ALLERGENOMICS AND ANALYSIS OF CAUSES OF UNINTENTIONAL INCORPORATION OF SUBSTANCES CAPABLE OF CAUSING IGE-MEDIATED FOOD ALLERGY INTO MEAT PRODUCTS

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Abstract
The article discusses the definition and mechanism of IgE‑mediated food allergy, provides an overview of the legal regulation of the production and labeling of allergen-containing food products. In order to prevent the inadvertent appearance of allergens in products during their production, an allergenomics procedure is required — a comprehensive assessment of the allergic potential of a food product: allergenicity of product ingredients, risk analysis, and the procedure for managing allergens in the production.

Introduction
Allergic diseases have gained an enormous scale in the world, both in developed and developing countries. According to the data of the World Health Organization, a number of patients with allergies have increased in Russia by 20% over the last decade. According to scientists’ forecasts, this number will grow as a majority of factors causing allergic reactions are linked with modern lifestyle. As the growth in the number of allergic reactions associated with food consumption is rapid, a food allergy is necessary to regard as one of the main public health problems [1,2,3,4]. The size of population with food allergies is different in various countries. For example, the prevalence of food allergies is 4.6% in Spanish population and 19.1% in Australian population [5]. According to various data, the prevalence of food allergies in the Russian Federation is 30% to 56% in children [6] and about 20% in adults suffering from atopic dermatitis [7].

At present, there are no available methods for prevention or treatment of food allergies: the only method to maintain remission in a patient is to exclude intake of a food allergen and treatment in the case of exacerbation includes only managing symptoms as they are revealed [9,10]. Individuals suffering from food allergies have to adhere to special diets to avoid allergic reactions [11]. Governments of many European countries acknowledged an importance of problems associated with food allergies and set requirements on the legislative level for managing allergens and labeling products containing allergens [12]. Nevertheless, the control of allergens in a processing enterprise and throughout the ingredient supply chain is a complex task for producers in the conditions of globalized economy [12]. When ingredients are obtained from suppliers of different regions and foreign suppliers, the risk of increasing a likelihood of unintentional presence of allergens in food products appears, which can lead to potential threats to food safety as well as economic threats, which is evident from large scale recalls affected many food companies [13,14,15,16].

Technologists and other specialists directly working with food products should have insight into forms of allergic diseases and their causes, as well as the causes of unintentional incorporation of allergens into products upon their production. It will be interesting for specialists of meat processing plants that, as a rule, meat per se does not cause allergic reactions, which, unfortunately, cannot be said about additives that are used in meat product manufacture [17].

At the same time, it is necessary to consider socio-psychological consequences of the food allergy risk, in particular, factors determining quality of life [18].

Main part

1. Definition and mechanism of IgE-mediated food allergy
A food allergy is an immune response to the contact of the body with food. A reaction can be mediated by IgE release, activation of T-cells or tissue basophils [19].

The IgE type I reactions are distinguished by recognition of IgE specific epitopes (linear or conformational) within a soluble antigen to trigger mast cell activation [20,21,22]. As a rule, the IgE-mediated food allergy is characterized by rapid onset: skin (urticarial, Quincke’s edema, exacerbation of atopic dermatitis/eczema), gastrointestinal (nausea, vomiting, diarrhea) and/or respiratory symptoms [23,24] appear in patients usually in an interval from several minutes to 2 hours.

