Impact of Processing on Thresholds of Elicitation

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1. Introduction

Management of food allergen risks requires assessment of the probability that a reaction will occur if an allergic population is exposed to a given amount of allergenic protein. This assessment requires knowledge of the distribution of the maximum doses that will provoke reactions in a population allergic to the allergen of concern, in other words, of thresholds. For any allergenic food, the amount of protein reported to provoke an objective allergic reaction ranges from perhaps a tenth of a milligram up to grams, and sometimes tens of grams; considerable individual variability thus exists among food-allergic individuals (Taylor et al 2002). Allergic reactions are immune responses, albeit aberrant ones, to foreign proteins. At the elicitation stage, one of the key events is recognition of the protein by IgE antibodies that the allergic person has produced in response to prior exposure. These IgE antibodies are produced against both three- and two-dimensional elements of the protein structure, for instance against linear epitopes as well as against conformational ones. It is accepted that, in general, (Taylor and Lehrer 1996) proteins with a molecular mass greater than 10kDa are most effective at generating IgE responses, although there are exceptions such as the lipid transfer proteins (Salcedo et al 2004). Conversely, the ability to generate IgE responses declines with lower molecular masses, and polypeptides below 1.5-2kDa generally lack this capacity, largely because they are poor immunogens anyway. Food processing takes many diverse forms, with a corresponding diversity of consequences for IgE responses to the allergenic proteins in foods. As a first approximation, any process that modifies the structure of a protein might be expected to interfere with its ability to be recognised by antibodies. The concentration of the modified protein that would need to be present to generate a given biological effect would thus be greater, sometimes to such an extent that no response would occur. This would translate biologically as an increase in the threshold of reactivity. However, this argument only considers possible changes of reactivity towards the original (unmodified) protein. It takes no account of the possibility that the allergic patient might actually have produced antibodies to the modified protein, and that analysing the response to the native protein may not be the most appropriate measure. A changed response to a

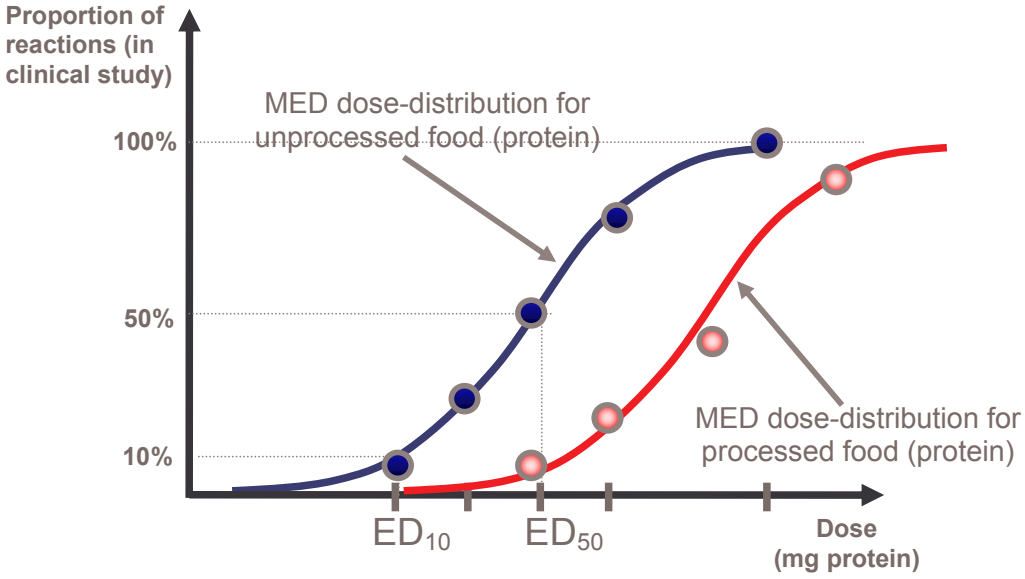
processed food may thus reflect a more complicated phenomenon than simply impaired recognition. Indeed it may also indicate altered immunogenicity of the processed protein compared to the native one, or even the inability to identify the appropriate entity with which to measure reactivity (e.g. with neo-allergens).

