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We publish original results of the fundamental and applied research in the field of:

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- targeted modification (selection, hybridization, operative manipulation);
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CONTENTS

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IMMUNOCHROMATOGRAPHIC FOOD CONTROL TOOLS: NEW DEVELOPMENTS AND PRACTICAL PROSPECTS

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Abstract
In the modern food production technologies, the tools and means of simple and rapid testing raw materials, intermediate products and the final ready-to-consume food products are in high demand. This monitoring allows determining the content of toxic and pathogenic contaminants and confirms the compliance of the objects being tested with the established regulatory requirements. Mobile tests tools and means (so called test systems) provide the opportunity of wide range monitoring without involving the specialized laboratories and highly qualified specialists. Thus, test systems for detection of toxic and pathogenic contaminants serve as the useful addition to confirming instrumental analytical methods. An actively developing approach for this field testing is the using of immunochromatographic test strips, in which strips all the necessary reagents are applied to the membrane components of the analytical system. Contact of the test strip with the sample being tested, initiates all further interactions and generates the recordable or visually assessable optical signal. The market of test systems based on immunochromatographic analysis is constantly growing, thus offering the permanently widening choice of solutions. However, in recent years there has been a real boom of new developments in immunochromatography field, thus offering various options for highly sensitive and information capacitive analytical systems. This study systematizes these developments and provides their comparative assessment in terms of prospects for their technological implementation and practical application in the coming years. The opportunities of designing the antibodies and alternative receptor molecules for controlling the affinity and the selectivity of recognition of the compounds being monitored are considered. The advantages and limitations of the new nanodispersed markers and non-optical methods for their registration in immunochromatography are discussed. The methods for quantitative assessment of the contaminants content via immunochromatography are characterized. The developed design options of the test systems for multiplex control — simultaneous detection of several compounds — are presented. Examples of integration of immunochromatographic tests with the systems of automatic registration, processing, transfer, storage and analysis of results of numerous tests are represented.

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Introduction

New data on the factors, which affect the quality and safety of food products, expand the range of contaminants that require control — natural and man-made compounds, as well as the microorganisms. Timely detection of these substances in food products allows for the effective prevention of negative consequences for the consumers' health, like acute poisoning, various diseases and long-term physiological disorders.

The public health protection is ensured by a set of analytical monitoring methods [1,2] made of two levels with different methodologies and instruments. Thus, when characterizing contamination of food products with toxic compounds, methods of primary screening testing and confirmatory identification characteristics are used [1,3]. At the first level, immunochemical and other bioreceptor methods based on the specific binding of controlled compounds by complementary biomolecules — antibodies, proteins of other classes, oligonucleotide receptors (aptamers), etc. — are widely represented [4,5]. The second level of the control involves the substantiated identification of the molecular structures of the detected contaminants. For this purpose, mass spectrometry or other means of detailed characterization of fragments (components) of

Table 1. Comparative evaluation of chromatography and immunoassay

	Chromatographic methods for monitoring toxic contaminants	Immunochemical methods for monitoring toxic contaminants
Instrumental implementation	Stationary equipment for specialized laboratories	Small devices for mass testing
Characteristics of the results obtained	Sample components are separated and identified individually	Target compounds bind selectively to the receptor. The resulting complex is detected
Advantages	➢ High sensitivity ➢ Unified procedures ➢ Grounded identification ➢ Supporting conclusions	➢ Quick testing ➢ Simple procedures ➢ Low cost ➢ Wide screening

target molecules and reconstruction of their structure are usually used [6,7].

These two groups of analytical approaches differ in their principles and, as a consequence, in the measurement results. Therefore, they cannot be considered as competitors or potential replacements for each other. Table 1 summarizes the main differences between chromatographic and immunochemical analytical methods.

It is extremely important that the methods of each control level ensure high efficiency, productivity and a minimum number of false positive and false negative results. This review presents the modern capabilities of immunochromatographic analytical systems (lateral flow test strips). These test systems are promising and actively developing means of preliminary screening control of toxic and pathogenic contaminants of food products. In addition, immunochromatographic test systems successfully detect various biomarkers that are significant for other areas — medicine, veterinary, agriculture, monitoring the state of environmental objects and biosafety [8].

Objects and methods

The object of the study was domestic and foreign developments in the field of immunochromatographic control of toxic and pathogenic contaminants of food products, described in articles and patents. The area of research included modern developments of immunochromatographic systems and methods for their use. The search was carried out in the ScienceDirect, PubMed, Google Scholar, eLibrary databases and other open electronic sources. The following keywords were used: immunochromatography, lateral flow assay, membrane test systems, nanodispersed markers, antibody–nanoparticle complexes, registration of labeled immune complexes. The keywords were used in English and Russian versions in a general search of databases and in groups of works related to the control of major food contaminants. In addition, thematically related articles were searched for by citation chains. Non-peer-reviewed, uninformative and duplicate sources, as well as sources that are indirectly related to the research topic, were excluded from the search results. For general assessments, we mainly used recent (last 5 years) review publications, as well as descriptions of experimental developments containing unambiguous quantitative comparative assessments of traditional and proposed new immunochromatographic systems.

General principles of immunochromatographic test systems arrangement and operation

In monitoring food technologies and final products, two groups of test systems based on antibody (immune) recognition of target objects are actively used — microplate enzyme immunoassay (EIA) kits and immunochromatographic tests. To date, immunochromatography is the most successful from the known analytical methods, effectively adapted to field testing without the involvement of equipment and other additional resources [9,10].

Figure 1 shows the processes occurring during immunochromatography. All reagents required for selective detection of controlled toxic or pathogenic objects are pre-applied and dried in certain areas of membrane components of the test strip. As a result, contact of the test strip with the liquid sample initiates:

1) movement of the sample along the membranes of the test strip;
2) interactions of an analyte potentially present in the sample with specific immunoreagents;
3) as a result, the formation of labeled specific immune complexes in certain areas of the test strip.

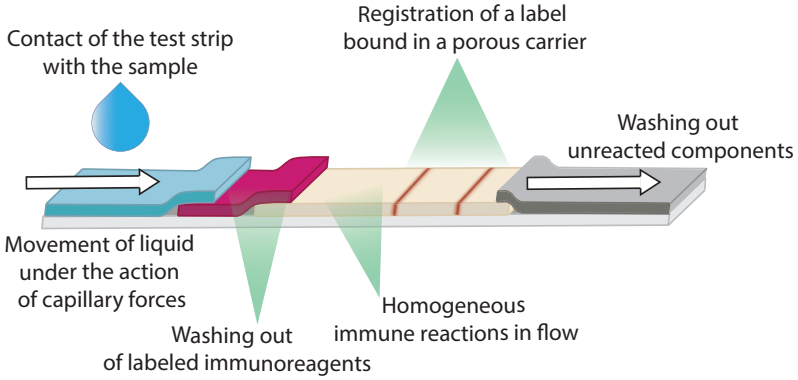

Figure 1. Scheme of the immunochromatographic test system and the processes occurring in it

The presence or absence of these complexes in the simplest version is controlled visually — by a colored label included in their composition. The corresponding coloring allows, based on the testing results, to make a prompt conclusion about the presence or absence of the controlled compound in the sample.

The most well-known problems solved using immunochromatography are pregnancy detection by changes in the concentration of chorionic gonadotropin, for which the very first tests were proposed [11], and monitoring antibodies to the causative agent of COVID-19, for which immunochromatography was successfully applied only recently [12]. This method is in high demand in medical diagnostic practice, which determines priority analytes and forms the basis for commercial demand for immunochromatographic test systems. In addition, immunochromatography is increasingly used in veterinary medicine, environmental monitoring, and food control [8]. However, an assessment of the prospects for technical re-equipment of this method should, first of all, focus on the current tasks of the largest consumers of test systems — medical diagnostics.

Despite the successful technological implementation of the immunochromatography principle, increase in publications describing its new variants occurs in recent years (Figure 2). These articles are focused, as a rule, not on extensive expansion (the application of known methods to new compounds), but on the description of the proposed changes in the testing format. This situation is not typical. As a rule, after the transition of new technical solutions to mass production, their improvement is fixed in the form of know-how or other documents protecting intellectual property. In the case of immunochromatography, there is a boom in new scientific is observed. It is clear that the significance of emerging scientific publications is not the same. Most of the author's proposals remain at the level of laboratory prototypes. However, research activity in the field of immunochromatography deserves attention primarily for assessing what immunochromatographic tests users can expect in the near future, which of the new ideas have the greatest chances of being transformed into reproducible and universal technological solutions.

The proposed review is based on a comparative analysis of the practical prospects of the developments according to uniform criteria, rather than on subjective assessments. What issues will need to be addressed to move from laboratory prototypes to mass production and use of test systems? We analyze which ideas are easily integrated with existing technologies, and which require more complex adaptation. Without pretending to make unambiguous forecasts (the success of implementation depends on many factors), we believe that this critical review of new developments will help users prepare for the analytical solutions of the future.

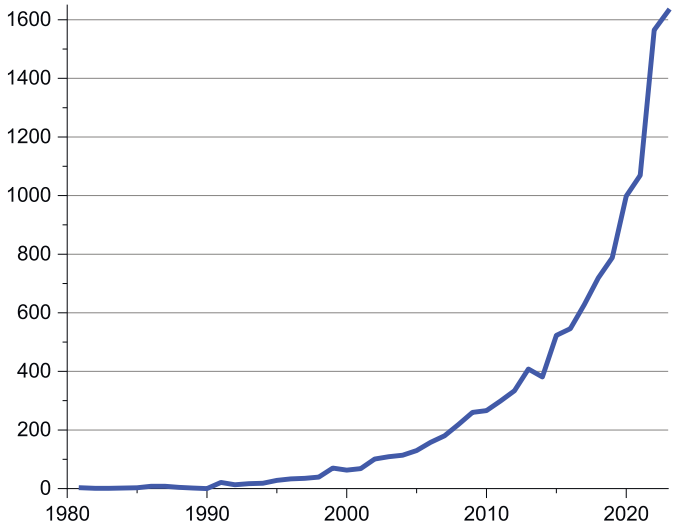

Figure 2. Dynamics (1980–2022) of Scopus indexed publications that meet the Theme criterion: (immunochromatogr* or (lateral and flow and immun*)). The information by state as of 20.10.2024

Main groups of developments for improving immunochromatographic analytical systems

It should be recognized that the same methodological solutions that provided the basic advantages of immunochromatography are currently becoming limitations in the progress of its capabilities. Table 2 summarizes the relationships between the advantages and disadvantages of immunochromatography.

It is clear from Table 2 that proposals for the progress of immunochromatographic test systems cannot be limited to one of their components, which limits the effectiveness of the test systems. Depending on the specific tasks to be solved, needs to change one or another design features of the test systems arise. In this regard, it seems convenient to divide new developments in the field of immunochromatography into five groups, shown in Figure 3. These groups simultaneously reflect both the successive stages of the testing procedure and the directions of changes in

Table 2. The main properties of immunochromatographic test systems, considered as their advantages and limitations

Property	Positive assessment	Negative assessment
All reagents are applied to the membranes of the test strip	Simple completion	Limited number of reagent combinations
Contact of the test strip with the sample initiates all subsequent processes	Easy-to-use	The reactants interact in a strictly fixed order
The duration of the analysis is determined by the movement of liquid along the pores of the membranes	Quick testing	The interactions of the reactants may not reach equilibrium
The colored label in the composition of specific complexes can be registered visually	Using outside the laboratory, without instrumentation support	Subjectivity of results assessment
Specific complexes can be detected immediately after their formation	Quick obtaining of results	No provision for signal development/ amplification

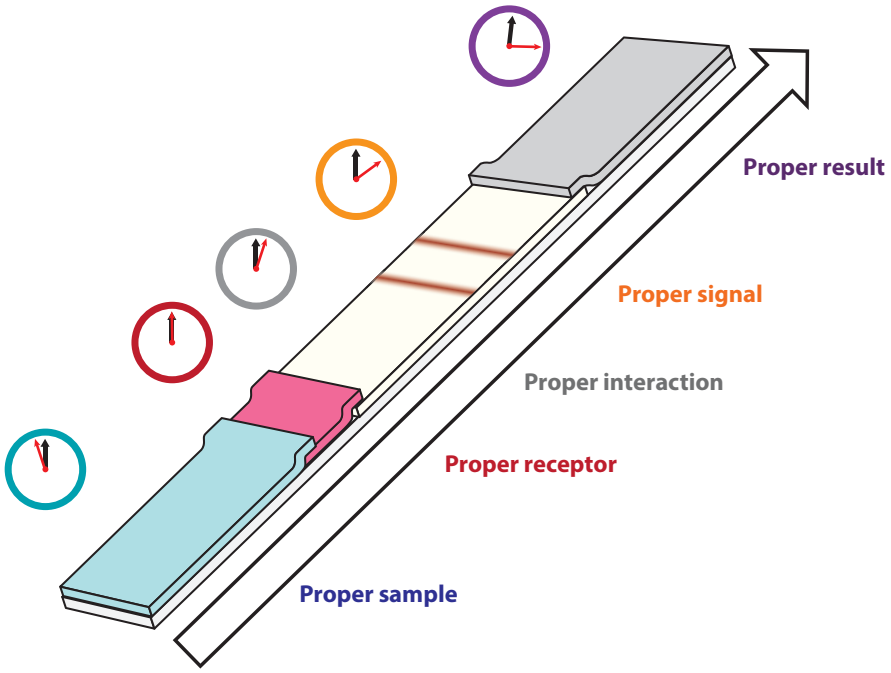

Figure 3. Five groups of methods of impact on the parameters of immunochromatography, localized in time and space

the components of the test systems or processes occurring during immunochromatography.

The analytical characteristics of test systems can be improved by changing the sample preparation, the receptor reagent used, the conditions of immunochromatographic interactions, signal generation, and the processing and interpretation of results. In general, approaches belonging to different groups can be effectively combined within a single test system. However, if we are talking about changes in the same stage of immunochromatography, then, as a rule, it is necessary to choose one of the proposed changes or, based on several ideas, to construct a new approach with possible additional features.

Most of the proposed developments are aimed at lowering detection limits. It should be noted that even with established and controlled maximum permissible levels of contaminants contents, test systems that detect the contaminants in significantly lower concentrations are in demand. They allow for simplified handling with samples and increased reliability of the results obtained. In addition, a lowering in the detection limit is usually accompanied by increased accuracy in measuring analyte concentrations.

However, quite often the improvements declared in publications are disproportionately greater than could be expected from the changes made. For example, with a small increase in the intensity of label staining, a decrease in the detection limit by tens to hundreds of times is described. It is important to take into account that the detection limits of immunoassay systems have theoretical limitations described in the previous century by Jackson and Ekins [13]. Therefore, achieving record levels should be associated with the use of either extremely high-affinity interactions (high binding constants in the antigen-antibody reaction) or methods for generating a detectable signal in the analysis that combine a high response per unit complex formed with extremely high reproducibility of measurements [14]. Confirmed super-record sensitivities [15,16] should be accompanied by fundamental changes in testing methods that ensure the possibility of overcoming the calculated chemical-kinetic thresholds.

Effective sampling and sample preparation techniques

It should be recognized that immunochromatographic tests, initially proposed for work with biological fluids (urine, blood), are most effectively compatible with liquid samples. Target compounds are uniformly distributed in the volume of such samples, and matrix components that can potentially affect the movement of liquid along the test strip and the implementation of specific interactions during this movement are usually successfully separated. This separation is ensured either by means of selected membrane elements of the test strip or by simple and rapid actions directed to the original sample, such as filtration or chemical precipitation of some of its components. Dilution of liquid samples is often proposed as a sample preparation. This idea is useful as a way to introduce components into the reaction mixture that promote washing out and uniform movement of immunoreagents on the membrane, as well as preventing non-specific interactions. However, significant dilution of samples reduces the sensitivity of testing (in terms of the unit volume of the original sample) and therefore cannot be considered a good solution [17].

When moving to solid samples, we should take into account the uneven distribution of contaminants in them and the regulatory requirements for sampling procedures that should compensate for this unevenness. Therefore, testing is forced to lose the compactness characteristic of

traditional immunochromatography variants. It is important that the complication of sampling and sample preparation does not lead to the loss of the advantages provided by subsequent testing. The requirements for simple and rapid analytical procedures should still be met [18]. For this purpose, sample preparation methods with rapid destruction of solid components and effective release of target compounds are being developed. The transfer of traditional extraction protocols with evaporation recommended for chromatographic analytical methods can hardly be considered acceptable, in contrast to protocols with rapid separation of the extract and solid components. However, when proposing new sample preparation methods, it is necessary to additionally confirm that they ensure complete extraction of target compounds.

It should be noted that in some cases there is no need for a strict quantitative assessment of the degree of contamination. In this case, simple methods that extract most (but not necessarily 100%) of the contaminant from samples are acceptable. In this regard, the idea of the participants of the recent European MycoKey project, who developed a compact kit for testing grain contamination with mycotoxins, seems beautiful and promising. It uses a compact vacuum cleaner to collect microparticles from the surface of grains. These particles contain toxigenic mold fungi that produce mycotoxins. The obtained test results are close to the data of generally accepted extraction protocols [19].

From the point of view of practical prospects, new immunochromatographic systems for monitoring food toxicants with a modified sample preparation stage should be assessed primarily based on whether they retain rapidity and ease of use. These requirements are taken into account when monitoring the quality of liquid food products, including commercially available immunoanalytical systems. Analysis of solid food products will require the additional instrumental support. At the same time, the question of effective and universal solutions acceptable for technological implementation remains open today.

Recognition of structurally related analytes (selectivity management)

Immunochromatographic testing involves the use of antibodies as bioreceptor molecules that selectively bind target compounds. Natural combinatorial mechanisms allow the creation of active sites of antibody capable of binding to various compounds with high affinity and specificity. However, the complexity of food contaminant control is due to the potential presence of structurally similar compounds in the tested samples — the original techno- or biogenic contaminants, the products of their transformation in living organisms and in the environment. Designing antibodies that recognize each such substance is difficult and often impossible. In addition, the need to work with a wide range of immunoreagents of different selectivity during testing increases the cost of analysis and makes it more complex.

As a rule, one of the requirements is set for immunoassay of structurally related compounds: either individual or group-specific testing. In the first case, antibodies are selected that recognize one substance — the most common, the most dangerous, etc., while binding to other substances of this family is minimized. In the second case, antibodies should bind the maximum number of toxic representatives of the controlled family of substances with comparable efficiency.

Initially, various approaches to the design of derivatives (haptens) used in the synthesis of immunogens were applied to manage the selectivity of antibodies [20,21]. In some cases, combinations of different haptens during immunization and analysis make it possible to significantly change the selectivity of testing and bring it closer to practical requirements [22,23]. Moreover, the selectivity of test systems is not a quantitative parameter that strictly corresponds to differences in the affinity of interaction of antibodies with different haptens. It has been shown that by varying the concentrations and ratios of reagents in a competitive immunoassay, the assay can be made either more highly specific or broadly specific [24]. This approach is most effective for nonequilibrium analytical methods, which include immunochromatography.

Another promising resource is immunodetection of small molecules using non-competitive interaction schemes. When selecting schemes for conducting immunoassay of low-molecular compounds, it is taken into account that binding of two antibody molecules to these substances is impossible due to steric hindrances. Therefore, so-called sandwich schemes with the formation of antibody-antigen-antibody complexes are excluded for them and competition between the antigen in the sample and the antigen-protein conjugate introduced into the system for binding to antibodies is realized. Unfortunately, for competitive immunochromatography schemes, detection of low analyte concentrations is pretty impeded. This leads to a slight decrease in the degree of binding of labeled antibodies to the antigen-protein conjugate, which within one test strip cannot be compared with binding in case of the analyte absence [14]. To overcome these limitations, variants of the so-called open sandwich and other immunoassay formats have been proposed [25,26]. Thus, the formation of an antibody-antigen complex and the recognition of this complex (but not its components) by the second antibody makes it possible to obtain direct dependences of the recorded signal on the concentration of the analyte and thereby eliminate the difficulties of competition described above.

In recent years, based on the established approaches to obtaining recombinant antibodies, computational methods for molecular design of their antigen-binding sites have been developed. As a result of these calculations and subsequent genetic substitutions, it is possible to increase affinity and eliminate undesirable cross-interactions [27–29]. Such improved antibody preparations may be a promising tool for immunochromatography.

From the point of view of practical application, the approaches described in this section do not require fundamentally new technological solutions and can be applied quite quickly to improve existing test systems. The only significant point that should be noted is the difference in the immobilization conditions on marker nanoparticles for recombinant and natural full-length antibodies.

Replacement of antibodies with alternative receptor molecules

Of interest are the developments of membrane test systems — analogues of immunochromatographic ones, in which other bioreceptors are used instead of antibodies. The most actively considered in this regard are oligonucleotide receptors — aptamers, which have already been selected for thousands of practically in-demand compounds and are successfully used in various analysis formats [30–33]. Researchers note a number of indisputable advantages of aptamers in comparison with antibodies:

- low cost of production;
- high degree of reproducibility of the properties of different drugs;
- ease of chemical modification with fixed stoichiometry of products;
- stability over a wide range of conditions, including thermal stability.

These advantages certainly deserve to be applied in new analytical systems. Despite the fact that a large number of prototype aptachromatographic test systems have already been described, they have not become the subject of wide manufacture. To a large extent, this is due to the difference in the structures of the analyte binding sites: conformationally stabilized components of the protein globule in the case of antibodies and very labile short nucleotide chains in the case of aptamers. This lability limits the efficiency of aptamer-analyte interactions when they are transferred to the kinetic regime and carried out in the near-surface flow in membrane pores. To realize the analytical potential of aptamers in membrane tests, it is apparently necessary:

- more detailed characterization of their structure and reactivity under different conditions;
- adaptation and modification of existing solutions for immobilization and stabilization of test system components in relation to aptamers.

It is also necessary to mention molecular reagents as promising analytical reagents. Molecularly imprinted polymers (MIPs) are the preparations obtained by polymerization of a mixture of monomers in the presence of target molecules for detection and subsequent leaching of the analytes. The polymer structure forms niches for analyte binding, which are characterized by geometric complementarity and affinity interaction of individual groups of the polymer and analyte. Traditionally, MIPs were used in columns for preliminary enrichment of tested samples. However, recent developments in the field of surface imprinting and the demonstrated capabilities of highly selective detection of molecules of different structures presenting in complex matrices indicate the prospects of MIPs as components of various bioanalytical systems [34,35]. Although the appearance of such commercial products is still a long way off, the extreme cheapness and stability of MIPs determine the advisability of continuing developments in this direction.

Variation of interaction conditions during immunochromatography

Traditional immunochromatography is based on the use of a standard set of reagents — a nanoparticles — antibodies conjugate and binding reagents in the test and control zones. In this case, the production of test systems includes the same actions for applying reagents to membranes, and in immunochromatography, the same order of processes is carried out, determined by the washing out and movement of specific reagents along the membranes.

However, such a standard order may not be optimal for a number of analytical tasks. Often users are ready to partially sacrifice rapidity and, by extending the testing by several minutes, obtain a significant (up to one or two orders of magnitude) gain in the detection limit. In this regard, the typical order of interaction in a number of new developments varies:

- the reaction of the analyte with the antibody-label conjugate is implemented outside the test strip and is carried out in a separate test tube, where, under homogeneous conditions, the formation of the immune complex is significantly accelerated [36,37];
- instead of directly conjugating specific antibodies with a label, a combination of native specific and conjugated anti-species antibodies is used, which allows for independent variation of the content of specific antibodies (for effective competition) and label (for an intense signal) in the system [38,39];
- the dynamics of release of reagents applied to membranes is modulated by the addition of various slowly soluble compounds [40,41];
- the reagents are applied to the working membrane, performing a barrier function and modulating the speed of movement of reagents or focusing them in certain areas of the membrane [42,43];
- to form immune complexes, several successive stages of movement of different immunoreagents along the test strip are used [44,45];
- to control the movement of reagents and their effective removal from the pores of the working membrane, test strips are fixed on microrotor systems [46,47];
- traditional test strip geometry is integrated with the additional means for concentrating sample components from a large volume [48–50].

The most critical issue in the scalable implementation of these ideas is the reproducibility of analytical procedures. In addition, a number of developments involve complicating the procedure for manufacturing test systems with

strict orientation of the application of reagents within a separate test strip.

Markers for immunochromatography

Nano- and submicron-sized particles are used in immunochromatography as carriers for immunoreagents and detectable markers. This choice simplifies analytical methods, allowing the formation of immune complexes on membranes to be recorded in real time — by an optical or other signal from the bound marker. Larger total area of the particle preparations accelerates interactions on their surface and ensures rapid testing.

The markers in mass-produced test systems are essentially limited to gold nanoparticles and colored latex particles. Although significant lowering in the detection limit have been shown for a number of alternative markers, the relationships between particle parameters and test system characteristics using them are considered in scattered studies and are not presented as universal conclusions. The choices of particle sizes, the composition of their conjugates with antibodies, and the conjugation method are based on a limited number of recommendations, the scope of which remains a subject of debate. Therefore, when considering ultrafine particles as reagents for immunochromatography, the characterization of new preparations is in demand. A useful tool for achieving this goal is the passportization of the properties of candidate nanoparticles, presented in Table 3. It allows one to focus on a number of a priori known properties of the materials used. In addition, it allows excluding unwarranted hopes for preparations if their analytically significant characteristics do not undergo significant changes.

From a variety of candidates nanoparticles — immunochromatographic carriers and markers proposed in developments in recent years and characterized in review publications [51–54], we will highlight two groups that appear to be the most promising.

The first group — quantum dots — are semiconductor metal particles of the core-shell structure, characterized by stable fluorescence, the peak of which is determined by the particle size. The advantages of quantum dots in comparison with traditional colorimetric markers:

- proportional increase in signal with increasing excitation light at an extremely low background signal;
- stability of glow (no fading);
- common excitation light for simultaneously used particles of different sizes;
- possibility of multi-color multi-assay;
- the possibility to select the composition of quantum dots and their spectral characteristics when working with different matrices, excluding autofluorescence of sample components.

The combination of these advantages leads to a reduction in the detection limits of immunochromatography by several dozen times [55,56]. Note that the need for radiation generating fluorescence is not a critical complication. Such radiation can be provided by a variety of simple devices, including hand-held detectors of currency authenticity operating in the same ranges of excitation and emission light. To date, a number of portable systems for working with immunochromatographic tests based on quantum dots have been described. In them, background radiation is excluded to increase the accuracy of measurements (the test strip is placed in a closed chamber), and the signal is recorded by a portable detector or a smartphone camera.

Recent developments have led to commercially available nanoparticle preparations with hydrophilic coatings and reactive surface groups for conjugation with antibodies. These capabilities are extremely valuable for the technological implementation of new highly sensitive immunochromatographic systems.

The second group of promising carriers and markers are magnetic ultradisperse particles. As follows from Figure 4, their use in immunochromatography leads to the integration of a number of advantages:

- elimination of diffusion limitations for immune interactions;
- increasing the area of contact of analyte molecules with antibodies;
- collection of analyte molecules from a large sample volume;
- simple and rapid separation of immune complexes by applying an external magnetic field;
- easy removal of sample matrix components;
- concentration of the re-solubilized drug in a small volume before running the immunochromatography.

As a result, specific immune complexes can be registered on the test strip membrane both by the intrinsic optical properties of magnetite particles and by using complexes with chromogens — organic dyes or other functionalized nanoparticles. The gains achieved in the detection limits, determined primarily by the concentration factor, amount to about two orders of magnitude [57,58].

The most critical issue in creating such serial tests is the choice of conditions for the effective movement of magnetic particles along immunochromatographic membranes. Potentially, the magnetic particles can be modified

Table 3. Key characteristics for the evaluation of new marker particles for immunochromatography

Quantitative characteristics	Qualitative characteristics
Limit of detection of the marker from the unit volume	Price and availability
Limit of detection of the marker per unit area	Ease of conjugation
Number of markers attached to one immunoreagent	Stability under storage
Changes in marker signal during conjugation	No influence of samples' matrix on the marker signal

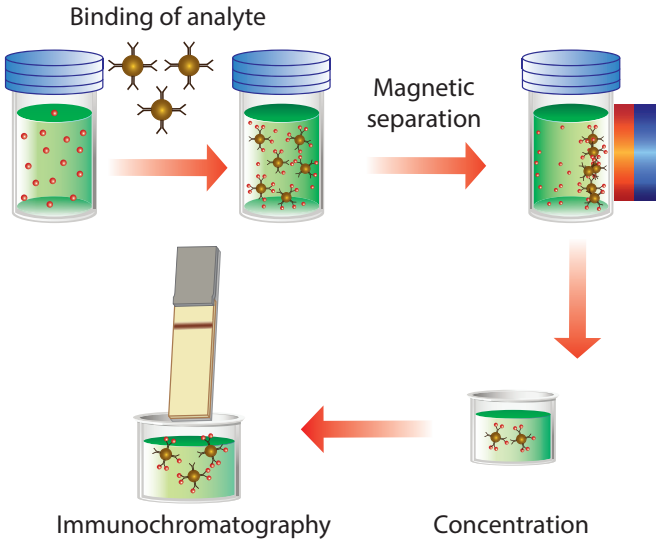

Figure 4. Integration of analytes' concentrating with the help of magnetic ultradisperse immunosorbents and immunochromatography

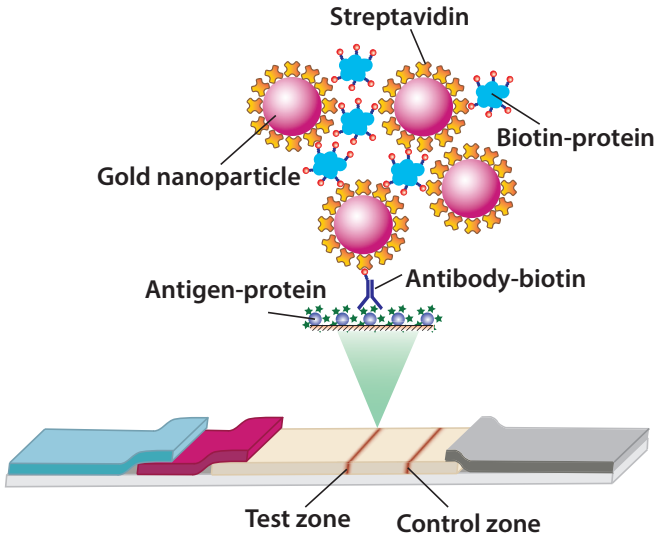

Figure 5. Signal amplification in immunochromatography via the functionalized nanoparticles aggregation

to form various surface coatings. However, the choice of the optimal coating for the immunochromatographic purposes has not yet been grounded.

Signal amplification methods in immunochromatography

A promising idea for reducing the detection limit in immunochromatography is to enhance the signal from the detectable marker after it has bound as part of the immune complex in a certain area of the test strip. However, the choice of marker is limited by the requirements for its size. Larger particles, although they give a larger registered signal, are less acceptable as participants in immunochromatography processes. With increasing size, the risks of incomplete washing out of the applied marker, its binding to the membrane before reaching the binding zone, etc. increase. However, we can carry out initial interactions with nanoparticles of a suitable size, and then increase them in the binding zone or ensure that they generate an additional amplifying signal.

Such amplifying processes can be the complex formation of several variants of conjugates. nanoparticles functionalized with complementary interacting combinations of molecules: biotin — streptavidin, additional pairs of antigens and antibodies, etc. The principle of such amplification is shown in Figure 5 and can provide gains in detection limits of up to two orders of magnitude.

Of the various options for increasing the size of nanoparticles, the most promising seems to be the reduction of metal salts on their surface. Such processes, quickly and effectively implemented on immunochromatographic membranes, include, in particular, the widely known silver mirror reaction. In recent years, many new solutions in the field of immunochromatography have proposed using nanozymes as markers — nanoparticles that have enzymatic activity. Advantages of nanozymes bioanalytical markers — ease of production and modification, low cost, high catalytic activity, stability during storage, resistance to inactivation. The most studied types of nanozyme catalysis are oxidase, peroxidase, catalase, superoxide dismutase, and for oxidation-reduction reactions that can be realized using a large number of chromogenic substrates. It should only be taken into account that the optimal conditions for transformations of chromogens (pH, composition of the reaction medium) can be different for natural enzymes and nanozymes. Literature data [59–64] allow asserting the possibility of using nanozyme amplification in 1–5 minutes to achieve a decrease in the detection limits of immunochromatography from one to three orders of magnitude.

When moving from laboratory prototypes to production technologies, the issue of reproducibility of amplification results becomes critical. Systems with multiple additional conjugates are associated with significant risks, since variability of results is significantly increased due to both differences in the properties of new reagents and discrepancies between strips in the dynamics and completeness of washout. For amplification by particle growth and nanozyme catalysis, the variants (not yet debugged) with amplifying reagents dried on additional membrane components, which are tightly pressed to the working membrane after immunochromatography, would be preferable.

Multianalytical immunochromatographic systems

An important opportunity to expand the information content of immunochromatographic testing is the simultaneous detecion of several compounds using one test strip. The use of several markers for this purpose, registered in one binding zone and distinguished by optical properties, is not very popular because minor nonspecific signals can significantly worsen the differentiation and quantitative assessment of specific signals. Variants with geometric separation of binding zones of different specificity are preferable for this purpose [65–68]. In this case, different

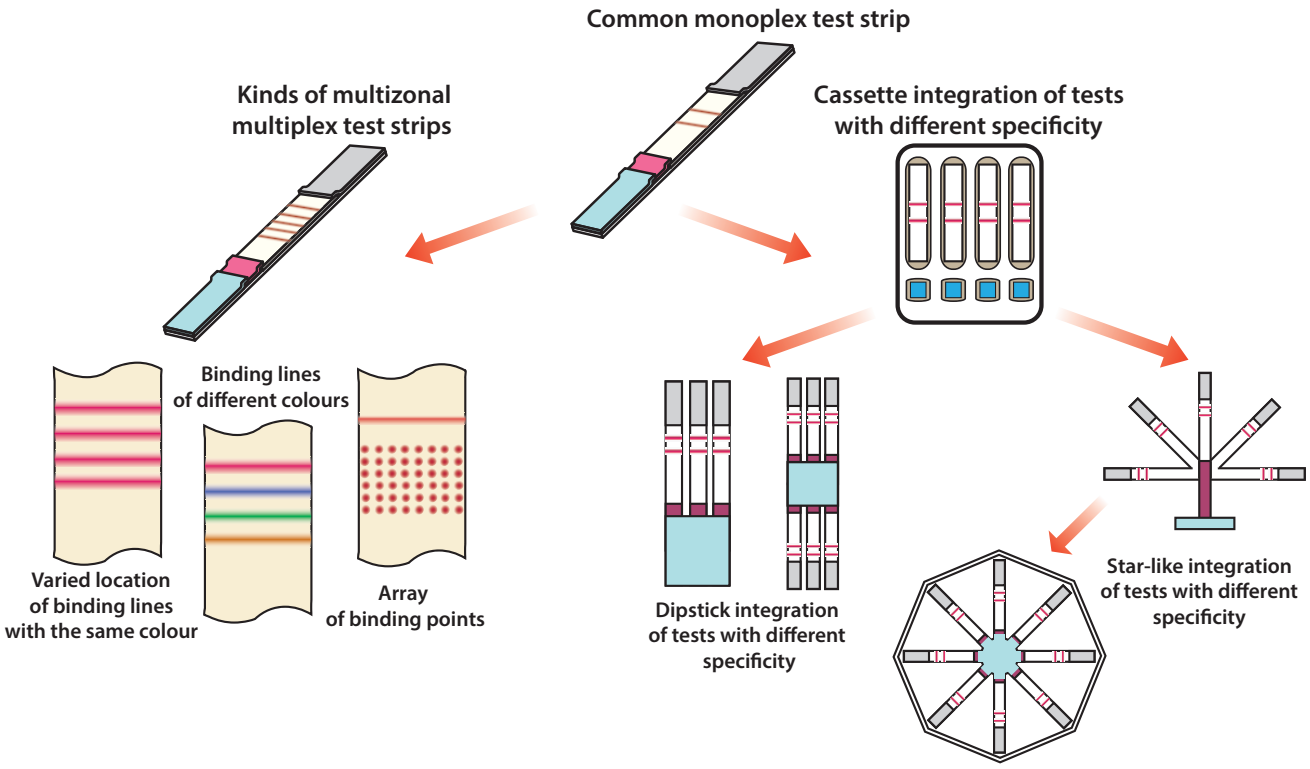

Figure 6. Variants of immunochromatographic test systems for simultaneous detection of several compounds

labels can be used in these zones for ease of identification. The geometric diversity of multiplex test systems is shown in the Figure 6.

In practice, variants with cassette combination of individual test systems and application of several lines with reagents of different specificity to the working membrane are already successfully used. It should be noted that combining immunoreagents of different specificity on one test strip is not a trivial task. Depending on the location of the binding zones, the duration of reagent interactions, the blurring of the liquid front moving along the membrane, and the dynamics of its intersection with the binding zones change. Therefore, it is necessary to evaluate the properties of the reagents and select their location on the membranes so that the analytical characteristics of the multitest do not deteriorate in comparison with the monotest.

A significant gain is achieved by transfer to a two-dimensional geometry for applying the immunoreagents of different specificity to a test strip [68]. Standard test strip sizes allow for the simultaneous, high-precision control of the content of approximately 30 analytes in a sample. However, the productivity of test system manufacturing in this variant is significantly reduced, even despite the availability of automation tools for this process.

Similar approaches can be used for semi-quantitative testing when monitoring a single analyte. By selecting the concentrations and reactivity of the immunoreagents applied to various binding zones on the working membrane, it is possible to ensure that the number of colored zones on it changes in accordance with the concentration of the analyte and is characterized by several threshold levels — Figure 7.

