ISSN 1330-9862
(FTB-3165)

minireview

Allergenic Proteins in Foods and Beverages

Ana Barros[1]* *and Fernanda Cosme*[2]

[1]CITAB – Centre for the Research and Technology of Agro-Environmental and Biological Sciences, Chemistry Department, University of Trás-os-Montes and Alto Douro, PT-5001-801 Vila Real, Portugal

[2]IBB/CGB-UTAD – Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, School of Life Sciences and Environment, Department of Biology and Environment, University of Trás-os-Montes and Alto Douro, P.O. Box 1013, PT-5001-801 Vila Real, Portugal

Received: August 16, 2012
Accepted: February 25, 2013

Summary

Food allergies can be defined as immunologically mediated hypersensitivity reactions; therefore, a food allergy is also known as food hypersensitivity. The reactions are caused by the immune system response to some food proteins. The eight most common food allergens are proteins from milk, eggs, peanuts, tree nuts, soya, wheat, fish and shellfish. However, many other foods have been identified as allergens for some people, such as certain fruits or vegetables and seeds. It is now recognized that food allergens are an important food safety issue. A food allergy occurs when the body's immune system reacts to otherwise harmless substances in certain foods. For these reasons, one of the requirements from the European Union is that allergenic food ingredients should be labelled in order to protect allergic consumers. According to the European Federation of Allergy and Airways Diseases Patients' Associations, about 8 % of children and 4 % of adults suffer from some type of food allergy.

Food allergies often develop during infant or early childhood ages, affecting mainly the gastrointestinal tract (stomach and intestines). In some cases, the allergy may persist in adult age, for example, coeliac disease, which is an abnormal immune response to certain proteins present in gluten, a type of protein composite found in wheat and barley. Almost all allergens are proteins, and highly sensitive analytical methods have been developed to detect traces of these compounds in food, such as electrophoretic and immunological methods, enzyme-linked immunosorbent assay (ELISA) and polyacrylamide gel electrophoresis.

The purpose of this review is to describe the allergenic components of the most common causes of food allergies, followed by a brief discussion regarding their importance in the food industry and for consumer safety. The most important methods used to detect allergenicity in food will also be discussed.

Key words: food allergy, allergen identification, food proteins

Introduction

The term food allergy is commonly used for any adverse reaction, immediate and abnormal, to a harmless food or food component normally tolerated (*1,2*). The substances that cause this abnormal reaction of the defence (immune) system are called allergens (*1*). There are two different types of allergic food reactions involving the immune defence system, namely reactions that are immunoglobulin E (IgE)-mediated and non-IgE-mediated, or a combination of both (*3,4*). The majority are IgE-mediated, making the non-IgE-mediated reactions to food scarce (*5*). The incidence of allergies continues to increase year after year, with a higher incidence in developed

*Corresponding author; Phone: ++351 259 350 283; Fax: ++351 259 350 480; E-mail: abarros@utad.pt

countries (*6,7*) and, apparently, involves not only diet and environmental factors, but also the interaction between these and genetic factors (*8*).

Food allergies can have a significant impact on life quality in a profoundly negative way, restricting food choices and increasing the cost, thus causing anxiety (*9,10*). Allergy sufferers sometimes have difficulty in managing their social life, a fact which has an important effect on family relationships, since they frequently face isolation (*9–11*).

Most common foods are generally safe at all levels of intake for most people (in a percentage of almost 95 %), and are nutritionally valuable (*12*). Food allergens as contaminants are only dangerous for people with a specific allergy, and can even be lethal (*13*). In general, the rates and the prevalence of food allergy are not precise; nevertheless, even in small amounts, it can sometimes induce a wide variety of hypersensivity reactions (*10*). Recent studies have shown that approx. 1 in 20 children under the age of 5 and approx. 1 in 25 adults are allergic to one type of food at least (*1*).

