Review:

Allergens of Animal Origin: Stability and Allergenicity of Processed Foods

Matthias BESLER (a, b), Hans STEINHART (a), Angelika PASCHKE (a)

(a) Institute of Biochemistry and Food Chemistry, University of Hamburg, Hamburg, Germany
(b) matthias besler ONLINE PUBLISHER, Hamburg, Germany

SUMMARY

This article reviews recent data on the stability of cow's milk, hen's egg, fish, crustaceae, and meat allergens during food processing. Generally, allergens of food origin are stable and highly potent. Most severe allergic reactions, including fatal events, can be due to ingestion of cow's milk, hen's egg, fish, and shrimp. The allergenicity could be altered potentially by various procedures such as washing, chopping, mincing, heating, canning, storage, and ripening. Separation methods may simply reduce the allergen content of a specific product by e.g. extraction, precipitation, or ultrafiltration. In comparison heating (dry heating, boiling or cooking) and enzymatic digestion affect the allergen structure. Cow's milk and hen's egg allergens retain their allergenicity after common industrial treatments. The production of hypoallergenic cow's milk infant formulas requires extensive conditions of hydrolysis, heating, and/or ultrafiltration. Fish and crustaceae are usually stable to heat treatment, while meat allergens are only partially heat stable. Fish is partially stable and meat is susceptible to enzymatic digestion. Future investigations should monitor the allergenicity of foods throughout manufacturing processes from source to shelf- products by various analytical and diagnostic methods such as DBPCFC, SPT, RAST, SDS-PAGE immunoblot, and inhibition tests. Especially products containing cow's milk and hen's egg should be evaluated since the latter are common as hidden allergens in various processed foods. Clinical evaluations of processed foods should be conducted in several countries with appropriate numbers of patients with convincing allergy to the native food allergen.
(Internet Symposium on Food Allergens 2000, 2(4):171-84)

KEYWORDS

food allergy
allergen stability
food technology
cow's milk
hen's egg
fish
crustaceae
meat

Matthias Besler

Correspondence:
Matthias Besler, PhD
matthias besler - ONLINE PUBLISHER
Mildestieg 17, D-22307 Hamburg, Germany

Phone: +49-40-6970-4097
Fax: +49-40-6970-4098
eMail: m.besler@food-allergens.de

INTRODUCTION

The prevalence of food allergy in children younger than 3 years of age can be up to 8% and in adults about 2% (Sampson 1999). The most important allergens of animal origin are milk, egg, fish, and crustaceae. The frequencies of self-reported allergy to milk, egg, and fish in adults representative of the general population were between 0.5 and 0.7% (Table 1). Allergy to milk and egg is most frequent in young children and has a good prognosis of outgrowing in the first six years of life. In contrast fish and shrimp allergy are not likely to be lost and tend to persist or develop in adulthood (Table 2). The most common symptoms of food allergy are gastrointestinal, cutaneous, and respiratory reactions. Anaphylactic reactions to foods are less frequent. However foods of animal origin are commonly seen among foods inducing anaphylactic reactions (Table 3). Moreover fatal cases of anaphylaxis after ingestion of cow's milk, hen's egg, fish, and shrimp have been reported (Yunginger et al. 1988, Sampson et al. 1992, European Commission 1999).

Table 1: Allergy prevalences to foods of animal origin in the general adult population of Great Britain (16420 men and women, > 15 years of age, interview survey) (Emmett et al. 1999)

Food	Prevalence
Milk	0.71 %
Eggs	0.69 %
Fish	0.50 %
Cheese	0.21 %

Table 2: Allergy prevalences to foods of animal origin in children and adolescents with DBPCFC-proven food allergy (Bock & Atkinson 1990)

Food	Children < 3 years (n=74)	Children 3-19 years (n=111)
Cow's milk	57 %	14 %
Hen's egg	43 %	27 %
Fish	4.1 %	4.5 %
Shrimp	0 %	1.8 %

Table 4 shows the threshold concentrations for elucidation of symptoms after ingestion of the offending food as determined by double- blind, placebo controlled food challenge (DBPCFC). Threshold concentrations ranged from 1 mg to 12 g of protein in the cited studies. They are strongly dependent on the patient's individual susceptibility and the allergenic potency of the particular food. According to Moneret-Vautrin et al. (1998) 30% of 40 egg allergic individuals reacted to doses of less than 11 mg, another 30% to doses between 11 and 110 mg, and 30% to 110 mg to 780 mg of hen's egg white protein. The most potent animal allergen sources seem to be hen's egg and fish. Although allergic reactions to lower amounts can not be excluded, the lowest dose elucidating allergic symptoms in DBPCFC was 1 mg of codfish protein.

Table 3: Frequency of foods of animal origin in episodes of food-induced anaphylaxis

France (Andre et al. 1994)	**Great Britain** (Pumphrey & Stanworth 1996)	**Spain** (Novembre et al. 1998)	**USA** (Kemp et al. 1995)
Adults and Children (n=60)	**Adults and Children (n=90)**	**Children (<16 years) (n=44)**	**Adults (12-75 years) (n=89)**
Crustaceae 17%	Eggs 4.4%	Fish 30%	Crustaceae 29%
Fish 13%	Cow's milk 3.3%	Cow's milk 22%	Beef 9%
Cow's milk 3.3%	Crustaceae 2.2%	Hen's egg 11%	Pork 4.5%
Eggs 1.7%	Fish 2.2%	Goat's milk 4%	Eggs 3.4%
Ewe's milk 1.7%			Tuna 1.1%
Beef 1.7%			

Table 4: Threshold concentrations for elucidation of symptoms in DBPCFC

Allergen	Dose at first reaction	Amount of protein*	Reference
Cow's milk	a) 0.5 - 8 g (dried milk) b) up to 206 g	a) 125 mg - 2 g b) up to 6.6 g	Bock et al. 1978
Cow's milk	5-52 g	165 mg - 1.65 g	Host & Samuelsson 1988
Cow's milk	5 - 250 g	165 mg - 8.25 g	Norgaard & Bindslev-Jensen 1992
Hen's egg	25 mg - 8 g (dried egg)	12 mg - 4 g	Bock et al. 1978
Hen's egg	50 mg - 50 g	6 mg - 6 g	Norgaard & Bindslev-Jensen 1992
Codfish	6 mg - 6.7 g	1 mg - 1 g	Hansen & Bindslev-Jensen 1992
Shrimps	4 - 64 g **	700 mg - 12 g	Daul et al. 1988
Beef	250 mg - 60 g	48 mg - 11.5 g	Werfel et al. 1997
Bovine serum albumin		90 mg**	Fiocchi et al. 1995a
Ovine serum albumin		63 mg**	Fiocchi et al. 1995a