Application of test strips outside the scope of immunochromatographic recognition: field molecular genetic diagnostics

In monitoring pathogenic contaminants of food products and raw materials, an effective tool is the possibility to highly selectively recognize regions of nucleic acids that are characteristic namely of these pathogens. The first kind for the successful implementation of this con-

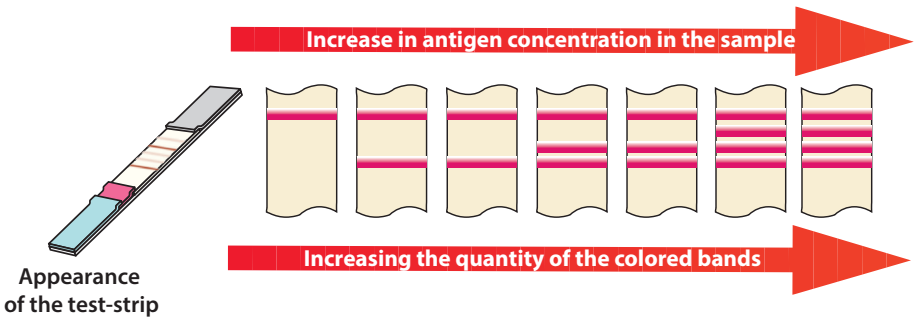

Figure 7. Immunochromatographic test system for semi-quantitative characterization of the target analyte content — changing number of visually observed colored binding zones

cept was the polymerase chain reaction (PCR) and analytical systems based on its application. Currently, PCR is widely used and is considered as the gold standard of molecular genetic diagnostics. Special devices, thermal cyclers, have been developed and are mass-produced to perform PCR in real time (with direct detection of the resulting target products). They provide cyclic modes for the formation and dissociation of nucleic acid complexes, enzymatic synthesis of complementary DNA chains, and registration of the reaction product by the glow of the fluorophore label.

PCR continues to be the main method for laboratory diagnostics, since it requires special sample preparation, the use of stationary equipment, and testing in specially equipped rooms with sterile conditions that exclude contamination of samples.

A promising alternative to PCR is isothermal amplification methods. In them, the production of multiple copies of target unique regions of nucleic acids is provided by special enzymes and combinations of oligonucleotide probes. The test sample, after simple and rapid sample preparation, is mixed with reagents for amplification, and all subsequent reactions occur simultaneously at a constant temperature. The exclusion of a thermal cycler in such testing brings it closer to field use. For this purpose, the use of simple non-device analytical means for the final stage of assessing the presence of a specific reaction product seems reasonable. Such proven means include immunochromatographic test strips, which is what determines the interest in their integration with isothermal amplification methods. The number of developments implementing this idea (the general principle of analytical processes is shown in Figure 8) has increased sharply in recent years [69–71], although there are only a few examples of commercialized products for quality control of agricultural products and food.

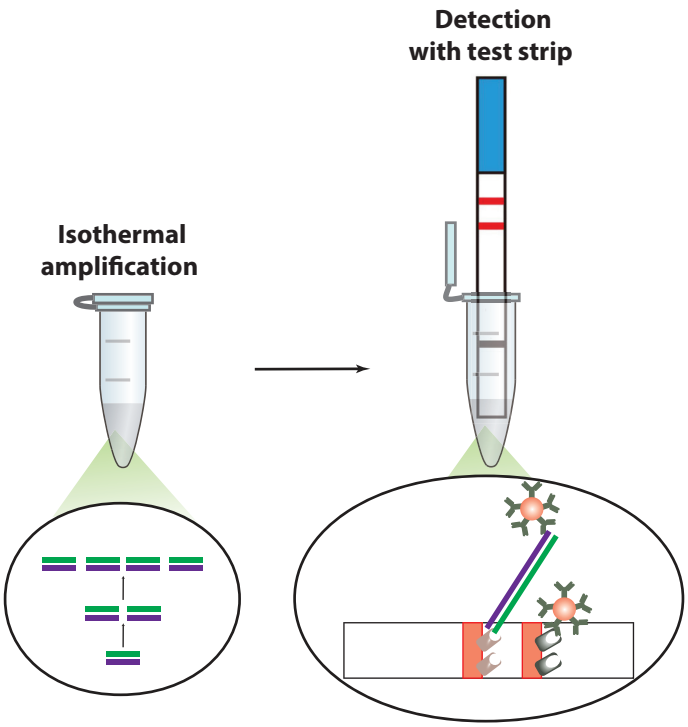

Figure 8. Scheme of the combination of isothermal amplification and detection of its products using immunochromatographic test strips

What features should be taken into account when considering the implementation of such analytical systems into practice?

1) The most promising testing protocols should be those that involve simple and rapid sample preparation at the initial stage. Today, there are a significant number of solutions developed for working with biosamples in medical diagnostics that can also be applied to various food matrices.
2) Despite the exclusion of the thermal cycler, when switching to isothermal amplifications, there remains a need for thermostatic instrumentation, since the enzymes used for these processes are capable of effective catalysis only at certain temperatures in the range from 37–42 °C to 60–70 °C. In this regard, low-temperature amplification variants are most interesting, such as recombinase polymerase amplification, a shift of interactions to suboptimal lower temperatures, or the search for/design of new enzyme preparations for amplifications.
3) Direct binding of the products of amplification processes on the test strip by complementary interactions of nucleic acids is impossible in most cases, since the product is a double-stranded DNA fragment without sticky ends. Therefore, most successful developments involve the use of primers with low-molecular labels, while additional receptors with high-affinity affinity to labels are used to form colored zones on the test strip — biotin — streptavidin, various combinations of antigens and antibodies specific to them.
4) A new promising tool for molecular genetic diagnostics are nucleases of the Cas family in complex with guide RNAs, activated strictly after highly selective recognition of nucleic acid regions complementary to the guide RNAs. The results of the investigations demonstrate the possibilities of their successful combination with isothermal amplification processes.

Registration of markers by various physical parameters

An actively developing trend in immunochromatographic developments in recent years is the creation of systems with marker's registration based on several physical parameters [72,73]. Thus, variants of test systems with combinations of colorimetric and fluorescent, optical and magnetic, optical and photothermal registration, a combination of traditional colorimetry and registration of giant Raman scattering have been described. Systems with three and even four registration methods are also proposed. Such an expansion can significantly reduce the detection limits of labeled immune complexes. Corresponding gains of several orders of magnitude in

comparison with traditional colorimetry are observed in a number of systems using the same markers and immunoreagents. In addition, magnetometry detects marker particles with equal efficiency regardless of their location in the pores of the test strip and the distance from the surface. This minimizes the dispersion of signals typical of colorimetric detection.

However, it is necessary to keep in mind that alternative methods of registration require the development of appropriate instrumentation — portable detectors [74,75]. Given the availability of various prototype solutions in the literature, the question of their serial production remains open (with the exception of photometric detectors). Technical solutions used for other tasks with large objects and large distances require significant adaptation for use in immunochromatography. The analytical characteristics of portable detectors can still be estimated approximately. Volumes of their production and cost will be determined by the variety of controlled analytes and the scale of demand.

The question of the practical implementation of the integration of two detection methods in one test system remains controversial. It is unclear how two types of measurements should be organizationally combined and how to solve problems arising from discrepancies in results. Moreover, when rechecking all negative results of a screening test with a more sensitive method, the role of the initial testing becomes unclear. Perhaps for some specific tasks such two-level control will be justified, but these issues have not yet been worked out.

As noted above, the situation with colorimetric registration and processing of immunochromatographic testing results can be considered successful today. There are a number of mass-produced (including in Russia) portable detectors, programs and attachments for smartphone cameras and other communication devices, software for transforming the resulting images of test strips into analyte concentrations [76]. So the practical prospects of this area of developments can be assessed optimistically. However, the question of the competitive advantages of specialized detectors compared to the adaptation of widely produced general-purpose communication devices remains open. With the formal economic preference of the second option, it remains uncertain how reproducible the results obtained using different cameras are. Metrological certification of detectors of standard configuration seems more realistic.

The development of modern information technologies and Internet resources makes it possible, in the near future, to consider field diagnostic tools (such as immunochromatographic tests) as elements of information systems that ensure the collection, processing and transmission of test results with their inclusion in integrated databases [77,78]. This makes it possible to conduct prompt comprehensive monitoring of the situation with products contaminations, analyze observed trends and formulate reasonable and well-grounded recommendations.

Control of toxic contaminants in food: assessment of the actual situation in Russia

After analyzing idealized systems, it is appropriate to move on to consideration of existing immunochemical analytical systems, their diversity and capabilities. The possibilities of their use in different countries vary significantly and require separate detailed research. Within the framework of this publication, we will limit ourselves to information characterizing the practical implementation of monitoring and application of immunochemical test systems in Russia.

Among the toxic contaminants of food products, the most popular objects of control are antibiotics. Chloramphenicol, the tetracycline group, streptomycin and zinc bacitracin are still widely used as antibacterial drugs in veterinary medicine due to their low cost and high efficiency. Immunochemical test systems for mycotoxin control are just beginning to be widely distributed, and immunodetection of pesticides is very limited.

The main method used to control antibiotics in food is chromatographic, and its results indicate the prevalence of situations with the simultaneous presence of several antibiotics in samples [79]. In this regard, in Russia, the legislatively established control of antibiotic residues is focused primarily on this method. The "List of regulatory means and methods of testing for the application and implementation of the requirements of the Technical Regulations of the Customs Union 021/2011"[1] contains mainly methods for determining antibiotics based on the principles of HPLC–MS/MS and enzyme immunoassay.

According to the decision of the Eurasian Economic Commission[2], all groups and classes of antibiotics previously regulated by Technical Regulation of the Customs Union 034/2013 "On the Safety of Meat and Meat Products"[3] are included in the Technical Regulation of the Customs Union 021/20114, the scope of which also concerns processed products. At the same time, verification of raw materials supplied by third-party organizations, although it assumes its assessment within the framework of production control, but not with such a frequent frequency as for microbiological indicators. Usually, raw materials are

[1] List of international and regional (interstate) standards, and in case of their absence — national (state) standards containing rules and methods of research (testing) and measurements, including rules for sampling, necessary for the application and implementation of the requirements of the technical regulations of the customs union and the implementation of conformity assessment of objects of technical regulation. For Technical Regulation of the Customs Union "On the Safety of Food Products" (TR CU 021/2011). Retrieved from https://www.gostinfo.ru/trts/List/45 Accessed October 09, 2024 (In Russian)

[2] "On amendments to some decisions of the Customs Union Commission and the Council of the Eurasian Economic Commission" Decision of June 23, 2023 No. 70 Retrieved from https://www.alta.ru/tamdoc/23sr0070/ Accessed October 09, 2024 (In Russian)

[3] TR CU 034/2013 Technical Regulations of the Customs Union "On the safety of meat and meat products" Retrieved from http://docs.cntd.ru/document/499050564. Accessed October 09, 2024 (In Russian)

[4] TR TU 021/2011. "Technical Regulations of the Customs Union On food safety (as amended as of July 14, 2021)" Retrieved from https://docs.cntd.ru/document/902320560#8Q20M0. Accessed October 20, 2024 (In Russian)

controlled once every three months. The decision of the Eurasian Economic Commission does not impose obligations on manufacturers to identify the current wide list of monitored antibiotics. Enhanced control remains the prerogative of state control carried out by specialized departmental laboratories.

Low levels of permissible antibiotic content in animal products necessitate the use of highly sensitive and high-throughput methods for their detection. However, the number of chromatographic methods that allow the simultaneous extraction and determination of a wide range of antibiotics in animal products is very limited. One of the causes for this limitation is the difficulty of simultaneous extraction of various antibiotics. Therefore, the development of a unified pre-treatment based on the general properties of the isolating antibiotics is in great demand [80].

High sensitive instrumental analytical methods such as high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC–MS) are not suitable for monitoring bulk samples due to the high cost of equipment, the complexity of pre-treatment, and the need for qualified personnel. This situation determines the demand for alternative simple and productive methods, such as immunochemical ones.

The "List…"[1] includes about 30 immunochemical methods, but almost all of them implement the principle of enzyme immunoassay and are oriented towards imported test systems. Rapid immunochemical analysis is practically not represented in the "List…". For effective monitoring of food contaminants, it is necessary to develop and start the production of immunochromatographic test systems, both in traditional formats and using the improvements described in the review.

The public interest in immunochromatographic tests for medical purposes caused by the COVID-19 pandemic has led to the expansion of manufacturing bases for large-scale production of such tests and increased awareness among potential end users about them [81]. This opens up great prospects for the expansion of the application of immunochromatography in the field of food safety in the nearest future.

Conclusion

The examples of new developments in the field of immunochromatographic systems, reviewed herein, demonstrate the opportunity of overcoming the limitations, traditionally attributed to these systems: low sensitivity and the inability to quantify the analytes content. This allows us assuming the new prospects for the immunochromatography application to solve the issues of food safety ensuring. The application of new markers, signal amplification systems, portable detectors and the immunochromatography results processing tools significantly increases the competitive potential of these test systems, their information output and objectivity of the test results. However, the capabilities, demonstrated in the laboratory developments of the prototype test systems, do not guarantee the success in starting the production and subsequent wide use. The inclusion of new components and stages into immunochromatographic devices requires additional time- and labor-consuming technological developments in order to ensure the reproducibility of the new test systems properties, their long-term stability, and confirmation of absence of the negative effects of the biosamples components. The approaches versatility is also important, allowing combination of the new testing principles with the monitoring of wide range of analytes — in food and agricultural technologies, in veterinary and medical diagnostics. The wide range of new developments makes it possible to assume with high degree of probability, the upgrading the immunochromatographic testing tools that will be capable to detect significantly lower analytes' content while maintaining the rapidity and methodological simplicity of the testing.

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CAMEL MEAT PERCEPTION AND THE FACTORS INFLUENCING ITS CONSUMPTION WILLINGNESS AMONG ALGERIAN CONSUMERS

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Abstract
The purpose of this research was to evaluate Algerian consumers' perceptions of camel meat and identify the variables driving their intention to consume it. A survey of 142 participants selected at random through in-person interviews and a self-administered questionnaire in El Oued district located in the southeast of Algeria assisted in this study. The data revealed that 93.7% of the participants had consumed camel meat previously at least once; however, merely 6.3% of participants had never consumed this meat before. The majority of participants (83.1%) held a favorable perception of camel meat and expressed a willingness to consume it again (80.3%). Conversely, 59.2% of the participants said that eating camel meat was often linked with particular occasions, like religious and sociocultural activities. Taste (65.5%) was the primary factor influencing customers' choice of red meat varieties, while tenderness (58.5%) was the most strongly correlated attribute with camel meat perception. Analysis of the determinant variables showed that males were willing to eat camel meat at a higher rate than females (92.1% vs. 66.7%; $\chi^2 = 14.440$; $p = 0.000$). A substantial beneficial impact was also evident due to prior consumption and the favorable perception of this meat among consumers ($\chi^2 = 29.043$; $p = 0.000$ and $\chi^2 = 52.857$; $p = 0.000$). The willingness to indulge in camel meat consumption was also significantly impacted by ascertaining how frequently consumers consume this meat. Altogether, this investigation offers a clear understanding of how consumers perceive the quality of camel meat and the factors that influence its consumption.

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Introduction

Camels are well adaptable in dry and semi-dry climates that are unsuitable for taming other livestock species and are thus considered as a valuable domestic creature serving as an excellent alternative for livestock production [1,2]. Camels are exceptional as they can endure crude environmental conditions with high temperatures, sustain amidst variable and poor food and water supplies, and thrive upon fibrous plants that have low nutritional benefits and are unpalatable to other animals [3,4]. Camel farming offers a feasible solution to protein scarcity in dry areas, given the implications of global warming and the spread of dry and semi-arid climates in numerous regions worldwide [5].

Camel meat consumption is typically prevalent in the Middle East and northeastern Africa [6,7]. Unlike other commercialized red meats like beef and sheep, it has a higher content of polyunsaturated fatty acids (PUFA) and a smaller proportion of fat and cholesterol, making it one of the finest meats with fewer detrimental implications to human health [8,9]. Conversely, the greater toughness of camel meat compared to other varieties of meat becomes its drawback [10]. According to Husain et al. [7], there is a high demand for fresh camel meat, which is anticipated to account for 22% of the global market share by 2022. Camel meat accounts for 45% of the market in Africa, the Middle East, and Europe. By 2022, it is expected that camel meat will also be highly popular in the Asia Pacific area [11]. The camel meat market is primarily concentrated in Africa, the Middle East, and Europe covering almost 45% share, with a projection of expansion in Asia Pacific countries in the years coming ahead [11]. Although camels may be raised more profitably compared to other livestock in dry and semi-dry environments, serving as a viable substitute for meat, camel meat is sometimes misinterpreted as having less nutritional content and poor quality than other red meats.

As a result, the majority of research conducted until now has revolved around evaluating consumer attitudes and behaviors regarding different kinds of red meat, particularly those derived from cattle, sheep, and goats [12,13,14]. Fur-

thermore, it is noteworthy to point out that there is a lack of research focusing on the global and Algerian consumers' perceptions of camel meat and the variables that may impact their purchase decisions concerning camel meat.

This emphasizes the necessity of conducting an investigation to have a deeper understanding of customers' perceptions of this meat. Thus, the current study sets out to evaluate consumers' opinions on camel meat and explore the variables influencing their propensity to eat it.

Objects and methods

Study area

The Ethics Committee of the Life and Natural Sciences Faculty at the University of El Oued in Algeria governed the execution of the study. The study participants were informed in advance regarding the confidentiality of the data shared by them that would be utilized exclusively for research purposes. El Oued situated in the southeastern regions of Algeria (located at 3322′16.823″ N latitude and 650′52.686″ E longitude) which is 630 kilometers away from the capital city, Algiers, served as the geographical location for this survey conducted from January to May 2024. Given the district's prominence in breeding and marketing activities pertaining to camels, it was deemed an ideal location for this investigation. Additionally, the socio-demographic characterization of the study location also aligned well with the research goals.

Survey and data collection

The current research was performed with the contributions of 142 survey participants from diverse socio-demographics who had undergone random sampling for this study (Table 1).

Table 1. Socio-demographic characteristics of the participants (n = 142)

Characteristics	Frequency (n)	Percentage (%)
Gender		
Female	66	46.5
Male	76	53.5
Age (years old)		
18–25	35	24.6
26–35	34	23.9
36–45	42	29.6
46–55	18	12.7
> 56	13	9.2
Region of residence		
Urban	67	47.2
Rural	75	52.8
Educational level		
Primary and lower	5	3.5
Middle	11	7.7
Secondary	39	27.5
Higher	87	61.3
Income level perception		
Low	73	51.4
Acceptable	69	48.6

The trivial overrepresentation of men in the sample (53.5%) compared to women (46.5%) falls in line with the findings of earlier surveys carried out in Algeria, which could be attributed to the societal context of the research area's population, within which men restrict women from participating in projects led by foreigners. Age distribution of participants was about equal across the board, with the exception of those between the ages of 46 and 55 (12.7%) and people older than 56 years (9.2%). The various demographic pyramids in the research region may be the root cause of this occurrence. Comparable demographics were observed with regard to the educational levels of survey participants. Higher-educated respondents made up 61.3 percent of the study sample, exceeding all the other educational level categories. A balanced representation of respondents from both urban and rural areas was observed while analyzing their residential demographics. Prior to the initiation of the survey, each participant was granted verbal authorization to participate in the study. The survey was conducted in close collaboration with participants who expressed enthusiasm for participating in the study and had attained 18 years of age in order to ensure legitimacy. In order to ensure the confidentiality and consistency of the participants (each person participated in the study only once), the questionnaires were flagged with unique codes devoid of any personal information. A self-managed, structured questionnaire was used in face-to-face interviews with the study subjects to collect data on consumer opinions regarding camel meat and the variables influencing their desire to consume it. There were 2 parts in the questionnaire, both with multiple-choice questions.

The initial segment of the survey asked questions to gather data concerning participants' sociodemographic characteristics, including gender, age, place of residence, degree of education, and household income. The questions in the second section sought information on customers' perceptions of camel meat and the variables that influence their propensity to purchase it. The questionnaire comprised both closed (single-choice and multiple-choice) and open-ended (questions without any prospective answers) options. For issues that demanded additional details and clarification, open-ended questions were used to enable participants to share their ideas in their own words. Nominal data, such as selecting "female" or "male", "yes" or "no" was gathered through closed-ended questions. For polytomous data, participants were allowed to choose from lists of options with greater complexity, such as "daily", "more than once a week", "festive" and "never".

Statistical analysis

The statistical analyses were conducted utilizing the Package SPSS, Version 27.0 software. In order to determine the variables influencing consumers' propensity to purchase camel meat, Fisher's exact test or Chi-square analysis was used to examine survey data. Fisher's test was run in cases wherein more than 20% of the cells have predicted

frequencies less than 5. Conversely, if less than 20% of the cells had anticipated frequencies <5, the Chi-square method was employed. A significance threshold of $p < 0.05$ was deemed appropriate for all tests.

Results

Table 2 depicts the participants' opinions regarding camel meat. The survey outcomes revealed the frequent consumption of camel meat by most of the participants (93.7%) in their lifetime. Alternatively, regardless of the camel meat being favorably perceived by the majority of the participants (83.1%), they expressed willingness for its repeated consumption (80.3%), whereas it was graded fourth in preference by 41.5% of participants ensuing sheep, beef, and goat meats, respectively. Additionally, for many participants (59.2%), camel meat intake was more closely associated with jubilant occasions. On the scale of quality attributes, the respondents ranked taste as the most significant factor (65.5%) during selection of red meats, whereas the soft texture of camel meat (58.5%) was largely responsible for the consumer's fulfillment and was seen as the biggest obstacle diminishing camel meat intake.

Table 2. Participants perception of camel meat (n = 142)

Questions	Responses	Frequency (n)	Percentage (%)
Did you consume the camel meat previously?	Yes	133	93.7
	No	9	6.3
Which rank was taken by the camel meat among the others: sheep, beef and goat?	1st	12	8.5
	2nd	20	14.1
	3rd	51	35.9
	4th	59	41.5
Are you willing to eat the camel meat again?	Yes	114	80.3
	No	28	19.7
How do you appreciate the camel meat?	Good	118	83.1
	Not good	24	16.9
What are the main attributes driving your decision toward meat?	Taste	93	65.5
	Fat level	12	8.5
	Habit	10	7.0
	Tenderness	7	4.9
	Health reasons	6	4.2
	Nutritional reasons	5	3.5
	Quality	5	3.5
	Religious reasons	2	1.4
	Availability	1	0.7
	Bone level	1	0.7
What are the main attributes limiting your choice for camel meat?	Tenderness	83	58.5
	Taste	51	35.9
	Color	7	4.7
	Odor	1	0.7
How often do you consume camel meat?	Daily	1	0.7
	More than once a week	9	6.3
	More than once a month	39	27.5
	Festive	84	59.2
	Never	9	6.3
How do you perceive the price of camel meat?	Affordable	65	45.8
	Not affordable	77	54.2

The impact of the sociodemographic variables on consumers' willingness to eat camel meat is shown in Table 3. The findings reflected that out of all the parameters, only the customers' gender had an important bearing ($p < 0.05$) on their camel meat consumption decision. It is also noteworthy to note that, in comparison to the females, the males exhibited a statistically significant higher inclination for camel meat consumption (OR = 5.833; $p = 0.000$). Nevertheless, age, place of residence, education, income level, and perception of pricing did not impact consumers' willingness to eat camel meat significantly ($p > 0.05$) (Table 3).

Table 4 provides a summary of the variables influencing willingness to consume camel meat in association with generic eating practices. Consequently, the participants' overall opinion of camel meat intake became the most crucial variable influencing their consumption decisions ($\chi^2 = 52.857$; $p < 0.05$). Respondents with an unfavorable perception were less inclined to consume camel meat in contrast to consumers perceiving it favorably (OR = 0.021; $p = 0.000$). The second most significant variable influencing consumers' willingness to eat camel meat was correlated to the experience of its intake ($\chi^2 = 29.043$; $p < 0.05$). Customers having previous experience of eating camel meat appeared to be more likely to have repeat consumption rather than those who had never tasted it (OR = 0.022; $p = 0.000$). Conversely, people who either never tasted camel meat or ate it only on rare occasion-specific days exhibited a lesser inclination to consume it ($p < 0.05$) than people who consumed it frequently (every month, every week, or every day). Lastly, consumers' willingness was not significantly impacted by camel meat availability ($p > 0.05$) (Table 4).

Discussion

Apparently, this research may indicate camel meat consumption by all the interview participants. This trend aligns with the findings of the study by Brahimi et al. [15] conducted to analyze the camel meat consumption patterns in regions with high prevalence of camel meat consumption, particularly dry and semi-dry parts of the nation, demonstrating that it was an attractive component that might be effectively enhancing the local population's access to food. Previous research endeavors elaborate on the autochthone background of the majority of the participants residing in the study area which justifies the traditions and practices associated with the use of camel meat [15]. Baba et al. [6] assert that the ethnic importance of camel meat is liable for its predominant consumption trend in the Middle East and northeastern Africa. According to Faye [16], an apparent sociocultural connection exists between dromedary and its derivatives and the inhabitants of desert nations. Additionally, recent investigation by Bahwan et al. [9] pointed out that camel meat is often consumed in Asian and African nations because of its scarcity. A total of 898.150 camels

Table 3. Impact of socio-demographic variables on camel meat consumption willingness (n = 142)

Variables	Consumption willingness (Yes) n (%)	Consumption willingness (No) n (%)	Odds Ratio	*F*-Value /χ^2	*p*-value
Gender					
Female	44(66.7)	22(33.3)	1	14.440	0.000
Male	70(92.1)	6(7.9)	5.833		
Age (years old)					
18–25	24(68.6)	11(31.4)	1	7.693	0.094
26–35	25(73.5)	9(26.5)	1.273		
36–45	37(88.1)	5(11.9)	3.391		
46–55	17(94.4)	1(5.6)	7.791		
> 56	11(84.6)	2(15.4)	2.520		
Region of residence					
Urban	53(79.1)	14(20.9)	1	0.111	0.739
Rural	61(81.3)	14(18.7)	1.150		
Educational level					
Primary and lower	4(80.0)	1(20.0)	1	3.582	0.283
Middle	8(72.7)	3(27.3)	0.666		
Secondary	35(89.7)	4(10.3)	2.187		
Higher	67(77.0)	20(23.0)	0.837		
Income level perception					
Low	59(80.8)	14(19.2)	1	0.028	0.868
Acceptable	55(79.7)	14(20.3)	0.932		
Price perception					
Affordable	54(83.1)	11(16.9)	1	0.592	0.442
Not affordable	60(77.9)	17(22.1)	0.718		

Table 4. Impact of variables related to consumption practice on camel meat consumption willingness (n = 142)

Variables	Consumption willingness (Yes) n (%)	Consumption willingness (No) n (%)	Odds Ratio	*F*-Value /χ^2	*p*-value
Consumption experience					
Yes	113(85.0)	20(15.0)	1	29.043	0.000
No	1(11.1)	8(88.9)	0.022		
Overall perception					
Good	109(92.4)	9(7.6)	1	52.857	0.000
Not good	5(20.8)	19(79.2)	0.021		
Consumption frequency					
Never	1(11.1)	8(88.9)	1	26.361	0.000
Festive	67(79.8)	17(20.2)	31.529		
More than once a month	37(94.9)	2(5.1)	148.000		
More than once a week	8(88.9)	1(11.1)	64.000		
Daily	1(100.0)	0(0.0)	/		
Availability					
Available	64(86.5)	10(13.5)	1	3.758	0.053
Not available	50(73.5)	18(26.5)	0.434		

were processed for meat production in Asian, African, and Middle East countries in 2020 [17].

Regardless of camel meat's fourth ranking on the preference list following sheep, beef, and goat meat, sheep meat still remains the most popular variety of meat among the participant population. These findings are consistent with the results of the study by Benaissa et al. [18], claiming that the consumption rate of camel meat was extremely low in Algeria, accounting for 33% of the country's red meat consumption with fluctuating patterns. In the same perspective, Brahimi et al. [15] noted that camel meat accounts only for 1.24% of overall red meat consumption by Algerians, regardless of its anticipated production of 6000 tons/year in 2017 [19]. In urban areas, butchers' primary focus is on marketing other red meat varieties, specifically beef, which remains the most favored choice among consumers. They consider the marketing of camel meat as a supplementary effort to approach a diverse range of red meat consumers, particularly in rural regions, still experiencing a demand for camel meat [15]. Nonetheless, the taste of sheep meat rendered it the most frequently chosen option by customers of rural areas in comparison to alternative kinds of red meat [15]. The issues raised by research participants, particularly with regard to the tenderness of camel meat, are

attributed to its latest rating against other red meat variants. Therefore, for the majority of people, camel meat's hardness is one of the main barriers to its consumption. As a result, it has been noted by Bahwan et al. [9] that camel meat is typically thought to be fibrous and rough. The findings of an additional study conducted recently reflect camel meat's greater hardness ratings along with more roughness in contrast to beef and mutton meat, respectively [5]. Thus, it is noteworthy to emphasize that camel meat from older animals tends to have higher shear force values and tougher texture than younger animals' meat [5,20]. Conversely, Brahimi et al. [15] reported consumers' perception of a higher meat-to-bone ratio in beef rather than camel meat, which renders camel meat less desirable to consumers.

Likewise, Mohamed Ali [21] highlighted camel meat's property of losing its volume by 50% during cooking which makes it bonier. As a result, buyers opine camel meat as a costly variety because of its larger percentage of bones and cooking shrinkage, thus forcing them to spend more money on beef for its larger edible portion [15].

The majority of the study participants held a favorable perception of camel meat and a greater proportion of them with a willingness to eat this meat pointed towards camel meat's increasing popularity owing to its unique attributes and health benefits. As a result, people experiencing chronic heart ailments, those undergoing weight loss programs, and health-conscious customers are likely to prefer camel meat over other red meat variants [6]. Osaili et al. [22] speculate that these advantages of camel meat may have contributed to its increased demand. Consistent with the above assertion, newer research in Somalia has revealed extensive consumption of camel meat by Somali customers in comparison to other red meat alternatives due to the health and nutritional benefits of camel meat [23]. According to Husain et al. [7], people in Saudi Arabia assumed that camels were immune to the majority of livestock-related illnesses, which justifies the heightened demand for camel meat.

Our study reflected that camel meat's taste was the determinant trait driving consumers' preference for its consumption over other red meat variants. This aligns with the findings of other studies conducted in the same area as the present study, establishing that consumers' delight after red meat intake was mostly driven by its flavor [15,24]. Previous studies conducted in different nations have also emphasized that the taste of red meat, particularly that of sheep, correlates with consumers' perceptions and propensity to consume it [13,25,26].

Consistent with our study findings, Manheem et al. [5] and Bahwan et al. [9] highlighted, that camel meat's hardness is still a common constraint that restricts customers' inclination toward its consumption. Brahimi et al. [15] specified that in contrast to aged camel meat, consumers frequently opt for the tender meat owing to its ease of cooking. Thus, some recent research recommended pretreatments and cooking techniques that have a good impact on camel meat's tenderness [27,28]. In order to increase the tenderness of the meat, an appropriate cooking procedure should be followed, especially for preserved camel meat [5]. In another study, Maqsood et al. [29] explored the use of plant proteases offered to increase the tenderness of camel meat.

Although camel meat holds an obvious position in the culinary culture of people residing in dry and semi-dry regions, most of the study participants acknowledged infrequent intake of camel meat. This could be the consequence of recently evolved dietary patterns as well as limited breeding and supply of camel meat in our study region. This was in line with study findings published by Bougherara et al. [30], claiming minimal consumption of camel meat at the study location because it is poorly marketed and manufactured. According to a recent study by Lamri et al. [31], including an online poll of 665 consumers, camel meat is not often consumed by customers of the Kabylia (Algeria) district with 54.3% of residents who had never tasted it, 1.6% who consumed it regularly, 35% occasionally, and 9.1% seldom. Brahimi et al. [15] claim that camel meat was largely bought and utilized to celebrate festivals by preparing the well-known traditional food known as couscous. The residents of the study location follow a custom of celebrating the onset of fall by serving couscous made of camel meat. Kadim and Mahgoub [32] highlighted a similar pattern in which the pastoral societies avoided slaughtering other than for ceremonial contexts.

While analyzing the variables impacting consumers' propensity to consume camel meat, our research revealed that out of all the sociodemographic attributes, only the consumers' genders had a substantial bearing. Although consumers' age, dwelling locations, educational attainment, family income, and perceived price do not have a considerable implication, the reduced frequency of camel meat intake among the survey participants may induce a minor influence on their propensity to purchase it. Women exhibited a low likelihood to consume camel meat compared to men, potentially due to their higher innate sensitivity regarding animal welfare concerns [33]. Consequently, Topcu and Elmi [23] noted that customer satisfaction resulting from camel meat intake may be significantly impacted by issues involving animal welfare. Studies analyzing consumer behavior have consistently shown a higher likelihood of women to exhibit animal-friendly thinking compared to men, along with different points of view among men and women pertaining to significant ethical concerns of animal care [34,35].

This investigation made it readily apparent that the general impression of the product was the primary element driving the consumption practice influencing the customers' inclination to consume camel meat. Furthermore, our study's findings validated that consumers favorably perceiving camel meat are more inclined to eat it rather than those with an unpleasant hedonistic experience of its

consumption. This was in line with the observations published by Topcu and Elmi [23], who discussed how satisfaction after eating camel meat is influenced by the feelings and actual quality attributes of the food. As per the preceding researchers, the term "sensory quality" refers to a broad spectrum of sensory experiences, including visual accuracy and genuineness of quality along with the area of genesis bolstering the consumers' fulfillment after camel meat intake [23]. Additionally, it was determined that the hedonic attractiveness successfully influenced customers' propensity to consume camel meat. It is worth noting that a recent study conducted in the same area correlated the desire to eat camel meat with a favorable view of its hedonistic features [24].

Undoubtedly, customer expectations regarding a food product's quality indicators serve as the primary factor driving their desire to consume it. Moreover, de Andrade et al. [13] have observed that customers' expectations stemming from their prior experience with a specific variety of meat can impact their consumption decisions favorably or unfavorably. Our study indicates that consumers' prior experiences of eating camel meat render it appropriate for their consumption. Apparently, participants with prior experience of camel meat consumption exhibited a higher propensity to eat it rather than those without any prior experience.

Our study critiqued that the frequency of consumption had a substantial impact on consumers' willingness to consume camel meat. Consequently, it was discovered that consistent and frequent intake of this meat was significantly and positively correlated with a favorable propensity to consume it. Consistent with this outcome, prior research has indicated that intermittent consumption of sheep and goat meat might act as a barrier to satisfying consumers' appetite for these meats [13,24,31]. This can be attributed to the familiarity aspect of this meat, which has been previously emphasized in several other papers and even in this investigation [12,25,31].

Conclusion

This research imparted a general summary of how people perceive camel meat and the variables affecting their purchase decisions. In a nutshell, the current research shows that camel meat continues to be widely consumed and holds a significant and integral spot in the dietary habits and lifestyles of the consumers residing in the research region, yet other red meat variants are preferred over this meat type. Additionally, the majority of the survey participants expressed favorable views of this meat and were likely to consume it repeatedly throughout the course of their lives. Although camel meat was less consumed in the study region in comparison to other red meat variants or demonstrated an infrequent routine of consumption, the majority of respondents acknowledged its occasional intake. However, the research revealed that the tenderness of camel meat was the main factor restricting its consumption. While analyzing the influential variables, the results showed that males exhibited more inclination toward camel meat consumption than females. Furthermore, a strong beneficial association has been observed across all the facets of consumption practices, including the willingness to eat camel meat, the frequency and experience of consumption, and the general perception of this meat. The study findings provide insightful data on the dietary intake of camel meat, which will be useful in identifying the best marketing tactics to increase consumer acceptability of camel meat and thus expand its commercial sale beyond traditional markets.

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EXPLORATION OF THE POTENTIAL RESERVOIRS OF *PSEUDOMONAS* SPP. BACTERIA AT MEAT PROCESSING FACTORIES AND POULTRY FARMS

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Abstract
One of the microorganisms that cause spoilage of meat during its storage is the bacteria Pseudomonas. To prevent contamination of the finished products with these bacteria, it is important to find the places at the enterprise where they aggregate. Within the framework of this study, the objects and premises of the production facilities at meat processing factories and poultry farms were explored to detect their contamination with bacteria of Pseudomonas spp. The potential reservoirs of those bacteria were defined at these plants. In addition, the species diversity of Pseudomonas was established at the production facilities environment at the enterprises. 27 production facilities environments (structures, equipment, package containers) were examined for the presence of bacteria with the method of washings. The samples were examined to detect Pseudomonas bacteria, with their subsequent identification with the method of time-of-flight mass spectrometry MALDI-Tof-MS. 487 strains of bacteria of the genus Pseudomonas were isolated, which strains are represented by 47 species. As a result of the study it was found that all 27 production facilities were contaminated with various species of Pseudomonas. From two to fourteen species of Pseudomonas bacteria were detected at all facilities. 12 facilities of the enterprise for slaughter and processing of broiler chickens were contaminated with Pseudomonas gessardii. Pseudomonas bacteria spp. (identification is traced down only to its genus) were found at 10 objects. Pseudomonas tolaasii and Pseudomonas brenneri were found at 9 and 8 objects, respectively. The surfaces of 6 objects demonstrated contamination with Pseudomonas chlororaphis ssp chlororaphis and Pseudomonas koreensis. Other Pseudomonas species were found at 1–5 sites. Pseudomonas fluorescens were detected at 8 pork processing plant sites, Pseudomonas gessardii were found at 5 sites. 4 sites were contaminated with Pseudomonas chlororaphis ssp. chlororaphis and Pseudomonas koreensis, 3 objects contained Pseudomonas tolaasii, Pseudomonas spp., Pseudomonas rhodesiae, Pseudomonas libanensis and Pseudomonas extremorientalis. The remaining species of Pseudomonas were found at one or two sites in the territory of the pork processing plant. It was found that all production environment sites, regardless of their distance from the raw materials and the finished products, were contaminated with Pseudomonas bacteria. At the same time, the sites that had no contact with the food products showed wider diversity of Pseudomonas species than in the places where the contact took place. Thus, all the explored objects of the production environment at the pork processing enterprises and the facilities for slaughter and processing of broiler chickens are the potential reservoirs of Pseudomonas bacteria.

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Introduction

Modern technologies of food production and sanitization have changed the distribution and spatial arrangement of bacteria within the enterprises, and have led to circulation and contamination of the objects with microorganisms in the production sites. This is particularly acute issue for the production environment objects and facilities. Some are very difficult to sanitize, and due to it the organic residues and moisture accumulate and build up there for a long time. Such objects serve as reservoirs of various microorganisms, and upon contact with them, food products are contaminated [1]. Microorganisms at processing plant facilities can be either accidental contaminants or can be those microorganisms that have survived after sanitation due to their resistance to various factors.

Scientists around the world keep studying reservoirs of bacterial contamination in the food processing plants.

It is necessary to take into account that the objects of the meat processing flow line (equipment, auxiliary inventory, structures, etc.), which are made mainly of stainless steel (pipelines, hooks, knives), plastic (teflon), (e. g. conveyor belts) and polymers (polymer self-leveling floors) can be colonized by microorganisms. Microorganisms contaminate solid objects or the areas inaccessible for cleaning, both inside and outside of the equipment, like cracks, slots, holes and hollow parts, gaskets, unpolished or worn mate-

rials. They are the potential hideouts for the microorganisms and the reservoirs of resistant bacteria [2,3].

