

Validation of novel recipes for double-blind, placebo-controlled food challenges in children and adults

B. J. Vlieg-Boerstra^{1*}, I. Herpertz², L. Pasker², S. van der Heide³, J. Kukler¹, C. Jansink¹, W. Vaessen⁴, B. J. Beusekamp⁵ & A. E. J. Dubois¹

¹Department Paediatric Pulmonology and Allergy; ²Department of Paediatrics; ³Laboratory of Allergy and Pulmonary Diseases, University Medical Center Groningen, University of Groningen, Groningen; ⁴Essensor, Ede; ⁵Department of Dietetics, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

To cite this article: Vlieg-Boerstra BJ, Herpertz I, Pasker L, van der Heide S, Kukler J, Jansink C, Vaessen W, Beusekamp BJ, Dubois AEJ. Validation of novel recipes for double-blind, placebo-controlled food challenges in children and adults. *Allergy* 2011; DOI: 10.1111/j.1398-9995.2010.02539.x.

Keywords

food allergens; food allergy; milk allergy.

Correspondence

Berber J. Vlieg-Boerstra, PhD, RD,
Department of Pediatric Respiratory
Medicine and Allergy, Emma Children's
Hospital, Amsterdam Medical Center,
Meibergdreef 9, 1105AZ Amsterdam, the
Netherlands.

Tel.: +31205662851

Fax: +31206917735

E-mail: b.j.vlieg-boerstra@amc.uva.nl

*At the time of the study.

Accepted for publication 9 December 2010

DOI:10.1111/j.1398-9995.2010.02539.x

Edited by: Antonella Muraro

Abstract

Background: In double-blind, placebo-controlled food challenges (DBPCFCs), the use of challenge materials in which blinding is validated is a prerequisite for obtaining true blinded conditions during the test procedure. Therefore, the aim of this study was to enlarge the available range of validated recipes for DBPCFCs to facilitate oral challenge tests in all age groups, including young children, while maximizing the top dose in an acceptable volume.

Methods: Recipes were developed and subsequently validated by a panel recruited by a matching sensory test. The best 30% of candidates were selected to participate in sensory testing using the paired comparison test.

Results: For young children, three recipes with cow's milk and one recipe with peanut could be validated which may be utilized in DBPCFCs. For children older than 4 years and adults, one recipe with egg, two with peanut, one with hazelnut, and one with cashew nut were validated for use in DBPCFCs.

Conclusions: All recipes contained larger amounts of allergenic foods than previously validated. These recipes increase the range of validated recipes for use in DBPCFCs in adults and children.

The double-blind, placebo-controlled food challenge test (DBPCFC) is characterized by several crucial features. One of these features is sufficient blinding of the allergenic food to avoid that expectation factors can influence the outcome of the test. When neither the patient nor the healthcare professionals involved in the test can identify when the placebo or active test food is being administered, the test is double blind.

Blinding is a prerequisite for obtaining unbiased observations by both patients and healthcare professionals during the test procedure. Without validation of blinding of challenge materials or recipes, true double-blind test conditions cannot be achieved.

To date, efforts to develop and validate challenge materials have been limited, even for studies in which DBPCFCs are used. We previously showed that validation of blinding of recipes for DBPCFCs could be achieved in a professional food laboratory using professional panelists (1). Sensory testing was used to demonstrate that the presence of the

allergenic food could not be detected in the active test food. Subsequently, several other authors have adopted this method (2, 3), including the Food Allergy Research & Resource Program (FARRP) of the University of Nebraska, and some of the recipes developed by Institute of Food Research (IFR) and used in the Europrevall study (personal communication Dr. S. Taylor (FARPP) and Dr. C. Mills (IFR), respectively).

Although several recipes have been published, there is a need for a broader range of validated recipes to cover all age groups, all allergenic foods, and to meet the preferences and dislikes of fussy eaters. Specifically for children younger than 4 years of age, there is a need for recipes containing egg, peanut, and nuts. Additionally, for older children, there is also a need for recipes with a higher top dose than have been validated to date. Finally, new or altered amino acid and hydrolyzed formulas have become available which could be used as vehicles or placebos. Therefore, the aim of this study was to

enlarge the available range of recipes for DBPCFCs with the most common allergenic foods such as cow's milk, egg, peanut, hazelnut, and cashew nut to facilitate oral challenge tests in all age groups, including young children, while maximizing the top dose in an acceptable volume using a panel of adults.