During the last two decades, food allergies have been recognized as a rising problem for public health (*14*). It is, therefore, essential to have access to information about potential allergens contained in a food product. Consequently, methods for allergen detection are needed and legislation for food product labelling must be improved (*15*). The declaration of certain allergenic ingredients is obligatory and clearly defined in Directives 2003/89/EC (*16*) and 2005/26/EC (*17*).

Allergenicity of Food Proteins

Although any food protein can be potentially allergenic, only some proteins cause allergic reactions. Therefore, food allergies can be caused by various food proteins, those from animal origin, such as from milk (casein, β-lactoglobulin, α-lactalbumin), eggs (ovomucoid, ovalbumin, conalbumin, lysozyme), fish (parvalbumin), and shellfish (tropomyosin), or from plant origin such as peanuts (7S seed storage globulin, 11S seed storage globulin, 2S albumin), tree nuts (2S albumin, 7S storage globulin, 11S seed storage globulin, non-specific lipid transfer proteins, Bet v 1 homologue), soya (7S seed storage globulin, 11S seed storage globulin, Bet v 1 homologue, inactive papain-related thiol protease), seeds (2S albumin) and wheat (seed storage prolamins, α-amylase/trypsin inhibitors, glycosylated peroxidase) (*11,18*). Furthermore, an allergenic protein can only induce an allergic reaction in individuals sensitive to this allergen. Additionally, allergic incidence is dependent on the consumer's age; the foods related to allergic reactions in children are mostly eggs and milk; adults usually experience allergic reaction to the food that tends to continue beyond infancy, these are mostly caused by peanuts, tree nuts, seafood and fruit (*7,11*).

Since the allergy to cow's milk has become the most common allergy in early childhood, it is important to establish the role of each of the milk proteins in allergic reactions, but this is still a controversial topic (*19*). Milk proteins are composed of αs_1-casein, αs_2-casein, β-casein, κ-casein, β-lactoglobulin and α-lactalbumin (*20*). Although the importance of the most abundant proteins is recognized, those found in smaller quantities have a role in determining the extent of allergies. Casein fractions are well-known allergenic proteins of cow's milk, but in this case the identification of individual fractions responsible for this aspect is still controversial (*19*). In the case of eggs, the allergy is most frequent in children. Regarding these two allergenic foods, milk and eggs, the allergy can be outgrown during school age. Ovomucoid and ovalbumin represent 10 and 50 % of egg white proteins, respectively, and are the major egg allergens. In the egg white, lysozyme is also to be considered, for it represents a high risk due to its wide employment in the food industry (cheese preparations and wine) and large populations might be allergic to this enzyme (*21,22*).

In the case of fish, more than 20 proteins, mainly parvalbumins, have been classified as the major allergens. These proteins are found in various fish species such as cod, salmon, mackerel and herring (*23,24*). Consumers that are allergic to fish must avoid not only all kinds of fish, but also fish products, as even a low amount in their diet can cause an allergic reaction (*25*). The major allergenic protein in shellfish and seafood is tropomyosin, a heat-stable protein. It is found in various types of seafood, for example, in squids, lobsters, crabs and shrimps.

In the case of peanuts, contrary to other allergens such as milk or eggs, the allergic reaction tends to persist for life (*25*), and no significant differences have been observed among peanut varieties concerning allergic properties. Additionally, during production processes, contaminations with peanuts are common; therefore, undeclared peanut traces can be found in processed food products (*26*).

Food processing can change the allergenicity of certain food proteins, particularly from fruits and vegetables, which become less allergenic when processed; however, the allergenicity of other foods remains unchanged. These differences are related to the thermostability of the proteins involved in the allergic reactions, some of which are sufficiently changed by heating so that they no longer cause an allergic reaction. According to Paschke (*13*), the combination of enzymatic and heat treatment decreased the allergic potential of hen´s egg about 100-fold. Different food processing methods have diverse effects on food protein structure; therefore, some food processing methods may increase, decrease or have no effect on allergenicity of specific food proteins, since chemical or conformational changes can be induced during industrial food processing. The degree of maturity of some fruits and vegetables can also affect their level of allergenicity.

