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

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ABSTRACT

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

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

Keywords: food allergens, mass spectrometry, peptide markers, ThRAI, incurred foods.

1. Introduction

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

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 constraints 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, NCBI nr.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 arise 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 ± 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 (α S1-casein, α S2-casein, β -casein, κ -casein),
264 while the soluble fraction constitutes the serum characterized by whey proteins (β -lactoglobulin,
265 α -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 β -lactoglobulin
272 and α -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 α 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 β -casein (Bos d 11), α S2-casein
290 (Bos d 10), and κ -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 β -lactoglobulin (β -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. β -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 α -lactalbumin (VGINYWLAHK, FLDDDLTDDIMCVK,
301 DDQNP HSSNICNISC 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,

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

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

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

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), β -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, β -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). β -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 β -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 α (67 kDa), α' (71 kDa) and β (50 kDa), and were assessed to combine at
467 different ratios to form the multimeric complexes. The α and α' subunits are approximately 82%
468 identical, whereas the β -subunit is only 76% identical to the two α subunits. The individual

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

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

genes encode the monomeric subunits (Nielsen et al., 1989) and the Gly m 6 allergen can be an arrangement of these five proteins, which have individual IgE binding properties (Holzhauser et al., 2009). Each monomer consists of two specific polypeptide chains, one acidic (40 kDa) (A) and one basic (20 kDa) (B), linked together by disulfide bonds. Each monomer can be one of five subunits, namely glycinin G1 (A_{1a}B_{1b}), glycinin G2 (A₂B_{1a}), glycinin G3 (A_{1b}B_{1a}), glycinin G4 (A₅A₄B₃) and glycinin (A₃B₄) (Wang et al., 2014), being renamed as five isoallergens Gly m 6.0101, Gly m 6.0201, Gly m 6.0301, Gly m 6.0401 and Gly m 6.0501, respectively, in 2009. Similarly to what was already discussed for the quantification of Gly m 5 allergen, the unpredictable composition of the Gly m 6 hexameric complex makes general conversion factors a hard task to achieve. The peptide marker selection carried out so far in current literature completely neglected the different sequence expression in known isoallergens. Indeed, the most frequently used markers, VFD, VLI, and EAF, are expressed uniquely in Gly m 6.0101, whereas the peptide SQS, cited in 8 independent investigations, is conserved across the three isoforms Gly m 6.0101, Gly m 6.0201, Gly m 6.0301, and the peptide IST (cited in 7 independent investigations) is common to the isoforms Gly m 6.0401 and Gly m 6.0501 (see Fig. S5).

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

Hazelnut and almond allergens were selected in this project as most relevant members of the tree nut category according to the European legislation. Similar to peanut and soybean, the main proteins involved in tree nut allergy belong to cupin (legumins-11S globulin and vicilin-7S globulin) and prolamin (conglutin-2S albumin and ns-LTPs) superfamilies. Several hazelnut allergens have been identified and are already partly applied in component resolved diagnosis (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 α -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

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

2.4. List of candidate markers agreed by ThRAI partners

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

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 19th 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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FIGURES AND TABLES

Figure 1 (color online version only)

