

Protein modification by thermal processing

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This paper is a review concerning the way in which heat treatment can modify the allergenicity of food proteins. Any food protein may be allergenic if it can be absorbed intact, or as substantial fragments, through the gut mucosa and then evoke an immune (allergic) response. The intrinsic properties of the protein, the overall composition of the food, and the past processing history (especially thermal processing) all have an effect on the allergic potential. When a protein is denatured by heat, most of the original tertiary structure is lost, so that many of the sites recognized by antibodies on the native molecule are destroyed. There are many examples of allergenicity being reduced, but not eliminated, by heating. But heat-denatured proteins can also present new antigenic sites, uncovered by the unfolding process or created by new chemical reactions with other molecules present in the food (e.g., β -lactoglobulin associating with α -lactalbumin in milk). We have found that heat-denatured β -lactoglobulin has at least one new epitope, not found in the native state. Therefore, thermal processing can be part of a procedure for making hypoallergenic food, but will rarely be sufficient on its own. Increased understanding will help in evaluating novel proteins and processes.

Key words: allergenicity; antigenicity; conformation; denaturation; epitope; folding; unfolding; heat treatment; unfolding.

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The thermal modification of proteins is important in food allergy because heat treatment can bring about substantial changes in their allergenic nature. Many factors contribute to the allergenicity of proteins, some of which are more easily modified than others. A degree of resistance to digestion, a property that can often be modified by heating, is a clear requirement for an allergenic dietary protein, for allergens must be absorbed intact (or as substantial fragments) through the gut mucosa, if they are to stimulate an allergic response (1, 2). Thermal processing might enhance or hinder the digestibility of a particular allergen, so easing or exacerbating (respectively) the associated allergic problem. Antigenicity is another essential factor readily modified by heating. Since an allergen is simply defined as “an antigen that induces allergy”, changes in the antigenic character of a food protein through heat treatment are important to the study of dietary allergy (3, 4). Heat-induced changes in overall allergenicity can, therefore, result from a variety of effects but mainly through digestibility (impact on transmucosal antigen passage) and antigenicity. This brief review is concerned with thermal denaturation only in relation to antigenic character.

Thermal denaturation

Thermal processing is not usually carried out to reduce allergenicity; rather, it is to build or enhance texture, flavour, digestibility, microbiologic safety, freedom from toxins, etc. (5–7). But for those concerned with food allergy, the crucial question is what such processing does to the antigenic potential of the proteins involved. To answer that question, it is first necessary to appreciate what happens to a protein, in molecular terms, during thermal processing. In its native state, a globular protein is folded into a particular, very compact, highly ordered configuration, ultimately determined by the primary structure (specific amino-acid sequence). There are regions of regular conformation, known as α -helix and β -sheet, arising mainly from the repeating geometry of hydrogen-bonding groups in the polypeptide backbone. These regions associate to build the overall 3-D conformation, driven and stabilized by a variety of factors. Hydrophobic groups are buried in the interior of the structure away from water, while hydrophilic groups interact with water on the surface of the protein. This native configuration is cemented by hydrophobic interactions, hydrogen

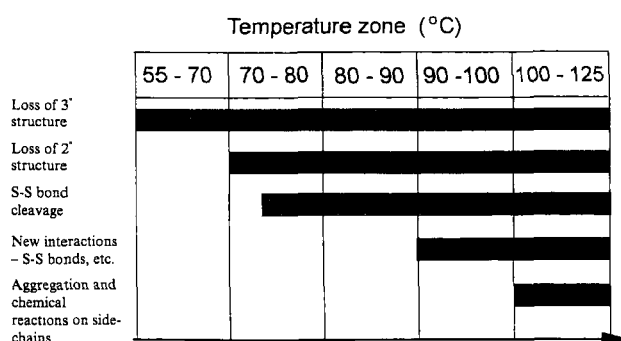


Fig. 1. Temperature dependence of major changes in protein structure (after Lee [14]).

bonding, electrostatic interaction, and disulphide bonds. Before heating, a dietary protein will be predominantly in this stable form, tending to inhabit the "global minimum" energy state (6, 8).

Proteins in this native state may be absorbed and exposed to the immune system when uncooked food is eaten, but what is different after heat denaturation? Thermal modifications start with increased "thermal motion" of the polypeptide chain, rupturing these delicately balanced intramolecular forces and drastically reorganizing all levels of protein structure (Fig. 1). Hydrophobic groups that had been inward-oriented are turned outward, exposing them to unfavourable interaction with solvent water. Unfolding increases with the extent of heating (6) until, at temperatures above 80°C, there is a loss of almost all secondary and tertiary structure, causing the protein to adopt a configuration that approaches a fully unfolded, random coil conformation (9). Initially, there is a reversible unfolding step followed by irreversible alterations (especially at higher pH), which include aggregation (intermolecular interactions), scrambling of disulphide bonds, and chemical modifications of side-chains (10-12). The most important chemical modifications are the deamination of asparagine, the Maillard reaction (between reducing sugars and primary amine groups), and a series of reactions with lysine leading through the N⁶ lysino intermediate (hydrogen loss from the ε-amino) to various derivatives. Intermolecular interactions, often leading to aggregation, are inevitable consequences of denaturation, for, as described above, heat-denatured proteins have their hydrophobic groups turned outward into the surrounding water. This unfavourable contact becomes a powerful force, driving the protein to adopt a lower energy state, most readily achieved by proteins coming together through mutual hydrophobic interaction. These interactions between proteins are, therefore, essentially the same as those that determine native protein

structure, and can amount to the formation of new antigenic structures, as well as the loss of some original antigenicity. For example, Baer et al. (13) reported that heat-denatured β-lactoglobulin (BLG) formed a stable complex with heat-denatured α-lactalbumin.