When studying the surface of the objects of the production environment in a meat processing plant, a high level of microorganisms of the phyla *Proteobacteria, Bacteroidetes, Firmicutes* and *Actinobacteria* was observed. It is necessary to clarify that the phyla *Proteobacteria, Bacteroidetes* and *Firmicutes* include cold-resistant microorganisms that cause food spoilage. Despite various hygiene practices, the microbiota in standard rooms and in the premises which require high level of hygiene, had no significant differences [4].

166 resistant mesophilic and psychrotrophic strains of *Pseudomonas* spp. were isolated from the surfaces of production environment objects (sinks, drains, walls, tables, equipment and floors) in a goat and sheep slaughter and processing premises (Jaén, Spain), in the cattle reception areas, slaughter premises, and all cold storage and cutting rooms [5].

The most contaminated objects in the production environment are the walls and floors. Floors are one of the main sources of microbial contamination, since their microbiota is transferred to various areas of the enterprise with the shoes of constantly moving employees, with the wheels of carts, and also during sanitization by high-pressure washing equipment. This method of floors cleaning spreads microorganisms because it sprays them into the air of the room in the form of aerosols [6].

Wastewater traps in the industrial premises is another hideout of microorganisms, in particular *Pseudomonas* spp. and *Aeromonas* spp., as these traps provide a favorable environment for microorganisms growth [7].

Another reservoir of microorganisms are the water supply hoses used for a long time at the enterprise (e. g. in meat processing ones). They contained biofilms with a complex and unique microbial community, represented by bacteria of the genus *Pseudomonas, Microbacterium* and *Psychrobacter.* The microbial composition of the biofilms in the hoses collected from one room was diverse and different. Opportunistic pathogens *Neochlamydia, Legionella* and *Pseudomonas* were detected. In two hoses *Pseudomonas* and *Microbacterium* were detected, and in water from them they found bacteria of the genus *Pseudomonas.* Very often the microbial composition of water (n = 5) correlated with the microbial composition of the water hoses internal surfaces. It is very important at food enterprises to ensure the supply of high-quality and safe water, since it is used at many stages of processing and for sanitization of food production facilities [8,9,10].

The constantly replenishable depots of microorganisms at the meat processing plants are the dirty skins of animals delivered for slaughter. Visually dirty skins and hides provided a large number of indicator microorganisms (total microbial count and *Enterobacteriaceae*) on the carcasses. Skins, in particular the cattle skins, are the main source of microbial contamination not only for the carcasses (during their skinning), but also of every object located within the animal slaughter line. It has been established that 1 cm^2 of cattle skin can contain up to 11 log_{10} CFU of aerobic bacteria [2].

Microbiome mapping at pigs processing premises has shown that bacteria like *Pseudomonas, Acinetobacter, Psychrobacter, Stenotrophomonas, Brevundimonas, Acidovorax* and *Microbacterium* dominate among the others. This microbiota is common to all studied objects and indoor structures of the premises [11].

Hundreds of various species may be found at a one food enterprise, but only a few taxa of resident microorganisms dominate. The taxonomic profile of various objects in the production environment was studied for over 18 months long. Bacteria of the genus *Pseudomonas,* which were the most numerous, gradually became secondary, although they still remained among the main microorganisms found in drain waters in the traps, on the equipment and on the tables. Bacteria of the genus *Pseudomonas* are of interest due to their acknowledged role in food spoilage processes. They were often detected after sanitization on the surfaces of the objects. Moreover, their resistance is probably explained by their low requirements for growth factors, their ability to grow at low positive temperatures and to form biofilms, as well as their ability to resist to biocides. Gram-negative bacteria, especially *Pseudomonas* spp., followed by *Enterobacteriaceae* and *Acinetobacter* spp., dominate over the surfaces of many objects in the production premises of food processing enterprises [12].

As for the pork processing, it is critical to understand on the example of slaughtering and processing poultry how the microflora of the final product is affected along the various stages of the production process. High microbial load is defined at a plant at the moment when the poultry is slaughtered. Microorganisms are distributed throughout the plant through various objects, staff and the air flows. All this factors, require continuous sanitization of equipment and the air environment. However, the producers consider speeding up the poultry processing line as a main priority, because it's necessary to satisfy consumers' increasing demand; this reduces the frequency of sanitizations at the plant. Broiler chicken meat can be contaminated with more than 280 microorganisms geni [13,14,15].

The next object that can be a reservoir of a large number of microorganisms is scalding vats water. On the surface of carcasses about 20 phyla of microorganisms still survive even after carcass scalding in hot water, respectively these same microorganisms remain in the water. About 60–70% of all types of microorganisms found in water were the phyla *Firmicutes, Proteobacteria* and *Actinobacteria.* High microbial contamination of scalding vats water was observed at the beginning of the working day. After several hours of water using, phylum-specific changes occurred. The number of microorganisms of the phylum *Proteobacteria* remained stable (it did not change), the number of microorganisms of the phylum *Bacteroidetes* decreased, the number of microorganisms of the phylum *Firmicutes,*

on the contrary, increased. It should be remarked that microorganisms of the phylum *Proteobacteria,* i. e. *Pseudomonas* and *Acinetobacter,* provided particularly negative effect on the poultry meat quality [16].

The hard rubber fingers of the carcass feather removal equipment serve as a depot for physical contaminants (faeces, dust and dirt) and the microorganisms they spread around. Feather removal can be considered one of the two main ways of poultry carcasses contamination, when bacteria from one poultry carcass are transferred to another one. The second way of contamination is poultry carcass evisceration. All objects of the production environment involved in these operations can be considered reservoirs of various microorganisms [16,17].

When carcasses are air-cooled, condensation drops are formed on the walls, which flows down onto the floor bringing along with them the microbial contaminants, in particular psychrophilic lactic acid bacteria, *Pseudomonas* and pathogenic *Listeria.* spp. During sanitization of the floor, they are spread around the room in aerosols. At the stage of cutting up the poultry carcass, employees and equipment serve as vectors of cross-contamination for these microorganisms [14,18].

The most common bacteria found on poultry meat while its storage under aerobic conditions are proteolytic species of *Pseudomonas* spp., which account for up to 58.5% of all isolated geni of microorganisms. More than 111 species of *Pseudomonas* have been identified on poultry meat [19,20].

Bacteria of the genus *Pseudomonas* are considered as the most heterogeneous group of gram-negative bacteria, they are represented by aerobic, motile, catalase-positive and non-spore-forming rods. These bacteria are all-pervasive and are found in soil, fresh water, humans, on the surface of the plants and animals, and in the animal and plant products. Bacteria of the genus *Pseudomonas* are opportunistic microorganisms. They cause various infectious diseases in humans and animals [21,22,23,24,25], as well as plants [26]. In addition, these bacteria cause spoilage of food products, including chilled ones [27,28]. They cause taste deterioration, rotting, rancid odor, liquefaction of the food product and the mucous deposits formation, for example on the cheese surface. They give an unpleasant taste to protein products with high water activity (meat, dairy products) and fish products due to the volatile compounds formation and amino acids breakdown; change the color of minimally processed vegetables due to their pectinolytic activity, and also start lipolysis and proteolysis of processed milk due to heat-resistant enzymes. *Pseudomonas fragi, Pseudomonas putida* and *Pseudomonas fluorescens* are particularly active in causing spoilage.

Based on the analysis of scientific publications, it was established that there is interest to studying the main sources of microbial contamination of food products at the food processing plants. The main foci of these researches are the study of a wide range of microorganisms, while only few publications are devoted to studying of a particular genus that causes spoilage and reduces the shelf life of the food products.

The purpose of this study is to explore the objects in the production environment of meat processing plants and poultry farms for their contamination with bacteria *Pseudomonas* spp., and to detect the potential reservoirs of these bacteria in these production facilities.

Objects and methods

Production environment objects of a pork processing plant

Wall in the receiving bin for pig half-carcasses; Wall of the raw material workshop (opposite the conveyor with half-carcasses); Conveyor (metal) for cutting pig half-carcasses next to the band saw; Band saw body (raw material workshop); Saw blade (metal) of the band saw in the raw material workshop; Trap/conveyor (metal) at the point of sawing half-carcasses into the cuts; Conveyor (polymer material) for clean containers delivery; Belt (polymer material) of the finished product feeding line; Manual pallet forklift (metal) for transporting pallets; Wall (metal) of the elevator in the container washing room for lifting dirty containers.

Objects of the production environment at the enterprise for slaughter and processing of broiler chickens (BC)

The outer surface (stainless steel) of the water cooling vat for chicken carcasses; The underside of the steps of the cooling vat trap (stainless steel); The ceiling above the cooling vat for BC carcasses, painted with paint; Roller (teflon) of the conveyor for moving BC carcasses during their cooling; Chain (metal) of the conveyor for hanging BC carcasses after water cooling; The frame (stainless steel) of the machine for weighing and re-hanging BC carcasses; Fixed parts (stainless steel) of the machine for deboning BC thighs, contaminated with meat juice; The frame of the machine for deboning BC thighs without visible contamination with meat juice; Chain (metal) of the machine for BC carcasses evisceration; Belt (polymer material) of the equipment for BC meat packing; Container (plastic) for collecting BC carcasses; Wall (tile) at a distance of 1.60 m from the floor; Table (stainless steel) for shaping and arranging BC carcasses before packaging; Two-tier trolley (stainless steel); Wheel (polymer material) of a floor trolley for transporting raw materials; Trap (stainless steel) in the BC carcasses evisceration workshop; Trap (plastic) in BC carcasses evisceration workshop.

Bacterial strains *Pseudomonas* spp. isolated from the objects in the production environment.

Swabs sampling

The swabs were sampled during the work process. In order to study objects for the presence of bacteria of genus *Pseudomonas,* the swabs were taken from 100 cm^2 area of the surface. In case of uneven surfaces, the swabs were collected without reference to the area. The swabs were sampled with sterile sponges (3M Dry-Sponge, USA), premoistened in 10 cm^3 of buffered peptone water. The spong-

es were transported to the lab study site in sterile packages at a temperature of 1 °C to 4 °C to prevent contamination.

Isolation and identification of strains

From a sponge bag aliquot of 100 µl of liquid volume was taken and distributed with sterile spatula onto the surface of CFC agar (cephalothin-sodium fucidin -citrimide agar) (Oxoid, UK) in a Petri dish. After 72 hours of culturing the crops at a temperature of 24 °C, colonies were selected for their species identification using the MALDI-Tof-MS time-of-flight mass spectrometry method on Autof MS1000 mass spectrometer (Autobio Diagnostics, China). For this purpose, the bacterial mass of colonies was applied onto a plate/target and dried at room temperature. Then, 1.2 µl of formic acid was applied to each cell with the dried bacterial mass for 10 min, dried up, 1.2 µl of the HCCA matrix (a-cyano-4-hydroxycinnamic acid, 99%) was applied onto dry mass and dried again. The MALDI target was placed in the device and the equipment for identification of microorganisms was launched using the FlexControl program (spectra acquisition). The obtained results were analyzed using the software: if the value was below 6.0, the result was considered unreliable and was not used for the further study. The result was considered as reliable and was taken into consideration when the values accounted for 6.0–9.0 — at the genus level, 9.0–9.5 — at the species level.

Results and discussion

To establish the presence of bacteria of the genus *Pseudomonas* at enterprises processing meat of the slaughtered animals (pigs) and poultry, the objects of the production environment were selected both being in direct contact with raw materials and finished products, and quite remote from them. At the pork processing enterprise 10 objects were assessed, at the BC slaughtering and processing enterprise — 17 objects. Microorganisms isolated from these objects were identified, and for the further analysis only data on bacteria of the genus *Pseudomonas* were used. The fact of the presence of the sought microorganisms was established on the basis of detection of 487 strains of genus *Pseudomonas* bacteria. Each of them was identified up to the species, or to the genus if the species profile of the identified strain in the device database was absent.

The study established that the bacteria *Pseudomonas* spp. were present in a pork processing plant and a poultry slaughter and processing plant, and were represented by 47 species.

Researches of many scientists show that pseudomonads may be present at the enterprises for several reasons. First of all, it happens due to constantly replenishable sources of these bacteria, such as animal skins, bird feathers, water and biofilms on various surfaces (e. g. in hoses, in hard-to-reach places) [2,8,9,10]. In addition, there are resistant "industrial" strains of these microorganisms. The mechanism of microorganisms' transmission is the presence of contaminated objects and/or their sanitization with high-pressure equipment, when microorganisms are transferred via long distances due to aerosols. In addition, after sanitization, the surface of the equipment remains wet, and water is the main reservoir of *Pseudomonas* genus bacteria.

To determine the sources of which species of *Pseudomonas* spp. is some particular object, a list of species isolated from the surface of each object was drawn up. The results are presented below in the Tables 1 and 2.

Table 1. Species diversity of *Pseudomonas* at the production facilities of a pork processing plant

	Objects of research	*Pseudomonas* species	*Pseudomonas* species number
1	Wall in a receiving bin for pig half-carcasses	*Pseudomonas brenneri* *Pseudomonas fluorescens* *Pseudomonas fragi* *Pseudomonas frederiksbergensis* *Pseudomonas gessardii* *Pseudomonas mandelii* *Pseudomonas* spp.	n = 7
2	Wall of the raw materials workshop (opposite the conveyor with half carcasses)	*Pseudomonas cedrina* *Pseudomonas fluorescens* *Pseudomonas koreensis* *Pseudomonas rhodesiae* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 6
3	Conveyor for cutting pig half-carcasses next to a band saw	*Pseudomonas fluorescens* *Pseudomonas gessardii* *Pseudomonas graminis* *Pseudomonas rhodesiae*	n = 4
4	Band saw body (raw materials workshop)	*Pseudomonas extremorientalis* *Pseudomonas fluorescens* *Pseudomonas proteolytica*	n = 3
5	Band saw blade (raw materials workshop)	*Pseudomonas azotoformans* *Pseudomonas chlororaphis* *Pseudomonas extremorientalis* *Pseudomonas fluorescens* *Pseudomonas frederiksbergensis* *Pseudomonas gessardii* *Pseudomonas libanensis* *Pseudomonas putida*	n = 8
6	Trap/conveyor at the point of cutting half-carcasses into the cuts	*Pseudomonas brenneri* *Pseudomonas chlororaphis* *Pseudomonas koreensis* *Pseudomonas tolaasii*	n = 4
7	Conveyor for clean containers delivery	*Pseudomonas chlororaphis* *Pseudomonas fluorescens* *Pseudomonas koreensis* *Pseudomonas libanensis* *Pseudomonas taetrolens*	n = 5
8	Finished product feed line belt	*Pseudomonas chlororaphis* *Pseudomonas extremorientalis* *Pseudomonas fluorescens* *Pseudomonas gessardii* *Pseudomonas grimontii* *Pseudomonas libanensis*	n = 6
9	Manual pallet forklift	*Pseudomonas fluorescens* *Pseudomonas fragi*	n = 2
10	Wall of the lift in the container washing room for lifting dirty containers	*Pseudomonas aeruginosa* *Pseudomonas gessardii* *Pseudomonas jinjuensis* *Pseudomonas koreensis* *Pseudomonas rhodesiae* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 7

Out of the 10 objects studied at the pork processing plant, only 5 had direct contact with raw meat and finished products. One of them was a band saw blade located in the raw materials workshop. It was found to contain the most types of bacteria *Pseudomonas* (n = 8). The saw blade may be a potential source of contamination, since it is not sanitized after each carcass cutting. On the body of the same band saw there were significantly fewer *Pseudomonas* species (n = 3). In similar studies by Japanese scientists, *Pseudomonas* spp. were also found in the swabs taken from the handle of a brisket saw, from the hooks and from the aprons of the staff in the boning workshop. At some sites they were found even after sanitization [29]. A conveyor for delivery of clean containers, which should be free of all types of contamination, was found to be contaminated with five species of *Pseudomonas*.

On objects that had no direct contact with raw materials, such as the wall of the storage bin, the wall of the elevator for lifting dirty containers to the container washing room, *Pseudomonas* (n = 7) was detected in lesser amounts, but not significantly lesser. Among the bacteria detected in the elevator, the bacteria pathogenic for humans were found — *Pseudomonas aeruginosa*. On the wall opposite the conveyor with pig half-carcasses, 6 species of *Pseudomonas* were found, including those that generate pigment when growing on meat. *Pseudomonas* bacteria were also found on the belt of the finished product feed line and on the conveyor for cutting up half-carcasses near the band saw and on the trap/conveyor of the point where half-carcasses are cut into the cuts.

Exploration at the pork processing plant found that all 10 production objects were contaminated with various species of *Pseudomonas* bacteria, which fact meant they were all sources of microbial contamination.

According to the research data presented in the work [30], the number of *Pseudomonas* spp. varies depending on the type of industrial activities of the enterprise. The samples collected from the dairy industry demonstrated the highest average count of *Pseudomonas* spp. among all other industries studied, followed by the samples taken from pork and poultry processing plants ($P < 0.05$).

We have obtained very interesting results of bacterial colonization by *Pseudomonas* spp. not only at pork processing plants, but also at the poultry processing ones, which proves the diversity and abundance of microorganisms in these industries.

The results of the study of the production environment objects at the enterprise for BC slaughter and processing are presented below in the Table 2.

To establish a deep fundamental understanding of bacterial relationships and changes in poultry meat and at production facilities (from breeding to distribution), it is necessary to have data on the microbiota along the entire poultry production chain.

Our findings can help in developing quality and safety management measures in poultry processing plants. It is

Table 2. *Pseudomonas* species diversity in the production environment at the enterprise for BC slaughter and processing

No	Objects of research	Types *Pseudomonas*	*Pseudomonas* species
	External surface (stainless steel) of the vat for water cooling of BC carcasses	*Pseudomonas brenneri* *Pseudomonas fluorescens* *Pseudomonas gessardii* *Pseudomonas graminis* *Pseudomonas* spp.	n = 5
	Underside of the stairs (stainless steel) of the cooling vat	*Pseudomonas lundensis* *Pseudomonas poae* *Pseudomonas proteolytica* *Pseudomonas* spp. *Pseudomonas trivialis*	n = 5
	Ceiling above the cooling vat of BC carcasses, painted with paint	*Pseudomonas chlororaphis ssp chlororaphis* *Pseudomonas lundensis*	n = 2
	Roller (teflon) of the conveyor for moving BC carcasses during their cooling	*Pseudomonas corrugata* *Pseudomonas gessardii* *Pseudomonas veronii*	n = 3
	Chain (metal) of the conveyor for hanging BC carcasses after water cooling	*Pseudomonas azotoformans* *Pseudomonas brenneri* *Pseudomonas chlororaphis* *Pseudomonas gessardii* *Pseudomonas grimontii* *Pseudomonas koreensis* *Pseudomonas marginalis* *Pseudomonas proteolytica* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 10
	Frame (stainless steel) for BC carcasses weighing and re-hanging machine	*Pseudomonas corrugata* *Pseudomonas mendocina* *Pseudomonas putida* *Pseudomonas* spp.	n = 4
	Fixed parts (metal) of the BC thigh deboning machine contaminated with meat juice	*Pseudomonas brenneri* *Pseudomonas chlororaphis* *Pseudomonas gessardii* *Pseudomonas koreensis* *Pseudomonas libanensis* *Pseudomonas lundensis* *Pseudomonas putida* *Pseudomonas synxantha* *Pseudomonas syringae* *Pseudomonas tolaasii*	n = 10
	Frame (without visible contamination with meat juice) of the machine for BC thighs deboning	*Pseudomonas azotoformans* *Pseudomonas brenneri* *Pseudomonas extremorientalis* *Pseudomonas flavescens* *Pseudomonas fragi* *Pseudomonas frederiksbergensis* *Pseudomonas gessardii* *Pseudomonas lundensis* *Pseudomonas putida* *Pseudomonas rhodesiae*	n = 10
	Chain (metal) of the machine for BC carcasses evisceration	*Pseudomonas chlororaphis* *Pseudomonas corrugata* *Pseudomonas extremorientalis* *Pseudomonas marginalis* *Pseudomonas* spp.	n = 5
	Tape (polymer material) for BC meat packaging equipment	*Pseudomonas brenneri* *Pseudomonas koreensis* *Pseudomonas gessardii* *Pseudomonas libanensis* *Pseudomonas mandelii* *Pseudomonas savastanoi ssp savastanoi* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 8

Table 2. End

No	Objects of research	Types *Pseudomonas*	*Pseudomonas* species
	Container (plastic) for collecting BC carcasses	*Pseudomonas azotoformans* *Pseudomonas brenneri* *Pseudomonas fluorescens* *Pseudomonas fragi* *Pseudomonas gessardii* *Pseudomonas graminis* *Pseudomonas orientalis* *Pseudomonas synxantha* *Pseudomonas tolaasii*	n = 9
	Wall (tile) at a distance of 1.60 m from the floor	*Pseudomonas aeruginosa* *Pseudomonas brenneri* *Pseudomonas frederiksbergensis* *Pseudomonas fuscovaginae* *Pseudomonas gessardii* *Pseudomonas grimontii* *Pseudomonas koreensis* *Pseudomonas libanensis* *Pseudomonas mendocina* *Pseudomonas mosselii* *Pseudomonas proteolytica* *Pseudomonas putida* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 14
	Table (stainless steel) for shaping and arranging BC carcasses before packaging	*Pseudomonas azotoformans* *Pseudomonas brenneri* *Pseudomonas gessardii* *Pseudomonas mucidolens* *Pseudomonas putida*	n=5
	Two-tier trolley (stainless steel)	*Pseudomonas agarici* *Pseudomonas corrugata* *Pseudomonas gessardii* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 5
	Wheel (polymer material) of a floor trolley for raw materials transporting	*Pseudomonas brenneri* *Pseudomonas chlororaphis ssp aurantiaca* *Pseudomonas corrugata* *Pseudomonas extremorientalis* *Pseudomonas fluorescens* *Pseudomonas fragi* *Pseudomonas gessardii* *Pseudomonas koreensis* *Pseudomonas libanensis* *Pseudomonas proteolytica* *Pseudomonas* spp. *Pseudomonas stutzeri* *Pseudomonas tolaasii*	n = 13
	Trap (stainless steel) in the BC evisceration workshop	*Pseudomonas fragi* *Pseudomonas frederiksbergensis* *Pseudomonas gessardii* *Pseudomonas libanensis* *Pseudomonas lundensis* *Pseudomonas* spp. *Pseudomonas tolaasii* *Pseudomonas trivialis* *Pseudomonas veronii*	n = 9
	Trap (plastic) in the BC evisceration workshop	*Pseudomonas chlororaphis* *Pseudomonas corrugata* *Pseudomonas fulva* *Pseudomonas koreensis* *Pseudomonas tolaasii*	n = 5

important to prevent cross-contamination during slaughter and processing via management of the employees' hygiene and the production environment.

Pseudomonas bacteria (n = 14) was found in wall swabs collected at the evisceration site at a height of 1.6 m from the floor at BC slaughter and processing plant. This premise does not come into contact with products, but its microbial composition may serve as a source of *Pseudomonas* spp. spreading over the other objects through high-pressure washing. Among the 14 species of *Pseudomonas,* two species pathogenic to humans were found: *Pseudomonas aeruginosa* and *Pseudomonas mendocina* [31]. It is possible that this object is not sanitized regularly and efficiently. In the case of condensation on the wall, along with the drops 14 species of *Pseudomonas* spp. will move down to the floor, and when the floor is sanitized with high-pressure washing, they will move up in aerosols through the air and spread over equipment and finished products. *Pseudomonas* bacteria can also move along the floor on the wheels of a cart, both inside and outside of the premises.

Pseudomonas bacteria (n = 13), predominantly from the group of *Pseudomonas fluorescens,* were also found on the wheel of the floor trolley used to transport the raw materials.

Third place in species diversity of *Pseudomonas* spp. in the slaughterhouse and processing plant is occupied by the chain of the conveyor for BC carcasses hanging (after water cooling), the stationary parts of BC thigh deboning machine contaminated with meat juice, and the frame of BC thigh deboning machine. On their surfaces 10 species of *Pseudomonas* were found, of which two were common to all three sites: *Pseudomonas brenneri* and *Pseudomonas gessardii. Pseudomonas brenneri* features proteolytic activity and when growing on a protein matrix generates an unpleasant odor of aldehydes, ketones and esters. *Pseudomonas gessardii* conduct lipolytic and proteolytic activity [27,28]. In case of cross-contamination of poultry products containing large amounts of protein and fat with these *Pseudomonas* species, spoilage processes are possible.

An analysis of the production environment confirmed our assumption about the possibility of contamination of food products upon their contact with the industrial objects. Nine species of *Pseudomonas* were found in the container for collecting BC carcasses, most of which are the microorganisms that contribute to spoilage process. This means that collecting BC carcasses into this container will contaminate them.

Two species of *Pseudomonas* were also found in the swabs from the ceiling above the cooling vat, which vat was painted with paint. If condensation formed on it, there was a risk of contamination of the food products.

The remaining 10 objects in the production environment of the slaughterhouse and processing plant were also contaminated with various species *of Pseudomonas.* These objects can be considered as potential depots *of Pseudomonas* spp.

If to sanitize only the objects of production environment that contact with raw materials and finished products, then the objects that do not come into contact can become depots of various microorganisms.

By analyzing the frequency of various species *of Pseudomonas* occurrence, it was found that out of 487 strains collected from industrial environment objects, 28 species *of Pseudomonas* were detected in no more than 1.0% (n = 50) of each. Among them were strains of bacteria pathogenic to humans — *Pseudomonas aeruginosa* (0.62%, n = 3) and *Pseudomonas mendocina.*

The remaining 19 species *of Pseudomonas* showed more strains. For example, the share of *Pseudomonas mosselii, Pseudomonas synxantha, Pseudomonas azotoformans, Pseudomonas frederiksbergensis, Pseudomonas mandelii* and *Pseudomonas rhodesiae* accounted for 1.03–1.85% (n = 44) of the total number of strains (n = 487). *Pseudomonas lundensis, Pseudomonas putida, Pseudomonas extremorientalis* and *Pseudomonas koreensis* were found in quantity of 2.26–2.87% (n = 50) of strains. *Pseudomonas proteolytica, Pseudomonas fluorescens* and *Pseudomonas libanensis* showed slightly bigger quantity — 4.11–4.93% (n = 67) of strains. The species of *Pseudomonas fragi, Pseudomonas tolaasii* and *Pseudomonas chlororaphis* represented 5.13–5.54% (n = 78) of the strains.

Most of the isolated strains were represented by two species: *Pseudomonas gessardii* (12.53%, n = 63) and *Pseudomonas brenneri* (20.53%, n = 100). However, for 7.19% (n = 35) of the strains, it was not possible to determine the species affiliation, since the device's software reliably identified them only down to the genus *Pseudomonas* spp.

All 47 *Pseudomonas species* were distributed across facilities, thus making it possible to determine which facilities in the enterprise are the reservoirs of certain *Pseudomonas species.*

According to foreign authors, bacteria of *Pseudomonadaceae* family, consisting of the genus *Pseudomonas* dominated over the place at the slaughter stage by an average of 14.71%, at the processing stage — by 29.05%, and at the sales stage — by 30.54%. They were detected in 27.23% of the floor swabs samples analyzed. The relative abundance *of Pseudomonas* remained high at the stage of poultry rearing, slaughter, processing and sale stages. Some *Pseudomonas* species, such as *P. fragi,* now are widely recognized as the dominant species associated with spoilage of meat products and are found in BC microbiota [30].

For assessing the frequency of particular species detection on the objects, the data on *Pseudomonas* species from the Tables 1 and 2 were presented in the form of diagrams. It was not possible to find a similar assessment of the data in the studies of the other researchers.

Figures 1 and 2 show the diagrams *Pseudomonas* species distribution over the objects of the production environment at enterprises.

In result of study of ten objects in the production environments of pork processing plant, it was found that eight of them (80.0%) were contaminated with *Pseudomonas fluorescens,* five (50.0%) — with *Pseudomonas gessardii.* Four objects (40.0%) were infected with *Pseudomonas chlororaphis* ssp. *chlororaphis* and *Pseudomonas koreensis.*

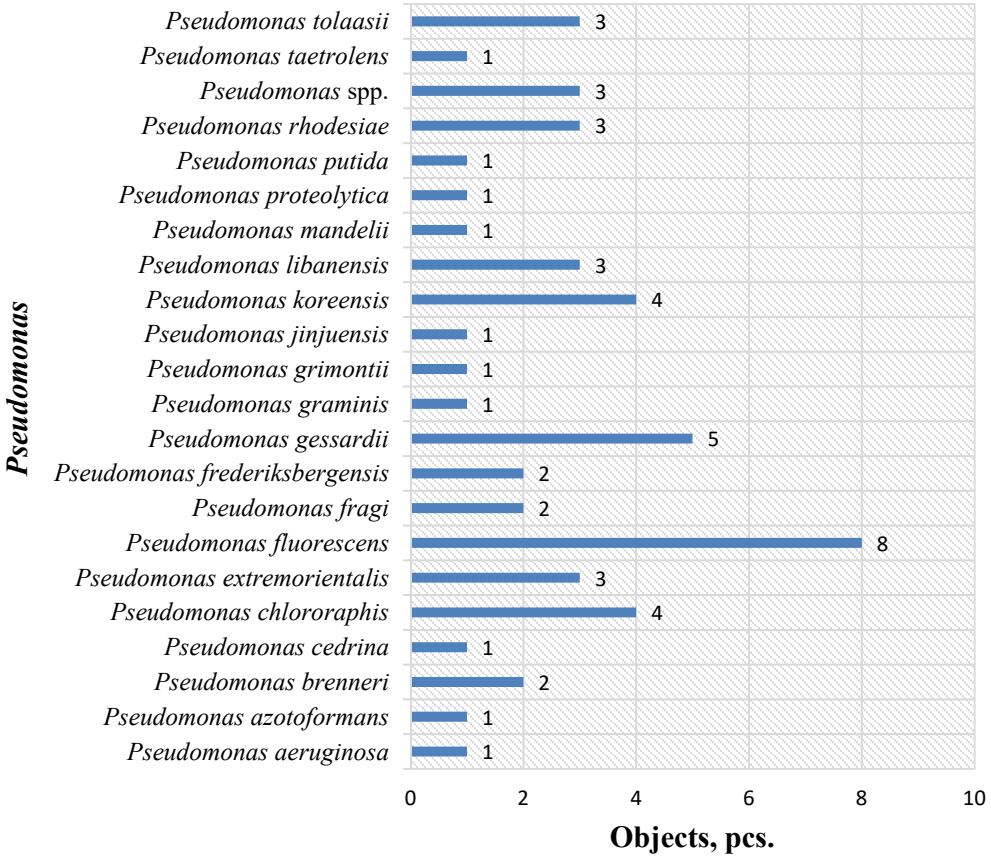

Figure 1. Distribution of various *Pseudomonas* species (n = 22) among the production objects of a pork processing plant

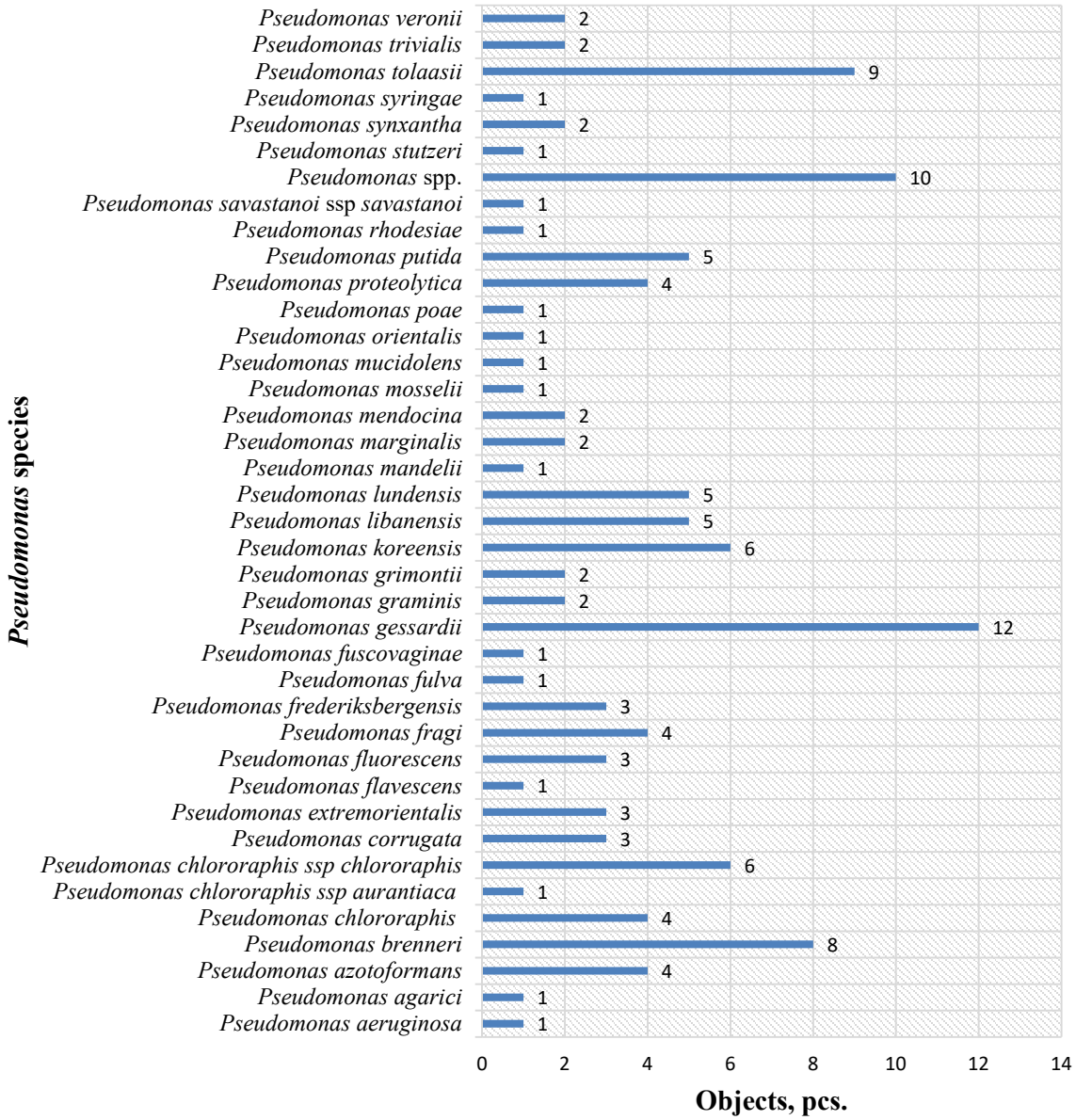

Figure 2. Distribution of various *Pseudomonas* species (n = 39) among the production objects of BC slaughtering and processing plant

Three objects (30.0%) contained *Pseudomonas tolaasii*, *Pseudomonas* spp., *Pseudomonas rhodesiae*, *Pseudomonas libanensis* and *Pseudomonas extremorientalis*. The rest of *Pseudomonas* species were detected at one or two sites, which made up 10.0 to 20.0% of their occurrence. At this pork processing facility *Pseudomonas fluorescens, Pseudomonas gessardii, Pseudomonas chlororaphis* ssp. *chlororaphis* and *Pseudomonas koreensis* were the most predominant species.

Out of 17 objects in the production objects of the enterprise for BC slaughtering and processing, 12 of them (70.6%) were contaminated with *Pseudomonas gessardii*. On the surface of 10 (58.8%) objects some strains could be identified only down to the genus *Pseudomonas*. *Pseudomonas tolaasii* were detected on 9 (52.9%) objects, and *Pseudomonas brenneri* were found on 8 (47.1%) objects. The surface of 6 (35.3%) objects contained *Pseudomonas chlororaphis* ssp *chlororaphis* and *Pseudomonas koreensis*. Other *Pseudomonas* species were detected at 1–5 (5.9% — 29.4%) sites.

At this slaughterhouse and processing plant most of the objects were contaminated with *Pseudomonas gessardii, Pseudomonas* spp., *Pseudomonas tolaasii* and *Pseudomonas brenneri*.

Conclusion

The results of the study showed that all 27 objects of the production environment at meat processing and poultry enterprises were contaminated with 47 species of bacteria of *Pseudomonas* genus. Many of these bacteria species affect the organoleptic properties of food products, even if the food products are stored in full compliance with storage conditions, and in this case meat products become a source of bacteria of *Pseudomonas* genus. The obtained data showed that *Pseudomonas gessardii* is the common *Pseudomonas* species among the industrials objects of the pork processing plant and BC slaughtering and processing enterprise. In addition to this microorganism, the pork processing plant features the dominating presence of *Pseudomonas fluorescens, Pseudomonas chlororaphis* ssp. *chlororaphis* and

Pseudomonas koreensis; at the slaughtering and processing plant *Pseudomonas* spp., *Pseudomonas tolaasii* and *Pseudomonas brenneri* dominate. It should be noted that all objects of the production workshops, regardless of their distance from raw materials and the finished products, are contaminated with *Pseudomonas* bacteria. At the same time on the surface of the objects that did not come into direct contact with the products the diversity of *Pseudomonas* species was higher than at those objects which had direct contact. The obtained data prove the necessity to revise the sanitization program in terms of the list of objects for sanitization, and to include the microbiological parameter "*Pseudomonas* spp. bacteria" into the check list of the production control program for the finished products monitoring.

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A REVIEW OF THE IRRADIATION EFFECT ON THE QUALITY AND SAFETY OF DIFFERENT TYPES OF MEAT

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Abstract
This review explores the benefits of irradiation in improving the quality and safety of different meat types. The process involves exposing meat in a shielded room using one source of radiation that can be gamma radiation, electron beam or X-radiation for a specified period of time. Through the use of this technology, parasites, viruses, insects and bacteria can be effectively reduced, which in turn increases the lifespan and quality of meat products. According to products to be irradiated and the bacteria to be eradicated, the radiation dose could be high, low or medium. Irradiating meat at an appropriate dose does not affect its sensory qualities such as taste, texture and color. The impact of irradiation on nutritional and chemical aspects of different types of meat is complex, since free radicals can cause lipid oxidation and alter vitamins, fatty acids, and amino acids. Furthermore, irradiation can also affect physical properties of meat, such as texture and tenderness. This review also summarizes the available information on the impact of irradiation on the extension of meat shelf life.