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

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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 Allergome	
					Ara h 2.0201	Yes	AAN77576	
					Ara h 2.0202	No	see Allergome	
	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
	Profilin	Cor a 2	14	NA	Cor a 1.0404	Yes	AAG40331	Q9FPK2
					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
Almond	Oleosin	Cor a 12	17	NA	Cor a 11.0101	Yes	AAL86739	Q8S4P9
	Oleosin	Cor a 13	14-16	NA	Cor a 12.0101	Yes	AAO67349	Q84T21
	2S albumin (Prolamin)	Cor a 14	10	NA	Cor a 13.0101	Yes	AAO65960	Q84T91
	Non-specific lipid transfer protein 1 (Prolamin)	Pru du 3	9	Disulfide bond	Cor a 14.0101	Yes	ACO56333	D0PWG2
	Profilin	Pru du 4	14	NA	Pru du 3.0101	Yes	ACN11576	C0L0I5
	60s acidic ribosomal prot. P2	Pru du 5	10	NA	Pru du 4.0101	Yes	AAL91662	Q8GSL5
					Pru du 4.0102	Yes	AAL91664	Q8GSL5
					Pru du 5.0101	Yes	ABH03379	Q8H2B9
					Pru du 6.0101	Yes	ADN39440	E3SH28
						No	CAA55009	Q43607
Soybean	Legumin-like, Amandin, 11S globulin (Cupin)	Pru du 6	ca. 360	Disulfide bond	Pru du 6.0201	Yes	ADN39441	E3SH29
					Pru du 6.02 variant	No	CAA55010	Q43608
					Pru du 6.03 variant	No	CAA55010	Q43608
	Profilin	Gly m 3	14	Initial Met Removal	Gly m 3.0101	Yes	CAA11756	O65809
	Pathogenesis-related protein, PR-10, (Bet v 1 like)	Gly m 4	17	NA	Gly m 3.0102	Yes	CAA11755	O65810
					Gly m 4.0101	Yes	CAA42646	P26987
					Gly m 5.0101	Yes	BAA23360	Q22120
	Vicilin, β-conglycinin, 7S globulin (Cupin)	Gly m 5	63	NA	Gly m 5.0201	Yes	BAA74452	Q9FZP9
			65	n° 1 Asn glycosylation	Gly m 5.0301	Yes	AAB23463	P25974 (variant F36L V51G F197L)
			48		Gly m 5.0302	Yes		P25974 (variant V51G)
			48	n° 1 Asn glycosylation		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
Bos d 9 – α-S1-Casein	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 ₈ ⁺	676.6	y ₆ ⁺	267.3	a ₂ ⁺	Ansari et al., 2011
			QqQ	692.9 (+2)	920.3	y ₈ ⁺	991.3	y ₉ ⁺	-	-	Heick et al., 2011a
			QqQ	692.9 (+2)	920.3	y ₈ ⁺	991.3	y ₉ ⁺	-	-	Heick et al., 2011b
			IT	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	1090.6	y ₁₀ ⁺	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 ₈ ⁺	676.4	y ₆ ⁺	295.2	b ₂ ⁺	Monaci et al., 2010b
			Dual LIT	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	-	-	Monaci et al., 2014
			QqQ	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	1090.6	y ₁₀ ⁺	Newsome & Scholl, 2013
			QqQ	692.9 (+2)	920.5*	y ₈ ⁺	991.5	y ₉ ⁺	1090.6	y ₁₀ ⁺	Parker et al., 2015
			Dual LIT / Orbitrap	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	-	-	Pilolli et al., 2014
			Dual LIT	692.9 (+2)	920.5*	y ₈ ⁺	991.5	y ₉ ⁺	545.8	y ₁₀ ⁺⁺	Pilolli et al., 2017a
