

REVIEW

Impact of dietary factors and food processing on food allergy

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Allergic reactions to food can significantly reduce the quality of life and even result in life-threatening complications. In addition, the prevalence of food allergy has increased in the last decades in industrialized countries and the mechanisms underlying (increased) sensitization are still not fully understood. It is believed that the development and maintenance of oral tolerance to food antigens is a process actively mediated by the immune system and that this reaction is essential to inhibit sensitization. Ongoing research indicates that different dietary factors also may contribute to immune homeostasis and oral tolerance to food and that food processing modulates allergenicity. One of the major questions in food allergy research is therefore which impact nutrition and food processing may have on allergenicity of food and perhaps on sensitization. We summarize in this review the different dietary factors that are believed to contribute to induction of oral tolerance and discuss the underlying mechanisms. In addition, the functional consequences of allergen modification will be emphasized in the second part as severity of allergic reactions and perhaps sensitization to food is influenced by structural modifications of food allergens.

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1 Introduction

Allergic reactions to food can result in a variety of symptoms which range from itching and milder reactions in the oral cavity (also referred to as “oral allergy symptom”) to systemic and even life-threatening reactions such as the anaphylactic shock. The prevalence of food allergy has increased significantly in industrialized countries during the past 50 years and is currently estimated 3% in adults and 3–8% in children [1–3]. According to the recently published international consensus, the term food allergy is defined as a “specific immune response that is directed toward food and occurs reproducibly on exposure to a given food” [4]. The immune reaction can be mediated by IgE (hypersensitivity type I with acute onset of symptoms) or other, non-IgE mechanisms such as the cell-mediated hypersensitivity type IV with delayed immune

reaction [5]. In type I reactions, onset of symptoms is within minutes to about 2 hours after uptake of the allergenic food and is elicited via cross-link of specific IgE antibodies on the surface of mast cells by the antigenic proteins. This reaction triggers the release of histamine, leukotrienes, and other mediators which are causative for the allergic symptoms. IgE production is induced by cytokines such as IL-4, -5, and -13 that are released by T helper (T_H) 2 effector cells leading to differentiation of B lymphocytes to IgE-producing plasma cells [4].

Under normal conditions, the gut mucosa reacts with anti-inflammatory responses to the contact with food antigens. This is a remarkable task as the gastrointestinal tract is supposed to differentiate “self” from “nonself” and “friend” from “foe” within an environment consisting of approximately 10¹⁴ commensal and pathogenic bacterial cells from about 1000 different species [6, 7] and perhaps a cumulative annual amount of 50 kg food protein [8]. One of the anti-inflammatory defense mechanisms also referred to as immune exclusion is the generation of secretory IgA antibodies to prevent colonization of (potentially dangerous) microorganisms and to avoid penetration of harmful antigens. Antigen-specific secretory IgA is produced in B lymphocytes located in Peyer’s patches after antigen delivery by dendritic cells (DCs) and transforming growth factor (TGF)- β -mediated IgA switching [9]. IgA is then exported to the epithelial cell

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Abbreviations: β -lg, β -lactoglobulin; CD, circular dichroism; CD, cluster of differentiation; DC, dendritic cell; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; TGF, transforming growth factor; T_H, T helper

