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ASSESSMENT OF THE MICROSCOPIC STRUCTURE - COMPLEMENTARY METHOD OF QUALITY CONTROL OF SAUSAGES

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Abstract

Information concerning the sources of raw material used in order to obtain meat products is of utmost importance, as many technologies permit the replacement of raw material with additives comprising proteins of various animal or plant origin. The purpose of this paper was to assess the quality of preserved sausages (by smoking, boiling, etc.) by using the routine histological examination. In this paper, a total of 22 sausages produced in various processing units were randomly acquired from supermarkets in Bucharest.

The samples were performed according to the routine histological procedure by embedding in paraffin, using a histoprocessor, sectioned and stained by HE (hematoxylin-eosin), and by Tricomic Masson staining. The results of the current study indicate that the studied products contain several types of tissues in varying proportions (muscle, connective and adipose tissues, blood vessels) and amorphous, anhistous structures. The morphological wholeness of the muscle tissue was assessed by the specific structural elements (sarcolemma, sarcoplasm, nuclei). The study supports the introduction of routine histological examination as an additional method for assessing the quality of sausages.

Key words*: sausages, quality, raw materials, integrity, microscopic structure.*

INTRODUCTION

The consumed products 'quality has a major impact on the general health of the population. In this regard, information on the sources of raw materials for the processing of meat products is of utmost importance, as many technologies permit the replacement of raw materials (for instance: muscle tissue) with various additives comprising proteins of different animal or plant origin. Moreover, cases of adulteration of meat products using animal species that are not covered by technology are becoming more widespread. (Natalya L. Vostrikova et al., 2019). Falsification of raw materials by altering the composition of the species modifies the properties of the final product and constitutes a danger for the consumer's health. Severe risks are associated with the replacement of raw materials with animal meat. The use of animal meat as replacement is prohibited or restricted due to the possibility of it being infected with prions or viruses. In some situations, the use of undeclared components like soy, mustard milk proteins, may trigger allergic reactions, a risk about which the consumer remains uninformed. Moreover, adulterating raw materials may infringe on the moral code of consumers whose local or religious views do not allow the consumption of meat obtained from certain animal species. (Natalya L. Vostrikova et al., 2019).

Many studies have shown the potential of histological techniques to evaluate the composition of sausages. This method can accurately assess the quality parameters of meat products, detecting (and quantifying) specific tissues of animal organs, extracellular connective tissue, fat content, bone tissue. (Ghisleni et al., 2010; Latorre et al., 2015; Malaskiene et al. 2016; Moghtaderi et al.,2019; Sadeghi et al., 2003; Sezer et al., 2013).

It is extremely important to verify the composition of meat products (namely: raw materials, intermediate products, and finished products), at all stages of production.

To guarantee the effectiveness of such verification, unfailing and productive analytical methods are needed, so as to grant researchers the detection of individual ingredients and molecular identifiers that depict the content of

different types of raw materials in the finished product (Natal'ya L. Vostrikova et al., 2019).
At present, in the Czech Republic, the microscopic analysis of food is monitored by experts from the Department of Plant-based Food and Plant Production, at the Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, in Moscow, Russia, at the V. M. Gorbatov Federal Research Center for Food Systems. RAS put in place a concept to ensure the quality and complete safety of meat products, The concept includes the use of barrier technologies, of predictive microbiology, the critical control points, the production management principles, the safety and product quality monitoring throughout the production, the transport, and the sales chain (Kalinova Yu., 2007; Pospiech et al., 2009).
Multi-stage technological treatments (e.g., fine grinding, salting, heat treatment,) can make it difficult to identify the structure of the muscle tissue, as recommended by traditional methods.
There are many methods to process and prepare specimens for microscopic examinations today, along with a variety of investigative techniques. These procedures include both conventional methods and methods that use the most recent equipment, but they all show advantages and disadvantages.
Nevertheless, histological analysis may point out a more comprehensive view of the composition of meat products. (Tremlová et al, 2002; Pospiech et al., 2009; Ghisleni et al., 2010; Doaa M. Mokhtar et al., 2018).
The purpose of this study is to establish the quality of preserved sausages (smoked, boiled etc) by using the histological analysis method.

MATERIALS AND METHODS

Throughout this paper, a total of 22 sausages produced in different processing units were randomly acquired from supermarkets in Bucharest. The sausages were selected from the following product categories: cold-smoked, boiled and smoked and smoked and boiled and double-smoked and each sample is a commercial type. The parameters of the samples were set down, including the type of sample and the date of sampling, then the samples were immediately transported and processed.
Samples fixed in 10% formaldehyde solution were subjected to the routine paraffin inclusion method, using a histoprocessor, initially sectioned and stained by the HE (hematoxylin-eosin) technique, and for better differentiation of the connective tissue from sausages was used Tricomic Masson staining. The sections were examined with the Olympus BX 41 Microscope with an integrated computer shooting system.

