ISSN 2414-438X (Print)
ISSN 2414-441X (Online)

ФНЦ ПИЩЕВЫХ СИСТЕМ ИМ. В.М. ГОРБАТОВА РАН

# THEORY AND PRACTICE OF MEAT PROCESSING

# ТЕОРИЯ И ПРАКТИКА ПЕРЕРАБОТКИ МЯСА

2020, vol.5, no.3

# ЦЕЛИ И ЗАДАЧИ ЖУРНАЛА

Приоритетной целью Журнала «Теория и практика переработки мяса» является распространение в мировом научном сообществе трудов по науке о мясе ученых научных центров, научно-исследовательских институтов и высших учебных заведений из России и стран СНГ, повышение уровня присутствия достижений представляемой ими науки на международной арене, знакомство Российских ученых с исследованиями за рубежом, освещение результатов перспективных направлений научно-исследовательской деятельности в мясной и птицеперерабатывающей промышленности.

К публикации в журнале приглашаются как отечественные, так и зарубежные ученые и специалисты.

Важнейшими задачами журнала являются: обобщение научных и практических достижений в области науки о мясе, повышения научной и практической квалификации как научных работников, так и представителей промышленности.

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The top priority goal of the Journal "Teoriâ i praktika pererabotki mâsa" (Theory and practice of meat processing) is to distribute in the world scientific community the results of the research in the field of meat science performed by the scientists from scientific centers, scientific-research institutes and institutions of higher education from Russia and the CIS countries, increase the level of presentation of the achievements of the respective science in the international arena, inform the Russian scientists about the research carried out abroad, highlight the results of the prospect directions of the research activities in the meat and poultry processing industries.

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**www.meatjournal.ru**

**Учредитель:**
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**Printing Office:**
Federal State Budgetary Scientific Institution "V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences"
Talalikhina str. 26, Moscow, Russia, 109316

Журнал зарегистрирован в Роскомнадзоре
Регистрационные данные:
ПИ № ФС77-71611 от 13.11.2017 года
ЭЛ № ФС77-71609 от 13.11.2017 года
Издается с 2015 года
Контент доступен под лицензией
Creative Commons Attribution 4.0 License
Свободная цена
Периодичность — 4 номера в год
Подписной индекс в каталоге «Пресса России» 38871
Подписано в печать 30.09.2020
Дата выхода из печати 10.10.2020
Тираж 1000 экз. Заказ № 311

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ISSN 2414-438X (Print)
ISSN 2414-441X (Online)
Префикс DOI: 10.21323/2414-438X

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Talalikhina str. 26, Moscow, Russia, 109316

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**Редакция:**
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**Printing Office:**
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Talalikhina str. 26, Moscow, Russia, 109316

Журнал зарегистрирован в Роскомнадзоре
Регистрационные данные:
ПИ № ФС77-71611 от 13.11.2017 года
ЭЛ № ФС77-71609 от 13.11.2017 года
Published in 2015
This work is licensed under a
Creative Commons Attribution 4.0 License
Free price
Frequency — 4 issues a year
Subscription index in the catalogue "Press of Russia" 38871
Signed print 30.09.2020
Released from press 10.10.2020
Circulation — 1000 copies. Order № 311
© FNCPS, 2020

ISSN 2414-438X (Print)
ISSN 2414-441X (Online)
DOI-prefix: 10.21323/2414-438X

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DOI: https://doi.org/10.21323/2414–438X–2020–5–3–4–11

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Available online at https://www.meatjournal.ru/jour
*Review paper*

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

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**Keywords:** *food allergy, allergens, causes, unintentional ingestion, meat products*

***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

FOR CITATION: Chernukha I. M., Kuzlyakina Yu.A., Zamula V. S., Kryuchenko E. V. Allergenomics and analysis of causes of unintentional incorporation of substances capable of causing IgE-mediated food allergy into meat products. *Theory and practice of meat processing.* 2020;5(3): 4–11. https://doi.org/10.21323/2414–438X–2020–5–3–4–11

binds to the surface of mast cells in different connective tissues (the gastrointestinal system, respiratory tract, skin) and basophils in blood. Cross-linking of allergens with IgE on the surface of mast cells or basophilic membrane triggers release of histamine and other chemotactic mediators responsible for clinical allergic symptoms. Histamine, prostaglandins and leukotrienes can promote contraction of smooth tissue in the blood vessels, gastrointestinal tract and respiratory tract and increase permeability and dilation of vessels, increase mucus secretion and increase chemotaxis of eosinophils, neutrophils and mononuclear cells. Mediators are released into the blood flow and can cause systemic reactions affecting several tissues and organs [24,25].

In addition to IgE-mediated food allergy, which symptoms are known and the mechanism is described, there is non-IgE-mediated food allergy, which pathogenesis is not fully understood.

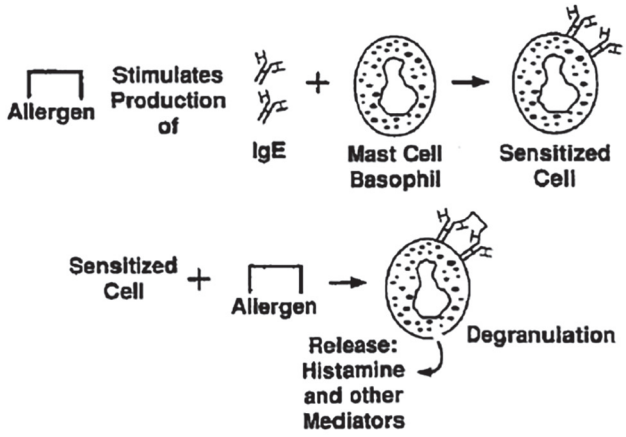


**Figure 1.** Mechanism of IgE-mediated food allergy

Almost all products with natural proteins can cause allergic reactions in certain individuals. However, an impact of food proteins not always leads to the production of protein-specific IgE antibodies and only a small percent of food proteins was identified as allergens [26]. A majority of food allergens are water- or salt-soluble glycoproteins with acidic isoelectric end points that are comparatively resistant to processing, food preparation, proteolysis and digestion processes [27].

*2. Prevalence of IgE-mediated food allergy*

As mentioned above, food allergy is a problem of public health worldwide as 5–10% of children and 3–4% of adults suffer in the western countries according to medical estimates. Awareness about food allergies is growing and up to 35% of people self-diagnose food allergies. The prevalence of food allergies is increasing in the world, although specific causes have not been revealed [28,29,30].

There are many theories about growing prevalence of food allergies: the genetic factors, number of caesarean sections, hygienic hypothesis, time and route of the first contact with food allergens, changes in nutrition habits, food processing and levels of vitamin D exposure [30]. No large-scale changes in population genetics can explain the rise in food allergies [31]. 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 the food allergic reaction. 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 attempt 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 planed 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 its 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].

**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;<br>— accompanying documents are not analyzed upon raw material entrance for obtaining corresponding information about an allergen or any changes;<br>— 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);<br>— mishandling of damaged containers, boxes, bags with allergens, which leads to cross-contamination upon receiving;<br>— absence of information about GMO ingredients;<br>— 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;<br>— nonuse of clear designation for separation zones of intermediate storage and transfer;<br>— absence of physical barriers;<br>— nonuse of special trays, containers, appliances;<br>— allergenic ingredients are not identified by labeling or color coding;<br>— closed containers are not used;<br>— procedures for cleaning from spillage or damaged containers with allergens are not used and documented;<br>— succession of manufacturing allergenic products after allergen free products is not planned;<br>— risk of allergenic dust migration during processing is not assessed;<br>— absence of control for reincorporation of a product into a process |
| Sanitary | — time schedule for sanitary treatment is not made;<br>— absence of instructions for equipment cleaning;<br>— absence of equipment cleaning immediately after manufacturing food products with allergens;<br>— cleaning quality is not controlled;<br>— absence of allocated tools for cleaning;<br>— absence of documented rules for cleaning of spillage;<br>— 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;<br>— hand washing is not controlled;<br>— special clothing is not allocated and its timely exchange is not controlled;<br>— control of product rework is absent;<br>— waste control is absent;<br>— control of appliances use is absent |
| Packaging and labeling | — absence of packaging control;<br>— change of packaging lines is not controlled;<br>— 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;<br>— failure to support decisions made in the course of hazard analysis;<br>— failure to assess a likelihood of hazard factor realization and severity of its consequences;<br>— failure to implement effectively control means to support decisions made in the course of hazard analysis |

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 of 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].

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

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 021/2011 "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 allergenic 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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The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

The authors declare no conflict of interest.

**Received 14.05.2020 Accepted in revised 02.09.2020 Accepted for publication 25.09.2020**

DOI: https://doi.org/10.21323/2414–438X–2020–5–3–12–17

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*Review paper*

# A SYSTEM APPROACH TO SIMULATION OF INDIVIDUAL FOOD PRODUCTS

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**Keywords:** *simulation of living systems, individual food product, system approach, protein cleavage*

***Abstract***
*There is no doubt that the further development in the field of nutrition is linked with personalization. Nutrition management with account for metabolism plays a key role in health strengthening and prevention of human diseases. The paper gives a review of studies associated with personalized nutrition. Personalized nutrition is inextricably linked with personalized food products. At present, however, mass production of personalized food products for individuals or small groups of people is unfeasible. The development of personalized food products requires both time and labor input, as well as multidisciplinary and profound knowledge in a wide spectrum of areas associated with biology, medicine, nutrition and food systems. Among the most important characteristics of modern science is the study of complex and super-complex organized objects such as the food system. These objects were studied previously but by the way of significant simplification of their structure. Investigation of objects with all variety and complexity of their organization requires not only new scientific ideas but also a new conceptual framework, new research methodology, new approaches to simulation of both products and physiological processes. In this study, the authors made an attempt to bring the theoretical view on an individual product closer to the complex task solution using the method of mathematical physiology. The intuitive conceptual model for a process of food design is shown with regard to the "health passport" of an individual, disease risk and gastrointestinal (GI) tract status. The differential equations of the concentration dynamics of protein, denatured protein and peptides in the human stomach are presented. The differential equations that describe the process of protein assimilation in the human stomach were solved in the simulation environment Simplex 3. The presented fragments of model realization show the possibility of virtual study on an effect of different indicators of the food nutritional value on the rate of digestion and the process of cleavage of complex components (proteins, fats and carbohydrates) to mono-structural elements depending on different state and influence factors.*

***Funding:*** *The article was published as part of the research topic No. 0585–2019–0008 of the state assignment of the V. M. Gorbatov Federal Research Center for Food Systems of RAS.*

## Introduction

The idea of personalized medicine was presented for the first time by American biochemist Roger John Williams in the book "Biochemical Individuality: The Basis for the Genetotrophic Concept" [1] in 1956. In this book, R. J. Williams stressed the uniqueness of each person in terms of metabolism and requirements in nutritional microelements. Due to the "biochemical individuality", persons have their own nutrition requirements.

However, this idea was implemented only after human genome mapping in 2003 [2].

