QqQ QqQ	634.4 (+2) 634.6 (+2)	991.6 991.8	y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup>	771.5 771.4	y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup>	529.3	y <sub>4</sub> <sup>+</sup>	Montowska & Fornal, 2019 Pavón-Pérez et al., 2019			
<b>Bos d 10 - αS2-Casein</b>	NAVPITPTLNR <i>Bos d 10.0101</i>	<i>Bos taurus</i> (9913) <i>Bubalus bubalis</i> (89462) <i>Bos motus</i> (72004)	Q-ToF	1195.7 (+1)	911.4	y <sub>8</sub> <sup>+</sup>	701.4	y <sub>6</sub> <sup>+</sup>	600.3	y <sub>5</sub> <sup>+</sup>	Gomaa & Boye, 2015			
			QqQ	598.3 (+2)	911.4	y <sub>8</sub> <sup>+</sup>	158.3	a <sub>2</sub> <sup>+</sup>	-	-	Heick et al., 2011a			
			QqQ	598.3 (+2)	911.4	y <sub>8</sub> <sup>+</sup>	158.3	a <sub>2</sub> <sup>+</sup>	-	-	Heick et al., 2011b			
			Orbitrap	598.3 (+2)	-	-	-	-	-	-	-	Monaci et al., 2013		
			Q-ToF	598.4 (+2)	-	-	-	-	-	-	-	Monaci et al., 2010a		
			QqQ	598.3 (+2)	911.5	y <sub>8</sub> <sup>+</sup>	456.3	y <sub>8</sub> <sup>++</sup>	285.2	b <sub>3</sub> <sup>+</sup>	Planque et al., 2016			
			QqQ	598.3 (+2)	911.5	y <sub>8</sub> <sup>+</sup>	456.3	y <sub>8</sub> <sup>++</sup>	285.2	b <sub>3</sub> <sup>+</sup>	Planque et al., 2017b			
			QqQ	598.3 (+2)	911.5	y <sub>8</sub> <sup>+</sup>	701.4	y <sub>6</sub> <sup>+</sup>	600.3	y <sub>5</sub> <sup>+</sup>	Gu et al., 2018			
			QqQ	598.3 (+2)	912.0	y <sub>8</sub> <sup>+</sup>	285.5	b <sub>3</sub> <sup>+</sup>	-	-	Ke et al., 2017			
			QqQ	598.3 (+2)	911.5	y <sub>8</sub> <sup>+</sup>	285.2	b <sub>3</sub> <sup>+</sup>	-	-	Planque et al., 2019			
QqQ	598.4 (+2)	911.6*	y <sub>8</sub> <sup>+</sup>	456.3	y <sub>8</sub> <sup>++</sup>	-	-	Qi et al., 2019						
	FALPQYLK <i>Bos d 10.0101</i>	<i>Bos taurus</i> (9913) <i>Bos motus</i> (72004)	QqQ	490.3 (+2)	120.1	a <sub>1</sub> <sup>+</sup>	648.4	y <sub>5</sub> <sup>+</sup>	-	-	Heick et al., 2011a			
			QqQ	490.3 (+2)	120.1	a <sub>1</sub> <sup>+</sup>	648.4	y <sub>5</sub> <sup>+</sup>	-	-	Heick et al., 2011b			
			IT	979.3 (+1)	-	-	-	-	-	-	-	Losito et al., 2013		
			QqQ	490.1 (+2)	761.5	y <sub>6</sub> <sup>+</sup>	648.4*	y <sub>5</sub> <sup>+</sup>	-	-	-	Lutter et al., 2011		
			Orbitrap	979.6 (+1)	-	-	-	-	-	-	-	Monaci et al., 2013		
			QqQ	490.3 (+2)	761.5	y <sub>6</sub> <sup>+</sup>	648.4	y <sub>5</sub> <sup>+</sup>	423.3	y <sub>3</sub> <sup>+</sup>	Gu et al., 2018			
			QqQ	490.2 (+2)	761.5	y <sub>6</sub> <sup>+</sup>	219.2	b <sub>2</sub> <sup>+</sup>	-	-	Ke et al., 2017			
QqQ	490.3 (+3)	648.4	y <sub>5</sub> <sup>+</sup>	219.1	b <sub>2</sub> <sup>+</sup>	-	-	Qi et al. 2019						
<b>Bos d 5 - β- Lactoglobulin</b>	TPEVDDEALEK <i>Bos d 5.0101</i>	<i>Bos taurus</i> (9913) <i>Bubalus bubalis</i> (89462) <i>Bos motus</i> (72004)	QqQ	623.3 (+2)	918.4	y <sub>8</sub> <sup>+</sup>	819.4*	y <sub>7</sub> <sup>+</sup>	-	-	Lutter et al., 2011			
			Dual LIT	623.3 (+2)	572.8	y <sub>10</sub> <sup>++</sup>	819.4	y <sub>7</sub> <sup>+</sup>	-	-	Monaci et al., 2014			
			QqQ	623.3 (+2)	572.8	y <sub>10</sub> <sup>++</sup>	819.4	y <sub>7</sub> <sup>+</sup>	918.4	y <sub>8</sub> <sup>+</sup>	Parker et al., 2015			
			QqQ	623.3 (+2)	-	-	-	-	-	-	-	Yang et al., 2014		
			QqQ-ToF	623.3 (+2)	1048.2	y <sub>9</sub> <sup>+</sup>	199.2	b <sub>2</sub> <sup>+</sup>	-	-	-	Ji et al. 2017		
			QqQ	623.3 (+2)	572.8	y <sub>10</sub> <sup>++</sup>	819.4	y <sub>7</sub> <sup>+</sup>	918.4	y <sub>8</sub> <sup>+</sup>	Boo et al., 2018			
			Q-Orbitrap	623.3 (+2)	-	-	-	-	-	-	-	Pilolli et al., 2018		
