ACNFP

Advisory Committee on Novel Foods and Processes

Proteins in novel foods: issues for consideration

1. The term 'novel food' covers a broad range of foods. In recent years the Committee has considered chemically synthesised products (e.g. phosphated distarch phosphate, chewing gum base polymer), fermentation products (black bean extract, glucosamine), fruits and vegetables (baobab fruit pulp, kiwi fruit) and extracts (DHA rich algal oil, magnolia bark extract). Different food products raise different safety concerns, including those related to the presence of potentially harmful proteins.

2. Applications for novel foods should always quantify the level of protein present. This could be limited to relatively basic nutritional information (for instance as part of a proximate analysis of major nutritional components) or there may be a requirement for additional analyses to confirm the presence or absence of proteins. It is quite possible that EU consumers will not have been exposed to some or all of the proteins present in novel foods, and the 'risk' that these proteins may pose to consumers requires review on a case-by-case basis. Unless the absence of protein can be demonstrated, it is reasonable to assume that there is the potential for an allergic response in certain individuals.

3. The Committee has advised on a number of occasions that the analytical methods used to quantify and/or identify the proteins present in novel foods have been insufficient. This has led to applicants underestimating the allergenic potential of their products and a requirement to provide more reliable evidence about the proteins present in novel foods, and whether these give rise to allergenic risk.

4. This paper is concerned with the detection of proteins in novel foods. Quantifying the risk associated with exposure to novel proteins is not straightforward, but there are detailed procedures for assessing the allergenic potential of foods[1] and these should also be employed in the assessment of novel foods. As a guide, and prior to carrying out an analysis of protein in novel foods, the following questions should be considered, together with the different **scenarios** detailed below.

[1] http://www.efsa.europa.eu/de/scdocs/doc/1700.pdf

Scenario 1 - No evidence that proteins are present

What methods were used to demonstrate absence? [Note 1]

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Are these adequate to demonstrate the absence of protein [Note 1] → Yes → Negligible allergenic risk

↓ ↑

No → Carry out additional analyses

Scenario 2 - Evidence that proteins are present

What methods were used to identify and/or quantify protein levels?

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Are these adequate to quantify the level of protein [Note 2] → Yes → Indicate likely allergenic risk and (if appropriate) propose suitable risk management strategy

↓ ↑ ↗

No → Carry out additional analyses

↓ ↗

Provide rationale why additional information not required [Note 3]

Note 1: The absence of protein should be demonstrated using appropriate analytical methods (see 'Issues to Consider'). A distinction should be drawn between "absence" and "absence at the limit of detection".

Note 2: What information is available regarding the methods employed – are these appropriate for test material, are they carried out by accredited laboratories, what is known about the proteins present?

Note 3: Could apply when the novel food is obtained from an existing ingredient with a known allergenic potential. Consideration needs to be given to existing allergen labelling requirements and whether the inclusion of the novel ingredient in a wide range of foods may lead to a restriction in choice for allergenic individuals.

- What is known about the source material e.g. allergenic potential, any relevant information about threshold levels?
- Does the production process minimise the presence of detectable protein in the final product e.g. crystallisation or other purification steps?
- What is known about the substances used in the production process e.g. enzymes, sources of raw materials?
- What methods is used to quantify and identify proteins present in the final product?
- Is the method the most appropriate for this novel food? Why?
- Are these methods carried out by an accredited laboratory and what are the limits of detection?
- What information is available regarding method optimisation e.g. extraction procedures, choice of control substances?

Methodology

5. If a novel food is obtained from a vegetable, microbial, or animal source, or materials from these sources are used in its production, then a key step is to understand the impact that this may have in terms of allergenic potential. It is also possible that, even if the source is taxonomically distinct from sources of known allergens, the novel food may contain proteins which could cross-react with a known allergen and cause an allergic response in sensitised individuals. Recent novel food applications (e.g. arracacha root, fungal glucosamine[2]) highlight the importance of these issues.