Nutrition management in chronic diet-related diseases can be improved based on data about the genome of a particular individual. To establish an interaction, the four-year Food4Me project (http://www.food4me.org/) financed by the European Commission was launched with the involvement of experts in the field of nutrition from different countries. The project envisaged individual recommendations on nutrition at three levels: 1) only a human diet; 2) a diet combined with the knowledge of human phenotype (measurable characteristics, such as height, weight, cholesterol level and so on); 3) a diet, phenotype and genotype (detection of the hereditary genetic identity of a person, for example, a gene variant linked with the weight gain). The European-wide study (more than 1500 adults from Germany, Greece, Ireland, the Netherlands, Poland, Spain and UK) showed that personalized recommendations on nutrition were more effective for improving dietary behavior than traditional recommendations for population.

The results were presented at the project's final conference "Personalised nutrition: paving a way to better public health?' in Brussels on February 26, 2015. The study demonstrates that remote collection of phenotypic data and data on food consumption through the Internet is possible and allows obtaining consistent and reliable data [3,4,5].

It is worth emphasizing that personalized nutrition is inextricably linked with personalized food products. At present, mass production of personalized food products for individuals or small groups of people is unfeasible. Therefore, the development of personalized food products requires both time and labor input, as well as multidisciplinary and profound knowledge in a wide spectrum of areas associated with food products.

The fundamental trends that shape the world are analyzed and discussed in [6,7]. One of the megatrends is the availability of right personalized food products to prevent, mitigate diet-related diseases especially for vulnerable population groups including the elderly. At the same time, King et al. [7]

FOR CITATION: Lisitsyn A. B., Chernukha I. M., Nikitina M. A. A system approach to simulation of individual food products. *Theory and practice of meat processing.* 2020;5(3): 12–17. https://doi.org/ 10.21323/2414–438X-2020–5–3–12–17.

believe that introduction of different types of microbiomes to cure people of certain diseases (personalized approach) will lead to food safety problems. Systematic research including the use of nutrigenomics, metabolomics and toxicogenomics is necessary to ensure safety of personalized products.

A process of designing personalized nutrition along the food chain is presented in [8] covering the following topics: 1) food raw materials and components; 2) food industry and gastronomy aspects; 3) digestion and microbiota; 4) food perception (Figure 1).

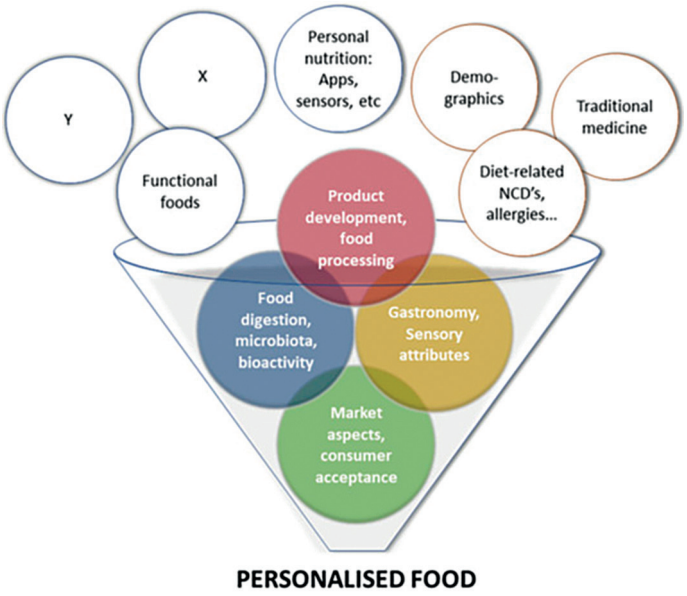


**Figure 1.** Individual approach to food [8]

The Biomolecula portal (https://biomolecula.ru) has the project "Science for life extension", which points out that developed countries have faced the consequences of irrational nutrition such as obesity and type 2 diabetes mellitus over the last years [9] (Figure 2). It was established that longevity and the development of "deadly three" — cardiovascular, neurodegenerative and oncological diseases — depend on a human diet [10].

Medical data show the interrelation between nutrition and the most prevalent non-communicable diseases. In 2019, the research results of the scientific team from the USA, Norway and Spain were published [11]. The scientists analyzed data on the health state of the population from 195 countries and territories for the period from 1990 to 2017. They identified 92 diagnoses that finally led to irreversible deterioration of physical or mental abilities of patients. Among them were cardiovascular diseases (13), chronic respiratory diseases (6), cancers (35), diabetes, chronic kidney diseases, injuries and neurodegenerative diseases as well as vision and hearing impairments.

Analysis showed that age-related diseases accounted for more than half of health problems in all adult population of the world; however, data differed between countries and senility began at a very different age (Figure 3).

According to the data of 2017, the lowest SDI was in the Republic of the Niger (0.19), Somali (0.23), Afghanistan (0.29). SDI was 0.79 in Russia, 0.77 in Belarus, 0.7 in

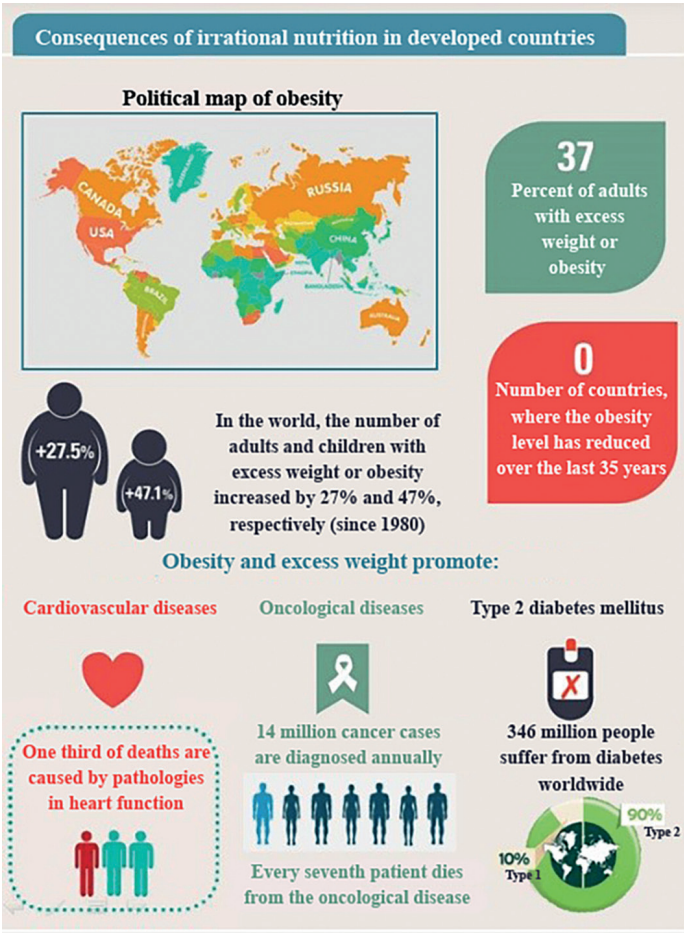


**Figure 2.** Consequences of unbalanced nutrition in developed countries: prevalence of obesity and type 2 diabetes mellitus. The excess weight and "western diet" contribute to the development of cardiovascular and oncological diseases, type 2 diabetes mellitus [biomolecula.ru]

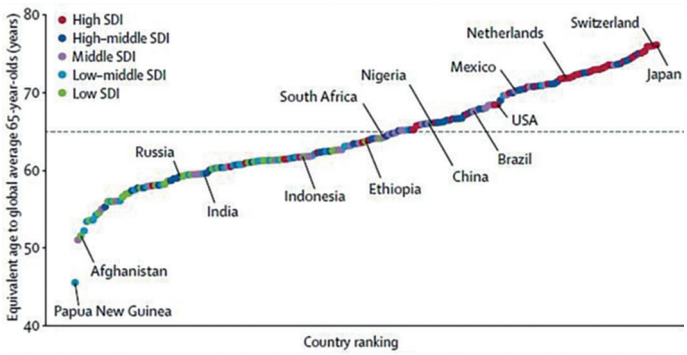


**Figure 3:** Comparison of the equivalent ages with global average index (65 years) across countries in 2017 [11]. SDI (Socio-demographic Index) — a summary measure of a degree of a country or region development expressed in values from 0 to 1 [12]

Armenia, 0.7 in Azerbaijan, 0.69 in Georgia, 0.74 in Kazakhstan, 0.6 in Kirgizstan, 0.62 in Uzbekistan. The highest SDI was in Denmark, Luxemburg (0.92), the Netherlands, Norway (0.91).

It is noted that although in developing countries (with low socio-demographic Index — SDI) longevity is considerably lower than in the developed ones, people still experience the burden of senility and accumulate age-related diseases. It just happens significantly earlier.

Chang A. et al. from the University of Washington Seattle have noticed that these incomparable results indicate that

an increase in longevity can be regarded in the old age both as additional opportunities and as a threat to well-being of a society in general depending on the age when the real age-related health problems arose in individuals and not on their actual age.

According to Ludwig von Bertalanffy [13], the system approach is an approach, in which any system (object) is regarded as an assembly of interrelated elements (components) having the output (target), input (resources), communication with the external environment and feedback. This is the most complex approach.

Among the most important peculiarities of modern science is the study (examination) of complex and super-complex organized objects. These objects were studied previously but by the way of significant simplification of their structure. Investigation of objects with all variety and complexity of their organization requires not only new scientific ideas but also a new conceptual framework, new research methodology [14].

The main advantage of simulation modeling compared to analytical is a possibility to solve more complex tasks. Simulation models allow quite easy consideration of factors such as the presence of discrete and continuous elements, non-linear characteristics of system elements, multiple random impacts and others, which often create difficulties in analytical investigations. At present, simulation modeling is the most effective method for investigation of complex systems and often is the only feasible method for obtaining information about system behavior especially at the stage of its design [14,15,16,17,18].

It should be mentioned that today an opportunity has arisen for the development and wider use of mathematical and information technologies and models in description of biological processes in particular linked with food production and consumption.

The authors of this paper propose using the system approach in simulation modeling of the development of individual food products with regard to the human "health passport", disease risk and gastrointestinal tract status.

The aim of the work is to use the system approach in the development of individual food products with detailing nutrient digestion in the human body.

**Methodology of the research**

The simulation modeling method enables solving tasks of complex system analysis including the tasks of assessing system structure variants, effectiveness of different algorithms of system management, effects of changes in different system parameters. Simulation modeling can also be used as the basis for structural, algorithmic and parametric synthesis of complex systems when it is necessary to create a system with given characteristics upon certain restrictions, which is optimal by several criteria of effectiveness assessment [14].

Simulation modeling was carried out in the program environment Simplex3 (http://www.simplex3.net/) [19,20].

**Results**

After human health screening and obtaining their "health passport", it is necessary to develop food products having regard to nutrient digestibility. To this end, it is necessary to formalize (translate to the mathematical language) processes occurring in the gastrointestinal tract using methods for modeling of living systems, including mathematical physiology methods [21,22].

The human gastrointestinal tract can be considered a natural biochemical reactor that ensures mechanical, thermal, enzymatic and microbiological processing of nutrients by their main components (proteins, fats, carbohydrates). The digestive process, therefore, consists in hydrolysis of nutrients (substrates) under the action of enzymes along the gastrointestinal tract and can be described by the system of Michaelis-Menten substrate-enzymatic reactions [23]. At the same time, it is necessary to take into account peculiarities of the human GI tract, first of all, pH, the presence of pathologies (for example, ulcer disease), food component composition, consistency and temperature.