			QqQ	623.3 (+2)	572.8	y <sub>10</sub> <sup>++</sup>	819.4	y <sub>7</sub> <sup>+</sup>	-	-	-	New et al., 2018		
			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)	-	-	-	-	-	-	-	Figeys et al., 1996
					QqQ	533.3 (+2)	853.4*	y <sub>7</sub> <sup>+</sup>	754.4	y <sub>6</sub> <sup>+</sup>	-	-	-	Lutter et al., 2011
QqQ	533.3 (+2)	853.4			y <sub>7</sub> <sup>+</sup>	754.4	y <sub>6</sub> <sup>+</sup>	641.3	y <sub>5</sub> <sup>+</sup>	641.3	Parker et al., 2015			
QqQ	533.3 (+2)	853.4			y <sub>7</sub> <sup>+</sup>	754.4	y <sub>6</sub> <sup>+</sup>	641.3	y <sub>5</sub> <sup>+</sup>	641.3	Planque et al., 2016			
QqQ	533.3 (+2)	853.4			y <sub>7</sub> <sup>+</sup>	754.4	y <sub>6</sub> <sup>+</sup>	641.3	y <sub>5</sub> <sup>+</sup>	641.3	Planque et al., 2017b			
QqQ	533.3 (+2)	853.4			y <sub>7</sub> <sup>+</sup>	754.4	y <sub>6</sub> <sup>+</sup>	-	-	-	Planque et al., 2019			
VYVEELKPTPEGDLEILLQK <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)	QqQ	771.5 (+3)	-	-	-	-	-	-	-	Figeys et al., 1996			
		QqQ	771.8 (+3)	912.0	y <sub>16</sub> <sup>++</sup>	790.9	y <sub>14</sub> <sup>++</sup>	627.9	y <sub>11</sub> <sup>++</sup>	627.9	Planque et al., 2016			
		QqQ	771.8 (+3)	912.0	y <sub>16</sub> <sup>++</sup>	790.9	y <sub>14</sub> <sup>++</sup>	627.9	y <sub>11</sub> <sup>++</sup>	627.9	Planque et al., 2017b			
LSFNPTQLEEQCHI <i>Bos d 5.0101</i>	<i>Bos taurus</i> (9913) <i>Bubalus bubalis</i> (89462)	QqQ	858.4 (+2)	928.4	y <sub>7</sub> <sup>+</sup>	815.3	y <sub>6</sub> <sup>+</sup>	627.8	y <sub>10</sub> <sup>++</sup>	627.8	Parker et al., 2015			
		QqQ	858.4 (+2)	928.4	y <sub>7</sub> <sup>+</sup>	1254.6	y <sub>10</sub> <sup>+</sup>	627.8	y <sub>10</sub> <sup>++</sup>	627.8	Planque et al., 2016			
		QqQ	858.4 (+2)	1254.6	y <sub>10</sub> <sup>+</sup>	815.3	y <sub>6</sub> <sup>+</sup>	627.8	y <sub>10</sub> <sup>++</sup>	627.8	Planque et al., 2017b			
		QqQ	858.4 (+2)	928.4	y <sub>7</sub> <sup>+</sup>	627.8	y <sub>10</sub> <sup>++</sup>	-	-	-	Boo et al., 2018			
		QqQ	858.6 (+2)	462.2*	b <sub>4</sub> <sup>+</sup>	685.1	y <sub>11</sub> <sup>++</sup>	-	-	-	Ke et al., 2017			
		QqQ	858.4 (+2)	1254.6	y <sub>10</sub> <sup>+</sup>	627.8	y <sub>10</sub> <sup>++</sup>	-	-	-	Planque et al., 2019			
<b>Gal d 2 - Ovalbumin</b>	GGLEPINFQTAADQAR <i>Gal d 2.0101</i>	<i>Gallus gallus</i> (9031) <i>Alcaligenes xylosoxydans</i> <i>xylosoxydans</i> (85698)	Q-ToF	-	-	-	-	-	-	-	Azarnia et al., 2013			
			LIT	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1331.7	y <sub>12</sub> <sup>+</sup>	-	-	-	Mattarozzi et al., 2014		
			Orbitrap	844.4 (+2)	-	-	-	-	-	-	-	Monaci et al., 2013		
			Dual LIT	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1121.5	y <sub>10</sub> <sup>+</sup>	-	-	-	Monaci et al., 2014		
			QqQ	844.4 (+2)	1007.5	y <sub>9</sub> <sup>+</sup>	1121.5	y <sub>10</sub> <sup>+</sup>	860.4	y <sub>8</sub> <sup>+</sup>	860.4	Parker et al., 2015		
			Dual LIT / Orbitrap	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1331.7	y <sub>12</sub> <sup>+</sup>	-	-	-	Pilolli et al., 2014		

			Dual LIT	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1121.5	y <sub>10</sub> <sup>+</sup>	732.4	y <sub>7</sub> <sup>+</sup>	Pioli et al., 2017a	
			QqQ	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1121.5	y <sub>10</sub> <sup>+</sup>	1331.7	y <sub>12</sub> <sup>+</sup>	Planque et al., 2016	
			Q-ToF	844.4 (+2)	-	-	-	-	-	-	Tolin et al., 2012b	