Methods

Development of the recipes

Recipes were developed for cow's milk, egg, peanut, hazelnut, and cashew nut. The development of the challenge materials was performed as previously described (1). Briefly, a placebo and an active test food were developed for each recipe. The active and placebo test foods were as similar as possible to a total amount of the allergenic ingredients equivalent to one food serving or, alternatively, to the maximum amount which could be disguised in the total challenge dose. Based on previous work (1) for liquid and mashed foods, the total challenge dose was limited to not more than 250 ml, whereas for the solid foods, the total challenge dose was limited to not more than 125 g. In the solid recipes, we aimed to keep the fat content of the recipes as low as possible (4). We aimed at masking a total amount of allergenic food ingredients equivalent to one food serving (5–7).

Food matrices and allergenic food ingredients

For cow's milk, the following formulas were chosen as a vehicle: Nutramigen AA, an amino acid formula which has recently become available, Nutramigen 2 LGG (Mead Johnson Nutrition, Nijmegen, the Netherlands), (the taste of this product has recently been changed by the manufacturer), and Friso Allergy Care, a casein hydrolysate available in the Netherlands. For the cow's milk recipes, powdered ingredients were used as much as possible to facilitate preparation, and thus a protein-enriched cow's milk powder (Protifar Plus, 88.5% protein) was used. Coconut cream was added to the placebo milk recipes to mask the fatty taste and texture of milk resulting in a slightly different taste of the formula. For the egg recipe, gingerbread was used as a food matrix, in which pasteurized and lyophilized whole egg powder (47.4% protein; Bouwhuis Enthoven, Raalte, the Netherlands) was disguised.

Several peanut recipes were developed, both for younger and for older children. For children of 4 years and older, gingerbread and minced meat were utilized as a vehicle. For the younger children, ready-to-use baby food (Olvarit, Nutricia, the Netherlands) served as a vehicle. Partially defatted and lightly roasted peanut flour was used as the allergenic ingredient (12% fat; 51.6% protein; Golden Peanut Company, Alpharetta, GA, USA). This peanut flour is easier to mask than ground peanuts because of its fine texture and reduced fat content. For the hazelnut and cashew nut recipes, gingerbread was also used as a vehicle. For the hazelnut recipe, we used unroasted nuts, as it has been shown that roasting reduces the allergenicity of hazelnuts (8). In some active and placebo test food recipes, artificial peanut and hazelnut

flavors (Internatio Möller, Mechelen, Belgium) were used to mask and/or match the typical taste and smell of peanut, hazelnut, and cashew nut.

Validation of the recipes and selection of the panel for sensory testing

The validation of the challenge materials was performed by sensory testing for difference as previously described (1). The objective of this test is to determine whether the active test food sample containing the allergenic food ingredient could be identified in a statistically significant way. When performing this test, panelists were asked to evaluate one placebo sample and one active sample of a recipe in comparison. Samples were coded by using three-digit numbers derived from a random table (9). Not only taste but all sensory aspects (including appearance, smell, texture, and after taste) were evaluated. The two sample combinations (verum-placebo and placebo-verum) were offered with equal frequency in random order to the panelists. Panelists were asked to identify the sample containing the allergenic food. The paired comparison test has a forced choice procedure, requiring panelists to guess the right answer when the presence of the allergenic food is not detectable.

A two-step procedure was used for validation. Once developed, recipes were first tested by at least 15–20 hospital volunteers and students in Nutrition and Dietetics. When the active test food sample could not be statistically significantly frequently identified, the recipe was considered as being preliminarily validated. Subsequently, the recipe was tested by panelists specifically selected for the sensory panel of the Food Challenge Unit of the University Medical Center Groningen (UMCG).

Ninety-nine candidate panelists were recruited by local advertisement to participate in a matching test (Essensor, Ede, the Netherlands) (Fig. 1). Sensory tests are used to determine a candidate's ability to discriminate differences among several taste and smell stimuli presented at intensities well above threshold level (9). To test their tasting ability, candidates are familiarized with an initial set of seven labeled taste solutions in water: sweet, sour, bitter, salty, astringent, metallic, and neutral. After familiarization, the candidates are asked, in a testing phase, to taste a set of 10 undisclosed (randomly numbered) taste stimuli and to label them with the previously learned taste names. On this basic taste test, a score between 0 and 10 can be obtained.