We presented the structural-parametric model of the GI tract and structural -parametric model of a product separately in [24,25,26]. In the system approach, they will be examined as components of a single model.

A model of an individual food product requires not only consideration for food design according to indices of the nutritional and biological value but also the understanding of what part of nutrients in a food product will be assimilated by certain individuals with regard to their physiological characteristics. For this purpose, mathematical models of biological processes and equations of mathematical physics are used as a rule.

We will show the possibilities of simulation modeling by the example of protein cleavage and assimilation. To this end, at the beginning, we will describe multiple subsystems, organs, variables and links between them in the form of graphic presentation — the IDEF0 scheme (Figure 4).

Changes in the variables of the state of the certain GI tract segments can be described in finite differences by a sum of influencing components in the system of algebraic equations:

$$\Delta X_i = \sum_j^N w_{ij} \cdot f_{ij} \cdot \Delta X_j;\ i = 1.20 \qquad (1)$$

where $f_{ij}$ is the influence function of the $j^{th}$ influencing factor (enzymes, chemical substances of the influencing environment, mechanisms of substance transport) on the $i^{th}$ parameter of the GI tract state; $w_{ij}$ is the sign function of the direction of the influence of the $j^{th}$ factor on the $i^{th}$, stimulating (+) or retarding (–).

Dynamics of the biochemical reactions of the enzymatic type as well as the biochemical and biomechanical processes of substance transport along the GI tract are described in [27] by the system of ordinary differential equations:

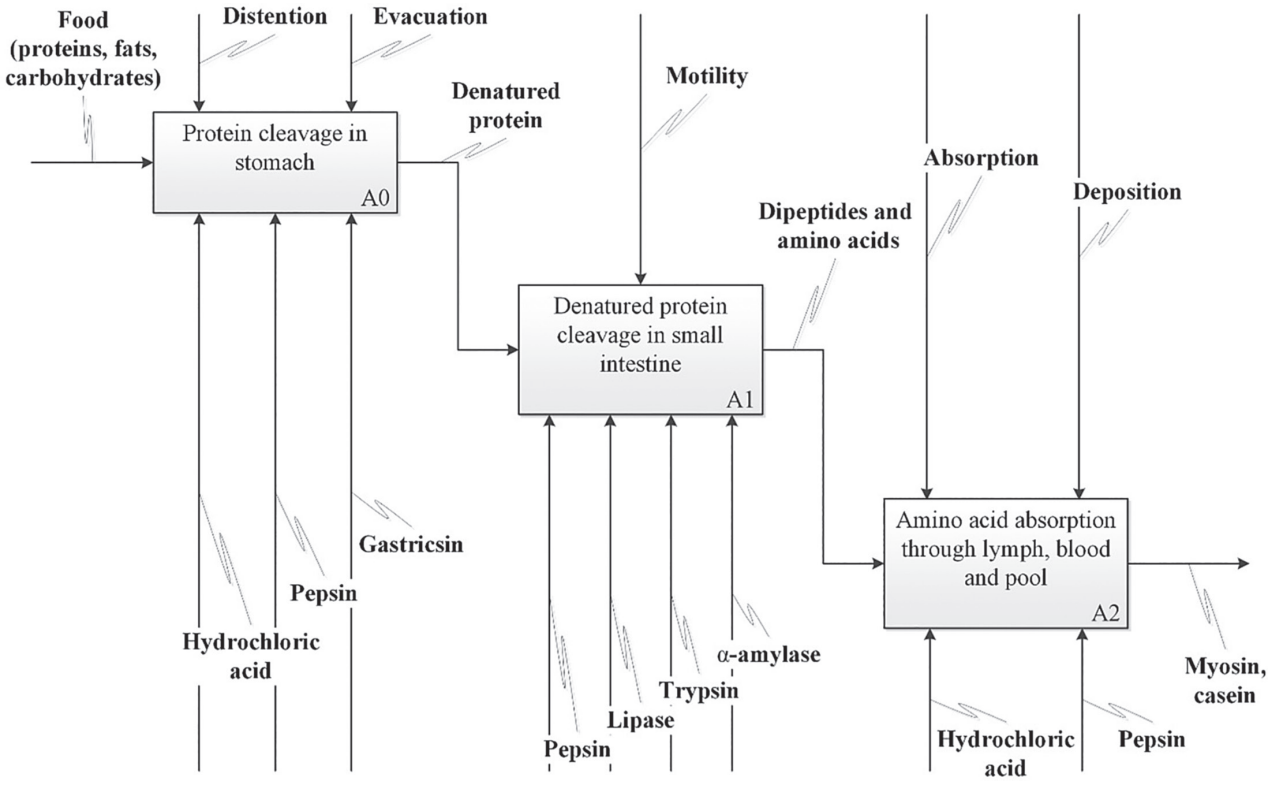


**Figure 4.** The scheme of protein cleavage; a blood pool — an organ or tissue with an ability to accumulate in its vessels a significant amount of blood, which can be used by the body when necessary (for example, liver, spleen, skin blood vessels and so on)

$$\frac{dX_i}{dt} = r\sum_{l=1}^{n_i}\sum_{j=1}^{23}\frac{w_{ij}\alpha_{jl}X_lY_j}{\left(1+\beta_{jl}X_l\right)};\ i=\overline{1.20} \qquad (2)$$

where $X$, $Y$ are variables of the system state and influencing factors of the biochemical environment, respectively; $\alpha_{jl}$ are coefficients of the intensity of the $j^{th}$ influence of enzymes and transport mechanisms on the $i^{th}$ parameter of the state; $\beta_{jl}$ are coefficients of chemical reactions or substance transport; $r$ are scale coefficients of rates of changes in variables in different GI tract segments.

Table 1 presents the used variables of the digestive system state and table 2 shows the factors of the biochemical environment of the GI tract and transport mechanisms.

**Table 1. An example of description of food components transformed in the digestive process**

| Symbols | Product components determining digestive system state | Symbols | Product components determining digestive system state |
|---|---|---|---|
| *In stomach* | | *In small intestine* | |
| $x_1$ | Proteins | $x_6$ | Denatured proteins |
| $x_2$ | Fats (triglycerides) | $x_7$ | Peptides |
| $x_3$ | Carbohydrates (polysaccharides) | $x_8$ | Dipeptides and amino acids |
| $x_4$ | Denatured proteins | $x_9$ | Fats (triglycerides) |
| $x_5$ | Peptides | $x_{10}$ | Emulsified fats |
| *In blood, lymph and pool* | | $x_{11}$ | |
| $x_{17}$ | Amino acids | $x_{12}$ | Fatty acids |
| $x_{18}$ | Fatty acids | $x_{13}$ | Carbohydrates (polysaccharides) |
| $x_{19}$ | Triglycerides | $x_{14}$ | oligosaccharides |
| $x_{20}$ | Glucose | $x_{15}$ | Disaccharides |
| | | $x_{16}$ | Glucose |

**Table 2. An example of analyzed factors of the GI tract environment**

| Symbols | Factors of the GI tract biochemical environment |
|---|---|
| $y_1$ | Hydrochloric acid |
| $y_2$ | Pepsin(ogens) |
| $y_3$ | Bicarbonates of Brunner's glands |
| $y_4$ | Bicarbonates of duodenum |
| $y_5$ | Bicarbonates of pancreatic juice |
| $y_6$ | Bile secretion (outflow to duodenum) |
| $y_7$ | Enterokinase |
| $y_8$ | Trypsinogen |
| $y_9$ | Proteolytic enzymes of gastric juice |
| $y_{10}$ | Peptidase |
| $y_{11}$ | Dipeptidase |
| $y_{12}$ | Lipase |
| $y_{13}$ | Monoglyceride lipase |
| $y_{14}$ | α-Amylase |
| $y_{15}$ | Oligosaccharidase |
| $y_{16}$ | Dissacharidase |
| $y_{17}$ | Electrolytes in intestine |
| $y_{18}$ | $Ca^{++}$ |
| | Transport mechanisms |
| $y_{19}$ | Gastric distention |
| $y_{20}$ | Gastric evacuation |
| $y_{21}$ | Intestinal motility |
| $y_{22}$ | Absorption |
| $y_{23}$ | Deposition |

In relation to the symbols (Table 1 and Table 2) and IDEF0 scheme, equation (2):

— for dynamics of the concentration of entered proteins ($x_1$) in the stomach with regard to the factors of protein denaturation by hydrochloric acid ($y_1$), gastric distention ($y_{19}$) and evacuation ($y_{20}$) is described in the expanded form by the following differential equation:

$$\frac{dx_1}{dt} = x_1 - \frac{\alpha_{1.19}x_1}{1+\beta_{1.19}x_1}y_{19} - \frac{\alpha_{1.1}x_1}{1+\beta_{1.1}x_1}y_1 - \frac{\alpha_{1.20}x_1}{1+\beta_{1.20}x_1}y_{20} \quad (3)$$

— for dynamics of the concentration of denatured protein in the stomach as a result of an impact of hydrochloric acid ($y_1$) on entered protein ($x_1$), reduction of the concentration of denatured protein ($x_4$) due to an impact of pepsin ($y_2$) and partial evacuation into small intestine ($y_{20}$) has the form:

$$\frac{dx_4}{dt} = \frac{\alpha_{1.1}x_1}{1+\beta_{1.1}x_1}y_1 - \frac{\alpha_{2.4}x_4}{1+\beta_{2.4}x_4}y_2 - \frac{\alpha_{20.4}x_4}{1+\beta_{20.4}x_4}y_{20} \quad (4)$$

— for dynamics of the peptide ($x_5$) concentration in the stomach reflects their accumulation upon cleavage of denatured protein ($x_4$) by pepsin ($y_2$) and reduction as a result of the further evacuation ($y_{20}$)

$$\frac{dx_5}{dt} = \frac{\alpha_{2.4}x_4}{1+\beta_{2.4}x_4}y_2 - \frac{\alpha_{20.5}x_5}{1+\beta_{20.5}x_5}y_{20} \quad (5)$$

The differential equations of the dynamics of fat and carbohydrate cleavage are formulated in a similar way.

The initial data of the task were the coefficients of enzyme activities and transport mechanisms $\alpha_1 = 0.03$, $\alpha_2 = 0.01$, $\alpha_{20} = 0.001$; the coefficients of the chemical reaction kinetics or substance transfer $\beta_1 = 1$, $\beta_2 = 1$, $\beta_{20} = 0.01$; the initial protein concentration was $x_1 = 0.1$ (Figure 5).

In the stomach, a decrease in the concentration of the protein substrate X1 is observed, which is transformed into the denatured form and/or evacuated from the stomach. At the same time, the concentration of denatured protein increases with the further cleavage to peptides X5.

The presented fragment of model realization in the universal simulation model Simplex 3 shows a possibility of virtual study on the influence of different food nutritional value indicators on the rate of digestion and the process of cleavage of complex components to monostructural elements depending on different state and influence factors.