			QqQ	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	-	-	-	-	Planque et al., 2017a	
			QqQ	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1121.5	y <sub>10</sub> <sup>+</sup>	1331.7	y <sub>12</sub> <sup>+</sup>	Planque et al., 2017b	
			QqQ	844.4 (+2)	1007.5	y <sub>9</sub> <sup>+</sup>	1121.5	y <sub>10</sub> <sup>+</sup>	860.4	y <sub>8</sub> <sup>+</sup>	Boo et al., 2018	
			Dual LIT	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1121.5	y <sub>10</sub> <sup>+</sup>	732.4	y <sub>7</sub> <sup>+</sup>	Pioli et al., 2017b	
			QqQ	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1121.5	y <sub>10</sub> <sup>+</sup>	-	-	New et al., 2018	
			Q-Orbitrap	844.4 (+2)	-	-	-	-	-	-	Pioli et al., 2018	
			Dual LIT	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1121.5	y <sub>11</sub> <sup>+</sup>	1331.7	y <sub>12</sub> <sup>+</sup>	De Angelis et al., 2017b	
			QqQ	844.4 (+2)	666.3	y <sub>12</sub> <sup>++</sup>	1121.5	y <sub>11</sub> <sup>+</sup>	-	-	Planque et al., 2019	
	HIATNAVLFFGR Gal d 2.0101	<i>Gallus gallus</i> (9031) <i>Alcaligenes xylosoxydans</i> <i>xylosoxydans</i> (85698)	Q-ToF	-	-	-	-	-	-	-	Azarnia et al., 2013	
			QqQ	673.4 (+2)	1095.6	y <sub>10</sub> <sup>+</sup>	223.2	a <sub>2</sub> <sup>+</sup>	-	-	Heick et al., 2011a	
			QqQ	673.4 (+2)	1095.6	y <sub>10</sub> <sup>+</sup>	223.2	a <sub>2</sub> <sup>+</sup>	-	-	Heick et al., 2011b	
			Orbitrap	673.4 (+2)	-	-	-	-	-	-	Monaci et al., 2015	
			QqQ	449.3 (+3)	639.4*	y <sub>5</sub> <sup>+</sup>	526.3	y <sub>4</sub> <sup>+</sup>	608.3	b <sub>6</sub> <sup>+</sup>	Parker et al., 2013	
			Q-ToF	-	-	-	-	-	-	-	Tolin et al., 2012*	
	ISQAVHAAHAEINEAGR Gal d 2.0101	<i>Gallus gallus</i> (9031) <i>Coturnix japonica</i> (93934) <i>Alcaligenes xylosoxydans</i> <i>xylosoxydans</i> (85698)	Q-ToF	-	-	-	-	-	-	-	Azarnia et al., 2013	
			Orbitrap	887.4 (+2)	-	-	-	-	-	-	Monaci et al., 2013	
			QqQ	887.5 (+2)	1138.6	y <sub>11</sub> <sup>+</sup>	1067.5	y <sub>10</sub> <sup>+</sup>	996.5	y <sub>9</sub> <sup>+</sup>	Planque et al., 2016	
			QqQ	887.5 (+2)	1138.6	y <sub>11</sub> <sup>+</sup>	1067.5	y <sub>10</sub> <sup>+</sup>	996.5	y <sub>9</sub> <sup>+</sup>	Planque et al., 2017b	
<b>Gal d 4 - Lysozyme C</b>	FESNFNTQATNR Gal d 4.0101	<i>Gallus gallus</i> (9031) <i>Coturnix japonica</i> (93934) <i>Rattus norvegicus</i> (10116) <i>Gallus lafayetii</i> (9032) <i>Gallus sonneratii</i> (9033)	QqQ	714.8 (+2)	277.1	b <sub>2</sub> <sup>+</sup>	-	-	-	-	Cryar et al., 2013	
			Orbitrap	714.8 (+2)	-	-	-	-	-	-	Monaci et al., 2013	
			QqQ	714.8 (+2)	1152.5	y <sub>10</sub> <sup>+</sup>	951.5	y <sub>8</sub> <sup>+</sup>	804.4	y <sub>7</sub> <sup>+</sup>	Parker et al., 2015	
			QqQ	714.8 (+2)	589.3	y <sub>5</sub> <sup>+</sup>	690.4	y <sub>6</sub> <sup>+</sup>	-	-	Pioli et al., 2014	
			QqQ	714.8 (+2)	1152.5	y <sub>10</sub> <sup>+</sup>	951.5	y <sub>8</sub> <sup>+</sup>	804.4	y <sub>7</sub> <sup>+</sup>	Boo et al., 2018	
		NTDGSTDYQILQINSR Gal d 4.0101	<i>Gallus gallus</i> (9031) <i>Anas platyrhynchos</i> (8839) <i>Catreus wallichii</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) <i>Pavo cristatus</i> (9049) <i>Phasianus colchicus colchicus</i> (9057) <i>Phasianus versicolor</i> (9055) <i>Syrnaticus soemmerringii</i> (9067) <i>Syrnaticus 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 colchicus</i> (9057) <i>Gallus lafayetii</i> (9032) <i>Gallus sonneratii</i> (9033) <i>Alopochen aegyptiaca</i> (30382)	Orbitrap	877.4 (+2)	-	-	-	-	-	-	Monaci et al., 2013