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Introduction

Nowadays, meat plays an important role in the human diet due to its protein, vitamins, and minerals [1]. However, meat is susceptible to microbial contamination, which can adversely affect its quality and safety [2]. Historically, Hippocrates (460 BC) recognized a direct connection between human illness and food consumption, based on his observation of how illness is directly linked to food consumption [3]. According to the World Health Organization (WHO), approximately 600 million people around the world are estimated to be ill due to consumption of contaminated food and 420000 people die every year [4,5]. Over 60 types of food have been approved by health and safety authorities for food irradiation, including meat, spices, grains, vegetables and fruits [6].

Ionizing radiation is used for treatment of meat as a physical process to ensure the safety and quality of food products. In addition to improving the safety of fresh meats by reducing food-borne pathogens, irradiation can also extend the shelf life of meat when stored at refrigeration temperatures or create shelf stable products without deterioration of the nutritive quality [7]. In the food industry, gamma radiations, electron beams and X-radiations are the main sources of radiation used to process food. Additionally, UV–C is widely used in the sterilization and microbial reduction of food, and was approved to delay the ripening and senescence of different fruits and vegetables. Microbes on surfaces of food can be quickly inactivated by pulsed ultraviolet (PUV) light. PUV light contains UV–C light, and its wavelength ranges between 180 and 1100 nm [8]. During food irradiation, radiation is not permitted to escape from a shielded room. When radiation is used at the prescribed energy level, food is not contaminated with radioactivity. The radiation dose absorbed by food treated by irradiation does not make it radioactive. Radiation damages deoxyribonucleic acid (DNA) or other critical molecules in bacteria, killing or preventing them from reproducing, and therefore, helps achieve the desired food safety and security objectives [9].

There are still concerns about the efficacy, safety, and impact of food irradiation on nutritional and sensory properties despite its obvious benefits. Some critics claim that food irradiation destroys vitamins and proteins or creates harmful compounds depending on a level of irradiation, which effects differ from food to food [10]. Irradiation can significantly extend the shelf life of various foods such as meat and fruits. Several studies have reported that irradiating fruits and meat before ripening extends their shelf life [11]. Singh and Singh [12] concluded that there is no significant effect of irradiation on the chemical composition of food. This process does reduce some vitamins; however, this is also common in other traditional food preservation methods, such as drying, canning and smoking. Temperature, oxygen, moisture, and pH are also important environmental factors that influence the consequences of food irradiation. For example, in the presence of oxygen, ionizing radiations have a greater lethal effect, while in the absence of oxygen and in wet environments, radiation resistance increases by 2–4 times [13].

Meat irradiation has been studied by many researchers regarding the chemical composition, microbiological safety, shelf life, physical and chemical properties, sensory properties

and nutritive values of foods. They have demonstrated that irradiation can inactivate microorganisms in any food product, such as fruits, vegetables or meat [14–16]. Gamma radiation was also shown to be a successful alternative remedy for inactivating microorganisms and extending shelf life [17]. Acceptance and use of irradiated food can be influenced by consumers' perception. Additionally, the flavor, nutritional content and presence or absence of additives determine the acceptance rate of products. The main purpose of this review is to investigate the principles, applications, technological and safety aspects of irradiated meat.

Objects and methods

A review of scientific literature published in English is included in this paper. Google Scholar, PubMed, Web of Science, Research Gate, Springer Link, ScienceDirect, Taylor & Francis, and Scopus, as well as the Google search engine, were elaborately reviewed to retrieve relevant keywords like food safety, meat irradiation, meat quality, pathogenic bacteria, chemical and physical properties, microbiological safety, and shelf life extension. As for the publications, they were as follows: published between 1975 and 2024 (72 references were selected for this review).

In this paper, we review the principles and applications of irradiation in food preservation. There is a schematic representation of the entire process of food irradiation using gamma rays, electron beams, and X-rays. Moreover, authors also discuss the benefits and limitations of food irradiation as a way to ensure that food is healthy and safe.

In addition, the Google search engine was used to capture more documents, reports, and theses from other reputable sources. Computers were used to store the information gathered. A title and abstract assessment was carried out on all papers collected for screening and analysis.

Some methods used for investigation of the effects of meat irradiation

Several authors have used several methods to analyze the effect of meat irradiation in some different ways. Asmarani et al. [18] employed a meta-analysis to investigate the effects of gamma irradiation on the quality of chicken meat and its products in Indonesia. The objective was to evaluate several parameters including oxidation, microbial activity, physicochemical characteristics, sensory properties, and nutrient quality. The study was carried out from August 2, 2023, to October 31, 2023. The study employed a mixed-model methodology where gamma irradiation treatment was treated as a fixed effect and the differences between experiments as random effects. A number of oxidation parameters were measured, including thiobarbituric acid-reactive substances (TBARS), total volatile base nitrogen (TVBN), and peroxide value. The microbial load parameters consisted of total aerobic bacteria, coliforms, lactic acid bacteria, enterobacteria, *Salmonella*, *Pseudomonas*, staphylococci, yeast, and mold. Physicochemical characteristics included pH, lightness (L*), redness (a*), and yellowness (b*). Moreover, parameters of sensory perception, such as acceptability, flavor and texture, were assessed.

In the study carried out in China, chicken breast samples were obtained from a local supermarket [19]. The samples were aseptically cut into uniform cubes (5 cm × 5 cm × 1.5 cm), weighing approximately 50 grams each. Three samples were packaged in a sterile polyethylene bag designed to prevent re-contamination. The samples were transported to the irradiation facility under chilled conditions (0–4 °C) immediately after preparation. The chicken breast samples were irradiated using an X-Rad 320 Biological Irradiator with a tungsten anode. The irradiation was performed at 160 kV and 25 mA, with doses of 0.0 (control), 0.75, 1.00, 1.25, 1.50, 1.75, and 2.00 kGy administered at a rate of 39.5 Gy/min. The X-ray source was capable of generating a high flux of up to 4 kW with a tunable broadband energy spectrum from 5 to 320 Ke V. The study evaluated various quality indicators of the irradiated chicken meat, including microbial counts, thiobarbituric acid reactive substances (TBARS), total volatile basic nitrogen contents (TVB-N), pH, color, texture, and sensory properties.

The study by Alahakoon et al. [20] investigated the effects of electron beam irradiation and high-pressure processing on the quality characteristics of marinated chicken breast meat, both with and without the addition of citrus peel extract (CPE). Fresh skinless chicken breast meat was purchased from a local market in Daejeon, Korea. The meat was transported in a polystyrene box with ice and stored at –18 °C until use. CPE was prepared by treating citrus peels with 70% ethyl alcohol (1:3, w/v) for 72 hours at room temperature (approximately 20 °C), followed by solvent evaporation and lyophilization. The marinade was created using water, salt, sugar, sodium pyrophosphate, and monosodium glutamate, with or without 2% CPE. Chicken meat (approximately 25 g) was mixed with the marinade by hand for 5 minutes to ensure maximum absorption. Each meat sample was vacuum packed before treatment. Samples were irradiated using a linear electron beam accelerator with doses of 1 kGy and 2 kGy. The beam power was set to 40 kW with a conveyor velocity of 10 m/min and a dose rate between 1.1 and 2.2 kGy/s. Samples were subjected to pressures of 300 MPa and 400 MPa. These methods provide a comprehensive approach to evaluating and enhancing the safety and quality of poultry products through innovative food processing techniques.

Sources of meat irradiation

An electromagnetic or particulate wave is a form of radiation that travels through matter or space. The term radiation refers to ionizing radiation, which can be divided into two types. First, directly ionizing radiations can ionize atoms of their target material through columbic interactions with the electrons of the material, and the amount of this columbic force depends on the kinetic energy of the particles, such as alpha radiation, beta radiation (high energy electron beams), and many other charged particles.

Secondly, indirectly ionizing radiations are neutral particles such as neutrons, gamma rays, X-rays that ionize atoms indirectly by ejecting energetic electrons called secondary electrons through their interactions [21]. Ionizing radiation sources can only be used in three main types of meat processing.

1) Gamma radiations are a type of the electromagnetic wave that have very short wavelengths and high energy photons emitted from Cobalt-60 (^{60}Co) or Cesium-137 (^{137}Cs) radioactive sources, which give off ionizing gamma radiations of 1.17 MeV, 1.33 MeV and 662 keV [22]. Both of them eject highly penetrating gamma radiations that can be normally applied for irradiation of meat and agriculture products. ^{60}Co is more commonly used than ^{137}Cs because it penetrates deeply into meat products and destroys harmful microorganisms [23]. Moreover, ^{60}Co is water insoluble and poses less or no environmental hazard. Using gamma rays at doses below 10 kGy can enhance meat safety by inactivating pathogenic microorganisms, such as *Salmonella* and *Campylobacter*, as well as extend the shelf-life of foods by removing microorganisms that cause food spoilage. Electron beams and X-rays are becoming more accepted due to the extensive shielding to prevent radiation and the potential health risks associated with gamma rays.
2) Electron beams are high energy electrons that are generated from an electron accelerator with the maximum energy level (8–10 MeV) [24]. The main disadvantages of electron beams are low dose uniformity and low penetration depth (approximately 2.5 cm). However, they have a lower cost compared to gamma rays and X-rays [25]. Additionally, a radioactive source is not required, and the device can also be turned off when not in use [26]. Applications for low energy electron beam of 0.1–1 MeV include packaging modifications, surface sterilization and aseptic packaging. Electron beams with medium energies of 1–5 MeV can be used to sterilize surfaces, pasteurize food in customized packaging, and modify packaging material. In contrast, applications for electron beams with high energies of 5–10 MeV can include food sterilization, waste treatment and phytosanitary treatment in the food industry and pasteurization [9]. All types of food can be irradiated with electron beams, including meat, fruits, seafood, vegetables, cereals and dairy products [27].
3) X-radiations are high-energy photons, which are emitted from orbital electrons when they interact with target material. Like electron beams, the device does not use a radioactive substance and can be easily shut off when not needed. The penetration depth of X-rays is high but they are expensive because only 8% of incident energy is converted to X-rays [28]. Recently, they have been applied to seafood, vegetables, and dairy products for the reduction of microbial contamination [29,30].

Applications of various doses in meat preservation

Irradiation of meat is determined by radiation absorbed dose represented as units of Grays (1 Gy = 100 rad) or kiloGray (kGy), with 1 kGy equal to 1000 joule of absorbed energy per kilogram. International health and safety authorities have endorsed the safety of irradiating food up to 10,000 Gy (10 kGy) [31]. There are three different principles involved in using radiation doses to preserve food.

1) Radurization involves decreasing the number of bacteria to the barest minimum on meat or seafood in order to enhance the keeping quality by using low doses of ionizing radiation (0.1–1 kGy) [32].
2) Radicidation uses middle doses of 1–10 kGy. Meat must be exposed to radiation doses necessary to reduce spoilage and microbial pathogens. The application of this dosage is similar to pasteurization, but since it does not use thermal energy, it is commonly used for frozen meat and other frozen foods [33].
3) Radappertization uses high doses (10–50 kGy) and is applied in the sterilization process since it can destroy microorganisms down to the level of spores in foodstuffs. In general, food irradiation sources and principles focus on using radiation to disrupt bacteria, enzymes, proteins, and microorganisms' genetic material, resulting in increased food safety and quality. For the safety and effectiveness of irradiation, national and international authorities regulate its use [34].

Principles of meat irradiation processing

Microorganisms and pests are controlled with a process, and the products are packaged before they are irradiated in order to avoid recontamination or re-infestation. Due to the exposure to radiation, packaging materials must be radiation resistant, and they should not transmit toxic substances into food, nor should they alter the texture or flavor of the food. Packaging materials are chosen based on radiation dose levels [33].

After meat has been prepared beforehand by packaging, it is exposed to a measured dose of ionizing radiation during the process as shown in Figure 1. The process is carried out in an irradiation room using a conveyor belt for a specified period of time. In the shielded room, meat passes through a defined amount of ionizing radiation for a certain

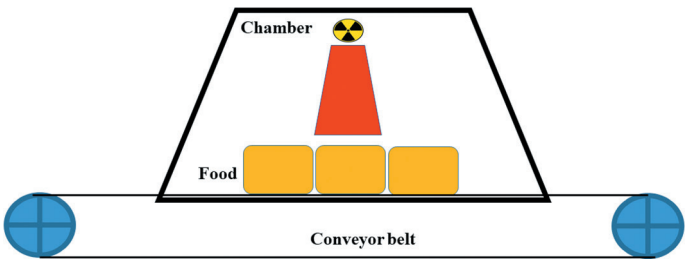

Figure 1. Meat irradiation process. First, meat is transported in a package to the irradiation chamber by the conveyor system, then meat is irradiated for a specified period of time, and finally it is unloaded and shipped to markets

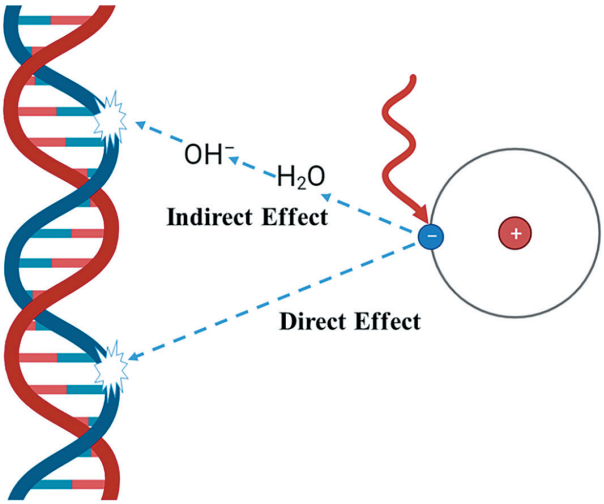

Figure 2. Radiation effects on microorganisms' genetic material DNA. Microorganisms can be killed or rendered inactive by radiation by damaging their DNA directly or by causing water molecules to undergo radiolysis to produce free radicals. As a result of these free radicals attacking DNA and other organic molecules in a microbe, a microbe may die, if it is not able to recover from the radiation-induced damage

amount of time before being redirected to the unloading area. The amount of time of exposure depends on meat type and sources of radiation being used. When radiation passes throughout meat, it breaks the chemical bonds, damaging food-borne pathogens or spoilage organisms by inactivating DNA in living cells, either directly or indirectly through the production of radicals and ions that damage DNA as shown in Figure 2. Gamma irradiation is mostly utilized for meat irradiation in commercial scale facilities such as the ^{60}Co source with high energy of 1.33 MeV [33]. The source is stored in a shielded container or under water to absorb all radiation, when the source is not used [35]. The second most used source are electron beams produced by an electron accelerator with high energy of 10 Me V. They are widely used to inactivate foodborne pathogens. X-rays, which are produced by striking a target material such as tungsten with high velocity electrons, are less commonly used for meat irradiation [33].

Effect of irradiation on the quality of different meat types

Sensory quality

The sensory qualities of meat can be affected both positively and negatively by irradiation. The sensory characteristics of meat including color, texture and taste, are not significantly affected by irradiation when an appropriate dose is used. However, high doses of irradiation can negatively impact sensory attributes, such as taste, odor and texture. Meat products may be perceived differently by consumers as a result of these changes. To minimize sensory changes that occur when meat is irradiated and improve sensory acceptability, storage conditions, packaging and flavor enhancers or additives can be used. Irradiation up to a dose of 10 kGy has no effect on the nutritional properties of meat or food safety [36]. According to Du et al., [37] radiation accelerates the oxidation and discoloration of lipids, and it decreases the levels of sensory properties in meat and meat products that cause off-flavors. It was found that the flavor score decreased with each doubling of the dosage by the same amount. It has been shown by Merritt et al. [38] that sensory acceptance can decrease as irradiation treatment dose and temperature increase. Shah et al. [7] showed that higher than 10 kGy may cause biochemical modifications and negatively affect the sensory qualities of food. In the study by Huang et al. [39] irradiation using gamma radiation at 2 kGy had no discernible effect on sensory attributes of smoked chicken breast. However, irradiation using gamma radiation at 3, 4 and 6 kGy led to a decrease in sensory qualities.

It is important for irradiated meat to have a good sensory quality, so strategies for improving meat sensory quality should be considered in the development and marketing of these products. Table 1 shows research findings on gamma irradiation regarding the sensory qualities of meat.

Microbiological safety

Irradiation has a significant effect on reducing the growth of microorganisms such as viruses, bacteria, as well as parasites, and therefore, reduces the risk of foodborne illness. Consequently, it enhances the quality and safety of meat

Table 1. Effect of gamma irradiation on the sensory qualities of meat

Meat	Radiation source	Dose rate (kGy)	Sample storage conditions	Sensory quality	References
Chicken meat	Gamma irradiation	1 and 2 kGy	Vacuum-packed; refrigeration storage at 4 °C; 14 days	Irradiation at 2 kGy did not affect sensory properties with turmeric powder	[40]
Camel meat	Gamma irradiation	0, 3, 5 and 7 kGy	Refrigeration storage at 1–4 °C; 42 days	Irradiated and non-irradiated camel meat had similar sensory properties	[41]
Ostrich meat	Gamma irradiation	0, 1 and 3 kGy	Air-packaged, under refrigerated storage at 4 °C; 9 days	Ostrich meat packaged in air quickly lost its qualities such as odor and texture	[14]
Rabbit meat	Gamma irradiation	0, 1.5 and 3 kGy	Packed in polyethylene pouches; refrigeration storage at 3–5 °C; 21 days	The sensory qualities of rabbit meat were not significantly affected by gamma irradiation	[42]
Turkey meat	Gamma irradiation	0, 0.5, 2 and 4 kGy	Refrigeration storage at 18 °C; 60 days	Sensory properties of irradiated and non-irradiated samples were similar and were equally accepted in terms of odor	[43]
Beef	Gamma irradiation	10, 25, 50 kGy	Refrigeration storage at 5 °C; 17 days	High doses of irradiation resulted in a brownish color of meat surface	[44]
Camel meat	Gamma irradiation	1.5, 3 and 4.5 kGy	12-month storage	Gamma rays had little sensory effects on odor and taste	[45]

products. At very low doses, irradiation is not effective in killing microbial toxins or viruses on meat. Therefore, a specific dose that is suitable for killing germs should be used according to a type of meat [46]. There are several food-borne bacterial pathogens that can cause severe illness or death in vulnerable populations, including *Salmonella*, *E. coli*, *Campylobacter* and *Listeria* [47].

Most current research has shown that ionizing radiation is used to irradiate meat, reducing microbial contamination and preserving nutritional value [48,49]. Arshad et al. investigated an effect of radiation doses on frozen duck meat using the electron beam. They concluded that at the dose of 3 kGy, the total bacterial and coliform counts were reduced by 1 log and 2 log cycles, respectively [50]. A study conducted by Sedeh et al. discovered that using 3 kGy gamma irradiation reduced mesophilic aerobic bacteria, coliforms, and *Staphylococcus aureus* in meat [51]. According to the research by Park et al., electron beams have less effect on meat products than gamma irradiation. Therefore, gamma irradiation is more effective in inhibiting the growth of microorganisms on meat [52].

Shelf life

It is very important to extend the shelf life of meat products using irradiation without losing safety and quality. Meat can have its shelf life doubled by applying relatively adequate doses of radiation without changes in color, flavor and texture. Certain factors affect shelf life extension such as a type of source, processing method, packaging and storage temperature [53]. Quality of meat products is determined by four parameters, such as nutritional quality, technological quality, sensory quality and safety quality. All those parameters affect the shelf life of meat types [54]. When ionizing radiation is applied to meat, it can cause reduction or elimination of microorganisms, which is one of the important factors that increase the shelf life of products [55].

Previous research has shown that irradiation is an appropriate process for meat types as shown in Table 2. In the recent study, the shelf life of boneless chicken was increased by 8 days using gamma irradiation. The results also showed that *Allium sativum* essential oil and gamma irradiation can extend shelf life of boneless chicken for 14 days [56]. Otoo et al. [57] reported that gamma irradiation at a dose rate of 0.74 kGy/hr can increase the shelf life of smoked guinea chicken under refrigeration. Generally, irradiation can extend the shelf life of meat types, but it is only one element of the comprehensive preservation strategy that also involves handling, storage, and other food safety measures. Previous studies show that irradiation has an effect of extending the shelf life of meat (Table 2).

Nutritional properties

Important components of meat are lipids, which are compounds crucial to cellular metabolism and several physiological functions [62]. Lipid oxidation can cause changes in the color, flavor and nutritional quality of meat. These changes depend on appropriate dose, meat type and packing conditions [63]. Earlier, some investigations showed that irradiation can lead to some changes in the nutritional composition of meat types.

Jai et al. demonstrated that lipidomics technology can be used to determine the composition of lipids of goat meat and their nutritional value [64]. As a result of two hours of gamma irradiation treatment, modified polyunsaturated fatty acids were found in goat meat after exposure to different radiation doses. Jia et al. applied five doses of gamma irradiation (0 kGy, 1 kGy, 2 kGy, 4 kGy and 6 kGy) to vacuum-packed goat meat for up to 2 hours. The results showed that irradiation increases the content of lipids that are rich in polyunsaturated fatty acids [64]. Another study examined an irradiation effect on the nutritional properties and lipid composition of marbled beef by using electron beam irradiation of 0, 2.5 and 4.5 kGy. The findings showed that irradiation of marbled beef had an effect on lipids, but with increasing the radiation dose from 2.5 kGy to 4.5 kGy little difference was observed [65].

In conclusion, it is important to note that irradiating meat may potentially affect its nutrient composition and quality, but it is a safe and effective way for protecting against foodborne illness.

Chemical properties

In irradiated meat, chemical properties refer to changes in the composition and constituents that occur due to exposure to ionizing radiation. These changes of chemical properties depend on a radiation dose and ambient oxygen when it reacts with

Table 2. Effect of irradiation on the shelf life of meat

Meat	Radiation source	Dose rate	Sample storage conditions	Shelf life	References
Turkey Bologna	Gamma irradiation	10 kGy	Vacuum-packed; 8 weeks at 4 °C	Irradiation at 10 kGy increased the storage life	[58]
Chicken wings	Gamma irradiation	0, 3, 5 and 7 kGy	Vacuum-packaged and cooked samples were stored at 2 and 7 °C; 7 weeks	Chicken wings had a shelf life of more than 7 weeks at 2 °C	[59]
Broiler meat	Gamma irradiation	0, 1, 2 and 3.5 kGy	Stored for 0, 30 and 60 days at –20 °C	Gamma irradiation at 2.0 kGy resulted in shelf life extension of broiler meat compared to non-irradiated meat	[60]
Mutton	Gamma irradiation	0, 1.5, 2 and 4 kGy	Stored for 0, 30 and 60 days at –20 °C	The shelf life of mutton was increased by 4 kGy irradiation	[16]
Chicken, salmon and beef fillets	UV–C irradiation	360 J/m^2	Vacuum-packaged storage; 5 days	The shelf life of chicken, beef and salmon fillets can be prolonged by 66.6% by irradiating with UV–C and vacuum sealing	[61]

meat [66]. Therefore, meat should be packaged after irradiation. When irradiated meat is exposed to free radicals, chemical changes occur, and a reaction between meat compounds and free radicals generates sulfur-containing volatiles. As a result of chemical reactions, fat oxidation, volatile production and gas production, meat loses sensory properties and is changed in quality [67].

Meat is rich in protein and vitamins (B1, B2, B6 and E), but irradiation can lower protein and vitamin content in meat. Using low radiation doses in combination with other mild treatments minimizes the effects of irradiation on organoleptic changes and vitamin loss in meat [68].

Physical Properties

Physical properties can also change, including water holding capacity (WHC), color and tenderness of meat. WHC refers to fresh meat ability to retain its own water during grinding, cutting and heating and also during transport, storage and cooking [69]. Changes in physical qualities of meat regarding tenderness will cause more tenderness or hardness in meat by the appropriate dose of radiation. However, sometimes meat loses its color. Poultry and beef are more prone to changes in color. The study by Bliznyuk et al. investigated the impact of electron beam irradiation at 0, 1.5, 3, 4.5 kGy on raw beef [70]. They found that electron beam irradiation decreased the color value (L*, a* and b*).

Meat irradiated with more than 10 kGy has lower myofibrillar protein content. As a result of changes in the secondary structure of the myofibrillar protein, the functional properties of the protein change, which affect the texture of meat. Several factors play a role in these changes, including radiation dose, pH, temperature, packaging conditions, muscle type and storage time. In addition, different protein types are sensitive to different effects of irradiation on meat texture [32,71]. Rane et al. analyzed changes in pH, TBA (thiobarbituric acid) values, and tyrosine levels in irradiated chicken nuggets over time with electron beam doses of 3, 3.5 and 4.5 kGy. They found that pH values of chicken nuggets increased gradually during storage, indicating possible spoilage or quality changes. TBA values, which measure lipid oxidation, also increased, suggesting oxidative changes in both control and irradiated samples over time [72]. Moreover, color changes were monitored using a color difference meter, noting variations in L*, a*, and b* values throughout the storage period.

Advantages of meat irradiation technique

1) Taste, color and texture of meat are not significantly changed by irradiation when an appropriate dose is used.
2) The shelf life of meat is extended by the use of irradiation, which can slow down the spoilage of meat.
3) Irradiation can replace chemical fumigants for disinfestation and microbial control as it causes no radioactivity in meat and leaves no harmful radioactive residues on meat.
4) Irradiation can reduce or kill viruses, bacteria, and parasites on meat depending on the radiation doses used.
5) When meat is irradiated, it helps companies and businesses to easily import meat to other countries as they ensure the safety and quality of meat.
6) Meat that has been irradiated does not have significant changes in its nutritional content. Physical and chemical properties of meat are preserved after irradiation. Furthermore, this technique can be used on packed meat to prevent recontamination.
7) A greater safety and quality of products can be achieved through irradiation in conjunction with other methods of meat preservation.

Conclusions

Irradiation technique is a beneficial technology in improving the safety and quality of meat types. The process can extend the shelf life of meat and prevent spoilage. In addition, a certain amount of radiation dose can reduce or completely destroy bacteria, viruses and parasites on meat. Taste, odor and color, which are the most important characteristics of meat according to most researchers, are not significantly affected when the right dose is used. When high radiation dose is used, it affects the chemical and physical qualities of meat and can reduce the vitamin content in meat or destroy its tender texture. Finally, it is important to perform further research in the future for products that have not been tested for worldwide acceptance.

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GAS DISCHARGE VISUALIZATION AS A PROMISING TOOL FOR MEAT ANALYSIS DURING ITS STORAGE

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Abstract

Recently a lot of analytical methods have been developed, however, only a few of them have found application in the meat industry, since they do not provide sufficient understanding of the processes that occur in meat during its storage. The use of the GDV method for analyzing the food products quality has got a number of advantages, since it allows for quick and non-invasive getting of information on the food product condition, which capability may be quite promising for meat analysis. The study described in this paper is based on the GDV method application for analyzing the condition of the chilled pork at various stages of its storage and for detecting the peculiar changes in its quality.

The study analyzed samples of Sus scrofa m. longissimus dorsi stored at a temperature of 0 to 4 °C for five days. Using the software ImageJ to analyze the gas-discharge glow of meat, its main parameters were obtained, such as an area, average radius of glow and color characteristics. The most significant characteristics of the gas-discharge glow were determined, among which the glow area, shape factor, uncertainty and dispersion were focused on. It was shown that synchronously with the development of rigor mortis, there was a decrease in the area of the gas-discharge glow, and with its resolution and further storage of meat — a noticeable increase. The dispersion of the radius of meat glow by the end of the storage period increased by 2.03 times in comparison with the original value, and the gas discharge was unstable and featured a large number of streamer branches.

The influence of histostructural changes and fractional composition of proteins on the properties of the electromagnetic field during GDV of meat has been proven. It has been shown that the method of gas discharge visualization, along with histological studies, can be used to analyze meat during its storage and defining the depth of autolytic changes that take place in the meat.

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Introduction

The analysis of meat quality via traditional physical (mechanical) and chemical methods is still reliable and still serves as "reference standard", but most of the methods are labor-consuming and possess a row of disadvantages, such as the necessity to violate the integrity of the sample as well as the pretty considerable duration of the analysis execution, which is not always acceptable in case of express monitoring or online monitoring. Therefore, to improve the efficiency of analysis and to minimize the associated losses, modern methods are being developed taking into account the reasons of speed, accuracy and non-invasiveness [1,2].

In most cases there are two main methods used for objective assessment of meat quality: subjective and objective ones. Subjective methods are usually based on sensory assessment, which includes visual analysis and taste sensations. The main disadvantage of these methods is their strong dependence on the expert's personal experience, low reproducibility and difficulties with quantitative assessment. Objective assessment methods traditionally include laboratory tests targeted at analyzing the physical and chemical properties of meat, as well as detecting the available microorganisms [3]. These methods provide high accuracy of results; however, they can vary in wide range due to the extreme complexity of the structure and composition of meat, as well as its biological origin, animal's growing conditions, transportation, duration of storage, etc. [4].

The issue of obtaining the reliable information on the meat quality and properties throughout the entire production process is one of the main problems that the meat industry encounters. Therefore, one of the tasks of food quality control is the development of reliable express methods of analysis that allow detecting the adulteration or quality reduction, which analysis, in its turn, can be implemented by electrophysical properties measuring. For example, measuring the specific electrical conductivity of meat allows for a high-precision assessment of its functional and

technological properties and detect the adulteration of the thermal condition [5], and application of electrical impedance spectroscopy allows determining the degree of freshness [6], predict the course of meat maturation and curing [7] and assess the influence of various defrosting methods on its quality [8]. In this regard the development of new methods for meat quality assessing through measuring its electrophysical properties is highly relevant and has high potential for its using in arbitration express-test of meat and meat products quality.

As is known [9,10], electric discharge is widely used to study the microrelief of a surface, the heterogeneity of the dielectric constant and studying the degradation of insulating materials. In emission spectroscopy, an electric discharge allows for the qualitative and quantitative determination of the composition of the sample under study [11]. Thus, one of the possible and appropriate electrophysical methods for analyzing meat quality may be the electrobioluminescence or gas-discharge visualization (GDV) method, which is based on the effect of air breakdown in result of the electric current impact on the object.

The research of the biological objects properties based on the analysis of the characteristics of gas discharge glow has a certain prospect. Absolute values of the parameters of gas discharge glow allow evaluation the properties of liquid-phase and solid-phase organic and inorganic objects (blood, water, plant crops, minerals, etc.) in order to perform quality control of plant materials, including fruits, seeds and grain crops, as well as the impact of various food products on the human health condition [12].

The GDV method is based on quantum biophysics and is exercised via Kirlian effect principle. During the process of GDV of the biological object being analyzed, a complex interaction of the applied pulsed electric field and the formed gas discharge takes places. The state of the surface structures and the electrical resistance of the internal structures of the object being analyzed determine the minimum value of the electrical breakdown voltage. Due to the heterogeneity, surface and volume properties of the object under analysis, the electromagnetic field is modulated, which influences on the gas discharge parameters, based on which a conclusion is made about the condition of the object. The processed GDV-gram (discharge image) allows analyzing a number of parameters which indicate the state of the object, and on this basis it is possible to make certain conclusions. The most important GDV parameters are intensity, perimeter, glow area, shape factor and entropy [13].

The scope of GDV method application is quite wide and nowadays there is a growing interest in its application in various areas of science and life [14], which interest is also proven by the growing number of publications on this topic, according to Science Direct, Google Scholar and PubMed.

As of today the hundreds of practical modifications of the GDV method have been developed worldwide depending on the geometric shape, parameters and physical properties of the studied objects of animate and inanimate nature. This method has shown a fairly high sensitivity and information content for assessing the physiological condition of plants and their antioxidant status [15], for finding pathologies in biological tissues [16], for analyzing blood and determining the properties of precious materials [17]. GDV analysis allows identifying the differences in the glow of electrolyte solutions of different concentrations and compositions, as well as to differentiate natural and synthetic essential oils with identical chemical composition [18].

In the works [19,20] it is shown that the gas-discharge visualization method in combination with automatic analysis of digital gas-discharge images can serve as an efficient additional tool for the prompt assessment of the heterogeneity and hidden defects of wheat seeds. The results presented in these works do not contradict to Kolesnikov et al. [21], who proved that GRV of soft wheat allows obtaining more complete characteristic of biological and economic suitability of the seed material. The results obtained by the authors allow prediction of field germination of the seeds and their potential yield, allow identifying the main defects of seeds, prediction of diseases development and probable changes of plant resistance to diseases.

It should be noted that despite the variety of areas of application of this method, it has found its greatest reflection in biology and medicine for diagnostics of various diseases [13], for assessment of the body functional state [22], for analysis of the antigen-antibody reaction [23], etc., since the GDV method combines non-invasiveness, safety, methodological simplicity, simplicity of use and high throughput [22]. However, the application of the GDV method in medicine still remains highly controversial, as the GDV glow characteristics have high variability and/or little information [24]. This suggests that the literature related to this area of research should be reviewed and questioned [25].

Based on the known fact about the ability of the GDV method to detect minor changes in the physicochemical characteristics of materials and organic and inorganic substances solutions, some researchers have tried to study the possibility of using the GDV to assess the quality of food products and meat. Thus, the authors [26] have established that the corona discharge of leaves and fruits provides useful information on the stressed state of plants and defines the variety of the plant. Laurent et al. in their study [27] presented the possibility of using the GDV to assess the impact of the fattening method on the quality of rabbit meat, and in [28] the effect of preliminary exposure of chicken meat to a structured aqueous solution of fructose on the organoleptic properties and parameters of the GDV was shown. The authors showed that after processing poultry meat in an aqueous solution of fructose, the number of photons increased and the energy of GDV luminescence rose up.

Despite the data obtained by the authors on the study of the GDV parameters of meat and food products, today the application of this method in the food industry is quite

limited due to insufficient study, the lack of scientifically substantiated information on the relation between the parameters of the GDV glow of meat and the physicochemical and other processes occurring there. It should also be noted that the lack of a standardized methodology for studying the meat properties via the GDV method leads to gross mistakes that cause errors and significant deviations of numerical values from the average, which makes this area of research deviant.

For this reason, further detailed studies are required to assess the influence of various factors such as storage duration, pH, fractional composition of proteins, the state of muscle tissue microstructures, fat content, etc. on the electrophysical properties of meat, with subsequent development of the standardized GDV method.

Thus, the purpose of this study is the adaptation of the GDV method for meat quality research, as well as the scientific substantiation of the influence of physicochemical processes in the meat during its storage on the GDV parameters.

Materials and methods

The chilled samples of the rib-eye — i. e. the longest back muscle of pork (*Sus scrofa M. longissimus dorsi*) 24 hours after slaughter were used as the objects of the study. Meat samples were taken from three different half-carcasses of two-breed crossbreed pork (large white × landrace) at Mogilev Meat-Processing Plant OJSC, Republic of Belarus, and were delivered to the laboratory in an isothermal bag within one hour from the time of collection. The meat was packed in polyethylene bags and stored at a temperature of 0 to plus 4 °C for 5 days. Sampling and sample preparation for testing were done in accordance with GOST 7269-2015[1] and GOST R 51447-99 (ISO 3100-1-91)[2]. Every day the samples of *longissimus dorsi* pork muscle were analyzed according to the following methods for 5 days of storage.

GDV of meat

From the meat pre-warmed to 20 °C the sample was cut out with a cylindrical knife so that the muscle fibers lay across the blade of the knife. The diameter of the samples was 20 mm, and the thickness was 5 mm.

Pieces of meat were carefully arranged on a clean glass electrode, which consisted of two 1 mm thick glass plates air-tightly sealed at a distance of 1.5 mm from each other, the space between which was filled with 3M of KCl solution. Grounding electrode was affixed to the meat, the GDV camera, which operating principle is shown in Figure 1, was covered to prevent access of light. To create the electrobioluminescence effect, high-frequency current and voltage generator was used, which included a signal

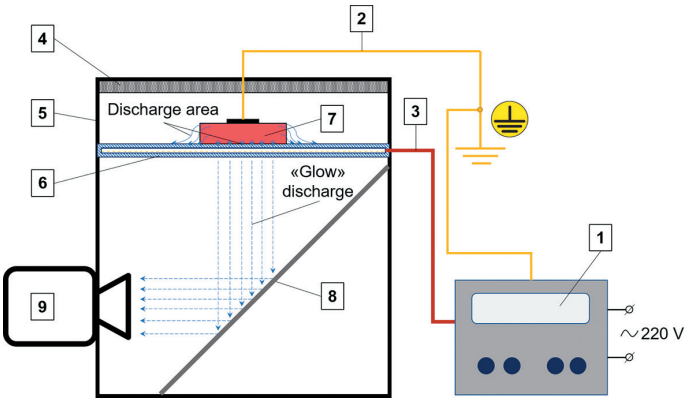


Figure 1. Schematic diagram of the GDV device for meat: 1 — generator of high-frequency current and voltage, 2 — grounding electrode, 3 — high-voltage wire, 4 — GDV camera cover, 5 –body of the GDV camera, 6 — glass electrode, 7 — object of study, 8 — mirror, 9 — photo camera

generator Power Pulse Modulator PWM -OCXi v3 (RM-Cybernetics LTD, UK) and ignition coil NGK 48342 (NGK, Germany), connected to high-voltage wire that was wired to the glass electrode. When the generator was turned on, the sample was exposed to high voltage (20–30 kV) and frequency (250–400 Hz) pulses, with a duty cycle of 80%. The total pulse exposure time was 30 s.

During the GDV of meat, a series of photographs were taken using a camera Canon SLR EOS400 D (Canon, Japan). The lens of the camera was directed at the special mirror (Figure 1). The shooting frequency was 1 frame per second. The images were analyzed with the software ImageJ.

To determine the measurement error and assess the degree of environmental factors influence on the GDV characteristics, a reference object was used which properties did not change throughout the experiment, unlike the object of study. For that a steel cylinder with a diameter of 20 mm and a height of 5 mm was used as the reference object.

To highlight various visual features of the obtained photo images, to reduce information redundancy, to provide contrast and better visualization of the discharges, the obtained images were pseudo-colored with the help of the software ImageJ, which process is based on dividing the brightness spectrum of the image into several K_i parts of equal area. Each section is assigned to a specific color $S_k(b) = \text{const}$, in result of which all points which luminescence intensity lies within the defined interval are displayed on the screen in the same color.