To test the candidates' odor recognition abilities, the following procedure was used: Every 45 s, the candidate is presented a fragrance stimulus (such as vanilla, peppermint, orange, ginger, and cinnamon) and asked to smell it and to tick the correct aroma on a score sheet on which 24 matching fragrances are given. Eighteen stimuli are given in one run. In total, the test consists of two runs of 18 stimuli, each run taking 13.5 min. Fragrances are presented in a wide-mouthed jar with tight cap, which is opened only during smelling (9). The test material is covered with many layers of cotton wool to preserve the fragrances in the headspace of the jar.

All correct answers are added up, and a score between 0 and 36 is given accordingly to each candidate. Both basic taste score and odor recognition scores are transformed to Z scores and added up into one total score. Candidates with scores in the top 30% of the group (30 panelists) were selected for the sensory panel of the UMCG.

Sensory testing took place in a test room environment at the UMCG having sensory evaluation booths in which the panelists can conduct difference testing individually. Each test was conducted by 22–40 panelists. Panelists were paid for their work (€ 10 per hour) and were instructed to abstain from wearing perfumes on the day of the testing and to abstain from coffee, eating, and smoking preceding and during the testing (1). When the active test food sample could not be identified statistically significantly more frequently than by chance of guessing, the recipe was considered to be validated.

Statistics

In SPSS for Windows 16.0 (SPSS Inc., Chicago, IL, USA), the binomial exact test was used to test the differences, with a significant level of 0.05 (one-tailed probability).

Results

In total, nine recipes were tested by the volunteer panel. Six of these recipes could be validated by the UMCG sensory panel (Table 1), and three recipes were rejected because the active test food sample was identified statistically significantly more frequently by the panelists (not shown). An additional three recipes were validated by the sensory panel of the UMCG without a preliminary validation procedure. The ingredients of the validated recipes are listed in Appendix 1.

In total, nine recipes for use in DBPCFCs were validated: three recipes with cow's milk, three recipes with peanut, one with egg, one with hazelnut, and one with cashew nut.

In Table 2, details regarding the total volume or weight of the recipe, the total amount of allergenic ingredients, the protein equivalent amounts of allergenic protein, the fat content and the age group in which the recipes can be applied are shown. For the solid test foods and the baby food, the fat proportion ranged from 16% to 35.3% of total energy. The

liquid milk recipes contained 41.4–44.6% fat, because of the high fat content of the infant formulas.

The milk and one of the peanut recipes can be used in young children; the other recipes are suitable for children above the age of four and adults. The total challenge dose can be adjusted by varying the total challenge volume. The maximum amounts of allergenic foods disguised in the test foods contained 2.8 g of cow's milk protein, 4.0 g of egg protein, 4.4 g of peanut protein, and 1.7–2.9 g of nut protein.

Discussion

In this study, we succeeded in validating several novel recipes in addition to those already available and published previously. The availability of a broad range of recipes will provide centers with the necessary tools to perform DBPCFCs with the most common allergenic foods in all age groups and thus to enhance their diagnostic capability.

It is important to use recipes guaranteed for adequate blinding in scientific studies and in clinical care in patients in whom a correct diagnosis is of vital importance. Once tested and validated by a panel that consists of well-selected panelists, the results of the blinding can be expected to minimize the psychological effects related to the recognition of test samples, both by patients and by their parents as well as the healthcare professionals performing the test.

Using volunteer panels in an uncontrolled environment is likely to overestimate blinding, and thus validity of recipes, as we showed in a previous study (1). This is further demonstrated in this study where three recipes which could not be decoded by the volunteer panel were subsequently decoded by the UMCG sensory panel. We, therefore, selected a panel of the 30% best tasters using established selection procedures for sensory ability (taste and odor recognition).

It could be argued that recipes used in DBPCFCs in children should be tested by a panel of children. However, there are several drawbacks to this proposal. First, it is known that the power of sensory testing is generally poor (9). To obtain a statistically adequate power, unattainably large numbers of panelists are needed (1, 9). Thus, when performing sensory tests using unselected children, very large test panels of hundreds of children would be needed. Alternatively, because

Table 1 Recipes validated by the University Medical Center Groningen sensory panel using the paired comparison test