* calculated

** no lower dose tested

Food allergens of animal origin are generally resistant to extremes of heat, pH and enzymatic degradation. Resistance to denaturation and degradation during food processing and passage through the digestive system enables the allergen to either sensitize the individual or to elucidate an allergic reaction. Only a few allergens are labile and do not survive processing. Several allergens of animal origin have been identified and characterized (Table 5), but little is known about their stability and the allergenicity of processed foods determined under standardized conditions.
During food processing the allergenicity can be altered by various procedures such as storage time, prolonged washing, separation techniques, heating, and texturizing. Moreover, various chemical interactions during the food manufacture between natural food ingredients and food additives can occur. The allergenic potential may be unaffected or decreased or even increased by food processing. Physico-chemical methods may simply reduce the allergen content of a specific product by e.g. extraction, precipitation, or ultrafiltration. The molecular basis of allergen alteration is the inactivation or destruction of IgE-binding epitope structures or the formation of new epitopes or better accessability of cryptic epitopes after denaturation of the native allergen. Heat treatment can induce the loss of the tertiary protein structure and induce aggregation of allergens affecting the conformational structure. In contrast, proteolytic or hydrolytic treatments affect the conformational structure as well as the linear amino acid sequence which may destroy sequential IgE-binding epitopes.

The majority of available studies examined the impact of heating (dry heating, boiling or cooking) and enzymatic digestion on native foods or allergen extracts of native foods. For recent reviews on the alteration of allergenicity by food processing see Moneret-Vautrin 1998 and Hefle 1999. This review summarizes available data on the stability of cow's milk, hen's egg, fish, crustaceae, and meat allergens during food processing as well as the allergenic potential of food additives of animal origin such as lecithins and gelatine.

Table 5: Characterized food allergens of animal origin (Larsen & Lowenstein 2000)

Animal Source	Species	WHO/IUIS Allergen Nomenclature	Protein name / family	Molecular mass (kDa)
Abalone (snail)	*Haliotis midae*	Hal m 1	Muscle protein	49
Cow's milk	*Bos domesticus*	Bos d 4 Bos d 5 Bos d 6 Bos d 7 Bos d 8	alpha-Lactalbumin beta-Lactoglobulin Serum albumin Immunoglobulin Caseins	14.2 18.3 67 160 20-30
Codfish	*Gadus callarias*	Gad c 1	Parvalbumin	12
Hen's egg	*Gallus domesticus*	Gal d 1 Gal d 2 Gal d 3 Gal d 4 Gal d 5	Ovomucoid Ovalbumin Ovotransferrin Lysozyme Serum albumin (alpha-Livetin)	28 42.7 80 14.3 69
Lobster	*Homarus americanus*	Hom a 1*	Tropomyosin	
Lobster	*Panulirus stimpsoni*	Pan s 1*	Tropomyosin	34
Salmon	*Salmo salar*	Sal s 1	Parvalbumin	12
Shrimp	*Metapenaeus ensis*	Met e 1	Tropomyosin	34
Shrimp	*Penaeus aztecus*	Pen a 1	Tropomyosin	36
Shrimp	*Parapenaeus fissurus*	Par f 1*	Serum albumin	39
Shrimp	*Penaeus indicus*	Pen i 1	Tropomyosin	34
Squid	*Todarodes pacificus*	Tod p 1	Tropomyosin	38

* designated names not listed in the official allergen list (Lin et al. 1993, Leung et al. 1998)

MILK ALLERGENS

Several milk proteins have been identified as allergenic in humans (Besler et al. 2000). The major allergens from cow's milk are constituted by caseins (Bos d 8) and whey proteins beta-lactoglobulin (Bos d 5), alpha-lactalbumin (Bos d 4) and serum albumin (Bos d 6) (see Table 5).

Raw Milk, Processed Milk, and Boiled Milk

Pasteurized and homogenized milk are as allergenic as raw milk, while boiling of milk for 10 min results in partial reduction of its allergenicity. Host & Samuelsson (1988) found comparable allergenic potencies of raw milk, pasteurized milk (75°C, 15s) and pasteurized and homogenized milk (60°C, 175kg/cm^2) in 5 cow's milk allergic children by skin prick test (SPT) and DBPCFC. They observed no positive reactions to an extensively hydrolysed casein-based infant formula. Pasteurized and homogenized whole milk was also capable of inducing allergic reactions in 3 adults in DBPCFC (Norgaard & Bindslev-Jensen 1992). Amounts of milk elucidating symptoms during DBPCFC range between 5 g and 250 g (Table 4).
A partial reduction of its allergenicity was observed after boiling of milk (100°C) for 10 min in SPT and dot-immunoblotting. In contrast, boiling for 2 and 5 min, respectively, induced no significant alterations (Norgaard et al. 1996, Werfel et al. 1997). Alpha- lactalbumin and caseins showed a 50% and 66% decrease in IgE-binding in scored crossed radio-immunoelectrophoresis (CRIE) after heating of skimmed milk for 10 min, while beta- lactoglobulin and serum albumin lost their IgE-binding potencies completely (Gjesing et al 1982). Norgaard et al. (1996) confirmed the inactivation of beta- lactoglobulin and serum albumin after boiling milk for 10 min, while caseins still induced positive reactions in SPT.

Cheese

Studies on allergenicity during the complicated manufacture of cheese have not been performed to date. Although there are more than 600 different kinds of cheese, cheese-making usually involves common procedures like pasteurization of milk, acidification by added starter cultures (change milk sugar into lactic acid), and production of curd by adding rennet (mainly chymosin). Hereby the structure of the caseins is changed to form a solid curd called para- casein. The whey fraction including alpha- lactalbumin and beta-lactoglobulin passes out and is usually lost. The ripening process is brought about through enzyme systems produced by bacteria which have grown in the curd.

Several cases of allergic reactions, including anaphylactic reactions after ingestion of different kinds of cheese (hard cheese, soft cheese, like mozarella and parmesan), have been reported (Wüthrich & Hofer 1986, Fiocchi et al. 1999). Goat's and sheep's cheese induced allergic reactions as well (Wüthrich & Johansson 1995, Umpierrez et al. 1999).

