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ABOUT THE JOURNAL

The journal "Theory and practice of meat processing" is a peer-reviewed scientific journal covering a wide range of issues: formation of the composition and properties of meat raw materials including various methods for raising animals and poultry; the main questions of meat raw material processing, improvement of technologies for meat product manufacture including functional foods, effects of meat and meat product consumption on human health.

The primary objectives of the journal "Theory and practice of meat processing" are to create content for distribution of international knowledge in the world scientific community, promote research performed by scientists from Russia and foreign countries, enhance presentation of their scientific achievements in the international arena and highlight promising research directions in the meat industry.

The main tasks of the journal consist in publishing results of theoretical and experimental studies carried out in Russian and foreign organizations, as well as on the authors' personal initiative; bringing together different categories of researchers, university and scientific professionals; creating and maintaining a common space of scientific communication, bridging the gap between publications at the regional, federal and international levels.

The editors strive to expand the pool of writers and welcome new authors.

The journal content areas reflect the modern state of different scientific and technical problems, the industrial implementation of scientific results and advanced national and foreign experience, such as regulation of feeding rations and keeping conditions, targeted modification (selection, hybridization, operative manipulations with animals and poultry), the study of effects of the main meat nutrients (proteins, lipids), micro- and macroelements, vitamins, their compatibility and preservation during meat and poultry product manufacture on meat quality and properties.

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- investigations in the field of biotechnology, biochemistry, physiology, lanimology, nutritiology
- investigation and development of new technologies of meat production
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- standardization, certification, systems of quality and safety management
- microbiology, sanitary and hygiene of meat and meat products
- automation and informatization of the technological processes in the meat sector

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IMPACT OF FEED SUPPLEMENTATION WITH BALSAM POPLAR BUDS ON PERFORMANCE OF YOUNG BULLS

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Keywords: *nutrition, additive, bioactive compounds, slaughter characteristics, meat quality*

Abstract
There is an urgent need to develop new strategies to minimize the environmental impact of animal production and support sustainability of food production and consumption. Feed additives have been for a long time used in animal nutrition to improve animal growth and performance as well as animal health. Balsam poplar plants (Populus balsamifera) is well known as a rich source of bioactive compounds with positive health effects, and might be used in agriculture as a feed additive for ruminants. The aim of the present study was to evaluate the effect of balsam poplar-based additives on growth and performance of fattening young bulls of Simmental breed. In the present study, we used 4 combinations of extract from balsam poplar buds or its components as a feed additives. The animals were given the supplements at the age of 15 months, 3 months before slaughter. The growth and slaughter characteristics of young bulls were studied. After the first and second month of feeding with dietary supplements, animals from the groups fed 10% balsam poplar buds extract and dry shredded balsam poplar buds had significantly higher live weight compared to the control animals fed a diet without any supplements ($P < 0.05$). At slaughter, group fed 10% balsam poplar buds extract had significantly higher live weight compared to control. Average daily gain was also greatest in that group. Major sensory as well as physical and chemical parameters were not affected by balsam poplar-based supplements ($p > 0.05$ for all) and were in line with regulatory meat hygiene requirements.

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Introduction

With continuous growth of world population, the major focus of agricultural sector is to ensure an adequate food supply and food security. Animal health and welfare are closely related to animal production efficiency and has therefore indirectly linked to reduced poverty, which is one of the UN sustainable development goals. Beef cattle is an important agricultural and economic resource in many countries including Kazakhstan. Thus, there is an increasing interest to improve feed conversion and increase growth rate without compromising meat quality and animal welfare. Reduction of negative environmental effects such as carbon emissions and contribute to sustainable land use is also important. Genetic selection, animal management, dietary modification, and application of new feeding techniques and use of feed additives [1–4] can enhance feed conversion and growth rate.

Nutritional needs of livestock animals with respect to energy, protein, minerals and vitamin requirements are well established. To optimize livestock production, antibiotic growth promoters had been used as dietary additive for decades. However, uncontrolled use of antibiotics contributes to increasing resistance in bacteria of human and animal origin [5]. Since 2006, marketing and use of antibiotics as growth promoters in animal production are prohibited in EU although hormonal growth promoters are still in use in US to increase daily weight gain and produce leaner meat [6]. Moreover, consumers prefer "hormone- and antibiotic-free" food because of potential health effects and environmental risks [7]. Over recent decades, much research has also been aimed to improve the quality of feed without use of hormonal growth promoters. This included research on forages and forage conservation [8], as well as the use of various supplements from natural sources [9–11].

There is an increasing interest in using plant bioactive compounds to enhance ruminant health and performance [12]. For this purpose, numerous studies have attempted to use supplements with bioactive compounds or plant extracts [13,14]. Although plant bioactive compounds have lower potency compared to pharmaceuticals, they might affect physiological processes in a positive way and showed numerous benefits for animal health. The existing knowledge on the use of plant bioactive compounds as growth promoters in livestock production was recently reviewed by Valenzuela-Grijalva [15]. Balsam poplar (Populus balsamifera) as a possible feed ingredient for cattle has been studied since they contain a wide range of bioactive compounds with antioxidant activity [16–18]. Cuttings from poplar were shown to have good digestibility [19]. Bark of aspen (*Populus tremuloides*), which belong to the same genus as balsam poplar, was suggested as a good feed for goats [20].

In the North Kazakhstan, there are approximately 1400 ha of balsam poplar plantations but utilization of the buds from balsam poplar is low. Moreover, poplars are early successional species with rapid growth rates. Several studies highlighted balsam poplar buds as an important source of chemically highly diverse bioactive substances such as phenolic compounds and essential oils [18,21]. These compounds are known for their biological functions but are still far from being fully investigated.

We hypothesized that the use of the Balsam poplar buds as a part of animal feed can facilitate digestion process and improve cattle growth and performance. Thus, in the present study we used extract from balsam poplar buds or its components as on growth rate and performance of Simmental bull.

Objects and methods

All procedures used in the study were conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) and "The Guide for Care and Use of Laboratory Animals" [22]. During the experiment, all efforts were made to avoid animal suffering.

Animals and feeding

In total, 25 fattening young bulls of Simmental breed, with live weight (LW) from 295 to 307.8 kg and age of approximately 15 months were included into the study. The animals were randomly divided into 5 groups (n=5 in each group). All animals were fed a commercial mixed diet containing wheat grass, hay barley and concentrate. Additionally, each experimental group received a supplement containing extract from balsam poplar buds or its components. The group without any supplement served as a control (group 1). Group 2 received supplement with only polyethylene pellets (once, 500 g /animal). The applied polyethylene pellets are chemically inert substances and do not provide energy. Group 3 received supplement with polyethylene pellets (once, 500 g /animal) in combination with 0.4% essential oil of balsam poplar buds (10 mL once a day for 5 days). Group 4 received supplement with polyethylene pellets (once, 500 g /animal) in combination with a 10% balsam poplar buds extract (10 mL once a day for 5 days). Group 5 received supplement with polyethylene pellets (once, 500 g /animal) in combination with dried shredded balsam poplar buds, containing 2% extract (5 g once a day per 5 days). The animals were given the supplements at the age of 15 months, 3 months (90 days) before slaughter.

Polyethylene pellets in the diet were mixed with a concentrate (1.5 kg of concentrated feed per animal) and fed animals after 18 hour fasting. The animals were slaughtered at a local slaughterhouse using standard procedures.

Measurements after slaughter

Dressing percentage was calculated by dividing the weight of warm carcass by the weight of the live animal and expressed as a percentage. Other measurements of the carcass and veterinary control were performed according to standard methods and veterinary (veterinary-sanitary) rules approved by RK Government No. 7–1/587 of June 29, 2015.

Reaction to the peroxidase (benzidine test) and pH

All laboratory analyses of meat were performed in accordance with the State Standard 23392–78 "Meat. Methods of freshness chemical and microscopic analysis".

Meat extract (2 ml) was added into the test tube, following by addition of 5 drops of 0.2% alcoholic benzidine and 2 drops of 1% hydrogen peroxide. The extract of fresh meat of healthy animals acquired a greenish-blue color, turning into brown in a few minutes. Such coloring is the evidence of peroxidase activity. The meat extract of sick, overworked, dead animals doesn't change the color.

pH of the meat extract was determined by potentiometric method with the use of ionomer 827 pH Lab Metrohm.

Microscopy of smears-marks

The method is based on determining the number of bacteria and degradation of the muscle tissue by microscopy of smears-marks using a binocular microscope Mikmed-5, a mirror digital camera Nikon D5100 with Camera Control Pro2 software and digital cameras Omax A3590U ToupView software. Meat was cut into pieces with a clean scissors from the deep layers of the muscle and attached to a glass slide. Smears were dried, fixed in the flame of the burner and stained as previously described [23].

Sensory test

Sensory tests were performed in accordance with the State Standard 7269–79, which includes evaluation of meat appearance, color and smell, condition of the muscles in the section, as well as the evaluation of broth clarity and flavor.

The appearance and color of the meat samples were evaluated by visual examination. Appearance and color of the muscles in the section were studied in the deep layers of muscle tissue in the fresh meat section. At the same time, there were ascertained presence of adhesiveness and moisture on the section surface of meat by attaching piece of absorbent paper to the section.

The flavor of the surface layer of the test sample was examined by sensory analysis. The sections were made with a clean knife and the flavor in the deep layers was immediately identified.

Evaluation of broth clarity and flavor was performed as follows: 20 g of minced meat were placed into a flask, filled with 60 ml of distilled water and mixed thoroughly. Then, the flask was covered with a watch glass and placed into a boiling water bath. The flavor was examined within the process of heating up to 80–85 °C. To determine clarity, 20 ml of the broth and were transferred into the cylinder and visually adjusted its degree of clarity.

Statistical analysis

All statistical analyses were conducted with Statistical Analysis System, Version 9.4 (SAS Inst., Cary, NC, USA). The mixed model included fixed factor of treatment when evaluating the effect on live weight. Live weight at the beginning of the experiment was included as covariate when evaluating the effect of treatment on other characteristics. Comparisons between the control group fed traditional diet only, and groups with dietary supplements were performed using probability differences. A p-value less than 0.05 was regarded as statistically significant.

Results and discussion

During the experiment span, veterinarians as healthy recognized all animals, and no morbidity and mortality were recorded in any of groups.

At the beginning of the experiment live weight of the young bulls did not significantly differ between the groups (Table 1; $P = 0.974$). After the first and second month of feeding with dietary supplements, animals from the groups 4 and 5 (fed 10% balsam poplar buds extract and dry shredded balsam poplar buds, respectively) had significantly higher live weight compared to the control animals (Table 1, $P < 0.05$). At slaughter, however, the overall effect of group did not reach the significant differences, although according to more specific probability differences test, group 4 fed 10% balsam poplar buds extract had significantly higher live weight compared to control (Table 1). Average daily gain was also greatest in this group.

The determination of the live weight at a given age is probably one of the most obvious and easiest way to access feed efficiency. To the best of our knowledge, this is the first study to investigate the effect of the Balsam poplar buds as a part of animal feed on cattle growth and performance. In the present study, only live weights of the animals in groups 4 and 5 differed from that in control group, indication that the supplements of 10% balsam poplar buds extract and dried shredded balsam poplar buds, containing 2% extract, were most promising in increasing feed efficiency. The loss of statistical significance between group 5 and control group at slaughter time suggests that possible adaptation of the animals to dried shredded balsam poplar buds. In future practical application, the time between providing supplements of balsam poplar-based product and slaughter can be reduced.

Carcass weight was greatest in the groups 2 and 4 (fed with only polyethylene pellets and fed 10% balsam poplar buds extract, respectively) (Table 2). No differences in carcass dressing percentage and other post-slaughter characteristics between treatments was observed ($P > 0.05$ for all, Table 2). Dressing percentage is one of factors defining the value of a livestock animal after slaughter. Numerically,

Table 1. Growth parameters of young bulls

Parameter	Groups					P-value, effect of group
	1 (control)	2	3	4	5	
Live weight before changes in the diet, kg	301 ± 13.6	304 ± 13.6	301 ± 13.6	308 ± 13.6	295 ± 13.6	0.975
Live weight after 1 month, kg	322^{a} ± 3.4	326^{a} ± 3.4	323^{a} ± 3.4	338^{b} ± 3.4	334^{b} ± 3.4	0.015
Live weight after 2 month, kg	341^{a} ± 6.0	356^{a} ± 6.0	351^{a} ± 6.0	366^{b} ± 6.1	371^{b} ± 6.1	0.017
Live weight at slaughter, kg	372^{a} ± 7.9	393^{a} ± 7.9	387^{a} ± 7.9	401^{b} ± 7.9	394^{a} ± 7.9	0.139
Average daily gain, g	777^{a} ± 87.4	1017^{a} ± 87.5	948^{a} ± 87.4	1103^{b} ± 87.4	1025^{a} ± 88.0	0.139

Data are presented as least squares (LS) means ± standard errors. LS means with different superscripts within row differs at $P < 0.05$

Table 2. Effect of dietary supplementation with balsam poplar buds on post-slaughter characteristics

Indicators	Groups					P-value, effect of group
	1 (control)	2	3	4	5	
Carcass, kg	194^{a} ± 5.7	211^{b} ± 5.7	206^{a} ± 5.7	218^{b} ± 5.8	209^{a} ± 5.8	0.086
Dressing, %	52.1 ± 1.15	53.7 ± 1.15	53.3 ± 1.15	54.4 ± 1.16	53.0 ± 1.16	0.714
Muscle, %	53.8 ± 0.97	53.3 ± 0.97	53.8 ± 0.97	52.7 ± 0.97	53.7 ± 0.97	0.918
Bones, %	19.2 ± 0.94	17.6 ± 0.94	18.4 ± 0.94	18.9 ± 0.95	18.3 ± 0.95	0.798
Connective tissue,%	11.6 ± 0.61	10.4 ± 0.61	11.0 ± 0.61	11.1 ± 0.61	11.3 ± 0.61	0.741
Fat, %	15.4 ± 0.88	18.6 ± 0.88	16.8 ± 0.88	17.2 ± 0.89	16.8 ± 0.89	0.191

Data are presented as least squares (LS) means ± standard errors. LS means with different superscripts within row differs at $P < 0.05$

the animals from group 1 (control) had the lowest dressing percentage even though these differences did not reach statistical significance.

The economic value of livestock animals depends on its composition. Nowadays in many countries, a progress was made in reducing the fat content of livestock animals because of consumer demand for lean meat. Generally, the relationship between live weight and fat content is affected by feeding, environmental factors as well as any subclinical diseases that might affect the animal growth rate. In the present study, the animals were kept in the same environment and differed only in feeding strategy. No statistically significant differences between fat content and muscle content were observed suggesting that balsam poplar-based supplements do not exert any effect on carcass composition.

According to organoleptic evaluation and external examination of carcasses and organs of the animals, no deviations were observed in appearance and colour of meat of all groups. Analysis of bacterioscopy of prints smears in meat revealed up to 2.6±0.51 mostly gram-positive coccal microorganisms in the surface of muscle tissue (Table 3). In the deep layers of the samples studied, no microorganisms were found.

The meat pH in all groups corresponded to meat obtained from healthy animals and was in the range from 5.8 ± 0.06 to 6.0 ± 0.05. No primary and secondary protein degradation products in the meat of all groups were observed. The benzidine reaction in all samples was positive.

Thus, major sensory as well as physical and chemical parameters were not negatively affected by balsam poplar-based supplementations ($p > 0.05$ for all).

In the recent years, plant-based bioactive supplements in animal diet received a growing attention as a means to increase production efficiency of ruminants. The phytopreparations from balsam poplar buds contain amino acids, unsaturated fatty acids (malic, tartaric, lemon, linoleic, linolenic, arachidonic), polyphenols (pinostrobin, pinocerbrin, chrysin, tektohrizin, apigenin, kaempferol, quercetin, myricetin, galangin, iszalpinin etc.) carbohydrates, minerals (zinc, manganese, cobalt, copper, iron) and vitamins (A, C, P, E) [16,18,24,25]. Previous studies on the chemical composition of balsam poplar buds also identified the presence of alkanes, terpenes, chalcones and prostaglandins [16,26,27]. This composition might lead to the improvement of fermentation in the rumen, improvement of metabolic processes and suppression of undesirable microflora of the rumen [28]. Additives based on bark from *Populus tremuloides* and leaves and stems from *Populus tremula* were shown have potential to decrease methane production during rumen fermentation [12, 29]. Additionally, many plant-based products represent potential alternative to treat various diseases due to the presence of bioactive compounds with therapeutic properties. Salicin, salicortin, salireposide, and populoside were also identified as bioactive components in balsam poplar responsible for the inhibition of adipogenesis and were suggested as complementary agents in antiobesity and antidiabetic therapies [30]. Currently, the use of the preparations from balsam poplar is limited. In Kazakhstan, balsam poplar buds are used to treat hemorrhoids and scurvy [31]. In Russia, fresh or dry balsam poplar buds are used in forms of an ointment or tincture and used in traditional medicine to treat rheumatism or cold [31]. Development of such preparations represents a complex effort demanding a highly integrated interdisciplinary approach. Recently, antioxidant, anti-inflammatory, hepatoprotective and vasorelaxant activities of *Populus nigra* flower buds ethanolic extract were evaluated using mice as an animal model [32]. It was shown that the extract had anti-inflammatory, hepatoprotective and vasorelaxant properties and no toxic effect was observed after administration of 200 mg/kg during 4 weeks [32].

We believe that our results open new research avenues for the development of novel and safer additives for the improvement of livestock production.

Conclusion

Balsam poplar plants is well known as a rich source of bioactive compounds with positive health effects, and might be used in agriculture to support sustainable animal production and animal health without compromising meat quality and generating environmental impact issues. Animals from the groups fed 10% balsam poplar buds extract and dry shredded balsam poplar buds had significantly higher live weight compared to the control animals fed traditional diet without any supplement ($P < 0.05$). At slaughter, group fed 10% balsam poplar buds extract had significantly higher live weight compared to control.

Table 3. Sensory, physical and chemical examination of meat

Group	Boiling test	Bacterioscopy		Physical and chemical indicators			
		Outer	Inner	Reaction with sulfuric acid copper*	Reaction to peroxidase*	Formaldehyde reaction*	pH
1	clear broth	1.5 ± 0.25	0	–	+	±	6.0 ± 0.07
2	clear broth	2.5 ± 0.25	0	–	+	±	5.8 ± 0.02
3	clear broth	2.0 ± 0.0	0	–	+	±	5.9 ± 0.04
4	clear broth	2.5 ± 0.25	0	–	+	±	6.0 ± 0.05
5	clear broth	2.0 ± 0.5	0	–	+	±	5.8 ± 0.04

* — *Negative, + Positive, ± Doubtful*

Average daily gain was also greatest in that group. Major sensory as well as physical and chemical parameters were not affected by balsam poplar-based supplementations ($p > 0.05$ for all) and were in line with regulatory meat hygiene requirements.

Our results indicated that the development of new balsam poplar-based additives for livestock can help to improve animal growth and performance. However, more research is required to establish the optimal dose and period of feeding.

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EVOLUTION OF METHODS FOR *IN VITRO* PRODUCT DIGESTIBILITY ANALYSIS: A SYSTEMATIC REVIEW

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Abstract

The inability to reproduce certain digestive processes in vivo, high research costs and ethical aspects have led to the development of a large number of in vitro digestion models. These models allow us to take into account various factors of modeling complex multistage physiological processes occurring in the gastrointestinal tract, which makes them promising and widely used. A significant part of in vitro methods includes assessment by enzymatic digestion and are based on the calculation of nitrogen remaining after digestion in relation to the initial total nitrogen (according to the Dumas, Kjeldahl method, spectrophotometric or chromatographic method). There are also a number of titrometric methods (pH-stat), which are mainly used to assess the digestibility of feed, most successfully for aquatic animals due to the simplicity of their digestive tract. Methods for assessing the digestibility of food products by enzymatic digestion have undergone various stages of evolution (since 1947) and have been widely modified by including various enzymes (pepsin, trypsin, pancreatin, erepsin, etc.) in model systems, indices for various products have been determined on their basis (pepsin-digest-residue (PDR) index, 1956; pepsin pancreatin digest (PPD) index, 1964; pepsin digest dialysate (PDD), 1989). As a result, a single protocol was formed to study the digestibility of food — INFOGEST (2014–2019), which includes three stages of digestion (oral, gastric and intestinal). It allows researchers to accurately reproduce the conditions of the human gastrointestinal tract and is widely used by scientists around the world.

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Introduction

Nowadays, the concept of food nutritional value includes a degree of the digestibility and assimilability, the presence or generation of minor biologically active compounds or anti-alimentary factors in the digestion process in addition to the main indicators (safety, energy and biological value).

The interest to the investigation and understanding of the food digestibility processes has increased over the last decade. Today, the food product digestibility has been studied using different *in vitro* and *in vivo* models. With that, to study questions linked with diet components, new ingredients and foods, the priority is given to models that include farm animals and humans as it is possible to obtain the most accurate results with their use [1]. However, it is technically difficult to analyze the complex multi-stage process that takes place during digestion in humans or animals, and it is not always possible to carry out such investigations from the ethical and financial points of view. In this connection, *in vitro* digestion models simulating processes in the gastrointestinal tract are proposed as an alternative to *in vivo* experiments [2]. There is a real need for the development and use of *in vitro* models that allow accurate simulation of the physiological processes during digestion taking into consideration factors such as the presence of certain digestive enzymes and salts and their concentration, pH value, digestion duration. To simulate the digestion processes, static and dynamic models were developed, which common features are correct simulation of the digestion processes and digestive liquids in animals and humans [3]. The main requirements for these models are flexibility, accuracy and reproducibility; they should be a decent alternative to animal and human models and allow rapid screening of food products and ingredients [4]. *In vitro* simulation of the digestive processes approximated to the *in vivo* physiological conditions is widely used

in pharmacology, feeding and nutrition sciences, food chemistry being useful tools for studying and understanding changes, interactions as well as bioavailability and metabolites of nutrients, medicines and anti-nutritional compounds.

Over the last decade, different *in vitro* digestion models have been used worldwide to analyze structural and chemical changes occurring in food and feed matrices. For example, using *in vitro* digestion models, the digestibility of feed and feed additives including those obtained from genetically modified plants has been studied. Models with simulation of rumen liquid [5,6,7], gastric and intestinal juices of pigs and poultry [8] with the use of the isolated porcine small intestine [9] and others are widely used. These studies allow analyzing the character of digestion of different substances for particular species of animals and poultry. Methods for *in vitro* determination of the food digestibility are aimed, first of all, to detection of the digestibility of the main substances that ensure anabolic processes and synthesis of metabolically active substances in the body.

Modern *in vitro* digestion models described in the literature are static or dynamic with the use of various enzyme systems. Due to the fact that dynamic models are complex computerized systems and 89% of all publications are based on the use of static models, we examined the latter in this paper.

The aim of this paper is to present a review of static *in vitro* digestion models by analysis of the evolution of these methods with regard to the development of digestion models (parameters, protocols, guidelines) and to study a possibility of their use in the field of food analysis.

Objects and methods

Study design: systematic review according to the protocol of the PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions [10].

The strategy for the search of publications is presented in Figure 1.

Criteria for inclusion are:

1. Correspondence to the theme of the systematic review by one of four modalities: *in vitro* method, static model, product digestion, use of enzymes.
2. Original research published in the peer-reviewed journal, patent for invention.
3. Data on the *in vitro* digestion method are given.
4. Publication is devoted to the study of the digestibility of products, feedstuff, main nutrients, in particular, protein.

Criteria for exclusion are:

1. Studies that envisage the investigation of the digestibility and assimilation of nutrients *in vivo* (including the use of laboratory rodents, farm animals, poultry, humans).
2. Studies that envisage the investigation using the model of digestion in rumen and intestine of ruminants (including the use of "artificial" rumen, rumen liquid, gastric and intestinal juice of pigs and poultry).
3. Studies that envisage the investigation of absorbability by the ideal method (in the small intestine of rats, pigs; terminal method).
4. Studies based on the *in situ* approach.
5. Studies of drug metabolism *in vitro*.

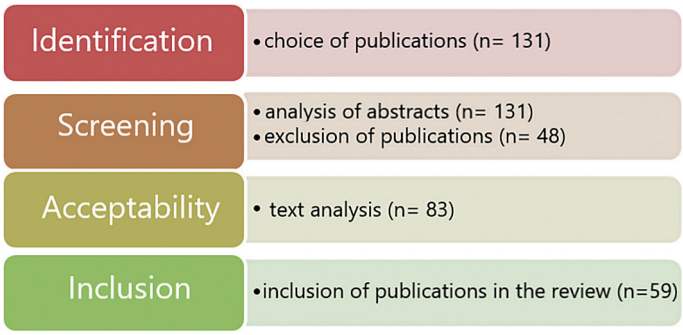

Figure 1. Strategy of selection and inclusion of publications into the systematic review

A search for relevant scientific publications was carried out using the Russian and foreign electronic databases: Web of Science, United States National Library of Medicine (pubmed.gov), Russian scientific electronic library (elibrary.ru), Russian State Public Scientific and Technical Library in Russian and English for a period of 1950 to 2021.

Titles of papers obtained in the course of search were analyzed; part of publications was excluded as inconsistent with the criteria for inclusion. Then, abstracts of selected papers were analyzed and the second exclusion was carried out. After that, the following data were selected from each publication included into the review: author(s), year of publication, country; aim and design of the research; verification of the statistical hypothesis; description of the methodology of the experiment; results. A detailed analysis of each publication included into the review was performed based on the specific elements of research questions and the aim of the review by double data extraction (two independent researchers worked on the review).

All data obtained by this way were used for analysis and systematization of the results.

Results and discussion

Over the last 40 years, more than 2500 studies with the use of digestion simulation by *in vitro* methods were published; among them, 80% of studies were published over the last 20 years [1]. More than 200 publications were examined within the framework of the paper. *In vitro* static models are described in the significant part of analyzed literature for investigating the digestibility of protein ingredients and protein feed additives. For ease of understanding, we conditionally divided widely used methods into groups, which are described in the sections below.

Methods with the use of enzyme systems

When developing these methods, the main emphasis is shifted to simulation of parameters of enzymatic cleavage that simulate processes of digestion in the stomach or both in the stomach and small intestine.

The general principle of these *in vitro* methods is the use of mono- and multi-enzyme systems; the product digestibility can be calculated as soluble nitrogen remained after digestion in relation to the initial total nitrogen in a product. In the experiment, additional stages (filtration, centrifugation or exclusion by a molecule size) can be included before analysis. Traditionally, nitrogen and protein amounts in the obtained samples are detected by the Dumas method (combustion in the atmosphere of pure oxygen), by the Kjeldahl method, spectrophotometric or chromatographic methods.

The development of these methods was initiated abroad at the end of the 1940s; with that, the peak of the research was in 1956–1985. Foreign researchers used mainly multi-enzyme systems, most often "pepsin-pancreatin" and chromatographic methods for detection of one or several bioavailable amino acids. In 1956, Sheffner A. L. et al. published the study [11], in which they determined changes in the composition of essential amino acids by *in vitro* pepsin digestion of protein products and the pepsin-digest-residue (PDR) amino acid index was described. The authors used this index to describe differences between the composition and structure of essential amino acids released as a result of pepsin digestion and the amino acid structure of the initial protein and undigested residue.

The essence of the method consisted in incubation of the analyzed product sample (contained 1 g of protein) in 30 ml of the pepsin solution (25 g) with addition of 0.1 N hydrochloric acid for 24 hours followed by assessment of the content of essential amino acids. Profiles of essential amino acids in a food product and its hydrolysate expressed as a percent of the total content of essential amino acids were compared obtaining data about essential amino acids in the unhydrolyzed protein residue. Then, results obtained for the hydrolyzate and for the residue were compared with the corresponding values for egg protein determining the geometric mean for the "egg ratio" and obtained results were multiplied by the coefficient of the relative amount of the digestible substance and residue generated by the pepsin action on the sample analyzed and egg protein.

It is not easy to calculate the PDR index; however, division of this index by the digestibility coefficient of the respective proteins gave values that predicted quite accurately the biological value of proteins under study in *in vivo* experiments [11]. In this connection, this method became quite widespread. Later on, researchers included enzymes such as pancreatin, trypsin and erepsin in addition to pepsin into the system [12], as well as antimicrobial preparations (Thiomersal) to prevent the microbial growth in the system "substrate-enzyme" and obtain more pure data.

In 1964, Akeson W. R. and Stahmann M. A. published a paper [13] introducing the concept of the pepsin pancreatin digest (PPD) index. The reaction of the enzymatic cleavage of protein samples (100 mg protein) included their incubation in the solution of pepsin (1.5 mg) with addition of 0.1 N hydrochloric acid at 37 °C for 3 hours, neutralization with 7.5 ml of 0.2 N sodium hydroxide, following addition of the pancreatin solution (type III, 4 mg in 7.5 ml of phosphate buffer with pH 8.0) into the system and incubation at 37 °C for 24 hours. Then, 10 ml of the obtained suspension were mixed with 50 ml of 1% solution of picric acid, centrifuged at 1000 G for 30 min to remove undigested protein and large peptides. The obtained supernatant in a volume of 50 ml was run through the chromatographic column with anion exchange resin (AG 2-X, 200–400 mesh with three portions of 0.02 N hydrochloric acid, 5 ml each). Obtained samples were dried by lyophilization, diluted (up to 10 ml using the buffer with pH 2.2) and amino acid analysis was carried out by the ion exchange method. The pepsin pancreatin digest (PPD) index was calculated similar to PDR.

Kennedy J. et al. [14] continued to develop the methodology and in 1989 proposed to carry out an experiment on pepsin digestion in the dialysis cell. As a result, they introduced a new index PDD (pepsin digest dialysate), which is calculated similar to the PDR index discussed above. The authors obtained the PDD values for protein ingredients such as soybean meal, gelatin, gluten, casein, egg protein, cow milk enriched with carbohydrates, protein and vitamins [14]. The following was mentioned as an advantage of using the PDD index compared to the PDR and PPD: firstly, the use of a simpler device; secondly, the use of only one enzyme; thirdly, the use of the modern equipment for analysis of amino acids; fourthly, higher reproducibility and, finally, a possibility of computerized calculation [15]. For instance, Gauthier S. F. et al. [16] proposed an *in vitro* method by the example of casein. It consisted in peptic proteolysis by pepsin (3152 units/mg protein, an enzyme: substrate ratio of 1:250) in the close system followed by hydrolysis with pancreatin (an enzyme: substrate ratio of 1:25) for 24 hours in the "digestion cell") with the continuous elimination of digested products by dialysis (the circulating buffer was 10 mM sodium-phosphate buffer, pH 7.5) [16].

In 1965, in Russia, A. A. Pokrovsky and I. D. Ertanov [17] developed a method for detection of attackability (a degree of cleavage and digestion) of proteins, which gained the widespread use later on and became the conventional routine method in many laboratories. In the experiment, a product was subjected to two-stage hydrolysis with acidic and alkaline proteases in conditions close to the natural process in the human gastrointestinal tract. An amount of the accumulated low molecular weight products of hydrolysis was determined by the Lowry color reaction [18] quantitatively expressing the mass fraction of tyrosine in hydrolysate to the mass fraction of tyrosine in protein of the initial sample in percentage terms (mg tyrosine/g protein). For comparison, proteins with the high degree of the digestibility in the human and animal bodies were used [19,20,21]. Besides the tyrosine method, accumulation of proteolysis products was also controlled by the reaction of amino acids with ninhydrin [22].

Protocol according to A. A. Pokrovsky and I. D. Ertanov is described quite accurately in [23] and consists in the following. Freshly prepared pepsin solution (concentration of 1 mg/ml: 50 ml of 0.02 N hydrochloric acid solution (pH=1.2) is mixed with 50 ml of crystalline pepsin) is added to 1g of a minced sample, thoroughly agitated and warmed up at 37 °C, holding at this temperature for 3 hours. To determine the digestibility by pepsin, proteolysis is terminated and undigested protein is precipitated by addition into the sample of 50 ml of the mixture containing the 20% trichloracetic acid solution and acetone in a ratio of 3:2, agitating and filtering after 30 min. Residues of samples after digestion by pepsin are neutralized upon agitation with 0.4 ml of 2 N sodium hydroxide; after that, 15 ml of 0.02 N sodium bicarbonate solution (pH 8.2) is added, the mixture is warmed up at 37 °C for 10 min. and 15 mg of crystalline pancreatin are added with the following incubation at 37 °C for 3 hours. A degree of attackability of proteins in the product under study is assessed by an amount of non-protein nitrogen.

Indicators obtained in the control experiments (I/control — suspension of the analyzed sample in 0.02 N hydrochloric acid; II/ control solution of enzyme(s)) are subtracted from a value characterizing hydrolysis. The data are presented in% to total nitrogen.

Calculations are carried out by the equation:

$$K = A - B - C \quad (1)$$

where:

K is an increase in hydrolysis products due to the action of enzyme(s);

A is the concentration of hydrolysis products in the sample analyzed;

B is the concentration of the same products in the food product suspension, I/control;

C is the concentration of the same products in the solution of enzyme, II/ control.

Currently, many variations of the method by A. A. Pokrovsky and I. D. Ertanov have been developed and used [17]: in modification of the V. M. Gorbatov All-Russian Research Institute for Meat Industry (now Gorbatov Research Center for Food Systems) (Moscow, Russia) [24]; Moscow State University of Applied Biotechnology (now Moscow State University of Food Productions) (Moscow, Russia) [25,26]; Institute for Meat and Dairy Industry (Minsk, Republic of Belarus). Specific features of modifications were a possibility to use dialysis bags, control of pH, periodicity of sampling without stopping proteolysis. For example, the use of dialysis after each stage allows removing hydrolysis products from the sphere of the reaction to avoid inhibition of digestive enzymes by low molecular weight peptides and free amino acids [27].

The specialists of the Moscow State University of Applied Biotechnology (now Moscow State University of Food Productions) (Moscow, Russia) developed the apparatus for hydrolysis of minced product samples (on the basis of the content of about 150 g protein in it). The apparatus consists of several cells that represent a system of internal and external vessels divided by the semipermeable membrane. A sample and 15 ml of the hydrochloric acid solution (0.02 mol/l) are placed into the internal vessel of the apparatus, while 60 ml of the hydrochloric acid solution with the same concentration and then 15 mg of crystalline pepsin (the enzyme concentration is 1 mg/ml) are placed into the external vessel. With that, the internal vessel is placed into the external one so that its bottom is dipped into the solution and the levels of liquids in both vessels are equal. The experiment is performed at a temperature of 37 °C. The reaction is carried out with agitation of the liquid using a mixer (rotation frequency $1s^{-1}$) and a sample (0.1 cm^3) is taken three times at hour intervals from the internal vessel. After that, a volume of hydrochloric acid equal to a volume of the sample taken is transferred to the vessel. The biuret reagent (1 ml) is added into each sample and the control sample (1 ml of distilled water and 1 ml of the biuret reagent) is prepared. The samples are placed into cuvettes, the latter are placed into a photoelectric colorimeter and absorbance is measured at a wavelength of 540 nm. For the further hydrolysis, the liquid from the external glass is replaced with 15 ml of $NaHCO_3$ (0.02 mol/l) and pepsin digest is neutralized with 0.4 ml of the NaOH solution (2 mol/l). Then, 15 ml of the $NaHCO_3$ solution (0.02 mol/l) are added and 15 mg of crystalline trypsin are introduced after temperature equalization. The subsequent procedures were conducted in a similar way measuring absorbance of three samples by the color reaction according to the Lowry method using a photoelectric colorimeter. Data determined by a calibration graph are recalculated with account for the total volume of liquid in the external and internal vessels and, then, these values are added up. Values obtained in the control experiments are subtracted from the tyrosine concentration, which characterizes a hydrolysis degree: an enzyme solution in the first experiment and a suspension of the analyzed product in the buffer solution in the second experiment. Accumulation of hydrolysis products detected by the Lowry method is expressed in micrograms of tyrosine per 1 g of dry matter.

The modification of the Institute for Meat and Dairy Industry (Minsk, Republic of Belarus) resides in the sequential exposure of protein substances of the product under study to the proteinase system consisting in pepsin and trypsin upon continuous agitation and removal of hydrolysis products from the reaction sphere with the subsequent photometric measurement of the product color intensity with the Folin's reagent, quantitatively expressing the tyrosine concentration (μg/cm^3) due to an effect of proteolytic enzymes on a product over 6 hours with a one-hour interval.

The integration of methods developed by Russian and foreign scientists is of special scientific interest. For example, Bologa et al. [28] obtained the pepsin-pancreatic index for whey proteins using the system "pepsin-trypsin- chymotrypsin".

Table 1. Systemized data about comparison of methods for assessment of the food product digestibility by enzymatic digestion

Analytic method*		AA	AA	AA	A/AA	L	L	L
Authors		Sheffner et al., 1956 [11]	Akeson, Stahmann 1964 [13]	Kennedy et al., 1956 [14]	Gauthier et al., 1986 [16]	Pokrovsky, Ertanov, 1965 [17]	Lipatov, Yudina, Lisitsyn, 1994 [27]	Institute for Meat and Dairy Industry (2020)
Gastric enzymes	Pepsin	×	×	×	×	×	×	×
	Trypsin	–	–	–	–	–	×	×
	Temperature 37 °C	–	×	×	×	×	×	×
	pH 1.2–3	×	–	–	–	×	×	×
	30–60 min	–	–	–	×	–	–	–
	120–180 min	–	×	–	–	×	×	–
	6 hours	–	–	–	–	–	–	×
	24 hours	×	–	×	–	–	–	–
	Dialysis	–	–	×	–	–	–	–
	Termination of proteolysis	–	–	–	–	–	–	×
Intestinal enzymes	Pancreatin	–	×	–	×	×	–	–
	Temperature 37 °C	–	×	–	×	×	–	–
	pH 8	–	×	–	–	×	–	–
	< 30–60 min	–	–	–	–	×	–	–
	120–180 min	–	–	–	–	–	–	–
	24 hours	–	×	–	×	–	–	–
	Dialysis	–	–	–	×	–	–	–

Note: * AA — amino acid analysis; A — determination of nitrogen content; L — Lowry method (reaction of Folin's reagent with phenol radicals of amino acids).

Among various methods, it is worth noting the method by N. M. Savich and G. M. Zholdaspaeva [29], in which protein under study is placed on a strip of filter paper in several replications, part of samples are processed with the proteolytic enzyme (pepsin/papain) and another part is held as a control. After incubation, all samples are colored with amido black 10B; then, the complex of protein with the dye is eluted and the protein content in the obtained colored solutions is determined by the spectrophotometric method. The digestibility is assessed as a proportion of hydrolyzed protein expressed in percent of the initial quantity [29]. Today, this method is used mainly for assessment of grain raw materials.

Table 1 presents variability of the existing static *in vitro* methods for studying cleavage of analyzed products with different nature using the enzyme systems.

Methods for assessing food protein digestibility based on changes in environmental pH (pH-stat, titration method)

Although this method also uses enzymes, it is based on the regression equation of calculation of changes in pH of the reaction medium that occur due to liberation of protons from cleaved peptide bonds in protein hydrolysis by digestive enzymes and has been used for many decades.

The beginning of the development of this method is considered to be the 1970s, when Maga J. A. et al [30] revealed a close interrelation with the initial rate of protein hydrolysis under the action of trypsin assessed as an indirect measure of pH in the reaction mixture over time and the digestibility of protein samples in *in vivo* experiments [28]. Later on, this method was subjected to various modifications in terms of using enzyme combinations and duration of the experiment [31]. In 1977, for example, Hsu H. W. et al. [32] published data on testing various enzyme combinations to improve the correlation coefficients between *in vitro* experiments (drop in pH) and *in vivo* protein digestibility (in rats). The result of the work was the pH-stat method, in which the multi-enzyme systems (trypsin, chymotrypsin and peptidase) were used [33]. The method validity was assessed by comparison of data with coefficients of the protein digestibility for rats. It was revealed that when using the new pH-stat method to assess 23 sources of food protein, the correlation coefficient with the apparent digestibility of protein for rats was 0.9; with that, an effect of the trypsin inhibitor, chlorogenic acid and thermal treatment on the digestibility was shown. Therefore, the authors established that the high content of ash affected the results of the digestibility of the protein under study and demonstrated that this method based on the pH decline depended on the buffering capacity of the protein analyzed [8].

The standard protocol of the *in vitro* pH-stat experiment for assessing the food protein digestibility according to Hsu H. W. et al. [32] consists in the following. Analyzed samples with the known protein content are comminuted into fine powder, which can pass through an 80-Mesh sieve (with a hole diameter of 0.18 mm). Then, a protein aqueous suspension is prepared on the basis of 6.25 mg protein/ml based on distilled water, 50 ml are taken, pH is brought to 8.0 with 0.1 N hydrochloric acid and /or sodium hydroxide and the mixture is placed into the water bath at 37 °C. The multi-enzyme solution containing (in 1 ml) 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase is prepared and held in the ice bath, pH is brought to 8.0 with 0.1 N hydrochloric acid and /or sodium hydroxide. The multi-

enzyme solution (5 ml) is added to the warmed protein suspension (50 ml) and the mixture is agitated in the water bath at 37 °C. Sodium caseinate is used as a standard. pH is recorded automatically over 10 min using a recording pH meter. During the reaction, a rapid pH drop is observed, which is caused by liberation of carboxyl groups of amino acids from the protein chain by proteolytic enzymes.

The following can be determined additionally: 1) graphical dependence of the pH drop on time for each enzyme individually according to the procedure described for the multi-enzyme system; 2) an effect of the trypsin inhibitor contained in the sample analyzed on digestion by trypsin or the multi-enzyme system by addition of soybean trypsin inhibitor (23.4 mg)/ trypsin inhibitor type II-S to 50 ml of casein suspension (6.25 mg/ml) by detecting the mixture digestibility using trypsin (1.6 mg/ml) or the multi-enzyme solution according to the procedure described above; 3) buffering abilities of different protein sources by the following way: 50 ml of the protein suspension (6.25 mg/ml) are brought to pH 8, with 0.1N NaOH or HCl at 37 °C; after that; the protein suspension is slowly titrated with 0.0096 N HCl to pH 6.45 for 10 min; the buffering capacity of a protein source is determined as an amount of used acid [32,33].

