SCIENTIFIC JOURNAL

ISSN 2466-2852

meat technology

62 Vol. 02 No. 2021 Belgrade

Founder and Publisher
Institute of Meat Hygiene
and Technology

"Meat Technology" is the scientific journal that publishes results of basic and applied research in the field of biotechnical sciences i.e. the following subcategories: veterinary sciences, food engineering and biotechnology.

Journal "Meat Technology" is abstracted in FSTA (Food Science and Technology Abstract). Full text is available in SCOPUS, CABI Database, EBSCO publishing, DOAJ, AGRIS, www.ifocus.my Database and www.inmes.rs. "Meat Technology" is an open access journal.

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ISSN 2466-2852

meat technology
scientific journal

Meat Technology Vol. 62 No. 2 P. 91–156 Belgrade 2021

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Based on the opinion issued by Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 413-00-00416/2000-01), this publication is of special interest for the science.

Subscription

Annual subscription rate for aboard is: 100 EUR. Orders should be sent to Institute for Meat Hygiene and Technology, P.O. Box 33-49, Kacanskog 13, 11000 Belgrade, R. Serbia.

Annual subscription rate for Republic of Serbia is 5000.00 RSD. Payments can be made to an account of Institute no 205-7803-56 at Commercial Bank with a note subscription to the journal.

www.journalmeattechnology.com

Computer processing and printing
„Naučna KMD“, Beograd
www.naucnakmd.com
Circulation 100 copies

PUBLICATION OF THIS JOURNAL IS FINNANCIALLY SUPPORTED BY:
Ministry of Education, Science and Technological Development of the Republic of Serbia

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 001.8
ID: 53362953
https://doi.org/10.18485/meattech.2021.62.2.1

How to increase your chances of publishing

David L. Hopkins[1*]

A b s t r a c t: Every scientist is required to publish their work, a process that gives creditability to their findings and provides a platform for the real-life application of the findings. Although the conduct of experiments is the core of much scientific work, there is sadly a percentage of studies that are based on flawed designs or written by authors who do not understand how to robustly analyse the data they generate. The consequence is that when they attempt to publish in reputable journals, they often have their papers rejected. In other cases, authors may fail to consider the scope of a target journal, their papers are poorly written, or not formatted according to the journal's guidelines. These again lead to rejection. Overall, this presents a large cost to the research and development (R&D) sector, as some work will never get published, and therefore, the investment has yielded zero returns. In addition, the time spent revising papers adds to the overall cost of undertaking R&D. In many cases, better training can help to reduce these costs and significantly improve the scientific output of scientists. This paper is designed to help authors to improve their success rate when attempting to publish.

Keywords: *scientific journal, publishing, chance.*

Introduction

The publication of research outcomes in the scientific literature is the approach that has been adopted by the community to give creditability to the reported findings. Given that public money is often expended to generate the findings, it is imperative that the resulting publications are of high quality and based on well designed and properly executed experiments. Having held editorial roles with three journals and in the light of several years as the Chief Editor of the international journal *Meat Science*, I have observed that there is a real need for scientists and their students to be better informed and taught about how to achieve the goal of scientific publication. Administrators often do not place enough emphasis on scientific publication and instead want the quick adoption of unpublished outcomes. This is a flawed approach. The process of publication gives confirmation that the findings have been independently scrutinized by experienced people (peer-reviewed) and they are ready to be translated to adopters. This point withstanding, scientists have a responsibility to produce robust findings and to produce high quality papers, yet this often does not occur and as a consequence journal editors see many papers that are rejected, with levels often higher than 75% for good quality journals. It is worth mentioning here that often sub-standard papers will be published, but in the long term, this practice reflects on the accepting journal. Publication for the sake of publication should never be the objective of a journal. This paper is designed to outline how researchers can be successful in publishing scientific papers and is based on my extensive experience as a reviewer, author, and editor.

Background

There are about 10,000 journal publishers globally, of which approximately 5,000 are found in the Scopus database (https://www.scopus.com/). These currently publish more than 23,000 peer-reviewed journals, a number which is growing and now includes an increasing number of Open access journals. Collectively, these will publish more than 3 million papers per year. If we focus on the 30 Food Science journals that were published by Elsevier, in 2020 there were more than 58,000 papers submitted to these journals, and these had an overall acceptance (success) rate of 21%. This immediately should raise concerns. It suggests that a significant amount of research will never be published or available in the public domain. The

**Paper was announced as plenary lecture on 61st International meat industry conference — meatcon2021 held on Zlatibor mountain on September 26–29th 2021.*

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percentage of submitted and subsequently unpublished manuscripts is in the order of 20% for Meat Science, given rejected papers are often subsequently published in other journals, but with extra cost reflected in salaries and time to revise those papers!

Problem areas

One of the most important factors that is often not commonly understood by scientists is the requirement for robust experimental designs. Repeatedly, as an editor, I deal with papers based on flawed designs, the most common being the lack of replication. It is blatantly obvious that many scientists do not understand this requirement and as a result, this is often reflected in the papers of their students. The critical factor is to understand what is an **"experimental unit"**, and thus, what is a replicate. A true replicate is the smallest unit to which an experimental manipulation is independently applied. For example, if you make a batch of "novel" sausage mixture and divide this up into sub-units to analyze or apply further treatments to, then to ensure you have replication you need to make further independent batches and repeat the process as the batch is the level of replication. The sub-units are not true replicates, but pseudo replicates. For a feeding experiment, the same consideration needs to be applied. In this case the best replication is to independently feed each animal so that the individual animal is the replicate. You could also have multiple pens of animals group fed the same diet and achieve replication at this level, but it is less powerful as you end up comparing only the mean data of the pen. To illustrate this point, 4 pens per feeding treatment would produce 4 means for comparison (and require a lot more feed than if you had, for example, 3 animals per pen), whereas to feed 4 individual animals would give the same number of replicates. It is imperative that the design of experiments is solidly constructed and authors are encouraged to create the design and then use "dummy" data to ensure the data can be analyzed before they start any experiments. This includes undertaking power tests to ensure there are enough replicates to detect any likely significant differences.

A related issue is the lack of understanding about how to analyze data and which type of analysis and models to apply. If we consider the example of the sausage mixture experiment, then this would require a model that had a fixed term for the treatment and a random term for the replication. This is a mixed model as it contains a fixed and random term. If say a storage component was part of the design, then it would be fitted as an additional fixed term and its interaction with the treatment effect would need to be included. Often scientists omit the fixed term interactions in the model and thus the interpretation of the results is incorrect. Another common issue is the failure to provide the model for the analysis of sensory data, which should account for additional sources of variation, such as the effects of session or panelist. Feedback to authors is often provided with a suitable reference to consult (e.g. *Biffin et al*, 2020).

The misuse of correlation analysis is often encountered, and some authors fail to understand that this metric only indicates collinearity (association) and **NOT** causation. In this respect, correlations need to be used carefully. For instance, if you undertake an experiment, create different treatments, pool the data, and then look at their correlations, you can get a false idea of the strength of the association because of the variance due to treatments. In terms of linear modelling, the following reference provides some guidance on how to approach this analysis (*Starkey et al*. 2017), noting that in both cases experienced biometricians are authors on these papers. Scientists are well advised to develop working relationships with such people to ensure their designs are robust along with the analysis. Fewer papers would be rejected if this policy was adopted and assurance made to ensure that replication is properly applied.

What do editors look for?

When assessing a manuscript, its originality is an important consideration. Authors need to be aware that all papers in reputable journals are screened to detect overlap with other sources of text and duplication with previously considered papers. If excessive levels of overlap with other texts are detected, then a manuscript will be automatically rejected if the paper is not authored by any of the current authors. If there is overlap with previous papers of the author(s) in areas like the methods this can be reduced by referring to previous papers provided they clearly give all the required detail. This aspect is related to the fact that editors want papers that will advance the knowledge in the area under consideration and not papers that repeat previous work without providing new insights. Methods need to be adequately described and, if animals are involved, approval from an Animal Ethics committee is mandatory for all credible journals. There is a difference between good research and good communication of research, i.e. a well written article cannot make

up for poor research, whereas a badly written article can diminish good research. For this reason, the flow of a paper is important. Authors should get others to read their papers to give a "fresh" perspective on its readability and flow. If you want editors to reject your paper without review, then submit poorly written papers that are full of mistakes and have poor grammar! An easy first step, that is frequently missed, is to run a spelling- or grammar-check of the manuscript prior to its submission. This is a major consideration for non-English speaking authors, with the major journals printed in English. In this case, my advice is to seek to develop relationships with those who are English speaking.

Paper structure

The first thing an author should do before writing a paper is to consult the author guidelines for the target journal. For ***Meat Science*** we see many papers that fall outside the scope of the journal – so you would not submit a paper with a title such as "The effect of dietary betaine on the meat quality, postmortem glycolysis and antioxidant capacity of partridge shank broiler chicken", because the journal does **NOT** publish papers on poultry meat! Further the formatting of the journal should be followed. You must remember that all editors and reviewers **HATE** wasting time on poorly prepared manuscripts and will reject them.

The structure of scientific papers is in many ways straight forward, but there are factors to consider for each element of a paper. The title is designed to succinctly indicate to readers what the paper is about, and it needs to be specific, concise, and not full of jargon and abbreviations. Importantly, the content of the paper must match the title. In some cases, authors have submitted papers with titles that do not match the content of the paper, and what the title says and what they did are inconsistent. Following the title, the abstract is the section that promotes the paper and in a succinct way describes the work undertaken and the major findings. If your article is published, the abstract could be the only part that is freely available to users and as such, a clear abstract will strongly influence whether your work is further considered. Connected with this are the key words – these are important because they will be used by search engines, so do not be too narrow, do not repeat words in the title and avoid abbreviations.

The introduction to a paper must contain three elements 1) an overall picture of the issue to be covered, 2) the current state of knowledge and 3) outline what the issue is and what the objective or hypothesis is that you are testing. For the materials and methods (M&M), include detailed information so that a knowledgeable reader can reproduce the experiment, but use references and supplementary materials to indicate previously published procedures. On this latter point, please reference the primary source that describes the method. Frequently authors will reference secondary sources resulting in a chain of references that must be followed to identify the primary source (if there is one at all!). Ensure there is a section that clearly outlines the design, with the number of samples tested and how replication was applied. The last section of the M&M should be a section that outlines the statistical analysis and including a sentence to the effect of 'we applied an ANOVA to the data' is just not sufficient, as outlined above.

When outlining the results, these should be presented in a logical way following the order of the M&M, are summarised using tables and figures and focus on the major findings which relate to the objective of the work. Some journals allow results and discussion to be intermingled, but my preference is to keep them separate as it helps the reader in my view to, more easily grasp the findings of the work.

The discussion is the section of the paper that relates the results of the experiment to the aims of the experiment. It does this in the context of what other studies have found, and without avoiding those who may have reported different findings to your own. Again, logical order is important. For example, if the paper covered the growth and carcase characteristics of a livestock species and the meat quality traits, then the results will be discussed in this order. This does not preclude discussion about how, for example, growth rate impacts on meat quality traits, but this would be integrated into the latter aspect. The notable findings should also be the heart of the discussion. There can be a tendency for authors to develop discussion on tangential findings, and this must be kept in check, because it is the hypotheses raised that must be addressed. A good discussion will also outline any identified limitations of the work and **SHOULD** not extend beyond the limits of the experiment and the data.

The conclusions in a paper must briefly cover the main findings of the work, put the results in context for the current state of knowledge and point the reader to the possible applications of the findings, which could include the need for further work. In this case try to be specific instead of including motherhood statements!

There are two important remaining components to a paper − the Acknowledgements and the References. For the former, ensure you thank all those who have helped with the work and how they have helped e.g. "Mr Right from Banks Farm is thanked for providing access to his land or Mr Right from the University of Australia is thanked for his technical work in assaying the meat samples for fatty acids". The other important acknowledgement should be to those who provided the funding for the work. Compiling the reference list takes care, with authors needing to ensure the format complies with the journal guidelines. Editors easily detect authors who have submitted a paper that has been rejected elsewhere as often authors fail to check on the in-text referencing and reference list requirements before submitting to another journal! It is very important that authors cite the core papers related to their work and not inflate the reference list. An experimental paper is normally expected to have 25–30 references. On this note, an author should avoid excessive self-citation and not quote every paper they have ever written!

Paper revision

If your paper makes it past the editor and is sent for review, pending a favorable outcome you will be asked to revise the paper. You should carefully study the reviewer's comments and amend the manuscript accordingly. You **MUST** respond to all comments even if you disagree with the reviewer. In this instance, provide a scientifically solid rebuttal, but do not ignore the comments. It can be useful to remember that there is a person at both ends of the reviewing process, and reviewer comments are, for the most part, given to be constructive rather than confrontational. With this in mind, avoid being dismissive or aggressive in your responses. When making changes to the manuscript I prefer these to be shown in colored font not as 'track changes', but different journals and editors will have other requirements. Aligned with the changes to the manuscript, a detailed response to the reviewer comments must be provided, and again, I prefer to see each point from the reviewer followed by a response from the author(s) in colored font. This approach makes it easier for editors to assess the responses and the changes, and making it easier for editors should be an objective if you want prompt feedback! The response comments do not have to be extensive; this is dependent on the point raised by the reviewer and for many straightforward changes all that is needed is to say "*Amended"* and give the line number. In other cases, there will be a requirement for a detailed response.

There will be times that the review process has rendered a reject decision for your paper. You must remember this happens to all people, despite their experience and the number of papers they have published. After taking a breath, try to understand why the paper has been rejected and decide if you can revise and address the comments and resubmit the paper. The other possibility is that you need to generate further data, or some re-analysis of the current data is required depending on what the comments were. Authors should note that rejected papers resubmitted to the same journal will now, for many journals, be identified as duplicates and this flags to editors that they need to take a close look to ensure the revised paper has dealt with all the previous comments. Whatever the decision **DO NOT** submit the paper to the same journal or elsewhere without addressing the reasons for rejection. As an editor I have had authors resubmit the paper without any revisions after a rejection and this is simply not acceptable, and in some cases this is unethical. Plus − your submission could end up with the same reviewer as previously, to the detriment of your reputation and reduce the likelihood of publication.

Further help

If you have questions or are not clear about something in the publication process, you can always email the editor for the target journal. Additionally, I run seminars/webinars for staff and students on how to publish with some practical exercises to teach about correct experimental design. This can also include how to write a review paper. Contact me if you wish to take up this option. See also https://www.researchgate.net/profile/David-Hopkins-3

Kako povećati svoje šanse za objavljivanje naučnog rada

Dejvid L. Hopkins

A p s t r a k t : Od svakog naučnika se traži da objavi svoj rad, što je proces koji daje kredibilitet njihovim rezultatima i pruža platformu za primenu tih rezultata u stvarnom životu. Iako je izvođenje eksperimenata srž naučnog rada, nažalost postoji procenat studija koje su zasnovane na pogrešnim oglednim dizajnima ili su ih napisali autori koji ne razumeju kako da robusno analiziraju podatke koji se na taj način generišu. Posledica toga je da kada pokušaju da objave u renomiranim časopisima, njihovi radovi često budu odbijeni. U drugim slučajevima, autori možda ne uzimaju u obzir obim ciljnog časopisa, njihovi radovi su loše napisani ili nisu formatirani u skladu sa smernicama časopisa. Ovo opet dovodi do odbijanja. Sve u svemu, ovo predstavlja veliki trošak za istraživački i razvojni sektor (R&D), jer neki radovi nikada neće biti objavljeni, pa stoga investicija nije donela nikakav povraćaj. Pored toga, vreme utrošeno na reviziju dokumenata doprinosi ukupnim troškovima preduzimanja samog istraživanja i razvoja. U mnogim slučajevima, bolja obuka može pomoći u smanjenju ovih troškova i značajno poboljšati naučne rezultate naučnika. Ovaj rad je osmišljen da pomogne autorima da poboljšaju svoju stopu uspešnosti kada pokušavaju da objave.

Ključne reči: *naučni časopis, objavljivanje, šansa.*

Disclosure statement: No potential conflict of interest was reported by authors.

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Paper received: June 26th 2021.
Paper accepted: July 2nd 2021.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 613.2:004/007
ID: 53393417
https://doi.org/10.18485/meattech.2021.62.2.2

Digital solutions for healthy eating

Marina A. Nikitina[1], Irina M. Chernukha[1], Andrej B. Lisitsyn*

A b s t r a c t: This study presents a computer system used for assessment of a healthy diet. Based on mathematical models, the system provides a solution for the problem of structural-parametric diet optimization, adjusted for a variety of constraints and conditions, and produces the optimal solution for the given utility functions. The information basis of the system is a database containing nine independent tables. Each table contains 15 fields. The structured query language (SQL) is used. An aggregate algorithm for implementing the solution of healthy diet composition, containing four stages, with due consideration for a "human health passport" is described. At the first stage, based on anthropometric data and biomarkers (hemogram, acidity of gastrointestinal tract) of a person's physiological state, the system generates a user model ("a human health passport"). The model considers the risk of disease and the gastrointestinal tract status. At the second stage, the system allows a choice of food products to be made, based upon the physiological state of a person and that proactively excludes undesirable food products, dishes, and culinary products. At the third stage, the developed diet is assessed, and the food nutrients (proteins, fats, carbohydrates, vitamins, macro— and microelements) in the diet are analyzed and compared with the recommended norms for this particular person. At the fourth stage, the adequacy of the diet is assessed according to the quality function.

Keywords: *computer system, health, diet.*

Introduction

According to the World Health Organization (WHO, https://www.who.int/ru), a number of diseases are associated with an insufficiency or excess of certain components in the daily human diet (Figure 1). In Western Europe, 77% of all diseases are non-communicable diseases, while in 86% of cases they are the cause of death. The same trend is observed in Russia.

Medical data indicate interrelation between nutrition and the most common noncommunicable diseases. Many cardiovascular diseases, different cancers, diabetes, gout, and obesity are directly linked with excess intake of calories due to fats, simple carbohydrates, table salt, diets with reduced content of vitamins and dietary fibers (*Bush et al*. 2020).

Maintaining and strengthening human health is impossible without adequate nutrition. Constant violation of dietary regime inevitably leads to pathological changes in vital functions. This is due to the deep impact of nutrition on all biochemical and physiological processes of the body. It is this fundamental influence that underlies the use of diet therapy − therapeutic, functional nutrition − for smoothing, treating and reducing the risk of various diseases. When recommending a particular diet, a dietitian should use not only biochemistry data (protein, carbohydrate, and lipid statuses, immune parameters, and biochemical blood analysis), physiology data (weight deficit, activity factors, and injuries), and food hygiene data (volume, weight, consistency, and temperature of food), but also take into account individual parameters (age, anthropometric, and caliper measure data). The mathematical apparatus is widely used in the analysis of nutrition problems (*Sukhatme*, 1961; *Edwardson*, 1974; *Anderson and Earle*, 1983; *Alpaslan*, 1996; *Kaldırım and Köse*, 2006; Lv, 2009; *Sahingoz and Sanlier*, 2011) as well as principles of food combinatorics when designing combined food products (*Lipatov*, 1985; *Ivashkin et al*, 2000; *Nikitina et al*, 2019; *Youbo*, 2009).

Designing an individual balanced diet considering many factors (individual intolerance to some components, anthropometric indicators, body mass index, indicators of nutritional and biological value) is impossible without the use of a mathematical tool and modern digital techniques.

The article (*Chen et. al*, 2018) summarizes scientific and practical prerequisites for creating

**Paper was announced on 61st International meat industry conference — meatcon 2021 held on Zlatibor mountain on September 26–29th 2021.*

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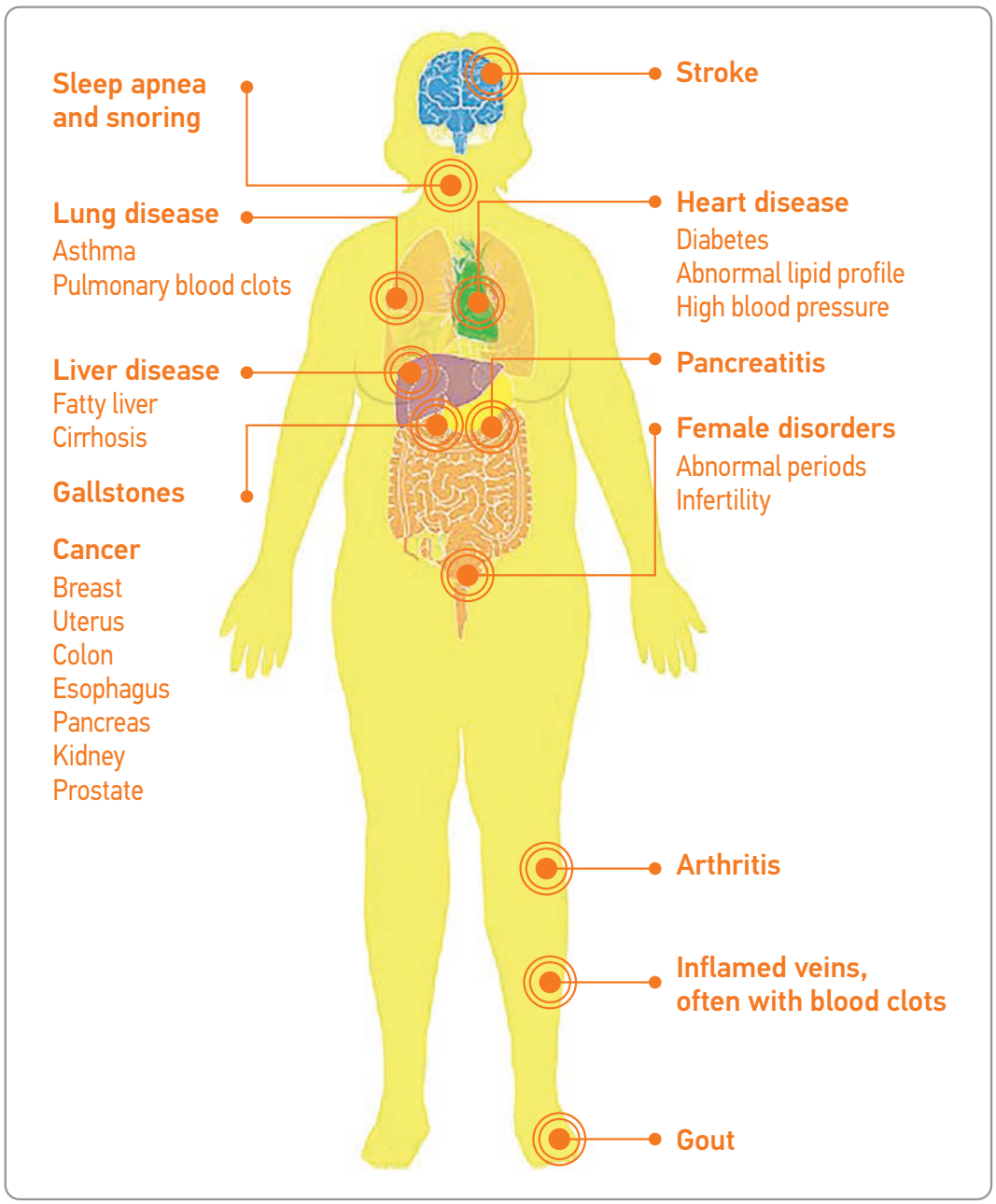

Figure 1. Medical complications of obesity
(https://www.cdc.gov/vitalsigns/adultobesity/infographic.html)

multicomponent foods with desirable quality characteristics and consumer properties. The Russian methodology of food design originated from *Lipatov* (1985). His six basic principles of designing balanced multicomponent foods are still relevant today. At the next stage, *Ivashkin* (2000) improved formulations using the methods of system analysis, modeling, and product range optimization. Modern food chemistry, food biotechnology, and information technologies allow for effective computer design and optimization of multicomponent food formulations for specific population groups. Authors (*Lisitsyn et. al* 2020; *Musina and Lisin*, 2015; *Musina et. al*, 2017) proposed a methodology for system modeling of multicomponent food products. They defined system modeling as a strategy for studying and creating biosystems, particularly food products, their formulations, and production technologies.

An approximate model for selecting a personalized diet is proposed in. The author views personalized nutrition as individually adapted nutrition. With this approach, the gender, age, level of physical activity, presence of different chronic diseases and personal food preferences are taken into account. Individually tailored nutrition is aimed at prevention and treatment of different diseases, reduction of the

negative effects of harmful environmental factors, and support of healthy lifestyle. It is impossible to choose a diet without relying on achievements of modern genetics and nutrition science.

To address the issue of adequate nutrition that meets the needs and capabilities of the human body and is balanced by all indicators of nutritional and biological value, it is necessary to process large amounts of data. First of all, Big Data (big arrays of information and knowledge about the subject area) is used (*Davis-Dusenbery,* 2017; *Wu et. al,* 2017). Our Big Data should contain information about dietary menus, functional and specialized products, nutritional characteristics of differentiated groups of people (students, pregnant women, retirees, etc.), norms of food nutrient consumption as per the region of residence, climatic zones, gender factors, anthropometric indicators (age, weight, body mass index), general and specific medical and biological requirements, characteristics of food consumption (whether there is an allergy or not), as well as information about the source of immune status disorders. It is also necessary to consider the combinability of foods in one diet, and various effects of the combination, such as synergy and antagonism.

In this regard, the issue of therapeutic, functional food can be solved by using a knowledge-oriented system of adequate nutrition, which will help the doctor to quickly and correctly design (select) an adequate diet, taking into account the state of nutrition, individual characteristics, and external factors.

The aim of this work is to develop a methodology and a computer system for assessing the quality of dietary structures for differentiated groups of population, as well as a specific person.

1. Information techniques for optimizing adequate personalized human nutrition

The development of a knowledge-oriented system involves building a knowledge base that contains basic concepts and relations between them. In the case under consideration:

- region of residence;
- activity factor;
- injury factor (operations, bone fractures, traumatic brain injuries, etc.);
- current state of nutrition;
- anthropometric data;
- caliper measure indicators;
- indicators of trophological status;
- indicators of micro– and macronutrient status;
- indicators of vitamin status;
- database on the chemical composition of food products;
- database of biomedical nutritional requirements depending on the disease.

For example, the intake of vitamin B1 influences carbohydrate metabolism, heart and lung functions; vitamin B6 content − metabolism of amino acids and fatty acids, nervous system functions; folic acid (folate) — maturation of red blood cells, DNA and RNA synthesis; calcium − blood clotting, nervous and muscular system functions, heart functions; phosphorus − muscle and nervous system functions; magnesium − energy production, acid-base balance, etc. (*The Merck Manual of Medical Information Whitehouse Station,* 2008).

These data in the knowledge base can be presented as structural and parametric matrices of relations that include three main blocks: patient's physiological state, diet indicators, environmental factors, and many relations within blocks and between blocks and groups of factors.