Lactic Acid Fermentation

Fermentation of sterilized cow's milk by meso- and thermophile lactic acid cultures decreased the antigenicity of alpha- lactalbumin and beta- lactoglobulin about 99% employing rabbit antibodies (ELISA). In contrast, the allergenicity of the fermented milk product was hardly affected in skin tests (Jedrychowski & Wroblewska 1999).

Hydrolysis with Digestive Enzymes

Purified milk proteins are more susceptible to enzymatic degradation than proteins in "crude" milk samples. In vitro digestions with duodenal fluid and human trypsin and elastase, respectively, showed the highest degradation rate for caseins followed by beta- lactoglobulin and alpha- lactalbumin, which were hydrolyzed at 100 and 500 times lower rates (Jakobsson et al. 1982, 1983).

Schmidt et al. (1995) combined an in vitro hydrolysis of whey proteins with pepsin (90 min) and a subsequent hydrolysis with a mixture of pancreatic enzymes (pH 7.5 for 150 min). Peptic digestions were performed at pH 2, 3 and 4 simulating pH values of the gastric fluids from adults and small children, respectively. Residual IgE-binding activities of whey proteins after hydrolysis at pH 2 and pH 3 were less than 14%. In contrast after digestion at pH 4 alpha- lactalbumin, serum albumin and bovine immunoglobulin still retained 48%, 58% and 91% of IgE-binding activity (RAST inhibition). Beta-lactoglobulin was hardly affected by pepsin hydrolysis, but was almost completely digested by pancreatic enzymes (Schmidt et al. 1995).

Astwood et al. (1996) found a high stability (>60min) of beta- lactoglobulin against peptic hydrolysis (pH 1.2), while caseins and serum albumin were completely hydrolyzed after 2 min and 30 s, respectively.

Hypoallergenic Infant Formulas

Infant formulas produced by enzymatic hydrolysis of cow's milk proteins are commonly used as milk substitutes in infant nutrition. Hydrolyzed formulas can be classified as either partially hydrolyzed or extensively hydrolyzed. Although the partially hydrolyzed cow's milk is reduced in allergenicity, it still contains full- length allergen and large allergen fragments capable of binding IgE. Therefore only extensively hydrolyzed formulas should be applied in the nutrition of cow's milk allergic infants (Bousquet et al. 1998). Hydrolyzed formulas are produced from caseins or whey proteins by heat denaturation and enzymatic hydrolysis, sometimes combined with ultrafiltration. Extensively hydrolyzed whey protein formulas (Schwartz et al. 1991, Halken et al. 1993) as well as extensively hydrolyzed casein formulas (Sampson et al. 1991) are well tolerated by most cow's milk allergic children (immunoblot, RAST, DBPCFC). Nevertheless, severe adverse reactions have been observed in a few patients. Anaphylactic reactions occurred either after ingestion of extensively hydrolyzed casein formulas (Lifschitz et al. 1988, Amonette et al. 1991, Saylor & Bahna 1991) and extensively hydrolyzed whey formulas (Businco et al. 1989).

In a study with 20 cow's milk allergic children 45% did not tolerate a partially hydrolyzed whey formula, while 10% and 13% did not tolerate extensively hydrolyzed formulas produced from caseins and whey proteins, respectively (Ragno et al. 1993).

Comparing the allergenicity in RAST inhibition and skin tests casein formulas were less allergenic than

whey formulas. Of 45 cow's milk allergic children 15% reacted to extensively hydrolyzed whey formula and 2.5% to extensively hydrolyzed casein formula in SPT (Oldaeus et al. 1991). Similarly according to Wahn et al. (1992) extensively hydrolyzed casein formulas had the least residual allergenic activity in skin tests and oral challenges of 6 different, tested formulas.
Traces of native milk proteins could be specifically detected in extensively hydrolyzed formulas using both monoclonal antibodies and serum from allergic subjects in SDS-PAGE immunoblotting (Restani et al. 1995, 1996).

Microparticulated Proteins
Simplesse, a fat substitute, is made from ultrafiltrated egg white and skimmed milk by heat and high-shear processing. The coagulated nanoparticles are 0.1-3 mm in diameter. Sampson and Cooke (1992) investigated the allergenic potential of Simplesse by SDS-PAGE immunoblotting. No differences in IgE-binding patterns between Simplesse proteins and native egg and cow's milk proteins were observed using 16 sera from egg and/or cow's milk allergic individuals.

Restructured Fish
A 30-year old woman experienced anaphylactic symptoms after eating a prepackaged bread with salmon (Koppelman et al. 1999). The patient was not sensitized to fish. Detailed examination of the product revealed that a new process for restructured meat had been introduced by the manufacturer. The salmon had been treated with the microbial enzyme transglutaminase to improve the structure of the meat by covalent linkage between added caseins and meat proteins. Assuming 10 to 50 g of salmon were ingested approximately 10 to 50 mg of caseins induced the allergic reactions (Koppelman et al. 1999).

EGG ALLERGENS

Ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), and lysozyme (Gal d 4) are the most important allergens in egg white protein (Barkholt et al. 2000). The major allergenic component of the egg yolk is alpha-livetin (identical to chicken serum albumin, Gal d 5) (Table 5).

Raw Eggs, Boiled Eggs and Heated Egg White
Challenges with dilutions of masked fresh, raw hen's egg induced symptoms in DBPCFC in 7 egg allergic adults (Norgaard & Bindslev-Jensen 1992). A dose of 50 mg whole egg induced symptoms of oral allergy syndrome (OAS), diarrhoea, asthma, and conjunctivitis in 2 patients. Doses of 500 mg and 2.5 g induced allergic reactions in 2 other patients. In the other patients doses of 50 g of whole egg were necessary for the elucidation of symptoms. The threshold doses were equivalent to 5 mg to 5 g of egg protein (Table 4).
Ovomucoid and ovalbumin could be detected in soft-boiled (100°C, 3 min) and hard-boiled eggs (100°C, 20 min) by radio- immunoelectrophoresis (RIEP) with rabbit antisera showing reduced, but clear residual antigenicity (Hoffmann 1983).
IgE-binding of egg white decreased about 58% in RAST after heating to 90°C for 10 min (Anet et al. 1985). In DBPCFC a decrease in positive reactions occurred after heating of egg white (90°C, 60 min). Only 55% of egg allergic patients with positive DBPCFC to freeze-dried egg white reacted to heated egg white (Urisu et al. 1997). Heated, ovomucoid depleted egg white was positive in only 6% (DBPCFC).