To overcome the sensitivity of the pH-stat method to the buffering ability of protein samples, Pedersen B. et al. (1983) [34] revised the method considering an amount of alkali consumed in the reaction medium as an indirect indicator of the true digestibility of protein in rats. The authors maintained pH of the reaction at the constant level of 8 during titration with alkali for 10 min, which allowed improving the correlation coefficient from c 0.9 to 0.96 with the residual error of 1.29 after assessment of 30 protein samples [33]. The authors assumed that an effect of the ash content on the test results was linked with differences in the content of mineral substances mainly, calcium. The authors proposed to use two different regression equations for accurate prediction of the digestibility of protein samples from plant and animal origin. However, even proposed equations for predicting the digestibility of the protein source type are unreliable due to the fact that it is necessary to know the average dissociation of α-amino groups and the number of peptide bonds in the structure of proteins present in the analyzed ingredient when measuring a degree of protein hydrolysis by this method [8].

Subsequently, the pH-stat method developed by Hsu H. W. et al. [32] was modified by Satterlee L. D. et al. [33]. The experiment was aimed to assessment of different feed ingredients with the high protein content using three variants of enzyme mixtures and compared with the results of the true protein digestibility in male broiler chickens subjected to cecectomy (endoscopic dissection) [35]. The high correlation of the performed experiment with the lysine digestibility was revealed in male broiler chickens for the ingredients tested. However, the experiment did not show any relation with the lysine digestibility and the protein efficiency ratio in the samples of feather and meat meal of different quality.

Therefore, the pH-stat method has the following disadvantages: low accuracy of prediction of protein bioavailability (correlation with *in vitro* experiments is 0.9 to 0.96); labor intensity and complexity of the method for routine quality control of samples analyzed; reproducible results are obtained only for easily digestible sources of pure protein with known data about average dissociation of α-amino acids and the number of peptide bonds.

The systemized data and comparison of the pH-stat methods (titration method) for assessing food protein digestion as well as objects of research are presented in Table 2.

Table 2. Systemized data about evolution of the pH-stat method (titration method) for assessing food protein digestion

Authors	Experimental conditions	Analyzed objects
Maga J.A. et al [30]	Enzyme — trypsin; incubation temperature and time — 37 °C, 10 min	Sodium caseinate, defatted peanut meal, defatted cotton meal, protein concentrate of fish meal, soybean isolate
Vavak, D.L.R. [31]	Enzyme — trypsin -chymotrypsin; incubation temperature and time — 37 °C, 10 min	Purified dried grain protein
Hsu H.W. et al. [32]	Enzymes — pork trypsin (type IX, 14190 BAEE / mg protein), bovine chymotrypsin (type II, 60 U/mg powder), pork peptidase (III, 40 U/ g powder); incubation temperature and time — 37 °C, 10 min; control- sodium caseinate.	Soybean isolate; cotton meal; durum semolina; whole-grain white wheat meal, whole-lactose defatted whey; partly purified milk whey; standard caseins; soybean meal of different thermal processing (90, 70 and 20 PDI); soybean protein concentrate
Satterlee L.D. et al [33]	Enzymes — pork trypsin (type IX), pork peptidase (I), bovine a-chymotrypsin (type II), bacterial pronase P or E; incubation temperature and time — 37 °C, 20 min; control- sodium caseinate.	Soybean isolate, protein concentrate from fermented wheat, protein concentrate from corn
Pedersen B., Eggum B. O. [34]	Enzymes — pepsin (7192,100 mU/mg powder), pork trypsin (type IX, 14190 BAEE/mg protein), bovine chymotrypsin (type II, 47 U/ mg powder), pork peptidase (I, 22 U/ mg powder); Streptomyces griseus protease (4.4 U/mg powder); incubation temperature and time — 37 °C, 10 min; control- sodium caseinate.	Lyophilized beef, cod fillet, eggs

Therefore, the pH-stat test is mainly used to predict the digestibility of easily digestible sources of pure proteins with known data about average dissociation of α-amino groups and the number of peptide bonds. It is worth noting that from the beginning of the 1990s, the pH-stat method has been used only to assess feed ingredients for aquatic animals, which is linked with the simplicity of their digestive tract, in particular, the enzyme composition, as well as with the wide use of sources of easily digestible proteins, such as fish meal, for their feeding [36]. Up to now, all accumulated data obtained by the pH-stat method especially using purified enzymes extracted from the stomach and intestine of the targeted animals have been in agreement with the *in vivo* digestibility analyses on the targeted aquatic animals [37,38]. In conclusion, it is necessary to note that the average constant of casein dissociation and the number of peptide bonds are used in calculation of the degree of hydrolysis of the objects analyzed as standards to eliminate restrictions of the method [8].

It is worth noting that over the indicated period, no methods based on the changes in the pH of the medium were revealed in the national literature even in the case of studying feedstuff and feeding meal. In the Russian Federation, two state standards have been approved and used for studying the feedstuff digestibility. The first standard GOST R55987–20141 describes the method that includes incubation of a preliminary defatted and dried sample of feeding meal in the solution of pepsin in diluted hydrochloric acid at a temperature of 45 °C for 16 hours, filtration of the suspension and detection of the mass fraction of the dry residue on a filter in relation to the mass of the initial defatted and dried sample (digestibility of feeding meal) or mass fraction of nitrogen in the dry residue on a filter (protein digestibility) in relation to the mass of nitrogen in the initial defatted and dried sample. When the mass fraction of fat in feeding meal is less than 10%, the digestibility is determined without defatting. The document includes the description of differences in process temperature and duration, centrifugation regimes, expression of the result in percent. The second standard GOST 24230–802 includes the method for detection of a degree of the digestibility (dissolution) of the dry matter using enzymes pepsin and celloviridine also with the description of differences in process temperature and duration, centrifugation regimes, expression of the result in percent.

Despite a large number of *in vitro* methods for studying the product digestibility, the main of which are described in this paper, all of them have limitations and drawbacks. To solve this problem and within the framework of the investigation of wholesome properties of foods by their assessment in the digestive process, COST Action network was created in 2011. Since 2015, it has turned into the international research network uniting more than 440 scientists from 150 institutes in 45 countries of Europe, USA, Canada, Argentina, Australia, New Zealand and others [39]. Scientists from the INFOGEST network [40] are guided by particular goals such as: to harmonize conditions of *in vitro* digestion; develop a static model that would be easily adjusted and used for various objects and a large research community; have a possibility to compare the results of the investigations.

In 2019, the standardized protocol for *in vitro* investigation of the food product digestibility INFOGEST was published as a result of the large-scale work. Published for the first time in 2014, the method has acquired the status of Highly Cited Paper for agricultural sciences over the last years and was cited in journals included into Web of Science more than 650 times. The method is widely used by scientists worldwide with multiple goals for various food products and endpoints [40]. It is an easy-to-use static digestion method, in which samples of food products are subjected to sequential oral, gastric and intestinal digestion; while parameters such as electrolytes, enzymes, bile, dilution, pH and digestion duration are based on available physiological data. However, the method is not suitable for simulation of digestion kinetics [39,40]. The altered and improved digestion method INFOGEST 2.0 [41] allows avoiding problems linked with the initial method, such as inclusion of the oral phase and the use in gastric lipase. The method is widely used in assessment of endpoints that arise as a result of food product digestion by analysis of digestion products (for example, peptides/amino acids, fatty acids, simple sugars) and assessment of release of food microelements from the food matrix. The whole protocol can be completed within about 7 days including about 5 days that are necessary to determine the activity of enzymes of the gastric and intestinal digestion [42]. This protocol was also used by Russian scientists [43].

Therefore, methods for studying cleavage of different products and the creation of *in vitro* models is a dynamically developing field of knowledge (Figure 2).

Today, methods developed in the period from 1964 that include one or two digestion phases using several enzymes (pepsin/trypsin/pancreatin) as well as the recent methods that simulate the digestion processes in one, two or three sequential phases (oral, gastric and intestinal) with the use of multi-component mixtures of enzymes are applied depending on objects of a study and aims of an experiment [1].

These methods are widely used to assess the digestibility of proteins [42], lipids [44] and carbohydrates [45], allergenicity of proteins and their resistance during the digestion process [46,47,48,49], release and bioavailability of nutrients from complex and simple food matrices [50,51], to study interaction of various compounds with nutrients [50].

[9] GOST R55987–2014 "Feeds, raw material for mixed feeds. Method for determination of digestibility of feather meal in vitro". Moscow: Standartinfopm, 2020. — 11 p.

[2] GOST 24230–80 "Vegetable feeds. Method for determination of digestibility in vitro". Moscow: IPK Publishing House of Standards, 2003. — 4 p.

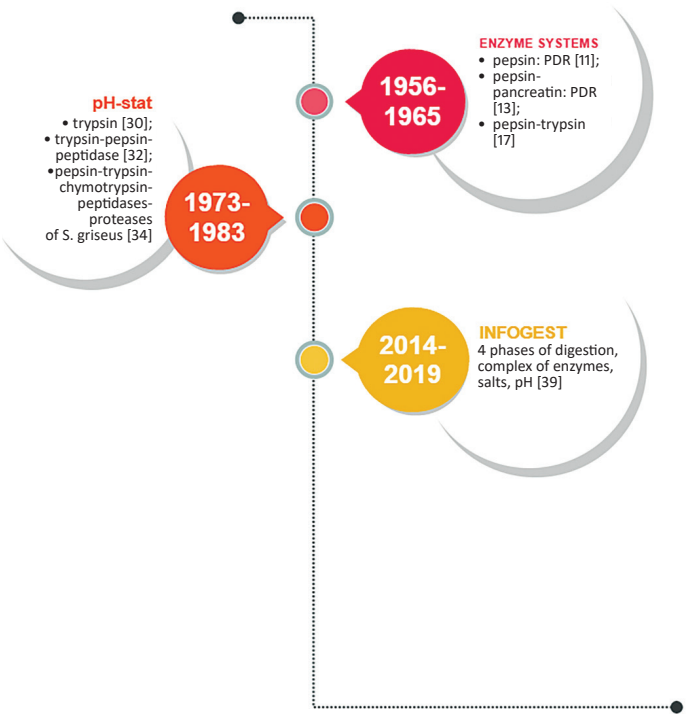

Figure 2. Evolution of *in vitro* static digestion models

The recent trend in the application of this method includes the study of the development and stability of bioactive molecules (including peptides) during digestion in the gastrointestinal tract [52,53,54], digestibility and bioavailability of phytochemical substances of plants and their metabolites with the antioxidant activities [55,56], bioavailability of pollutants to evaluate the health risks [57]; to assess viability of incapsulated beneficial microorganisms [58].

Conclusion

1. It was established that *in vitro* digestion models are promising and alternative options of *in vivo* experiments to simulate complex multi-stage physiological processes in the gastrointestinal tract that allow taking into account such factors as the presence and concentration of digestive enzymes, pH values in the gastric and intestinal phase, digestion duration, salt concentration and so on.
2. It was found that the used means for studying *in vitro* the food product digestibility include the assessment methods using enzymatic digestion that can be based on the calculation of nitrogen remained after digestion in relation to the initial total nitrogen (by the Dumas method, Kjeldahl method, spectrophotometric or chromatographic methods) and include additional stages such as filtration, centrifugation or exclusion by a molecule size, as well as methods (pH-stat, titration method) that are based on changes in pH of the reaction medium (occurring due to liberation of protons from cleaved peptide bonds in protein hydrolysis by digestive enzymes) and depend on the buffering capacity of the protein under study, which are mainly used for prediction of the digestibility of easily digestible sources of pure proteins with known data about average dissociation of α-amino groups and the number of peptide bonds.
3. It was revealed that the pH-stat methods are mainly used to assess the digestibility of feedstuff for aquatic animals, which is linked with the simplicity of their digestive tract, in particular, the enzyme composition, as well as with the wide use of sources of easily digestible proteins for their feeding, while methods for assessment of the digestibility by enzymatic digestion are widely used also for analysis of different food product types.
4. It was established that methods for assessment of the food product digestibility by enzymatic digestion have undergone different stages of evolution (since 1947) and have been used in the wide modification by inclusion of various enzymes (pepsin, trypsin, pancreatin, erepsin, etc.) as well as antimicrobial preparations into model systems, which allowed introducing concepts of pepsin-digest-residue (PDR) index (1956); pepsin pancreatin digest (PPD) index (1964); pepsin digest dialysate (PDD) (1989). Among these indices, the latter has several advantages compared to the other above mentioned indices such as the use of a simpler device, only one enzyme, modern equipment for analysis of amino acids, higher reproducibility and a possibility of computerized calculation. At the same time, the development of methods based on changes in pH of the medium began from 1973 when a close interrelation with the initial rate of protein hydrolysis under the action of trypsin was revealed with the following analysis of various combinations of enzymes (1977) and the study of a possibility to overcome method sensitivity to the buffering ability of protein samples (1983) and comparison with the results of the true protein digestibility in farm animals (1984).
5. Currently, in the Russian Federation and Republic of Belarus, several methods for *in vitro* detection of the food product digestibility have been developed and used, which allow detailed assessment of quality indicators of these products: with the use of mono- and multi-enzyme systems, the modified apparatus of MGUPB, the model system of the Institute for Meat and Dairy Industry and others, which characteristic features are a possibility to use dialysis bags, control of pH, parameters of proteolysis termination and precipitation of undigested protein. In addition, several state standards for studying the feedstuff digestibility are in force (GOST R55987–2014 and GOST 24230–80), which methods are based on incubation of a preliminarily defatted and dried sample in the solution of pepsin in diluted hydrochloric acid as well as detection of a degree of the digestibility of the dry matter using enzymes pepsin and celloviridine.
6. The INFOGEST (2019) method allows quite accurate reproduction of the conditions in the gastrointestinal

tract due to the sequential use of digestive enzymes in the physiological concentrations; simulation of a suitable environment for the action of enzymes (temperature, pH, presence of co-factors), duration of each phase that is close to the physiological (for humans) and removal of digestive products. In this connection it is widely used by scientists when studying food products.

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COLOR MEASUREMENT OF ANIMAL SOURCE FOODS

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Abstract
Rapid and objective assessment of food color is necessary in quality control. The color evaluation of animal source foods using a computer vision system (CVS) and a traditional colorimeter is examined. With the same measurement conditions, color results deviated between these two approaches. The color returned by the CVS had a close resemblance to the perceived color of the animal source foods, whereas the colorimeter returned not typical colors. The effectiveness of the CVS is confirmed by the study results. Considering these data, it could be concluded that the colorimeter is not representative method for color analysis of animal source foods, therefore, the color read by the CVS seemed to be more similar to the real ones.

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Introduction

Animal source foods are an essential source of numerous components [1–3]. Nowadays, the color represents a decision-making criterion in the food purchasing [4]. It is a crucial tool in the food marketing [5].

In terms of meat color, lighter appearance is preferred by consumers due to their association of dark meat with quality lack [6]. This sensory property also can be an indicator of some defects in milk, such as adulteration [7], spoilage [8] and the long-term storage conditions [9]. Regarding eggs, yolk [10] and shell color [11] make the valuable quality attribute for consumers' judge. In general, customers rather desire the yellow-orange egg yolk than off-white yolk [12–13]. In case of the eggshell, consumer priority for color differs worldwide [14]. Furthermore, white-shell eggs are desired in Japan, North and Central America, Middle East, India, Taiwan, and Philippines, whereas brown-shell eggs are opting for Latin America, Europe and China [15].

Instrumental color evaluation is vital for food technology and can be performed using instruments such as colorimeters. Commonly the meat, milk and egg color measurements are evaluated using Minolta colorimeters [16–19]. These devices offer a simple and fast food color analysis, moreover, they are easy to handle and calibrate. Each colorimetric instrument has several settings influencing food color parameters such as color system, illuminant, observer, port size and calibration procedure. However, only a few percentages of papers reported all the procedures and technical parameters used for animal source foods (meat, milk and egg) color determination as stated by Tapp et al. [16], Tomasevic et al. [17], Milovanovic et al. [18], Milovanovic et al. [19,20].

On the other hand, the colorimeter has a various number of shortcomings concerning failure to capture broad spectral information in terms of internal characteristics of objects [21] as well as the incapability to measure a extend surface, with non-homogenous color [22]. To achieve consistent analysis these color instruments require the homogeneous and uniform samples [23]. Furthermore, to overcome shortcomings of colorimeters, it has developed a new alternative method known as a computer vision system (CVS). By applying the CVS, the advantage of ability to determine color readings for each pixel of a sample image provides the rapidness, budget and simplicity [24]. Additionally, CVS has been widely performed for color measurement of animal source foods [25–29].

Material and methods

Sample preparation, color evaluation equipment used, sensory tests by a trained panel and statistical analysis performed were all explained in previous publications [19] and [25–29].

Results and discussion

Meat and meat products

Instrumental color data (L*a*b*, hue and chroma) for meat and meat products were significantly different [25–28].

The instrumental color assessments acquired by the colorimeter for chicken and turkey (lighter colored poultry) are in line with the previously published color results

for chicken [30] and turkey breast meat [31] acquired with other colorimeters. Furthermore, the appearance of chicken and turkey meat returned from CVS was lighter, whereas duck and goose were darker [25]. Therefore, with the total color difference of $\Delta E = 18.5$ for chicken and $\Delta E = 22.04$ for turkey meat, it can be concluded that the two systems performed their color significantly different and even contrasting (Figure 1). The color of duck and goose breasts (darker colored poultry meat) measured with the CVS was darker and more "red" than the colour obtained with the colorimeter (Figure 1). When comparing the colorimeter-generated color readings obtained, they are in concurrence with the data available in previously published papers [32,33]. However, the total color differences between the two color devices, for goose and duck were half the values calculated for chicken and turkey [25].

The instrumental color data read by colorimeter for wild boar and deer (darker colored game meat) are in concurrence with Borilova et al. [34] and Kudrnáčová et al [35] for wild boar and deer meat, respectively. The color of wild boar and deer meat read by CVS had lower L* and higher a* values (brighter and redder color) than colorimeter (Figure 2). However, quail, pheasant and rabbit (lighter colored game meat) indicated that the color acquired by CVS had the higher lightness than the colorimeter. All redness values were much higher when measured with CVS compared to colorimeter, meant that the color acquired by CVS was more "red" (or less "green"). Total color difference was in range from 9.7 (pheasant) to 19.0 (rabbit) [26].

Regarding pork meat, the color traits measured with CVS and colorimeter were significantly different with the exception of b* reading [27]. The high lightness (L*), a less redness (a*), and relatively high yellowness (b*) indexes of pork meat were read by colorimeter in comparison to the CVS. In case of meat and fat pork parts, total color difference was 16.7 and 10.8, respectively, indicating that for meat parts even contrasting. These results are in good agreement with Girolami et al. [36], who confirmed using CVS as more precise and closer to the exact color value. Furthermore Sun et al. [37] concluded that the CVS has potential to be used as a tool in predicting pork color attributes. In addition, Sun et al. [38] also postulated that CVS can accurately evaluate pork color, a major advantage over traditional subjective evaluation and/or colorimeter devices which have their own.

Beef lightness read by the colorimeter was higher than the color obtained using the CVS. On the contrary, the color attributes such as a*, b*, chroma values, gathered through the CVS, were higher [27]. Meat and fat parts were assessed in darker colors when measured with CVS compared to the colorimeter device (Figure 3). Girolami et al. [36] assessed that the light from a colorimeter illuminates about 15–20 mm, and about 5 mm from the CVS. Similarly, Trinderup et al. [39] found that light penetrates about 20 mm from a colorimeter, and a few mm from the CVS. With regard to the results of pork and beef, they are

Figure 1.
Color of poultry meat as evaluated by the two methods [25]

Figure 2.
Color of game meat as evaluated by the two methods [26]

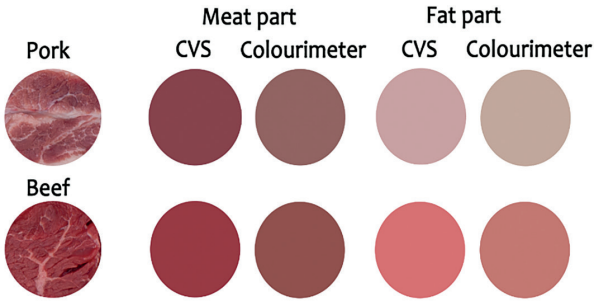

Figure 3. Color of pork and beef meat as evaluated by the two methods [27]

in good agreement with findings from previous investigation of Girolami et al. [36] that the color predicted with the CVS is closer to the sample than the color read by colorimeter, making CVS more representative for beef color analysis.

Considering meat products, uniformly-colored meat products revealed that the color gathered through CVS had higher lightness value than that obtained with the colorimeter. All the a* values were higher when measured with CVS compared to colorimeter, therefore, the color obtained with CVS was more "red" (Figure 4). This investigation is in concurrence with the conclusions of Valous et al. [40] that CVS is a tool that can objectively specify color of cooked-hams. Regarding bi-colored meat products, the total color differences between the two methods of the meat segments were in a range from 7.3 up to 14.6 and for the fat parts in a range from 7.7 up to 12.9 [28]. Meat segments were assessed in darker and fat segments in lighter colors when obtained by CVS compared to colorimeter (Figure 5a).

In terms of non-uniformly colored meat products, the color of meat parts read by CVS was significantly darker, had greater intensity and was more saturated, compared to colorimeter-measured equivalents (Figure 5b). The opposite was observed for CVS-generated fat color. Girolami et al. [36] also concluded that CVS is a method that can objectively evaluate the color of fermented sausages.

In addition, the possible reason for the color deviations between these two systems could be the interaction light source with the surface of meat which is translucent [22]. This caused light diffusion from light source resulting in less accurate analysis by the colorimeter.

Sensory tests also showed differences between these two color devices. Frequency of similarityassessed by the panelists was 100% for all poultry, game, beef and pork meat samples (Table 1).

Frequency of similarity (the first test) was very high and ranged from 85.7% for rabbit meat, 92.9% for chicken pate, beef sausage, smoked bacon, dry pork neck and pancetta, to 100% for all the other meat products. The second test (CVS vs. colorimeter) demonstrated that the CVS-produced squares were more resemble to the sample of the poultry, game, pork, beef and meat products visualized on the monitor, compared to colorimeter-produced color square in all (100%) individual trials conducted. The third test (level of difference) regarding meat products revealed that, as assessed by the assessors, the magnitude of differences between the color chips generated by CVS and colorimeter and displayed on the monitor, ranged from 1.0 („very low") for deer meat to 4.7 („high") for turkey breast meat [25–28].

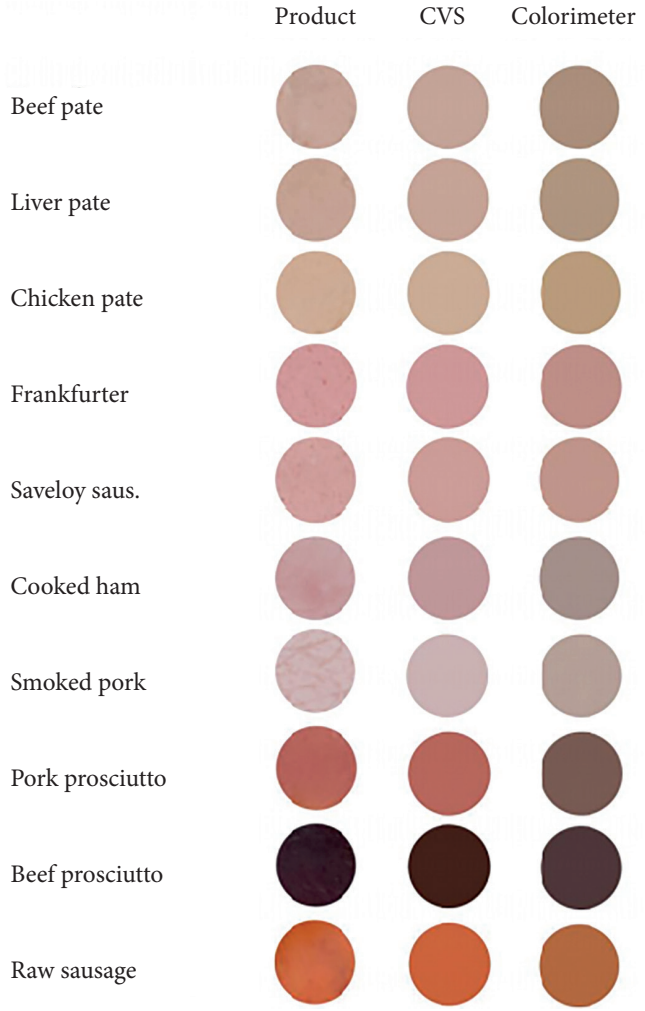

Figure 4. Color of uniformly colored meat porducts as evaluated by the two methods [28]

Figure 5. a) Color of bi-colored and b) non-uniformly colored meat products as evaluated by the two methods [28]

Table 1. Similarity test results of meat and meat products [25–28]

	Frequency of similarity	Level of similarity	CVS vs. Colorimeter	Level of difference test
Beef pate	100.0%	3.4 ± 1.4$^{a, b}$	CVS (100.0%)	3.0 ± 1.1$^{a, b, c}$
Liver pate	100.0%	3.6 ± 1.1$^{a, b}$	CVS (100.0%)	2.4 ± 1.1$^{a, b, c}$
Chicken pate	92.9%	3.5 ± 1.0$^{a, b}$	CVS (100.0%)	2.1 ± 1.0$^{a, b, c}$
Beef fermented sausages	92.9%	3.6 ± 1.0$^{a, b}$	CVS (100.0%)	3.2 ± 0.4$^{a, b, c}$
Pork fermented sausages	100.0%	4.0 ± 0.8$^{a, b}$	CVS (100.0%)	2.3 ± 0.5$^{a, b, c}$
Frankfurter	100.0%	4.0 ± 1.1$^{a, b}$	CVS (100.0%)	1.7 ± 0.5$^{a, b}$
Saveloy sausage	100.0%	3.8 ± 0.9$^{a, b}$	CVS (100.0%)	1.2 ± 0.5^{a}
Mortadella	100.0%	2.9 ± 1.2^{a}	CVS (100.0%)	2.1 ± 1.1$^{a, b, c}$
Cooked ham	100.0%	3.0 ± 1.2$^{a, b}$	CVS (100.0%)	3.6 ± 0.3$^{b, c}$
Smoked cooked bacon	92.9%	3.1 ± 1.3$^{a, b}$	CVS (100.0%)	2.2 ± 0.4$^{a, b, c}$
Smoked cooked pork	100.0%	3.5 ± 1.0$^{a, b}$	CVS (100.0%)	2.8 ± 1.2$^{a, b, c}$
Pork prosciutto	100.0%	4.1 ± 0.8$^{a, b}$	CVS (100.0%)	4.2 ± 1.0^{c}
Beef prosciutto	100.0%	3.6 ± 0.9$^{a, b}$	CVS (100.0%)	3.1 ± 1.8$^{a, b, c}$
Dry pork neck	92.9%	3.5 ± 1.3$^{a, b}$	CVS (100.0%)	3.0 ± 0.7$^{a, b, c}$
Pancetta	92.9%	2.8 ± 1.5^{a}	CVS (100.0%)	2.7 ± 1.5$^{a, b, c}$
Pork hamburger	100.0%	2.8 ± 1.0^{a}	CVS (100.0%)	2.0 ± 1.0$^{a, b}$
Beef hamburger	100.0%	3.4 ± 1.3$^{a, b}$	CVS (100.0%)	2.7 ± 1.0$^{a, b, c}$
Raw sausage	100.0%	4.4 ± 0.8^{b}	CVS (100.0%)	3.2 ± 1.5$^{a, b, c}$
Chicken breast	100.0%	1.7 ± 0.8^{a}	CVS (100.0%)	3.8 ± 1.4^{a}
Duck breast	100.0%	2.4 ± 1.0$^{a, b}$	CVS (100.0%)	1.8 ± 0.4^{b}
Goose breast	100.0%	3.1 ± 0.8^{b}	CVS (100.0%)	1.4 ± 0.5^{a}
Turkey breast	100.0%	2.9 ± 1.03^{b}	CVS (100.0%)	4.7 ± 0.7^{b}
Quail	100.0%	2.7 ± 1.3^{a}	CVS (100.0%)	3.6 ± 1.4^{a}
Wild boar	100.0%	3.4 ± 1.3^{b}	CVS (100.0%)	1.9 ± 0.9$^{b, c}$
Rabbit	85.7%	2.7 ± 1.2^{a}	CVS (100.0%)	4.2 ± 1.2^{a}
Deer	100.0%	4.1 ± 0.8^{b}	CVS (100.0%)	1.0 ± 0.0^{c}
Pheasent	100.0%	3.2 ± 1.2$^{a, b}$	CVS (100.0%)	3.4 ± 1.3$^{a, b}$
Pork	100.0%	2.6 ± 0.8^{a}	CVS (100.0%)	4.2 ± 0.7^{a}
Beef	100.0%	4.1 ± 0.5^{b}	CVS (100.0%)	4.0 ± 0.7^{a}

Means in the same column with different small letters are significantly different (P < 0.05)
Five-point scale ranks from 1 "very low", 2 "low", 3 "moderate", 4 "high" to 5 "very high".

Milk and milk products

The color coordinates of milk and milk products were statistically different as reported by Milovanovic et al. [29].

Regarding milks, samples seemed lighter and redder when CVS was considered, meaning they were in the red space. In contrast, all the milk samples showed higher yellowness readings read by the colorimeter as compared with the CVS, denoting more yellow milk appearance (Figure 6). Total color difference provided well perceptible difference, ranged from 4.3 (cows' milk and goats' milk) to 5.6 (sheep's milk). The color parameters of raw milks read by colorimeter are in line with the literature data reported by Milovanovic et al. [18].

The color of white chesses assessed by colorimeter was lighter than color acquired by CVS. White cheeses were closer to the red and blue region as compared to the green and yellow region read by the colorimeter (Figure 6). Color difference was according to the scale in the range of 11.3–11.8 [29]. These instrumental results obtained with the colorimeter are in agreement with findings from previous investigations determining the color of fresh cheese [41] and brined cheese [42].

As regards to the fermented products, all L* and b* readings read by Minolta were higher than by CVS, whereas a* readings were in the redness region compared with colorimeter-produced color (Figure 7). The color variations are in line with ΔE, ranged from 5.8 (yoghurt) to 6.6 (kefir) [29]. Concerning fermented products, color readings obtained by the colorimeter are in line with previously published results of color measurement for yoghurt [43], set-style yoghurt [44] and kefir [45] acquired with other colorimeters.

Color determinations using two devices for color detection of sour cream and heat treated cream were significantly different. Moreover, using the colorimeter is obtained brighter, greener and yellower appearance as compared to the color read by CVS (Figure 7). The total color difference ranged from 6.7 (heat treated cream) to 11.0 (sour cream) [29].

When it comes to the skim milk powder, there is a significant difference between colorimeter and CVS color readings. On the contrary, all a* values obtained by CVS were higher (more "red") than those measured by the colorimeter (Figure 7). Yellowness values acquired by the colorimeter were higher (yellower appearance) compared with those gained by the CVS. Total color difference was 15.4 [28].

With regard to the lightness observations of kajmak spread, the colorimeter had higher values (brighter appearance) than CVS. All a* values observed using CVS were less "green" in contrast to the colorimeter-observed color, whereas all the b* observations indicated more "yellow" color with the colorimeter, in comparison to the CVS

(less "yellow" color of kajmak spread) (Figure 7). The overall color difference was 9.5, indicating the difference in color perceptible at a glance [29].

All yellow cheeses except Grana Padano indicated that color assessed with CVS was darker than the color acquired with the colorimeter. Regarding a* observations, CVS resulted in more "red" appearance or colors obtained by the colorimeter were less "green" (Figure 8). The total color difference was in a range from 6.0 for pasta filata up to 14.9 for processed cheese resulting in great color difference detection. The instrumental color measurements obtained with the colorimeter for these samples are similar to the previously published results for semi-hard cheese [46], cheese with mould [47], Pasta Filata — Mozzarella [48], Grana Padano [49], processed cheese [50].

Regarding butter color, observed a* reading using CVS was higher than by the colorimeter indicating less "red" appearance (Figure 9). In contrast, yellowness data

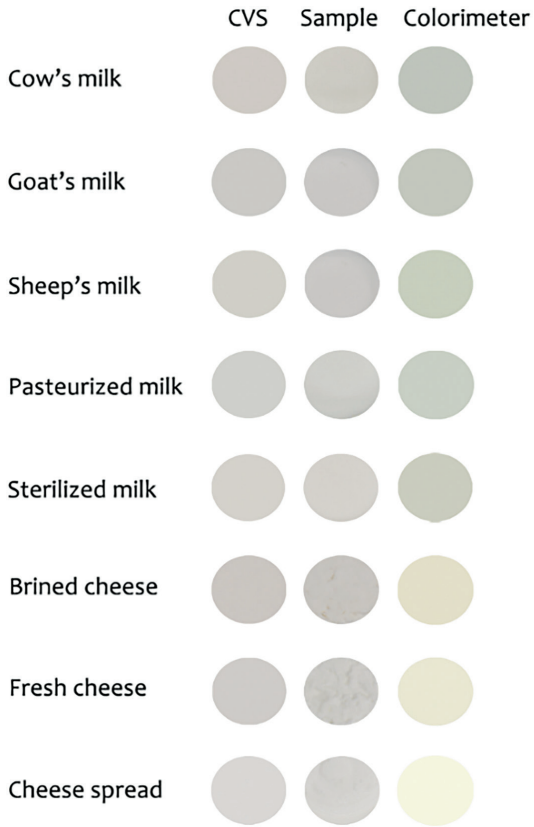

Figure 6. Color of milks and white cheeses as evaluated by the two methods [28]

Figure 7. Color of fermented products, cream products, skim milk powder and kajmak spreads evaluated by the two methods [28]

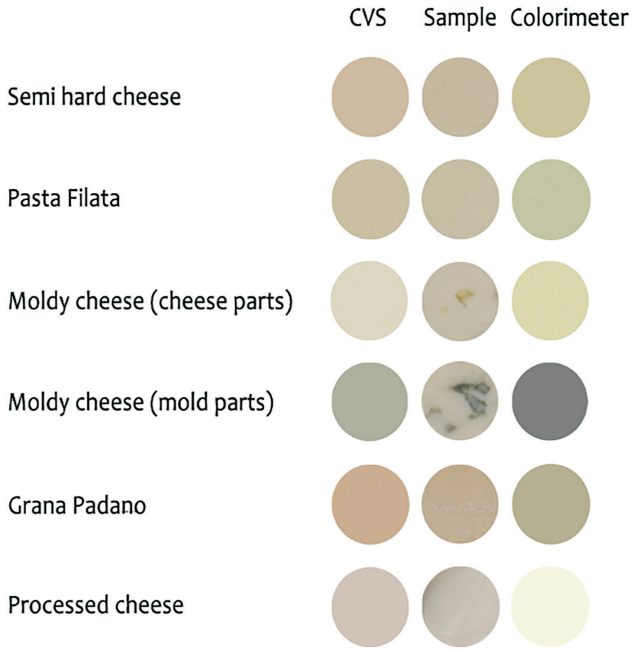

Figure 8. Color of yellow cheeses as evaluated by the two methods [29]

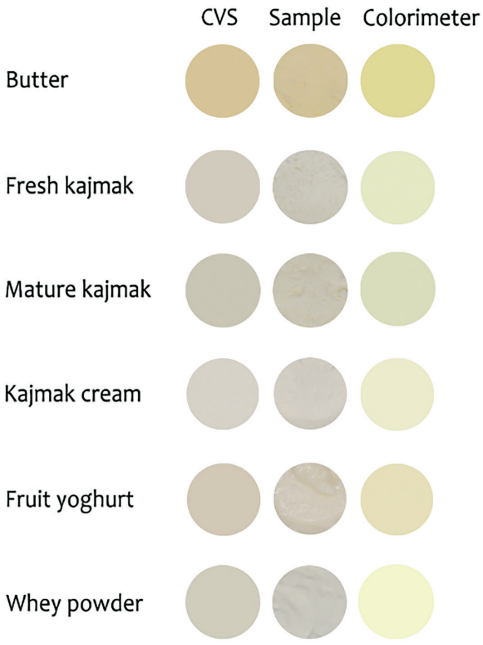

Figure 9. Color of butter, kajmak products, fruit yoghurt and whey powder as evaluated by the two methods [29]

were higher with the colorimeter than by CVS. There is a great color difference regarding the total color difference ΔE = 11.8 [29]. The color results read by colorimeter are in line with the study conducted by Truong, Palmer [51], whereas the color results of butter obtained by CVS are in the concurrence with the Tarlak, Ozdemir [52].

Furthermore, a* values obtained using CVS, were higher giving more "red" color for kajmak samples in contrast to the more "green" color obtained by colorimeter (Figure 9). In contrast, b* values were higher with the colorimeter, denoting yellower color than CVS, which were more "blue" [29].

Fruit (apricot) yoghurt had different color coordinates according to the colorimeter and CVS. Colorimeter-generated color was brighter. In contrast, the redness parameter was higher with CVS device than with the colorimeter. CVS-generated color was more in the redness region than greenness (Figure 9). Yellowness was higher with the colorimeter than CVS [29].

The color of whey powder measured with CVS was significantly darker, more "red" and less "yellow" compared with colorimeter-measured appearance (Figure 9). The total color difference was 17.1, indicating a large color difference [29].

The color deviations between two color systems could be affected, among other factors, by the penetration depth of the light, which is different between a colorimeter (placed on the sample surface) and CVS (a lamp located far). This, therefore, caused scattering from the illumination source, thereby colorimeter assessments were less representative [29].

CVS-produced color on display showed that the assessors found products with the same color inside a box as the samples presented on display. Frequency of similarity was 100.0% for all milk and dairy products. The level of similarity ranged from "moderate" to "high". The second test showed that CVS-observed color was more resemble to those of the actual milk product in comparison with the colorimeter-observed color [29]. Triangle test revealed that there was the difference between color returned by CVS and the colorimeter, and this is a good agreement with the instrumental data. The color difference between these two devices was ranged from 1.7 ("low") to 4.3 ("high") (Table 2).

Eggs

The color parameters of egg samples measured by the two approaches were statistically different with some exceptions (L* reading for quail's egg shell and WI for turkey's egg shell) [19].

The color of eggshell gathered through the Minolta depicted brighter, less „red" and more „yellow" appearance than CVS (Figure 10). According to ΔE scale, these two

Table 2. Sensory test results for milk and milk products [29]

	Frequency of similarity	Level of similarity	CVS vs. Colorimeter	Level of difference test
Butter	100.0%	3.3 ± 1.1^{a}	CVS (100.0%)	$3.7 \pm 0.6^{e-f}$
Semi hard cheese	100.0%	3.1 ± 0.7^{a}	CVS (100.0%)	$3.0 \pm 0.4^{c-e}$
Pasta Filata	100.0%	3.4 ± 0.8^{a}	CVS (91.7%)	$2.6 \pm 0.7^{a-d}$
Brined cheese	100.0%	3.7 ± 1.1^{a}	CVS (100.0%)	$3.7 \pm 0.9^{e-f}$
Fresh cheese	100.0%	3.2 ± 1.3^{a}	CVS (100.0%)	$3.3 \pm 1.1^{c-f}$
Moldy cheese	100.0%	3.1 ± 1.0^{a}	CVS (100.0%)	$3.7 \pm 0.9^{e-f}$
Grana Padano	100.0%	3.6 ± 0.8^{a}	CVS (100.0%)	$3.7 \pm 0.7^{e-f}$
Processed cheese	100.0%	3.4 ± 1.2^{a}	CVS (91.7%)	$3.7 \pm 0.8^{d-f}$
Cheese spread	100.0%	3.1 ± 1.1^{a}	CVS (83.3.%)	$2.7 \pm 1.1^{a-e}$
Fresh kajmak	100.0%	3.0 ± 1.0^{a}	CVS (100.0%)	$3.4 \pm 0.8^{c-f}$
Mature kajmak	100.0%	3.2 ± 0.6^{a}	CVS (100.0%)	$3.4 \pm 0.8^{c-f}$
Kajmak cream	100.0%	2.9 ± 1.2^{a}	CVS (100.0%)	$3.0 \pm 0.9^{c-e}$
Kajmak spread	100.0%	3.4 ± 1.1^{a}	CVS (100.0%)	$3.2 \pm 0.9^{c-f}$
Cow's milk	100.0%	3.7 ± 1.1^{a}	CVS (100.0%)	1.7 ± 0.5^{a}
Goat's milk	100.0%	3.2 ± 1.0^{a}	CVS (100.0%)	$2.5 \pm 0.7^{a-c}$
Sheep's milk	100.0%	3.2 ± 0.7^{a}	CVS (100.0%)	$2.6 \pm 0.5^{a-d}$
Pasteurized milk	100.0%	3.7 ± 1.3^{a}	CVS (100.0%)	$1.8 \pm 0.8^{a, b}$
Sterilized milk	100.0%	3.7 ± 1.1^{a}	CVS (100.0%)	$2.6 \pm 0.7^{a-d}$
Yoghurt	100.0%	2.9 ± 1.2^{a}	CVS (91.7%)	1.7 ± 1.0^{a}
Set style yoghurt	100.0%	3.3 ± 0.9^{a}	CVS (83.3%)	1.7 ± 0.6^{a}
Kefir	100.0%	3.7 ± 1.1^{a}	CVS (83.3%)	$2.6 \pm 0.8^{a-d}$
Fruit yoghurt	100.0%	2.7 ± 1.1^{a}	CVS (100.0%)	$3.6 \pm 0.7^{c-f}$
Heat treated cream	100.0%	3.7 ± 1.0^{a}	CVS (100.0%)	$2.9 \pm 0.5^{b-e}$
Sour cream	100.0%	3.7 ± 1.1^{a}	CVS (100.0%)	$3.0 \pm 0.8^{c-e}$
Skim milk powder	100.0%	2.9 ± 0.9^{a}	CVS (100.0%)	$3.6 \pm 0.5^{c-f}$
Whey powder	100.0%	3.2 ± 0.8^{a}	CVS (100.0%)	4.3 ± 0.6^{f}

Means in the same column with different small letters are significantly different (P < 0.05)
Five-point scale ranks from 1 "very low", 2 "low", 3 "moderate", 4 "high" to 5 "very high".