Allergies have increased in the last years, particularly in the developed countries. The main reason for the increase is that the human immune system is less exposed to infection agents during infancy, as a consequence of more hygienic sanitary environment and modern medical practices such as immunizations. Therefore, our immune system does not need to recognize and fight the infection agents, as it would have to do if it was exposed to them. This theoretical explanation is given in the 'hygiene hypothesis' (*27*).

Allergenicity of Protein Processing Aids Used in Beverages

Beverage production usually involves protein fining, one of the many processing techniques used to clarify and stabilize beverages. Proteins derived from bovine milk (casein and potassium caseinate), hen eggs (egg albumin and lysozyme), and fish (fish gelatine and isinglass from fish swim bladder) are used as processing aids. These proteins coagulate with the colloids present in the beverages, resulting in flocculation and sedimentation of these substances. They also eliminate the insoluble and unstable colloidal substances and thus improve the beverage sensorial properties. Phenolic compounds such as tannins and monomeric flavonols responsible for astringency or bitterness are also removed. Through the European Union (EU) legislation, Directives 2000/13/EC (*28*) and 2003/89/EC (*16*), the listing of all allergenic ingredients specified in the Annex IIIa that are used in processed food became mandatory in order to get a higher level of protection for allergic individuals. After that, the European Community issued Directive 2007/68/EC (*29*), which states that 'any substance used in production of a foodstuff and still present in the finished product' has to be declared on the label, especially if it originates from allergenic ingredients. This was initially mandated to take effect on 31 May 2009, but had been suspended until 30 June 2012 due to the limited scientific data available concerning their actual permanence as residual proteins (*30*,*31*).

Milk casein is a heterogeneous group of four major phosphoproteins and phosphoglycoproteins whose molecular mass (M_r) ranges from 11.6 to 24.1 kDa with an average isoelectric point (pI) of 4.6 (*32*,*33*). Likewise, egg white is composed of diverse proteins, with ovalbumin (phosphoglycoprotein) representing the main protein, having a M_r of 45 kDa and an isoelectric point of 4.6 (*34*,*35*). Isinglass, a product obtained from fish swim bladder, is used as fining agent, comprising three hydroxyproline-rich polypeptide chains in a helical conformation, mainly composed of collagen with a high molecular mass (M_r=300 kDa) (*36*). It seems that due to the anatomical location and tissue composition of the fish swim bladder of different species, isinglass probably does not contain the major allergenic fish protein, parvalbumin (M_r=10–13 kDa) (*37*). Lysozyme (M_r=14.3 kDa; pI=10.7) can also present a risk for consumers allergic to hen's egg (*22*,*35*). The major allergenic component of bovine milk is casein, as described by Docena *et al.* (*38*) and Lam *et al.* (*39*). Nevertheless, there is no indication of the existence of detectable casein residues in the final wines able to cause allergic reactions. It is known that casein is insoluble at the wine pH. Consequently, casein is considered to be totally coagulated and sedimented (*40*). Kirschner *et al.* (*41*) demonstrated that wines treated with fining agents containing proteins from egg, milk or fish in commercial concentrations were tolerated by consumers allergic to these proteins. Rolland *et al.* (*42*) investigated if wines treated with fining agents containing proteins from egg, milk or fish could incite allergic reactions (anaphylaxis) in consumers with confirmed immunoglobulin E–mediated food allergy. After performing a double-blind, placebo-controlled trial, these authors concluded that no allergic reaction was induced by the consumption of wine produced with the proteins mentioned previously, according to good manufacturing practice, which suggests that the traces of fining agents remaining in the wine after treatments are insignificant. Vassilopoulou *et al.* (*43*) also concluded that even if traces of residues of casein, isinglass or egg proteins are found in the treated wine, the risks for allergic consumers are very low. It is known that if the wine is manufactured according to good oenological practices, the amount of processing aids that remain in the finished wine is negligible. Nevertheless, the production of allergen-free food products has become important due to consumer safety concerns and new international labelling regulations. Therefore, research has been carried out in order to search for alternatives to allergenic proteins (*43*–*47*).