Recipe	Number of sensory tests performed	No. of correct responses (active food sample identified)	Critical no. for significance (9)	P-value
Protifar Plus in Nutramigen AA	26	12	18	0.578
Protifar Plus in Nutramigen 2 LGG	22	10	16	0.584
Protifar Plus in Friso Allergy Care	26	13	18	0.500
Hen's egg in gingerbread	32	16	22	0.500
Peanut in Olvarit baby food (carrot-rice-chicken)	40	9	26	0.999
Peanut in gingerbread	32	18	22	0.299
Peanut in minced meat	36	16	24	0.691
Hazelnut in gingerbread	40	13	26	0.981
Cashew nut in gingerbread	40	8	26	1.000

Table 2 Amount of allergenic food and total volume or weight of the test food recipes

Recipe	Total challenge volume or weight	Total amount of allergenic ingredient in recipe	Equivalent amount of fresh food	Protein content (g)	Fat content (%)	Age group
Protifar Plus in Nutramigen AA	250 ml	3.1 g of Protifar Plus	78 ml of cow's milk	2.8	44.6	Infants and toddlers
Protifar Plus in Nutramigen 2 LGG	250 ml	3.1 g of Protifar Plus	78 ml of cow's milk	2.8	41.4	Infants and toddlers
Protifar Plus in Friso Allergy Care	250 ml	3.1 g of Protifar Plus	78 ml of cow's milk	2.8	43.8	Infants and toddlers
Hen's egg in gingerbread	≈125 g	8.4 g of dried whole egg powder	31 g of hen's egg (≈0.62 median size hen's egg)	4.0	24.4	Children above 4 and adults
Peanut in Olvarit baby food (carrot-rice-chicken)	≈215 g	8.6 g of defatted peanut flour	1 serving of peanut butter	4.4	18.9	Children above 1 year
Peanut in gingerbread	≈122 g	8.6 g of defatted peanut flour	1 serving of peanut butter	4.4	16.0	Children above 4 and adults
Peanut in minced meat	≈107 g	8.6 g of defatted peanut flour	1 serving of peanut butter	4.4	33.4	Children above 4 and adults
Hazelnut in gingerbread	≈120 g	12.4 g of hazelnuts (≈12 nuts)	12 hazelnuts	1.7	35.3	Children above 4 and adults
Cashew nut in gingerbread	120 g	15 g of cashew nuts (≈22 nuts)	22 cashew nuts	2.9	31.3	Children above 4 and adults

this is not feasible, it is of most importance to increase power by optimizing the conditions of sensory testing such as the selection of the best tasters. It is doubtful if it is feasible to select a panel of children consisting of the best tasters, representing all ages. The child should be able to understand the purpose of the testing sufficiently, should be able to concentrate for sufficiently long periods of time on the task, and maybe even more importantly, should be able to recognize and describe different kinds of taste and smell of foods. Second, for nonallergic children, the milk recipes based on hydrolyzed infant formulas are quite disgusting and hence we expect that children would not want to taste these foods and thus would not be able to test them thoroughly.

The major challenge in developing and validating recipes for use in DBPCFCs is to disguise sufficiently great amounts of allergenic foods in an acceptable volume (1, 10) to avoid false-negative test results. We aimed at masking a total amount of allergenic food ingredients equivalent of one food serving (5–7), or alternatively, the maximum amount that could be disguised in an acceptable volume of 250-ml liquid test food, ready-to-use baby food or 125 g of solid test food. This is based on the observation that individual patients may react to amounts of allergenic food up to a normal food serving and also reflects the maximum amounts of test foods which can be consumed by young children during DBPCFCs.

In an earlier study (4), it was shown that the threshold in peanut challenges using recipes with a fat content of 22.9% of total energy was significantly lower than in recipes with a fat content of 31.5%. Therefore, we tried to keep the fat content of the solid recipes below 25% of total energy.

A limitation of this study is that although we have calculated the amount of allergenic protein incorporated in the recipes,

we did not measure the IgE-binding capacity of the allergens in the recipes after preparation. It is known that heating and the presence of other ingredients such as wheat may reduce IgE-binding capacity to proteins (11) and the importance of this effect should be addressed in future studies.

