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.4

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.

FOCUS AND SCOPE

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.

Both Russian and foreign scientists and experts are invited for publication in the journal.

The main tasks are generalization of scientific and practical achievements in the fields of meat science, increase scientific and practical qualifications as researchers and industry representatives.

РАЗДЕЛЫ ЖУРНАЛА

- Исследования в области биотехнологии, биохимии, физиологии, ланималогии, нутрициологии
- Исследование и создание новых технологий мясных производств
- Процессы, оборудование и аппараты мясных производств
- Стандартизация, сертификация, системы управления качеством и безопасность
- Микробиология, санитария и гигиена мяса и мясных продуктов
- Экономика
- Автоматизация и информатизация технологических процессов

SECTION POLICIES

- Investigations in the field of biotechnology, biochemistry, physiology, lanimology, nutritiology
- Investigation and development of new technologies of meat production
- Processes, equipment and apparatus of meat production
- Standardization, certification, systems of quality and safety management
- Microbiology, sanitary and hygiene of meat and meat products
- Economics
- Automation and informatization of the technological processes in the meat sect

Министерство науки и высшего образования Российской Федерации
Minister of Science and Higher Education of the Russian Federation

Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
Federal State Budgetary Scientific Institution "V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences"
(Gorbatov Research Center for Food Systems)

Теория и практика переработки мяса
"Teoriâ i praktika pererabotki mâsa"
Theory and practice of meat processing
www.meatjournal.ru

Учредитель:
Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
109316, Россия, Москва, Талалихина, 26

Founder:
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

Издатель:
Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
109316, Россия, Москва, Талалихина, 26

Publisher:
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

Редакция:
Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
109316, Россия, Москва, Талалихина, 26
Тел.: +7–495–676–93–51
e–mail: a.zakharov@fncps.ru

Editorial 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
Tel.: +7–495–676–93–51
e–mail: a.zakharov@fncps.ru

Типография:
Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
109316, Россия, Москва, Талалихина, 26

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

Свободная цена
Периодичность — 4 номера в год
Подписной индекс в каталоге «Пресса России» 38871
Подписано в печать 25.12.2020
Дата выхода из печати 30.12.2020
Тираж 1000 экз. Заказ № 322.

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

Министерство науки и высшего образования Российской Федерации
Minister of Science and Higher Education of the Russian Federation

Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
Federal State Budgetary Scientific Institution "V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences"
(Gorbatov Research Center for Food Systems)

Теория и практика переработки мяса
"Teoriâ i praktika pererabotki mâsa"
Theory and practice of meat processing
www.meatjournal.ru

Учредитель:
Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
109316, Россия, Москва, Талалихина, 26

Founder:
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

Издатель:
Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
109316, Россия, Москва, Талалихина, 26

Publisher:
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

Редакция:
Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
109316, Россия, Москва, Талалихина, 26
Тел.: +7–495–676–93–51
e–mail: a.zakharov@fncps.ru

Editorial 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
Tel.: +7–495–676–93–51
e–mail: a.zakharov@fncps.ru

Типография:
Федеральное государственное бюджетное научное учреждения «Федеральный научный центр пищевых систем им. В. М. Горбатова» РАН
109316, Россия, Москва, Талалихина, 26

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
Published in 2015

Free price
Frequency — 4 issues a year
Subscription index in the catalogue "Press of Russia" 38871
Signed print 25.12.2020
Released from press 30.12.2020
Circulation — 1000 copies. Order № 322.

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

CONTENTS

Andrey N. Ivankin, Natal'ya L. Vostrikova, Olga N. Krasulya,
Ekaterina V. Kazakova, Andrey V. Kulikovskii, Aleksandra S. Knyazeva
THE FORMATION OF FLAVORING CHARACTERISTICS
OF MEAT PRODUCTS BY CHANGING THE CHEMICAL
COMPOSITION OF FOOD COMPOSITIONS 4

Aleksandr Yu. Prosekov
THE METHODOLOGY OF FOOD DESIGN.
PART 1. THE INDIVIDUAL ASPECT 13

Branko Velebit
FOODBORNE VIRUSES — AN EMERGING PATHOGENS 18

Natalia V. Rudometova, Irina S. Kim
RESEARCH OF THE PHYSICAL AND CHEMICAL PROPERTIES
AND METHODS OF RED RICE (FOOD COLOUR) DETERMINATION
IN SAUSAGE PRODUCTS 23

Anton G. Belozerov, Yuriy M. Berezovskiy,
Igor A. Korolev, Taras A. Sarantsev
A CALCULATION MODEL FOR THE HEAT CAPACITY
OF BEEF WITH DIFFERENT MOISTURE DURING FREEZING
TAKING INTO ACCOUNT FREE WATER CRYSTALLIZATION 29

Vladimir Yu. Kornienko
ANALYSIS OF REQUESTS FOR JOURNALS *NATURE FOOD*
AND *NPJ SCIENCE OF FOOD* BY THE DATA OF THE SCI-HUB
SERVICE FOR THE FIRST HALF OF 2020 35

DOI: https://doi.org/10.21323/2414–438X–2020–5–4–4–12

Available online at https://www.meatjournal.ru/jour
Original scientific paper

THE FORMATION OF FLAVORING CHARACTERISTICS OF MEAT PRODUCTS BY CHANGING THE CHEMICAL COMPOSITION OF FOOD COMPOSITIONS

Andrey N. Ivankin[1,2], Natal'ya L. Vostrikova[2],* Olga N. Krasulya[3], Ekaterina V. Kazakova[3], Andrey V. Kulikovskii[2], Aleksandra S. Knyazeva[2]
[1] Bauman Moscow State Technical University (National Research University), Moscow, Russia
[2] V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia
[3] Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, Russia

Keywords: *taste, aroma, meat products, amino-, fatty acid composition, inosine, carnosine, yeast extract, soy hydrolysate*

Abstract
The article presents the results of the study of changes in flavour characteristics when using corrective additives. Monosodium glutamate, ribotide, yeast extract and hydrolysate of vegetable soy protein were used as flavoring additives (FA). To assess the effect of composition of meat product recipe, as well as the method of FA-introduction on taste intensity, the recipes of model meat systems with partial replacement of meat raw materials were used. Pork fat, soy protein and potato starch were used as meat substitutes. The effect of recipe composition on the content of non-volatile substances of aroma was accessed. It is shown that replacement of pork by pork fat in the recipe by 20–40% led to a sharp decrease in the concentration of aromatic substances and a decrease in intensity of taste of the finished product several times. The ways for taste correction using FA was studied. For this, a chopped semi-finished product — minced meat was prepared from chilled whole-muscle pork and 0.05% of each FA was added. It is shown that the dynamics of changes in the content of free amino acids is the most pronounced when using monosodium glutamate not as a mono-additive, but in compositions: monosodium glutamate with yeast extract and monosodium glutamate with ribotide. A pool of chemical compounds involved in the formation of taste and aroma of products was detected. The main components were derivatives of C_6–C_{24} fatty acids, as well as a significant number of other biochemical compounds, mainly substituted amines, amides, alcohols and ketones, with a content ranging from 0.001 to 0.2 mg/kg. The results of organoleptic analysis showed that the most delicious and attractive samples were those containing monosodium glutamate with yeast extract and monosodium glutamate with ribotide.

Introduction

The quality of meat products depends on the composition and properties of raw materials, as well as the conditions of its technological processing. The influence of natural factors, conditions of livestock rearing, transportation, pre-slaughter animal treatment, slaughtering conditions, the state of primary meat processing, parameters of autolysis of the obtained raw materials and their further cold storage is significant [1,2].

The main consumer indicators of raw material quality, including tenderness, pH level, and the degree of development of muscle tissue elements, are largely inherited and can be corrected by various food additives [3].

Feeding diets have a decisive influence on the quality of raw materials of animal origin and, ultimately, on its chemical composition. Feeding diets largely determine the composition and ratios of biochemical substances formed in the flesh of the animal, which later form flavour characteristics [4,5].

The lack of essential components in the feeding diets leads to an increase in water content and causes a decrease in the mass fraction of protein and fat, as well as an increase in the coarseness of fiber structures [6].

As a result of disorders in the type of feeding diets, as well as the presence of stress factors, animal raw materials may have parameters of lower quality with a predominance of a specific smell and taste. Microbiological additives in feed, as well as additives of processed seafood waste, also contribute to the appearance of an undesirable oil or fishy off-flavor [7].

Disorders in the type of feeding diets, increased susceptibility of animals at mass management, stress during the slaughter result in production of raw materials, primarily of lower morphological quality. In this case, it is significant that meat with non-traditional quality characteristics is formed: the so-called PSE (Pale, Soft, Exudative) watery, flabby and exudative meat with a pH < 5.4 and coarse, dark DFD (Dark, Firm, Dry) meat with a pH > 6.2. Recently, red non-standard watery meat RSE (Red, Soft, Exudative) is also distinguished. Meat with abnormal characteristics has technological properties that are not typical of NOR (normal) raw materials and, most important, different taste, aroma, consistency, i. e. other organoleptic properties. The amount of PSE, DFD, and RSE can be from a quarter to half of all processed volumes. This significantly complicates the production of "delicious" meat products

FOR CITATION: **Ivankin A. N., Vostrikova N. L., Krasulya O. N., Kazakova E. V., Kulikovskii A. V., Knyazeva A. S. The formation of flavoring characteristics of meat products by changing the chemical composition of food compositions. *Theory and practice of meat processing.* 2020; 5(4): 4–12. https://doi.org/10.21323/2414–438X–2020–5–4–4–12**

and should be taken into account when making food systems recipes [8,9,10].

Formation of PSE, RSE, and DFD properties in raw meat correlates with the release of adrenaline in muscle tissue under stressful management of animals, an increased content of which leads to an increased breakdown of ATF to inosine with the simultaneous formation of a large amount of lactic acid in muscle tissue, which can acquire PSE properties. Each factor in this chain is related with the intensive formation of a number of biochemical substances, which are manifested in the flavor traits of the finished product [11].

All of the above highlights the main problems of lifetime formation of acceptable quality of raw materials of animal origin, which, in many ways, determines the final consumer properties of food products. However, the achievements of food chemistry make it possible to correct the flavour range of products at the final stages of production, to smooth or completely eliminate unsatisfactory taste and aroma of finished products [12,13].

The most important economic parameter of the flavour formation problem is the need of imparting the so-called characteristics of "drinkability" and "eatability" to the products being sold. In this case, the task of producers becomes the necessity to form such properties in the product that cause its inordinate buying and corresponding consumption. For example, the industry producing beverages such as Coca-Cola, Pepsi-Cola, 7 Up, Orangeade, and a whole host of others is aimed at production of drinking products that can't slake the thirst. This is achieved by introducing a number of substances of natural and synthetic origin into the food recipes [14].

Similarly, for meat and fish products, the use of monosodium glutamate, inosine derivatives and guanidines allows not only correcting the taste, but also causes increased "eating" of products. Monosodium glutamate is widely used, but inonisate and sodium guanylate separately and in mixture can enhance the taste ten times better than monosodium glutamate [15].

The task of forming flavour characteristics of meat products is largely the art of chefs and industrial technologists.

It is known that raw meat has almost no pronounced taste and aroma, although it may have a slight specific smell and a slightly sweet, light-salty taste. A noticeable specific taste and aroma appears after temperature treatment as a result of formation of the products by the Maillard reaction, which appear when the raw material components interact with amino groups of peptides, amino acids or amines with carboxyl groups of aldehydes, ketones and sugars [16].

So, for example, the content of free glutamic acid in beef, pork and mutton during heat treatment may decrease, respectively, before and after, mg%: from 4.6 to 2.2; from 2.0 to 1.2; from 6.1 to 2.8. Similarly, the amount of carnosine dipeptide (beta-alanyl-L-histidine), respectively: from 90 to 38; from 68 to 58; from 25 to 15 [12,17].

The role of glutamic acid in the flavour spectrum is very significant. It and its sodium salt, even in a small amount, about 0.03%, impart the product a meat taste. The presence of free amino acids such as valine, methionine and glycine also contributes to the unique aroma of meat products. The content of inosinic, cytidylic, uridine and guanylic acids in beef and pork in the process of heat treatment is changed almost in the same manner. Their decrease may be 25–40% of the initial value [15,18].

Substances that are flavor precursors are of great importance. Substances that determine the taste and aroma of meat have a molecular weight of less than 200 Da and are extractive. Native meat extracts contain natural amino acids and short peptides, as well as glucose, glucosamine, fructose and ribose. After heat treatment, a greater amount of lactic acid appears, amines, inosine monophosphate, inosines, carbonyl and sulfur-containing compounds are formed, as well as significant amounts of free and derived amino acids. An important role here is played by the flavor precursors contained in raw meat. These include: carnitine, lysine, carnosine, methionine, creatinine, methylhistidine, cysteine, isoleucine, cystine, leucine, glucose-6-phosphate, glutamic acid, glutamine, nicotinamide adenine dinucleotide (NAD), glutathione, ornithine, glycerophosphoethanolamine, glycine, phenylalanine, glycoproteins, phosphoethanolamine, histidine, phosphoserine, hydroxyproline, fructose, hypoxanthine, fructose-6-phosphate, inosine-5-monophosphate, proline, nucleotides, purine nucleotides, purine nucleosides, ribose, ribose-5-phosphate, serine, methylhistidine, taurine, tyrosine, threonine, isoleucine. Each of these substances has a certain influence on the variety of flavour characteristics [19].

According to information sources, the aromatic composition of meat can contain up to 0.5% of ketones and esters, 1.5% of hydrocarbons, 1.5–2% of sulfur-containing compounds, 4–5% of alcohols, 12–15% of aldehydes, 28–30% of furans and up to 50% of pyrazines. Pyrazines were detected in heat-treated meat, for example, 2,3-diethyl-5-methylpyrazine, 2-methylpyrazine, 2,3-diethyl-2-methylpyrazine, 2,5-dimethylpyrazine, 2,3,5-triethylpyrazine, 2-acetyl-3-methylpyrazine, 1-pyrazinyl-2-propanone, etc., in total a few tens of substituted pyranosides [20,21].

Pyrazines have a sensory effect in very low concentrations and play a significant role in creating the aroma of fried food. Methoxyalkylpirazines convey the aroma of greenery. The presence of a substituent in the third position of the ring causes the aroma of green beans, substitution of hydrogen atoms in the ring causes the characteristic aroma of green bell pepper. Threshold concentrations of sensory properties of these compounds are, ppb: 2-ethylpyrazine (nutty, burnt) — 400.0; 2-ethyl-3,6-dimethylpyrazine (aroma of fried, spicy boiled potato) — 0.002; 2-ethyl-6-vinylpyrazine (buttered, baked) — 0.002; dimethylhexahydroxydicyclopyrazine (fried beans) — 0.002; 2-methylamino-3-methylpyrazine (roasted, greenery) — 0.002; 2-methyl-5-thiomethylpyrazine (meat,

vegetables) — 0.002; 2-methoxy-3-isobutylpyrazine (bell pepper) — 0.002; 2-methoxy-3-hexylpyrazine (bell pepper) — 0.001; 2-methoxy-3-isopropylthiopyrazine (pepper, raw potatoes) — 0.002; 2-methoxy-3-methylpyrazine (roasted nuts) — 4.0; 2-isobutyl-3-methoxy-6-methylpyrazine (mint-camphor) — 2.6; 2.5-dimethylpyrazine (aroma of boiled potatoes) –1800.0 [22].

The mechanisms of forming taste and aroma of meat are determined by a complex of organic components, which can be contained in small amounts, up to 0.001%. However, a fundamental role in the formation of the aromatic "bouquet" of meat is played by a small group of key substances that form the four main tastes — salty, sweet, sour and bitter. In meat, the sour taste is formed mainly by lactic, phosphoric and pyruvic acids; salty — by salts of the same acids and chlorides, bitter — by nitrogen-containing carboxylic acid creatine, some free amino acids, such as L-tryptophan and L-isoleucine, and nitrogenous extractives. The sweet taste is determined by glucose, ribose, trioses, and some amino acids, such as a mixture of D — and L-tryptophan, as well as L-glycine, L-alanine, L-serine, L-proline, D-valine, D-leucine, D-threonine, D-methionine, and D-histidine [23,24].

Recently, some Asian countries (Japan, China) have distinguished the fifth taste, the so-called Umami, which means meat, spicy and delicious taste with a long aftertaste. Umami taste receptors are proteins with a high content of glutamine and glutamic acid, as well as glutamates. This aroma and taste become noticeable after 2–4 days after slaughter at low positive temperatures and becomes well-pronounced on 5th day and the highest intensity is reached after 10–14 days.

The described above approaches to the formation of flavour characteristics of food products need to be understood from the point of view of safety and usefulness for humans. This is especially true for the most biologically useful, but also the most expensive food products based on animal origin raw materials.

In this regard, the purpose of the work was to determine a set of possible ways to correct taste and aroma of meat and meat products for improving their flavor characteristics.

Objects and methods

The NOR semifat pork, part of the carcass — carbonade according to GOST 31476–2012, chilled, with a temperature in the muscle thickness of 0–4°C was used in the study as the object of research. Chemical composition of raw material — semifat pork, chilled: moisture — 67.5±6.7%, fat — 9.9±1.5%, protein by Kjeldahl — 20.25± 2.90%, ash — 1.01± 0.2%, carbohydrates — 1.34%, pH — 6.2. Background content of free monosodium glutamate, inosine and carnosine,% wt, is respectively 0.005±0.001, 0.0015±0.0002 and 0.18 0±03. Amino acid composition of protein, g/100 g of raw material: Asp 1.32 ±0.20; Glu 1.55±0.23; Ser 0.71±0.11; His 0.65±0.10; Gly 1.50 ±0.23; Thre 0.66±0.10; Arg 1.00± 0.15; Ala1.12±0.17; Tyr 1.58±0.24; Cys 0.11±0.02; Val 0.75±0.11; Meth 0.32±0.05; Phen 0.66±0.10; Ile 1.58± 0.24; Leu 2.24±0.34; Lys 1.25± 0.34; Pro 0.98± 0.15 (total 20.08±2.85).

The following food additives were used as FA:

— monosodium glutamate produced by "Ajinomoto do Brasil Industriae Comercio de Alimentos Ltda" (Brazil) with formula $C_5H_8NO_4Na$ and basic substance content > 95%, an average particle size of 150 μm, pH of 1% solution — 7.0;
— ribotide Ajitide, Ribotide (I+G) produced by Ajinomoto (Japan), which is a 1:1 mixture of sodium inosinate (E627) and sodium guanylate (E631), containing > 98% of basic substance, pH of 1% solution — 7.8;
— yeast extract containing,%: dry matter > 95; ash — 9.5; nitrogen of free amino groups — 5.5; total nitrogen — 10.7; potassium — 5.7; calcium — 0.1; magnesium — 0.12; sodium — 0.3; amino acids: alanine — 8.7; histidine — 2, proline — 4, arginine — 5, isoleucine — 5.6; serine — 4.7; aspartic acid — 9.7; leucine — 7.6; threonine — 4.4; cystine — 0.8; lysine — 8, tryptophan — 1.2: glutamic acid — 16.1; methionine — 1.3; tyrosine — 2.3; glycine — 4.9; phenylalanine — 3.8; valine — 5.8; pH of 2% solution — 6.8.
— hydrolysate of vegetable soy protein "HVP 2M-P1" produced by Vitana (Czech Republic), containing > 95.0% of dry matter, 26% of protein, 2.0% of fat, 7% of natural glutamate, pH of 10% solution — 5.6.

Determination of the mass fraction of moisture, fat, protein, ash, carbohydrates, and pH was performed using standard methods [25].

The content of amino -, fatty acids, free monosodium glutamate, inosine and carnosine was carried out by chromatographic method [26].

Model systems were prepared according to the following scheme. At the first stage, semifat pork pre-minced in a meat grinder was added to the stirrer, and the ingredients listed above were added one by one. The finished minced meat was shaped in the form of cutlets. Heat treatment was carried out without adding oil in the convection steamer until the temperature 72–75°C inside was reached. 4 hours after the heat treatment, 100 g of the sample of each model system was minced using a blender. Non-volatile substances (NVS) were isolated by extraction 1:4 with hexane, and the composition was analyzed by the method of HPLC in the conditions: the volume of introduced sample 20 μl, gradient of eluent at the start 98% of *A* and 2% of *B* (*A* – 0.1% aqueous solution of formic acid, *B* — acetonitrile), 10 min — 80% of A and 20% of B, 20–30 min 10% of A and 90% of B, flow rate 1 ml/min, pressure 1500 psi, UV detector 355 nm. Identification was performed in automatic mode using mathematical statistics methods, estimating the sum of one hundred most significant peaks on the HPLC chromatogram.

The chemical composition of aroma components was analyzed by gas chromatography on a 7890A chromato-

Table 1. Model systems

№	Raw materials and materials	Recipe of the control sample, %	Model system, %					
			1	2	3	4	5	6
1	Pork, semifat whole muscle	90.0	70	45	70	60	80	60
2	Pork fat	–	20	45	–	–	–	–
3	Hydrated (1:3) soy protein	–	–	–	20	30	–	–
4	Hydrated (1:1) starch	–	–	–	–	–	10	30
5	Onion	10	10	10	10	10	10	10
6	TOTAL:	100	100	100	100	100	100	100
7	Water	5.0	5.0	5.0	5.0	5.0	5.0	5.0
8	Common salt	1.8	1.8	1.8	1.8	1.8	1.8	1.8
9	Monosodium glutamate	0.05	0.05	0.05	0.05	0.05	0.05	0.05
10	TOTAL:	132.4	127.4	117.4	126.4	120.4	126.4	120.4

graph with a mass-selective detector 5975C VLMSD Agilent Technologies (USA) [27].

Results and discussion

Recipes of model meat systems with partial replacement of meat raw materials and method of FA introduction was developed to assess the effect of recipe composition of meat products and method of FA introduction on the flavor intensity. Pork fat, soy protein, and potato starch were used as substitutes for the part of meat (Table 1).

The effect of the recipe composition on the content of non-volatile components of NVS aroma was accessed. As it can be seen from Table 2, replacement of pork with pork fat in the recipe by 20% and 40% (model systems 1 and 2) led to a sharp decrease in the concentration of NVS, i. e., to a decrease in the taste intensity of the finished product 6.25 and 14.2 times, respectively. At the same time, replacing pork with soy protein or starch only slightly reduced the taste intensity of the product: when replacing pork with 20 and 30% of soy protein (model systems 3 and 4), the taste intensity of the product decreased by 5% and 9%, respectively. A similar replacement with starch (model systems 5 and 6) resulted in a decrease in the taste intensity of the product, respectively, by 3 and 5%.

The obtained results can be explained by the fact that with a significant increase in the fat content in the recipe, there is a deficiency of protein for binding fat drops and, as a result, there is an increase in the proportion of fat drops without protein shells. These drops actively absorb fat-soluble NVS, and that leads to a noticeable decrease in the taste of the finished product. In addition, protein deficiency can lead to reduced stability of meat emulsions and formation of broth-fat pockets.

It is also necessary to note the deterioration of the quality of pork fat sold on the Russian market. So, its fatty-acid composition included (%): C4:0–0.02; C6:0–0.11; C8:0–0.1; C10:0–0.17; C12:0–0.43; C14:0–0.61; C15:0–0.08; C16:0–19.8; C17:0–1.4; C18:0–17.4; C19:0–1.3; C20:0–0.1; C22:0–1.47; C14:1–0.12; C15:1–0,1; C16:1–4,6; C17:1–0.5; C18:1n9c — 27.3; C20:1–0.6; C22:1n9–0.35; C18:2n6c — 4.1; C18:3n6–0.48; C18:3n3–0.29; C20:2–0.35; C20:3n6–0.62; C20:4n6–1.35; C22:2–0.48; C20:5n3 — 0.22; C22:5n3–0.11; C22:6n3–0.02.

Its fatty acid profile is mainly represented by unsaturated fatty acids, which are quickly oxidized during storage, and that, in turn, negatively affects the meat taste. Due to the ongoing reduction in the fattening period of pigs from 12 to 5 months, i. e. 2.5 times, changes occur in the fatty acid profile, in particular, during this period there is no sufficient accumulation of saturated fatty acids, which are not capable of rapid oxidation.

