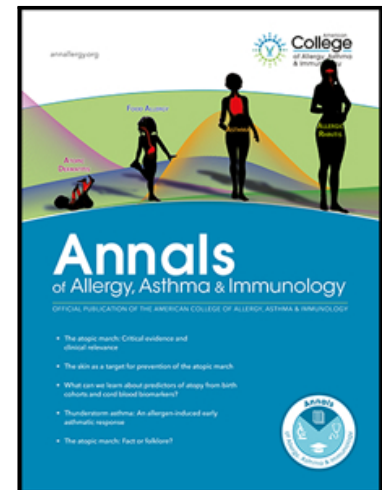


Validation of Novel Recipes for Masking Peanuts in Double-Blind, Placebo-Controlled Food Challenges

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Title: Validation of Novel Recipes for Masking Peanuts in Double-Blind, Placebo-Controlled Food Challenges

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Background: double-blind, placebo-controlled, oral food challenges (DBPCFCs) are the gold standard in food allergy diagnosis. However, proper masking of peanuts is particularly complex due to their intense flavor and odor. Thus, it is important to use validated recipes to ensure their adequate masking during oral food challenges.

Objective: the aim of this study is to design and validate recipes containing masked peanuts for DBPCFCs.

Methods: two types of products (cookies and a custard-type dessert) containing the masked peanuts and other ingredients with low allergenic potential were designed and validated. For this purpose, out of the 24 initial cookie recipes and 12 initial custard recipes developed, those that did not exhibit significant differences in their texture were selected for sensory validation.

Results: similarity triangle tests were performed using a panel of 36 selected tasters, enabling the validation of one pair cookie recipes and one pair custard-type dessert recipe, both with low allergenic potential and suitable for celiacs and vegans.

Conclusion: the validated recipes are of clinical and research interest, as they allow to confirm a peanut allergy and detect a wide range of tolerated threshold doses, which makes it possible to provide specific indications for each patient.

Keywords: peanut, allergy, food allergy, food allergy, validation, recipes, DBPCFC, oral food challenge

Abbreviations

DBPCFC: double-blind, placebo-controlled food challenge.

ED10: dose eliciting a reaction in 10% of allergic patients.

USDA: United States Department of Agriculture.

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1. INTRODUCTION

The double-blind, placebo-controlled, oral food challenge (DBPCFC) is the gold standard in food allergy diagnosis (1). Food masking is not always easy, as the intense flavor, color, or odor of certain foods make it difficult for them to be adequately masked within a food matrix. Thus, it is important to use validated recipes to ensure adequate food masking during oral food challenges. Although the use of different foods for oral challenges has already been validated in previous studies (2,3), more validation recipes including some of the foods that are most commonly implicated in food allergies, such as peanuts, are required in order to conduct these tests in both young and adult patients (3).

Data published in the literature (4,5) describe the increasing prevalence of food allergy in recent decades, with peanut allergy being perceived as a particularly important social concern in many countries with a high prevalence of this condition (6). Following the diagnosis of a peanut allergy, complete avoidance of peanuts with a view to prevent severe reactions can be difficult due to their ubiquity and adversely affect patients' quality of life (1,7). Due to these reasons, we believe that it is crucial to have the necessary tools to facilitate the early and accurate diagnosis of this allergy in order to introduce peanuts into the diet of patients who tolerate this food. Hence, the aim of this study is to develop and validate masking recipes for placebo-controlled, oral food challenges with peanuts.

2. METHODS

2.1 Food Matrices: Ingredients and Preparation

Two types of products containing the masked peanuts and other ingredients with low allergenic potential were prepared: cookies and a custard-type dessert. The allergen was included in both matrices in the form of peanut flour (ML2351, Common-baits FUTTERMITTEL/Preise inkl. MwSt., Deutschland).

2.1.1 Cookies

These were prepared using organic gluten-free whole-grain oat flour (Sol Natural, Vipasana Bio S.L.), gluten-free chickpea flour (NaturGreen), white sugar (Azucarera), fat-free pure cocoa powder (Valor), coconut flakes (Vahiné), baking powder (Royal), salt, gluten-free egg substitute (Orgran), water, sunflower oil (Urzante), lactose-free milk chocolate (Valor), and vanilla essence (Vahiné). The cookie dough was prepared using a Thermomix TM5 food processor (Vorwerk) and consecutively rolled out to a thickness of 6 mm. Subsequently, the dough was cut with a cookie cutter measuring 4 cm in diameter and baked in an electric oven (Rational SCC 61) for 14 minutes at 180 °C. Finally, the baked cookies were coated with melted chocolate and left to set at room temperature.