2. Thresholds, their measurement and significance

The Concise Oxford English Dictionary (9th Edition) defines threshold (Physiology) as "a limit below which a stimulus causes no reaction". Thresholds can be understood at both an individual and a population level. For any one allergic individual, it will usually be possible to establish a value experimentally through challenge. However, population thresholds cannot formally be determined experimentally. Crevel et al (2006) have suggested "minimum eliciting dose" to designate the amount of allergen predicted to produce a reaction in a defined proportion of the allergic population to distinguish it from thresholds determined experimentally in challenge studies. In this context the minimum eliciting dose can be considered as a threshold for a defined proportion of the allergic population.

Food challenges, in particular double-blind placebo-controlled food challenges (DBPCFC), remain the only scientifically accepted way to establish or rule out allergy to a food (Bindslev-Jensen 2004) and to determine thresholds of reactivity. The DBPCFC was first developed in the 1970s (Bock 1988) as a diagnostic test for food allergy but more recently, low dose challenge protocols have emerged, designed specifically to generate information on thresholds (Taylor et al, 2004). From this perspective, a consensus emerged that in future low dose challenge studies, each food should be tested in at least 29 patients to ensure adequate statistical power. Clearly the actual threshold will depend on the criteria used to define a response, with a continuing debate about whether only independently observable ("objective") signs should be considered, or whether symptoms reported by the patient, but not capable of independent verification ("subjective") should be taken into account. There is also debate about how both the threshold (no effect level) and the lowest observed adverse effect level (LOAEL) should be derived from the challenge results. Threshold studies are performed by titrated food challenges with increasing doses applied at intervals of usually 15 to 30 minutes, chosen mainly for reasons of practicality. There is currently no agreement on whether the cumulative dose ingested up to the point of reaction, or merely the last discrete dose should be taken as the LOAEL.

The ideal study for comparing the allergenicity of a food or food protein before processing and after a defined process would involve challenging an appropriately selected study population with the materials of interest, generating thereby a frequency - dose-distribution of minimum eliciting doses and/or of individual thresholds for the unprocessed and unprocessed foods. The results could be represented as in the figure below, the effect of processing being described quantitatively by the ratio between the ED50s, for instance.



Apart from the inherent difficulties of undertaking studies in man, using challenge studies to evaluate the effects of processing also raises a number of issues with respect to interpretation. As the allergenic activity resides in the protein component of the food, this should at first sight form the basis of the comparison. While this would be logical in foods where the protein content of the unprocessed food is not too dissimilar from that of the processed food, it would be neither practicable nor appropriate where processing removes most of the protein, such as in edible oil refining (Crevel et al 2000) and where the question posed is whether the derived food has significant residual allergenicity.

Conventional toxicological risk assessment derives a safe intake or safe level for a food component, for instance, by considering the Lowest Observed Adverse Effect Level (LOAEL) (usually obtained in an animal study) in conjunction with the exposure of the population to that material. Thresholds for food allergens, as determined in food challenges, are analogous to the LOAEL. They characterise the allergenic hazard and thus form a critical

part of a quantitative risk assessment of the food allergen in question. Knowledge of an individual patient's threshold can help the physician to tailor advice. At the population level, knowledge of the distribution of thresholds provides a key element to to inform public health decisions on allergen risk management both for regulatory authorities and for risk managers in industry.