Egg Protein Additives: Meat Pastes
Leduc et al. (1999) tested the IgE-binding activity of 3 experimental pork meat pastes containing 2% dried egg white. Egg white allergens could be detected by SDS-PAGE immunoblot and EAST in raw and in pasteurized meat pastes (70°C, 2 h), while no IgE-binding to the sterilized paste (115°C, 90 min) was observed.

Hydrolysis with Digestive Enzymes
Ovalbumin and phosvitin resisted peptic digestion at pH 1.2 (>60min), while ovotransferrin was

immediately degraded and ovomucoid was degraded after 8 min of treatment (Astwood et al. 1996). In contrast, Urisu et al. (1999) determined significant IgE-binding by pepsin, chymotrypsin, and trypsin digested ovomucoid in DBPCFC positive egg allergic children (RAST inhibition).

Egg Lecithins

Palm et al. (1999) proved the allergenic potential of egg lecithins in a 15-month old girl in DBPCFC. After ingestion of 50 mg of egg lecithins allergic symptoms of the skin occurred within an hour (erythema of neck and shoulders). The protein content of the lecithin preparation was 11.3%.

Microparticulated Proteins

See cow's milk allergens.

FISH ALLERGENS

The first food allergen characterized in detail was "Allergen M" (Gad c 1, 12 kDa) from codfish (Aas & Elsayed 1969). Gad c 1 belongs to the family of Ca-binding parvalbumins, which are present in muscle tissue of amphibians and fish. Proteins with cross- reactivity to Gad c 1 have been identified in several fish species: salmon (Sal s 1), tuna, perch, carp, eel, catfish, dogfish, and snapper (James et al. 1997, Bugajska-Schretter et al. 1998). Additional fish allergens are present in the molecular mass range from 15 to 200 kDa (Bugajska-Schretter et al. 1998).

Raw and Boiled Fish

The capability of fresh, raw codfish meat to induce allergic reactions was proven in 10 fish allergic adults by DBPCFC (Table 4). Amounts as low as 6 mg to 6.7 g elucidated allergic symptoms. More severe reactions including anaphylaxis occurred with relatively high amounts of 25-50 g codfish (Hansen & Bindslev-Jensen 1992).

The allergenic potential of boiled fish was confirmed by Bernhisel-Broadbent et al. (1992a) testing 10 different fish species in DBPCFC. SDS-PAGE experiments revealed denaturation of some protein bands and formation of high-molecular bands in boiled fish as compared to protein extracts from raw fish. A relatively strong decrease in IgE-binding to allergens from boiled fish could be observed in immunoblotting. Bernhisel-Broadbent et al. (1992a) found that in vitro tests were frequently not in agreement with the clinical relevance of fish hypersensitivity.

Allergens in codfish, herring, and plaice were heat- stable after boiling for 6 min, 1 h, and 4 h tested with sera from 2 patients with codfish allergy (Hansen et al. 1994). With the exception of mackerel the activity of histamine release was unchanged for all protein extracts from boiled fish. Protein bands > 40 kDa were found to be heat labile, while proteins with lower molecular mass proved to be stable. Still, after 4 h of boiling IgE-binding fish proteins could be detected in SDS-PAGE immunoblot.

Canned Fish Products

Studies of Bernhisel-Broadbent et al. (1992b) indicate that canning fish results in the loss of its allergenic activity. DBPCFC with canned tuna was negative in 18 fish allergic patients as well as with canned salmon in 2 salmon allergic patients.

SDS-PAGE proved a significant loss of distinct protein bands in canned fish as compared to raw and boiled fish. The IgE-binding activity of canned fish was weak in immunoblotting and about 100 to 200 times less as compared to boiled fish in EAST inhibition (Bernhisel-Broadbent et al. 1992b).

Surimi

Thoroughly washing of fish meat eliminates the water- soluble proteins and retains water- insoluble proteins. Therefore surimi, which is produced from minced, thoroughly washed fish meat from different species, presents an allergen pattern different from native fish species. Allergens in fresh codfish were detected in the range of 13 to 63 kDa, while surimi retained only a 63 kDa allergen in SDS-PAGE (Mata

et al. 1994). In SPT 2 out of 6 patients with codfish allergy reacted to surimi, while all 6 sera were positive to surimi in RAST. IgE-binding to codfish extract was inhibited by surimi extracts with maximum inhibition of 60 to 94% (RAST inhibition). Helbling et al. (1992) proved the IgE-binding potential of surimi with sera from fish allergic patients in RAST inhibition. Surimi may contain other ingredients like starch, egg white, and other food additives giving a gelateous structure after heating.

"Hidden" Allergens in Used Fat

Frying foods using the same fat which was previously used for fish may threaten fish allergic subjects. Yunginger et al. (1988) reported a fatal anaphylactic reaction in a fish allergic individual after eating fried potatoes. The fat had been previously used for frying fish.

Hydrolysis with Digestive Enzymes

Aas & Elsayed (1969) examined the stability of the muscle myogene fraction from codfish against extensive enzymatic digestion with trypsin, pepsin, subtilisin, and pronase (48h, 37°C), respectively. After the treatment the allergenic activity in skin testing was lost. In contrast after digestion with elastase approximately 50% of allergenic activity was retained. Some allergenic activity was present in partial hydrolysed protein fractions after digestion with pronase for 24 h and with trypsin and pepsin for 2 h, respectively.

CRUSTACEAE ALLERGENS

The family of crustaceae includes shrimps, lobster, crab, and crayfish. The major allergens of crustaceae are tropomyosins which were identified in several shrimp species (Met e 1, Pen a 1, Pen i 1) and lobsters (Pan s 1, Hom a 1) as well as in squids (Tod p 1), which belong to the family of molluscs (Table 5).

Boiled Shrimps

The heat stability of shrimp allergens was evidenced by several studies. 30% of 30 patients with a history of shrimp allergy reacted to boiled shrimps after open oral challenge (Daul et al. 1988). Six of these patients were positive in DBPCFC too. Challenge tests were performed with doses of 1 to 16 shrimps corresponding to 4 to 64 g of shrimps (Table 4).
Naqpal et al. (1989) isolated a 34 kDa allergen (Pen i 1) from boiled shrimps which retained its allergenic activity. Daul et al. (1994) detected similar allergenic activity of the major allergen Pen a 1 in shrimp extracts as well as in the cooking water by SDS-PAGE immunoblot.
One patient was described who experienced an anaphylactic reaction after ingestion of boiled shrimps while having a negative SPT to raw shrimps. The evaluation by SPT revealed exclusive sensitivity to boiled shrimps in this particular patient (Rosen et al. 1994).
Yunginger et al. (1988) reported a fatal case of anaphylaxis after ingestion of crab.