Figure 10. Color of yellow cheeses as evaluated by the two methods [19]

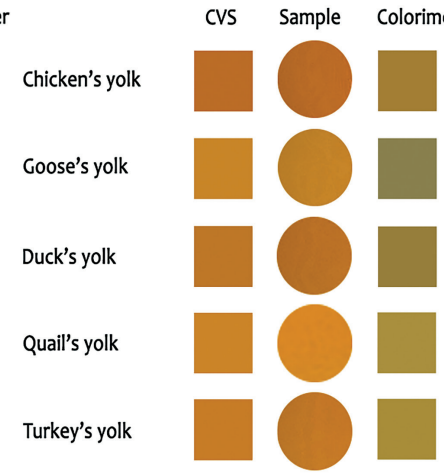

Figure 11. Color of egg yolks as evaluated by the two methods [19]

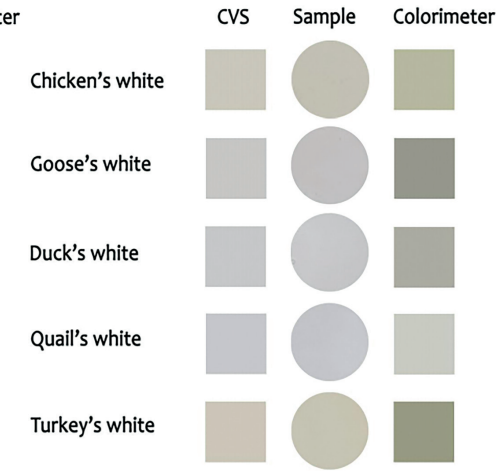

Figure 12. Color of egg white as evaluated by the two methods [19]

different devices provided greatly perceptible total color difference, ranged from 8.0 to 22.0 for quail's egg shell and quail's egg shell (spots), respectively.

Regarding the color of yolk samples, Minolta had a lighter (except the goose's yolk), more "green" and less "yellow" color (Figure 11), whereas CVS indicated the appearance of albumen as lighter (except quail's), more "red" and less "yellow" than colorimeter (Figure 12). Total color difference was in the range from 6.9 (quail's egg white) to 18.7 (goose's egg white). Those results read by Minolta showed a non-real color of egg samples, whereas CVS-obtained color was highly similar to the actual egg color sample [19].

Frequency of similarity was 75.0% (goose's egg white), 83.3% (hen's egg white, duck's egg white and turkey's egg white), 91.7% (quail's egg white) and 100% for all other egg samples. The level of similarity ranged from 1.2 ("very low") for turkey's egg white and hen's egg white to 4.3 ("very high") for duck's egg yolk and turkey's egg yolk. The second test showed that CVS was highly similar to the all egg samples in 100% all trials performed. The final test showed that the difference was large, in the range 2.2 (quail's egg white) to 4.8 (goose's egg yolk) (Table 3) [19].

Conclusion

From the above mentioned results, it can be concluded that even if the same parameters for color evaluation was conducted, significant differences were observed. Taken together, the data clearly demonstrated that the Minolta methodology is less representative and precise for measuring the color of the animal source foods, resulting in non-real appearance. Although using colorimeter for color evaluation of all samples was reliable, it proved to be less accurate. This can be ascribed the fact that Minolta requires opaque food mediums. Furthermore, the penetration depth of the illumination source could be influencing factors on the measurements carried out using two color systems. Therefore, the efficiency of a CVS should be seriously taken into account as a more powerful alternative and non-contact tool for measuring the color of the animal source foods.

Table 3. Sensory test results for eggs [19]

	Frequency of similarity	Level of similarity	CVS vs. colorimeter	Level of difference test
Hen's eggshell	100.0%	3.9 ± 0.7^{c}	CVS (100.0%)	2.7 ± 1.3^{a}
Goose's egg shell	100.0%	$3.5\pm1.3^{b,c}$	CVS (100.0%)	$3.2\pm0.5^{a,b}$
Duck's egg shell	100.0%	$2.9\pm1.2^{a,b}$	CVS (100.0%)	3.1 ± 0.5^{a}
Quail's egg shell	100.0%	2.3 ± 1.1^{a}	CVS (100.0%)	4.2 ± 0.7^{c}
Turkey's egg shell	100.0%	$3.3\pm0.8^{b,c}$	CVS (100.0%)	$3.8\pm0.7^{b,c}$
Hen's egg yolk	100.0%	3.8 ± 0.9^{a}	CVS (100.0%)	4.2 ± 0.4^{a}
Goose's egg yolk	100.0%	4.1 ± 0.8^{a}	CVS (100.0%)	4.8 ± 0.4^{b}
Duck's egg yolk	100.0%	4.3 ± 0.5^{a}	CVS (100.0%)	4.7 ± 0.4^{b}
Quail's egg yolk	100.0%	3.9 ± 1.0^{a}	CVS (100.0%)	4.5 ± 0.5^{ab}
Turkey's egg yolk	100.0%	4.3 ± 0.8^{a}	CVS (100.0%)	4.6 ± 0.5^{ab}
Hen's egg white	83.3%	1.2 ± 0.7^{a}	CVS (100.0%)	3.7 ± 0.6^{b}
Goose's egg white	75.0%	1.8 ± 1.3^{a}	CVS (100.0%)	4.2 ± 0.9^{b}
Duck's egg white	83.3%	2.2 ± 1.3^{a}	CVS (100.0%)	3.8 ± 0.8^{b}
Quail's egg white	91.7%	2.2 ± 1.1^{a}	CVS (100.0%)	2.2 ± 0.6^{a}
Turkey's egg white	83.3%	1.2 ± 0.7^{a}	CVS (100.0%)	4.5 ± 0.5^{b}

Means in the same column with different small letters are significantly different ($P < 0.05$)
Five-point scale ranks from 1 "very low", 2 "low", 3 "moderate", 4 "high" to 5 "very high".

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COMPARATIVE PROTEOMIC STUDY OF PIG MUSCLE PROTEINS DURING GROWTH AND DEVELOPMENT OF AN ANIMAL

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Keywords: *two-dimensional electrophoresis, muscle proteins, 2-DE, ontogenesis, proteomics*

Abstract

The production of high-quality pork is closely related to the growth and development of muscle tissue. The present article provides a comparative proteomic research of l. dorsi, b. femoris, m. brachiocephalicus during the pigs' growth and development (at age of 60 days and 180 days). This work was supported by data of electrophoretic methods: one-dimensional electrophoresis according to Laemmli with densitometric assessment in the ImageJ software and two-dimensional electrophoresis according to O'Farrell method with its further processing on the software ImageMaster. The mass spectrometric identification was conducted with the help of the high-performance liquid chromatography (HPLC) system connected to a mass spectrometer; further the data were interpreted by search algorithm Andromeda. When comparing frequency diagrams of one-dimensional electrophoregrams of all three muscle tissues of weaned pigs, the greatest difference was observed for the muscle sample l. dorsi. Comparison of diagrams of muscle tissue samples taken for mature pigs showed a great similarity of all three studied muscles samples. Within the framework of the research, the Fold indicator was calculated. The exceeding its value by more than 2 units is generally considered to be a statistically significant difference. When analyzing two-dimensional electrophoretograms of weaned pigs' muscles, 18 protein fractions were revealed with Fold > 2. When examining the muscle tissue of mature pigs, 15 of those proteins were found; the differences were mostly detected in the minor protein fractions. The mass spectrometric analysis of the cut bands with well-pronounced differences from the one-dimensional electrophoretogram revealed 214 proteins involved to a greater extent in cellular and metabolic processes, physical activity and localization. Growth and development protein — semaphorin-6B (96.78 kDa) — was revealed in muscle tissue of l. dorsi, a. Also in l. dorsi and b. femoris the growth and development proteins were found: cadherin-13 (78.23 kDa), cadherin-7 (87.01 kDa), the F-actin-cap protein beta subunit (30.66 kDa), and two uncharacterized proteins at 65.60 kDa and 63.88 kDa.

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Introduction

The way of application of existing and new technologies for forecasting of some definite characteristics in many food products, including meat, is now of a great interest [1.2]. Proteomic methods enable successful forecasting the quality of meat, and make it possible to improve stability of food parameters and increase the nutrition value of branded products [3]. For instance, the tenderness of pork is known to be difficult to forecast, probably because it is influenced by many factors, like pH [4.5], lipids [6], content of collagen [7] and muscle proteins degradation [8.9]. The researchers also found differences in the expression of metabolic and stress response proteins in tough and tender beef [10] and pork [11]. The studies run by various scientists demonstrate quite significant relationship between muscle metabolism and the quality of meat [12]. At the same time, differences in the proteomic profile of muscle tissues correlate with differences in meat quality [13]. Carlson et al. [14] have documented that even if pH, color and lipid content are the same, the tenderness of meat can vary quite significantly. These differences are associated with postmortem degradation of troponin-T, desmin, filamin and titin. Thus, there is a suggestion that the tenderness variation and the observed postmortem proteolysis in pork of the similar pH, lipid and color is explained by differences in the proteomic profile of sarcoplasm [15]. This research examines the proteome of pig muscles during pig's growth and development in order to determine the differences in protein composition to find additional potential biomarkers of meat quality.

Recently it has become popular to use biomarkers-based food tests to verify the authenticity of food and ensure its quality. The metabolic parameters of meat products were checked by several analytical platforms, mainly electrophoretic methods, chromatography-mass spectrometry (HPLC–MS and GC–MS) [16] and isotope ratio mass spectrometry (IRMS) [17], Fourier transformation infrared spectroscopy (FT-IR) [18] and nuclear magnetic resonance (NMR) [19]. In this article the proteome of pig muscles in the process of growth and development was studied with

the help of electrophoretic methods (one- and two-dimensional gel electrophoresis) in combination with bioinformatic processing and mass spectrometric identification.

Objects and methods

The samples of muscle tissue: *l. dorsi, b. femoris, m. brachiocephalicus* of pigs (aged 60 days and 180 days) of the Vietnamese pot-bellied breed were used as an object of research.

One-dimensional gel electrophoresis (1-DE)

One-dimensional gel electrophoresis was conducted in 12.5% polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS-PAGE) in an electrophoresis chamber (Helicon, USA) at a constant current amperage and voltage of 55V and 130V for 2 hours. As standard solution, a marker was used. The marker consisted of standard preparations with a molecular weight of 250, 150, 100, 70, 50, 40, 30, 20 kDa (Thermo, USA).

Two-dimensional gel electrophoresis (2-DE)

The samples were studied by two-dimensional electrophoresis [20]. At the first stage, isoelectric focusing (IEF) was run at 3,650 V/h in tubular gels of 2.4 mm × 160 mm. 0.01 M orthophosphoric acid and 0.02 M sodium hydroxide were used as anodic and cathodic buffers, respectively. After IEF, the gels were incubated for 10 minutes in 2.5 ml of equilibration buffer solution I (6 M urea, 20% glycerol, 2% SDS, and 1% DTT in 50 mM Tris-HCl buffer, pH 8.8), then in equilibration buffer II (6 M urea, 20% glycerol, 2% SDS, and 4% iodoacetamide in 375 mM Tris-HCl buffer, pH 8.8) [21].

Next, electrophoresis was run using a buffer solution, containing 25 mM Tris-HCl, 192 mM glycine, and 0.1% SDS; for this, the equilibrated gels were transferred into a 12.5% polyacrylamide gel (170 mm×180 mm×1.5 mm). The process was conducted at a current of 30 mA per gel, until the front of the dye reached the edge of the gel.

Visualization of protein fractions and image analysis

Protein stains were visualized by their staining with Coomassie brilliant blue dye G-250 (PanReac, Spain). When determining the density of stains (ImageJ, USA) [22, 23], at least 3 one-dimensional electrophoretograms of equal application were used.

The computerized densitometry of 2D-electrophoregrams was conducted in a wet condition. Their digital images were obtained via a Bio-5000 plus scanner (Serva, Germany). The scanned images were analyzed using *ImageMaster ™ 2D Platinum* software powered by Melanie 8.0 (GE Healthcare and Genebio, Switzerland).

Statistical analysis

Experimental data were analyzed using Student's t-criteria and one-way analysis of variance (between gels of various samples) using *ImageMaster ™ 2D Platinum* software based on Melanie 8.0 (GE Healthcare and Genebio, Switzerland). If P value is less than 0.05 (P <0,05), it is considered as a sign of significant difference. As part of the work, protein stains were compared by their volume, and the Fold index was calculated. If Fold index value is exceeded by more than 2 units, it is generally considered to be a statistically significant difference. All results are presented as mean ± standard deviation, obtained from at least three independent experiments.

Mass spectrometric analysis

The analysis was conducted using the equipment of the Human Proteome Core Facility: the peptides obtained by trypsinolysis were analyzed using an Ultimate 3000 RSLCnano HPLC system (Thermo Scientific, USA) connected to a Q-exactive HFX mass spectrometer (Thermo Scientific, USA)) in positive ionization mode, using an NESI source (Thermo Scientific, USA). Proteins were revealed, using the software *MaxQuant v.1.6.3.4*, search algorithm *Andromeda* using the *Uniprot* database with restrictions relevant to the species of the organism.

Results and discussion

Three different muscles of *l. dorsi, b. femoris* and *m. brachiocephalicus* of weaned pigs were studied by electrophoretic methods in order to reveal significant differences in composition of proteins. At the initial stage one-dimensional electrophoretograms of muscle tissue samples were obtained. Those samples were subsequently analyzed in the *ImageJ* software. During data processing of one-dimensional electrophoregrams of *l. dorsi* samples (Figure 1) a wide range of compounds was revealed of various molecular weights ranging from 13 kDa and more. The major peaks were found on the diagrams corresponding to high molecular weight fractions of 250 kDa and more, 150 kDa, 110–115 kDa, as well as smaller proteins: 93–98 kDa, 78–83 kDa, 62–66 kDa, 41–46 kDa, 40 kDa, 37–38 kDa, 34–35 kDa, 30 kDa, 24 kDa, 17 kDa. The distinct peaks of lower height should also be noted: 95–97 kDa, 70–72 kDa, a number of peaks, incl. minor peaks within the range of 50–62 kDa, 36 kDa, 33–34 kDa, 28–29 kDa, 23 kDa, 20 kDa and 14 kDa. The high sensitivity of the software used during densitometric analysis also made it possible to detect the smallest peaks corresponding to the bands 135–137 kDa, 103–106 kDa, 51–52 kDa, 39 kDa, three peaks within the range 31–33 kDa, 22–23 kDa, 21 kDa, as well as several peaks of 13–15 kDa. The above-listed compounds were generally typical for both mature animals and for young animals with some exceptions. Thus, the mentioned smallest peaks of 103–106 kDa and 51–52 kDa were observed in the longest muscle of only young individuals, while peaks at 44 and 80 kDa were found in tissues of mature animals only. The samples also differed in strength of some individual bands. A high degree of protein expression, which manifested itself in increase in the peaks height, was peculiar for the samples taken from

adult animals. This is evidenced and confirmed by increase in saturation of the molecular weight fractions of 250 kDa, 60 kDa, 39–40 kDa, 36 kDa, 33–34 kDa, 30 kDa, 28–29 kDa, 20 kDa, 17 kDa, 15 and 14 kDa (Figure 1, No. 2). The decrease in the bands expression was not peculiar for the process of animal's growth, and was expressed as decrease in the height of a single peak of 78–83 kDa. This fact can be explained by the absence of the need for expression of proteins included in the above-specified fraction, which leads to a decrease in proteins concentration in tissues.

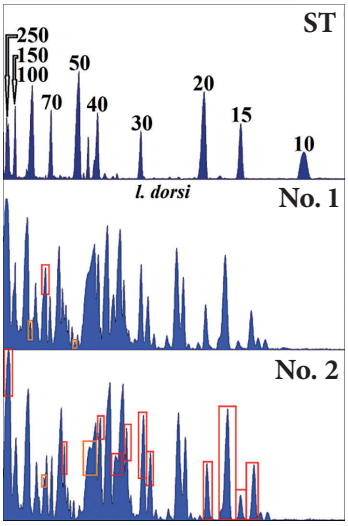

Figure 1. Diagrams of protein fractions frequency in the samples of *l. dorsi*.

Legend: ST — standard of molecular weights, expressed in kDa; No. 1 — sample of *l. dorsi* taken from young pigs; No. 2 — sample of *l. dorsi* taken from adult pigs. Red frames indicate the increase in the corresponding peaks height; changes in the fractional composition are shown in orange

Basing on the data obtained from densitometric analysis of the diagrams, that represent the muscle densities of *brachiocephalicus* (Figure 2), a large difference in the proteomic profile and intensity of individual fractions manifestation was revealed. Both diagrams were characterized by the peaks of 250 kDa and more, 145 kDa, 105 kDa, a series of minor peaks of 85–97 kDa, a peak with an average height of 73–78 kDa, minor bands of 69 kDa and 66–67 kDa, a series of peaks within the ranges 53–63 kDa and 48–52 kDa, major fractions 40–45 kDa, 39 kDa and 36 kDa, as well as the spectrum of lower peaks, incl. the smallest, within the range less than 35 kDa down to the last manifested peak that appeared within 11–12 kDa. Comparative analysis of the diagrams of the protein fractions saturation in the samples showed the proteomic profile peculiar for the muscle proteins of young animals with a molecular weight of less than 31 kDa, namely 16–17 kDa, 18–19 kDa, 20–21 kDa, 22 kDa, 25 kDa and 31 kDa. (Figure 2, # 1, marked in orange). In a sample taken from mature pigs (Figure 2, No. 2), there was a general trend to an increase in intensity of manifestation of most of minor and major peaks. High-molecular-weight proteins remained, in general, identical in both cases; and a striking difference was noted medium- and low-molecular-weight bands in a greater degree.

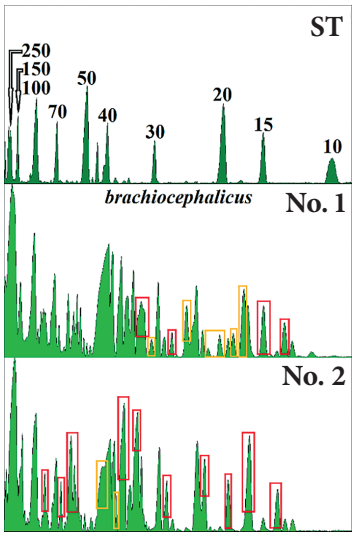

Figure 2. Diagrams of protein fractions frequency in the samples of *brachiocephalicus*.

Legend: ST — standard of molecular weights, expressed in kDa; No. 1 — a sample of *brachiocephalicus* taken from young pigs; No. 2 — a sample of *brachiocephalicus* taken from adult pigs. Red frames indicate the increase in the corresponding peaks height; changes in the fractional composition are shown in orange

Comparison of results of software processing of *b. femoris* electrophoregrams (Figure 3) showed almost identical qualitative protein composition of muscle samples taken from pigs at the age of 60 and 180 days, as well as a wide profile of compounds. The major peaks of 250 kDa and more, 150 kDa, 98 kDa, as well as 39–43 kDa, 37–38 kDa, 36 kDa and 33–34 kDa were detected. In addition, there were lower peaks, including minor peaks in the entire presented range of molecular weights. It is necessary to note these peaks at 130–140 kDa, 76–85 kDa, 68–69 kDa, 65–66 kDa, as well as compounds within the range of 51–58 kDa and 11–49 kDa. The fractional composition of the considered samples differed by the smallest peak of 61–62 kDa in young animals;

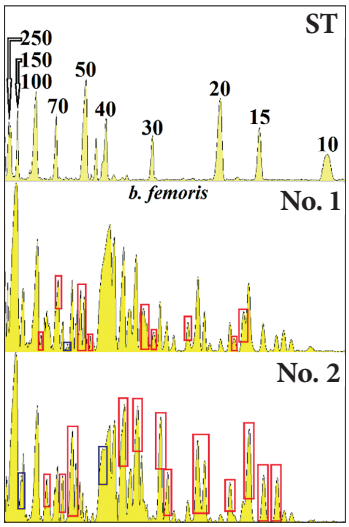

Figure 3. Diagrams of frequency of protein fractions of samples *b. femoris*.

Legend: ST — standard of molecular weights, expressed in kDa; No. 1 — sample of *b. femoris* of young pigs; No. 2 — sample of *b. femoris* of adult pigs. Red frames indicate the increase in the corresponding peaks height; changes in fractional composition are shown in blue

and more distinct bands of 143–145 kDa and 41–43 kDa in mature animals respectively. A distinctive factor when comparing the diagrams was, as in the previously considered cases, precisely the change in the height of the peaks, or the saturation of the corresponding protein fractions. So, on the diagram of the protein fractions density in reference to the young animals (Figure 3, No. 1), the peaks 84–90 kDa, 68–69 kDa, 53–54 kDa, 51 kDa, 32 kDa, 17 kDa, and also some others to a lesser extent (47–48 kDa, 31–32 kDa, 25 kDa and 18 kDa). In the case of mature pigs, an increase in the number of peaks was observed (58 kDa, 57 kDa, 36 kDa, 33–34 kDa, 29 kDa, 28 kDa, 24 kDa, 22–23 kDa, 18–19 kDa, 16 kDa, 14–15 kDa and 13 kDa, etc.), which witnesses a greater degree of accumulation of the relevant proteins in tissues of *b. femoris*.

When comparing the diagrams of the densities of all three samples (Figure 4), obviously the greatest difference was observed for muscle *l. dorsi* taken from other weaned piglets. This fact was confirmed by the clear peak observed at 93–98 kDa compared to a number of peaks within this range in the samples of *brachiocephalicus* and *b. femoris*; a higher peak 62–66 kDa and subsequent peaks within the range 58–61 kDa, as well as bands 34–35 kDa, 37–38 kDa, 29 kDa and 24 kDa; a distinctive 33 kDa peak, corresponding to less pronounced 32–33 kDa bands in the other two muscles. At the same time, a clear difference was determined in the low molecular weight proteins fraction in the sample of 11–20 kDa (Figure 4, *l. dorsi* No. 1). *Brachiocephalicus* and *b. femoris* tissue samples, according to the corresponding diagrams, were the most similar among

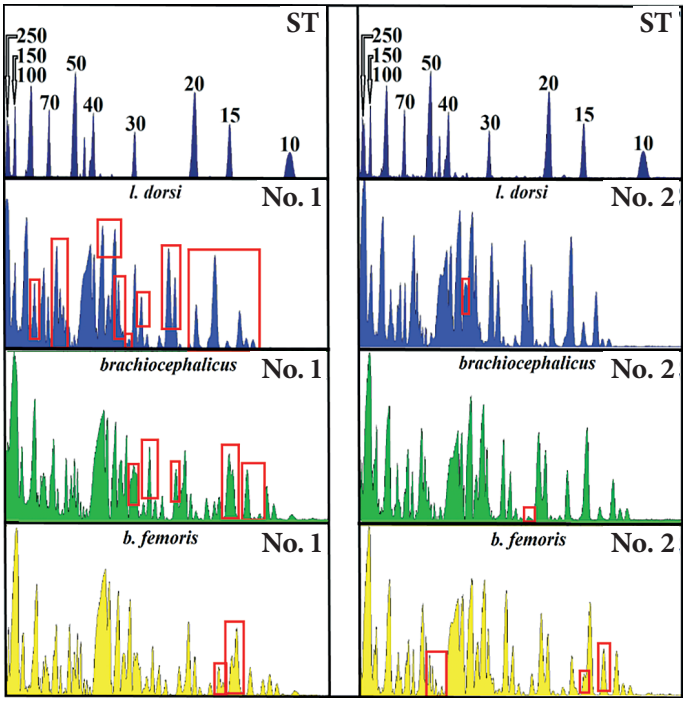

Figure 4. Frequency diagrams of protein fractions of samples l. dorsi, brachiocephalicus, b. femoris.

Legend: ST — standard of molecular weights, expressed in kDa; No. 1 — tissue sample taken from young pigs; No. 2 — tissue sample taken from adult pigs. Red frames show peculiar differences on the diagram of the corresponding sample

the young piglets. The difference was found only in higher peaks of 32–34 kDa, 29 kDa, 25 kDa, and 15 kDa in the case of *brachiocephalicus* muscle, as well as in the fractional composition of the low-molecular proteins fraction in both samples. Thus, the diagram for *brachiocephalicus* showed better visible bands of 20 kDa, 18 kDa, 17–18 kDa, 15 kDa, and 13 kDa, while in tissue of *b. femoris* the bands were more distinct at18–19 kDa, 16–17 kDa and 14 kDa. Comparison of diagrams of muscle tissue samples taken from mature pigs (Figure 4, No. 2) showed a great similarity of all three muscles under consideration. The only exceptions were some separate peaks within the range 53–57 kDa, 17 kDa, and 14–15 kDa in case of *b. femoris*, 35 kDa — in case of *l. dorsi*, and the absence of an expressed peak of 25 kDa in the diagram for *brachiocephalicus*.

For a more detailed analysis of muscle tissues proteome, a study was conducted by the method of two-dimensional electrophoresis of three different muscles: *l. dorsi, b. femoris* and *m. brachiocephalicus*. Fragments of two-dimensional electrophoretograms of weaned pigs muscles, which display 18 fractions with Fold > 2, are presented below in the Figure 5, and their integrated optical densities are shown in the Table 1.

Biological information analysis of the revealed proteins stains suggests that the fractions numbered 1 and 2 in the Figure 5 correspond to myosin light chains of fast (MLC1f) and slow (MLC1s / v) skeletal muscle. These fractions were quite distinct in *l. dorsi*. It is interesting to note that in samples of *b. femoris* tissues, only a slow chain was detected, while in *m. brachiocephalicus* a vaguely expressed fraction of MLC1s was found. The authors V. Montowska and Pospiech [24] used MLC in their studies as a marker to authenticate the meat products made from pork and other kinds of meat.

An interesting distribution of integral optical density was observed in a group of proteins within the range of molecular weights from 50 to 60 kDa, marked with numbers 3–7 in the Figure 5 below. Fractions No. 4 and No. 7 were more pronounced in muscles *b. femoris*, were less pronounced in *m. brachiocephalicus* and muscles of *l. dorsi*. Protein compounds No. 3 and No. 6 were 4 times more pronounced in the muscles *l. dorsi* in comparison with and *m. brachiocephalicus*.

The intensity of staining of protein No. 8 (Figure 5) decreased evenly from *l. dorsi* to *m. brachiocephalicus*. The same trend was observed in fractions No. 13 — No. 17, among which troponin fractions are presumably present. The skeletal muscle protein troponin I has already been characterized as a potential thermostable and species-specific biomarker of mammalian muscle tissue in raw meat and meat products [25], which makes it promising for identification of muscles of various localization. Possibly a lesser staining in *m. brachiocephalicus* was related to the biological function of this muscle, since it is less active in comparison with to *l. dorsi* and *b. femoris*. An interesting distribution of protein stains was observed in the fractions

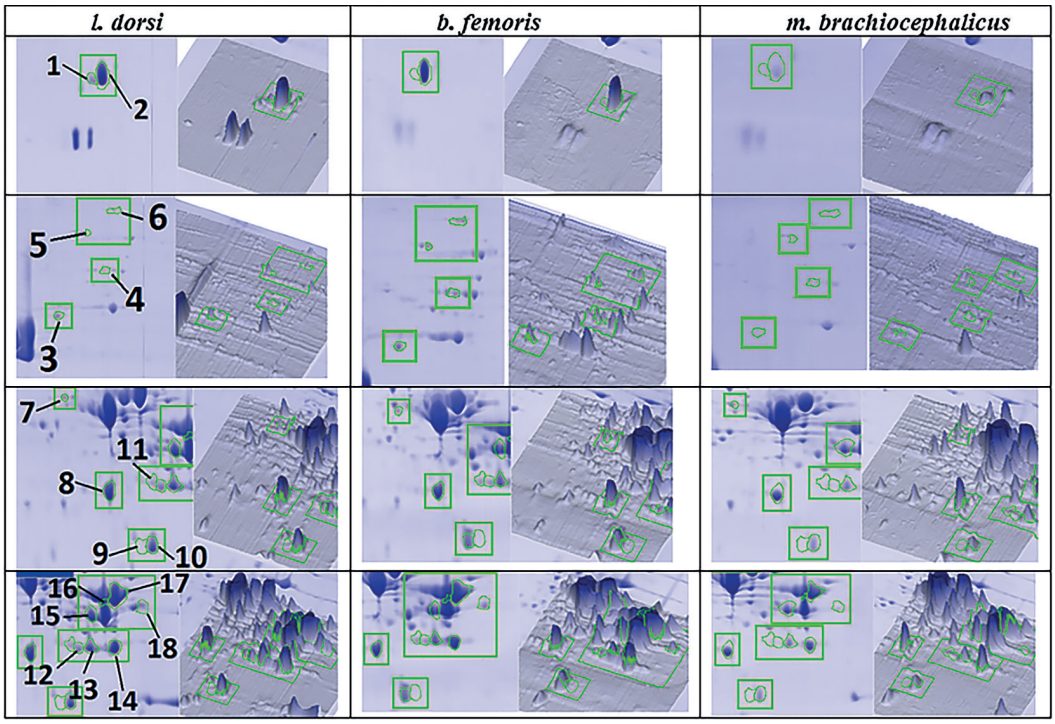

Figure 5. Fragments of 2-DE muscle tissue gels of weaned pigs

Table 1. Results of densitometric analysis of protein fractions of a piglet's muscle tissue (vol ± SD)

No. of a protein spot	l. dorsi	b. femoris	m. brachiocephalicus
1	$9.07 \pm 1.19 \times 10^6$	$2.60 \pm 1.63 \times 10^6$	$1.46 \pm 0.36 \times 10^6$
2	$0.11 \pm 1.35 \times 10^7$	$8.93 \pm 1.13 \times 10^7$	$2.78 \pm 1.77 \times 10^7$
3	$3.84 \pm 0.67 \times 10^6$	$2.74 \pm 1.15 \times 10^6$	$1.02 \pm 0.37 \times 10^6$
4	$1.15 \pm 0.34 \times 10^6$	$2.63 \pm 0.52 \times 10^6$	$1.18 \pm 0.57 \times 10^6$
5	$2.55 \pm 0.63 \times 10^5$	$1.18 \pm 0.29 \times 10^6$	$2.72 \pm 0.67 \times 10^5$
6	$6.88 \pm 3.57 \times 10^5$	$2.42 \pm 0.40 \times 10^6$	$1.42 \pm 0.47 \times 10^6$
7	$1.49 \pm 0.79 \times 10^6$	$5.96 \pm 1.67 \times 10^6$	$3.49 \pm 0.22 \times 10^6$
8	$6.21 \pm 0.29 \times 10^7$	$5.16 \pm 0.83 \times 10^7$	$1.75 \pm 1.52 \times 10^7$
9	$4.90 \pm 2.28 \times 10^6$	$1.86 \pm 1.21 \times 10^7$	$2.08 \pm 0.16 \times 10^6$
10	$3.33 \pm 0.48 \times 10^7$	$1.27 \pm 0.92 \times 10^7$	$8.07 \pm 5.80 \times 10^6$
11	$5.93 \pm 1.10 \times 10^6$	$5.78 \pm 1.72 \times 10^6$	$2.03 \pm 0.49 \times 10^6$
12	$1.77 \pm 0.45 \times 10^7$	$8.42 \pm 2.46 \times 10^6$	$1.69 \pm 0.54 \times 10^6$
13	$4.37 \pm 0.32 \times 10^7$	$3.38 \pm 1.30 \times 10^7$	$1.24 \pm 0.34 \times 10^7$
14	$5.99 \pm 0.19 \times 10^7$	$5.19 \pm 1.02 \times 10^7$	$1.84 \pm 1.53 \times 10^7$
15	$5.31 \pm 0.88 \times 10^7$	$4.18 \pm 0.62 \times 10^7$	$2.52 \pm 0.99 \times 10^7$
16	$1.56 \pm 0.28 \times 10^7$	$7.86 \pm 2.20 \times 10^6$	$3.90 \pm 2.56 \times 10^6$
17	$1.40 \pm 0.12 \times 10^8$	$1.16 \pm 0.05 \times 10^8$	$6.86 \pm 1.51 \times 10^7$
18	$1.07 \pm 0.23 \times 10^7$	$1.21 \pm 0.17 \times 10^7$	$3.71 \pm 1.17 \times 10^6$

Note. The spot volume (Vol) was normalized to the total actual volume of the spot and to the mean for duplicate gels, in triplicate. The presented data are mean ± SD of three independent experiments.
*** Spot volume is the sum of the gray values minus the background of all pixels limited by the spot.**

No. 9 and No. 10. Thus, in *b. femoris* only protein No. 9 was detected, but the fraction No. 10 (adenylate kinase) was present in a small amount. Moreover, in the longest muscle of the back *longissimus dorsi*, its intensity was stronger than in *m. brachiocephalicus*. The volume of stain No. 11 in *m. brachiocephalicus* was three times smaller than that of *l. dorsi* and *b. femoris*. The highest value of the Fold index was observed in the fraction No. 12, which is probably phosphoglycerate mutase, and was equal to 10.5; also it was maximally expressed in *l. dorsi*. Protein No. 18 was more strongly expressed in *b. femoris*, less expressed in *l. dorsi* and was found in small quantities in *m. brachiocephalicus* [26].

The changes in the above described intensity of staining in the selected protein fractions may reflect the intensity of the growth of muscle tissue in growing animals. For example, for *l. dorsi*, the strongest muscle of the spinal column, which determines the movement of the trunk and

head, the maximum amount of intensely stained protein fractions was revealed. The fractions 1, 3, 6, 8, 10–17 can be candidate markers for *l. dorsi* of piglets (Figure 5, Table 1). For muscle *b. femoris*, which is functionally active: hip extensor and hock and knee flexor, muscle fractions 2, 4, 7, 9, and 18 can serve as markers (Figure 5, Table 1). The hip muscle and its protein profile have been researched quite well, since various types of dried ham (jamon, prosciutto, prosciutto) are made from pork ham. It is already known that peptides produced in this muscle have biological functionality (some peptides from proteins MLC1, CK, MYO, TNT and MHC7 showed the highest functionality).

Thus in the composition of *l. dorsi* 12 proteins were identified, *b. femoris* — 5 proteins, and for *m. brachiocephalicus* — 1 protein. In addition, the latter revealed an inclination for a lower intensity of protein fractions, some of which are smaller by one order of magnitude in comparison with *l. dorsi*. The lowest intensity of staining of protein fractions was noted in the tissues of *m. brachiocephalicus*, which is possibly related to low metabolic processes in this muscle due to low functional load.

The electrophoretic study of muscles tissues of different localization taken from mature pigs (Figure 6, Table 2), the differences were mostly found in minor protein fractions.

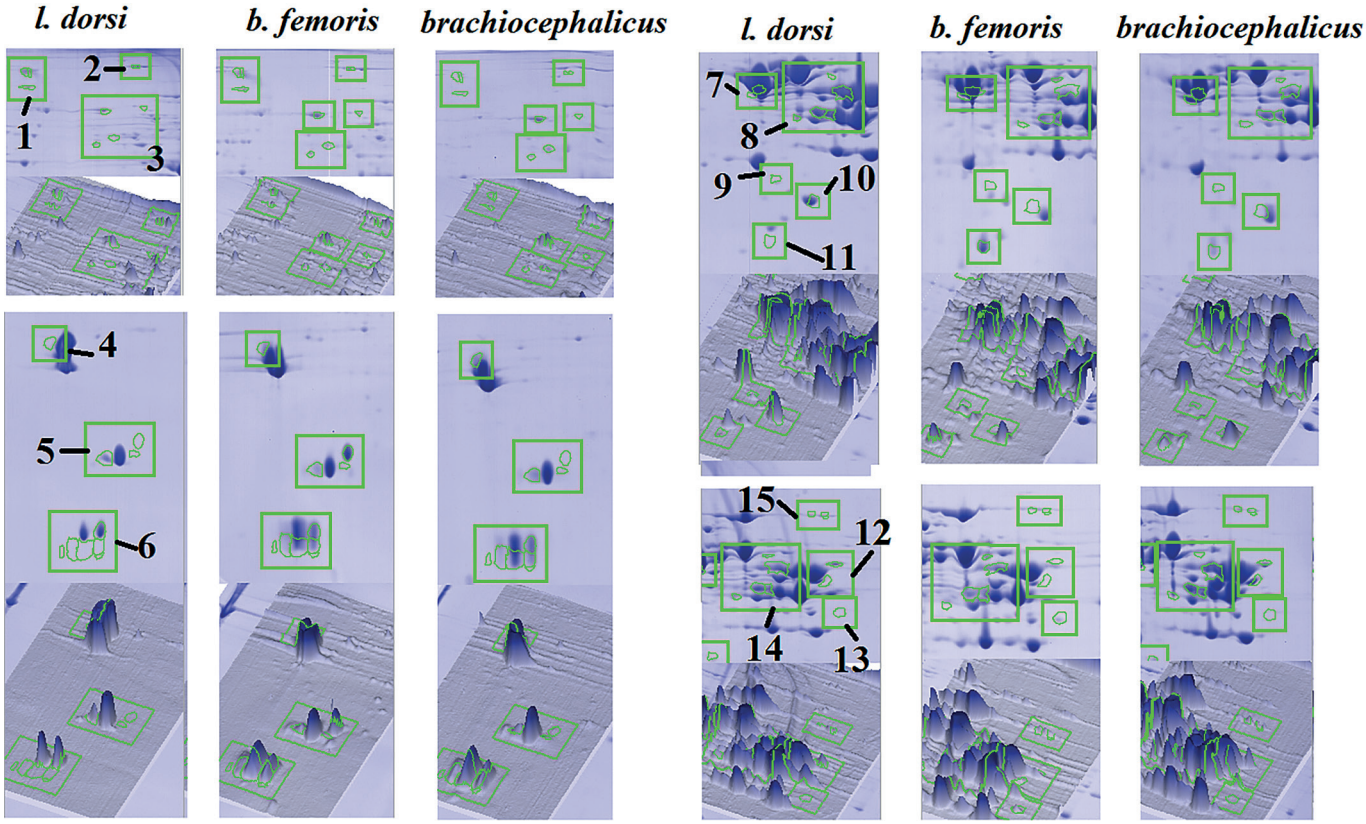

Figure 6. Fragments of 2-DE gels of muscle tissue taken from the mature pigs

Table 2. Results of densitometric analysis of protein fractions of muscle tissue taken from the mature pigs (volume ± SD)

No. of a spot	l. dorsi	b. femoris	m. brachiocephalicus	Fold
1	$9.03 \pm 0.24 \times 10^5$	$6.54 \pm 0.10 \times 10^5$	$2.00 \pm 0.11 \times 10^6$	3.05
2	$7.29 \pm 0.27 \times 10^5$	$0.97 \pm 0.06 \times 10^5$	$3.65 \pm 0.18 \times 10^5$	7.45
3	$3.95 \pm 0.42 \times 10^5$	$3.20 \pm 0.22 \times 10^5$	$1.10 \pm 0.17 \times 10^6$	3.43
4	$2.78 \pm 0.69 \times 10^6$	$1.06 \pm 0.14 \times 10^7$	$7.11 \pm 1.28 \times 10^6$	3.82
5	$8.03 \pm 2.50 \times 10^6$	$2.20 \pm 0.41 \times 10^7$	$7.73 \pm 0.91 \times 10^6$	2.85
6	$2.76 \pm 0.09 \times 10^7$	$1.24 \pm 0.02 \times 10^7$	$1.35 \pm 0.42 \times 10^7$	2.23
7	$1.86 \pm 0.07 \times 10^7$	$4.80 \pm 0.11 \times 10^7$	$4.70 \pm 0.15 \times 10^7$	2.58
8	$2.69 \pm 0.29 \times 10^7$	$2.35 \pm 0.23 \times 10^7$	$1.35 \pm 0.15 \times 10^7$	2.13
9	$8.32 \pm 0.86 \times 10^5$	$2.24 \pm 0.46 \times 10^6$	$1.86 \pm 0.46 \times 10^6$	2.68
10	$2.31 \pm 0.18 \times 10^7$	$1.17 \pm 0.05 \times 10^7$	$5.76 \pm 2.44 \times 10^6$	4.01
11	$3.84 \pm 1.57 \times 10^6$	$1.02 \pm 0.70 \times 10^7$	$3.75 \pm 1.38 \times 10^6$	2.70
12	$5.13 \pm 1.25 \times 10^6$	$1.93 \pm 0.81 \times 10^6$	$2.53 \pm 0.12 \times 10^6$	2.66
13	$1.03 \pm 0.06 \times 10^6$	$1.36 \pm 0.01 \times 10^6$	$3.22 \pm 0.51 \times 10^6$	3.13
14	$4.55 \pm 0.57 \times 10^7$	$4.25 \pm 0.06 \times 10^7$	$1.80 \pm 0.17 \times 10^7$	2.52
15	$8.50 \pm 0.16 \times 10^5$	$1.57 \pm 0.16 \times 10^6$	$1.87 \pm 0.25 \times 10^6$	2.20

A group of proteins (No. 1–3, figure 6) within the range of more than 70 kDa, such as alpha-1.4-glucan phosphorylase, amine oxidase, actin-depolymerizing factor and heat shock protein HSP 90-alpha in significantly larger quantities (Table 2, Fold > 3.05) was found in *m. brachiocephalicus*. Also the we noted the predominance of *m. brachiocephalicus* proteins CFL2b variant 1 and troponins C of fast and slow skeletal muscles, which proteins are responsible for the binding of actin filaments. As far as the structural muscle proteins are concerned, the significant differences there were found in fragments of tropomyosin, myosin light chains 1 and αβ-crystallin (No. 4, No. 5, No. 11 in Figure 6), which were at least twice as intense in tissues of *b. femoris*. For *l. dorsi* the fractions No. 6, No. 10 and No. 12 are predominant, which are myosin light chains 2, acireductone dioxygenase and gamma actin-2 in accordance with mass spectrometric identification. It is worth and interesting to note, that creatine kinase (subunit M) and long-chain 3-ketoacyl-CoA thiolase were found there only in minimal quantities compared to *b. femoris* and *m. brachiocephalicus*. At the same time, L-lactate dehydrogenase A chain and porin are less pronounced in *b. femoris*.

Mass spectrometric analysis of the cut out bands with expressed differences, taken from the one-dimensional electrophoretogram (Figure 7), revealed 214 proteins, involved to a greater extent in the cellular and metabolic processes, motor activity and localization.

In the tissue of *l. dorsi* muscle, the protein responsible for growth and development was identified by mass spectrometry through binding of the semaphorin receptor and chemorepellant activity — semaphorin-6B (96.78 kDa). Development proteins were also identified in *l. dorsi* and *b. femoris* — cadherin-13 (78.23 kDa), cadherin-7 (87.01 kDa), the F-actin-capping protein *beta* subunit (30.66 kDa), and two uncharacterized proteins at the band of 65.60 kDa and 63.88 kDa.