6. In all cases the most appropriate, which may not be the simplest or most readily available, analytical methods should be employed, and due consideration needs to be given to sample preparation (e.g. protein solubility) and the use of appropriate controls (e.g. whether the 'default' standard, often bovine serum albumin, is appropriate). A variety of analytical methods can be employed to quantify and identify proteins present in foods and these are reviewed in detail elsewhere (e.g. Noble and Bailey, 2009).

7. The presence of protein can be confirmed by the use of analytical methods such as **Kjeldahl** or **Dumas**, which detect the presence of nitrogen. Both of these methods provide accurate determination of nitrogen and are appropriate for the analysis of foods. It is also common to convert nitrogen content of biological materials to protein by multiplying by a factor of 6.25[3]. However, although proteins are the major nitrogen-

[2] http://acnfp.food.gov.uk/assess/fullapplics/glucosamine and http://acnfp.food.gov.uk/meetings/acnfpmeet09/acnfp16sep09/acnfpmin160909

[3] This figure can vary – eg a figure of 5.7 is widely used for determination of protein content in cereals

containing compounds in most foods it cannot be assumed that all of the nitrogen is derived from proteins (e.g. glucosamine). Analysis of nitrogen content should therefore be accompanied by separate analysis which specifically determines protein content.

8. Several colorimetric methods which can be used to estimate the level of protein, but as quantification requires comparison with standards of known quantity, care must be taken in the experimental design. Many methods are based on chemical reactions involving the peptide backbone, including the historic **Lowry** method and more recent methods employing bicinchoninic acid (BCA) or the highly sensitive fluorimetric assay based on 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde (CBQCA). In contrast the **Bradford** Assay utilises the dye reagent Coomassie Blue to bind to protein and reacts with larger peptides and intact proteins. This means the assay performance can be affected by treatments that affect protein integrity. As a consequence, the Bradford method is not itself sufficient to confirm the absence of protein from novel foods.

9. **Gel-electrophoresis** (e.g. SDS-PAGE) can be used to provide additional information regarding the range of proteins present in the sample. A range of staining methods can be employed to optimise detection, However, limitations related to the limit of detection also apply to this method, particularly when the protein size is small (<6kDa) or large (>200kDa).

10. **Mass-Spectroscopy** (e.g. LC-MS, MALDI-ToF) has been used to provide additional assurance that a novel food does not contain protein[4], and the use of this technology could also be extended to provide additional qualitative information in response to concerns about specific proteins. This technique is better suited for mass profiling smaller proteins and peptides not readily amenable to SDS-PAGE analysis, especially in the region of 2-6kDa.

Threshold levels

11. The European Food Safety Authority's opinion on the evaluation of allergenic foods for labelling purposes suggests that whilst it is possible to identify clinical thresholds for food allergens at the time of publication data were incomplete and hence threshold doses below which foods would be safe for allergic consumer to consume could not be identified[5].

12. Population level threshold data are still required to support efforts to derive agreed allergen management levels or action levels for food allergens. Ongoing work to bring together clinical threshold data to derive dose distribution data at a population level,

[4] http://www.food.gov.uk/multimedia/pdfs/magbarkextractopinion.pdf and http://acnfp.food.gov.uk/assess/fullapplics/glucosamine

[5] http://www.efsa.europa.eu/en/efsajournal/doc/opinion_nda_04_en1,1.pdf

together with the application of appropriate safety margins will assist in the development of agreed allergen action levels in future. Analytical methods must be able to detect the allergenic food effectively at levels around the agreed action levels across various matrices and work is being carried out to assess their effectiveness. This research may, in time, provide additional information regarding the detection of proteins in food matrices which will be applicable to novel foods. However, current data on thresholds relate to existing known allergens and it is uncertain what their applicability is to novel foods.

13. There are indications that extensively hydrolysed protein preparations are less allergenic since they can provide benefit in the treatment of infantile cows milk allergies where hydrolysed hypoallergenic formulas are widely used. In general, the longer the peptides in a hydrolysed formula, the greater its allergenicity, with a whey hydrolysate containing peptides of greater that 15,000Da having significant allergenic activity (Wahn et al 1992) although some formulas based on extensively hydrolysed caseinate can be as effective as elemental formulas which can be tolerated by >90% of cows' milk allergic infants (Bahna 2008).

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