Table 2. Change in the total content of non-volatile substances

№	Variant	Content of NVS, %
1	Control sample (pork semifat)	100
2	Model system:	
3	1 (replacement — 20% of pork fat)	26
4	2 (replacement — 45% of pork fat)	18
5	3 (replacement — 20% of soy protein)	89
6	4 (replacement — 30% of soy protein)	85
7	5 (replacement — 10% of starch)	75
8	6 (replacement — 30% of starch)	45

From the obtained data, it can be concluded that it is expedient to replace high-quality meat raw materials with non-fat-containing ones in the recipes of meat products, and use hydrated soy protein or starch. In this case, there is a less significant decrease in the flavour characteristics of the finished product.

To study the effect of the method of introduction of food additives that affect the taste of finished products, the following experiment was conducted. In order to simulate standard conditions of introduction, monosodium glutamate was added to the meat system at the first stage of mixing in the model meat system (variant 1, Table 3). For comparison, sodium glutamate was added to the model system (variant 2, Table 3) at the second stage of the minced meat mixing.

Table 3. The effect of monosodium glutamate on the taste of model systems

Variant	Operation	Content of NVS, %
1	Introduction of monosodium glutamate at the beginning of the mixing process	92
2	Introduction of monosodium glutamate at the end of the mixing process	109

As the results show, the introduction of monosodium glutamate at the second stage was more effective, since the amount of substances responsible for the taste of meat and meat products increases by more than 18%.

Ways of correction of taste of meat and meat products in order to improve them were studied. To do this, a chopped semi-finished product — minced meat was made from chilled whole-muscle pork. From the total mass of minced meat, samples of minced meat weighing 100 g each were selected and flavouring additives were added one by one according their compositions:

— sample № 1 (control);
— sample № 2 sodium glutamate (dosage of 0.05%);
— sample № 3 sodium glutamate (dosage of 0.05%) + yeast extract (dosage 0.05%);
— ample № 4 sodium glutamate (dosage of 0.05%) + ribotide (dosage of 0.05%);
— sample № 5 sodium glutamate (dosage of 0.05%) + vegetable protein hydrolysate (dosage 0.05%).

The results of the analysis of the chemical composition of the samples with the added FA are presented in Table 4.

The results presented in Table 4 indicate the identity of the chemical composition of the studied samples. The indicator "carbohydrate content" draws attention. In samples № 3 and № 4, their amount increased almost 3 and 4.3 times, respectively, compared to the control sample. This fact can be explained by the presence of increased carbohydrate content in sample № 3 (yeast extract) and sample № 4 (ribotide).

From the above data, it can be seen that the addition of sodium glutamate leads to its adequate increase almost 4 times in the minced meat, while the content of inosine and carnosine remains almost unchanged compared to the original sample.

Addition of the composition consisting of monosodium glutamate and yeast extract (sample № 3) contributes to the increase in the content of aroma precursors — inosine and carnosine, respectively, 2.2 and 1.7 times, which positively affects the taste of meat products. Besides, it is known that there is a synergistic effect between monosodium glutamate and the nucleotides contained in the yeast extract.

In sample № 4, containing a composition of monosodium glutamate and ribotide (inosinic acid: guanoic acid = 50:50), a significant increase in all analyzed indicators was noted: monosodium glutamate — 5.8 times; inosine — 4.7 times; carnosine — 1.6 times.

It can be assumed that the above composition will be the most impactful on improving the taste of the finished meat product.

Sample № 5, containing monosodium glutamate and vegetable protein hydrolysate also provides an increase in the level of all the studied indicators: monosodium glutamate 5.6 times; inosine — 1.2 times; carnosine — 1.5 times.

The obtained results confirm the fact of significant influence of the used NVS on the characteristics of the food composition.

The composition of samples containing various types of flavor enhancers after heat treatment in convection steamer was studied. Table 5 shows the characteristics of heat-treated products.

Table 4. Analysis of the chemical composition of samples

№	Sample	Moisture, %	Fat, %	Protein, %	Ash, %	Carbohydrates, %	pH, units	Content, g/100g		
								Monoodium glutamate	Inosine	Carnosine
1	Control	67.8 ± 7.1	8.9 ± 1.2	21.5 ± 1.7	1.1 ± 0.2	1.17	5.7	Background (0.005)	0.001	0.17
2	№ 2	67.2 ± 6.7	9.0 ± 1.3	21.4 ± 1.7	1.11 ± 0.2	1.19	5.8	0.021	0.0015	0.175
3	№ 3	66.5 ± 6.6	7.0 ± 1.0	21.2 ± 1.7	1.34 ± 0.3	3.96	5.7	0.02	0.0032	0.303
4	№ 4	62.4 ± 6.2	9.9 ± 1.5	20.7 ± 1.6	1.21 ± 03	5.79	6.0	0.029	0.007	0.287
5	№ 5	65.5 ± 6.5	8.9 ± 1.3	20.8 ± 1.7	1.52 ± 0.4	3.28	5.8	0.028	0.0017	0.271

Table 5. Physical and chemical characteristics of heat-treated products

№	Sample	Moisture, %	Fat, %	Protein, %	Ash, %	Carbohydrates, %	pH, units
1	№ 1	47.5 ± 3.1	12.0 ± 1.9	29.25 ± 3.9	3.21 ± 0.5	8.04	6.2
2	№ 2	47.6 ± 3.1	11.9 ± 1.7	31.1 ± 4.7	3.16 ± 0.5	6.24	5.8
3	№ 3	46.5 ± 3.6	17.0 ± 2.0	31.2 ± 2.7	3.34 ± 0.4	8.08	5.7
4	№ 4	42.2 ± 3.4	13.9 ± 1.9	30.7 ± 3.4	3.51 ± 0.5	9.69	6.0
5	№ 5	45.7 ± 3.5	11.9 ± 1.7	30.5 ± 3.7	3.58 ± 0.8	8.32	5.8

Table 6. Comparison of free amino acid content before and after heat treatment of minced pork samples, mg/100 g of product

№	Amino acid	Control		Monosodium glutamate		Monosodium glutamate + yeast extract		Monosodium glutamate + ribotide		Monosodium glutamate + soy protein hydrolysate	
		raw	boiled	raw	boiled	raw	boiled	raw	boiled	raw	boiled
1	aspartic acid	18.06	58.12	58.07	94.88	162.65	165.19	99.94	115.55	128.17	122.13
2	glutamic acid	19.12	35.51	69.56	76.09	87.41	149.98	88.98	139.22	119.72	138.71
3	serine	27.85	40.19	125.86	53.54	129.79	62.88	107.34	72.66	123.57	119.43
4	histidine	115.72	129.58	375.44	412.09	780.68	772.34	567.98	677.78	307.72	791.11
5	glycine	28.33	68.12	125.67	126.38	214.39	199.68	134.87	159.66	121.06	144.75
6	threonine	42.45	64.22	82.47	160.85	283.66	187.24	154.86	167.67	193.05	118.46
7	arginine	10.95	21.72	72.97	59.42	55.32	147.97	34.98	47.99	115.21	130.89
8	alanine	6.16	18.73	57.14	55.83	27.35	128.64	12.56	28.69	15.18	128.75
9	tyrosine	4.42	15.58	43.45	20.00	21.51	40.03	24.99	30.00	15.34	27.39
10	cystine	4.14	25.27	34.18	86.96	66.14	121.81	44.78	51.81	130.45	152.79
11	valine	10.85	51.05	77.84	103.81	151.16	80.21	67.98	77.21	18.16	29.16
12	methionine	5.35	37.06	56.35	97.11	45.29	149.18	73.34	89.18	19.19	43.65
13	phenylalanine	6.15	33.71	47.12	96.68	126.99	83.74	90.44	103.78	143.52	199.14
14	isoleucine	21.46	96.61	131.91	206.13	142.57	242.09	234.93	292.09	15.26	39.13
15	leucine	7.24	55.14	124.27	106.46	496.83	188.52	342.12	388.57	118.14	110.56
16	lysine	7.58	39.87	78.68	80.27	132.16	94.33	98.66	104.36	117.12	135.19
17	proline	5.96	39.18	98.01	108.44	298.04	265.83	498.12	515.11	128.17	122.03

The results presented in Table 5, show that due to the heat treatment there is a change in the balance of moisture, fat and protein because of thermal loss during steam-boiling. High carbohydrate content of the samples after heat treatment should be noted; its level increased more than 6 times, that, apparently, can be explained by the intensive interaction of amino acids contained in the flavouring compositions with the proteins of the muscle tissue of the studied samples.

It is known that glutamic and aspartic acids, histidine, serine, cystine and methionine have the most significant influence on the taste of meat and meat products, which have the ability to correct the taste of the product according the sweet — bitter scale [27,28].

Amino acids are contained in food in a free and bound state, and free amino acids influence the taste and aroma more significantly [29].

A comparative analysis of the content of free amino acids before and after heat treatment in the studied samples is given in Table 6.

From the results shown in Table 6 it can be seen that the addition of monosodium glutamate, as a flavor enhancer, allows increasing the content of glutamic acid in sample № 2 almost 2 times after heat treatment, compared with its content in the control sample (semifat pork), in samples № 3, 4, 5 — almost 4.0 times. The addition of flavor enhancers allowed increasing the content of aspartic acid after heat treatment in sample № 2, almost 1.6 times, in sample № 3–2.85 times, and in sample № 4.5 — almost 2 times.

The content of serine in sample № 2 increased 1.35 times, in samples № 3, 4–1.6 times, in sample № 5–3 times.

The amount of histidine in sample № 2 increased about 3.2 times compared to its content in the control sample, in samples № 3, 4, 5 — almost 6 times.

The content of cystine in sample № 2 increased approximately 3.5 times, in sample № 3 — almost 5 times, in sample № 4–2 times, in sample № 5–6 times.

The content of methionine increased in sample № 2–2.6 times, in sample № 3–4 times, in sample № 4–2.4 times, and in sample № 5 remained almost unchanged.

Analysis of the dynamics of changes in the content of amino acids conditioning the maximum degree of taste of meat and meat products shows that their maximum accumulation is characteristic of samples № 3, 4 and 5, which contain compositions having a synergistic effect. It can be stated that the addition of monosodium glutamate as a mono-additive is less effective than its use as part of the studied compositions.

According to the expert assessment, the "enhanced taste" of the meat product most clearly appeared in the case of using a combined mixture of monosodium glutamate and soy protein hydrolysate.

Mass spectrometric analysis of aromatic substances revealed a pool of free fatty acids involved in the formation of taste and aroma of products. The basic components were derivatives of fatty acids. Their composition in products with monosodium glutamate and soy protein hydrolysate, before and after heat treatment, in the form of methyl esters is shown in Figure 1, in concentrations, mg/kg:

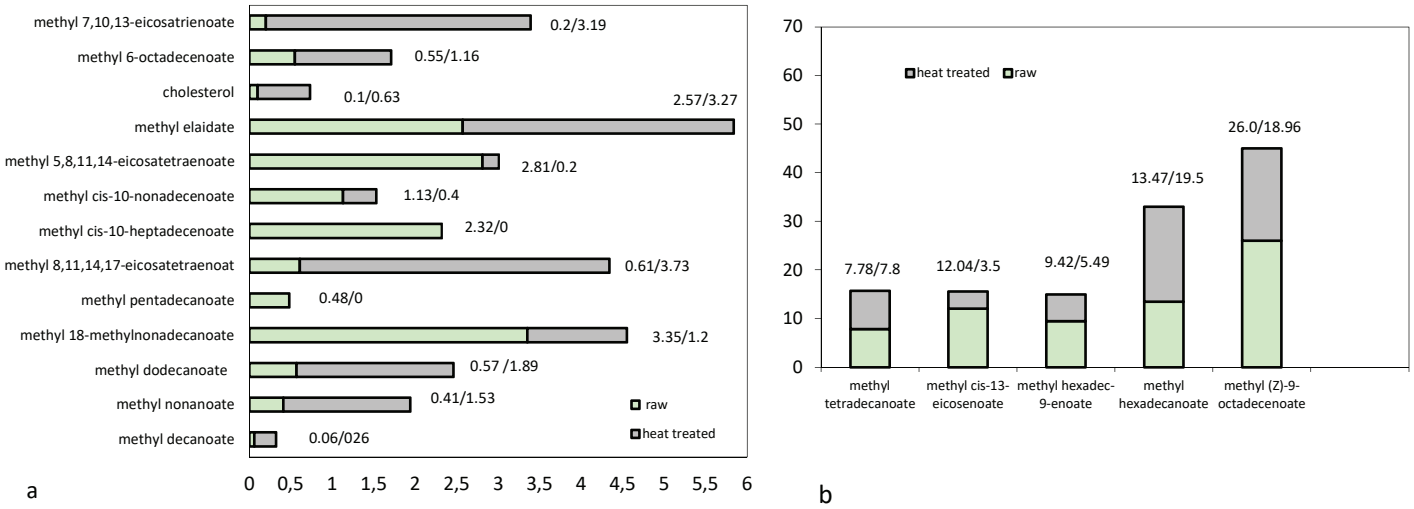


Figure 1. Content of methyl esters of fatty acids in concentrations, mg/kg: a — minor components, b — major components

Most of the major compounds were methyl esters of myristic (methyl tetradecanoate), erucic (methyl cis-13-eicosenoate), palmetoleic (methyl hexadec-9-enoate), palmitic (methyl hexadecanoate) and oleic acids (methyl (Z)-9-octadecenoate), which are mainly monounsaturated fatty acids.

In the composition of aromatic substances of the product with the addition of monosodium glutamate and soy protein hydrolysate were found more than 200 components, including those with the highest content is presented in Table 7.

A significant proportion of the aroma components was present in quantities from 0.001 to 0.2 mg/ kg. And only 38 components were present in higher concentrations. It is obvious that for a more complete assessment of the influence of these substances on the aroma profile, more in-depth research is needed, which was not the purpose of this work.

Table 7. Aromatic substances of the product with the addition of monosodium glutamate and soy protein hydrolysate

№	Compound name	Content, mg/kg, without heat treatment/ with heat treatment	Compound name	Content, mg/kg, without heat treatment/ with heat treatment
1	cyclopentyl ester	2.82 (ND)	5,6-dimethyl-phenanthridinium	2.22 (ND)
2	10-pentadecen-5-yn-1-ol	0.85 (ND)	1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene	0.94 (ND)
3	6-tetra-O-methyl-octanoic acid ethyl ester	0.89 (0.33)	1-undecene	1.71 (ND)
4	trenbolone	0.93 (005)	1-phenyl-4-(2-cyano-2-phenylethenyl) benzene	0.54 (ND)
5	2-ethylacridine	2.31 (1.56)	heneicosane	3.62 (3.3)
6	eicosane	2.14 (1.45)	tetratriacontane	2.47 (ND)
7	pyridine	2.26 (0.76)	2-hexen-1-ol	0.31 (ND)
8	10-methylnonadecane	4.01 (2.5)	7-methoxy-3,7-dimethyl-octanal	0.56 (0.1)
9	n-nonadecanol-1	7.53 (ND)	3-methyl-tridecane	0.94 (0.45)
10	octadecane	2.16 (ND)	2-naphthyl-p-tolyl sulfone	0.40 (ND)
11	trans-2,3-methylenedioxy-b-methyl-b-nitrostyrene	0.29 (ND)	nexahydro-2H-pyrido(1,2-a)pyrazin-3(4H)-one	0.69 (ND)
12	(3,4-dimethoxy-benzyl)-(4-morpholin-4-yl-phenyl)-amine	0.46 (ND)	5-(4-ethoxyphenyl)-3-(4-pyrrol-1-ylphenyl)-[1,2,4]oxadiazole	0.41 (0.16)
13	N-(2-chloroethoxycarbonyl)-l-methionine, propyl ester	0.45 (ND)	2,3-dihydro-2,8-dimethyl-benz[b]-1,4-oxazepine-4(5H)-thione	0.55 (ND)
14	ethanethioic acid	0.21 (ND)	paroxetine	0.41 (ND)
15	ethyl ester decanoic acid	3.86 (ND)	1-acetyl-4-[1-piperidyl]-2-butynone	0.35 (ND)
16	5-ethyl-2-methyl-octane	7.13 (ND)	2-methylaminomethyl-1,3-dioxolane	0.36 (ND)
17	triacontane	3.37 (2.55)	1-dodecene	0.67 (ND)
18	(3s)-pentanol	1.02 (ND)	4-methyl-2-hexanone	0.34 (ND)
19	docosane	1.13 (1.25)	tetratriacontane	2.61 (0.95)

Some components found in the composition of aromatic substances of the starting raw material, after heat treatment together with the used NVS, were found to be in significantly smaller quantities or were present in the form of other chemical derivatives of these components. Generally, the overall aroma seems to be due to the synergistic effect of all the detected substances.

The results of organoleptic analysis obtained by ten tasters: appearance, color index of the product (in the section), taste, aroma and texture of the studied products showed that the most delicious samples were those containing monosodium glutamate with yeast extract (sample № 3), and monosodium glutamate with ribotide (sample № 4). The results of a positive tasting assessment correlate with the dynamics of changes in the amino acid composition of samples after heat treatment, confirming the significance of the influence of free amino acids on the flavour characteristics of food products.

It can be concluded that the above-mentioned compositions are effective correctors for enhancing the flavour of meat and meat products.

Conclusion

The results of the study of the mechanism of formation of flavour properties of meat products show the effectiveness of introducing FA containing monosodium glutamate at the final stage of mixing/cutting, after the introduction of fat-containing raw materials.

The results of chromatographic analysis of nonvolatile substances responsible for the taste of semi-finished meat indicate that when replacing a part of raw meat, the dominant factor, deteriorating the taste of the product when introducing additives, is the quality and quantity of pork fat containing a substantial amount of unsaturated fatty acids that undergo rapid oxidation and, as a consequence, deteriorate the taste of meat product. Carbohydrate-containing components (soy protein and starch) have almost no negative impact on taste when replacing part of the meat, as evidenced by almost no decrease in quality indicators of the studied meat products based on pork raw material.

Using more fat-containing raw materials leads to absorption by fat droplets of non-volatile substances responsible for taste that can cause deterioration of the flavour profile of the product. Noticeable effect of known meat taste precursors — mono-additives inosine and carnosine, when they were added together with monosodium glutamate, was not detected.

The most effective compositions of food additives that enhance the taste of meat products were mixtures of sodium glutamate with yeast extract and sodium glutamate with ribotide in dosages of 0.05% of each component by weight of raw materials.

REFERENCES

1. Hygreeva, D., Pandey, M.C. (2016). Novel approaches in improving the quality and safety aspects of processed meat products through high pressure processing technology — A review. *Trends in Food Science & Technology*, 54, 175–185. https://doi.org/10.1016/j.tifs.2016.06.002
2. Reyes-Padilla, E., Valenzuela-Melendres, M., Camou, J.P., Sebranek, J.G., Alemán-Mateo, H., Dávila-Ramírez J.L., Cumplido-Barbeitia, G., González-Ríos, H. (2018). Quality evaluation of low fat bologna-type meat product with a nutritional profile designed for the elderly. *Meat Science*, 135, 115–122. https://doi.org/10.1016/j.meatsci.2017.09.007
3. Auqui, S.M., Egea, M., Peñaranda, I., Garrido, M.D., Linares, M.B. (2019). Rustic Chato Murciano pig breed: Effect of the weight on carcass and meat quality. *Meat Science*, 156, 105–110. https://doi.org/10.1016/j.meatsci.2019.05.022
4. Zhou, B., Shen, Z., Liu, Y., Wang, C., Shen, Q.W. (2019). Proteomic analysis reveals that lysine acetylation mediates the effect of antemortem stress on postmortem meat quality development. *Food Chemistry*, 293, 396–407. https://doi.org/10.1016/j.foodchem.2019.04.122
5. Atela, J.A., Mlambo, V., Mnisi, C.M. (2019). A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chickens. *Animal Nutrition*, 5(6), 179–184. https://doi.org/10.1016/j.aninu.2018.08.002
6. Chernukha, I., Kovalev, L., Mashentseva, N., Kovaleva, M., Vostrikova, N. (2019). Detection of protein aggregation markers in raw meat and finished products. *Foods and Raw Materials,* 7(1), 118–123. http://doi.org/10.21603/2308–4057–2019–1–118–123
7. Gómez-Cortés, P., Guerra-Rivas, C., Gallardo, B., Lavín, P., Mantecon, A.R., de la Fuente, M.A., Manso, T. (2018). Grape pomace in ewes diet: Effects on meat quality and the fatty acid profile of their suckling lambs. *Food Research International,* 113(11), 36–42. https://doi.org/10.1016/j.foodres.2018.06.052
8. Rogov, I.A., Zharinov, A.I., Tekutieva, L.A., Shepel, T.A. (2009). Biotechnologies of meat and meat products. Moscow: DeLi print. — 296 p. (in Russian)
9. Shipulin, V.I. (2006). Quality of raw meat and problems of its processing. *Bulletin of the North Caucasus State Technical University*, 1, 58–61. (in Russian)
10. Krasnov, A.E., Krasulya, O.N., Bolshakov, O.V., Shlenskaya, T.V. (2001). Information technology of the food industry in conditions of uncertainty. — Moscow: VNIIMP. — 496 p. (in Russian)
11. Dokmanović, M., Velarde, A., Tomović, V., Glamočlija, N., Marković, R., Janjić, J., Baltić, M. Z. (2014). The effects of lairage time and handling procedure prior to slaughter on stress and meat quality parameters in pigs. *Meat Science*, 98(10), 220–226. https://doi.org/10.1016/j.meatsci.2014.06.003
12. Ravindran, V., Tancharoenrat, P., Zaefarian, F., Ravindran, G. (2016). Fats in poultry nutrition: Digestive physiology and factors influencing their utilization. *Animal Feed Science and Technology*, 213(1), 1–21. http://dx.doi.org/10.1016/j.anifeedsci.2016.01.012
13. Aaslyng, M.D., Meinert, L. (2017). Meat flavour in pork and beef — From animal to meal. *Meat Science*, 132, 112–117. https://doi.org/10.1016/j.meatsci.2017.04.012
14. Lisitsyn, A.B., Kriger, O.V., Mitrokhin, P.V. (2016). Study of chemisrty and hydrolysates drying parameters of feather-downy raw material. *Foods and Raw Materials*. 4(1), 44–50. http://doi.org/10.21179/2308–4057–2016–1–44–50
15. Campagnol, P.C.B., dos Santos, B.A., Terra, N.N., Pollonio, M.A.R (2012). Lysine, disodium guanylate and disodium inosinate as flavor enhancers in low-sodium fermented sausages. *Meat Science*, 91(7), 334–338. https: //doi.org/10.1016/j.meatsci.2012.02.012
16. Zhao, J., Wang, T., Xie, J., Xiao, Q., Du, W., Wang, Y., Cheng, J., Wang, S. (2019). Meat flavor generation from different composition patterns of initial Maillard stage intermediates formed in heated cysteine-xylose-glycine reaction systems. *Food Chemistry,* 274(2), 79–88. https://doi.org/10.1016/j.foodchem.2018.08.096
17. Farouk, M.M., Yoo, M.J.Y., Hamid, N.S.A., Staincliffe, M., Davies, B., Knowles, S.O. (2018). Novel meat-enriched foods for older consumers. *Food Research International*, 104, 134–142. https://doi.org/10.1016/j.foodres.2017.10.033
18. Ramalingam V., Song Z., Hwang I. (2019). The potential role of secondary metabolites in modulating the flavor and taste of the

meat. *Food Research International*, 122, 174–182. https://doi.org/10.1016/j.foodres.2019.04.007
19. Shahidi, F. (1994). Flavor of Meat and Meat Products. Boston: Springer. — 296 p. ISBN978–1–4613–5911–1, https://doi.org/10.1007/978–1–4615–2177–8
20. Realini, C. E., Bianchi, G., Bentancur, O., Garibotto, G. (2017). Effect of supplementation with linseed or a blend of aromatic spices and time on feed on fatty acid composition, meat quality and consumer liking of meat from lambs fed dehydrated alfalfa or corn. *Meat Science*, 127, 21–29. https://doi.org/10.1016/j.meatsci.2016.12.013
21. Bogdanović, T., Pleadin, J., Petričević, S., Listeš, E., Sokolić, D., Marković, K., Ozogul, E., Šimat V. (2019). The occurrence of polycyclic aromatic hydrocarbons in fish and meat products of Croatia and dietary exposure. *Journal of Food Composition and Analysis*, 75, 49–60. https://doi.org/10.1016/j.jfca.2018.09.017
22. Flores, M., Mora, L., Reig, M., Toldrá, F. (2019). Risk assessment of chemical substances of safety concern generated in processed meats. *Food Science and Human Wellness,* 8(3), 244–251. https://doi.org/10.1016/j.fshw.2019.07.003
23. Khan, M. I., Jo, C., Tariq, M.R. (2015). Meat flavor precursors and factors influencing flavor precursors. A systematic review. *Meat Science,* 110(12), 278–284. https://doi.org/10.1016/j.meatsci.2015.08.002
24. Schwartz, C., Chabanet, C., Lange, C., Issanchou, S., Nicklaus, S. (2011). The role of taste in food acceptance at the beginning of complementary feeding. *Physiology & Behavior,* 104(4), 646–652. https:// doi.org/10.1016/j.physbeh.2011.04.061
25. Lisitsyn, A.B., Ivankin, A.N., Nekludov, A.D. (2002). Methods of practical biotechnology. Moscow: VNIIMP. — 402 p. (in Russian)
26. Miller, R. K. (2014). Chemical and physical characteristics of meat. Palatability. Book Chapter in Encyclopedia of Meat Sciences (Second Edition). Boston: Elsevier. pp. 252–261. https://doi.org/10.1016/b978–0–12–384731–7.00085–4
27. Kuznetsova, T.G., Chernucha, I.M., Bogdanova, A.V., Figovsky, O.L., Ivankin, A.N., Evdokimov, Yu.M. (2012). Nano sensory analysis of volatile components in the differentiating of animal objects. *Scientific Israel–Technological Advantages*, 14(1), 13–20.
28. Schilling, M. W., Suman, S. P., Zhang, X., Nair, M. N., Desai, M.A., Cai, K., Ciaramella, M.A., Allen, P. J. (2017). Proteomic approach to characterize biochemistry of meat quality defects. *Meat Science,* 132, 131–138. https://doi.org/10.1016/j.meatsci.2017.04.018
29. Hygreeva, D., Pandey, M.C. (2016). Novel approaches in improving the quality and safety aspects of processed meat products through high pressure processing technology. A review. *Trends in Food Science & Technology*, 54, 175–185. https://doi.org/10.1016/j.tifs.2016.06.002