The mechanism involved in the IgE-mediated food allergic reactions is shown in Figure 1. There are two stages in the development of the IgE-mediated food allergy: the sensitization phase and manifestation phase [24]. Sensitization can occur at any age and does not always emerge at the first allergen exposure. Sensitization does not have symptoms and consists of adsorption, processing and presentation of an allergen, activation of T-cells and B-cells, development of oral tolerance or allergic sensitivity and synthesis of antigen-specific IgE-antibodies by plasma cells. Allergen-specific IgE
Epigenetics is the study of heritable and non-inherited changes in the gene function, which occur without changes in the DNA nucleotide sequence. Epigenetic changes caused by changes in a diet and environmental impact were associated with the development of asthma and allergic rhinitis, but not with food allergy [32,33]. The hygienic hypothesis assumes that an increase in the infection level at an early age has a protective action on the development of allergies, asthma and other atopic diseases [34,35,36,37]. The hypothesis about the double action of an allergen states that tolerance emerges due to peroral food exposure and allergic sensitization due to skin exposure [38]. Inflammation caused by eczema reduces the effectiveness of epidermal barrier protein and opens a possibility of allergen protein impact and production of food allergen-specific T-cells in unprotected skin [39]. Low levels of peanut are accessible to infants in household conditions after cleaning providing skin exposure for individuals at risk [40]. The time of peanut introduction into a diet influenced significantly the prevalence of peanut allergy among Israeli schoolchildren [41]. Israeli children consumed more peanut during the first year of life compared to UK children and the prevalence of peanut allergy was 0.17% in Israel and 1.85% in the UK. Changes in atopy, social class or genetic background did not have a significant effect [41]. In the USA, the number of people with peanut allergy doubled over four years (2006–2010). With that, the incidence of anaphylactic shock caused by peanut doubled over the five-year period [42]. In addition, the form of peanut consumption can determine the appearance of allergic reaction. Stability and allergenicity of allergenic proteins can be altered upon food processing. For example, peanut roasting affects the stability of peanut allergens via the Maillard reaction and modified peanut allergens have an increased ability to bind IgE [43,44]. Nevertheless, there is no reliable evidence that allows linking changes in nutrition habits or food industry with the rise in the food allergy prevalence [41].

In the USA, milk, eggs and peanut are the most common allergenic food among children, while adults more often suffer from allergies to shellfish, peanut and tree nuts [30]. Many children will outgrow food allergies and become more tolerant to milk, eggs, soybean and wheat. Allergies to peanut, and tree nuts and shellfish seldom reduce with age [30,45,46]. Allergies to milk and eggs are prevalent worldwide; however, other main food allergens will vary among regions depending on cultural and dietetic habits [47]. Food allergies are potentially dangerous for life. Once ingested, food allergens can cause anaphylactic shock and human death. Using the developed method for studying possible cases of fatal anaphylaxis by the tryptase level from mast cells and allergen-specific antibodies to immunoglobulin E (IgE) in serum of people died from anaphylactic shock, John W. Yunginger et al. [48] established increased serum tryptase levels (12 ng/ml to 150 μg/ml) in nine of nine fatal cases caused by food. Serum IgE antibodies were increased in eight of eight studied fatal cases due to food allergy. According to the medical statistics data, 100 to 200 deaths from anaphylactic shock due to food allergies are recorded in the USA each year [9].
Catering establishments and educational institutions remain to be the most common places of fatal allergic reactions, and peanut accounts for more than 50% of deaths linked with food allergies in the USA [49].

The problem of the allergic response to innovative food additives and products with them deserves close attention. These are GMO food ingredients. Studies show that albumin, globulin, gluten of transgenic wheat varieties can cause asthma and IgE food allergy [50]. It is also of interest to assess an allergenicity risk of protein from non-traditional sources such as insects.

3. Legislative requirements

Initially, the list of top priority allergens was published in Codex Alimentarius in 1999. Later on, this list became a starting point for the European Commission and other state organizations to publish the special legislative act that regulates labeling of food products containing the ingredients from the list [51].

In foreign countries, legislative requirements that included a list of allergens and the processes of their control were developed:

- The Regulation (EU) no 1169/2011 on the provision of food information to consumers. The Annex II of this document lists 14 groups of food products that cause allergies, which should be mandatory on a product label if they are used as ingredients irrespective of their quantity [52].
- Directive 2003/89/EC as regards indication of the ingredients present in foodstuffs (European Union)
- Directive 2005/26/EC on allergens (European Union)
- Federal Legislation. Section 201–210 (USA)
- Food Allergen Labeling and Consumer Protection Act of 2004
- Australia New Zealand Food Standards Code — Standard 1.2.3 [2].

Recommendation 24–2017 of the Scientific Committee of the Federal Agency for the Safety of the Food Chain (FASFC) (Belgium) regarding reference doses of allergens listed in Annex II of Regulation (EC) No. 1169/2011 of October 25, 2011.

In November 2015, USDA’s Food Safety and Inspection Service (FSIS) issued guidelines to assist producers of meat, poultry and processed egg products in attempting to reduce side reactions to food allergens. This guidance includes measures for prevention and control of potentially allergenic ingredients, packaging, labeling, control lists and training [53].