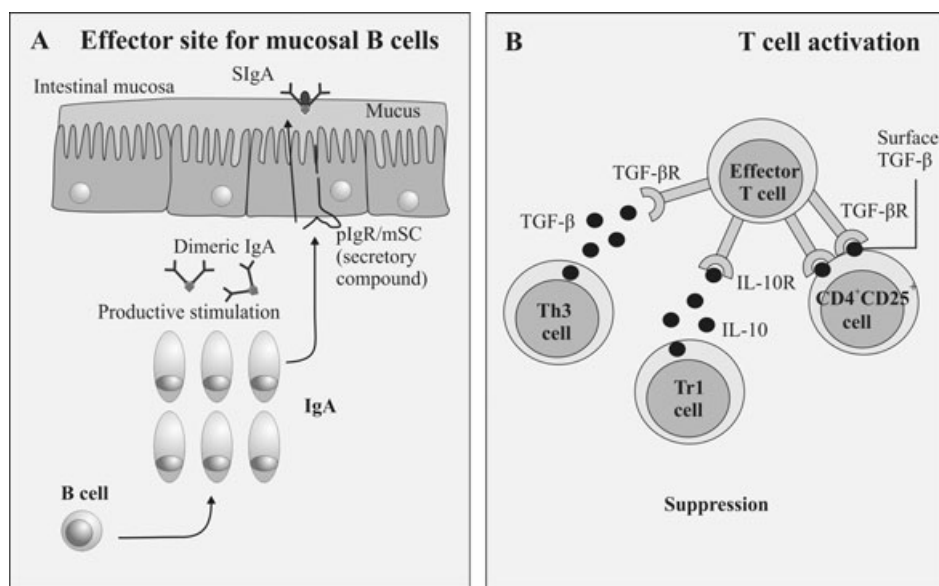


Figure 1. (A) Mechanism of immune exclusion by secretion of sIgA. Activated naïve B cells differentiate to plasma cells or memory cells. IgA⁺ plasma cells secrete IgA which is exported via secretory compound (pIgR/mSC) to the epithelium where it binds to the mucus layer. Figure according to Brandtzaeg [12]. (B) Hyporesponsiveness via regulatory T cells. Activation of regulatory T cells suppresses immune response through the cytokines IL-10 and TGF-β. Figure according to Chehade [9].

surface where it binds to the mucus layer via the secretory compound [10, 11] (Fig. 1A). In addition, the response to nonpathogenic antigens such as food proteins is actively suppressed by regulatory T cells or T-cell anergy, leading to induction of oral tolerance [12]. Hyporesponsiveness via regulatory T cells (low-dose tolerance) is mainly mediated by three subgroups: T_H3 cells that suppress immune response by TGF-β secretion, T_R1 cells which secrete the regulatory cytokine IL-10 and CD4⁺ CD25⁺ cells that are believed to block T_H1 and T_H2 induction via their transcription factor forkhead box P3 (Fig. 1B) [9, 13]. Loss or inability to develop oral tolerance by the above-mentioned mechanisms leads to food allergy by shifting the immune response to overactivation of T_H2 [14].

It becomes increasingly evident that dietary factors also influence the maintenance and perhaps development of oral tolerance. We will focus in this review therefore on current aspects of oral tolerance in relation to dietary factors and discuss the potential underlying mechanisms. In addition, severity of allergic reactions is influenced by modification of allergens in food. The structural and functional consequences of allergen modification will be emphasized in the second part of this review.

2 Impact of dietary factors on food allergy

2.1 Retinoic acid

Vitamin A is taken up primarily via animal-based food as retinal or retinol and can be metabolized to retinoic acid by retinal dehydrogenases [15]. Besides several other regulatory effects, retinoic acid promotes secretory immunity by inducing IgA switch of B lymphocytes. Depletion of vitamin A in rats has been shown to lead to pronounced loss of IgA⁺ plasma cells resulting in attenuation of mucosal immune defense [16].

In addition, together with TGF-β and IL-10, retinoic acid mediates the development of forkhead box P3⁺ regulatory T cells in mesenteric lymph nodes via CD103⁺ DCs [17]. Both mechanisms are believed to contribute to noninflammatory responses against innocuous antigens maintaining intestinal homeostasis.

It has been shown that deficiency of vitamin A goes along with impaired immune responses in the intestine and increased gastrointestinal infections probably because of reduced sIgA levels [18]. This attenuated immune response is associated with increased mortality in developing countries [19]. On the contrary, there has been no association observed concerning vitamin A intake and outcome of allergic diseases in industrialized countries [20]. The role of dietary vitamin A intake might therefore be negligible in the formation of atopic diseases. Interestingly, a recent study provided evidence that retinol is transported from the liver together with bile to small intestinal DC where metabolism to retinoic acid occurs. The ability of CD103⁺ DC to induce regulatory T-cell differentiation was consequently dependent on systemic but not on dietary retinol levels [21]. Together, this indicates that systemic retinol deficiency (that might occur in developing countries) but not marginal dietary retinol deficiency (which is seen in industrialized countries) influences the immunomodulatory effect of retinoic acid.