Methods to Detect the Allergenicity and Antigenicity of Protein Residues in Food and Beverages

The precise and sensitive methods for the detection and quantification of food allergens are essential to the food industry to guarantee the correct labelling of their products in order to protect allergic consumers. All substances purposely added to food products have to be labelled according to the European Food Labelling Directive. At the same time, in order to control allergens in foods, it is also important to know what quantity of an allergen can trigger an allergic reaction in an individual. However, the threshold at which all allergens can cause allergic reactions (lowest adverse effect level observed for peanuts: 0.25–10 mg of protein, soya: 88–522 mg of protein, tree nuts: 0.02–7.5 mg of protein, egg: 0.13–1 mg of protein, milk: 0.36-3.6 mg of protein, fish: 1–100 mg of protein (*48*)) is not well known; therefore, it is not clear how sensitive the detection methods need to be. Additionally, allergens frequently exist in trace quantities, making detection and quantification in food products difficult, and this difficulty is increased in processed food products as the food matrix frequently camouflages them.

To detect traces of allergens, highly sensitive analytical methods have been developed, using either enzyme immunoassay methods based on antibodies or, to a lesser extent, polymerase chain reaction (PCR) or the electrophoretic method (sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)) (*6*,*49*). Allergenic proteins able to induce allergic reaction in their native structural state or after chemical or conformational changes induced by the manufacturing treatments can nowadays be identified by using immunochemical methods such as enzyme-linked immunosorbent assay (ELISA). Sandwich and competitive ELISA methods, and dipstick assays or lateral-flow devices (LFD) have been developed for several food allergens (*37*,*50*). Concerning the detection of peanut, tree nut and soya bean proteins, real time PCR and sandwich ELISA methods have been developed with a limit of detection lower than 10 ppm for peanuts in processed food (*26*). For allergenic fish protein, electrophoretic techniques, ELISA, PCR and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) are currently used

(*37*). Regarding wine samples, the method of widespread use for the detection of fining proteins is based on antibody recognition. Antibody-based ELISA is commercially used for different allergenic targets, and this method has the advantage of being fast and usually appropriate for routine analysis. However, this technique suffers from numerous restrictions since the target protein is indirectly detected. In numerous matrices antibodies recognize analogous structures not important for food allergy, which give a positive and indistinguishable signal from those of the target allergen. This is called cross-reactivity and can lead to false negative results (*51*). Several ELISA test formats have recently been developed for detection of casein, wheat gluten, parvalbumin, peanut and ovalbumin residues (*37,40,52–56*), with the lowest limit of detection equal to 8 ng/mL for casein, 100 ng/mL for parvalbumin, 8 ng/mL for peanut and 1 ng/mL for ovalbumin (*52*). A quantitative indirect ELISA method for the determination of casein, in the range of 0.01–10 mg/L, has been reported by Weber *et al.* (*54*). Lifrani *et al.* (*53*) developed animal models with allergy to ovalbumin, caseinate and isinglass, and also designed sandwich ELISA tests specific to each protein, with the purpose of detecting their residue antigenicity. A weakness of ELISA test kits is that they only detect one allergen in each test. Nowadays, several authors have developed procedures for the identification of allergenic proteins in food samples using mass spectrometry (MS), expecting to overcome certain limitations of the immunochemical assays (*56*). The advantages of MS over the ELISA method are that it is a direct detection method and can detect multiple allergens in the same analysis. Monaci and van Hengel (*57*) developed a method using solid-phase extraction and liquid chromatography coupled with mass spectrometry to detect traces of three allergenic cow's milk proteins (lactalbumin and lactoglobulins α and β) in mixed fruit juice samples. The same authors developed, for the first time, a method based on capillary liquid chromatography combined with electrospray ionization tandem mass spectrometry (capLC–ESI-MS/MS) for the detection and identification of casein-derived peptides in fined white wine (*58,59*). More recently, a method based on LC-ESI-high-resolution (HR)/MS analysis, using a single-stage Orbitrap mass spectrometer, for the quantification of casein allergens (*60*) has been presented. Heick *et al.* (*15*) developed a multi-method for the simultaneous detection of seven allergens (milk, egg, soya, hazelnut, peanut, walnut and almond) based on liquid chromatography and triple quadrupole tandem mass spectrometry in a multiple reaction mode with a detection concentration ranging from 10 to 1000 mg/g. At the same time, other authors (*61*) adopted the combinatorial peptide ligand library (CPLL) technology for the identification of casein traces present in white wines. The authors demonstrated that the detection limit of this technique for casein is around 1 μg/L (*62*). A fast detection method without sample preparation was developed by Zhou *et al.* (*63*) using an extractive electrospray ionization mass spectrometry to determine the traces of egg lysozyme present in white wine. With this method it is possible to detect lysozyme at 5 μg/mL, this concentration being lower than the quantity required to cause an allergic reaction.