## Conclusion

An advantage of personalized nutrition is indisputable. Nowadays, however, mass production of personalized food products for individuals or small groups of people is unfeasible. The development of personalized food products requires not so much time and labor input as multidisciplinary and profound knowledge in a wide spectrum of areas associated with nutrition, biochemistry, physiology and food technologies. It is necessary to use the system approach with an integrated and detailed examination of a complex food system. The authors made an attempt to bring the theoretical view on an individual product closer to a complex task solution using the method of mathematical physiology. The process of protein cleavage in the form of the IDEF0 scheme is presented. The possibility to simulate the process of protein assimilation in the human stomach is shown in the simulation environment Simplex 3.

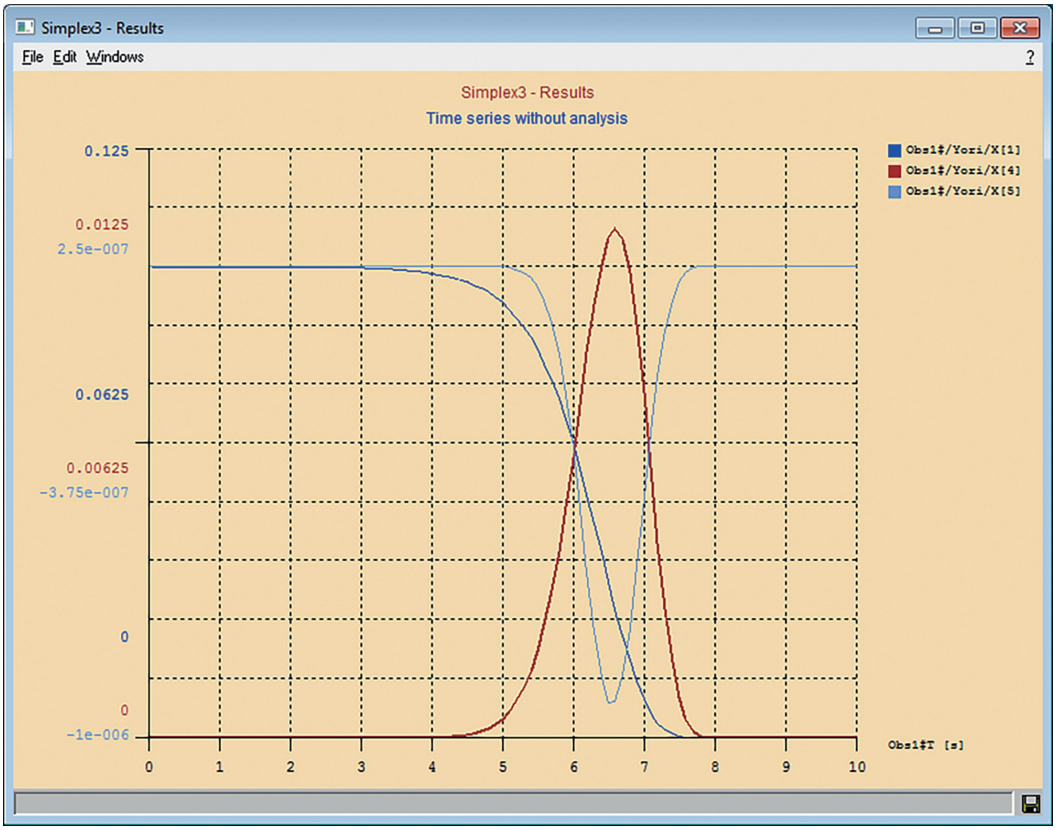


**Figure 5.** Changes in the concentration of proteins X1, denatured protein X4, and peptides X5 in the stomach during the digestive process

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The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

The authors declare no conflict of interest.

**Received 09.08.2020 Accepted in revised 15.09.2020 Accepted for publication 25. 09.2020**

DOI: https://doi.org/10.21323/2414–438X–2020–5–3–18–21

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# STUDY OF THE FUNCTIONAL PRODUCT'S PROTEIN COMPOUNDS DIGESTION FEATURES

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**Keywords:** *digestion, biocorrecting meat product, proteins, peptides, one-dimensional electrophoresis*

***Abstract***

*The aim of the study was to investigate the transformation of meat product's proteins from pig hearts and aortas during enzymatic hydrolysis in an in vitro model of the gastrointestinal tract. The model consisted of three phases simulating digestion processes: "oral cavity" phase (a-amylase, pH 7.0; 2 min), "stomach" phase (pork pepsin, pH 3.0; 120 min), "intestine" phase (pork pancreatin, pH 7.0; 130 min). The product was sequentially subjected to hydrolysis, at the end of each phase, samples were taken to determine the protein concentration (biuret method) and visualize the protein fractions (one-dimensional electrophoresis). A significant increase in protein concentration at the "stomach" phase was revealed by 3.2 times, and the absolute content by 4.6 times. At the "intestine" phase, a decrease in the number of peptide complexes with copper ions by 1.8 times, the absolute protein content by 8.5% was revealed. The noted tendency was confirmed by electrophoretic studies — at the stage, simulating digestion in the stomach, the products of meat product's proteins hydrolysis were visualized; at the "intestine" phase, a low expression of protein fractions in the range of more than 10 kDa is shown. The maximum hydrolysis of protein compounds at the "stomach" phase to poly- and oligopeptides was confirmed, continuing at the "intestine" stage with the accumulation of free amino acids. This methodology makes it possible to visualize the products of hydrolysis of proteins in a meat product at all stages of the model and to monitor changes in protein concentration in the system.*

***Funding:*** *The research was supported by state assignment of V. M. Gorbatov Federal Research Centre for Food Systems of RAS, scientific research No. 013.20.05.*

## Introduction

Animal food products' proteins are of particular interest today due to the fact that such compounds are the main source of biologically active components.

Today bioactive components of foods are considered dietary elements that have a measurable biological effect (for example, antioxidant, immunomodulatory, antihypertensive, osteoprotective, hypolipedemic, opiate, etc.) and have a beneficial effect on human health [1,2,3]. During different phases of industrial processing and digestion, bioactive peptides with a wide range of biological effects can be released from protein [4,5].

It is known that bioactive peptides are naturally formed in mammals in the gastrointestinal tract during the metabolism of dietary meat proteins. Consumed proteins derived from raw meat are attacked by digestive enzymes secreted by the stomach, such as pepsin, and, in the small intestine, trypsin, chymotrypsin, elastase, and carboxypeptidase undergo proteolysis. To study peptides of meat products that potentially have functional effects, a simulated digestive system of the gastrointestinal tract can be used to reproduce the processes of generating peptides similar to those released during physiological digestion. The process that simulates digestion of the gastrointestinal tract is based on enzymatic hydrolysis using various commercial exogenous proteinases obtained from animal tissues (pepsin and trypsin), plants (papain, ficin and bromelain) and microbial sources (alkalase, collagenase or proteinase, etc.) [6,7].

The number of studies that use the methodology of modeling gastrointestinal digestion *in vitro* to study bioactive peptides from food proteins are increasing [8,9,10,11,12,13]. Using these methods, researchers are trying to isolate bioactive peptides that can be produced in our body after eating a certain food or dietary protein, and determine its activity.

In this article, we will attempt to understand meat products' proteins transformation using the example of a biocorregating product based on pigs' hearts and aorts after passing through the digestive tract.

## Materials and methods

The object of the study was an experimental development — a meat product made on the basis of pig hearts and aortas in accordance with [14,15], containing functional proteins with biocorregulating properties [16,17,18].

The functional product is a sterilized canned minced meat, it contains 17.5 ± 0.9% protein, 3.8 ± 0.1% fat, 0.30 ± 0.01% sodium chloride and 2.3 ± 0.2% starch.

To simulate the digestion process of the experimental product, a static model of digestion was used in accordance with [19]. Pate crushed in a mortar to a particle size of 3 ± 0.5 mm in amount of 10 g was placed in I imitating liquid ("oral cavity", pH 7, volume 7 ml), consisting of 0.5 ml of α-amylase (30 U/mg, "PanReac AppliChem", Germany), 25 μl of calcium chloride (0.3 m), 10.6 ml of phosphate-salt buffer ("Sigma-Aldrich", USA) and 0.09

FOR CITATION: Vasilevskaya E. R., Akhremko A. G., Polishchuk E. K., Fedulova L. V. Study of the functional product's protein compounds digestion features. *Theory and practice of meat processing.* 2020;5(3): 18–21. https://doi.org/10.21323/2414–438X-2020–5–3–18–21.

ml of hydrochloric acid (6 M, "Sigma-Aldrich", USA). The mixture was then thoroughly mixed for 2 minutes and a sample was taken for further studies. Next, 10 ml of the mixture was placed in II imitating liquid ("stomach", pH 3, volume 15 ml), consisting of 1.6 ml of pig pepsin solution (250 U / mg, "PanReac AppliChem", Germany), 5 ml of calcium chloride (0.3 M, "Sigma-Aldrich", USA)), 0.8 ml of hydrochloric acid (1 M) and 10.0 ml of phosphate-salt buffer. The mixture was stirred using a magnetic stirrer for 120 minutes, and samples were taken for further studies at the end of the incubation time. Then 20 ml of gastric hummus was added to the III imitating liquid ("intestines", pH 7, volume 20 ml) containing 5.0 ml of porcine Pancreatin solution (50.5 U / mg, "PanReac AppliChem", Germany), 1.5 ml of canned bovine bile ("Samson-med", Russia) and 40 ml of 0.3 M calcium chloride, 10.6 ml of phosphate-salt buffer. To neutralize the mixture of a neutral pH value, 0.6 ml of 1 M sodium hydroxide (Sigma-Aldrich, USA) and 986 µl of distilled water were added. The mixing time was 130 minutes, then samples were also taken. In parallel, similar procedures were performed for all stages of the model without adding the test sample to obtain control samples.

All samples were taken in plastic tubes of the Eppendorf type and placed in a freezer at a temperature of minus (40 ± 1)°C to inactivate the enzymatic activity for at least 60 minutes. After defrosting, the samples were centrifuged using a CM-6M centrifuge (ELMI, Latvia) at 3500 rpm for 5 minutes.

The protein concentration in the supernatant of the samples after centrifugation was measured by biuret method in accordance with [20] on Biochem SA photometer (HTI, USA).

For electrophoretic analysis, 50,00 ± 0.05 ml of the sample's supernatant was taken into a microcentrifuge tube, 50 µl of solubilizing solution was added (Glycerol 10%, β-mercaptoethanol 0.01 M, Bromphenol blue 0.02%, Tris-HCl 0.5 M, SDS2% — "Sigma-Aldrich", USA) and kept in a thermostat at 95 °C for 5 minutes. The resulting solution was centrifuged in an Eppendorf 5402r centrifuge at 14,000 rpm for 8 minutes. The supernatant was used for further analysis.

Analysis of the fractional composition was carried out by denaturing electrophoresis in 10% polyacrylamide gel in the presence of sodium dodecyl sulfate using a Hellicon "VE-10" electrophoretic chamber, at a constant current and voltage of 55 V and 130 V, for 2 hours. As a solution of standards, a marker consisting of standard preparations with a molecular weight is used 250, 150, 100, 70, 50, 40, 30, 20 kDa ("Thremo", Latvia). Visualization was performed with Coomassie R250 solution and silver nitric acid ("Sigma-Aldrich", USA).