MEAT ALLERGENS

Meat allergens are serum albumins (66 kDa), gamma- globulins (160kDa), and actins (Restani et al. 1997) as well as several additional proteins (14, 18, 20, 45 und >60 kDa). Cross-reactivity to whey proteins from milk are due to serum albumins and gamma-globulins. Most recently Ayuso et al. (2000) obtained complete inhibition of IgE-binding to bovine IgG with lamb, venison, and milk. Denatured type I collagen (beef) was identified as the major allergenic component of gelatine.

Poultry and Mammals

According to a recent study (Ayuso et al. 1999) 75% of patients with sensitivity to meat from mammals were sensitive to poultry at the same time (IgE-binding in SDS-PAGE immunoblot). Vice versa only 50% of poultry sensitive patients showed IgE-binding to meat extracts from mammals. Sensitivity in SDS-

PAGE immunoblot was associated to clinically relevant poultry meat allergy in 64% of patients, while only 29% of patients with beef sensitivity had a clinically relevant allergy to beef (Ayuso et al. 1999).
Restani et al. (1998) found no IgE-binding to serum albumins from turkey and chicken in 6 children allergic to bovine serum albumin (SDS-PAGE immunoblot).

Heated Meat

A case of adverse reactions to raw meat without sensitivity to cooked meat was described by Fisher (1982). A patient experienced anaphylactic symptoms after eating rare-cooked beef, while well-cooked beef was tolerated.
In a DBPCFC study with 11 children (aged 16 months to 14 years) with beef allergy 8 of them reacted against well-cooked beef (brown), while 3 children reacted to rare-cooked meat (red centrally and pink peripherally) (Werfel et al. 1997). Reported adverse reactions were predominantly cutaneous symptoms, whereas severe systemic reactions were not observed.
Heat treatment induces denaturation of meat proteins which lose their state of water solubility. The analysis of allergens by SDS-PAGE immunoblotting revealed a 17.8 kDa and 19 kDa allergen and four additional weakly detected bands (14, 20, 45 and >60 kDa) in minced beef which was heated (85°C) up to 2 h (Werfel et al. 1997). These protein bands were identified in well-cooked minced beef (20 min, up to 80°C) as well. The strongest IgE-binding activity was observed to the 17.8 kDa allergen by sera from patients with positive DBPCFC to cooked beef. Bovine serum albumin and gamma-globulin bands vanished in SDS-PAGE after heating (80°C) of minced beef for 10 and 3 min, respectively. Purified serum albumin was stable for 15 min at 95°C purified, while gamma-globulin was stable for 15 min at 65°C (Werfel et al. 1997).
Seven of 11 children with beef allergy and positive SPT to native bovine serum albumin also showed a sensitivity in SPT to heated bovine serum albumin. And 4 children had positive challenge with heated bovine serum albumin in DBPCFC (Fiocchi et al. 1998).

Restani et al. (1998) found that purified bovine serum albumin was still capable of IgE-binding after treatment at 100° for 10 min. In these conditions, bovine serum albumin produced aggregates but both monomers and polymers were antigenic. It was suggested that milk could protect bovine serum albumin from aggregation with easier denaturation, thus thermostability could depend on the medium.

Of 57 patients with suspected meat allergy to beef, pork, lamb, rabbit, or poultry only 2 showed in vitro reactivity to tropomyosins from pork and chicken, respectively (Ayuso et al. 1999). IgE-binding to allergens from raw meat was stronger than to allergens from heated meat (20 min, 140°C) in 75% of sera with the exception of chicken meat. 6 of 24 sera reacted strongly to proteins in heated chicken meat, while IgE-binding to proteins from raw meat was weak. Several neoallergens were detected in heated chicken meat at a molecular mass range of 14 to 90 kDa. Heat labile allergens with 45 and 150 kDa were identified in raw chicken meat. Heat stable allergens simultaneously detected in raw and heated chicken meat had molecular masses of 17, 20, 24, 28, 31, and 66 kDa in SDS-PAGE.

IgE-binding in SDS-PAGE immunoblots disappeared completely in cooked beef extracts (20 min, 140°C) and raw beef extracts separated under reducing conditions using sera from 12 patients with convincing history of beef allergy and IgE-binding to raw beef extracts indicating heat labile and conformational epitopes, respectively (Ayuso et al. 2000).

Homogenization and Lyophilization

Fiocchi et al. (1998) studied the impact of freeze-drying and homogenization on the allergenicity of beef. 10 children with positive SPT to raw and heated beef and positive DBPCFC with 180 g heated beef (5 min, 100°C) were tested. All children reacted to native bovine serum albumin in SPT and 5 children reacted to native bovine serum albumin in DBPCFC.
Only one child had a positive reaction to freeze-dried beef in SPT, while no positive reaction to freeze-dried beef was observed in DBPCFC. With homogenized beef neither positive SPT nor positive DBPCFC

results could be observed in any of the children. In an earlier study (Fiocchi et al. 1995b) weak allergenicity of homogenized and freeze-dried beef and freeze-dried sheep's meat could be observed in 2 of 12 children with clinically relevant beef allergy by SPT.

Hydrolysis of Meat Proteins

Fiocchi et al. (1995b) performed SPT with pepsin digested serum albumins from beef and sheep in 12 children with clinically relevant symptoms of beef allergy and positive SPT to native serum albumin. After 5 min of digestion 4 children showed positive SPT to digested bovine serum albumin, while after 2 and 4 h of pepsin digestion 2 positive SPT were obtained, respectively. Pepsin preparations of ovine serum albumin digested for 5 min elucidated positive reactions in SPT in 3 children, while preparations digested for 2 h elucidated no skin reactions in any of the children. The pepsin digestions all were negative in RAST.

Gelatine

Cases of anaphylaxis to gelatine containing vaccines have been frequently reported in the literature, while reports of allergic events after ingestion of gelatine containing foods are rare. According to a study of Sakaguchi et al. (1995), 7 of 26 children who experienced systemic allergic reactions induced by gelatine containing vaccines showed allergic reactions after ingestion of gelatine containing foods. The cross-reactive potential of gelatines from various mammalian species was proven by RAST inhibition with sera from 12 children with bovine gelatine allergy. In one child a strong sensitization to fish gelatine could be observed (Sakaguchi et al. 1999a). The alpha 2 chain of type I collagen from beef was identified as the major IgE-binding fraction of gelatine in SDS-PAGE immunoblot and RAST (Sakaguchi et al. 1999b). Wahl and Kleinhans (1989) reported a case of oral allergy syndrome after ingestion of fruit gums containing gelatine. SDS-PAGE immunoblotting revealed allergens in the molecular mass range of 40 to 120 kDa.