AUTHOR INFORMATION

Andrew N. Ivankin — doctor of chemical sciences, professor, Head of the Department of Chemistry, Bauman Moscow State Technical University
141005, Mitishi, 1-st Institutskaya str., 1. Tel.: +7–498–687–36–00. e-mail: aivankin@inbox.ru
https://orcid.org/0000–0002–6557–2697

Natal'ya L. Vostrikova — doctor of technical sciences, Head of the Laboratory Center for food and feed testing, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences
109316, Moscow, Talalikhina str., 26. Tel.: +7–495–676–79–81. E-mail: n.vostrikova@fncps.ru
https://orcid.org/0000–0002–9395–705X
* corresponding author

Olga N. Krasulya — doctor of technical sciences, professor, Department "Technology of storage and processing of animal products", Russian State Agrarian University — Moscow Agrarian Academy by K. A. Timiryazev
127550, Moscow, Timiryasvskaya str., 49. Tel.: +7–499–154–79–79. E-mail: okrasulya@mail.ru
https://orcid.org/0002–8096–5210

Ekaterina V. Kazakova — candidate of agricultural sciences, docent, Department "Technology of storage and processing of animal products", Russian State Agrarian University — Moscow Agrarian Academy by K. A. Timiryazev
127550, Moscow, Timiryasvskaya str., 49. Tel.: +7–499–903–70–74.E-mail: kazakova.ev@rgau-msha.ru
https://orcid.org/0000–0001–6082–4771

Andrey V. Kulikovskii — candidate of technical sciences, head of laboratory Scientific and methodical work, biological and analytical research, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences
109316, Moscow, Talalikhina str., 26. Tel.: +7–495–676–60–11. E-mail: a.kulikovskii@fncps.ru
https://orcid.org/0000–0002–9140–5390

Aleksandra S. Knyazeva — junior researcher of the Laboratory "Scientific and methodical work, biological and analytical research", V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences
109316, Moscow, Talalikhina str., 26. Tel.: +7–495–676–79–61. E-mail: a.knyazeva@fncps.ru
https://orcid.org/0000–0002–0038–9744

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

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

The authors declare no conflict of interest

Received 29.09.2020 Accepted in revised 01.12.2020 Accepted for publication 15.12.2020

DOI: https://doi.org/10.21323/2414–438X–2020–5–4–13–17

Available online at https://www.meatjournal.ru/jour
Original scientific paper

THE METHODOLOGY OF FOOD DESIGN.
PART 1. THE INDIVIDUAL ASPECT

Aleksandr Yu. Prosekov
Kemerovo State University, Kemerovo, Russia

Keywords: *nutrigenetics, eating behavior, genotype, polymorphism, functional product, personalized nutrition*

Abstract
Innovative technologies for food raw material processing and food production are becoming globally important within the framework of modern biotechnology. The need to create a universal methodology for food design and the importance of its implementation in different lines of human life activity are obvious. Within the paradigm of modern biotechnology, personalized diets that take into consideration the genetic characteristics of consumers are becoming more and more popular. Nutrition science deals with the development of this direction. It is divided into nutrigenetics and nutrigenomics. Nutrigenetics investigates an effect of modifications in genes on absorption of metabolites, nutrigenomics investigates how food components affect the work of genes. In this work, we consider mutations that influence the assimilation of metabolites and contribute to nutrigenetic research. The work is aimed at finding and studying genes responsible for eating behavior. Methods of analysis of genetic polymorphisms and modern achievements of nutrigenetics in the development of personalized nutrition are considered. The review allowed us to find and describe the genes that influenced human eating behavior: the role of genes, their localization, polymorphisms affecting the metabolism of nutrients and food preferences are indicated. Thirty four genes that influence eating behavior were identified, and significant shortcomings of current methods / programs for developing personalized diets were indicated. Weaknesses in the development of nutrigenetics were identified (inconsistency of data on SNP genes, ignoring population genetics data, information that is hard for consumers to understand, etc.). Taking into consideration all shortcomings, an approximate model for selecting a personalized diet is proposed. In the future, it is planned to develop the proposed model for making up individual diets.

Introduction

Interrelation between nutrition and health is a cornerstone of human life. Questions of nutrient interaction and their effect on the human body allow the complex study of regularities of biophysical, biochemical and energy mechanisms ensuring life activity.

Nutrition is one of the most important factors of health support. Medical data indicate interrelation between nutrition and the most common noncommunicable diseases. Many cardiovascular diseases, different cancers, diabetes, gout, obesity are directly linked with excess intake of calories due to fats, simple carbohydrates, table salt, diets with reduced content of vitamins and dietary fibers.

Innovative technologies of food raw material processing and food production are becoming globally important within the framework of modern biotechnology [1]. At the current stage of the biotechnology development, nutrition science is not only the topical interdisciplinary research direction, but also rapidly developing. Personalized nutrition is individually adapted nutrition. With this approach, the gender, age, level of physical activity, presence of different chronic diseases and personal food preferences are taken into account. Individually tailored nutrition is aimed at prevention and treatment of different diseases, reduction of a negative effect of harmful environmental factors, support of healthy lifestyle. It is impossible to choose a diet without relying on achievements of modern genetics and nutrition science.

The paper is devoted to the study of food design principles. A necessity to create a universal methodology for food design, an importance of its realization in different directions of human life activity are obvious. The presented problem has different history of highlighting questions about methods for food design. There is a certain tradition of studying theoretical foundations and specific methods for using principles of balanced food design. Traditionally, food design is linked with formalization of qualitative and quantitative concepts about the rational use of food products [2]. The nutritional value of one or another product (proteins, fats, carbohydrates and their ratio) is placed in the center of the traditional design methods. At present, not only nutritional and biological value, but also a variety of medical, technological, economic, social and many other factors are taken into account when designing food products. The center of the modern biotechnological research is an individual [3, 4].

We presume that there is a relation between biotechnological, nutrition factors and a human diet. For complex examination of eating behavior and prescription of a corresponding diet with consideration for personal preferences of consumers, it is necessary to study methods for food design, including complete analysis of the human genome for polymorphisms in different gene groups. The scientific novelty of this work resides in the fact that there are no available studies that examine systemically and in detail genes and their polymorphisms influencing human eating behavior.

FOR CITATION: Prosekov A. Yu. The methodology of food design. Part 1. The individual aspect. *Theory and practice of meat processing.* 2020;5(4): 13–17. https://doi.org/10.21323/2414–438X-2020–5–4–13–17.

In our previous studies, we have already dealt with the indicated problem [5, 6, 7]. Principles and regularities of formation of dispersed food systems with functional properties are demonstrated from the current viewpoint. The peculiarities that should be taken into account in the development of functional food recipes are shown in [6, 7, 8]. This paper will actualize specific methodological aspects of the product design process in the personalized aspect.

The aim of this study is to assess the effectiveness of food design methods and detect genes influencing food preferences by analysis of publications on the indicated theme. In particular, our research focus includes the genes that are responsible for fat and carbohydrate assimilation, food intolerance, vitamin metabolism, taste sensation, oxidation of xenobiotics, food preferences and food addiction.

Materials and methods

The search of the literature carried out in November 2019 and updated in September 2020 considered papers published from January 1, 2015 up to now. For cross-validation, we used databases of papers from Scopus, Web of Sciences, Google Scholar, PubMed, LitVar, GeneCards, SNPedia, 1000 genomes (1KGP), Russian scientific electronic libraries (https://www. elibrary.ru; https://cyberleninka.ru). Search queries were formed by key words 'genes', 'gene polymorphism', 'genetic diseases', 'eating habits' and so on.

Results and discussion

In the national research, the development of functional products is based on the principles of food combinatorics (exclusion, fortification, replacement of a certain nutrient according to a human health state). A food product itself, its nutritional value, different recipe modifications are in the center of the traditional methodology. Modern methods for food product design have the anthropocentric direction. They are guided by requirements of individuals, their social-economic status, place of residence, peculiarities of the life activity of the body and genetic "memory" in general. The improvement of recipe design of multicomponent food products is largely linked with the use of one or another method of linear, experimental-statistical programming or the object-oriented approach.

For example, A. B. Lisitsyn and colleagues proposed a system modeling methodology for multi-component food products [9]. The essence of such tasks is selection of an optimal option from multiple possible recipe options by a targeted feature. O. N. Krasulya et al. (2015) examined the question of designing multi-component food products with consideration for information about actual values of functional-technological properties (FTP) of main raw materials and ingredients, kinetics of biochemical and colloid processes, analytical and empirical dependencies [10]. The study [11] proposes to use neural network technologies. A program in the high-level language Object Pacal was developed to design gerodietetic bread compositions [12]. M. A. Nikitina et al. (2018) proposed to use the multi-criteria optimization method — the Pareto method [13].

A special place in modern biotechnology and food combinatorics is given to nutrition science research linked with the development of nutrition systems, diets and so on. The nutrigenetics field includes the genetic basis of different reactions of individuals to the same nutrients. Creation of an individual diet is based on analysis of genetic information, which needs a list of genes. Genes participating in gaining an excess weight take an important place in the nutrition research [14].

— ***Genes responsible for carbohydrate and fat assimilation.*** There are nine genes responsible for carbohydrate and fat assimilation: ADRB2 (polymorphisms rs1042714, rs1042713), TCF7L2 gene (rs12255372, rs7903146), FABP2 gene (rs1799883), PPARG gene (rs1801282), CETP gene (rs5882), ADRB3 gene (rs4994), ApoA5 gene (rs662799, rs3135506), LEPR gene (rs1137101) and ApoE gene (rs429358, rs7412).

— ***Genes responsible for food intolerance.*** The list includes the HLA-DQ and MCM6 (rs4988235) genes, which cause monogenic diseases. The HLA genes are part of the immune response mechanism; that is, they help the immune system to differentiate self-proteins of the body from foreign proteins — viruses and bacteria.

— ***Genes responsible for vitamin metabolism.*** They include BCMO1 (rs7501331, rs12934922, rs119478057), Alpl (rs1256335) and NBPF3 (rs4654748), MTNFR (rs1801133), FUT2 (rs602662), VDR (rs1544410) and GC (rs2282679), F17ADS1 (RS14547).

— ***Genes responsible for taste sensation.*** GLUT2 (rs5400) is responsible for sweet taste sensitivity, TAS2R38 (rs1726866) for bitter taste, CD36 (rs1761667) is linked with taste sensitivity and preference for fat. ADD1 (rs4961) and CYP11B2 (rs1799998) are associated with salt sensitivity. The GLUT2 (or SLC2A2) gene encodes protein that transports glucose through the cell membrane; as a result, the gene is a good "sensor" of glucose sensitivity [15].

— ***Genes responsible for metabolism of xenobiotics.*** MnSOD (rs4880), GSTP1 (rs947894) and CYP1A2 (rs762551) take part in oxidation of xenobiotics entering the body with food.

— ***Genes responsible for eating behavior.*** The list of genes influencing food preferences includes FTO (rs9939609), MC4R (rs17782313), DRD2 (rs1800497). In this study, food preferences mean a tendency to overeat caused by genetic polymorphisms. The FTO gene encodes the protein that takes part in energy metabolism, oxidation reactions and metabolism of fatty acids.

— ***Genes responsible for food addiction.*** Genes responsible for the develop ment of food addiction include ADH1B (rs1229984) and ALDH2 (rs671), CHRNA5 (rs16969968) and CHRNA3 (rs1051730). The ADH1B and ALDH2 genes are responsible for sensitivity to alcohol [16].

Definitely, available data are somewhat contradictory. It is impossible to give the decisive answer to the question about roles of genes, their polymorphisms in food preferences and several diseases. It is due to several reasons. The first one resides in the fact that a small number of people from different ethnic groups and different life conditions participated in the research [17, 18, 19, 20]. Sampling results are not fully relevant. The complex study of the human genome and use of population genetics data are necessary for system assessment. The second reason resides in the character of material under study. For analysis and assessment, we used data from already published papers and not from the initial data presented by authors. This focus of analysis significantly narrowed the review.

Today, it is possible to find databases that combine information for research in the field of nutrigenomics, for example, the studies of Oxford scientists NutriGenomeDB [21]. The authors' materials allow entering a gene or genes of interest and obtaining information about their expression. Comparatively recently, a model of a personalized diet has been developed, which includes individual restrictions (past medical history, DNA, habitat, climate, life style and energy expenditure), the purpose of a diet (to maintain health or physical fitness, longevity, taste preferences, a balanced diet that promotes fast saturation with a small portion) [22]. The presented model is based on the following criteria: information architecture, service technologies, production technology [23].

In addition, a method for the formation of personalized nutrition based on DNA analysis with an emphasis on excess weight and food intolerance was developed [24]. The method includes the study of the polymorphic sites of the LCT, PPARG, ADRB2, FABP2, TCF7L2 genes and identification of the HLA-DQ haplotype. A corresponding diet is recommended depending on how the polymorphism influences the excess weight and/or food intolerance, and/or the presence of HLA-DQ haplotype. The examined method is effectively used by our national colleagues to select an individual diet. The authors believe that the method is well suited for the indicated genes (LCT, PPARG, ADRB2, FABP2, TCF7L2, HLA-DQ), but these are not the only genes that can influence excess weight and food intolerance.

T. Matsuo et al. [25] demonstrated that a diet with the high/low fat content influences the PPARG gene expression. It is noted that if there is a polymorphism in the gene, a body weight decreases. I. Arkadianos et al. [26] used a nutrigenetic test to optimize nutrients in a human diet. They performed genetic testing (one of gene analyses was PPARG) and modified the Mediterranean diet according to the individual requirements of the body according to the test results. Therefore, nutrigenetics is a tool for improvement and optimization of adequate nutrition. It is an effective means for long-term changes in the lifestyle.

Weaknesses of the available methods and apps for the development of personalized diets include the following:

— they do not take into account genetic data;
— they are difficult regarding adherence to a diet (both in a product choice and in a regime), therefore, a consumer often has to quit, which is harmful for the body;
— applications are not translated into a corresponding language;

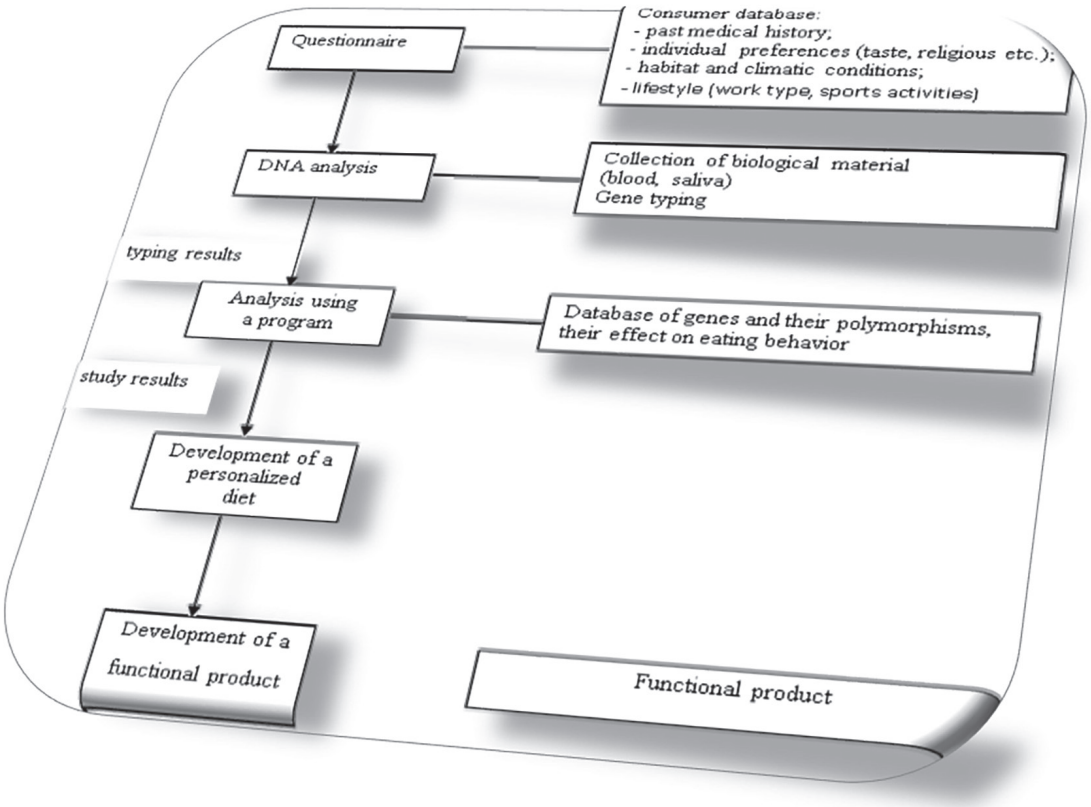


Figure 1. The model for the development of a personalized diet, which ultimate goal is production of a functional product [5,8].

— they should be paid for;
— information about a genetic predisposition is difficult to understand;
— population genetics is not taken into consideration.

Based on the presented weaknesses, a model for performing a nutrigenetic study was generated to create a personalized human diet (Figure 1).

The presented model consists of the following components. First, consumer survey questionnaire. It includes the past medical history, individual preferences (taste, religious), habitat, climatic zone of residence and lifestyle. All information should be entered into a protected database, which later on will be used for analysis of DNA testing results.

Second, DNA analysis (a gene or a set of genes that corresponds to the specified purpose is chosen with consideration for population genetics data). Materials (venous blood, buccal epithelium, saliva) are sampled, DNA is extracted and necessary polymorphisms are determined.

Third, analysis of data obtained using programs. A program analyzes data obtained upon extracted DNA typing, information from an individual questionnaire, data on genes and their influence on food behavior. In the end, the program gives a formed result;

Fourth, creation of personalized nutrition. An expert in nutrigenetics processes data obtained using a program and forms an individual diet for a consumer.

Fifth, development of a functional product. Based on obtained data and with a permission from consumers, optimal food components and nutrients are selected to develop a functional product of new quality based on genetic information and psycho-emotional preferences of a consumer [27].

Conclusion

The analyzed methods for formation of personalized diets have several weaknesses: some methods do not take into account genetic data; many diets are difficult regarding adherence (expensive products, tough regime), therefore, a consumer often quit a prescribed diet; the majority of available programs are not adapted to foreign users; open access information about a genetic predisposition is difficult for understanding and interpretation of results; population genetics is not taken into consideration in designing diets. A significant drawback is analysis of a small number of genes. It is necessary to carry out complete analysis of the human genome for polymorphisms in different gene groups for complex consideration of eating behavior regularities, prescription of a corresponding diet. It is also important to take into account personal preferences of a consumer.

As a result of the analytical review, thirty eight genes responsible for food behavior were revealed. The obtained data indicate that the number of polymorphisms causing monogenic diseases is lower than the number of genes leading to appearance of polygenic diseases. It is possible to identify genes, which mutations can lead to the development of obesity (ADRB2, FABP2, PPARG, ADRB3, LEPR, FTO, MC4R); type 2 diabetes (ADRB2, TCF7L2, FABP2, PPARG, CETP, ADRB3, MTHFR, GLUT2, CD36); cardiovascular diseases (CETP, ApoA5, ApoE, MTHFR, GLUT2, CD36, ADD1, CYP11B2, MnSOD); oncology (MnSOD, GSTP1, CYP1A2, CHRNA5, CHRNA3); central nervous system diseases (CETP, ApoE, ALPL, NBPF3, MTHFR, TAS2R38, CD36).

Creation of a personalized diet envisages consideration for all genes influencing human eating behavior. It was established that substantiated dietetic recommendations based on a wide study of genes are not given in scientific literature despite the importance of nutrigenetic studies. It is linked with complexity of nutrigenetics as a science because there are many contradictory data about a role and effect of SNP genes responsible for eating behavior. In this sphere, there is a need for experts competent not only in genetics but also in dietetics. The future of the indicated multidisciplinary direction of studying methods for the development of personalized nutrition models resides in creation of qualitatively new technologies of functional food production that play an important part in human nutrition and life activity.