In 2018, the Proposed Draft Code of Practice on Food Allergen Management for Food Business Operators was placed on the official site of the Food and Agriculture Organization of the United Nations (FAO) for public discussion; it was planned to consider the possibility of its adoption at the 43th session of the Codex Alimentarius Commission on July 6–11 2020 in Rome (Italy).

In Russia, the list of most common food allergens, the consumption of which may cause allergic reactions or is contraindicative in certain types of diseases, is given in the Technical Regulation of the Customs Union “Food products in part of their labeling” (TR CU022/2011); it is fully harmonized with the EC legislation and contains the following products:

1) peanut and products of its processing;
2) aspartame and aspartame-acesulfame salt;
3) mustard and products of its processing;
4) sulphur dioxide and sulphites if their total content exceeds 10 milligrams per 1 kilogram or 10 milligrams per one liter in terms of sulphur dioxide;
5) cereals, containing gluten and products of their processing;
6) sesame and products of its processing;
7) lupin and products of its processing;
8) molluscs and products of their processing;
9) milk and products of its processing (including lactose);
10) nuts and products of their processing;
11) crustaceans and products of their processing;
12) fish and products of its processing (excluding fish gelatin used as a basis in preparations containing vitamins and carotenoids);
13) celery and products of its processing;
14) soya and products of its processing;
15) eggs and products of its processing [54].

In addition, according to the requirements of TR CU022/2011 “Food products in part of their labeling”, components capable of causing allergic reactions are indicated in the food product composition irrespective of their quantity. These measures are aimed at ensuring provision of timely information to consumers with food allergies for correct composition of their diets.

4. Causes of unintentional incorporation of allergens into meat products upon their production

When a problem with meat product safety linked with incorrect handling of allergenic ingredients arises, not only consumer health but also reputation and economic stability of meat industry enterprises are threatened. To avoid realization of such risks, specialists of meat processing enterprises should analyze causes of unintentional incorporation of allergens into meat products upon their production. It is necessary to assess every technological stage supporting the process where realization of the risk of the undeclared allergen presence in meat products is possible, and analyze information related to unintentional incorporation of allergens into products during their processing [23,55,56,57, 58, 59].

As a result of the analysis of the likelihood of allergen realization, the following causes of unintentional incorporation of allergens into meat products can be identified (Table 1).

Activities on meat product manufacture are different by their character and not all causes of allergen incorporation into meat products highlighted in table 1 are applicable to a particular enterprise or process. The common denominator of all examined causes is a requirement for hazard analysis and absence of information on realization of this requirement [60].
To minimize possible unintentional incorporation of allergens into food products, food industry enterprises develop and introduce a complex of measures within a framework of allergen management programs [55].

Advanced companies that have been working for many years according to the international food safety and quality standards determined years ago the ways of the development with regard to designing allergen management programs. As for small and medium-sized businesses, the scantiness of information resources in the sphere of implementation tools does not allow them to compete with giant manufacturers [61].

Introduction of allergen management should be regarded as an extension of the existing system of food safety management.

At the initial stage of work in this direction, it is expedient to analyze the following factors:

- total quantity of allergens that can provoke a reaction in sensitive people (these data are relative as different people can have different levels of sensitivity and sensitivity of a person can vary under different circumstances);
- how frequent population consuming allergen-containing food products has general adverse reactions;
- whether there are any subgroups of the population that are in the special risk zone (infants and children). These subgroups include people who restrict food choices due to diets;
- relative allergenicity of the component being used; moreover, if a product was processed, a corresponding protein can be absent and, therefore, it will not present a risk of cross-contamination with an allergen;
- origin of particular ingredients, their geographic and manufacturing environment [57].

