

SHORT REPORT

Evaluation and standardisation of different matrices used for double-blind placebo-controlled food challenges to fish

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Abstract

Background: Fish allergens represent one of the most common causes of adverse reactions to food worldwide. Double-blind placebo-controlled food challenges (DBPCFC) are the gold standard for food allergy diagnosis. However, no standardised recipes are available for common food allergens such as fish, and a well trained dietitian is essential for creating and standardising them. The present study aimed to create and standardise recipes for use in DBPCFCs to fish.

Methods: Three recipes were prepared. Employing a standardised procedure, a total of 35 panelists evaluated the different matrices using an evaluation form. A paired comparison test was used to estimate total evaluation's outcome. Fish allergic patients were challenged with different fish species blinded with the selected matrix and evaluated the recipe using the same form.

Results: From a base recipe and step-by-step modifications, a low fat recipe was selected among other recipes tested, which proved to be appropriate for fish blinding, in terms of taste, odour, appearance and blinding. Patients challenged with the final matrix found it acceptable, no matter which fish type was used.

Conclusions: In this pilot study, a recipe with satisfactory organoleptic characteristics was developed and validated for DBPCFC to fish.

Introduction

Fish allergens represent one of the most frequent causes of adverse reactions to food worldwide and, in many cases, clinical symptoms can develop just by touching fish or on inhalation of vapour in areas where fish is cooked (Van Do *et al.*, 2005).

Although various fish species have been reported as allergenic, codfish is the most extensively studied and its major allergen (Gad c1) is identified and characterised (Swoboda *et al.*, 2007). Parvalbumins from different fish species show high homology, explaining their high cross reactivity.

A double-blind placebo-controlled food challenge (DBPCFC) is considered to be the only method of establishing or ruling out an adverse reaction to a food

(Gellerstedt *et al.*, 2007). Nevertheless, the challenge procedures are not fully developed and standardised, apart for food with mild characteristics such as milk, although this would be helpful for the safety of allergic individuals and the accuracy of allergy diagnosis and treatment by health professionals (van Odijk *et al.*, 2005; Devenney *et al.*, 2006; Crevel *et al.*, 2008).

In particular, fish blinding appears difficult as a result of both its smooth texture and intense smell. It is not accidental that, so far, fish gelatine or lyophilised fish has been used only in attempts to blind fish (Bernhisel-Broadbent *et al.*, 1992; Hansen *et al.*, 1996). Furthermore, the use of capsules as a vehicle for DBPCFC is not always feasible. Standard easy recipes have to be developed for masking fish that should be the least time consuming and applicable in everyday clinical practise.

The present study aimed to develop and validate recipes for use in DBPCFCs to fish.

Materials and methods

Development of challenge materials (recipes)

For each recipe, a placebo and an active test food recipe was developed, aiming to fulfil the following requirements: acceptable organoleptic characteristics (taste, smell, texture, appearance); allowance of a challenge dose high enough to elicit allergic reactions in an acceptable volume (approximately 9 g of protein); good matching of sensory properties of placebo and active matrices; and the use of optimum low allergenic matrix ingredients, for use also in polyallergic patients (Vlieg-Boerstra *et al.*, 2004; Lim *et al.*, 2008).

Recipes

Three developed recipes (Table 1) were freshly prepared on the day of challenge and tested at room temperature (RT) in the food laboratory where they were prepared (Allergy Research Centre, Second Paediatric Clinic, University of Athens, Greece).

Sensory testing

Thirty-five volunteer healthy nonfood allergic panellists (medical doctors, nurses, dietitians) validated the recipes. Ethical approval was obtained from local ethics committee on Medical Research Ethics.

Panellists were asked to test a pair of a placebo and an active sample of each recipe, coded with a one-digit random number derived from a random table and allowed

to compare samples as often as desired (Vlieg-Boerstra *et al.*, 2004). An evaluation form was individually filled for each matrix with a value in the range -2 to 2 (0 reflects the baseline of acceptance in terms of each organoleptic characteristic). Comparison of each recipe's pair was recorded as +1 if panellists found them similar and -1 if not.

DBPCFC

Patients' selection criteria

Seven patients were included in the study on the basis of diagnosed fish allergy (positive skin prick test and radio-allergosorbent test), confirmed either by previous positive challenge to fish or a convincing history (Table 2). Anti-histamine medication was discontinued 1 week prior to testing and challenge, to avoid any effect on their outcomes. Patients' challenges were performed with various fish species (cod, tuna and swordfish) blinded with the selected recipe. The total amount of fish used on challenge was approximately 40 g (total protein intake 9.132 g) (<http://www.ars.usda.gov/main/>) (Fig. 1). Negative DBPCFCs were followed by open challenges with boiled fish in a moderate service amount (approximately 90 g).