As a result of the analysis of the obtained images (GDV-grams), the following parameters were calculated:

1) the luminescence area (S), which was determined using the software ImageJ;
2) the average radius of the glow (R), which was determined using the software ImageJ as the arithmetic mean of the glow radii, which values are equal to the distance between the first and last points of non-zero intensity lying on the beam from the center of the glow

[1] GOST 7269-2015 "Meat. Methods of sampling and organoleptic methods of freshness test." Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200133105 Accessed August 20, 2023 (In Russian)
[2] GOST R 51447-99 "Meat and meat products. Methods of primary sampling." Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200028183 Accessed August 19, 2023 (In Russian)

at an angle $\alpha \in [0;2 \cdot \pi)$ to the vertical axis;

3) the shape coefficient (K) (dimensionless value equal to 4π for a circle and increasing along with rising complexity of the figure shape), which was determined by formula (1)

$$K = L / 2\pi R, \quad (1)$$

where L is the length of the outer glow contour perimeter;

4) color parameters of luminescence in the CIE coordinate system L*a*b*, which was determined using the software ImageJ;
5) uncertainty (N) associated with the estimate is the experimental standard deviation of the mean value, and is equal to the positive square root of the experimental dispersion of the mean value.

The uncertainty $N(x_i)$ for the measurement result $x_i = \overline{x}_i$, calculated as the arithmetic mean, was determined using the following formula (2)

$$N(x_i) = u_A(x_i) = \sqrt{\frac{1}{n(n-1)} \sum_{g=1}^{n} \left(x_{ig} - \overline{x_i}\right)^2}. \quad (2)$$

6) the dispersion of the glow radius (D) was calculated as the arithmetic mean deviation of the squares of the difference in the glow radii of the general totality from their mean value R.

The deviation of environmental parameters did not exceed 8%, so their influence was neglected in order to simplify the calculations.

Analysis of molecular weight distribution of the protein fractions by one-dimensional electrophoresis method

100 mg of sample was taken and 2000 µl lysing solution (9M urea, 5% β- mercaptoethanol, 2% triton X-100, 2% ampholines with a pH of 3–10) was added. The resulting homogenate was clarified by centrifugation at 14,000 rpm for 20 minutes. After that, the supernatant was separated and protein buffer was added to it in a ratio 1:1. The protein buffer was prepared by 1 ml of sodium dodecyl sulfate (SDS) 10%, 250 µl of concentrated β-mercaptoethanol, 625 µl of Tris-HCl 0.5 M, 1.5 g of urea, added to Eppendorf tubes, then bromophenol blue was added until reaching a dark color and brought to a volume of 5 ml with water, and then the samples were heated in a boiling water bath for 5 minutes.

To perform vertical gel electrophoresis, a VE-20 chamber (Helikon, Russia) was used and filled with 12.5% polyacrylamide gel. 6% gel was poured over its top, and the wells were made in its surface for nesting the samples. The sample to be studied was added in amount of 10 µl. Solution containing 25 mM of tris-HCl, 192 mM of glycine and 0.1% SDS was used as a buffer. Electrophoresis was run under the following parameters: the first 30 minutes — at 60 V, and then at 120 V until the dye front (bromophenol blue) reached the lower edge of the gel plates.

The proteins were dyed with Coomassie G-250 in a solution of the following composition: 10% acetic acid, 25% isopropanol, 0.05% Coomassie G-250. To remove the unbound dye, 10% acetic acid was used.

For computer densitometry, one-dimensional electropherograms in a wet state were used. Their full digital images were obtained with scanner Bio-5000 Plus (Serva, Germany) in 600 ppi 2D-RGB mode. The obtained digital images were edited in the graphic editor ImageJ.

Histological examination of meat

Histological examination was implemented in accordance with GOST R31479-2012[3] and GOST 19496-2013[4]. The sections were evaluated with the microscope Micromed-1 var.2-20 (Micromed, Russia). The muscle fiber diameter were measured with the software ImageJ.

Determination of shear force

The shear force values were obtained via Warner-Bratzler method according to [29].

Statistical analysis

Statistical analysis of the results was run using software Excel 2019 (Microsoft, USA). The results were considered significant at $p < 0.05$. To assess any correlations between various factors, r-Pearson correlation coefficients were calculated.

Results and discussion

Analysis of meat GDV-grams

As a result of the analysis of the gas discharge glow of the chilled meat and the reference object, significant differences were detected in the structure of their corona discharge (Figure 2). Glow of the reference object (Figure 2a) showed the uniform distribution of the streamers5 and the absence of their branching. The corona discharge was stable throughout the entire period of exposure to electrical pulses.

In turn, the figures formed during electrobioluminescence of meat, in comparison with the reference standard, featured more chaotic character, which changed synchronously with the lengthening of the samples storage duration (Figure 2b and 2c).

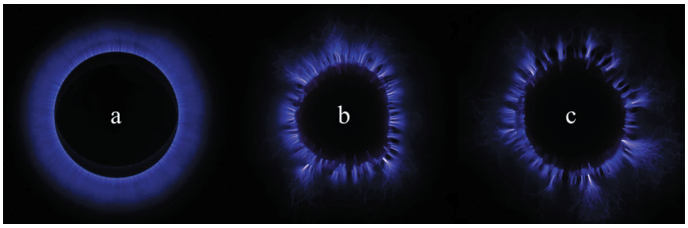


Figure 2. Gas discharge glow of the reference object (a), meat in the first day (b) and meat in the fifth (c) day of storage

[3] GOST R 31479-2012 "Meat and meat products. Method of histological identification of composition." Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200097485 Accessed August 19, 2023 (In Russian)

[4] GOST 19496-2013 "Meat and meat products. The method of histological investigation." Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200107317 Accessed August 20, 2023 (In English)

[5] Streamer is a set of thin branched channels through which electrons and ionized gas atoms move, being organized in peculiar streams.

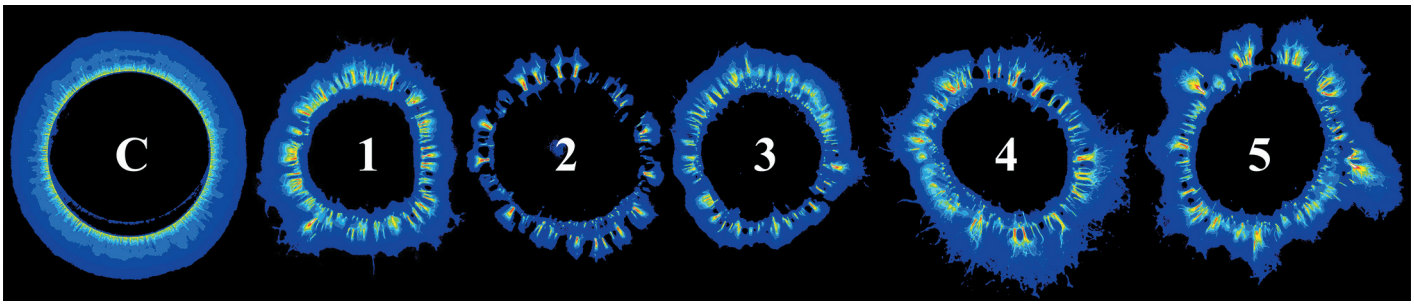


Figure 3. GDV-grams after pseudo-staining: reference object (C); chilled meat on: the 1st day of storage (1); the 2nd day of storage (2); the 3rd day of storage (3); the 4th day of storage (4); the 5th day of storage (5)

As can be seen from Figure 2, the glow structure formed by sliding and avalanche discharges is more symmetrical and uniform in the meat at the initial stage of storage. During further storage of meat, sharp spikes and more complex Lichtenberg figures were observed, the gas discharge glow became more chaotic and non-uniform, which was expressed in a change in the corona discharge geometry, formation of breaks and thickened streamers of various lengths (Figure 3).

During the first day of meat storage, when the meat was placed into a high-intensity and high-frequency electric field, its gas-discharge glow was characterized by a stable, uniform, and bright corona structure, without large breaks and defects in the inner and outer contour lines. The gas discharge was represented by closely spaced streamers with a dense and clearly defined structure. The zone with the highest discharge density was distributed in the meat evenly and continuously. The streamer channels did not exceed 7 mm in length and were stable for 30 s.

On the second day of meat storage, its gas-discharge glow was stable and without sharp bursts. However, in the GDV glow the clear signs of changes in the discharge geometry were found. As can be seen from the Figure 4, the glow area of the samples on the second day decreased down by 15.3% in comparison with the initial value. Increase of the dispersion values in the glow radius by 9.6% indicated the formation of heterogeneity and chaos in the discharge. Streamer channels became thicker and shorter by 20.8%, which was detected by the average glow radius decrease. The location of the discharge excitation points was uniformly distributed, but their number significantly decreased in comparison with the same parameter on the first day of storage, which is visible in the Figure 3.

As can be seen from the Table 1, the spectral characteristics of the discharge changed synchronously with the change in the geometry. The gas-discharge glow of meat on the second day of storage became dimmer in comparison with its glow on the first day, which clearly shows the decrease in the lightness L* and saturation *C* values * of the corona discharge color by 48.5% and 53.2%, respectively.

When analyzing the shape factor (Table 1), which value indirectly characterizes the density and uniformity of the streamers distribution, it was defined that intensity of this parameter insignificantly changed during storage, and practically did not go beyond the limits of reliable difference ($p < 0.05$). On the second day of storage, this parameter increased by 7.7%, and on the third it decreased down by 14.7% in comparison with the initial value on the first day. In its turn, on the third day of chilled pork storage, a partial restoration of the corona discharge structure up to its original condition was observed, while the area of the gas discharge glow S and the luminosity of the gas discharge L* were 1.2 times less in comparison with the first day, which evidenced a lower intensity of the glow.

On the fourth day of pork samples storage, sharp change in the corona discharge outlines was observed. When high-voltage and high-frequency currents passed through muscle tissue, the resulting gas discharge turned to be unstable, with sharp surges and breaks. The streamers became thicker and longer, and the corona discharge outlines were more chaotic and unstable, as evidenced by a statistically significant increase in the dispersion of the glow radius by 1.57 times and uncertainty by 1.23 times. At the same time, the area of meat glow became the same as it was on the first day of the meat storage (Figure 4).

On the fifth day of storage, an increase in the average rate of change in the dispersion of the glow radius was found from 52 units / day (on the first day of storage) to 247 units / day. The gas-discharge glow of chilled meat was unstable and had a large number of streamer branches (Figure 3), while the glow area changed insignificantly in

Table 1. Changes in the parameters of GDV-grams of the meat during its storage

Shelf life, days	L*	a*	b*	*C**	*K*
1	3.01 ± 0.12	6.44 ± 0.26	–22.15 ± 0.88	23.07 ± 0.92	6.65 ± 0.30
2	1.55 ± 0.06[a]	2.70 ± 0.10[a]	–10.45 ± 0.38[a]	10.80 ± 0.39[a]	7.16 ± 0.29
3	2.48 ± 0.09[a]	5.28 ± 0.19[a]	–18.87 ± 0.68[a]	19.59 ± 0.71[a]	5.67 ± 0.23[a]
4	2.67 ± 0.10	5.59 ± 0.21	–20.12 ± 0.75	20.88 ± 0.78	5.91 ± 0.25
5	2.79 ± 0.13	5.81 ± 0.27	–21.89 ± 1.02	21.66 ± 1.01	6.35 ± 0.30

L*, a*, b* — color parameters; *C** — saturation; *K* — form factor;
[a] — the mark of the values that are statistically significantly different ($p < 0.05$) from the previous one.

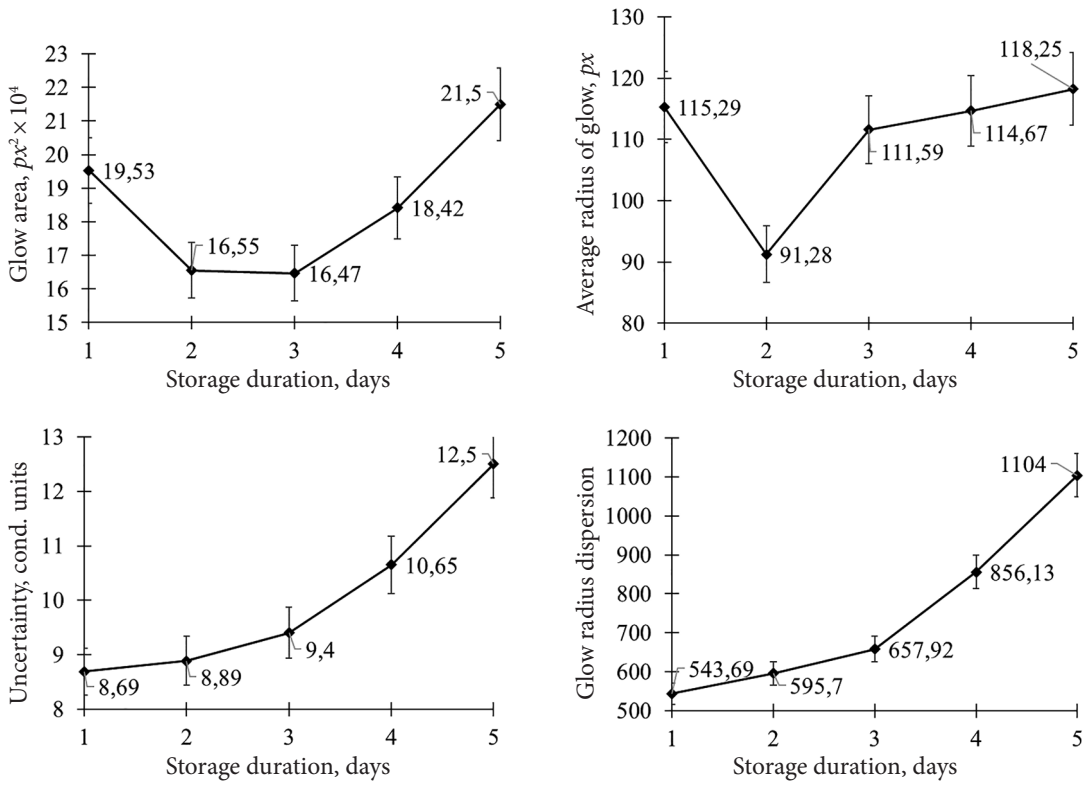


Figure 4. Dynamics of geometric parameters changes of GDV glow of chilled meat during its storage

comparison with that on the first day of storage. The uncertainty and dispersion of the glow radius of meat, indicating the heterogeneity of the glow contour, reached the maximum value (Figure 4).

It should also be noted that the uncertainty and dispersion of the luminescence of the metal reference standard throughout all studies did not exceed 2 ± 0.4 units and 50 ± 15 units, respectively, depending on environmental conditions.

Based on the obtained data, a hypothesis was offered that biochemical and microstructural changes in meat that take place during storage [30] have a strong influence on the structure and geometry of the gas discharge.

To confirm or decline this hypothesis, simultaneously with the study of gas discharge glow of meat, the studies were conducted on the fractional composition of proteins and the histostructure of muscle tissue of chilled pork during its storage, and the correlation analysis was conducted.

Analysis of the fractional composition of proteins

Analysis of 1D electropherograms of meat proteins during the storage allowed detecting the significant differences in the fractional composition of proteins and their concentration, determined by the intensity of protein zones staining.

The proteins contained in the identified zones, based on the database [31], had different origins and were identified as: connective tissue, myofibrillar and metabolic proteins.

Based on the densitometric analysis of 1D electropherograms (Figure 5), it is visible that during the first day of storage, the relative content of the high-molecular fraction of proteins within the range of 291–51 kDa and the medium-molecular fraction within 51–42 kDa increased against the background of the fraction decrease within the range 42–20 kDa.

These changes evidenced that autolysis processes took place in the meat, thus resulting in parallel aggregation and partial disintegration of various protein structures.

Based on the color intensity changes in the protein zones with a molecular weight of 239–248 kDa and 205–213 kDa, it is possible to assume changes in myosin fractions. Thus,

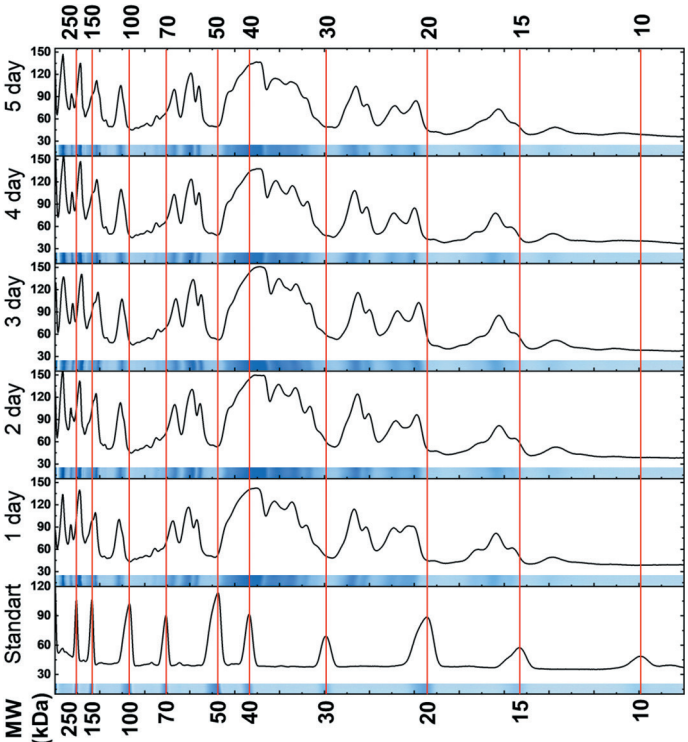


Figure 5. Results of densitometry of 1D electropherograms of *Sus scrofa M. longissimus dorsi* proteins during storage

in result of autolytic processes, on the second day of storage a decrease was observed in the relative amount of the myosin fraction by 13.9%. This could be caused by the development of *rigor mortis* and the formation of complexes between F-actin and myosin, which process is peculiar for *rigor mortis*. The following increase of relative content of low-molecular protein fractions up to the fourth day obviously indicated the destruction of actomyosin molecules and myosin aggregates down to its heavy (200–223 kDa) and light (16–20 kDa) chains.

As a result of bioinformatics analysis, it was established that the desmin fraction (50–53 kDa) went through strong destruction starting from the third day of storage, which coincides with the beginning of the *rigor mortis* resolution, which was confirmed by the results of histological analysis. On the third day after slaughter, the mass content of protein substances with a molecular weight of 51–52 kDa reached its maximum value of 3.33%. With further storage the mass content of this fraction decreased down to 3.12% on the fifth day. As a result of the weakening of the myofibril structure due to the degradation of desmin and intramuscular connective tissue under the action of calcium ions, the meat structure got looser and softer, as evidenced by the shear force decrease.

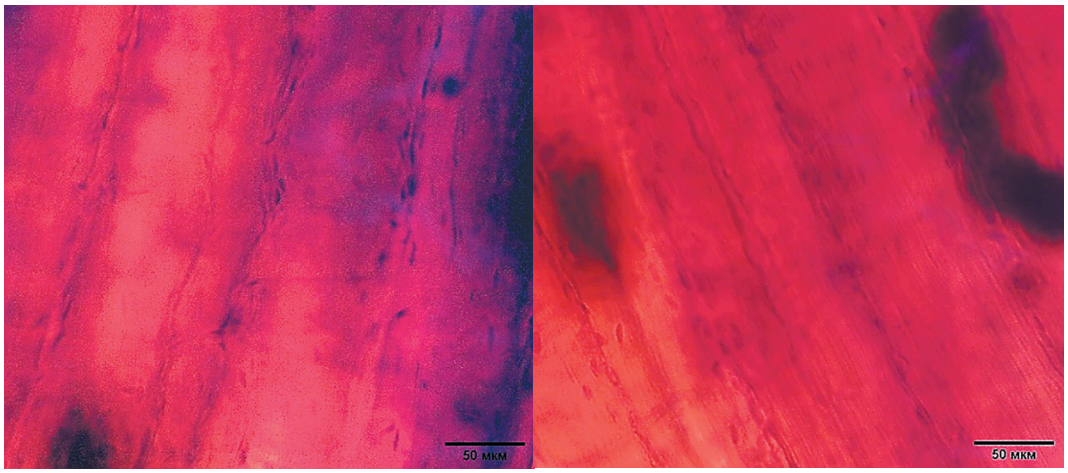
During meat storage, there was an increase in the amount of low-molecular proteins, as indicated by intensity changes of protein zones of the fraction with a molecular weight within the range of 10–18 kDa. Thus, from the data presented above, it is clear that during storage, high-molecular protein substances partially or completely decomposed with the formation of medium- and low-molecular fractions, which is also consistent with the data presented in the review of Warner et. al. [32]. The above-indicated changes in the proteins fractional composition entailed the relevant histostructural changes described below.

Study of meat histostructure

As it was already noted, the formation of high-molecular protein complexes in the first two days of meat storage was the main cause of microstructural changes in muscle tissue. Thus, in the first day of storage, asynchronous contraction of muscle fibers was observed with weakening of transverse striation and enhancing of longitudinal striation with oval contraction nodes formation (Figure 6). The muscle fibers featured an irregular shape due to deformation changes during *rigor mortis*. The fibers were wavy, tightly adjacent to each other. In some places partial relaxation of muscle fibers and restoration of transverse striation were detected. In the contraction nodes, there were ruptures in the muscle fibers sarcolemma with preservation of the fiber content and its internal structure. The diameter of muscle fibers in the first day was 80 ± 20 μm. The fibers were wavy, tightly adjacent to each other. The shear force was equal to 47.2 ± 0.11 N. On the second day of storage, the diameter of muscle fibers decreased by 18.7% and was 65 ± 15 μm, and the shear force decreased by 1.63 times in comparison with the initial value.

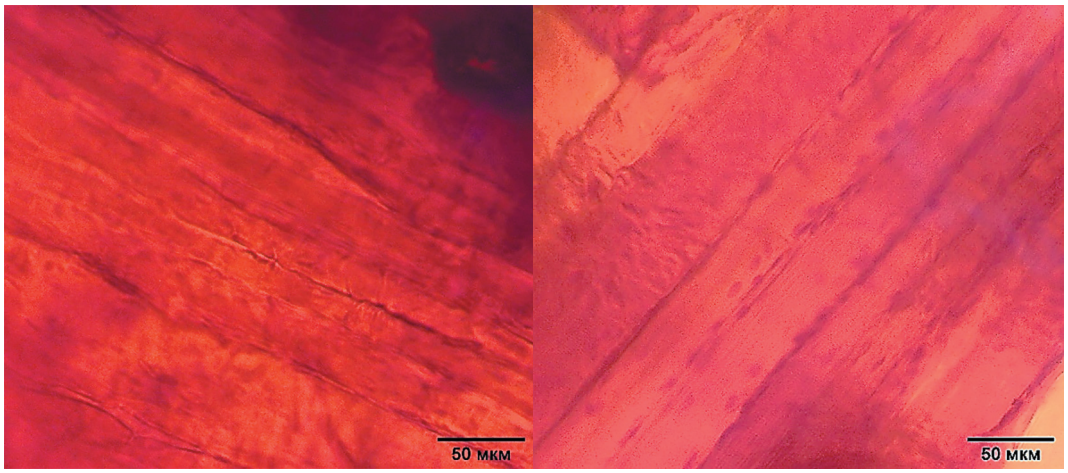
On the third day of meat storage, the histological sections of muscle tissue showed *rigor mortis* resolution and the beginning of meat maturation (Figure 7a). Histological changes in muscle tissue were expressed by the destructive processes development in meat, which became more and more intense depending on the storage time of the meat. The beginning of fiber fragmentation and loosening of connective tissue fibrous elements with their further detachment from muscle fibers were observed. No signs that evidenced the presence of contracted muscle fiber were detected. Widening of the interfiber space and increasing number of microcracks was noticeable in the sections. In some cases, separation of the sarcolemma fibers and its granular disintegration were observed. The fiber diameter was 40 ± 10 μm, and the shear force was 19.9 ± 0.9 N.

On the fourth day of storage the muscle fibers were loose and unevenly stained (Figure 7b). Local lysis was observed in some spots. The number of transverse-slit-like breaks of the muscle fibers integrity increased, while the structure of nuclei, transverse and longitudinal striations in the fragments still partially preserved. The fibers slightly increased in diameter up to 55 ± 15 μm. The shear force was 16.9 ± 0.9 N.



(a) (b)

Figure 6. Microstructure of meat on the first day (a) and second day (b) of storage (40× magnification)



(a) (b)

Figure 7. Microstructure of the meat in the third day (a) and fourth day (b) of storage (40× magnification)

On the fifth day, the muscle fibers became more fragmented, the number and size of transverse-slit-like lesions observed in the sections rose up (Figure 8). The sections got a basophilic color. Granular disintegration of individual fragments, fibers separation and local destruction of the sarcolemma were observed. Longitudinal striation was slightly distinguishable, and transverse striation was practically absent. The fiber diameter and shear force, in comparison with the meat samples on the 4th day of storage, remained almost the same and amounted to 57 ± 15 μm and 16.7 ± 0.9 N, respectively.

Thus, it was established that during the pork storage the irreversible changes in the muscle tissue microstructure occurred, caused by autolytic breakdown of myofibrils and destruction of muscle fibers, which is consistent with the materials presented in the review by Warner et. al. [32] and the results obtained by Soldatova et. al [33].

Correlation analysis and discussion of results

Correlation analysis of the characteristics of GDV-grams, meat histostructure and protein fractional composition (Figure 8) allowed establishing that the structure and geometry of the gas discharge of meat were closely related to post-slaughter changes in protein fractions, which led to the meat microstructure and cell membranes destruction. As a result of the analysis of data obtained during the study, a high correlation was established between the parameters of GDV-grams and the relative content of high-molecular and medium-molecular protein fractions. The change of color intensity in the protein zones with molecular weights of 281–291 kDa, 239–248 kDa, 130–140 kDa, 65–67 kDa, 51–42 kDa, 33–25 kDa and 17–19 kDa, which, based on the database [31] corresponded to various structural and myofibrillar proteins fractions, took place synchronously with the geometric and spectral characteristics change of the meat gas-discharge glow.

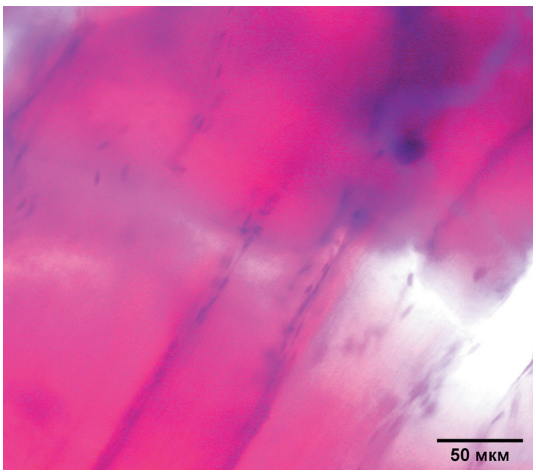


Figure 8. Microstructure of the meat in the fifth day of storage (40× magnification)

Among the most significant characteristics of meat GDV-grams presented for analysis, were the average glow radius and shape coefficient, as well as the dispersion of the glow radius and uncertainty, which reliably correlated with the change in the meat shear force during its storage.

Based on the detected changes in the structure of the meat gas discharge, it is possible to define several main stages depending on autolysis stages, which are described below.

1) During the *rigor mortis* development, the GDV-grams of meat were characterized with uniform and stable corona discharge. The more expressed microstructural changes in meat at *rigor mortis* stage were, the less intense its GDV glow was.
2) When *rigor mortis* resolved, a decrease in the area and an increase in the dispersion of the glow radius were observed, which indirectly evidenced the muscle fiber destruction onset. The significant decrease of the corona discharge brightness and saturation could also evidence the disintegration of protein structures and changes in the meat physical properties.
3) After the *rigor mortis* resolution, the corona discharge structure was partially restored, but remained less intense. This loss of intensity could be caused by complex and irreversible microstructural changes.
4) During the meat maturation and its deep autolysis, sharp changes in the corona discharge contours and uncertainty increase were observed, which happened simultaneously with decrease in the high-molecular proteins fractions and the increase in medium-molecular and low-molecular proteins fractions.

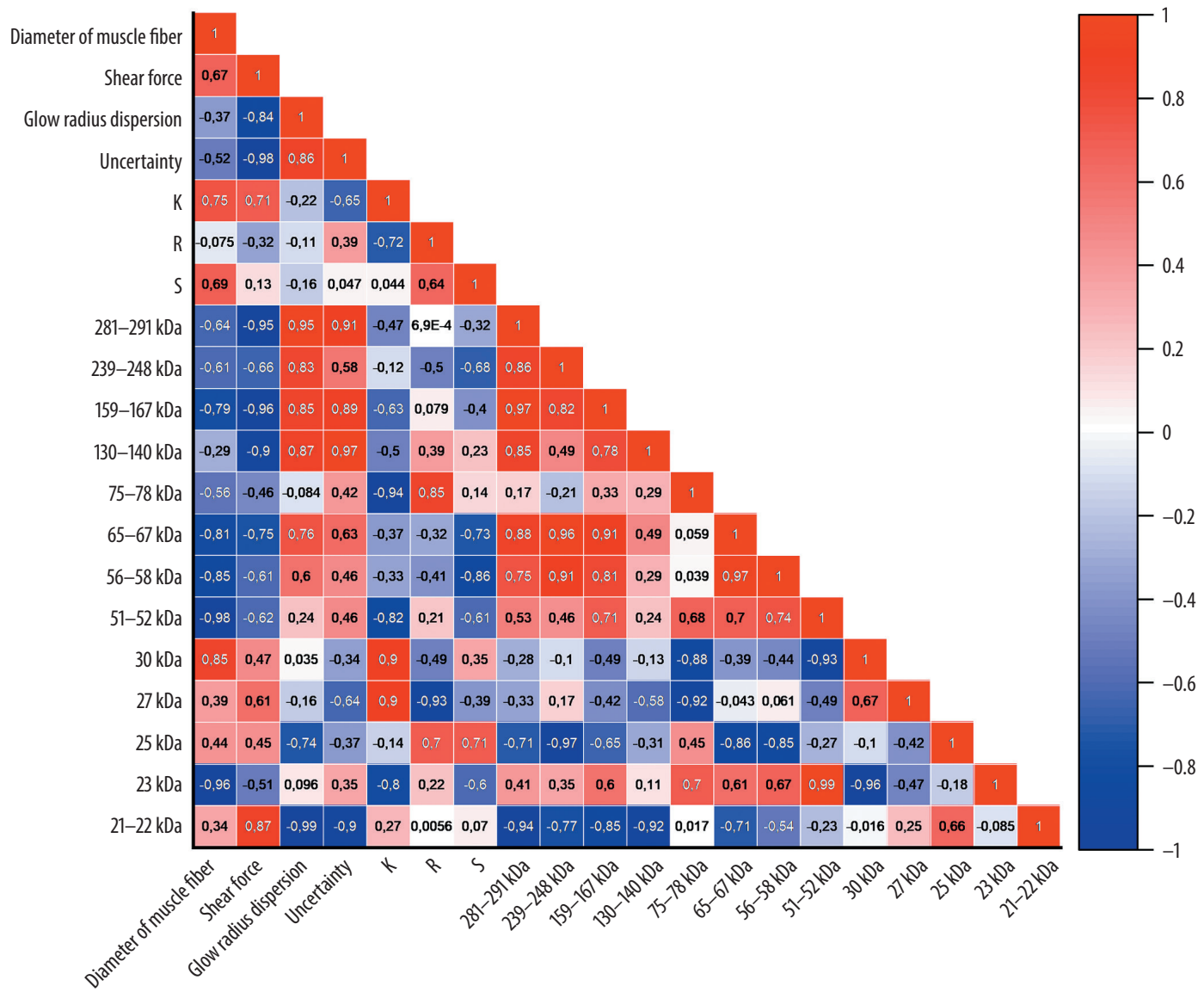


Figure 9. Correlation matrix of the characteristics of the meat GDV-grams, the results of histological studies and the molecular weight distribution of protein fractions

The high correlation between the GDV parameters and the relative content of some protein fractions confirms that changes in the fractional composition of proteins are closely related to physical changes that occur in the meat. The influence of protein fractions on the structure of the gas discharge of meat can be evaluated through several key aspects, since the proteins state plays crucial role in maintaining the physical and chemical structure of muscle tissue [34].

High-molecular proteins such as myosin and actin provide for muscle contractile activity and provide muscle contractions. Desmin maintains the structural and mechanical integrity of the cell during contraction, while helping to transmit force and withstand the longitudinal load. When they disintegrate during autolysis, the mechanical strength of the fibers decreases, the orderliness and homogeneity of muscle tissue decrease, which probably led to a more chaotic and unstable gas-discharge glow and a change in the geometry and intensity of GDV-grams.

The increase in the number of microcracks and muscle fibers fragmentation, caused by autolytic changes, obviously affect the ionic composition and contribute to increase of tissue electrical conductivity [35]. This leads to variations in the electrical properties of meat and provided the decisive effect on initiation of gas discharge when pork samples are placed in a high-intensity electromagnetic field, which reason does not contradict the results obtained by Arkhipov et. al. [19]. Structural changes could also affect the degree of tissue polarization [36], which could change the electric field distribution and the pattern of the GDV [37].

Thus, along with an increase in the storage duration of the meat, changes occurred in the structure of the gas discharge glow against the background of peculiar changes in the fractional composition of proteins and the microstructure of muscle tissue. The heterogeneity of the surface and volume of muscle tissue that undergo changes during storage, provide for a significant effect on the electromagnetic field parameters, which does not contradict the data presented in the work of Priyatkin et. al. [20].

Gas discharge glow can serve as a peculiar indicator of meat quality and the autolytic changes degree. However, for deeper understanding of the processes occurring in meat during GDV, it is necessary to consider the possibility

of application of the other methods of analysis that take into account biophysical and electrochemical changes, and to evaluate the effect of various storage conditions on the meat quality.

It is worth noting that at the time of this work writing, no publications were found on the topic being studied that described the application of GDV for analyzing meat quality during its storage and the effect of autolytic changes within on the gas discharge formation. Since there was no reference point of comparison, the authors' conclusions on the work results were based on researches in the other fields of science.

These findings may be useful both for scientific researches in food processing and for their practical application in the meat industry, especially within the framework of quality control and the storage conditions optimization.

Conclusion

As the result of the implemented work, possibility of adjusting the GDV method for quality research meat was considered. The correlation between the parameters of GDV glow of meat and the development of autolytic processes and the physicochemical processes which take place in the meat during its storage was discovered and scientifically substantiated. The influence of physicochemical changes in the meat on the topographic features of the streamers distribution, the structure of the gas discharge as well as its geometric and color parameters, such as area, uncertainty, dispersion and average radius of glow were presented.

Depending on the meat autolysis stage, certain changes in the glow area and shape factor were observed. During the development of *rigor mortis*, its resolution and subsequent maturation of the meat, significant increase in the glow radius dispersion was observed. The characteristics of GDV-grams that describe changes in the state of meat raw materials during their storage are confirmed by the results of studies of histological structure and molecular mass distribution of the meat proteins.

The obtained data highlight the potential of the GDV method for monitoring the changes in the meat quality and changes in its properties related to the storage processes. However, for the effective implementation of GDV in the meat processing industry, further research is required to standardize the methodology and establish the correlation between GDV parameters and changes in the physicochemical characteristics of the meat. This will allow increasing the safety and improve the quality of meat products, which is an important issue for the food processing industry.

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All authors made an equal contribution to the work.

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INFLUENCE OF NATURAL PLANT SUBSTANCES ON QUALITY INDICATORS OF BROILER CHICKEN MEAT

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Keywords: *broiler chickens, chemical composition, essential elements, cinnamaldehyde*

Abstract

The research was conducted in the vivarium of the Federal Research Centre of Biological Systems and Agrotechnologies in 2023 to study the effect of biologically active substances isolated from medicinal plants on the productivity and meat quality indicators. Week-old broiler chickens were divided into 4 groups (N = 180, n = 45). Broilers in the control group received the basic diet; animals from experimental groups I, II and III, along with the basic diet, were fed cinnamaldehyde in the amount of 15, 30 and 55 mg/kg of feed, respectively. At the age of 42 days, the animals were slaughtered and the chemical, amino acid and mineral composition of the muscles was determined. Broilers that, in addition to the basic diet, received cinnamaldehyde in the amount of 30 and 55 mg/kg of feed, exceeded the control group in pre-slaughter live weight by 4.50% and 7.27%, respectively. Similarly, the mass of muscle tissue and edible part of carcass in group III increased by 8.67% and 8.40% relative to the control group values, respectively. It was found that the mass fraction of protein in the breast was higher in young animals from group II than in the poultry from groups C, CA-I and CA-III by 1.57%, 2.16% and 1.35%, respectively. In terms of calcium content in the thighs, broilers from groups CA-II and CA-III exceeded the control group by 2 times. Young animals from the experimental groups CA-I and CA-III exceeded the poultry from group C in the accumulation of the essential amino acid arginine in the thighs by 0.4% and 0.2%, respectively. Thus, the use of cinnamaldehyde as a feed additive for agricultural poultry has a positive effect on the chemical composition of meat, the content of essential elements and amino acids.

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Introduction

Poultry production is one of the fast-growing and most flexible sectors of the livestock industry. In particular, due to very high demand, it has expanded, consolidated and become global in countries with different income levels. Broiler meat production has increased significantly in recent decades, especially through intensive production systems, increasing its importance for the global animal protein production and economics. Since antibiotic growth promoters are still used in many countries to improve poultry productivity, their use has simultaneously increased. However, they contribute to the development and increase of antibiotic-resistant bacteria, such as extended-spectrum beta-lactamase-producing bacteria in poultry. As a result of these negative consequences of antibiotic use and the prohibition against the use of antibacterials as growth promoters in the European Union (2006), their use as a feed additive was banned [1,2]. Currently, feed additive manufacturers face two challenges: production of additives that have a bactericidal effect and stimulate the growth of high-quality muscle tissue [3,4]. There are more than a dozen solutions on the market that have the above-mentioned properties with varying degrees of effectiveness, such as probiotics [5], prebiotics [6], symbiotics [7], phytobiotics [8,9] and plant extracts [10,11]. They are widely used worldwide due to their unique properties and positive effects on productivity. Phytobiotics play a growing role as potential alternatives to antibiotic growth promoters, since they are natural, readily available, non-toxic and contain no residues [12]. Phytobiotic additives may stimulate appetite, increase the secretion of digestive enzymes, stimulate immunity and have bactericidal, antiviral and antioxidant effects, as well as improve growth performance and the quality of animal products [13,14].