Conclusions

Food allergies result from the reactions that are caused by the immune system response to some food proteins. The most common allergenic proteins are from milk, eggs, peanuts, tree nuts, soya, wheat, fish and shellfish. To guarantee the safety of allergic consumers, the European Union requires labelling of all allergenic food ingredients. For that reason, highly sensitive and expeditious analytical methods have been developed to detect traces of these compounds in food.

References

1. J.A. Boyce, A. Assa'ad, A.W. Burks, S.M. Jones, H.A. Sampson, R.A. Wood *et al.*, Guidelines for the diagnosis and management of food allergy in the United States: Report of the NIAID-sponsored expert panel, *J. Allergy Clin. Immunol. 126* (2010) S51–S58.
2. J.J.S. Chafen, S.J. Newberry, M.A. Riedl, D.M. Bravata, M. Maglione, M.J. Suttorp *et al.*, Diagnosing and managing common food allergies: A systematic review, *JAMA, 303* (2010) 1848–1856.
3. A. Fiocchi, H.J. Schünemann, J. Brozek, P. Restani, K. Beyer, R. Troncone *et al.*, Diagnosis and Rationale for Action Against Cow's Milk Allergy (DRACMA): A summary report, *J. Allergy Clin. Immunol. 126* (2010) 1119–1128.
4. A. Urisu, M. Ebisawa, T. Mukoyama, A. Morikawa, N. Kondo, Japanese guideline for food allergy, *Allergol. Int. 60* (2011) 221–236.
5. N.H. Eshuis: *Adverse Reactions to Food*, European Federation of Asthma and Allergy Associations, Leusden, The Netherlands (1997).
6. J. Ring, K. Brockow, H. Behrendt, Adverse reactions to foods, *J. Chromatogr. B: Biomed. Sci. Appl. 756* (2011) 3–10.
7. H.A. Sampson, Update on food allergy, *J. Allergy Clin. Immunol. 113* (2004) 805–819.
8. J. Shimada, H. Yano, K. Mizumachi, Trends in food allergy research, *Sci. Tech. Trends*, *16* (2005) 26–35.
9. M. Fernández-Rivas, S. Miles: Food Allergies: Clinical and Psychosocial Perspectives. In: *Plant Food Allergens*, E.N.C. Mills, P.R. Shewry (Eds.), Blackwell Publishing Ltd, Oxford, UK (2004) pp. 1–23.
10. E.N.C. Mills, A.R. Mackie, P. Burney, K. Beyer, L. Frewer, C. Madsen *et al.*, The prevalence, cost and basis of food allergy across Europe, *Allergy, 62* (2007) 717–722.
11. E.N.C Mills, H. Breiteneder, Food allergy and its relevance to industrial food proteins, *Biotechnol. Adv. 23* (2005) 409–414.
12. S.K. Sathe, G.M. Sharma, Effects of food processing on food allergens, *Mol. Nutr. Food Res. 53* (2009) 970–978.
13. A. Paschke, Aspects of food processing and its effect on allergen structure, *Mol. Nutr. Food Res. 53* (2009) 959–962.
14. R. Crevel, Industrial dimensions of food allergy, *Biochem. Soc. Trans. 30* (2002) 941–944.
15. J. Heick, M. Fischer, B. Pöpping, First screening method for the simultaneous detection of seven allergens by liquid chromatography mass spectrometry, *J. Chromatogr. A*, *1218* (2011) 938–943.
16. Commission Directive 2003/89/EC of the European Parliament and of the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs, *Off. J. Eur. Comm. L308* (2003) 15–18.
17. Commission Directive 2005/26/EC of 21 March 2005 establishing a list of food ingredients or substances provisionally