2.2 Dietary fiber and prebiotics

One attempt to describe the mechanisms that lead to increase in allergy prevalence is the hygiene hypothesis. Nonpathogenic microorganisms are believed to contribute to immune homeostasis either by T_H1 immunostimulation in favor of the IgE-inducing T_H2 cell responses (original hygiene hypothesis) [22] or by the induction of regulatory T cells (extended hygiene hypothesis) [23]. Modification or

reduction of gut-derived microbial immunostimulation is postulated to contribute to the allergic march in industrialized societies. This is underscored by the finding that the microbiota in allergic and asthmatic children is altered compared to nonatopic children [24] and that commensal microbiota indeed can induce CD25⁺ CD4⁺ T_{reg} cells [25]. In addition, evidence emerges that dietary factors have significant influence on the composition of gut microbiota [26] and thereby influence inflammatory responses and immune homeostasis [27]. The gut microbiota is able to generate short chain fatty acids (SCFA) such as butyrate and propionate from the degradation of dietary fiber. SCFA are important energy sources for colonocytes and have been shown to be involved in maintenance of the epithelial barrier function which is vital for oral tolerance [28]. Besides this, a recent study demonstrated direct effects of SCFA on immune cells beyond the gut. SCFA have been identified as (the currently only) ligands of the G protein-coupled receptor GPR 43 of immune cells (neutrophils, macrophages, and eosinophils) and deficiency of G protein-coupled receptor has lead to poorly resolving inflammatory responses, e.g. in allergic airway inflammation models [27]. Together, this indicates that gut microbiota is able to directly signal immune cells via SCFA. Besides the degradation of dietary fiber to SCFA, the commensal *Bacteroides fragilis* are able to synthesize glycans such as polysaccharide A from dietary fiber. Polysaccharide A has been shown to have a strong anti-inflammatory effect via the induction of regulatory T cells and IL-10 production [29].

Apart from the beneficial effects of dietary fiber on the gut microbiome composition and subsequent immunomodulatory effects, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) seem to have direct positive effects on immune homeostasis. GOS and FOS have been shown to cross epithelial layers in vitro and to exert suppression of T_H2 cytokine production in a peanut allergy model [30]. A recent study provided first evidence for a potential mechanism by which GOS and FOS might downregulate allergic reactions. Following dietary intervention with GOS/FOS de Kivit et al. observed upregulation of the soluble lectin galectin-9 in intestinal epithelial cells and increase in galectin-9 serum levels [31]. Furthermore, galectin-9 has been shown to enhance T_H1 and T_{reg} cell development and to suppress mast cell degranulation [31].

2.3 Vitamin E and polyphenols

There is some evidence that higher concentrations of vitamin E have positive effects on IgE-mediated disorders [32]. On molecular level it has been demonstrated that vitamin E suppresses the expression of IL-4 on mRNA level by blocking transcription factor binding sites and interference with promoter activity [33]. IL-4 is essential for the IgE isotype switching in B lymphocytes and mediates differentiation of naïve T cells to T_H2.

Several epidemiological studies have shown a beneficial effect of fruit and vegetable consumption on the incidence of asthmatic diseases [20]. It is however not clear whether these effects can be ascribed to the elevated polyphenol uptake or to a generalized healthier lifestyle. The positive role of polyphenols is supported by one study showing a reduction of food allergy symptoms in mice receiving a polyphenol-enriched diet [34]. More specific, decrease in mediator release and reduction of T_H2-associated cytokine gene expression was observed particularly when the diet was given until the challenge indicating that the underlying effects are rather attenuation of allergic symptoms than induction of oral tolerance. Structural details of the supplemented polyphenols were not provided in this study which consequently does not allow estimating the effect of different polyphenols. Overall, the potential molecular mechanisms of polyphenol supplementation on allergic responses as well as structure–function relationship are still elusive.

2.4 Polyunsaturated fatty acids

Based on the lipid hypothesis, the increase of allergic diseases might in part be related to the increased intake of ω -6 polyunsaturated fatty acid (PUFA) (present mainly in vegetable oils) and a decreased intake of ω -3 PUFA (mainly found in seafish) [35]. ω -6 PUFA can be metabolized via synthesis of arachidonic acid to prostaglandin E₂. Prostaglandin E₂ has been reported to suppress T-cell differentiation to T_H1 [36] and promotes direct isotype switching of B lymphocytes to IgE production resulting in elevated IgE levels [37]. In addition, Oh et al. recently identified a G protein receptor (GPR120) for ω -3 fatty acids that directly induces anti-inflammatory effects [38]. Besides this direct anti-inflammatory effect, a novel ω -3 fatty acid-derived mediator termed resolvin E1 has been identified in coculture of epithelial cells and neutrophils. Resolvin E1 reduced the production of IL-12 and blocked transendothelial migration of polymorphonuclear leukocytes [39]. The above-mentioned studies may explain, at least partially, the anti-inflammatory properties of ω -3 PUFA. It remains to be investigated whether the outcome of allergic diseases can also be influenced via these pathways.