Enzymatic digestion with collagenase degraded pork gelatine (heat- denatured collagen) to peptides <10 kDa which retained very low IgE-binding potentail (Sakai et al. 1998).

CONCLUSIONS

Food allergens of animal origin are generally highly resistant to common treatments during food processing, with limitations to some extent as regards meat. The impact of heat and enzymatic treatments on the allergenicity of food allergens are summarized in Table 6. Cow's milk allergens retain their allergenicity after conventional industrial treatments. In order to produce "hypoallergenic" (less allergenic) cow's milk infant formulas, extensive conditions employing hydrolysis, heating, and sometimes ultrafiltration are needed. Allergens from hen's egg are stable to heat and enzymatic digestion nonetheless. Moreover, milk and egg products are common ingredients of many convenience foods and therefore hidden potential allergens in processed foods. The significance of fish and crustaceae as hidden allergens is low, while they are usually stable to heat treatment. The allergenic potential of meat is partially heat stable, while the stability to enzymatic digestion is relatively weak. Gelatine may be important as a hidden allergen, while its prevalence is very low.

Table 6: Stability of important food allergens of animal origin

Allergen	Heat Treatment	Enzymatic Hydrolysis
Cow's milk	stable	partially resistant
Hen's egg	stable	resistant
Fish	stable	partially resistant
Crustaceae	stable	no information
Meat	partially stable	susceptible

The allergic individual is usually advised to avoid all forms of the food allergen to which he is sensitized. On one hand this situation reflects the lack of data on the allergenicity of processed foods and food additives. On the other hand it shows the need for the development of well characterized less or non-allergenic foods.
Future research should investigate the allergenic potentials of foods, especially cow's milk and hen's egg, throughout manufacturing processes from source to shelf- products. Modern food quality management should take into account standardized food specifications with respect to the state of allergenicity. Clinical evaluations of processed foods with an appropriate number of patients with convincing allergy to the native food allergen are also needed. Ideally food products should be monitored simultaneously by various analytical and diagnostic methods like DBPCFC, SPT, RAST, SDS-PAGE immunoblot, and inhibition tests.

REFERENCES

- Aas K, Elsayed SM (1969) **Characterization of a major allergen (cod). Effect of enzymic hydrolysis on the allergenic activity** *J Allergy 44:333-43*
- Amonette MS, Schwartz RH, Mattson L, Peers LB, Eldredge DM (1991) **Double- blind, placebo- controlled food challenges (DBPCFC) demonstrating acute IgE-mediated allergic reactions to Good Start, Ultrafiltered Good Start, Alfaré, Nutramigen, and Alimentum in a seven- year- old** *Pediatr Asthma Allergy Immunol 5:245-51*
- Andre F, Andre C, Colin L, Cacaraci F, Cavagna S (1994) **Role of new allergens and of allergens consumption in the increased incidence of food sensitizations in France** *Toxicology 93:77-83*
- Anet J, Back JF, Baker RS, Barnett D, Burley RW, Howden MEH (1985) **Allergens in the white and yolk of hen's egg. A study of IgE binding by egg proteins** *Int Arch Allergy Immunol 77:364-71*
- Ayuso R, Lehrer SB, Tanaka L, Ibanez MD, Pascual C, Burks AW, Sussman GL, Goldberg B, Lopez M, Reese G (1999) **IgE antibody response to vertebrate meat proteins including tropomyosin** *Ann Allergy Asthma Immunol 83:399-405*
- Ayuso R, Lehrer SB, Lopez M, Reese G, Ibañez MD, Martin Esteban M, Ownby DR, Schwartz H (2000) **Identification of bovine IgG as a major cross- reactive vertebrate meat allergen** *Allergy 55:348-54*
- Barkholt V, Besler M, Sampson HA (2000) **Allergen data collection: Hen's egg white *(Gallus domesticus)*** *Internet Symposium on Food Allergens 2(Suppl.1):1-29*
- Bernhisel-Broadbent J, Scanlon SM, Sampson HA (1992a) **Fish hypersensitivity. I. In vitro and oral challenge results in fish-allergic patients** *J Allergy Clin Immunol 89:730-7*
- Bernhisel-Broadbent J, Strause D, Sampson HA (1992b) **Fish hypersensitivity. II: Clinical relevance of altered fish allergenicity caused by various preparation methods** *J Allergy Clin Immunol 90(4Pt1):622-9*
- Besler M, Eigenmann P, Schwartz RH (2000) **Allergen data collection: Cow's milk *(Bos domesticus)*** *Internet Symposium on Food Allergens 2:9-74*
- Bock SA, Atkins FM (1990) **Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food** challenges *J Pediatr 117:561-7*
- Bousquet J, Björkstén B, Bruijnzeel-Koomen CAFM, Huggett A, Ortolani C, Warner JO, Smith M (1998) **Scientific criteria and the selection of allergenic foods for product labelling** *Allergy 53:3-21*
- Bugajska-Schretter A, Elfman L, Fuchs T, Kapiotis S, Rumpold H, Valenta R, Spitzauer S (1998) **Parvalbumin, a cross-reactive fish allergen, contains IgE-binding epitopes sensitive to periodate treatment and Ca2+ depletion** *J Allergy Clin Immunol 101(1Pt1):67-74*
- Businco L, Cantani A, Longhi MA, Giampietro PG (1989) **Anaphylactic reactions to a cow's milk whey protein hydrolysate (Alfa-Re, Nestle) in infants with cow's milk allergy** *Ann Allergy 62:333-5*
- Daul CB, Morgan JE, Hughes J, Lehrer SB (1988) **Provocation- challenge studies in shrimp- sensitive individuals** *J Allergy Clin Immunol 81:1180-6*
- Daul CB, Slattery M, Reese G, Lehrer SB (1994) **Identification of the major brown shrimp *(Penaeus aztecus)* allergen as the muscle protein tropomyosin** *Int Arch Allergy Immunol 105:49-55*
- Emmett SE, Angus FJ, Fry JS, Lee PN (1999) **Perceived prevalence of peanut allergy in Great Britain and its association with other atopic conditions and with peanut allergy in other household members** *Allergy 54:380-5 (erratum 54:891)*
- European Commission (1998) **Reports on tasks for scientific cooperation. The occurrence of severe food allergies in the EU** *European Commission, Directorate-General III, Brussels*
- Fiocchi A, Restani P, Riva E, Qualizza R, Bruni P, Restelli AR, Galli CL (1995a) **Meat allergy: I - Specific IgE to BSA and OSA in atopic, beef sensitive children** *J Am Coll Nutr 14:239-44*