Conclusion

The data obtained in result of comparative analysis of piglets' and mature pigs' muscles showed a high degree of tissue differentiation in the young animals, with subsequent change in the fractional composition of proteins during the growth and development of the animals. In case of *longissimus dorsi* the most significant changes mainly affect the proteins fractions with a molecular weight of less than 31 kDa; and in the samples of *brachiocephalicus* and *biceps femoris*, the entire presented range of molecular weights was also affected with this change. Using the method of two-dimensional electrophoresis, it was shown that in the process of pig's growth and development, the number of fractions of troponins, light myosin chains and proteins of the actomyosin complex increases, but their quantitative content decreases. The changes revealed in protein compounds reflect the intensity of processes of muscle tissue development in animals, which changes contribute to capability to monitor the patterns of formation of the above-specified parameters of meat and meat products quality.

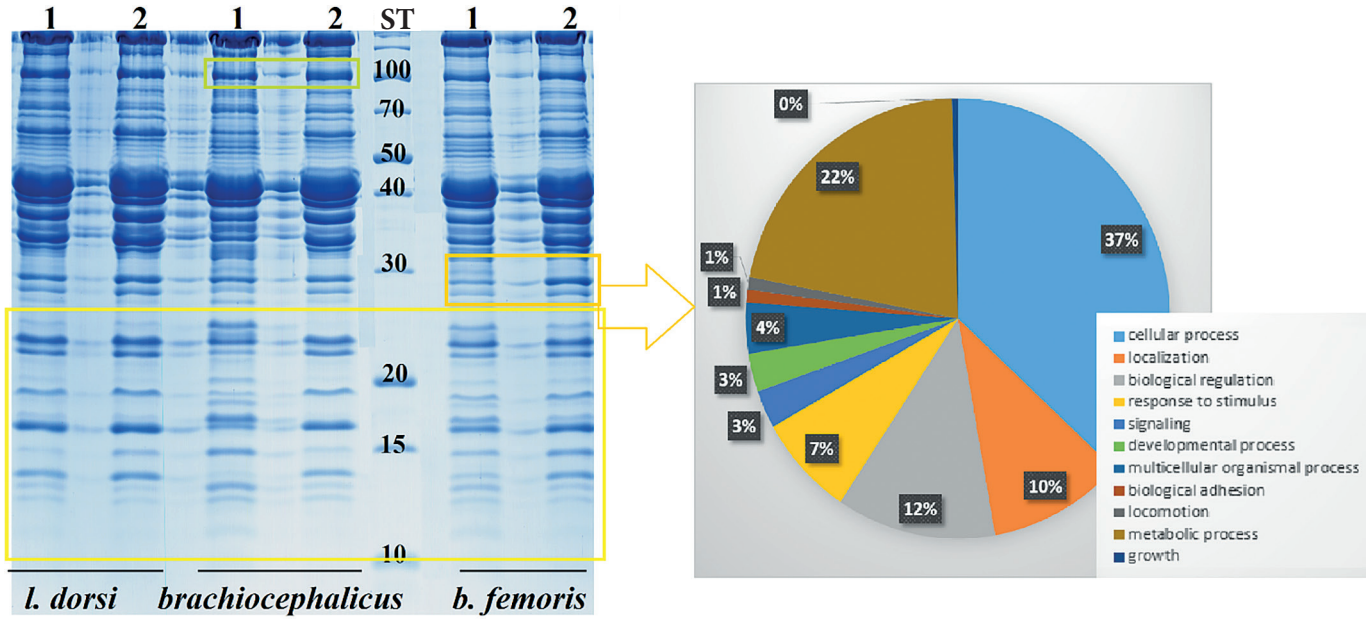

Figure 7. One-dimensional electrophoregram of pig muscles during growth (1 — piglets, 2 — mature pigs)

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METHODOLOGY OF FOOD DESIGN. PART 2. DIGITAL NUTRITIOLOGY IN PERSONAL FOOD

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Keywords: *digital nutrition, personalized nutrition, atherosclerosis, bioinformatics, nutritional genetics, FoodTech, preclinical trials*

Abstract

Atherosclerosis (the main cause of a wide range of cardiovascular diseases) and other multifactorial diseases depend on several nutrition factors, defined in general by lifestyle that directly and constantly affects the human body. The modern level of science and technology development is able to form a diet, taking into account all personal characteristics in such a way that makes nutrition an effective preventive measure against diseases in order to keep a person healthy. The purpose of this article is to define and study all the limitations (the scope of its coverage in the scientific literature) that arose in the process of research aimed to formation of an integrated personal approach to designing of nutrition to prevent atherosclerosis. The object of the study was scientific literature, which is available in open source and free access databases: PubMed, ScienceDirect, eLIBRARY.RU, www.fips.ru, Patentscope. The language of search is Russian and English, search depth is 12 years. In the course of the research two food design concepts were found that affect process of digitalization in the food sector: the concept "FoodTech" (food technology) and digital nutritiology. It was established that in Russia only one company — LLC "City Supermarket" (Moscow), that works with the brand "Azbuka Vkusa" — acts in the sphere of "FoodTech" on the Russian market. This company selects personalized food, taking into account the results of personal nutrigenetic tests, in cooperation with LLC "Genotech" (Moscow). There is a need to use biological information, statistical information processing (nutrigenetic studies, nutrigenomic research) and machine-aided data processing (machine learning) for further generation of automatic algorithm that compiles personal recommendations. The relevance of generation of a national domestic database on chemical composition of food products (presented in the market) to simplify the preparation of individual personal diets is observed. We underline the necessity to use the test-organisms, i. e. dorio fish / zebrafish (Danio rerio) and nematodes (Caenorhabditis elegans)), which were used to determine the activity of candidate substances — the biologically active substances that feature antiatherosclerotic properties. In the future the authors plan to conduct a nutrigenomic and nutrigenetic study, using digital achievements. To collect information about consumers, it is necessary to apply digital devices, and use biological informatics to process the results; after that it is necessary to generate the algorithm for automatic selection of personalized dietary recommendations.

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Introduction

The development of "4P" concept (product, place, price, promotion) in healthcare and the modern level of "omics" technologies led to the emergence of personalized nutrition [1]. The relevance of this idea is ensured by the fact that nutrition is an environmental factor that directly and constantly affects the consumer's health. Regulation of nutrition is capable to change the state of health of the body [2]. Personal nutrition takes into account the entirety of the individual consumer's characteristics, helps prevent the development of a range of chronic diseases, and therefore it features prophylactic function [3,4]. The general goal of the authors' research is to form an integrated approach to nutrition, including the selection of personal dietary recommendations and the development of individual functional food products (FFP) and biologically active additives (BAA) to prevent diseases [5–7]. In particular, for the prevention of atherosclerosis, as the root cause of a number of cardiovascular diseases, which occupy a leading position in the structure of human mortality throughout the world [8]. To achieve this goal, in their previous works the authors [9–11] emphasize the importance of a personal approach to nutrition to prevent development of chronic, multifactorial diseases, and draw their attention to:

- identified genes whose mutations affect nutrient metabolism and are associated with a predisposition to the development of a number of socially important diseases;
- identified genes influencing the development of atherosclerosis and associated with nutrition;
- presented an algorithm for developing a personalized diet;
- highlighted the strengths and weaknesses of personal nutrition.

In the course of the study, a number of limitations arose that hindered the formation of an integrated approach to personal nutrition. For example, to form personal dietary recommendations, it is necessary to process a huge amount of information about the consumer: history data, family diseases, nutritional characteristics (intolerance, dependence, preference), body composition data (anthropometry, etc.) and nutrigenetic tests data [12]. Therefore, it is necessary to turn to the field of mathematical and bioinformatic data processing, etc. It's possible also to use modern gadgets to collect the necessary data. In the course of the research it became clear that is necessary to consider digitalization in personal nutrition planning.

The purpose of this work was to study and work out the limitations that arose during the study; in particular, during the study of the role of digital nutritionology in the modern concept of personalized nutrition. The research was conducted by analyzing scientific literature on this issue.

Objects and methods

The object of the research was scientific literature (articles, patented inventions), that covers the role of the digital approach in science of nutrition. The scientific literature was analyzed in the following databases: PubMed (United States National Library of Medicine), ScienceDirect, Scopus (Elsevier publishing house), eLIBRARY.RU electronic library, www.fips.ru search systems (Federal Institute of Industrial Property), Patentscope (World Intellectual Property Organization). The search covered the literature in English and Russian. The depth of search was 12 years.

Results and discussion

Digitalization is the introduction of modern digital systems and technologies into various spheres of human life [13]. In the course of a literature analysis, it was found that digital technologies are used in nutrition in "FoodTech" sphere, and in the field of nutrition directly.

"FoodTech" (Food technology) is a new branch of nutrition science associated with the new innovative technologies, namely biotechnology, information and digital technologies. This is an aggregate set of operations and activities, starting from growing of raw materials, processing, food production, packaging, storage till the moment of sale and disposal of waste [14]. Consequently this industry branch is subdivided into two components:

1. Orientation towards the end consumer (B2C), towards the production of traditional food products, towards personalized nutrition.
2. Orientation towards businesses (B2B).

The world market of FoodTech consists of five areas of activity: development of technologies for obtaining alternative sources of protein (insects, algae, microorganisms-protein producers) [15,16] and innovative food products (lactose-free, gluten-free food products, food with a low sugar content, etc.) [17], table 1; development and application of biotechnology in the field of agriculture (application of synbiotic additives for normalizing the digestive system of livestock, producing strains, strains for plants growth stimulation, etc.) [18]; application of digital technologies in the field of agriculture (application of machine-aided data processing algorithms, automation, introduction of unmanned technologies, etc.) [19,20]; development of "smart packaging" (environmentally friendly and sustainable packaging, packaging that displays shelf life of the food products, etc.); development of personalized nutrition (nutrigenetic testing, study of the microbiota of the gastrointestinal tract, individual FFP) [14]. FoodTech provides for not production of food only, but also for their delivery (from catering businesses, shops) [21], which is in a great demand now due to the spread of coronavirus infection [22,23].

The Russian market for innovative products is mainly represented by functional drinks and food additives produced from alternative sources of nutrients by the companies located in the European part of the country. There is only one competitor on the market today that selects personalized food products based on genetic tests results. This information proves that it is possible to enter the market

Table 1. The example of a number of domestic companies engaged in innovative nutrition in FoodTech sphere

Category	Food product	Producer, city	reference
Innovative food products	"NEORON" drink	LLC "SINCOR", Moscow	[24]
	"Octa" milk drink	LLC "Dairy Naro-Fominsk Plant", Moscow	[25]
	Chlorella drink "Be. Live.Organic"	LLC "Holdingvest", Tver	[26]
Nutritional supplements	Fine powders	LLC "EVOFOOD", Perm	[27]
	Wolffia-based food additive "BIOVOLF"	LLC "BIOVOLF RUS", Moscow	[28]
	Sweetening proteins "Hi-Protein"	JSC "EFKO", Alekseevka	[29]
	Vegetable salt replacement	LLC "SALICORNIA NUTRITION", Astrakhan	[30]
Alternative protein source	Protein product from sunflower kernel "SFP-60"	LLC "ECO PRODUCT", Moscow	[31]
Alternative meat products	Herbal food products "Greenwise"	CJSC "PARTNER-M", Maloyaroslavetz	[32]
	Vegetable meat "Hi!"	JSC "EFKO", Alekseevka	[29]
Personal food	Range of food products from the private supermarkets chain "Azbuka Vkusa" ("Alphabet of Taste") based on nutrigenetic tests conducted by the company "Genotech"	LLC "City Supermarket", Moscow	[33]

with individually selected dietary supplements and produced FFP. However this process is quite costly, which is explained by various difficulties in the field of food personalization. It is important that some of these difficulties (i. e. restrictions) will be eliminated by the introduction of digitalization in nutrition and preclinical researches.

The term "digital nutritiology" was presented for the first time in the Russian scientific literature in the paper of Yu. N. Orlov and his colleagues [34]. The paper developed an automatic algorithm for calculating the size of helping (food portion), taking into account the data on the daily intake of essential nutrients. There are three blocks that enable software implementation in the field of nutritional science:

- the first block — an aggregate set of models of food digestion by a body, that is taking into account all body's basic features (gender, age, weight, level of physical activity, etc.) and data on the health of the consumer, as well as his / her cultural and religious preferences;
- the second block — an aggregate set of data on chemical composition of food product (before and after its cooking by various cooking methods);
- the third block — an aggregate set of computational algorithms compiled for analysis of information from the first two blocks. Those algorithms select the composition and size of helping based on individual data about the consumer, and are able to evaluate the menus made according to consumer preferences, etc.

The role of digitalization in this case is that the collection and aggregation of information on the consumer (i. e. the first block) is possible thanks to modern gadgets used in real time mode (cell phones with special applications, wearable technologies devices — smart watches, etc.) [35–38]. On the modern market there is a huge variety of mobile applications that assist in weight management, control the therapeutic diets and give advice for people with diabetes, gastrointestinal tract issues, allergic reactions to certain food components, etc. [39,40]. To calculate the algorithms that define helping sizes and so on, statistical and bioinformatic data processing is required. Traditional elements of statistical analysis of nutritional data include: t-tests, analysis of variance (ANOVA), analysis of covariance (ANCOVA) [41,42], chi-square, regression [43], etc. These operations play an important role in describing nutritional information; they are able to transform the received data into analytical variables. But they cannot interpret the large amount of data collected during the formation of personal dietary recommendations, when analyzing the relation between intake of nutrients and the health of a consumer. Therefore it is necessary to supplement traditional statistical methods with machine-aided processing of data[44,45]. Machine-aided processing of datarefers to a computer system capable of describing the solution to a given problem and creating an algorithm based on this solution. More details about machine-aided processing of datamethods are presented in the work of L. Khorramine-zhad, [46]. You also need to use programs like Python (Python), R, RStudio, Statistics, etc. to process the collected data.

For the formation of an individual diet for the prevention of both atherosclerosis and any other disease, it is important to create a domestic database on the chemical composition of food products. The demand for this database is caused by the fact that its availability will simplify the development of personal dietary recommendations by specialists and individual dietary supplements, food additives and FFPs for prevention of diseases. This topic is considered in more detail in the paper of V. V. Bessonov and his colleagues [47]. For a detailed study of the chemical composition of food products, it is necessary to improve the methods used in the "Foodomics" approach. This approach is the discipline of studying food, using "omics" technologies (that is genomics, transcriptomics, proteomics and metabolomics) to improve the quality of food, functional food activity and assessment of the food safety [48]. Due to necessity to process a large amount of information this area is also closely related to bioinformatics [49]. Methods of analytical chemistry play a significant role in Foodomics technologies, namely chromatographic analysis, mass spectrometry, nuclear magnetic resonance, as well as methods of sample preparation, extraction and purification of biologically active substances (BAS) from the researched raw materials [50], methods of genetic analysis to detect food pathogens [51].

In order to organize an experiment *in silico*, the initial data from experiments *in vivo* and / or *in vitro* are required. So an important problem is to assess the efficiency of the developed FFP and dietary supplements assigned to prevent atherosclerosis. During the review of the scientific literature it was noted that it was possible to evaluate on model test systems the following parameters in *in vitro* conditions:

1. The ability of cell lines to accumulate cholesterol. Cellular models are presented in the work of A. N. Orekhova and his colleagues [52]. Thus, to assess the anti-atherosclerotic potential of the studied biologically active substance, it is necessary to cultivate human aortic cells under conditions of gradual adding atherogenic serum / atherogenic serum plus biologically active substances.
2. The ability to transform foam cells on human monocytes (THP-1). Monocytes were transferred into macrophages, then into foam cells (using oxidized lipoprotein of low density) in the presence / absence of the studied BAS. The technique is described in the paper of X. W. He [53].
3. The ability of probiotics, synbiotics to change the level of cholesterol in the nutrient medium. The technique is thoroughly described in the work of D.K Lee [54].

To assess the efficiency of dietary supplements / food additives and FFP *in vitro*, Figure 1 below is compiled to show all test models being researched in comparison with the model of a human [55].

	PARAMETERS	Nematodes	Drosophila	Zebrafish	Frogs	Mice	Human
ORGANS	Fat tissue	–	Thick body	+	+	+	+
	Liver	–	Thick body	+	+	+	+
	Pancreas	–	Insulin producing cells	+	+	+	+
	Placenta	–	–	–	–	+	+
	Lacteal gland	–	–	–	–	+	+
CONTENT	Venue of the experiment	Any vessel	Flasks	Water vessels	Water vessels	Supervised cages (1–4 mice per cage)	Variable, controlled
	Nutritive substrate	Growth medium	Wet powder	Pellets / flakes (added to water)	Pellets (added to water)	Dry powder or granules	Variable
	Keeping costs	Low	Low	Moderate	Moderate	Very high	Very high
OFF-SPRINGS	Interval of generations	4 days	1 week	Months	Months	Months	Decades
	Number of offsprings in the brood	Hundreds	Hundreds	Hundreds	Hundreds	< 12	1–2

Figure 1. Model test organisms suitable for Nutrigenetic Research

The genetic information of all the test organisms, shown in the figure above, has been decoded. That made them suitable for research in the field of nutritional genetics. But zebrafish (*Danio rerio*) and nematodes (*Caenorhabditis elegans*) were of particular interest as they feature genetic similarity to the human genome, they feature rapid development, high fertility; they are easy to conduct genetic manipulation on, as well as the costs of their keeping are relatively low, as well as fewer ethical limitations in comparison with models of mammals and large amphibians [56]. In the scientific literature, zebrafish are the model organisms of scientific priority in studies related to nutritional genetics and development of obesity [57]. The zebrafish can also be used for researches related to cardiovascular diseases and metabolic syndrome [58,59]. Due to the fact that nematodes (*Caenorhabditis elegans*) have a transparent body, they can be used to study the accumulation of lipids — their body accumulates fats in the form of droplets in the subcutaneous and intestinal cells, which are clearly visible to the observer [60]. Nematodes are also used for research targeted to finding candidate genes related with human obesity [61]. Consequently, these test models can be used for further research targeted at finding the relation between nutritional genetics and development of atherosclerosis.

Conclusion

Nowadays the personalization in the healthcare system is an urgent preventive system; in particular, the personalization is expressed in a way of individual nutrition. This article briefly summarizes the authors' findings within the research conducted in the nutritional genetics, and represents the revealed limitations. Limitations are related to digitalization in the nutrition system and preclinical studies, which serve as a source of data for further machine-aided processing of data, for statistical forecasting, etc. In this paper it was found that:

- all "omics" technologies are aimed at prevention of diseases development and ensuring the normal well-being of the consumer;
- processing of data obtained via "omics" technologies is not possible without the introduction of digital technologies;
- the role of digitalization in the modern world keeps growing, and its achievements can be used to develop personalized nutrition;
- in the sphere of nutrition, digitalization is implemented in the form of "FoodTech" and in nutritional science directly;
- development of algorithms for the automatic selection of appropriate food, according not only to its composition and size of helping, but also for drawing of dietary recommendations for the prevention of diseases (in particular atherosclerosis) is of high relevance now;
- in order to develop these algorithms, it is necessary to involve data on basic characteristics of consumers. Some of that data can be collected via mobile applications, smart watches and other digital devices;
- in order to develop there algorithms, it is necessary to involve data on chemical composition of food products and its biologically active features;
- in order to study the chemical composition and safety of existing food products and developed FFP, dietary supplements, it is necessary to develop Foodomics technology;

- to assess the efficiency of the selected nutrition, FFP and dietary supplements, it is necessary to conduct preclinical studies on the model test systems;
- nematodes (*Caenorhabditis elegans*) and zebrafish (*Danio rerio*) are the promising test organisms;
- the researches on cell models *in vitro* are of relevance too;
- bioinformatics methods are necessary to process the obtained data of "omics" (nutrigenomic and nutrigenetic research) analysis;
- on the domestic market there is a service for selection of personal food products, taking into account the results of nutrigenetic testing. This service is provided by only one company, that is LLC "City Supermarket" (that operates under the brand "Azbuka Vkusa") in cooperation with LLC "Genotek".

Within the course of further research the authors plan to form personal dietary recommendations, aimed at the prevention of atherosclerosis, on the basis of nutrigenetic tests; also to make a list of BAS-candidates which feature anti-atherosclerotic properties; to run preclinical tests — i. e. nutrigenomic research; to evaluate the efficiency of the selected biologically active substances on the model systems *in vitro*, *in vivo*. The authors plan to conduct research *in silico*, on the basis of the collected data; further to form an integrated approach to nutrition to prevent atherosclerosis, as well as to develop individual dietary supplements and FFP.

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MEAT CONSUMPTION: THEORY, PRACTICE AND FUTURE PROSPECTS

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Keywords: *authenticity, modern meat market, nitrate, meat replacement, non-thermal processing, nutritive value, consumer preference*

Abstract

This research reviewed human meat consumption and highlighted associated history, challenges and benefits. Selected literature for the manuscript was from relevant titles and reliable international sources. From early times of the mankind meat consumption and animal husbandry were inseparable parts of living, and with similar consequences as dramatic influence on environment. Human need for meat consumption fueled development of large world markets with incredible trade, processing and consumption. This overconsumption has caused health problems associated with high intake of cholesterol and sodium chloride. Another problem with meat consumption is the use of additives in processed products. In modern time these problems are tackled by the use of additives from plants that have health benefits. Thermal processing is yet another problem with meat consumption that food industry and science addresses by non-thermal replacements (e. g. high-pressure processing and electrotechnologies). Recently, interesting alternatives for meat processing included 3D Printing that is able to engineer admirable meat products from by-products. However, this technology might need to employ enzymes such as transglutaminase, associated with potential health problems and misleading the customers. Unfortunately, fraudulent activities are common for meat products and it would be prudent to organize enforcement centers with at least police and analysts skilled in chemometrics and various laboratory techniques for food defense. It seems as humankind expands it will seek more proteins from plant, insects, unicellular biomass, and synthetic meat than from the animal origin, however all of the alternatives must be carefully evaluated against consumer acceptance, public health and environmental concerns.

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Introduction

Human cravings for animal meat probably dates back to the origins of humankind [1]. The earliest archeological evidence of early humans includes common stone tools as well as animal remains. The earliest human cave art, dating back 44,000 years ago, depicts scenes of hunting native animals [2]. Even in human physiology, there are specific metabolic pathways, such as for iron metabolism, to better utilize nutrients from animal meat [3]. For instance, the transport of dietary iron in the human body occurs through two pathways, one for non-heme iron and one for the heme-iron. Needless to say, that the historically hemoglobin in the human diet is mostly of animal origin or derived from meat.

Objects and methods

The subject of the study was to provide an overview of human meat consumption and highlight the challenges and benefits associated with it. The focus was on a brief anthropological and historical review, reasons for the popularity of meat and its nutritional value, describing modern production and processing, providing current trends in meat science, and finally projections of future trends related to meat production, processing, and consumption. The literature review included the electronic databases Web of Science, PubMed, Scopus, ScienceDirect, and Google. References were selected for the manuscript whose titles and descriptions were relevant to the topic and were from reliable international sources.

Development of International Meat Market

The human population grew, so did the need for reliable meat production. As hunting proved to be an inadequate source of meat, a new branch of agronomy, animal husbandry, emerged [4]. The earliest domesticated animals included sheep, goats, pigs, cows and chickens, probably more than 10,000 years ago [4]. Since ancient civilizations, the Egyptians, Romans etc., the breeding of animals for meat increased as the human population grew [4, 5]. However, with the decline of the Romans in Middle Ages, the systematic breeding of animals for meat declined until Agricultural Revolution in Britain around the 18th century. What was new here was the selective breeding of animals, which improved the production of meat and paved the way for modern meat production [6].

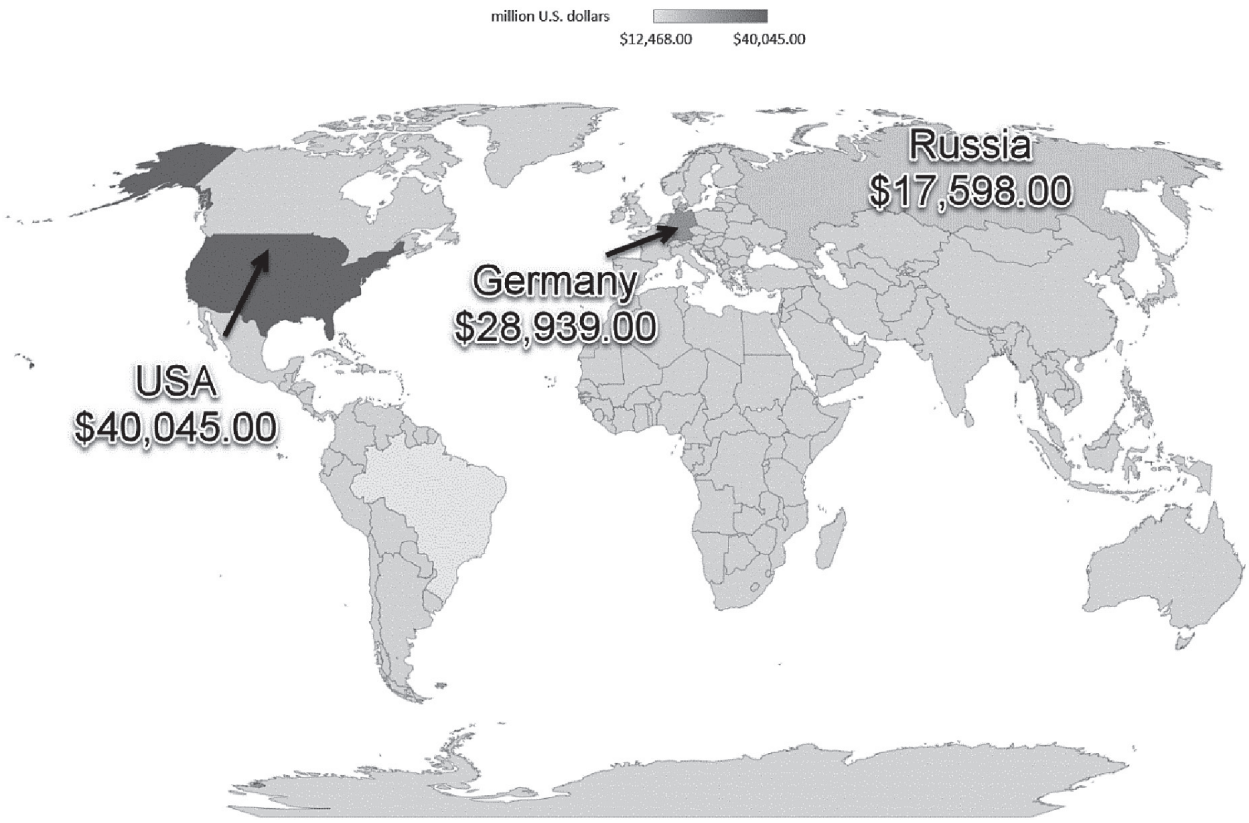

Figure 1. Top 3 worldwide revenues from meat products and sausages in 2021 (in millions of U.S. dollars) [7]

The value of modern meat market was $838 billion in 2020, and it is expected to increase to $1 trillion by 2025. In 2020, 328 million tons of meat were produced worldwide, equivalent to an annual per capita consumption of 35 kilograms of meat. The largest meat markets in the world (Figure 1.) are the US, Germany, Russia, France and Brazil [7]. The US is the largest meat producer and exporter in the world, especially for beef. Currently, most meat is produced in Asia (136 million tons), with pork and poultry being the most produced, while lamb is the least produced. The largest companies producing meat are Tyson, Hormel Foods, and National Beef [7]. It is hard to believe but in 2014 alone, humanity consumed 1.47 billion pigs, 545 million sheep, 444 million goats and 300 million cattle [8]. By 2021, that number will be 1 billion cattle, 0.75 billion pigs, and 0.1 billion chickens [9]. All of the data presented shows the sheer magnitude of modern meat production. However, even though modern meat production is one of the largest industrial sectors in the world, there are still many meat production challenges that need to be addressed.

Environmental concerns

As in prehistory, the main problem with meat consumption is the growing human population, as livestock requires more land, which in turn suffers from desertification [10]. Even more, over 65% of infectious diseases are transmitted to humans through livestock production, in an area that covers 70% of the total arable land mass [10]. In 2017, Stockholm International Water Institute stated that 70% of water is used for agriculture [11], with animal farming consuming most of it [10]. Additionally, global food systems are responsible for 30% of all greenhouse gas emissions produced, with 60% of that coming from animal agriculture [12].

Nutritive value and importance of meat

The need for meat consumption has always been a strain on natural resources, from prehistoric times until today, as extinctions of entire species (e. g. Mammoths) have been linked to human expansion on the planet after the period of the Ice Age [13,14]. The changes affecting the environment have now taken different forms, but the effects are similar to those experienced thousands of years ago. Unsurprisingly, modern meat production has faced problems that include sociocultural issues, negative impacts on human health (e. g. the introduction of antibiotics into the environment during animal farming, which can create new strains of pathogenic bacteria that are resistant to antibiotics), deforestation of land, pollution of water, global warming and so on [11]. So what drives people to consume meat at these costs throughout the history?

In the past, animals were hunted to consume their fat to increase the chances of survival during the cold seasons. Some carnivorous species have continued this behavior today, such as polar bears, which eat mainly fats from seals to build-up energy reserves for the harsh winter time. Researchers suggest that this type of diet may have played an important role in the evolution of humans as a whole [15]. Furthermore, meat is known to be an excellent source of proteins, lipids, minerals and vitamins that humans need to survive [16]. On average, an adult human requires 50–80 g of protein per day with all essential amino acids [17].

Albeit protein content may vary depending on the type of carcasses, it is generally similar among different mammals, birds, and fish [16]. Undeniably, meat is an important source of protein, Fe, Zn, Se, and vitamins D and B12 for many people [18]. However, in order to get nutrients from meat, it is important to process it, because even simple cooking over fire greatly increases the amount of nutrients available compared to raw alternatives. Thus, the discovery of fire and cooking allowed the human body access to more energy, and thus running more powerful brain that allowed our species to become the dominant on the planet [19]. Cooking with fire was the earliest form of thermal processing of meat, which along with drying, ensured the survival of early humans and was used into modern times.

Production and Processing

Apart from the environmental problems, there are also problems with meat production in terms of production and processing. According to the World Health Organisation (WHO), some meat products are classified as Group 1 carcinogens, due to the use of nitrates, which are a common additive in meat processing [20]. Nitrates are added to meat as an important antioxidant that prevents the growth of anaerobic bacteria (e. g., *Clostridium botulinum*), lipid oxidation, and off-flavors while preserving the appealing color of the products [21]. On the other hand, nitrates also promote the production of N-nitrosamines, which have been associated with an increased risk of cancer [20]. In addition to nitrates, several groups of synthetic additives such as butylated hydroxytoluene, butylated hydroxyanisole, tertiary butylhydroquinone, and propyl gallate, which are used to inhibit microbial growth and oxidation of meat [22], have negative health effects because they pose a cancer risk [23].

Current efforts in meat science are primarily concerned with the replacement of additives with natural alternatives and the use of non-thermal processes. This mainly involves the aforementioned nitrate salts, which are replaced by natural additives (antioxidants) from aromatic plants and their oils, bioactive peptides from eggs [24] fish, milk and meat [25], nuts [26]; polyphenols from fruits such as berries [20,27], and others [28].

Another alternative to synthetic additives that can provide similar technological functions is probiotics, which function primarily as starter cultures. In addition to microbial inhibition, probiotics have the ability to regulate fermentation, shorten product ripening, and improve food preservation [29]. They are excellent raw materials for various (functional) foods and are generally considered safe for consumption. Nevertheless, their consumption may have adverse consequences for some consumer groups, as their use in the public diet is not sufficiently regulated by law, which is certainly a concern due to the potential lack of consumer safety when consuming such products [30,31]. Advantages and disadvantages can be enumerated in all these approaches, as no approach is perfect. Nevertheless, positive changes can be expected in the future, which can already be observed on grocery shop shelves. We are seeing an increasing number of foods (and meat products) that contain natural additives that are less harmful and even helpful to health (e. g., they contain functional components such as plant polyphenols).

Another, more dietary problem with meat consumption is the ingestion of cholesterol. This infamous molecule, similar to unsaturated lipids is susceptible to oxidation [32], and tends to agglomerate on the walls of blood vessels, which can lead to blood flow blockage and consequent cardiovascular problems [33,34]. Besides cholesterol, processed meat contains larger amounts of salt (NaCl), which in large amounts increases blood pressure. In addition, meat is naturally rich in carnitine, which when broken down by human physiology, increases the risk of atherosclerosis by generating trimethyl amine N-oxide [18].

Replacing fattening calories and cholesterol from meat is a somewhat complicated task to accomplish. For this purpose, industry and academia use gels and emulsions made from marine organisms or plants [35–38]. Here, researchers are concerned with finding the best alternative that increases the nutritional value of a product (reducing saturated fats) while maintaining all the benefits of naturally occurring fats from meat, namely sensory value, texture, technological properties, etc. [39]. As with the replacement of synthetic antioxidants, fat substitutes also have various (dis)advantages that science and industry are meticulously trying to document in order to meet consumers demands, health requirements, regulatory requirements and market value of the products.

Even the simple thermal processing and smoking of meat has a similar result, where the heat generates the production of toxic N-nitrosamines in addition to polycyclic aromatic hydrocarbons [20]. Needless to say, meat oxidation is a major problem for both consumers and industry, as it negatively affects health and lowers the economic profitability of production [32]. It has been documented that oxidized products in meat (e. g., carbonylated proteins) are associated with several diseases as cancer, Alzheimer's disease, chronic renal failure, and diabetes [20].

In order to reduce the thermal exposure and the amounts of additives in meat (and food in general), food scientists have turned to the use of non-thermal technologies that are able to provide the obligatory inactivation of microorganisms while preserving nutritional value of the meat. Thermal processing, such as grilling, produces harmful carcinogenic compounds and an appealing, juicy flavor that is appreciated by many. Grilling is one of the most common cooking methods for meat, and the taste is achieved by denaturing the meat on the surface, creating the familiar crunchy coating [40]. It is also probably the oldest cooking method as it was used in prehistoric times. The main reasons for thermal processing are enzymatic and microbial inactivation to extend the shelf-life of meat

[41], and improvements in digestibility of nutrients. Other ways of preparing meat include boiling, blanching, microwaving, oven cooking, and roasting, each of which has its own advantages and disadvantages.

An alternative to conventional cooking is the *sous-vide* method, which is preferred by many restaurant professionals [42], because it better preserves the taste and natural characteristics (juiciness) of the meat for their customers [43]. This technology involves the use of plastic bags used in *sous-vide* equipment to hold vacuumed meat that is systematically subjected to a very slow cooking process (up to 48 hours) [44]. Initially, the meat can be semi-cooked or raw, depending on the occasion, and is then placed in water baths at 65–95 °C. In this way, ready-to-eat food of high quality and with low production costs can be produced. The meat obtained in this way retains its fresh quality [45,46].

Among the increasingly growing number of alternatives to conventional thermal meat processing, the most notable alternatives are non-thermal treatments such as high pressure based technologies, electrotechnologies, e. g. pulsed electric fields (PEF), or the use of ultrasound energy which alleviates many of the concerns associated with thermal treatments. High pressure processing (HPP) is one of the most successful advanced technologies for meat [47], accounting for 20–30% of all food processed with this technology. In 2019, 400,000 tons of meat were processed using this HPP, mainly in the United States. This technology offers minimal changes in nutritional and sensory quality and is suitable for various meats [47]. Testament to the potential of high pressure processing is the U. S. Food and Drug Administration (FDA) approval for use in industry for cold pasteurization [48].

As already known, in pulsed electric field technology, high-intensity electric fields (> 0.1 kV/cm) are applied to the meat between two electrodes for a very short time (in the range of milliseconds to microseconds) [49]. The most important phenomenon associated with PEF is the occurrence of electroporation, which increases the juiciness of the meat [50]. Another advantage is the minimal effect on the microstructure of the meat [50], while shortcomings include the strong dependence on intrinsic factors of the treated food/meat which may reduce its effectiveness [49].

Both of the above technologies treat meat at lower temperatures (even room temperature) while ensuring microbial safety and product quality [50]. However, sometimes these technologies are not able to inactivate microbes efficiently, and they are combined under the concept of 'Hurdle technology' that essentially encompasses combination(s) of different food preservation factors that synergistically ensure food protection and microbial inactivation [51].

Next comes ultrasound technology, which is useful for inactivating microorganisms, but is more commonly used as a mechanical aid in meat processing. This technology is based on acoustic energy, which provides a non-ionizing, non-invasive, and non-polluting form of mechanical energy [52]. It is suitable for tenderizing meat, inactivating microbes on the surface of poultry, and accelerating the cooking process [53]. Although this technology has significant potential for meat processing, it has not yet been massively used in production.

Another interesting advanced technology that is not designed to solve food safety issues is 3D printing [54]. This technology has the ability to utilize remnants of meat from processing which lacks aesthetics and market value, as an entirely new raw material for legitimate food products [54]. The greatest strength of this technology is the endless aesthetic possibilities for product design, limited only by the imagination, consistency, and texture of the material used in the device. Moreover, it is an additive technology in which a specific product is shaped by patiently making a single slice at a time [55]. Since this technology is capable of using virtually any type of paste-like material for food production, it can utilize various by-products of the food industry that are otherwise considered waste. Therefore, it is referred to as an environmentally friendly approach, however, in this case, food safety must be ensured by other means, such as hurdle technology. Recently this amazing technology allowed researchers to print the famous Wagyu beef which resembles the natural characteristics of the real steak [56].

Another enzyme associated with 3D printing of meat in this particular research is the controversial transglutaminase, also known as "meat glue." This enzyme enables the joining of different pieces of meat into a larger structure that looks like a normal slice of meat [57]. In 2010, the use of transglutaminase was banned in the EU due to concerns about labeling and potential to fraud the customers into believing they were buying a usual piece of meat. Transglutaminase is able to catalyze the binding of acyl transfer between the γ-carboxyamide side of the peptide chain and primary amines. As a consequence, the linked peptides form larger polymers with high molecular weight, thus altering the structural properties of food [58]. Although this enzyme has the potential to reduce the amounts of by-products from production, it has also been linked to health problems, namely autoimmune diseases, intestinal permeability, celiac disease, Alzheimer's disease and Huntington's disease [57].

Legal issues

Given the high demand in meat markets, it is not surprising that numerous offenders tend to adulterate meat products [59]. Unfortunately, meat adulteration is a widespread problem with numerous incidents, material losses, and dire consequences for human health [60,61]. Terminologically, food crimes of any kind are activities that focus on the mistreatment of consumers by various groups or individuals with clear (criminal) motives and intentions [62,63]. One of the first reported cases of food fraud involved the replacement of beef with horsemeat and pork,

which led to legislative changes in the EU and Germany [64]. This scandal revealed weaknesses in public food control, traceability and origin of food in a supply chain, while adulteration of meat products (and others) remains a serious problem even today. Detailed explanations of the problem of authenticity have been published elsewhere [65].

The problems with the aforementioned food frauds related to meat can be addressed in various ways, but mainly through increased legal controls, governmental monitoring of food markets enforced with the police and other representatives of the state monitoring system, the establishing legal framework for the most affected fraudulent activities that are not yet in place, the introduction of food protection systems along the entire food chain, and vigilant control of raw materials and their traceability [66]. This should include the establishment of centers with analytical support and techniques capable of quickly detecting tempering of products and working closely with the police [60,67,68]. In addition to "wet" laboratory techniques, such centers should have data analysts skilled in chemometrics and management of large datasets, as fingerprinting of food commodities in most cases relies on myriad data points to draw useful conclusions that are difficult to extract unless managed by experienced professionals [69]. Among the most useful chemometric techniques are multivariate analyses such as factor analysis, different data clusters, mathematical modeling, multivariate analysis of variance etc. [70,71].

Meat replacements and alternative protein sources

Clearly, the habitable space on the planet is shrinking due to the ever-increasing human population and animal agriculture. Therefore, humans must either figure out how to use the uninhabitable parts of the world or opt for food solutions that are more compatible with environmental constraints. Among the most likely solutions already available are alternative sources of protein that do not come from farming livestock. In first place are proteins from single-celled organisms (e. g. bacteria or yeasts), followed by marine organisms (algae seaweed), insects and plants [17].

Bacteria have the highest protein content in the biomass (50–80%), followed by yeasts (30–75%) and molds (20–45%). Most of the unicellular biomasses contain abundant lysine but lack methionine and cysteine. However, the addition of methionine makes this biomass equivalent to animal proteins [72]. In addition, this biomass is a good dietary source of vitamin B and minerals, while yeasts also have a useful probiotic effects [17]. On the other hand, the main disadvantage of unicellular biomass is the high content of nucleic acids, which can lead to gout if consumed in excess of 30 g/day. Seaweed and algae are protein alternatives that do not pollute the environment, and also provide good proteins, vitamins and minerals. Nevertheless, their use is not widely accepted in the industry due to the high cost of production and the development of products with appealing taste [73]. Some other organisms such as krill are a viable protein source, but their biomass contains chitin, which is an allergen [74]. Insects are another alternative (or supplement) to meat processing, with digestibility of their proteins comparable to egg proteins or meat [17]. Farming insects for protein requires less land area, has lower greenhouse gas emissions per unit of biomass than farming livestock, and requires fewer economic resources. Apart from human nutrition, insect biomass can also be used for fishmeal, which is also an agricultural approach that is less polluting than livestock farming [10]. Unfortunately, insects are not well accepted as part of the diet in Western countries because they are considered disgusting [73] and their biomass also contains chitin, which is considered an allergen [74].

Compared to meat proteins, plant-based counterparts are preferable because of their lower environmental impact. One of the most important sources of plant proteins are cereals (e. g. wheat and barley) and legumes (e. g. soy, beans, lupins and peas). Ecology aside, there are no ethical animal welfare issues involved in the production of plant proteins. However, there is another controversy associated with legumes, namely soy, and genetically modified organisms that are, for better or worse, poorly accepted by customers. Plant products do not naturally contain cholesterol, but other limitations include lower digestibility compared to meat and risk of celiac problems due to the presence of gluten [17]. In any case, plant proteins will be an indispensable part of the diet in the future and their share is only expected to increase.

In vitro "cultured" or "synthetic" meat is an interesting alternative for people who do not want to give in to their carnivores urges and become vegetarians, but can still be environmentally conscious and reduce their environmental footprint on the planet [75]. But is this really the case?