Each individual block has its own parameters and attributes, for example, the block Patient's physiological state includes age, gender, anthropometric indicators — body weight, height, body mass index, shoulder circumference; caliper measure indicators — fat content, thickness of the fold 2 centimeters above the navel; thickness of the fold over the biceps, etc. Nationality and religious traditions are important. Expert estimates, correlation, and simple and multiple regression coefficients influence estimates found as a result of active experiments, while possible functions, relations, and conversion algorithms can be used as formalized characteristics of relations between parameters of structural and parametric characteristics.

General diagnostics of the person's condition and decision-making in the knowledge-oriented system of adequate nutrition is carried out using the following algorithm. In the first stage, 69 physiological indicators of the patient (trophological status indicators, macro- and micronutrient composition) and 5 indicators of external factors (region of residence, religion, nationality, activity, injuries) are entered. In the second stage, the prognostic risk index, main energy exchange and actual energy expenditure are calculated in order to further determine the person's (patient's) needs for proteins, fats, carbohydrates, etc. The main diagnostic procedure is then further conducted. It forms a structural and parametric situational model of the abnormal human condition being measured and searches for deviations from normal values (normative indicators from the Institute of Nutrition, FAO/WHO, etc.).

1.1. Identification algorithm

The main mechanism of the diagnostic procedure is a cyclic process of moving the matrix of structural and parametric characteristics along the diagonal (non-diagonal elements reflect relations between the patient's state indicators) with a comparison of values of diagonal elements with the norm levels. If an indicator does not match the reference value, values of the indicators with which it is associated are tracked. Upon the occurrence of a loop (i.e. repeated repetition in the cycle of the same indicator) all links of this diagonal element are artificially broken, while the above-mentioned procedure is repeated until the next indicator. All abnormal values of indicators are stored in an array of deviations from the norm. Based on its interaction with the knowledge base, the system user is provided with the conclusion of the diagnostic procedure.

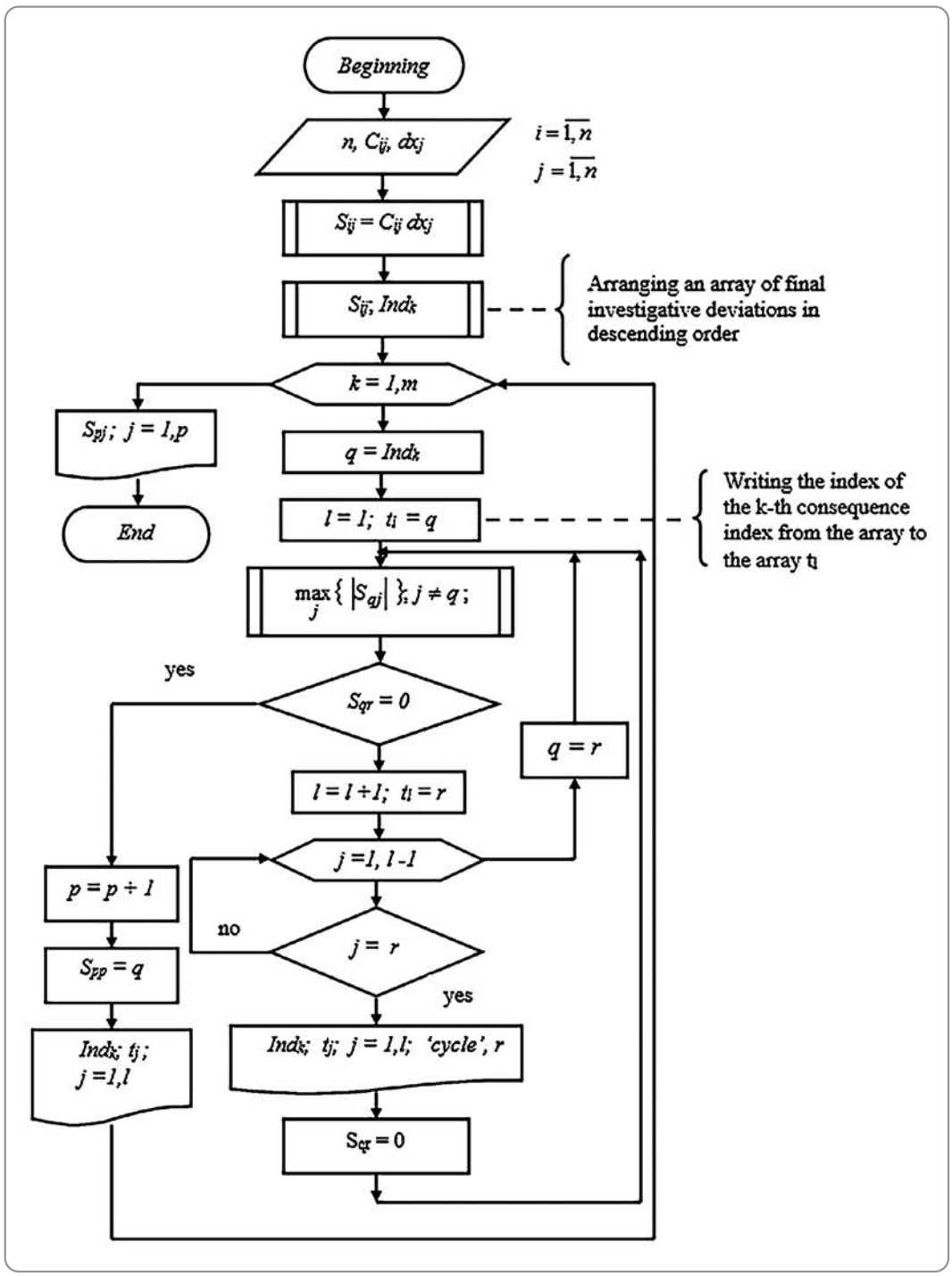

Figure 2. Block diagram of the algorithm for diagnosing the abnormal state of a nutrition system

The identification algorithm contains (Figure 2) a block of the situation matrix formed and the procedure for finding the causes of the anomalous state of the system.

The procedure is a cycle of iteration of independent deviations, within which the maximum element in a line is searched for, its ordinal number p is remembered, and transition to the *p*-th line takes place followed by a new search for the maximum element of this line (Ivashkin, 2005).

To detect possible looping of cause-effect relationships, an array of indices of diagonal elements included in the interaction trajectory is formed, and when two elements of this array match, it is followed by a "cycle" signal. In this case, the cause may be inside or outside the cycle circuit. To exit the cause-effect cycle and continue to search for the original cause, the last link of feedback is broken, i.e. element $S_{gp} = 0$, with its value $f_{gp} = S_{gp}$ and addresses memorized in index arrays Ind_{g_1}; Ind_{g_2}. Then when iterating over the elements of the *g*-th line, the procedure will either stop at the last link of the cycle (if the reason lies within the cycle circuit) or go further through the steps of a new cycle (Figure 2). When moving to the detection of the causal chain of the next *k+1*-th consequence, the interrupted link of the *j*-th cycle of the previous trajectory of the links is restored, i.e. $S_{Ind_{j_1},Ind_{j_1}} = f_j$.

To find the influence of other factors on the next *k*-th consequence, the first maximum contribution to its deviation is set equal to zero and the next largest element of the *k*-th line is selected, i.e. the next largest contribution to the *k*-th consequence.

All abnormal values of indicators are kept in the array of deviations from the norm, and based on the knowledge base, the person is provided with an initial selection of products for their recommended diet. The diet compensates for existing deviations and takes into account individual characteristics of the patient and social conditions (personal perception of certain product, presence of allergies, and availability of specific products due to material or geographical factors).

If there is insufficient compensation for deviations by selecting the desired products and dishes included in the diet, a search should be made for their optimal quantitative ratios (structural optimization) with the possible introduction of additional products and dishes, depending on the current deviations of parameters from the norms, or an individual combined product that minimizes residual deviations could be developed.

Composed function of the dietary structure quality

The quality and sufficiency of the dietary structure are determined, first of all, by its compliance with the requirements for the physiological characteristics of the body and the recommended norms for consumption of biochemical elements. To assess the sufficiency and quality of the daily ration, a composed function is proposed (*Nikitina*, 2020), reflecting the weighted average total deviation of the state parameters from actual to normative values. With regard to the weight coefficients and defining certain groups of factors, the quality functional looks like:

$$F(x) = 1 - \sqrt{\frac{1}{n}\sum_{i=1}^{n} a_i \sum_{j=1}^{n_i} b_{ij}} \left(\frac{x_{ij} - x_{ij}^0}{\Delta x_{ij}^k}\right) \quad (1)$$

where *n* is the number of combined indicators;

x_{ij}, x_{ij}^0 are actual and required values;

Δx_{ij}^k is maximum deviation from the required value for the *k*-th quality level;

b_{ij} is weight coefficient of the *j*-th parameter in the *i*-th group;

a_i is group significance factor.

The quality functional value range is presented in the form of the following graduated scale. If the quality functional value is equal to 1, this means complete coincidence of food nutrients and energy with the recommended ones, i.e. the best quality is achieved. If the quality functional value is equal to 0 or takes negative values, the recommended values are not achieved, so the diet does not meet the specified quality level.

To determine weight coefficients, a complete factorial experiment can be used, where the following values are entered into columns of the response function of the *r*-th repetition in the *k*-th experiment: 1–0.7 − when the product is classified as having a very good level of quality; 0.7–0.3 − good; 0.3–0 — satisfactory; 0 – (–0.2) — bad; less than (–0.2) − very poor quality level.

Information basis of the system

The information basis of the system is a database with nine independent tables. These tables correspond to different types of dishes, namely: "zakuska" (snacks), "sladost" (desserts), "hleb" (baked goods), "napitok" (drinks), "kasha" (cereals), "salat" (salads and vinaigrettes), "sup" (first courses), "myasoriba" (main courses, meat and fish dishes), and "garner" (side dishes).

Each table contains 15 fields. The Id field contains a simple index, which is used to retrieve the required record from the database. The "bludo" fields contain the names of various dishes the user can select from the drop-down list. Next are the fields with physicochemical indicators of dishes per 100 grams: "gir" (the field contains the fat content in the dish), "belki" (proteins), "uglevod" (carbohydrates), "cennost" (energy value of the dish), "C" (vitamin C), "B2" (vitamin B2), "B1" (vitamin B1), "PP" (vitamin PP), "E" (vitamin E), "Ca" (calcium), "Fe" (iron), "P" (phosphorus), "Mg" (magnesium).

Each dish is given a specific record.

Work within the system

Working with a computer system can be represented as an aggregate block diagram (Figure 3). At the first stage, based on anthropometric data and biomarkers (hemogram, acidity of gastrointestinal tract) of the person's physiological state, a user model ("a human health passport") is generated in the system. The model considers the risk of disease and the gastrointestinal tract status. The model description includes such descriptors as individual nutritional needs, biomedical requirements, dietary habits, and available allergies.

At the second stage, the system allows one to make a choice of products considering the person's

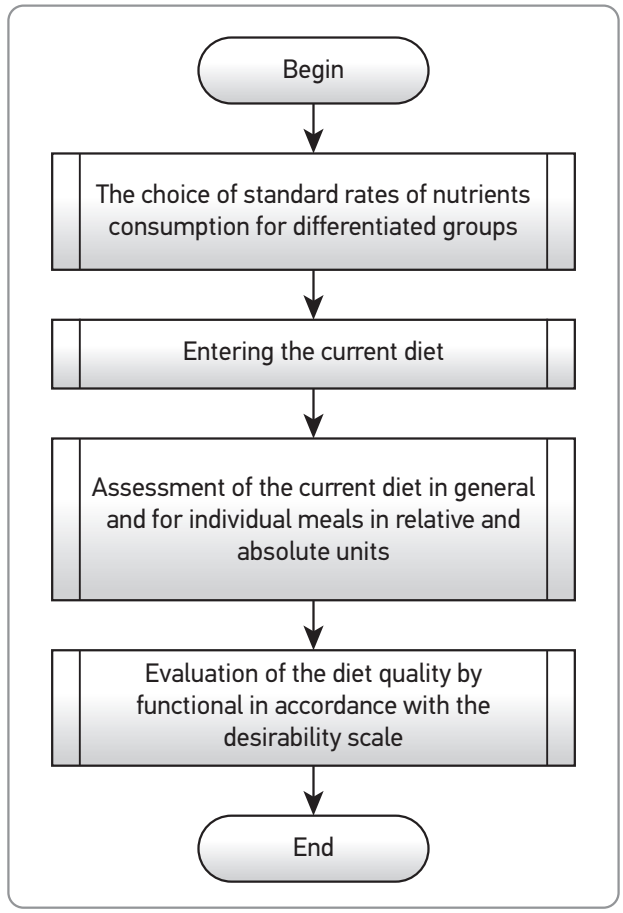

Figure 3. An aggregate block diagram of a healthy diet based upon a person's "health passport"

Breakfast

	Yield, g	Proteins, g.	Fat, g.	Carbo-hydrates, g.	Energy value, kcal	C, mg	B2, mg.	B1, mg.	PP, mg.	E, mg.	CA, mg.	Fe, mg.	P, mg.	Mg, mg.
Boiled egg	70	8.05	8.925	0.525	110.25	0.14	0.315	0.126	0.35	1.05	38.5	1.351	336	21
'Magdalena' cake	60	3.27	14.346	31.452	236.184	21.27	0.456	0	0	0	0	0	0	0
Doctor's crispbread	60	1.56	4.92	27.78	145.2	0	0.03	0.096	1.98	1.02	10.2	0.84	51.6	21.6
Plain black coffee	200	0.1	0.4	0.4	14	0	0	0	1.2	0	10	0	14	0
"Health" Muesli Pear (25% of nuts and fruits)	50	4.3	1.75	32	133	28.515	0.055	0.12	0	0.3	33	0	0	45
Total for breakfast		17.28	30.341	92.157	638.634	49.925	0.856	0.342	3.53	2.37	91.7	2.191	401.6	87.6
As a percentage of the recommended daily ration	Young men	15.29	29.46	20.43	19.35	62.41	42.8	21.38	17.65	23.7	11.46	21.91	33.47	21.9
	Girls	18	33.71	24.06	22.81	62.41	57.07	26.31	19.61	29.63	11.46	12.17	40.16	21.9

Figure 4. Estimated indicators of food nutrients for "Breakfast"

physiological state. For example, if a person has celiac disease, then products, dishes and culinary products containing gluten are automatically excluded from their menu.

At the third stage, the developed diet is assessed, and the food nutrients (proteins, fats, carbohydrates, vitamins, macro- and microelements) in the diet are analyzed and compared with the recommended norms for this particular person (Figure 4). The assessment is carried out for individual meals (breakfast, lunch, dinner) and for the whole diet during the day in absolute and relative units.

As we can see in Figure 4, the first column displays the names of selected dishes, culinary and food products; the second column indicates the weight of the serving. This is the data the user enters at the second stage, when they make up their diet based on their preferences. The remaining columns display physical and chemical indicators cal culated by the system. All indicators are presented at three levels.

- The first level: calculation of indicators for each dish, culinary product and food product separately;
- The second level: calculation of complex indicators for breakfast, lunch and dinner in general;
- The third level: calculation of daily outcome indicators.

In addition to summarizing the overall results (Figure 4), the system compares the indicators with the recommended daily rates as a percentage.

At the fourth stage, the composed function of quality is calculated. If the obtained value of the quality functional corresponds to the acceptable level of the desirability scale, then the user receives their daily ration with differentiation in time and recommendations for food intake. Otherwise, if the diet does not meet their physiological needs, then a return to the second stage to adjust the diet is made. At that stage, products can be replaced if desired.

Conclusion

The developed computer system based on mathematical models provides a solution for the problem of structural-parametric diet optimization, adjusted for a variety of constraints and conditions, and produces the optimum solution for the given utility functions. It 1) is easy to use, allowing the daily diet to be assessed quickly and reliably, and; 2) establishes whether any imbalance (excess/deficiency) of nutrients exists in the diet. The knowledge available in the system builds a cause-and-effect relation for the statement “If there is a shortage or excess of this or that food nutrient in the body, then this will lead to such and such a disease.” Dietary structure (diet) is a health factor that can be changed easily to improve one’s health.

Digitalna rešenja za zdravu ishranu

Marina A. Nikitina, Irina M. Chernukha, Andrej B. Lisitsyn

A p s t r a k t : U ovom istraživanju predstavljen je kompjuterski sistem koji se koristi za procenu zdrave ishrane. Na osnovu matematičkih modela, sistem daje rešenje za problem strukturno-parametarske optimizacije ishrane, prilagođene različitim ograničenjima i uslovima, i proizvodi optimalno rešenje za date funkcije korisnosti. Informaciona osnova sistema je baza podataka koja sadrži devet nezavisnih tabela. Svaka tabela sadrži 15 polja. Koristi se jezik strukturiranih upita (structured query language SQL). Opisan je agregatni algoritam za implementaciju rešenja sastava zdrave ishrane, koji sadrži četiri etape, uz dužno razmatranje „ljudskog zdravstvenog pasoša“. U prvoj fazi, na osnovu antropometrijskih podataka i biomarkera (hemogram, kiselost gastrointestinalnog trakta) fiziološkog stanja osobe, sistem generiše korisnički model („ljudski zdravstveni pasoš“). Model uzima u obzir rizik od bolesti i status gastrointestinalnog trakta. U drugoj fazi, sistem omogućava izbor prehrambenih proizvoda na osnovu fiziološkog stanja osobe, i koji proaktivno isključuje nepoželjne prehrambene proizvode, jela i kulinarske proizvode. U trećoj fazi procenjuje se razvijena ishrana i analiziraju hranljive materije (proteini, masti, ugljeni hidrati, vitamini, makro i mikroelementi) u ishrani i upoređuju sa preporučenim normama za ovu osobu. U četvrtoj fazi, adekvatnost ishrane se procenjuje prema funkciji kvaliteta.

Ključne reči: *kompujterski sistem, zdravlje, ishrana.*

Disclosure statement: No potential conflict of interest was reported by authors.

Acknowledgments: This article is published as part of scientific research theme No. 0585-2019-008 under the state assignment of the federal state budgetary scientific institution V.M. Gorbatov’s Federal Research Centre for Food Systems of RAS.

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Paper received: June 26th 2021.
Paper accepted: July 7th 2021.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 637.524.053/.054
ID: 53253129
https://doi.org/10.18485/meattech.2021.62.2.3

Original scientific paper

Histological and histochemical analysis of dry fermented sausage of kulen composition

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A b s t r a c t: The application of histological methods in meat composition analysis and identification of prohibited tissues and organs added to meat and meat products is still in the research phase, although there have been some promising results. The aim of this work was to assess the possibility of using histological and histochemical methods for analysis of kulen composition. In this research, six samples of kulen were examined, one of which was produced in domestic conditions, while the rest were commercial products sampled from local markets. The samples were carried through classical histological preparation. The obtained slides were stained with haematoxylin/eosin, Masson-Goldner, toluidine blue and periodic acid-Schiff/alcian blue. The content of muscle, fat and connective tissue was evaluated using histomorphometric analysis. Histological analysis of kulen composition determined the presence of the following structures: muscle, adipose and connective tissues, blood vessels, glandular epithelium, peripheral nerve, cartilage and plant tissue. The histomorphometric analysis showed that the kulen products contained on average 56±2.52% muscle tissue, 7.27±1.38% connective tissue and 19.82±3.24% adipose tissue. The results show that by applying histological methods it is possible to identify different permitted and prohibited animal tissues in kulen, and hence, it is possible to analyse the composition of kulen. It is also possible to confirm the presence of various plant tissues, but for their precise identification, additional histological methods are needed.

Keywords: *fermented sausage, histology, animal tissue, plant tissue, histomorphometry*

Introduction

Kulen is a fermented dry sausage that is produced in Serbia according to a specific recipe and technology, depending on whether it is produced domestically (recognised as domestic kulen) or industrially (when it is recognised as kulen). According to the relevant Serbian regulation (*Official Gazette of RS*, 50/2019), kulen is defined as a product made from first and second category pork meat, with the addition of solid fat, salt or pickling salt, sugar, additives, ground red pepper, red pepper extract and other spices and starter cultures. The stuffing of kulen is medium to coarsely ground and is filled into natural and artificial large diameter casings.

The meat protein content is at least 22%, while the collagen content in meat proteins is limited to maximum 15%. Domestic kulen is homemade from first category meat, solid adipose tissue, salt or brine, ground red pepper, red pepper extract and other spices and starter cultures. The stuffing of domestic kulen is medium to coarsely ground and is filled into pig appendix or colon. The content of meat protein in domestic kulen is at least 24%, and the collagen content in meat proteins is up to 10% (*Official Gazette of RS*, 50/2019). According to the same regulation, first category meat includes skeletal muscles that naturally contain little connective and adipose tissue, or meat in which the portion of connective and adipose tissue is reduced to an appropriate level by processing. Second category meat has a naturally higher proportion of adipose and connective tissue than first category meat, from which connective tissue and larger deposits of adipose tissue have been coarsely separated.

Taking the production method of fermented sausages into account, which involves grinding the raw material, it is possible for various components to be added, be it on purpose or accidentally. Some examples include different types of connective tissue, epithelial and glandular tissue, nerve tissue and different types of plant tissue (*Harem and Altun*, 2018). The possibility that inadequate carcass processing or inadequate processing of raw input material leads to the appearance of unauthorised components or the presence of permitted components above the limit

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values cannot be excluded. Recognising this problem, the Food and Agriculture Organization (FAO) has prepared a set of guidelines for proper processing and preparation of raw materials in order to obtain foods that are in accordance with regulations in terms of composition (*FAO,* 1991). Raw materials that contain forbidden components are of lower quality because they have lower nutritional values, and on the other hand, can lead to faster spoilage of the finished product.

Microbiological, physicochemical, sensory and chemical analyses are performed when evaluating the quality parameters of kulen. Chemical analyses are used in assessing product quality, among other things. Chemical analysis of kulen composition includes determination of moisture, fat, meat protein, content of collagen in meat protein, ash, chloride, nitrate and nitrite (*Official Gazette of RS,* 50/2019). Chemical analysis of moisture content shows whether water above the permitted limits has been added to the finished product. Meat protein analysis involves the determination of total nitrogen (*SRPS ISO 937:1992*), while the collagen content is determined by examining the hydroxyproline content. (*SRPS ISO 3496:2002*).

In addition to classical chemical methods, numerous additional methods (molecular, chromatographic, electrophoretic, immunochemical) have been developed in order to detect animal and plant tissue in meat products (*Kesmen et al.,* 2007*; Rao and Hsieh,* 2007). While these methods are very precise and provide valuable information, they can be quite costly and some can be very time consuming for everyday use. Additionally, it is difficult to quantify the presence of plant tissue (*Castro et al.,* 2007). Chemical methods that are used in routine examination of the quality parameters of kulen and other meat products cannot be used to determine the presence of various animal and plant tissues. Additionally, routine methods cannot determine the origin of proteins, i.e., meat proteins cannot be distinguished from other nitrogen-containing compounds, such as plant proteins (*Moore et al.,* 2010).

Numerous researchers have shown that the use of histological methods in combination with routine methods would give more accurate results when evaluating meat quality (*Horn and Seidler,* 1978*; Ugurlu,* 1991*; Tremlova and Starha,* 2003*; Mokhtar et al.,* 2018). These studies have shown that histological methods are one of the most accurate ways to determine various tissues in fermented sausages because they give a comprehensive view of the components that are part of the product. Determination of various tissues and organs using histological methods can be a useful method for detecting counterfeit products (*Ince and Ozfiliz,* 2018). Histological examination of the composition of fermented sausages allows direct determination of the presence of illicit animal and plant tissues (*Ghisleni et al.,* 2010). Histological methods can also be used to estimate the percentage of muscle and connective tissue in fermented sausages (*Koolmees and Bijker,* 1985*; Ghisleni et al.,* 2010). Some authors have shown that the application of these methods can relatively easily detect the presence of plant allergens in meat products (*Rencova,* 2007). Other researchers have applied histological methods to determine the differences between fresh and frozen meat (*Zhu et al.,* 2004). One report shows that the best results when analysing fermented sausage or other meat products are obtained by combining chemical and histological methods, which implies that these two methods are complementary (*Mokhtar et al.,* 2018). The aim of this study was to evaluate the usefulness and practicality of histological and histochemical methods to analyse kulen composition.

Materials and Methods

Six different samples of kulen were studied, one of which was produced in domestic conditions, while the rest were commercial products acquired on the local market. The commercial products were chosen at random from different stores. The samples were marked with unique codes, whereby the one produced in domestic/traditional conditions was labelled DK1, while the commercial products were labelled KK1-KK5. Ten tissue blocks 1 x 0.5cm of each product were randomly selected and were fixed in 10% buffered formaldehyde for 48 hours. The samples were put through standard histological procedure and embedded in paraffin blocks. Six micron-thick sections were obtained from each block. The six sections were stained with haematoxylin/eosin (*MerckMillipore, Darmstadt, Germany*), periodic acid- Schiff/alcian blue *(MerckMillipore, Darmstadt, Germany*), toluidine blue and Masson-Goldner (*Masson-Goldner staining kit, MerckMillipore, Darmstadt, Germany*).

Histomorphometry

All histomorphometric measurements were performed on two sections from each examined sample. For each sample, muscle, connective and adipose tissue content was measured. Four micrographs

were taken from each section at 10x magnification, using a microscope equipped with a digital camera and adequate software (*Olympus CX31 with UC50 Soft Imaging Solutions camera and SensEntry 1.13 software, Münster, Germany*). All histomorphometric measurements were performed on these micrographs using Photoshop^{R}CS3 (*Adobe, San Jose, California*). In Photoshop^{R}CS3 suitable analysis steps were taken for each image captured from tissue sections stained with haematoxylin/eosin. The software enabled the analyst to choose locations of interest in the image, and calculation and export as a .txt file of the measurements for each tissue type. The muscle and connective tissue were individually measured, while adipose tissue content was calculated by subtracting muscle and connective tissue content and artefacts from the total area size of the image analysed. The standard error was calculated for all samples.

Results and Discussion

Histological analysis of the kulen composition resulted in identification of the following structures: striated muscle tissue, adipose and connective tissue, blood vessels, glandular epithelium, peripheral nerves, cartilage and plant tissues. These structures were found in different amounts in the different kulen samples. The striated muscle tissue was identified by the finding of longitudinally or transversely cut muscle cells, which in some places merged into a homogeneous mass, while in others, muscle cell striation and numerous peripherally located nuclei were observed (Fig. 1a-f). Unilocular adipose tissue was recognised by the finding of adipocytes filled with a large fat droplet bordered by a thin layer of cytoplasm and a cell membrane (Fig. 1 (a, b, d, e, f)).

The presence of connective tissue was confirmed by identifying collagen and elastic fibres, which were particularly highlighted on samples stained with Masson-Goldner because they acquired an intensely green colour. A more detailed analysis revealed that some samples contained loose connective tissue, while other samples had a large amount of dense connective tissue (Fig. 1a). The irregular and wavy fibre distribution suggested it was irregular, dense connective tissue.