An increasing number of studies have shown that new additives such as phytobiotics may be used as an alternative to in-feed antibiotics. However, the effectiveness of alternative additives depends on many factors such as the concentration of digestible substances, the diet, the method of additive administration or the rearing conditions. Lee et al. [15] demonstrated in their studies that feeding cinnamaldehyde (125 mg/kg) increased the levels of intestinal mRNA encoding IL-1β, IL-6, IL-15 and interferon-γ and reduced body weight loss induced by *Eimeria acervulina* and *Eimeria maxima*. The anticoccidial

and antiparasitic activities were probably related to the immunomodulatory capacity of cinnamaldehyde. A recent study showed that dietary supplementation with encapsulated cinnamaldehyde (100 mg/kg) improved growth performance, reduced intestinal lesions caused by *Eimeria* spp. and *C. perfringens*, and modulated the cecal microbiota of broiler chickens vaccinated against coccidiosis [16].

Among feed additives, another possibility is the use of pure bioactive compounds found in plants, such as chemically synthesized analogues of these bioactive substances, referred to as nature-identical compounds or pure plant (phytochemical) components. Natural feed products are increasingly preferred because they are believed to have fewer undesirable side effects than synthetic analogues. The difference in using nature-identical compounds rather than plant extracts is that the former are single, pure molecules. By using pure molecules or mixtures of molecules whose composition is known, the amount of inclusion in the feed is therefore precise. In addition, products may be formulated by selecting the most effective compounds and combining them to obtain potential synergies [17].

Cinnamaldehyde is the main component of cinnamon essential oil (up to 90%) and cassia essential oil (up to 75%), which determines their odor. Cinnamaldehyde has been used for many years to produce food flavors and medicines, without taking into account its properties as an alternative to antimicrobial drugs for broilers [18]. Despite the fact that studies have been conducted to replace growth promoters with plant components, including cinnamaldehyde, it remains unknown what levels of their inclusion give the best results in terms of broiler meat quality and how do they act.

The aim of our study was to evaluate the effect of organic substances isolated from medicinal plants on the productivity and quality indicators of broiler chicken meat.

Objects and methods

The study was conducted in the vivarium of the Center for Shared Use of Scientific Equipment of the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences; the period of the study was February to August 2023. The objects of the study were broiler chickens of the Arbor Acres cross, cinnamaldehyde (chemical formula C9H8O, molar weight 132.16 g/mol, manufacturer: Acros Organics BVBA, Belgium). The objects of the study, 7-day-old broiler chickens, in the amount of 180 animals were divided into 4 groups (n = 45) using the analog method. The control group (C) received the basic diet (BD), experimental group I (CA-I) received BD + cinnamaldehyde 15 mg/kg feed/day, experimental group II (CA-II) received BD + cinnamaldehyde 30 mg/kg feed/day, experimental group III (CA-III) received BD + cinnamaldehyde 55 mg/kg feed/day. During the experiment, all animals were kept in the same conditions. The formulation of basic diet (BD) for the experimental poultry during the studies was carried out taking into account the recommendations by the All-Russian Scientific Research and Technological Institute of Poultry (VNITIP)[1]. The experiment used industrial compound feed from CJSC "Ptitsefabrika Orenburgskaya", consisting of sunflower meal, corn, wheat, soybean meal, vitamin and mineral premix. The experimental animal was fed 2 times a day; the consumption was recorded daily. Decapitation of poultry using pentobarbital ether was performed on the 42nd day (n = 5).

The analysis of meat chemical composition was carried out according to standardized methods in an independent certified Testing Center of the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences.

Post-slaughter anatomical cutting of carcasses

The cutting was carried out according to the VNITIP method. Eviscerated broiler carcasses, weighed on Mercury 327 ACP LCD scales (MERCURYWP TECH GROUP CO., LTD., Republic of Korea) with an acceptable scale division error of ±2 g, were cut into the primal cuts: breast, legs + thighs, wings, anterior part and back. The obtained cuts were anatomically deboned, the constituent tissues (muscles, skin, veins, fat, bones with and without residual trim) were isolated, and their yield was determined by weighing on MW-II laboratory scales (CAS Corporation, Republic of Korea) with an acceptable weighing error of ±0.01 g.

Eviscerated carcasses had all internal organs, head (between the second and third cervical vertebrae), neck (without skin) at the level of the shoulder joints, feet up to the tarsal joint or below it, but not more than 20 mm, removed. Eviscerated carcasses with a set of giblets and a neck are eviscerated carcasses, in the cavity of which a set of processed giblets (liver, heart, muscular stomach) and a neck are placed, packed in a polymer film approved for contact with similar food products.

Determination of moisture mass fraction[2]

A sample of at least 200 g was selected from a representative sample. The sample was stored in such a way as to prevent spoilage and changes in chemical composition. Samples were freed from fasciae or skins, ground in a homogenizer or passed through a meat grinder twice and mixed thoroughly. In this case, the sample temperature should not exceed 25 °C. Then, 8 to 10 g of purified sand and a glass rod were placed in a weighing cup and dried for 30 minutes in a drying cabinet at a temperature of 150 ± 2 °C. Next, the weighing cup was covered with a lid, cooled in a desiccator to room temperature and weighed. Weighing results were recorded to the third decimal digit. Then, 2 to 3 g of the prepared sample were placed in

[1] Fisinin, V.I., Egorov, I.A., Lenkova, T.N., Okolelova, T.M., Ignatova, G.V., Shevyakov, A.N. et al. (2009). Guidelines for optimizing compound feed recipes for agricultural poultry. VNITIP, Moscow. 2009.

[2] GOST 9793–2016 Meat and meat products. Method for determination of moisture content. Retrieved from https://docs.cntd.ru/document/1200144231 Accessed March 06, 2024

a weighed weighing cup, re-weighed, thoroughly mixed with sand using a glass rod and dried in a drying cabinet in an open weighing cup at a temperature of 150 ± 2 °C for 1 hour. Then the weighing cup was covered with a lid, cooled in a desiccator to room temperature and weighed. The mass fraction of moisture X (%) was calculated using the equation:

$$X(\%) = \frac{(m_1 - m_2)}{m_1 - m} \times 100 \quad (1)$$

where:
X is the moisture mass fraction, %;
m_1 is the weight of a weighing cup with sample, glass rod and sand, g;
m_2 is the weight of a weighing cup with sample, glass rod and sand after drying, g;
m is the weight of a weighing cup with glass rod and sand, g; 100 is the percentage conversion factor.

Determination of fat mass fraction[3]

The method is based on multiple extraction of fat with a solvent from a dried sample in a Soxhlet extraction apparatus, followed by removal of the solvent and drying of the separated fat to a constant weight. About 5 g of the prepared sample was weighed and the result was recorded to the fourth decimal digit. The analyzed sample was dried on a watch glass in a drying cabinet at a temperature of 103 ± 2 °C for 1 hour. The dried sample was quantitatively transferred to a sleeve made of filter paper, on the bottom of which a piece of cotton wool was placed. The watch glass was wiped with cotton wool soaked in a solvent (diethyl ether), which was also placed in the sleeve. The sleeve was carefully closed and placed in the extractor of the Soxhlet apparatus. The extraction flask was placed in a heating mantle or a water bath. The extraction duration was 5 to 7 hours with the extract draining rate being 5 to 8 times per hour. The completeness of degreasing was checked by applying a drop of the extract flowing from the extractor to filter paper. No greasy stain should remain on the paper. After the extraction was complete, the solvent was distilled off from the extraction flask. The extraction flask with the fat remaining after extraction was dried in a drying cabinet at a temperature of 103 ± 2 °C until constant weight.

The mass fraction of fat X (%) was calculated using the equation:

$$X(\%) = \frac{(m_2 - m_1) \times 100}{m} \quad (2)$$

where:
X is the fat mass fraction, %;
m_2 is the weight of extraction flask with fat, g;
m_1 is the weight of extraction flask, g;
100 is the percentage conversion factor;
m is the weight of analyzed sample, g.

[3] GOST 23042–2015 Meat and meat products. Methods of fat determination. Retrieved from https://docs.cntd.ru/document/1200133107. Accessed March 06, 2024

Determination of protein mass fraction[4]

The method is based on mineralization of organic substances in the sample with subsequent determination of nitrogen by the amount of formed ammonia. About 15 g of anhydrous potassium sulfate and 0.5 g of sulfate were placed in a Kjeldahl flask. About 2 g of the prepared sample was weighed on a piece of ash-free filter paper to an accuracy of 0.001 g and carefully placed in the Kjeldahl flask. Then, 25 cm^3 of sulfuric acid were added to the Kjeldahl flask. The contents of the flask were carefully mixed by slightly rotating the flask with the liquid. The flask was placed in an inclined position at an angle of about 40° relative to the vertical position on a heating device. First, the flask was carefully heated until foaming appeared and until the sample was completely dissolved. Then, mineralization was continued with vigorous boiling, turning the flask from time to time until the liquid became absolutely transparent and acquired a light green-blue color. After the contents of the flask had completely cleared, boiling was continued for another 90 minutes. The total duration of mineralization should be at least 2 hours. The Kjeldahl flask with the contents was cooled to a temperature of 40 °C; 50 cm^3 of distilled water were carefully added, mixed and cooled to room temperature. The mass fraction of protein X (%) was calculated using the equation:

$$X(\%) = \frac{0{,}0014 \times (V_1 - V_2) \times K \times 100}{m} \times 6.25 \quad (3)$$

where:
X is the protein mass fraction, %;
0.0014 is the amount of nitrogen equivalent to 1 cm^3 of 0.1 mole/dm^3 hydrochloric acid solution, g;
V_1 is the volume of 0.1 mole/dm^3 hydrochloric acid solution spent on titration of the test sample, cm^3;
V_2 is the volume of 0.1 mole/ dm^3 hydrochloric acid solution spent on titration of the control sample, cm^3;
K is the correction factor to the nominal concentration of hydrochloric acid solution;
100 is the percentage conversion factor;
m is the weight of the sample, g;
6.25 is the protein conversion factor.

Determination of meat amino acid composition

The analysis was carried out by capillary electrophoresis on Kapel-105 equipment, manufactured by Lumex (Russia) (GOST R 55569–2013[5]). The method is based on the decomposition of the sample by acid hydrolysis with the conversion of amino acids into free forms, obtaining FTC derivatives of amino acids, their further separation and quantitative determination by capillary electrophoresis. To determine the amino acids, the analyzed sample of 0.100 ± 0.001 g was placed in a hydrolysis vial; 10.0 cm^3

[4] GOST 25011–2017 Meat and meat products. Protein determination methods. Retrieved from https://docs.cntd.ru/document/1200146783 Accessed March 06, 2024

[5] GOST R 55569–2013. Feedstuffs, compound feeds, feed raw materials. Determination of proteinogenic amino acids using capillary electrophoresis. Retrieved from https://docs.cntd.ru/document/1200105562 Accessed March 06, 2024

of hydrochloric acid was added. The hydrolysis vial was hermetically sealed with a screw cap and mixed. The hydrolysis vials were placed in a drying cabinet. Hydrolysis was carried out at a temperature of 110 °C for 14 to 16 h. After hydrolysis, the hydrolysis vials were removed from the cabinet and cooled to room temperature. After cooling, the contents of the hydrolysis vials were filtered through blue ribbon filters, discarding the first portions and collecting the filtrates in vessels with lids to prevent evaporation. Then the obtaining of FTC derivatives was carried out. The prepared solutions were transferred to Eppendorf tubes and centrifuged for 5 minutes at a rotation speed of 5000 rpm. The capillary was prepared for work. The mass fraction of each amino acid in the sample X (%) was calculated using the equation:

$$X(\%) = \frac{V_{hydr} \times V_{fin} \times C_{mes} \times 100}{m \times V_{al} \times 1000} \quad (4)$$

where:
X is the mass fraction of amino acids, %;
V_{hydr} is the total volume of hydrolysate, cm³ (10 cm³);
V_{fin} is the volume of the final (analyzed) solution, cm³ (0.5 cm³);
C_{mes} is the measured value of the amino acid concentration in the solution prepared as 7.4, mg/dm³;
100 is the percentage conversion factor;
m is the weight of the analyzed sample, mg (100 mg);
V_{al} is the volume of an aliquot portion of the hydrolysate taken to obtain FTC derivatives, cm³ (0.05 cm³);
1000 is the coefficient for converting volume units.

Determination of meat chemical element composition

The analysis was carried out by atomic emission spectrometry and mass spectrometry (ICP-AES and ICP-MS) on Elan 9000 (Perkin Elmer, USA) and Optima 2000 V (Perkin Elmer, USA) equipment. The prepared sample introduction into the spectrometer, as well as the measured of atomic radiation of the elements and the concentration of the elements being determined were carried out under environmental conditions, taking into account the requirements of the spectrometer operating manual. The optimal mode for recording spectra and measurements was set. The intensity of the characteristic radiation was recorded by a photosensitive detector after this radiation passed through a monochromator. The intensity and position of the spectral lines were measured and processed by the spectrometer computer system.

Ethics statement

The experiments were carried out in accordance with the requirements of the Federal Law of the Russian Federation[6], the Declaration of Helsinki[7], the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123, Strasbourg, 1986)[8].

Statistical analysis

Statistical processing was performed using "SPSS Statistics Version 20" software by calculating the mean value (M), standard deviation (σ), and standard deviation error (m). The significance level was considered reliable at $p \le 0.05$.

Results and discussion

Broilers from the experimental groups CA-II and CA-III were characterized by high pre-slaughter live weight; according to this indicator, they exceeded group C by 4.50% and 7.27%, respectively (Table 1).

Al-Kassie [19] found that broilers receiving dietary supplements of thyme and cinnamon mixture had a significantly higher body weight gain than the control.

The young animals from group CA-III had the highest eviscerated carcass weight. Poultry from group C were 6.53% lower in this indicator than animals from group CA-III. Similarly, the weight of muscle tissue and edible part in the experimental group III significantly differed from the control values by 8.67% and 8.40%, respectively ($p \le 0.05$). Similar data were obtained by foreign colleagues, so the weight of the carcass (by 10.4 and 7.4%), weight of the breast and the relative percentage of breast increased ($p \le 0.05$) when Tecnaroma PL herbal mixture containing essential oils was added to the diet compared to the diet of poultry fed the control diet [11]. This may be due to the effect of cinnamaldehyde on the increase in villi width and surface area, which contributed to the improvement of nutrient absorption. Lower productivity of animals receiving a larger amount of microencapsulated carvacrol and cinnamaldehyde mixture may be explained by possible irritability of the intestinal mucosa with a decrease in the intestinal surface and, as a result, a smaller absorption area. Addition of phytobiotics did not have a significant effect on the slaughter yield of carcasses [20,21]. In this experiment, the slaughter yield of young poultry from the control group exceeded the similar indicator of broilers from groups CA-I and CA-III by 0.7 and 0.5%, respectively. Poultry, which received cinnamaldehyde with the basic diet in the amount of 30 mg/kg of feed per day, had an advantage over the control group by 0.9% in terms of slaughter yield of carcasses.

According to laboratory data, the mass fraction of dry matter in the keel bone was maximum in poultry from group CA-II; according to this parameter they reliably exceeded the control group (C) by 2.22% ($p \le 0.05$) (Table 2).

Similar results were obtained by İpçak et al. [22], who noted that the addition of secondary metabolites to the

[6] Federal Law of the Russian Federation dated December 27, 2018 No. 498-FZ "On the responsible treatment of animals and on amendments to certain legislative acts of the Russian Federation". Retrieved from https://docs.cntd.ru/document/552045936 Accessed March 06, 2024

[7] WMA Declaration of Helsinki — ethical principles for medical research involving human subjects Retrieved from https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ Accessed March 06, 2024

[8] European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Retrieved from https://rm.coe.int/168007a67b. Accessed March 06, 2024

Table 1. Slaughter indicators of broiler chickens at the end of the experiment ($n = 5$)

Indicator	Treatment			
	C	CA-I	CA-II	CA-III
Pre-slaughter live weight, g	2974.8 ± 222.82	2928.0 ± 170.10	3108.8 ± 154.06	3191.3 ± 53.12*
Eviscerated carcass, g	2185.0 ± 161.24	2131.0 ± 138.72	2312.4 ± 123.05	2327.7 ± 16.76
Muscle tissue, g	1074.9 ± 82.64	1047.5 ± 62.19	1137.0 ± 60.59	1168.2 ± 3.92
Bone tissue, g	619.5 ± 42.35	622.1 ± 36.27	664.4 ± 32.46	647.8 ± 13.30
Edible part, g	1922.2 ± 106.03	1884.9 ± 98.44	2033.4 ± 104.70	2083.7 ± 15.04*
Inedible part, g	880.2 ± 70.02	876.4 ± 50.55	933.5 ± 46.09	916.8 ± 27.11
Edible part/inedible part ratio	2.18 ± 0.017	2.15 ± 0.016	2.18 ± 0.042	2.27 ± 0.051
Slaughter yield,%	73.5 ± 0.43	72.8 ± 1.00	74.4 ± 0.63	73.0 ± 0.77

Note: C is the control group, CA-I is the experimental group I, CA-II is the experimental group II, CA-III is the experimental group III (here and below) * P-value ≤ 0.05; ** P-value ≤ 0.01 relative to the control group.

Table 2. Chemical composition of muscles from broiler chickens, % ($n = 5$)

Indicator	Treatment			
	C	CA-I	CA-II	CA-III
Breast				
Moisture mass fraction	77.22 ± 2.51	76.44 ± 2.44	75.0 ± 2.34	76.3 ± 2.25
Dry matter mass fraction	22.78 ± 0.55	23.56 ± 0.65	25.0 ± 0.54*	23.7 ± 0.51
Fat mass fraction	0.96 ± 0.04	2.34 ± 0.07**	1.62 ± 0.06**	1.67 ± 0.05**
Ash mass fraction	0.99 ± 0.02	0.98 ± 0.03	0.98 ± 0.02	0.98 ± 0.02
Protein mass fraction	20.83 ± 0.44	20.24 ± 0.64	22.4 ± 0.52*	21.05 ± 0.73
Thighs				
Moisture mass fraction	74.38 ± 2.37	75.23 ± 2.46	74.82 ± 2.39	76.06 ± 2.26
Dry matter mass fraction	25.62 ± 0.51	24.77 ± 0.52	25.18 ± 0.40	23.94 ± 0.46*
Fat mass fraction	3.24 ± 0.05	3.05 ± 0.04*	3.84 ± 0.07***	2.66 ± 0.05***
Ash mass fraction	0.97 ± 0.01	0.97 ± 0.02	0.96 ± 0.02	0.97 ± 0.01
Protein mass fraction	21.41 ± 0.37	20.75 ± 0.39	20.38 ± 0.29	20.31 ± 0.44

Note: *P-value ≤0.05; **P-value ≤0.01 relative to the control group.

diet was effective in terms of crude fat and crude ash content in the breast as well as dry matter, crude fat and crude ash content in leg meat.

In terms of fat content in the breast, broilers from group C were inferior to the animals from group CA-I by 1.38% ($p \leq 0.01$), group CA-II by 0.66% and group CA-III by 0.71%. This was probably due to the dose-dependent effect of aldehyde on carbohydrate-fat metabolism in the body of broiler chickens, in part by increasing glucose absorption and improving insulin sensitivity in adipose and skeletal muscle tissue. It was found that protein mass fraction in the breast was higher in group CA-II than in groups C, CA-I and CA-III by 1.57%, 2.16 and 1.35%, respectively. Foreign colleagues also found a slightly lower protein content when introducing medium doses of essential oils (20.22 g/100 g), while in the control group this value was significantly ($p < 0.05$) lower (18.51 g/100 g) [23].

The highest amount of fat in the thighs was found in the young animals from group CA-II, which exceeded the similar indicator in the control group by 0.6% ($p \leq 0.01$). Broilers in the control group had superiority over the experimental groups CA-I and CA-III in the concentration of fat in the thighs by 0.19 and 0.58%, respectively ($p \leq 0.01$).

The introduction of cinnamaldehyde into the broiler diet contributed to a slight decrease in the concentration of essential and nonessential amino acids in the breast (Table 3).

Table 3. Amino acid content in the breast of broiler chickens, % ($n = 5$)

Indicator	Treatment			
	C	CA-I	CA-II	CA-III
Arginine	6.0 ± 0.12	5.4 ± 0.18*	5.5 ± 0.16*	6.0 ± 0.16
Lysine	7.4 ± 0.20	7.3 ± 0.13	7.1 ± 0.14	7.3 ± 0.17
Tyrosine	4.9 ± 0.19	4.3 ± 0.15*	3.9 ± 0.18**	4.6 ± 0.20
Phenylalanine	3.3 ± 0.08	3.2 ± 0.07	3.1 ± 0.06	3.3 ± 0.11
Histidine	3.3 ± 0.05	2.8 ± 0.06***	2.7 ± 0.07***	3.2 ± 0.06
Leucine + isoleucine	11.1 ± 0.11	10.7 ± 0.18	10.2 ± 0.22**	10.8 ± 0.13
Methionine	2.7 ± 0.06	2.1 ± 0.09***	2.1 ± 0.10**	2.4 ± 0.07*
Valine	4.7 ± 0.10	4.4 ± 0.11	4.1 ± 0.09**	4.4 ± 0.13
Proline	3.1 ± 0.07	3.0 ± 0.08	2.9 ± 0.05*	2.8 ± 0.05**
Threonine	3.8 ± 0.12	3.3 ± 0.15*	3.3 ± 0.07**	3.5 ± 0.09
Serine	3.4 ± 0.12	2.9 ± 0.05**	2.9 ± 0.09*	3.1 ± 0.13
Alanine	7.8 ± 0.20	7.3 ± 0.17	7.0 ± 0.14*	7.5 ± 0.15
Glycine	3.7 ± 0.11	3.6 ± 0.09	3.6 ± 0.10	3.7 ± 0.08

Note: * P-value ≤ 0.05; ** P-value ≤ 0.01 relative to the control group.

Thus, in the muscles of animals from groups CA-I and CA-II, the lowest concentration of arginine, methionine, valine was noted, on average lower by 0.6% relative to group C. A similar pattern was observed for the content of tyrosine, histidine, and serine, which was lower by 0.6% to 1%, 0.5% to 0.6%, and 0.5% relative to the control, respectively. Interestingly, in animals that were fed cinnamaldehyde in the amount of 100 mg, a lower content of dry

matter, protein and amino acids such as lysine and tryptophan was found, compared to young animals that received the supplement with a dosage of 50 mg. This may be due to high concentrations of chemical compounds (aldehyde), which may block lysine and tryptophan residuals in digestive enzymes [24].

Supplementation of broiler chickens with cinnamaldehyde resulted in minor changes in major element composition of breast (Table 4).

Thus, the sodium level increased significantly in group CA-II by 70.25% ($p \leq 0.01$) and in group CA-III by 19.48% ($p \leq 0.01$) relative to group C. The control broilers were inferior in terms of accumulation of iron in the breast by 36.23% and zinc by 17.94% compared to the animals from groups CA-I and CA-III. The smallest amount of essential and conditionally essential elements was deposited in breast from group CA-II, such as manganese by 19.75%, cobalt by 50%, nickel by 47.61% and chromium by 30.24% compared to group C.

Young animals from the experimental groups CA-I and CA-III exceed the animals from group C in accumulation of the essential amino acid arginine in the thighs by 0.4 and 0.2%, respectively (Table 5).

The proportion of essential amino acids (histidine, serine, glycine) in the thighs of poultry from group CA-I decreased by 0.3% relative to group C. It has been proven that phytoncides improve not only the quality of meat, but also its amino acid composition and fatty acid profile [25,26]. The proportion of polyunsaturated fatty acids (PUFA) in the total fat of the breast and legs, as well as in the abdominal fat, was positively affected by additives containing 2% and 2.5% *Boswellia serrata*, improving the dietary parameters of meat (n-3/n-6, S/P, TI, AI and HH) [27]. Gomathi et al. [28] recommend including cinnamon oil in the diet of domestic poultry, which will help to increase the content of unsaturated fatty acids and reduce the content of saturated fatty acids in the carcass. The introduction of encapsulated cinnamaldehyde improved meat quality and intestinal health by reducing the Warner-Bratzler shear force in meat, increasing the villi-to-crypt ratio in the intestine, and favorable microbiota composition in the ileum and cecum [29].

In terms of calcium content in the thighs, broilers from groups CA-II and CA-III exceeded the control by 1.2 times or 16.6% (Table 6).

Similar data were obtained in the experiments by Popović et al [23] and Galli et al. [30], with the content of Ca and P significantly improving in the groups in which the essential oil mixture was used as a food additive. The introduction of cinnamaldehyde in low and medium doses contributed to an increase in the nickel content by 63.63% and 51.51% compared to the control. In terms of copper content in thighs, the young animals from group CA-I had an advantage over group C of 0.23 mg/kg (by 11.79%). The maximum concentration of chromium was observed in group CA-II, which was 10.69% higher than in group C.

Table 4. Chemical element content in the breast of broiler chickens ($n = 5$)

Element	Treatment			
	C	CA-I	CA-II	CA-III
Major elements, g/kg				
Ca	0.26 ± 0.01	0.23 ± 0.01	0.29 ± 0.01	0.27 ± 0.01
P	9.33 ± 0.28	8.98 ± 0.29	8.28 ± 0.28	8.45 ± 0.32
K	16.31 ± 0.57	14.95 ± 0.45	13.78 ± 0.50	14.23 ± 0.63
Na	1.95 ± 0.07	2.18 ± 0.07	3.32 ± 0.10*	2.33 ± 0.10*
Mg	1.17 ± 0.04	1.14 ± 0.04	1.04 ± 0.03	1.08 ± 0.05
Essential and conditionally essential elements, mg/kg				
B	1.58 ± 0.05	1.47 ± 0.11	1.57 ± 0.06	1.55 ± 0.12
Fe	41.42 ± 1.86	56.43 ± 1.75*	35.39 ± 1.59	50.13 ± 1.55
Zn	27.42 ± 1.34	30.96 ± 1.18	32.34 ± 1.33*	28.05 ± 0.90
Se	0.69 ± 0.155	0.68 ± 0.230	0.76 ± 0.126	0.62 ± 0.284
Mn	0.81 ± 0.03	0.76 ± 0.02*	0.65 ± 0.02*	0.69 ± 0.02*
Co	0.02 ± 0.001	0.02 ± 0.001	0.01 ± 0.001*	0.02 ± 0.001
Ni	0.42 ± 0.017	0.41 ± 0.016	0.22 ± 0.013*	0.32 ± 0.021*
Cu	1.39 ± 0.04	1.38 ± 0.06	1.32 ± 0.05	1.11 ± 0.04*
Cr	2.48 ± 0.082	1.94 ± 0.163*	1.73 ± 0.052*	1.77 ± 0.195*

Note: * P-value ≤ 0.05 relative to the control group.

Table 5. Amino acid content in the thighs of broiler chickens, % ($n = 5$)

Indicator	Treatment			
	C	CA-I	CA-II	CA-III
Arginine	5.1 ± 0.08	5.5 ± 0.07**	5.1 ± 0.05	5.3 ± 0.05*
Lysine	6.8 ± 0.12	7.0 ± 0.14	6.6 ± 0.15	6.8 ± 0.12
Tyrosine	3.3 ± 0.06	3.2 ± 0.05	3.0 ± 0.08**	3.2 ± 0.12
Phenylalanine	3.1 ± 0.07	3.0 ± 0.08	2.9 ± 0.05	3.0 ± 0.10
Histidine	2.6 ± 0.05	2.3 ± 0.08*	2.4 ± 0.06*	2.5 ± 0.05
Leucine + isoleucine	10.0 ± 0.22	10.0 ± 0.25	9.5 ± 0.24	9.8 ± 0.19
Methionine	2.1 ± 0.06	2.2 ± 0.07	2.1 ± 0.07	2.3 ± 0.10
Valine	3.9 ± 0.10	4.0 ± 0.09	3.8 ± 0.11	4.0 ± 0.12
Proline	2.9 ± 0.09	3.0 ± 0.07	2.8 ± 0.08	2.9 ± 0.09
Threonine	3.1 ± 0.11	3.3 ± 0.07	3.1 ± 0.06	3.3 ± 0.08
Serine	2.8 ± 0.07	3.1 ± 0.06*	2.8 ± 0.09	3.0 ± 0.10
Alanine	6.1 ± 0.14	6.3 ± 0.11	5.8 ± 0.15	6.1 ± 0.12
Glycine	3.5 ± 0.09	3.8 ± 0.13*	3.3 ± 0.08	3.6 ± 0.11

Note: * P-value ≤ 0.05; ** P-value ≤ 0.01 relative to the control group.

Table 6. Chemical element content in the thighs of broiler chickens ($n = 5$)

Element	Treatment			
	C	CA-I	CA-II	CA-III
Major elements, g/kg				
Ca	0.30 ± 0.01	0.27 ± 0.01	0.25 ± 0.01*	0.25 ± 0.01*
P	8.16 ± 0.30	8.09 ± 0.29	7.89 ± 0.25	8.28 ± 0.29
K	14.43 ± 0.48	13.72 ± 0.47	13.95 ± 0.43	14.93 ± 0.48
Na	2.46 ± 0.08	2.48 ± 0.07	2.45 ± 0.08	2.57 ± 0.10
Mg	1.00 ± 0.03	0.98 ± 0.03	0.98 ± 0.03	1.03 ± 0.03
Essential and conditionally essential elements, mg/kg				
B	1.63 ± 0.10	1.58 ± 0.09	1.40 ± 0.06	1.46 ± 0.06
Mn	0.78 ± 0.03	0.78 ± 0.02	0.73 ± 0.03	0.65 ± 0.02*
Co	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.002	0.01 ± 0.001*
Fe	46.41 ± 1.53	46.32 ± 1.53	43.52 ± 2.09	38.48 ± 1.65
Zn	54.95 ± 1.87	62.20 ± 2.18	58.63 ± 2.70	54.98 ± 1.81
Ni	0.22 ± 0.009	0.36 ± 0.012*	0.39 ± 0.020*	0.23 ± 0.012
Cu	1.95 ± 0.10	2.18 ± 0.09*	1.68 ± 0.06	1.50 ± 0.05
Cr	1.87 ± 0.060	1.96 ± 0.074	2.07 ± 0.089*	1.31 ± 0.039

Note: * P-value ≤ 0.05 relative to the control group.

Conclusion

Based on the experimental data obtained on determining the efficiency of using cinnamaldehyde as a feed additive for agricultural poultry, a positive effect on the chemical composition of meat, the content of essential elements and amino acids was established. Thus, the introduction of cinnamaldehyde into the basic diet in the amount of 30 mg/kg of feed contributed to an increase in slaughter yield by 0.9%, the mass fraction of protein and fat in the breast by 1.5% and 0.66%, and the amount of zinc by 17.9% relative to the control.

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NUTRITIONAL ASSESSMENT AND ANTIOXIDANT POTENTIAL OF SELECTED MEAT TYPES CONSUMED IN OWHELOGBO, DELTA STATE, NIGERIA

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Abstract

The present study aims at evaluating the nutritional value and antioxidant potential of various meat types (beef, pork, chicken, catfish and snails) consumed in Owhelogbo, community in Isoko Local Government, Delta State, Nigeria. The different meat samples were analyzed for nutritional composition and antioxidant properties. The results obtained show that the protein content varied from 20.09 to 61.74%, while the fat content varied from 2.00 to 12.08%. The calcium content ranged from 10.30 to 143.73 mg/100 g, while the phosphorous content ranged from 100.85 to 300.11 mg/100g. The linoleic acid content was in a range from 10.91 to 29.54%, while the linolenic acid content ranged from 0.84 to 5.53%. The content of vitamin A and vitamin D varied from 4.61 to 110.69 μg/100g and 2.15 to 18.05 μg/100g, respectively. The DPPH free radical scavenging ability and FRAP inhibitory activities of the different meat types ranged from 50.84 to 65.64% and 0.88 to 1.59%, respectively. The levels of high density lipoprotein and low density lipoprotein were in a range from 13.34 to 21.90 mg/dL and 2.30 to 5.59 mg/dL, respectively. The level of low density lipoprotein was the lowest in snail meat (SN), which suggests that it may be useful in managing obesity and preventing disorders linked to lipids. Consequently, the results conclude that snail meat may be a more valuable and innovative source of animal protein than beef, pork, chicken, and catfish.

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Introduction

Consuming enough protein is crucial for growth and well-being. Because of its amino acid composition and ease of digestion for humans, animal-derived protein is frequently of higher quality [1]. Although consuming too much protein has been connected to a higher risk of developing diabetes mellitus, milk and seafood are excellent sources of taurine and other amino acids that help with blood pressure and glucose metabolism. When consumed moderately, animal proteins are especially important for the health of those who are more vulnerable [2].

However, excessive consumption of animal foods high in protein is associated with poor health outcomes and a higher risk of metabolic and physiological abnormalities as well as diet-related non-communicable diseases (NCDs). These effects are partially attributed to several food ingredients such as potential carcinogens and saturated fats of processed meat, as well as the atherogenic methionin

metabolite homocysteine. Consuming foods high in saturated and trans fatty acids (TFA and SFA) is linked to the risk of cardiovascular diseases (CVDs) [2,3]. Around 70% of mortality and mobility worldwide are attributed to NCDs, and over 75% of annual deaths in Nigeria are caused by NCDs [4].

Polyunsaturated fatty acids (PUFA), such as omega-3 (n-3 PUFA) and omega-6 (n-6 PUFA), are crucial because of the diversity of fatty acids present in food. Two types of important fatty acids (EFA) are linoleic acid (LA) and alpha-linolenic acid (ALA) that are not synthesized by the human body and are included in the omega fatty acid group [5]. Furthermore, naturally occurring sterols (mostly phytosterols and cholesterol) that are important for human health make up the unsaponified fat fraction in food. To avoid chronic diseases linked to nutrition, the World Health Organization advised consuming no more than 300 mg of cholesterol per day to avoid chronic diseases linked to nutrition [6].

Blood plasma of all animals contains cholesterol, a lipid — waxy (fat-like) steroid that is present in cell membranes [7]. It is a crucial component of body cells and is involved in the synthesis of several hormones as well as fat digestion. When exposed to sunshine, a specific type of cholesterol found in the skin called 7-dehydrocholesterol can transform into vitamin D [8]. Two distinct types of cholesterol exist: serum cholesterol, or blood cholesterol, which is largely produced by the body and circulates in the blood and dietary cholesterol, derived from animal-based foods and beverages [9]. It is a necessary component of mammalian cell membranes to establish appropriate membrane permeability and fluidity [10]. Awareness of the link between dietary cholesterol and human disease has fueled research in food cholesterol content since the relationship between plasma cholesterol levels and atherosclerosis was shown in rabbits in 1913 [11]. Cholesterol has consequently gained significance in composition research on meat and poultry products. One essential lipid component that has been viewed as bad for human intake is cholesterol, which is thought to have harmful impacts on health. More precisely, meat products, especially red meat are a source of public concern [12].

Red meat, which is a popular product among most people globally, comprises edible animal muscle from sheep, pigs, cows, and several other animal species. Some groups, however, have been advocating for the consumption of plant-derived foods in place of animal-derived foods in recent years [13]. Red meat is regarded as a source of high-quality protein in addition to numerous other healthful nutrients like fatty acids, vitamins, minerals, and molecules regulating different biological responses [14]. On the other hand, excessive consumption of red meat may also lead to lipid metabolic abnormalities, inflammatory reactions, and maybe chronic disorders. Excessive ingestion of cholesterol and saturated fats alters serum total cholesterol levels. High serum cholesterol builds up in macrophages and triggers the NLRP3 inflammasome via the NF-κB signaling pathway [15]

Dyslipidemia is gaining global attention and has been shown to be a significant risk factor for cardiovascular and metabolic diseases, as well as the underlying cause of stroke and other life-threatening illnesses [16]. It has been demonstrated in recent years that aberrant lipid metabolism is caused by chronic inflammation [17]. Inflammation is caused by oxidative stress, and a study on red meat consumption found that eating red meat may alter oxidative stress, which in turn may cause inflammation and related disorders [18]. Furthermore, red meat — especially the cuts with a high myoglobin content — is the main source of serum iron. The overabundance of iron ions in the body can exacerbate an inflammatory response and cause oxidative stress [19]. Low-density lipoprotein cholesterol (LDL–C) and other lipoproteins in the blood can reach the artery intima through circulation, and an accumulation of lipoproteins in the arterial intima can cause pathological alterations and inflammation that endanger people's lives and health [20].

About 30% of pork flesh is fat, with a substantial proportion of saturated fats. Consuming excessive amounts of saturated fatty acids raises cholesterol levels and causes concerns about the possibility of hyperlipidemia, which can lead to cardiovascular disorders [21]. In other words, of all meats from farm animals, pork is the hardest to digest if not properly cooked. Moreover, the red-meat derived non-human sialic acid, N-glycolylneuraminic acid (Neu5Gc), is present in pig meat. Several studies demonstrated a relationship between consumption of red-meat derived Neu5Gc and inflammation, cancer, cardiovascular and autoimmune diseases [22].

Pork poses a health risk to humans due to its high cholesterol, high fat content, and high levels of bacteria, toxins, viruses, and parasites, all of which can cause a variety of illnesses and disorders [23]. For instance, approximately 40 different kinds of parasites and at least 30 diseases can be carried by feral pigs, which has an impact on both human and animal health. Pigs can spread numerous waterborne or foodborne illnesses to humans [24].

Among the most popular muscle foods consumed worldwide are turkey and broiler chickens in particular. They are a significant source of nutrients and dietary energy, including critical fatty acids, vitamins, high-quality proteins, and minerals that are highly bioavailable [25]. Due to their delicious flesh, ducks and geese also play a significant role in Eastern and Western Europe, as well as in Southeast and Eastern Asia.

According to Kim et al. [26], 40% of meat consumed globally in 2022 was poultry. Global poultry consumption increased by 287% during the period from 1990 to 2022. The main initiatives to enhance the quality of poultry carcasses and the nutritional content of meat are the rise in muscle percentage and the decrease in carcass fat content through rigorous restriction and selection [27].

The high fat content, especially saturated fatty acids, which significantly boost the possibility of cardiovascular illnesses and various types of cancer, has drawn criticism [28].

Catfish is nutrient-dense and extremely high in unsaturated fatty acids, vitamins, proteins, and minerals. Customers place a great priority on the nutritious content of fish meat. In addition, it contains a high concentration of fat-soluble vitamins and microelements, and omega-3 fatty acids, among other nutrients [29]. Consequently, there is a constant increase in the market for fish meat. Conversely, there is a declining global supply of fish and seafood. As a result, the interest in fish farming and aquaculture has increased [30].

The pulmonate, nocturnal, hermaphrodite African land snails (*Archachatina marginata*) belong to the *Achatinidae* family of gastropods. They are native to Africa and can be found throughout sub-Saharan Africa, from the eastern region of Lake Chad to the western Gambia. Their range reaches the Orange River in South Africa [31] in the south. Achatina (Lamarck) and Archachatina (Albers) are the two primary genera that comprise the enormous African land snails. The former is found across Africa, whilst the latter is only found in the sub-region of West Africa [32].