Challenges were stopped when allergic symptoms occurred and appropriate medication was given. In the case of doubtful reactions, the last dose was repeated (Vassilopoulou *et al.*, 2008).

Skin prick testing

Skin prick tests to various commercial fish extracts, and prick to prick to boiled cod fish fillet before DBPCFC

Table 1 Recipes developed for fish blinding

Recipe I	Recipe II	Recipe III
Active		
1 part of cod fish	1 part of cod fish	1 part of cod fish
1 part of chicken breast	1 part of chicken breast	1 part of chicken breast
1/8 part of starch flour	1 part of mince meat from beef	2 parts of potato powder
1/16 part of spice mix for chicken containing salt, pepper, carry, starch, onion, basil, oregano, thyme, marjoram, tarragon	1 part of traditional bread (Pain Campagnard, Dr. Schär GmbH, Burgstall, Italy)	1/8 part of salt
1/8 part of salt	1/4 part of salt	1/8 part of pepper
1 part of olive oil	1/2 part of olive oil	spearmint, dill
2 parts of water	2 parts of water	2 parts of water
		1/2 part of vinegar

In all cases, ingredients were blended in a commercial blender until a homogenous mixture was obtained, and then meat balls were formed. Olive oil was used for frying. Analogy between ingredients is mentioned as parts of final total mixture; for example, 40 g of fish in recipe III is mixed with 40 g of chicken (one part), 80 g of water (two parts) and 5 g of salt (1/8 part).

Placebo matrices were prepared in the same way as the active matrices, except cod fish was replaced with an equal quantity of chicken breast.

Table 2 Patients characteristics that underwent fish double-blind placebo-controlled food challenges with matrix III

Age (years)	8	9	11	6	12	22	12
Sex	Male	Male	Male	Male	Male	Female	Female
Asthma	Moderate, postviral	Mild atopic	(-)	(-)	Mild atopic	(-)	Moderate, postviral
Rhinitis	Mild, persistent	Mild, interminant, obstructive	(-)	Mild, interminant, obstructive	(-)	(-)	Mild, persistent
Atopic Dermatitis	(+)	(+)	(+)	(-)	(-)	(+)	(-)
Other food allergy	Cow's milk, sensitised to egg	Peanut, tree nut	Tree nut	Egg, tree nut	(-)	(-)	Cow's milk
Type of reaction after consuming fish	CU	U; GI	U;GI	U	U; GI	U; AE	OAS; U; AE
SPTs to CF on challenge day (mm)	3	3.5	3.6	8	5.5	6.7	3.5
Fish sIgE on challenge day	CF: 0.7	CF: 0.8	CF: 1.2	CF: 8.91 T: 2.84 SW: 1.77	CF: 17.2 T: 4.45 SW: ND	CF:8.91 T: >100 SW:ND	CF: 1.18 T: ND SW: ND
DBPCFC	CF: (-)	CF: (-)	CF: (-)	CF: ND T: (+) SW: (-)	CF: ND T: (+) SW: (+)	CF: ND T: (-) SW: (+)	CF: (+) T: (-) SW: (-)
Open challenge	CF: (-)	CF: (-)	CF: (-)	SW: (-)	ND	T: (-)	T: (-) SW: (-)

Open challenges were only performed when double-blind placebo-controlled food challenges (DBPCFCs) were negative.

AE, atopic eczema; CU, contact urticaria; GI, gastrointestinal symptoms; OAS, oral allergy symptoms; U, urticaria; ND, not done; CF, cod; T, tuna; SW, swordfish.

were performed, according standardised protocols, with wheal diameter greater than 3 mm² considered as positive (Vassilopoulou *et al.*, 2007).

Specific immunoglobulin (Ig) E

Patients' specific IgE antibodies for various fish species were determined (cod, tuna, whiff, hake, sardine, trout,

swordfish, mackerel, herring, anchovy) (CAP FEIA; Pharmacia Upjohn, Uppsala, Sweden).

Statistical analysis

The binomial exact test (SPSS software, 10th edition; SPSS Inc., Chicago, IL, USA) was used to evaluate the differences between the active and placebo matrix (one-side testing), with $P < 0.05$ (one-tailed probability) to be considered significant. With the paired comparison test, which uses the forced choice procedure, panellists were required to identify the active sample, even when fish was not detectable (Ronteltap *et al.*, 2004).