The presence of cartilage was confirmed by finding chondrocytes in cartilage lacunae and extracellular matrix characteristic of cartilage (Fig. 1b). In the extracellular matrix, the presence of a darker coloured territorial and lighter interterritorial matrix was confirmed. Careful observation of the interterritorial matrix revealed the presence of connective tissue fibres that suggested that it was elastic cartilage.

Nerve tissue was identified by finding parts of peripheral nerves and observing cross, oblique and longitudinal sections of myelinated nerve fibres (Fig. 1c). All connective tissue components of the peripheral nerve, endoneurium, perineurium and epineurium were observed, especially on the samples stained with Masson-Goldner.

The presence of arteries and veins was determined by minutely analysing the observed blood vessels (Fig. 1d, 1e). The three-layer wall structure of muscular arteries was preserved. Endothelial cells, especially their nuclei, were very prominent in the *tunica intima*, and the *lamina elastica interna* was present in some of the samples. The *tunica media* also retained a recognisable structure dominated by smooth muscle cells and sparse elastic fibres. The *tunica adventitia* had also been preserved. In one sample, a structure resembling glandular tissue was observed, but for definitive confirmation, additional histological analyses would be needed (Fig. 1f).

The histological analysis showed the presence of several types of plant structures (Fig. 3). One such observed structure resembled the palisade structure of soybean seeds in its appearance and structure (Fig. 3c), although additional analyses are required to definitely determine that it is a soybean seed. In some samples, starch grains were identified, and they occurred in two forms: 1) small grains, irregular in shape with a lighter central zone and darkly coloured edges (Fig. 3d) and 2) oval, larger, homogeneously coloured grains with a characteristic central crack (Fig. 3e). Starch grains were especially prominent on samples stained with the periodic acid-Schiff/alcian blue method, when they had an intensely pink colour.

In all the commercial kulen samples, rod-shaped and coccoid bacteria were observed, with the rod-shaped bacteria being the predominant type (Fig. 3 (a, c, d, e, f)). Bacteria were observed in the form of small clusters located between muscle and adipose tissue. The size of the clusters, as well as the density of bacteria in the clusters, varied between samples. Bacteria were most clearly observed on samples stained with periodic acid-Schiff/alcian blue, where they had a clear blue colour due to the presence of proteoglycans in their cell walls. Histomorphometric analysis revealed that the products contained an average of 54.45±2.52% muscle tissue, 7.27±1.38% connective tissue and 19.82±3.24% adipose tissue.

Muscle, adipose, connective and plant tissues were found in all samples. Peripheral nerve was found in KK5. Cartilage was identified in KK1, while blood vessels (arteries and veins) were observed in KK2. Starch grains and the structure resembling glandular tissue were observed in KK4. A tabular presentation of the structures found in all examined kulen is given in Table 1.

In all kulen, the most common finding was striated muscle tissue, followed by adipose tissue, which was expected given that these two tissues are the main raw materials for the production

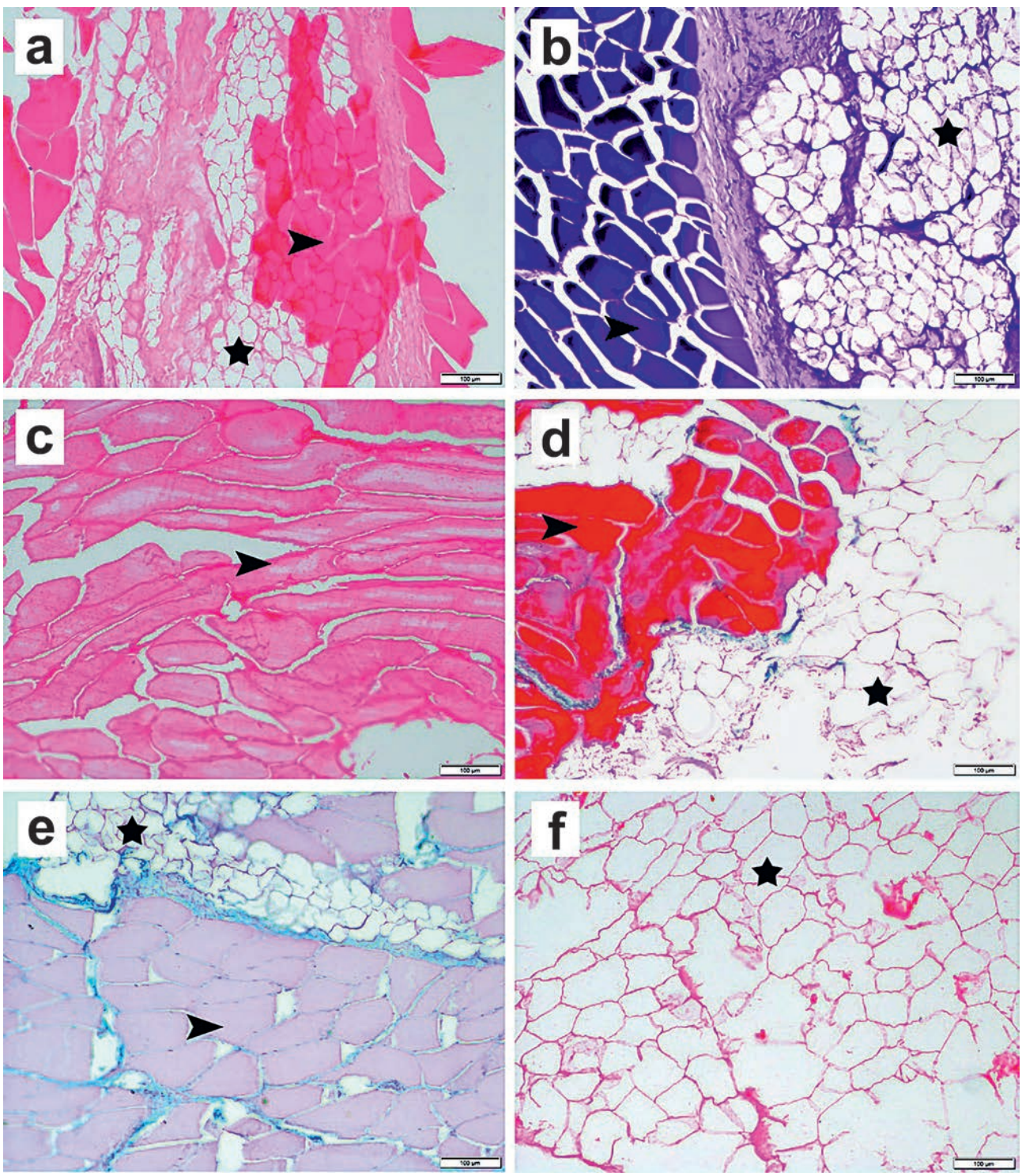

Figure 1. Microphotographs of skeletal muscle tissue (arrowheads) and fat tissue (stars) in commercial and domestic kulen stained with haematoxylin/eosin (a, c, f), toluidine blue (b), Masson-Goldner (d) and periodic acid-Schiff/alcian blue (e). Bars: 100 μm.

of kulen. The production of kulen involves grinding and mixing the raw material followed by fermentation. Although these procedures lead to changes in tissue structure, clear visualisation of the muscle and adipose tissue was possible with the histological methods used. Similar observations were made by researchers who performed histological analyses of other types of fermented sausages (*Harem and Altun*, 2018). Small amounts of connective tissue are expected given that it is not possible to completely

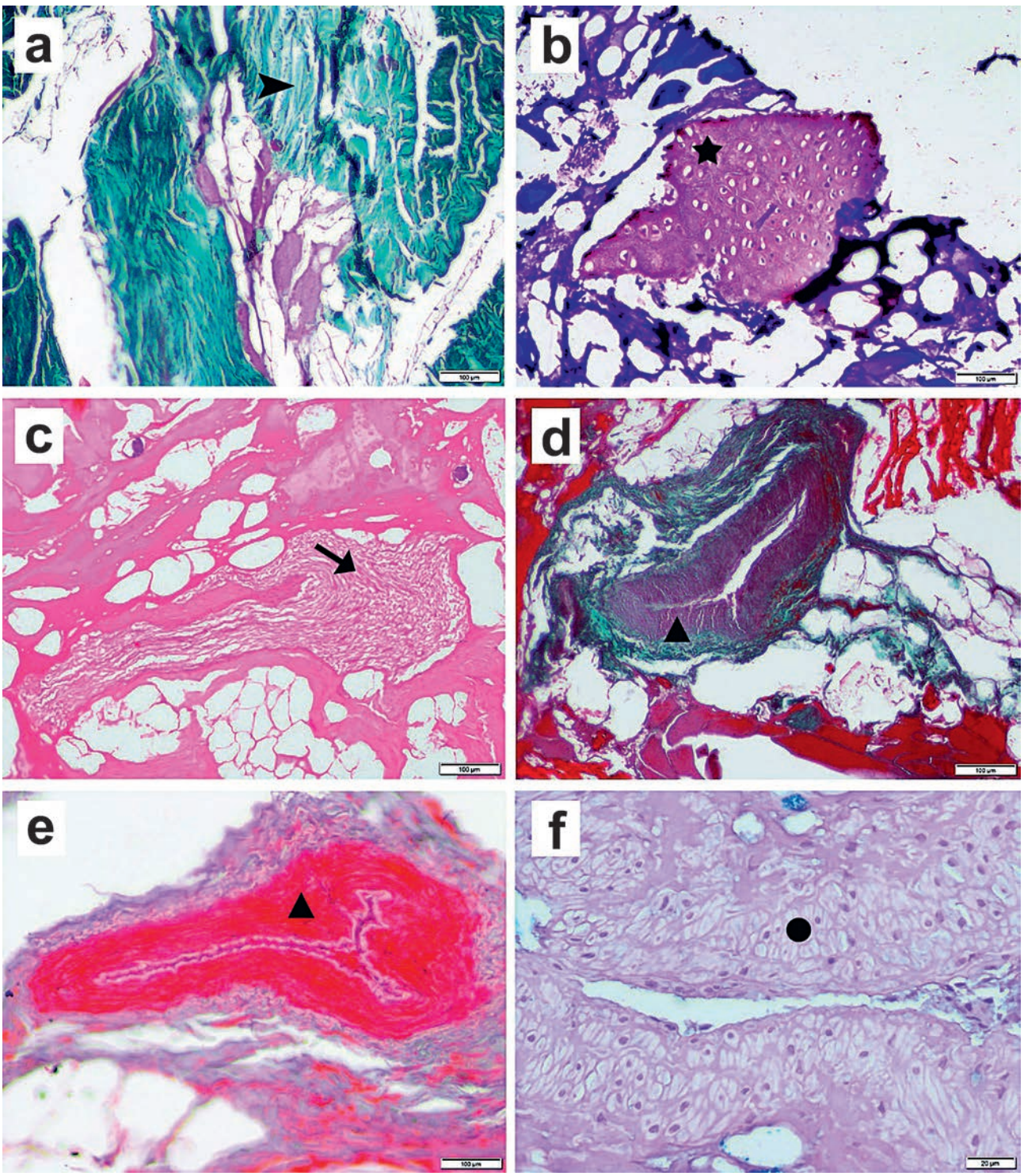

Figure 2. Representative micrographs of domestic and commercial kulen showing: (a) connective tissue marked with arrowhead; (b) cartilage – asterisk; (c) peripheral nerve – arrow; (g, d) arteries of muscle type - triangle and; (f) glandular epithelium – circle. Micrographs stained with haematoxylin/eosin (c), toluidine blue (b), Masson-Goldner (a, d, e), periodic acid-Schiff/alcian blue (f). Bars: 100 μm (a, b, c, d, e), 20 μm (f).

separate the connective from the muscle tissue during carcass processing. Connective muscle and adipose tissues were relatively easily discernible and easy to identify.

The findings of the peripheral nerve, blood vessels and cartilage in Kulen are not in accordance with the relevant Serbian regulation (*Official Gazette of RS*, 50/2019). The identification of these tissues was relatively simple, which suggests that histological methods could be used to confirm the presence of illicit tissues in kulen sausages. Many researchers have used histological methods to identify various

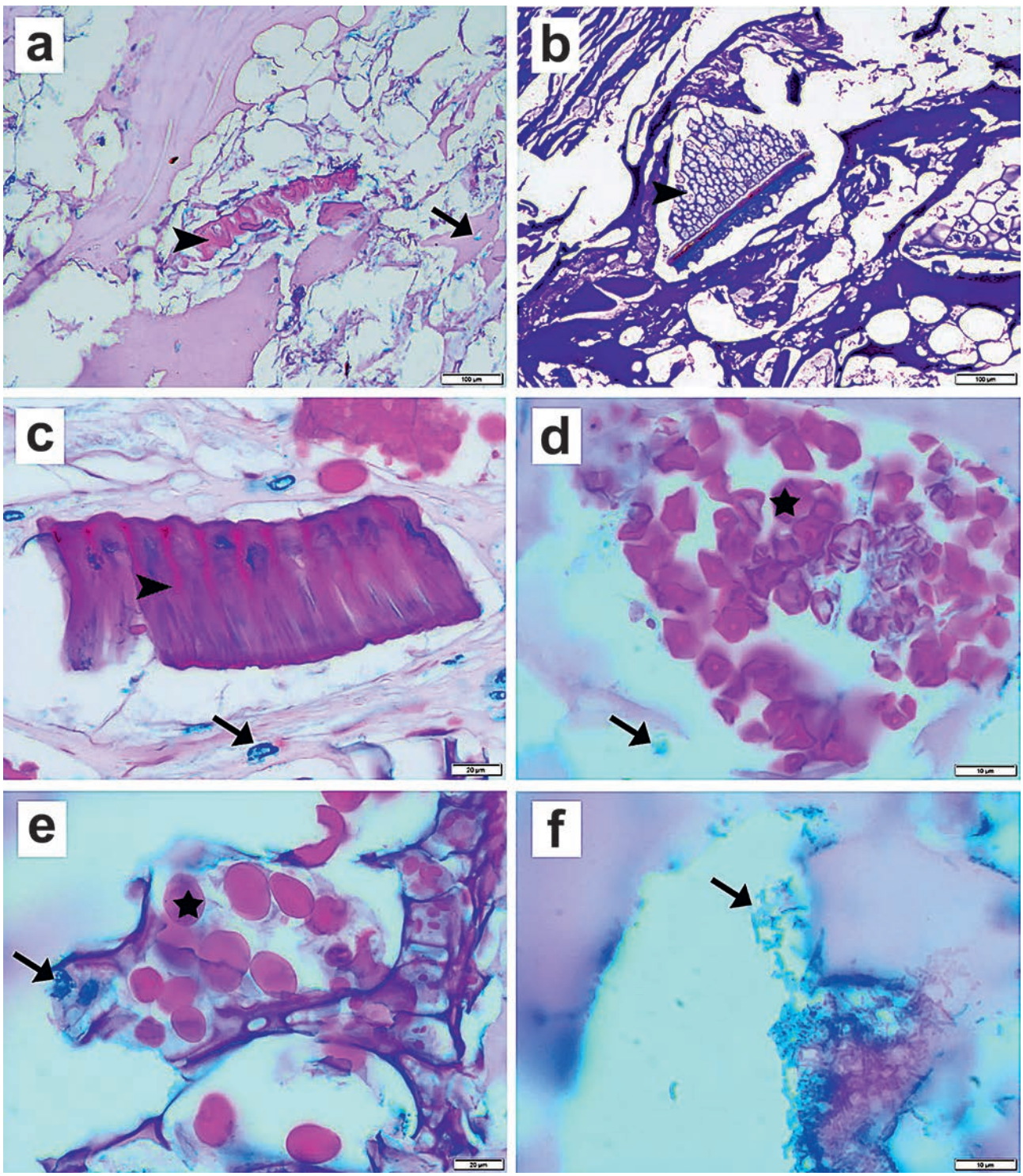

Figure 3. Representative micrographs of domestic and commercial kulen showing different plant tissues marked with arrowheads (a, b, c); starch grains – asterisks (g, d) and; bacteria – arrows (a, v, g, d, f). Micrographs stained with toluidine blue (b) or periodic acid-Schiff/alcian blue (a, c, d, e, f). Bars: 100 μm (a, b), 20 μm (c, e), 10 μm (d, f).

Table 1. Plant and animal tissues identified in the examined kulen

Tissue	Kulen					
	DK1	**KK1**	**KK2**	**KK3**	**KK4**	**KK5**
Adipose	+	+	+	+	+	+
Muscle	+	+	+	+	+	+
Cartilage	–	+	–	–	–	–
Glandular	–	–	–	–	+*	–
Blood vessels	–	–	+	–	–	–
Nerve	–	–	–	–	–	+
Plant	+	+	+	+	+	+
Connective	+	+	+	+	+	+

+ indicates a positive finding

– indicates a negative finding

+* indicates suspicion of the presence of that tissue

illicit tissues in meat products: cartilage, nerves, blood vessels, lungs, bone, glands, internal organs, tendons and skin (*Sezer et al.,* 2013; *Malakauskiene et al.,* 2016; *Migaldi et al.,* 2016*; Harem and Altun,* 2018*; Moghtaderi et al.,* 2018*; Mokhtar et al.,* 2018*; Abdel-Maguid et al.,* 2019). In our study, some tissues had an altered structure, so it was not possible to accurately identify them, as was the case with the glandular tissue. Plant tissue was found in all samples, which complies with the relevant Serbian regulation, according to which the use of ground red pepper and other spices in the production of kulen is allowed (*Official Gazette of RS,* 50/2019). However, a palisade plant tissue structure was found in one kulen sample, and this structure was earlier referred to by others as soybean seed (*Mokhtar et al.,* 2018). The presence of structures characteristic of starch grains in commercial kulen samples could be explained by the addition of various spices or some other plant components. However, additional histological staining is required for accurate identification of the various plant tissues and structures. The presence of rod-shaped and coccoid bacteria can be explained by the fact that starter cultures responsible for the fermentation of the product are used in kulen production.

Conclusion

Based on the results and their critical consideration, histological methods can be used to identify various permitted and illicit animal tissues in kulen fermented sausages, and the kulen composition can also be analysed. Various plant tissues can be determined, but for their precise identification, additional histological or chemical methods are required. One of the limiting factors of this technique is the lack of precise identification of individual tissues due to structural damage which arises during the production process.

Histološka i histohemijska analiza sastava fermentisane kobasice u tipu kulena

Vladimir Gajdov, Anita Radovanović, Tijana Lužajić Božinovski, Danica Marković, Silvana Stajković, Ivan Milošević

A p s t r a k t: Primena histoloških metoda u analizi sastava mesa i utvrđivanju prisustva nedozvoljenih tkiva u mesu i proizvodima od mesa je još uvek u fazi istraživanja, iako su postojeći rezultati u ovoj oblasti obećavajući. Cilj našeg istraživanja bio je ispitivanje mogućnosti korišćenja histoloških i histohemijskih metoda u analizi sastava fermentisane kobasice u tipu kulena. U ovom istraživanju ispitano je šest uzoraka kulena, od kojih je jedan proizveden u domaćim uslovima, dok su ostali komercijalni proizvodi poticali iz lokalnih marketa. Uzorci su sprovedeni klasičnom histološkom procedurom. Dobijeni isečci bojeni su metodama: hematoksilin/eosin,Masson-Goldner, toluidin plavo i perjodna kiselina/Schiff/alcijan plavo. Sadržaj mišićnog, masnog i vezivnog tkiva procenjen je primenom histomorfometrijskih analiza. Histološkom analizom sastava kulena utvrđeno je prisustvo sledećih struktura: mišićno, masno i vezivno tkivo, krvni sudovi, epitel žlezdanog tipa, periferni nerv, hrskavica i biljno tkivo. Histomorfometrijska analiza je pokazala da proizvodi sadrže u proseku 56±2,52% mišićnog tkiva, 7,27±1,38% vezivnog tkiva i 19,82±3,24% masnog tkiva. Rezultati pokazuju da je primenom histoloških metoda moguće identifikovati različita dozvoljena i nedozvoljena životinjska tkiva u kulenu, pa je samim tim moguće analizirati sastav kulena. Takođe je moguće potvrditi prisustvo različitih biljnih tkiva, ali je za njihovu preciznu identifikaciju neophodno primeniti dodatne metode.

Ključne reči: *fermetisane kobasice, histologija, životinjska tkiva, biljna tkiva.*

Disclosure statement: No potential conflict of interest was reported by authors.

Acknowledgment: The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract number 451-03-9/2021-14/200143).

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Paper received: October 11th 2021.

Paper corrected: November 10th 2021.

Paper accepted: November 5th 2021.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 637.524.053/.054
ID: 53257225
https://doi.org/10.18485/meattech.2021.62.2.4

Original scientific paper

Chemical and sensory properties of household and industrially produced Bosnian sudzuk

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A b s t r a c t: Sudzuk, a Bosnian dry fermented sausage, is traditionally made from beef, beef tallow, table salt, garlic and pepper. In this paper, the chemical and sensory properties of household- and industrially-produced Bosnian sudzuk were investigated. The technological processes in both cases of preparation and production of sudzuk were carried out in a manner specific to the given product. Chemical analyses (moisture, total ash, sodium chloride, total acids, fat, protein and pH) showed the different sudzuk produced by three households had some statistically significantly ($p<0.05$) different parameters. Among the industrially produced sudzuk (from four different companies) the moisture content, total ash, sodium chloride, total acids, protein and pH were different ($p<0.05$), while the fat content did not significantly differ between the producers ($p>0.05$). Instrumental colour measurement indicated statistically significant differences ($p<0.05$) for a between the sudzuk produced in the households, for a* between those produced in industrial conditions, and for L* between the industrially produced sudzuk. No statistically significant difference ($p>0.05$) was determined for b* between the sudzuk produced in households or between those produced in industrial conditions. In sensory evaluation, differences ($p<0.05$) were found in the overall grades of the sudzuk produced by households, while the overall grades of the industrially produced sudzuk did not statistically differ ($p>0.05$).*

Keywords: *Bosnian sudzuk, traditional and industrial production, chemical and sensory properties.*

Introduction

Sudzuk is a dry fermented sausage, very popular in Turkey and in Middle East countries, as well as in Europe (*Ercoskun and Özkal*, 2011). In Bosnia and Herzegovina, it is traditionally produced in small plants or village households in autumn and winter when weather conditions (temperature and relative humidity) are favourable.

According to Bosnian law (*Official Gazette BiH*, 2013), sudzuk is a shelf-stable sausage that is produced according to the manufacturer's specification. Earlier, it was produced only in households and artisanal slaughterhouses, while in the last few decades, sudzuk has been made by meat companies for the urban population.

Sudzuk is produced from a mixture of meat and fat; the mixture includes beef, sheep and/or buffalo meat, beef tallow and sheep tallow with the addition of salt, sugar, garlic, pepper and some other spices. After mixing, the stuffing is filled into a casing and the sausage is subjected to fermentation under specified conditions to produce the final result: a semi dry or dried meat product (Özgal and Ercoksun, 2016). According to *Toldra and Reig* (2011), sudzuk produced by natural fermentation are nutritionally valuable due to their high quality protein, B group vitamins, mineral elements, trace elements and some bioactive compounds. *Erkmen and Bozkurt* (2004) concluded that lipid oxidation in sudzuk could have significant influence on the qualities of colour, flavour, texture and nutritional value. *Operta et al.* (2007) state that frozen beef of III category was used in industrial conditions for the production of sudzuk, and Čengić et al. (2008) used beef meat of I and II categories.

Previous research and determination of physical-chemical and sensory properties based on moisture content, fat, protein, NaCl, ash, pH, water activity (a_w), and the sensory properties showed sudzuk can have rather variable values (*Operta et al.*, 2012). Therefore, in this study, the most important physico-chemical parameters and the most important sensory properties were investigated in Bosnian sudzuk produced traditionally and in industrial conditions from fresh, chilled and frozen beef of II and III category.

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Materials and Methods

Sudzuk were produced in households or in industrial conditions, according to the producer's specification. For the needs of chemical and sensory analyses, several sudzuk were taken from four different household producers and at retail from three different industrial producers in the Una-Sana Canton. Sudzuk were coded and harmonized for further research needs in the winter of 2020, and three samples (horseshoes) were taken from each producer. Producers were: A1, domestic producer 1; A2, domestic producer 2; A3, domestic producer 3; A4, domestic producer 4; B1, industrial producer 1; B2, industrial producer 2 and; B3, industrial producer 3. Samples of household-produced sudzuk were taken in a bulk package, and samples of industrially produced sudzuk were taken packed in a polyamide-polyethylene bags and vacuumed. Chemical and sensory analyses were performed in the Laboratory for Control and Quality of the Biotechnical Faculty.

Selected frozen beef from suppliers, beef tallow, 55 mm diameter collagen, black pepper, fresh garlic and nitrite salt were used for the production of Bosnian sudzuk in industrial conditions. The meat was partially cut to a 5 cm thickness and 30 cm width, and chopped to a granulation of 5 mm. The meat was further minced in a cutter together with the spice mixture and nitrite salt. After cutting, the filling was transferred to a vacuum filler where it was filled into collagen wrappers and closed by clipping. The formed horseshoe shaped sausages were hung on poles on a cart and left to drain for 12 h. The drained sudzuk was transported to a conditioned chamber for ripening, drying and smoking in controlled temperature and humidity conditions. The process lasted for 20 days (depending on the piece and the position in the chamber), until the desired sensory properties of the product were achieved. The initial ripening temperature in the chamber was from 14°C to 15°C, and the relative humidity was maintained at about 72%. After ripening and drying, the sudzuk were packed in polyamide-polyethylene bags, vacuumed and stored in a warehouse (temperature range from 4 to 5°C, maximum up to 15°C).

For household sudzuk, the following ingredients were used: beef meat, beef tallow, table salt, black pepper and garlic. Meat and beef tallow were chopped manually with a knife into small pieces with addition of table salt, black pepper and garlic. After mixing meat and tallow together with spices, the mixture was left to ripen for 5 days in a cold place (about 5°C). In the next step, the ripened mixture was ground in a meat grinder. After grinding, the filling was stuffed into thin beef casings. Before use, casings were salted and left in warm water to become elastic. A sausage stuffer was used to stuff the filling into the casings, which were stuffed well, in order to become firm. After stuffing, the sudzuk were tied up into ring shapes and placed on a pole without touching each other. Then, sudzuk were stored in a room with average temperature about 10°C for one day, and then, the sausages were arranged on rounded poles to be equally smoked and dried. The sausages were smoked and dried in a typical smokehouse with an open furnace for 10 days without the possibility to control the temperature or humidity. Beech wood was used to create flameless smoke. After smoking, sudzuk were left to ripen 5 days in a room with average temperature of 10°C.