REFERENCES

1. Bush, C.L., Blumberg, J.B., El-Sohemy, A., Minich, D.M., Ordovas, J.M., Reed, D.G., Behm, V.A.Y. (2020). Toward the definition of personalized nutrition: A proposal by the American nutrition association. *Journal of the American College of Nutrition*, 39(1), 5–15. https://doi.org/10.1080/07315724.2019.1685332
2. Lipatov, N.N. (1985). Methods for quantitative assessment and modeling of amino acid balance of meat products. *XXXI European Congress of Scientific Workers of the Meat Industry, Sofia,* 158.
3. Martín-Hernández, R., Reglero, G., Ordovás, J.M., Dávalos, A. (2019). Nutri GenomeDB: A nutrigenomics exploratory and analytical platform. *Database (Oxford),* baz097. https://doi.org/10.1093/database/baz097
4. Neeha, V.S., Kinth, P. (2013). Nutrigenomics research: A review. *Journal of Food Science and Technology*, 50(3), 415–428. https://doi.org/10.1007/s13197–012–0775-z
5. Vesnina, A., Prosekov, A., Kozlova, O., Atuchin, V. (2020).Genes and Eating Preferences, Their Roles in Personalized Nutrition. *Genes*, 11(4), 357. https://doi.org/10.3390/genes11040357.
6. Prosekov, A. Yu. (2005). Scientific foundations of food production. Kemerovo: KemTIPP. — 234 p. ISBN 5–89289–324–3 (in Russian)
7. Prosekov, A. Yu. (2019). Fundamentals of food technology Kemerovo: Kemerovo State University. — 498 p. ISBN 978–5–83532–275–6 (in Russian)
8. Vesnina, A. D. Research of genes responsible for eating behavior (Master's thesis in biotechnology) Kemerovo State University, Kemerovo, 2020. — 185 p. (accepted for publication). (in Russian)
9. Lisitsyn, A.B., Nikitina, M.A., Zakharov, A.N., Sus, E.B., Nasonova, V.V., Lebedeva, L.I. (2016). Prediction of meat product quality by the mathematical programming methods. *Theory and practice of meat processing,* 1(1), 75—90. https://doi.org/10.21323/2414–438X-2016–1–1–75–90 (in Russian)
10. Krasulya, O.N., Nikolaeva, S.V., Tokarev, A.V., Krasnov, A.E., IPanin, I.G. (2015). Modeling of recipes for food products and technologies for their production: theory and practice. Saint-

Petersburg: GIORD. — 320 p. ISBN978–5–98879–164–5 (in Russian)
11. Koneva, M.S., Usatikov, S.V., Bugaets, N.A., Tamova, M.Y. (2017). Neu ral Network and Regression Analysis of the Dependence of the Ranking Score of Organoleptic Characteristics on the Food System Composition. *Asian journal of pharmaceutics*, 11(2), Appl. S., S308-S319. https://doi.org/10.22377/ajp.v11i02.1270
12. Berezina, N.A., Artemov, A.V., Nikitin, I.A., Budnik, A. A. (2019). The Method of Computer-Aided Design of a Bread Composition with Regard to Biomedical Requirements. *International Journal of Advanced Computer Science and Applications,* 10(5), 137–143. https://doi.org/10.14569/ijacsa.2019.0100517
13. Nikitina, M.A., Chernukha, I.M. (2018). Multi–criteria optimization of a product recipe composition. *Theory and practice of meat processing,* 3(3), 89—98. https://doi.org/10.21323/2414–438X-2018–3–3–89–98 (in Russian)
14. Musina, O., Putnik, P., Koubaa, M., Barba, F.J., Greiner, R., Granato, D., Roohinejad, S. (2017). Application of modern computer algebra systems in food formulations and development: a case study. *Trends in food science & technology,* 64, 48—59. https://doi.org/10.1016/j.tifs.2017.03.011
15. Ruggiero, J.E., Northrup, H., Au, K.S. (2015). Association of facilitated glucose transporter 2 gene variants with the myelomeningocele phenotype. *Birth Defects Research Part A: Clinical and Molecular Teratology*, 103(6), 479–487. https://doi.org/10.1002/bdra.23358
16. Hashimoto, M., Watanabe, M., Uematsu, Y., Hattori, S., Miyai, N., Utsumi, M., Oka, M., Hayashida, M., Kinoshita, K., Arita, M., Takeshita, T. (2016). Relationships of alcohol dehydrogenase 1B (ADH1B) and aldehyde dehydrogenase 2 (ALDH2) genotypes with alcohol sensitivity, drinking behavior and problem drinking in Japanese older men. *Environmental Health and Preventive Medicine*, 21(3), 138–148. https://doi.org/10.1007/s12199–016–0507–5
17. Mozafarizadeh, M., Mohammadi, M., Sadeghi, S., Hadizadeh, M., Talebzade, T., Houshmand, M. (2019). Evaluation of FTO rs9939609 and MC4R rs17782313 polymorphisms as prognostic biomarkers of obesity: A population-based cross-sectional study. *Oman Medical Journal,* 34(4), 56–62. https://doi.org/10.5001/omj.2019.09
18. Drabsch, T., Gatzemeier, J., Pfadenhauer, L., Hauner, H., Holzapfel, C. (2018). Associations between single nucleotide polymorphisms and total energy, carbohydrate, and fat intakes: A systematic review. *Advances in Nutrition,* 9(4), 425–453. https://doi.org/10.1093/advances/nmy024
19. Sun, X., Luquet, S., Small, D.M. (2017). DRD2: Bridging the genome and ingestive behavior. *Trends in Cognitive Sciences*, 21(5), 372–384. https://doi.org/10.1016/j.tics.2017.03.004
20. Sevgi, M., Rigoux, L., Kühn, A.B., Mauer, J., Schilbach, L., Hess, M.E., Gruendler, T.O.J., Ullsperger, M., Stephan, K.E., Brüning, J.C., Tittgemeyer, M. (2015). An obesity-predisposing variant of the FTO gene regulates D2R-dependent reward learning. *Journal of Neuroscience*, 35(36), 12584–12592. https://doi.org/10.1523/jneurosci.1589–15.2015
21. NutriGenomeDB platform. [Electronic resource: http://nutrigenomedb.org/home Access date 24.02.2020]
22. Layman, D.K. (2014). Eating patterns, diet quality and energy balance. *Physiology & Behavior,* 134, 126–130. https://doi.org/10.1016/j.physbeh.2013.12.005
23. Sukhikh, S., Astakhova, L., Golubcova, Yu., Lukin, A., Prosekova, E., Milent`eva, I., Kostina, N., Rasshchepkin, A. (2019). Functional dairy products enriched with plant ingredients. *Foods and Raw Materials*, 7(2), 428–438. https://doi.org/10.21603/2308–4057–2019–2–428–438
24. Volobuev, V.V., Polunovskiy, V.V., Tsvetkovich, A.V., Seledtsova, L.A. The method of forming individual dietary recommendations based on DNA analysis. Patent RF, no.2691145, 2019.
25. Matsuo, T., Nakata, Y., Katayama, Y., Iemitsu, M., Maeda, S., Okura, T., Kim, M.K., Ohkubo, H., Hotta, K., Tanaka, K. (2009). PPARG genotype accounts for part of individual variation in body weight reduction in response to calorie restriction. *Obesity*, 17(10), 1924–1931. https://doi.org/10.1038/oby.2009.199
26. Arkadianos, I., Valdes, A.M., Marinos, E., Florou, A., Gill, R.D., Grimaldi, K.A. (2007). Improved weight management using genetic information to personalize a calorie controlled diet. *Nutrition Journal*, 6(1), 29. https://doi.org/10.1186/1475–2891–6–29.
27. Trihina, V.V., Spirichev, V.B., Koltun, V.Z., Avstrievskih, A. N. Nutritional factor in ensuring health and reliability increase of professional activities of industrial workers. *Foods and Raw Materials*, 3(1), 86–96. https://doi.org/10.12737/11242

AUTHOR INFORMATION

Aleksandr Yu. Prosekov — doctor of technical sciences, professor, rector, Kemerovo State University.
650000, Kemerovo, Krasnaya str., 6 Tel.: +7–923–502–00–22, E-mail: a.prosekov@rambler.ru
ORCID: https://orcid.org/0000–0002–5630–3196

Completely prepared the manuscript and is responsible for plagiarism.

The author declare no conflict of interest.

Received 24.09.2020 Accepted in revised 29.11.2020 Accepted for publication 15.12.2020

DOI: https://doi.org/10.21323/2414–438X–2020–5–4–18–22

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

FOODBORNE VIRUSES — AN EMERGING PATHOGENS

Branko Velebit
Institute of Meat Hygiene and Technology, Belgrade, Serbia

Keywords: *foodborne viruses, norovirus, hepatitis A virus, hepatitis E virus*

Abstract
Viral foodborne illnesses which have become a significant cause of all reported foodborne illnesses in recent years and considered as an emerging risk in veterinary public health. Foodborne transmission can occur by contamination of food by infected food handlers, by contamination of food during the production process and by consumption of products of animal origin harboring a zoonotic virus. Microbiological genomics studies discovered that noroviruses and hepatitis A viruses were primarily associated with food-handler transmission and sewage-contaminated foods while hepatitis E was associated with consumption of raw or undercooked meat of pig or wild animals. Routine harmonized surveillance of viral outbreaks, and surveillance of virus occurrence in food commodities, in combination with systematic strain typing, and joint expertise from veterinary, food, and clinical microbiologists would be recommended to aid source attribution studies and identify risk prevention measures.

Introduction

Over the last decades, due to rise of discretionary incomes in Europe and North America, increased urbanization and altered eating habits, worldwide food industry has changed from being locally oriented and supply-driven to become globalized and demand-driven. In order to satisfy consumer groups demanding safety, fair trade, "green" production, and animal welfare-related changes in production practices, policy makers imposed high hygienic standards and various control strategies for pathogenic bacteria, viruses, and parasites. These measures primarily concerned with common causes of food-borne diseases such as unsafe raw food, abused temperature, poor storage infrastructures, inadequate cooking, poor personal hygiene, improper handling methods, and cross-contamination of cooked food with uncooked raw food. Although contamination prevention and control strategies are mostly successful in reduction of food-borne diseases, they also demonstrate the weakness of the global food supply: if there is a fault in the process, then contamination may occur with pathogens from across the globe, including those that have recently emerged [1]. This proved to be challenging for diverse and complex food systems, especially those in less-developed countries.

In this paper we will address viral foodborne illnesses which have become a significant cause of all reported foodborne illnesses in recent years and considered as an emerging risk in veterinary public health.

Food-borne viruses and their role in food safety

Currently known viruses that can infect humans are grouped into 22 families. In addition to this, the recent advances in molecular techniques that allow characterization of all genetic material in a given sample has led to the identification of several new viruses in recent years, most of which remain to be fully characterized [2, 3]. Viruses are strict intracellular parasites and cannot replicate outside a specific living cell. The host cell treats viral genetic information as if it were its own. Replication of viruses occurs by transcription and translation of the viral genome using host cell mechanisms. It is not possible to culture them in an environment free of living cells, and therefore the number of viral particles does not increase in food and water during production, processing, transport, and storing. Sensory characteristics of products containing these pathogens and those of non-contaminated food are identical [4, 5]. Transmission of the virus does not only depend on its interaction with the host, but also on the influence of the external environment. Outside the host organism, viruses are inert particles without their own metabolism. The longer they survive in the infectious state environment, the higher is the probability of transmission and spread of infection [6].

Foodborne transmission has been documented for viruses belonging to at least 10 taxonomic families, and the diseases associated with these infections range from mild diarrheal illness to severe encephalitis. Foodborne transmission can occur by:

- contamination of food by infected food handlers (frequently),
- by contamination of food during the production process (frequently — in bivalve molluscan shellfish or berry fruit production),
- by consumption of products of animal origin harboring a zoonotic virus (very rare).

The first and second mean of transmission applies to viruses that are transmitted by the faecal-oral route, hence infect their host after ingestion, followed by invasion of cells in the gut epithelium, and subsequent replication in the same site or elsewhere in the body. WHO and FAO [7] have ranked noroviruses (NoV), group A rotaviruses, and hepatitis A viruses (HAV) as priority hazards. When it comes to emerging hazards, hepatitis E virus (HEV), Nipah viruses, H5N1 avian influenza viruses and SARS

FOR CITATION: Velebit B. Foodborne viruses — an emerging pathogens *Theory and practice of meat processing.* 2020; 5(4): 18–22. https://doi.org/10.21323/2414–438X-2020–5–4–18–22

coronavirus were considered to be of greatest importance. They have also linked risk of specific virus to a specific commodity, and the importance of that commodity in causing viral foodborne illness and found following virus-commodity combinations for which prevention and control measures should be thoroughly considered:

- NoV and HAV in bivalve molluscan shellfish;
- NoV and HAV A in fresh produce;
- NoV and HAV in prepared foods;
- Rotaviruses in water for food preparation;
- Emerging viruses in selected commodities.

Foodborne NoV outbreaks are often linked to food handlers who infect foods that are eaten raw or not further processed (ready to eat (RTE) foods) prior to consumption [8]. In many of these outbreaks, a sick food-handler or food-handler with a recent history of gastroenteritis was noticed. Workers have often been in contact with ill family members including children before the worker handled food. The most common food worker errors identified in relation to outbreak of NoV and HAV are food handling by an infected person or carrier of virus together with bare-hand contact by handler (RTE foods) and failure to properly wash hands (9). Poor personal hygiene was also identified as a contributing factor in outbreaks with NoV assigned as the causative agent. Food handlers can contaminate food either with particles from vomit (NoV) or from faeces (NoV/HAV) when employing insufficient personal hygiene after using toilets. Asymptomatic food workers are implicated more frequently than symptomatic workers, which helps explain the difficulty in detecting and stopping an outbreak by excluding ill food workers [9].

Food contamination at site happens when food is contaminated during the primary production of risky commodities, such as berries, green vegetables or bivalve molluscan shellfish. In these cases sewage or wastewater contamination are the primary source of food-borne viruses, and NoV and HAV were considered to be priority concerns according to aforementioned WHO/FAO opinion [7]. Sewage or contaminated water frequently contain multiple RNA viruses, opposite to cases in which food handler contamination occurred. In this case, cohabitation of different (ss+) RNA viruses and subsequent co-infection of a human cells by genetically distinct viral strains can lead to the generation of recombinant viruses shuffling their individual mutations and thus making unpredictable effects on viral behavior and virulence.

Zoonotic food-borne infection occurs when meat, organs, or other products from an infected animal are consumed [10]. For viruses, this is the very rare mode of transmission, although in every emerging disease outbreak this should be investigated. This is especially case with hepatitis E virus since infected pig liver (of both domestic pig and wild boar) consumed raw or undercooked is the main source of infection/contamination. Also, severe acute respiratory syndrome (SARS) and Nipah virus have been transmitted through food-related incidents [11, 12].

Common foodborne viruses

Norovirus

NoV belong to the Family *Caliciviridae*, that is divided into five genera. NoV and Sapovirus are the two genera of the family *Caliciviridae* that contain viruses that cause infections in humans. NoV have also been detected in pigs, cattle, mice, cats, dogs, and sheep, and sapoviruses in pigs. The other genera of the family Caliciviridae are Lagovirus, Vesivirus, and Nebovirus encompassing viruses infecting rabbits, and brown hares (lagoviruses), sea lions, swine, cats, dogs, fish, seals, other marine animals, cattle and primates (vesiviruses), and cattle (Nebovirus) [13]. In humans, NoV infection typically causes acute gastroenteritis, with the most common symptoms being nausea, vomiting, diarrhea, and stomach pain. Symptoms usually develop 12 to 48 hours after infection. The disease normally lasts between 1 and 3 days.

NoV can be divided into five distinct genogroups, based on phylogenetic analyses of the capsid protein (GI-GV). Viruses of GI, GII and GIV are known to infect humans. GII viruses have additionally been detected in pigs, and GIV viruses have been detected in a lion cub and a dog. GIII viruses infect cattle and sheep and GV viruses infect mice. Recombination between viruses from different genogroups is rare suggesting that this constitutes a species level in taxonomy. Within each genogroup, viruses are further divided into genotypes [14].

NoV illness prevalence is highest in young children (< 5 years) and the elderly [15]. Factors that contribute to the significant impact of noroviruses include a large human reservoir, low infection dose (only 10 to 100 viral particles), their environmental robustness, the short-lived immunity to noroviruses (18 months at most), and the ability to be transmitted by various routes. Majority of incriminated foods includes shellfish which feed by filtration of surrounding water, then berry fruit and green vegetables contaminated during soil fertilizing shortly before picking or watered by contaminated municipal water [5, 16, 17].

Most NoVs can also escape the receptor-blocking activities from antibodies triggered by earlier infections due to accumulated mutations in genome [18, 19]. Viruses are present in faeces and vomitus of diseased people at extremely high levels, up to 10^{10} viral particles per gram of stool [20]. The major obstacle to research human noroviruses has been the lack of a robust and reproducible in vitro cultivation system. Such a system is critical to achieve a full mechanistic understanding of human noroviruses replication, stability, evolution and pathogenesis. However, recently stem-cell derived, non-transformed human intestinal enteroid (HIE's) cultures validated as an appropriate pre-clinical model for clinically important enteric infections have been reported [21].

When it comes to prevalence, WHO estimates that Norovirus is the most common cause of foodborne illness in the European region with close to 15 million cases each year, causing more than 400 deaths. In the Netherlands,

Norovirus remains the key pathogen causing food-related outbreaks in 2016 as in previous years, followed by *Salmonella* and *Campylobacter* [22].

EFSA, ECDC and FVO have been systematically monitoring whole picture of the state of affairs concerning the Norovirus issue. European Union-coordinated monitoring program on the prevalence of norovirus in raw oysters was initiated. The objective of the study was to estimate the European prevalence of norovirus-contaminated oysters at production areas and batches of oysters at dispatch centers, with a 95% level of confidence and a level of precision of 5% considering an expected prevalence of 50%. The survey started in November 2016 and finishes in October 2018 [23]. The EFSA delivered a scientific opinion on the evaluation of heat treatments, different from those currently established in the EU legislation that could be applied to live bivalve molluscs. Of particular relevance are the achievement of at least 90 °C for at least 90 s in the molluscs flesh and the inactivation of viruses [24].

Currently, EU regulatory authorities are focusing in following areas in Norovirus combat: (i) whole genome sequencing for the characterization of Norovirus and other foodborne viruses; (ii) surveillance to generate more information about levels of Norovirus occurring in food; (iii) refinements to current RT-PCR to improve detection of low numbers of norovirus particles in all food matrices; (iv) the binding properties and possible methods of inactivation of norovirus; (v) the effectiveness of depuration (or alternatives such as high pressure, UV, ozone, irradiation) in removing norovirus from oysters; and (vi) establishment of the infectious dose in different food commodities including shellfish and fresh produce (lettuce and berries).

Hepatitis A virus

Hepatitis A is caused by the hepatitis A virus (HAV) which belongs to genus Hepatovirus within family *Picornaviridae*. Hepatoviruses have only been found in humans and primates, suggesting there is no introduction from any other reservoir. Based on genetic diversity, hepatitis A viruses are divided into six lineages or genotypes, of which genotypes I–III infect humans [25]. It consists of a non-enveloped icosaedral capsid of around 30 nm in diameter containing a positive ssRNA genomic molecule of 7.5 Kb [26]. HAV is a unique picornavirus because it does not inhibit host-cell protein synthesis to allow a regulated ribosome traffic rate thus ensuring the proper protein folding [27]. Capsid folding is critical to permit a long period-environmental stability for a virus transmitted through the faecal-oral.

Since proper sanitation and good hygienic conditions greatly reduce transmission rate of HAV its prevalence is significantly lower than prevalence of NoVs, [28]. In highly endemic regions, HAV is one of the childhood infections that, in the majority of cases, runs an asymptomatic course, while triggering a protective immune response that lasts long, possibly even lifelong [29]. HAV is quite stable outside a host and, therefore, can persist on contaminated environments, food, and water for a quite long time. Food- and water-borne outbreaks have been documented, although again, as for NoVs, the most common mode of transmission occurs between persons. Incidence risk of food-borne HAV at present comes from introduction through food into regions where population immunity is relatively limited. Foods commodities susceptible to contamination during the production phase, such as bivalve filter-feeding oysters, clams, mussels or commodities that are irrigated with water that may be contaminated (lettuce, green onions, and soft fruits, such as raspberries and strawberries). These foods should be considered the principal targets for virological analysis. Nevertheless, in roughly 40% of the reported cases of hepatitis A the source of infection cannot be identified [30]. The first documented shellfish-borne outbreak of "infectious hepatitis" occurred in Sweden in 1955, when 629 cases were associated with raw oyster consumption. However, the most significant outbreak of HAV infection occurred in Shanghai, China, in 1988, in which almost 300,000 cases were caused by consumption of clams harvested from a sewage-polluted area. A specific problem with shellfish is that the current microbiological quality control criteria are based on quantitative testing for *E. coli* contamination, which often fails to predict the presence or absence of viruses. Water-depurated shellfish have been associated with outbreaks of norovirus, hepatitis A, gastroenteritis, and other viral diseases [31].

Hepatitis E virus

HEV is a non-enveloped icosahedral virus with a diameter of 35 nm, classified into the unassigned genus Hepevirus. The genome consists of one positively oriented single-stranded RNA molecule and around 7 kb in length. The major ORFs are ORF-1, which encodes a non-structural polyprotein, ORF-2 encoding the capsid protein and ORF-3 encoding a phosphoprotein.

The HEV strains can be grouped into 4 genotypes, with different spatiotemporal distribution and different host. Genotypes 1 and 2 have been found solely in humans, i. e. genotype 1 is endemic in Asia and Africa where inhabitants are exposed to the virus due to poor sanitary conditions and sewage overspill that results from heavy rainfall [32]. In these conditions surface water is contaminated that is used for drinking water production or as source for water used for household tasks, so this explains the magnitude of outbreak. Genotype 2 is endemic in Mexico and Western Africa. However, beside in humans, genotypes 3 and 4 have been detected in pigs and other animal species. Genotype 3 is distributed worldwide and genotype 4 is mostly restricted to Southeast Asia. Endemic strains found in Europe are usually of genotype 3.

The epidemiology of HEV is rather complex, and a foodborne transmission of HEV from animal products to humans is an emerging risk, especially in the European developed countries. A few research studies indicated the

following food commodities present risk factors for onset of HEV infection: pork pies, liver pate, wild boar, undercooked or raw pork, home-made sausages, meat (in general), unpasteurized milk, shellfish and ethnic foods [33]. Nevertheless, these factors were not adequately proven due to scarce data obtained from very few systematic studies. One systematic case-control study has been performed in Germany, in which eating of any offal or wild boar meat was identified as risk factor for autochthonous hepatitis E [34]. In addition, another recent small-scaled case-control study identified eating of raw pig liver sausage as a risk factor for hepatitis E in France [35]. Earlier publications from Japan indicate direct HEV transmission by eating raw or undercooked meat from wild boar or deer by detailed analysis of small outbreaks [36]. No detailed information on hepatitis E cases, including the proportion of foodborne cases, is available for the EU which is the reason why EFSA in July 2017 advised national competent authorities to commence gathering data on HEV prevalence and/or possible HEV outbreaks [37]. Despite rough estimations that approximately 2 billion people could have been exposed to HEV [38], majority of HEV cases occurred in the endemic regions in Asia, Africa and Central America, where transmission is mainly due to faecally contaminated water. Europe is not a endemic region, but sporadic hepatitis E cases have been described in France, The Netherlands, Spain, Hungary, the UK, Denmark, Norway (39), indicating an EU-wide distribution of the virus. In Germany, HEV is notifiable as of 2001 and their data indicate that a total of 40 to 220 cases (mostly non-travelers in endemic area) per year are registered, with increasing tendency (39). In France the disease is also notifiable and 218 cases have been identified in 2008. Among these cases 146 have been identified as autochthonous cases, 23 to travels and no epidemiological data was available for 49 cases [39].

Conclusion

NoV and HAV have been recognized as priority concerns in viral food-borne transmission. However, proper diagnosis of infection caused by these agents is often hindered due to sharing general symptoms with other diseases (fatigue, dehydration, nausea, vomiting, diarrhea, and some stomach cramping), failure of notification and relatively quick resolution of signs of illness. The most important role in transmission route is attributed to infected food handlers and sewage-contaminated foods. In the latter category, complex mixtures of human and animal viruses and other pathogens may be present in a single food item, causing possible genetic recombination and subsequent uncontrolled expansion of the diversity of these pathogens. Routine harmonized surveillance of viral outbreaks, and surveillance of virus occurrence in food commodities, in combination with systematic strain typing, and joint expertise from veterinary, food, and clinical microbiologists would be recommended to aid source attribution studies and identify risk prevention measures.