Epidemiologic studies on the effect of PUFA on allergic diseases are however inconsistent. Several studies provide data indicating either beneficial or contradictory results concerning PUFA intake and outcome of allergic disease in children. For adults, no association for the consumption of PUFA and allergy endpoints is observed (for a detailed overview see [20]). Notably, the effects of early supplementation with ω -3 fatty acids are more promising. Furuholm et al. [40] observed that ω -3 PUFA supplementation during pregnancy and lactation leads to a reduction of IgE-associated diseases and milder allergic phenotypes. In addition, the induction of neonatal oral tolerance in rats was significantly influenced by the ω -3/ ω -6 fatty acid ratio of the maternal diet during late gestation and lactation. Differences in maternal diet resulted

in significant shifts of serum ω -3/ ω -6 fatty acid composition in the offspring and a reduced hypersensitivity reaction of the ω -3 population [41]. Together, these data indicate that an ω -3-rich diet might support early oral tolerance induction but shows no (or only minor) effects in established allergy.

3 Impact of processing on food allergen structure and allergenicity

Food is subjected to numerous processing techniques such as thermal treatment in different matrices (e.g. boiling, frying, or roasting) and novel processes such as high-pressure treatment, extrusion, or ultrasound. These processes can significantly alter structural characteristics of allergens and thereby increase or attenuate their antigenic potential [42]. Modifications of processed proteins include partial unfolding, aggregation, cross-linking with food matrix or chemical modifications such as lactosylation, oxidation, or the numerous products of the Maillard reaction [43]. In terms of food safety, processing can also lead to a pronounced reduction of analytical sensitivity when using immunochemical methods such as ELISA. This way false-negative results might be obtained that discriminate modified but allergenic proteins [44, 45].

One of the major questions in the investigation of food allergy is what makes some proteins more allergenic than others. The exact physicochemical properties that render a protein particularly allergenic are still elusive but one parameter is the stability against gastrointestinal degradation to retain the linear or conformational epitopes that cross-link IgE on the surface of mast cells [46]. It has been demonstrated that peptides from whey hydrolysates smaller than ~3500 Da are unable to elicit mast cell degranulation indicating that the minimal sequence length for immunogenicity in context of food allergy is about 30 residues [47]. Food processing can modulate digestibility and thereby influence the antigenic structures presented to intestinal immune cells [48]. At present, different *in vitro* digestion models have been developed to study gastric and intestinal stability of allergens [49]. Notably, the individual differences concerning influence of processing on digestibility are pronounced. While the digestibility of the egg allergen ovomucoid is not influenced by heating or glycation, the same study reported an increased digestion upon heating and decrease of digestibility after glycation for ovalbumin [50]. In contrast, De Jongh et al. showed a decreasing digestibility of the codfish allergen parvalbumin after heat treatment (probably due to aggregation caused by exposure of hydrophobic residues) whereas glycation reduced aggregation propensity leading to increased digestibility [48]. An example for extreme stability against gastrointestinal digestion is the 2S albumin from sesame Ses i 1 [51].

Besides this indirect influence on immunogenicity, food processing can also directly modulate the allergic reaction to proteins. Conformational epitopes can be destroyed by partial unfolding of proteins resulting in loss of allergenicity. Sequential epitopes are however only affected when the

respective primary structure is modified which occurs during extended Maillard reaction. Conformational changes that expose buried structures of the native fold can lead to the formation of neoepitopes [52]. In addition, a recent study demonstrated that extended Maillard modification of ovalbumin resulted in increased uptake in DCs [53]. Allergenicity is also affected by interactions with other ingredients of the food matrix that can influence the availability to the intestinal immune cells as well as the digestibility [54, 55]. For example, the allergenic potential of nonspecific lipid transfer proteins is in parts attributable to their ability to interact with lipids thus reducing digestibility [56]. Together, the net effect of food processing on immunogenicity or digestibility is far from being predictable (though some general rules do exist) [52]. The following paragraphs are therefore devoted to describe in more detail the observed modifications, functional consequences in terms of allergenicity and analytical strategies to elucidate process-induced modifications.