Chemical analysis

Water content (drying at 105°C to constant mass) was determined according to BAS ISO 1442 (*ISO*, 2007a), fat content (according to the Soxhlet method) using BAS ISO 1443 (*ISO*, 2007d), and protein content (according to the Kjeldahl method) using BAS ISO 937 (*ISO*, 2007b). Determination of total ash/mineral matter in sausages was by the method of dry incineration BAS ISO 936 (*ISO*, 2007c) and the content of sodium chloride was determined according to the Mohr method (*ISO*, 1996). The content of organic acids expressed as malic acid and was determined according to *Trajković et al*. (1983).

pH measurement

The pH was determined according to the reference method (*ISO*, 2004). Measurements were made using a digital pH meter (TESTO 206, Germany). Before and during reading the pH, the pH meter was calibrated using standard buffer solution (pH buffer calibration was 7.00 and 4.01 at 20°C). The result is expressed as the arithmetic mean of eight measurements.

Instrumental colour measurement

Instrumental colour measurement was performed using a colorimeter LCC-A11 (LABTRON, United Kingdom), with 8 mm port size, illuminant D65 and a 10° standard observer, and after standardization of the instrument with respect to the white calibration plate. Colour parameters, expressed as CIE L*, a* and b* values, were determined as indicators of lightness, redness and yellowness. For the

colour determination, measurement was done immediately after cutting the sudzuk to prevent colour degradation, which can occur as a result of light and oxygen. The mean of five measurements was recorded for each colour parameter.

Descriptive sensory analysis

Descriptive sensory analysis was used to evaluate sudzuk quality. The panel consisted of a group of five trained evaluators, and the overall sensory quality of Bosnian sudzuk was assessed by the quantitative descriptive analysis method (*ISO*, 1985) in order to identify the relative quality of selected product properties. In the sensory evaluation, the coefficient of significance (Cs) was determined for each selected sensory attribute/parameter (there were 20 of these). Appropriate Cs were multiplied by the sensory evaluation score for each selected attribute. The most important sensory attributes were evaluated: outside appearance and/or casing (Cs=2), cut appearance (Cs=5), cut colour (Cs=3), smell, aroma and flavour (Cs=7); consistency (Cs=3).

Statistical analysis

The results of this study are presented as the mean values accompanied by standard deviations. One factor analysis of variance (ANOVA) was performed using statistical software SPSS (VER.20). When the main impact was significant, averages were split by Tukey's test of the smallest significant deviations at 5% level.

Results and Discussion

Tables 1 and 2 show the results of chemical analyses of the Bosnian sudzuk.

Table 1. The average chemical composition and pH of Bosnian sudzuk produced in households (n=12)

Parameters (%)	Producers			
	A1	A2	A3	A4
Water content	26.02±4.35^{d}	37.77±3.57^{ab}	41.35±2.88^{ac}	41.46±4.04^{bc}
Total ash	4.54±0.34^{c}	6.82±0.40^{a}	6.44±0.32^{ab}	5.93±0.48^{b}
NaCl	4.20±0.05^{c}	6.74±0.07^{ab}	6.39±0.31^{a}	6.29±0.34^{b}
Total acids	0.06±0.01^{a}	0.08±0.02^{ab}	0.13±0.03^{c}	0.09±0.01^{ab}
Protein	24.77±0.71^{c}	28.99±0.70^{b}	33.35±1.17^{a}	32.03±1.36^{a}
Fat	47.96±2.64^{c}	33.04±1.84^{b}	23.52±1.31^{a}	20.80±3.65^{a}
pH	6.28±0.05^{a}	6.17±0.03^{b}	5.75±0.03^{c}	5.51±0.02^{d}

Legend: Data are expressed as mean±standard deviation. Means with the same letter in the same row do not differ statistically at 5% (p>0.05). A1 = household producer 1, A2 = household producer 2, A3 = household producer 3, A4 = household producer 4.

Table 2. The average chemical composition and pH of Bosnian sudzuk produced in industrial conditions (n=9)

Parameters (%)	Producers		
	B1	B2	B3
Water content	28.63±4.39^{a}	28.29±2.48^{a}	34.55±2.26^{b}
Total ash	5.87±0.62^{b}	4.43±0.28^{a}	5.13±0.32^{a}
NaCl	4.72±0.05^{a}	4.17±0.12^{b}	4.34±0.11^{c}
Total acids	0.06±0.02^{ab}	0.04±0.01^{a}	0.06±0.01^{b}
Protein	25.19±0.65^{a}	22.28±3.96^{b}	24.73±0.64^{a}
Fat	41.29±3.54^{a}	46.77±3.34^{a}	37.25±9.62^{a}
pH	5.50±0.01^{b}	5.81±0.04^{a}	5.77±0.08^{a}

Legend: Data are expressed as mean±standard deviation. Means with the same letter in the same row do not differ statistically at 5% (p>0.05). B1 = industrial producer 1, B2 = industrial producer 2, B3 = industrial producer 3.

For the Bosnian sudzuk produced in households, the lowest moisture content was found in that from producer A1 (26.02%), and the highest moisture content was found in that from producer A4 (41.46%). *Jahić and Pračić* (2018) determined an average moisture content of 43.58% for samples of domestic Bosnian sudzuk with beef, and *Ganić et al.* (2018) determined the moisture content of high beef sudzuk to be 35.01%. For the Bosnian sudzuk produced in industrial conditions, the moisture content ranged from 28.29% in sausage from producer B2 to 34.55% in sausage from producer B3. *Siriken et al.* (2009), for Turkish sudzuk, reported moisture content ranging from 29.80% to 47.60%. *Operta et al.* (2012) recorded the moisture content in sudzuk made from chilled meat as 32.87%, and in sudzuk made from frozen meat as 30.11%. *Kurćubić et al.* (2016) recorded the moisture content was 19.44% in industrially-produced sudzuk, and 38.74% in domestically-produced sudzuk. According to *Operta* (2018), the average moisture content in Bosnian sudzuk produced in a ripening chamber ranged from 31.40% to 34.17%. Due to drying in uncontrolled conditions in domestic production, the finished sudzuk product remains as "raw" (*Kurćubić et al.*, 2016). According to *Dučić et al*. (2018), the higher moisture content in one sample of industrial sudzuk was due to the use of chilled meat. According to Bosnian legislation (*Official Gazette BiH,* 2013), fermented dry sausages after drying and ripening contain a maximum 40% of moisture.

The ash content in the Bosnian sudzuk produced in households ranged from 4.54% A1 to 6.82 A2. *Jahić and Pračić* (2018) determined an ash content of 6.85% for the samples of domestic sudzuk made from beef, and *Ganić et al.* (2018) determined an ash content of 6.29% for Visoko sudzuk (this is from Visoko, BiH). In this study, the ash content in the Bosnian sudzuk produced in industrial conditions ranged from 4.43% B2 to 5.87 B1. The content of mineral substances in sudzuk produced in industrial conditions was earlier determined to be 5.16% (*Kurćubić et al.*, 2016), 5.85% in cooled meat sausages (*Operta et al*., 2012), and 5.56% in frozen meat sausages. According to *Dučić et al.* (2018), the ash content was in the range from 4.70% to 5.50% on the 15th day of sudzuk production by three different industrial producers.

The lowest NaCl content in the samples of Bosnian sudzuk produced in households was 4.20% in the sausage from producer A1, and the highest NaCl content was 6.74% in sausage from producer A2. *Ganić et al*. (2018) also recorded a relatively high NaCl content in Visoko domestic sudzuk (5.27%), and *Jahić and Pračić* (2018) recorded a NaCl content of 3.00% in domestic beef sudzuk. *Kurćubić et al.* (2016) found a slightly lower NaCl content for sudzuk produced in industrial conditions compared to sudzuk produced in households (4.70% and 5.12%, respectively). In this study, the NaCl content in industrial sudzuk ranged from 4.17% B2 to 4.72% B1. *Operta et al.* (2012) reported the NaCl content in industrial sudzuk: 4.74% for sudzuk made from chilled meat, and 4.41% for sudzuk made from frozen meat, levels in accordance with the results of this study. *Dučić et al.* (2018) recorded the NaCl contents in industrially produced sudzuk ranged from 3.60% to 4.10% on the 15th day of production.

The lowest content of total acids (expressed as malic acid) in Bosnian sudzuk produced in households was found in sausage from producer A2 (0.08%), and the highest in sausage from producer A3 (0.13%). The lowest pH was recorded in sudzuk from producer A3 (pH 5.75), and the highest in sudzuk from producer A1 (pH 6.28). The content of total acids in sudzuk from producer A1 was 0.06%. *Kurćubić et al.* (2016) recorded pH 5.02 in domestic sudzuk, and pH 4.80 in industrial sudzuk. *Jahić and Pračić* (2018), found dried domestic sudzuk were pH 5.44 and 5.72, and *Ganić et al.* (2018) recorded pH 5.21 for Visoko sudzuk. In this study, the following pH values were found in Bosnian sudzuk produced in industrial conditions: B1 5.50, B3 5.77 and 5.81 B2. *Operta* (2018) recorded the average pH of 5.10 (with variation from 4.8 to 5.2). According to *Dučić et al.* (2018), the lowest pH was found in sausages with higher amounts of glucono-delta-lactone, and the highest pH values were in sausages where glucono-delta-lactone was not added. The pH of factory and butcher's sudzuk varied from pH 4.53 to 5.77, and pH 4.83 to 6.74, respectively (*Erkmen and Bozkurt*, 2004).

The protein contents in Bosnian sudzuk produced in households were: 24.77% A1, 28.99% A2, 32.03% A4 and 33.35% A3. Slightly lower protein contents were recorded in sudzuk produced in industrial conditions: 22.28% B2, 24.73% B3, 25.19% B1. According to *Operta* (2018), the average protein content in sudzuk was 32.42%, and ranged from 30.20% to 35.15%. *Kurćubić et al.* (2016) recorded protein contents in domestic sudzuk of 21.12% and in industrial sudzuk of 20.60%.

In this study, the fat content of domestic sudzuk varied from 20.80% A4 to 47.96% A1. In industrial sudzuk, the fat content ranged from 37.25% B3 to 46.77% B2. *Kurćubić et al.* (2016) recorded a

Table 3. Instrumental colour measurements on the surface of Bosnian sudzuk produced in households (n=12)

Parameters (n=5)	Producers			
	A1	A2	A3	A4
L*	48.82±5.75[a]	45.24±7.68[a]	41.39±5.67[a]	43.32±8.49[a]
a*	1.32±1.72d	10.61±3.74[ab]	8.01±1.40a[c]	8.66±3.84b[c]
b*	9.97±0.82[a]	7.58±2.02[a]	10.61±2.6[1a]	7.48±2.55[a]

Legend: Data are expressed as mean±standard deviation. Means with the same letter in the same row do not differ statistically at 5% (p>0.05). A1 household producer 1, A2 household producer 2, A3 household producer 3, A4 household producer 4.

Table 4. Instrumental colour measurements on the surface of Bosnian sudzuk produced in industrial conditions (n=9)

Parameters (n=5)	Producers		
	B1	B2	B3
L*	39.35±2.93[a]	47.21±3.09[b]	42.91±4.27[ab]
a*	15.54±1.04b	8.00±1.92a	10.84±3.67[a]
b*	10.28±2.5[4a]	11.12±1.4[8a]	8.14±1.94[a]

Legend: Data are expressed as mean±standard deviation. Means with the same letter in the same row do not differ statistically at 5% (p>0.05). B1 Industrial producer 1, B2 Industrial producer 2, B3–Industrial producer 3.

high fat content in industrial sudzuk (47.05%), and in domestic sudzuk (27.98%). *Operta* (2018) obtained an average fat content in sudzuk of 28.46%, ranging from 24.0% to 32.41%.

Values of the colour parameters L* (lightness), a* (redness) and b* (yellowness) on the surface of sudzuk produced in households are shown in Table 3, and those for sudzuk produced in industrial conditions are in Table 4. No statistically significant difference (p>0.05) was found for L* for the Bosnian sudzuk produced in the four households, while for the Bosnian sudzuk produced in industrial conditions, we found statistically significant differences (p <0.05) in L* between producers B1 and B2. Values of a* were statistically significantly different (p <0.05) for the Bosnian sudzuk produced by the four households, and also for the sudzuk produced in industrial conditions. Redness a* is often use as an indicator of meat products' colour stability (*Hromiš et al.*, 2013). No statistically significant differences (p >0.05) were found for b* between the sudzuk produced in the four households or between those produced in industrial conditions. According to *Bozkurt and Bayram* (2006), the formation of colour in industrial sudzuk is due to the reduction of nitrate to nitrites by bacteria, while red pepper and sugar also contribute to the desired colouring of sudzuk.

Tables 5 and 6 show the results of sensory assessment of the Bosnian sudzuk.

The overall grades of sausages made in the households were significantly different (p <0.05), while those of sausages produced in industrial conditions were not statistically significantly different (p >0.05) between producers. The sudzuk from producer A1 had a fatty taste during chewing in the mouth, while saltiness was satisfactory, and the products had characteristic flavour and aroma. These sudzuk had the lowest table salt content of all the sausages evaluated. The sudzuk from producer A2 was found to have a uniform colour and mosaic sections, a stronger flavour of smoke than other products, gently pronounced acidity and a pronounced saltiness. The stuffing was filled firmly and evenly in the casings. According to the results of chemical analysis, these sudzuk had the highest NaCl content. In the sudzuk from producer A3, the cross-sectional colour was lighter and with darker edges than the other household products. Toughness was felt during chewing, so the evaluators concluded these products were

Table 5. The results of sensory assessment of Bosnian sudzuk produced in households (n=12)

Sensory properties	Producers			
	A1	A2	A3	A4
Outside appearance	9.08±0.23a[b]	8.12±0.42d	9.44±0.26a[c]	9.56±0.09b[c]
Cut appearance	19.4±0.22[a]	22.90±0.8[9b]	17.50±1.9[7a]	23.20±0.5[7b]
Cut colour	10.14±0.3[9a]	13.8±0.56[b]	10.26±0.9[3a]	14.04±0.3[3b]
Smell, aroma and flavour	32.48±1.06[b]	22.72±1.37[a]	22.54±0.31[c]	26.88±2.30[a]
Consistency	13.20±1.70[ab]	12.36±0.78[ac]	10.02±0.27[c]	12.12±0.59[b]
Overall grade	84.30±1.2[7a]	84.90±1.1[9ab]	69.76±2.9[0c]	85.80±3.1[1cb]

Legend: Data are expressed as mean±standard deviation. Means with the same letter in the same row do not differ statistically at 5% (p>0.05). A1 household producer 1, A2 household producer 2, A3 household producer 3, A4 household producer 4.

Table 6. The results of sensory assessment of Bosnian sudzuk produced in industrial conditions (n=9)

Sensory properties	Producers		
	B1	B2	B3
Outside appearance	8.36±0.39a	9.16±0.17b	8.56±0.43a
Cut appearance	21.10±1.5[6ab]	23.40±0.6[5a]	20.20±1.7[2b]
Cut colour	12.96±0.4[5ab]	13.74±0.2[5a]	12.00±1.2[2b]
Smell, aroma and flavour	31.08±0.38[a]	29.12±0.63[ab]	28.84±2.07[b]
Consistency	13.26±0.39[a]	12.60±0.30[a]	12.12±1.15[a]
Overall grade	86.76±2.5[5a]	88.02±1.2[2a]	81.72±6.2[9a]

Legend: Data are expressed as mean±standard deviation. Means with the same letter in the same row do not differ statistically at 5% (p>0.05). B1 Industrial producer 1, B2 Industrial producer 2, B3–Industrial producer 3.

not sufficiently dried. The evaluators agreed that the taste of pepper was more pronounced in these samples than in the other household sudzuk, with a slight note of smoke that resulted in a sour taste, while the consistency of the sudzuk was quite hard. Also, for these household sudzuk, differences in cross-sectional mosaicism were found. According to the results of *Jahić and Pračić* (2018), homemade Bosnian beef sudzuk had a pronounced, dark cross-section, with a pronounced mosaic and visible pieces of beef tallow, without cracks in the interior and with good consistency and satisfactory taste.

Sudzuk from producer B2, in comparison to the other industrial products, had a uniform surface appearance and a uniform mosaic in cross-section, with a lighter cross-sectional colour, more pronounced fat content and pronounced aromaticity due to the presence of a larger amount of spices. According to the evaluators, the sudzuk from producer B3 had a visible amount of adipose tissue of higher granulation and more pronounced fat content compared to the other assessed sausages. During chewing, greater toughness was felt, and the aromaticity of these sudzuk was more pronounced due to the presence of a large amount of black pepper. According to *Operta et al.* (2012), similar sensory properties were found for sudzuk made from chilled and frozen meat. However, sudzuk made from frozen (rather than chilled) meat had a better connection between muscle

and fat tissue, a characteristic white colour of fat tissue and a more pronounced presence of bark. Both types of sudzuk were moderately fatty, with noticeable discrete acidity and a moderate garlic aroma. According to *Kurćubić et al.* (2016), sudzuk produced in households ranked better for external appearance and surface colour than those produced in industrial conditions. When evaluating product consistency, sudzuk obtained in industrial conditions was evaluated as better, but when evaluating the cross-section colour, smell and taste, no statistically significant differences were found between sudzuk produced in household and industrial conditions (*Kurćubić et al*., 2016). Two samples of sudzuk from producer B1 were found to have uneven surface colour, with significant separation of adipose tissue during cutting the sausage and softer consistency. The smell and taste of sudzuk from producer B1 were characteristic and satisfactory.

Conclusion

Based on the results obtained in this study, it can be concluded that the chemical properties of sudzuk produced domestically and industrially were not uniform, and variations existed in the moisture, protein, sodium chloride and fat contents. The pH of Bosnian sudzuk from different producers also differs. The sensory properties of the Bosnian sudzuk are quite uneven in terms of consistency, while deviations exist in terms of aroma and smell of the products. The largest differences in sensory properties were recorded for external appearance, cross-sectional appearance, and cross-sectional colour. These results are probably due to differences in sudzuk recipes, the origin of the raw materials, and the different approaches in the technological process of production. Therefore, the results of chemical and sensory analyses in this study should be used to help achieve uniform product quality, which in the future could result in Bosnian sudzuk being legally designated as of protected geographical origin or as a traditional specialty.

Hemijske i senzorne osobine domaćeg i industrijski proizvedenog bosanskog sudžuka

Suzana Jahić, Sebila Rekanović

A p s t r a k t: Sudžuk, bosanska suva fermentisana kobasica tradicionalno se proizvodi od goveđeg mesa, goveđeg loja, kuhinjske soli, belog luka i bibera. U ovom radu su istražene hemijske i senzorne osobine domaćeg i industrijski proizvedenog bosanskog sudžuka. U oba slučaja, tehnološki proces pripreme i proizvodnje sudžuka je izveden na način specifičan za dati proizvod. Dobijene vrednosti određene hemijskom analizom (vlaga, ukupan pepeo, natrijum hlorid, ukupne kiseline, masti, proteini i pH vrednost) u uzorcima domaćeg proizvedenog bosanskog sudžuka su bile statistički značajne ($p<0.05$). U uzorcima industrijski proizvedenog sudžuka za sadržaj vlage, ukupnog pepela, natrijum hlorida, ukupnih kiselina, proteina i pH vrednosti je ustanovljena statistički značajna razlika ($p<0.05$), dok za sadržaj masti nije ustanovljena statistički značajna razlika između uzoraka ($p>0.05$). Rezultati instrumentalnog merenja boje su pokazali statistički značajne razlike ($p<0.05$) za vrednosti parametra a u uzorcima bosanskog sudžuka proizvedenog u domaćinstvu i u industrijskim uslovima, te za vrednost parametra L* u uzorcima industrijski proizvedenog sudžuka. Nije utvrđena statistički značajna razlika ($p>0.05$) za vrednost parametra b* u uzorcima bosanskog sudžuka proizvedenog u domaćinstvu i u industrijskim uslovima. U senzornoj evaluaciji je ustanovljena statistički značajna razlika ($p<0.05$) za ukupnu ocenu za uzorke bosanskog sudžuka proizvedenog u domaćinstvu, dok za ukupnu ocenu industrijski proizvedenog sudžuka nije utvrđena statistička značajnost ($p>0.05$).*

Ključne reči: *bosanski sudžuk, tradicionalna i industrijska proizvodnja, hemijske i senzorne osobine.*

Disclosure statement: No potential conflict of interest was reported by authors.

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Paper received: October 11th 2021.
Paper corrected: November 11th 2021.
Paper accepted: November 16th 2021.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 637.5.037:636.2
ID: 53342729
https://doi.org/10.18485/meattech.2021.62.2.5

Original scientific paper

Investigation of the physico-chemical and microstructure changes of beef meat during frozen storage at −23°C

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A b s t r a c t: Freezing beef meat is the most effective way to extend its storage life. However, there is little information about whether this practice alters the microstructure of beef and its effects on meat quality. For this reason, the object of our research was to determine the effect of frozen storage (one year at −23°C, with meat examined every two months) on physical, chemical and microstructural properties of beef in cuts of 20 Biceps femoris muscles. Significant physical changes were detected at different frozen storage durations, including increases in pH and yellowing (b), as well as decreases in water activity, lightness (L*), and redness (a*). In terms of chemical characteristics, the protein solubility in the beef reduced, but lipid oxidation (TBARS) values considerably rose with frozen storage duration. The width of ice crystals in frozen beef steadily increased as storage time was extended to 12 months, indicating structural changes in the frozen meat.*

Keywords: *beef, physico-chemical parameters, microstructure, freezing, storage time.*

Introduction

Meat is an important part of a well-balanced and varied diet, since its nutritional components fulfil the demands of the majority of people for essential nutrients, including protein, minerals and vitamins (*Rahman et al.,* 2015). The worldwide beef meat export industry is valued over US$ 13 billion, and demand is growing at a rate of 3.5 % each year (*Leygonie et al.,* 2012a). Freezing is frequently used in the food industry to preserve perishable substances for lengthy periods of time (*Wang et al.,* 2020). Freezing, in particular, is important for keeping meat safe, allowing the meat industry to tailor its production to customer demand, adapt meat output to processing rates, and export meat to all parts of the world. The freezing technique employed in meat preservation is mainly concerned with slowing the growth and proliferation of meat spoilage microorganisms as well as delaying other deleterious changes to colour, odour, texture, moisture and ultrastructure (*Zhang et al.,* 2019).

As a result of freezing, ice crystals cause mechanical damage, structural changes and protein denaturation in meat (*Jeong et al.,* 2011). Ice crystals disrupt the ultrastructure of meat and concentrate solutes, causing metabolic changes and influencing the meat's physical qualities (*Leygonie et al.,* 2012b). One of these changes is a reduction in water-holding capacity that reduces the tenderness and the overall eating quality of the meat, lowering its commercial value (*Kim et al.,* 2015)frozen only, and 3 or 4. weeks ageing at −1.5°C then frozen. Protein oxidation also lowers meat quality by causing fragmentation or aggregation of proteins and diminishing protein solubility *(Sun et al.,* 2002). Phospholipids containing a wide variety of fatty acids including polyunsaturated fatty acids are the lipid component of meat that is most sensitive to oxidation. As a result, radical secondary lipid oxidation can be produced during meat freezing (*Medić et al.,* 2018), resulting in undesired alterations in meat quality (*Deng et al.,* 2021). Therefore, researchers must have a complete understanding of the physicochemical and structural changes caused by frozen storage of meat (*Bertram et al.,* 2007).

Numerous studies have reported on the impact of long-term preservation on physicochemical and biochemical changes in meat of different animal species (*Muela et al.,* 2015; *Medić et al.,* 2018;)although the influence of frozen holding temperatures was negligible. LL carbonyl, and nitrate and nitrite content responses were variable and yet broadly reflected an increased incidence of protein oxidation

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across increasing chilled storage and ensuing frozen storage periods — this aspect meriting future exploration. Total myoglobin content and the estimated myoglobin redox fractions (metmyoglobin, deoxymyoglobin, and oxymyoglobin. Regardless, there are just a few studies in the literature on the impact of ice crystal development on physico-chemical changes during long-term frozen beef storage. It appears important to highlight the consequences of these significant alterations due to freezing, in order to anticipate the quality of frozen beef. The aim of this study was to investigate the effect of prolonged frozen storage on the quality of beef by looking at the physicochemical changes of the meat and its tissue structure.

Materials and methods

Twenty randomly selected beef carcasses from a commercial slaughterhouse in the Algerian city of Batna were sampled. At 24 h postmortem, sections of the femoral biceps were selected. Each sample was divided into portions of equal approximate weight that were individualy vacuum packed and frozen at −23°C in a freezer (CRF-NT64GF40, Condor, Algeria). The temperature during frozen storage was monitored with an infrared thermometer (TIA 101, China). Fresh samples (n = 20) were analysed before freezing then portions from the same muscle were analysed after 2, 4, 6, 8, 10 and 12 months of storage.

Determination of physico-chemical changes on fresh and frozen/thawad beef

Meat pH

Meat pH was measured according to the procedure of *Zhu et al.* (2020). A sample of beef (fresh or frozen/thawed) was minced, and 5 g of the mince was mixed for 1 min in 50 mL distilled water. The pH of the mixture was measured using an INOLAB digital pH meter.

Water activity (a_w)

Water activity ($\mathbf{a_w}$) was measured using a BT-RS1 Rotronic Hygroscope as indicated by (*Lakehal et al.*, 2019). The beef was cut into small pieces and placed in a three-quarter capacity sample cup. The probe was immediately placed in the sample cup. The result was read as soon as the humidity and temperature readings stabilized.

Colour measurement

The surface colour of beef samples was determined by a computer vision system (CVS) as described by *Tomasevic et al.* (2019), using a digital camera (Canon DS126621) mounted in a black box supplied with standard illumination (6500 K) positioned at an angle of 45° from the sample to obtain uniform lighting. The colour was analysed quantitatively using Adobe Photoshop CS3 software to assess lightness (L*), redness (a*) and yellowness (b*).

Protein solubility

Protein solubility was measured according to the method of *Zhang et al.* (2017) with minor modifications. Total protein was extracted from a 1 g sample of the beef using 10 mL of ice-cold solution (1.1 M potassium iodide in 0.1 M phosphate buffer, pH 7.2). The samples of beef meat were minced, homogenised in the ice-cold solution in an ice bath, and then refrigerated for 20 h. Following centrifugation (2600 *g*, 30 min, 4°C), the protein in the supernatant was quantified using the Biuret assay. Protein solubility was calculated in milligrams of protein per kilogram of beef.

Thiobarbituric acid reactive substances (TBARS)

TBARS measurements indicated fat oxidation in the frozen meat according to the protocol described by *Buege and Aust*, (1978). Minced beef (4 g) and 40 ml distilled water were mixed for 1 min. After that, 1 ml of the solution was combined with 2 ml of a solution containing TCA-TBA-HCI reagent (15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25 N hydrochloric acid). The mixture was boiled for 20 min in a water bath. The resulting solution was cooled for 10 min nnder running water. The absorbance of the resulting top layer was measured at 532 nm. The amount of TBARS was expressed as nmol of malondialdehyde/g of beef using a molar extinction value of 1.56×10^{-5} M^{-1} cm^{-1}.