REFERENCES

1. Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., van der Giessen, J., Kruse, H. (2010). Food-borne diseases — the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*, 139 (Suppl. 1), S3–15. https://doi.org/10.1016/j.ijfoodmicro.2010.01.021
2. Allander, T., Tammi, M.T., Eriksson, M., Bjerkner, A., Tiveljung-Lindell, A., Andersson, B. (2005). From The Cover: Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proceedings of the National Academy of Sciences of the United States of America*, 102(36), 12891–12896. https://doi.org/10.1073/pnas.0504666102
3. Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, 451(7181), 990–993. https://doi.org/10.1038/nature06536
4. Cook, N. (2001). Viruses in food. *CPD Infection*, 2, 98–101.
5. Koopmans, M., Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90(1), 23–41. https://doi.org/10.1016/s0168-1605(03)00169-7
6. Rzezutka, A., Cook, N. (2004). Survival of human enteric viruses in the environment and food. *FEMS Microbiology Reviews*, 28(4), 441–453. https://doi.org/10.1016/j.femsre.2004.02.001
7. WHO (World Health Organization). (2008). Viruses in food: Scientific advice to support risk management. Microbiological Risk Assessment Series, No. 13. [Electronic resource: http://www.who.int/foodsafety?/publications?/micro/mra13/en/index.html. Access date 20.10.2020]
8. Baert, L., Mattison, K., Loisy-Hamon, F., Harlow, J., Martyres, A., Lebeau, B., Stals, A., Van Coillie, E., Herman, L., Uyttendaele, M. (2011). Review: Norovirus prevalence in Belgian, Canadian and French fresh produce: A threat to human health? *International Journal of Food Microbiology*, 151(3), 261–269. https://doi.org/10.1016/j.ijfoodmicro.2011.09.013
9. Todd, E.C., Greig, J.D., Bartleson, C.A., Michaels, B.S. (2008). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 4. Infective doses and pathogen carriage. *Journal of Food Protection*, 71(11), 2339–2373. https://doi.org/10.4315/0362-028x-71.11.2339
10. Koopmans, M. (2012). Food-borne viruses from a global perspective. Chapter in a book: Improving Food Safety Through a One Health Approach: Workshop Summary. Washington (DC): National Academies Press (US). A9. https://doi.org/10.17226/13423
11. 11.Trostle, J. A., Hubbard, A., Scott, J., Cevallos, W., Bates, S. J., Eisenberg, J. N. S. (2008). Raising the Level of Analysis of Food-Borne Outbreaks: Food-Sharing Networks in Rural Coastal Ecuador. *Epidemiology*, 19(3), 384–390. https://doi.org/10.1097/ede.0b013e31816a9db0
12. Luby, S. P., Rahman, M., Hossain, M. J., Blum, L. S., Husain, M. M., Gurley, E., Khan, R., Ahmed, B.-N., Rahman, S., Nahar, N., Kenah, E., Comer, J., Ksiazek, T. G. (2006). Foodborne Transmission of Nipah Virus, Bangladesh. *Emerging Infectious Diseases*, 12(12), 1888–1894. https://doi.org/10.3201/eid1212.060732
13. EFSA (European Food Safety Authority). (2011). Scientific Opinion on an update on the present knowledge on the occurrence and control of foodborne viruses. *EFSA Journal*, 9(7), 2190. https://doi.org/10.2903/j.efsa.2011.2190
14. Kroneman, A., Verhoef, L., Harris, J., Vennema, H., Duizer, E., van Duynhoven, Y., Gray, J., Iturriza, M., et al. (2008). Analysis of integrated virological and epidemiological reports of norovirus outbreaks collected within the Foodborne Viruses in Europe network from 1 July 2001 to 30 June 2006. *Journal of Clinical Microbiology*, 46(9), 959–2965. https://doi.org/10.1128/jcm.00499-08
15. de Wit, M.A., Koopmans, M.P., Kortbeek, L.M., Wannet, W.J., Vinjé, J., van Leusden, F., Bartelds, A.I., van Duynhoven, Y.T. (2001). Sensor, a population-based cohort study on gastroenteritis in the Netherlands: Incidence and etiology. *American Journal of Epidemiology*, 154(7), 666–674. https://doi.org/10.1093/aje/154.7.666
16. Dubois, E., Agier, C., Traore, O., Hennechart, C., Merle, G., Cruciere, C., Laveran, H. (2002). Modified concentration method

for the detection of enteric viruses on fruits and vegetables by reverse transcriptase-polymerase chain reaction or cell culture. *Journal of Food Protection*, 65(12), 1962–1969. https://doi.org/10.4315/0362–028x-65.12.1962
17. Votava, M., Cerhnohorska, L., Heroldova, M., Hola, V., Mejzlikova, L., Ondrovcik, P., Ruzicka, F., Dvorackova, M., Woznicova, V., Zahradnicek, O. (2003). Special Medical Microbiology. Brno: Neptun. 237–365. (in Czech).
18. Lindesmith, L.C., Donaldson, E.F., Lobue, A.D., Cannon, J.L., Zheng, D.P., Vinje, J., Baric, R.S. (2008). Mechanisms of GII.4 norovirus persistence in human populations. *PLoS Medicine*, 5(2), e31. https://doi.org/10.1371/journal.pmed.0050031
19. Siebenga, J.J., Beersma, M.F., Vennema, H., van Biezen, P., Hartwig, N.J., Koopmans, M. (2008). High prevalence of prolonged norovirus shedding and illness among hospitalized patients: A model for in vivo molecular evolution. *Journal of Infectious Diseases*, 198(7), 994–1001. https://doi.org/10.1086/591627
20. Lee, N., Chan, M. C. W., Wong, B., Choi, K. W., Sin, W., Lui, G., Chan, P.K.S., Lai, R.W.M., Cockram, C.S., Sung, J.J.Y., Leung, W. K. (2007). Fecal Viral Concentration and Diarrhea in Norovirus Gastroenteritis. *Emerging Infectious Diseases*, 13(9), 1399–1401. https://doi.org/10.3201/eid1309.061535
21. Costantini, V., Morantz, E. K., Browne, H., Ettayebi, K., Zeng, X., Atmar, R. L., Estes, M.K., Vinjé, J. (2018). Human Norovirus Replication in Human Intestinal Enteroids as Model to Evaluate Virus Inactivation. *Emerging Infectious Diseases*, 24(8), 1453–1464. https://doi.org/10.3201/eid2408.180126
22. Papapanagiotou, E. P. (2017). Foodborne Norovirus State of Affairs in the EU Rapid Alert System for Food and Feed. *Veterinary Sciences*, 4(4), 61. https://doi.org/10.3390/vetsci4040061
23. Abrahantes, J.C., Richardson, J., O'Mahony, M., Pare, A., Bruckers, L., Johne, R., Keaveney, S., Ianni, I., Lowther, J., Suffredini, E., et al. (2017). European baseline survey of norovirus in oysters. *In Proceedings of the 11th International Conference on Molluscan Shellfish Safety (ICMSS) 2017*, Galway, Ireland, 14–18 May 2017.
24. EFSA Panel on Biological Hazards. (2015). Scientific opinion on the evaluation of heat treatments, different from those currently established in the EU legislation, that could be applied to live bivalve molluscs from B and C production areas, that have not been submitted to purification or relaying, in order to eliminate pathogenic microorganisms. *EFSA Journal*, 13(12), 4332. https://doi.org/10.2903/j.efsa.2015.4332
25. Cella, E., Golkocheva-Markova, E. N., Trandeva-Bankova, D., Gregori, G., Bruni, R., Taffon, S., Angeletti, S., et al. (2018). The genetic diversity of hepatitis A genotype I in Bulgaria. Medicine, 97(3), e9632. https://doi.org/10.1097/md.0000000000009632
26. Fauquet, C., Fargette, D. (2005). International Committee on Taxonomy of Viruses and the 3,142 unassigned species. *Virology Journal*, 2, 64.
27. Aragones, L., Guix, S., Ribes, E., Bosch, A., Pintó, R. M. (2010). Fine-Tuning Translation Kinetics Selection as the Driving Force of Codon Usage Bias in the Hepatitis A Virus Capsid. PLoS Pathogens, 6(3), e1000797. https://doi.org/10.1371/journal.ppat.1000797
28. Jacobsen, K.H., Wiersma, S.T. (2010). Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine*, 28(41), 6653–6657. https://doi.org/10.1016/j.vaccine.2010.08.037
29. Hollinger, F.B., Emerson, S.U. (2007). Hepatitis A virus. In: Knipe DM, Howley PM, editors. Fields virology. 5. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; pp. 911–947.
30. Walker, C.L.F., Perin, J., Aryee, M.J., Boschi-Pinto, C., Black, R.E. (2012). Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. *BMC Public Health*, 12, 220. https://doi.org/10.1186/1471–2458–12–220
31. Ueki, Y., Shoji, M., Suto, A., Tanabe, T., Okimura, Y., Kikuchi, Y., Saito, N., Sano, D., Omura, T. (2007). Persistence of caliciviruses in artificially contaminated oysters during depuration. *Applied and Environmental Microbiology*, 73(17), 5698–5701. https://doi.org/10.1128/aem.00290–07
32. Purcell, R.H., Emerson, S. U. Hepatitis E virus. (2001). In: Knipe DM, Howe PM, editors. Fields' Virology. New York: Raven Press; 2001. pp. 3051–3061.
33. Lewis, H. C., Wichmann, O., Duizer, E. (2010). Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: a systematic review. *Epidemiology and Infection*, 138(2), 145–166. https://doi.org/10.1017/s0950268809990847
34. Wichmann, O., Schimanski, S., Koch, J.V., Kohler, M.G., Rothe, C., Plentz, A., Jilg, W., Stark, K. (2008). Phylogenetic and case-control study on hepatitis E virus infection in Germany. *The Journal of infectious diseases*, 198(12), 1732–1741. https://doi.org/10.1086/593211
35. Colson, P., Borentain, P., Queyriaux, B., Kaba, M., Moal, V., Gallian, P., Heyries, L., Raoult, D., Gerolami, R. (2010). Pig liver sausage as a source of hepatitis E virus transmission to humans. *The Journal of infectious diseases,* 202(6), 825–834. https://doi.org/10.1086/655898
36. Tei, S., Kitajima, N., Takahashi, K., Mishiro, S. (2003). Zoonotic transmission of hepatitis E virus from deer to human beings. *The Lancet*, 362(9381), 371–373. https://doi.org/10.1016/s0140–6736(03)14025–1
37. Panel on Biological Hazards. (2017). Public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen. *EFSA Journal*, 15(7), 4886. https://doi.org/10.2903/j.efsa.2015.4330
38. Aggarwal, R., Jameel, S. (2008). Hepatitis E vaccine. *Hepatology International*, 2, 308–315. https://doi.org/10.1007/s12072–008–9071–4
39. Aspinall, E. J., Couturier, E., Faber, M., Said, B., Ijaz, S., Tavoschi, L., Takkinen, J., Adlhoch, C. (2017). Hepatitis E virus infection in Europe: surveillance and descriptive epidemiology of confirmed cases, 2005 to 2015. *Eurosurveillance*, 22(26). https://doi.org/10.2807/1560–7917.es.2017.22.26.30561

AUTHOR INFORMATION

Branko Velebit — M. Sc. DVM, Principal Research Fellow, Head of Department of Microbiology and Molecular Biology, Institute of meat hygiene and technology
Kaćanskog 13, 11040 Belgrade, Serbia. Tel.: +381–11–2650–722. E-mail: branko.velebit@inmes.rs
https://orcid.org/ 0000–0002–7577–8074

Completely prepared the manuscript and is responsible for plagiarism.

The author declare no conflict of interest.

Received 15.11.2020 Accepted in revised 10.12.2020 Accepted for publication 22.12.2020

DOI: https://doi.org/10.21323/2414–438X–2020–5–4–23–28

Available online at https://www.meatjournal.ru/jour
Original scientific paper

RESEARCH OF THE PHYSICAL AND CHEMICAL PROPERTIES AND METHODS OF RED RICE (FOOD COLOUR) DETERMINATION IN SAUSAGE PRODUCTS

Natalia V. Rudometova, Irina S. Kim
All-Russia Research Institute for Food Additives — Branch of V. M. Gorbato Federal Research Center for Food Systems of RAS, St. Petersburg, Russia

Keywords: *food colours, monascus, red rice, sausage products, extraction, thin layer chromatography, high performance liquid chromatography, identification, spectrophotometric analysis*

Abstract

In Russia, in the production of meat and sausage products, the food colour, named as Red rice, is use. Red rice is obtain by the cultivation the strains of the Monascus fungus on various carbohydrate substrates, for example rice. That Red rice may contain the mycotoxin citrinin, but neither the purity of the food colour nor the safety profile are regulated. The aim of this work was to study the physical and chemical properties of Red rice and to develop method for its determination in sausage products. The experiments were carried out on model and commercial samples of sausages. The samples were analyzed using spectrophotometry and high-efficiency planar and liquid chromatography. Spectrophotometric analysis revealed differences in solubility, coloring power, spectral characteristics, composition and ratio of pigments in commercial samples of Red rice. The extraction parameters of colouring substances from sausages model samples were determined. It has been established that Red rice is extracted with chloroform, acetone, ethanol and its aqueous solutions. Petroleum ether was proposed for degreasing sausages. Red rice from sausages was extracted with acetone because it did not extract synthetic colours and carminic acid. It was found, that 90% of Red rice is extracted from samples of sausages by double extraction with ultrasonic treatment with a power of 128 W. High performance thin layer chromatography method and high performance liquid chromatography method for the Red rice identification was proposed. The content of Red rice in the extracts was determined by spectrophotometric method. The developed method for the determination of Red rice was tested on commercial samples of sausage products.

Introduction

The current situation on the Russian market is characterizing by a significant amount of falsified food products and various food additives, including colours [1]. Most food colours are xenobiotics, therefore, it is necessary to control the use of colors in food production for safety reasons. In Russia red food colours Red rice, Carmines E120 and Ponceau 4R E124 are used in production of some meat and sausage products [2].

Red rice is obtained by fermenting various carbohydrate substrates, most often rice, by the strains of the fungus *Monascus*, which produce yellow and red substances: $C_{21}H_{26}O_5$ (Monascine), $C_{23}H_{30}O_5$ (Ankaflavine), $C_{21}H_{22}O_5$ (Rubropunctatine), $C_{23}H_{26}O_5$ (Monascorubine), $C_{21}H_{23}O_4N$ (Rubropunctamine) and $C_{23}H_{27}O_4N$ (Monascorubramine), whose structural formulas are shown in Figure 1 [3,4].

Various genus of the *Monascus* are used to obtain the colour: *Monascus purpureus, Monascus pilosus, Monascus anka* and *Monascus ruber.* The strain-producer and the conditions of its cultivation affect the content and composition of colour substances. But technical information about *Monascus* colours production is a commercial secret and therefore rarely published [5]. The colours are highly soluble in ethanol and slightly soluble in water. The color of its solutions depends on pH: orange at pH 3÷4, red at pH 5÷6, purple at pH 7÷9. *Monascus* colouring substances are fairly stable in 70% aqueous solution of ethanol. It is resistant to light, high temperatures and oxidation. Red rice is use in China as a food additive for over 2000 years [6].

It was found that Red rice contains the mycotoxin citrinin [7], but neither the criteria for its purity, nor the safety indicators in TR CU029/2012 are regulated, as well as the method for the determination of this food colours [2]. China is the main manufacturer and supplier of Red rice. However, the current Chinese National Standard does not set a maximum limit for citrinin in pigments produced by *Monascus*. Extensive testing of Red rice has not been conducted in the USA, but consumers are warned to avoid dietary supplements based on it due to possible myopathy and renal failure [5].

In the European Union the maximum level of citrinine (2 mg / kg) in Red rice preparations is regulate. This level ensures the possible exposure of citrinine from these drugs to well below the level of its nephrotoxicity (0.2 μg / kg body weight) [8]. However, the results of Red rice various samples study showed that the content of citrinin can reach 7 μg / kg of the product [9]. Acceptable daily intake of Red rice for humans has not been established. As the uncertainty about the carcinogenicity and genotoxicity of Red rice remains, it was decided to revise its maximum allowable level in foods [8, 10, 11].

FOR CITATION: Rudometova N. V., Kim I. S. Research of the physical and chemical properties and methods of Red rice (food colour) determination in sausages products. *Theory and practice of meat processing.* 2020; 5(4): 23–28. https://doi.org/10.21323/2414–438X2020–5–4–23–28

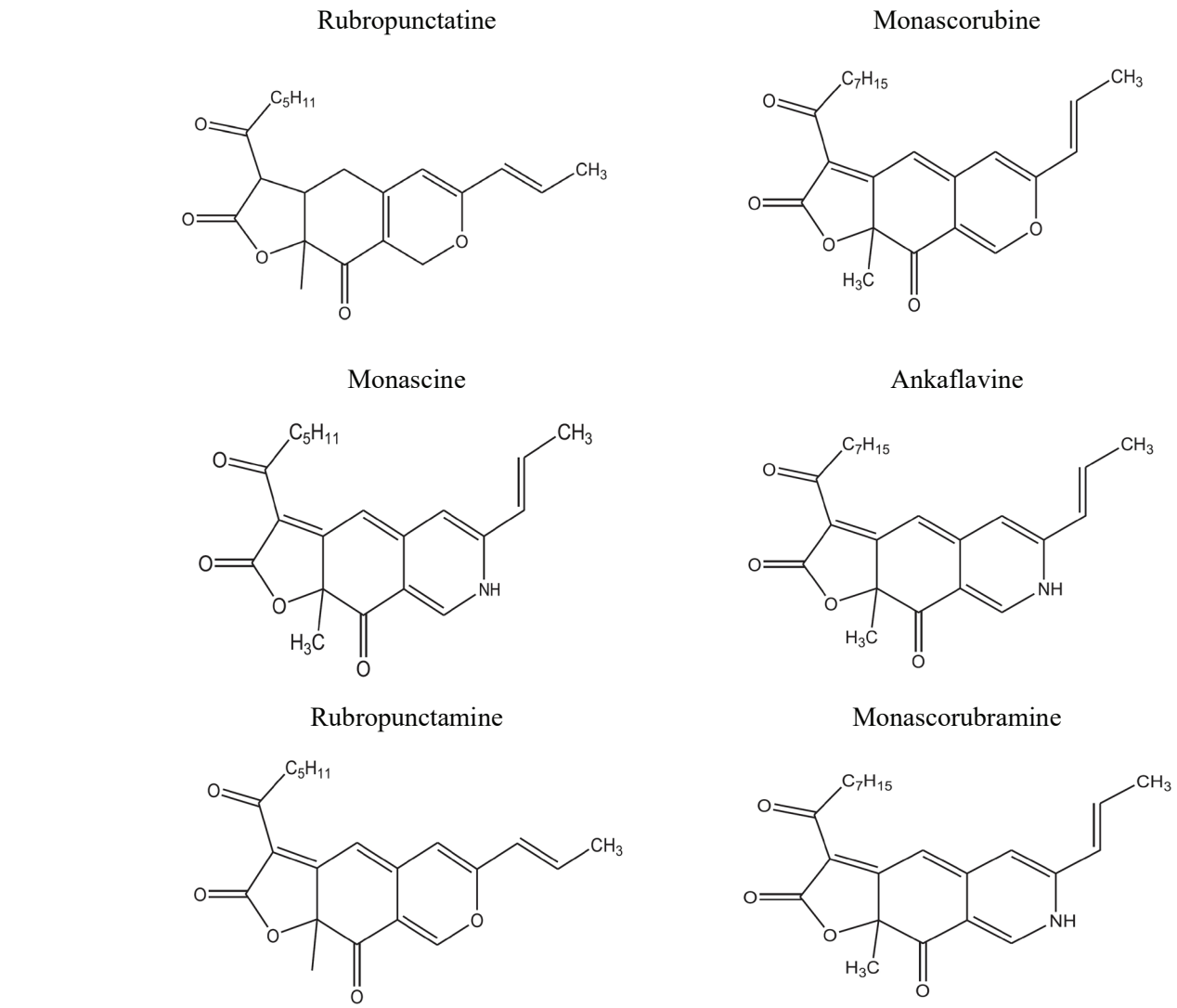


Figure 1. The structural formulas of Red rise colouring substances

The inadequate level of researching Red rice, as well as the possibility of falsification, which consists in replacing one colour with another, dictates the need to develop determination methods of colours in food products. The aim of this work was to study the physical and chemical properties of Red rice and to develop method for its determination in sausage products.

Materials and methods

The objects of the study were commercial samples of food colours Red Rice, Carmines E120, Ponceau 4R E124 and cooked smoked sausages: «Cervelat Muskatny», «Cervelat Konyachny», «Cervelat Gubernsky», which were produced in accordance with the manufacturers' specifications.

The content of colours in the samples and extracts was determined by measuring the optical density of solutions at the maximums of light absorption at characteristic wavelengths by the spectrophotometric (SPh) method. The measurement was carried out on a SHIMADZU UV-1800 double-beam scanning spectrophotometer in the wavelength range of 300–700 nm against the solvent. Mass fraction of colours in the object of study (in %) was calculated according to the formulas in the reference source [12]. The colours were identified by high performance thin layer chromatography (HPTLC) method on «Sorbfil» PET sheet plates (Table 1) and high performance liquid chromatography (HPLC) method.

Table 1. Conditions for colours assay by HPTLC method

№	Eluent composition, v/v	Values Rf for colours		
		Ponceau 4R E124	Carmines E120	Red rice
1	pyridine: 3-methyl-1-butanol: 2-methylpropanol-1: ethanol: ammonia 25% (3: 3: 3: 4: 4)	0.33 ± 0.03	0.00	0.43 ± 0.03 0.53 ± 0.03 0.64 ± 0.03 0.79 ± 0.03
2	acetone: ethanol: ammonia 25%: water (7: 3: 0.05: 3)	0.89 ± 0.03	0,00 0.49 ÷ 0.64 0.74 ± 0.03	0.76 ± 0.03 0.84 ± 0.03 0.93 ± 0.03

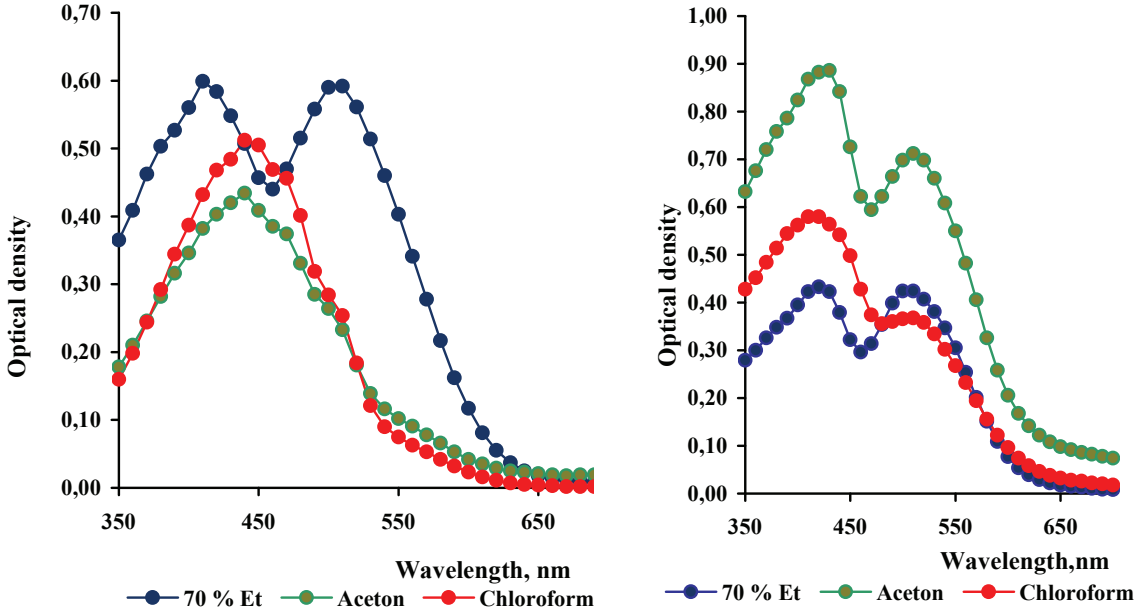
Also the colours were identified by reverse phase HPLC on a chromatograph VARIAN920 — LC with a diode array detector: column Polaris C8A 150×4.6 mm (5μm), temperature 28 °C, flow rate 0.6 cm^3/min. Eluent is a mixture of 0.02 M solution of sodium acetate and acetonitrile in a volume ratio of 90:10. Sample volume is (10 ÷ 50) mm^3, detector wavelength at 500 nm, time of analysis is 10 minutes. The samples were filtered through a filter with a pore diameter of 0.45 μm. The obtained chromatograms were processed using the GALAXIE program.

Sample preparation for the determination of colours in cooked smoked sausages was carried out in accordance with GOST R ISO 13496–2013. Samples were defatted with chloroform or petroleum ether and centrifuged. The colours were extracted from defatted cooked smoked sausages with constant stirring at a temperature from 20 °C to 60 °C and varying the extraction time from 10 to 60 min. Extraction was performed in a stirring device with a rotation speed 120 rpm, in a Bandelin Sonorex ultrasonic bath at a power of 128 W and 160 W, and on a magnetic stirrer with a stirrer rotation speed of 400 to 1200 rpm. For extraction we used: chloroform, petroleum ether, cyclohexane, hexane, acetone, ethyl alcohol, its aqueous solutions with a mass fraction of ethyl alcohol 50% and 70%, as well as mixtures of these solvents with the ratio sample: extractant 1:4. The resulting extracts were centrifuged for 15 minutes at 6000 rpm. In the supernatant the colours content was determined by the spectrophotometric method.

All experimental measurements were performed three times. Analysis of variance of the obtained data was carried out by Microsoft Excel with a significant difference at *P* 0.05. Graphical dependencies were obtained using Microsoft Excel software.

