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Commentary

Allergenicity assessment strategy for novel food proteins and protein sources

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ABSTRACT

To solve the future food insecurity problem, alternative and sustainable protein sources (e.g. insects, rapeseed, fava bean and algae) are now being explored for the production of food and feed. To approve these novel protein sources for future food a comprehensive risk assessment is needed according to the European food legislation. Allergenicity risk assessment might pose some major difficulties, since detailed guidance on how to assess the allergenic potential of novel foods is not available. At present, the approach relies mostly on the guidance of allergenicity assessment for genetically modified (GM) plant foods. The most recent one was proposed by EFSA (2010 and 2011); "weight-of-evidence approach". However this guidance is difficult to interpret, not completely applicable or validated for novel foods and therefore needs some adjustments. In this paper we propose a conceptual strategy which is based on the "weight-of-evidence approach" for food derived from GM plants and other strategies that were previously published in the literature. This strategy will give more guidance on how to assess the allergenicity of novel food proteins and protein sources.

1. Introduction

Strategies are being developed to change the current agricultural practices by creating more sustainable and new climate-resistant crops and to ensure an adequate, safe, sustainable and nutritious food supply (e.g. alternative protein sources) in the near future. Before novel food proteins or protein containing products can be brought to market, we *need to take precautions to avoid that* novel products will add to the burden of food allergy. At least we have to take care that we will not introduce allergens as potent as the major allergenic foods such as peanut. On the other hand, we also have to be aware that any (novel) protein might have some risk of allergenicity. Therefore we need to take care that we will not exclude promising new protein sources with low or virtually absent allergenic potential from the market. The EU novel food law requires that a comprehensive food safety assessment (addressing nutritional value, microbiological, toxicological, and allergenic risks) has to be performed for all novel foods or food ingredients that were not commonly consumed in the EU before May 1997, before they can be launched onto the food market (EC regulation No 258/97 and EU recommendation 97/618 EC; http://eur-lex.europa.eu/). For the assessment of nutritional, microbiological and toxicological risks, standard and well defined methods do exist. The assessment of allergy risks for a novel protein source is less straight forward. At present, the approach relies mostly on the guidance of allergenicity assessment for genetically modified (GM) plant foods. The most recent one was proposed by EFSA (2010 and 2011): the so-called "weight-of-evidence approach". The purpose of these guidelines was to prevent the introduction of an allergenic protein into a food source, which might pose a risk for consumers allergic for this protein or to prevent the introduction of a protein that is similar to an allergenic protein, so that cross reactivity might occur. The applicability of these guidelines for the assessment of new and modified proteins or protein containing products (e.g. insects, algae, alternatively processed products) is hampered, since there is no generally accepted, validated and broadly applicable method available for allergenicity hazard and risk assessment. The shortcomings of the current guidelines for this latter purpose will be

Abbreviations: BAT, Basophil Activation Test; EFSA, European Food Safety Authority; DBPCFC, Double Blind Placebo Controlled Food Challenge; GM, Genetically Modified; GMO, Genetically Modified Organism; LC-MS/MS, Liquid Chromatography and mass spectrometry; OFC, Open Food Challenge; SPT, Skin Prick Test.

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discussed in this paper.

Food allergy is an adverse reaction of the human immune system to an otherwise harmless food component and the prevalence of food allergy in Europe is up to 3% according to the EAACI food allergy and anaphylaxis guidelines group (Nwaru et al., 2014). Food allergy develops in two phases. In the first phase susceptible subjects become sensitised to specific food proteins after dietary exposure, or possibly via other routes of exposure (inhalation and/or skin contact). This may result in the production of specific IgE to the food protein (Johnston et al., 2014; Kimber and Dearman, 2002). When sensitised subjects subsequently encounter the respective allergen(s) again, cellular bound specific IgE will recognize the allergens and an allergic reaction may be elicited. Allergic symptoms may vary considerably and can range from mild, local and transient effects to potential fatal reactions like systemic anaphylaxis (Sicherer and Sampson, 2014; Sicherer and Wood, 2013).

Generally, food allergens are proteins but the vast majority of food proteins are weak or virtually non-allergenic (Metcalfe et al., 1996; Radauer et al., 2008). Most cases (90%) of food allergic reactions are caused by a limited range of products; milk, egg, peanut, tree nuts, fish, soy, wheat and crustaceans (Boyce et al., 2010; Hefle et al., 1996; Young et al., 1994). Furthermore, the manifestations of food allergies can be dependent on geography, dietary habits, food preparation and age at which food is first consumed (Lucas et al., 2004). It is therefore possible that a food product that was not reported to be common or known as allergenic in Asia can be an allergenic food in Europe, for example kiwi fruit (Lucas et al., 2004). Another example is the allergy to peach, a member of the Rosacea family which is attributed to birch pollen in Central and Northern Europe (Pru p 1, the Bet v 1 homologue, PR-10) and leads to mild reactions (oral allergy syndrome), while in the Mediterranean areas where birch trees are less common, peach allergy may result from sensitisation to Pru p 3 (lipid transfer protein, LTP) and/or Pru p 4 (profilin) which more commonly leads to severe allergic reactions (Andersen et al., 2011).