**Results and discussion**

Proteins' biotransformation in the product was evaluated by quantifying the formation of protein peptide bonds with divalent copper ions during modeling and visualization of protein compounds after gradual digestion by digestive enzymes.

According to the study results, it was found that during modeling stage I, which recreates the conditions of product's digestion in the "oral cavity", there were no significant changes in the protein concentration (Figure 1). The main transformations were revealed after the second phase corresponding to digestion in the "stomach" — the protein concentration increased by 3.2 times, and the absolute protein content increased by 4.6 times. At stage III, after passing the simulated "intestine" fluid, the protein concentration in the system decreased by 1.8 times, but the absolute protein content decreased by only 8.5%, relative to stage II.

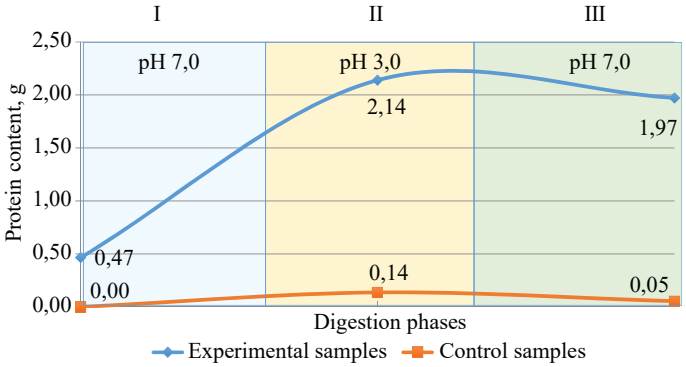


**Figure 1.** Protein content after digestion model phases

When one-dimensional electrophoresis was stained with Coomassie solution, the preservation of product's major fractions with molecular weights in range from 35 to 37 kDa and 13 kDa was shown (Figure 2A, track 1), and fractions corresponding to oral enzymes were also identified. When visualizing with silver nitrate, the presence of an almost complete spectrum of the initial product can be noted, with the addition of oral enzymes fractions (Figure 2B, track 1).

On the second track of the electrophoregram, the result of enzymatic hydrolysis of product proteins was demonstrated. Coomassie staining revealed a protein front in the range of molecular weights less than 12 kDa, which was not previously detected in the product, as well as minor fractions (42–48 kDa) corresponding to "stomach" enzymes (Figure 2A, track 2). Nitric silver as a dye allowed us to detect the presence of protein fractions in the range of molecular weights from 250 to 80 kDa, from 40 to 25 kDa, and from 17 to 14 kDa, which correspond to the proteins initially present in the product (Figure 2B, track 2).

Similar results were found when visualizing product's protein profile at the end of the third phase of digestion ("intestine"). After Coomassie staining, low-molecular compounds (less than 12 kDa) and enzymatic minor bands in the molecular weight range of 28–30 kDa were also visualized (Figure 2A, track 3). The dye with silver nitric acid also showed the presence of protein bands fixed in the sample obtained after the "intestine" stage (Figure 2B, tracks 2 and 3).

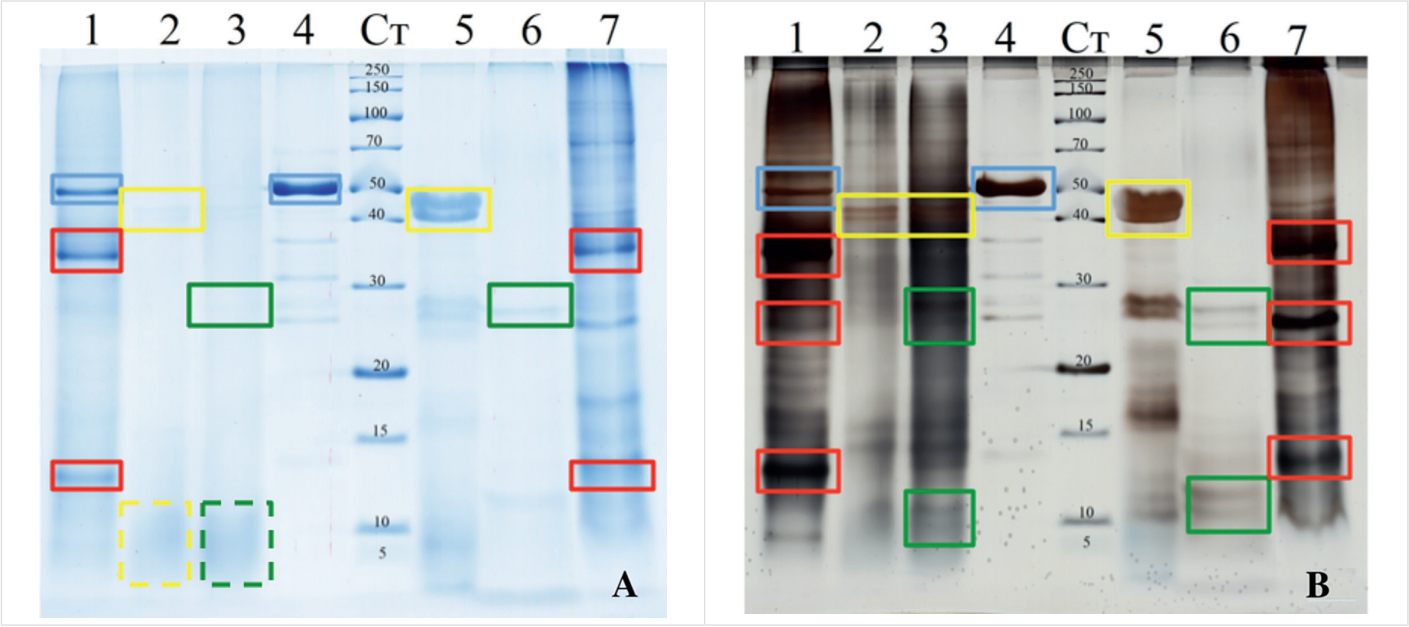


**Figure 2.** One-dimensional electrophoregrams of the studied samples. A — Coomassie color; B — silver nitric acid color.
Symbols:
1 — I phase — imitation of digestion in the " oral cavity»;
2 — II phase — imitation of digestion in the " stomach»;
3 — III phase — imitation of digestion in the " gut»;
4 — control sample for phase I;
Ст — standard for molecular weights: 250, 150, 100, 70, 50, 40, 30, 20 kDa (top to bottom)
5 — control sample for phase II;
6 — control sample for phase III;
7 — product before the experiment.

The revealed tendency to decrease the concentration of protein and its absolute content is due to the fact that the "stomach" phase is active hydrolysis of product proteins by pig pepsin, which cleaves the central peptide bonds in protein molecules. At this phase, a large number of poly- and oligopeptides and a small amount of free amino acids are formed. The decrease in the number of peptide complexes with divalent copper ions at the "intestine" stage is due to the specificity of the action of trypsin and chymotrypsin, which destroy them with the formation of carboxyl groups of amino acids, as well as free amino acids [21,22].

As expected, the one-dimensional electrophoregram also visualized hydrolysis of meat product proteins at the stage simulating digestion in the stomach. When simulating digestion in the"intestine", the marked decrease in the number of peptide complexes with copper ions (by 8.5%) correlates with a low expression of protein fractions in the range of more than 10 kDa and confirms the further metabolism of protein compounds — the conversion of peptides to free amino acids.

## Conclusion

The prospects of using a complex technique for studying the digestibility of functional products' proteins, including a three-stage enzymatic model of digestion with sequential sampling to determine the protein concentration at each stage and visualization of individual components on one-dimensional electrophoresis, were proved. The demonstrated results of the study indicate the accumulation of a peptide pool during digestion, and special attention should be paid to "stomach" and "intestine" phases.

The next step in the study of bioactive peptides obtained in the meat product proteins' proteolysis process, that can be identified, and its' absorption study at the intestinal level in the *in vitro* system using cell cultures.

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The authors declare no conflict of interest.

**Received 08.08.2020 Accepted in revised 15.09.2020 Accepted for publication 25. 09.2020**

DOI: https://doi.org/10.21323/2414–438X–2020–5–3–22–27

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*Original scientific paper*

# THE CHANGES IN THE AMINO AND FATTY ACID PROFILES IN THE SEMIFINISHED FOODSTUFFS BASED ON BROILER MEAT AND COMPONENTS OF CHICKEN EGGS AFTER DIFFERENT TYPES OF THERMAL TREATMENT

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**Keywords:** *broiler meat, coagulated chicken egg melange, semifinished foodstuff, thermal treatment, amino and fatty acid profiles, nutrient balance*

***Abstract***

*The changes in the amino and fatty acid profiles in the semifinished foodstuffs (SFFs) based on broiler meat and coagulated chicken egg melange after different types of thermal treatment (water or steam boiling, braising, baking, frying) were studied. The amino acid profiles were determined on Knauer analyzer; tryptophan by standard method. The biological value of the treated products was assessed using amino acid balance coefficients calculated by the method of N. N. Lipatov. It was found that the changes in the initial amino acid profiles of the SFFs were the least after water and steam boiling; braising and baking were found to increase the contents of the essential amino acids. The amino acid profiles in the treated SFFs were close to the reference values. The best criteria of their biological value (coefficient of rationality of amino acid composition, comparable redundance) were found after water and steam boiling. It was found that all types of thermal treatments insignificantly affected the parameters of fatty acid balance within the SFFs; the changes found were primarily related to slight increase in total content of saturated fatty acids and increase in total content of polyunsaturated fatty acids (PUFAs) in compare to initial profiles, by 2.64–3.88% depending on the treatment type. The changes in ω-6/ω-3 PUFAs ratios were more substantial especially after braising.*

## Introduction

The development and reinforcement of the health status in human of different ages cannot be achieved without consumption of the foodstuffs with high content of essential nutrients (well-balanced protein and fat, etc.). The increasing demand for these foodstuffs is a constant trend of the food market worldwide.

The everyday consumption of poultry meat can provide consumers with vitally important essential amino acids (tryptophan, lysine, and methionine) in the most beneficial ratio 1:3:3 [1,2]; these amino acids take part in the biosynthesis of different tissue proteins and also play certain special functions in human. However, the protein of broiler meat is known to be valine-deficient and hence the foodstuffs fully balanced for all essential amino acids should be additionally corrected for the valine content since the deficit (or absence) of this amino acid can result in the severe dysfunctions of the central nervous system and myasthenia [3].