excluded from Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council, *Off. J. Eur. Comm. L75* (2005) 33–34.

18. R.K. Bush, S.L. Hefle, Food allergens, *Crit. Rev. Food Sci. Nutr.* (Suppl.), *36* (1996) 119–163.
19. M. Natale, C. Bisson, G. Monti, A. Peltran, L.P. Garoffo, S. Valentini *et al.*, Cow's milk allergens identification by two dimensional immunoblotting and mass spectrometry, *Mol. Nutr. Food Res. 48* (2004) 363–369.
20. H.E. Swaisgood, Review and update of casein chemistry, *J. Dairy Sci. 76* (1993) 3054–3061.
21. S. Frémont, G. Kanny, J.P. Nicolas, D.A. Moneret-Vautrin, Prevalence of lysozyme sensitization in an egg-allergic population, *Allergy, 52* (1997) 224–228.
22. P. Weber, H. Kratzin, K. Brockow, J. Ring, H. Steinhart, A. Paschke, Lysozyme in wine: A risk evaluation for consumers allergic to hen's egg, *Mol. Nutr. Food Res. 53* (2009) 1469–1477.
23. L.K. Poulsen, T.K. Hansen, A. Nørdgaard, H. Vestergaard, P.S. Skov, C. Bindslev-Jensen, Allergens from fish and egg, *Allergy* (Suppl. 67), *56* (2001) 39–42.
24. Y. Hamada, H. Tanaka, S. Ishizaki, M. Ishida, Y. Nagashima, K. Shiomi, Purification, reactivity with IgE and cDNA cloning of parvalbumin as the major allergen of mackerels, *Food Chem. Toxicol. 41* (2003) 1149–1156.
25. S.L. Taylor, S.L. Hefle, C. Bindslev-Jensen, S.A. Bock, A.W. Burks, L. Christie *et al.*, Factors affecting the determination of threshold doses for allergenic foods: How much is too much?, *J. Allergy Clin. Immunol. 109* (2002) 24–30.
26. O. Stephan, S. Vieths, Development of a real-time PCR and a sandwich ELISA for detection of potentially allergenic trace amounts of peanut (*Arachis hypogaea*) in processed foods, *J. Agric. Food Chem. 52* (2004) 3754–3760.
27. D.P. Strachan, Family size, infection and atopy: The first decade of the 'hygiene hypothesis', *Thorax* (Suppl. 1), *55* (2000) 2–10.
28. Directive 2000/13/EC of the European Parliament and of the Council of 20 March on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs, *Off. Eur. Comm. L109* (2000) 29–42.
29. Comission Directive 2007/68/EC amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients, *Off. J. Europ. Union, L310* (2007) 11–14.
30. P.L. Teissedre, Allergens in oenological practices, *Rev. Fr. Oenol. 138* (2011) 7–8 (in French).
31. S. Taylor, Chemistry and detection of food allergens, *Food Technol. 46* (1992) 148–152.
32. E.W. Evans: Use of Milk Proteins in Formulated Food. In: *Developments in Food Proteins – 1*, B.J.F. Hudson (Ed.), Applied Science Publishers, London, UK (1982) pp. 131–169.
33. P.F. Fox, P.A. Morrissey, D.M. Mulvihill: Chemical and Enzymatic Modification of Food Proteins. In: *Developments in Food Proteins – 1*, B.J.F. Hudson (Ed.), Applied Science Publishers, London, UK (1982) pp. 1–60.
34. J.C. Cheftel, J.L. Cuq, D. Lorient: *Food Proteins: Biochemistry, Functional Properties, Nutritional Value*, Technique et Documentation Lavoisier, Paris, France (1985) pp. 306–308 (in French).
35. G.W. Froning: Nutritional and Functional Properties of Egg Proteins. In: *Developments in Food Proteins – 6*, B.J.F. Hudson (Ed.), Applied Science Publishers, London, UK (1988) pp. 1–34.
36. R.V. Leather, M. Sisk, C.J. Dale, A. Lyddiatt, Analysis of the collagen and total soluble nitrogen content of isinglass finings by polarimetry, *Inst. Brew. 100* (1994) 331–334.
37. P. Weber, H. Steinhart, A. Paschke, Competitive indirect ELISA for the determination of parvalbumins from various fish species in food grade fish gelatins and isinglass with PARV-19 anti-parvalbumin antibodies, *J. Agric. Food Chem. 57* (2009) 11328–11334.