Results

Recipe validation

Panellists regarded recipe I (Table 1) acceptable in terms of appearance, odour, taste and texture for both active and placebo matrix (Fig. 2), but most identified the active matrix (30/35) (Fig. 3).

The modified recipe II (starch flour replaced with gluten/starch free flour; spices mix removed to minimise other potential allergens; beef mince meat added as a result of stronger organoleptic characteristics compared to chicken alone) was found to be improved in terms of odour (Fig. 2), but blinding showed no improvement (30/35) (Fig. 3).

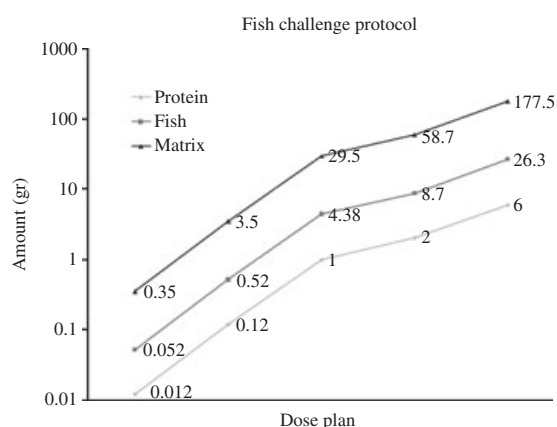


Figure 1 Fish challenge protocol and dose planning with recipe III. Forty grams of boiled fish, containing 9.132 g of fish protein, are blinded in a total final matrix of 270 g, which results in a mixing of the specific ingredients in the analogy detailed on Table 1.

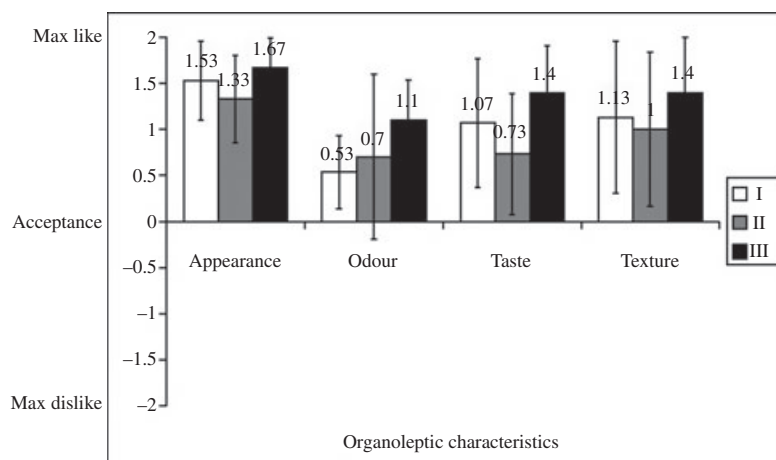


Figure 2 Panellists' average evaluation of active matrices prepared with all recipes I, II and III in terms of appearance, odour, taste and texture. Evaluation was performed with a value in the range from -2, corresponding to major dislike; 0, corresponding to baseline acceptance; and +2, corresponding to major like.

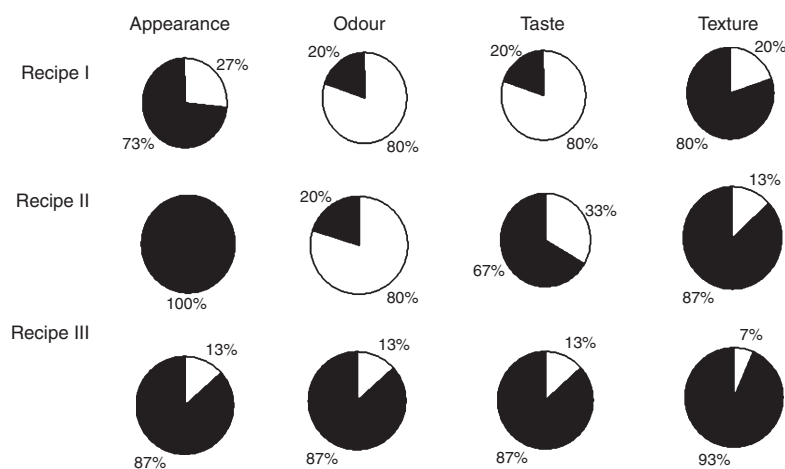


Figure 3 Panellists' comparison of the pair of active and placebo matrices resulting from each recipe in terms of their organoleptic characteristics (appearance, odour, taste and texture) expressed as a percentage of similarity (solid part of the pie).