3. Food processing

Food processing covers an almost infinitely wide range of activities which a food or food ingredient can undergo before it is consumed. At its origins, processing aimed either to improve the range or palatability of what could be made out of a raw material (for instance, milling of wheat, cooking of meat) or to preserve perishable foodstuffs (e.g. salting of fish). Modern processing has added considerably to this, for instance, by imparting the properties of one type of food material to another by altering its physical structure (e.g. use of microparticulated protein in Simplesse (Sampson et al 1992)), developing novel ways of using certain foods (e.g. textured soy protein, mycoprotein). Food allergenicity is largely, if not exclusively, a function of the protein component of the food, even where it involves non-protein structures such as carbohydrate epitopes (Lüttkopf et al 2000). Insofar as processing alters the protein component, as well as the matrix in which the protein resides, and therefore could affect recognition of that protein by antibodies, it is unsurprising that food processing can affect allergenicity. It is equally unsurprising that it has proved difficult to develop any useful rules to predict the effect of defined types of processing on the allergenic potential of foods.

The effects of processing on the allergenicity of foods were first documented by Prausnitz and Küstner (1921) when they demonstrated the passive transfer to Prausnitz of Küstner's allergy to cooked, but not raw fish. Most work since that time has continued to use endpoints such as IgE antibody binding and other in vitro measures. The findings have been extensively reviewed recently (Poms et al 2004, Hefel 1999, Sathe et al 2005) and also form the subject of other papers in this workshop. They will not therefore be discussed extensively here. However, from the perspective of their effects on allergenic potential of whole foods, it may be useful to group processes into those that considerably reduce the protein content, those that cleave the proteins to a greater or lesser extent and those that can alter the conformation of proteins. Edible oil refining and the manufacture of wheat starch are examples of the first. Full oil refining reduces the protein concentration of the final product by over 250,000-fold

compared to the original seed oils, such that the oils are very unlikely to provoke allergic reactions (reviewed by Crevel et al 2000). Processes such as fermentation to produce soy sauces, for instance, involve the hydrolysis of much of the protein component of the raw materials and might be expected to result in reduced ability to trigger allergic reactions, compared to the starting materials. However, the extent of any reduction would depend on factors such as process parameters as well as the resistance of individual proteins to hydrolytic cleavage. The differing allergenicity of various hypo-allergenic infant formulae illustrate this well (Kleinman 1992). The third category of process includes the greatest range of techniques and is thus the one for which predictions are most difficult. Numerous examples exist of apparently unchanged allergenicity (microparticulated egg and milk proteins (Sampson et al 1992), mango nectar (Dube et et al 2004), chickpea (Clemente et al 1999)), diminished allergenicity (kiwi fruit concentrate (Chen et al 2006), lupine (Alvarez-Alvarez et al 2005), increased allergenicity (peanuts (Maleki and Hurlburt 2004), wheat (Simonato et al 2001). In addition, food processing may result in the formation of new allergenic entities, not present in the original food, which do not necessarily trigger reactions in individuals allergic to the native food. The reaction of Küstner (Prausnitz and Küstner 1921) to cooked, but not raw fish represents one of the first documented examples. More recent ones include wheat protein isolates that produced an allergic reaction in a patient who could tolerate wheat flour (Leduc et al 2003).

4. Effect of processing on thresholds of elicitation

Allergy to a food is defined by clinical reactivity to that food, in other words by the allergic individual suffering an adverse reaction on exposure to, or contact with that food. A diagnosis of food allergy requires a DBPCFC, and to date no other test has proved able to substitute for it. Similarly, determining thresholds requires DBPCFC with the relevant food in the appropriate form. It follows that, in order to establish whether processing affects the minimum dose of a particular food required to trigger a reaction, data from DBPCFC are needed for both the processed and unprocessed food. Indeed, as already mentioned, the ideal study would investigate the processed and unprocessed food in the same group of well-characterised allergic individuals using foods and processing methods that had been well-defined. Few published studies meet these criteria. The question arises, therefore, whether conclusions about changes in thresholds can be drawn from other types of data. These data could include challenge studies in which different forms of the food had been examined in different populations, the numerous studies using serological methods to investigate

alterations in allergenicity, as well as epidemiological data. Two illustrative examples will serve to highlight the possibilities and limitations of the available data.