3.1 Partial loss of conformation

Protein unfolding leads to alteration of the native tertiary structure where protein fold is retained only partially and buried reactive residues might become accessible to form intra- or intermolecular cross-links [57]. This is exemplified by β -lactoglobulin (β -lg) that upon heating unfolds revealing Cys¹²¹. Thereby a nonnative intermediate fold is stabilized which is reactive to other proteins via the exposed Cys¹¹⁹ [58, 59]. This enhanced surface activity of the partially folded β -lg can lead to the formation of high molecular weight aggregates by intermolecular disulphide bonds [60]. Circular dichroism (CD) spectroscopy and Fourier transform infrared spectroscopy can be used for the investigation of protein unfolding as alterations in secondary structure elements can be measured (see info boxes Fig. 2, Fig. 3 and [61]). In the study from Carrotta et al., heating leads to loss of helical and β -strand elements and increase of random coil structures [60]. Surface accessibility

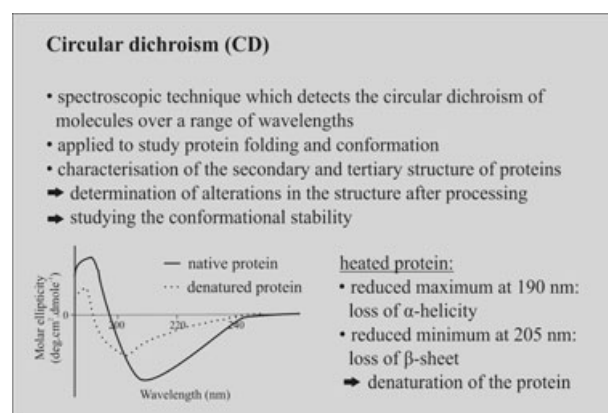


Figure 2. Info box: Circular dichroism (CD) spectroscopy.

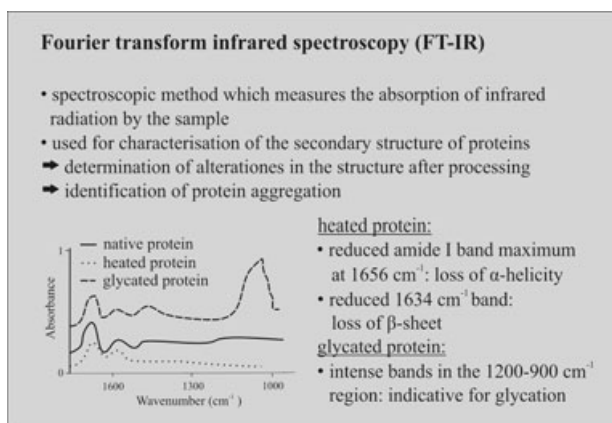


Figure 3. Info box: Fourier transform infrared spectroscopy.

of reactive cysteines has been investigated in a neat study using *N*-ethylmaleimide as a thiol-reactive label and MS to elucidate surface exposure of different Cys residues in β -lg under native and nonnative conditions [58]. In addition, unfolding of proteins can be directly analyzed as the charge distribution of proteins in ESI-MS is broadened upon this modification [62].

The stabilization of nonnative folds via disulphide shuffling and the propensity for further aggregation depends on the allergenic structure. The 2S albumin Ses i 1 shows slight loss of helical structure and increase in β -sheet elements when heated. This structural modification is however reversible on cooling further underlining the stability and compact fold of Ses i 2 [51]. Despite well-defined compact tertiary structures some allergens contain disordered and mobile structural elements. Two prominent examples for such intrinsically disordered proteins are the prolamins from wheat (which form the gluten fraction) and casein from cow's milk [63]. These proteins possess linear rather than conformational IgE epitopes and consequently do not respond to heating with decrease in IgE-binding capacity [64, 65].

3.2 Chemical modification

One of the major reactions of proteins during heating is the Maillard reaction. This nonenzymatic reaction also referred to as glycation occurs when proteins are thermally treated in the presence of reducing sugars [66] leading to formation of glycosylamines by reaction of the protein amino groups with the sugar that rearrange to Amadori products. Prolonged heat treatment leads to a complex mixture of different reaction products and to the formation of advanced glycation end products such as carboxymethyllysine or pentosidine [42]. Further chemical modifications include oxidative modifications that lead to carbonylation of proteins such as 4-hydroxy-2-nonenal [67] and lactosylation especially for milk allergens [68].