			QqQ	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	676.4	y ₆ ⁺	Planque et al., 2016
			QqQ	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	676.4	y ₆ ⁺	Planque et al., 2017a
			QqQ	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	676.4	y ₆ ⁺	Planque et al., 2017b
			QqQ	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	676.4	y ₆ ⁺	Gu et al., 2018
			QqQ	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	1090.6	y ₁₀ ⁺	Boo et al., 2018
			QqQ	692.9 (+2)	920.5	y ₈ ⁺	-	-	-	-	Groves et al., 2018
			QqQ	692.9 (+2)	295.1*	b ₂ ⁺	394.2	b ₃ ⁺	-	-	Ke et al., 2017
			Q-Orbitrap	692.9 (+2)	-	-	-	-	-	-	Pilolli et al., 2018
			Dual LIT	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	1090.6	y ₁₀ ⁺	De Angelis et al., 2017b
			QqQ	692.9 (+2)	920.5	y ₈ ⁺	991.5	y ₉ ⁺	-	-	Planque et al., 2019
			QqQ	692.9 (+2)	460.8*	y ₈ ⁺⁺	496.3	y ₉ ⁺⁺	-	-	Qi et al., 2019
			QqQ	692.8 (+2)	1090.6	y ₁₀ ⁺	676.4	y ₆ ⁺	450.3	y ₄ ⁺	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 ₈ ⁺	771.8	y ₆ ⁺	249.4	a ₂ ⁺	Ansari et al., 2011
			QqQ	634.3 (+2)	991.3	y ₈ ⁺	249.2	a ₂ ⁺	-	-	Heick et al., 2011a
			QqQ	634.3 (+2)	991.3	y ₈ ⁺	249.2	a ₂ ⁺	-	-	Heick et al., 2011b
			IT	634.4 (+2)	991.6	y ₈ ⁺	771.5	y ₆ ⁺	658.4	y ₅ ⁺	Lamberti et al., 2016
			IT	1267.6 (+1)	-	-	-	-	-	-	Losito et al., 2013
			LIT	634.8 (+2)	991.8	y ₈ ⁺	771.4	y ₆ ⁺	-	-	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 ₈ ⁺	771.5	y ₆ ⁺	249.2	a ₂ ⁺	Monaci et al., 2010b
			Dual LIT	634.4 (+2)	991.6	y ₈ ⁺	771.5	y ₆ ⁺	-	-	Monaci et al., 2014
			QqQ	634.4 (+2)	991.6	y ₈ ⁺	771.5	y ₆ ⁺	-	-	Newsome & Scholl, 2013
			QqQ	634.4 (+2)	991.6*	y ₈ ⁺	771.5	y ₆ ⁺	658.4	y ₅ ⁺	Parker et al., 2015
			Dual LIT / Orbitrap	634.4 (+2)	991.6	y ₈ ⁺	771.5	y ₆ ⁺	-	-	Pilolli et al., 2014
			Dual LIT	634.4 (+2)	991.6	y ₈ ⁺	771.5	y ₆ ⁺	658.4	y ₅ ⁺	Pilolli et al., 2017a
			QqQ	634.4 (+2)	934.5	y ₇ ⁺	771.5	y ₆ ⁺	658.4	y ₅ ⁺	Planque et al., 2016
			QqQ	634.4 (+2)	934.5	y ₇ ⁺	771.5	y ₆ ⁺	658.4	y ₅ ⁺	Planque et al., 2017b
			QqQ-ToF	634.6 (+2)	991.3	y ₈ ⁺	249.2	a ₂ ⁺	-	-	Ji et al., 2017
			QqQ	634.4 (+2)	991.6	y ₈ ⁺	771.5	y ₆ ⁺	658.4	y ₅ ⁺	Gu et al., 2018
			QqQ	634.4 (+2)	991.6	y ₈ ⁺	771.5	y ₆ ⁺	658.4	y ₅ ⁺	Boo et al., 2018
			QqQ	634.4 (+2)	991.6	y ₈ ⁺	249.2	a ₂ ⁺	-	-	New et al., 2018
			QqQ	634.4 (+2)	991.6	y ₈ ⁺	-	-	-	-	Groves et al., 2018
			Q-Orbitrap	634.4 (+2)	-	-	-	-	-	-	Pilolli et al., 2018
			Dual LIT	634.4 (+2)	991.6	y ₈ ⁺	771.5	y ₆ ⁺	658.4	y ₅ ⁺	De Angelis et al., 2017b
			QqQ	634.4 (+2)	934.5	y ₇ ⁺	771.5	y ₆ ⁺	-	-	Planque et al., 2019
			QqQ	634.4 (+2)	552.8	y ₉ ⁺⁺	249.1	b ₄ ⁺⁺	-	-	Qi et al., 2019