### Table 1. Causes of unintentional incorporation of allergens into meat products

| Life cycle processes, supporting processes, management processes | Causes of unintentional incorporation of allergens |
|---------------------------------------------------------------|-------------------------------------------------|
| **Purchase of raw materials, specifications (incoming control)** | — absence of procedures for assessing suppliers; — accompanying documents are not analyzed upon raw material entrance for obtaining corresponding information about an allergen or any changes; — absence of information on a passport of every pallet/box/bag about the presence of an allergen (enterprises can use color coding, labeling or other means for identification of allergenic ingredients); — mishandling of damaged containers, boxes, bags with allergens, which leads to cross-contamination upon receiving; — absence of information about GMO ingredients; — absence of information about non-traditional protein sources |
| **Planning of production** | — joint storage and transfer across an enterprise of ingredients containing allergens and ingredients free from allergens; — nonuse of clear designation for separation zones of intermediate storage and transfer; — absence of physical barriers; — nonuse of special trays, containers, appliances; — allergenic ingredients are not identified by labeling or color coding; — closed containers are not used; — procedures for cleaning from spillage or damaged containers with allergens are not used and documented; — succession of manufacturing allergenic products after allergen free products is not planned; — risk of allergenic dust migration during processing is not assessed; — absence of control for reincorporation of a product into a process |
| **Sanitary** | — time schedule for sanitary treatment is not made; — absence of instructions for equipment cleaning; — absence of equipment cleaning immediately after manufacturing food products with allergens; — cleaning quality is not controlled; — absence of allocated tools for cleaning; — absence of documented rules for cleaning of spillage; — absence of documented rules for disassembly of equipment when cleaning |
| **Training and instruction of personnel** | — absence of training on allergen awareness and control of personnel according to their job responsibilities; — hand washing is not controlled; — special clothing is not allocated and its timely exchange is not controlled; — control of product rework is absent; — waste control is absent; — control of appliances use is absent |
| **Packaging and labeling** | — absence of packaging control; — change of packaging lines is not controlled; — high level of problems with mislabeling |
| **Food safety system** | — one of hazard types (allergen) was not considered when analyzing a likelihood of hazard factor realization and severity of its consequences; — failure to support decisions made in the course of hazard analysis; — failure to assess a likelihood of hazard factor realization and severity of its consequences; — failure to implement effectively control means to support decisions made in the course of hazard analysis |
Then, a likelihood of cross-contamination at each stage of food production process is assessed beginning from the incoming control of food raw materials to finished product sale. With that, it is necessary to assess a physical form of used allergens, for example, a liquid and powder present a different degree of the cross-contamination risk. For instance, during powder milk weighing, it can be introduced into a product through a ventilation system or from personnel clothes; while introduction of liquid milk is less likely when adhering to certain measures (separation with physical barriers, a distance between products).

When an unacceptable contamination risk was identified, it is necessary to apply measures aimed at reduction of unintentional presence of allergens in a product to the fullest extent possible. To this end, Good Manufacturing Practice (GMP) has been successfully used in the framework of production process organization. To ensure food safety, GMP requires maintenance of strict discipline by all personnel [63]. Key aspects of allergen management in meat product manufacture are presented in Figure 2.

![Figure 2. The main areas for consideration when creating allergen management system](image)

A manufacturer should know about the presence of allergens in all used raw materials, which is achieved when working with suppliers and upon incoming control of accompanying documents on raw materials. A manufacturer should request information from suppliers regarding the content of food allergens in raw materials in a form of:

- a) the main components indicated in the composition (for example, soy plant protein in the composition of the complex food additive);
- b) auxiliary components (for example, a food additive produced from an allergenic source, for example, amylase from wheat);
- c) undeclared components introduced due to cross-contamination with an allergen upon production.

Raw material suppliers should be aware about risks that may realize as a result of product contamination with allergens and provide corresponding information. Components should be fully described on a label and in specifications; the use of generalized names of used ingredients such as “plant oils and fats” is unacceptable [53,56].

When placing in a manufacturer’s warehouse after incoming control, raw materials containing allergens should be identified; it is expedient to provide separate storage of such ingredients.

The only approach to full exclusion of cross-contamination with allergens during production process is the use of separate production areas; however, it is often impossible. There are other measures for separating products with allergens from products without allergens:

- separation of production into zones; establishment of physical barriers between production lines;
- provision of allocated equipment, appliances and containers;
- minimization of unnecessary material movement; proper planning of production cycles including equipment cleaning between production cycles;
- organization of individual air supply where it is possible, and so on.