2.1.2 Custard-type Dessert

This dessert was prepared with hydrolyzed rice (Blemil plus 2, Ordesa), white sugar (Azucarera), fat-free pure cocoa powder (Valor), cornflakes (Corn flakes Special line), instant decaffeinated coffee (Nescafé), xanthan gum (E415, Leila's Deli), and black powder coloring (E153, Rainbow dust). All ingredients were weighed and mixed to obtain a powdered product that was subsequently hydrated and homogenized with 125 ml of water (15–18 °C) immediately before its consumption.

2.2 Physicochemical Analysis to Select the Recipes to be Validated

2.2.1 Cookies

Texture assay: A cut analysis was performed using a TA-XT PLUS texture analyzer (Stable Micro Systems). Each assay was performed with eight replicates.

Color assay: A colorimeter (RS – 232C, KONICA MINOLTA) was used to determine the International Commission on Illumination L^*a^*b (CIELAB, *Commission internationale de l'éclairage*) color coordinates. Each assay was performed with five replicates.

2.2.2 Custard-type Dessert

Color assay: A Verivide Digieye image capture equipment was used to determine the CIELAB coordinates. Each assay was performed with three replicates.

Viscosity assay: Viscometer model ST-DIGIT L (spindle no. 4) was used to analyze the viscosity of the product at a temperature of 18 °C.

2.3 Sensory Analysis

A panel of tasters was first selected following the provisions of standard UNE EN ISO 8586 for the sensory validation of the recipes. This panel comprised a total of 41 candidates between the ages of 20 and 58.

Three triangle tests (UNE EN ISO 4120) and two ranking tests (UNE EN ISO 8587) were carried out in two different sessions to select the panel. The concentrations of the solutions used for the tasting are shown in Table I.

The final selected panel was formed by a total of 36 tasters (14 men and 22 women). Similarity triangle tests ($\beta = 0.05$ and $pd = 30$ percent) were conducted to ensure the sensory validation of the samples under study, with a maximum number of correct answers of 13 for a total of 36 being established to confirm the similarity between two samples. The sensory tests were carried out in the certified tasting room of the Public University of Navarra.

2.4 Statistical Analysis

Analyses of variance (ANOVAs) were performed using statistical software Statgraphics Centurion XVI version 16.2.04. Differences with a p value below 0.05 were considered statistically significant.

3. RESULTS

3.1 Cookies

3.1.1 Adjustment of the Formulations to Obtain Cookies of Similar Appearance and Texture

The flours used were selected based on their impact on the cookies' texture (8,9). Cocoa powder and coconut flakes were used to mask the color and flavor of the peanuts. The initial recipes were subsequently modified using a full factorial experimental design (8×3) to adjust the formulation. The factors selected for the study comprised the type of flour (level 1 = 100% chickpea flour [C], level 2 = 100% oat flour (O), level 3 = 50% O and 50% peanut flour (P), level 4 = 60% C and 40% O, level 5 = 60% O and 40% P, level 6 = 70% C and 30% O, level 7 = 70% O and 30% P, and level 8 = 90% C and 10% O) and the cocoa percentage (level 1 = 2.5%, level 2 = 3.1%, and level 3 = 3.7%). A total of 24 formulations were obtained (S1 to S24, not shown), of which nine corresponded to active formulations.

The cookies that were baked with the active formulations did not differ significantly from those baked with the peanut-free formulations in terms of their size, which was expressed as an expansion rate (diameter [cm]/thickness [cm]). In relation to their color, the active samples were darker (with lower L^* values) and had higher a^* values than the inactive samples ($p < 0.05$).

The texture analysis enabled the selection of two recipes: active recipe S17 (70% O, 30% P, and 3.1% cocoa powder) and inactive recipe S24 (90% C, 10% O, and 3.7% cocoa powder), with no significant differences being detected in terms of their hardness (61.4 ± 13.3 and 62 ± 11.9 N, respectively; $p > 0.05$). In contrast, from a sensory point of view, the graininess of inactive sample S24 was greater than that of the cookie containing peanut flour.

To attempt to match the graininess, flavor, and color of these cookies, both the proportion of flours and the amount of water used in the inactive recipe were modified (10), sugar was added to the active recipe, vanilla essence was added to both formulations, and all cookies were coated with chocolate. Four formulations were selected for the sensory validation (Table II).

3.1.2 Sensory Validation of the Recipes

Two similarity triangle tests were performed. Formulations AF1 and IF1 were analyzed during the first test, obtaining a total of 11 correct answers, and formulations AF2 and IF2 were analyzed during the second triangle test, with a total of 24 tasters detecting the correct sample.

Therefore, the differences between the samples were confirmed. Based on these results, the pair formed by formulations AF1 and IF1 was deemed validated from a sensory point of view.