Protein modifications via the Maillard reaction are known to modulate the allergenicity of food proteins. One of the first indications for the appearance of neoantigens upon Maillard reaction was provided in a case study reporting that an allergic patient reacted to roasted but not to raw pecan nuts [69]. The change in allergenicity is of course dependent on the studied allergen. In general, allergenicity of fruit allergens is not enhanced by glycation. As an example, the Bet v 1 homologue from cherry, Pru av 1 showed reduced IgE-binding capacity when heated with different sugars and complete loss of IgE binding after incubation with reactive carbonyl compounds such as glyoxal or glyceraldehyde [70].

The peanut allergen Ara h 1 reacts on roasting with the formation of covalently linked trimers that show enhanced resistance to gastrointestinal digest and increased IgE-binding capacity [71]. This different behavior was originally linked to glycation during the roasting process. A recent study challenged this view showing that heating of Ara h 1 in the presence of glucose did not enhance IgE-binding capacity but rather showed differences in the formed aggregates when compared to roasted Ara h 1 [72]. It has to be noted that due to the complex nature of highly modified proteins neither study investigated glycation on molecular level. Despite the morphologically distinct aggregates formed by roasting versus boiling in the presence of glucose, variations in glycation pattern might contribute to the observed differences and remain to be investigated.

Notably, a recent study demonstrated that the consequences of glycation on allergenicity not only depends on the modified proteins but also shows significant individual differences in the allergic population [73]. Glycation of hazelnut proteins consistently reduced basophil activation in patients with oral allergy syndrome, indicating that the allergic reaction to the more labile Bet v 1 homologue Cor a 1 (and probably Cor a 2) was abolished by glycation. In contrast, allergic patients with systemic reactions to hazelnut either showed reduced, increased, or unchanged basophil activation to glycated hazelnut proteins. This indicates that modification of allergens might play a role for some allergic patients in the sensitization phase [73].

Interestingly, a recent study provided evidence that Maillard modification also can result in enhanced T-cell immunogenicity [74]. Glycated ovalbumin showed increased activation of CD4⁺ T cells in coculture with mesenteric DCs. The uptake of glycated ovalbumin in DCs was significantly enhanced and mediated via specific receptor interaction [53, 74].

3.3 Novel processing techniques

Emerging techniques such as high-pressure treatment or pulsed electrical field can be used for pasteurization or sterilization of food products instead of prolonged heating processes. These alternative treatments offer the possibility to reduce negative effects such as off-flavor or loss in texture which can occur upon thermal pasteurization. Johnson

et al. investigated the impact of these techniques on the conformation of the peanut allergen Ara h 2, 6 and the apple allergens Mal d 1b and Mal d 3. Together, pulsed electrical field treatment had no effect on any of the investigated allergens, whereas pressure treatment at elevated temperatures lead to changes in CD spectra of the apple allergens [75]. The peanut allergens were not affected by pressure treatment. On the contrary, high-pressure microfluidization of Ara h 2, 6 resulted in modification of secondary structure and reduction of antigenicity [76]. The more pronounced impact of microfluidization on Ara h 2, 6 structure in comparison to high-pressure treatment might be due to increased shear force during microfluidization.

4 Conclusion

The investigation of the impact of dietary factors on development and perhaps maintenance of oral tolerance is still in its infancy and mechanisms for observed effects are mostly poorly understood. It is however becoming increasingly clear that timing of supplementation with immunomodulatory factors is essential for the beneficial effect. Especially the perinatal period and the first months of life seem to be a critical phase as the gut microbiota is not fully established and the epithelial barrier is developing. Dietary factors such as GOS/FOS or ω -3 fatty acids seem to be promising candidates for further research.

The second question that is of central importance in food allergy research is what makes an allergen and how does food processing influence allergenicity. The increasing number of studies demonstrates that allergenicity can be increased or decreased by different processing techniques and that although structural homology is an indicator for the stability of allergens, it is not reliably predictable which effect processing might have on allergenicity. This is complicated by the fact that besides the processing also matrix components such as polyphenols, lipids, or carbohydrates can strongly influence allergen modification. Processing can also lead to impaired analytical sensitivity for allergens that are not significantly reduced in their allergenicity. Lastly, the impact of allergen modification on sensitization potential is still not understood but the currently available publications indicate that future studies need to take this aspect into account.

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5 References

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