Cow's milk recipes

The milk recipes we validated can be used in DBPCFCs in young children. We could not disguise more than 3.1 g of Protifar, equivalent to 78 ml of cow's milk, in 250-ml test food. Although this amount is lower than one serving of cow's milk of 150 ml, in our experience, this challenge amount is sufficient in most children to avoid false-negative test results. However, older children and adults are able to consume larger amounts of test food and hence 480 ml of test food can be administered (containing an equivalent amount of 150 ml of cow's milk) by changing the total test food volume. Although we would have preferred to use a powdered coconut cream, we used a liquid form because, to our knowledge, a suitable coconut cream powder is not commercially available. We would have preferred to use all powdered ingredients for several reasons. First, as cow's milk is the food for which challenges are most frequently performed in the Netherlands, we wanted to facilitate the preparation and in this way to enhance the use of validated recipes in DBPCFC with cow's milk, also in centers and practices where sophisticated kitchen tools and staff are not available to prepare test foods for DBPCFCs. Second, powder recipes have an advantage of greater storability and transportability and hence they may be dispensed from the hospital for use in the patient's home when this is appropriate. Third, powdered ingredients lend themselves more easily to commerciali-

zation in the form of validated ready-to-use test kits for the use in DBPCFCs with cow's milk, for which these recipes could be used. Several test kits have been marketed in the Netherlands, but no validated recipes have been used for these kits to date.

Egg recipe

The recipe with egg is not suitable for DBPCFCs in young children because of the texture and total test volume of the test food. However, this recipe can be applied in children of approximately 4 years and older as well as in adults. As the egg powder is baked at 150°C (302°F) for 30 min, we can ascertain for the tolerance of baked egg and not for raw egg. We were unsuccessful in our attempt to validate a recipe with egg for younger children using a higher total dose than we were able to validate previously (approximately 17 g of egg). (1) We could not mask more than 8.4 g of dried egg powder (equivalent to 31 g of fresh egg) in an acceptable volume of approximately 125 g of test food. However, this recipe still contains a considerable amount (4.0 g) of egg protein. The same strategy may thus be used as for the milk recipes for older children and adults who are able to consume larger amounts of test food: the total amount of test food can be increased to 200 g, containing 13.4 g of dried egg powder equivalent to one medium-sized fresh egg of 50 g. We succeeded in keeping the fat content of the egg recipe low (24.4%).

Peanut recipes

The recipe with peanut in baby food can be utilized in young children and hence the diagnosis of peanut allergy can be established reliably early in life to prevent unnecessary long-term avoidance. This may become increasingly important as studies suggest that the delayed introduction of peanut in young children may counteract the induction of tolerance (12). As the risk of severe reactions also increases with age (13, 14), diagnosing peanut allergy in infancy will also help optimize the safety of the procedure. The other two peanut recipes can be utilized in children of 4 years or older and in adults. We succeeded in masking 8.6 g of peanut flour which is equivalent to

one serving of peanut butter in an acceptable test volume. The criterion of a low fat percentage was met for the baby food and gingerbread recipe, but not for the peanut in (lean) minced meat recipe (33.4% fat). Further studies are needed to compare threshold results using different recipes.

Nut recipes

As for the other gingerbread recipes with egg and peanut, the recipes with hazelnut and cashew nut can be used in children from 4 years and older and in adults. We succeeded in masking amounts of nuts equivalent to 1–2 handfuls of hazelnuts and cashew nuts, respectively, in an acceptable test volume. The fat percentages of 31.3% and 35.3% of the hazelnut and cashew recipe are higher than 25%, but this is caused by the natural high fat content of allergenic foods in question, i.e., hazelnut (89% of total energy) and cashew nut (74% of total energy). Therefore, the fat percentages of these recipes cannot be compared to recipes where other food ingredients from the food matrix result in a high fat percentage.

In conclusion, the recipes validated in this study add to the availability of validated recipes for use in DBPCFCs, in young children as well as in older children and adults. Professional validation of challenge materials is important to establish true blinding which is a necessary prerequisite for reliable diagnosis.

Acknowledgments

We thank the students in Nutrition and Dietetics, Marjolein de Nie-Vermeulen, Dalia Yousif, Christine van Vuuren and Anda Toonder for their dedicated assistance in developing and validation of the recipes. We also thank the panelists for participating in the sensory testing and definite validation of the recipes and for their willingness to taste the bitter hydrolysates and amino acid formula samples without complaining.

Funding

This study was funded by Mead Johnson and Friso Infant Feeding (Hero, the Netherlands).