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

Gal d 4 - Lysozyme C			Dual LIT	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1121.5	y ₁₀ ⁺	732.4	y ₇ ⁺	Pilolli et al., 2017a
			QqQ	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1121.5	y ₁₀ ⁺	1331.7	y ₁₂ ⁺	Planque et al., 2016
			Q-ToF	844.4 (+2)	-	-	-	-	-	-	Tolin et al., 2012b
			QqQ	844.4 (+2)	666.3	y ₁₂ ⁺⁺	-	-	-	-	Planque et al., 2017a
			QqQ	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1121.5	y ₁₀ ⁺	1331.7	y ₁₂ ⁺	Planque et al., 2017b
			QqQ	844.4 (+2)	1007.5	y ₉ ⁺	1121.5	y ₁₀ ⁺	860.4	y ₈ ⁺	Boo et al., 2018
			Dual LIT	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1121.5	y ₁₀ ⁺	732.4	y ₇ ⁺	Pilolli et al., 2017b
			QqQ	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1121.5	y ₁₀ ⁺	-	-	New et al., 2018
			Q-Orbitrap	844.4 (+2)	-	-	-	-	-	-	Pilolli et al., 2018
			Dual LIT	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1121.5	y ₁₁ ⁺	1331.7	y ₁₂ ⁺	De Angelis et al., 2017b
			QqQ	844.4 (+2)	666.3	y ₁₂ ⁺⁺	1121.5	y ₁₁ ⁺	-	-	Planque et al., 2019
	HIATNAVLFFGR	<i>Gallus gallus</i> (9031)	Q-ToF	-	-	-	-	-	-	-	Azarnia et al., 2013
	Gal d 2.0101	<i>Alcaligenes xylosoxydans</i> (85698)	QqQ	673.4 (+2)	1095.6	y ₁₀ ⁺	223.2	a ₂ ⁺	-	-	Heick et al., 2011a
			QqQ	673.4 (+2)	1095.6	y ₁₀ ⁺	223.2	a ₂ ⁺	-	-	Heick et al., 2011b
			Orbitrap	673.4 (+2)	-	-	-	-	-	-	Monaci et al., 2013
Gal d 4 - Lysozyme C			QqQ	449.3 (+3)	639.4*	y ₅ ⁺	526.3	y ₄ ⁺	608.3	b ₆ ⁺	Parker et al., 2015
			Q-ToF	-	-	-	-	-	-	-	Tolin et al., 2012°
	ISQAVHAAHAEINEAGR	<i>Gallus gallus</i> (9031)	Q-ToF	-	-	-	-	-	-	-	Azarnia et al., 2013
	Gal d 2.0101	<i>Coturnix japonica</i> (93934)	Orbitrap	887.4 (+2)	-	-	-	-	-	-	Monaci et al., 2013
		<i>Alcaligenes xylosoxydans</i> (85698)	QqQ	887.5 (+2)	1138.6	y ₁₁ ⁺	1067.5	y ₁₀ ⁺	996.5	y ₉ ⁺	Planque et al., 2016
			QqQ	887.5 (+2)	1138.6	y ₁₁ ⁺	1067.5	y ₁₀ ⁺	996.5	y ₉ ⁺	Planque et al., 2017b
	FESNFNTQATNR	<i>Gallus gallus</i> (9031)	QqQ	714.8 (+2)	277.1	b ₂ ⁺	-	-	-	-	Cryar et al., 2013
	Gal d 4.0101	<i>Coturnix japonica</i> (93934)	Orbitrap	714.8 (+2)	-	-	-	-	-	-	Monaci et al., 2013
		<i>Rattus norvegicus</i> (10116)	QqQ	714.8 (+2)	1152.5	y ₁₀ ⁺	951.5	y ₈ ⁺	804.4	y ₇ ⁺	Parker et al., 2015
		<i>Gallus lafayetii</i> (9032)	QqQ	714.8 (+2)	589.3	y ₅ ⁺	690.4	y ₆ ⁺	-	-	Pilolli et al., 2014
		<i>Gallus sonneratii</i> (9033)	QqQ	714.8 (+2)	1152.5	y ₁₀ ⁺	951.5	y ₈ ⁺	804.4	y ₇ ⁺	Boo et al., 2018
	NTDGSTDYQILQINSR	<i>Gallus gallus</i> (9031)	Orbitrap	877.4 (+2)	-	-	-	-	-	-	Monaci et al., 2013
	Gal d 4.0101	<i>Anas platyrhynchos</i> (8839)	QqQ	585.3 (+3)	489.3	y ₄ ⁺	617.3	y ₅ ⁺	730.4	y ₆ ⁺	Parker et al., 2015
		<i>Catreus wallichii</i> (9085)	Dual LIT / Orbitrap	877.4 (+2)	489.3	y ₄ ⁺	617.3	y ₅ ⁺	-	-	Pilolli et al., 2014
		<i>Chrysolophus amherstiae</i> (9088)	Dual LIT	877.4 (+2)	489.3	y ₄ ⁺	617.3	y ₅ ⁺	730.4	y ₆ ⁺	De Angelis et al., 2017b
Vitellogenin-1	YLLDLLPAAASHR	<i>Gallus gallus</i> (9031)	QqQ	480.6 (+3)	709.4	y ₇ ⁺	582.3	y ₁₁ ⁺⁺	355.2	y ₇ ⁺⁺	Planque et al., 2016
	(lipovitellin-1 chain)		QqQ	480.6 (+3)	709.4	y ₇ ⁺	582.3	y ₁₁ ⁺⁺	355.2	y ₇ ⁺⁺	Planque et al., 2017b
			QqQ	480.6 (+3)	709.4	y ₇ ⁺	355.2	y ₇ ⁺⁺	-	-	New et al., 2018
			QqQ	480.6 (+3)	709.4	y ₇ ⁺	582.3	y ₁₁ ⁺⁺	-	-	Planque et al., 2019
	ALLLSEIR	<i>Gallus gallus</i> (9031)	QqQ	457.8 (+2)	617.4	y ₅ ⁺	730.5	y ₆ ⁺	-	-	New et al., 2018
	(lipovitellin-1 chain)	<i>Fundulus heteroclitus</i> (8078)									