At the moment, novel foods such as insects and rapeseed are entering the market without a proper allergenicity risk assessment. For mealworms, larval stage of the yellow mealworm beetle, it was recently demonstrated in a double blind placebo controlled food challenge (DBPCFC) that 87% of a shrimp allergic patients population showed allergic reaction upon eating Yellow mealworm and that *de novo* sensitisation to Yellow mealworm proteins is possible (Broekman et al., 2015a) (Broekman, JACI, in press). In case of rapeseed, which was formally in use in the EU only in the form of rapeseed oil, the EFSA panel concluded, that a risk of sensitisation to rapeseed protein isolate cannot be excluded and that it is likely that rapeseed will trigger allergic reactions in mustard allergic subjects (EFSA NDA Panel (EFSA NDA Panel, 2013)). This conclusion was based on a food challenge and a skin prick tests with crushed rapeseed (not protein isolate) in atopic Finnish children with atopic dermatitis and suspected food allergies. 10.9% of the children showed sensitivity in the SPT and 89% of these children reacted positive in the food challenge. Cross reactivity with mustard seeds was demonstrated using IgE binding tests with serum form mustard allergic patients. Furthermore, structural homology of 95% of seed storage proteins of various members of the *brassicaceae*, incl. mustard was shown. In this assessment, clinically relevant studies were performed with crushed rapeseed but not with rapeseed protein isolate. In the latter, a higher protein concentration can be expected and furthermore, the effect of processing was not taken into account. Other novel food dossiers submitted in the last five years for approval by the EFSA (e.g. Chia seed, Lentinus edodos and alfalfa) were lacking properly conducted clinically relevant tests (e.g. SPT, or basophil activation tests (BAT)) and in most cases no formal proof of absence of allergenicity using double blind placebo controlled food challenge (DBPCFC) was given, nor was the effect of processing or the sensitising potency tested (EFSA NDA Panel, 2009, 2010a, 2010b). Food challenges are essential for determining if IgE binding measured with techniques such as immunoblot, BAT and SPT is clinically relevant. IgE binding or IgE cross reactivity does not automatically indicate that an allergic reaction will occur. For instance, some proteins have cross reactive carbohydrate determinants (mostly found in plants) that bind to IgE but do not elicit an allergic reaction (Mari et al., 1999). Furthermore, cross reactivity between taxonomically related foods, such as the legume family (peanut, soy, lupine, white bean) does not automatically indicate clinical cross reactivity (Peeters et al., 2007). Ibáñez et al. showed that white bean and overall green bean are well tolerated by children allergic to other legumes (Ibanez et al., 2003).

It is in the interest of the producer of novel food products to predict allergenicity in an early stage of product development to avoid withdrawal of the novel food from the food market after introduction. For this reason, it is necessary to assess the allergenic potential of novel foods before a well-informed decision can be made on the allergenic potential of a novel food and to guide the implementation of risk managements tools such as labelling. Risk management aspects are not addressed in this paper.

In this paper the current risk assessment strategy and guidelines will be discussed and a conceptual strategy is suggested, aimed to give better guidance in how to assess the allergenicity of novel food proteins and protein sources.

2. Current strategy and guidelines

As already mentioned above there is no predictive and validated method to assess the allergenicity of novel proteins (sources) or protein containing products. In most recently filed novel food dossiers, parts of the allergenicity risk assessment guideline for Genetically Modified Organisms (GMO) which was drafted in 2010 by the EFSA's Genetically Modified Organisms (GMO) Panel (EFSA GMO Panel, 2010) and updated in 2011 (EFSA GMO Panel, 2011),were used. The in this guidelines suggested weight-of-evidence approach (Fig. 1) involves an integrated case-by-case approach to be used in the allergenicity risk assessment of newly expressed proteins in genetically modified (GM) feed and foods.

The safety evaluation mainly focusses on:

1) Evaluation of the source of the gene
2) Sequence homology with known allergens
3) Binding to IgE from allergic individuals
4) Stability of the protein in a pepsin resistance test.

2.1. Source of the gene

Allergenicity assessment of GM food starts with the evaluation of the source of the gene. If the source of the gene has a proven allergenic potential then a careful assessment is mandatory to ensure that the gene of interest does not encode for an allergen. The relevance of this evaluation is apparent from the incidence that a GM soybean was produced that contained a gene from Brazil nut. This GM soybean showed allergenic reactions in Brazil nut sensitive individuals (Hefle et al., 1996).

2.2. Sequence homology to known allergen(s)

Bioinformatic tools are used to compare the amino acid sequence of the newly expressed protein with the sequences of known allergens to determine sequence homology. High sequence

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