38. G.H. Docena, R. Fernandez, F.G. Chirdo, C.A. Fossati, Identification of casein as the major allergenic and antigenic protein of cow's milk, *Allergy, 51* (1996) 412–416.
39. H.Y. Lam, E. van Hoffen, A. Michelsen, K. Guikers, C.H.W. van der Tas, C.A.M. Bruijnzeel-Koomen *et al.*, Cow's milk allergy in adults is rare but severe: Both casein and whey proteins are involved, *Clin. Exp. Allergy, 38* (2008) 995–1002.
40. P. Weber, H. Steinhart, A. Paschke, Investigation of the allergenic potential of wines fined with various proteinogenic fining agents by ELISA, *J. Agric. Food Chem. 55* (2007) 3127–3133.
41. S. Kirschner, B. Belloni, C. Kugler, J. Ring, K. Brockow, Allergenicity of wine containing processing aids: A double-blind, placebo-controlled food challenge, *J. Investig. Allergol. Clin. Immunol. 19* (2009) 210–217.
42. J.M. Rolland, E. Apostolou, K. Deckert, M.P. de Leon, J.A. Douglass, I.N. Glaspole *et al.*, Potential food allergens in wine: Double-blind, placebo-controlled trial and basophil activation analysis, *Nutrition, 22* (2006) 882–888.
43. E. Vassilopoulou, E.A. Karathanos, G. Siragakis, S. Giavi, A. Sinaniotis, N. Douladiris *et al.*, Risk of allergic reactions to wine, in milk, egg and fish-allergic patients, *Clin. Transl. Allergy, 1* (2011) 10–14.
44. S.L. Walker, M.C.D. Camarena, G. Freeman, Alternatives to isinglass for beer clarification, *J. Inst. Brew. 113* (2007) 347–354.
45. S. Lefebvre, P. Restani, B. Scotti, The utilization of vegetable proteins in oenology: Focus on the authorization and the risk of allergy, *Rev. Fr. Oenol. 202* (2003) 10–14 (in French).
46. S. Lefebvre, N. Sieczkowski, F. Vidal, Food security in oenology: The case of vegetable proteins, *Rev. Fr. Oenol. 210* (2005) 23–30 (in French).
47. F. Cosme, I. Capão, L. Filipe-Ribeiro, R.N. Bennett, A. Mendes-Faia, Evaluating potential alternatives to potassium caseinate for white wine fining: Effects on physicochemical and sensory characteristics, *LWT – Food Sci. Technol. 46* (2012) 382–387.
48. Approaches to Establish Thresholds for Major Food Allergens and for Gluten in Food, Food and Drug Administration, Silver Spring, MD, USA (2011) pp. 1–21.
49. P. Restani, B. Beretta, C. Ballabio, C.L. Galli, A.A.E. Bertelli, Evaluation by SDS-page and immunoblotting of residual antigenicity in gluten-treated wine: A preliminary study, *Int. J. Tissue React. 24* (2002) 45–51.
50. R.E. Poms, E. Anklam: Tracking and Tracing for Allergen--Free Food Production Chain. In: *Allergy Matters: New Approaches to Allergy Prevention and Management*, *9*, L.J.W.J. Gilissen, H.J. Wichers, H.F.J. Savelkoul, R.J. Bogers (Eds.), Springer, Dordrecht, The Netherlands (2006) pp. 79–85.
51. A.J. van Hengel, Food allergen detection methods and the challenge to protect food-allergic consumers, *Anal. Bioanal. Chem. 389* (2007) 111–118.
52. J.M. Rolland, E. Apostolou, M.P. de Leon, C.S. Stockley, R.E. O'Hehir, Specific and sensitive enzyme-linked immunosorbent assays for analysis of residual allergenic food proteins in commercial bottled wine fined with egg white, milk, and nongrape-derived tannins, *Agric. Food Chem. 56* (2008) 349–354.
53. A. Lifrani, J. Dos Santos, M. Dubarry, M. Rautureau, F. Blachier, D. Tome, Development of animal models and sandwich-ELISA tests to detect the allergenicity and antigenicity of fining agent residues in wines, *J. Agric. Food Chem. 57* (2009) 525–534.