In vitro meat is essentially laboratory-grown tissue from muscle stem cells of an animal in fetal bovine serum (FBS) derived from bovine blood [75]. With cellular proliferation and interlinking final product looks like usual meat without fats and with sensory quality similar to real meat. Originally a very expensive process, this currently has the potential to be used in meat production on an industrial scale [75]. Opponents of this approach point out that there are a number of problems with this method of meat production. Firstly, the main problem is the use of FBS, as it supposedly has to come from dead calves. While this reduces ethical issues related to the protection of animal rights, still they remain unsolved as animals continue to be killed in the process but to a lesser extent [76]. An alternative is the use of plant-based serums, which are claimed to be a sufficient substitute. The next problem is the use of antibiotics, fungicides, and growth hormones that are necessary to prevent contamination and proliferation of *in vitro* cell lines. Yet another issues that needs to be addressed is the objective and quantifiable assessment of the environmental, health and legislative impacts of conventional and synthetic meat. Even though this approach has

the potential to solve a number of problems related to meat consumption, there are also many (un)known caveats that need to be considered, especially now that this technology is still in its early stages of development. In other words, mass application in production should be carefully waged prior any rushing into actions that could be detrimental to public health and the general population.

Conclusion and Future trends

Humans always had preferences for meat consumption and this is unlikely to change in the future despite the fact that such preferences have negative repercussions on the environment. Testament to this is the enormous size of the current meat market and industrial production, which is expected to increase in the future. Meat provides high quality protein, vitamins and minerals while offering pleasure when consumed. The high level of interest in the trade of meat and its products has led (and may still lead) to fraudulent activities that have resulted in public health damages and economic losses for producers. Consequently, four actions are available to the food industry to address the above challenges, namely, improving the current regulatory framework to protect the authenticity of food, hiring more laboratory and data analysts, improving food production with healthier and less toxic additives, improving production to produce fewer undesirable compounds in meat products, and finding a meat substitute that meets all the benefits that meat provides for human nutrition while avoiding all the disadvantages of animal agriculture. Solutions to address the overconsumption of meat and the associated health risks also include nutritional education by public health officials and other key players in the food chain (e. g. government, consumer protection non-governmental organizations, industry, etc.).

It is expected that in the future proteins from insects and plants will account for a higher proportion of total dietary proteins in the market, but one problem that still needs to be solved is consumer acceptance due to imperfect taste. Social marketing campaigns may be helpful to improve the public image of "insect meat" and additional research may address this sensory acceptance problem. The innovative approach of growing meat in a petri dish is a possible solution that can theoretically solve most animal husbandry problems, but the current literature shows that there is still a long way to go from theory to practice. Finally, it remains to be seen how meat science and industry can satisfy the many conflicting sides of an individual who is clashed between a preference for eating meat and protecting the environment and is not a fan of slaughtering animals for consumption. This is indeed no easy task.

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ANALYSIS OF THE EFFICIENCY OF PRODUCTION OF WHOLE-MUSCLE TURKEY PRODUCTS WITH VEGETABLE SPRINKLES

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Abstract
Human health is largely determined by the nature, level and structure of nutrition. A promising direction in the development of new food products is the expansion of the base of used ingredients used to partially replace meat raw materials with vegetable ones, in order to maximize the saturation of products with nutrients that contribute to the maintenance of normal life of the consumer. The use of new food ingredients contributes to the actual task set by the State policy in the field of healthy eating — expanding the range of enriched and functional food products. The work is devoted to the study of baked whole-muscle products using turkey meat and vegetable dressing as sources of high protein content, which solves the problem of deficiency of this component in the diet. A recipe for brine with the addition of the food additive "Glimalask", which has a complex effect on the products under study, has been presented. The evaluation of quality indicators of finished products has been carried out, the article presents the results of a comparative analysis of baked whole-muscle turkey meat products, calculations of the product cost price. The comparative analysis has shown that, in comparison with the control sample, the baked whole-muscle products from turkey meat with vegetative dressing have improved physical and chemical properties, outstanding organoleptic characteristics, the yield of the product increases by 9.0–12.0%, depending on the formulation. Differences in the dynamics of microbial growth in the experimental and control samples were insignificant, the vegetable dressing helps to slow the growth of microorganisms on the surface of the product. The presented baked whole-muscle turkey meat products using optimized brine and vegetable sprinkles of chickpeas, sesame and paprika are recommended to a wide range of consumers of different age groups.

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Introduction

Today, issues related to healthy and rational nutrition are becoming more and more popular. At present, much attention is paid to human nutrition — new food products regularly appear on the shelves, and consumers are increasingly paying attention to their composition and nutritional value [1]. Human health is largely determined by the nature, level and structure of nutrition, which has a number of serious violations. Lack of essential substances in nutrition is one of the most important problems in Russia. One of the directions of the State policy in the field of healthy nutrition of Russians is to increase domestic production of food raw materials and food products, including enriched products and products with functional and therapeutic properties [2]. Unbalanced nutrition is a pressing problem in our country. Modern lifestyle forces people to eat on the go, which leads to various diseases [3].

Nutrition has the most significant impact on human health and life. Lack of vitamins, macro- and microelements in the diet negatively affects the body. Proper nutrition contributes to the prevention of many diseases, including nutritional diseases, and also contributes to increasing the average human life expectancy [4,5]. The creation of meat products designed to treat and prevent disease is a progressive trend in the food industry that has extremely important practical and social significance [6].

To improve the quality of food products, their appearance, structure, storability, for their enrichment are currently used in many ways. These include, for example, the use of various food additives, vitamin complexes, dietary fiber of plant origin, salt mixtures, etc. Today, the priority area of food technology is the production of enriched meat products characterized by high nutritional and biological value. Innovative food products made from natural raw materials, not only provide consumers with a healthy diet, but also guarantee the producers an increased competitive status and access to the world class market. One of the modern trends in expanding the range and improving the quality of food

products is the integrated use of raw materials of animal and plant origin [7].

For the production of enriched product turkey meat can be used very effectively. As a product of animal origin, meat contains animal protein, which is most fully assimilated by the body, as well as many nutrients necessary for the body [6].

Turkey meat is considerably superior to duck and goose meat in terms of high-protein content. It is a source of such amino acids as tryptophan, lysine, arginine. Low enough fat content. Turkey meat is rich in B vitamins, niacin and riboflavin, which has medicinal value, as well as fat-soluble vitamins A, D. Using such meat in the diet does not lead to an increase in blood cholesterol. Turkey is rich in various minerals, especially calcium and phosphorus. At the same time, the presence of collagen and elastin in the proteins of poultry leads to stiffness of the product and difficulty in digestion. Therefore, the creation of products with specified properties by introducing cheap plant materials into the product makes it possible to reduce the final cost of production [8].

Enrichment, or the process of optimizing raw materials and then the final product, is an important tool for functional and specialized nutrition [9]. Currently, the use of food additives of plant origin is widely used in the technology of meat products, which not only expand the range, but also increase the biological value, improve the organoleptic characteristics of the finished food products. Also, the use of plant ingredients leads to optimization of functional and technological properties of the product [10].

The relevance of this study is to study and develop new technologies and recipes for the food industry, which are aimed at expanding the range of products and improving its quality. The work is devoted to the study of baked whole muscle products using turkey meat and vegetable crumbles as sources of high protein content; the development of the recipe for brine optimized composition for the production of baked product, as well as analyzing the effectiveness of the use of crumbles of vegetable raw materials in the production of this product. The development of the method of production of baked meat product according to the recipes using regional raw materials is also very relevant and appropriate in terms of rational use of raw materials of food industry [11–13].

The aim of the work is to study the technology and qualitative characteristics of whole-muscle baked turkey products with a vegetable sprinkling. The objectives of the study are to select ingredients, formulation development, brine injection of meat raw materials (turkey) and the selection of vegetable raw materials for the sprinkling of baked products, optimization of technological regimes, evaluation of quality parameters of the product.

Objects and methods

The work was carried out on the basis of the department "Technologies of food production" of Volgograd State Technical University and complex analytical laboratory of the "Volga Region Scientific Research Institute of Meat-and-Milk Production and Processing" and consisted of the following stages: selection and preparation of raw materials, production of experimental samples of whole baked turkey products, conducting organoleptic, physical and chemical and microbiological studies to assess the quality of the products.

The research scheme is shown in Figure 1. The objects of the research work were samples of whole baked turkey products, which were produced using the above components with salt and spices.

The control sample was a baked turkey product, injected with brine of standard composition. Experimental samples were produced using brine with the food additive "Glimalask": sample № 1 — baked turkey product with a mixture of black and white sesame; sample № 2 — baked turkey product with a spicy coating; sample № 3 — baked whole-muscle turkey product with a coating of extruded chickpeas.

Optimization of the formulation of the developed product was carried out using the Excel program included in the package MS Office 2019. Production of the studied samples of baked product was carried out in accordance with the current regulatory and technical documentation (GOST 34159–2017)[1]. Sampling and preparation of samples for laboratory studies were carried out according to a unified methodology in accordance with the requirements of GOST R51447–99 (ISO 3100–1–91)[2]. Determination of organoleptic characteristics was carried out according to the requirements of GOST R53159–2008 (ISO 4120:2004)[3], GOST R53161–2008 (ISO 5495:2005)[4]. Mass fraction of fat was determined by extraction of total fat with a mixture of chloroform and ethyl alcohol in a filtering separating funnel; protein by mineralization of the sample by Kjeldahl and photometric measurement of color intensity of indophenol blue, which is proportional to the amount of ammonia in the mineralizer. Determination of microbiological indicators — the number of mesophilic aerobic, facultatively anaerobic microorganisms — in accordance with the requirements of GOST 54354–2011[5]; nutrient composition — using tabular data from the Guide to the chemical composition of Russian food products edited by I. M. Skurikhin; energy value — calculation method in accordance with the standards of SanERR2.3.2.1078.01[6].

[1] GOST 34159–2017 "Products from meat. General specifications". Moscow: Standartinform, 2017. — 12 p. (In Russian)

[2] GOST R51447–99 "Meat and meat products. Methods of primary sampling". Moscow: Standartinform, 2001. — 6 p. (In Russian)

[3] GOST R53159–2008 "Organoleptic analysis — Methodology — Triangle test". Moscow: Standartinform, 2010. — 16 p. (In Russian)

[4] GOST R53161–2008 "Organoleptic analysis — Methodology — Paired comparison test". Moscow: Standartinform, 2010. — 20 p. (In Russian)

[5] GOST 54354–2011 "Meat and meat products. General requirements and methods of microbiological testing". Moscow: Standartinform, 2013. — 38 p. (In Russian)

[6] Sanitary and epidemiological rules and regulations SanERR2.3.2. 1078–01 "Hygienic requirements for the safety and nutritional value of food products". Retrieved from https://docs.cntd.ru/document/901806306/titles/LSES2M Accessed August 15, 2021. (In Russian)

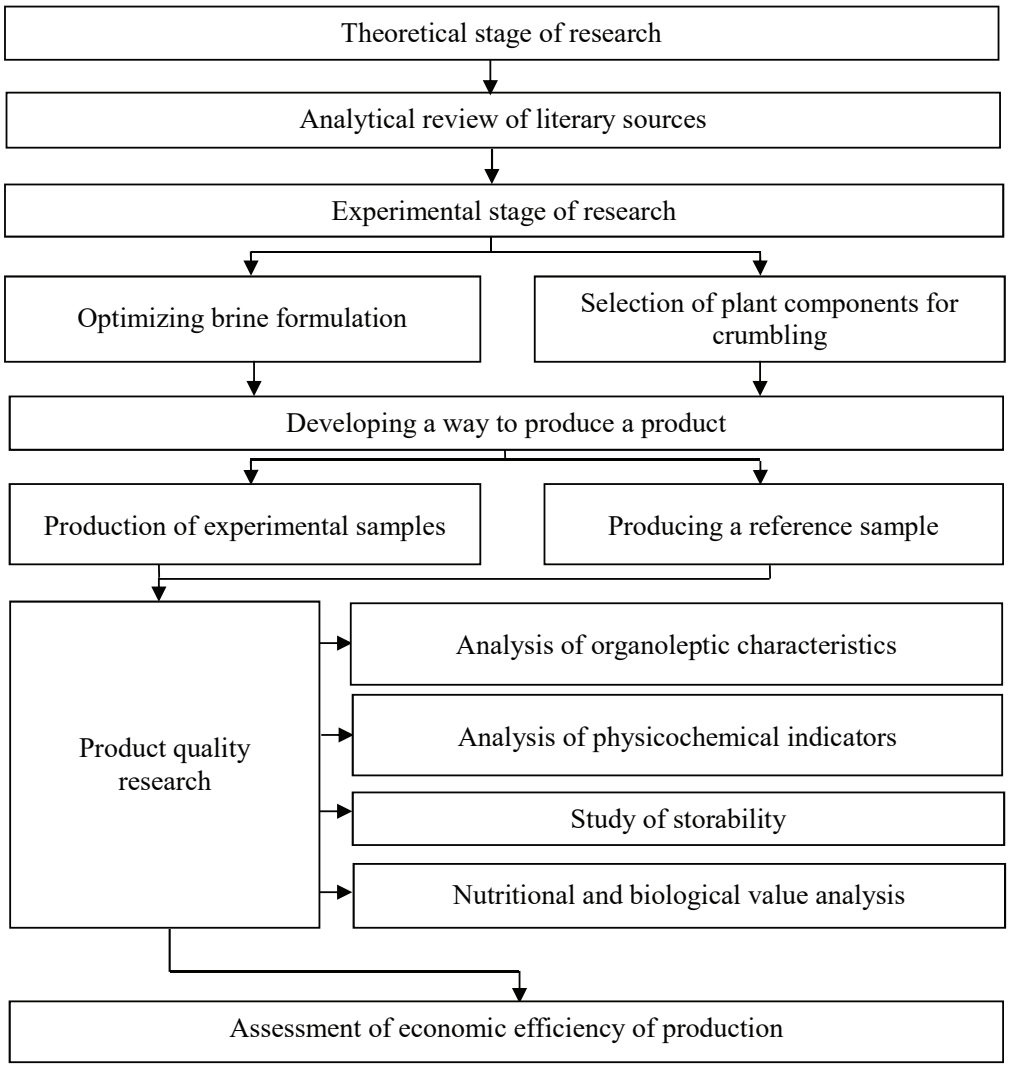

Figure 1. Scheme of work

Mineral composition of experimental samples was determined on spectrophotometer "Kvant-2AT" (KORTEK, Russia); amino acid composition — analyzer ARACUS Amino Acid Analyzer (MembraPure GmbH, Germany); vitamins — using device "Fluorat-02–5M" (Lumex, Russia).

The equipment for sample production is presented in Table 1.

Table 1. List of equipment used for sample production

№ pos.	Name of equipment	Type, brand	Manufacturer, country
1	Brine preparation unit	Techtron + CM 300	Techtron, Russia
2	Injector	Dorit PSM 10	Dorit, Germany
3	Vacuum Massager	IPCS –107–200 (H)	Russia
4	Industrial blender	Sirman ORIONE FIVE VV	Sirman, China
5	Universal heat chamber	Thermostar SLT 2000	Schaller, Germany
6	Packaging vacuum line	Multivak R-105	Multivak, Germany
7	Electronic table scales	CAS AD-10H	CAS, South Korea

The study materials were processed by variation statistics according to Plokhinsky N. A. using the "Microsoft Office" program package on a PC [14]. The difference in the indices was considered reliable at $P > 0.95$.

Results and discussion

Sample production

In the course of this research the technology of baked turkey product was developed. Whole-muscle roast meat product is a meat product made from different parts of the carcass (half carcass) in the form of cuts or individual muscles, pieces of meat subjected to salting in the manufacturing process, bringing it to readiness for consumption through heat treatment. The technology of the baked product includes the following stages: reception of raw materials, injection of brine, massaging, ageing, molding, heat treatment, cooling, quality control, packaging, labeling and sales.

As a result of the optimization of the formulation was developed a way of producing a balanced product, using brine for injection, the formulation of which is presented in Table 2. The recipe of the product is presented in Table 3.

Table 2. Recipe of brines

Brine components	Consumption, kg	
	for the control sample	for experimental samples
Water	94.25	94.2
Phosphate complex	1.0	1.0
Nitrite salt	2.0	2.0
Soy protein isolate	2.25	2.25
Granulated sugar	0.5	0.5
Nutritional supplement "Glimalask»	—	0.05
ИТОГО	100.0	100.0

In the brine used in the manufacture of test samples of the product, added a complex food additive "Glimalask". The food additive is a complex of organic acids: aminoacetic acid (glycine), ascorbic acid and malic acid. Recipe of the food additive contains per 100 kg of product: glycine — 80 kg, ascorbic acid — 12 kg, malic acid — 8 kg[7]. The expected effects of the food additive "Glimalask": firstly, reducing the pH of the brine, which will inhibit the growth of putrefactive microorganisms in the salting process. Secondly, obtaining a product enriched with the above organic acids, with improved organoleptic characteristics (soft consistency, taste, aroma). Thirdly, reducing the pH of the brine will reduce the active acidity of whole-muscle baked products, which will increase the shelf life of the finished product.

Table 3. Product Recipe

Ingredient name	Weight of the ingredient according to formulation 1, kg (control sample)	Weight of the ingredient according to the formulation 2, kg (sample № 1)	Weight of the ingredient according to the formulation 3, kg (sample № 2)	Weight of the ingredient according to the recipe 4, kg (sample № 3)	Ingredient's raw material affiliation
Turkey. Brisket, (boneless meat)	100.0	100.0	100.0	100.0	meat
Sprinkle with a mixture of black and white sesame	—	10.0	—	—	plant
Spicy sprinkles	—	—	8.0	—	plant
Chickpea shells	—	—	—	10.0	plant
Brine	10.0	10.0	10.0	10.0	—

For the production of baked turkey product the following method of salting was used: injecting meat raw material with brine in an amount of 10% of the weight of unsalted raw material, massaging in a massager with a drum rotation frequency of 18 r / min for 15 minutes. Salt has a positive effect on the yield of the finished product. As a result, the meat has an increased water-binding capacity, which markedly improves the consumer properties of the finished product.

[7] Gorlov I. F., Polyakov V. A. Food additive. Patent RF, no.2519777, 2014. (In Russian)

As an additional food ingredient, crumbles are used. It gives the product an attractive appearance, taste and aroma. To apply crumbles used breading drum. Its work consists of shaking pieces of meat in a mixture for crumbling. Sprinkles are applied in an amount of 7–10% to the mass of unsalted raw meat.

Thermal processing is baking. Baking the product at 100–120 °C for 1–2 hours. When the temperature in the center of the product reaches 70–72 °C, the product is considered ready. The next step is cooling, which is carried out in special chambers until the temperature reaches 8 °C in the thickness of the product.

Features of the used sprinkles

Sesame seeds

Particular attention should be paid to the coating. Sesame seeds are high in protein (Table 4). Easily digestible vegetable protein accounts for about 19%. Sesame is a source of essential and substitutable amino acids: 36% and 27% per 100 grams of protein, respectively. Thus the content of all the amino acids found in the seeds (in 100 grams) exceeds 10% of the daily requirement. In sufficient quantities in sesame are amino acids such as valine, tryptophan and isoleucine. Arginine and glycine are the most abundant among the substitutable amino acids [15].

Also in abundance, sesame includes dietary fiber, which, in turn, contributes to the removal of toxins from the body. Fiber can bind fat molecules and improve organ motility [16].

Sesame seeds are high in fatty acids, including polyunsaturated omega-6 fatty acids (namely linoleic acid): 100 g contains 214% of the daily requirement. Omega-6 and omega-3 in sesame have a ratio of 5.7:1, this value is the best for the human body [17,18]. At the same time, sesame seeds are also rich in monounsaturated omega-9 fatty acid, up to 19 grams in 100 grams. This fatty acid protects blood vessels from the formation of cholesterol plaques and is a prevention of atherosclerosis, which suggests the use of a sample with this coating as a gerodietic product [19].

Table 4. Nutritional value of sesame seeds

Indicator	Quantity, g/100 g	Percentage of recommended daily allowance*
Proteins	19.40 ± 0.08	21.09%
Fats	48.70 ± 0.15	72.69%
Carbohydrates	12.20 ± 0.10	8.70%
Dietary fiber	5.60 ± 0.12	28.0%

*** — the average vitamin and mineral levels for adults were used for the calculation.**

Sesame seeds influence hormone levels through their significant phytosterol content (campesterol, beta-sitosterol, and stigmasterol). Their content reaches 714 mg, which is 1298% of the recommended daily allowance. They regulate estrogen levels in their excess or deficiency [20].

Sesame has antioxidant properties. Having in its composition lecithin, sesamin and squalene, it protects tissues from

environmental influences. These substances also stimulate the growth of collagen and elastin fibers [20].

Sesame seeds have beneficial properties due to their high content of vitamins A, B, C and E. Niacin (PP) is involved in carbohydrate and lipid metabolism. Thiamine (B_1) and riboflavin (B_2) in sesame have beneficial effects on the nervous system [21]. These vitamins support nerve cell function. The vitamin content of sesame is shown in Table 5.

Table 5. Vitamin content of sesame per 100 grams

Indicator	Quantity, mg	Percent of the recommended daily rate*
Vitamin B_1	0.79 ± 0.09	46.9%
Vitamin B_2	0.79 ± 0.09	39.5%
Vitamin B_3	0.10 ± 0.006	24.3%
Vitamin B_6	4.52 ± 0.14	22.6%
Vitamin B_9	2.44 ± 0.12	16.3%
Vitamin E	0.25 ± 0.008	12.5%

*** — the average vitamin and mineral levels for adults were used for the calculation.**

100 grams of sesame seeds contain a significant amount of essential minerals (Table 6). It is in the composition of sesame that calcium is best absorbed [21,22]. All these micronutrients characterize the high benefits of white and black sesame for the body.

Table 6. Mineral content of sesame per 100 grams

Indicator	Quantity, μg	Percent of the recommended daily allowance*
Silicon	199 110 ± 1250	663.3%
Copper	4100 ± 150	410.6%
Nickel	190 ±35	126.7%
Manganese	2460 ±220	123.0%
Calcium	1070 000 ± 45000	113.6%

*** — the average vitamin and mineral levels for adults were used for the calculation.**

Spicy Sprinkles

The spice coating includes paprika. This spice is the dried and ground fruits of the mildly spicy capsicum annuum pepper. Paprika is valuable not only for its characteristic taste and aroma, but also for its unique composition of biologically active substances [23]. The nutritional value of paprika is presented in Table 7.

Table 7. Nutritional value of dried paprika per 100 grams

Indicator	Quantity, g	Percent of the recommended daily allowance*
Proteins	15.4 ± 0.12	18.19%
Fats	13.80 ± 0.20	16.74%
Carbohydrates	23.20 ±0.22	20.50%

*** — the average vitamin and mineral levels for adults were used for the calculation.**

The consumption of such a spice has a positive effect on the work of organs and tissues, this is achieved due to the content of many different biologically active substances [24]. It is important to note that paprika, which is produced from sweet varieties of pepper, when used in various dishes is safe for the body even in large quantities.

Paprika stimulates the immune system, is characterized by high content of carotene and vitamin C. Paprika has a favorable effect on the circulatory system, helps to prevent blood clotting and purifies it from excess cholesterol, is used to prevent thrombosis, which is an important element in the prevention of acute heart disease. Spice affects blood vessels and heart muscle and has a general strengthening effect [25].

Paprika normalizes metabolic processes in the body. Spice promotes not only faster but also more effective absorption of useful substances. Spice prevents gastrointestinal disorders.

This spice contains large amounts of lutein, which makes it especially beneficial for the eyes. Spice keeps the retina in a healthy state, has a beneficial effect in violation of the integrity and structure of the optic nerve. Paprika prevents damage to the cells of the eye, due to oxidative stress, reduces the accumulation of some pigments that provoke the development of maculodystrophy [25].

Chickpeas and chickpea extrudate

The application of chickpea extrudate wrappings leads to increased nutritional and biological value, increased shelf life, reduced caloric content, and reduced losses during thermal processing of finished products [26]. Chickpeas have about 28% (in terms of dry matter) of protein. The extrudate has a very high water-binding capacity (WBC), which in turn leads to an increase in fat-retention capacity (FTR) and antibacterial activity. The nutritional value of chickpeas is presented in Table 8.

Table 8. Nutritional value of chickpeas per 100 grams

Indicator	Quantity, g	Percent of the recommended daily allowance*
Protein	20.10 ± 0.80	26.4%
Fats	4.32 ± 0.16	7.7%
Carbohydrates	46.16 ± 0.58	21.1%
Dietary fiber	9.90 ±0.90	49.5%

*** — the average vitamin and mineral levels for adults were used for the calculation.**

Chickpeas contain nicotinic and ascorbic acids. It is also rich in Omega-3 and Omega-6 acids [27]. It is worth noting the high starch content — up to 43%. It contains about 60% of high quality carbohydrates, about 5% of minerals (Table 9) and various vitamins such as A, K, B_1, B_2, B_4, B_6, B_9, E [28].

Table 9. Mineral content of chickpeas

Indicator	Quantity, mg	Percent of the recommended daily allowance *
Potassium	968.0 ± 9.0	38.7%
Calcium	193.0 ± 7.0	19.3%
Magnesium	126.0 ± 5.0	31.5%
Phosphorus	444.0 ± 8.0	55.5%
Manganese	3.0 ± 0.2	107.0%
Silicon	92.0 ± 5.0	306.7%

* — the average vitamin and mineral levels for adults were used for the calculation.

Chickpea consumption helps to regulate blood sugar levels, which indicates the benefits for people suffering from diabetes. The regular use of the product improves the digestive processes and the state of the gastrointestinal tract. Chickpeas are digested long enough, while giving a large amount of energy to the human body.

To reduce the fat content of the crumbling mixture, as well as possible rancidity, you can use chickpea whole-grain flour as a raw material for extrusion. Due to the fact that the vegetable raw material is rich in carbohydrates and protein, a Maillard reaction is possible; extrusion reduces the lysine content of the product [29]. In turn, such legumes as chickpeas are rich in this amino acid. The use of chickpea extrudate from whole-grain flour as a breading mixture makes it possible to use it in meat products subject to heat treatment, in dietary nutrition, provides a reduction of losses during heat treatment, increases the shelf life, and increases the nutritional and biological values of the finished products [30].

Organoleptic evaluation of the product

As a result of research, the formulation of brine and product samples were optimized. Prototypes were developed with subsequent organoleptic evaluation (Table 10), which showed high consumer qualities. Tasting evaluation was carried out (Table 11). Profilogram of organoleptic indicators of experimental samples is shown in Figure 2.

In terms of consistency, color on the cut, as well as appearance, the samples of whole-muscle baked products do not differ from each other; the exception may be the use of different sprinkles. Tasting evaluation of the products

Table 10. Organoleptic indicators

Item name	Control sample	Baked turkey product with sesame sprinkles	Baked turkey product with spicy sprinkles	Baked turkey product with chickpea sprinkles
1. Shape, surface	Shape peculiar to part of the carcass or other configuration according to the casings, nets, films and molds used. Surface clean, without slips, broth and fatty swellings, without mucus, mold	Shape peculiar to part of the carcass or other configuration according to the casings, nets, films and molds used. Surface clean, without slips, broth and fatty swellings, without mucus, mold	Shape peculiar to part of the carcass or other configuration according to the casings, nets, films and molds used. Surface clean, without slips, broth and fatty swellings, without mucus, mold	Shape peculiar to part of the carcass or other configuration according to the casings, nets, films and molds used. Surface clean, without slips, broth and fatty swellings, without mucus, mold
2. Structure, consistency	Dense. The meat is tender, easy to cut	Dense. The meat is tender, easy to cut	Dense. The meat is tender, easy to cut	Dense. The meat is tender, easy to cut
3. Taste and smell	A pronounced taste and odor typical of this product, moderately salty	The distinct taste and smell of added food additives, sesame, moderately salty	The distinct taste and smell of added food additives, paprika, moderately salty	The distinct taste and smell of added food additives, chickpeas, moderately salty
4. Color on the cut	Uniformly colored muscle tissue of light pink color	Uniformly colored muscle tissue of light pink color	Uniformly colored muscle tissue of light pink color, without gray spots	Uniformly colored muscle tissue of light pink color, without gray spots

Table 11. Tasting evaluation of organoleptic characteristics

Indicator	Characteristic							
	Control sample	score	Baked turkey product with sesame sprinkles	score	Baked turkey product with spicy sprinkles	score	Baked turkey product with chickpea sprinkles	score
Consis-tency	Dense. The meat is tender, easy to cut	5	Dense. The meat is tender, easy to cut	5	Dense. The meat is tender, easy to cut	5	Dense. The meat is tender, easy to cut	5
Color on the cut	Uniformly colored muscle tissue of light pink color	4	Uniformly colored muscular tissue of light pink color. No gray spots	5	Uniformly colored muscle tissue of light pink color	4	Uniformly colored muscular tissue of light pink color. No gray spots	5
Taste	Moderately salty	2	Moderately salty, with a distinct sesame flavor	4	Moderately salty, spicy	5	Moderately salty, with a nutty flavor	5
Smell	Fresh meat product, with no extraneous odor	3	Fresh meat product, nutty, sweet smell	5	Fresh meat product, with a spicy smell	5	Fresh meat product, with a pronounced legume flavor	4
Appea-rance	Shape peculiar to the part of the carcass Surface clean, without slips, clots, broth and fatty swellings, without mucus, mold	3	Shaped peculiar part of the carcass with a uniform sprinkling of black and white sesame seeds. Without mucus and mold	5	Shaped peculiar part of the carcass with a coating of a mixture of spicy herbs, paprika. No mucus, no mold	5	Shaped like a part of the carcass with a sprinkling of chickpea extrudate. No mucus, no mold	5

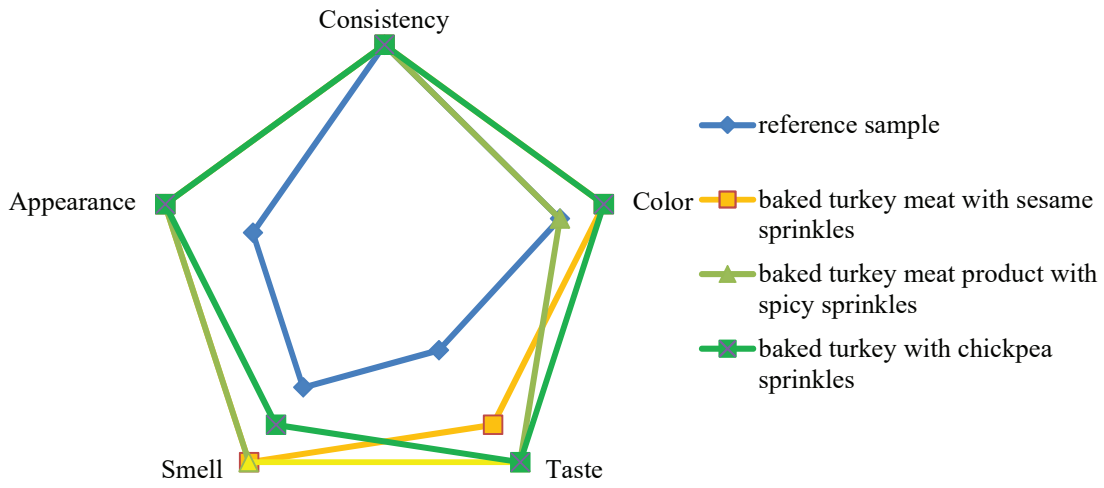

Figure 2. Profilogram of tasting evaluation of experimental samples

showed that the use of sprinkles has a positive effect on the perception of products: compared with the control, where there is no sprinkles, whole turkey meat baked products with sprinkles acquire a presentable appearance of the delicacy, the smell and taste become more expressive due to the added ingredients: sesame seeds, spicy herbs, paprika, chickpea extrudate (Figure 2).

Physical and chemical parameters of the finished product

In terms of physical and chemical parameters, the product must meet the requirements specified in Table 12.

As can be seen from the table, the content of sodium chloride, nitrite and phosphate does not change with the addition of vegetable dressing to the recipe of baked whole-muscle products. Sesame, chickpea and paprika do not contain these substances. Sesame and chickpea extrudate crumbles increased the protein content of the finished product, with the chickpea crumbled sample having the highest protein content. The chickpea extruded sample ranked first among the samples in terms of fat content. These variations are directly related to the type of crusting used: chickpea is rich in protein, while sesame is high in fatty acids.

The results of determining the moisture-binding capacity (MBC) of the finished products are shown in Figure 3. Baked whole-muscle products with extruded chickpea sprinkles had the highest MBC; the control sample had the lowest water-binding capacity. Consequently, the use of the sprinkles contributes to a noticeable increase in the water-binding capacity.

Table 12. Physico-chemical parameters of baked whole-muscle products

Indicator	Rate per position, %			
	Control sample	Baked turkey meat with sesame sprinkles	Baked turkey meat product with spicy sprinkles	Baked turkey with chickpea sprinkles
Mass fraction of protein	20.5 ± 0.3	22.0 ± 0.6*	20.2 ± 0.3	26.0 ± 0.4***
Mass fraction of fat	12.0 ± 0.5	13.0 ± 0.3	12.0 ± 0.5	12.2 ± 0.6
Mass fraction of table salt (sodium chloride)	3.0 ± 0.01	3.0 ± 0.01	3.0 ± 0.01	3.0 ± 0.01
Mass fraction of nitrite	0.004 ± 0.0001	0.004 ± 0.0001	0.004 ± 0.0001	0.004 ± 0.0001
Mass fraction of added phosphorus (in terms of P_2O_5)	0.4 ± 0.005	0.4 ± 0.005	0.4 ± 0.005	0.4 ± 0.005

* — P>0.95; ** — P>0.99; *** — P>0.999 — reliability of the difference compared to the control sample.

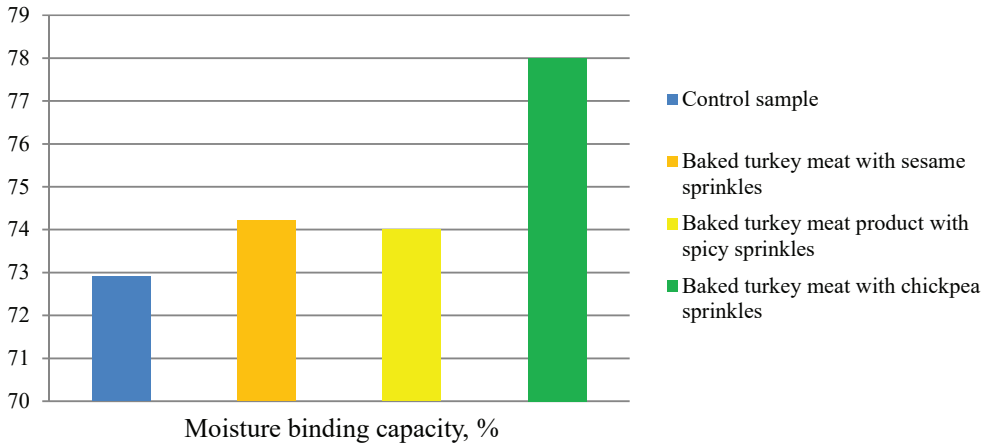

Figure 3. Results of determining the moisture-binding capacity of whole-muscle baked products

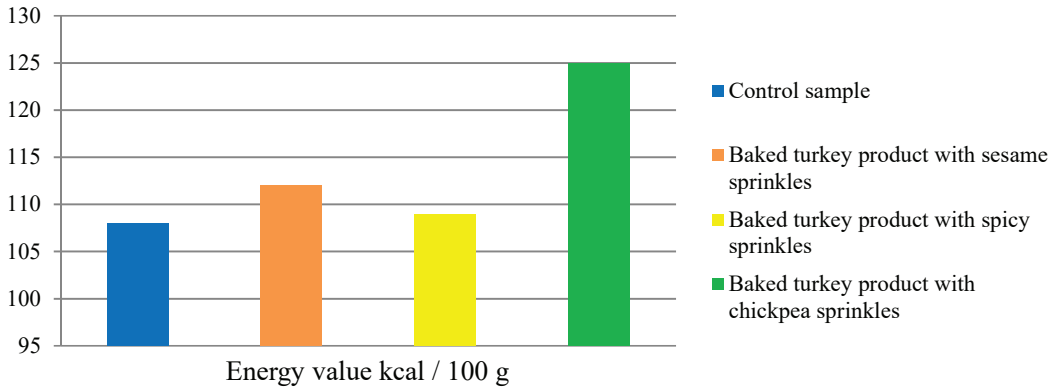

Figure 4. Energy value of whole-muscle baked products

Energy value

The results of calculating the energy value of the developed products are shown in Figure 4. As can be seen from the above data, the sample with a coating of extruded chickpeas has the highest caloric value. This is explained by the fact that chickpea extrudate contains more protein than raw meat. The increase in carbohydrate content also contributes to the increase in the energy value of the sample using chickpea sprinkles.

Product yield

During sample production, we evaluated the yield of the finished product after brine injection, crumbling, and heat treatment operations. The results are presented in Table 13. More clearly the growth of the yield of finished products depending on the used brine and crumbling is shown in Figure 5.

In the production of the assortment of baked whole-muscle turkey meat products the same parameters of salting of raw meat and heat treatment were used. The difference was in the composition of the brine and dressing used. The histogram shows that the control sample had the lowest yield, while the sample using brine with "Glimalask" food additive and extruded chickpea crumbles had the highest yield. The difference in yield between the product using the different types of sprinkles is minimal and is only 3%. Consequently, the use of a combination of the food additive "Glimalask" as part of the brine and plant crumbles contributes to an increase in the yield of the finished product.

Table 13. Effect of heat treatment on mass yield of samples

Sample	Weight of samples before treatment, kg	Weight of specimens with the sprinkling after brine injection, kg	Weight of samples after heat treatment, kg
Control sample	100.0 ± 0.01	109.4 ± 0.50	105.0 ± 2.50
Baked turkey meat with sesame sprinkles	100.0 ± 0.01	118.4 ± 0.70	114.0 ± 2.80***
Baked turkey meat product with spicy sprinkles	100.0 ± 0.01	116.8 ± 0.30	116.0 ± 1.90***
Baked turkey meat with chickpea sprinkles	100.0 ± 0.01	119.2 ± 0.90	117.0 ± 3.20***

*** — P>0.95; ** — P>0.99; *** — P>0.999 — reliability of the difference compared to the control sample.**

Product storage

At the final stage, the dynamics of microbial growth during storage of baked whole-muscle turkey meat products were studied by counting the quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMA&OAMO) [31].

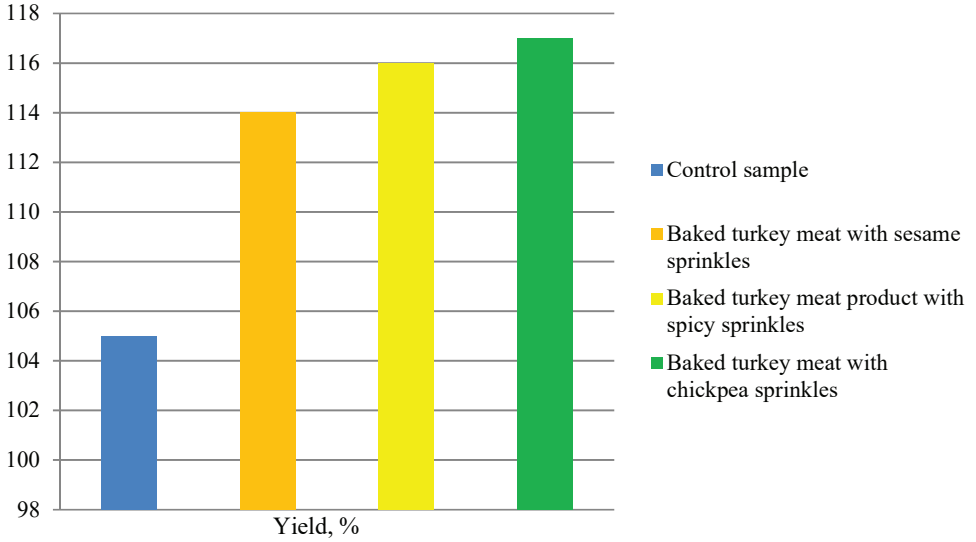

Figure 5. Yield of finished products

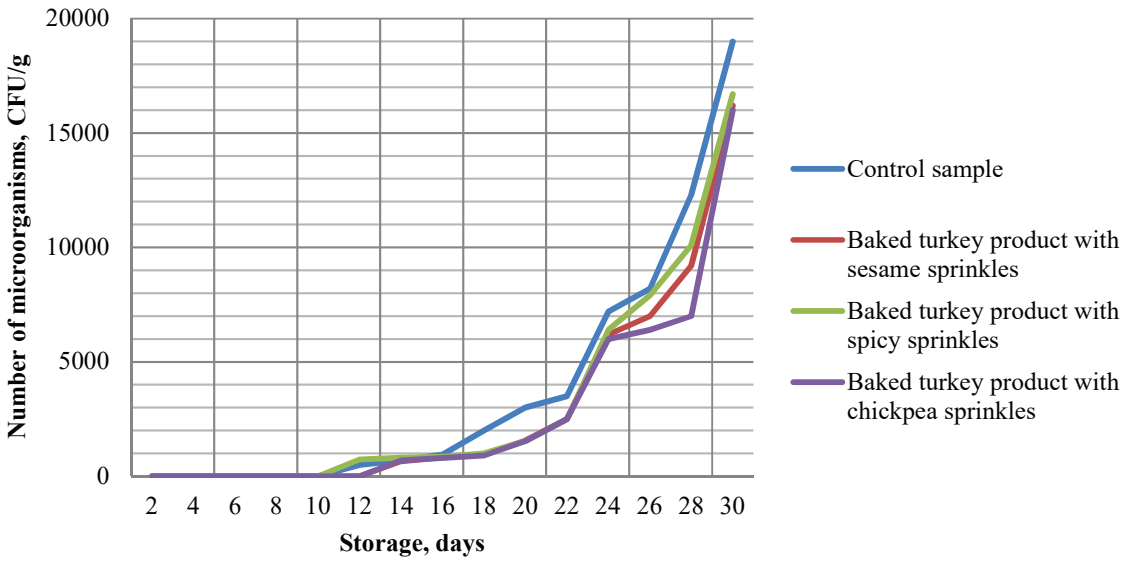

Figure 6. Dynamics of growth of QMA&OAMO indicator during storage

The growth of the total number of bacteria in the control and experimental samples had a similar character, which is clearly shown in Figure 6. However, the control sample showed accelerated growth of QMA&OAMO indicator, and at day 30 had the highest value.