Microstructure of meat

The microstructure of meat was studied according to the method of *Su et al.* (2014) fresh shrimp and porcine liver were frozen by PSF at 100 MPa (−8.4°C. To begin, meat samples were fixed in Clarke's solution (75% absolute ethanol and 25% glacial acetic acid) at −23°C for 24 h. After that, the samples were equilibrated to room temperature, then dehydrated with ethanol gradients according to the protocols and techniques defined previously (*Luna,* 1968) and then were immersed in xylene and paraffin at 58°C. After that, each sample was paraffin-embedded and trapped

in paraffin blocks. Blocks were sliced into 6 µm thick slices with a microtome (Leica, Jung-histocut 820) and resultant sections were placed on glass slides, dewaxed, and stained with Calleja solution for later microscopic analysis. Finally, a microscope (Zeiss Axioscope) equipped with a digital camera (SLS-Mvision) was used to examine all of the prepared the sectioned tissues. The intracellular location of each ice crystal, as well as the number and average diameter of ice crystals in each cell, was computed after the intracellular ice crystals (white voids) were delimited.

Statistical analysis

Results were statistically evaluated using the SPSS software version 20 (IBM SPSS Statistics v22). Analysis of variance (one-way ANOVA) techniques and Tukey's multiple comparison tests were employed to examine for differences among the data acquired. Results are presented as means with standard deviations.

Results and discussion

Beef meat pH

It was observed that the pH of beef samples increased with the extended frozen storage period, as shown in (Figure 1). According to *Ho et al.* (2020), accumulation of free amino acids, ammonia and organic sulphides derived from the hydrolysis of proteolytic amines might be considered to be the primary cause of the elevated pH. In this study, amino acids were not measured, but in another study, the changes of free amino acids during frozen storage of lamb meat were measured, and authors concluded that the increase in pH was due to the increase in free basic amino acids (*Braggins et al.,* 1999). Commonly, the increase of pH could be mainly associated with the increase of alkaline substances (*Farouk and Swan,* 1998).

Water activity (a_w)

Figure 2 shows the water activity (a_w) in the beef changed during frozen storage. The a_w in fresh samples averaged 0.945, but a_w in all beef samples decreased dramatically as storage duration increased after two months. Although the a_w dropped after freezing, it rose for about ten months before dropping again at the conclusion of storage (12 months). Variations in a_w are linked to fluid movement and ice crystallization (*He et al.*, 2015). Our results are in agreement with those of others (*Medić et al.*, 2018), who detected a change in pork a_w after 18 months' freezing. Nonetheless, one study (*Coombs et al.*, 2017)at 24 h post-mortem, and assigned to five chilled storage periods (0, 2, 4, 6 and 8 weeks found no changes in a_w in frozen lamb meat for two freezing temperatures (−12 and −18°C) during a 52-week period. It has been demonstrated that unfavourable reactions are frequently linked to a food's a_w rather than its water content (*Black and Jaczynski,* 2008) chicken breast meat, and trout fillets was modified to intermediate (aw 0.98–0.99.

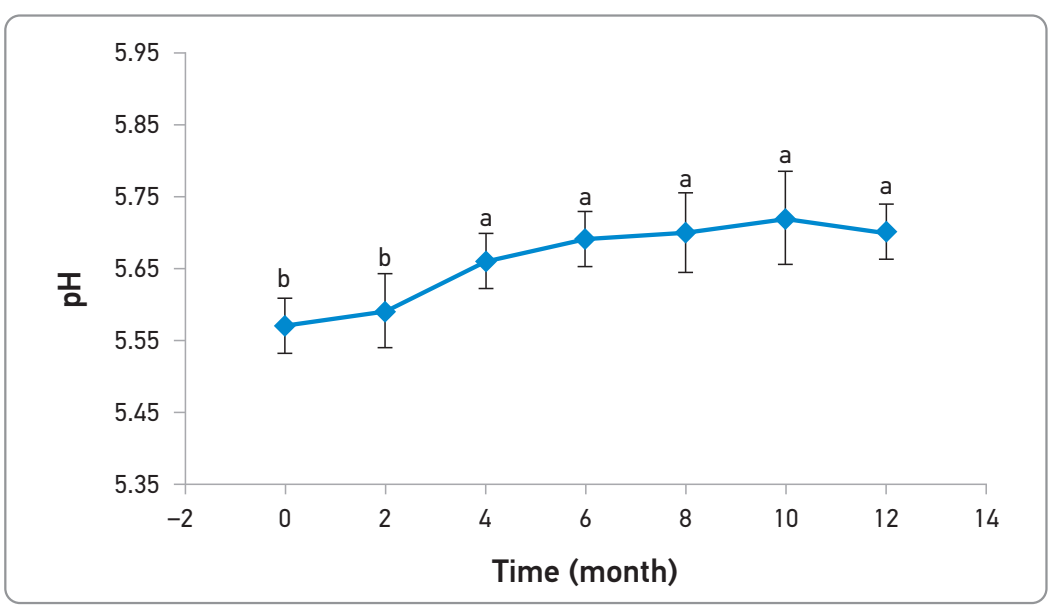

Figure 1. Beef pH during frozen storage.

Legend: a, b different letters show a statistically significant difference ($p<0.05$)

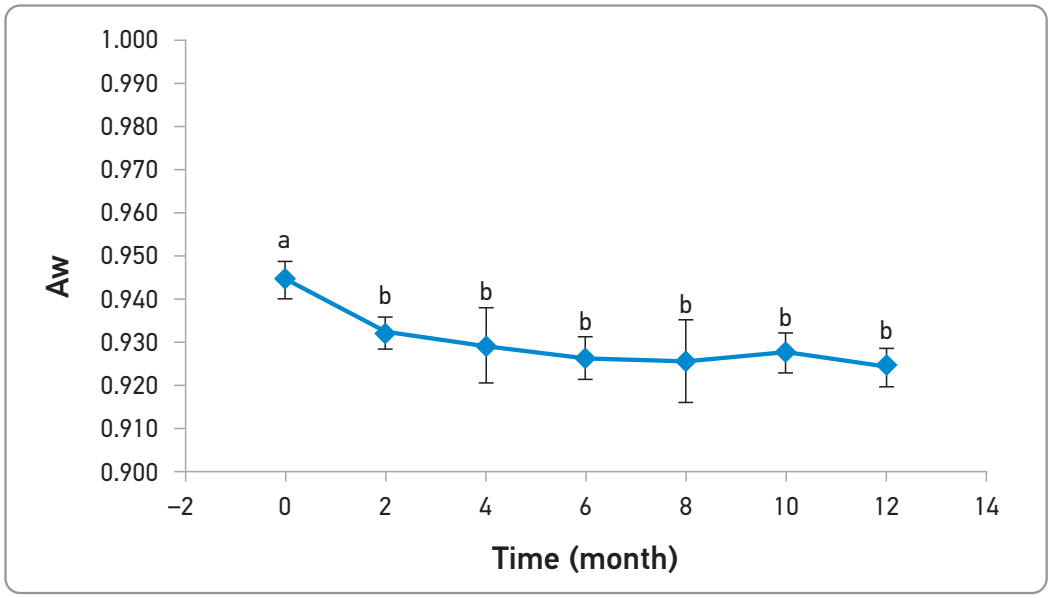

Figure 2. a_w of frozen beef during storage.

Legend: [a, b] different letters show a statistically significant difference ($p<0.05$)

Colour evaluation

Table 1 shows colour value changes (CIE L*, a*, and b*) occurred during frozen storage. Our results showed that from the fourth month of frozen storage, a significant decrease of (L*) occurred. Decrease in meat lightness due to freezing was reported earlier (*Muela et al., 2015*; *Hou et al.,* 2020) and was explained by the structural changes in the meat caused by myofibrillar protein degradation during the storage (*Wang et al.,* 2020). After 11 months of frozen storage, the values of (a*) were significantly lower than in the fresh meat ($p<0.05$). *Hansen et al.* (2004) also found that, after 30 months, (a*) levels decreased with frozen storage. According to *Alonso et al.* (2016), the lowering in (a*) values could be related to myoglobin denaturation during frozen processing. On the other hand, in our study, (b*) values were obviously increased by frozen storage from the 6th month ($p<0.05$). Several previous studies reported an increase in meat yellowness (b*) (*Hansen et al.,* 2004; *Vieira et al.,* 2009; *De Paula Paseto Fernandes et al.,* 2013; *Coombs et al.,* 2017). This change could be explained by the oxidation of fat and protein degradation during frozen storage (*Estévez,* 2011; *Muela et al.,* 2015). Meat colour is a crucial factor for consumers when evaluating meat quality, and it is also the most popular determinant of whether or not to buy frozen meat (*Zhang et al.,* 2019).

Protein solubility

With increasing frozen storage duration, protein solubility in frozen/thawed beef samples showed a significant tendency to decrease ($p<0.05$) (Figure 3). Protein solubility dropped from 111.65 m/g to 77.25 mg/g by the end of storage. *Nahar et al.* (2014) and *Farouk and Swan* (1998) found similar reductions in protein solubility in frozen chicken and beef meat, respectively. In the food sector, protein solubility is an extremely important parameter. Protein solubility has been frequently employed as a good indicator of

Table 1. Changes i CIE L*a*b* of froze beef uscles during frozen storage

	Frozen storage time (Months)						
	Fresh meat	**2**	**4**	**6**	**8**	**10**	**12**
CIE L*	42.83[a]	42.67[a]	40[ab]	38.50[b]	38.33[b]	39.17[ab]	38.16[b]
CIE a*	15[a]	15[a]	15.67[a]	15.67[a]	15.5[a]	12.83[b]	12[b]
CIE b*	5.17[c]	5.5[c]	7[bc]	9.43[a]	9.67[a]	8.93[a]	9.69[a]

Legend: CIE L*a*b* — light reflectance scores; [a,b,c] different letters in the same row show statistically significant difference ($P<0.05$).

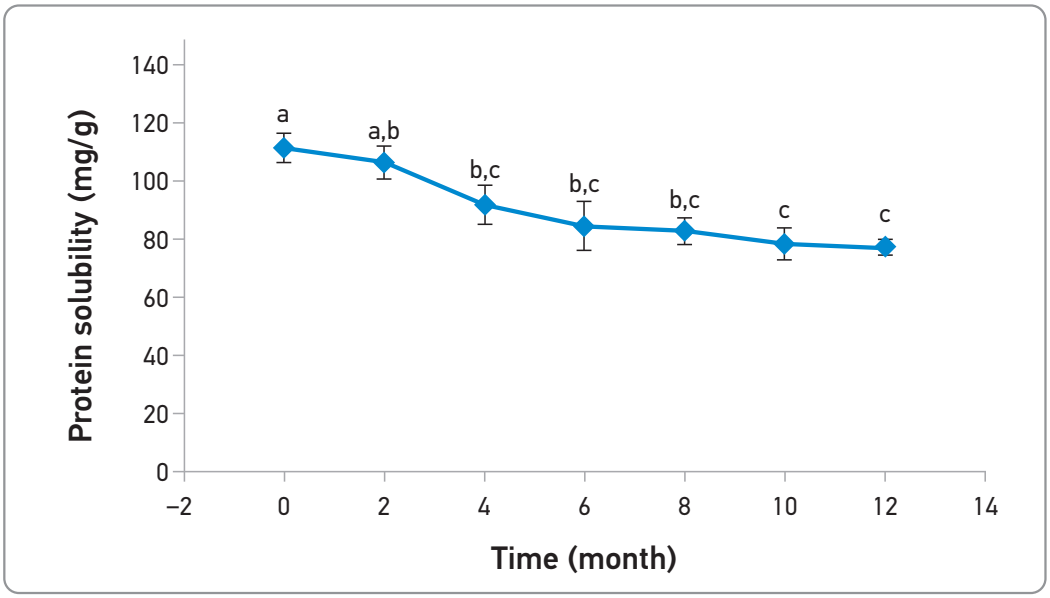

Figure 3. Protein solubility of beef during frozen storage.

Legend: [a, b, c] different letters show a statistically significant difference ($P < 0.05$).

protein denaturation (*Nahar et al*., 2014) because it is easy to test and has a high association with meat texture (*de Koning and Mol*, 1991). According to *Chan et al.* (2011), protein solubility is significantly reduced during freezing storage, and this reduction could be associated with sensitivity of proteins to temperature-induced aggregation. It is also worth noting that the dynamics of ice formation had an impact on the solubility of proteins (*Farouk and Swan*, 1998). In addition, the freezing process effectively contributes to protein damage, as it is the main culprit in the appearance of intracellular ice crystals and, thus, it mechanically weakens meat's protein structures and causes their further fragmentation (*Zhou et al.*, 2018).

TBARS evaluation

Figure 4 shows the mean TBARS value was 0.172 mg/kg on day 0 and it increased significantly ($p<0.05$) during frozen storage up to a maximum value at 12 months (0.870 mg/kg). Despite the fact that no sensory analysis was undertaken in this

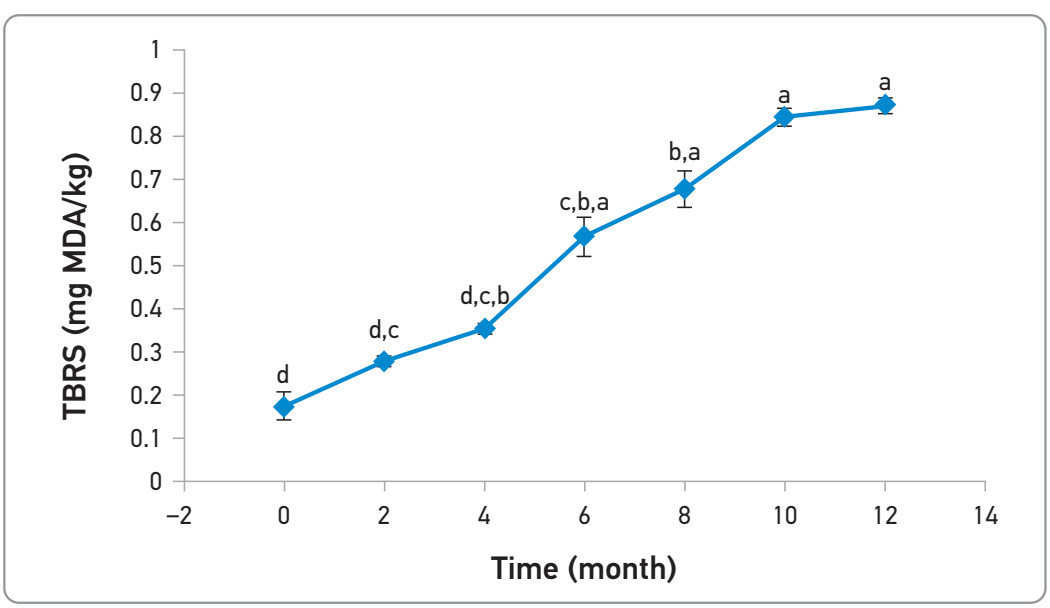

Figure 4. TBRS values in beef during frozen storage.

Legend: [a, b, c, d] different letters show a statistically significant difference ($P < 0.05$)

study, the final TBARS values achieved in this work did not reach the flavour criterion (>1.0 MDA mg/kg) above which an undesirable odour and a rancid taste could result (*Ripoll et al.*, 2011). The TBARS parameter indicates secondary oxidation products, which result from the hydrolysis of polyunsaturated fats, and which relate to unpleasant flavours in meat and meat products (*Sun et al.*, 2019). Also, oxidation of lipids can cause denaturation of proteins, which changes their structure, causes peptide scission (*Saeed and Howell*, 2002) and changes their functional qualities, such as their water holding capacity (*Cao et al.*, 2018; *Zhou et al.*, 2018). The TBARS value is the main indicator of fat oxidation in food (*Turgut et al.*, 2017).

Microscopic observation

Figure 5(A–G) shows microscopic images of the beef meat at different frozen storage durations. Figure 5A shows a typical microscopic image of the beef meat before freezing for the purpose of comparison

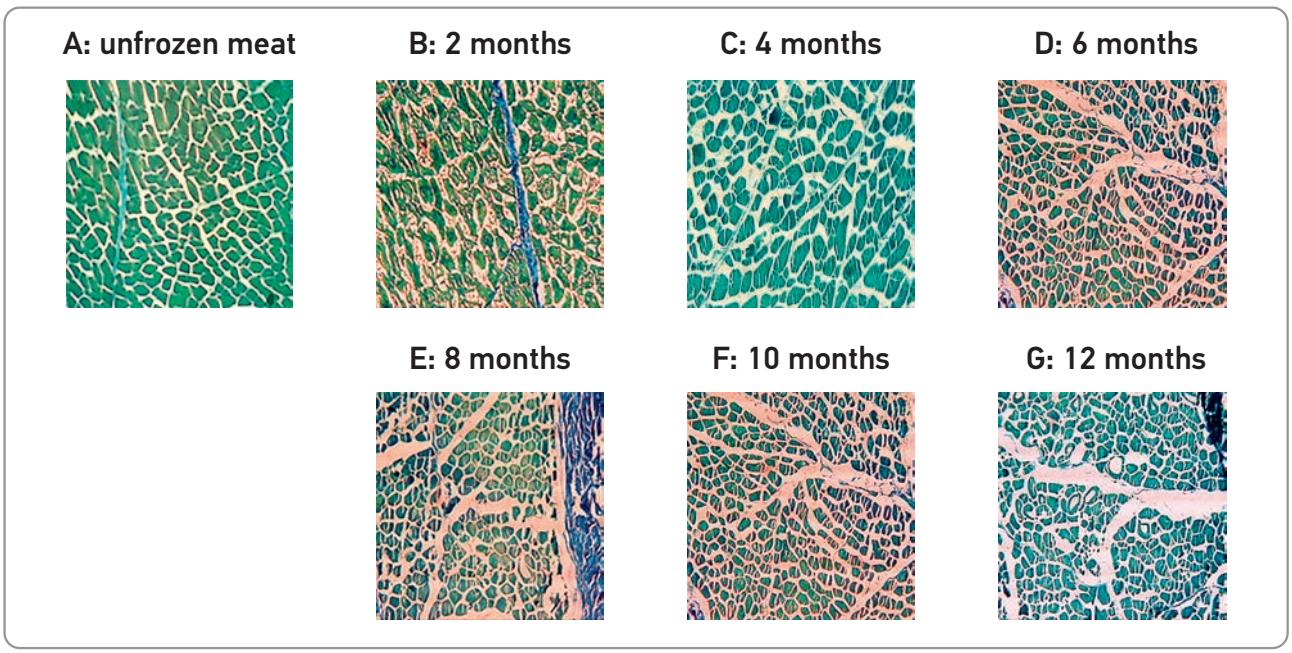

Figure 5. Changes in microstructural cross section of frozen beef meat with time. *Calleja stain*; ×100.

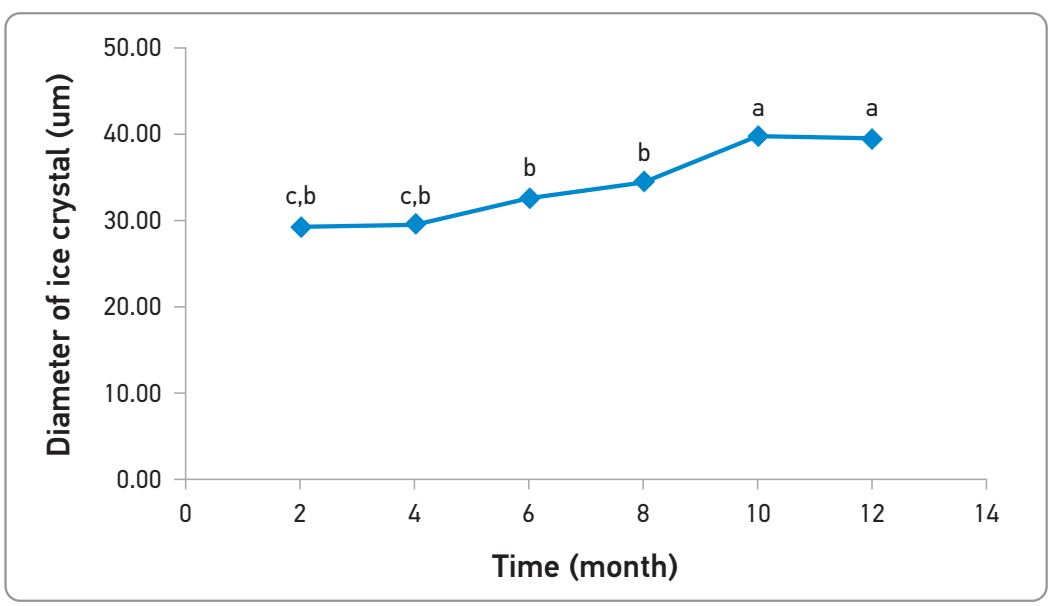

Figure 6. Mean diameter of ice crystals during frozen storage

Legend: [a, b, c,] different letters show a statistically significant difference ($p<0.05$)

with frozen meat (Figure 5B-G). The fresh, unfrozen beef (Figure 5A) had uniform distribution of regularly formed fibres. At the end of two months of frozen storage, small vacuoles, formed from ice crystals in the meat, were observed (Figure 5B). After 12 months of storage, the intracellular ice crystals appeared larger and the space between fibres was greater (Figure 5G). The main factor affecting the texture of meat is structural changes related to the relationship between proteins and water molecules (*Nakazawa et al.,* 2019). Generally, lowering the storage temperature is a good technique to keep meat fresh for a long time (*Zhang et al.,* 2019). However, the crystallization of water in the tissue might result in an increase in the solute concentration during frozen storage, followed by the separation of the endomysium and the expansion of the perimysium, which results in a decrease in cell size and an expansion of the extracellular space (*Shi et al., 2018*; *Tolstorebrov et al.,* 2016).

By analysing the data obtained from microscopic images (Figure 5), we noted that ice crystal diameters showed an ascendant trend as storage duration prolonged. The diameter of ice crystals increased from 29.09 μm after 2 months to 39.34 μm after 12 months, with significant differences ($p > 0.05$) between them (Figure 6). *Jiang et al.* (2020)distribution of water and freshness properties of grass carp during frozen storage was investigated. The freezing methods contained air-blast freezing (AF demonstrated that recrystallization during frozen storage could cause expansion of voids inside muscle fibres.

Conclusion

In general, our results showed that the physical properties of beef meat are significantly affected by frozen storage, and these effects evolve during the meat's frozen storage at −23°C. Freezing increased the pH and yellowing (b*) of the meat, while the lightness (L*), redness (a*) and a_w decreased. Based on TBAS values, it was deduced that the beef was of a quality that was adequate for processing after 12 months of frozen storage. However, a continuous decrease in protein solubility was observed during the 12 months of storage. The microstructure of the beef visually showed a gradual increase in the diameter of intra-tissue ice crystals with increased frozen storage duration, which means the muscle tissue was undergoing fracture.

Ispitivanje promena fizičko-hemijske i mikrostrukture goveđeg mesa tokom skladištenja na niskoj temperaturi

Saliha Lakehal, Omar Bennoune, Ammar Ayachi

A p s t r a k t: Zamrzavanje goveđeg mesa je najefikasniji način da se produži dužina odn. trajanje njegovog skladištenja. Međutim, malo je informacija o tome da li ova praksa menja mikrostrukturu govedjeg mesa, kao i o njenom uticaju na kvalitet. Iz tog razloga, cilj našeg istraživanja je bio da se utvrdi uticaj šest perioda skladištenja u zamrznutom stanju na fizička, hemijska i mikrostrukturna svojstva goveđeg mesa u komadima mišića biceps femoris (20). Značajne fizičke promene su otkrivene u različitim vremenima skladištenja, uključujući povećanje pH i intenziteta žute boje (b), kao i smanjenje aktivnosti vode, svetlost (L*) i crvene boje (a*). Zatim, u pogledu hemijskih karakteristika, rastvorljivost proteina u uzorcima govedjeg mesa je smanjena, ali su vrednosti oksidacije lipida (TBA) znatno porasle. Širina ledenih kristala u smrznutim uzorcima stalno se povećavala kako se vreme skladištenja produžavalo na 12 meseci, što ukazuje na strukturne promene u smrznutim mišićima.*

Ključne reči: *goveđe meso, fizičko-hemijski parametri, mikrostruktura, zamrzavanje, vreme skladištenja.*

Disclosure statement: No potential conflict of interest was reported by authors.

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Paper received: October 11th 2021.
Paper corrected: October 14th 2021.
Paper accepted: November 9th 2021.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 637.5.05:598.182.223
ID: 53267209
https://doi.org/10.18485/meattech.2021.62.2.6

Original scientific paper

Development and characterization of low fat cooked yacare (*Caiman yacare*) meat sausages

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A b s t r a c t: The limited consumption of yacare (Caiman yacare) is due to cultural and economic factors, beyond a limited availability of products based on this meat. Here cooked sausages were developed from yacare meat shavings and fat substitutes (inulin and soy protein), and characterized. Moisture ranged from 63.90% (T1) to 59.89% (T3), a decrease with the increase in the inulin content (T1 had the lowest, T3 the highest inulin content). The protein content decreased from 27.67 (T1) to 25.32% (T3). The highest lipid content was 5.36% (T2) and the lowest 1.69% (T3). The ash content ranged from 4.50 to 4.62%. The highest luminosity value was obtained for T2 (59.69) and the lowest for T3 (57.24). The highest average shear force (18.01 N) was obtained for T3. Good sensory characteristics were obtained for all treatments, with acceptability indexes varying from 68.67 to 87.11%. However, the highest purchase intention was declared by 72% of panelists who certainly or probably would purchase T1.

Keywords: *yacare meat, sausage, inulin, soy protein.*

Introduction

Considered a reptile with high population density, the yacare (Caiman yacare) lives in different aquatic environments, between salt pans, freshwater lagoons, perennial and temporary rivers, and swamps, the proportions and stability of which vary from region to region (*Campos et al.*, 2010). The breeding of yacare, if well managed, can contribute to the economic and ecological evolution of a region by the production of an alternative source of proteins from animals intensively adapted to the natural conditions of that specific environment (*Carreira & Sabbag*, 2015).

Yacare meat is considered a rich source of proteins of high digestibility and biological value. It contains insignificant amounts of cholesterol and great technological potential for the elaboration of derived products. The meat processing, in addition to the noble cuts, generates shavings that are sold as baits. The preparation of meat products with this waste is an option to develop food products with high added value and obtain further economic gains (*Romanelli et al.*, 2002). Among a range of potential meat products that can be obtained from the processing of yacare, cooked sausage can be underlined as an innovative technological option because this product is not found on the market.

The addition of pork fat is allowed in the production of cooked sausages. Due to the low fat content of yacare meat, fat replacers would be required in cooked yacare meat sausages. Literature reports several ingredients that can act as substitutes for animal fat in meat products (*Colmenero et al.*, 2012; *Cavenaghi-Altemio et al.*, 2013). Different product categories can be utilized as fat replacers: non-meat proteins (milk and soy proteins), base carbohydrates (carrageenan, starches and fibers such as inulin) and mixtures of ingredients (*Yashini et al.*, 2019). These fat substitutes are commonly used by the sausage meat industry, mainly for their ability to form gels, contributing consistency to the final product.