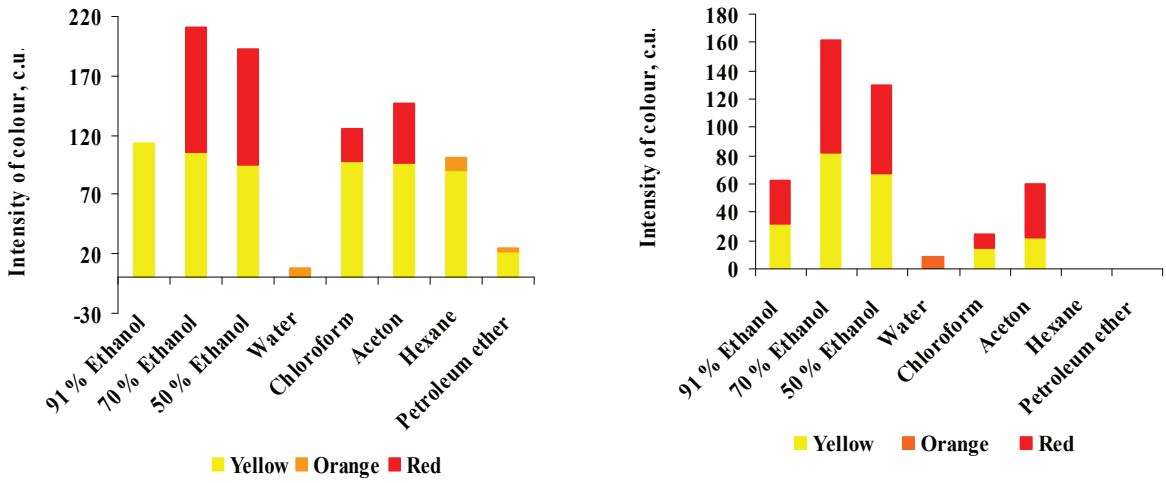
Results and discussion

SPh analysis of commercial samples of Red rice, carried out in accordance with the method [12], shows that the samples differ in their coloring power, spectral characteristics (Figure 2), composition and quantitative ratio of pigments (Figure 3). The pigment content varied from



Sample 1 Sample 2

Figure 2. Absorption spectra of different Red rice commercial samples



Sample 1 Sample 2

Figure 3. Colour composition of different Red rice commercial samples

(60 ± 2)% to (90 ± 2)%. Experiments show that chloroform and acetone extract all of the Red rice coloring substances. Petroleum ether extracts only a small amount of yellow and orange pigments, which will not affect the subsequent identification of the Red rice. Therefore, the petroleum ether is the best for degreasing of the sausages. From the reference source it is known that the isolation of Red rice from various matrices is carried out with water-ethanol solutions at the temperature range from 30 °C to 60 °C [8, 11, 13]. However, a study of the solubility of colours show, that Carminic acid and Ponceau 4R, as well as Red rice, dissolve in water-ethanol solutions, and these colours have very similar spectral characteristics (Figures 4, 5).

Carminic acid and Ponceau 4R is not extracted in acetone, therefore, it was proposed to isolate Red rice from defatted sausages with acetone.

The extraction process of colours was studied on model samples. Analysis of the sample «Cervelat» Muscatny» showed that it does not contain any colours, therefore, model samples were made on its basis. Colours, celite or sodium sulfate, or quartz sand were added to the model samples. Then the sample was triturated until it became homogeneous. It was shown that celite and sodium sulfate was adsorbed colours irreversibly. Therefore, quartz sand was chosen for grinding the samples. It's known, that ultrasonic action is increasing the extraction of different substances from plant and animal matrices [14, 15]. The results (Figure 6) showed that 90% of the Red rice was extracted from the samples in two stages of extraction with ultrasonic treatment.

According to the reference sources, the quantitative determination of Red Rice, as a rule, is carried out by SPh and HPLC methods [3,4,5,7,8,9,10,16,17,18]. Based on the results of the identification of colours by HPTLC, two elution systems were selected (Table 1). These eluents correctly identify Red rice in the presence of synthetic colours and Carmine E120 (Figure 7).

According to the experimental data obtained, proposed HPLC method also allows correct identification of colours in sausage products (Figure 8). The retention time of the colours was (4.5 ± 0.2) min for Carminic acid, for Ponceau 4R — (7.3 ± 0.2) min, for Red rice — (3.3 ± 0.2) min. Thus, the methods of identification of Red rice, Carmines and Ponceau 4R with their possible simultaneous presence in the analyzed sample have been determined.

The developed method was tested on samples of boiled smoked sausages "Cervelat" Konyachny" and "Cervelat "Gubernsky". Food colours Carmine E120 in the amount of (4.0 ± 0.4) mg/kg and Red rice in the amount of (32 ± 3) mg/kg were found in the sample "Cervelat" Konyachny ". No colours were found in the sample "Cervelat" Gubernskiy ". Sample analysis results are consistent with the information on the sample label.

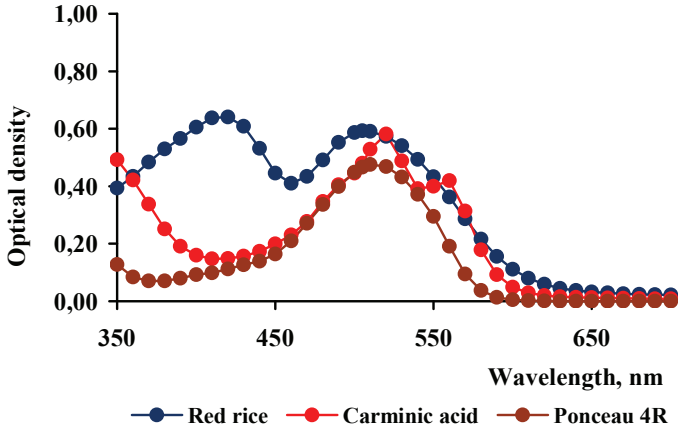


Figure 4. Absorption spectra of colours in 50% water-ethanol solution

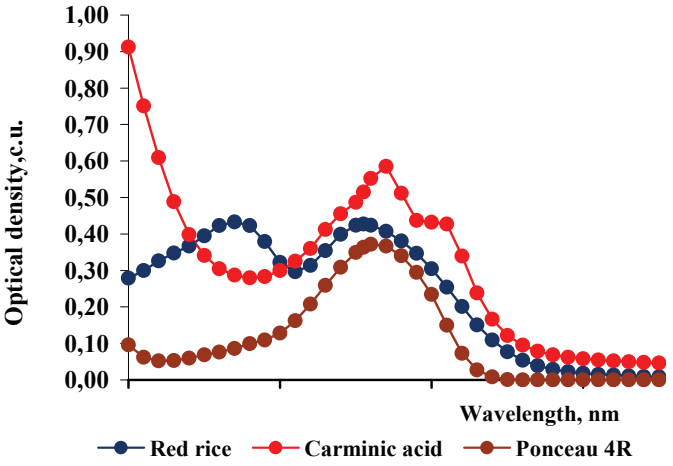
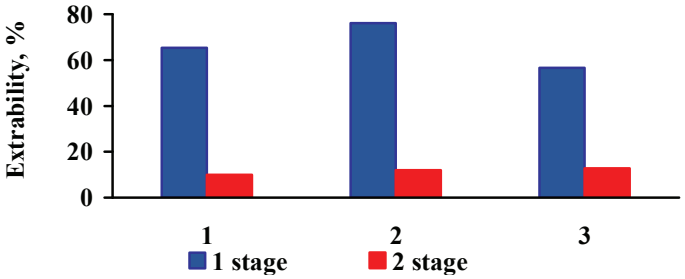


Figure 5. Absorption spectra of colours in 70% water-ethanol solution



1 — without ultrasonic treatment,
2 — ultrasonic treatment with a power of 128 W,
3 — ultrasonic treatment with a power of 160 W

Figure 6. Influence of ultrasonic treatment on the acetone extraction of Red rice from model samples

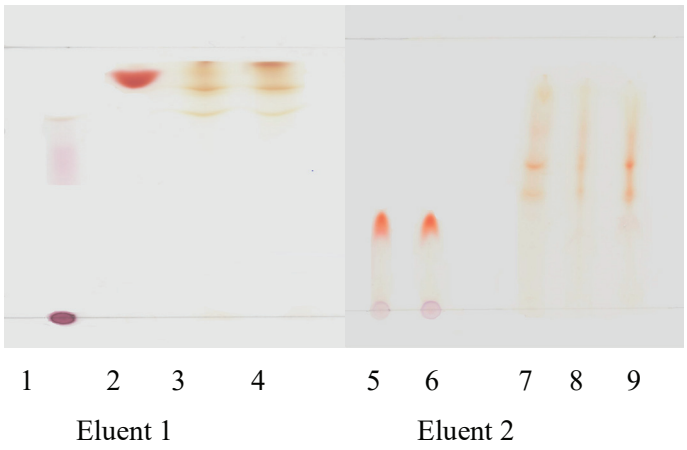


Figure 7. The identification results of Carminic acid (1), Ponceau 4R (2), Red rice (3,4,7,8,9) and a mixture of Carminic acid and Ponceau 4R (5,6) obtained by HPTLC method

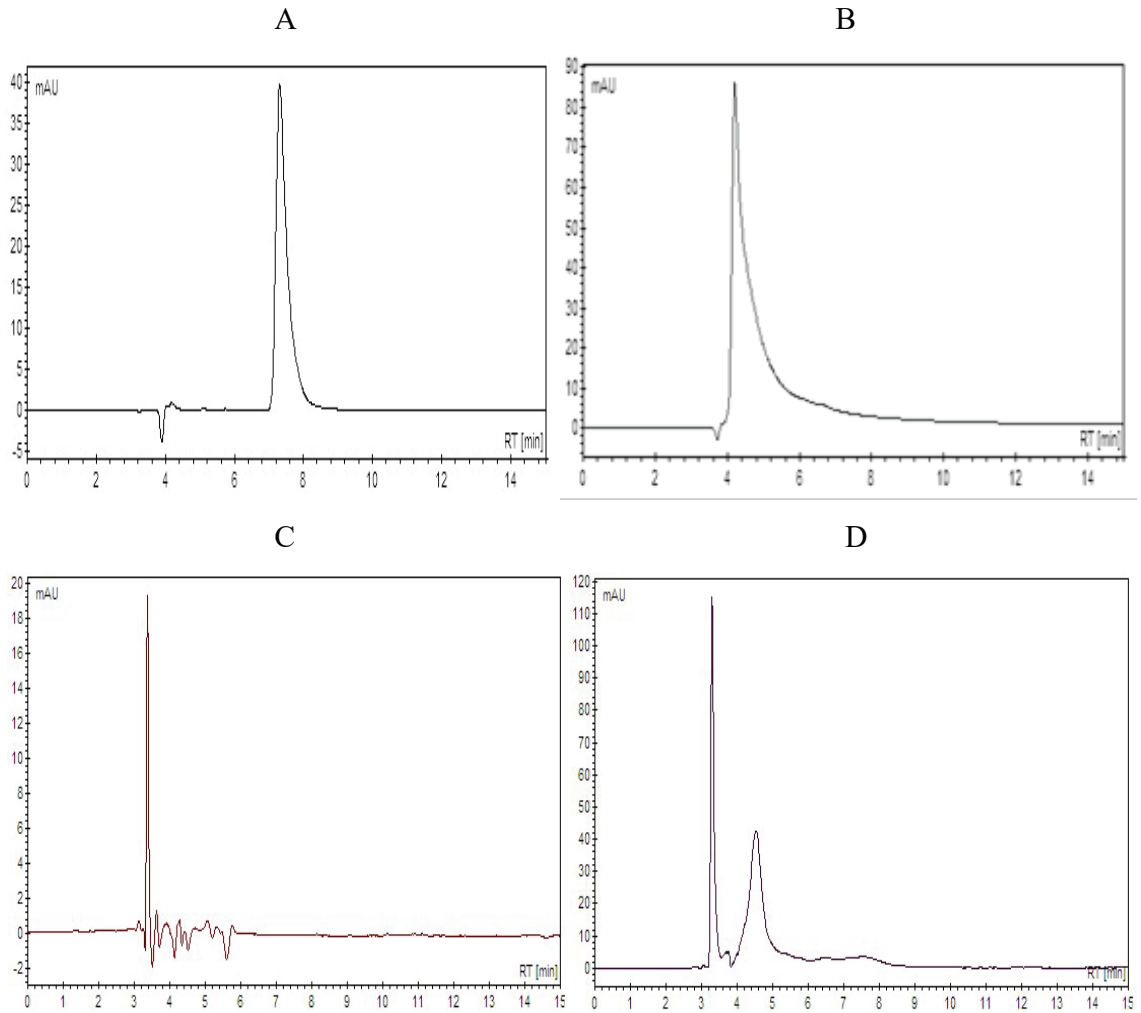


Figure 8. The identification results of Ponceau 4R (A), Carminic acid (B), Red rice (C) and a mixture of Carminic acid and Red rice (D), obtained by HPLC method

Conclusion

The process of extracting food colors has been studied on model samples of cooked smoked sausages. The extraction parameters of Red rice from sausages are determined. Spectrophotometric analysis revealed differences in solubility, coloring power, spectral characteristics, composition and ratio of pigments in commercial samples of Red rice. It is proposed to use petroleum ether for defatting sausages, and acetone for the extraction of Red rice. Methods of spectrometric and chromatographic analysis have been developed to determine the Red rice in cooked smoked sausages. The developed method was tested on commercial samples of cooked smoked sausages.

REFERENCES

1. Rudometova, N.V. (2014). Food additives and colorants: methodological support of product safety. *Production quality control*, 8, 13–19.
2. TR TS029/2012 Customs Union Technical Regulations "Safety requirements of food additives, flavorings and processing aids" [Electronic resource: http://www.eurasiancommission.org/ru/act/texnreg/deptexreg/ tr/Pages/bezopPischDobavok.aspx / Access date 09.09.2020]
3. Agboyibor, C., Kong, W.-b., Chen, D., Zhang, A.-m., Niu, S-q. (2018). Monascus pigments production, composition, bioactivity and its application: A review. *Biocatalysis and Agricultural Biotechnology*, 16, 433–447. https://doi.org/10.1016/j.bcab.2018.09.012
4. Mhalaskar, S.R., Thorat, S.S., Deshmukh, Y.R. (2017). Standardization of fermentation parameters for the production of food bio-colours through submerged bio-reactor fermentation. *Ecology, Environment and Conservation*, 23(1), 211–216.
5. Srianta, I., Ristiarini, S., Nugerahani, I., Sen, S. K., Zhang, B. B., Xu, G. R., Blanc, P. J. (2014). Recent research and development of monascus fermentation products. *International food research journal*, 21(1), 1–12.
6. Hendry, G.A.F., Houghton, J.D. (1996). Natural food colorants. Blackie Academic & Professional. — 350 p. ISBN978–0–7514–0231–5, https://doi.org/10.1007/978–1–4615–2155–6
7. Xu, B.-j., Jia, X.-j., Gu, L.-j., Sung, C.-k. (2006). Review on the qualitative and quantitative analysisof the mycotoxin citrinin. *Food Control*, 17(4), 271–285. https://doi.org/10.1016/j.foodcont.2004.10.012
8. Commission Regulation (EU) № 212/2014 of 6 March 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of the contaminant citrinin in food supplements based on rice fermented with red yeast Monascus purpureus. (2014). *Official Journal of the European Union*, 67/3.
9. Priatni, S., Damayanti, S., Saraswaty, V., Ratnaningrum, D., Singgih, M. (2014). The Utilization of Solid Substrates on Monascus Fermentation for Anticholesterol Agent Production. *Procedia Chemistry*, 9, 34–39. https://doi.org/10.1016/j.proche.2014.05.005
10. Kumari, H. P. M., Naidu, K. A., Vishwanatha, S., Narasimhamurthy, K., Vijayalakshmi, K.G. (2009). Safety evaluation of monascus purpureus red mould rice in albino rats. *Food and chemical toxicology*, 47(8), 1739–1746. https://doi.org/10.1016/j.fct.2009.04.038

11. Dikshit, R., Tallapragada, P. (2014). Collective effects of stress on optimization of pigment production by monascus purpureus. *Chiang mai journal of science*, 41(3), 524–530.
12. Food additives code. Ministry of Food and Drug Safety Regulation (2019). [Electronic resource: http://extwprlegs1.fao.org/docs/pdf/kor190402.pdf /Access date 09.09.2020.]
13. Velmurugan, P., Kamala-Kannan, S., Balachandar, V., Lakshmanaperumalsamy, P., Chae, J. — C., Oh, B.-T. (2010). Natural pigment extraction from five filamentous fungi for industrial applications and dyeing of leather. *Carbohydrate Polymers*, 79(2), 262–268. https://doi.org/10.1016/j.carbpol.2009.07.058
14. Kalinina, I.V., Fatkullin, R.I. (2016). Implementation of effects of ultrasonic cavitation influence as a factor of intensification of extraction of functional elements. *Bulletin of the South Ural state university. Series: food and biotechnology*, 4(1), 64–70.
15. Rudometova, N. V., Kim, I.S. (2019). Capsaicin extraction from hot pepper of capsicum genus. *Processes and food production equipment*, 1, 62–73. https://doi.org/10.17586/2310–1164–2019–12–1–62–73
16. Singgih, M., Saraswaty, V., Ratnaningrum, D., Sri Priatni, S., Damayanti, S. (2014). The influence of temperature and ethanol concentration in monacolin K extraction from monascus fermented rice. *Procedia Chemistry*, 9, 242–247. https://doi.org/10.1016/j.proche.2014.05.029
17. Liang, B., Du, X.-J., Li, P., Sun, C.-C., Wang, S. (2018). Investigation of Citrinin and Pigment Biosynthesis Mechanisms in Monascus purpureus by Transcriptomic. *Frontiers in Microbiology*, 9. https://doi.org/10.3389/fmicb.2018.01374
18. Maľa, P., Baranová, M., Marcinčáková, D., Nagy, J. (2010). Organoleptic evaluation of poultry meat products with wheat protein — seitan, coloured by microbial natural pigment. *Assam university journal of science and technology: biological and environmental sciences*, 5, 1–5.

AUTHOR INFORMATION

Natalia V. Rudometova — candidate of chemical sciences, leading research scientist, laboratory of physical and chemical research methods, All-Russia Scientific Research Institution for Food Additives — Branch of V. M. Gorbatov Federal Research Center for Food Systems of RAS, 191014, Sankt-Petersburg, Liteyniy Prospect, 55. Tel. +7–812–272–75–78. E-mail: natrudjob@mail.ru
ORCID: https://orcid.org/0000–0002–9414–6806
* corresponding author

Irina S. Kim — junior research scientist, laboratory of physical and chemical research methods, All-Russia Scientific Research Institution for Food Additives — Branch of V. M. Gorbatov Federal Research Center for Food Systems of RAS, 191014, Sankt-Petersburg, Liteyniy Prospect, 55. Tel. +7–812–272–56–26. E-mail: vniipakk55@mail.ru

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

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

The authors declare no conflict of interest.

Received 09.09.2020 Accepted in revised 06.12.2020 Accepted for publication 20.12.2020

DOI: https://doi.org/10.21323/2414–438X–2020–5–4–29–34

Available online at https://www.meatjournal.ru/jour
Original scientific paper

A CALCULATION MODEL FOR THE HEAT CAPACITY OF BEEF WITH DIFFERENT MOISTURE DURING FREEZING TAKING INTO ACCOUNT FREE WATER CRYSTALLIZATION

Anton G. Belozerov[1], Yuriy M. Berezovskiy[2],* Igor A. Korolev[2], Taras A. Sarantsev[2]
[1] V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia
[2] All-Russian Scientific Research Institute of Refrigeration Industry — Branch of V. M. Gorbatov Federal Research Center for Food Systems of RAS, Moscow, Russia

Keywords: *beef freezing, free water, water crystallization model, model parameters, beef heat capacity calculation*

Abstract
The paper proposes a model for the process of free moisture crystallization in beef within the framework of the Debye concept with establishment of dependencies of model parameters on the initial moisture content. Model adequacy was validated by comparison of the calculation results with the results of the experiments on determination of values of heat capacity and phase transition enthalpy in beef with different initial moisture obtained by the differential scanning calorimetry method. It is shown that the end of free water phase transition in beef with initial moisture in a range of 37% to 80% occurs at a temperature of 243 K. Calculation dependencies of parameters of the model used for calculation of beef heat capacity are presented.

Introduction

The knowledge of product thermophysical properties is extremely important in designing and realization of processes of their transportation, storage and technological processing linked with freezing and thawing. This determines a large number of studies conducted by several foreign and national scientists [1,2,3,4,5,6,7,8,9,10,11,12,13] and summarized largely in [14]. Meat is one of such products. Meat freezing is accompanied by crystallization of water contained in it. From the viewpoint of studying heat exchange processes in meat refrigerated processing, it is conventional to classify water contained in it into free and bound. There is still no strict definition of the term "bound water" [13]. It is noted that bound water does not freeze at a temperature of minus 40 °C. It is shown in [2,15] that freezing of free water and, consequently, crystallization are ended when refrigerating at minus 30 °C (beef) and minus 31 °C (pork) (243.15 K and 242.15 K, respectively). Riedel pointed at this fact for the first time by the example of beef [2].

When studying the process of phase transition, the basic thermophysical characteristics of a product are the cryoscopic temperature T_f and initial moisture content w. At the cryoscopic temperature in a range of 273 K to 268 K, the crystallization process is accompanied by the release of 90% of latent heat of crystallization [12]. Specific isobaric heat capacity measured in this area is a sum of heat capacities: true heat capacity [11] and heat capacity conditioned by released latent heat of crystal formation [15].

It is generally agreed that the most reliable results are obtained upon measurement using a low-temperature adiabatic vacuum calorimeter [8,16,17]. Figure 1 presents a graph of the dependence of beef heat capacity measured using such instrument according to the data obtained by Latyshev [8].

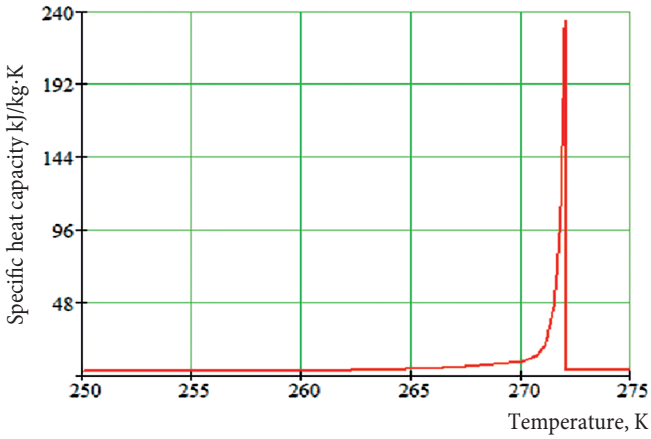


Figure 1. Dependence of beef specific heat capacity measured using a low-temperature adiabatic vacuum calorimeter [8]

Peculiar features of the indicated measurements are discreteness and duration of the measurement process upon the absence of the possibility to manage the rate of refrigeration. Due to this, discreteness of results, as a rule, is equal to or higher than 1 K.

The studies appeared that noticed the significance of the effect of physico-chemical processes occurred in meat in a range of the subcryoscopic temperatures ($T_{kr} \pm 0.5$ K) on its consumer properties [18]. For such investigations of meat refrigeration in a narrow range of the subcryoscopic temperatures, higher frequency of temperature measurement using the DSC method is necessary [16,17,19].The use of the differential scanning calorimetry method allows minimizing discreteness for specific heat capacity calculation.

FOR CITATION: **Belozerov G. A., Berezovskiy Y. M., Korolev I. A., Sarantsev T. A. A calculation model for the heat capacity of beef with different moisture during freezing taking into account free water crystallization. *Theory and practice of meat processing.* 2020; 5(4): 29–34. https://doi.org/10.21323/2414–438X-2020–5–4–29–34.**

At present, however, there is no method that enables predicting the character of meat heat capacity dependence on a temperature in a range of the phase transition temperatures.

Several studies of the crystallization process consider two phenomena: the crystal growth and diffusion of the intercrystalline liquid phase to the crystal surface. With that, they analyze the crystal growth rate, which changes the sizes of channels between them and hydrodynamics of the intercrystalline liquid flow. This approach is realized in [20,21,22]. The calculated dependencies presented in these studies are markedly inconsistent with the data obtained using an adiabatic calorimeter [8]. A significant difference in the nature of these processes from meat free water crystallization does not allow using any elements of these studies in this research.

The aim of this research is to develop a model for crystallization process based on the Debye concept, which enables predicting by calculation the specific isobaric heat capacity depending on a temperature and initial moisture as applied to beef freezing.