			QqQ	585.3 (+3)	489.3	y <sub>4</sub> <sup>+</sup>	617.3	y <sub>5</sub> <sup>+</sup>	730.4	y <sub>6</sub> <sup>+</sup>	Parker et al., 2015	
			Dual LIT / Orbitrap	877.4 (+2)	489.3	y <sub>4</sub> <sup>+</sup>	617.3	y <sub>5</sub> <sup>+</sup>	-	-	Pioli et al., 2014	
			Dual LIT	877.4 (+2)	489.3	y <sub>4</sub> <sup>+</sup>	617.3	y <sub>5</sub> <sup>+</sup>	730.4	y <sub>6</sub> <sup>+</sup>	De Angelis et al., 2017b	
<b>Vitellogenin-1</b>	YLLDLLPAAASHR (lipovitellin-1 chain)	<i>Gallus gallus</i> (9031)	QqQ	480.6 (+3)	709.4	y <sub>7</sub> <sup>+</sup>	582.3	y <sub>11</sub> <sup>++</sup>	355.2	y <sub>7</sub> <sup>++</sup>	Planque et al., 2016	
			QqQ	480.6 (+3)	709.4	y <sub>7</sub> <sup>+</sup>	582.3	y <sub>11</sub> <sup>++</sup>	355.2	y <sub>7</sub> <sup>++</sup>	Planque et al., 2017b	
			QqQ	480.6 (+3)	709.4	y <sub>7</sub> <sup>+</sup>	355.2	y <sub>7</sub> <sup>++</sup>	-	-	New et al., 2018	
			QqQ	480.6 (+3)	709.4	y <sub>7</sub> <sup>+</sup>	582.3	y <sub>11</sub> <sup>++</sup>	-	-	Planque et al., 2019	
	ALLLSEIR (lipovitellin-1 chain)	<i>Gallus gallus</i> (9031) <i>Fundulus heteroclitus</i> (8078)	QqQ	457.8 (+2)	617.4	y <sub>5</sub> <sup>+</sup>	730.5	y <sub>6</sub> <sup>+</sup>	-	-	New et al., 2018	

		<i>Galdieria sulphuraria</i> (130081) <i>Nicrophorus vespilloides</i> (110193)									
Vitellogenin-2	NIPFAEYPTYK (lipovitellin-1 chain)	<i>Gallus gallus</i> (9031) <i>Larus argentatus</i> (35669) <i>Anas platyrhynchos</i> (8839) <i>Cuculus canorus</i> (55661) <i>Buceros rhinoceros silvestris</i> (175836) <i>Charadrius vociferus</i> (50402) <i>Merops nubicus</i> (57421) <i>Pelecanus crispus</i> (36300) <i>Mesitornis unicolor</i> (54374) <i>Colinus virginianus</i> (9014) <i>Meleagris gallopavo</i> (9103) <i>Callipepla squamata</i> (9009) <i>Haliaeetus albicilla</i> (8969) <i>Mesitornis unicolor</i> (54374)	QqQ QqQ QqQ	671.8 (+2) 671.8 (+2) 671.8 (+2)	1115.5 1115.5 1115.5	y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup>	508.3 508.3 558.3	y <sub>4</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> y <sub>9</sub> <sup>++</sup>	558.3 558.3 -	y <sub>9</sub> <sup>++</sup> y <sub>9</sub> <sup>++</sup> -	Planque et al., 2016 Planque et al., 2017b Planque et al., 2019
	NIGELGVEK (lipovitellin-1 chain)	<i>Gallus gallus</i> (9031) <i>Larus argentatus</i> (35669) <i>Anas platyrhynchos</i> (8839) <i>Nipponia nippon</i> (128390) <i>Calypte anna</i> (9244) <i>Nestor notabilis</i> (176057) <i>Buceros rhinoceros silvestris</i> (175836) <i>Charadrius vociferus</i> (50402) <i>Pelecanus crispus</i> (36300) <i>Merops nubicus</i> (57421) <i>Aptenodytes forsteri</i> (9233) <i>Patagioenas fasciata monilis</i> (372326) <i>Phalacrocorax carbo</i> (9209) <i>Antristomus carolinensis</i> (279965) <i>Chlamydotis macqueenii</i> (187382) <i>Opisthocomus hoazin</i> (30419)	QqQ QqQ QqQ	479.8 (+2) 479.8 (+2) 479.8 (+2)	731.4 731.4 731.4	y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup>	674.4 674.4 228.1	y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> b <sub>2</sub> <sup>+</sup>	545.3 545.3 -	y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> -	Planque et al., 2016 Planque et al., 2017b New et al., 2018