Textured soy protein is obtained industrially through the extrusion of defatted white soy bran. It is an essential ingredient in the preparation of some meat products and can be added in amounts up to 20% without changing the flavor (*Masson & Gelinski*, 2014). Soy proteins are the main functional components of some meat product technologies. Several

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authors mention that soy in meat products improves texture and emulsifying capacity, intensifies its appearance, firmness, juiciness, sliceability, and cooking efficiency, and reduces the formulation cost (*Xiong*, 2005; *Youssef & Barbut*, 2011).

Inulin is a natural storage oligosaccharide from several plants, including chicory, dahlia, and Jerusalem artichoke (*Barclay et al.*, 2010). After it is extracted and dried, inulin is identified as a white, hygroscopic powder, with a neutral odor and flavor, which can be included in foods without changing the appearance, viscosity and flavor of the formulations (*Franck*, 2002). It is characterized by its low caloric value and the formation of opaque gels in high concentrations, when mixed with water or other aqueous liquid, forming a compound similar to fat. In this sense, inulin has been used industrially in foods as a fat replacer to reduce the caloric value, promote water retention, and enrich foods with fiber (*Franck*, 2002; *García et al.*, 2006).

Thus, this work aimed to develop cooked sausages containing yacare (*Caiman yacare*) meat residues with added fat replacers (texturized soy protein and inulin), and characterize the obtained products through chemical, physical, microbiological, and sensory analyses.

Material and methods

Yacare (Caiman yacare) meat

Yacare (*Caiman yacare*) meat shavings were donated by Caimasul Ltda (Corumbá, MS, Brazil). They were transported to the Laboratory of Food Technology, Federal University of Grande Dourados, Dourados, MS, Brazil, under refrigerated conditions. Meat shavings were stored up to two weeks under freezing until processing.

Cooked sausages obtained from yacare meat

For the preparation of the cooked sausages, the meat shavings were milled in a grinder with a 12 mm disc (Weg, Jaraguá do Sul, SC, Brazil) at 1.5°C. Then, the ingredients of the formulations were added, according to the three treatments listed in Table 1, and the sausage batters were manually homogenized for 10 min at 4°C. Subsequently, the sausage batters were stuffed into natural bovine casing in horseshoe format. Next, the sausages were submerged in liquid smoke for 1 min. (Figure 1). Then they were cooked until the internal temperature reached 72°C, when they received a thermal shock with cold water below 5°C. After

Table 1. Formulations utilized for the cooked yacare (*Caiman yacare*) meat sausages

Ingredient	Formulation treatment (g/100g)		
	T1	T2	T3
Yacare meat shavings	83.94	82.94	81.94
Inulin	1.00	2.00	3.00
Cold water	8.095	8.095	8.095
Textured soy protein	2.50	2.50	2.50
Refined sodium chloride	2.00	2.00	2.00
Spices	1.10	1.10	1.10
Fat emulsifier	0.50	0.50	0.50
Sugar	0.40	0.40	0.40
Smoke aroma	0.40	0.40	0.40
Ascorbic acid	0.05	0.05	0.05
Sodium nitrite	0.015	0.015	0.015

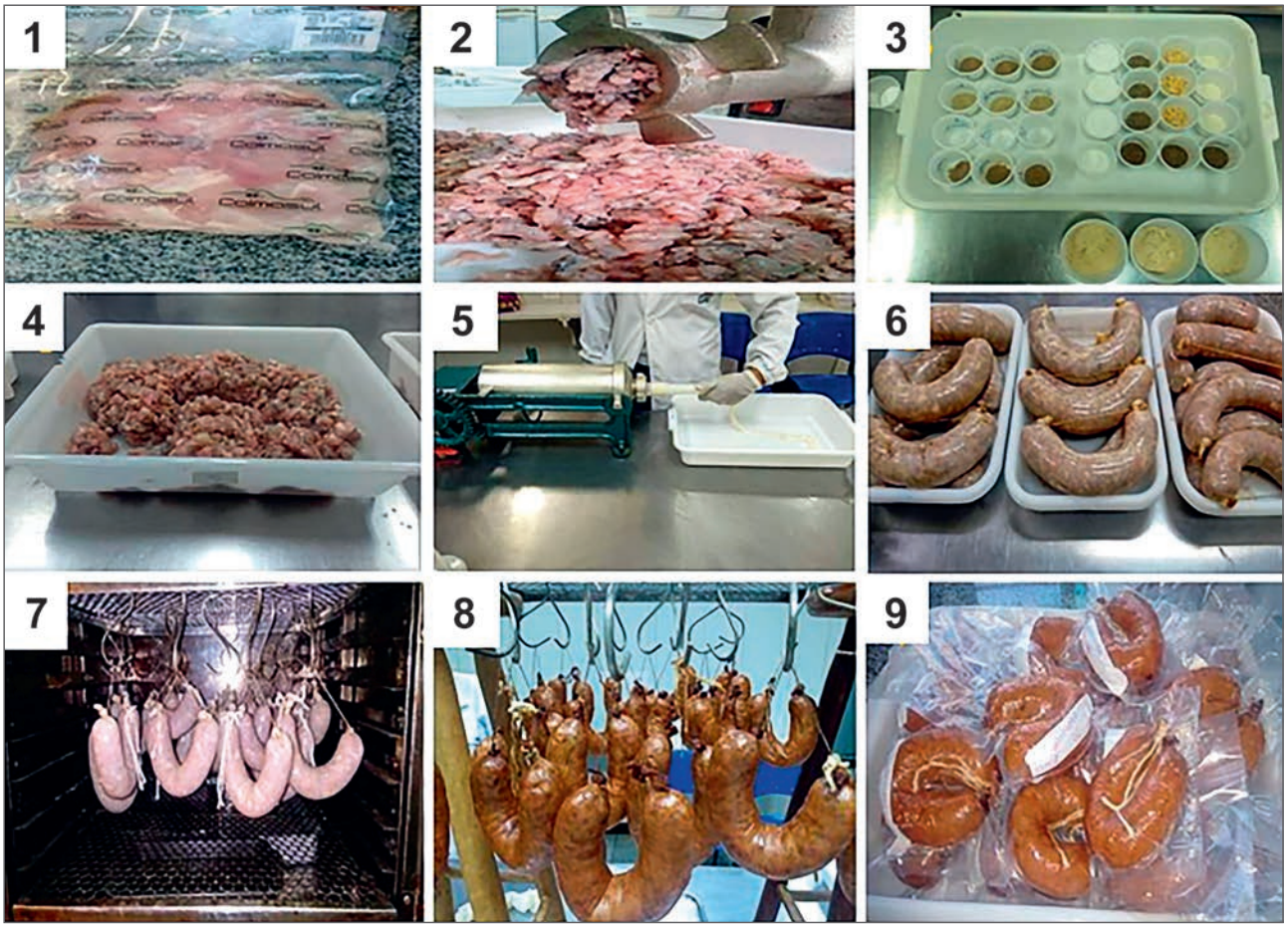

Figure 1. Development of cooked sausages of yacare (*Caiman yacare*) meat shavings. (1) Raw material; (2) Grinding; (3) Weighing of the ingredients; (4) Homogenization; (5) Stuffing the meat batter into casing; (6) After submersion in liquid smoke; (7) Cooking; (8) Drying; (9) Vacuum packaging

cooling, they passed through a varnish bath and drying at room temperature (28–30°C). The cooked sausages were identified as T1, T2, and T3, according to their formulation, vacuum packed, and then stored at 4°C for further analysis. Additives and condiments were supplied by Cavenaghi Eireli (Dourados, MS, Brazil).

Chemical analysis

Proximate composition

Moisture, crude protein, and crude ash contents of the cooked sausages were determined in triplicate according to the methods described by *AOAC* (2012). Moisture was determined by the oven drying method at 105°C until constant weight (method 950.46B), protein by the Kjeldahl method (method 928.08) and ash by the muffle oven technique (method 920.153). The lipid content was obtained in triplicate by the extraction method with cold organic solvent (*Bligh & Dyer*, 1959). The carbohydrate content was estimated by difference.

pH of the cooked sausages was measured in triplicate using a digital pH meter (Instrutherm model pH-2000, São Paulo, Brazil) by mixing 25 g of the sample and 10 ml of distilled water, according to the method described elsewhere (*Spitzer & Werner*, 2002).

Water activity

Water activity of the cooked sausages was determined in triplicate in a hygrometer (Aqualab, São José dos Campos, SP, Brazil) at 25°C with 1 g of sample.

Physical analysis

Instrumental color

The color [CIE L*(lightness), a* (redness), b* (yellowness)] of the cooked sausages was evaluated using a colorimeter (Minolta Chroma Meter CR 410), with measurements standardized with respect to the white calibration plate (*Jiménez & Gutiérrez*,

2001). The analysis was performed in triplicate in the internal part of the sausages.

Shear force

Texture analysis of the cooked sausages was carried out using a texture analyzer Model TAXT-plus (Stable Micro Systems, Surrey, England) calibrated with a standard weight of 5 kg. Products kept at 2°C were equilibrated at room temperature (28–30°C) before analysis. Samples of 15 × 15 × 115 cm were cut, placed in the texture analyzer and submitted to a cutting/shearing test (speed of 1.0 mm/s, distance of 30 mm) using a Warner-Bratzler shear blade (1 mm thick) to determine the shear force (N), which indicated the firmness of the sample. A minimum of 10 replicates of each treatment were analyzed (*Kang & Chen*, 2014).

Microbiological analysis

Microbiological analyses of the cooked sausages were performed for thermo-tolerant coliforms at 45°C, coagulase positive *Staphylococcus aureus* (CPS), and *Salmonella* sp. in accordance with the methodology described elsewhere (*USDA/FSIS*, 1998).

Sensory analysis

Sensory analyses of the cooked sausages were conducted by 50 trained panelists ranging in age from 20 to 51 years. A nine-point hedonic scale (9=like extremely; 1=dislike extremely) was used for evaluation of the attributes color, odor, texture and taste. The treatments were heated in microwave ovens for 5 s, then they were cut transversely 2 mm thick, and served in disposable containers, coded with three-digit random numbers. Overall acceptability was evaluated in terms of purchase intention using a 5-point scale, where 5 = certainly would purchase, 4 = probably would purchase, 3 = perhaps would purchase/perhaps would not purchase, 2 = probably would not purchase and 1 = certainly would not purchase, which was expressed as the percentage of total score (*Cavenaghi-Altemio et al.*, 2018). The acceptability index (AI) was calculated according to the following equation: AI = (average of the attributed grades/maximum attributed grade) x 100. The sample was considered acceptable if the AI was greater than 70% (*Stone & Sidel*, 1993).

Statistical analysis

Statistical results were evaluated through analysis of variance (ANOVA) and the Tukey's test for comparison of means, at a level of 5% of significance, using the statistical software Statistica 7.0. The sensory attributes and the purchase intention results were analyzed in percentages.

Results and Discussion

Chemical analysis

Proximate composition

The proximate compositions carried out for the cooked sausages prepared with yacare (*Caiman yacare*) meat shavings according to treatments T1 (1% inulin), T2 (2% inulin) and T3 (3% inulin) are shown in Table 2.

A significant difference ($p<0.05$) in moisture was observed between treatments, ranging from 63.90% for T1 to 59.89% for T3, showing the moisture decreased with the increase in the inulin content (Table 2). This can be explained due to the high water binding capacity of the inulin (*Rashid et al.*, 2018). Moreover, the addition of oligosaccharides in foods acts as a moisture reducer, limiting the water available in foods and preparations (*Gomes et al.*, 2007).

Table 2. Proximate composition of the cooked yacare (*Caiman yacare*) meat sausages

Determination (%)	T1	T2	T3
Moisture	$63.90^{a} \pm 0.30$	$61.26^{b} \pm 0.40$	$59.89^{c} \pm 0.38$
Protein	$27.68^{a} \pm 0.52$	$26.10^{a} \pm 0.82$	$25.33^{a} \pm 0.83$
Lipids	$3.34^{b} \pm 0.24$	$5.36^{a} \pm 0.67$	$1.69^{c} \pm 0.16$
Ash	$4.50^{a} \pm 0.03$	$4.62^{a} \pm 0.12$	$4.58^{a} \pm 0.07$
Carbohydrates	0.58	2.66	8.51

Legend: Means with the same letter in the same row do not differ statistically at 5% (P>0.05). Treatments (T1, T2, and T3) according to Table 1.

The moisture content in yacare meat decreases with the animal age and body portion. For example, average values of 77.18% of moisture were reported for yacare back meat in animals aged 14 months, which was higher than values obtained for tail meat and animals aged 26 months (*Vicente Neto et al.*, 2007). This value is higher than those found in the present study mainly because of the water-absorbing ingredients *e.g.* texturized protein soy and inulin that were added to the cooked sausages.

The protein content decreased from 27.67 (T1) to 25.32% (T3), without significant difference ($p>0.05$) between the samples (Table 2). The Brazilian Technical Regulation on Sausage Identity and Quality establishes that the minimum protein content for cooked sausages must be 14% (*MAPA*, 2000). Protein values ranging from 18.39 to 19.44% were reported elsewhere for the yacare meat (*Romanelli et al.*, 2002). These differences may be related to the type of breeding, sex, and the age of the animal (*Vicente Neto et al.*, 2007; *Fernandes et al.*, 2017).

The lipid content was 3.34% for T1, 5.36% for T2, and 1.69% for T3, with a significant difference between all treatments ($p<0.05$) (Table 2). Lipid contents around 3.94% were obtained elsewhere for yacare carcasses (*Fernandes et al.*, 2015). Despite the difference between treatments, the lipid content of the cooked sausages was quite similar to that obtained for yacare meat. Lipid concentration can vary depending on the sex and age of the animal, and the meat cut. The tail, for example, has muscles that execute more excessive physical activities due to locomotion in aquatic environments, and the tail also contains energy reserves that accumulate in the muscular tissues in the form of fat that is available for use when there is food shortage (*Vicente Neto et al.*, 2007).

The ash content ranged from 4.50 to 4.62%, without a significant difference ($p>0.05$) between treatments (Table 2). Ash contents of 1.00–1.05 and 0.70–0.95% were reported for yacare meat by *Romanelli et al.* (2002) and *Vicente Neto et al.* (2007), respectively. The ash content is equivalent to the mineral material present in the product and is influenced by the raw material (*Oliveira Filho et al.*, 2012). Thus, the high ash contents of the sausages (Table 2) could be explained by the addition of condiments and salts with inorganic residues (*Nascimento et al.*, 2007; *Cavenaghi-Altemio et al.*, 2013), which did not vary for the different formulations. In accordance with this, literature reports ash contents of 3.92 and 8.21% for unsmoked and hot smoked sausages, respectively (*Fernandes et al.*, 2013).

Carbohydrates were obtained by difference, and amounted to 0.58, 2.65, and 8.51% for T1, T2, and T3, respectively. The contribution of carbohydrates from the yacare tissue is minimum (0.07%) (*Fernandes et al.*, 2015). Thus, variations in carbohydrate contents are also related to the sausage composition, so T1 contained the lowest amount of carbohydrate, which was likely due to it having the lowest inulin content (1%), while more carbohydrate was found in T2 (2% inulin) and T3 (3% inulin), explained by the addition of greater amounts of oligosaccharide inulin, *i.e.*, the more inulin added, the greater the carbohydrate content.

Water activity and pH

The water activity was 0.960 for T1, 0.951 for T2, and 0.954 for T3, so there was a significant difference between all treatments ($p<0.05$) (Table 3). The water activity results confirmed that cooked sausages are considered high water activity foods,

Table 3. Water activity, pH, instrumental color, and shear force of the cooked yacare (*Caiman yacare*) meat sausages

Determination	T1	T2	T3
Water activity	$0.960^{a} \pm 0.000$	$0.951^{b} \pm 0.000$	$0.954^{c} \pm 0.001$
pH	5.61a ± 0.02	5.53a ± 0.09	5.60a ± 0.04
L*	58.52a.b ±1.05	59.69a ± 1.28	57.24b ± 1.37
a*	$6.08^{a} \pm 1.34$	$4.93^{b} \pm 1.04$	$4.93^{b} \pm 0.88$
b*	11.7^{9}a ± 1.39	$10.5^{7a} \pm 1.49$	10.2^{9}a ± 0.95
Shear force (N)	14.33b ± 1.07	15.33b ± 0.93	18.01a ± 0.63

Legend: Means with the same letter in the same row do not differ statistically at 5% (P>0.05). L*: lightness); a*: redness, b*: yellowness. Treatments (T1, T2, and T3) according to Table 1.

favoring the growth of microorganisms (*de Alcantara et al.*, 2012). However, there are conditions that guarantee the safety of these products such as the addition of preservatives, *e.g.* sodium nitrite, and storage under refrigeration temperature.

pH values ranged between 5.53 to 5.61, with no significant difference (p>0.05) between treatments (Table 3). These pH values were similar to those obtained for yacare meat, which reached pH values of 5.5–5.7 at 36–48 h after slaughter (*Taboga et al.*, 2003; *Vicente Neto et al.*, 2007).

Physical analysis

Instrumental color

Instrumental color was determined for the parameters luminosity (L*), chroma a*, and chroma b* (Table 3). The highest L* value was obtained for T2 (59.69) and the lowest for T3 (57.24). These treatments differed from each other (p<0.05). However, neither T2 nor T3 differed from T1 (p>0.05).

Rodrigues et al. (2007) reported L* values for yacare meat ranging from 54.01 to 56.02. The obtained values were slightly superior to these values (Table 3). However, it must be considered that other ingredients contribute not only to the luminosity but all color parameters. Another aspect that must be considered is that small differences in the size of the meat fragments used to elaborate the sausages could have occurred due to manual homogenization of the meat batter. In relation to inulin, it has luminous characteristics close to those of fat, which means inulin's ability to reflect light is similar to that of fat (*Menegas et al.*, 2013). Therefore, inulin should not affect drastically the luminosity when it is used as a fat replacer (*Menegas et al.*, 2013).

Low values of chroma a* (redness) ranging from 4.93 to 6.08 were obtained for the three treatments, without significant difference between them (p>0.05) demonstrating that the sausages had a light color (Table 3) because the higher the value of chroma a*, the redder the color of the meat evaluated (*Trindade et al. 2005*). Regarding chroma b* (yellowness), the values ranged from 10.29 (T3) to 11.79 (T1). However, there were significant differences observed between the treatments (p> 0.05) (Table 3).

Shear force

The instrumental texture was evaluated in terms of shear force (Table 3). Results showed that the highest average shear force (18.01 N) was obtained for T3 (3% inulin), differing (p> 0.05) from T1 (14.33 N) and T2 (15.33 N), the values of which did not differ from each other (p>0.05).

Thus, the increase in the inulin content in the T1-T3 series positively influenced the shear force. This fact could be related to the gel strength of inulin, as it depends mainly on inulin concentration (*Mensink et al.*, 2015). This same tendency of increasing hardness by replacing fat with different concentrations of fiber was observed in other sausage meat products (*Selgas et al.*, 2005).

Microbiological analysis

Microbiological evaluations of the cooked yacare (*Caiman yacare*) meat sausages for coliforms at 45ºC, coagulase positive Staphylococci (CPS) and *Salmonella* sp. were carried out in order to confirm the microbiological safety of the sausages consumed by panelists during sensory analysis. The results of these determinations are shown in Table 4. Results showed that all sausages met the standards of Brazilian legislation for meat sausages (*ANVISA*, 2001). Thus, sensory analysis was performed for sensory attributes, acceptability indexes and purchase intention.

Sensory analysis

The means and standard deviations for the sensory attributes of appearance aroma, color, taste, texture, and overall impression of the cooked sausages by the acceptance tests are expressed in Table 5.

Table 4. Microbiological analyses of the cooked yacare (*Caiman yacare*) meat sausages

Microbiological analyses	T1	T2	T3
Coliforms at 45ºC	< 1.0^0×102 CFU/g	< 1.0^0×102 CFU/g	< 1.0^0×102 CFU/g
CPS	< 1.0^0×103 CFU/g	< 1.0^0×103 CFU/g	< 1.0^0×103 CFU/g
Salmonella sp.	Absence in 25 g	Absence in 25 g	Absence in 25 g

Legend: CFU: colony forming units; CPS: coagulase positive *Staphylococcus*. Treatments (T1, T2, and T3) according to Table 1.

Table 5. Sensory analysis of the cooked yacare (*Caiman yacare*) meat sausages

Attribute	T1	T2	T3
Appearance	6.30[a] ± 1.84 (70.00)	6.36[a] ± 1.71 (70.67)	6.60[a] ± 1.82 (73.33)
Aroma	7.52[a] ± 1.23 (83.56)	7.32[a] ± 1.38 (81.33)	7.08[a] ± 1.47 (78.67)
Color	6.18[a] ± 1.90 (68.67)	6.20[a] ± 1.90 (68.89)	6.60[a] ± 1.71 (73.33)
Taste	7.84[a] ± 1.11 (87.11)	7.52[a,b] ± 1.39 (83.56	7.06b ± 1.95 (78.44)
Texture	7.08[a] ± 1.50 (78.67)	7.04[a] ± 1.38 (83.56)	6.44[a] ± 1.41 (78.44)
Overall impression	7.28[a] ± 1.37 (80.89)	6.98[a] ± 1.36 (77.56)	6.84[a] ± 1.87 (76.00)

Legend: Means with the same letter in the same row do not differ statistically at 5% (P>0.05). Values in parenthesis are the acceptability index (%). Treatments (T1, T2, and T3) according to Table 1.

The average scores for the appearance and color ranged from "I did not like it" to "I liked it moderately" in all treatments. The same was observed for the texture of T3 and the overall impression of T2 and T3. For the aroma and flavor, the average scores ranged from "I liked it moderately" to "I liked it very much" in all treatments. The same was observed for the texture of T2 and T3 and for the overall impression of T1 (Table 5).

The increase in shear force for T3 (3% inulin) was not noticed by the panelists in the acceptance test for the texture attribute, as there was no significant difference (p>0.05) between treatments for this attribute (Table 5). The properties of inulin related to the texture could be improved by adding other ingredients such as gums in the formulations until the desired effect on the product is achieved (*da Silveira et al.*, 2015).

Similar results were reported for different yacare sausage treatments (*Fernandes et al.*, 2013). However, that study revealed significant decreases in some sensory attributes of hot smoked sausages due to excessive dehydration and the highest salt concentration (*Fernandes et al.*, 2013).

The acceptability indexes ranged from 70.00 to 87.11% across all the sensory attributes, except for the color of T1 and T2, which attained 68.67 and 68.89%, respectively (Table 5). When the acceptability index is equal to or greater than 70%, the product is considered accepted (*Stone & Sidel*, 2004).

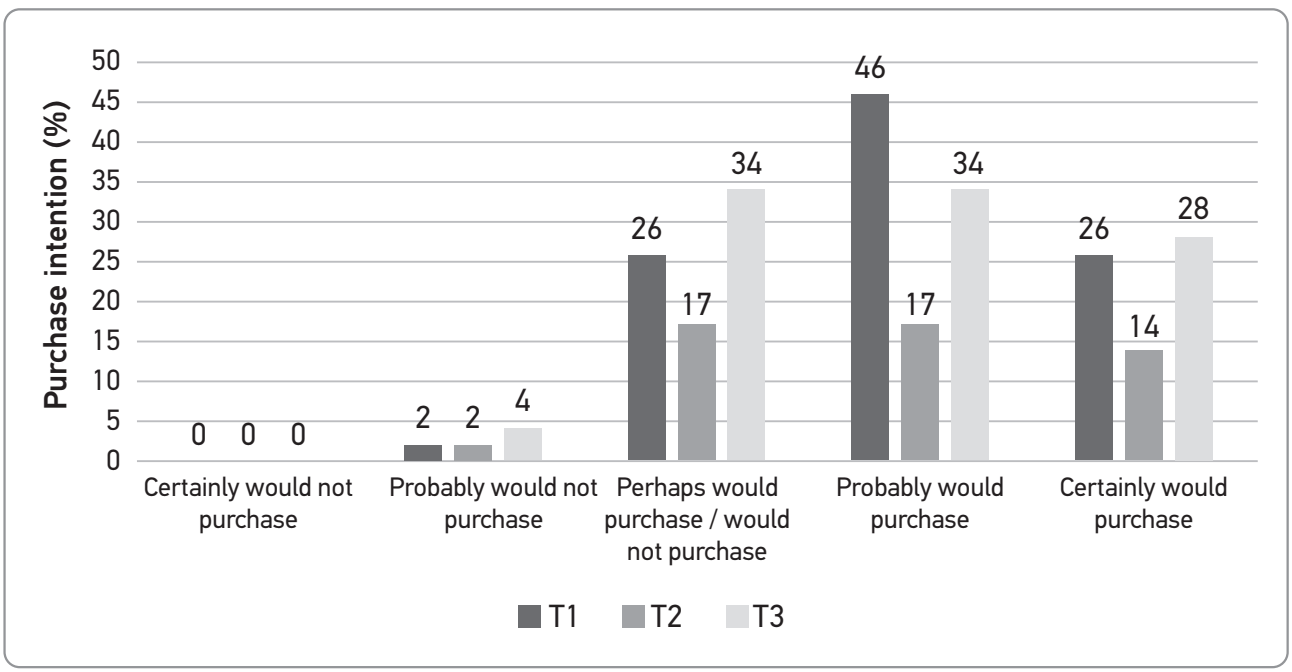

Figure 2. Panelists' purchase intentions for cooked yacare (*Caiman yacare*) meat sausages. Treatments (T1, T2, and T3) according to Table 1

Thus, T1 and T2 would not be accepted in relation to color. In comparison, an average acceptance of 78% for a smoked product prepared with yacare meat was reported, which is between the hedonic terms "I liked it moderately" and "I liked it a lot" (*Romanelli et al.*, 2002), similar to the results obtained in the current study.

Figure 2 shows the panelists' purchase intentions with regard to the cooked sausages prepared with yacare meat shavings. The summed purchase intentions "certainly and probably would buy" were 72% (T1), 62% (T2), and 64% (T3). Therefore, panelists indicated they were more likely to buy products with lower inulin content in relation to the other treatments. Other authors have achieved good results in the overall acceptability of meat products made with higher concentrations of inulin, *e.g.* 4% inulin in beef sausages (*Devereux et al.*, 2003) and up to 7.5% inulin in bologna sausages with conventional and reduced fat content (*García et al.*, 2006).

Conclusions

Cooked sausages were successfully developed from yacare (*Caiman yacare*) meat shavings through three different treatments involving the addition of inulin as a fat replacer. All treatments presented low levels of lipids and high protein contents. T1 (1% inulin) had also the lowest carbohydrate content, which is desired for low-carbohydrate and low-fat diets. In testing, the products were considered microbiologically safe. Thus, sensory analyses indicated good sensory characteristics for all treatments. However, the purchase intention revealed the highest value of 72% of panelists certainly or probably would like to purchase T1.