		<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 ₉ ⁺ y ₉ ⁺ y ₉ ⁺	508.3 508.3 558.3	y ₄ ⁺ y ₄ ⁺ y ₉ ⁺⁺	558.3 558.3 -	y ₉ ⁺⁺ y ₉ ⁺⁺ -	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>Anstroctomus 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 ₇ ⁺ y ₇ ⁺ y ₇ ⁺	674.4 674.4 228.1	y ₆ ⁺ y ₆ ⁺ b ₂ ⁺	545.3 545.3 -	y ₅ ⁺ y ₅ ⁺ -	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 ₉ ⁺⁺	725.4	y ₇ ⁺	-	-	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>Antrusotomus carolinensis</i> (279965) <i>Chlamydotis macqueenii</i> (187382) <i>Colinus virginianus</i> (9014) <i>Opisthocomus hoazin</i> (30419)									
Ara h 1 Cupin (Vicillin-Type, 7S Globulin)	DLAFPGSGEQVEK Ara h 1.0101 (clone P41B) Ara h 1 - clone P17	<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 ₉ ⁺ y ₉ ⁺ y ₉ ⁺ y ₉ ⁺ y ₉ ⁺ y ₉ ⁺ y ₉ ⁺ y ₉ ⁺	- 300.2 300.2 833.4 833.4 833.4 300.2 300.2 300.2 447.2	- a ₃ ⁺ a ₃ ⁺ y ₈ ⁺ y ₈ ⁺ y ₈ ⁺ b ₃ ⁺ b ₃ ⁺ b ₄ ⁺	- - - 1077.5 776.4 776.4 229.1 - -	- - - y ₁₀ ⁺ y ₇ ⁺ y ₇ ⁺ b ₂ ⁺ - -	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
	VLLEENAGGEQEER Ara h 1.0101 (clone P41B) Ara h 1 - clone P17	<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 ₇ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺ - y ₇ ⁺ - -	875.4 875.4 875.4 875.4 213.2 - 1118.5 - -	- y ₈ ⁺ y ₈ ⁺ y ₈ ⁺ b ₂ ⁺ - y ₁₀ ⁺ - -	989.4 989.4 680.8 989.4 989.4 - - - -	y ₉ ⁺ y ₉ ⁺ y ₁₂ ⁺⁺ y ₉ ⁺ y ₉ ⁺ - - - -	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 Ara h 1.0101 (clone P41B) Ara h 1 - clone P17	<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 ₆ ⁺ y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ -	557.5 557.5 557.4 557.4 557.4 -	y ₅ ⁺ y ₅ ⁺ y ₅ ⁺ y ₅ ⁺ y ₅ ⁺ -	- 799.5 444.3 799.5 -	- y ₇ ⁺ y ₄ ⁺ y ₇ ⁺ -	Heick et al., 2011a Heick et al., 2011b Parker et al., 2015 Gu et al., 2018 Boo et al., 2018 Pilolli et al., 2018
Ara h 2 - Conglutin (2S Albumin)	CCNELNEFENNQR Ara h 2.0101, Ara h 2.0201 Ara h 2.0202	<i>Arachis ipaensis</i> (130454) <i>Arachis hypogaea</i> (3818) <i>Arachis duranensis</i> (130453)	QqQ 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 ₈ ⁺ b ₇ ⁺ y ₆ ⁺ y ₉ ⁺ y ₉ ⁺ y ₉ ⁺	- 807.4 1050.5 1050.5 1050.5 1050.5	- y ₆ ⁺ y ₈ ⁺ y ₈ ⁺ y ₈ ⁺ y ₈ ⁺	- 660.3 660.3 936.4 936.4 936.4	- y ₅ ⁺ y ₅ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺	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 Ara h 2.0101, Ara h 2.0201 Ara h 2.0202	<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 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) 543.3 (+2) 543.8 (+2)	858.4 858.4 858.4 858.4 858.2 858.4 858.4 858.4 858.4 858.4 858.4	y ₇ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺ y ₇ ⁺	761.4 761.4 761.4 761.4 429.8 [*] 429.7 761.4 429.7 429.7 633.3	y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ y ₇ ⁺⁺ y ₇ ⁺⁺ y ₆ ⁺ y ₇ ⁺⁺ y ₇ ⁺⁺ y ₅ ⁺	633.3 633.3 633.3 633.3 - 633.3 633.3 - - -	y ₅ ⁺ y ₅ ⁺ y ₅ ⁺ y ₅ ⁺ - y ₅ ⁺ y ₅ ⁺ - - -	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
	LITLAIPVKNKGR Gly m 5.0101	<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 ₇ ⁺ y ₇ ⁺ y ₇ ⁺ -	583.4 [*] 583.4 583.4 -	y ₁₁ ⁺⁺ y ₁₁ ⁺⁺ y ₁₁ ⁺⁺ -	476.3 [*] 476.3 476.3 -	y ₉ ⁺⁺ y ₉ ⁺⁺ y ₉ ⁺⁺ -	Houston et al., 2011 Planque et al., 2016 Planque et al., 2017b Chen et al., 2019