In most humid tropical locations, snails can be found in freshwater, marine, and terrestrial settings. Although a small number of land species and several marine species may be omnivores or carnivores, the majority of them are herbivores that graze on green vegetation, such as fruits and vegetables, on farms [33]. The habitat of land snails in Nigeria spans from the southern region's dense tropical high rainfall forest to the derived Guinea savannah's fringe riparian forests [31]. In Nigeria and certain other parts of Africa, land snails constitute a traditional source of protein for wildlife. The mollusks' protein content and chemical score are superior to those of the egg [34].

Snail flesh is considered a delicacy and is rich in nutrients. Due to its unique flavor, it is a top choice in lodging facilities and dining establishments. Many reports have said that it is a unique meat that can be used to cure kidney-related illnesses and hypertension [35]. According to [36], snails are a rich source of protein, omega-3 fatty acids and vitamins. In addition, the necessary amino acids lysine, leucine, isoleucine, and phenylalanine are all present in good balance in snail meat. Due to the widespread belief in its effectiveness in treating specific ailments, haemolymph, a bluish liquid obtained from snails, is frequently used in African traditional medicine. According snail is abundant in iron and copper, which are critical for oxidative phosphorylation and the synthesis of cellular energy [37].

The aim of this current investigation was to compare the nutritional and cholesterol level of selected meat/fish/ commonly consumed in Owhelogbo community, Delta State.

Materials and methods

Sources of material

The meat samples, namely beef, pork, chicken, catfish and snails, used in this study were bought from Owhelogbo market in Delta State, Nigeria. The reagents used for the analyses were of a high grade and quality, and were purchased from Pascal Scientific limited, Akure, Ondo State.

Study area and sites

Owhelogbo is an Isoko town located in Delta State's Isoko North Local Government Council between latitudes 50 351 N and 50 401 N and longitudes 60 181 E and 60 241 E. Its borders are as follows: Abbi to the south, Otor Owhe to the north, Ozoro to the east, and Orogun to the west.

Sample preparation

Processing of beef, pork, chicken meat and catfish meat

Samples of beef (BF), pork (PK), chicken meat (CHK) and catfish meat (CF) were rinsed with clean water to remove blood. Then, they were deboned, cut into small slices and weighed to about 4 g each. After that, the meat samples were boiled in clean water for 30 min. The boiled meat samples were dried in an oven at 62 °C for 60 min. and stored in an air tight zip lock polymer sack at a temperature of 0 °C in a refrigerator for further analysis.

Processing of snails

Snails (SN) were killed by breaking the shell and the content were removed carefully. Then, the snails were washed with alum and clean water to remove the slimy material. After that, the snails were cut into small slices and weighed to about 4 g each. The snails were boiled in clean water for 30 min. The boiled snails were dried in an oven at 62 °C for 60 min. and stored in an air tight zip lock polymer sack at a temperature of 0 °C in a refrigerator for further analysis.

Determination of proximate composition

The levels of moisture, ash, fat, and protein in the popcorn samples were estimated using methods from the Association of Official Analytical Chemists [38]. To calculate total amount of carbohydrates, the percentages of fat, protein, and ash were deducted from 100%. When determining the energy value, the percentages of crude protein, crude fat, and carbohydrate were multiplied by the required factors (2.44, 8.37, and 3.57, respectively), as shown by [39], to calculate the calorific value (in kcal/g) of the sample.

Determination of mineral composition

Using an Atomic Absorption Spectrophotometer AAS Model SP9, (Scitek, China), the values of calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), and zinc (Zn) were determined. NaCl and KCl were used as standards to calculate the amounts of sodium (Na) and potassium (K) in the meat samples using a flame emission photometer Sherwood Flame Photometer 410 (Sherwood Scientific

Ltd., Cambridge, UK) [40]. Using the Vanado-molybdate colorimetric method, phosphorus was determined. Additionally, the molar ratios of Na/K, Ca/Mg, and Ca/P were computed.

Determination of fatty acid composition

The different meat samples were extracted using a 2:1 v/v ratio of chloroform to methanol, and any solid material was eliminated using filtration. After the solvent was removed, the entire extracted lipid content was recovered in a nitrogen stream. After being redissolved in anhydrous chloroform/methanol (19:1 v/v), the samples were centrifuged at 10,000 x g for 10 minutes to remove any remaining particles (Centrifuge 5702 R, Eppendorf, United States). A methanol solution containing 14% (w/v) boron triflouride (BF3) was used to achieve tranmethylation. A 15 mL Teflon-lined screw-cap tube was filled with an aliquot of each sample and fifty nanograms of heptadecanoic acid (the internal standard). Following nitrogen gassing to remove the solvent, the samples were combined with 0.5 ml of 14% w/v BF3 reagent, warmed to 100 °C for 30 mins, and then cooled [40]. Hexane was used to extract the transmethylated fatty acids following the addition of saline solution. Parallel processing was done on a calibration mixture of fatty acid standards. Gas chromatography was used to evaluate aliquots from the hexane phase. A Hewlett-Packard gas chromatograph (5890 Series II, Gentech, United States) fitted with a flame-ionization detector was used to separate and quantify fatty acids. An Omegawax (30 m x 0.32 mm ID, Supleco, Bellefonte, PA) fused silica capillary column was injected with a two microliters split-mode aliquot of the hexane phase. Temperature settings for the injector were 200 °C, detector 230 °C, oven 120 °C at first, and 120–205 °C for eighteen minutes. Helium served as the carrier gas, and the flow rate was roughly 50 cm/sec. The constant flow mode of electronic pressure control was employed. The quantification of fatty acids in the lipid extracts was done using the internal standard (heptadecanoic acid, C17:0) and the calibration standards (NuCheck, Elysian, MN). The fatty acids listed are the mean of the three calculations. Total saturated fat (SFA), monounsaturated fat (MUFA), polyunsaturated fat (PUFA), PUFA/SFA, n-6/n-3, and MUFA/SFA were among the other fatty acid characteristics that were computed [41].

Determination of vitamin composition

Vitamins A, D, E and K were determined using UV-visible spectrophotometer (SP-UVG752, Scitek, China) [42].

Cholesterol estimation

Determination of cholesterol content

A 1-g homogenized sample was combined with 9 ml of ethanol and 1 mL of 33% KOH solution, and the mixture was well stirred for 20 secs. Afterwards, the sample was allowed to cool, 5 ml of deionized water was added, and it was agitated for a duration of two minutes in order to saponify the non-sterol lipids. The contents were shaken and centrifuged (Centrifuge 5702 R, Eppendorf, United States) for three minutes at 358xg after 10 mL of hexane was added. With caution, the top layer was detached and moved to a fresh flask. Following the addition of 10 mL hexane to the residual portion, each sample underwent a 3-minute centrifugation at 358xg. After being separated, the top layer was moved to the appropriate flasks. Each sample tube was then filled with 6 mL of Liebermann-Burchard reagent. After 30 minutes at room temperature, the absorbance of the tubes was evaluated in comparison to a blank that had been made in a similar manner but did not contain the sample [43].

Determination of antioxidant properties

Using a stirring plate (HPS-280, China), 10 g of the ground sample was hydrated in 100 ml of double-distilled water for 24 hrs to produce an aqueous extract of the various meat samples. After centrifuging the sample for 20 minutes at 9000 g, the supernatant was filtered and kept at 4 °C for additional analysis.

Determination of 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) radical scavenging activity

Using the procedure outlined by [44], the scavenging activity of the extract from the various meat samples against the DPPH radical was assessed. Samples were combined with 1% (w/v) Triton-X in 0.1 M sodium phosphate buffer (pH 7.0). Methanol was added to DPPH until it reached a final concentration of 100 µM. In a 96-well plate, microplate (DR-200B, Diatek, China), a 100 µl aliquot of each sample was combined with 100 µl of the DPPH radical solution, and the mixture was incubated for 30 mins at room temperature in the dark. The positive control in the experiment was reduced glutathione (GSH), and the blank was the buffer. Using a microplate reader (DR-200B, Diatek, China), absorbance was measured at 517 nm, and the following equation was used to calculate the percentage of DPPH radical scavenging activity.

$$DPPH\ radical\ scavenging\ activity\ (\%) = \\ = \frac{Absorbance(blank) - Absorbance(sample)}{Absorbance(blank) \times 100} \quad (1)$$

Determination of hydroxyl (OH) free radical scavenging activity

The ability of the extracts to scavenge hydroxyl radicals was assessed using the technique [45]. One milliliter of various extract concentrations (2–10 mg/ml), 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate, 0.26% EDTA), 0.5 ml of 0.018% EDTA, 1.0 ml of DMSO (0.85% in 0.1 M phosphate buffer pH 7.4), and 0.5 milliliters of 0.22% ascorbic acid were included in the reaction mixture. After sealing the tubes securely and heating them in a water water bath WB-1R2H-7 (Infitek, China) bath at 80 to 90 °C for 15 min, 1.0 ml of ice-cold TCA (17.5%) was added to

stop the reaction. 3.0 ml of the Nash reagent (75.0 g of ammonium acetate, three milliliters of glacial acetic acid, and 2.0 ml of acetyl acetone) was added to the reaction mixture mentioned above. Distilled water was added to make a total volume of 1 L. The mixture was then incubated at room temperature for 15 mins to allow for the development of color. The intensity of the yellow color was evaluated at 412 nm using a UV-visible spectrophotometer SP-UVG752 (Scitek, China) in relation to a blank for the reagent. Gallic acid and ascorbic acid were utilized as benchmarks [46].

Determination of Ferric reducing antioxidant power (FRAP) assay

With some modifications, the ferric reducing antioxidant power (FRAP) test was carried out according to [47] instructions. After heating 30 ml of freshly prepared FRAP reagent to 37 °C and reading at 593 nanometers for the reagent blank, 100–150 microliters of each sample were added, and the volume was made up to a total volume of 1ml with distilled water. After 0 sec and 4 min, absorbance (A) values were obtained using a Jenway Vis Spectrophotometer 6305 (Fisher scientific, UK). After that, the absorbance (A593 nm) change for each sample between the final reading chosen and the blank reading was calculated calculated for each sample and correlated with the absorbance (A593 nm) of a Fe^{2} standard solution that was tested concurrently. The 4-minute data were chosen so that the FRAP values could be computed [46].

Determination of Fe^{2+} chelating ability

The chelating activity of the proteins and extracts on Fe^{2}+ was determined [48], methodology with minor adjustments. An amount of 200 µL sample aliquots was combined with 740 µL of deionized water and 20 µL of the 2 mM $FeCl_2$ solution, then allowed to stay at room temperature for 30 minutes. Following the incubation period, 200 µL of 5 mM ferrozine was added, and the mixture was left standing under the same conditions for a further 10 min. The absorbance was measured at 562 nm using a UV-visible spectrophotometer (SP-UVG752, Scitek, China). Instead of using the sample, distilled water without sample aliquots was used as a control. The capacity to chelate metals was computed [46].

Determination of 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS)

With a few minor adjustments, the scavenging activity was calculated using the methodology outlined by [49]. The ABTS solution had 2.5 mM potassium persulfate and 7 mM ABTS and had been made 12 hours before it was used. After obtaining an absorbance of 0.7 ± 0.02 at 734 nm, the obtained solution was diluted with 200 mM phosphate buffer pH 7.4 and 4 mL of the diluted solution was combined with 40 mL of extract solution 0.1 mg/mL using a UV-visible spectrophotometer SP-UVG752 (Scitek, China). After ten minutes, the absorbance at 734 nm was measured using water as a control instead of the sample [46,49].

Determination of total phenols

Gallic acid was used as a reference when determining the total phenol content (TPC) using the Folin–Ciocalteu test [50]. A test tube was filled with 50 µl of an aqueous extract solution containing 0.5 milligrammes of aqueous extract. The test tube was well shaken after adding 500 µL of Folin-Ciocalteu reagent and 50 µL of distilled water. After three minutes, 400 µL of a 7.5% sodium carbonate solution was added, and the mixture was incubated for 40 min at 45 °C in a water bath (WB-1R2H-7, Infitek, China). At 765 nm, the absorbance was measured in relation to a blank. Using the normal 0.1 mg/mL gallic acid solution, the process was repeated. 400 µL of 7.5% sodium carbonate, 500 µL of Folin-Ciocalteu reagent, and 100 µL of distilled water are combined to create the blank. Using the gallic acid calibration curve, the total phenolic content was determined and represented as mg of gallic acid equivalent per gramme of sample (mg of GAE/g sample).

Ethical approval

30 Wister rat was used in the study. Before performing the experiment, it was approved ethically. The protocol for managing laboratory animals during the study was adhered to. Ethical approval was obtained from the Faculty Research Ethics Committee, Faculty of Science, Delta State University of Science and Technology, Ozoro (Ethical approval number: FOS/DSUST/24/0117). The research was carried out according to the guidelines of the ethics committee and the protocol was approved by FOS/DSUST/24/0117.

Statistical analysis

Three separate analyses were conducted, and one-way analysis of variance (ANOVA) was employed to examine the data using SPSS (21.0) software. The means were compared using the New Duncan's Multiple Range Tests (NDMRT), and significance was declared at the 5% level. Graphs were plotted using GraphPad Prism 6.

Results and discussion

Proximate composition and energy value of the different meat types

The proximate composition of the different meat types is shown in Table 1. The composition of moisture, ash, fat, fiber, protein, carbohydrates and energy content of the different meat types ranged from 5.88 to 9.04%, 0.54 to 2.99%, 2.00 to 12.08%, 0.38 to 1.34%, 15.61 to 61.74%, 23.73 to 70.31% and 362.94 to 417.20% respectively with significant (at $p < 0.05$) differences among them. These results are consistent with previous research. The result from moisture content showed that SN had the lowest moisture content, while the highest value was in BF. All of the meat samples had low moisture content, which indicates their strong potential stability [51]. With correct packaging, meat samples can be kept fresher for longer. In such

Table 1. Proximate composition (g/100 g) and energy value (kcal/ 100 g)

Parameters	BF	PK	CHK	CF	SN
Moisture	9.04 ± 0.02^{a}	8.78 ± 0.04^{b}	8.55 ± 0.03^{c}	7.04 ± 0.04^{d}	5.88 ± 0.05^{e}
Total ash	1.70 ± 0.03^{b}	0.79 ± 0.02^{d}	1.10 ± 0.10^{c}	0.54 ± 0.02^{e}	2.99 ± 0.05^{a}
Crude fat	2.02 ± 0.03^{d}	12.08 ± 0.05^{a}	2.00 ± 0.02^{d}	5.66 ± 0.04^{b}	4.32 ± 0.04^{c}
Crude fibre	1.05 ± 0.03^{c}	1.23 ± 0.03^{b}	0.38 ± 0.01^{e}	0.84 ± 0.05^{d}	1.34 ± 0.03^{a}
Crude protein	20.09 ± 0.20^{c}	20.11 ± 0.03^{c}	28.15 ± 0.50^{b}	15.61 ± 0.04^{d}	61.74 ± 0.06^{a}
Carbohydrates	66.10 ± 1.21^{b}	57.01 ± 1.10^{c}	59.82 ± 1.04^{c}	70.31 ± 1.02^{a}	23.73 ± 1.11^{d}
Energy	362.94 ± 3.21^{e}	417.20 ± 2.11^{a}	369.88 ± 2.11^{d}	394.62 ± 2.22^{b}	380.76 ± 2.03^{c}

Note: Means (± SEM) with different alphabetical superscripts in the same row are significantly different at P<0.05.
Key: BF: beef; PK: pork; CHK: chicken (broilers); CF: catfish; SN: snails.

circumstances, water-catalyzed or facilitated biological and chemical modification reactions are prevented [51].

The result from ash content analysis showed that CF had the lowest value, while SN had the highest value. The high ash content of SN may be due to the fact that snails are a rich source of minerals [52]. Since ash is the inorganic residue left over after water and organic matter are removed through heating in the presence of an oxidizing agent, the amount of ash in food materials can be utilized as an indicator of the mineral elements in those materials [53]. The result from crude fat determination showed that CHK had the lowest value, while PK had the highest value. The high fat content of PK may be attributed to the high fat content of pork meat. Pork is known to be very rich in fat [54]. The low fat content of the SN samples may be due to the low fat content in snails, which might be a reason for its recommendation as a safe animal fat and protein for those with kidney disease and blood-related illnesses [55].

Interestingly, the result from crude fiber determination showed that CHK had the lowest value while SN had the highest value. The fiber contents of all the different meat in this study were lower when compared to 2.75–3.56% previously reported by [56]. The consumption of food with adequate dietary fibre has been linked with the reduced risk of obesity, diabetes and coronary heart diseases [57].

The result from the determination of the crude protein content showed that SN had the highest value, while CF had the lowest value. The percentage of protein in all the meat samples, particularly the snail meat, shows that it is a good source of protein. According to [34], snails are high in omega-3 fatty acids, protein, and vitamins. Futhermore, the necessary amino acids lysine, leucine, isoleucine, and phenylalanine are all present in good balance in snail meat.

Meanwhile, the result from carbohydrates content showed that CF had the highest value while SN had the lowest value and this may be attributed to the high carbohydrate content of catfish [58]. The findings were consistent with those of [59]. The obtained carbohydrate contents in this study (23.73–70.31%) agreed with the previous finding of [60] that snail flesh is a delicate delicacy that is easy to eat and appetizing for the elderly without posing a health risk. The result from the energy content analysis showed that PK had the highest value, while BF had the lowest value. Pork meat is known to have the high energy content [61]. This result aligns with the result of other authors [62]. This opinion is also shared by [63], who said that eating snail meat lowers the chance of developing chronic illnesses, prevents clinical inadequacies in the elderly, and promotes overall health.

Mineral composition of the different meat types

The data in Table 2 show the mineral composition of the different meat types. The outcome demonstrated that the various meats had significantly different mineral compositions ($p < 0.05$). The portion of ingested nutrients that can be used for storage and regular bodily processes is known as nutrient bioavailability [64]. The mean values of the calcium, magnesium, phosphorous, potassium, sodium, iron, zinc, copper and manganese content of the different meat types ranged from 10.30 to 143.73 mg/100 g, 34.73 to 309.59 mg/100g, 100.85 to 300.11 mg/100g,

Table 2. Mineral composition (mg/100 g)

Parameters	BF	PK	CHK	CF	SN
Ca	15.10 ± 0.10^{d}	10.30 ± 0.08^{e}	127.51 ± 0.20^{b}	143.73 ± 0.11^{a}	103.62 ± 0.20^{c}
Mg	53.00 ± 0.11^{c}	34.73 ± 0.12^{e}	114.55 ± 0.31^{b}	41.87 ± 0.11^{d}	309.59 ± 0.19^{a}
P	117.37 ± 0.13^{d}	300.11 ± 0.51^{a}	221.90 ± 0.43^{c}	100.85 ± 0.21^{e}	271.41 ± 0.16^{b}
K	200.76 ± 0.83^{b}	302.53 ± 0.51^{a}	118.20 ± 0.01^{d}	190.21 ± 0.33^{c}	112.31 ± 0.51^{e}
Na	30.15 ± 0.13^{e}	76.41 ± 0.42^{a}	55.78 ± 0.19^{c}	41.89 ± 0.15^{d}	64.61 ± 0.30^{b}
Fe	30.24 ± 0.11^{c}	41.21 ± 0.71^{b}	57.15 ± 0.30^{a}	15.48 ± 0.22^{e}	17.23 ± 0.32^{d}
Zn	19.68 ± 0.09^{a}	7.44 ± 0.55^{c}	6.44 ± 0.41^{d}	9.22 ± 0.11^{b}	7.59 ± 0.62^{c}
Cu	0.12 ± 0.01^{d}	0.41 ± 0.04^{b}	0.15 ± 0.03^{d}	0.31 ± 0.02^{b}	0.87 ± 0.02^{a}
Mn	0.64 ± 0.02^{a}	0.30 ± 0.01^{b}	0.32 ± 0.01^{b}	0.20 ± 0.01^{c}	0.62 ± 0.01^{a}
Na/K	0.15^{e}	0.25^{c}	0.47^{b}	0.22^{d}	0.57^{a}
Ca/P	0.13^{d}	0.03^{e}	0.57^{b}	1.43^{a}	0.38^{c}

Note: Means (± SEM) with different alphabetical superscripts in the same row are significantly different at P < 0.05.
Key: BF: beef; PK: pork; CHK: chicken (broilers); CF: catfish; SN: snails.

112.31 to 302.53 mg/100g, 30.15–76.41 mg/100g, 15.48 to 57.15 mg/100g, 6.44 to 19.68 mg/100g, 0.12–0.87 mg/100g and 0.20 to 0.62 mg/100g respectively. The result showed that the magnesium, copper and manganese content was the highest in SN, while the phosphorus, potassium and sodium content was the highest in PK. This result aligns with the data of the other author who reported the high potassium and sodium content in snails [65]. The result also of this study showed that snail meat is rich in potassium and phosphorus.

The content of iron was highest in CHK and the lowest in CF. The result showed that all the meat types were rich sources of minerals but did not exceed the recommended value [66]. Calcium is responsible for formation of bone, clotting of blood, control of heartbeat and contraction of muscle. It has also been reported with potential to prevent type-2 diabetes [67]. Potassium and sodium are responsible for maintenance of body fluid, regulation of body pH, muscle and nerve signals [68]. Magnesium, zinc and phosphorus are responsible for metabolism of carbohydrate, bone and hemoglobin formation. Magnesium helps with the regulation of zinc level in the body. Hence, zinc and magnesium are reported as co-factors for management of diabetes through the initiation of insulin receptor [69].

The presence of copper and zinc in the various meat samples may be caused by the usage of pesticides, fertilizers, and herbicides in animal feed, as well as the dumping of waste and municipal sewage [70]. This is similar to the work reported by [71]. When copper levels rise above the maximum allowable limit, harmful consequences arise. Damage to the kidneys and liver might also result from high concentrations [72]. According to the study's findings, some heavy metal concentrations were higher than the permitted levels recommended by EC, WHO, and FAO committees [73].

The content of zinc was the highest in BF and the lowest in CHK. Drozd et al. [74] found that the meat of snails has a higher iron content (1.25 mg/100g) than that of broiler (1.25 mg/100g), goat (0.80 mg/100g), and tilapia fish (0.55 mg/100g). The content of copper and manganese was the highest in SN, while the copper content was the lowest in PK and the manganese content was the lowest in CF. Rygało-Galewska et al. [75] reported that snail meat is rich in copper and iron, which are important in oxidative phosphorylation and cellular energy production. According to scientific research, zinc, magnesium, and calcium are crucial for glucose metabolism because they act as components or cofactors for the enzymes involved in glucose metabolism, which improves the action of insulin by activating the insulin receptor [76]. Zinc is an important mineral element during pregnancy for normal development [77]. Zinc is an important co-factor for more than 70 enzymes and plays a central role in cell division, protein synthesis and growth [78].

Additionally, red meat contains iron. It is commonly recognized that iron is a redox-active metal that catalyzes the Fenton reaction, which produces hydroxyl free radicals. Excessive iron intake can cause oxidative stress, which is characterized by increased lipid peroxidation, altered proteins, and DNA damage. Long-term oxidative stress brought on by iron can result in the emergence of numerous illnesses, including cardiovascular diseases, type 2 diabetes, atherosclerosis, neurological disorders and chronic inflammation [79]. High iron intake (161 mg/wk vs. 100 mg/wk) was linked to an increased risk of ischemic stroke, per [80]. Foods with a calcium to phosphorus ratio of >1.0 are clearly rated good, whereas those with a ratio of less than 0.5 are clearly rated poorly. Reports state that children's growth, development, and tooth development are dependent on calcium and phosphorus [81].

The Ca/P molar ratios (0.03–1.43) of the samples were within the recommended value (>1). Hence, they may be adequate to prevent rickets and osteoporosis that were associated with calcium deficiency. Also, the result revealed that Na/K molar ratios (0.15–0.57) of the different meat samples were within the value (<1.0) recommended by the National Research Council (NRC, 1989) [82].

Fatty acid composition of the different meat types

The fatty acid content of the different meat is given in Table 3. The following ranges correspond to the composition of total saturated fatty acid (∑SFA), monounsaturated fatty acid (∑MUFA), and polyunsaturated fatty acid (∑PUFA); 26.92 to 54.29%, 14.47 to 42.02% and 18.22 to

Table 3. Fatty acid composition (%)

Samples	BF	PK	CHK	CF	SN
Saturated (SFA)					
Caprylic acid (C8:0)	0.30^{b}	0.39^{a}	0.10^{d}	0.20^{c}	0.11^{d}
Capric acid (C10:0)	0.72^{b}	0.88^{a}	0.66^{b}	0.51^{c}	0.42^{d}
Lauric acid (C12:0)	0.05^{d}	0.20^{c}	0.30^{b}	4.20^{a}	0.20^{c}
Myristic acid (C14:0)	1.21^{c}	2.41^{b}	1.09^{d}	3.26^{a}	0.02^{e}
Palmitic acid (C16:0)	18.20^{d}	26.39^{a}	23.30^{b}	19.03^{c}	8.91^{e}
Margaric acid (C17:0)	7.22^{b}	9.56^{a}	1.92^{d}	5.66^{c}	0.02^{e}
Stearic acid (C18:0)	17.07^{a}	8.19^{d}	9.50^{c}	4.99^{e}	10.15^{b}
Arachidic acid (C20:0)	0.25^{e}	2.66^{b}	0.30^{d}	1.71^{c}	2.71^{a}
Behenic acid (C22:0)	0.61^{d}	1.22^{b}	0.61^{d}	1.09^{c}	1.29^{a}
Lignoceric acid (C24:0)	1.78^{c}	2.39^{b}	0.90^{e}	1.50^{d}	3.51^{a}
∑SFA	47.41^{b}	54.29^{a}	38.68^{d}	42.15^{c}	26.92^{e}
Monounsaturated (MUFA)					
Palmitoleic acid (C16:1)	1.47^{d}	5.21^{b}	2.30^{c}	6.59^{a}	1.00^{e}
Oleic acid (C18:1)	10.55^{e}	31.92^{b}	37.30^{a}	25.42^{c}	15.87^{d}
Erucic acid (C22:1)	2.45^{b}	4.89^{a}	1.05^{d}	1.64^{c}	1.00^{e}
∑MUFA	14.47^{e}	42.02^{a}	40.65^{b}	33.65^{c}	17.87^{d}
Polyunsaturated (PUFA)					
Linoleic acid (C18:2)	12.74^{d}	29.54^{a}	18.09^{b}	10.91^{e}	17.89^{c}
Linolenic acid (C18:3)	5.41^{b}	2.61^{c}	0.87^{d}	0.84^{e}	5.53^{a}
Arachidonic acid (C20:4)	2.01^{e}	3.95^{c}	2.39^{d}	6.47^{b}	11.27^{a}
∑PUFA	20.16^{d}	36.10^{a}	21.35^{c}	18.22^{e}	34.69^{b}
MUFA/PUFA	0.72^{d}	1.16^{c}	1.90^{a}	1.85^{b}	0.52^{e}
PUFA/SFA	0.43^{d}	0.66^{b}	0.55^{c}	0.43^{d}	1.29^{a}
(PUFA+MUFA)/SFA	0.73^{e}	1.44^{c}	1.60^{b}	1.23^{d}	1.95^{a}

Note: Means (± SEM) with different alphabetical superscripts in the same row are significantly different at P < 0.05.
Key: BF: beef; PK: pork; CHK: chicken (broilers); CF: catfish; SN: snails.

36.10% respectively. The results showed that the saturated fatty acids namely caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0) ranged from 0.11 to 0.39, 0.42 to 0.88%, 0.05 to 4.20%, 0.02 to 3.26%, 8.91 to 26.39%, 0.02 to 9.56%, 4.99 to 17.07%, 0.25 to 2.71%, 0.61 to 1.29%, 0.90 to 3.51% and 26.92 to 54.29% respectively. The monounsaturated fatty acids ranged from 1.00 to 6.59%, 10.55 to 37.30% and 1.00 to 4.89% for palmitoleic acid (C16:1), oleic acid (C18:1) and erucic acid (C22:1) respectively. The polyunsaturated fatty acids had the following ranges: 10.91 to 29.54%, 0.84 to 5.53%, 2.01 to 11.27% for linoleic acid (C18:2), linolenic acid (C18:3), arachidonic acid (C20:4) respectively. CF had the lowest content of PUFA and PK had the highest. Snails showed lower ($P < 0.001$) levels of unsaturated (UFA) to saturated (SFA) fatty acids ratio in comparison with the other meat types. The findings from this study on saturated fatty acids agrees with the findings of [83] which reported (66 32% in pork, 82.14% in beef, and 65.64% in rabbit). Futhermore, the leading causes of death in Western cultures are coronary heart disease and atherosclerosis, which are closely linked to diets high in cholesterol and saturated fat [84].

The research by Azemi et al. [85] indicates that linoleic acid, an unsaturated fatty acid, is likely to have its cholesterol-lowering impact through metabolism of a metabolite rather than linoleic acid itself. According to research by Astrup et al. [86], stearic acid, a long-chain saturated fatty acid, seems to have no effect on low density lipoprotein (LDL), or bad cholesterol, or total cholesterol. Scientific studies have also shown that in both normo-cholesterolemic men and women who ate a typical western diet and non-human primates, palmitic acid, lauric acid, and myristic acid raise LDL cholesterol, high density lipoprotein, total blood cholesterol, and the LDL/HDL ratio [87]. Studies have found that oleic acid has beneficial effect on total cholesterol, LDL and HDL compared with saturated fats [88]. The present study provides evidence that the oleic acid content was high in CHK and PK.

The findings of the present study demonstrated that the PUFA/SFA molar ratios in SN were higher than the FAO/WHO-recommended threshold of >1 [89]. This suggests that the meat of snails contains a higher proportion of polyunsaturated fatty acids than saturated fatty acids. Consuming a diet with this ratio has health benefits, including reducing the risk of cardiovascular diseases [34].

The PUFA+MUFA/SFA ratio was > 1.0 in all tested samples from different types of meat, namely, SN, CF, CHK and PK, which implies that the meat samples contain more good fats than the bad fats [90]. Since the meat samples were rich in monounsaturated fatty acids (MUFA) (14.47–42.02%) and polyunsaturated fatty acid (PUFA) (18.22–36.10%), they might have the ability to reduce LDL cholesterol while possibly increasing HDL cholesterol [91].

In general, the fatty acid profile can act as a vehicle for human health protection based on the ratio of their profile. The fatty acids ω-6 and ω-3, along with PUFA, MUFA, and SFA, are regarded as markers of primary significance for the nutritional assessment of fat [92]. For the prevention of atherosclerosis, a dietary ratio of ω-6/ω-3 less than 4:1 is advised [93]. Additionally, ω-3 fatty acids have anti-inflammatory qualities and are a crucial component of cell membranes [94].

They are therefore regarded as helpful compounds in the treatment of numerous chronic and inflammatory illnesses. In the current investigation, snails had the highest UFA/SFA ratio. This elevated lipid content of the diet is most likely what caused it. The higher ratio that was observed suggests a better profile of fatty acids, which is further supported by the high PUFA/MUFA ratio. The result from this study suggests that snail meat may be better for prevention of lipid-related diseases.

Vitamin composition of the different meat types

Data in Table 4 show the content of fat soluble vitamins in the different meat types. The content of vitamins A, D, E and K ranged from 4.61 to 110.69 µg/100 g, 1.79 to 18.05 µg/100 g, 0.30 to 0.39 µg/100 g and 10.70 to 70.07 µg/100 g, respectively. The present study showed that vitamin A and vitamin E were more abundant in CHK, while vitamin D was more abundant in SN. Vitamin K was more abundant in BF. These vitamins have a variety of distinct, essential roles in metabolism, and when they are either overly abundant or deficient, they can lead to health issues [94]. Hrubša et al. [95] reported that beef, pork, chicken and fish are rich source of vitamin B. The proper ratio of these vitamins is preserved by vitamin A, which is carried via fat and kept in the liver and fat tissue [96]. A vitamin D shortage can cause weak, fragile, or misshapen bones in addition to osteomalacia in adults and rickets in children [97]. Muscle lipid oxidation after slaughter may negatively impact the quality, flavour, and nutritional value of fresh, frozen, and cooked meat and meat products, making vitamin E a crucial part of the antioxidant defence

Table 4. Vitamin composition

Parameters	BF	PK	CHK	CF	SN
Vitamin A (µg/100 g)	4.61 ± 0.01^{e}	5.50 ± 0.02^{d}	110.69 ± 0.03^{a}	9.96 ± 0.02^{c}	54.99 ± 0.02^{b}
Vitamin D (µg/100 g)	4.14 ± 0.01^{c}	1.79 ± 0.05^{e}	2.15 ± 0.02^{d}	6.06 ± 0.01^{b}	18.05 ± 0.06^{a}
Vitamin E (mg/100 g)	0.03 ± 0.01^{c}	0.31 ± 0.07^{b}	0.39 ± 0.03^{a}	0.04 ± 0.01^{c}	0.30 ± 0.05^{b}
Vitamin K (µg/100 g)	70.07 ± 0.01^{a}	20.30 ± 0.00^{c}	11.00 ± 0.05^{d}	65.07 ± 0.01^{b}	10.70 ± 0.03^{e}

Note: Means (± SEM) with different alphabetical superscripts in the same row are significantly different at $P < 0.05$.
Key: BF: beef; PK: pork; CHK: chicken (broilers); CF: catfish; SN: snails.

system in live tissues [98]. Vitamin E content varies among meats, but it is generally low. It has been noted that chicken meat has more vitamin E than beef and pork. Except for chicken meat, meat is not a great source of vitamin E [99]. It has been noted that vitamin E helps to stabilise the colour of meat [100]. Meat quality metrics, particularly beef softness, can be enhanced by vitamin E concentration [101]. There are two forms of vitamin E: tocopherols and tocotrienols [102]. Tocotrienols in oil have also been shown in human studies to reverse platelet aggregation and carotid artery blockage, hence reducing the risk factors for arteriosclerosis, stroke, and ischemic heart disease [103].

Because tocopherols can scavenge free radicals, they can inhibit or postpone the initiation of the lipid peroxidation process in conjunction with other natural antioxidants such ascorbic acid and β-carotene. As an antioxidant, vitamin E shields fat in the membranes surrounding cells-including muscles, neurons, heart, and red blood cells — from oxygen-induced oxidative damage [104].

In addition to being vital for maintaining healthy bones and preventing heart disease, vitamin K is also necessary for other biological functions. Its biological function involves directing calcium into the right places in the body, such as the teeth and bones, and removing calcium from arteries and soft tissues [105].

Cholesterol content of the different meat

The cholesterol content of the different meat types is given in Table 5. Bile acids are produced from cholesterol, and fats cannot be metabolized without bile acids. The absorption of fat-soluble vitamins, including vitamins A, D, E, and K, depends on bile acids. The results of this study showed that the quantity of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL–C), low density lipoprotein cholesterol (LDL–C) and very low density lipoprotein cholesterol (VLDL–C) ranged from 17.45 to 30.10 mg/dL, 89.03 to 130.67 mg/dL, 13.34 to 21.90 mg/dL, 2.30 to 5.59 mg/dL and 10.90 to 28.77 mg/dL, respectively. The highest level of TC was in PK, while the lowest was in SN. PK had the highest level of TG, while BF had the lowest. PK showed the highest level of HDL–C, while the lowest level was determined in SN. The level of LDL–C was the highest in PK and the lowest in SN. As for VLDL–C, PK had the highest level and BF had the lowest. It can be seen from the results obtained that the low density lipoprotein in the different meat samples was in the following order: SN > CF > CHK > BF > PK. In vertebrates, including humans, triglycerides make up the majority of body fat. They are also found in blood, where they facilitate the transport of blood glucose from the liver to adipose tissue in both directions [106].

It has been shown that dietary cholesterol causes an increase in blood levels of low-density lipoprotein (LDL) cholesterol in certain people. Dietary cholesterol may have less harmful effects than saturated and trans fatty acids [107], and the effect of decreased consumption of saturated fatty acids is lessened. According to some research, eating more cholesterol raises your chance of developing cardiovascular disease [107]. Extensive research, encompassing a substantial number of stroke cases, shows a significant positive correlation between the risk of both ischemic stroke and total stroke and the consumption of fresh red meat, processed meat, and red meat in general [108]. An 11%, 13%, and 11% higher risk of stroke overall was linked to increased consumption of one serving per day of fresh red meat, processed meat, and total red meat, respectively [109]. The risk of stroke can be elevated by consumption of red meat through a number of possible mechanisms [110]. Cholesterol and saturated fats can be found in red meat. High consumptions of saturated fats have been shown to increase plasma levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides, all of which may increase the risk of stroke [111].

A study has indicated that consuming a high-fat diet might result in raised cholesterol levels in the tissues, which may enhance the tissues' vulnerability to lipid peroxidation. However, this effect can be mitigated by the presence of sufficient antioxidants [112]. Increased triglycerides may be a factor in artery hardening or pancreatitis. Heart disease, stroke, and heart attacks are now more likely as a result [113]. Because an increase in high density lipoprotein cholesterol (HDL-c) is associated with improved cardiovascular health, it is referred to as "good cholesterol". Since the early to mid-1960s, cholesterol has been referred to as the "oily killer", particularly after a number of studies revealed that it is the primary cause of atherosclerotic lesions, which are the primary causes of coronary heart disease [114].

Due to growing awareness of the detrimental consequences of eating a diet high in cholesterol on one's health, most people today opt to consume foods that are cholesterol-free [115]. People consume a wide range of fatty meals, either as the main course or as ingredients in other dishes.

Table 5. Cholesterol content

Parameters	BF	PK	CHK	CF	SN
TC (mg/dL)	23.17 ± 0.06^{b}	30.10 ± 0.05^{a}	22.89 ± 0.04^{c}	19.23 ± 0.08^{d}	17.45 ± 0.09^{e}
TG (mg/dL)	89.03 ± 0.04^{e}	130.67 ± 0.04^{a}	121.50 ± 0.03^{b}	109.91 ± 0.09^{c}	90.30 ± 0.05^{d}
HDL–C (mg/dL)	16.89 ± 0.05^{b}	21.90 ± 0.05^{a}	16.85 ± 0.02^{c}	14.33 ± 0.06^{d}	13.34 ± 0.06^{e}
LDL–C (mg/dL)	4.50 ± 0.03^{b}	5.59 ± 0.04^{a}	3.61 ± 0.05^{c}	2.71 ± 0.06^{d}	2.30 ± 0.03^{e}
VLDL–C. (mg/dL)	10.90 ± 0.01^{e}	28.77 ± 0.03^{a}	17.69 ± 0.10^{d}	18.93 ± 0.09^{c}	19.42 ± 0.02^{b}

Note: Means (± SEM) with different alphabetical superscripts in the same row are significantly different at $P < 0.05$.
Key: BF: beef; PK: pork; CHK: chicken (broilers); CF: catfish; SN: snails; TC: total cholesterol; TG: triglyceride; HDL–C: high density lipoprotein; LDL–C: low density lipoprotein; very low density lipoprotein.