At the stage of incoming control of the main raw materials and auxiliary materials, they are checked on correspondence to normative technical documentation including information about the presence of allergens. Training of responsible employees on allergen awareness and their control according to job responsibilities are carried out. The control of corresponding documents, identification of incoming raw materials and other materials regarding correspondence to information, visual assessment are performed. Then, clear labeling is carried out indicating whether there is a potential allergen (enterprises can use color coding or other means for identification of allergenic ingredients) and batches of incoming raw materials and other materials are placed separately [62]. At the planning stage, it is necessary to segregate zones for storage, production of the main raw materials, auxiliary materials with and without allergens; however, if there is no such possibility, other methods are used. Zones for allergen storage are prepared and established. Special transport containers (marked or color coded) are used. Allergenic raw materials are placed in an allocated and marked warehouse zone separately from raw materials without allergens; physical barriers are used. Instructions have been developed with regard to prevention of cross contamination and are distributed in necessary locations [58, 59]. When transporting allergenic raw materials from a warehouse to the area of spices composition, special marked closed containers are used.

Routes for transportation of allergens and non-allergens, finished products and waste are separated by time (space) to prevent cross-contamination. After transportation, facilities are cleaned along the route of transportation and sanitary treatment of transport equipment is applied. When storing and using allergens, racks, weighing scales, appliances (shovels, small containers, bag), places for storage...
of cleaning appliances and cleaning appliances per se are marked. Personnel use special clothes; the control of its timely exchange is performed. Operation of a ventilation system is controlled. In meat product manufacture, production of allergenic products after products that are free from allergens is planned. After the end of manufacturing process, equipment and appliances are thoroughly cleaned. It is necessary to make schedules of sanitary treatment and instructions, control quality of equipment cleaning, allocate tools, develop rules for cleaning of spillage and disassembly of equipment upon cleaning. Also, it is necessary to carry out identical measures and control upon packaging products with allergens and products free from them. All allergenic ingredients should be indicated on a label, product labeling is carried out according to TR CU022/2011[54]. It is necessary to control secondary processing and utilization of food waste. It is stated in article 10 clause 2 of Technical Regulation of the Customs Union 02/1/2011 “On safety of food products” that a manufacturer should develop, introduce and maintain procedures based on the HACCP principles. In the system framework, it is necessary to analyze risks of the likelihood of hazard factor realization and severity of its consequences. Previously, three hazard types (biological, chemical, physical) were examined; now allergens are also analyzed.

At present, specialists of the V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences have been developing a draft of GOST R “Meat industry. Order of development of allergen management program for meat industry”. The present standard gives recommendations to producers on the development of procedures for determination of allergens in the process of production as well as on realization of measures for allergen management including control measures for:

— managing a level of a hazard for meat product safety, which is characteristic for a product and environment where it is produced;
— managing a likelihood that a production environment will become a source of emergence of hazards for food safety;
— assurance of correct labeling of allergens for packaged finished products.

Meat industry enterprises have a big responsibility in product manufacture regarding the correspondence to the requirements of the legislation and regarding consumers’ health. In this connection, it is necessary to develop, introduce and maintain a program for allergen management, analyze the causes of allergen realization and organize resource management to minimize unintentional incorporation of allergens into finished products.

**Conclusion**

Food allergy is a developing problem of public health, which can have a serious consequences for health of consumers sensitive to food allergens and can even lead to death. There are IgE and IgG food allergies. IgE food allergy is an acute reaction that occurs in 2–3% of population. Any food product that is considered allergen can cause this type of allergy. About 20% of world population have IgG food allergy. It is characterized by the delayed allergic reaction with lower risk of a severe disease or death. As today the only method to stop a food allergy is complete exclusion of an ingredient that causes an allergy from a diet, the food industry, in particular, its meat branch, has to provide a consumer with reliable information on a product label, as well as exclude unintentional incorporation of allergens into products upon their manufacture. When a threat for meat product safety linked with mishandling of allergenic ingredients arises, specialists of meat processing enterprises should analyze causes of unintentional incorporation of allergens into meat products upon their manufacture. In analysis, it is necessary to assess every technological stage supporting a process, where the realization of the risk of the presence of undeclared allergens in meat products is possible, and analyze information linked with causes of unintentional incorporation of allergens into products upon their manufacture. To control allergens, modern analytical methods such as mass spectrometry are necessary. It is necessary to develop databases of protein sequences to simplify identification of allergenic protein in proteomic investigations.

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