Ingredients in recipe III (potato powder instead of flours, spearmint and vinegar added as a result of their strong aroma, olive oil removed from basic mixture to minimise the oily feeling) significantly improved fish blinding compared to previous recipes (10/35; $P = 0.029$) (Fig. 3). Furthermore, acceptance increased in terms of appearance ($P = 0.041$), taste ($P = 0.05$), odour ($P = 0.028$) and texture ($P = 0.05$).

Patients' evaluation and reactions

Recipe III was acceptable by all of the patients challenged with fish blinded with this recipe (Fig. 4) and 2/7 (28%) recognised the active sample. Among the four patients challenged to different fish species, one of four was positive to cod, two of four were positive to tuna, and two of four were positive to swordfish, confirming that active matrices retained the allergenicity of the fish, regardless of the fish species used (Table 2).

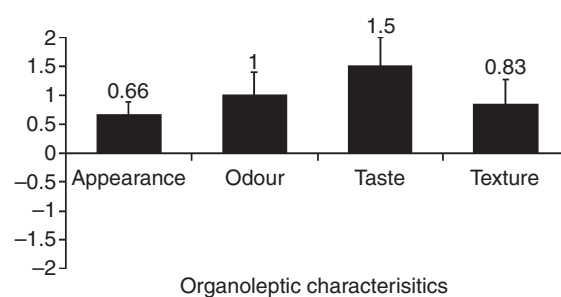


Figure 4 Patients' average evaluation of active matrices prepared with recipe III in terms of appearance, odour, taste and texture. Evaluation was performed with a value in the range from -2, corresponding to major dislike; 0, corresponding to baseline acceptance; and +2, corresponding to major like.

Discussion

The organoleptic characteristics of fish make it difficult to blind for DBPCFCs. A simple recipe suitable for fish

challenges is proposed containing the fewest possible hypoallergenic materials and minimum fat content.

In the present study, three different recipes were developed and compared. Cod fish was used as a model in initial trials as a result of the known cross reactivity between fish species (Helbling *et al.*, 1996; Hansen *et al.*, 1997; Sten *et al.*, 2004; Van Do *et al.*, 2005).

Fish organoleptic characteristics make it difficult to blind and therefore, to date, fish gelatine or lyophilised fish have been used for DBPCFCs. Nevertheless, alterations of fish allergenicity as a result of processing, and therefore false challenge results, have been reported (Bernhisel-Broadbent *et al.*, 1992; Hansen *et al.*, 1996, 2004). Adequate fish protein (approximately 9 g) was blinded, in a final matrix with minimum quantities of other ingredients, to simplify preparation (e.g. exclusion of beef meat and gluten-free flour of the recipe II) and to facilitate the process even for young children because they cannot consume a more than 10–50 g of solid food in a single dose (Sicherer *et al.*, 2000).

To avoid potential side effects upon challenge, allergenic ingredients were excluded from the matrix (e.g. starch and spice mix containing mustard (Figueroa *et al.*, 2005; Chartrand *et al.*, 1997).

Minimising the fat content was critical because trials conducted both *in vivo* and *in vitro* have shown that it prevents IgE-epitope binding, with the risk of a negative challenge outcome or delayed reactions, and further serious implications for the patient (Grimshaw *et al.*, 2003; van Odijk *et al.*, 2005).

The chosen recipe retained the allergenicity of fish because some patients reacted upon challenge, whereas negative DBPCFCs were confirmed by negative open challenges to a higher protein amount (approximately 21 g). Furthermore, the recipe proved appropriate to blind fish other than cod (swordfish and tuna). Most patients found matrices tasteful and declared they could not identify fish's presence. The ones who identified the active matrix reported uncertainty about their decision, but explained their decision as a result of the slightly softer texture of the active matrix or fish after tasting.

Although the recommended recipe was appropriate and acceptable by both panellists and patients, it might not be of use for other individuals because of specific personal or cultural eating habits.

Further testing with this promising fish challenge material is required in a larger population of age-matched fish allergic versus healthy or food-nonfish allergic individuals. Sustained trials will ensure the generalisability and allergenicity of the challenge material.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflict of interests.

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All authors had a valuable role in conducting and fulfilling the study: EV was responsible for designing and writing the protocol, trying out all different recipes, organising challenges and sensory testing with panellists and fish allergic patients, collecting and analysing data, and providing nutritional support to the fish allergic patients. ND, AS, SVC and MFR were responsible for patient selection and medical testing for inclusion in the study. SV also prepared challenge materials according to the final selected recipe for challenging some fish allergic patients and verifying the recipe's good appliance. AS performed the statistical analysis. NGP as a head of the research centre followed up the study and organised the collaboration of all researchers. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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