Materials and methods

The development of a model for free water crystallization in beef is based in this study on the publication [19], in which the authors (being also the authors of the present paper) showed the possibility to use the Debye concept by the example of NOR beef. The dependence has the following form:

$$c = 3\mu \cdot N \cdot k \cdot \left(\frac{\theta}{T_{kr} - T + \delta} \right)^2 \cdot e^{-\left(\frac{\theta}{T_{kr} - T + \delta} \right)} + B \cdot 10^{-3} \cdot T, \qquad (1)$$

where

c is meat specific heat capacity, kJ/kg·K;

3 is the nondimensional coefficient;

$\mu \cdot N$ is the number of crystallization centers in a meat sample;

$N = 10^{25}$ kg^{-1} is the order of determining the number of crystallization centers;

$\mu = 0.0725 \div 1.035$ is the coefficient that depends on the sample moisture content;

T_{kr} is the beef cryoscopic temperature (the temperature of the beginning of free water freezing with crystal formation);

$\theta = h\nu/k$, K is the characteristic temperature;

h is the Planck constant, $h = 6.626 \cdot 10^{-34}$ J · s;

ν is the vibration frequency of atoms in a crystal, s^{-1};

k is the Boltzmann constant, J/K; $k = 1.38 \cdot 10^{-23}$ J/K;

T is Kelvin temperature;

δ is the coefficient corresponding to a deviation of the temperature of the water crystallization onset in the process of transformation into ice from the temperature of the heat capacity peak in the process of phase transition, K;

B is the coefficient characterizing the contribution of heat capacity of components not containing free water, $B = 7.5 \cdot 10^{-3}$ kJ/kg·K.

By investigating beef heat capacity in a wide range of moisture (37% — 75%), the possibility to use dependence (1) to calculate specific isobaric heat capacity using the calculated dependencies of the parameters μ, θ, δ and T_{kr} obtained below is shown in this paper.

The most important characteristic of the crystallization process is the cryoscopic temperature T_{kr}. Methods for measuring this parameter are given in [15, 19]. For beef, the temperature, when free water is finally frozen out, is 243 ±0.25 K (–30.15 ±0.25 °C), which corresponds to the end of free water phase transition in beef. The above mentioned paper [19] presents dependence (2) by the results of the experimental study of a decrease in heat capacity of a frozen sample lower than the temperature of the end of phase transition.

$$c_{fr.b.} = 0{,}548 + 1{,}85 \cdot 10^{-3} \cdot T + 1.68 \cdot 10^{-5} \cdot T^2, \qquad (2)$$

where $c_{fr.b.}$ is specific heat capacity of frozen beef not including heat capacity determined by latent heat of crystallization (melting) kJ/kg · K.

It is necessary to note that the intersection point of the phase transition curve (1) with curve (2) corresponds to the end of moisture crystallization process in beef. Fulfilment of the indicated statement by dependence (1) for different moisture levels in beef can be another criterion of the model adequacy to the real process of phase transition.

The experimental base of this study aimed at validation of the dependence (1) adequacy are the results of the detection of heat capacity of beef with different moisture content obtained by the differential scanning calorimetry (DSC) method using a DSC204 F1 NETZSCH instrument.

Detection of phase transition enthalpy in beef freezing by the indicated method ensured the error of not more than ±3%. When processing the results of the DSC experiments with the method of τ — R correction [17], dependencies of beef heat capacity on temperature were practically in the complete agreement with the data on heat capacity obtained using the adiabatic instrument by Latishev at a meat moisture level of 74.1% [8].

Latent heat of water crystal formation in the samples was calculated as the integral difference of total heat capacity of the sample $c_{TS.S}$ and specific heat capacity of the frozen sample $c_{fr.b.}$ by (2):

$$\Delta H_{LH} = \int_{T_1}^{T_2} (c_{TS.S} - c_{fr.b.})\, dt \qquad (3)$$

where:

ΔH_{LH} — enthalpy (latent heat) of crystallization of free water in a beef sample, J/kg;

T_1, T_2 are temperatures of the beginning and end of the melting peak, respectively ($T_1 = 243$ K; $T_2 = T_{kr}$ is the cryoscopic temperature) °C;

$c_{fr.b.}$ is the line determined by the values of beef specific heat capacity by (2);

$c_{TS.S}$ is the line that characterizes total (effective) specific heat capacity of a sample.

The method of heat capacity determination proposed in the work [19] in correspondence with the concept of heat capacity by Debye can be used for analysis of the association of

obtained dependency (1) parameters with indicators of the freezing regime and different meat initial moisture.

Different moisture content in the samples was achieved by freeze drying, after which they were placed into a crucible for DSC measurements. After DSC measurements of heat capacity values, the crucibles were opened and moisture of the samples was determined by drying in a thermostat at an air temperature of 100oC and the following weighing.

Values of the cryoscopic temperature necessary for investigations were detected using an osmometer-cryoscope OSCR-1. The instrument was entered into the RF State Register of measuring instruments under the number of 42519-09. The technical characteristics are given in Table 1.

The absolute thermodynamic scale, according to which $T = 273.15\ K + t\,°C$ [14], is used in the work.

Table 1. Main specifications of OSKR-1

Parameter	Error
Range of freezing temperature measurement:	**0 to –3.720 °C**
Limits of allowable fundamental absolute error in temperature measurement	
— in the range of 0 to –0.930 °C:	**±0.002 °C**
— in the range of –0.930 to –3.720 °C:	**±0.010 °C**
Sample volume, not less than:	**0.3 ml**

The development of the model of heat capacity by (2) in dependence on a temperature and moisture of the studied beef sample is realized by selection of the model parameter investigation depending on the initial moisture by minimizing the integral dependencies (3) and (4). The results are given in table 2, as well as in the form of polynomial dependencies (6–10). The final correction of correspondence of the calculated values of beef specific heat capacity to the values obtained by the empirical way is carried out using the coefficient B.

The parameter T_{kr} was determined using the above mentioned instrument with account for the instrument error and random errors with the overall error of ± 0.05 K.

The parameters μ; θ; and δ were found based on the following considerations:

1 — It has been noticed that the parameter μ is determined by the maximum value of the peak of the experimental curve of heat capacity, which enables using this parameter as a reference point when determining the parameter μ. The sequence of these points obtained at different moisture levels in beef can be described as a polynomial of type (8), Figure 3.

2 — The characteristic temperature θ, as was shown above upon its definition, depends on the character of heat removal (the phonon flow according to Debye with the frequency of $\nu \sim 10^{11}$); with that, the value of the parameter θ increases with reduction of the water content in a sample; several reference points of the parameter θ value for the experimental curves of beef heat capacity allowed obtaining the empirical polynomial dependence of the parameter on moisture (9), Figure 6.

3 — The parameter δ (see the definition above). This value practically compensates errors in the measurement of the cryoscopic temperature and corrects the position of the peak of the heat capacity curve in the area of phase transition. The sequence of values of this parameter obtained upon correction by bringing into proximity the position of the empirical peak of the phase transition curve to the calculated one by dependence (2) is approximated by dependence (10), Figure 5.

Verification of the obtained calculated expressions of specific heat capacity for samples with all moisture levels was carried out by several criteria:

1 — minimization of the difference between the calculated and experimental values of phase transition enthalpy; that is, minimization of the difference between the results of the calculation by (3) and calculation of enthalpy using dependencies (1) and (2):

$$\Delta H_{LH}^{exp} - \Delta H_{LH}^{calc} = \int_{T_1}^{T_2} (c_{TS.S} - c_{fr.b.})\,dt - \int_{T_1}^{T_2} (c_1 - c_2)\,dt, \qquad (4)$$

where: c, $c_{fr.b}$ are specific heat capacities calculated by dependencies (1) and (2).

2 — minimization of the difference between the sequence of measurement results for specific heat capacity by the DSC method and by equation (1).

$$\Delta c = c_1 - F(T_{DSK}), \qquad (5)$$

See Figure 2. The experimental curves of heat capacity $F(T_{DSK})$ are marked in the figure by the numbers with the 'e' index.

Results and discussion

In the final form, the mathematical model of free water crystallization upon beef freezing is a system of equations (1), (2), (6–10) plus correcting equation (11).

Approximating dependencies of the parameters T_{kr}, ΔH, μ, θ, δ on the initial moisture content in beef have the following form (6–10):

$$T_{kr}(w) = 257.1 + 34 \cdot w - 18 \cdot w^2;\ \Delta_{error} \le 0{,}1\% \qquad (6)$$

$$\Delta H_{LH} = L \cdot w \cdot (1 - 0{,}35 \cdot (1 - w)/w);\ \Delta_{error} \le 5\% \qquad (7)$$

$$\mu(w) = 0{,}014 + 1{,}85 \cdot w^4 + 3 \cdot w^5;\ \Delta_{error} \le 14\%; \qquad (8)$$

$$\theta(w) = 3{,}66 - 4{,}35 \cdot w;\ \Delta_{error} \le 20\% \qquad (9)$$

$$(w) = 1{,}94 - 2{,}25 \cdot w;\ \Delta_{error} \le 10\%. \qquad (10)$$

It is necessary to note that the error of dependence (7) in the area of low moisture levels (< 60%) is ± 5%, in the area of >60% of moisture, the deviation of calculated values is ~3%. The use of dependence (7) by Riedel [2] to calculate beef enthalpy is significantly easier than the use of dependencies (1 and 2) without decreasing accuracy.

Dependencies of beef heat capacity by equation (1) using the experimental data presented in Table 2 are given in a form of graphic dependencies in Figure 2. It is necessary

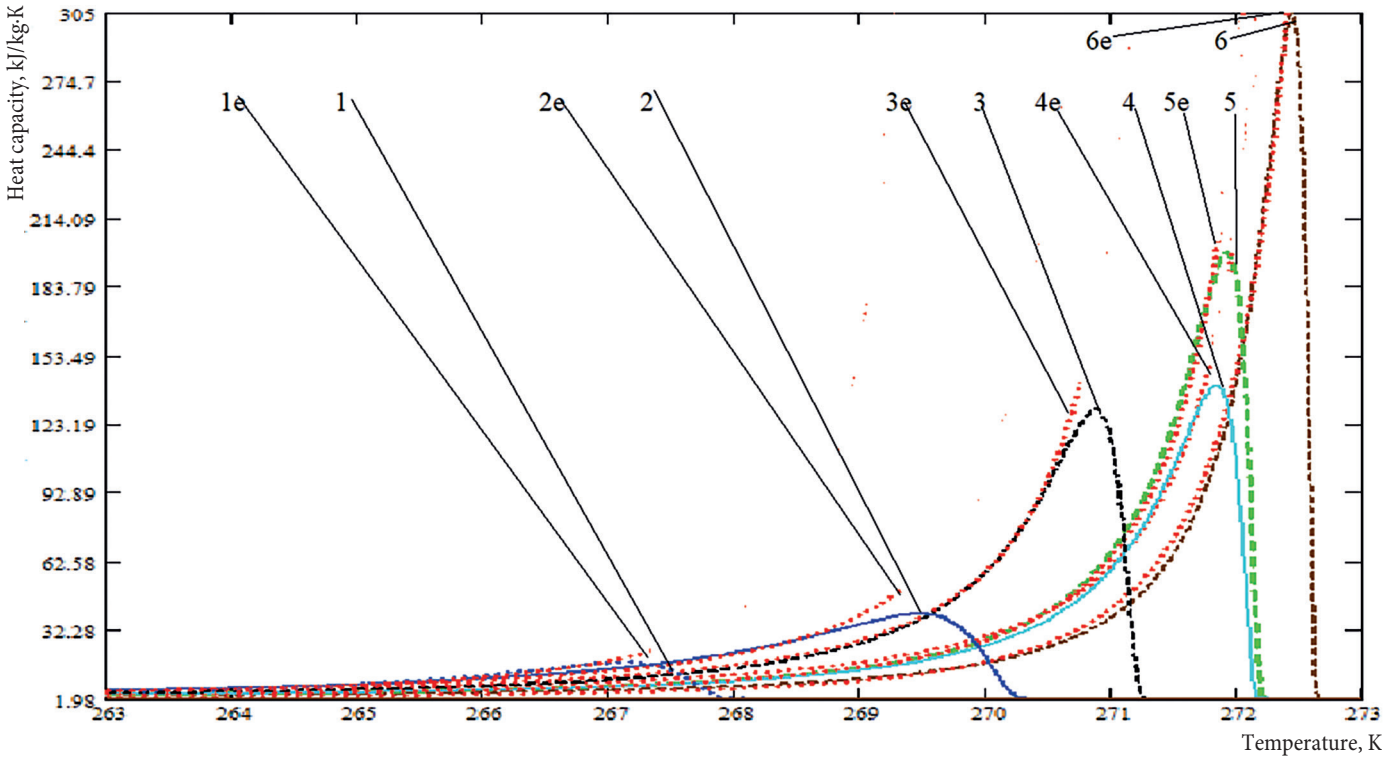


Figure 2. Dependencies of beef heat capacities at different initial moisture levels presented in Table 2

Table 2. Moisture, cryoscopic temperature, enthalpy and parameters μ, θ, δ of the equation of phase transition in beef samples

Number of meat samples	Initial beef moisture w, mass fraction	T_{kr} ±0.05 K	H_{LH}, by (3) (exp.) kJ/kg	H_{LH} (calc.) by (1), kJ/kg	H_{LH}, (calc.) by (5) kJ/kg	μ, non-dimensional	θ, K	δ, K
1	0,370	267,14	45	44.998	49,843	0,0725	2,130	1,100
2	0,450	269.14	91	90,136	85,850	0,2980	1,800	0,96
3	0,600	270,73	interp. 155	155,420	153,364	0,6300	0,920	0,600
4	0,651	271.84	168,5	169.837	169,837	0,7130	0,820	0,400
5	0,700	271,85	198	198.213	198,213	0,8780	0,710	0,416
6	0,751	272,4	220	220.07	220,007	1,0350	0,497	0,28

to take into account that all calculations are true in a range of 243 K ≤ T_{kr} K.

Numbers of curves correspond to the sequential numbers of the rows in Table 2; with that, the numbers with the "e" index correspond to approximations of the experimental values of heat capacities and without the index to calculated values by equation (1).

Figures 3–7 present the graphs of dependencies (6–10) with account for data of Table 2.

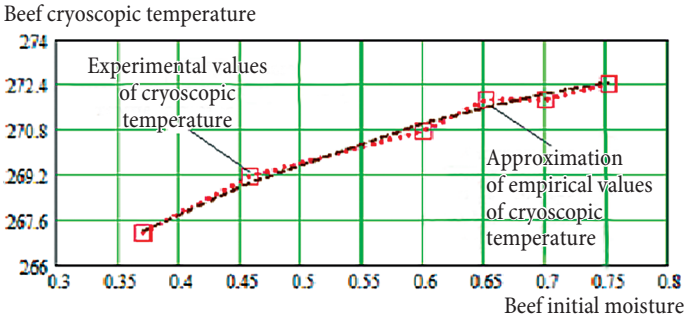


Figure 3. Dependence of the beef cryoscopic temperature by the experimental results and by approximating equation (6)

Upon condition of postulation of the circumstance that at the temperature of the end of phase transition all curves by equation (1), which were calculated for different moisture levels by equations (6–10), should converge in one intersection point at T_{kr} = 243 K with the freezing curve of a beef frozen sample (11) (Figure 8), calculated by equation (2), it is necessary to assign a value to constant B that ensures the condition of the postulate in equation (1) for each moisture level. Table 3 gives these values and the polynomial approximation of the set of values (11).

Table 3. Adjusted values of the parameter B for equation (1)

w	0.37	0.45	0.600	0.625	0.700	0.752
B·10⁻³. J/kg·K	7.55	6.911	7.197	7.385	7.325	7.557

$$D(w) = 9{,}125 - 8{,}5w + 8{,}5w^2, (\Delta_{error} \pm 5\%) \quad (11)$$

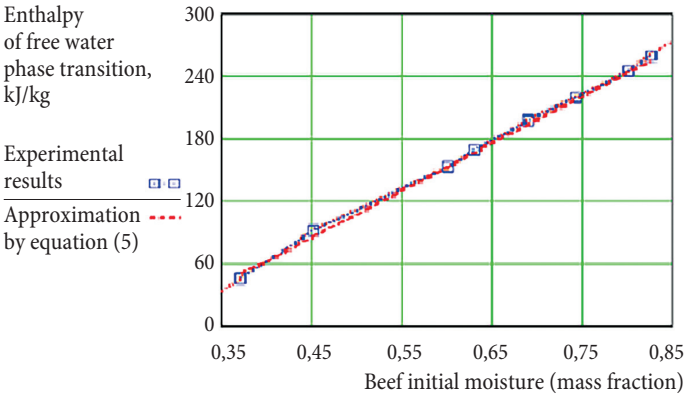


Figure 4. Dependence of enthalpy of phase transition (crystal formation) on the initial moisture of the beef sample by equation (7)

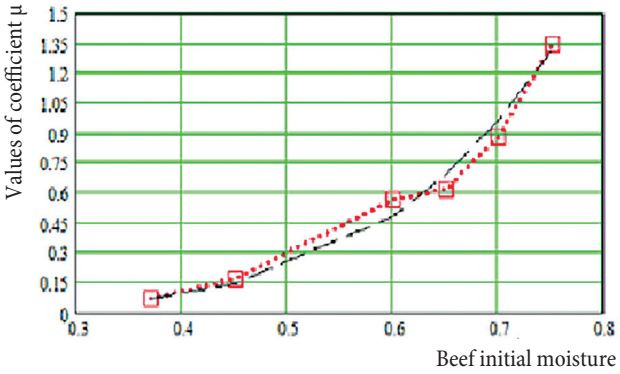


Figure 5. Dependence of the coefficient μ, which characterizes the number of crystals in the mass unit of freezing water, on the initial moisture content by equation (8)

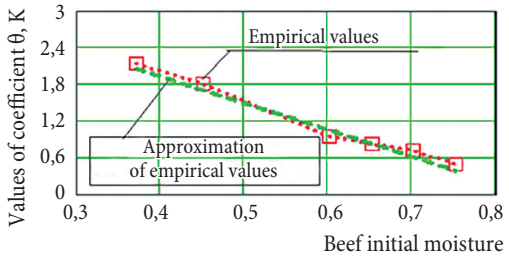


Figure 6. Dependence of the characteristic temperature θ on the initial moisture of beef sample, approximation by equation (9)

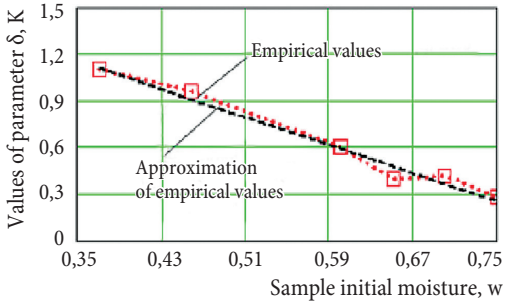


Figure 7. Empirical values of the parameter δ and their approximation depending on the beef initial moisture by equation (10)

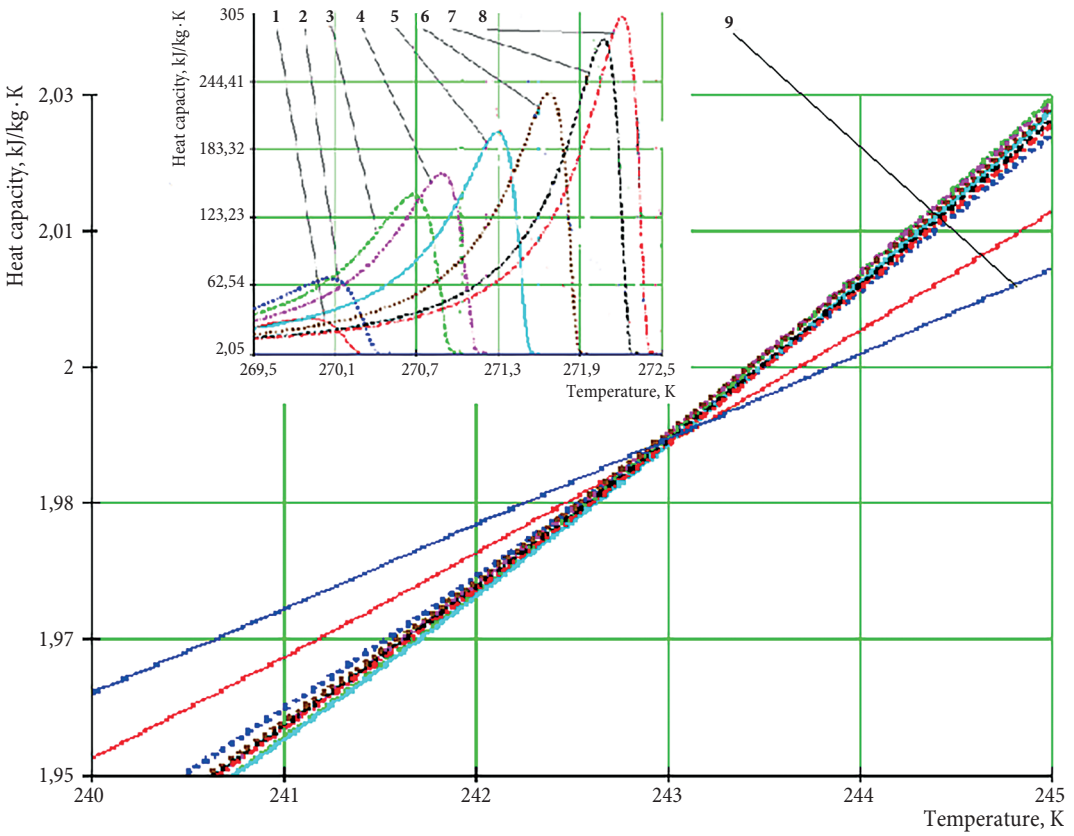


Figure 8. The intersection point of phase transition curves according to equation (1) with account for dependencies (6–10) with freezing curve of beef frozen sample (11) by equation (2)

Conclusion

The calculated model was developed for the beef freezing process in a range of temperatures of free water phase transition realized by the way of crystallization described by the system of equations (1–2, (6–11), linking beef specific heat capacity, temperature and initial moisture.

The proposed model allows predicting beef heat capacity values in a range of the most energy-intensive freezing process.

The development method can be used for similar computational simulation of freezing processes for other meat raw materials and semi-finished products as well as fish.