References

1. Vlieg-Boerstra BJ, Bijleveld CMA, van der Heide S, Beusekamp BJ, Wolt-Plompen SAA, Kukler J et al. Development and validation of challenge materials for double blind, placebo-controlled food challenges in children. *J Allergy Clin Immunol* 2004; **113**:341–346.
2. Ronteltap A, van Schaik J, Wensing M, Rynja FJ, Knulst AC, de Vries JH. Sensory testing of recipes masking peanut or hazelnut for double-blind placebo-controlled food challenges. *Allergy* 2004; **59**:457–460.
3. Ballmer-Weber BK, Holzhauser T, Scibilia J, Mittag D, Zisa G, Ortolani C et al. Clinical characteristics of soybean allergy in Europe: a double-blind, placebo-controlled food challenge study. *J Allergy Clin Immunol* 2007; **119**:1489–1496.
4. Grimshaw KE, King RM, Nordlee JA, Hefle SL, Warner JO, Hourihane JO. Presentation of allergen in different food preparations affects the nature of the allergic reaction – a case series. *Clin Exp Allergy* 2003; **33**:1581–1585.
5. Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M et al. Double blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol* 1988; **82**:986–997.
6. Niggemann B, Wahn U, Sampson HA. Proposals for standardization of oral food challenge tests in infants and children. *Pediatr Allergy Immunol* 1994; **5**:11–13.
7. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J et al. Standardization of food challenges in patients with immediate reactions to foods – position paper from the European Academy of Allergology and Clinical Immunology. *Allergy* 2004; **59**:690–697.
8. Hansen KS, Ballmer-Weber BK, Luttkopf D, Skov PS, Wüthrich B, Bindslev-Jensen S

- et al. Roasted hazelnuts: allergenic activity evaluated by double-blind, placebo-controlled food challenge. *Allergy* 2003;**58**:727–729.
9. Meilgaard MC, Civille GV, Carr BT. Sensory Evaluation Techniques. CRC Press LTD, Boca Raton, London, New York, Washington, DC, 1999.
10. Carter C. Double-blind food challenges in children: a dietitian's perspective. Current medical literature (Allergy). *The Royal Society of Medicine* 1995;**3**:95–99.
11. Kato Y, Oozawa E, Matsuda T. Decrease in antigenic and allergenic potentials of ovomucoid by heating in the presence of wheat flour: dependence on wheat variety and intermolecular disulfide bridges. *J Agric Food Chem* 2001;**49**:3661–3665.
12. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol* 2008;**122**:984–991.
13. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001;**107**:191–193.
14. Pumphrey R. Anaphylaxis: can we tell who is at risk of a fatal reaction? *Curr Opin Allergy Clin Immunol* 2004;**4**:285–290.

Appendix 1: ingredients of validated recipes

Ingredients are listed in the order in which they are incorporated in the recipe

Recipes	Active test food ingredients	Placebo test food ingredients
Protifar Plus in Nutramigen AA	250 ml of Nutramigen AA (34 g powder + 225 ml water) 3.1 g Protifar Plus (Nutricia)*	250 ml of Nutramigen AA (34 g powder + 225 ml water) 9.5 ml of coconut cream (Kara)†
Protifar Plus in Nutramigen 2 LGG	250 ml of Nutramigen 1 LGG (37 g powder + 225 ml water) 3.1 g Protifar Plus (Nutricia)*	250 ml of Nutramigen 1 LGG (37 g powder + 225 ml water) 9.5 ml of coconut cream (Kara)†
Protifar Plus in Friso Allergy Care	250 ml of Friso Allergy Care (37 g powder + 225 ml water) 3.1 g Protifar Plus (Nutricia)*	250 ml of Friso Allergy Care (37 g powder + 225 ml water) 9.5 ml of coconut cream (Kara)†
Hen's egg in gingerbread‡	50.5 g of soft white sugar 8.5 g of dairy-free margarine 42 g of self-raising wheat flour 8.4 g of dried whole egg flour§ 42 ml of rice milk 0.08 g of salt 8.5 g of 'dethmers® koekkruiden'*** (gingerbread spice mixture) 6.7 g of desiccated coconut 6.7 g of beet sugar syrup††	42 g of soft white sugar 8.5 g of dairy-free margarine 59 g of self-raising wheat flour 8.5 ml of soy cream (Alpro soja cuisine)¶ 34 ml of rice milk 0.17 g of salt 3.4 g of 'dethmers® koekkruiden'*** (gingerbread spice mixture) 8.4 g of desiccated coconut 10.1 g of beet sugar syrup††
Peanut in Olvarit® Baby food (carrot–rice–chicken)	152 g of Olvarit® Babyfood: carrot, rice and chicken* 39 g of mashed potato (containing 5% rice milk) 48 g of apple sauce 8.6 g of defatted peanut flour‡‡ 6.4 g of soft white sugar	152 gram of Olvarit® Babyfood: carrot, rice and chicken* 39 g of mashed potato (containing 5% rice milk) 27 g of apple sauce 0.54 ml of peanut flavor QL 35189§§ 6.5 g of soft white sugar
Peanut in gingerbread‡	31 g of soft brown sugar 6.2 g of dairy-free margarine 43 g of self-raising wheat flour 8.6 g of defatted peanut flour‡‡ 0.025 ml of hazelnut flavor QL 13849§§ 37 ml of rice milk 0.12 g of salt 1.25 g of 'dethmers® koekkruiden'*** (gingerbread spice mixture) 7.4 ml of beet sugar syrup††	31 g of soft brown sugar 6.2 g of dairy-free margarine 43 g of self-raising wheat flour 0.09 ml of peanut flavor QL 35189§§ 0.025 ml of hazelnut flavor QL 13849§§ 31 ml of rice milk 0.12 g of salt 1.25 g of 'dethmers® koekkruiden'*** (gingerbread spice mixture) 6.4 ml of beet sugar syrup††