Another important aspect of healthy nutrition is the balance in the fatty acid profile of the dietary lipids since saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids are the main structural and functional components of cell membranes. The special attention should be paid to the essential PUFAs which cannot be synthesized by animals and human and should be provided via feed or food, primarily linolic (LA, C18:2, ω-6) and α-linoleic (ALA, C18:3, ω-3) acids. These PUFAs are the precursors of a wide range of different eicosanoids, hormone-like substances protecting and maintaining structural and functional integrity of cells and cell components [4,5,6]. The ω-3 and ω-6 PUFAs are the necessary dietary nutrients for all vertebrates since their presence and ratio influence the status of lipid metabolism, susceptibility to the cardiovascular diseases, disturbances in neural and ophthalmic functions, allergic diseases, inflammations [5,7,8,9,10]. On the gene level these PUFAs control gene expression in different organs [8,9] and tissues [11,12,13]. The substantial part (ca. 50–70%) of dietary PUFAs is catabolyzed to meet energy requirements in human [14,15]. Only minor part of the dietary essential PUFAs undergoes the biotransformation to eicosahexaenic and docosahexaenic acids [4,16], main fatty acids in the retinal membranes and the precursors of local cell-regulating hormones affecting the inflammation, bloodstream regulation, prepartum fetal loss, etc. [17].

Poultry meat contains large amounts of the PUFAs; however, the ω-6 acids are predominant and the ω-6/ω-3 PUFAs ratio is relatively large (12:1 in lipids of broiler meat and 25:1 in turkey) in compare to the recommended level (no more than 1:10).

With all aforementioned taken into account, the development of new technologies or modification of the existing

FOR CITATION: Stefanova I. L., Shakhnazarova L. V., Klimenkova A.YU., Sorokina I. M. The changes in the amino and fatty acid profiles in the semifinished foodstuffs based on broiler meat and components of chicken eggs after different types of thermal treatment. *Theory and practice of meat processing.* 2020; 5(3): 22–27. https://doi.org/10.21323/2414–438X-2020–5–3–22–27.

foodstuffs involving the additional supplementation with ingredients with high biological and nutritive value is as urgent task for modern food industry.

The enrichment of foodstuffs with protein and fat ingredients usually involves the supplementation with collagen-rich animal meat products [18] and butter or vegetable (mostly sunflower) oils, respectively. All these ingredients are rich in ω-6 PUFAs and the resulting dietary ω-6/ω-3 PUFAs ratio is far from optimal. The use of the ingredients based on poultry eggs and their components can be the valuable alternative since these ingredients could be the source of high-quality protein containing almost all essential amino acids [19]. The content of valine and some other essential amino acids in chicken eggs is higher in compare to protein of broiler meat.

Egg lipids contain simple fats, phospholipids (including lecithin, cephalin, sphingomyelin), cerebrosides, sterides, sterines (cholesterol). Choline presents up to 75% of egg phospholipids; concentration of lecithin is 6-fold higher in compare to cholesterol promoting the better bioavailability of egg lipids. However, the most value ingredients within the eggs are essential PUFAs deficient in other standard foodstuffs in human diets.

Eggs also have certain important technological properties like foam and gel production, emulsifying ability, ability to make a product "airy". The most important technological factor is the gel producing ability of the albumen [20].

The coagulation of egg components is a prospective technology of maximal preservation of the useful properties of the eggs. The studies on the changes in the latter induced by different factors resulted in the development of protein-rich food ingredients based on the coagulated egg components (melange, albumen, yolk) with grainy texture [21]. The use of these ingredients as the additives to meat can increase the contents of full-value protein and fat in standard and functional food commodities to enhance their biological value.

The use of chopped meat-based semifinished foodstuffs (SFFs) in human diets requires the knowledge on the changes in nutritional and biological value which occur in the SFFs during the last stage of cooking, different types of thermal treatment. These types are known to differently affect the composition of the SFFs [21,22]. However, there is a lack of available data on the changes in the biological value of the SFFs after the thermal treatment; this knowledge is necessary for the formulation of diets for different categories and ages of consumers, receipts of special and functional nutrition. There is also a lack of information regarding the effects of thermal treatments on the combined SFFs containing poultry meat and egg-based ingredients.

The aim of the study presented was the investigation of the effects of different types of thermal treatment (cooking) on the parameters of biological value of the combined SFF containing broiler meat and coagulated chicken egg melange (CCEM).

**Materials and methods**

The SFF in this study was the cutlet-like semifinished product contained chopped broiler meat (55%), CCEM (20%), milk powder (12%), wheat bread (9%), dried onion (3%), salt (0.8%), and spices.

The CCEM was preliminary produced via the heating of acidified melange of chicken eggs until the formation of a clot with the texture similar to grainy curd [23]. The yield of CCEM was 82.7% of initial melange; CCEM produced contained 14.3% of protein and 12.1% of fat.

The types of the thermal treatment used for the cooking of the SFF included water boiling (water/SFF ratio 0.5:1.0), steam boiling, traditional techniques of braising, baking, and frying; the amino and fatty acid profiles of cooked SFF were compared to the respective parameters before the treatments.

The amino acid profiles were determined on Knauer analyzer; tryptophan by standard method according to Russian standard GOST 34132. The biological value of the protein within the treated products was assessed using amino acid balance coefficients calculated by the method of N. N. Lipatov [24]. The following criteria were used: amino acid score (AS); coefficient of rationality of amino acid composition (Rc); "comparable redundance" of essential fatty acids (σ), utilitarian coefficient (αj).

Fatty acid profiles were determined via capillary gas-liquid chromatography. The total amounts of SFAs, MUFAs, and PUFAs were determined; their ratios and ω-6/ω-3 PUFAs ratios were calculated.

The percentages of moisture, protein, and fat in the products were determined according to GOST 31470.

The temperature of the thermal treatments was controlled by the thermometer with the scale from –50 to +300 °C and maximum relative measurement error ±0.5 °C.

**Results and discussion**

The preliminary comparative investigation of the amino and fatty acid profiles in the intact SFF with CCEM in compare to the SFF without CCEM supplementation has revealed the significant increases in the contents of essential amino acids: methionine by 35%, valine and isoleucine by 22–23%, threonine, leucine, phenylalanine, and lysine by 14–15% while the content of tryptophan in the CCEM-supplemented SFF has been slightly lower; the ratio of essential ω-6/ω-3 PUFAs has been better in the CCEM-supplemented SFF. The conclusion has been made that the balance of the amino and fatty acids has been better in the supplemented product. The σ criterion has been lower in the CCEM-supplemented SFF while the Rc criterion has been higher; these findings have evidenced the better biological value of this product.

The comparative investigation of the amino and fatty acid profiles in the CCEM-supplemented SFF after different types of thermal treatment (cooking) in compare to the intact one revealed that all treatments affected the contents of moisture, fat, and protein (Table 1).

**Table 1. The changes in basic nutritive parameters in the SFF depending on cooking (thermal treatment) type**

| Treatment type | Content, % | | |
|---|---|---|---|
| | Moisture | Fat | Protein |
| Raw (untreated) | 70.9 | 6.7 | 14.3 |
| Water boiled | 70.9 | 6.3 | 14.5 |
| Steam boiled | 69.4 | 6.5 | 15.3 |
| Braised | 69.6 | 6.4 | 15.3 |
| Baked | 62.7 | 10.0 | 18.1 |
| Fried | 65.2 | 10.3 | 16.1 |

The moisture content underwent little changes during the thermal treatments. Moisture loss after braising, steam boiling, and frying was 1.3; 1.5 and 5.7%, respectively; the highest moisture loss was found for baking (8.2%).

The thermal treatments resulted in the increases in protein content within the cooked SFF: baking by 3.8%, frying by 1.8%, braising and stream boiling by 1.0%, water boiling by 0.2%.

The changes in fat content in cooked SFF were different: braising, water and stream boiling resulted in the slight decreases in fat content while baking and frying increased this parameter by 3.0 and 3.3%, respectively.

The study of amino acid profiles of the proteins of intact and cooked SFF revealed the trend to higher contents of essential amino acids after all types of cooking (Figure 1).

The limiting amino acid in raw SFF was lysine with AS criterion 0.68 units. Different types of thermal treatment resulted in different changes in the amino acid profiles of protein within the SFF. The least affected amino acid profiles (in compare to the raw SFF) were found for water and steam boiling. Braising and baking resulted in higher contents of essential amino acids; AS for essential amino acids in these cases were close to the reference levels (close to 1.0) with the exception of tryptophan (AS1.82). No limiting amino acids were found in baked SFF though AS for certain essential amino acids (leucine, lysine, threonine, tryptophan, phenylalanine, and tyrosine) were substantially higher than 1.0 (Table 2). The utilitarian coefficients αj (the content of essential amino acids in relation to the respective physiologic requirements of consumer) in the cooked SFF were close to the intact one.

The calculated criteria of biological value (coefficient of rationality of amino acid composition Rc and "comparable

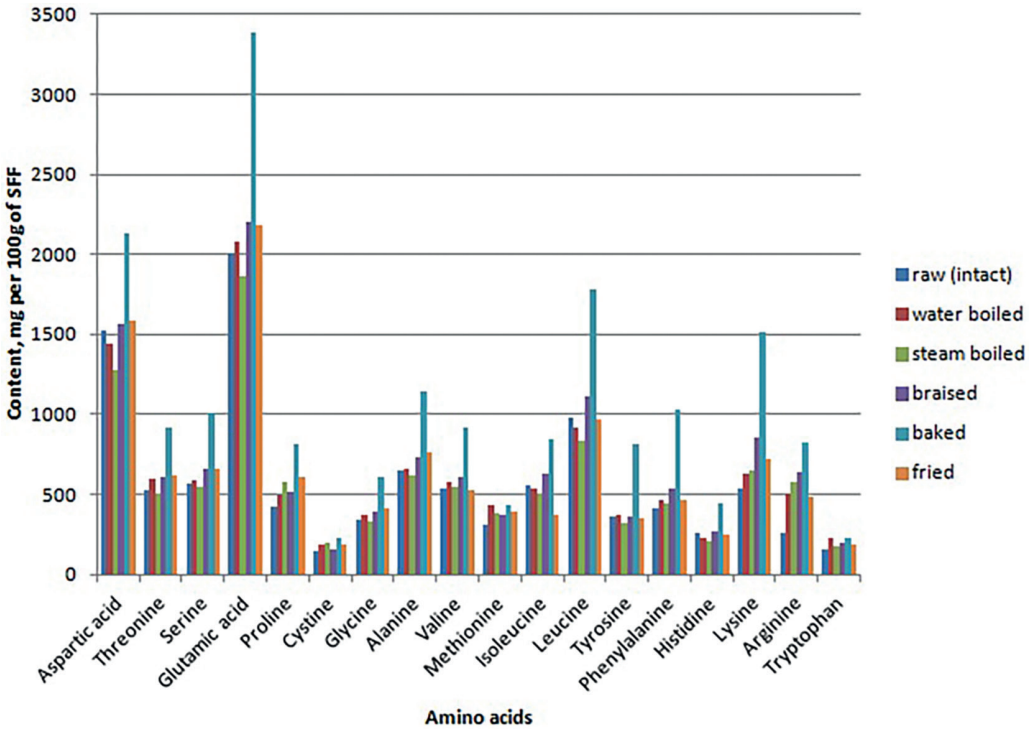


**Figure 1.** Amino acid profiles of the SFF depending on the type of the thermal treatment

**Table 2. The essential amino acid scores (AS) and utilitarian coefficients (αj) in raw and cooked SFF**

| Amino acids | Raw | | Water boiled | | Steam boiled | | Braised | | Baked | | Fried | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AS | αj | AS | αj | AS | αj | AS | αj | AS | αj | AS | αj |
| Valine | 0.76 | 0.89 | 0.79 | 1.00 | 0.72 | 1.00 | 0.80 | 1.00 | 1.02 | 1.00 | 0.65 | 0.89 |
| Isoleucine | 0.98 | 0.69 | 0.92 | 0.86 | 0.83 | 0.87 | 1.03 | 0.78 | 1.17 | 0.87 | 0.58 | 1.00 |
| Leucine | 0.98 | 0.69 | 0.91 | 0.87 | 0.78 | 0.92 | 1.04 | 0.77 | 1.41 | 0.72 | 0.86 | 0.67 |
| Lysine | 0.68 | 1.00 | 0.79 | 1.00 | 0.78 | 0.92 | 1.02 | 0.78 | 1.52 | 0.67 | 0.82 | 0.71 |
| Methionine+cystine | 0.91 | 0.75 | 1.23 | 0.64 | 1.08 | 0.67 | 0.98 | 0.82 | 1.03 | 0.99 | 1.42 | 0.41 |
| Threonine | 0.92 | 0.74 | 1.03 | 0.77 | 0.83 | 0.84 | 1.00 | 0.80 | 1.26 | 0.81 | 0.96 | 0.60 |
| Tryptophan | 1.11 | 0.61 | 1.54 | 0.51 | 1.19 | 0.61 | 1.82 | 0.44 | 1.29 | 0.79 | 1.15 | 0.50 |
| Phenylalnine+tyrosine | 0.90 | 0.76 | 0.96 | 0.82 | 0.83 | 0.87 | 0.98 | 0.82 | 1.70 | 0.60 | 0.85 | 0.68 |