Increasing the preservation period of the quality of experimental samples of the product due to the presence of crumbling and food additive "Glimalask" in the composition of the brine. As is known, during storage on the surface of the product is the development of bacteria, and as a consequence — spoilage of products and at its consumption in food — infectious diseases, poisoning, etc. [32]. The content of antioxidants in the casing can slow the rate of oxidative processes and, accordingly, reduce the amount of oxidation products. At the same time, due to the hygroscopic properties of the sprinkles, moisture does not accumulate on the surface of the product, which reduces the likelihood of active bacterial development.

Calculation of the cost of the product

The cost of production was calculated, as well as the recommended retail price (RRP) for the studied products (Table 14). The purpose of this stage was to determine how much more expensive the products would be if the brine of optimized composition and plant fillings were used in the production.

When calculating the cost of production, we relied on the results of the output of finished products. The recommended retail price was obtained taking into account the possible manufacturer's markup of 40%. Naturally, the control sample will have the lowest recommended price, since the production of the samples under study did not involve replacing meat raw materials as the most expensive ingredient with cheaper components. The increase in price is mainly due to the use of the complex food additive "Glimalask" in the brine. The most expensive product is whole-muscle turkey product with sesame sprinkles.

Table 14. Calculation of the cost and recommended price per 1 kg of finished product

Name of raw material	Price per kg, rubles	Quantity, kg			
		Control sample	Baked turkey product with sesame sprinkles	Baked turkey product with spicy sprinkles	Baked turkey product with chickpea sprinkles
Main raw materials					
Turkey (brisket)	316	1.0	1.0	1.0	1.0
Spices and materials					
Phosphate complex	110	0.01	0.01	0.01	0.01
Nitrite salt (0,6% $NaNO_2$)	120	0.02	0.02	0.02	0.02
Soy protein isolate	200	0.0225	0.0225	0.0225	0.0225
Sugar sand	49	0.005	0.005	0.005	0.005
Nutritional supplement «Glimalask»	1000	—	0.05	0.05	0.05
Water	15	0.9425	0.942	0.942	0.942
Black and white sesame mixture	198	—	0,1	—	—
Spice sprinkle mix	210	—	—	0,08	—
Chickpeas extruded	64	—	—	—	0,1
Raw material costs, rubles		338.4	408.7	405.2	394.8
The cost of the finished product, rubles / kg		322.3	358.1	349.3	337.5
RRP, rubles/kg		451.2	501.4	489.1	472.5

The cheaper product is the extruded chickpea crumble. However, it is still recommended to keep all three positions of baked turkey meat products with sprinkles, since the main objective of our study was to expand the range of enriched food products using a combination of meat and vegetable raw materials. In addition, the recommended prices for these products are much lower than the market average, the value of which is 710.0 rubles / kg.

Conclusion

In the course of the study an optimized composition of brine for meat raw material injection was developed. The use of brine for injecting the product containing the complex food additive "Glimalask" as well as flakes of vegetable raw materials in the technology of whole muscle baked products increases the functional and technological properties, product yield, the content in the product of nutrients necessary for the body, improves organoleptic indicators and moisture-binding capacity.

During the study of organoleptic characteristics of the experimental samples it was found that the best results have the samples with the use of sprinkles from a mixture of sesame and chickpea. The use of sprinkles in the recipe contributed to improving the appearance, as well as the taste and aromatic properties of the product. The use of brine of optimized composition allowed to improve the consistency of the product, color on the cut.

The produced samples according to physical and chemical indices correspond to the norms established by scientific and technical documentation. The sample with chickpea sprinkles has the highest energy value, its value is 125 kcal/100 g of product, this is a consequence of increased content of proteins, fats and carbohydrates in the finished product. The increased protein content in the experimental samples with sprinkles is achieved through the rational use of vegetable raw materials with high protein content. The presence of crumbles allows us to enrich the products with vitamins such as A, K, B group, fatty acids and dietary fiber.

Samples with sesame and spice crumbles do not have great differences in terms of energy value, and are 111.7 and 108.9 kcal / 100 g of product, respectively. The energy value of the control sample — 107.7 kcal / 100 g. The control sample does not contain carbohydrates in its composition. Compared with the control sample, the yield of the products increased by 9.0–12.0%, the maximum increase was noticed when using chickpea extrudate filling.

The best storability is in the samples with chickpea and sesame extrudates, the products with spice extrudates are slightly inferior to their QMA&OAMO indices. The decrease in the rate of oxidative processes due to the content of antioxidants in the sprinkles, as well as its hygroscopic properties, can increase the preservation period of the quality of the finished product.

The average market price of turkey products is 710 rubles per kg. The production of this product is economically profitable, as manufactured products have a lower recommended retail price. The RRP of three experimental samples is — 501.4, 489.1 and 472.5 rubles per kg, respectively.

The produced samples of the product are recommended to a wide range of consumers of different age groups, which represents an important part in the implementation of the product. Thus, the development of technology of baked turkey product with the use of multicomponent brine of optimized composition and vegetable dressing contributes to the expansion of the range of enriched meat products, reducing the cost, has practical and social significance.

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All authors made an equal contribution to the work.

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APPLICATION OF CONCAVE INDUCTION COOKING TO IMPROVE THE TEXTURE AND FLAVOR OF BRAISED PORK

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Abstract

Long-term cooking may reduce the eating and nutritional quality attributes of meat products due to excessive oxidation. This study aimed to investigate the feasibility of concave induction to improve the quality of braised pork belly. Pork belly cubes were subjected to concave induction cooking (2000 W) or plane induction cooking (2000 W, traditional) for 60 min, 90 min, 120 min or 150 min. Then texture, fatty acid profile, lipid and protein oxidation, volatile flavor and sensory test in braised meat were evaluated. Compared with traditional method, concave induction cooking showed higher heating performance with shorter time to achieve a setting temperature. Compared with traditional cooking for 150 min, concave induction cooking for 60 min did not only produce a comparable volatile flavor and sensory scores, but also give better quality attributes, including lower hardness, chewiness, thrombogenicity values, PUFA/SFA value, lipid and protein oxidation. E-nose results showed that samples cooked by concave induction for 60 min and 90 min showed a great similarity to those cooked by plane induction for 150 min. Concave induction cooking for 60 min also showed advantages to retain higher abundances of other volatile compounds including 2-pentylfuran, (E, E)-3,5-octadien-2-one, 2, 3-octanedione, 2-decahydro-1,6- dimethylnaphthalene when compared with plane induction cooking for 150 min.

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Introduction

Induction heating is a technology that generates an electric current in the heated material using electromagnetic induction [1]. It has been widely applied to kitchens and food industry because of good uniformity, high efficiency and high safety [2,3] observed that the protein and fat contents were higher in roast beef, baked beans and steamed salmon by induction heating than by traditional cooking. Induction heating also showed higher efficiency in extracting pectin from plants than traditional methods [4], and sterilization for ketchup [5].

Induction heating equipment used for cooking mainly includes plane induction cooker [6] and concave induction cooker [7]. The plane induction cooker is suitable for family and small-scale food preparation, while the concave induction cooker is more suitable for factory processing and has a promising industrial application. It has been shown that the concave induction cooker has higher heating efficiency and uniformity compared with plane induction cooker [8]. However, it is still little studied how concave induction cooking affect food quality and flavor.

Braised pork is a typical meat product that is cooked at a high temperature (usually 100 °C) for at least 150 min. During such a long-time cooking, heavy lipid and protein oxidation may occur, which further has a great impact on meat texture, flavor and decreased protein digestion [9,10,11]. Li et al. [12] used a plane induction cooker to optimize the process and found that the texture and sensory scores of braised pork were the highest after pre-frying combined with stewing for 150 min. However, there are some difficulties in applying such a plane induction cooker to a large-scale processing practice of braised pork in meat industry because of low-energy efficiency. The concave induction cooker could be a good alternative. Given concave induction cooker has higher heating efficiency compared to plane induction cooker, it may reduce the protein and lipid oxidation in meat and improve meat quality by shortening the cooking time.

The purposes of this study were to evaluate the feasibility of concave induction cooking to improve the heating efficiency and meat quality attributes of braised pork compared with the plane induction cooking.

Objects and methods

Sample preparation

Pork belly samples (the upper part of the abdomen of pigs of 110 kg slaughter weight at 6 months old) containing 2.29% of lipid (determined by the Soxhlet method) and 72.72% of moisture (determined by the direct drying method) were obtained at 24 h postmortem from a commercial company (Sushi, Jiangsu, China). Pork belly samples were collected from eight Duroc × Landrace × Yorkshire crossbred pigs that had similar feeding conditions and body weights. The belly was cut into 4 cm strips. The strips were bleached in boiling water for 5 min to remove blood residues and make the meat samples easy to cut. Then the strips were cut into smaller cubes (3×3×5 cm).

Cooking

The cubes (about 1.5 kg, 28–32 pieces) were stir fried for 20 min with soybean oil (20 g per kg meat) in a plane induction cooker (Jiuyang, Shandong, China) or a concave induction cooker (Kerun, Shandong, China) at a power of 1.4 kW. Then the oil was removed, and water, wine (40 g per kg meat), vinegar (4 g per kg meat), soy sauce (20 g per kg meat), sugar (40 g per kg meat) and salt (5 g per kg meat) were added and cooked at 2 kW for 60 min, 90 min, 120 min or 150 min, respectively. The ratio of water to meat depended on the cooking time, including 0.7, 1.2, 1.4 and 1.9 for plane induction cooker at 60 min, 90 min, 120 min or 150 min, respectively, and 1.5, 1.9, 2.7 and 3.65 for concave induction cooker at 60 min, 90 min, 120 min or 150 min, respectively. The water was added for two times to the concave induction cooker for each group but once to the plane induction cooker. For the former, the amount of added water at the first time was same as that of the plane induction cooker and the remainder water was added when the water in the cooker was completely evaporated. There were 8 groups and each group had 8 repeats. Induction cookers were shown in Figure 1. The ingredients and fatty acid composition of soybean oil were shown in Tables 1and 2.

Table 1. Information of ingredients

Ingredients	Brand	Raw material
Soybean oil	Jinlongyu	soybean oil (49%), canola oil (21%), sunflower seed oil (14%), corn oil (9%), peanut oil (3%), rice oil (3%), sesame oil (0.6%), sesame oil (0.4%)
Wine	Shuita	drinking water, yellow rice wine, white wine, monosodium glutamate, edible salt, onion juice, ginger juice, caramel color
Vinegar	Shuita	drinking water, sorghum, bran, barley, peas, edible salt, spices, caramel color, sodium benzoate
Soy sauce	Haitian	water, soy beans, edible salt, caramel color, wheat, granulated sugar, sodium glutamate, mushroom
Sugar	Suguo	white crystal sugar
Salt	Huaiyan	refined salt, potassium iodate, ammonium ferric citrate

Table 2. Fatty acid composition (mg/g oil) of soy bean oil

Fatty acid	Content
C16:0	9.51±6.84
C18:0	3.41±2.45
SFA	12.93±9.28
C18:1n9c	33.51±24.12
MUFA	33.51±24.12
C18:2n6c	99.4±72.6
C18:3n3	5.99±4.34
PUFA	105.39±76.93

Cooking performance evaluation

The cubes (about 1.5 kg) were cooked in water (2.7 kg) in a plane induction cooker (2 kW) or a concave induction cooker (2 kW) until all water was lost. The center temperature of meat cubes was tracked by a thermal probe (Yuwese, Shenzhen, China) and time to reach the cooking endpoint (when water was lost) was recorded. The cooking procedures were repeated for eight times (n=8 each).

A B

Figure 1. Pictures of plane and concave induction cooker used: A — plane induction cooking; B — concave induction cooking

Texture profile analysis

The samples were cut into 1 cm × 1 cm × 1 cm cubes. Texture of lean meat (muscle tissue) portions of braised pork cubes was determined by a TA.XT plus texture analyzer (XT Plus, Stable Micro systems Ltd, Godalming, UK) as previously described by Li et al. [12]. The parameters were set as follows: probe, 50 mm stainless cylinder; pre-test speed, 2 mm/s; test speed, 1 mm/s; a compression rate, 50%; post-test speed, 5 mm/s; trigger force, 5 g; testing interval time, 5 s. Hardness, springiness, cohesiveness and chewiness were recorded. The results were analyzed with the Texture Expert Exceed software (Stable Micro Systems Ltd). Eight replications were prepared for each treatment.

Fatty acid profiling

Lipid was extracted from the lean parts of braised pork as previously described by Li et al. [12]. Briefly, the lean (6 g) was mixed with chloroform/methanol 2:1 (v/v) solution (40 mL). Then the solution was filtered. The filtrate was mixed with 0.9% NaCl (8 mL) and centrifuged at 3000 rpm for 15 min. The organic phase (the lower part) was dried in a rotary evaporator at 44 °C water bath and the remainder was lipid. The lipid was saponified in a sodium hydroxide methanol solution and methylated in a 14% boron trifluoride methanol solution as described by Chen et al. [13]. The mixtures were analyzed by gas chromatography (GC2010 plus, Shimadzu, Kyoto, Japan). The volatile compounds were separated in a SP2560 column (100 mm × 0.25 mm × 0.25 mm, Supelco, Bellefonte, PA). The chromatography conditions were set as follows: injection volume, 1 µL; inlet temperature, 270 °C; FID temperature, 280 °C. A temperature program was set as follows: 100 °C for 13 min → an increase to 180 °C with a rate of 10 °C/min → 100 °C for 6 min → an increase to 200 °C at a rate of 1 °C/min → 200 °C for 20 min → an increase to 230 °C at a rate of 4 °C/min → 230 °C for 10.5 min. The carrier gas was highly pure N_2 with a flow rate of 1 mL/min, and the split ratio was 100:1. A mixed standard containing 37 fatty acids (CEM 47885, Supelco, Bellefonte, PA) was applied as external standard. Fatty acids in samples were quantified by an internal standard (methyl nonadenoate, C19:0). Atherogenicity index (AI) and thrombogenicity index (TI) were calculated according to the previous study [14]:

$$AI = \frac{C12:0 + C14:0 + C16:0}{n-3PUFA + n-6PUFA + MUFA} \quad (1)$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5 \times MUFA + 0.5 \times n-6PUFA + 3 \times n-3PUFA + n-3PUFA/n-6PUFA} \quad (2)$$

Lipid oxidation

Lipid oxidation was determined according to the method of Soladoye et al. [15] with minor modifications. Briefly, meat samples (5 g) were homogenized for 30 s in trichloroacetic acid (TCA, 7.5%, 25 mL). The homogenate was centrifuged at 12000 g for 5 min to remove the protein and other materials in meat. Two milliliters of the supernatant were taken and mixed with 2 mL of thiobarbituric acid (0.02 M). The mixture was well vortexed and heated at 95 °C (TW 20, Julabo Labortechnik GmbH, Germany) for 30 min. The absorbance was measured at 532 nm and the concentration of thiobarbituric acid reactive substances (TBARS) was calculated from a standard curve (1,1,3,3-tetra ethoxypropane, 0–1.5 µg/mL, $R^2 > 0.999$). The results were expressed in mg malonaldehyde (MDA) per kg of meat samples.

Protein carbonyl groups

Meat samples (1 g) were homogenized for 60 s in 5 mL of buffer solution [10 mM K_2HPO_4, 0.1 M NaCl, 2 mM $MgCl_2$, 1 mM ethylene bis (oxyethylenenitrilo) tetraacetic acid (EGTA)] at pH 7.0 and then centrifuged at 10000 g for 20 min. The supernatants were removed, and pellets were collected. After two repeated cycles of homogenization and centrifugation, the resulting pellets were suspended in 5 mL of 0.1 M NaCl. The suspended samples were centrifuged again at 10000 g for 20 min. The pellets were re-suspended in 5 mL of 0.6 M NaCl and filtered through four layers of gauze. The filtrate was collected as protein solution.

The protein concentration was determined using a bicinchoninic acid protein assay kit (Thermo Scientific, Waltham, MA) with bovine serum albumin as the standard. The carbonyl content was determined according to Oliver *et al.* [16] with minor modifications. Briefly, 1 mL protein solution was mixed with 2 M HCl (control) or 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl and incubated in dark at room temperature for 1 h. Then 1 mL of 20% TCA solution was added and the mixture was centrifuged at 8000 g for 10 min. The pellets were washed three times with 1 mL ethanol-ethyl acetate (1:1, v/v) and then suspended in 3 mL of 6 M guanidine HCl at 37 °C for 30 min. The suspension was centrifuged at 8000 g for 5 min. The carbonyl concentration was calculated using the absorption of 21000 M^{-1} cm^{-1} at 370 nm. The absorbance is determined using a spectrophotometer (Molecular Devices, California, USA).

Protein thiol group

Protein thiol content was determined according to Lund et al. [17]. Briefly, meat samples (1 g) were homogenized for 30 s in 25 mL of 5% sodium dodecylsulphate (SDS) in 0.10 M Tris buffer (pH 8.0) and then heated in a 90 °C water bath for 30 min. Then the solution was cooled and centrifuged at 1200 g for 20 min. The supernatants were filtered and protein concentration in the filtrate was determined using a BCA protein assay kit (Thermo Scientific, Waltham, MA). The filtrate (0.5 mL) was mixed with 2 mL 0.10 M Tris buffer (pH 8.0) and 0.5 mL 10 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) in 0.10 M Tris buffer (pH 8.0). Absorbance at 412 nm was measured after reacting for 30 min against a reference solution of 0.50 mL 5% SDS and 2.50 mL 0.10 M Tris buffer (pH 8.0). The thi-

ol concentration was calculated using the absorption of 13600 M^{-1} cm^{-1}.

E-nose measurement

Meat samples (1 g) were transferred to 20 mL headspace bottles and immediately sealed. Samples were preheated in a 70 °C water bath (TW 20, Julabo Labortechnik GmbH, Germany) for 10 min. The headspace bottle was inserted by a syringe needle with a hollow tube and headspace gas was sucked out. The headspace gas entered into the E-nose through a water filtration membrane, then the same needle was inserted into the same headspace bottle and the air was sucked to replenish volatile gas. The data collection time was 120 s, and the clean time was 100 s [18]. The performance of the PEN3 portable E-nose sensors (Win Muster Airsense Analytics Inc, Schwerin, Germany) was shown in Table 3.

Table 3. Performance description of PEN3 portable electronic nose sensors

Sensor name	Performance description
W1C	Aromatic compounds
W5S	Broad range
W3C	Ammonia, aromatic compounds
W6S	hydrocarbons
W5C	Alkanes and aromatics
W1S	Methane, broad range of compounds
W1W	Sulfur compounds, pyrazines and terpenes
W2S	Broad range, alcohols, aromatic compounds
W2W	Aromatics and organic sulfur compounds
W3S	Methane and aliphatic compounds

GC–MS

Volatile compounds were identified by a Thermo GC–MS system comprising of a TRACE GC ULTRA gas chromatograph and a DSQ II mass selective detector (Thermo Scientific, Waltham, MA). Briefly, lean samples (5 g) were taken and transferred into a 20 mL headspace bottle and then immediately sealed. An aged 50/30 μm CAR/PDMS/DVB solid-phase microextraction fiber (Supelco, Bellefonte, PA) was inserted into the 20 mL headspace bottle. The volatile compounds in the headspace bottle were collected at 60 °C for 30 min with the fiber, and the fiber was injected into the GC inlet. The fiber was desorbed at 250 °C for 3 min.

Gas chromatography was performed with an inlet temperature of 250 °C and a DB-WAX capillary fiber (30 m × 0.25 mm × 0.25 μm, Agilent, Santa Clara, CA) was used for separation. The carrier gas was helium, and the flow rate was set at 0.8 mL/min. The gas chromatographic temperature conditions were programmed as follows: the furnace temperature was maintained at 40 °C for 3 min, then increased to 90 °C at a rate of 5 °C/min, further to 230 °C at a rate of 10 °C/min and kept at 230 °C for 7 min.

Mass spectrometry was done under the conditions of EI source as ion source with ionization mode of EI + and electron energy of 70 eV. The ion source temperature was 200 °C, and interface temperature was 250 °C.

The retention time (RI) of the volatile compounds was converted to a linear retention index by n-Alkanes (C7-C26). The retention indices were compared to those in the NIST database (https://webbook.nist.gov/chemistry/name-ser/) and the matching factor was over 800. The retention index is calculated as described by Xu et al. [19] as follows:

$$RI = \left[\frac{Rt(x) - Rt(n)}{Rt(n+1) - Rt(n)} + n\right] \times 100 \quad (3)$$

Where $Rt(x)$, $Rt(n)$ and $Rt(n+1)$ are the retention times of the volatile compounds to be tested, and the normal paraffin containing n carbon atoms and the n-alkane of $n+1$ carbon atoms, respectively. Cyclohexanone was used as the internal standard to conduct semi-quantitative analysis by comparing the peak area of volatile compounds with the peak area of the internal standard.

Sensory evaluation

Sensory evaluation was performed according to the method described by Wang et al. [20] with some modifications. A professional panel of 12 (6 males and 6 females) members assessed the samples. Samples were evaluated for odor, color, texture and taste using the 9-point hedonic scale (1 = very unpleasant and 9 = very pleasant).

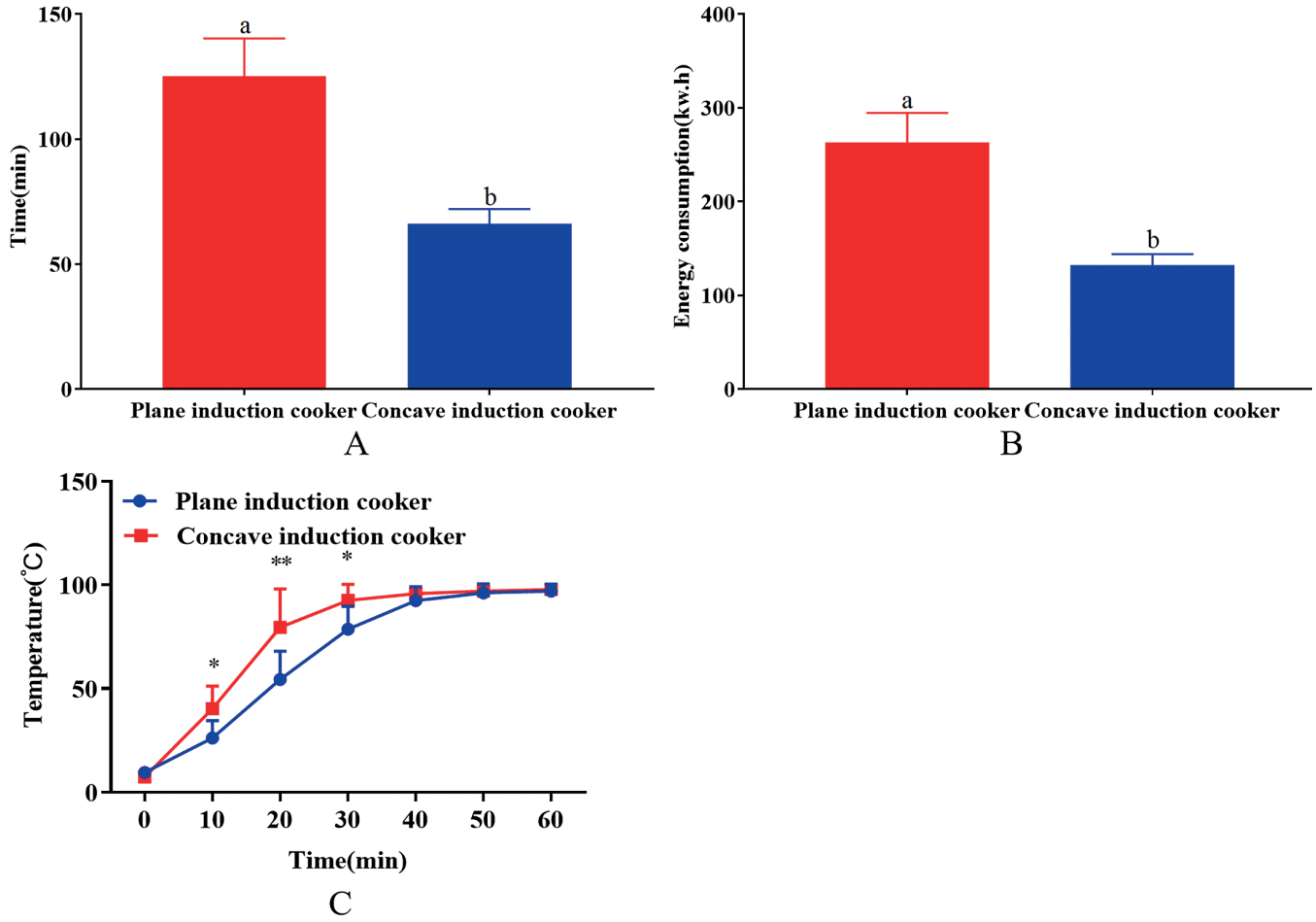
Statistical analysis

Cooking performance was evaluated by t-test in which cooking method was set as the independent. Eight repeats were performed. For texture attributes, fatty acids, lipid and protein oxidation, volatile compounds, and sensory test, factorial analysis of variance (ANOVA) with a mixed model was applied, in which cooking method, cooking time and their crosses were set as fixed effects, and sampling batch was set as a random effect. Least-squares means were compared by the Tukey's t test. The above statistical analyses were done by the SAS software (SAS Institute Inc, Cary, CA). E-nose data were analyzed by principal component analysis to discriminate the measured samples using the Winmuster software (Win Muster Airsense Analytics Inc, Schwerin, Germany). The significance level was set at 0.05.

Results and discussion

Performance evaluation and texture profile analysis

Compared with plane induction cooking, concave induction cooking had higher energy efficiency with shorter cooking time and lower energy consumption to achieve the same setting endpoint where the added water was completely evaporated (125 min vs. 66 min for cooking time; 4.38 kw·h vs. 2.2 kw·h for energy consumption, $P < 0.05$, Figure 2, A and B). The center temperatures of the meat samples near the center of the cookers were higher at 10 min, 20 min and 30 min in the concave induction cooker than those in the plane induction cooker ($P < 0.05$, Figure 2, C). This could be due to the fact that



a, b means differed significantly among cooking time points ($P < 0.05$). *, significant differences existed between concave induction cooking and plane induction cooking at a certain time point ($P < 0.05$).

Figure 2. Performance of two cooking methods: A — time to reach the end point that added water was lost; B — energy consumption to reach the end point; C — the center temperature of meat

concave induction cooker has a variable turn pitch coil (concave coil) making it have larger heating area and better heating performance [21]. Therefore, it is feasible to improve the texture and flavor of braised pork by concave induction cooking for shorter time than by plane induction cooking.

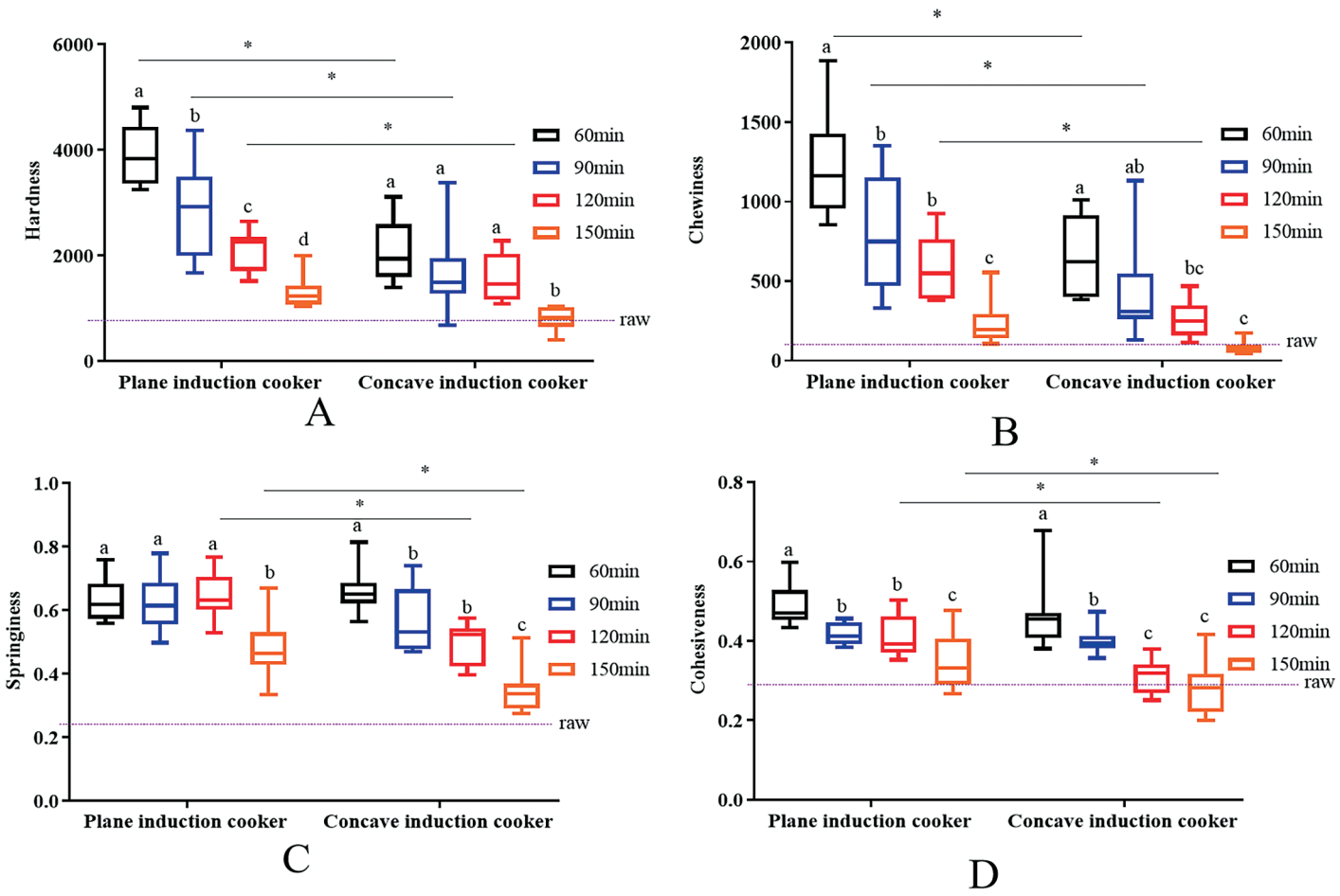
Texture is an important aspect for the sensory acceptance of meat. The main factors affecting meat texture are cooking temperature and time. Jiang et al. [22] observed that hardness and chewiness of bighead carp (*aristichthys nobilis*) muscle showed two peaks during heating, but springiness, adhesiveness, and cohesiveness declined.

As cooking time increased, the hardness, chewiness, springiness and cohesiveness of braised pork decreased greatly ($P < 0.05$, Figure 3), which gave stronger responses to concave induction cooking than to plane induction cooking. Generally, concave induction cooking resulted in much lower hardness and chewiness, at 60 min, 90 min and 120 min, and lower springiness and cohesiveness at 120 min and 150 min, compared with plane induction cooking ($P < 0.05$). In a previous study, we observed that braised pork cooked in a plane induction cooker for 150 min had the best texture [12]. Consumers usually prefer tender braised pork. In the present study, hardness of braised pork cooked for 60 min in the concave induction cooker reached the values of those cooked for 120 min in the plane induction cooker, but the latter had higher chewiness, springiness and cohesiveness ($P < 0.05$). This indicates that concave induction cooking may improve the texture of braised pork with shorter cooking time. Concave induction cooking for 60 min may be considered as a better cooking method for braised pork among the applied cooking parameters.

The decrease in hardness is caused by the fracture of myofibrillar structure. The lower hardness of the braised pork prepared by concave induction cooking could be because the temperature of concave induction cooker at early cooking time is significantly higher than that of the plane induction cooker, resulting in greater damage to myofibrillar structure. Similar results showed that the hardness, springiness and chewiness of *Volutharpaampullacealperryi* (commonly known as fake abalone) would be more obviously reduced by high temperature [23].

Fatty acid profile

Pork lean fractions contain different types of fatty acids. In raw and cooked lean samples of braised pork, the fatty acids predominantly comprise of myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid (Table 4). Cooking method showed a certain



a, b, c means differed significantly among cooking time points ($P < 0.05$). *, significant differences existed between concave induction cooking and plane induction cooking at a certain time point ($P < 0.05$).

Figure 3. Texture profile of braised pork: A — Hardness; B — Springiness; C — Chewiness; D — Cohesiveness

impact on medium chain fatty acids (MCT), saturated fatty acids (SFA), unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and TI value ($P < 0.05$, Figure 4) but did not affect the PUFA/SFA ratio and AI value ($P > 0.05$). At 150 min of cooking, concave induction cooking increased the contents of MCT, SFA, UFA, MUFA and PUFA ($P < 0.05$). Cooking time showed a greater effect on the above variables. MCT content and PUFA/SFA ratio increased with cooking time in the plane induction cooker ($P < 0.05$). In contrast, the AI values decreased with cooking time. However, in concave induction cooker, the contents of MCT, SFA, UFA, MUFA, PUFA and TI value increased with cooking time ($P < 0.05$). In addition, significant differences were observed between the two cooking methods in MCT, SFA, UFA, MUFA and PUFA contents at 150 min of cooking time ($P < 0.05$, Figure 4, A-E). MCT are healthy fatty acids, which may inhibit fat deposition by enhancing the thermogenesis and oxidation of human body. In addition, MCT have a certain therapeutic effect on type 2 diabetes [24]. MUFA could reduce the risk of cardiovascular disease and inflammation-related diseases [25]. The increase in saturated fatty acids, including MCT, may be due to the oxidation of some unsaturated fatty acids to saturated fatty acids after prolonged cooking. Several studies suggest that PUFA are structural lipids that are released during cooking [26,27].

Significant differences were observed between the two cooking methods in UFA at 150 min of cooking time ($P < 0.05$, Figure 4, C), but no significant difference existed at other time points ($P > 0.05$). The greatest changes in SFA content occurred for C14:0 and C16:0. Significant differences were observed between the two cooking methods in C14:0 and C16:0 contents at 150 min of cooking time ($P < 0.05$, Table 4). Higher C14:0 and C16:0 may cause higher concentrations of total and LDL cholesterol in plasma [28,29].

The value of PUFA/SFA of concave induction cooking at 60 min was greater than 0.40 that is recommended to prevent cholesterol elevation and reduce the risk of coronary heart disease [30]. The TI value of concave induction cooked samples for 60 min was lower than that of plane induction cooked samples at 60 min ($P < 0.05$, Figure 4, F-H). AI is a good indicator for assessing the risk of atherosclerosis, while TI is an indicator for assessing the possibility of platelet aggregation [31]. In this case, concave induction cooking for 60 min may produce healthier braised pork compared with plane induction cooking. Several fatty acids could not be detected in a part of samples, which may be due to their low abundance in pork [32]. In addition, the **n-6/n-3** ratio was higher than the values of 1 to 4 as recommended by Simopoulos et al. [33]. This is because the abundance of n-3 fatty acids is low in pork [34,35].

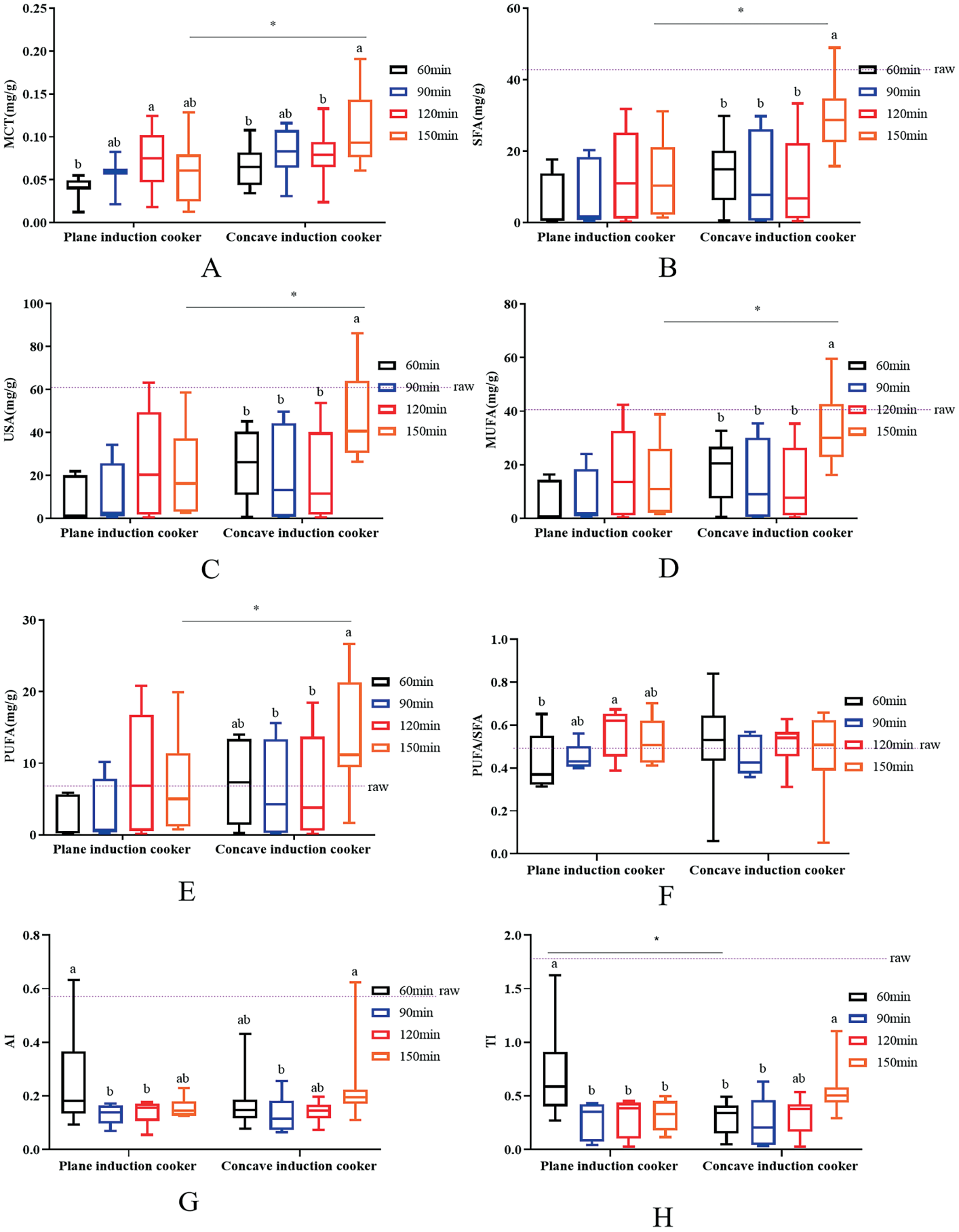
Table 4. Fatty acid composition (mg/g meat) of braised pork

Fatty acids	Raw	Plane induction cooking				Concave induction cooking				P values		
		60 min	90 min	120 min	150 min	60 min	90 min	120 min	150 min	method	time	method *time
C4:0	n.d.	n.d.	0.03 ± 0.01*	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01^{ab}	0.05 ± 0.01^{a**}	0.03 ± 0.01^{b}	0.02 ± 0.01^{c}	0.10	0.01	< 0.01
C10:0	n.d.	0.02 ± 0.01^{b}	0.03 ± 0.01^{ab}	0.04 ± 0.02^{a}	0.04 ± 0.02^{ab*}	0.04 ± 0.01^{b}	0.05 ± 0.02^{ab}	0.05 ± 0.02^{ab}	0.06 ± 0.03^{a**}	< 0.01	0.02	0.28
C12:0	n.d.	0.02 ± 0.01^{b}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a*}	0.03 ± 0.01^{b}	0.04 ± 0.01^{b}	0.03 ± 0.01^{b}	0.05 ± 0.02^{a**}	< 0.01	0.01	0.37
C14:0	0.47 ± 0.36	0.21 ± 0.18	0.23 ± 0.28	0.39 ± 0.36	0.37 ± 0.34*	0.43 ± 0.27^{b}	0.37 ± 0.40^{b}	0.33 ± 0.36^{b}	0.86 ± 0.32^{a**}	0.02	0.02	0.12
C15:0	n.d.	0.02 ± 0.01^{b}	0.04 ± 0.03^{a*}	n.d.	0.02 ± 0.01^{b}	0.02 ± 0.01	0.02 ± 0.01**	0.02 ± 0.00	0.03 ± 0.01	0.06	0.40	0.04
C16:0	7.78 ± 6.93	3.43 ± 4.48	4.73 ± 5.70	8.26 ± 7.87	7.77 ± 7.12*	8.94 ± 5.78^{b}	7.61 ± 8.13^{b}	6.92 ± 7.68^{b}	18.37 ± 6.60^{a**}	0.01	0.02	0.10
C17:0	n.d.	0.07 ± 0.04	0.06 ± 0.03	0.10 ± 0.03	0.09 ± 0.05	0.09 ± 0.04	0.12 ± 0.04	n.d.	0.15 ± 0.06	< 0.01	0.02	0.24
C18:0	4.74 ± 4.14	1.95 ± 2.58	2.63 ± 3.12	4.08 ± 3.82	4.12 ± 3.56*	4.57 ± 3.14^{b}	4.14 ± 4.41^{b}	3.88 ± 4.31^{b}	9.87 ± 2.97^{a**}	0.01	0.01	0.12
C20:0	n.d.	n.d.	n.d.	0.12 ± 0.03^{a}	0.08 ± 0.03^{b*}	0.09 ± 0.03^{b}	0.11 ± 0.02^{ab}	n.d.	0.14 ± 0.05^{a**}	< 0.01	0.01	
C21:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04 ± 0.01			
C16:1	1.02 ± 0.82	0.31 ± 0.33	0.38 ± 0.47	0.71 ± 0.69	0.69 ± 0.68*	0.80 ± 0.51^{b}	0.66 ± 0.74^{b}	0.59 ± 0.54^{b}	1.59 ± 0.73^{a**}	0.01	0.02	0.12
C17:1	n.d.	n.d.	0.05 ± 0.01^{b*}	0.08 ± 0.03^{a}	0.08 ± 0.04^{a}	0.07 ± 0.03^{b}	0.09 ± 0.03^{a**}	n.d.	0.09 ± 0.05^{a}	0.03	0.09	0.19
C18:1n9c	12.85 ± 12.08	5.37 ± 6.85	7.69 ± 9.30	15.48 ± 15.23	13.47 ± 12.90*	16.63 ± 10.44^{b}	12.91 ± 13.99^{b}	11.84 ± 12.88^{b}	30.64 ± 12.98^{a**}	0.02	0.03	0.10
C18:2n6c	6.77 ± 4.85	2.28 ± 2.84	3.27 ± 3.97	7.59 ± 7.37	6.00 ± 6.04	6.48 ± 5.32^{ab}	5.54 ± 5.97^{b}	5.93 ± 6.63^{b}	11.91 ± 7.54^{a}	0.08	0.10	0.31
C18:3n6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02 ± 0.01			
C20:1	n.d.	0.19 ± 0.08^{b}	0.27 ± 0.11^{b}	0.42 ± 0.15^{a}	0.30 ± 0.16^{ab*}	0.32 ± 0.14^{b}	0.4 ± 0.08^{ab}	0.35 ± 0.17^{b}	0.52 ± 0.2^{a**}	0.01	0.02	0.04
C18:3n3	n.d.	n.d.	0.29 ± 0.18^{b*}	0.59 ± 0.18^{a}	0.42 ± 0.24^{ab*}	0.43 ± 0.19^{b}	0.55 ± 0.11^{ab**}	0.39 ± 0.26^{b}	0.66 ± 0.25^{a**}	0.09	0.25	< 0.01
C20:2	n.d.	n.d.	n.d.	0.46 ± 0.16	0.29 ± 0.19*	0.33 ± 0.14^{ab}	0.38 ± 0.07^{ab}	0.30 ± 0.21^{b}	0.49 ± 0.23^{a**}	0.72	0.83	0.01
C20:3n6	n.d.	n.d.	n.d.	n.d.	0.06 ± 0.02	0.05 ± 0.01^{b}	n.d.	n.d.	0.08 ± 0.03^{a}	0.24	0.01	
C20:3n3	n.d.	n.d.	n.d.	n.d.	n.d.	0.06 ± 0.02^{ab}	0.06 ± 0.01^{b}	n.d.	0.08 ± 0.03^{a}		0.08	
C20:4n6	n.d.	n.d.	n.d.	0.30 ± 0.12	0.26 ± 0.16	0.20 ± 0.08	0.25 ± 0.04	n.d.	0.30 ± 0.13	0.56	0.39	
C24:1	n.d.	n.d.	n.d.	0.08 ± 0.03	0.08 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	n.d.	0.09 ± 0.04	0.55	0.26	
C22:6n3	n.d.	n.d.	n.d.	n.d.	0.09 ± 0.04	0.06 ± 0.02	0.05 ± 0.01	n.d.	0.07 ± 0.03	0.09	0.16	
MCT	n.d.	0.04 ± 0.01^{b}	0.06 ± 0.02^{ab}	0.07 ± 0.03^{a}	0.06 ± 0.04^{ab*}	0.07 ± 0.02^{b}	0.08 ± 0.03^{ab}	0.08 ± 0.03^{b}	0.11 ± 0.04^{a**}	< 0.01	0.03	0.23
SFA	42.45 ± 4.95	5.60 ± 7.34	7.71 ± 9.16	12.94 ± 12.23	12.45 ± 11.15*	14.16 ± 9.30^{b}	12.33 ± 13.11^{b}	11.22 ± 12.39^{b}	29.57 ± 10.00^{a**}	0.01	0.01	0.10
USA	61.19 ± 4.02	8.01 ± 10.11	11.69 ± 14.16	24.88 ± 24.44	21.26 ± 20.67*	25.20 ± 16.50^{b}	20.02 ± 21.66^{b}	18.96 ± 20.89^{b}	46.53 ± 20.99^{a**}	0.02	0.04	0.13
MUFA	41.36 ± 5.88	5.73 ± 7.32	8.23 ± 9.98	16.53 ± 16.27	14.48 ± 13.89*	17.81 ± 11.19^{b}	13.85 ± 15.01^{b}	12.54 ± 13.69^{b}	32.93 ± 13.88^{a**}	0.02	0.03	0.10
PUFA	6.77 ± 4.85	2.28 ± 2.84	3.45 ± 4.20	8.35 ± 8.18	6.78 ± 6.83*	7.39 ± 5.83^{ab}	6.18 ± 6.68^{b}	6.42 ± 7.20^{b}	13.60 ± 8.04^{a**}	0.05	0.07	0.26
n-6	6.77 ± 4.49	2.28 ± 2.84	3.27 ± 3.97	7.89 ± 7.44	6.26 ± 6.15*	6.68 ± 5.32^{ab}	5.78 ± 5.98^{b}	5.93 ± 6.63^{b}	12.23 ± 7.62^{a**}	0.07	0.08	0.27
n-3	n.d.	n.d.	0.29 ± 0.18*	0.59 ± 0.18	0.51 ± 0.22	0.54 ± 0.21^{ab}	0.66 ± 0.12^{a**}	0.39 ± 0.26^{b}	0.81 ± 0.29^{a}	0.20	0.12	0.06
n-6/n-3	n.d.	n.d.	12.17 ± 9.88	13.39 ± 10.53	10.75 ± 6.96	11.68 ± 7.24	8.92 ± 8.80	15.65 ± 9.56	15.16 ± 6.58	0.65	0.59	0.44
PUFA/SFA	0.47 ± 0.07	0.43 ± 0.13^{b}	0.45 ± 0.06^{ab}	0.57 ± 0.11^{a}	0.52 ± 0.11^{ab}	0.52 ± 0.08	0.45 ± 0.09	0.51 ± 0.10	0.52 ± 0.10	0.79	0.30	0.40
AI	0.57 ± 0.12	0.21 ± 0.10^{a}	0.13 ± 0.04^{b}	0.14 ± 0.04^{b}	0.16 ± 0.04^{ab}	0.16 ± 0.03^{ab}	0.13 ± 0.07^{b}	0.14 ± 0.04^{ab}	0.19 ± 0.04^{a}	0.98	0.05	0.16
TI	1.86 ± 0.45	0.60 ± 0.23^{a*}	0.27 ± 0.17^{b}	0.29 ± 0.18^{b}	0.32 ± 0.14^{b}	0.30 ± 0.15^{b**}	0.25 ± 0.23^{b}	0.33 ± 0.17^{ab}	0.55 ± 0.24^{a}	0.46	0.01	< 0.01

n.d., not detectable.

a, b, c means with different lowercase letters differed significantly in plane induction cooking or concave induction cooking ($P < 0.05$).

