

**Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies
on a request from the Commission related to a notification from Givaudan
Schweiz AG on fish gelatine used as carrier for flavour pursuant to
Article 6 paragraph 11 of Directive 2000/13/EC**

(Request EFSA-Q-2004-126)

(adopted on 2 December 2004)

SUMMARY

Fish is one of the most important allergenic foods, and allergic reactions to fish can be severe. The major allergen of fish is the muscle protein parvalbumin. Gelatine is made by denaturation of collagen. Fish gelatine is used in foods and pharmaceuticals, and the present application concerns use of fish gelatine for flavour encapsulation carrier system.

Gelatine for the present application is produced from cold and warm water fish skins. No analytical data regarding possible residual levels of the major fish allergen parvalbumin in the fish gelatine preparation are provided. Typical levels of fish gelatine in industrially processed foods are indicated to be up to around 1000 mg/kg. Levels of intake of fish gelatine under the conditions of use specified by the applicant are likely to be ranging from tens to hundreds of mg per day.

There is published evidence that some fish allergic individuals have specific serum IgE reactive with fish collagen and its denatured form gelatine, but there are no reports on clinical reactions attributed to gelatine in commercial foods. Two clinical provocation studies with fish gelatine are referred to by the applicant. In one single-blind oral provocation study three fish-allergic patients with IgE binding to fish gelatine did not react clinically upon ingestion of 5 g gelatine. In a double-blind placebo-controlled food challenge (DBPCFC) study of 30 patients with clinical allergy to fish, no patient reacted to a cumulative dose of 3.6 g of fish gelatine.

The scientific data provided by the applicant are insufficient to predict the likelihood of adverse reactions in fish allergic individuals. Nevertheless, taking all the information into account the Panel considers that it is not very likely that fish gelatine, under the conditions of use specified by the applicant, will cause a severe allergic reaction in the majority of fish allergic individuals.

However, appropriate analytical methods to determine residual levels of parvalbumin in fish gelatine preparations are needed. More data from clinical challenge studies with gelatine of fish allergic individuals having specific IgE or positive skin prick tests to fish collagen or gelatine are needed.

KEY WORDS

Fish gelatine, food allergy, flavour encapsulation systems.

BACKGROUND

In November 2003, the European Parliament and the Council adopted Directive 2003/89/EC¹ amending Directive 2000/13/EC, as regards indication of the ingredients present in foodstuffs.

Annex IIIa of the Directive specifies a list of ingredients that are known to trigger allergic reactions or intolerances for which no labelling exemptions are allowed. Whenever the listed ingredients or their derivatives are used in the production of foodstuffs, they must be labelled.

Article 1, paragraph 11 of the Directive establishes a procedure allowing for temporary labelling exemption of derivatives from ingredients listed in Annex IIIa for which it has been scientifically established that it is not possible for them to cause adverse reactions. In accordance with this provision, submissions of request for temporary labelling exemption were notified to the Commission before 25 August 2004. The Commission shall, not later than 25 November 2004, and after consultation with the European Food Safety Authority, adopt a list of those ingredients which shall be temporarily excluded from Annex IIIa, pending the final results of the notified studies, or at the latest until 25 November 2007. Therefore, the European Food Safety Authority is asked to provide scientific opinions on the submissions in accordance with the present terms of reference.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) N° 178/2002, the European Commission requests the European Food Safety Authority to evaluate the scientific data submitted by Givaudan Schweiz AG in the framework of the procedure laid down for temporary labelling exemptions in Article 6 paragraph 11 of Directive 2000/13/EC. On the basis of that evaluation, EFSA is requested to issue an opinion on the information provided, and particularly, pending the final results of the studies undertaken, to consider the likelihood of adverse reactions triggered in susceptible individuals by the consumption of the following ingredients/substances used under the conditions specified by the applicant: Fish gelatine used as carrier for flavour.