The whole situation would be much easier to understand if the protein simply unfolded, and then remained in that state on returning to ambient temperature. But most heat-denatured proteins do not remain completely unfolded, retaining some degree of ordered structure resulting from interactions between the numerous amino-acid side-groups and between these and the solvent (6, 9, 15). G-actin, for example, retained some 60% of its original helical structure after it had been heated to more than 30°C above its denaturation temperature (16). At the other end of the spectrum, however, thermally denatured α-lactalbumin was found to be practically devoid of folding, prompting the author to warn against overestimating "residual structure" retained after thermal unfolding (17). Therefore, heated food contains some proteins that have partially unfolded, and others that have irreversibly denatured to attain a virtually random coil state.

Heat-induced changes in antigenicity

Antibodies recognize and interact with allergenic proteins through distinctive molecular shapes (epitopes) on the molecule. There are two types - those made from a few amino acids of the polypeptide chain in their linear order (linear epitopes), and those made of a few amino acids from different parts of the sequence, brought together by the folding of the polypeptide chain (conformational epitopes). Immune responses to globular proteins (including IgE antibody responses) involve both types. Heating destroys most conformational epitopes by unfolding native proteins, leaving only the linear epitopes. Refolding might allow the reconstruction of some native conformational epitopes, while "residual structure" may either create new ones or retain some of the originals. Troublesome allergenic epitopes are mainly linear, because these are more likely to survive denaturation in the gut, as well as heating. Thus, heating can increase or decrease allergenicity, depending upon the protein involved and on the individual patient. Prausnitz & Küstner (18) reported the first example of new, heat-induced allergenic epitopes as far back as 1921, in a classic paper on "supersensitivity" that stands as the first meaningful investigation of food allergy. A fish-allergic patient (Küstner) was found to be totally unaffected by raw fish, even though he was

violently allergic to cooked fish – an intriguing paradox when so little was known of protein structure. The thermal process of cooking had created a new epitope or epitopes.

We have found that heat-treated BLG (htBLG) presents at least one new epitope, which is not found in the native state. The antibody response of rabbits to parenteral immunization with htBLG (90°C for 10 min in aqueous solution) included a population of antibodies that strongly bound the heat-treated protein, but not the native form. The epitope specificity of these antibodies was analysed by means of a random nonapeptide library displayed on protein VIII of M13 bacteriophage (kindly supplied by Prof. Ricardo Cortese, Institute of Molecular Biology, Pomezia, Rome) and by means of PEPSCAN linear peptide arrays (19). Linear epitopes and (sometimes) mimics of conformational epitopes can be identified with the phage-displayed peptide library (20), while PEPSCAN identifies only linear epitopes. The new epitope was not identified with PEPSCAN, strongly suggesting that it was not linear, but a mimic (with the amino-acid sequence ARQQVSLNA) was identified with the phage-displayed peptide library. To prove that the mimic and the new epitope were recognized by the same antibodies, a competition assay was set up, in which the binding of antibodies to the mimic was inhibited in the presence of htBLG. Native BLG could not inhibit the binding, and antibodies generated in response to native BLG did not recognize the mimic. All of this is clear proof that in the residual structure of htBLG there is a potentially allergenic epitope, created not simply by exposing the linear amino-acid sequence, but by the new interactions and folding occurring after heat denaturation.

In contrast, Urisu et al. (21) found that heat treatment reduced the allergenic activity of egg white, to the extent that many (but not all) patients who were allergic to freeze-dried egg white could tolerate the thermally processed material. Casein, the major protein of cow's milk, provides another type of example, for it already exists in a random coil state with very little secondary structure and strong intermolecular interactions (22). Thus, it is not surprising that the antigenicity of casein was not changed after heating at 121°C for 15 min (3, 4).

Implications of thermal processing in food allergy

Thermal processing can be part of a procedure for making hypoallergenic food, but will rarely be sufficient on its own. Properly understood and

controlled, it might be rationally combined with other process options (e.g., enzymic proteolysis) to produce the required effects on antigenicity and transmucosal passage. However, the importance of heat treatment goes beyond the question of whether any given protein is more or less antigenic, for it has an impact on novel dietary proteins, new ingredients, and new processes. The safety evaluation of novel food proteins must include heat-denatured as well as "native" forms. Moreover, food proteins are seldom heat-treated in isolation, for they are normally present in the complex mixture that constitutes a food where many interactions can occur between other proteins and food components (5, 6). Thus, any new formulation or individual new ingredient could cause the formation of new allergens, while new processing conditions could also increase or decrease overall antigenicity.

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