Gly m 6 - Glycinin (Legumin, 11S Globulin)	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 ₉ ⁺ -	870.5 -	y ₇ ⁺ -	613.4 -	y ₅ ⁺ -	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 ₈ ⁺	746.4	y ₇ ⁺	618.3	y ₆ ⁺	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 ₆ ⁺	546.3	y ₅ ⁺	374.2	y ₃ ⁺	Gu et al., 2018
	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 ₈ ⁺ y ₈ ⁺ y ₈ ⁺ y ₇ ⁺ y ₇ ⁺ -	219.2 219.2 489.2 602.3 602.3 -	a ₂ ⁺ a ₂ ⁺ y ₄ ⁺ y ₅ ⁺ y ₅ ⁺ -	- - 788.5 789.4 - -	- - y ₇ ⁺ b ₇ ⁺ - -	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 ₃ ⁺ y ₃ ⁺ y ₃ ⁺ y ₆ ⁺ -	1235.4 1235.4 716.8 643.3 -	y ₁₀ ⁺ y ₁₀ ⁺ [M+2H] ⁺⁺ -H ₂ O y ₅ ⁺ -	- - 1235.4 480.3 -	- - y ₁₀ ⁺ y ₄ ⁺ -	Heick et al., 2011a Heick et al., 2011b Pilolli 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 ₉ ⁺ y ₉ ⁺ y ₁₀ ⁺ -	870.5 870.5 670.4 -	y ₈ ⁺ y ₈ ⁺ y ₆ ⁺ -	783.5 783.5 456.4 -	y ₇ ⁺ y ₇ ⁺ y ₄ ⁺ -	Planque et al., 2016 Planque et al., 2017b Montowska et al., 2019 Chen et al., 2019
	FYLAGNQEQEFLK <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 ₁₁ ⁺⁺ y ₁₁ ⁺⁺	1163.6 424.2	y ₁₀ ⁺ b ₃ ⁺	1092.5 283.1	y ₉ ⁺ a ₂ ⁺	Pilolli 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 ₉ ⁺ y ₉ ⁺ y ₉ ⁺ y ₉ ⁺ y ₉ ⁺ y ₉ ⁺ -	484.4 484.4 484.4 484.4 899.5 484.3 -	y ₄ ⁺ y ₄ ⁺ y ₄ ⁺ y ₄ ⁺ y ₈ ⁺ y ₄ ⁺ -	- - 228.2 228.2 812.5 - -	- - b ₂ ⁺ b ₂ ⁺ y ₇ ⁺ - -	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 ₈ ⁺ y ₈ ⁺ y ₈ ⁺ y ₈ ⁺ y ₈ ⁺ - y ₈ ⁺ y ₈ ⁺	1019.5 1019.5 185.2 185.2 835.4 - 1019.6 1019.6	y ₉ ⁺ y ₉ ⁺ b ₂ ⁺ b ₂ ⁺ y ₇ ⁺ - y ₉ ⁺ y ₉ ⁺	- - 175.2 175.2 723.4 - 723.4 -	- - y ₁ ⁺ y ₁ ⁺ y ₁₃ ⁺⁺ - y ₁₃ ⁺⁺ -	Heick et al., 2011a Heick et al., 2011b Ansari et al., 2012 Costa et al., 2014 Pilolli et al., 2017a Pilolli et al., 2018 Planque et al., 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 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)	689.4 689.4 689.0 689.0 689.4 689.4 689.4 689.4 - 689.4	y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ y ₆ ⁺ - y ₆ ⁺	852.5 852.5 567.0 567.0 567.3 852.4 852.4 852.4 - 852.4	y ₇ ⁺ y ₇ ⁺ [M+2H] ⁺⁺ -H ₂ O [M+2H] ⁺⁺ -H ₂ O [M+2H] ⁺⁺ -H ₂ O y ₇ ⁺ y ₇ ⁺ - y ₇ ⁺ -	- - - - 852.5 588.3 588.3 - - -	- - - - y ₇ ⁺ y ₅ ⁺ y ₅ ⁺ - - -	Heick et al., 2011a Heick et al., 2011b Bignardi et al., 2010 Bignardi et al., 2013 Pilolli et al., 2017a Planque et al., 2017b Gu et al., 2018 Pilolli 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 ₁₀ ⁺ y ₁₀ ⁺ y ₈ ⁺ y ₈ ⁺ y ₁₀ ⁺ -	874.6 874.6 186.2 186.2 437.7 -	y ₈ ⁺ y ₈ ⁺ b ₂ ⁺ b ₂ ⁺ y ₈ ⁺⁺ -	- 314.2 314.2 - -	b ₃ ⁺ b ₃ ⁺ - -	Heick et al., 2011a Heick et al., 2011b Ansari et al., 2012 Costa et al., 2014 Korte & Brockmeyer, 2016b Pilolli et al., 2018