	LPLSLPVGPR (lipovitellin-2 chain)	<i>Gallus gallus</i> (9031) <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> (9009) <i>Phaethon lepturus</i> (97097) <i>Falco sparverius</i> (56350) <i>Buceros rhinoceros silvestris</i> (175836) <i>Charadrius vociferous</i> (50402) <i>Amazona aestiva</i> (12930) <i>Ficedula albicollis</i> (59894) <i>Merops nubicus</i> (57421) <i>Aptenodytes forsteri</i> (9233) <i>Hydroprogne caspia</i> (425641)	QqQ	524.8 (+2)	468.3	y <sub>9</sub> <sup>++</sup>	725.4	y <sub>7</sub> <sup>+</sup>	-	-	New et al., 2018

		<i>Patagioenas fasciata monilis</i> (372326) <i>Mesitornis unicolor</i> (54374) <i>Balearica regulorum gibbericeps</i> (100784) <i>Phalacrocorax carbo</i> (9209) <i>Anstroctomus carolinensis</i> (279965) <i>Chlamydotis macqueenii</i> (187382) <i>Colinus virginianus</i> (9014) <i>Opisthocomus hoazin</i> (30419)									
<b>Ara h 1   Cupin (Vicillin-Type, 7S Globulin)</b>	DLAFPGSGEQVEK <i>Ara h 1.0101 (clone P41B)</i> <i>Ara h 1 - clone P17</i>	<i>Arachis hypogaea</i> (3818) <i>Arachis duranensis</i> (130453)	Q-ToF QqQ QqQ QqQ QqQ QqQ QqQ QqQ QqQ	688.9 (+2) 688.8 (+2) 688.8 (+2) 688.8 (+2) 688.8 (+2) 688.9 (+2) 688.8 (+2) 688.8 (+2) 688.8 (+2)	- 930.6 930.6 930.5 930.5 930.5 930.5 930.5 929.4	- y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup>	- 300.2 300.2 833.4 833.4 833.4 300.2 300.2 447.2	- a <sub>3</sub> <sup>+</sup> a <sub>3</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> b <sub>3</sub> <sup>+</sup> b <sub>3</sub> <sup>+</sup> b <sub>4</sub> <sup>+</sup>	- - - 1077.5 776.4 776.4 229.1 -	- - - y <sub>10</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> b <sub>2</sub> <sup>+</sup> -	Chassaigne et al., 2007 Heick et al., 2011a Heick et al., 2011b Pedreschi et al., 2012 Sayers et al., 2016 Sayers et al., 2018 Shefcheck et al., 2006 New et al., 2018 Zhang et al., 2019
	VLEENAGGEQEER <i>Ara h 1.0101 (clone P41B)</i> <i>Ara h 1 - clone P17</i>	<i>Arachis hypogaea</i> (3818) <i>Arachis duranensis</i> (130453)	QqQ QqQ Dual LIT QqQ QqQ Q-Qorbitrap QqQ Q-ToF LIT-Orbitrap	786.9 (+2) 786.9 (+2) 786.9 (+2) 786.9 (+2) 786.9 (+2) 786.8 (+2) 786.9 (+2) 786.9 (+2) 786.9 (+2)	804.3 804.3 804.3 804.3 804.4 - 804.4 - -	y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> - y <sub>7</sub> <sup>+</sup> - -	875.4 875.4 875.4 875.4 213.2 -	- y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> b <sub>2</sub> <sup>+</sup> y <sub>10</sub> <sup>+</sup> -	989.4 989.4 680.8 989.4 989.4 -	y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>12</sub> <sup>++</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> -	Pedreschi et al., 2012 Sayers et al., 2016 Pilolli et al., 2017a Sayers et al., 2018 Shefcheck et al., 2006 Pilolli et al., 2018 New et al., 2018 Chassaigne et al., 2007 Korte et al., 2016*
	GTGNLLELVAVR <i>Ara h 1.0101 (clone P41B)</i> <i>Ara h 1 - clone P17</i>	<i>Arachis hypogaea</i> (3818) <i>Arachis duranensis</i> (130453)	QqQ QqQ QqQ QqQ QqQ Q-Qorbitrap	564.4 (+2) 564.4 (+2) 564.8 (+2) 564.8 (+2) 564.8 (+2) 564.8 (+2)	686.6 686.6 686.4 686.4 686.4 -	y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> -	557.5 557.5 557.4 557.4 -	y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> -	- -	- -	Heick et al., 2011a Heick et al., 2011b Parker et al., 2015 Gu et al., 2018 Boo et al., 2018 Pilolli et al., 2018