RESULTS AND DISCUSSIONS

The outcomes of our study indicate that the studied products contain several types of tissues. This diversity of observed tissue types is not completely different from that found by the american researchers in the analysis of meat products (Prayson et al., 2008a; Prayson et al., 2008b; Richard et al., 2013).
Sausages from the category of boiled and double-smoked products and from the category of boiled and smoked products present fragments of striated muscle tissue with moderately preserved cell morphology, with partial homogenization of the sarcoplasm and frequently with destruction of the sarcolemma (Figure1). These tissue fragments are bordered by an amorphous mass, sometimes with an oxyphilic fibrillar character that frequently includes vegetal tissue fragments (spices). Also, in the entire thickness of the product, there are large areas of adipose tissue with a partially preserved morphology with intact cell membranes, but without nuclei (Figure 2).
Other sections of the same category, present masses of connective tissue in the thickness of the muscle, the connective tissue has lost its normal morphology; it is homogeneous blue - light blue (Figure 3) in which rare shadows of nuclei are observed.
Sausages in the category of cold-smoked products have longitudinal and cross sections through striated muscle tissue with moderate preservation of cell morphology, the sarcolemma is occasionally detached, the sarcoplasm is homogeneous. Muscle tissue is frequently embedded in connective-adipose tissue, where fat cells have preserved only their cellular contour. In some sections there are

many granular areas of intense basophilic colour (salt), the plant fragments are more discreet but distributed throughout the product (Figure 4).

The histological method occasionally detected glandular tissues, (Figure 6), lymphoid tissue (Figure 7), nerve threads and blood vessels (Figure 8) in the examined sausage samples. In addition, the present study showed, in addition to muscle tissue, adipose, fibrous, and serous glandular tissue, the presence of parasitic structures *Sarcocystis* spp. (Figure 5),

To compare the morphological structure of the product categories analysed by microscopic examination, we resorted to the quantification of different aspects, shown in Tables 1, 2, and 3. According to the records, muscle tissue is intact in cold-smoked sausages and boiled and double-smoked products at least the architecture of the muscle tissue is preserved.

We note that no conclusion can be drawn on this issue due to the small number of samples analysed, but the method of quantifying tissue integrity can provide important data on the quality of the sausages analysed.

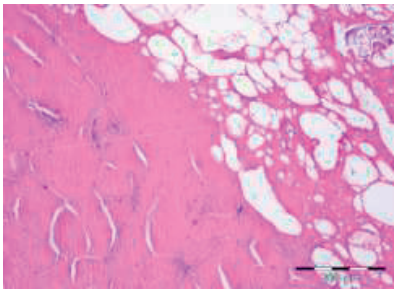

Figure 1. Sausage - boiled and double smoked product. Sections through muscle and adipose tissue with partially preserved morphology. Inaccurate delimitation of muscle fibers by sarcolemma HE stains (ob. 10x)

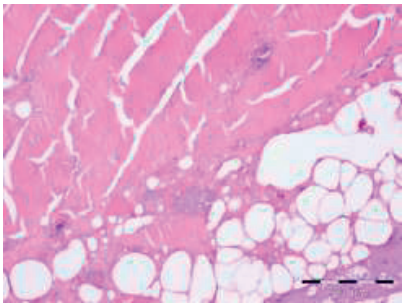

Figure 2. Sausages, boiled and double smoked product, adipose tissue with the preservation of cell membranes, but most of them without nuclei HE stains (ob. 10x)

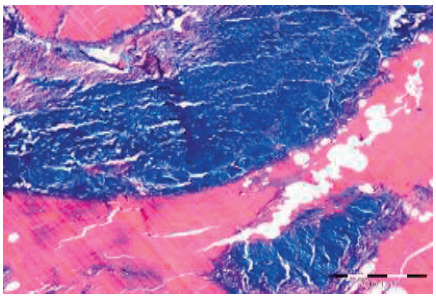

Figure 3. Sausage - boiled and double smoked product. Muscle tissue and adipose tissue with preserved structure and amorphous connective tissue. Tricomic Masson stains (ob. 10x)

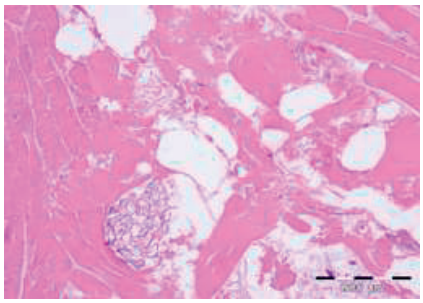

Figure 4. Sausage cold smoked product. Homogenized and fragmented sarcoplasm, plant fragments HE stains (ob. 10x)