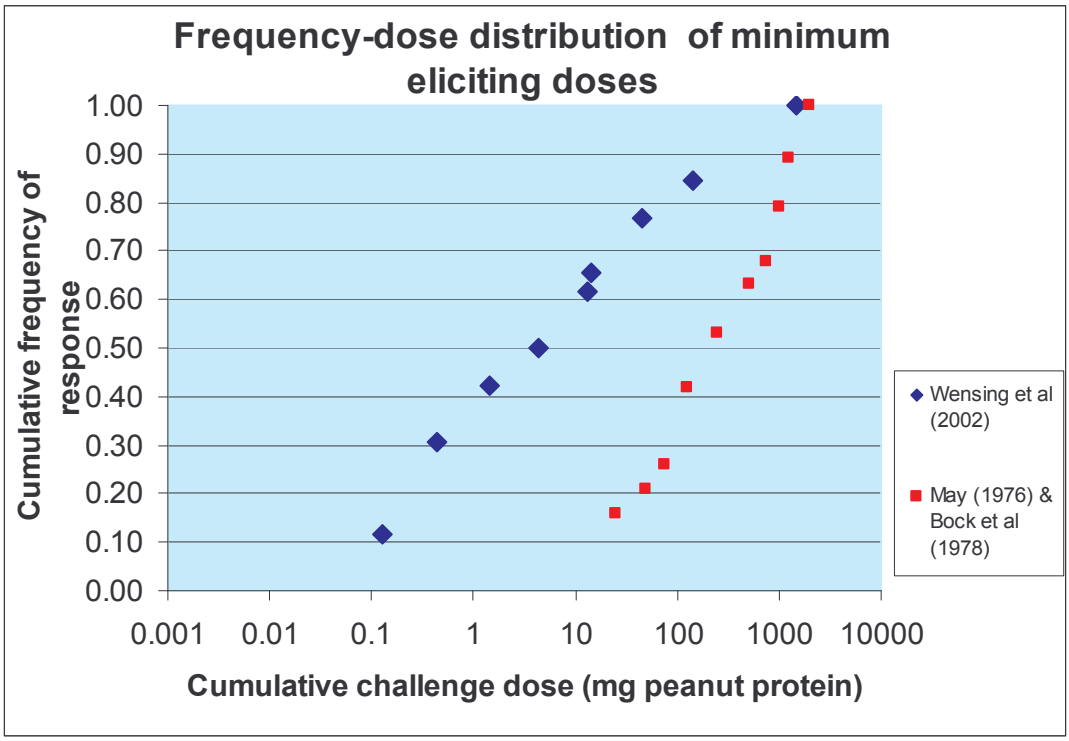
The effect of roasting on the minimum eliciting dose for hazelnut protein

Skamstrup Hansen and colleagues (2003) investigated the effect of roasting hazelnuts on the reactivity of individuals, whose hazelnut allergy was the result of cross-reactivity to Bet v1. The study included 17 patients, based in two centres (Copenhagen and Zurich). In addition to DBPCFC with hazelnut, masked in a pudding, other clinical assessments included specific IgE (CAP-RAST and EAST inhibition), skin prick tests and ex vivo basophil histamine release. In the DBPCFC, all subjects showed a positive response to challenge with raw hazelnut, while only 5/17 showed a response to the roasted. In parallel, median minimum eliciting dose increased from 2-3 g for raw hazelnut to about 7g when they were roasted. This study thus showed a clear effect of processing. However, it also provided information about the value of other measures to determine minimum eliciting doses. The skin prick test was positive in 16/17 subjects to raw hazelnut, but in only 4/17 to roasted hazelnut. Furthermore, of those 4 reactions, only one was positive in an individual that was positive to DBPCFC. A similar picture emerged with IgE binding, although it was less sensitive with raw hazelnut anyway, possibly due to the lability of the allergen itself. Histamine release showed more promise, with a significant reduction in 9 out of 10 tested, and an estimated 50% reduction in allergenic eliciting potency overall. However, none of the in vitro assays were able to discriminate predictively those who would react in DBPCFC and those who would not.