redundance" of essential fatty acids σ) showed that water and steam boiling resulted in the best parameters of biological value of protein within the FFS (Table 3).

**Table 3. The criteria of biological value of raw and cooked SFF**

| Treatment type | Coefficient of rationality of amino acid composition Rc | "Comparable redundance" of essential fatty acids σ | Biological value |
|---|---|---|---|
| Raw (untreated) | 0.77 | 10.61 | 77.5 |
| Water boiled | 0.83 | 7.12 | 76.9 |
| Steam boiled | 0.86 | 5.57 | 84.0 |
| Braised | 0.80 | 9.04 | 71.6 |
| Baked | 0.76 | 11.17 | 70.7 |
| Fried | 0.67 | 17.79 | 66.9 |

The study of the individual fatty acid profiles in the cooked CCEM-supplemented SFF (Table 4) and total contents of SFAs, MUFAs, and PUFAs (Table 5) revealed that water boiling, steam boiling, braising, and baking slightly increase the total SFA content (by 1.37; 1.89; 4.32 and 2.29%, respectively); the most substantial changes were found after braising. The total contents of MUFAs with these treatment types were similar to the level of the raw product.

**Table 4. Fatty acid (FA) profiles in raw and cooked SFF**

| FA | FA type | Content, % of total FA | | | | | |
|---|---|---|---|---|---|---|---|
| | | Raw | Water boiled | Steam boiled | Braised | Baked | Fried |
| Butyric | C4:0 | 0.13 | 0.10 | 0.09 | 0.13 | 0.09 | 0.12 |
| Capronic | C6:0 | 0.09 | 0.07 | 0.07 | 0.08 | 0.07 | 0.08 |
| Caprylic | C8:0 | 0.06 | 0.05 | 0.05 | 0.06 | 0.05 | 0.06 |
| Caprinic | C10:0 | 0.14 | 0.13 | 0.14 | 0.15 | 0.14 | 0.12 |
| Lauric | C12:0 | 0.17 | 0.18 | 0.18 | 0.18 | 0.17 | 0.14 |
| Tridecanoic | C13:0 | 0.03 | 0.03 | 0.03 | 0.13 | 0.03 | 0.05 |
| Myristinic | C14:0 | 0.89 | 0.93 | 0.97 | 1.02 | 0.94 | 0.75 |
| Myristoleic | C14:1 | 0.14 | 0.14 | 0.13 | 0.07 | 0.14 | 0.10 |
| Pentadecanoic | C15:0 | 0.17 | 0.17 | 0.20 | 0.47 | 0.18 | 0.22 |
| Palmitic | C16:0 | 22.43 | 23.53 | 24.20 | 25.49 | 24.12 | 19.42 |
| Palmitoleic | C16:1 | 3.93 | 3.93 | 3.94 | 3.77 | 3.85 | 3.11 |
| Margaric | C17:0 | 0.14 | 0.14 | 0.15 | — | 0.15 | 0.11 |
| Margaroleic | C17:1 | 0.05 | 0.05 | 0.06 | — | 0.07 | 0.05 |
| Stearic | C18:0 | 6.50 | 6.79 | 6.96 | 7.67 | 7.11 | 6.07 |
| Oleic | C18:1 | 35.33 | 36.31 | 36.71 | 36.31 | 35.52 | 32.95 |
| Elaidinic | C18:1(t9) | 1.62 | 1.64 | 1.66 | 1.57 | 1.63 | 1.39 |
| Linolic | C18:2(n6) | 25.76 | 24.24 | 23.06 | 22.28 | 23.32 | 33.72 |
| γ-linoleic | C18:3(n6) | 0.18 | 0.13 | 0.13 | — | 0.12 | 0.15 |
| α-linoleic | C18:3(n3) | 0.42 | 0.35 | 0.32 | 0.28 | 0.33 | 0.33 |
| Arachidonic | C20:0 | 0.08 | 0.08 | 0.09 | — | 0.09 | 0.11 |
| Gondoinic | C20:1 | 0.34 | 0.38 | 0.38 | — | 0.38 | 0.35 |
| Eicosatrienoic | C20:3 | 0.43 | 0.41 | 0.31 | 0.34 | 0.33 | 0.30 |
| Geneicosaenoic | C21:0 | 0.22 | 0.23 | 0.20 | — | 0.20 | 0.14 |
| Begenic | C22:0 | — | — | — | — | — | 0.18 |
| Docosenoic | C22:1 | 0.72 | — | — | — | — | — |

**Table 5. The contents of total SFAs, MUFAs, and PUFAs in raw and cooked SFF, % of total FA**

| Total | Cooking type | | | | | |
|---|---|---|---|---|---|---|
| | Raw | Water boiled | Steam boiled | Braised | Baked | Fried |
| SFAs | 31.05 | 32.42 | 33.34 | 35.37 | 33.34 | 27.56 |
| MUFAs | 42.16 | 42.45 | 42.83 | 41.72 | 41.59 | 37.94 |
| PUFAs | 26.79 | 25.13 | 23.83 | 22.90 | 24.10 | 34.49 |

The slight changes in the fatty acid profiles in cooked SFF were primarily related to the decreases in the PUFAs contents; in water-boiled, steam-boiled, braised and baked SFF this decrease was 2.64; 2.96; 3.88 and 2.68%, respectively. The ω-6/ω-3 PUFAs ratios were affected more substantially especially in the case of braising; the least change in this ratio was found in water-boiled SFF. Generally, all types of cooking (thermal treatments) had little influence on the fatty acid balance.

## Conclusion

The amino and fatty acid profiles in the SFF supplemented with coagulated chicken egg melange after different types of cooking (thermal treatments) were studied.

Different types of cooking differently affected the contents of protein, fat, amino and fatty acids within the product.

The least changes in the parameters of biological and nutritive value of the SFF was found with water- and steam-boiling. Braising and baking were found to increase the contents of essential amino acids; the resulting contents of the latter were close to the respective reference levels (AS close to 1.0) with the exception of tryptophan (AS1.82). No limiting amino acids were found in baked SFF though AS for certain essential amino acids (leucine, lysine, threonine, tryptophan, phenylalanine, and tyrosine) were substantially higher than 1.0 (i. e. higher in compare to the reference levels).

The highest biological value (as revealed by the coefficient of rationality of amino acid composition Rc and "comparable redundance" of essential fatty acids σ) had steam-boiled SFF; overall index of biological value for this treatment was the highest (84.0) in compare to the raw SFF (77.5).

The effects of different types of thermal treatment on the fatty acid profiles of SSF were insignificant. The slight changes in the fatty acid profiles in cooked SFF were primarily related to the increases in the SFAs and decreases in the PUFAs contents; in water-boiled, steam-boiled, braised and baked SFF this decrease was 2.64; 2.96; 3.88 and 2.68%, respectively. The ω-6/ω-3 PUFAs ratios were affected more substantially especially in the case of braising.

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The authors declare no conflict of interest.

**Received 25.06.2020 Accepted in revised 15.09.2020 Accepted for publication 28.09.2020**

DOI: https://doi.org/10.21323/2414–438X–2020–5–3–28–31

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*Original scientific paper*

# DEVELOPMENT OF HIGH SENSITIVE REAL-TIME PCR TO DETECT MUSTARD AND OTHER ALLERGENS OF THE FAMILY *BRASSICACEAE* IN FOOD SAMPLES

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**Keywords:** *real-time PCR, Brassica juncea, Brassica nigra, Sinapis alba, Brassicaceae, allergens, food products*

***Abstract***
*Mustard is a commonly used condiment including in production of other food products. As mustard is an allergen, it is necessary to control its presence. The development of PCR test-systems for its detection is complicated by the fact that this condiment can be made from seeds of various plant species (Brassica juncea, Brassica nigra, Sinapis alba) of the family Brassicaceae that are not closely related. This family includes other plant species such as white cabbage (Brassica oleracea) and rapeseed (Brassica napus), which can cause the allergic reaction, although seldom. In this connection, many authors use primers specific to many species of this family, including to allergens, to detect mustard. In this work, we used the similar strategy. To increase sensitivity, primers for the mitochondrial COX gene were selected. To increase PCR stability in analysis of deeply processed products, primers were selected for a region with a length of 61 base pair. In the work, the specificity and sensitivity of the developed PCR method was confirmed. Analyses of different products, including those that underwent deep technological processing, were carried out with these primers. Also, primers were selected to detect white mustard (S. alba). When analyzing products on the presence of white mustard, characteristic regional preferences were demonstrated: this species is used in manufacturing products mainly in the UK and USA.*

## Introduction

Mustard is one of the most commonly used condiments. It is used in many food types, such as spice mixtures, condiments, sauces, marinades, including for meat baking, finished meat products and gastronomy products. Mustard can be prepared from plant seeds of different species: brown mustard (*Brassica juncea*), black mustard (*Brassica nigra*) and white mustard (*Sinapis alba*). Irrespective of a species, which seeds were used for production, mustard is an allergen. Information about its content should be indicated on a label according to TR CU022/2011 [1] and Regulation (EU) No 1169/2011 of the European Parliament and of the Council [2]. More than half of patients with an allergy to mustard also have an increased sensitivity to several other plant-derived foods and pollen [3]. It is necessary to note that the family *Brassicaceae* also includes such allergens as rapeseed, cabbage and broccoli.