* means differed between plane induction cooking and concave induction cooking at the same cooking time point ($P < 0.05$).



a, b means differed significantly among cooking time points ($P < 0.05$). *, significant differences existed between concave induction cooking and plane induction cooking at a certain time point ($P < 0.05$).

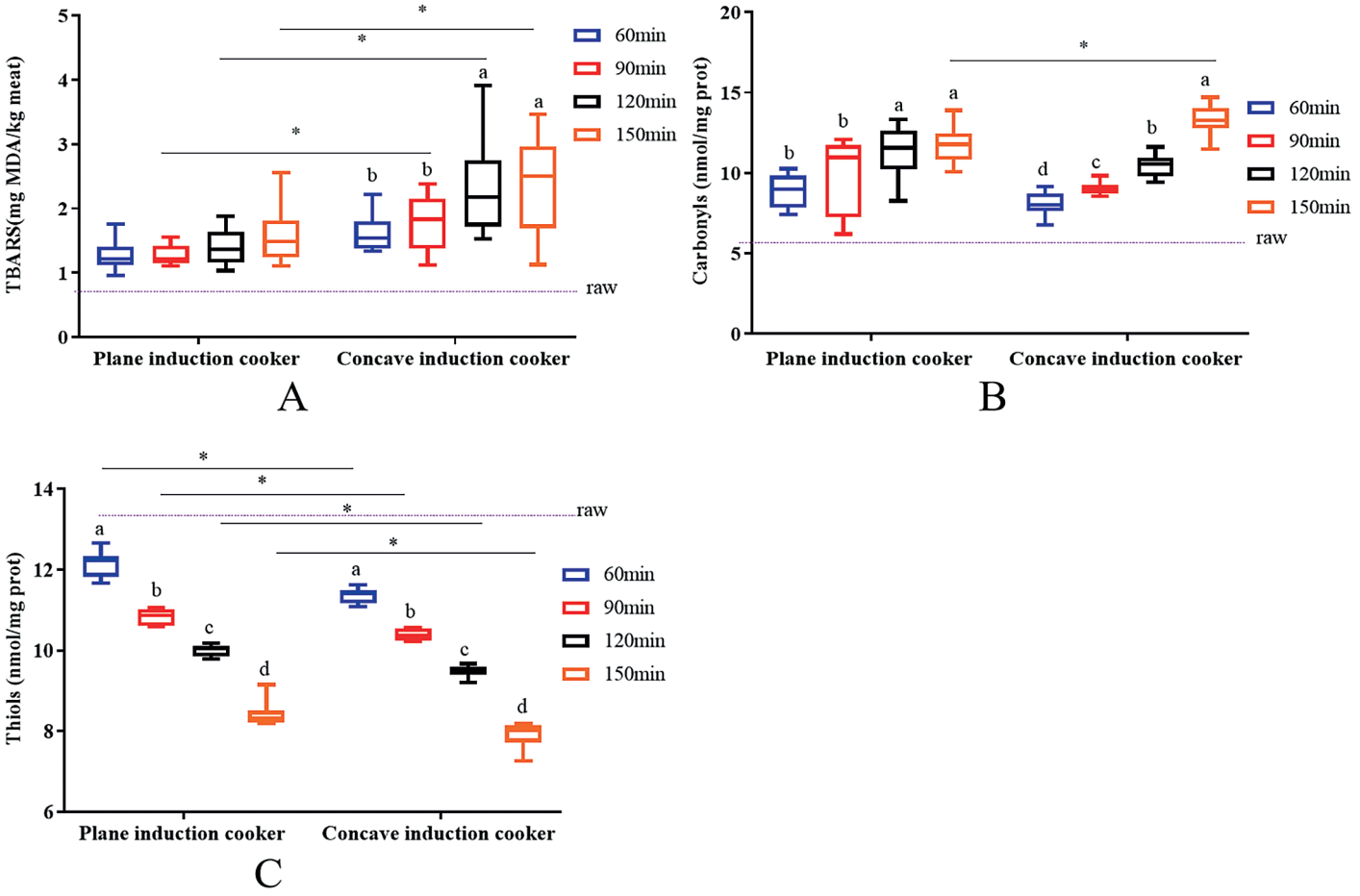
Figure 4. Fatty acids profile of braised pork: A — medium chain fatty acids (MCT); B — saturated fatty acids (SFA); C — unsaturated fatty acids (USA); D — monounsaturated fatty acids (MUFA); E — polyunsaturated fatty acids (PUFA)

Lipid and protein oxidation

Lipid and protein oxidation showed great changes with cooking method and time ($P < 0.05$, Figure 5). MDA is the secondary product of lipid oxidation. The MDA content in braised pork increased with cooking time in concave induction cooked pork but did not alter too much in plane induction cooked samples (Figure 5, A). The MDA value in meat samples cooked by concave induction for 60 min was similar to the values of plane induction cooked samples for 150 min. The carbonyl content increased greatly with cooking time. The values at 150 min were higher in concave induction cooked samples than in plane induction cooked samples ($P < 0.05$, Figure 5, B). Correspondingly, the thiol content decreased greatly with cooking time and the values were always lower in concave induction cooked samples than in plane induction cooked samples ($P < 0.05$, Figure 5, C). These results indicate that concave induction cooking may induce stronger lipid and protein oxidation. However, the carbonyl content of braised pork in concave induction cooker for 60 min was lower than that in plane induction cooker for 150 min ($P < 0.05$, Figure 5B), and the thiol content was higher in concave induction cooker for 60 min than that in plane induction cooker for 150 min ($P < 0.05$, Figure 5, C). This indicates that much shorter cooking time of concave induction cooking can compensate its negative impacts on meat quality attributes.

E-nose

E-nose is a sensitive technology to discriminate volatile compounds by different sensors. Principal component analysis showed that the first and second principal components (PCs) accounted for 94.22% and 3.55% of total variance of samples, respectively (Figure 6). Great differences were observed among samples cooked for different methods and times (ellipses A, C, E and G for plane induction cooking for 60 min, 90 min, 120 min and 150 min, respectively; ellipses B, D, F and H for concave induction cooking for 60 min, 90 min, 120 min and 150 min, respectively). PC1 mainly explained the variations caused by cooking method and cooking time. PC2 explained the variations from concave induction cooked samples for 120 min. Samples cooked by concave induction for 60 min and 90 min showed a great similarity to those cooked by plane induction for 150 min. The sensor signals indicate that the relative abundance of volatile compounds increased at the early stage due to chemical reactions such as lipid oxidation. Samples cooked by concave induction for 150 min overlaps with those cooked by plane induction for 60 min, 90 min and 120 min (Table 5), which may be because some volatile compounds of braised pork in concave induction cooker were evaporated with moisture. Once again, cooking method affected E-nose metrics, which have to some degree been associated with flavor attributes. However,



a, b, c, d means differed significantly among cooking time points ($P < 0.05$). *, significant differences existed between concave induction cooking and plane induction cooking at a certain time point ($P < 0.05$).

Figure 5. Lipid and protein oxidation of braised pork

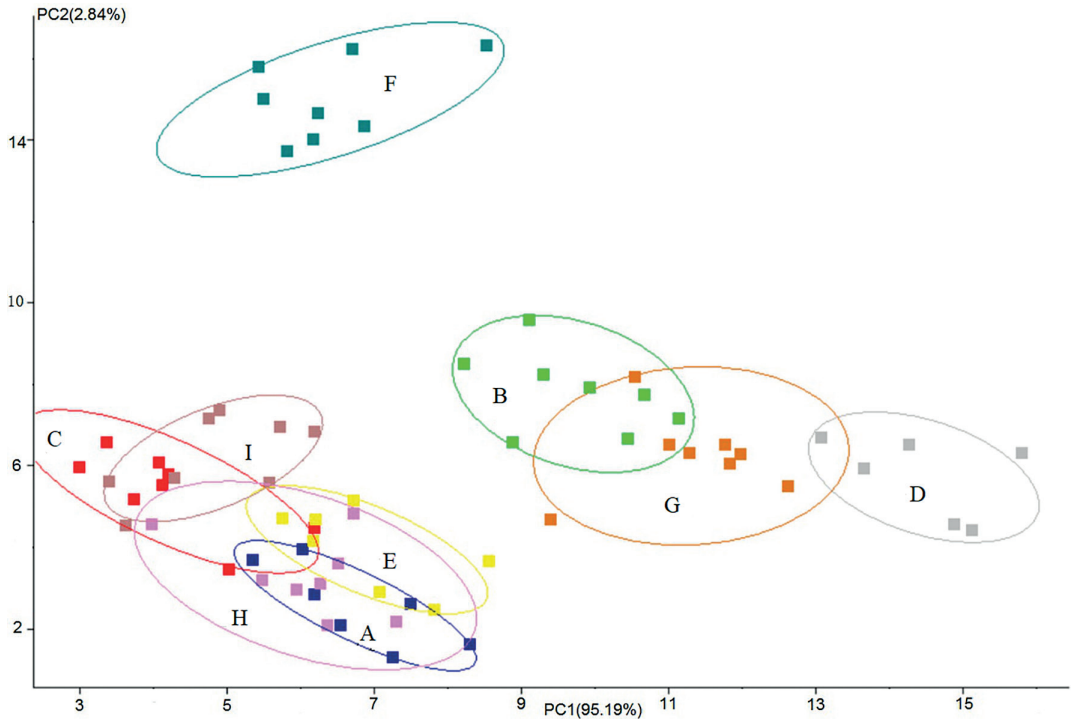


Figure 6. Principal component analysis scores plot for electronic nose data: A, C, E, G — concave induction cooking for 60 min, 90 min, 120 min and 150 min, respectively; B, D, F, H — plane induction cooking for 60 min, 90 min, 120 min and 150 min, respectively; I — raw meat

the specific volatile compounds still need to be further identified by GC–MS.

Volatile compounds

During meat processing, heat-induced lipid oxidation and Maillard reaction of proteins are the main sources of meat flavor compounds. Lipid oxidation may produce aldehydes, ketones, esters, carboxylic acids, and aromatic hydrocarbons. Maillard reaction may produce pyrroles, pyrazines, furans, oxygen-containing heterocyclic compounds, Strecker aldehydes and carbonyl compounds [36].

Cooking methods have significant effect on the formation of volatile compounds in meat. For example, in pork loin, frying produces more pyrazines than hot air or an electric stove [37]. In cooked pork cheeks, cooking temperature and time have significant effects on volatile flavor compounds derived from lipid degradation and Maillard reactions [38]. In pork jerky, infrared grills produce more volatile flavor compounds at 200 °C than at 150 °C [39].

In the present study, we identified 72 volatile compounds in the lean part of braised pork that differed with cooking method or time ($P < 0.05$, Table 6), including alcohols, nitrogen-containing compounds, aromatic hydrocarbons, phenols, furans, aldehydes, acids, ketones, aliphatic hydrocarbons and esters. Aldehydes were the most abundant volatile compounds in meat samples (Table 6). The identified aldehydes include nonanal, hexanal, benzaldehyde, (E, E)-2,4-decadienal, (2E)-2-octenal, (2E)-2-nonenal, octanal, pentadecanal, 5-ethylcyclopentene-1-carbaldehyde, hexadecanal and (E, E)-2,4-nonadienal. The abundance of most aldehydes decreased with cooking time. One exception is the hexadecanal whose abundance increased with cooking time ($P < 0.05$, Table 6). The relative abundances of aldehydes in the concave induction cooker for 60 min were similar to those cooked by plane induction for 150 min. This may be due to the stronger oxidation of fatty acids in concave induction cooked samples. The low thresholds of aldehydes contribute significantly to the flavor of braised pork [40]. Nonanal was one of the most abundant aldehydes in this study, which is a major oxidation product of oleic acid [41] and has fat aroma [42]. Benzaldehyde, which is derived from Strecker degradation of amino acids [43,44], was also highly abundant and its content in samples cooked by concave induction for 60 min was similar to those cooked by plane induction for 150 min (Table 6).

Concave induction cooking for 60 min also showed advantages to retain higher abundances of other volatile compounds including 2-pentylfuran, (E, E)-3,5-octadien-2-one, 2, 3-octanedione, 2-decahydro-1,6- dimethylnaphthalene when compared with plane induction cooking for 150 min (Table 6). 2-Pentylfuran has been reported to contribute to the flavor of meat [45], was the only detectable furan. (E, E)-3,5-octadien-2-one and 2, 3-octanedione may contribute to a butter aroma in meat products. 2-Decahydro-1,6-dimethylnaphthalene has grass-like aroma. In the present study, such compounds could be derived from vegetable oil, soy sauce or wine. Concave induction cooking can retain higher volatile compounds, which should be attributed to higher cooking temperature. Higher cooking temperature may produce more volatile flavor compounds through Maillard reaction and Strecker degradation [46] and improve the taste and volatile flavor of stewed pork [47]. Taken together, the volatile compound profile in braised pork prepared in a concave induction cooker for 60 min may be better than that in a plane induction cooker for 150 min.

Table 5. Response signals of E-nose sensors

Sensors	Raw	Plane induction cooking				Concave induction cooking				P values		
		60 min	90 min	120 min	150 min	60 min	90 min	120 min	150 min	method	time	method *time
W1C	1.00±0.06	0.76±0.04^{b}	0.78±0.04^{ab}	0.81±0.02^{a}	0.80±0.04^{a}	0.74±0.05^{c}	0.79±0.03^{b}	0.85±0.07^{a}	0.79±0.04^{b}	0.60	<0.0001	0.26
W5S	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00*	1.00±0.00^{ab}	1.00±0.00^{a}	1.00±0.00^{ab}	1.00±0.00^{b**}	0.27	0.36	0.06
W3C	1.02±0.07	0.76±0.05^{b}	0.79±0.04^{ab}	0.82±0.03^{a*}	0.80±0.05^{a}	0.75±0.05^{c}	0.81±0.04^{b}	0.87±0.07^{a**}	0.81±0.04^{b}	0.07	<0.0001	0.35
W6S	0.98±0.03	1.07±0.02	1.07±0.03	1.07±0.02	1.08±0.02	1.06±0.03	1.06±0.02	1.06±0.04	1.10±0.16	0.92	0.47	0.80
W5C	1.05±0.09	0.78±0.06^{b}	0.81±0.04^{ab}	0.84±0.04^{a*}	0.83±0.05^{a}	0.80±0.04^{c}	0.85±0.05^{b}	0.91±0.06^{a**}	0.85±0.05^{b}	0.00	<0.0001	0.62
W1S	0.95±0.11	1.44±0.11^{a*}	1.38±0.09^{ab*}	1.32±0.12^{b*}	1.39±0.12^{ab*}	1.28±0.07^{a**}	1.23±0.09^{ab**}	1.16±0.08^{b**}	1.23±0.18^{ab**}	<0.0001	0.03	1.00
W1W	1.18±0.21	3.34±0.53	3.37±0.81	3.32±0.48	3.33±0.30	3.05±0.32	3.08±0.20	3.06±0.48	3.51±0.57	0.18	0.50	0.44
W2S	0.99±0.02	1.38±0.33^{a*}	1.10±0.08^{b}	1.11±0.10^{b*}	1.10±0.11^{b}	1.17±0.08^{a**}	1.07±0.07^{a}	1.02±0.02^{b**}	1.10±0.07^{a}	0.02	0.00	0.12
W2W	1.17±0.25	3.27±0.51	3.26±0.77	3.20±0.48	3.20±0.27	2.86±0.29	2.97±0.25	3.04±0.45	3.34±0.56	0.13	0.64	0.39
W3S	1.00±0.02	1.13±0.05^{ab}	1.10±0.05^{b}	1.08±0.05^{b}	1.18±0.12^{a*}	1.08±0.05	1.08±0.05	1.05±0.02	1.07±0.04**	0.00	0.03	0.11

Table 6. Volatile compounds of braised pork

Volatile compounds (ng/g)	Raw	Plane induction cooking				Concave induction cooking				P values			RI
		60 min	90 min	120 min	150 min	60 min	90 min	120 min	150 min	method	time	method *time	
Alcohols	795.02±215.57	6841.05±1923.23^{a}	4896.23±2352.10^{b}	4106.01±1060.21^{b}	4592.29±1218.70^{b}	7388.01±2349.65^{a}	6150.55±3122.21^{ab}	3664.70±1085.55^{c}	4644.08±1714.81^{bc}	0.46	<0.0001	0.62	
1-Hexanol	n.d.	343.74±211.22	246.16±131.66*	237.15±204.61	266.62±292.01	605.39±283.59^{b}	1635.73±2316.79^{a**}	314.57±205.89^{b}	857.59±1029.71^{ab}	0.01	0.23	0.20	1361.38
1-Heptanol	n.d.	n.d.	347.99±102.86	n.d.	346.40±80.16	447.28±142.49^{a}	n.d.	294.24±24.37^{b}	286.50±55.68^{b}	0.19	<0.01		1461.33
1-Octanol	243.54±46.23	603.49±138.81^{a}	484.18±155.94^{a}	328.20±96.74^{b}	395.10±126.34^{ab}	637.01±216.39^{a}	455.26±149.87^{b}	334.42±77.80^{b}	365.09±110.68^{b}	0.89	<0.0001	0.90	1562.48
1-Dodecanol	n.d.	254.33±84.06^{a*}	168.19±34.35^{b*}	n.d.	147.35±69.39^{b*}	94.60±22.03**	101.82±47.39**	n.d.	77.88±35.19**	<0.0001	<0.01	0.01	1972.14
(E)-2-Octen-1-ol	n.d.	684.03±131.40^{a}	566.67±135.42^{ab}	448.93±71.55^{b}	442.91±95.70^{b}	746.77±260.05^{a}	551.68±126.29^{b}	n.d.	n.d.	0.65	<0.01	0.47	1619.07
1-Octen-3-ol	581.93±169.79	3012.13±657.88^{a}	2541.14±627.61^{ab}	1971.66±337.44^{b}	2076.85±579.96^{b}	3281.06±1164.89^{a}	2210.00±795.02^{b}	2074.89±495.77^{b}	2086.90±581.37^{b}	0.94	<0.0001	0.66	1455.68
4-Ethylcyclohexanol	n.d.	616.65±213.24^{a*}	319.66±121.47^{b}	355.85±232.05^{b}	280.04±81.95^{b}	409.91±112.14**	n.d.	300.28±101.90	389.76±132.42	0.24	<0.01	0.02	1527.60
Phenylethyl alcohol	n.d.	645.17±171.34	422.28±62.94	340.09±120.51	396.65±99.39	345.26±106.66^{b}	850.23±1321.51^{a}	296.70±135.33^{b}	231.18±84.71^{b}	0.87	0.18	0.17	1921.30
2,4-dimethylcyclohexan-1-ol	n.d.	120.10±43.60	135.99±49.79	90.38±39.00	128.75±39.76	154.58±77.27^{a}	n.d.	n.d.	93.28±36.31^{b}	0.98	0.13	0.05	1478.42
1-Pentanol	n.d.	548.78±125.42^{a}	414.48±124.62^{b}	295.79±67.35^{c}	318.77±89.83^{bc}	521.85±161.60^{a}	387.26±106.70^{b}	295.79±77.64^{b}	314.97±96.68^{b}	0.59	<0.0001	0.97	1258.62
2,4-dimethyl-2,6-Heptadien-1-ol	n.d.	311.20±89.34^{a}	206.93±42.51^{b}	240.04±29.42^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.01		1664.32
4,4-Dimethyl-cyclohex-2-en-1-ol	n.d.	159.66±51.15^{a*}	148.88±72.71^{ab}	59.70±38.02^{c}	122.22±56.00^{b}	249.50±98.05^{a**}	96.09±29.09^{b}	85.27±23.70^{b}	97.71±84.73^{b}	0.54	<0.0001	0.01	1343.70
2-Butyl-2,7-octadien-1-ol	n.d.	n.d.	81.39±33.45	n.d.	71.23±45.18	107.35±18.31	n.d.	n.d.	n.d.	0.97	0.20		1671.12
Nitrogen-containing compounds	n.d.	130.05±43.83	126.07±48.73	117.71±73.71	98.25±46.54	150.22±43.86^{ab}	155.54±92.02^{a}	97.47±35.03^{b}	104.75±38.79^{ab}	0.52	0.09	0.62	
2-Pentylpyridine	n.d.	130.05±43.83	126.07±48.73	117.71±73.71	98.25±46.54	150.22±43.86^{ab}	155.54±92.02^{a}	97.47±35.03^{b}	104.75±38.79^{ab}	0.52	0.09	0.62	1580.67
Aromatic hydrocarbons	n.d.	303.05±98.80^{a*}	364.07±146.62^{a*}	274.82±83.91^{ab}	250.51±78.62^{b*}	694.52±102.99^{a**}	485.68±124.79^{b**}	352.90±77.72^{c}	382.64±81.78^{c**}	<0.0001	<0.0001	<0.01	
Decahydro-1,6-dimethylnaphthalene	n.d.	303.05±98.80^{a*}	364.07±146.62^{a*}	274.82±83.91^{ab}	250.51±78.62^{b*}	694.52±102.99^{a**}	485.68±124.79^{b**}	352.90±77.72^{c}	382.64±81.78^{c**}	<0.0001	<0.0001	<0.01	1897.63
Phenols	168.72±73.04	61.08±28.37	58.71±13.51	60.87±11.69	52.77±21.16	67.72±22.77	n.d.	62.77±7.15	65.11±13.34	0.19	0.87	0.72	
2,4-Di-tert-butylphenol	168.72±73.04	61.08±28.37	58.71±13.51	60.87±11.69	52.77±21.16	67.72±22.77	n.d.	62.77±7.15	65.11±13.34	0.19	0.87	0.72	2317.09
Furans	172.67±88.62	1959.52±613.25^{a}	1649.76±494.85^{ab}	1346.72±224.68^{b}	1308.55±362.44^{b}	1789.80±695.19	1370.89±552.87	1421.34±300.37	1392.63±722.38	0.58	0.02	0.70	
2-Pentylfuran	172.67±88.62	1959.52±613.25^{a}	1649.76±494.85^{ab}	1346.72±224.68^{b}	1308.55±362.44^{b}	1789.80±695.19	1370.89±552.87	1421.34±300.37	1392.63±722.38	0.58	0.02	0.70	1233.72
Aldehydes	6511.37±1471.26	23883.54±5999.94	20784.37±6352.64	13395.72±3701.82	17388.04±5393.83	25864.87±8474.56	15910.51±6196.93	13393.84±3596.89	17610.93±7652.71	0.74	0.23	0.63	
Hexadecanal	2030.07±878.24	2446.59±948.22	2784.06±875.16	2457.63±1138.53	3093.51±1266.10*	2940.26±867.93^{b}	3192.82±1092.51^{b}	3149.31±763.74^{b}	4608.93±1587.10^{a**}	0.01	0.02	0.47	2136.83
Nonanal	1840.71±514.59	3830.22±1926.79^{a}	3182.68±1671.09^{ab*}	1914.29±378.24^{c}	2146.30±638.25^{bc}	3371.96±1226.35^{a}	2025.32±634.39^{b**}	1934.91±310.35^{b}	2093.96±600.98^{b}	0.13	<0.01	0.40	1393.06
Benzaldehyde	2147.78±315.75	2921.29±682.09^{a}	2242.21±556.78^{ab}	1862.50±560.97^{b}	2059.45±648.32^{b}	2345.81±950.58	1723.96±865.42	1630.62±499.68	1860.83±943.50	0.04	0.01	0.84	1522.56
Hexanal	171.11±65.09	2264.03±424.35^{a}	1830.94±464.90^{b}	1380.62±496.66^{b}	1446.15±598.32^{b}	2491.43±985.65^{a}	1718.56±521.49^{b}	1453.96±384.68^{b}	1329.63±580.79^{b}	0.90	<0.0001	0.81	1096.09
(E, E)-2,4-decadienal	159.83±69.78	3181.64±558.36^{a}	2303.56±747.22^{ab}	2357.17±2303.41^{ab}	1787.26±679.13^{b}	3340.40±857.21	3237.90±1991.47	2194.33±804.52	2121.19±902.46	0.32	0.03	0.66	1796.04
(2E)-2-Octenal	219.07±71.52	2218.08±634.61^{a}	1507.32±359.11^{b}	1241.85±259.92^{b}	1285.21±330.58^{b}	1864.29±786.52^{a}	1219.97±432.28^{b}	1198.24±297.26^{b}	1153.67±273.57^{b}	0.08	<0.0001	0.77	1428.93
(2E)-2-Nonenal	315.97±67.04	1200.30±349.69^{a}	854.39±216.17^{b}	655.63±190.10^{b}	721.67±185.21^{b}	1178.85±422.06^{a}	869.65±206.72^{b}	745.83±139.23^{b}	728.38±163.88^{b}	0.72	<0.0001	0.93	1536.62

Table 6. Ending

Volatile compounds (ng/g)	Raw	Plane induction cooking				Concave induction cooking				P values			RI
		60 min	90 min	120 min	150 min	60 min	90 min	120 min	150 min	method	time	method *time	
(2E)-2-Decenal	n.d.	1883.15±1145.50	1421.80±1288.27	n.d.	1061.04±274.34	1408.94±482.57	n.d.	n.d.	813.54±200.01	0.22	0.06	0.70	1646.52
2-Undecenal	n.d.	1441.62±213.06	1765.35±1936.71	n.d.	1050.90 ±183.83	1825.96±510.76	n.d.	n.d.	1042.56±313.79	0.57	0.10	0.55	1758.25
Octanal	n.d.	1156.92±730.67^{a*}	1019.35±622.54^{a}	n.d.	434.77±494.71^{b}	35.05±36.42**	n.d.	15.84±16.03	490.08±627.50	0.01	0.43	<0.01	1289.53
Pentadecanal	n.d.	967.23±271.49^{a*}	936.74±207.86^{a}	n.d.	673.82±254.47^{b*}	1268.08±348.42^{a**}	918.55±191.45^{b}	n.d.	958.58±209.55^{b**}	0.01	0.06	0.14	2035.41
Tetradecanal	n.d.	808.78±222.72*	839.51±163.27	n.d.	656.05±122.21*	1055.23±292.37^{a**}	784.07±220.84^{bc}	602.81±175.16^{c}	868.54±176.66^{ab**}	0.03	<0.01	0.08	1927.56
5-ethylcyclopentene-1-carbaldehyde	n.d.	756.33±170.46^{a*}	503.39±143.24^{b*}	387.48±79.43^{b}	434.73±135.46^{b}	568.02±201.31^{a**}	356.01±139.01^{b**}	351.83±108.93^{b}	423.54±129.87^{b}	0.01	<0.0001	0.24	1414.30
(Z)-2-heptenal	n.d.	767.79±345.00^{a}	505.67±227.12^{b}	441.58±172.96^{b}	588.35±360.38^{ab*}	693.60±272.91^{a}	279.50±109.32^{b}	306.02±89.28^{b}	319.74±48.71^{b**}	<0.01	<0.01	0.63	1322.81
(E, E)-2,4-Nonadienal	n.d.	506.98±110.58^{a*}	418.67±169.27^{ab}	280.93±142.39^{b}	309.54±141.01^{b}	657.58±163.78^{a**}	n.d.	n.d.	314.56±110.92^{b}	0.13	<0.0001	0.15	1704.30
Heptanal	n.d.	225.22±88.68^{a*}	171.02±240.54^{ab}	99.97±95.08^{ab}	76.51±36.89^{b}	80.84±135.36**	n.d.	n.d.	n.d.	0.04	0.12		1185.53
(E, E)-2,4-Heptadienal	n.d.	210.82±37.49^{a}	132.98±34.58^{b*}	118.47±42.47^{b}	151.99±29.86^{b}	175.43±64.09	194.89±55.45**	n.d.	n.d.	0.42	0.02	<0.01	1492.49
cis-4-Decenal	n.d.	206.70±56.45^{a*}	189.48±56.08^{ab}	143.00±37.23^{bc}	126.91±56.10^{c}	290.10±101.60^{a**}	191.30±34.78^{b}	166.40±15.92^{b}	140.29±44.31^{b}	0.03	<0.0001	0.17	1541.16
4-pentylbenzaldehyde	n.d.	148.71±32.14*	140.39±32.62	n.d.	n.d.	194.60±48.04^{a**}	147.75±54.80^{b}	119.43±36.45^{b}	136.77±44.39^{b}	0.07	0.01	0.19	2018.70
Pentanal	n.d.	137.06±104.82^{a}	81.18±81.69^{ab}	50.28±55.46^{b}	85.31±64.62^{ab}	74.92±56.30	53.85±45.39	n.d.	n.d.	0.08	0.13	0.49	976.46
Undecanal	n.d.	124.23±26.23^{a}	100.19±32.51^{ab}	62.43±16.70^{c}	72.17±19.46^{bc}	117.21±47.53^{a}	88.42±32.04^{ab}	74.20±19.61^{b}	88.28±42.92^{ab}	0.77	<0.01	0.52	1605.25
2-methylheptanal	n.d.	91.35±86.02*	99.98±69.95	80.42±58.27	62.76±64.48	236.04±88.36**	n.d.	n.d.	n.d.	<0.01	0.77		975.69
(Z)-octadec-9-enal	n.d.	n.d.	220.86±51.67	n.d.	255.24±63.64	215.17±24.46^{b}	280.66±74.20^{a}	255.93±61.64^{ab}	315.40±81.42^{a}	0.01	0.01	0.99	2383.97
Octadecanal	n.d.	n.d.	153.78±16.34	132.97±45.27	156.37±49.97*	136.02±28.33^{b}	n.d.	149.94±32.50^{b}	248.73±123.47^{a**}	0.01	0.01	0.09	2357.42
Acids	n.d.	340.39±118.15^{a*}	357.74±47.04^{a}	n.d.	204.87±55.28^{b}	440.32±118.66^{a**}	352.58±98.79^{a}	217.23±54.67^{b}	n.d.	0.13	<0.0001	0.10	1978.33
cis-8,11,14-Eicosatrienoic Acid	n.d.	340.39±118.15^{a*}	357.74±47.04^{a}	n.d.	204.87±55.28^{b}	440.32±118.66^{a**}	352.58±98.79^{a}	217.23±54.67^{b}	n.d.	0.13	<0.0001	0.10	1978.33
Ketones	n.d.	330.91±163.89^{b*}	902.34±310.77^{ab}	1104.72±141.67^{a}	771.48±372.16^{a}	1347.84±939.26^{a**}	1108.50±556.60^{ab}	822.24±323.17^{b}	1222.99±713.42^{ab}	<0.01	0.77	<0.01	
2-Pentadecanone	n.d.	106.06±41.30^{b}	154.60±69.39^{b*}	346.59±120.31^{a}	258.73±138.42^{a}	180.47±97.01^{b}	298.20±58.91^{a**}	305.11±115.31^{a}	284.53±119.54^{a}	0.05	<0.0001	0.08	2027.68
(E, E)-3,5-Octadien-2-one	n.d.	275.24±52.72*	254.06±100.97	n.d.	245.33±45.95	425.58±153.84^{a**}	321.89±99.44^{b}	n.d.	228.20±58.11^{b}	0.02	<0.01	0.05	1572.38
2,3-Octanedione	n.d.	n.d.	642.81±195.77^{ab*}	762.90±107.04^{a}	493.85±183.82^{b}	1112.70±522.69^{a}	929.26±100.92^{a**}	620.55±210.89^{b}	n.d.	0.41	0.01	0.02	1326.55
3-(4-hydroxybutyl)-2-methyl-cyclohexanone	n.d.	45.27±22.13^{a}	40.29±13.10^{ab}	27.37±6.30^{b}	34.68±5.71^{ab}	n.d.	n.d.	n.d.	n.d.		0.07		1434.69
Aliphatic hydrocarbons	829.99±452.93	3928.92±1902.27^{a}	3717.99±4196.57^{ab}	1929.38±1158.36^{b}	1595.79±460.03^{b}	2800.15±943.40	2080.76±1071.63	1370.36±638.55	2196.33±1139.24	0.14	0.03	0.35	
Dodecane	50.45±17.76	74.65±23.99	50.46±15.79*	75.88±57.98	44.47±20.69	63.53±24.12	82.45±31.16**	57.46±18.25	69.58±38.61	0.39	0.72	0.06	1191.16
Hexadecane	186.06±110.98	426.16±834.04	412.95±787.27	86.31±26.16	91.49±12.66	103.44±30.52	119.04±66.35	n.d.	100.48±17.74	0.11	0.37	0.49	1597.95
Tridecane	55.89±22.82	667.62±328.87^{a*}	491.74±370.37^{ab}	314.04±195.61^{bc}	238.09±96.36^{c}	401.45±169.33**	383.20±236.17	218.35±120.27	378.91±212.19	0.16	0.01	0.12	1297.96
Tetradecane	190.50±92.20	1074.12±1421.19^{a*}	766.24±1409.95^{ab}	207.46±102.64^{b}	135.18±34.04^{b}	290.63±78.75**	233.65±138.84	159.96±32.89	392.48±299.72	0.13	0.20	0.18	1398.71
Pentadecane	386.87±122.94	507.16±109.75^{ab}	1142.04±1984.09^{a}	425.91±337.63^{ab}	315.25±99.57^{b}	588.65±187.58	467.69±252.75	360.83±107.99	486.75±180.56	0.51	0.35	0.36	1470.08
Z, Z, Z-4,6,9-Nonadecatriene	n.d.	69.80±18.28^{a}	n.d.	39.34±8.78^{b}	47.10±7.68^{b}	90.49±37.43^{a}	n.d.	n.d.	65.46±22.10^{b}	0.02	<0.01	0.88	1687.03
4-Ethyl-3-nonen-5-yne	n.d.	1441.13±373.18^{a}	1050.04±341.56^{b}	957.22±406.68^{b}	830.32±266.79^{b}	1353.70±402.65^{a}	1018.56±379.79^{ab}	900.62±307.72^{b}	988.08±328.06^{b}	0.96	<0.01	0.76	1855.05
Esters	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	66.34±22.42	63.81±10.75	53.35±8.01		0.21		
5-Decanolide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	66.34±22.42	63.81±10.75	53.35±8.01		0.21		2216.64

n.d., not detectable.
a, b, c means with different lowercase letters differed significantly in plane induction cooking or concave induction cooking ($P < 0.05$).
* means differed between plane induction cooking and concave induction cooking at the same cooking time point ($P < 0.05$).

Table 7. Sensory evaluation scores of braised pork

	Plane induction cooking				Concave induction cooking				P values		
	60 min	90 min	120 min	150 min	60 min	90 min	120 min	150 min	method	time	method *time
odor	6.74 ± 0.16^{b*}	6.90 ± 0.13^{a}	6.86 ± 0.14^{ab*}	6.74 ± 0.12^{b}	7.38 ± 0.12^{a**}	6.86 ± 0.15^{c}	7.05 ± 0.10^{b**}	6.70 ± 0.16^{d}	<0.0001	<0.0001	<0.0001
color	7.13 ± 0.15^{a*}	6.79 ± 0.18^{b*}	6.84 ± 0.13^{b*}	6.46 ± 0.13^{c*}	7.80 ± 0.15^{a**}	7.03 ± 0.15^{c**}	7.33 ± 0.17^{b**}	6.64 ± 0.16^{d**}	<0.0001	<0.0001	<0.0001
texture	7.01 ± 0.22^{a*}	7.02 ± 0.12^{a}	6.55 ± 0.06^{b*}	6.36 ± 0.16^{c}	7.48 ± 0.11^{a**}	7.05 ± 0.26^{b}	6.83 ± 0.13^{c**}	6.39 ± 0.09^{d}	<0.0001	<0.0001	0.0002
taste	6.77 ± 0.17^{b*}	7.14 ± 0.10^{a*}	7.00 ± 0.13^{a*}	6.53 ± 0.17^{c*}	7.49 ± 0.18^{a**}	6.95 ± 0.21^{c**}	7.20 ± 0.19^{b**}	6.90 ± 0.13^{c**}	<0.0001	<0.0001	<0.0001

a, b, c means with different lowercase letters differed significantly in plane induction cooking or concave induction cooking ($P < 0.05$).
* means differed between plane induction cooking and concave induction cooking at the same cooking time point ($P < 0.05$).