REFERENCES

1. Eucken, A. (1940). Thermal conductivity of different types of substances and physical states. *Forschung auf dem Gebiet des Ingenieurwesens*, 11(1), 6–20. https://doi.org/10.1007/bf02584103 (in German)
2. Riedel, L. Zum problem des gebundennen Wassers in Fleisch // Kältetechnik. — 1961. — № 3. — P. 122–128. (in German)
3. Fennema, O.R. (1985). Water and Ice. Charter in the book: Food Chemistry New York: Marcel Dekker. — P. 23–67.
4. Franks, F. (1982). The Properties of Aqueous Solutions at Subzero Temperatures. Charter in the book: Water and Aqueous Solutions at Subzero Temperatures. pp. 215–338. https://doi.org/10.1007/978–1–4757–6952–4_3
5. 2014 ASHRAE Handbook — Refrigeration. American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc. ISBN 978–1–936504–72–5
6. Reid, D., Saettone, L.P.-A. (2006). The effect of average storage temperature and temperature fluctuation on the rate of moisture migration in a model frozen food. *13th World Congress of Food Science & Technology*. https://doi.org/10.1051/iufost:20060151
7. Alyamovskiy, I.G. (1968). Теплофизические характеристики пищевых продуктов при замораживании. *Kholodilnaya Tekhnika*, 5, 35–36. (In Russian)
8. Latyshev, V.P., Tsyrulnikova, N.A. (1992). Recommended reference materials for thermal calculations of food products. — Moscow: VNIKHI. — 86 p. (In Russian)
9. Chizhov, G.B. (1979). Thermophysical processes in food refrigeration technology. Moscow: Food industry. — 272 p. (In Russian)
10. Goff, H.D. (2005). Quality and Safety of frozen dairy products. Charter in the book: Handbook of Frozen Food Processing and Packaging. pp. 441–457. https://doi.org/10.1201/9781420027402.ch20
11. Ginsburg, A.S., Gromov, M.A., Krasovskaya, G.I. (1990). Thermophysical characteristics of food. Moscow: Agropromizdat. — 289 p. (In Russian)
12. Evance, G. A. (2010). Frozen foods. Production and sales. St. Petersburg: Profession. — 440 p. ISBN: 978–5–904757–02–1 (In Russian)
13. Zorrilla, S.E., Rubiolo, A.C. (2005). Mathematical modeling for immersion chilling and freezing of foods. *Journal of Food Engineering*, 66(3), 329–338. https://doi.org/10.1016/j.jfoodeng.2004.03.026
14. Encyclopedia of Food Technology V.16. Technologies for refrigeration processing and storage of food products. Moscow: V. M. Gorbatov Federal Research Center for Food Systems of the Russian Academy of Sciences. —2019. — 334 p. (In Russian)
15. Berezovskiy, Yu. M., Korolev, I.A., Agafonkina, I.V., Sarantsev, T.A. (2018). Investigation of the effect of beef moisture content on the amount of bound moisture with the calorimetric method. *Proceedings of the Voronezh State University of Engineering Technologies*, 80(4(78), 25–29. https://doi.org/10.20914/2310–1202–2018–4–25–29 (In Russian)
16. Drebushak, V.A., Shvedenkov, G. Yu. (2003). Thermal analysis. Novosibirsk: Novosibirsk State University — 114 p. (In Russian)
17. Höhne, G. W., Hemminger, W.F., Flammersheim, H.-J. (2003). Differential Scanning Calorimetry. Springer Verlag Berlin Heidelberg GmbH. — 298 p. ISBN: 978–3–662–06710–9, https://doi.org/10.1007/978–3–662–06710–9
18. Dibirasulaev, M.A., Belozerov, G.A., Dibirasulaev, D.M., Orlovsky, D.E. (2016). Effect of subcryoscopic storage temperature on the quantity of frozen-out water in NOR and DFD beef. *Theory and practice of meat processing*, 1(2), 18–25. https://doi.org/10.21323/2414–438X-2016–1–2–18–25
19. Berezovskiy, Yu.M., Korolev, I.A., Sarantsev, T.A. (2020). Calculation of heat capacity in meat during its freezing considering phase change. *Theory and practice of meat processing*, 5(1), 22–26. https://doi.org/10.21323/2414–438X-2020–5–1–22–26
20. ancheva, M., Dromenko, O., Potapov, V., Grinchenko, O., Zhelieva, T. (2018). Development of a physical-mathematical model for the process of crystallization of meat systems. *Eastern-European Journal of Enterprise Technologies,* 1(11(91), 50–55. https://doi.org/10.15587/1729–4061.2018.120793
21. Semenov, E.V., Slavyanskiy, A.A. (2009). The crystallization process of sugar solutions. Charter in the book: Theoretical foundations of food technology. Moscow: KolosS. pp. 930–943. ISBN: 978–5–9532–0766–9 (In Russian)
22. Kulev, D. Kh. (2009). Food grade citric acid crystallization process. Charter in the book: Theoretical foundations of food technology. Moscow: KolosS. pp. 943–961. ISBN: 978–5–9532–0766–9 (In Russian)

AUTHOR INFORMATION

Anton G. Belozerov — candidate of technical sciences, deputy director, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences. 109316, Moscow, Talalikhina str., 26 Tel: +7–495–676–92–14, E-mail: a.belozerov@fncps.ru
ORCID: https://orcid.org/0000–0002–1021–1026

Yuriy M. Berezovskiy — doctor of technical sciences, the head of the laboratory, research laboratory of Food Products Thermophysical Properties, All-Russian Scientific Research Institute of Refrigeration Industry — Branch of V. M. Gorbatov Federal Research Center for Food Systems of the Russian Academy of Sciences. 127422, Moscow, Kostyakova str. 12, Tel.: +7–909–685–49–83, E-mail: birjuza1@mail.ru
ORCID: https://orcid.org/0000–0003–1002–2580
*corresponding author

Igor A. Korolev — junior researcher, research laboratory of Food Products Thermophysical Properties, All-Russian Scientific Research Institute of Refrigeration Industry- Branch of V. M. Gorbatov Federal Research Center for Food Systems of the Russian Academy of Sciences. 127422, Moscow, Kostyakova str. 12, Tel.: +7–916–423–42–17, E-mail: korolev.vnihi@mail.ru
ORCID: https://orcid.org/0000–0003–3166–2827

Taras A. Sarantsev — research engineer, research laboratory of Food Products Thermophysical Properties, All-Russian Scientific Research Institute of Refrigeration Industry- Branch of V. M. Gorbatov Federal Research Center for Food Systems of the Russian Academy of Sciences. 127422, Moscow, Kostyakova str. 12, Tel.: +7–915–282–18–24, E-mail: codyjeps@gmail.com
ORCID: https://orcid.org/0000–0001–9755–3047

All authors are responsible for the work and data presented.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

The authors declare no conflict of interest.

Received 31.07.2020 Accepted in revised 10.12.2020 Accepted for publication 20.12.2020.

DOI: https://doi.org/10.21323/2414–438X–2020–5–4–35–38

Available online at https://www.meatjournal.ru/jour
Original scientific paper

ANALYSIS OF REQUESTS FOR JOURNALS *NATURE FOOD* AND *NPJ SCIENCE OF FOOD* BY THE DATA OF THE SCI-HUB SERVICE FOR THE FIRST HALF OF 2020

Vladimir Yu. Kornienko
V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia

Keywords: *Sci-Hub*

Abstract
This paper analyses the history of Sci-Hub service requests for two food industry journals ***Nature Food*** *and* ***npj Science of Food*** *for the period from 1.01.2020 to 29.06.2020. Trends in the development of the food industry and the most popular papers are discussed. Ten of the most popular papers from the journals* ***Nature*** *and* ***Science*** *according to Sci-Hub are presented to compare the popularity of papers related to the food industry and papers from other fields. Based on the analysis of the obtained data we made a conclusion that the popularity of papers related to the food industry is relatively low compared to other papers, which is a paradoxical situation. The data for this paper were provided by the developer of Sci-Hub.*

Acknowledgment
The author thanks Aleksandra Elbakyan, a founder of the current largest service of open access to scientific information Sci-Hub for presented information about the most frequent queries from users.

Introduction

Sci-Hub is a popular Internet service, which provides an access to scientific information. Despite the fact that this service positions itself as a "pirate", it is popular among scientific community [1]. The founder of Sci-Hub is Aleksandra Elbakyan [2]. This Internet resource has been in existence since 2011 and today its database covers more than 85% of all existing scientific papers [3].

From the moment the service was launched and up to now, the audience of Sci-Hub has been constantly growing and a significant percent of users is from the developed countries [1]. With that, Sci-Hub goes beyond distribution of only licensed content but distributes scientific literature without consideration for authors' rights, including scientific publications with open access. The founder of this Internet project was sued by rights holders several times; however, the activities of Sci-Hub were not stopped and, in 2016, Aleksandra Elbakyan entered the Nature's 10 list, which included people who had the highest influence on science in that year [2].

Therefore, the "pirate" activity of Sci-Hub can be perceived differently; however, the coverage of the broad scientific audience gives value to data on requests of one or another scientific paper, as these data are a kind of cross section of interests and activities of scientific community. It is necessary to note that many countries envisage liability for violation of author's rights. In this paper, we analyze data on the most requested papers for two journals related to the food industry — *Nature Food* and *npj Science of Food*.

Objects and methods

The initial data contained 50548903 requests of papers on different directions. We analyzed requests for two journals (*Nature Food* and *npj Science of Food*) that publish papers on themes linked to the food industry. For these journals, data for the period from 1.01.2020 to 29.06.2020 were analyzed and processed using a bash script.

Results and discussion

As a result of data processing, we identified fifteen papers that were more frequently requested on the Sci-Hub service for the journals *Nature Food* and *npj Science of Food*. These data are presented in Table 1. In general, papers presented in Table 1 have a review character. The first four positions in the table are occupied by papers devoted to genetic modifications of plants, including genetic modification of soybean [4,5,6,7]. We also would like to note the presence of papers devoted to production of edible gels and gelatin scaffolds for artificial meat fibers [8, 9]. Other papers are also of interest. For example, Herrero et al. discuss the ways of changes, prospects of optimization and general prospects of some processes associated with the food industry [10]. Mozaffarian et al. discuss peculiarities of different diets and questions of balanced substances in food to solve the problem of malnutrition and maintain the health of consumers [11]. Gibney et al. give recommendations for studying the nutrition process [12]. McClements et al. discuss the safety of using nanoparticles in semi-prepared food products [13]. Lessard et al. describe an interesting effect of feeding ill chickens with genetically modified corn that contained a region of an antibody to interleukin-10. It is noted that chickens had the same weight as those in the group received the corresponding pharmaceutical preparation [14]. Potentially, the same approach can be used, for example, in medicine. Cottrell et al. studied an effect of new aquafeeds on aquaculture growth [15]. Cui et al. present data on the demand for genetically modified products in all Chinese provinces [16].

FOR CITATION: Kornienko Yu. V. Analysis of requests for journals *Nature Food* and *npj Science of Food* by the data of the SCI-HUB service for the first half of 2020. *Theory and practice of meat processing.* 2020; 5(4): 35–38. https://doi.org/10.21323/2414–438X-2020–5–4–35–38

To compare how often papers related to the food industry are requested compared to other journals, we present the data for journals *Nature* and *Science* for the same time period (Table 2, Table 3).

According to the data of Yu-Ming Liao, the journals *Science* and *Nature* have the high impact factor and occupy leading positions in the journal ranking presented by the author [29]. It should be noted that, in general, the journals *Nature* and *Science* contain many papers linked with medical themes.

Analyzing data from Tables 2 and 3, we noticed that papers related to highly ranked journals of the food industry are requested relatively seldom compared to the most frequently requested papers from the journals *Science* and *Nature* (Figure 1)

Table 1. The most popular requests of papers for journals *Nature Food* and *npj Science of Food* according to the data from Sci-Hub for the indicated period of time

№	Paper title	Number of requests	Journal title	Reference
1	A CRISPR way for accelerating improvement of food crops	684	*Nature Food*	4
2	Local food crop production can fulfil demand for less than one-third of the population	531	*Nature Food*	5
3	Textured soy protein scaffolds enable the generation of three-dimensional bovine skeletal muscle tissue for cell-based meat	475	*Nature Food*	6
4	Crop biotechnology and the future of food	292	*Nature Food*	7
5	Innovation can accelerate the transition towards a sustainable food system	277	*Nature Food*	10
6	Dietary metabotype modelling predicts individual responses to dietary interventions	256	*Nature Food*	17
7	Design principles of food gels	215	*Nature Food*	8
8	Dietary and policy priorities to reduce the global crises of obesity and diabetes	194	*Nature Food*	11
9	Uncertainty in human nutrition research	171	*Nature Food*	12
10	Publisher Correction: The unmapped chemical complexity of our diet	150	*Nature Food*	18
11	Is nano safe in foods? Establishing the factors impacting the gastrointestinal fate and toxicity of organic and inorganic food-grade nanoparticles	146	*npj Science of Food*	13
12	Improved performance of Eimeria-infected chickens fed corn expressing a single-domain antibody against interleukin-10	124	*Nature Food*	14
13	Muscle tissue engineering in fibrous gelatin: implications for meat analogs	123	*npj Science of Food*	9
14	Global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030	110	*Nature Food*	15
15	Public perception of genetically-modified (GM) food: A Nationwide Chinese Consumer Study	108	*npj Science of Food*	16

Table 2. The most popular requests of papers for the journal *Nature* according to the data from Sci-Hub for the indicated period of time

№	Paper title	Number of requests	Journal title	Reference
1	Deep learning	14258	*Nature*	19
2	Proteomics identifies new therapeutic targets of early-stage hepatocellular carcinoma	9352	*Nature*	20
3	A photophoretic-trap volumetric display	7705	*Nature*	21
4	Restoration of brain circulation and cellular functions hours post-mortem	7624	*Nature*	22
5	CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy	6466	*Nature*	23
6	Dermatologist-level classification of skin cancer with deep neural networks	6267	*Nature*	24
7	A pneumonia outbreak associated with a new coronavirus of probable bat origin	6155	*Nature*	25
8	Electrochemical Photolysis of Water at a Semiconductor Electrode	6067	*Nature*	26
9	Prioritization of cancer therapeutic targets using CRISPR–Cas9 screens	5936	*Nature*	27
10	Search-and-replace genome editing without double-strand breaks or donor DNA	5680	*Nature*	28

Table 3. The most popular requests of papers for the journal *Science* according to the data from Sci-Hub for the indicated period of time

№	Paper title	Number of requests	Journal title	Reference
1	Thermal Barrier Coatings for Gas-Turbine Engine Applications	76618	*Science*	30
2	Culturally inclusive STEM education	11057	*Science*	31
3	Electric Field Effect in Atomically Thin Carbon Films	10059	*Science*	32
4	Plastic waste inputs from land into the ocean	7960	*Science*	33
5	The biology, function, and biomedical applications of exosomes	7001	*Science*	34
6	The Chemistry and Applications of Metal-Organic Frameworks	6665	*Science*	35
7	Combining theory and experiment in electrocatalysis: Insights into materials design	6108	*Science*	36
8	The global tree restoration potential	5641	*Science*	37
9	A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity	5444	*Science*	38
10	A bacterium that degrades and assimilates poly(ethylene terephthalate)	5237	*Science*	39

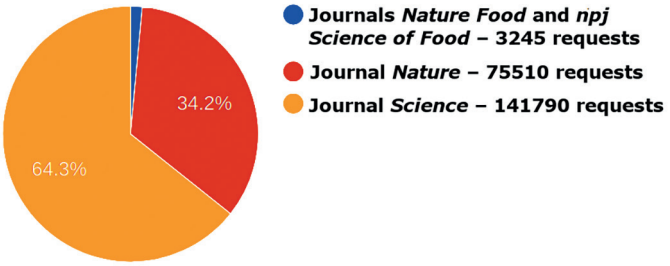


Figure 1. Comparison of the total number of requests for the first ten journals from each of the Tables 1, 2 and 3. The presented diagram reflects popularity of food industry papers compared to scientific papers from journals *Science* and *Nature*

Conclusion

Studies related to genome modification were most frequently requested on Sci-Hub during the period from 1.01.2020 to 29.06.2020. Apparently, attention of researches is focused in this direction, which will likely lead to appearance of new genetically modified agricultural cultures in the future. However, it should be noted that there are no papers dedicated to genetic modification of animals in Table 1. At the same time, part of presented papers touches on the question of interrelation between food quality and population health in the context of the excess weight and diabetes problems, but papers on such important direction as pathogen detection are absent in the presented table.

It is worth noting that there are many papers on the medical theme in Table 2 with high frequency of requests. Therefore, the attention of the world community is focused to a great extent on a search for new medical approaches (therapies), but at the same time, the attention to the work of the food industry is generally relatively low despite the fact that food quality directly influences population health. It should be emphasized that higher attention to the food industry can lead to reduction in the number of diseases among population.

REFERENCES

1. Jack, E. J.n(2020). Pirate open access as electronic civil disobedience: Is it ethical to breach the paywalls of monetized academic publishing? *Journal of the Association for Information Science and Technology,* 71(12), 1500–1504. https://doi.org/10.1002/asi.24351
2. Himmelstein, D.S., Romero, A.R., Levernier, J.G., Munro, T.A., McLaughlin, S.R., Tzovaras, B.G., Greene, C.S. (2018). Sci-Hub provides access to nearly all scholarly literature. *eLife.* 7, e32822. https://doi.org/10.7554/eLife.32822
3. Kornienko, V. Yu. (2020). Analysis of the history of Sci-Hub requests for the first half of 2020 for biologic journals. *Vsyo o myase,* 4, 3–5. https://doi.org/10.21323/2071-2499-2020-4-3-5
4. Zhang, Y., Pribil, M., Palmgren, M., Gao, C.(2020). A CRISPR way for accelerating improvement of food crops. *Nature Food,* 1(4), 200–205. https://doi.org/10.1038/s43016-020-0051-8
5. Kinnunen, P., Guillaume, J.H.A., Taka, M., D'Odorico, P., Siebert, S., Puma, M.J., Jalava, M., Kummu, M. (2020). Local food crop production can fulfil demand for less than one-third of the population. *Nature Food,* 1(4), 229–237. https://doi.org/10.1038/s43016-020-0060-7
6. Ben-Arye, T., Shandalov, Y., Ben-Shaul, S., Landau, S., Zagury, Y., Ianovici, I., Lavon, N., Levenberg, S. (2020). Textured soy protein scaffolds enable the generation of three-dimensional bovine skeletal muscle tissue for cell-based meat. *Nature Food,* 1(4), 210–220. https://doi.org/10.1038/s43016-020-0046-5
7. Steinwand, M.A., Ronald, P.C. (2020). Crop biotechnology and the future of food. *Nature Food,* 1(5), 273–283. https://doi.org/10.1038/s43016-020-0072-3
8. Cao, Y., Mezzenga, R. (2020). Design principles of food gels. *Nature Food,* 1(2), 106–118. https://doi.org/10.1038/s43016-019-0009-x
9. MacQueen, L.A., Alver, C.G., Chantre, C.O., Ahn, S., Cera, L., Gonzalez, G.M., O'Connor, B.B., Drennan, G.J., Peters, M.M., Motta, S.E., Zimmerman, J.F., Parker, K.K. (2019). Muscle tissue engineering in fibrous gelatin: implications for meat analogs. *npj Science Food,* 3(1), 20. https://doi.org/10.1038/s41538-019-0054-8
10. Herrero, M., Thornton, P.K., Mason-D'Croz, D. et al. (2020). Innovation can accelerate the transition towards a sustainable food system. *Nature Food,* 1(5), 266–272. https://doi.org/10.1038/s43016-020-0074-1
11. Mozaffarian, D. (2020). Dietary and policy priorities to reduce the global crises of obesity and diabetes. *Nature Food,* 1(1), 38–50. https://doi.org/10.1038/s43016-019-0013-1
12. Gibney, M., Allison, D., Bier, D., Dwyer, J. (2020). Uncertainty in human nutrition research. *Nature Food,* 1(5), 247–249. https://doi.org/10.1038/s43016-020-0073-2
13. McClements, D.J., Xiao, H. (2017). Is nano safe in foods? Establishing the factors impacting the gastrointestinal fate and toxicity of organic and inorganic food-grade nanoparticles. *npj Science Food,* 1(1), 6. https://doi.org/10.1038/s41538-017-0005-1
14. Lessard, P.A., Parker, M., Bougri, O., Shen, B., Samoylov, V., Broomhead, J., Li, X., Raab, R.M. (2020). Improved performance of Eimeria-infected chickens fed corn expressing a single-domain antibody against interleukin-10. *Nature Food,* 1(2), 119–126. https://doi.org/10.1038/s43016-020-0029-6
15. Cottrell, R.S., Blanchard, J.L., Halpern, B.S., Metian, M., Froehlich, H.E. (2020). Global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030. *Nature Food,* 1(5), 301–308. https://doi.org/10.1038/s43016-020-0078-x
16. Cui, Kai, Shoemaker, S.P. (2018). Public perception of genetically-modified (GM) food: A Nationwide Chinese Consumer Study. *npj Science Food Food,* 2(1), 10. https://doi.org/10.1038/s41538-018-0018-4
17. Garcia-Perez, I., Posma, J.M., Chambers, E.S., Mathers, J. C., Draper, J., Beckmann, M., Nicholson, J. K., Holmes, E., Frost, G. (2020). Dietary metabotype modelling predicts individual responses to dietary interventions. *Nature Food,* 1(6), 355–364. https://doi.org/10.1038/s43016-020-0092-z
18. Barabási, A., Menichetti, G., Loscalzo, J. (2020). Publisher Correction: The unmapped chemical complexity of our diet. *Nature Food,* 1(2), 140. https://doi.org/10.1038/s43016-020-0030-0
19. LeCun, Y., Bengio, Y., Hinton, G. (2015). Deep learning. *Nature,* 521(7553), 436–444. https://doi.org/10.1038/nature14539
20. Jiang, Y., Sun, A., Zhao, Y. et al. (2019). Proteomics identifies new therapeutic targets of early-stage hepatocellular carcinoma. *Nature,* 567(7747), 257–261. https://doi.org/10.1038/s41586-019-0987-8
21. Smalley, D., Nygaard, E., Squire, K. *et al.* (2018). A photophoretic-trap volumetric display. *Nature,* 553(7689), 486–490. https://doi.org/10.1038/nature25176
22. Vrselja, Z., Daniele, S.G., Silbereis, J. *et al.* (2019). Restoration of brain circulation and cellular functions hours post-mortem. *Nature,* 568(7752), 336–343. https://doi.org/10.1038/s41586-019-1099-1
23. Barkal, A.A., Brewer, R.E., Markovic, M. *et al.* (2019). CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature,* 572(7769), 392–396. https://doi.org/10.1038/s41586-019-1456-0
24. Esteva, A., Kuprel, B., Novoa, R. *et al.* (2017). Dermatologist-level classification of skin cancer with deep neural networks. *Nature,* 542(7639), 115–118. https://doi.org/10.1038/nature21056
25. Zhou, P., Yang, X., Wang, X. *et al.* (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature,* 579(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7

26. Fujishima, A., Honda, K. (1972). Electrochemical Photolysis of Water at a Semiconductor Electrode. *Nature,* 238(5358), 37–38. https://doi.org/10.1038/238037a0
27. Behan, F.M., Iorio, F., Picco, G. *et al.* (2019). Prioritization of cancer therapeutic targets using CRISPR–Cas9 screens. *Nature,* 568(7753), 511–516. https://doi.org/10.1038/s41586–019–1103–9
28. Anzalone, A.V., Randolph, P.B., Davis, J.R. *et al.* (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature,* 576(7785), 149–157. https://doi.org/10.1038/s41586–019–1711–4
29. Liao, Yu-M. (2019). Top 500 Journals in 2019. JCR Journal Impact Factor Released in 2019. [Electronic resource: https://www.researchgate.net/publication/333972052_Top_500_Journals_in_2019_JCR_Journal_Impact_Factor_Released_in_2019 Access date 05.10.2020]
30. Padture, N.P., Gell, M., Jordan, E.H. (2002). Thermal Barrier Coatings for Gas-Turbine Engine Applications. *Science,* 296(5566), 280–284. https://doi.org/ 10.1126/science.1068609
31. Zellmer, A.J., Sherman, A. (2017). Culturally inclusive STEM education. *Science,* 358(6361), 312–313. https://doi.org/10.1126/science.aaq0358
32. Novoselov, K.S., Geim, A.K., Morozov, S.V., Jiang, D., Zhang Y., Dubonos, S.V., Grigorieva, I.V., Firsov, A.A. (2004). Electric Field Effect in Atomically Thin Carbon Films. *Science,* 306(5696), 666–669. https://doi.org/10.1126/science.1102896
33. Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L. (2015). Plastic waste inputs from land into the ocean. *Science,* 347(6223), 768–771. https://doi.org/10.1126/science.1260352
34. Kalluri, R., LeBleu, V.S. (2020). The biology, function, and biomedical applications of exosomes. *Science,* 367(6478), eaau6977. https://doi.org/10.1126/science.aau6977
35. Furukawa, H., Cordova, K.E., O'Keeffe, M., Yaghi, O.M. (2013). The Chemistry and Applications of Metal-Organic Frameworks. *Science,* 341(6149), 1230444. https://doi.org/10.1126/science.1230444
36. Seh, Z.W., Kibsgaard, J., Dickens, C.F., Chorkendorff, I., Nørskov, J. K., Jaramillo, T.F. (2017). Combining theory and experiment in electrocatalysis: Insights into materials design. *Science,* 355(6321), eaad4998. https://doi.org/10.1126/science.aad4998
37. Bastin, J.-F., Finegold, Y., Garcia, C., Mollicone, D., Rezende, M., Routh, D., Zohner, C. M., Crowther, T.W. (2019). The global tree restoration potential. *Science,* 365(6448), 76–79. https://doi.org/10.1126/science.aax0848
38. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., Charpentier, E. (2012). A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science,* 337(6096), 816–821. https://doi.org/10.1126/science.1225829
39. Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., Oda, K. (2016). A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science,* 351(6278), 1196–1199. https://doi.org/10.1126/science.aad6359

AUTHOR INFORMATION

Vladimir Yu. Kornienko — candidate of biological sciences, senior researcher, Laboratory of molecular biology and bioinformatics, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences
109316, Moscow, Talalikhina str., 26. Tel.: +7–495–676–79–81. E-mail: unipraim@yandex.ru
https://orcid.org/0000–0001–9068–9814

Completely prepared the manuscript and is responsible for plagiarism.

The author declare no conflict of interest.

Received 22.10.2020 Accepted in revised 30.11.2020 Accepted for publication 15.12.2020