<b>Ara h 2 - Conglutin (2S Albumin)</b>	CCNELNEFENNQR <i>Ara h 2.0101,</i> <i>Ara h 2.0201</i> <i>Ara h 2.0202</i>	<i>Arachis ipaensis</i> (130454) <i>Arachis hypogaea</i> (3818) <i>Arachis duranensis</i> (130453)	QqQ QqQ QqQ QqQ QqQ QqQ	807.0 (+2) 576.2 (+3) 863.8 (+2) 863.9 (+2) 863.8 (+2) 863.8 (+2)	1050.5 920.3 807.4 1163.5 1163.5 1163.5	y <sub>8</sub> <sup>+</sup> b <sub>7</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup>	- 807.4 1050.5 1050.5 1050.5 1050.5	- y <sub>6</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup>	- 660.3 660.3 936.4 936.4 936.4	- y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup>	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
	NLPQQCGLR <i>Ara h 2.0101,</i> <i>Ara h 2.0201</i> <i>Ara h 2.0202</i>	<i>Arachis ipaensis</i> (130454) <i>Arachis hypogaea</i> (3818) <i>Arachis duranensis</i> (130453)	QqQ QqQ QqQ QqQ QqQ QqQ QqQ QqQ QqQ QqQ	543.3 (+2) 543.3 (+2) 543.3 (+2) 543.3 (+2) 543.6 (+2) 543.3 (+2) 543.3 (+2) 543.3 (+2) 543.3 (+2) 543.8 (+2)	858.4 858.4 858.4 858.4 858.2 858.4 858.4 858.4 858.4 858.4	y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup>	761.4 761.4 761.4 761.4 429.8* 429.7 761.4 429.7 633.3	y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>7</sub> <sup>++</sup> y <sub>7</sub> <sup>++</sup> y <sub>6</sub> <sup>+</sup> y <sub>7</sub> <sup>++</sup> y <sub>5</sub> <sup>+</sup>	633.3 633.3 633.3 633.3 -	y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> -	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 Planque et al., 2019 Zhang et al., 2019
<b>Gly m 5 - β-Conglycinin (Vicilin, 7S Globulin)</b>	LITLAIPIVKNPGR <i>Gly m 5.0101</i>	<i>Glycine max</i> (3847) <i>Glycine soja</i> (3848)	QqQ QqQ QqQ Q-Qorbitrap	464.7 (+3) 464.6 (+3) 464.6 (+3) 464.63 (+3)	767.5* 767.5 767.5 -	y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> -	583.4* 583.4 583.4 -	y <sub>11</sub> <sup>++</sup> y <sub>11</sub> <sup>++</sup> y <sub>11</sub> <sup>++</sup> -	476.3* 476.3 476.3 -	y <sub>9</sub> <sup>++</sup> y <sub>9</sub> <sup>++</sup> y <sub>9</sub> <sup>++</sup> -	Houston et al., 2011 Planque et al., 2016 Planque et al., 2017b Chen et al., 2019

	QQQEEQPLEVR <i>Gly m 5.0201</i>	<i>Glycine max (3847)</i> <i>Glycine soja (3848)</i>	QqQ Q-Orbitrap	692.3 (+2) 692.34 (+2)	1127.6 -	y <sub>9</sub> <sup>+</sup> -	870.5 -	y <sub>7</sub> <sup>+</sup> -	613.4 -	y <sub>5</sub> <sup>+</sup> -	Gu et al., 2018 Chen et al., 2019
	DSYNLQSGDALR <i>Gly m 5.0201</i>	<i>Glycine max (3847)</i> <i>Glycine soja (3848)</i>	QqQ	669.8 (+2)	859.5	y <sub>8</sub> <sup>+</sup>	746.4	y <sub>7</sub> <sup>+</sup>	618.3	y <sub>6</sub> <sup>+</sup>	Gu et al., 2018
	DSYNLHPGDAQR <i>Gly m 5.0301</i> <i>Gly m 5.0302</i>	<i>Glycine max (3847)</i>	QqQ	458.2 (+3)	643.3	y <sub>6</sub> <sup>+</sup>	546.3	y <sub>5</sub> <sup>+</sup>	374.2	y <sub>3</sub> <sup>+</sup>	Gu et al., 2018
<b>Gly m 6 - Glycinin (Legumin, 11S Globulin)</b>	VFDGELQEGR <i>Gly m 6.0101</i>	<i>Glycine max (3847)</i>	QqQ QqQ QqQ QqQ QqQ Q-Orbitrap	575.2 (+2) 575.2 (+2) 575.3 (+2) 575.3 (+2) 575.3 (+2) 575.28 (+2)	903.2 903.2 903.2 788.4 788.4 -	y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> -	219.2 219.2 489.2 602.3 602.3 -	a <sub>2</sub> <sup>+</sup> a <sub>2</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> -	- - 788.5 789.4 - -	- - y <sub>7</sub> <sup>+</sup> b <sub>7</sub> <sup>+</sup> - -	Heick et al., 2011b Heick et al., 2011a Huschek et al., 2016 Planque et al., 2017b Planque et al., 2019 Chen et al., 2019