3.2 Custard-type Dessert

3.2.1 Adjustment of the Formulations to Obtain Custards with Similar Color, Flavor, and Viscosity Characteristics

Rice hydrolyzate was used instead of milk—a common ingredient in commercial custards. Peanut flour (50% protein, USDA) was included in this recipe as well. Xanthan gum was used as a thickener. Fat-free cocoa powder and crushed cornflakes were used to match the color and texture of the active and inactive recipes. Instant decaffeinated coffee was used to ensure similarity in the taste of both recipes. Once the ingredients were selected, and in order to adjust the formulations subject to sensory validation, the initial recipes were modified using a full factorial experimental design ($3 \times 2 \times 2$). The factors selected for the study comprised the type of flour (level 1 = 100% peanut flour [20 g], level 2 = 0% peanut flour and 6% corn flour (7.5 g), level 3 = 0% peanut flour and 8% corn flour [10 g]), the cocoa percentage (level 1 = 4% [5 g] and level 2 = 6% [7.5 g]), and the coffee percentage (level 1 = 0.4% [0.5 g] and level 2 = 0.8% [1 g]) in relation to 125 ml of water. A total of 12 formulations were obtained (S1 to S12, not shown), of which four corresponded to active formulations.

Given that significant differences ($p < 0.05$) were found between the active and allergen-free formulations that were tested in terms of their color and texture, black food coloring was added to active formulation S1 and inactive formulations S6 and S9, and crushed cornflakes were added to active formulation S1. Following the implementation of these modifications, no significant differences were found in the CIELAB color coordinates and, in the viscosity analysis, all samples exhibited a non-Newtonian and pseudoplastic rheological behavior, with no significant differences being detected between the viscosity of the inactive (S6 and S9) and active (S1) formulations. The final recipes subject to sensory validation are shown in Table III.

3.2.2 Sensory Validation of the Recipes

Two similarity triangle tests were performed. Formulations S1 and S6 were analyzed during the first test, obtaining a total of 16 correct answers, and samples S1 and S9 were analyzed during the second triangle test, obtaining a total of 13 correct answers. Thus, the pair formed by active formulation S1 and inactive formulation S9 was deemed validated.

4. DISCUSSION

The interdisciplinary collaboration between the food technology experts, the selected tasters, and the allergists who participated in this research enabled the efficient masking of peanuts in two different types of recipes that can be used in DBPCFCs performed on adults and young people. We believe that the interest of these recipes lies in the fact that we have highlighted the clinical and health care need to develop easy-to-prepare recipes with low allergenic potential, owing to the characteristics and nature of the ingredients used in them, to perform food challenges on subjects under study for a suspected peanut allergy. In addition, given the wide range of doses that can be administered using the validated recipes, these formulations are also useful for determining the peanut dose threshold tolerated by patients.

As reported by Arkwright et al. (11) following their study based on an international multicenter survey that involved over 1600 adult (≥ 18 years) patients with peanut allergy, there is significant variability in the peanut tolerance threshold among peanut-sensitized subjects. In fact, they found that 10% of the sensitized subjects tolerated only 20 mg of peanuts, 50% of them experienced a reaction starting at a dose of 300 mg of peanuts, and 38% of them could tolerate up to 1 g of peanuts. The tolerance or clinical reactivity threshold of each patient can only be determined by performing an oral challenge test, and the use of recipes validated from a chemical, physical, and sensory point of view guarantees proper masking for the DBPCFC. The recipes described in this paper allow for calculating the dose of peanut protein administered to a patient and, therefore, to accurately determine the tolerated dose. For example, each cookie contains 0.62 g of peanut protein, whereas the custards allow a dosage starting at 1.5 mg of peanut protein, with this starting dose being lower than the ED₁₀, which is estimated to correspond to a dose of 2.8 mg of peanut protein that would make it possible to detect the

response threshold in over 90% of patients (5, 11). Consequently, this makes it easier to adjust the amount administered to the minimum dose eliciting symptoms in patients, thereby reducing the likelihood of occurrence of a severe reaction in each patient, as the dose administered is an important factor in the onset of anaphylaxis in patients presenting with less severe symptoms with lower doses of the allergen (12).

In a previous study conducted to validate certain recipes containing different foods for DBPCFCs, including low doses of peanuts, Vielg-Boerstra et al. (2) concluded that it is essential to rely on professional panelists in the sensory validation tests and harped on the importance of these tests being carried out in a food laboratory in order to increase the validation's reliability. In our study, we used a number of selected tasters that we deemed sufficient for the sensory validation of the prepared recipes by conducting triangle discrimination tests, which do not require intense training beforehand.