ASSESSMENT

1. Manufacturing process

Fish gelatines are produced by extraction and hydrolysis of fibrous, insoluble collagen from skins or bones. Regarding the present application, the skins of cold or warm water fish which have been filleted in high speed fish processing plants are used. Typical fish used are Tilapia, tuna, and perch. The skins are separated, frozen during storage and transport and thoroughly washed before gelatine is extracted by treatment with hot water.

Fish gelatines obtained are used with other food ingredients for the production of microcapsules using a coacervation process, a special technology by which microcapsules are produced. The subsequently applied encapsulation technology incorporates, according to the applicant, flavours into the corresponding capsules (protein shell), protecting the flavour

¹ Directive 2003/89/EC of the European Parliament and of the Council amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs. OJ L 308. 25.11.2003, p. 15.

ingredients from loss during thermal processing and interaction with other food ingredients during industrial processing or domestic cooking. The capsules are loaded with up to 75% flavour substances. The final flavour containing product is called Flavorburst®.

2. Characterisation of the products and their use

2.1 Characterisation of fish gelatine

Gelatine is denatured collagen, modified by the separation of three alpha chains comprising the triple helix of collagen and by some degree of hydrolysis of the resultant alpha chain polypeptides. Physicochemical characteristics of the gelatine are indicated by the applicant. Fish gelatines have high iso-electric points making them beneficial for the production of microcapsules, which are then loaded with flavours. Release of the full flavour profile is obtained by gradual diffusion or by the shearing action of chewing when the product is consumed.

2.2 Conditions of use and exposure levels

2.2.1 Conditions of use

The applicant states that typical applications for fish gelatine in the form of Flavorburst® include bakery products (biscuits, cakes), fat, margarine, pasta dishes, extruded snacks and sweet goods, fillings, chewing gum, soups, sauces, and meat coating. The Flavorburst® technology is selectively used in finished food applications in which liquid flavours or otherwise encapsulated flavour systems (e.g. spray-drying) do not effectively perform. Therefore, according to the applicant, Flavorburst® is used in only a limited range of food categories and to a limited extent.

2.2.2 Exposure levels

For the gelatine microcapsules, a flavour load from 10.2-75.7% typically applies, according to the applicant. The fish gelatine content in the microcapsules is indicated to be between 2.1 and 10.4%. Consequently, according to the applicant, the Flavorburst® flavour-loaded microcapsule contains between 2.5 and 9.3% of fish gelatine. The Panel cannot follow the applicant's calculation.

Dosage levels of loaded microcapsules in the final food application are determined by the organoleptic strength of the flavour and properties of the food matrix. The applicant provides the following indicative values for calculated ranges of fish gelatine in the flavoured foods (mg/kg) in commercial practice: processed vegetables 15-20, dry soups 8-194, extruded snacks 40-731, sauces 7-120, biscuits and cakes 14-971, chewing gum 864-1356, marinade 950-1000, and fats and margarines 57-1122. Based on these concentrations, exposure levels are expected to be up to 1 g per day.

3. Evidence of non-allergenicity

3.1. History of non-allergenicity of the product

3.1.1 Literature search strategy

No literature search is reported in the application.

3.1.2 Historical evidence of safe use

This issue is not raised by the applicant.

3.2 Laboratory-based tests

3.2.1 Residual amounts of known fish allergen (parvalbumin) in gelatine

No data or arguments are given by the applicant, except for research being conducted (see below).

The major fish allergen is parvalbumin (NDA, 2004), a protein structurally markedly different from collagen and gelatine. Mostly the parvalbumins have been isolated from fish muscle. However, there is one report in which parvalbumin was detected in the brain and kidney, and it is uncertain whether parvalbumin has been looked for in fish skin and fish scales. Other minor allergens have also been reported. Moreover, the allergenicity of fish parvalbumin is not easily destroyed by heat, proteolytic activity or denaturation with chemicals.