	SQSDNFEYVSFK <i>Gly m 6.0101</i> <i>Gly m 6.0201</i> <i>Gly m 6.0301</i>	<i>Glycine max (3847)</i>	QqQ QqQ Dual LIT QqQ Q-Orbitrap	725.7 (+2) 725.7 (+2) 725.8 (+2) 725.8 (+2) 725.83 (+2)	381.2 381.2 381.2 772.4 -	y <sub>3</sub> <sup>+</sup> y <sub>3</sub> <sup>+</sup> y <sub>3</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> -	1235.4 1235.4 716.8 643.3 -	y <sub>10</sub> <sup>+</sup> y <sub>10</sub> <sup>+</sup> [M+2H] <sup>++</sup> -H <sub>2</sub> O y <sub>5</sub> <sup>+</sup> -	- - 1235.4 480.3 -	- - y <sub>10</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> -	Heick et al., 2011a Heick et al., 2011b Piolli et al., 2017a Gu et al., 2018 Chen et al., 2019
	ISTLNSLTLPALR <i>Gly m 6.0401</i> <i>Gly m 6.0501</i>	<i>Glycine max (3847)</i> <i>Glycine soja (3848)</i> <i>Glycine microphylla (45693)</i>	QqQ QqQqQqQ Q-Orbitrap	699.9 (+2) 699.9 (+2) 699.9 (+2) 699.92 (+2)	984.6 984.6 1097.7 -	y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>10</sub> <sup>+</sup> -	870.5 870.5 670.4 -	y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> -	783.5 783.5 456.4 -	y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> -	Planque et al., 2016 Planque et al., 2017b Montowska et al., 2019 Chen et al., 2019
	FYLAGNQEQLFK <i>Gly m 6.0101</i> <i>Gly m 6.0201</i>	<i>Glycine max (3847)</i>	Dual LIT QqQ	793.9 (+2) 793.9 (+2)	638.8 638.8	y <sub>11</sub> <sup>++</sup> y <sub>11</sub> <sup>++</sup>	1163.6 424.2	y <sub>10</sub> <sup>+</sup> b <sub>3</sub> <sup>+</sup>	1092.5 283.1	y <sub>9</sub> <sup>+</sup> a <sub>2</sub> <sup>+</sup>	Piolli et al., 2017a Hoffmann et al., 2017
	INTVNSNTLPVLR <i>Cor a 9.0101 (Q8W1C2)</i> <i>Cor a 9 (A0A0A0P7E3)</i>	<i>Corylus avellana (13451)</i>	QqQ QqQ QqQ QqQ QqQ QqQ LIT-Orbitrap	720.9 (+2) 720.9 (+2) 721.1 (+2) 721.1 (+2) 720.9 (+2) 720.9 (+2) 720.9 (+2)	1013.6 1013.6 1013.7 1013.7 1013.6 1013.6 -	y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> -	484.4 484.4 484.4 484.4 899.5 484.3 -	y <sub>4</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> -	- - 228.2 228.2 812.5 - -	- - b <sub>2</sub> <sup>+</sup> b <sub>2</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> - -	Heick et al., 2011a Heick et al., 2011b Ansari et al., 2012 Costa et al., 2014 Planque et al., 2017b New et al., 2018 Korte et al., 2016*
	ALPDDVLANAFQISR <i>Cor a 9.0101 (Q8W1C2)</i> <i>Cor a 9 (A0A0A0P7E3)</i>	<i>Corylus avellana (13451)</i>	QqQ QqQ QqQ QqQ Dual LIT Q-Orbitrap QqQ QqQ	815.5 (+2) 815.5 (+2) 815.6 (+2) 815.6 (+2) 815.4 (+2) 815.4 (+2) 815.4 (+2) 815.4 (+2)	906.6 906.6 906.5 906.5 906.5 - 906.5 906.5	y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> - y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup>	1019.5 1019.5 185.2 185.2 835.4 - 1019.6 1019.6	y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup> b <sub>2</sub> <sup>+</sup> b <sub>2</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> - y <sub>9</sub> <sup>+</sup> y <sub>9</sub> <sup>+</sup>	- - 175.2 175.2 723.4 - 723.4 -	- - y <sub>1</sub> <sup>+</sup> y <sub>1</sub> <sup>+</sup> y <sub>13</sub> <sup>++</sup> - y <sub>13</sub> <sup>++</sup> -	Heick et al., 2011a Heick et al., 2011b Ansari et al., 2012 Costa et al., 2014 Piolli et al., 2017a Piolli et al., 2018 Planque et al., 2017b Planque et al., 2019