In addition, the same authors (2) concluded that the adequate statistical analysis of the data obtained with these tests guarantees the validation of the ingredients used in the challenge, as is the case for our study. All aforementioned aspects enabled us to achieve adequate masking of the peanuts, which is an extremely difficult food to mask because of its organoleptic characteristics. In addition, we used ingredients with low allergenic potential in our matrices, thereby avoiding the use of eggs, milk, and wheat, and using gluten-free products and vegan ingredients instead with a view to cover the majority of the peanut-sensitized population regardless of their comorbidities and dietary preferences. Moreover, the fat amount of both recipes was also carefully controlled by adding low fat ingredients in order to avoid high fat foods that could delay the absorption of the allergen (13).

Furthermore, a high cumulative dose (higher than 8 g) of peanut protein can be achieved with either of the two formulations—cookies or custards. In fact, the cumulative dose of the allergen that can be administered with our recipes is greater than that offered by other previously validated recipes (3), which makes them useful when the administration of higher doses is required in the case of subjects reporting subjective symptoms with low doses of the food

during an oral challenge with peanut and who require the ingestion of higher doses to manifest objective clinical reactivity. Moreover, the use of custards, which are easy to reconstitute and store, allows the administration of high doses of peanut protein to patients in an acceptable volume equivalent to that of a commercial custard. In the case of cookies, to achieve the same accumulated dose, up to eight cookies must be consumed, which could represent a limitation in the case of children or subjects with a poor appetite. In short, the validated recipes make it possible to cover a wide range of doses in different scenarios, such as the determination of the tolerated threshold dose and the maximum sensitivity, considering the patients' age and even their taste preferences and intake capacity. Therefore, for determination of lower triggering doses of peanut the custards are recommended whereas the cookies would be preferable when higher starting doses of peanut protein are required.

We believe that the recipes validated in this study constitute a relevant contribution to the growing issue of food allergy and, specifically, peanut allergy, considering the clinical and research interest in conducting DBPCFCs as the gold standard in food allergy diagnosis. Controlled oral challenges allow to provide specific indications to each patient to favor the maintenance of peanuts in their diet in case of tolerance or their avoidance if a peanut allergy diagnosis is confirmed. In this sense, it would be desirable to also have validated recipes containing other foods that often cause food allergies.

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Table I. Solutions used for the tasting panel selection tests

	Solutions
Triangle test 1	Sugar 6 g/L
Triangle test 2	Caffeine 0.2 g/L
Triangle test 3	Salt 1.3 g/L
Ranking test 1	Sugar 6 g/L, 5 g/L, 4 g/L, 3 g/L
Ranking test 2	Caffeine 0.25 g/L, 0.2 g/L, 0.15 g/L, 0.10 g/L

Table II. Selected cookie recipes for validation

Ingredients	AF 1	AF 2	IF 1	IF 2	AF 1	AF 2	IF 1	IF 2
	(% with respect to the total amount of flour)				(grams of each ingredient)			
Oat flour	70	70	60	60	175	175	150	150
Chickpea flour	0	0	40	40	0	0	100	100
Peanut flour	30	30	0	0	75	75	0	0
Coconut	23.1	23.1	23.1	23.1	57.8	57.8	57.8	57.8
Egg substitute	3.7	3.7	3.7	3.7	9.3	9.3	9.3	9.3
Oil	55.4	55.4	55.4	55.4	138.5	138.5	138.5	138.5
Baking powder	1.9	1.9	1.9	1.9	4.6	4.6	4.6	4.6
Sugar	30.8	30.8	24.6	24.6	77.0	77.0	61.5	61.5

Salt	2.5	2.5	2.5	2.5	6.3	6.3	6.3	6.3
Cocoa powder	3.1	3.1	3.7	3.7	7.8	7.8	9.3	9.3
Water	46.2	46.2	33.9	33.9	115.4	115.4	84.6	84.6
Vanilla essence	3.5	4	3.5	4	8.8	10.0	8.8	10.0

Abbreviations: AF = active formulation, IF = inactive formulation.

Table III. Ingredients of the Recipes Used for the Sensory Validation

Ingredients	S1	S6	S9	S1	S6	S9
	(% of weight of the unhydrated mixture)			(grams of each ingredient)		
Peanut flour*	38.2	0	0	20	0	0
Hydrolyzed rice	19.1	27	27	10.0	10.0	10.0
Sugar	19.1	27	27	10.0	10.0	10.0
Cocoa powder	14.3	13.3	20	7.5	4.9	7.4
Crushed cornflakes	5.7	27	20	3.0	10.0	7.4
Instant decaffeinated coffee	1.9	2.7	2.7	1.0	1.0	1.0
Xanthan gum	1.4	2.7	2.7	0.7	1.0	1.0
Black powder coloring	0.30	0.27	0.27	0.16	0.10	0.10