3.2.2 Allergenicity of fish gelatine

3.2.2.1 Serological and immunochemical studies of fish collagen and gelatine

3.2.2.1.1 Collagen and gelatine

Experimental studies (Hamada *et al.*, 2001 and 2003) found that the IgE reactivity of fish collagen was very thermostable and was preserved also in peptide fragments. When collagen was denatured to gelatine by heating in boiling water for 120 minutes, the collagen (gelatine) retained 90% of its original binding ability to the IgE in three human sera (Hamada *et al.*, 2001). Collagen allergenicity must, therefore, be considered in the discussion of gelatine allergenicity.

3.2.2.1.2 Mammalian and fish gelatines

Allergic reactions to mammalian (bovine, porcine) gelatines used in vaccines and medical devices are well documented. Also, a few cases of food allergic reactions to mammalian gelatine can be found in the literature. These cases may often be a consequence of sensitisation due to medical applications of gelatine.

Mammalian and fish gelatines have some similarities, and the possibility of allergic cross-reactivity must be considered. However, several published studies support the absence of serological cross-reactivity between mammalian and fish gelatines. In contrast, fish collagens from different species appear to be broadly cross-reactive, and it seems reasonable to treat fish

collagens from different species as one entity. No data have been found regarding cross-reactivity between collagens from different organs (e.g. skin and muscle) from the same species of fish.

3.2.2.1.3 Serological and immunochemical evidence for fish collagen allergenicity

A number of scientific papers report that serum IgE from some fish allergic individuals binds to fish collagen and gelatine. The applicant refers to Sakaguchi *et al.* (2000) who found specific IgE to fish gelatine in 3/10 patients with fish allergy and specific IgE to fish meat; 2/2 patients with fish meat and bovine gelatine allergy and specific IgE to fish meat and to bovine gelatine; and 5/15 patients with atopic dermatitis and specific IgE to fish meat. IgE from the pooled serum of the patients reacted with both the alpha-1 and alpha-2 chains of collagen in immunoblots. All patients with specific IgE to gelatine also had specific IgE to fish meat. This could be due to reactivity against collagen/gelatine which according to the authors comprises up to 12% of the protein in fish meat, or it could be due to concomitant reactivities against different allergens. It is somewhat unclear how several of the gelatines used by Sakaguchi *et al.* were prepared, and therefore also whether they may have been contaminated by fish flesh allergen.

The applicant cites a personal communication from André (2000, no further details provided), stating that the overall prevalence of sensitization to tuna fish gelatine can be estimated to be 3 per 10⁵.

The presence of IgE binding to gelatine does not mean that clinical allergy is present. Food challenges with gelatine or collagen were not performed in the various studies reporting fish collagen and gelatine IgE binding, except for the two clinical provocation studies discussed below.

3.3 Clinical studies

3.3.1 DBPCFC (double-blind placebo-controlled food challenge) study with fish gelatine

The applicant refers to a DBPCFC study performed in Denmark (Hansen *et al.*, 2004).

Patients: Thirty fish allergic patients age 9 to 50 years were included. All were fish allergic according to the European Academy of Allergology and Clinical Immunology (EAACI) Guidelines. Fifteen patients had reacted in DBPCFC with codfish (no information regarding how long before testing with gelatine), 12 of the remaining patients had experienced “classical systemic type 1 reactions to ingestion of very small amounts of codfish within few weeks to less than 24 months prior to this study”, and the last 3 patients all had experienced “relevant reaction to fish more than two years ago” and “still had a large skin prick test (SPT) to codfish and all had recently experienced rhinoconjunctivitis and asthma due to vapour from preparation of fish meals”. These latter patients considered highly sensitive had not been challenged with fish for ethical reasons.

Protocol: SPT and histamine release tests (HR) were performed with fish gelatine made from codfish skin and with fresh raw codfish. Codfish specific IgE was measured. All patients underwent DBPCFC (seven dose steps increasing from 10 mg to 7 g with a cumulative dose of 14.61 g fish gelatine).