- Fiocchi A, Restani P, Riva E, Restelli AR, Biasucci G, Galli CL, Giovannini M (1995b) **Meat allergy: II - Effects of food processing and enzymatic digestion on the allergenicity of bovine and ovine meats** *J Am Coll Nutr 14:245-50*
- Fiocchi A, Restani P, Riva E, Mirri GP, Santini I, Bernardo L, Galli CL (1998) **Heat treatment modifies the allergenicity of beef and bovine serum albumin** *Allergy 53:798-802*
- Fiocchi A, Restani P, Decet E, Travaini M, Mirri GP, Bernardo L, Riva E (1999) **Evolution from ewe's milk to cow's milk allergy** *Allergy 54:401-2*
- Fisher AA (1982) **Allergic contact urticaria to raw beef, histopathology of the specific wheal reaction at the scratch test site** *Contact Dermatitis 8:425*
- Gjesing B, Osterballe O, Schwartz B, Wahn U, Lowenstein H (1986) **Allergen- specific IgE antibodies against antigenic components in cow milk and milk substitutes** *Allergy 41:51-6*
- Halken S, Host A, Hansen LG, Osterballe O (1993) **Safety of a new, ultrafiltrated whey hydrolysate formula in children with cow milk allergy: a clinical investigation** *Pediatr Allergy Immunol 4:53-9*
- Hansen TK, Bindslev-Jensen C (1992) **Codfish allergy in adults. Identification and diagnosis** *Allergy 47:610-7*
- Hansen TK, Skov SP, Poulsen LK, Bindslev-Jensen C (1994) **Allergenic activity of processed fish** *ACI News Suppl 2:445*
- Hefle SL (1999) **Impact of processing on food allergens** *Adv Exp Med Biol 459:107-19*
- Helbling A, Lopez M, Lehrer SB (1992) **Fish allergy: is it a real problem with surimi-based products?** *Int Arch Allergy Immunol 99:452-5*
- Hoffmann DR (1983) **Immunochemical identification of the allergens in egg white** *J Allergy Clin Immunol 71:481-6*
- Host A, Samuelsson EG (1988) **Allergic reactions to raw, pasteurized, and homogenized/pasteurized cow milk: a comparison. A double- blind placebo- controlled study in milk allergic children** *Allergy 43:113-8*
- Jakobsson I, Lindberg T, Benediktsson B (1982) **In vitro digestion of cow's milk proteins by duodenal juice from infants with various gastrointestinal disorders** *J Pediatr Gastroenterol Nutr 1:183-91*
- Jakobsson I, Borulf S, Lindberg T, Benediktsson B (1983) **Partial hydrolysis of cow's milk proteins by human trypsins and elastases in vitro** *J Pediatr Gastroenterol Nutr 2:613-6*
- James JM, Helm RM, Burks AW, Lehrer SB (1997) **Comparison of pediatric and adult IgE antibody binding to fish proteins** *Ann Allergy Asthma Immunol 79:131-7*
- Jedrychowski L, Wroblewska B (1999) **Reduction of antigenicity of whey proteins by lactic acid fermentation** *Food Agric Immunol 11:91-9*
- Kemp SF, Lockey RF, Wolf BL, Lieberman P (1995) **Anaphylaxis. A review of 266 cases** *Arch Intern Med 155:1749-54*
- Koppelman SJ, Wensing M, de Jong GA, Knulst AC (1999) **Anaphylaxis caused by the unexpected presence of casein in salmon** *Lancet 354(9196):2136*
- Larsen JN, Lowenstein H (2000) **Official list of allergens: IUIS Allergen Nomenclature Subcommittee** *Internet: ftp://biobase.dk / pub / who-iuis / allergen.list*
- Leduc V, Demeulemester C, Polack B, Guizard C, Le Guern L, Peltre G (1999) **Immunochemical detection of egg-white antigens and allergens in meat products** *Allergy 54:464-72*
- Leung PS, Chen YC, Mykles DL, Chow WK, Li CP, Chu KH (1998) **Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen** *Mol Mar Biol Biotechnol 7:12-20*
- Lifschitz CH, Hawkins HK, Guerra C, Byrd N (1988) **Anaphylactic shock due to cow's milk protein hypersensitivity in a breast- fed infant** *J Pediatr Gastroenterol Nutr 7:141-4*
- Lin RY, Shen HD, Han SH (1993) **Identification and characterization of a 39 kd major allergen from *Parapenaeus fissurus*** *J Allergy Clin Immunol 92:837-45*
- Mata E, Favier C, Moneret-Vautrin DA, Nicolas JP, Han-Ching L, Gueant JL (1994) **Surimi and native codfish contain a common allergen identified as a 63-kDa protein** *Allergy 49:442-7*
- Moneret-Vautrin DA (1998) **Modifications of allergenicity linked to food technologies** *Allerg Immunol (Paris) 30:9-13*
- Moneret-Vautrin DA, Rance F, Kanny G, Olsewski A, Gueant JL, Dutau G, Guerin L (1998) **Food allergy to peanuts in France - evaluation of 142 observations** *Clin Exp Allergy 28:1113-9*
- Naqpal S, Rajappa L, Metcalfe DD, Rao PV (1989) **Isolation and characterization of heat- stable allergens from shrimp *(Penaeus indicus)*** *J Allergy Clin Immunol 83:26-36*
- Norgaard A, Bindslev-Jensen C (1992) **Egg and milk allergy in adults. Diagnosis and characterization** *Allergy 47:503-9*
- Norgaard A, Bernard H, Wal JM, Peltre G, Skov PS, Poulsen LK, Bindslev-Jensen C (1996) **Allergenicity of individual cow milk proteins in DBPCFC-positive milk allergic adults** [abstract 218] *J Allergy Clin Immunol 97(1Pt3):237*
- Novembre E, Cianferoni A, Bernardini R, Mugnaini L, Caffarelli C, Cavagna G, Giovane A, Vierucci A (1998) **Anaphylaxis in children: clinical and allergological features** *Pediatrics 101(4):e8*
- Oldaeus G, Bjorksten B, Einarsson R, Kjellman NIM (1991) **Antigenicity and allergenicity of cow milk hydrolysates**