Appendix 1: (Continued)

Recipes	Active test food ingredients	Placebo test food ingredients
Peanut in minced meat	92 g of lean fine ground minced beef (6% fat) 25 ml of rice milk 8.6 g of defatted peanut flour†‡ 0.65 g of salt 0.3 g of pepper	92 g of lean fine ground minced beef 18.5 ml of rice milk 0.09 ml of peanut flavor QL 35189§§ 13 g of breadcrumbs (free of egg/milk) 0.56 g of salt 0.3 g of pepper
Hazelnut in gingerbread‡	24.6 g of soft brown sugar 4.8 g of dairy-free margarine 34.5 g of self-raising wheat flour 12.4 g of ground blanched unroasted hazelnuts 0.06 ml of peanut flavor QL 35189§§ 29.6 ml of rice milk 0.12 g of salt 2.4 g of 'dethmers® koekkruiden'** (gingerbread spice mixture) 6 g of desiccated coconut 7.2 ml of beet sugar syrup††	24.6 g of soft brown sugar 4.5 g of dairy-free margarine 34.5 g of self-raising wheat flour 0.06 ml of peanut flavor QL 35189§§ 0.36 ml of hazelnut flavor QL 13849§§ 24.9 ml of rice milk 0.12 g of salt 1.8 g of 'dethmers® koekkruiden'** (gingerbread spice mixture) 6 ml of beet sugar syrup††
Cashew nut in gingerbread‡	30 g of soft brown sugar 6 g of dairy-free margarine 42 g of self-raising wheat flour 15 g of ground cashew nuts 0.12 ml of peanut flavor QL 35189§§ 36 ml of rice milk 0.12 g of salt 3.6 g of 'dethmers® koekkruiden'** (gingerbread spice mixture) 7.2 g of desiccated coconut 7.2 ml of beet sugar syrup††	30 g of soft brown sugar 6 g of dairy-free margarine 42 g of self-raising wheat flour 0.12 ml of peanut flavor QL 35189§§ 0.36 ml of hazelnut flavor QL 13849§§ 30 ml of rice milk 0.12 g of salt 2.4 g of 'dethmers® koekkruiden'** (gingerbread spice mixture) 7.2 g of desiccated coconut 7.2 ml of beet sugar syrup††

'Dethmers koekkruiden®' contains: cinnamon, coriander, nutmeg, clove, cardamom, ginger.

*Nutricia/Danone, Zoetermeer, the Netherlands.

†<http://www.kara.com.sg>, Indonesia.

‡These recipes are baked in 2 paper baking forms.

§Bouwhuis Enthoven, Raalte, the Netherlands. <http://www.bouwhuis-enthoven.com>.

¶N.V. Vandemoortele, Roosendaal, the Netherlands. <http://www.soya.be>.

**Unifine Food & Bake Ingredients, Breda, the Netherlands. <http://www.unifine.nl>.

††'Zeeuwse boerin' keukenstroop. <http://www.dutchepicure.com>.

‡‡Golden Peanut Company, Alpharetta, GA, USA.

§§Internatio Möller, Mechelen, Belgium: to be obtained at <http://www.allergiesupermarkt.nl>.