Results: In all 30 patients SPT, HR, and specific IgE to codfish were positive. In SPT with fish gelatine 3/30 were positive, and in HR with fish gelatine 7/30 were positive. One patient showed a mild objective reaction to placebo and no reaction to active substance challenge. Two patients reported mild subjective reactions to active challenge. They were re-challenged, and one patient with positive SPT/HR to fish gelatine and a high level of codfish-specific IgE (477 kUA/L) described subjective symptoms to active challenge with no reaction to placebo, while the other patient experienced mild subjective symptoms to placebo and nothing to active challenge. Thus, one strongly fish allergic patient showed a mild subjective reaction (“irritation in the throat” of which the patient according to the investigators was unsure [1st challenge], and “itching in the mouth” [2nd challenge]) to a cumulative dose of 7.61 g of fish gelatine, but without reappearance of the symptoms with the final dose (cumulative 14.61 g dose) in any of the two challenges. None of the 30 patients reacted adversely to the ingestion of 3.61 g cumulative dose of fish gelatine.

Conclusions: One patient had a confirmed subjective reaction at a cumulative dose of 7.61 g of fish gelatine at DBPCFC, without reaction to the following higher dose. The investigators conclude that the NOAEL for fish gelatine was a cumulative dose of 3.61 g. However, the applicant claims with reference to the study protocol (“reaction to fish gelatine not present at the maximal dose given” to be regarded as “no reactions”) that the NOAEL observed was 14.61 g fish gelatine. Statistically, the results according to the investigators indicate that there is 90% certainty that 95% of fish allergic consumers will not react to the ingestion of a cumulative dose of 3.61 g of fish gelatine. The applicant claims that “(the) results demonstrate that fish gelatine is unlikely to trigger adverse reactions”.

3.3.2 *Other clinical studies*

André *et al.* (2003) analysed serum samples from 100 consecutive adults and children (age 1 to 76 years) with documented fish allergy or sensitisation without clinical allergy and tested for IgE antibodies to hydrolyzed and non-hydrolyzed (gelforming) tuna (yellowfin) skin-derived gelatine, tuna skin, tuna flesh, and bovine and porcine gelatines. In SDS-PAGE and immunoblotting three of 100 serum samples showed evidence of reactivity to tuna skin-derived gelatine. Pre-incubation of the serum with gelatine, tuna skin or tuna flesh all removed the band corresponding to gelatine in immunoblotting. There was no evidence for cross-reactivity between fish gelatine and bovine and porcine gelatines. The three patients with IgE binding to tuna skin-derived gelatine had negative skin prick tests for the gelatine, and did not react upon ingestion of 5 g of tuna skin-derived gelatine (single-blind testing with one dose).

3.4 *Ongoing studies*

The applicant indicates, without providing further details, the following studies being conducted: i) further characterisation of the fish gelatine for flavour encapsulation carrier system to substantiate the absence of parvalbumins (Gad c 1 and homologous allergens); ii) validation of the conclusions by consultation with clinical experts; iii) further literature searches.

CONCLUSIONS AND RECOMMENDATIONS

Gelatine for the present application is produced from cold and warm water fish skins. No analytical data regarding possible residual levels of the major fish allergen parvalbumin in the

fish gelatine preparation are provided. Typical levels of fish gelatine in some industrially processed foods are indicated to be up to around 1000 mg/kg. Levels of intake of fish gelatine under the conditions of use specified by the applicant are likely to be ranging from tens to hundreds of mg per day.

In a DBPCFC study of 30 patients with clinical allergy to fish, no patient reacted to a cumulative dose of 3.6 g of fish gelatine.

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However, appropriate analytical methods to determine residual levels of parvalbumin in fish gelatine preparations are needed. More data from clinical challenge studies with gelatine of fish allergic individuals having specific IgE or positive skin prick tests to fish collagen or gelatine are needed.

DOCUMENTATION PROVIDED TO EFSA

Dossier submitted by Givaudan Schweiz AG to the European Commission pursuant to Article 6 Paragraph 11 of Directive 2000/13/EC as amended by Directive 2000/89/EC, on 25 August 2004.

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