intended for infant feeding*Pediatr Allergy Immunol 2:156-64*
- Palm M, Moneret-Vautrin DA, Kanny G, Denery-Papini S, Fremont S (1999) **Food allergy to egg and soy lecithins** *Allergy 54:1116-7*
- Pumphrey RS, Stanworth SJ (1996) **The clinical spectrum of anaphylaxis in north-west England** *Clin Exp Allergy 26:1364-70*
- Ragno V, Giampietro PG, Bruno G, Businco L (1993) **Allergenicity of milk protein hydrolysate formulae in children with cow's milk allergy** *Eur J Pediatr 152:760-2*
- Restani P, Velona T, Plebani A, Ugazio AG, Poiesi C, Muraro A, Galli CL (1995) **Evaluation by SDS-PAGE and immunoblotting of residual antigenicity in hydrolysed protein formulas** *Clin Exp Allergy 25:651-8*
- Restani P, Plebani A, Velona T, Cavagni G, Ugazio AG, Poiesi C, Muraro A, Galli CL (1996) **Use of immunoblotting and monoclonal antibodies to evaluate the residual antigenic activity of milk protein hydrolysed formulae** *Clin Exp Allergy 26:1182-7*
- Restani P, Fiocchi A, Beretta B, Velona T, Giovannini M, Galli CL (1997) **Meat allergy: III - Proteins involved and cross-reactivity between different animal species** *J Am Coll Nutr 1997 16:383-9*
- Restani P, Fiocchi A, Beretta B, Velona T, Giovannini M, Galli CL (1998) **Effect of structure modifications on IgE binding properties of serum albumins** *Int Arch Allergy Immunol 117:113-9*
- Rosen JP, Selcow EJ, Mendelson LM, Grodofsky MP, Factor JM, Sampson HA (1994) **Skin testing with natural foods in patients suspected of having food allergies: Is it a necessity?** *J Allergy Clin Immunol 93:1068-70*
- Sakaguchi M, Nakayama T, Inouye S (1995) **Food allergy to gelatin in children with systemic immediate-type reactions, including anaphylaxis, to vaccines** *J Allergy Clin Immunol 98(6 Pt 1):1058-61*
- Sakaguchi M, Hori H, Ebihara T, Irie S, Yanagida M, Inouye S (1999a) **Reactivity of the immunoglobulin E in bovine gelatin-sensitive children to gelatins from various animals** *Immunology 96:286-90*
- Sakaguchi M, Hori H, Hattori S, Irie S, Imai A, Yanagida M, Miyazawa H, Toda M, Inouye S (1999b) **IgE reactivity to alpha1 and alpha2 chains of bovine type 1 collagen in children with bovine gelatin allergy** *J Allergy Clin Immunol 104(3Pt1):695-9*
- Sakai Y, Yamato R, Onuma M, Kikuta T, Watanabe M, Nakayama T (1998) **Non- antigenic and low allergic gelatin produced by specific digestion with an enzyme-coupled matrix** *Biol Pharm Bull 21:330-4*
- Sampson HA, Cooke S (1992) **The antigenicity and allergenicity of microparticulated proteins: Simplesse** *Clin Exp Allergy 22:963-9*
- Sampson HA, Bernhisel-Broadbent J, Yang E, Scanlon SM (1991) **Safety of casein hydrolysate formula in children with cow milk allergy** *J Pediatr 118(4Pt1):520-5*
- Sampson HA, Mendelson L, Rosen JP (1992) **Fatal and near-fatal anaphylactic reactions to food in children and adolescents** *N Engl J Med 327:380-84*6
- Sampson HA (1999) **Food allergy. Part 1: Immunopathogenesis and clinical disorders** *J Allergy Clin Immunol 103:717-28*
- Saylor JD, Bahna SL (1991) **Anaphylaxis to casein hydrolysate formula** *J Pediatr 118:71-4*
- Schmidt DG, Meijer RJ, Slangen CJ, van Beresteijn EC (1995) **Raising the pH of the pepsin- catalysed hydrolysis of bovine whey proteins increases the antigenicity of the hydrolysates** *Clin Exp Allergy 25:1007-17*
- Schwartz RH, Peers LB, Amonette MS, Witherly SA (1991) **Usefulness of a cow's milk whey infant formula (Good Start) in the management of children with Group 1A (Topical Reactors) IgE-mediated cow's milk allergy** *Pediatr Asthma Allergy Immmunol 5:305-12*
- Umpierrez A, Quirce S, Maranon F, Cuesta J, Garcia-Villamuza Y, Lahoz C, Sastre J (1999) **Allergy to goat and sheep cheese with good tolerance to cow cheese** *Clin Exp Allergy 29:1064-8*
- Urisu A, Ando H, Morita Y, Wada E, Yasaki T, Yamada K, Komada K, Torii S, Goto M, Wakamatsu T (1997) **Allergenic activity of heated and ovomucoid- depleted egg white** *J Allergy Clin Immunol 100:171-6*
- Urisu A, Yamada K, Tokuda R, Ando H, Wada E, Kondo, Y, Morita Y (1999) **Clinical significance of IgE- binding activity to enzymatic digests of ovomucoid in the diagnosis and the prediction of the outgrowing of egg white hypersensitivity** *Int Arch Allergy Immunol 120:192-8*
- Wahl R, Kleinhans D (1989) **IgE-mediated allergic reactions to fruit gums and investigation of cross- reactivity between gelatine and modified gelatine- containing products** *Clin Exp Allergy 19:77-80*
- Wahn U, Wahl R, Rugo E (1992) **Comparison of the residual allergenic activity of six different hydrolyzed protein formulas** *J Pediatr 121(5 Pt 2):S80-4*
- Werfel SJ, Cooke SK, Sampson HA (1997) **Clinical reactivity to beef in children allergic to cow's milk** *J Allergy Clin Immunol 99:293-300*
- Wüthrich B, Hofer T (1986) **Food allergies. III. Therapy: elimination diet, symptomatic drug prophylaxis and specific hyposensitization** *Schweiz Med Wochenschr 116:1401-10* (in German)
- Wüthrich B, Johansson SGO (1995) **Allergy to cheese produced from sheep's and goat's milk but not to cheese**

produced from cow's milk *J Allergy Clin Immunol 96:270-3*

- Yunginger JW, Sweeney KG, Sturner WQ, Giannandrea LA, Teigland JD, Bray M, Benson PA, York JA, Biedrzycki L, Squillace DL, et al (1988) **Fatal food- induced anaphylaxis** *JAMA 260:1450-2*

[Summary] [Abbreveations]