Razvoj i karakterizacija nisko-masnog kuvanog punjenja od mesnih strugotina poreklom od kajmana (*Caiman iacare*)

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A p s t r a k t: Ograničena potrošnja mesa kajmana (Caiman iacare) je posledica kulturnih i ekonomskih faktora, kao i ograničene dostupnosti proizvoda na bazi ovog mesa. U ovom istraživanju je razvijeno i okarakterisano/ocenjeno kuvano punjenje (poput kobasica) od strugotina mesa kajmana i zamene masti (inulin i sojini proteini). Sadržaj vlage se kretao od 63,90% (T1) do 59,89% (T3), što ukazuje na smanjenje sa povećanjem sadržaja inulina. Sadržaj proteina je pao sa 27,67 (T1) na 25,32% (T3). Najviši sadržaj lipida bio je 5,36% (T2), a najniži 1,69% (T3). Sadržaj pepela se kretao od 4,50 do 4,62%. Najviša vrednost osvetljenosti utvrđena je kod T2 (59,69), a najniža za T3 (57,24). Najviši prosek za silu presecanja (18,01 N) dobijen je za T3. Dobijene su dobre senzorne karakteristike kod svih tretmana, sa indeksima prihvatljivosti od 68,67 do 87,11%. Međutim, najizraženija namera kupovine od 72% ispitanika koji bi sigurno želeli da kupe utvrđena je za T1.

Ključne reči: *meso kajmana, kobasica, inulin, sojini protein.*

Disclosure statement: No potential conflict of interest was reported by authors.

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Paper received: October 11th 2021.
Paper accepted: October 19th 2021.

UDK: 637.5.06:636.4]:595.132(497.11)"2017/2021"
616.995.132-036.22(497.11)"2017/2021"
ID: 53263113
https://doi.org/10.18485/meattech.2021.62.2.7

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

Original Scientific Paper

Serbian external quality assessment for *Trichinella* detection in meat in 2021 compared to 2017

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A b s t r a c t: In 2021, the Serbian National Reference Laboratory for Trichinellosis, Serbian Institute for the Application of Nuclear Energy, organized external quality assessment (EQA 2021) for the detection of Trichinella larvae presence in meat by the magnetic stirrer method (MSM). The aims of this study were to examine the performance of the accredited laboratories over time and to compare the participants' performance. EQA 2021 was organized according to ISO 17043, and the test panel consisted of three meat balls, two of which were spiked with four Trichinella spiralis L1 larvae. Evaluation of the qualitative results showed that 90.91% (100% in 2017) of participants successfully passed the EQA. Quantitative evaluation showed that, on average, 71.59% (in 2017 only 60%) of the spiked Trichinella larvae were detected. This study enabled comparisons of laboratories over time (2017 and 2021) and across the country. The results obtained should serve as motivation for improvement of laboratory performance. All official laboratories with accredited MSM for Trichinella detection should participate in an EQA every second year and all other laboratories that perform Trichinella testing should participate annually in EQAs organized at national level. Regular participation will bring improvement in sensitivity of the test method used and will promote the important one health concept.

Keywords: *Trichinella, external quality assessment 2021, meat.*

Introduction

Zoonotic foodborne parasites are important human health hazards. One of these pathogens is the helminth *Trichinella* (*Pozio,* 2020). Parasites of the genus *Trichinella* are widespread across the world, and domestic and wild animals can be infected (*Pozio and Zarlenga,* 2013*; Noeckler et al.,* 2019). Humans are infected after consumption of meat containing viable *Trichinella* larvae (*Pozio et al.,* 2003*; Mayer-Scholl et al.,* 2017*; Noeckler et al.,* 2019).

Examination of susceptible animals, especially pigs, is the main method for preventing trichinellosis in humans. In order to protect the health of European Union meat consumers, the Commission Regulation (EU) No. 2015/1375 (*European Union,* 2015) proclaims the rules for control of *Trichinella* in meat, and the magnetic stirrer method (MSM) for pooled sample digestion is stated as a reference method (*Mayer-Scholl et al.,* 2017). All the laboratories that perform official controls for *Trichinella* presence in meat need to participate regularly in comparative inter-laboratory or proficiency testing (PT) (*European Union*, 2017).

In EU member states, the National Reference Laboratories for *Trichinella* provide PTs to laboratories in order to evaluate the quality, competence and performance of the laboratory tests (*Marucci et al.,* 2009*; Riehn et al.,* 2013*; Marucci et al.,* 2016). The Serbian National Reference Laboratory for Trichinellosis organized successfully in 2017 the first Serbian external quality assessment (EQA) for the detection of *Trichinella* larvae in meat for accredited laboratories only (*Vasilev et al.,* 2019). Bearing in mind that Serbia is in the process of harmonizing its regulations with the EU, the regulations regarding the EQA should also agree with EU regulations. Therefore, all official, accredited laboratories for *Trichinella* detection need to participate in national PTs with the valuable aims of comparing their own performance with others and of improving their competence. As the next step towards these goals, the *Trichinella* EQA 2021 was recently conducted.

Materials and methods

Animals and parasite. Trichinella spiralis (strain *ISS 161*) was maintained at the Institute for the Application of Nuclear Energy (INEP), and the animal use complied with national regulations and institutional policies. Use of animals was approved by the Veterinary

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Directorate (Consent No 323-07-00758/2021-05 issued on 05.02.2021).

EQA. As described in detail in *Vasilev et al.* (2019), the EQA for detecting the presence of *Trichinella* larvae in meat was organized according to ISO 17043 (*ISO/IEC 17043*, 2010**)**. The *Trichinella* EQA 2021 was announced to accredited laboratories early in 2021. We offered the laboratories the opportunity to assess their own performance through a confidential system of testing the samples and to determine their ability to perform the analytical procedure. The laboratory's aim within EQA 2021 was to correctly identify each sample in a test panel as *Trichinella*-positive or -negative. The EQA 2021 test panel consisted of three samples with identical numbers of viable *Trichinella* larvae for the participants. The procedure for preparation of EQA samples was described by *Marucci et al*. (2016) and *Vasilev et al.* (2019). Briefly, the test panel consisted of three meat balls, two of which were spiked with four viable *Trichinella spiralis* L1 larvae, while one meatball was not spiked (negative control). Larvae were obtained by digestion of *T. spiralis* infected rat carcasses. Larvae were counted under a stereo-microscope and transferred individually to each meat ball. The glass was examined twice under the stereo-microscope to ensure that no larvae remained untransferred. Every single meat ball was enclosed in a bag and sealed under vacuum. For each participant laboratory, one coded envelope containing the three meat balls was then stored in a refrigerator until forwarding. EQA 2021 packages were forwarded the same day by courier in a thermo box containing ice packs with the aim to maintain a temperature of 4–15°C during the transport. The ice packs were separated from the meat balls by separators to avoid direct contact. All participating laboratories were invited to fill in forms to provide information about the package content and its condition at the moment of opening.

To check the test panel stability over time, one EQA test panel was stored at room temperature and tested by staff at INEP two days later.

The MSM for pooled sample digestion was used by all participants to analyse the EQA 2021 test panel of three meat balls (*European Union*, 2015).

Criteria for the result evaluation. According to European regulation (*European Union*, 2015), *Trichinella* evaluation is qualitative, so the task was to identify the samples in EQA 2021 as positive if *Trichinella* larvae were present or negative if larvae were not found. Final evaluation for the participant

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EPK je organizovan u skladu sa zahtevima standarda ISO 17043

IZVEŠTAJ *TRICHINELLA* EPK 2021

Ukupni rezultati učešća u eksternoj proceni kvaliteta (EPK) dijagnostike prisustva larvi *Trichinella spiralis* u mesu primenom metode magnetne mešalice

Dizajn

Svrha	Provera kvaliteta rada laboratorija koja pregledaju meso na prisustvo larvi Trichinella	
Tip PT šeme	Pojedinacno, istovremeno vise učesnika	
Učesnici	Laboratorije	
Broj učesnika	Zavisi od broja zahteva	
Metod	Metod veštačke digestije pomoću magnetne mešalice	
EPK uzorak	Matriks	Svinjsko meso
	Test materijal	*Trichinella* žive larve
	Broj uzoraka	Tri za svakog učesnika
	Distribucija	Odmah posle pripreme
Evaluacija rezultata	Kvalitativna	

Realizacija

Broj učesnika	11	EPK uzorci	Svinjsko meso (100 grama svaki)	Ukupan broj distribuiranih uzoraka 36
NIV	2		Sastav EPK panela	2 pozitivna sa po 4 larve, 1 negativan
VSI	8			
Druge laboratorije	1		Sastav dodatnog EPK panela	2 pozitivna sa po 4 i 5 larvi, 1 negativan
			Kurir	Post expres
Datum slanja		06.04.2021.		
Datum slanja dodatnog panela		01.06.2021.		

Organizator EPK
Nacionalna referentna laboratorija za trihinelozu
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EPK je organizovan u skladu sa zahtevima standarda ISO 17043

Analiza rezultata

U skladu sa direktivama EU predviđeno je da se rezultati veštačke digestije iskazuju samo kvalitativno, tj kao pozitivan ako su larve *Trichinella* prisutne u uzorku ili negativan ako larvi nema u uzorku. Za uspešan rezultat učešća potrebno je da svi pozitivni i negativni uzorci budu ispravno određeni.

Oznaka laboratorije	Broj uzoraka ispravno identifikovanih	Broj uzoraka koji NISU ispravno identifikovani	Rezultat učešća
1	3	0	USPEŠNO
2	3	0	USPEŠNO
3	3	0	USPEŠNO
4	3	0	USPEŠNO
5	3	0	USPEŠNO
6	1	2	NEUSPEŠNO
7	3	0	USPEŠNO
8	3	0	USPEŠNO
9	3	0	USPEŠNO
10	3	0	USPEŠNO
11	3	0	USPEŠNO
6*	3	0	USPEŠNO

*Ponavljanje testa posle sprovedenih odgovarajućih korektivnih mera

Rezultati zbirno

Broj laboratorija koje su učestvovale	11 +1 *Ponavljanje testa
Broj učesnika koji su uspešno prošli EPK	10 +1 *Ponavljanje testa
Broj učesnika koji nisu prošli EPK	1 + 0 *Ponavljanje testa

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Figure 1. Example of final evaluation report supplied to laboratories participating in *Trichinella* EQA 2021.

was positive if all samples in EQA 2021 test panel were properly identified.

Quantitative evaluation, with the number of spiked and recovered L1 larvae for all samples, was also conducted. To compare the results between the EQA 2021 participants, the number of recovered larvae was presented as a percentage of spiked number of larvae in the sample (detected/spiked × 100).

EQA report. Each participant laboratory was provided with an EQA report. The overall summary of participant performance in *Trichinella* EQA 2021 contained only anonymized laboratory codes to guarantee confidentiality (Figure 1).

Results and discussion

Participation in *Trichinella* EQA 2021 remained on a voluntary basis. A total of 11 (from 13) accredited laboratories that cover the epizootiological area across the country (two Scientific Veterinary Institutes, located in Novi Sad and Belgrade, the Laboratory of the Food Testing Center (Centar za ispitivanje namirnica — CIN) and eight Veterinary Specialist Institutes located in the cities of Subotica, Pozarevac, Zajecar, Jagodina, Nis, Sombor, Kraljevo, Zrenjanin) agreed to participate (Figure 2). All the packages (11) with the EQA samples (33)

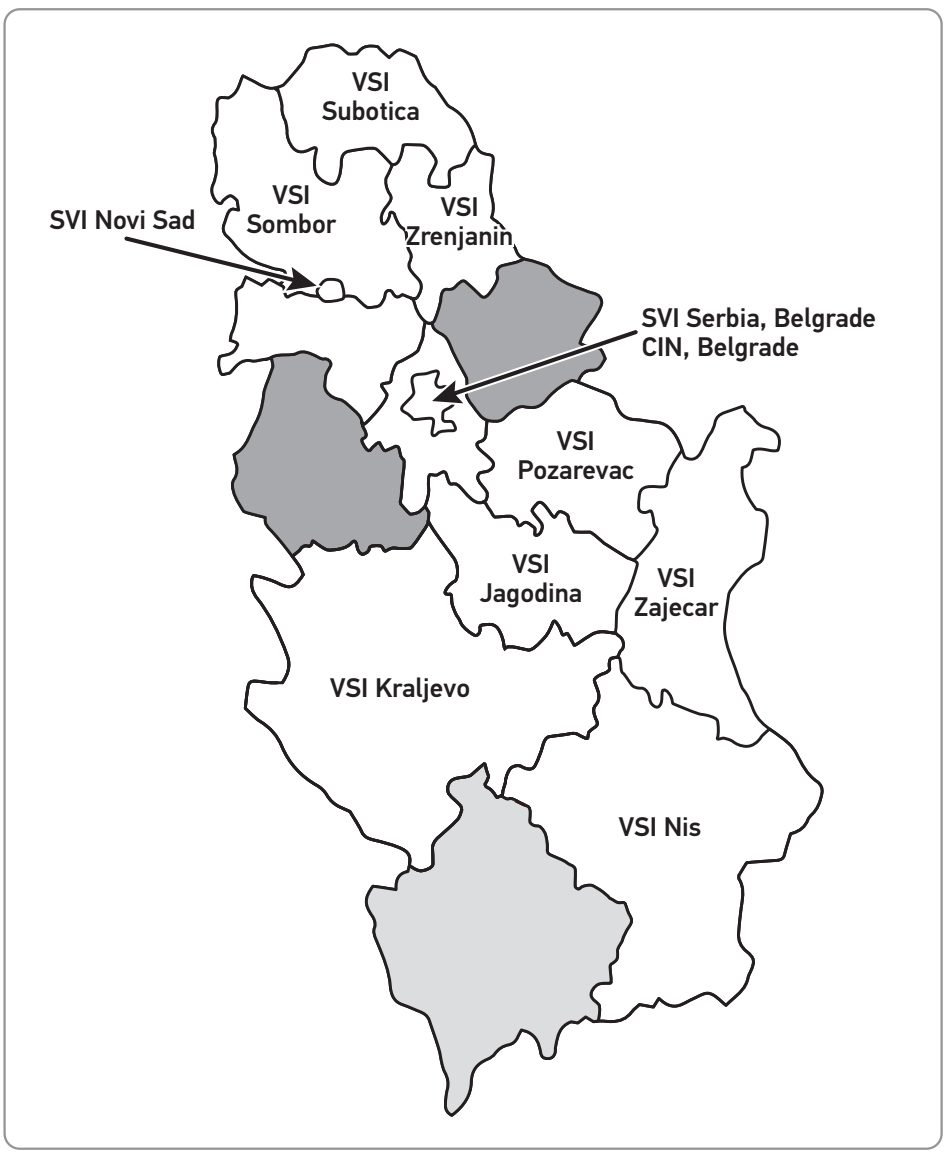

Figure 2. Epizootiological area in Serbia covered by participants in the EQA 2021 for *Trichinella* larvae detection

Legend: White – Epizootiological area covered by the Veterinary Specialist Institutes (VSIs) that participated in EQA 2021 (the name of the institute corresponds to the name of the city in which it is located). Dark grey – epizootiological area covered by a VSI that did not participate in EQA 2021. Light grey – Kosovo; no data. Note: The Scientific Veterinary Institute (SVI) Serbia, located in Belgrade, is a scientific institution that covers the whole territory of Serbia, while SVI Novi Sad, located in Novi Sad, is a scientific institution that covers the territory of Vojvodina province.

were delivered within 24 h to the participating laboratories. At the time of delivery, the internal temperature of all the packages was less than 15°C. Each participating laboratory performed controls on the package's content within 1 h of package arrival.

Qualitative results. One laboratory (with one false negative and one false positive result) did not pass the *Trichinella* EQA 2021. Ten of 11 (90.91%) participating laboratories passed the *Trichinella* EQA 2021 (Table 1). Analysis of the data obtained from the first two Serbian *Trichinella* EQAs, conducted in 2017 and the current one in 2021, showed that the ability of the participants to classify test samples as true-positive or true-negative was satisfactory (Table 2). The specificity of the test method depends on the analyst's skills, and the results were satisfactory except for one accredited laboratory. The overall rate of correct analysis by the participants in 2017 was 100%, but in 2021, it was 93.94%. This is in agreement with the results achieved in the

Table 1. EQA 2021. Qualitative and quantitative results

Lab code	Number of larvae		Difference	Results	Final Evaluation
	spiked	found			
1	0	0	0	positive	
	4	3	1	positive	**positive**
	4	3	1	positive	
2	0	0	0	positive	
	4	3	3	positive	**positive**
	4	4	1	positive	
3	0	0	0	positive	
	4	3	1	positive	**positive**
	4	2	2	positive	
4	0	0	0	positive	
	4	4	0	positive	**positive**
	4	3	1	positive	
5	0	0	0	positive	
	4	4	0	positive	**positive**
	4	4	0	positive	
6	0	3	3	negative	
	4	0	4	negative	**negative**
	4	3	1	positive	
7	0	0	0	positive	
	4	2	2	positive	**positive**
	4	2	2	positive	
8	0	0	0	positive	
	4	3	1	positive	**positive**
	4	3	1	positive	
9	0	0	0	positive	
	4	3	1	positive	**positive**
	4	1	3	positive	
10	0	0	0	positive	
	4	1	3	positive	**positive**
	4	3	1	positive	
11	0	0	0	positive	
	4	3	1	positive	**positive**
	4	3	1	positive	
6*	0	0	0	positive	
	5	3	2	positive	**positive**
	4	3	1	positive	

*Repetition of the EQA after in-house analysis and applied corrective measures

Table 2. Comparison of participation in EQA 2017 and EQA 2021

Laboratory code	EQA 2017	EQA 2021
1	positive	positive
2	positive	positive
3	positive	positive
4	positive	positive
5	positive	positive
6	positive	negative
		positive*
7	positive	positive
8	positive	positive
9		positive
10		positive
11		positive

*Repetition of the EQA after in-house analysis and applied corrective measures

PTs organized by the European Union Reference Laboratory for Parasites (EURLP) for the period 2007–2021. The percentage of EURLP participants which passed the PT successfully for the mentioned period varied between 83.3% and 100% (*Marucci et al., 2016; EURLP web site*).

Quantitative results. In the *Trichinella* EQA 2021, one of the participant laboratories successfully detected all of the spiked L1 larvae. Figure 3 presents the percentage of larvae detected in the first two Serbian EQAs. Three laboratories were very successful (laboratories 2, 4 and 5) since the level of L1 larvae detection was 87.5 %, 87.5% and 100 % respectively. For laboratories 1, 8 and 11, the level of L1 larvae detection was 75%. Laboratories 7, 9 and 10 successfully detected 50% of the L1 larvae, while laboratory 6 detected 37.5% of the L1 larvae spiked into the meat balls, with one false negative and one false positive result, resulting in this laboratory failing the EQA. Interestingly, among the three laboratories that detected 50% of the spiked L1 larvae, there were clear differences in the number of larvae recovered from the individual meat ball samples. Laboratory 7 detected 50% of the larvae in

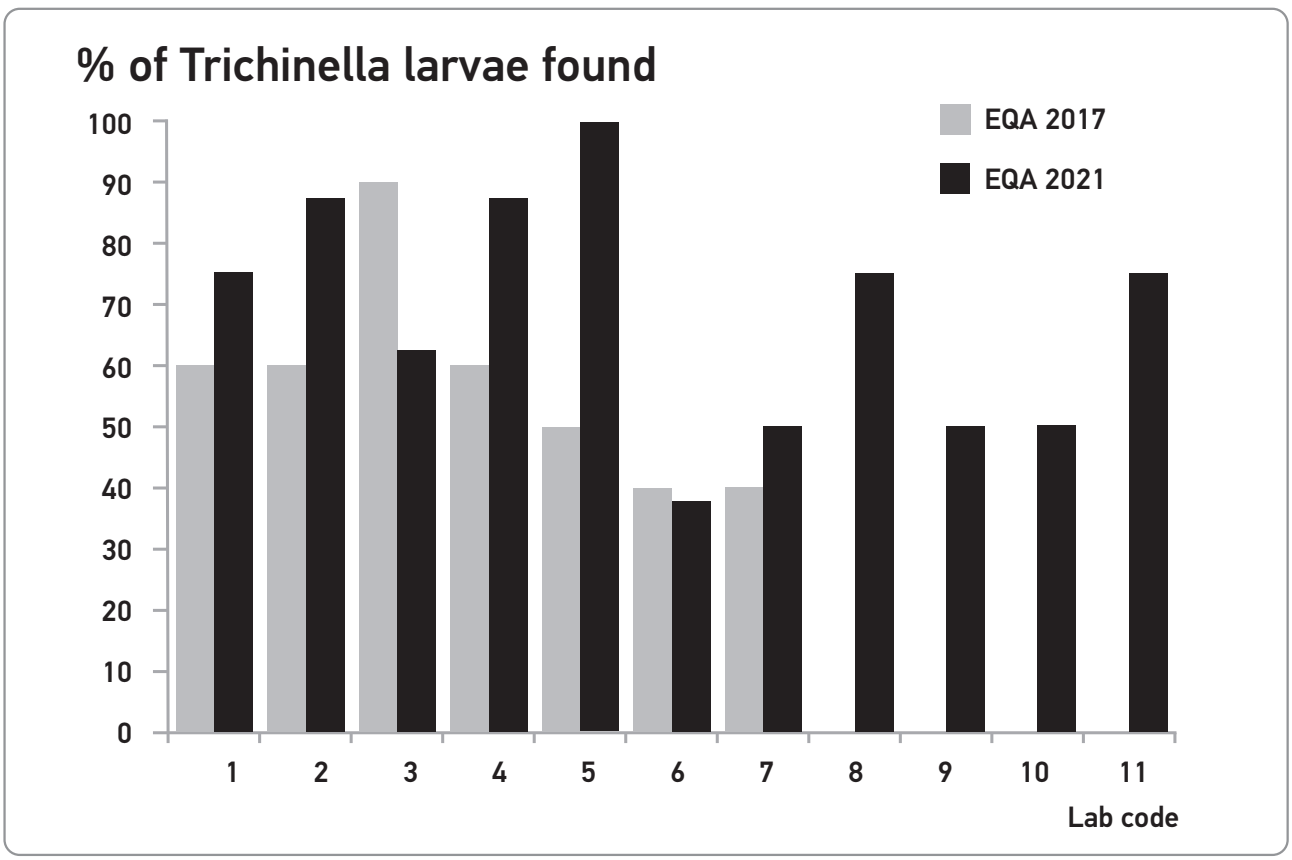

Figure 3. Quantitative results for the participating laboratories in *Trichinella* EQA 2017 and EQA 2021 in Serbia. Values are expressed as percentage of larvae detected in relation to the total number of larvae spiked per meat panel. One meat panel containing multiple samples was examined per year.

each sample, while laboratories 9 and 10 detected 75% of the larvae in one sample and 25% from another sample. Therefore, laboratories 9 and 10 were close to obtaining a false negative result (in one sample they detected only one larva from the four that were spiked). *Marucci et al*. (2016) showed that for the quantitative evaluation of samples containing less than six larvae, the Z-score should not be used. According to that, results with at least two larvae recovered should be acceptable for test samples with 4–5 larvae. If this criterion was applied to our *Trichinella* EQA 2021, laboratory 7 would meet the requirements (by finding two larvae of the four spiked, 50%), while laboratories 9 and 10 would not.

The finding of only one larva in samples with 4–5 L1 larvae initiates revision of laboratory standard operation procedure and monitoring of both the critical control points and the performance of the analyst. Comparing EQA 2017 with EQA 2021 indicated the performance of the laboratories is now worse than before, because in 2017, one laboratory had to apply in-house analysis after the EQA, but in 2021, three laboratories had to analyse their participation (one failed the EQA, and two found only one of four larvae in one sample). After in-house analyses and checking their critical control points, laboratory 6 requested a second EQA test panel. This panel consisted of two positive samples (meat balls with four and five larvae spiked) and one negative sample. Evaluation of results showed that this laboratory did then pass the requested test, with all three samples correctly identified and 75% of the larvae recovered.

The overall success in MSM performance on average was 60% in 2017, but in 2021 it was improved, with 71.59% of the larvae recovered. Quantitative results obtained in Serbian EQAs for *Trichinella* larvae detection (in 2017, 40–90% and in 2021, 50–100% depending on the participating laboratory) were similar to results from EU PTs (*Marucci et al.,* 2009*; Marucci et al.,* 2016). The current results are also similar to results from German PTs when laboratories found 60% of the L1 larvae (*Riehn et al.,* 2013). *Marucci et al.* (2016) and *Riehn et al.* (2013) showed that participating in PTs year by year improves laboratory performance (it is considered as good performance if more than 80% of larvae are recovered, and there are no false negative or false positive results). In relation to this, the results obtained by EQA 2021 should be considered as improvement of the MSM performance over time in the participating laboratories. The goal that laboratories should try to achieve could be set to 50% or more larvae recovery from each individual sample. It is important to keep in mind the critical control points for the MSM are a way to improve laboratory performance (*Djordjevic et al.,* 2013*; Mayer-Scholl et al.,* 2017*; ICT guidelines*). Detection of fewer L1 larvae than were spiked could be explained by errors related to critical control points (in each step 2–3% of the larvae are lost) (*Riehn et al.,* 2013).

Before our *Trichinella* EQA, some Veterinary Specialist Institutes participated in PTs organized by different EU providers. For laboratories in Serbia, these PTs have many disadvantages, especially long transport, very high prices and inability to compare performances (personal communication). We participated in 11 PTs organized by EURLP from 2009 to 2021 and successfully passed them all (even when samples were spiked with 1 larva). The National Reference Laboratory for Trichinellosis in INEP has been a member of the EURLP Network from 2008. In *Trichinella* EQA 2021, the criterion for positive evaluation of participants was the correct detection of *Trichinella* presence or absence in the sample. The numbers of spiked larvae in EQA 2021 samples was similar to the numbers spiked by EURLP after 2015 (*Marucci et al.,* 2016). According to *Forbes et al*. (1998), in the test panel samples, one larva can be detected by artificial digestion, but a limit of three larvae in PT samples for participating routine laboratories is more appropriate. With that knowledge, we decided to spike the samples with four larvae. The results of *Trichinella* EQAs clearly confirm our capability as PT provider to conduct this very important activity, as well as the knowledge and skills of participants.