The effect of roasting on the minimum eliciting dose for peanut

No studies equivalent to the Kamstrup Hansen study exist for peanut. However, comparing the results of two early DBPCFC studies on unroasted peanuts with those of a recent study on roasted peanuts might provide at least the upper limits of a quantitative estimate of the difference in allergenic potency between them. The studies in question are those by May (1976), Bock et al (1978) and Wensing et al (2002). The early studies included a total of 20 patients with a mean age of 9, most of them suffering from asthma, but none of which had experienced anaphylaxis. They were challenged with increasing doses of peanut flour equivalent to 25mg to 2000mg of peanut protein, presented as capsules, except where the patient was under 5, where a pudding-type vehicle was used. In contrast Wensing et al's study examined adults (mean age 25), 11 of whom had experienced severe symptoms, including in 3 cases anaphylaxis. Challenge doses ranged from 30 μg to 1000 mg peanut

protein in half-log 10 increments, giving a maximum cumulative dose of 1443mg. The peanut flour was incorporated into a pudding type of matrix. The frequency-dose distribution of minimum eliciting doses for these studies is illustrated in the figure below.



Comparison of the amount of allergen that would provoke reactions in half the tested population shows that roasted peanuts appear to be of the order of 100-fold more potent than their unroasted counterparts. However, the actual difference is likely to be considerably less because of differences in the design of the more recent study compared to the earlier ones. The Wensing study included a large proportion of patients who had experienced a severe reaction, whereas severe reactors tended to be excluded from the earlier studies. Critically, capsules were used to administer the allergen in the early studies. This method would have prevented early warning signs of a reaction in some individuals, resulting in a higher NOAEL. Wensing et al also used a different basis for determining minimum eliciting doses, citing both those for "subjective" symptoms, as well as the dose that gave the first "objective" reaction. Consequently it is difficult to draw conclusions about how much greater the allergenic potency of roasted peanuts is, compared to unroasted ones.

5. Concluding remarks: processing and its implications for allergen risk management

Clear evidence exists that food processing can affect the minimum amount of allergenic protein or allergenic food needed to provoke a response. However, risk management of allergens requires quantitative estimates to guide action. DBPCFC studies comparing the processed and unprocessed food in the same population currently constitute the only methodology which will produce such quantitative estimates. Unfortunately, very few relevant studies exist, and new ones are are likely to remain scarce because of their logistics, as well as the multiplicity of foods and food processing technologies. Serological studies comparing IgE binding to native and processed proteins can be valuable in indicating whether an issue exists, particularly if coupled with more functional in vitro tests such as histamine release. However, the example of the roasted hazelnuts shows emphatically that such studies contribute little to a quantitative estimate of allergenic potency, since 100-fold reductions were observed in IgE binding, but the minimum eliciting dose only increased by a relatively insignificant 2-fold. Such a small change, while useful, would not be sufficient to justify altering allergen management practices in a food manufacturing environment, nor the advice that would be given to the at-risk population. Assessing thresholds of elicitation for even a small proportion of processes will likely prove difficult, making impracticable a general requirement to assess such an effect without consideration of the extent of risk mitigation that could be obtained.

The aim of allergen risk management is to ensure that the risk of allergic reactions, both in terms of number and severity, remains at or below a level which is tolerable from a societal point of view. The effects of processing clearly need inclusion as one of the variables that may affect the risk. However, formulation of rules applicable to categories of processes remains a challenge, and consequently so does definition of appropriate testing methodologies and strategies. An effective approach could start from a reasoned consideration about the likely effect of processing, using this basis to develop an experimental strategy to confirm or modify predictions.

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