			QqQ LIT-Orbitrap QqQ QqQ	807.5 (+2) 807.5 (+2) 807.5 (+2)	1088.6 - 1088.6	y ₁₀ ⁺ - y ₁₀ ⁺	987.6 - 874.5	y ₉ ⁺ - y ₈ ⁺	874.5 - -	y ₈ ⁺ - -	Planque et al., 2017b Korte et al., 2016a Planque et al., 2019 Korte et al. 2019
Cor a 11 - 7S Seed Storage Globulin (Vicilin-Like)	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 ₇ ⁺ y ₇ ⁺ y ₇ ⁺	565.4 565.4 227.2	y ₄ ⁺ y ₄ ⁺ b ₂ ⁺	199.3 199.3 -	a ₂ ⁺ a ₂ ⁺ -	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 ₆ ⁺ y ₆ ⁺ y ₈ ⁺	402.3 402.3 743.5	y ₄ ⁺ y ₄ ⁺ y ₇ ⁺	191.3 191.3 -	a ₂ ⁺ a ₂ ⁺ -	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] ⁺⁺ -H ₂ O	-	-	Bignardi et al., 2010
Pru du 6 - Amandin, 11S Globulin Legumin-Like Protein	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 ₉ ⁺ - y ₈ ⁺	879.5 - 732.4	y ₈ ⁺ - y ₇ ⁺	732.4 - -	y ₇ ⁺ - -	Planque et al., 2017b Korte et al., 2016a Planque et al., 2019