54. P. Weber, H. Steinhart, A. Paschke, Determination of the bovine food allergens casein in white wines by quantitative indirect ELISA, SDS-PAGE, Western Blot and immunostaining, *J. Agric. Food Chem. 57* (2009) 8399–8405.

55. K. Tomkova, P. Cuhra, J. Rysova, P. Hanak, D. Gabrovska, ELISA kit for determination of egg white proteins: Interlaboratory study, *J. AOAC Int. 93* (2010) 1923–1929.

56. B. Simonato, F. Mainente, S. Tolin, G. Pasini, Immunochemical and mass spectrometry detection of residual proteins in gluten fined red wine, *J. Agric. Food Chem. 59* (2011) 3101–3110.

57. L. Monaci, A.J. van Hengel, Development of a method for the quantification of whey allergen traces in mixed-fruit juices based on liquid chromatography with mass spectrometric detection, *J. Chromatogr. A, 1192* (2008) 113–120.

58. L. Monaci, A. Visconti, Mass spectrometric-based proteomic methods for analysis of food allergens, *Trends Anal. Chem. 28* (2009) 581–591.

59. L. Monaci, I. Losito, F. Palmisano, A. Visconti, Identification of allergenic milk proteins markers in fined white wines by capillary liquid chromatography–electrospray ionization-tandem mass spectrometry, *J. Chromatogr. A, 1217* (2010) 4300–4305.

60. L. Monaci, I. Losito, F. Palmisano, M. Godula, A. Visconti, Towards the quantification of residual milk allergens in caseinate-fined white wines using HPLC coupled with single-stage Orbitrap mass spectrometry, *Food Addit. Contam. A, 28* (2011) 1304–1314.

61. A. Cereda, A.V. Kravchuk, A. D'Amato, A. Bachi, P.G. Righetti, Proteomics of wine additives: Mining for the invisible via combinatorial peptide ligand libraries, *J. Proteomics, 73* (2010) 1732–1739.

62. A. D'Amato, A.V. Kravchuk, A. Bachi, P.G. Righetti, Noah's nectar: The proteome content of a glass of red wine, *J. Proteomics, 73* (2010) 2370–2377.

63. Z. Zhou, J. Jiang, M. Li, Z.F. Zhao, J. Fu, Fast screening of chicken egg lysozyme in white wine products by extractive electrospray ionization mass spectrometry, *Chem. Res. 28* (2012) 200–203.