The current analysis showed that the cholesterol levels in the snail flesh were considerably lower. The result from this present study suggests that eating snail meat may be beneficial for controlling obesity and preventing lipid-related illnesses.

2, 2-diphenyl-1-picryl hydrazyl radical (DPP free radical scavenging ability of the different meat types

A stable free radical called 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) is frequently utilized to quickly assess the antioxidant activity of a material. As illustrated in Figure 1, the DPPH scavenging activity ranged from 50.84 to 65.64%. The most effective inhibitor of the DPPH was SN. Moreover, PK (48.44%), CHK (55.66%), and CF (56.39%) showed very high radical scavenging activities. Nonetheless, there were notable differences between the samples at $p > 0.05$. When the meat samples underwent the antioxidant reaction with hydrogen donors, they all showed notable antioxidant activity by scavenging the DPPH radical and reducing it to the equivalent hydrazine (Figure 1). The capacity of a component to donate hydrogen may be a source of its free radical-scavenging action. Each component demonstrated the significant antioxidant activity by scavenging free radicals such as DPPH. In line with the observation of Aouji et al. [55], SN have a higher inhibitory power against free radicals than CF, CHK, PK, and BF. The capacity of snail meat to reduce Fe^{3+} to Fe^{2+} provided evidence of its significant reducing characteristics. According to the antioxidant activities, the polyphenolics in the different meat types may function as reduction agents by giving free radicals an electron, thereby stopping chain reactions that are mediated by free radicals [116].

It is vital for human health to have antioxidants. The balance between oxidants and antioxidants is preserved throughout regular metabolism. It has been shown that the best defense against different oxidative stresses may involve combining natural antioxidant supplements with a balanced diet that includes adequate antioxidants [117].

Hydroxyl (OH) free radicals scavenging activity of the different meat

The results of the hydroxyl radical scavenging activity as illustrated in Figure 2 ranged from 43.39 to 63.44% with SN exhibiting significantly the highest and BF exhibiting the least ability to inhibit the hydroxyl radical. CF (52.93%), CHK (57.21%) and PK (56.50%) also exhibited high OH scavenging activity which implies that the different meats may serve as practical hydroxyl radical scavenger. Better antioxidant and free radical scavenging qualities were discovered when OH free radical scavenging and Fe^{2+}chelation were compared to other experimental materials, such as whole leaf, extract, and residue powder samples. This discovery could be explained by the separated protein's potent free bioactive peptides, which scavenge free radicals and function as antioxidants. In contrast, the wild lettuce leaf's capacity to scavenge free radicals and act as an antioxidant aligned with findings from earlier studies that examined the health benefits and antioxidant activity of various meat types [118]. It is well recognized that substances referred to as antioxidants, which inhibit the oxidation process by lowering the generation of free radicals, play a significant role in the prevention of long-term conditions like cancer, diabetes, obesity, and hypertension [119].

FRAP inhibitory activities of the different meat types

Figure 3 displays the ferric reducing antioxidant power (FRAP) of the various meat samples. Higher FRAP values were observed SN compared to the other meat samples, which showed a substantial diversity in their reducing activity. The FRAP value varied from 0.88 to 1.59% Fe^{2+}/mg. The reason for the increased ability of the different meat types to reduce Fe^{3+} to Fe^{2+} with increases from SN, CF, CHK, PK, and BF could be due to the inhibitory power of the meat samples to produce reductants that may react with the free radicals, stabilizing and finishing the radical chain [120]. The strong antioxidant qualities in snails may have contributed to their potential bioactive capabilities, as seen by the significantly ($p < 0.05$) higher antioxidant

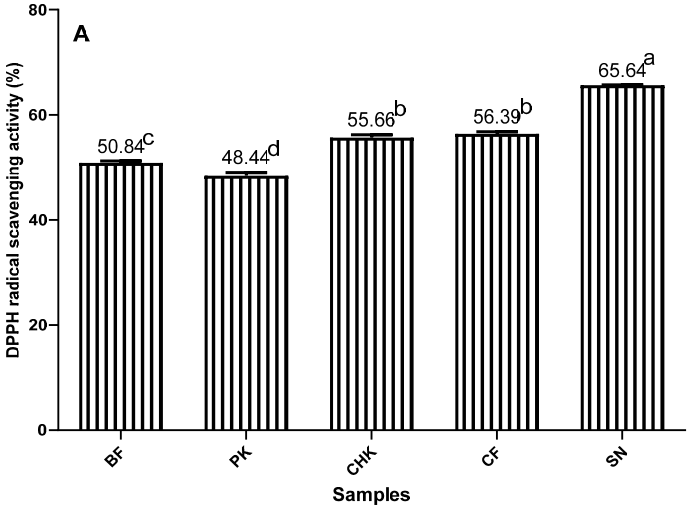

Figure 1. DPPH free radical scavenging ability

Note: Bars with different alphabetical superscripts are significantly different at $P < 0.05$.

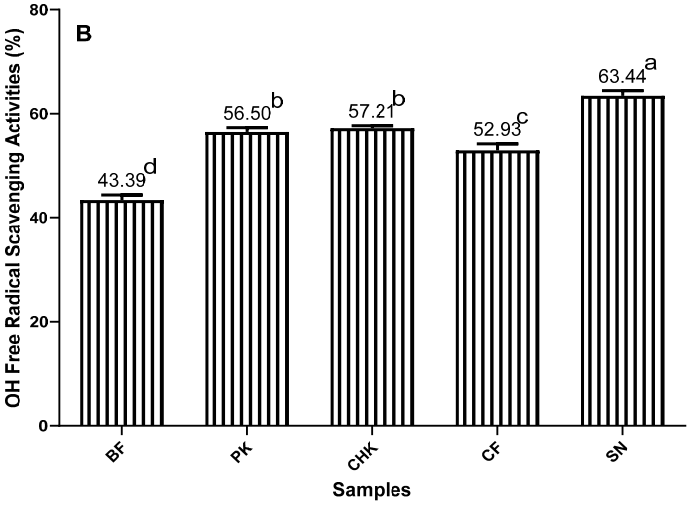

Figure 2. OH free radical scavenging ability

Note: Bars with different alphabetical superscripts are significantly different at $P < 0.05$.

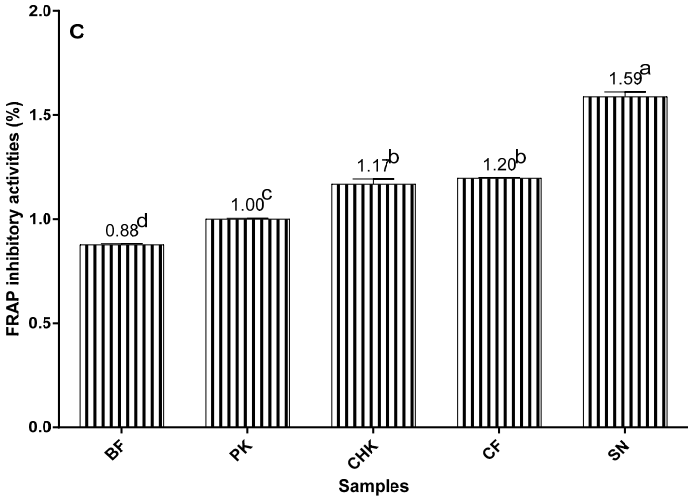

Figure 3. FRAP inhibitory activities

Note: Bars with different alphabetical superscripts are significantly different at $P < 0.05$.

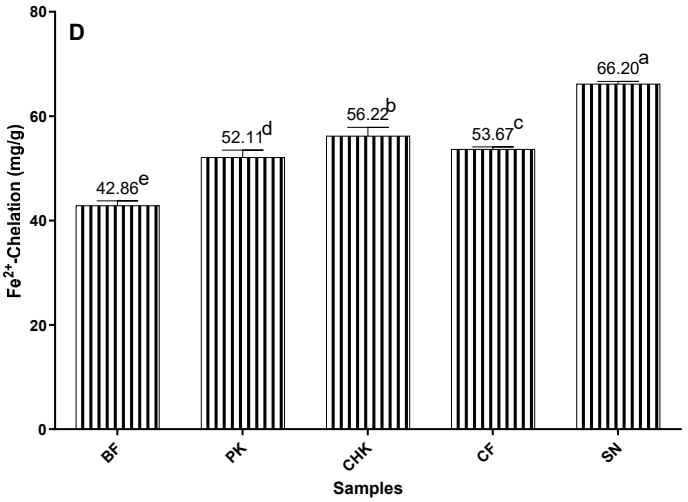

Figure 4. Fe^{2+} chelating ability

Note: Bars with different alphabetical superscripts are significantly different at $P < 0.05$.

properties observed in SN. Overall, the findings of SN antioxidant activities point to the possibility that snail meat can work as a useful scavenger of free radicals, preventing major degenerative diseases linked to them while also functioning as a useful snack during dietary interventions.

Fe^{2+} chelating ability of the different meat types

Figure 4 displays the antioxidant power of Fe^{2+}chelation for each of the meat samples. The maximum reduction ability of the various meat samples to reduce Fe^{3+} to Fe^{2+} was recorded in SN (66.20 mg/mL), and the lowest reduction ability was recorded in BF (42.86 mg/mL). The chelation power assay was used to evaluate the chelating ability of the various meat samples. The results showed that, in comparison to (CF, CHK, PK, and BF), the different meat samples had an impressive chelation power (42.86 mg/mL to 66.20 mg/mL in SN) [121].

ABTS scavenging ability of the different meat types

Antioxidant properties of the different meat types are presented in Figure 5. The ABTS values show that there were significant different meat samples ($p < 0.05$). The results demonstrated the scavenging capacity of the components against ABTS radicals in the range of 0.03 to 0.08 mMol/g (Figure 5). When it comes to scavenging free radicals, SN utperformed CF, CHK, PK, and BF. Compounds known as antioxidants shield cells from free radical damage. Despite being normal byproducts of cellular metabolism, free radicals have the ability to bind to healthy cells and cause illness within the body [122]. One of the spectrophotometric techniques used to assess the antioxidant activity of pure material solutions, aqueous solutions, and drinks Christodoulou et al. [123] is based on the production of the radical ABTS+.

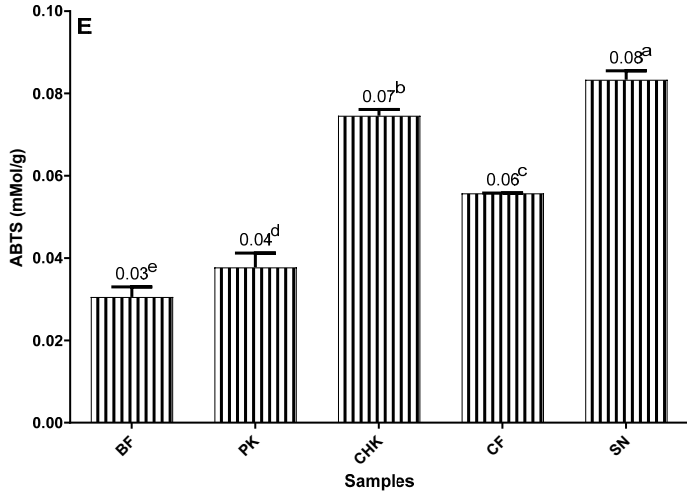

Figure 5. ABTS scavenging ability

Note: Bars with different alphabetical superscripts are significantly different at $P < 0.05$.

Total phenols of the different meat types

Figure 6 illustrates the total phenol content of the different meat samples. Total phenols were in a range from 46.53 to 73.57 mg GAE/g with SN having the highest and BF having the lowest levels. The result showed that all the meat possesses good antioxidant properties. The high polyphenol content may be the cause of the antioxidant action. Phenols have been shown to reduce the production of oxidized low-density lipoprotein (LDL), which is thought to be a causal factor of cardiovascular disease, by inhibiting the

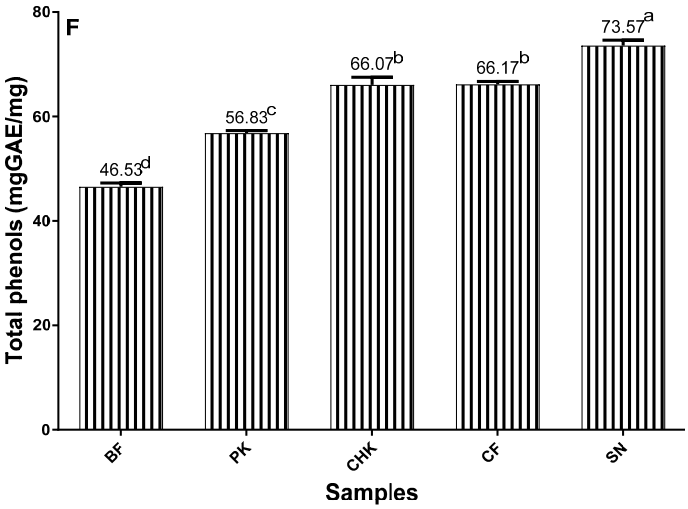

Figure 6. Total phenols

autoxidation of unsaturated lipids. Numerous prior investigations involving fruits or vegetables have also discovered a favorable relationship between total phenolic components and antioxidant activity, leading to the conclusion that high total phenol contents boost antioxidant activity [124].

Conclusion

The results of this study showed that the various meat samples had significant nutritional potential. However, SN exhibited better nutritional and antioxidant properties. From the foregoing, the results showed that SN had the highest protein content, magnesium content, PUFA+MUFA)/SFA ratio, vitamin D content, DPPH, OH free radical scavenging ability, FRAP inhibitory activities, Fe^{2+} chelating ability, ABTS scavenging ability, total phenols and had the lowest level of low density lipoprotein, which potentially aid in the control of obesity and the avoidance of illnesses linked to fat.

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COMPARATIVE CHARACTERISTICS OF DIFFERENT GRILLED BEEF CUTS AND APPLICATION OF TORCH GINGER (*ETLINGERA ELATIOR*) FLOWER IN SEASONING

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Keywords: *beef cuts, dipping sauce, grilled beef, marinade, torch ginger flower*

Abstract
Grilled beef processed with high temperature has the potential to form toxic compounds that are mutagenic and carcinogenic. This can be prevented by using beef cuts with low-fat content and antioxidant-rich spices, such as torch ginger (Etlingera elatior) flower. This study is aimed to analyze the physicochemical characteristics and antioxidant activity of grilled beef using different beef cuts and application of torch ginger flower in seasoning. Tenderloin and brisket cuts were chosen to represent the low fat and high fat content of the meat. The beef was seasoned with torch ginger flower as one of ingredients for marinade and for dipping sauce, and cooked using grilling method. This study was designed using randomized block design (CRD) with 2 × 6 factorial pattern, consisting of two beef cuts and six seasoning methods with three blocks of the samples manufacturing periods. The differences in beef cuts used to cook grilled beef were related to pH value, water, protein, fat, and malondialdehyde (MDA) content. The use of tenderloin cuts featured lower MDA levels ($p \leq 0,05$) than brisket cuts. Tenderloin cuts MDA content was 3,76 mg/kg while brisket cuts had MDA content of 1,67 mg/kg. The application of torch ginger flower in seasoning can increase water content and antioxidant activity while also reduce MDA levels in grilled beef. Soaking of beef in seasoning marinade without torch ginger flower and dipping sauce with the addition of torch ginger flower has resulted to the highest ($p \leq 0,05$) antioxidant capacity (135,19 mg EVC g^{-1}), which is more effective compared to other seasoning modes.

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Introduction

Beef is one of the animal products relied upon to meet the nutritional needs of the Indonesian people due to its high protein content with a balanced amino acid composition [1]. The interest to beef consumption is influenced by the rapid development of the food processing industry, certain lifestyles and people's consumption patterns [2]. It is also relied upon in fulfilling the consumers' diets as a great source of proteins, zinc, iron, selenium, phosphorus, also vitamins of A- and B-complex [3]. Processed meat products favored by Indonesian people generally involve heating through grilling, one of which product is grilled beef [4]. Food processed with roasting can form toxic compounds which are mutagenic and carcinogenic to the body if consumed for a long period of time [5]. High-temperature heating processes have the potential to produce free radical compounds affected by the lipids naturally present in meat, resulting in lipid oxidation reactions [6]. The fat content in processed meat is one of the factors that influences the risk level of carcinogenic compounds formation when cooked [7,8,9]. One of the by-products of lipid oxidation reactions is malonaldehyde (MDA), which is a known carcinogen that can provide a negative impact on human health [10]. The formation of carcinogenic compounds can be prevented if the fat content in meat is reduced. One of the methods is selecting meat cuts that have low-fat content [4]. Beef cuts that are often used to make grilled beef include brisket and tenderloin cuts [11]. Brisket cuts tend to have higher fat content than other cuts [12], including tenderloin cuts, thus the two cuts were used to represent different uses of beef cuts with different fat content.

The formation of toxic substances due to roasting can also be prevented by using food ingredients that are rich in antioxidants, such as torch ginger. *Etlingera elatior*, commonly known as torch ginger, is a source of natural antioxidants that are widely cultivated throughout Southeast Asia [13]. The flower of torch ginger, which is rich in active substances such as saponins, flavonoids and polyphenols, is frequently utilized for medical purposes and serves as a seasoning in many dishes in Indonesia, such as urab, pecel, chili sauce, and many others [14]. Moreover, flavonoids and phenolics in torch ginger flowers are known to reduce free radical levels in the human body [15]. Torch ginger flower can be used as an ingredient in grilled beef seasoning, one method that can be used is by marinating. Marinades have been increasingly used for processing meat products over the years among the many types of natural preservatives [16]. Adding compounds that have antioxidant properties to the meat marinating process can reduce the possibility of toxic compounds formation during the cooking process.

However, there are assumptions that high heating temperatures may reduce the antioxidant activity of natural ingredients due to its instability [17,18], including antioxidant compound in torch ginger flower [19]. Apart from marinating, seasoning a food can also be done after the cooking process, such as using dipping sauce. Dipping sauce as a condiment can be used as a means of applying torch ginger to grilled beef [14].

There have been many studies related to the physicochemical characteristics and antioxidant activity of processed meat products treated with torch ginger through marination [19,20,21]. However, there is no research related to the comparison of physicochemical characteristics and antioxidant activity of torch ginger before and after the cooking process of processed meat products, as well as the relevance of selecting low-fat beef cuts to inhibit the formation of carcinogenic compounds in grilled beef. The use of torch ginger as a natural antioxidant used in grilled beef seasoning and attention to the use of meat cuts in beef is expected to reduce the level of consumption of toxic substances contained in grilled beef. This study is aimed to analyze the physicochemical characteristics and antioxidant activity of grilled beef on the examples of different beef cuts and application of torch ginger flower in seasoning.

Materials and methods

Materials

Meat in this study was obtained from brisket and tenderloin of Brahman cross-breed cattle and were bought from meat supplier in Bogor, West Java. The ingredient used for production of marinade and dipping sauce were bought from local market in Indonesia.

Marinade preparing

The marinade formula used was the result of trial and error method on the pre-research stage using the limited number of the panelists; each ingredient was weighed in ratio based on the weight of beef used. The marinade ingredients include garlic (27.5%), chili (10.5%), onion (7%), ginger (2.5%), honey (36.5%), sesame oil (8%), vinegar (2.5%), salt (3.5%), and pepper (2%) were weighed, then pureed with a blender (Miyako BL-152, Kencana Gemilang, Indonesia) so that the marinade without the addition of torch ginger flower was obtained. The marinade without the addition of torch ginger flower then was supplemented with torch ginger flower (10%) and pureed with a blender to obtain the marinade with the addition of torch ginger flower. The marinade was weighed in a ratio of 2:1 as per the meat to each marinade.

Production of dipping sauce

The dipping sauce formula used was the result of trial and error method on the pre-research stage using the limited number of the panelists; each ingredient was weighed in ratio based on the weight of beef used. The dipping sauce was made by stir-frying garlic (15%) and onion (13%) that had been pureed using a blender (Miyako BL-152, Kencana Gemilang, Indonesia) for 3 minutes, then put in a pot along with brown sugar (14%), soy sauce (19%), sesame oil (9%), soy sauce (13%), and honey (17%), and heated until boiling (100 °C) for 5 minutes. The flame of the stove was turned off and the sauce was set aside until the temperature decreased up to room temperature (± 27 °C), resulting to obtaining the dipping sauce without the addition of torch ginger flower. The dipping sauce without the addition of torch ginger flower then was supplemented with torch ginger flower (10%) and pureed with a blender to obtain the dipping sauce with the addition of torch ginger flower. The dipping sauce was weighed in a ratio of 2:1 as per the meat to each sauce.

Production of grilled beef

Beef brisket and tenderloin was thinly sliced using a meat slicer (MSC-HS10, Astro Pandu Perkasa, Indonesia) with a thickness of 3.5 mm. The sliced beef were grouped into 5 parts according to the treatment of torch ginger flower application in the marinade. The sliced meat was marinated using immersion technique in a closed container at refrigerator temperature, which was about 4 °C for 1 hour, then grilled evenly using a grill pan on a stove (Rinnai, Rinnai Corporation, Japan) at 150–180 °C for 5 minutes with flipping the meat every 1 minute. After the grilling process, the meat was dipped in the dipping sauce according to the predetermined treatment for 10 seconds and prepped for later analysis.

Analysis of physicochemical characteristics

The physicochemical analysis of grilled beef samples covered the measurement of pH [22], water content [22], fat content [22], protein content [22], and browning intensity [23].

Analysis of antioxidant activity and capacity

Compounds from 1 g of the sample were extracted using 2.5 mL of 100% methanol at room temperature for 24 hours. After filtration, the filtrate was collected into a separate tube, and another 2.5 mL of methanol was added to the remaining retentate. The first filtrate was stored in a capped tube at –25 °C for 24 hours. The second filtrate was separated and combined with the first filtrate in a 10 mL volumetric flask, with methanol added until the total volume reached 10 mL. 0.15 mL of filtrate was taken and reacted with 0.9 mL of 0.1 mM DPPH solution in a vial tube. The solution was incubated in a waterbath (Memmert, Memmert GmbH, Germany) at 37 °C for 20 minutes. The absorbance was measured with a spectrophotometer (UV–Vis Agilent 8453, Agilent, USA) ($\lambda = 517$ nm). Antioxidant activity was assessed by measuring the percentage of DPPH radical scavenging activity, while antioxidant capacity was evaluated based on the inhibition of vitamin C impact at various concentrations on the DPPH radical. Antioxidant capacity was expressed as mg of vitamin C equivalent (VCE) per 100 g of the sample [24].

Analysis of malondialdehyde (MDA)

Analysis of malondialdehyde in grilled beef was carried out using analysis of thiobarbituric acid reactive substances (TBARS) content. A total of 10 g of sample was put into an Erlenmeyer flask and extracted with 97.5 mL distilled solution containing 0.1% propyl gallate (PG) and 0.1% ethylenediamine tetra acetate (EDTA). The sample was then supplemented 2.5 mL of 4 N HCl solution with a ratio of HCl and distilled water of 1:2 and 0.5 mL of antifoam drops. The mixture was then distilled until 50 mL of distillate was obtained from each sample. A total of 5 mL of distillate sample was supplemented with 5 mL of 0.02 M TBA solution. The samples were then incubated on waterbath at 100 °C for 40 minutes. Afterward, the samples were removed and cooled under running water. Determination of malondialdehyde levels was carried out using spectrophotometry (UV–Vis Agilent 8453, Agilent, USA) with a wavelength of 532 nm [25].

Experimental design and data analysis

The study was designed using randomized block design (CRD) with 2×6 factorial pattern consisting of two beef cuts and six methods of grilled beef seasoning with three blocks of samples manufacturing periods. The beef cuts included brisket and tenderloin cuts, while the grilled beef seasoning method consists of as follow:

- B0: control sample, without marinade and dipping sauce;
- B1: marinade with torch ginger flower;
- B2: dipping sauce with torch ginger flower;
- B3: marinade and dipping sauce without torch ginger flower;
- B4: marinade with torch ginger flower and dipping sauce without torch ginger flower;
- B5: marinade without torch ginger flower and dipping sauce with torch ginger flower.

The data were analyzed using analysis of variance to determine the effect of each mode, and the difference of means were analyzed using Tukey test.

Results and discussion

The mode of seasoning application by marination and/or dipping sauce and the use of different beef parts provided a significant effect ($p \leq 0.05$) on the pH value, but there was no significant interaction between the two modes, as shown in Table 1. The pH value of grilled beef processed with marinade and/or dipping sauce was significantly lower ($p \leq 0.05$) compared to grilled beef cooked without marinade or dipping sauce. This decrease in pH value may occur due to the influence of the acid content found in the ingredients added to the marinade. The use of seasonings for soaking meat can reduce the pH value of the processed meat final product [26,27].

Muhammad et al. [28] stated the torch ginger flower can reduce the acidity of meat because it has a low pH, which ranges from 4.18 to 4.92. This opinion differs from the results of the study which showed that the addition of torch ginger flower to the marinade and dipping sauce did not result to pH values in grilled beef which were statistically different from the seasoning without torch ginger flower, possibly due to the small amount of addition of torch ginger flower in the seasoning formula. Grilled beef from tenderloin cuts featured a higher pH value and water content ($p \leq 0.05$) compared to brisket cuts (Table 1). This is presumably related to the higher protein content in the tenderloin which can cause the pH value to be higher due to the protein's ability to bind water and maintain a more alkaline environment. Protein has a higher isoelectric point so it can absorb water more effectively and cause the muscle fibers to become tighter and the pH to be higher [29]. The moisture content of grilled beef processed with marinade without torch ginger flower and dipping sauce with torch ginger flower was higher ($p \leq 0.05$) compared to the control sample of the grilled beef. Moisture absorption from seasoning applied to beef contributed to the increase of grilled beef moisture content [30,31]. The addition of marinade and/or dipping sauce provided a significant effect ($p \leq 0.05$) on the absorbance value of the browning intensity of grilled beef (Table 1). The browning that occurs during cooking process

Table 1. pH value, water content, and browning intensity of grilled beef

Treatment	Beef cuts		Means
	Brisket	Tenderloin	
pH value			
B0	5.74 ± 0.47	5.83 ± 0.54	5.79 ± 0.06^{b}
B1	5.11 ± 0.17	5.30 ± 0.17	5.19 ± 0.16^{a}
B2	5.15 ± 0.14	5.33 ± 0.26	5.24 ± 0.07^{a}
B3	5.13 ± 0.23	5.33 ± 0.16	5.23 ± 0.14^{a}
B4	5.08 ± 0.10	5.21 ± 0.14	5.14 ± 0.11^{a}
B5	5.12 ± 0.17	5.22 ± 0.16	5.17 ± 0.07^{a}
Means	5.21 ± 0.26^{a}	5.37 ± 0.23^{b}	
Water content (%)			
B0	30.58 ± 2.78	34.19 ± 5.44	32.38 ± 2.55^{a}
B1	35.51 ± 4.33	39.58 ± 6.96	37.55 ± 2.87^{ab}
B2	31.38 ± 3.47	35.76 ± 7.18	33.57 ± 3.10^{ab}
B3	36.27 ± 5.16	42.91 ± 5.08	39.59 ± 4.70^{ab}
B4	34.98 ± 4.01	42.00 ± 4.16	38.49 ± 4.96^{ab}
B5	35.89 ± 3.57	45.85 ± 1.61	40.87 ± 7.04^{b}
Means	34.10 ± 2.47^{a}	40.05 ± 4.44^{b}	
Browning intensity			
B0	0.13 ± 0.03	0.12 ± 0.02	0.13 ± 0.005^{a}
B1	0.19 ± 0.08	0.18 ± 0.05	0.18 ± 0.01^{ab}
B2	0.20 ± 0.06	0.17 ± 0.03	0.19 ± 0.03^{ab}
B3	0.22 ± 0.06	0.20 ± 0.03	0.21 ± 0.01^{b}
B4	0.21 ± 0.04	0.19 ± 0.04	0.20 ± 0.02^{b}
B5	0.24 ± 0.04	0.22 ± 0.03	0.23 ± 0.01^{b}
Means	0.20 ± 0.04	0.18 ± 0.03	

Note: Different letters in the same row and column indicates significant differences (P < 0.05), B0: control sample, without marinade and dipping sauce; B1: marinade with torch ginger flower; B2: dipping sauce with torch ginger flower; B3: marinade and dipping sauce without torch ginger flower; B4: marinade with torch ginger flower and dipping sauce without torch ginger flower; B5: marinade without torch ginger flower and dipping sauce with torch ginger flower.

contributes significantly to the flavor, aroma, and appearance of the cooked meat [32,33,34]. Heating can cause non-enzymatic browning as indicated by Maillard reaction, due to reducing sugars or aldehyde groups from other sources reacting with amino groups obtained from amino acids, peptides and proteins [35,36]. The sugar contained in honey in the marinade may contributes to the Maillard reaction [37] when the beef is being cooked, thus increasing the browning process of the grilled beef.

Table 2 shows that protein and fat content of grilled beef were significantly ($p \leq 0.05$) affected by the interaction of applying the seasoning and the use of different beef cuts. The protein content of grilled beef on the example of tenderloin cuts and seasoning application was lower ($p \leq 0.05$) compared to the processing mode without applying the seasoning. This indicates that the seasoning application can reduce the protein content of grilled beef. Rahman et al. [31] explained that marinating the meat can cause protein-rich sarcoplasmic fluid to escape from the meat tissue. This dilution effect can cause reductions in meat protein content. The results of this study are also in line with Kumar et al. [38] which states that lemon-marinated and ginger-marinated chicken tikkas featured lower protein levels compared to the control mode. There was a decrease in fat content in grilled beef brisket pieces with applying the seasoning compared to the control sample ($p \leq 0.05$) (Table 2). This is likely due to the properties of fat that contradict with the moisture of food products, whereas the fat content in processed meat products can be significantly reduced when the water content increases [39]. Meanwhile, the addition of torch ginger flower to the seasoning did not resulted to a significant difference in protein content or fat content of grilled beef. This condition is in line with Fitri et al. [40] which stated that the addition of torch ginger extract had no significant effect on the protein and fat content of beef sausage.

Table 2. Protein content and fat content of grilled beef

Treatment		Protein content (%)	Fat content (%)
Beef cuts	Seasoning		
Brisket	B0	22.87 ± 2.16^{abc}	39.52 ± 1.76^{d}
	B1	23.18 ± 0.16^{abc}	23.79 ± 5.34^{bc}
	B2	22.57 ± 1.28^{abc}	24.29 ± 4.77^{c}
	B3	18.49 ± 4.27^{ab}	15.82 ± 4.77^{b}
	B4	20.52 ± 1.67^{ab}	18.81 ± 2.74^{bc}
	B5	16.66 ± 2.05^{a}	20.58 ± 1.01^{bc}
Tenderloin	B0	37.66 ± 2.66^{d}	3.10 ± 0.39^{a}
	B1	28.31 ± 1.94^{c}	5.05 ± 1.19^{a}
	B2	28.72 ± 2.03^{c}	3.76 ± 0.83^{a}
	B3	23.31 ± 3.32^{abc}	4.82 ± 0.91^{a}
	B4	29.29 ± 2.66^{c}	4.55 ± 0.70^{a}
	B5	24.99 ± 1.27^{bc}	4.83 ± 0.30^{a}

Note: Different letters in the same row and column indicates significant differences ($P < 0.05$), B0: control sample, without marinade and dipping sauce; B1: marinade with torch ginger flower; B2: dipping sauce with torch ginger flower; B3: marinade and dipping sauce without torch ginger flower; B4: marinade with torch ginger flower and dipping sauce without torch ginger flower; B5: marinade without torch ginger flower and dipping sauce with torch ginger flower.

Table 3 shows the results of DPPH inhibitory activity and antioxidant capacity, which results presented that there were significant differences ($p \leq 0.05$) between control sample of grilled beef and grilled beef processed with marinade and/or dipping sauce, as well as the using torch ginger flower in the seasoning. The grilled beef soaked with seasoning generally featured higher antioxidant activity ($p \leq 0.05$) than control sample griled beef. Various antioxidant compounds obtained from natural ingredients used for seasoning applications such as saponins, flavonoids and polyphenols from torch ginger flower [14], allicin and diallyl sulfides from garlic [41,42], quercetin from onion [43], gingerol from ginger [44,45], also flavonoids and phenolic acids from honey [46] has contributed to increasing the antioxidant activity of grilled beef. Zhang et al. [47] stated that antioxidants from natural food ingredients can counteract free radicals by replacing hydrogen from phenolic groups and are able to produce stable products. These natural antioxidants demonstrate a variety of biological activities, such as anti-inflammatory, antibacterial, anti-aging, and anticancer properties [48,49]. Moreover, grilled beef processed with adding marinade without torch ginger flower and dipping sauce with torch ginger flower (B5) had significantly higher antioxidant activity value ($p \leq 0.05$) than grilled beef processed with adding marinade with torch ginger flower and dipping sauce without torch ginger flower (B4). This suggests that exposure to high temperature heat during grilling process on the beef may affect the antioxidant activity of torch ginger flower applied in marinade, which is in line with previous study [19]. Heat treatment, as one of step in manufacturing certain products for human consumption, may lead to degradation which is attributed to

Table 3. Antioxidant activity and capacity of grilled beef

Treatment	Beef cuts		Means
	Brisket	Tenderloin	
DPPH radical scavenging activity (%)			
B0	25.76 ± 7.91	27.51 ± 5.05	26.64 ± 1.23^{a}
B1	48.88 ± 3.05	53.94 ± 13.90	51.41 ± 3.57^{bc}
B2	54.12 ± 7.25	59.68 ± 9.99	56.90 ± 3.93^{c}
B3	40.49 ± 2.30	47.50 ± 8.87	43.99 ± 4.95^{b}
B4	62.14 ± 8.37	58.99 ± 5.97	60.56 ± 2.23^{cd}
B5	74.79 ± 3.70	71.14 ± 5.77	72.96 ± 2.58^{d}
Means	51.03 ± 17.04	53.13 ± 14.76	
Antioxidant capacity (m g EVC g^{-1})			
B0	43.55 ± 14.66	47.39 ± 9.75	45.47 ± 2.71^{a}
B1	87.66 ± 5.05	97.14 ± 27.30	92.40 ± 6.71^{bc}
B2	99.55 ± 14.19	110.20 ± 18.88	104.88 ± 7.53^{c}
B3	71.57 ± 4.55	85.74 ± 17.04	78.65 ± 10.02^{b}
B4	112.20 ± 14.95	105.94 ± 10.04	109.07 ± 4.42^{c}
B5	138.53 ± 5.70	131.85 ± 10.34	135.19 ± 4.73^{d}
Means	92.18 ± 32.90	96.38 ± 28.48	

Note: Different letters in the same column indicate significant differences ($P < 0.05$), B0: control sample, without marinade and dipping sauce; B1: marinade with torch ginger flower; B2: dipping sauce with torch ginger flower; B3: marinade and dipping sauce without torch ginger flower; B4: marinade with torch ginger flower and dipping sauce without torch ginger flower; B5: marinade without torch ginger flower and dipping sauce with torch ginger flower.

the breakdown of the flavonoid structure, and can lead to a reduction in their bioactivity [18,50,51].

The results of malondialdehyde (MDA) content of grilled beef are shown in Table 4. The MDA level of grilled beef was significantly affected ($p \leq 0.05$) by the use of different beef cuts and seasoning treatment. Grilled beef samples using the brisket cuts had higher MDA levels ($p \leq 0.05$) than grilled beef with tenderloin cuts. According to Utrera et al. [52], fat content is one of the factors that can cause the formation of MDA. The higher fat content in meat leads to a higher MDA levels.

Grilled beef processed with marinade without torch ginger flower and dipping sauce with torch ginger flower (B5) and grilled beef processed with marinade with torch ginger flower and dipping sauce without torch ginger flower (B4) featured significantly lower MDA value ($p \leq 0.05$) than control sample of grilled beef. Natural ingredients that have antioxidant components, including kecombrang flowers, are able to inhibit oxidation reactions, thus resulting to lower MDA levels. The decrease in MDA levels can occur through inhibition of oxidation reactions that take place in case of increase in antioxidant activity [53]. According to Campo et al. [54], the limit of malondialdehyde value of meat products that are considered not rancid by the trained panelists is 2.28 mg/kg. Based on this limit, the grilled beef processed with both marinade and dipping sauce for the seasoning mode has a value of rancidity lower than the perceptable threshold limit.

Tabel 4. Malondialdehyde (MDA) formation intensity of grilled beef

Treatment	Beef cuts		Means
	Brisket	Tenderloin	
B0	5.04 ± 1.67	2.54 ± 0.46	3.79 ± 1.86^{b}
B1	3.81 ± 0.64	1.36 ± 0.44	2.58 ± 1.43^{ab}
B2	4.33 ± 0.92	1.16 ± 0.31	2.74 ± 1.84^{ab}
B3	3.65 ± 1.10	1.86 ± 0.31	2.76 ± 1.22^{ab}
B4	2.87 ± 0.59	1.51 ± 0.37	2.19 ± 0.87^{a}
B5	2.84 ± 1.01	1.58 ± 0.25	2.21 ± 0.95^{a}
Means	3.76 ± 1.21^{b}	1.67 ± 0.62^{a}	

Note: Different letters in the same column indicate significant differences ($P < 0.05$), B0: control sample, without marinade and dipping sauce; B1: marinade with torch ginger flower; B2: dipping sauce with torch ginger flower; B3: marinade and dipping sauce without torch ginger flower; B4: marinade with torch ginger flower and dipping sauce without torch ginger flower; B5: marinade without torch ginger flower and dipping sauce with torch ginger flower.

Conclusion

The use of beef tenderloin and brisket cuts resulted in differences in pH value, water content, protein content, fat content, and MDA levels of grilled beef. Tenderloin cuts featured lower MDA levels than brisket cuts. The application of torch ginger flower in seasoning can increase water content and antioxidant activity, and can reduce MDA levels in grilled beef. Based on the decrease in antioxidant activity of torch ginger flower due to its exposure to high temperature caused by grilling the beef, the application of torch ginger flower is reasonable and effective to use after the grilling process via its addition to the dipping sauce.

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