ADIYTEQVGR <i>Cor a 9.0101 (Q8W1C2)</i> <i>Cor a 9 (A0A0A0P7E3)</i>	<i>Corylus avellana (13451)</i>	QqQ QqQ LIT LIT Dual LIT QqQ QqQ Q-Orbitrap QqQ LIT-Orbitrap QqQ	576.3 (+2) 576.3 (+2) 577.0 (+2) 577.0 (+2) 576.3 (+2) 576.3 (+2) 576.3 (+2) 576.3 (+2) 576.3 (+2) 576.3 (+2) 576.3 (+2) 576.3 (+2)	689.4 689.4 689.0 689.0 689.4 689.4 689.4 689.4 689.4 689.4 689.4	y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup>	852.5 852.5 567.0 567.0 567.3 852.4 852.4 - 852.4 - 588.3	y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> [M+2H] <sup>++</sup> -H <sub>2</sub> O [M+2H] <sup>++</sup> -H <sub>2</sub> O [M+2H] <sup>++</sup> -H <sub>2</sub> O y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> - y <sub>7</sub> <sup>+</sup> - y <sub>5</sub> <sup>+</sup>	- - - - 852.5 588.3 588.3 - - - -	- - - - y <sub>7</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> y <sub>5</sub> <sup>+</sup> - - -	Heick et al., 2011a Heick et al., 2011b Bignardi et al., 2010 Bignardi et al., 2013 Piolli et al., 2017a Planque et al., 2017b Gu et al., 2018 Piolli et al., 2018 New et al., 2018 Korte et al., 2016a Planque et al., 2019	
QQQVLTIPQNFVAK <i>Cor a 9.0101 (Q8W1C2)</i> <i>Cor a 9 (A0A0A0P7E3)</i>	<i>Corylus avellana (13451)</i>	QqQ QqQ QqQ QqQ QqQ Q-Orbitrap	807.5 (+2) 807.5 (+2) 807.8 (+2) 807.8 (+2) 807.5 (+2) 807.5 (+2)	1088.6 1088.6 874.6 874.6 1088.6 -	y <sub>10</sub> <sup>+</sup> y <sub>10</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> y <sub>10</sub> <sup>+</sup> -	874.6 874.6 186.2 186.2 437.7 -	y <sub>8</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup> b <sub>2</sub> <sup>+</sup> b <sub>2</sub> <sup>+</sup> y <sub>8</sub> <sup>++</sup> -	314.2 314.2 - - -	b <sub>3</sub> <sup>+</sup> b <sub>3</sub> <sup>+</sup> - -	Heick et al., 2011a Heick et al., 2011b Ansari et al., 2012 Costa et al., 2014 Korte & Brockmeyer, 2016b Piolli et al., 2018	

			QqQ LIT-Orbitrap QqQ QqQ	807.5 (+2) 807.5 (+2) 807.5 (+2)	1088.6 - 1088.6	y <sub>10</sub> <sup>+</sup> - y <sub>10</sub> <sup>+</sup>	987.6 - 874.5	y <sub>9</sub> <sup>+</sup> - y <sub>8</sub> <sup>+</sup>	874.5 - -	y <sub>8</sub> <sup>+</sup> - -	Planque et al., 2017b Korte et al., 2016a Planque et al., 2019 Korte et al. 2019
<b>Cor a 11 - 7S Seed Storage Globulin (Vicilin-Like)</b>	LLSGIENFR <i>Cor a 11.0101</i>	<i>Corylus avellana (13451)</i>	QqQ QqQ QqQ	524.9 (+2) 524.9 (+2) 524.8 (+2)	822.4 822.4 822.4	y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup>	565.4 565.4 227.2	y <sub>4</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> b <sub>2</sub> <sup>+</sup>	199.3 199.3 -	a <sub>2</sub> <sup>+</sup> a <sub>2</sub> <sup>+</sup> -	Ansari et al., 2012 Costa et al., 2014 New et al., 2018
	AFSWEVLEAALK <i>Cor a 11.0101</i>	<i>Corylus avellana (13451)</i>	QqQ QqQ QqQ	682.7 (+2) 682.7 (+2) 682.4 (+2)	644.4 644.4 872.5	y <sub>6</sub> <sup>+</sup> y <sub>6</sub> <sup>+</sup> y <sub>8</sub> <sup>+</sup>	402.3 402.3 743.5	y <sub>4</sub> <sup>+</sup> y <sub>4</sub> <sup>+</sup> y <sub>7</sub> <sup>+</sup>	191.3 191.3 -	a <sub>2</sub> <sup>+</sup> a <sub>2</sub> <sup>+</sup> -	Ansari et al., 2012 Costa et al., 2014 New et al., 2018
	QQGQEQQQER <i>Pru du 6.0101</i>	<i>Prunus dulcis (3755)</i>	LIT	694.0 (+2)	677.0		685.0	[M+2H] <sup>++</sup> -H <sub>2</sub> O	-	-	-
<b>Pru du 6 - Amandin, 11S Globulin Legumin-Like Protein</b>	TDENGFTNTLAGR <i>Pru du 6.0201</i>	<i>Prunus dulcis (3755)</i>	QqQ LIT-Orbitrap QqQ	698.3 (+2) 698.3 (+2) 698.3 (+3)	936.5 - 879.5	y <sub>9</sub> <sup>+</sup> - y <sub>8</sub> <sup>+</sup>	879.5 - 732.4	y <sub>8</sub> <sup>+</sup> - y <sub>7</sub> <sup>+</sup>	732.4 - -	y <sub>7</sub> <sup>+</sup> - -	Planque et al., 2017b Korte et al., 2016a Planque et al., 2019