The main mustard allergens are 2S albumins of white and brown mustard seeds (Sin a 1 and Bra j 1, respectively) [4,5]. These two albumins have the similar structure and immunological properties. Therefore, when studying mustard as an allergen in a product composition, the species origin of raw materials is of no importance. For *S. alba*, several other allergens were revealed: 11S globulin of seeds (Sin a 2) [6], non-specific lipid transfer protein (Sin a 3) and profilin (Sin a 4) [7]. It is not unlikely that new allergens will be established for *B. juncea*. An allergy to pollen of white cabbage (*Brassica oleracea*) is rare [8]. With that, about half of patients showed cross-sensitivity to mustard. In addition, the search for potential allergens of *B. nigra* has been performed [9].

At present, to control the mustard presence in foods, several methods based on enzyme-linked immunosorbent assay (ELISA) [10,11] and polymerase chain reaction (PCR) are used. With that, ELISA methods showed false positive reactions when analyzing rapeseed [10] or egg protein and soy [11]. When developing PCR methods, the authors propose primer pairs for detection of individual species *B. juncea* and *S. alba*, as well as multiplex test-systems to detect these species together with other allergens. For example, Palle-Reisch *et al.* selected a primer pair for detection of *B. juncea* and *B. nigra* [12]. However, the reaction efficiency was different (100.6% and 85.3%, respectively) when studying DNA of each of these species. In this connection, the limit of detection for *B. nigra* was lower (0.005% DNA in a sample). In another work, Palle-Reisch *et al.* proposed a primer-probe system for the duplex real-time PCR assay for detection of all three mustard species [13]. Sensitivity of this system was 0.0005%. The use of multiplex test-systems is in high-demand for detection of allergens. For example, Köppel et al. proposed hexaplex real-time PCR to detect allergens including mustard [14]. In this work, primers proposed by Mustorp *et al.* were used, which were specific not only to possible mustard species, but also to rapeseed (*Brassica napus*), cabbage (*B. oleracea*), radish (*Raphanus sativus*). The detection limit of this reaction was 0.0032%.

The models of sausage meat heated at 75–78 °C for 15 min. were used in [12,13]. Nevertheless, mustard is used in the composition of deeply processed products including canned foods and marinades. This impact leads to a significant DNA degradation. One of the approaches that increase stability

**FOR CITATION:** **Kurbakov K. A., Zhulinkova V. N., Minaev M. Yu. Development of high sensitive real-time PCR to detect mustard and other allergens of the family *Brassicaceae* in food samples. *Theory and practice of meat processing.* 2020; 5(3): 28–31. https://doi.org/10.21323/2414–438X-2020–5–3–28–31**

of a PCR method in analysis of degraded DNA is reducing a length of the obtained amplicon [15].

Taking into account the presence of several allergens in the family *Brassicaceae*, impossibility to select a DNA region specific only to species *B. juncea*, *B. nigra* and *S. alba*, and the experience of several researchers, we proposed a primer pair specific to several species of this family. To increase PCR reliability in analysis of non-degraded DNA, primers were selected for a region with a length of 61 base pair.

It is considered that mainly species of the genus *Brassica* are used in Europe and Asia for mustard production, while *Sinapis alba* is used in the USA and Canada. In this work, we also assessed the share of mustard produced using seeds of *Sinapis alba* on the Russian market.

## Materials and methods

*Objects of investigation*

Seeds of white mustard, brown mustard and white cabbage were taken as positive controls. Soy, corn and wheat were used as the negative control.

Samples of mustard available on the Russian market at retail and produced in Russia (3), the Netherlands (1), Germany (1), Poland (1), France (1), USA (3) and UK (1) were studied. The following samples of products containing mustard seeds were taken: marinated vegetables (3), sauce (3), baking chicken marinade (1) and mustard oil (1). Mustard seeds and brine were taken from samples of marinated vegetables.

*Sampling and DNA extraction*

Seeds were ground in a homogenizer LB20E (Waring Commercial, Torrington, Connecticut, USA). When necessary, food products were minced in a homogenizer GRINDOMIX GM 200 (Retsch, Haan, Germany). Mustard oil (5 ml) was settled for one hour in a centrifuge SIGMA 3–18K (Sigma Zentrifugen, Osterode am Harz, Germany) with acceleration of 29700g at a temperature of 10 °C. After that, the supernatant was removed and sediment was mixed in the 20% Tween 80 solution. For DNA extraction, 100 mg of food products and control samples were taken. Liquid samples were taken with a volume of 200 µl. DNA extraction was carried out using Sorb-GMO-B kit (Syntol, Moscow, Russia) according to the instruction.

*Primer design*

The primer pairs Brass_fam and Sin_alb were complimentary to the mitochondrial COX gene and retrotransposon Sal-T1 (Table 1), respectively. The choice of the mitochondrial DNA region for identification of the family Brassicaceae was conditioned by its multi-copy nature, which enhanced sensitivity of the method. The DNA regions were available in the GenBank database [16]. For system design, the programs Primer-BLAST [17] and Oligo-Analyzer v. 3. were used [18].

*Conditions for real-time PCR*

Real-time PCR was carried out using an amplifier ANK-32 (Syntol, Moscow, Russia). The reaction mixture (30 µl) contained primers with a concentration of 300 nM, 2.5 mM MgCl2, dNTPs with a concentration of 0.25 mM each, SynTaq polymerase with a concentration of 2.5 activity units and 5 µL of extracted DNA. The components of the reaction mixture were produced by Syntol. The parameter of PCR was as follows: initial denaturation at 95 °C for 7 min and 45 cycles of amplification (60 °C, 40 s and 95 °C, 15 s). All samples were investigated in triplicate. The obtained data were analyzed using the software ANK-32 (Syntol).

Based on the results of PCR with primers Brass_fam, semi-quantitative analysis of the mustard content in the test samples was carried out by the equation:

$$x = E^{(Cq_K - Cq_T)} \quad (1),$$

where

$E$ is the reaction efficiency;

$Cq_K$ is the threshold cycle of the amplification curve of the positive control;

$Cq_T$ is the threshold cycle of the amplification curve of the sample.

Data analysis was carried out using Microsoft Excel 2016 [19].

## Results and discussion

*Detection of efficiency, specificity and cut-off cycles of positive results*

The short length of the PCR amplicon was used for increasing method stability when analyzing samples with degraded DNA.

To detect the reaction parameters and limit of detection, PCR with the selected primer pairs was carried out.

For analysis of primers for the Sin_alb gene, DNA of white mustard seeds and its decimal dilutions of up to 0.0001% were used. For analysis of primers Brass_fam, we used a dilution of DNA of white mustard seeds with a concentration of 100% to 0.0001%.The detection limit of PCR

**Table 1. Sequences and positions of the primers used in the study**

| Primer | Primer sequence (5'–3') | Amplification region | Amplification size, bp |
|---|---|---|---|
| Brass_fam-F | GCCGAGATCAAGGTTCAAACAAA | COX | 61 |
| Brass_fam-R | CTTAAAATGTCCTTCTTCCCCGC | | |
| Sin_alb-F | GTACGTCTCTAATCGGCATGGAT | Retrotransposn Sal-T1 | 107 |
| Sin_alb-R | CTGCTGTTCTCTGTTTCGTGAAG | | |

bp = base pairs

with primers for Brass_fam was 0.001% of the target template in a sample. This value was not lower than in similar test-systems [12,13,14]. The calculated coefficient of correlation of PCR was $R^2 = 0.99$; PCR efficiency was $E = 2.01$. The equation of linear regression is the following:

$$y = -3{,}317\,x + 44{,}97 \quad (2)$$

The calculated coefficient of correlation of PCR with primers Sin_alb was $R^2 = 0.99$; PCR efficiency was $E = 2.03$. The equation of linear regression is the following:

$$y = -3{,}2\,x + 51{,}145 \quad (3)$$

When using Brass_fam and Sin_alb, no non-specific annealing with samples of soy, corn, wheat was found. Non-specific annealing was absent in the reaction between primers Sin_alb and DNA of white cabbage.

*Analysis of mustard condiments*

The PCR result for the sample of the US organic mustard powder was expectedly close to the Cq values of the control. Relative quantitative analysis of condiments showed that in the samples of domestic mustard, its content was in a range of 0.5 to 4.5% in reference to ground seeds. This value was 0.22%, 1.8% and 13.9% in mustard produced by Germany, the Netherlands and Poland, respectively. The highest content of the mustard powder was in the condiment produced by France (29.6%) and the UK (71%). In one US sample, a mustard proportion was 5.9%, in another 0.004%. Such a low value can be explained by replacement of the mustard powder with some other aromatic raw materials. It is necessary to note that product pH influences the ultimate value of the PCR result [20]. As vinegar is used, as a rule, in production of this condiment, the obtained values can be insignificantly lower than real.

*Analysis of other food products*

The authors of previous studies tested their methods on different products: cooked sausage models, sauces, spice mixes, meat spreads, fried noodles, nuggets and so on [13,14]. However, there were no deeply processed products among these objects. In our work, we took samples of brine from canned marinated vegetables and mustard oil besides sauces and marinades.

The PCR method allowed detecting mustard in the sauce compositions: 0.6–0.4% of the content relative to the positive control. The mustard content was 10% in the sample of marinade for baking.

The result of the quantitative analysis of the mustard oil composition was 0.005% relative to the positive control, which is explained by the technology of vegetable oil purification. This result shows that it is impossible to detect mustard oil in the composition of food products using this method. Nevertheless, this method can be used for establishing vegetable oil falsification.

Mustard DNA was revealed in the samples of marinade of canned vegetables at a level of 0.001–0.0015%. With that, it is necessary to note that whole mustard seeds were added. Therefore, it was shown that sensitivity of the developed method allows identifying mustard in the composition of deeply processed products, in particular, canned foods.

*Analysis of the use of S. alba seeds in production of ready condiments and other food products*

Samples with the mustard content of not lower than 0.1% were used for analysis. It was linked with lower sensitivity of PCR with primers Sin_alb. Whole mustard seeds were taken from the brine of canned products. S. alba was qualitatively detected in the products from Poland and the Netherlands as well as USA and UK. In the US and UK mustard samples, mainly white mustard was detected. With that, it should be noted that the condiment sample from the USA was denoted as Braun Mustard. In the European products *S. alba* was detected in lower quantities. It is connected with the fact that these samples belonged to the recipe of "French" mustard, which composition envisages the use of both white mustard and brown or black mustard.

**Conclusion**

In this work, we proposed a primer pairs to detect mustard in food samples by the PCR method. High sensitivity of the method was shown. This, in particular, allowed detecting mustard traces in deeply processed samples such as canned foods. Also, successful PCR with the sample of DNA extracted from mustard oil was carried out. Therefore, this method can be used for detection of vegetable oil falsification.

Ready condiments and products were compared regarding the use of *S. alba* seeds. Despite availability of raw materials, European manufacturers traditionally use brown and black mustard, while in the UK and USA, white mustard has been detected in condiments declared as brown mustard. Nevertheless, there are condiment types, in which seeds of the genera *Brassica* and *Sinapis* are used. In this connection, it is impossible to rely on the geographical origin of a product and use one primer pair specific only to the genus *Brassica* or *Sinapis*, when analyzing food on the mustard presence.

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The authors declare no conflict of interest.

**Received 13.08.2020 Accepted in revised 01.09.2020 Accepted for publication 25.09.2020**