In Serbia, *T. spiralis* infected pork is usually the source of *Trichinella* infection (*Sofronic-Milosavljevic et al.,* 2013, *NRLT unpublished dat*a). In last ten years, the rate of pig infection significantly decreased from 0.02% to 0.003% (*unpublished data*) because of improvements in pig production, better control measures and public education. In Serbia, the sources of outbreaks are usually meat from untested infected backyard pigs (*Sofronic-Milosavljevic et al.,* 2013, *Vasilev et al.,* 2019) and, rarely, wild boars (*Pavic et al.,* 2020). Also, testing for *Trichinella* larvae in meat is regularly performed in laboratories of the Scientific Veterinary Institutes, Veterinary Specialist Institutes, veterinary hospitals, veterinary practices and slaughterhouses. However, the EQA in 2017 and especially the current one in 2021 (with one laboratory that failed to fulfil requirements) pointed to the need for training personnel in official laboratories for *Trichinella* detection, control of equipment used and regular participation in national PTs.

Conclusion

Ten of 11 participants successfully passed the Serbian *Trichinella* EQA 2021. These EQA results demonstrated the overall good performance of participants, but at the same time pointed to the need for training, control of equipment and regular participation in national PTs. An important feature regarding this study is that it enabled comparison of participant performance over time and also with the anonymized results of the other accredited Serbian *Trichinella* testing laboratories. The current study strongly indicates the need for all *Trichinella* laboratories in Serbia to participate annually in EQAs organized at national level. Regular participation could improve their performance, provide valuable and useful data for the Veterinary Directorate, and be of help in promoting the one health concept.

Eksterna provera kvaliteta otkrivanja prisustva larvi *trichinella* u mesu u Srbiji u 2021 i poređenje sa 2017

Saša Vasilev, Ivana Mitić, Natasa Ilić, Ljiljana Sofronić-Milosavljević

A p s t r a k t: U 2021 godini Nacionalna referentna laboratorija za trihinelozu (NRLT) je organizovala eksternu proveru kvaliteta (EPK) za otkrivanje larvi trihinela u mesu korišćenjem metode magnetne mešalice za zbirni uzorak (MMM). Cilj je bio da se omogući zainteresovanim akreditovanim laboratorijama da uporede kvalitet rada tokom vremena, ali i uporede svoj rezultat sa drugim učesnicima. EPK je organizovan prema standardu ISO 17043. Test panel poslat u 11 laboratorija se sastojao od po 3 loptice od mesa sa identičnim brojem larvi. U dva uzorka bilo je dodato po četiri živih mišićnih L1 larvi Trichinella spiralis a jedan uzorak je bio bez larvi. Procena kvalitativnih rezultata je pokazala da je 90,91% (2017 je bilo 100%) učesnika uspešno prošlo testiranje. Prosecan prinos larvi iznosio je 71,59% (dok je 2017 bilo 60%). Ovaj rad je po prvi put omogucio poredjenje rezultata laboratorija tokom vremena, ali i poređenje izmedju laboratorija. Učešće u EPK treba da posluži za poboljšanje izvodjenja metode magnetne mešalice za zbirni uzorak. Zato bi bilo poželjno da ove ali i druge laboratorije u Srbiji učestvuju u narednim EPK ili PT šemama organizovanim na nacionalnom nivou. Cilj je da ove aktivnosti dovedu do poboljšanja senzitivnosti metode, pruže korisne informacije kolegama ali i Upravi za veterinu i da budu od značaja za promociju koncepta „jedno zdravlje".

Ključne reči: *reč, Trichinella, eksterna procena kvaliteta, svinjsko meso.*

Disclosure statement: No potential conflict of interest was reported by authors.

Acknowledgement: Blagoje Milosavljevic, INEP technician, for help in laboratory work. Ministry of Education Science and Technological Development of Serbia, contract number: 451-03-9/2021-14/ 200019.

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Paper received: October 21st 2021.

Paper corrected: November 2nd 2021.

Paper accepted: November 1st 2021.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 637.56.04:639.3:597.551.214
ID: 53374729
https://doi.org/10.18485/meattech.2021.62.2.8

Original scientific paper

Quantitative deposition of nutrients in dorsal muscle, adipose tissue and liver in common carp (*Cyprinus carpio* L.) in a semi-intensive farming system

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A b s t r a c t: Carp is the dominant species grown in Serbia and makes up over 80% of the total fish production. The aims of the present study were to analyze changes of protein, lipid, ash and moisture in dorsal muscle, adipose tissue and liver in common carp additionally fed complete pellets during four months in natural carp ponds. Twenty fish from four ponds were sampled. Analysis of variance showed that protein content was the highest in dorsal muscle and adipose tissue and was the smallest in liver ($P < 0.05$). The percentage of protein was quite stable and reached a plateau value (18.42–19.49%) in dorsal muscle. Total lipid content in common carp was the highest in liver (14.79–17.24%) and smaller in dorsal muscle (1.92–5.42%) ($P<0.05$). More interested were how the fish mass increased during breeding. The proximate composition of fish tissues was expressed as absolute content by weight of each fish. Simple regression resulted in relationships between protein content (g/fish) and body weight (g) indicating strong association ($r = 0.965$). Simple regression resulted in not strong relationships between lipid content (g/fish) and body weight (g ($r = 0.784$). There was a strong relationship between moisture content (%) and lipid content (%) ($r = 0.962$). The protein content (g/fish) was strongly associated with body weight in dorsal muscle and adipose tissue since coefficients of regression were high (>0.95), as were t-tests of significance (13.69, 18.04), and in the liver there was also an association since the coefficient of regression was 0.952 and the t-test was high (11.72).

Keywords: *proximate composition, protein, fish weight, growth curve.*

Introduction

Cyprinids are by far the largest family of farmed finfish (20.4 million t or 71.1%). These are mostly produced by Asian family enterprises and consumed locally (*FAO*, 2020). Cyprinids are the most important cultivated species of fish in central-eastern Europe, contributing 75% of the production of freshwater fish (*Váradi et al.*, 2011). Carp is the dominant species grown in Serbia and makes up over 80% of the total fish production. Carp production is mostly in semi-intensive production systems based on a combination of natural and supplementary feed, cereals or complete extruded or pelleted feed. Recently, more than 50% of the carp were additionally fed by complete, primarily extruded feed (*Markovic & Poleksić*, 2011), which enabled more intensive carp production and the development of aquaculture in Serbia. With an increase in the fish weight come increases in the levels of body components such as moisture, protein, phospholipids, triglycerides, nucleic acids etc. (*Bureau et al.*, 2000; *Dumas et al.*, 2010) since fish use these as building blocks and energy sources for maintenance of life processes. Specific growth rate is a widely accepted model in aquaculture in spite of its disadvantages, and is based on determining the natural logarithm of the increase in the total weight of fish over a certain period of time. The main disadvantage of this model is that the growth rate varies with the size of the fish and also with the ambient temperature, which often leads to underestimation of weight gain (*Bureau et al.*, 2000; *Dumas et al.*, 2010). Daily growth coefficient (DGC), which is the cube root of the weight increase of fish within a certain time, better describes fish growth under optimal conditions. The best way to discover the relationship between the processes that were taking place in the body of the fish depending on the change in mass is a graphic representation of a variable size versus body mass using the equation $Y = aX^b$, where Y is the variable that needs to be determined, X is body weight, *a* and *b* are empirical constants derived from regression.

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Exponent *b* is of particular importance because it provides a scaling relationship between the size such as metabolism and body weight. This mathematical technique is called allometric analysis (*Gayon,* 2000) and is widely used to describe the rate at which changes are taking place in various tissues of animals as well as in fish (*Shearer,* 1994; *Azevedo et al.,* 1998; *Lupatsch et al.,* 1998; *Bureau et al.,* 2000; *Dumas et al.,* 2007). Common carp has been studied in basic as well as applied research (*Vandeputte et al.,* 2008; *Turkowski & Lirski,* 2010).

The aims of the present study were to analyze changes of protein, lipid, ash and moisture in dorsal muscle, adipose tissue and liver in common carp additionally fed complete pellets during four months in natural carp ponds rather than fully controlled fish tanks to make results directly applicable to real world aquaculture.

Since in the literature there is not enough data to explain how diet composition influences the retention of protein, lipid and ash in several tissues of farm-raised two year old common carp, this study was designed assuming that dietary supply is tissue specific to make results applicable to aquaculture.

Materials and methods

Samples

The study was carried out in four earthen ponds at the Despotovo fish farm (near the city of Backa Palanka, Vojvodina province, decimal degree 45.44 19.54) from July to October. Four fish ponds (J1, J3, J4 and J6) were used with surface area of 100 ha, 25 ha, 25 ha and 16 ha, respectively. The depth was the same in ponds, around 1.5 m. All ponds were stocked with 550 specimens of two-year-old common carp ha^{-1} in June, with an average weight of 750 g. Fish were fed with standard supplemental pelleted feed containing a mixture of soybean, maize, wheat with 25% protein (plant origin) and 7% lipid, at a feeding rate of 2.5% of total fish body mass. Fish were collected monthly from ponds. Twenty fish from four ponds were sampled; each fish was weighed and measured, then the dorsal muscle, adipose muscle and liver were separated and frozen immediately. Samples were kept at –20°C until analysis.

Proximate chemical analysis of fish

Analysis of moisture (ISO 1442:1997), lipid (ISO 1443:1973) and ash (ISO 936:1998) were performed according to standard ISO methods. Protein (Kjeldahl nitrogen) was analyzed by using a semi-automatic distillation unit (Kjeltec Auto 1030 Analyzer), with block-digestion apparatus (Digestion System 20, Tecator, Höganäs, Sweden) according to the manufacturer's instructions (Tecator Manual Rev. 2.2).

Statistical analysis

The obtained data are reported as the mean values ± the standard deviations. Analysis of variance (ANOVA) and the Tukey-Kramer test were used to analyze the data at the level of significance of 0.05 ($P \leq 0.05$). For statistical analysis and regression analysis, XLSTAT Free version (Addinsoft, NY, USA) was used. All the allometric equations were obtained by applying linear regression analysis to the logarithmic transformation. The antilog of this expression produces the final equation: $y = aX^b$.

Results and discussion

Proximate composition (protein, moisture, total lipids and ash) in dorsal muscle, adipose tissue and liver determined in July, August, September and October are given in Table 1.

Analysis of variance showed that protein content was the highest in dorsal muscle and adipose tissue and was the smallest in liver ($P < 0.05$). The percentage of protein was quite stable and reached a plateau value (18.42–19.49%) in dorsal muscle. This profile has also been reported for other cyprinid and salmonid species (*Shearer,* 1994; *Fauconneau et al.,* 1995). In reared *Diplodus puntazzo* fillets, the protein percentage was around 18% (*Orban et al.,* 2000). Very minor protein content changes are observed when fish are fasted (*Shimeno et al.,* 1990) or fed an imbalanced diet (*Venugopal & Keshavanath,* 1984), whereas sexual maturation has been reported to strongly affect this component (*Dhawan & Toor,* 1990). Total lipids in common carp were the highest in liver and were smaller in dorsal muscle ($P < 0.05$). Total lipids were lower than in the study of *Urbánek et al.* (2010). The moisture content follows a similar picture as lipid content (*Turchini et al.,* 2004; *Lupatsch et al.,* 2008). In the current study, the moisture content was slightly lower in liver than in dorsal muscle and adipose tissue ($P < 0.05$). With respect to the two remaining components, water and lipids, water is easiest to determine, so the lipid content can be calculated by subtraction (*Hernández et al.* 2003). The

Table 1. Proximate composition (% of wet weight) of dorsal muscle, adipose tissue and liver of carp from the four carp fish farms (n=20) from July to October

Parameter	Muscle /period	July	August	September	October
Protein	Dorsal muscle	19.49±0.30^{a}	20.22±1.11^{a}	19.74±1.21^{a}	18.42±0.66^{a}
	Adipose tissue	18.61±0.48^{a}	19.25±0.63^{a}	18.88±0.19^{a}	18.11±0.33^{a}
	Liver	12.45±0.77^{b}	13.85±1.04^{b}	13.72±0.43^{b}	12.44±0.37^{b}
Moisture	Dorsal muscle	77.42±0.61^{a}	77.65±1.54^{a}	76.12±0.23^{a}	73.41±1.66^{a}
	Adipose tissue	75.77±0.37^{a}	76.04±1.67^{a}	73.48±1.27^{a}	72.62±0.68^{a}
	Liver	68.29±2.30^{b}	68.98±2.13^{b}	68.01±3.08^{b}	67.77±3.53^{a}
Total lipids	Dorsal muscle	1.92±0.56^{b}	2.84±0.87^{b}	2.93±0.96^{b}	5.42±1.10^{b}
	Adipose tissue	3.63±0.27^{b}	3.20±0.68^{b}	5.27±1.65^{b}	6.85±1.17^{ab}
	Liver	17.34±3.25^{a}	16.57±3.51^{a}	15.52±4.02^{a}	14.79±5.38^{a}
Ash	Dorsal muscle	1.25±0.13^{ab}	1.15±0.08^{a}	1.19±0.24^{a}	1.33±0.10^{a}
	Adipose tissue	1.46±0.17^{a}	1.50±0.56^{a}	1.38±0.32^{a}	1.25±0.22^{a}
	Liver	1.09±0.19^{b}	1.11±0.04^{a}	0.98±0.13^{a}	1.06±0.06^{a}

Legend: n – number of samples; a, b, c Means within the same column sharing the same letter are not significantly different ($p > 0.05$)

ash content did not statistically significantly differ between tissues ($P > 0.05$).

More revealing was how the fish mass increased during breeding. The proximate composition of fish tissues was expressed as absolute content by weight for each fish. Plots of carp body weight by sampling day of are presented in Figure 1.

Simple regression resulted in relationships between protein content (g/fish) and body weight (g) indicating strong association (Figure 2) according to *Dumas et al.* (2007) and *Bureau et al.* (2000).

Simple regression resulted in not strong relationships between lipid content (g/fish) and body weight (g) (Figure 3) according to *Dumas et al.* (2007) and *Bureau et al.* (2000). Lipids were more affected by feeding regime (*Dumas et al.*, 2007; *Bureau et al.*, 2000; *Turchini et al.*, 2004; *Cook et al.*, 2000; *Hernández et al.*, 2003).

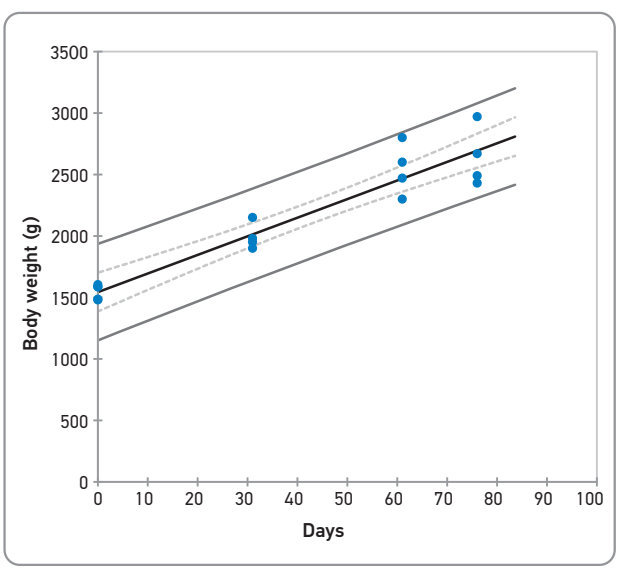

Figure 1. Growth curve of carps in four farms during rearing (r = 0.942) from July to October)

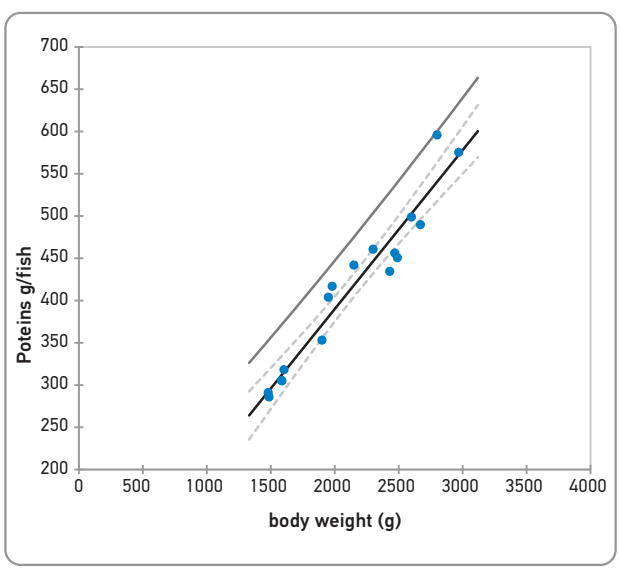

Figure 2. Quantitative deposition of protein in dorsal muscle (r = 0.965)

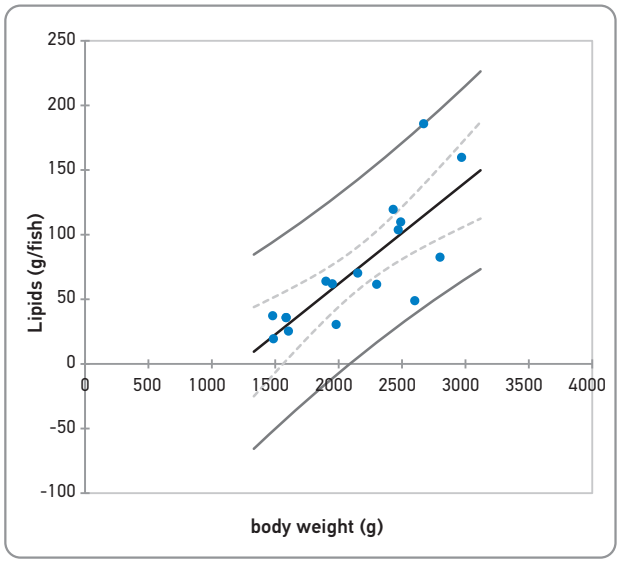

Figure 3. Quantitative deposition of lipids in dorsal tissue (r = 0.784)

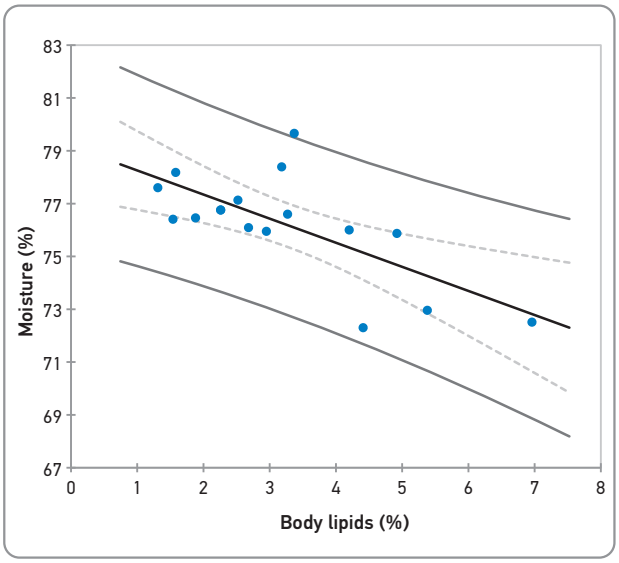

Figure 5. Relationship between relative content of lipid (%) and moisture (%) in the whole-body of common carp of dorsal muscle (r = 0.692)

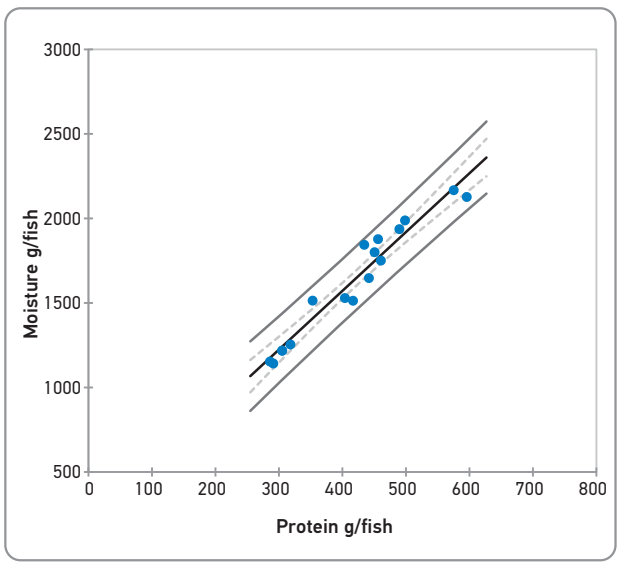

Figure 4. Relationship between moisture (g/fish) and protein (g/fish) in the whole-body of common carp of dorsal muscle (r = 0.902)

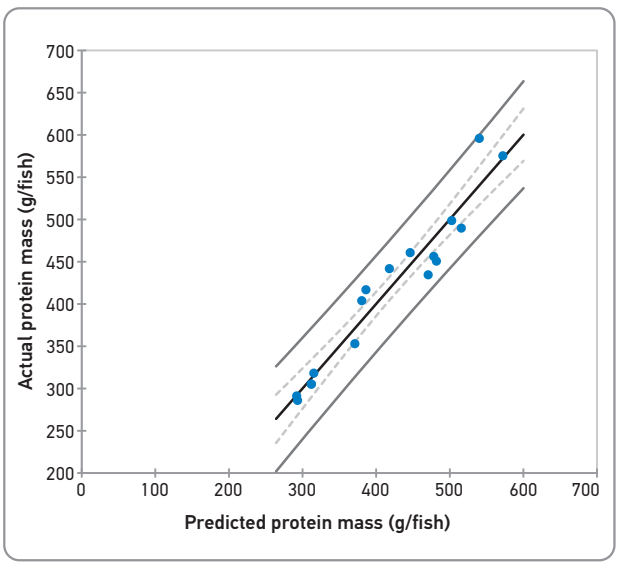

Figure 6. Experimentally obtained values of protein mass gain depending on the theoretical value of protein mass gain in the dorsal carp muscle (r = 0.965) (from July to October)

Table 2. Coefficients of regression of protein content (g/fish), Y, of carp weight, X (g):

Y (g/fish)		a	b	r	*t*	p
Protein content	Dorsal muscle	0.188	13.81	0.964	13.69	***
	Adipose tissue	0.174	24.03	0.980	18.64	***
	Liver	0.129	4.02	0.952	11.72	***

Legend: a and b – empirical constants; r – coefficient of regression; t – t test, p – level of significance *** significantly different (p > 0.001)

There was a strong relationship between moisture content (g/fish) and protein content (g/fish) (Figure 4) according to *Dumas et al.* 2007, *Bureau et al.* (2000) and *Breck* (2014).

There was a strong relationship between moisture content (%) and lipid content (%) (Figure 5) according to *Dumas et al.* (2007), *Bureau et al.* (2000), *Breck* (2014) and *Mohseni et al.*, (2007).

The effect of body size on protein content (g/fish) in dorsal muscle, adipose tissue and liver is given in Table 2.

From Table 2 it can be seen that protein content (g/fish) was strongly associated with body weight in dorsal muscle and adipose tissue since coefficients of regression were high (> 0.95) as was *t*-test of significance, but in the liver there was a slightly weaker association, since the coefficient of regression was 0.952, while the *t*-test was high. This is in accordance with *Turchini et al.* (2004) for a slightly different study. Figure 6 shows graphically the protein prediction in fish, depending on the actual prediction. The data in Figure 6 were obtained by calculating the protein content (g/fish) representing predicted protein for the same mass of fish from the equation.

Conclusion

Cyprinids are the most important cultivated species of fish in central-eastern Europe with a 75% contribution to the production of freshwater fish. The aims of the present study were to analyze changes of protein, lipid, ash and moisture in dorsal muscle, adipose tissue and liver in common carp additionally fed complete pellets during four months in natural carp ponds rather than in fully controlled fish tanks, and to make the results directly applicable to real world aquaculture. Since in the literature there is not enough data to explain how diet composition influences the retention of protein, lipid and ash in several tissues of farm-raised two year old common carp, this study was designed assuming that the dietary supply is tissue specific to make results applicable to aquaculture. The proximate composition of fish tissues was expressed as absolute content by weight of each fish. Simple regression resulted in relationships between protein content (g/fish) and body weight (g), indicating a strong association. There is a strong relationship between moisture content (%) and lipid content. Simple regression resulted in not strong relationships between lipid content (g/fish) and body weight (g). There is a strong relationship between moisture content (g/fish) and protein content (g/fish).

Kvantitativno taloženje hranljivih sastojaka u dorzalnom mišiću, masnom tkivu i jetri kod šarana (*Cyprinus carpio* L.) u poluintenzivnom sistemu uzgoja

Dejana Trbović, Ivana Živić, Marko Stanković, Vesna Đorđević, Radivoj Petronijević, Zoran Marković

A p s t r a k t: Šaran je dominantna vrsta koja se uzgaja u Srbiji i čini preko 80% ukupne proizvodnje ribe. Ciljevi ove studije bili su analiza promena proteina, masti, pepela i vlage u dorzalnom mišiću, masnom tkivu i jetri kod šarana dodatno hranjenih kompletnim peletima tokom četiri meseca u prirodnim ribnjacima. Uzorkovano je 20 riba iz četiri ribnjaka. Analiza varijanse pokazala je da je udio proteina najveći u mišićima dorzalnog tkiva i masnom tkivu, a najmanji u jetri (P < 0,05). Procenat proteina je prilično stabilan i dostiže vrednost (18,42–19,49%) u dorzalnom mišiću. Ukupni sadržaj lipida u šaranu bio je najveći u jetri (14,79–17,24%) i manji u dorzalnim mišićima (1,92–5,42%) (P < 0,05). Više je interesantno kako se riblja masa povećavala tokom uzgoja. Prosečan sastav ribljih tkiva izražen je kao apsolutni sadržaj mase svake ribe. Jednostavna regresija pružila je odnos između sadržaja proteina (g/riba) i telesne mase (g) što ukazuje na jaku povezanost (r = 0,965). Jednostavna regresija pruža slabu vezu između sadržaja lipida (g/riba) i telesne mase (r = 0,784). Postoji snažna veza između sadržaja vlage (%) i sadržaja lipida (%) (r = 0,962). Prema našem saznanju može se videti da je sadržaj proteina (g/riba) snažno povezan sa telesnom masom u dorzalnom mišiću i masnom tkivu s obzirom da je koeficijent regresije visok (> 0,95), kao i t test značajnosti (13,69, 18,04), ali i u jetri je postojala povezanost, jer je koeficijent regresije bio 0,952, a t test visok (11,72).

Ključne reči: *prosečan sastav, proteini, masa ribe, kriva rasta*

Disclosure statement: No potential conflict of interest was reported by authors.

Acknowledgements: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, according to the provisions of the Contract on research financing in 2021 (No 451-03-9/2021-14/200050 dated 05. 02. 2021).

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Paper received: November 2nd 2021.
Paper corrected: November 22th 2021.
Paper accepted: November 19th 2021.

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MEAT technology : scientific journal / editor in chief Vesna Z. Djordjevic. - Vol. 57, No. 1 (2016) - . - Belgrade : Institute of Meat Hygiene and Technology, 2016- (Beograd : Naučna KMD). - 30 cm

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Tehnologija mesa = ISSN 0494-9846
ISSN 2466-4812 = Meat technology (Belgrade)
COBISS.SR-ID 225196812