Sensory evaluation

For plane induction cooked samples, the odor and taste scores increased from 60 min to 90 min but decreased afterwards ($P < 0.05$, Table 7). The color and texture scores decreased as cooking time increased ($P < 0.05$). For concave induction cooked samples, the color and texture scores decreased during the whole cooking period. The odor and taste scores decreased from 60 min to 90 min with a small increase from 90 min to 120 min, and subsequent decrease from 120 min to 150 min ($P < 0.05$). The greatest values were observed in concave induction cooked samples for 60 min ($P < 0.05$, Table 7). Such a difference was in accordance with the results of sensory evaluation. In previous studies, E-nose sensor signals were shown to have a certain correlation with sensory attributes [48,49].

As mentioned above, concave induction cooking had higher energy efficiency and lower energy consumption. The center temperatures of the meat samples near the center of the cookers were higher in the concave induction cooker. The higher temperature may cause greater changes in myofibrillar proteins to produce better texture. The higher heating efficiency also leads to the release of more structural lipids (mainly PUFA). In addition, the lipid oxidation and protein oxidation were not serious in concave induction cooked samples for a shorter time. Volatile compounds are mainly derived from lipid oxidation and Maillard reaction. Higher cooking temperature for a short time can also increase the content of volatile compounds and sensory scores. Therefore, concave induction cooking can improve the texture and flavor of braised pork in a short time, which may be due to better heating efficiency.

Conclusion

In this study, concave induction cooking was shown to have higher cooking efficiency and exhibited a significant impact on the texture, fatty acid composition, lipid and protein oxidation, volatile flavor and sensory evaluation in braised pork compared with plane induction cooking. At a power of 2000 W, concave induction cooking for 60 min produced a comparable or better level of texture, fatty acid profile, lipid and protein oxidation, flavor and sensory scores to plane induction cooking for 150 min. Thus, concave induction cooking is a promising alternative for traditional long-term and high-temperature cooking in meat products.

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PROMOTION OF CULTURAL HERITAGE — REGIONAL AND TRADITIONAL POLISH MEAT PRODUCTS

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Keywords: *regional meat product, traditional meat product, consumer, cultural heritage*

Abstract
The diverse culinary heritage of various countries in the European Union (EU) has been attracting attention for a very long time. This type of high-quality traditional food should be fully exploited and promoted as a common good that is part of the history of given countries. In order to distinguish individual products and their value (not only cultural, but also qualitative), the EU created special awarding signs (quality schemes) that conform to the quality of traditional products: Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), or Traditional Speciality Guaranteed (TSG). One of the first associations with Polish cuisine would undoubtedly be meat dishes, which play an important role in preserving the tradition. The most popular types of meat in Poland are pork, beef, and then poultry. In addition, game animals are very popular, including wild birds (black grouse and larks). This type of dishes is prepared according to traditional recipes handed down from generation to generation. Products typical of the region obtained from local crops and animal breeding are used in their preparation. Thanks to this, traditional dishes acquire specific taste values, which cannot be recreated in other parts of the country.

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Introduction

According to the study «Apetyt na region» (Appetite for the region), carried out by Citybell Consulting and ARC Rynek Opinia, about 67% of Poles bring in from their trips regional food products, such as cheese (35%), honey (14%), fish (14%), cold cuts (13%) or bread (8%). Each country has its own specific agricultural products and foodstuffs that characterize a given region and are part of the tradition and culinary culture of the people living there [1]. For example, mutton and lamb meat (mostly in the form of smoked ribs) dominated in one of Polish regions — Podhale. Kindziuk or dried sausage is an important dish for people from another part of the country — Podlasie. In the Kurpie region, cuisine was dominated by venison. Some of the dishes were known in several regions. An excellent example is «czernina», i. e. goose or duck blood soup. In Małopolska voivodship, tripe and duck were characteristic meat dishes. Also, other meat products play an important role in Polish regional culinary culture [2]. Polish cold cuts, sausages, hams, and smoked meats are an inexhaustible range of valued flavors and aromas. Depending on the culinary regions, these products can be prepared in different ways, using different culinary techniques and methods of serving which make each product really special. It all adds up to the uniqueness of the culinary traditions of a given region.

The definition of a regional and traditional product was included in Regulation (EU) No 1151/2012 of the European Parliament and of the Council of November 21, 2012 on quality schemes for agricultural products and foodstuffs [3]. The purpose of this law is to help producers of agricultural products and foodstuffs to inform their buyers and consumers about the characteristics of their goods, thereby ensuring:

a) fair competition among farmers and producers of agricultural products and foodstuffs with added-value characteristics and properties;
b) consumers' access to reliable information about products;
c) compliance with intellectual property rights;
d) the integrity of the internal market.

The measures set out in this Regulation are intended to support agro-processing activities and farming systems linked to high-quality products and thus contribute to the achievement of the objectives of rural development policy. The Regulation establishes quality schemes that provide the basis for the definition and, where appropriate, protection of names and terms which, in particular, indicate or describe agricultural products characterized by:

a) value-added features, or
b) value-added characteristics as a result of the agricultural production or processing methods used in their production, or of the place of their production or marketing [3,4].

Food described as «traditional» covers different categories of products that are characterized by specific raw materials, processing methods and place of origin. According

to EU Regulation No. 1151/2012 [3], «traditional» product should have a documented history on the market for a period indicating its transmission from generation to generation, and it should correspond to the time usually assigned to one generation [5,6]. It includes goods whose quality or unique ingredients and properties result from the use of traditional production or processing methods which were used for at least 25 years. Another important factor is to possess an element of the cultural heritage of the region, in which they are produced, and to be part of the identity of the local community [7]. In addition, the product should have specific characteristics which distinguish it from other similar products of the same category in terms of «traditional taste» [5,8].

The first regulations preventing counterfeiting of regional and traditional products throughout the European Union (EU) were issued as early as 1992 and 1993 [9,10]. They were aimed at standardizing the procedures of verification and identification of unique products in the territory of the EU. On their basis, a system of Protection of Designation of Origin and Geographical Indication of products was created. Traditional speciality guaranteed (TSG) highlights the traditional aspects, such as the way the product is made or its composition, without being linked to a specific geographical area [11].

The European quality system distinguishes three product categories: Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG) (Figure 1).

The basis for PDO registration is the manufacturing tradition and the special and unique quality associated with historically established production and processing methods for at least 25 years, which guarantee the high quality of the product obtained. Moreover, the PDO quality scheme is granted to a product, in which all production stages take place in the same geographical area [3,13]. Similarly, for PGI registration, the certification scheme indicates the above, but the link with the territory is less, and at least one of the production phases must take place in a specific region.

None of Polish meat products are registered as PDO. However, three meat products have obtained PGI certification: 1. jagnięcina podhalańska (a. k. a. podhalańska lamb), 2. kiełbasa biała parzona wielkopolska (a. k. a. «Wielkopolska» white steamed sausage), and 3. kiełbasa piaszczańska (a. k. a. piaszczańska sausage) [3,12,12,13].

In addition, there are four meat products registered as TSG: 1. kiełbasa myśliwska staropolska (a. k. a. staropolska hunter's sausage), 2. kabanos, 3. kiełbasa jałowcowa staropolska (a. k. a. staropolska juniper sausage), and 4. kiełbasa krakowska sucha staropolska (a. k. a. dry krakowska staropolska sausage).

Description of some Polish meat products registered as PGI or TSG

«Wielkopolska» white steamed sausage
(Kiełbasa biała parzona wielkopolska) (PGI)

Steamed sausages have been well known in Poland for a long time. The recipes depend on the availability of meat. In Wielkopolska, the production rule for white raw sausages and later on for white cooked sausages were well documented in the 90s of the 20th century.

The popularity of the sausage increased so much that butchers started producing it on a large scale. The sausage was available all year long and gave rise to various forms of its use in preparing dishes.

The tastiness of «kiełbasa biała parzona wielkopolska» is created by the taste of cooked pork with a gentle aroma of garlic and pepper. The sausage will not taste properly without the addition of marjoram. The uniqueness of the sausage is due to the use of fresh, chilled, uncured meat, 70% of which is derived from ham.

«Kiełbasa biała parzona wielkopolska» is used in more and more new recipes for meals published, among others, in magazines and on Internet forums. The unique character of the sausage successfully combines the traditions of the region with the present day and is protected by geographical indication [12].

Hunter's sausage
(kiełbasa myśliwska staropolska) (TSG)

For the production of Hunter's («myśliwska») sausage, meat from pigs with a greater weight and fat content than typical pigs (body weight of up to 120 kg, above 3% intramuscular fat content) is used. More fatty type of meat gives a distinctive flavor of the product and is based on native Polish breeds of pigs: Wielka polska biała, Polska biała zwisłoucha, Puławska or Złotnicka. Fattening is based on traditional mixtures of cereal and other natural components [14]. Energy is provided by feed mixture components including cereal like wheat, rye, oat and corn. Lupin, fava bean, soy, rape cake, fodder yeast and dried green fodder are used as protein components. The raw material used for the production of the described product is 50%

Figure 1. Graphic symbols of (from left) Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG) [12]

beef and 50% pork. The unique smell and taste distinguish «Myśliwska» sausage from other types of sausages. This is due to the use of different spices like juniper, pepper and garlic. Juniper is a traditional spice used in Polish cuisine and occurs frequently in forests. Its addition to the sausage enhances the taste and smell of the product and influences its specificity [15]. «Myśliwska» sausage is warm-smoked and heat-treated to an internal temperature of at least 70 °C. These processes of production give the product the characteristic color and unique taste. The internal temperature of 70 °C inactivates microorganisms present in the stuffing. The final stages of the production process are cooling to <10 °C and drying at 14–18 °C for 5–7 days to achieve the appropriate quality characteristics [4,16].

Kabanos (TSG)

The biggest advantages of kabanos are the specific taste and extended shelf life, which are ensured by drying and smoking processes. It is a thin, dried and smoked pork sausage in sheep intestine. The typical color of the surface is dark red with different shades of cherry. Kabanos has a specific taste of heat-treated corned pork, and a light aftertaste of caraway and pepper. The specificity of the product is due to pork used in production process. Meat from more fatty type of pigs (weight of up to 120 kg) gives characteristic features of kabanos [17]. Pork with an intramuscular fat content above 3% ensures characteristic taste and appropriate technological properties of the obtained products [4]. Meat from Polish purebred pigs like Wielka polska biała or Polska biała zwisłoucha and traditional production methods (in particular the most important stages: grinding, curing, smoking) provide unique taste of kabanos and exceptional crispiness. The characteristic feature of kabanos is a snapping sound produced when it is broken to half. Due to the effect of properly conducted drying and smoking processes, meat acquires a peculiar fragility. Smoking and heat-treating give the right color of the skin and appropriate taste qualities. The heating process destroys any pathogens present in the stuffing. Also, specific taste and smell of kabanos are the result of spices: natural pepper, nutmeg, and cumin [17].

Other traditional and regional products in Poland

Consumer choice of certain foods is strongly influenced by cultural heritage. Nowadays, young generations are showing a progressive interest in their cultural heritage, including traditional cuisine. This interest affects the satisfaction of various needs, including mental and social needs, and is related to the desire to preserve and display the values of cultural heritage [18,19]. In Poland, traditional food products are perceived by consumers as extremely tasty and of high quality [5,18,20].

At the national level, the registration of traditional products is carried out by the Ministry of Agriculture and Rural Development, and the entry on the lists is made after evaluation by the competent voivodship marshal (the head of the provincial-level government). The list of traditional products of the Ministry of Agriculture and Rural Development includes 1972 different food products (as of May 30, 2020). The presence in this list favors increasing interest in a product within a given community at the provincial, district or municipal levels (voivodship, poviat, commune, respectively), as well as among tourists, and it becomes a kind of local showcase [21].

Table 1. Number of different edible products (e. g. meat products, honeys, drinks, sweets, etc.) on the traditional product list issued by voivodships in 2020 [22]

Name of voivodship	Number of products
Dolnośląskie	52
Kujawsko-Pomorskie	92
Lubelskie	223
Lubuskie	82
Łódzkie	150
Małopolskie	224
Mazowieckie	154
Opolskie	68
Podkarpackie	246
Podlaskie	74
Pomorskie	179
Śląskie	145
Świętokrzyskie	94
Warmińsko-Mazurskie	46
Wielkopolskie	98
Zachodniopomorskie	55

Meat products occupy an important place in Polish cuisine. This type of products, especially highly processed and consumed in excess, may have an adverse effect on human health and well-being. However, as meat and meat products are a very important part of the diet, they should not be eliminated from it. Polish cuisine is divided into several culinary regions. In many of them, similar raw materials are used for the production of food, but the specificity of the regional cuisine lies in the unique method of combining them, the use of different culinary techniques, the method of serving or naming. It all adds up to the uniqueness of the culinary traditions of a given region.

Description of other traditional Polish meat products

Bacon stewed in beer

The bacon stewed in beer is produced in the village of Łazy in Polish voivodships — Małopolska (Figure 2). Due to good soil and favorable climatic conditions conducive to the production of healthy and high-quality raw materials, this region has a rich agricultural and breeding tradition that is still cultivated. A record of the recipe for bacon stewed in beer has been preserved in the family notebook with recipes since the 1950s. The bacon stewed in

beer is prepared from lean, raw, boneless bacon. In the past, the dish was usually prepared after pig slaughter, which took place on farms several times a year. It was served at the festive table, e. g. for a cold Easter breakfast with horseradish and bread. The bacon is prepared in several stages. First, it is necessary to clean the bacon. The next step is the preparation of the marinade, which consists of garlic, salt, oil, marjoram and pepper. The marinade should be rubbed thoroughly into the bacon, which is then placed into the fridge for a minimum of five hours. Prepared meat is put into a gooseneck (or a heat-resistant dish), beer is added, the dish is placed into the oven preheated to a temperature of about 160–170 °C and simmered covered with a lid for about 1.5 hours. After that, the lid is removed; bacon is sprinkled with caraway seeds and baked for about 15–20 minutes [23].

Figure 2. Part of Poland where bacon stewed in beer is known as a traditional dish

«Kindziuk» or «skilandis» — Traditional specialty from eastern Poland

It is a very tasty and durable product from eastern part of Poland called «Podlasie» (Figure 3), and thanks to the addition of salt, it can hang in a cool place, even in hot weather. This meat is an example of how to manage keeping the product fresh without the use of preservatives and chemicals that are used nowadays. The history of its creation is related to the need to provide food for the harvest season in a time when refrigerators were not known. Meat and cold cuts were prepared in winter, so that they were ready for the time of intensive work in the field (spring and summer). The meat of the ham, sirloin and shoulder blades was cut into 1–3 cm pieces, salted and seasoned abundantly with garlic and pepper, then firmly stuffed into a cleaned pork stomach and sewn up. The product was hung for a short time in a warm place by the stove to be dried, and then taken to the attic, where kindziuk dried in an airy place. Sometimes it was smoked. Kindziuk cut into smaller pieces, served with home-made bread and butter is considered a delicacy. In summer, kindziuk can be used to cook very tasty borscht and sour soups with a specific smell [24].

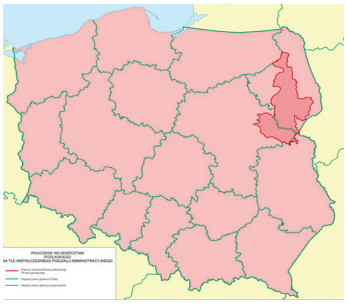

Figure 3. Part of Poland where kindziuk is known as a traditional dish

The modern method of making kindziuk resembles the old one, except that today it is more often made in a pork bladder than in the stomach. Thanks to this, the product has the smaller size. Also, nowadays more spices are added to cure the meat (e. g. allspice, coriander, mustard) [25].

Biłgorajska sausage

Biłgorajska sausage is produced in family farms using local, traditional recipes passed down from generation to generation. From the 60–70s, the recipe, composition of raw materials, production and smoking methods have never been changed [26].

Figure 4. Part of Poland where Biłgorajska sausage is known as a traditional dish

Biłgorajska sausage consists mainly of crushed meat and fat raw materials. The natural casing sticks tightly to the stuffing, is brown to dark brown and evenly wrinkled. Jelly and looser binding of meat raw materials is allowed. It is a crispy and brittle sausage, and has the taste and smell characteristic of pork, smoked and roasted sausages. The smell of smoking is strongly felt in a traditional smokehouse with the use of alder wood and spices, especially garlic. Biłgorajska sausage is based on raw materials obtained exclusively in the region, from the ecologically clean areas of Roztocze. These are pork, garlic and alder wood for smoking [27]. Throughout the production process, the most important factor influencing the quality of the final product is the quality of meat and spices. The raw materials prepared after cutting are mixed, and cooked pork skins are also added. In winter and spring, about 10% more garlic is given due to the loss of aroma during storage. Alder wood is also an important factor, which gives a specific flavor and aroma bouquet. Thermal treatment consists of smoking with hot smoke in a special smokehouse made of burnt bricks fired with alder wood. The smoking process takes about 4–6 hours, then the sausage is cooled down with air [26].

Smoked ham from Wisznice

A typical ham from Wisznice has the form of an irregular cylinder, up to 30 cm long and up to 10 cm in diameter. The finished ham has a brown surface color with a shade of dark cherry, a soft and slightly juicy texture, a salty taste and the smell of alder smoke. It owes its characteristic taste and juiciness to the high-quality raw material from local pig farms, as well as the addition of natural spices and proper smoking. Other stages of production are equally important. The production process is performed as follows. First, the necks of young pigs are rubbed with salt and placed tightly in special pools for 2–3 days. After this time, they are soaked in hot water for 2–3 hours, washed and put to drain. The hams are injected with the brine containing a small amount of peclosalt, covered with spinning nets, tied and hung on smoking

sticks to dry. The hams are smoked for 3–4 hours with the smoke of dry alder wood in closed smokers made of burnt red brick. Then, they are boiled in a steam kettle at the temperature of 80–82 °C for 60–150 minutes and hung up until evaporation of moisture [28].

Thin black pudding (Kaszanka Cienka)

The Rzgów commune is located in the central part of Poland. Due to the poor quality of the soil in this area, the inhabitants mainly deal with cattle and pig farming. One of the characteristic products prepared in the Rzgów commune is thin black pudding. It has the brown to dark brown color, visible pieces of gray-brown liver and light points of groats and fat. Its taste is characteristic of giblets steamed with the addition of blood and buckwheat, moderately salty, with noticeable spices such as pepper. For its preparation, pork raw materials, i. e. masks from heads, center, liver, pork skin, fat, lungs, greaves and blood are used. The distinguishing feature of thin black pudding is the use of white buckwheat cooked in meat broth. The delicate taste of the groats prepared in this way and liver cut into thick cubes gives the product a specific taste. Black pudding is cooked in large kettles, where the product is placed in hot water for about 30 minutes [29].

Figure 5. Part of Poland where thin black pudding is known as a traditional dish

«Salceson polski» from the Karkonosze Region

This product entered the list of traditional meat products in 2019 and it comes from part of Poland, which is called «Dolny Śląsk». To prepare salceson, pork stomachs or beef bladders are filled with a stuffing made of pork head meat, tongues, hearts, meat from pork knuckle with jelly. The spices used to make it are mainly salt, cumin and black pepper. The meat is cured in brine with bay leaf, allspice, garlic and pepper. Then, the stomachs are tied at the ends with yarn, boiled and placed on nets, pressed with a heavy object, in order to obtain the characteristic kidney-shape. Salceson contains clear, amber-colored jelly. The cross-section shows pieces of meat, masks of heads and offal. In consistency, it is compact, resilient, slightly greasy. The taste of garlic, marjoram, pepper and cumin is noticeable and product aroma is typical of cured meat and offal l [30].

Figure 6. Part of Poland where salceson polski is known as a traditional dish

Goose pate

Goose breeding and processing have played one of the important roles in the nutrition of the inhabitants of Zachodniopomorskie voivodship. The recipe for this meat product was brought to this region almost 70 years ago. In spring, the local population bought young geese at fairs that functioned in almost every city. They were most often used as a pate. Currently, older residents of this region are less likely to produce this product, passing the tradition of its production to the younger generation. A modern recipe for goose pate describes the several steps involved in preparing this food product. To begin with, necks of geese should be separated from the bodies. The geese are boiled in a pot, adding vegetables to the water. The liver is cooked separately. Pork bacon is baked on a greased baking tray. The onion is fried. All meat is ground with onions and dried mushrooms. Eggs, a soaked wheat roll and spices are added. The obtained mass is thoroughly mixed, put on a baking tray and sprinkled with marjoram. Goose pate is baked for about 40–50 minutes. The color of this meat product is dark brown, gray-brown in cross-section. Its consistency is firm, soft and spreadable and the taste is described as reminiscent of roasted meat, salty with the addition of pepper and herbs. The product has a delicate aroma, characteristic of roasted meats [32].

Figure 7. Part of Poland where goose pate is known as a traditional dish

Mazurska smoked lard

The cuisine of the Mazury region has always been based on high-quality ingredients in this land. These include the products of animal breeding: pork, beef, less often veal and mutton, and all kinds of poultry. Pigs have been bred for a long time, and traditional fattening methods have been widely used for centuries. Only such meat gives and guarantees an unforgettable taste experience. The basis for the success of a butcher was efficient slaughter, proper processing and preservation of meat. Pigs were most often slaughtered at the farmer's house, and meat was used to make various kinds of cold cuts, often in the homes of their later owners.

Mazurska smoked lard does not contain any preservatives or fillers. One kilogram of raw material is required to produce 0.6–0.7 kilograms of the product. If Mazurska

Figure 8. Part of Poland where Mazurska smoked lard is known as a traditional dish

smoked lard is hung in appropriate airy places at 10 ÷ 12 °C and 75–85% air humidity, it can be a cured meat product suitable for long-term consumption [32].

Conclusion

In Poland, the interest of producers and consumers in traditional food has increased significantly in recent years. This may be caused by the fact that the high quality of traditional products is preserved through the use of natural raw materials and additives, uncomplicated production methods and direct distribution. The growing interest in this type of food means that more consumers choose traditional products, assuming that «traditional» is synonymous with «high-quality».

Recently, a positive food trend has been observed. Polish consumers are more and more willing to reach for healthier, more natural food products. On the other hand, there is a noticeable lack of knowledge about regional and traditional products, even among people brought up in a given tradition. One of the reasons for this situation is the difficulties in reaching this type of products, as reported by consumers.

The promotion of traditional and regional products is supported by projects, including those of the Ministry of Agriculture and Rural Development and implemented by universities such as the University of Life Sciences in Lublin. Also, it should be emphasized that the development of the regional and traditional food market may contribute to an increase in the attractiveness of regions. The continuous trend of consuming regional products can be the basis for activating the community.

The production, protection and promotion of high-quality food play an increasingly important role in the European Union countries. The benefit of promoting regional products is to show consumers and producers how rich various regions of Poland are in terms of traditional food production and cultural heritage.

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NEW APPLICATION OF MICROBIAL L-GLUTAMINASE AS A FLAVOR ENHANCING AGENT IN BEEF BURGERS

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Keywords: *microbial L-glutaminase, beef burger, sensory evaluation, monosodium glutamate, flavor enhancer*

Abstract

L-glutaminase (L-glutamine amidohydrolase EC3.5.1.2) is the key enzyme in enhancing the taste and aroma of oriental fermented foods by increasing their glutamic acid content and as a result imparting a palatable taste. Beef burgers were prepared using different levels of the partially purified L- glutaminase (2.0 to10.0 U/100 g) prepared from Aspergillus oryzae NRRL 32567. Beef burgers treated with 6.0 U/100g and the others treated with monosodium glutamate (5000 ppm) were chemically, sensory and microbiologically evaluated and compared to untreated control during frozen storage at –18 °C for 3 months. Treatment with L-glutaminase (6 U/100g) resulted in an increase of 443% in glutamic acid and a reduction of 63% in glutamine contents resulting in an enhanced preferable taste and odor of the prepared beef burgers. Burgers treated with 6.0 U/100g exhibited the best odor, texture, taste and overall quality scores when compared to the untreated control and samples treated with monosodium glutamate (5000 ppm). During the frozen storage of all samples, an expected slight, but significant ($p \leq 0.05$), increase in the total mesophilic bacterial count was evident and such increase was quite acceptable since numbers did not exceed the limit of $5.7x10^3$ cfu/g. Similarly, the total psychrotrophs did not exceed $3.7x10^2$ cfu/g.

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Introduction

L-Glutaminase (L-glutamine amidohydrolase EC3.5.1.2) catalyzes the hydrolysis of L-glutamine to L-glutamic acid and ammonia [1,2]. L-glutaminase is generally regarded as a key enzyme in enhancing the taste and aroma of oriental fermented foods such as soy sauce by increasing their glutamic acid content and thereby imparting a palatable taste [3,4,5]. Amino acids that are produced by the enzymatic degradation of the proteins contained in the raw materials are well known as basic flavor components of fermented condiments. L-glutamic acid is one such flavor enhancing amino acid produced by the hydrolytic action of L-glutaminase on L-glutamine [6,7].

Monosodium glutamate (MSG) gives the taste "umami", which has been widely recognized as the fifth basic taste besides sweet, acid, salty and bitter. It has been widely used as a flavor enhancer in the food industry. However, there is some questions about its safety, since it may cause some side effects for some people such as wheezing, changes in the heart rate and difficulty in breathing [8,9]. Therefore, the need to develop a safer natural flavor enhancer as an alternative to MSG has been increased.

Glutamic and aspartic acids are well known amino acids contributing not only fine taste, "umami" and sharp sour taste but also nutritional effects to food [10,11]. Therefore, in the present study, microbial L-glutaminase has replaced the use of monosodium glutamate to enhance flavor in beef burgers. Besides, the effect of such replacement on the chemical, sensory and microbiological quality of the produced burger were evaluated.

Materials and methods

Enzyme

Partially purified *Aspergillus oryzae* NRRL 32567 L-glutaminase was utilized in this study [12,13].

Raw materials

Frozen lean beef and fat were purchased from the local market in Giza, Egypt. Soy flour was obtained from Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

Formulation of beef burgers

Beef burgers were prepared, in accordance with the Egyptian standard specification for beef burgers (ES: 1688–2005) [14], as follows: 60% frozen lean beef, 20% fat, 10% soy flour, 1.3% spices mixture, 1.7% salt, 1% corn starch, 1% casein and 5% onion. All ingredients were well mixed and then the mixture was divided into two batches. The first batch was divided into six treatments (a control and five treatments with partially purified L-glutaminase at different concentrations, from 2.0 to 10.0 U/100 g). Each treatment was separately mixed for 5 min at medium speed to obtain homogeneous mixture. This mixture was shaped using a commercial burger maker to obtain burgers of approximately 9 cm diameter, 50 g in weight and 0.5 cm thick. Burgers were then cooked by frying in

sunflower oil and evaluated by sensory analysis. The second batch was divided into three portions; the first was used as control, the second (treatment A) was mixed with 5000 ppm of monosodium glutamate, while, the third batch (treatment B) was mixed with 6.0 U/100g of the partially purified glutaminase (as the best enzyme concentration). Each treatment was processed as described before to obtain beef burgers that were packed in foam plates and stored at –18 °C for 3 months. After storing, samples from the three treatments were chemically and microbiologically evaluated (after thawing) every month. Sensory evaluation was performed on the cooked burgers only at the end of storage.

Enzyme assay

The glutaminase activity was assayed according to Imada et al. [15]. One unit of glutaminase was defined as the amount of enzyme that liberates 1 µmol of ammonia under optimal assay conditions.

Chemical analyses

Moisture, protein, fat, crude fibers, total ash and total carbohydrates of beef burger samples were determined according to the official methods [16]. Free amino acids (FAA) were analyzed by HPLC [17]. The pH value of beef burger samples was measured by homogenizing 10 g of sample with 100 ml distilled water for 30 sec. The pH of the prepared sample was measured using a pH meter (Orion 301, USA) at 20 °C [18].

Microbiological quality

Samples (30 g) were aseptically taken from each beef burger and homogenized with peptone water (0.1%) in a Lab-Blender for 3 min to have a final dilution of 1:10. Serial decimal dilutions were made using the same diluent and then plated in duplicate for bacterial counts. Aerobic mesophilic bacteria were determined on plate count agar (Merck, Darmstadt, Germany) after 48 h incubation at 30 °C. Mold and yeast were counted on acidified potato dextrose agar (Merck, Darmstadt, Germany) after 48 h incubation period at 28 °C. Coliform group was determined on MacConkey agar (Merck, Darmstadt, Germany) after 48 h incubation at 37 °C. Psychrotrophs were determined on plate count agar (Merck, Darmstadt, Germany) after ten-day incubation at 7 °C [19].

Sensory evaluation

Burgers were assessed for a number of sensory characteristics by ten members from the Food Science Department, Faculty of Agriculture, Cairo University, Egypt. They were selected on the basis of interest and experience in sensory evaluation and availability. Panelists were instructed to evaluate color, texture, taste, odor and overall quality using 10-point hedonic scale for grading the quality of samples where, 10 points indicated the highest acceptability and 5 was the acceptance boundary. On the other hand, 4 points indicated unacceptable samples [20].

Statistical analysis

Results were subjected to one way analysis of variance, ANOVA [21], and data were presented as the mean of three experiments.

Results and discussion

Chemical composition of frozen beef

The chemical composition of the frozen beef meat was as follows (g/100 g fresh weight): 75.0, moisture; 18.5, protein; 5.56, fat; 1.0, ash and zero total carbohydrates. These results were in accordance with those of Gehan and Emara [22] and Kassem et al. [23].

Chemical composition of frozen beef burgers

The moisture content of prepared beef burgers at zero time was 62.51, 62.49 and 63% for control, treatment A and treatment B samples, respectively. A slight reduction in the moisture content during burger frozen storage was expected and it was due to the evaporation of moisture through the polyethylene bags [24,25]. The protein content was 15.2, 15.18 and 15.5% for the control, treatment A and treatment B samples, respectively at zero time. A very slight decrease in the protein content during frozen storage at –18 °C was noted and might be a result of slight protein degradation by meat enzymes [26]. Also, at zero time, the fat content was 16.72, 16.70 and 16.3%, ash content was 1.73, 1.75 and 1.77%, crude fibers content was 1.26, 1.33 and 1.00% and carbohydrate content was 2.58, 2.55 and 2.45% for the control, treatment A and treatment B samples, respectively. On the other hand, during storage at –18 °C, storage such values were slightly increased for all samples due to a decrease in the moisture content [25,27,28]. The hydrolysis of meat proteins generates polypeptides that can be further degraded to smaller peptides and free amino acids. This degradation can be produced by endogenous and microbial enzymes as reported by different authors [29,30,31]. The results for free amino acids generated during beef burger processing of both the control and treatment B are presented in Table 1. Mainly, results clearly show an increase in the amount of glutamic acid from 23 to 125 mg/100 g and a decrease in glutamine content from 119 to 75 mg/100 g in the control and treatment B samples, respectively. These results were the direct effect of the added L-glutaminase. Such findings were confirmed when both odor and taste scores increased by the addition of L-glutaminase up to 6.0 U/100 g (Table 2). It was reported that the large amounts of hydrophobic amino acids (such as methionine, valine, leucine and tryptophan, which are usually associated with bitter taste) were generated during processing [32,33]. Some of these amino acids, especially the branched-chain amino acids, have been proved to be metabolized by *Debaryomyces* sp. generating volatile compounds in dry fermented sausage [34]. Meanwhile, high quantities of alanine and glutamic acid caused a sweet taste and umami sensation, respectively, the final sausages [35]. Therefore, the balance of these free amino acids will affect the sensory characteristics of the product [36,37].

Table 1. Free amino acid (FAA, mg per 100 g dry matter) concentration in beef burgers at zero time

FAA	Control	Treatment B
Asparagine	3.4	3.9
Glutamic acid	23.0	125.0
Serine	8.3	9.2
Glycine	20.6	25.0
Glutamine	119.0	72.0
Alanine	58.0	61.0
Arginine	7.1	7.4
Proline	8.2	9.4
Tyrosine	5.3	10.0
Histidine	4.2	6.1
Threonine	6.7	8.2
Valine	8.5	10.0
Methionine	3.4	9.2
Tryptophan	2.1	4.1
Leucine	9.0	13.0

Sensory evaluation of beef burgers as affected by different levels of partially purified glutaminase

Beef burgers were prepared using different levels of partially purified glutaminase (2.0 to 10.0 U/100 g) and data are presented in Table 2. Results indicate that, odor scores ranged from 6.5 to 9.0 and the best odor was observed for the sample with enzyme treatment of 6.0 U/100g. Meanwhile, increasing the enzyme concentration above 6.0 units caused a gradual decrease in the odor attribute. Increasing the enzyme concentration, up to 8.0 U/100 g, in burger treatments caused an increase in texture scores. This increase in texture scores may be due to the increase in the proteases content contaminating the partially purified glutaminase. These proteases improved tenderness of beef burgers as compared with the control sample. Regarding taste, it was noticed that by increasing the glutaminase level, the taste scores increased reaching the highest level of 9.0 for samples treated with 6.0 U/100 g followed by a gradual decrease where scores of 7.5 and 6.0 were obtained for samples treated with 8.0 and 10.0 U/100 g, respectively. Such decrease in scores was due to the appearance of bitter taste (as distinguished by panelists) which was probably due to the degradation of protein and an increase in the bitter amino acids such as: methionine, valine, leucine and tryptophan [32]. The highest color score (8.8) was recorded for the control sample. By increasing the enzyme level, the color attribute decreased due to the increase in the undesirable dark color, which was probably due to the formation of the Maillard reaction between reducing sugars and the formed amino acids and the lowest score (6.0) obtained at 10.0 U/100 g. Also, the highest overall quality score of 8.6 was given by the panelists for the sample treated with 6.0 U/100 g followed by the score of 8.0 for the sample treated with 4.0 U/100 g. Therefore, the concentration of 6.0 U/100 g was selected as the best enzyme concentration and was used for the further experiments.

Data in Table 3 indicate that the pH values of different beef burgers (control, treatment A and treatment B) at zero time ranged from 6.15 to 6.60 with significant differences (5% level) between them. Similarly, the pH values of all samples during storage showed significant differences ($p \leq 0.05$). A slight but significant decrease in pH values for all samples was noted after one month of storage. This might be due to the breakdown of glycogen to lactic acid [24]. Then, pH values were stable until the end of storage.

Table 3. pH values of beef burgers during frozen storage at –18 °C for 3 months.

Treatments	Storage time (month)			
	Zero	1	2	3
*C	**6.15^{c}_{a}	6.05^{c}_{b}	6.02^{c}_{b}	6.01^{c}_{b}
A	6.25^{b}_{a}	6.15^{b}_{b}	6.12^{b}_{b}	6.11^{b}_{b}
B	6.60^{a}_{a}	6.47^{a}_{b}	6.46^{a}_{b}	6.45^{a}_{b}

* C = Control, A = treatment with monosodium glutamate at 5000 ppm, B = treatment with partially purified glutaminase (6.0 U/100 g)
** Means followed by different superscripts (within each column) and different subscripts (within row) are significantly different ($p \leq 0.05$).

Microbiological quality of beef burger

The total bacterial count has been used to assess sanitary quality and safety of various meat products. High microbial load leads to certain undesirable changes in color, flavor and accumulation of their toxins in meat [38]. The results (Table 4) indicate that at zero time all samples including control were acceptable in terms of microbiological quality since the microbial load range was $3.1–3.3 \times 10^3$ cfu/g, which was much lower than the limit (10^5 cfu/g) of the Egyptian Standard Specification (ES: 1688–2005) of frozen beef burgers [14]. It is essential to start with clean raw materials to get high quality products even over a period of long-term storage. During storage, an expected slight, but significant ($p \leq 0.05$), increase in the microbial count was evident and such increase was quite acceptable since numbers did not exceed the limit of 10^3. This could be due

Table 2. Sensory evaluation of beef burgers as affected by different levels (2.0 to 10.0 U/100 g) of partially purified glutaminase ± SD

Characteristic	Treatments					
	*C	2U	4U	6U	8U	10U
Odor	**$7.0^{d} \pm 0.00$	$7.5^{c} \pm 0.01$	$8.2^{b} \pm 0.03$	$9.0^{a} \pm 0.00$	$7.2^{d} \pm 0.01$	$6.5^{e} \pm 0.01$
Color	$8.8^{a} \pm 0.02$	$8.5^{b} \pm 0.00$	$8.2^{c} \pm 0.01$	$7.5^{d} \pm 0.02$	$7.0^{e} \pm 0.01$	$6.0^{f} \pm 0.00$
Texture	$7.5^{d} \pm 0.00$	$8.0^{c} \pm 0.01$	$8.5^{b} \pm 0.02$	$8.9^{a} \pm 0.00$	$9.0^{a} \pm 0.00$	$8.0^{c} \pm 0.02$
Taste	$6.5^{d} \pm 0.01$	$7.5^{c} \pm 0.01$	$8.0^{b} \pm 0.00$	$9.0^{a} \pm 0.01$	$7.5^{c} \pm 0.02$	$6.0^{e} \pm 0.01$
Overall quality	$7.0^{d} \pm 0.03$	$7.7^{c} \pm 0.02$	$8.2^{b} \pm 0.01$	$8.6^{a} \pm 0.02$	$7.7^{c} \pm 0.03$	$6.1^{e} \pm 0.00$

* C = Control ** Means followed by different superscripts within each row are significantly different ($p \leq 0.05$).

to an increase in the amounts of free nitrogen compounds as well as fatty acids due to the slow activity of proteases and lipases during storage, which allow for better microbial growth.

Microorganisms that can grow at refrigerated conditions have usually been called psychrotrophic microorganisms. They are a subgroup of mesophilic microorganisms and when presented in large numbers can cause a variety of off-flavors as well as physical damage to refrigerated food [39]. Psychrotrophic bacterial counts (cfu/g) in beef burgers (treatment A and B as well as control) were monitored during storage at — 18 °C for three months (Table 5).

Table 4. Total bacterial count (cfu/g) in beef burgers during frozen storage at –18 °C for 3 months.

Treatments	Storage time (month)			
	Zero	1	2	3
*C	$^{**}3.3\times10^{3a}{}_{d}$	$3.9\times10^{3a}{}_{c}$	$4.8\times10^{3a}{}_{b}$	$5.7\times10^{3a}{}_{a}$
A	$3.0\times10^{3a}{}_{bc}$	$3.2\times10^{3b}{}_{b}$	$3.6\times10^{3c}{}_{b}$	$4.5\times10^{3b}{}_{a}$
B	$3.1\times10^{3a}{}_{d}$	$3.7\times10^{3a}{}_{c}$	$4.2\times10^{3b}{}_{b}$	$5.7\times10^{3a}{}_{a}$

* C = Control, A = treatment with monosodium glutamate at 5000 ppm, B = treatment with partially purified glutaminase (6.0 U/100 g).
** Means followed by different superscripts (within each column) and different subscripts (within each row are significantly different ($p \le 0.05$).

Table 5. Psychrotrophic count (cfu/g) in beef burgers during frozen storage at –18 °C for 3 months.

Treatments	Storage time (month)			
	Zero	1	2	3
*C	$^{**}1.6\times10^{2a}{}_{d}$	$2.1\times10^{2a}{}_{c}$	$2.5\times10^{2a}{}_{b}$	$3.7\times10^{2a}{}_{a}$
A	***<10	<10	<10	<10
B	<10	$1.8\times10^{2a}{}_{c}$	$2.2\times10^{2a}{}_{b}$	$3.5\times10^{2a}{}_{a}$

* C = Control, A = treatment with monosodium glutamate at 5000 ppm, B = treatment with partially purified glutaminase (6.0 U/100 g).
** Means followed by different superscripts (within each column) and different subscripts (within row) are significantly different ($p \le 0.05$).
*** Estimated Standard Plate Count (ESPC/g).

Counts in the control and treatment B show a similar trend, where a slight increase in the psychrotrophic count was noted during storage and did not exceed 10^2 cfu/g. On the other hand, samples A showed no growth at all and this was probably due to the inhibiting effect of monosodium glutamate [40]. Similarly, a slight but significant increase, in the mold and yeast count was observed during storage with no significant differences between control and sample B. Sample A showed lower counts and this was probably due to the inhibitory effect of monosodium glutamate [40,41] and in all cases numbers did not exceed 3.6×10^3 cfu/g, which is very acceptable. The Egyptian Standard (ES: 1688–2005) [14] for frozen beef burgers indicates that the coliform group count should not exceed 10^2 cfu/g. However, there was no evidence of the presence of the coliform group in any treatment as well as the control.

Sensory evaluation of beef burgers

The results for sensory evaluation of beef burgers after three months of storage are presented in Table 6. Most of beef burger characteristics have been affected by the enzymatic treatment (B). The highest odor, texture and taste scores (8.9, 8.9 and 8.5, respectively) were recorded for treatment B. Meanwhile, the lowest color score (7.0) was given by the panelists for treatment B; the color score decreased due to the increment in the undesirable dark color, which was probably due to the formation of the Maillard reaction between reducing sugars and the formed amino acids. Also, the highest overall quality score (8.6) was for treatment B. Therefore, it can be concluded that, the partially purified glutaminase improved the overall quality of frozen beef burgers especially, the enhancement in odor and taste.

Table 6. Sensory evaluation of beef burgers at the end of 3 month of frozen storage at –18 °C (±SD).

Characteristics	Treatments		
	*C	A	B
Odor	$^{**}7.0^{c}\pm0.01$	$7.5^{b}\pm0.00$	$8.9^{a}\pm0.02$
Color	$8.8^{a}\pm0.00$	$8.7^{a}\pm0.00$	$7.0^{b}\pm0.01$
Texture	$7.9^{b}\pm0.02$	$8.1^{b}\pm0.01$	$8.9^{a}\pm0.00$
Taste	$6.5^{c}\pm0.00$	$7.7^{b}\pm0.02$	$8.5^{a}\pm0.01$
Overall quality	$7.3^{c}\pm0.03$	$7.9^{b}\pm0.02$	$8.6^{a}\pm0.00$

* C = Control, A = treatment with monosodium glutamate at 5000 ppm, B = treatment with partially purified glutaminase (6.0 U/100 g).
** Means followed by different superscripts within each row are significantly different ($p \le 0.05$).

Conclusion

The extracellular L-glutaminase of *Aspergillus oryzae* NRRL 32567 was successfully utilized as a flavor enhancing agent in beef burgers. The produced burgers showed high sensory scores as well as high microbiological quality. As a result, L-glutamiase from this source could be considered as a potential flavor improver in food industries replacing monosodium glutamate. However more applications on other products should be also tested.

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