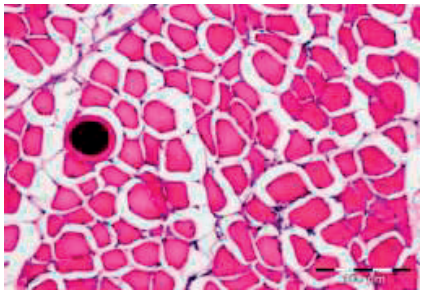

Figure 5. Sausage - cold smoked product. *Sarcocystis* spp. In cross-sectioned muscle fibers; preservation of muscle tissue structure HE stains (ob. 20x)

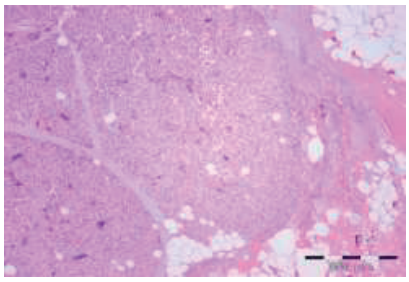

Figure 6. Sausage - boiled and smoked product. Glandular tissue integrated in the product HE stains (ob. 4x)

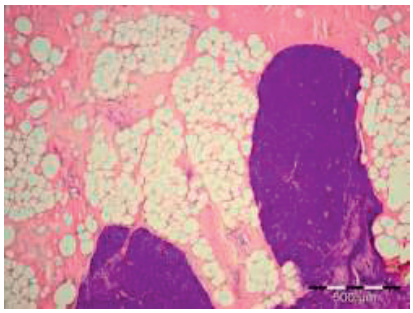

Figure 7. Sausage - cold smoked product. Fragment of lymphoid tissue in the composition of the product. HE stains (ob. 4x)

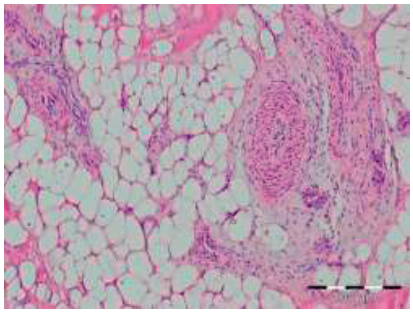

Figure 8. Sausage - cold smoked product. Preserved blood vessels in a mass of adipose tissue. HE stains (ob. 10x)

The utilization of unapproved animal tissue in meat products is due to the economic value of meat itself. The problem of authentication in meat products could include the replacement of meat species, tissues, vegetable proteins, organic compounds, as well as the replacement of vegetable fats with animal fats. (Ballin N.Z., 2010).

Some studies attest to the importance of introducing histological examination, which could be a useful tool for government authorities in fraudulent and quality control of meat products. (Ballin NZ., 2010).

Non-compliant tissues, such as glandular tissue and lymphoid cells, were also highlighted in the current study.

Cetin et al., stated that 21 of the 127 ready-for - sale meat samples held a large quantity of calcium, suggesting the addition of non-meat materials like: scrapings of bone and mechanically deboned meat. (Cetin O., 2016)

Rokni et al., presented salivary gland tissue in boiled sausages, which reflects the use of meat obtained from the heads of slaughtered animals, in meat products. (Rokni et al.,1997)

Sepehri Erayi observed additive tissue consisting of chicken skin, peritoneal fat, hyaline cartilage, and kidney in 30 samples of three different types of sausages. (Sepehri Eraei et al., 2008). Similar aspects were identified in the samples analysed in this study.

Moghtaderi, et al. examined 20 samples of sausages and revealed unauthorized tissues, including conective tissue (6.66%), cartilage (28.30%), bone (8.30%), skin (51,60%), and blood vessel (11.66%), in addition, plant tissues were recognized in 97,70% of the samples. (Moghtaderi, et al., 2019)

Sadeghinezhad and his team focused on the qualitative and quantitative accuracy of the histological examination for the detection of unauthorized plant and animal tissue content in minced beef where the minced meat composition contained between 5 and 20% soybeans and chicken organs (Sadeghinezhad et al., 2015).

The results obtained by Pospiech (2009) indicated the addition of plant additives to meat products, which reduces the quality of meat and affects food safety as allergens (Pospiech et al., 2009).

What is certain is that only the standard physicochemical determinations usually applied to meat products cannot provide sufficient information on the quality and integrity of muscle tissue or the presence of non-compliant tissues.

From this point of view, we consider the microscopic investigation in this paper useful, and we advocate for its introduction as an additional method of surveilling the quality of foodstuff of animal origin.

Table 1 Morphological Characteristics of the different tissue types detected in the category of boiled and double-smoked sausages, by histological methods

Product type	Integral muscle fibers, delimited by the sarcolemma	Presence of muscle fiber interruptions	The presence of striations	The presence of nuclei	Connective tissue	Presence of anhistic material	The presence of spices
Sample A1	inconstant	+	+	inconstant	+	+	+
Sample A2	–	–	–	–	+	+	+
Sample A3	–	+	–	–	+	+	+
Sample A4	–	+	–	–	+	+	+
Sample A5	–	+	–	–	+	+	+
Sample A6	inconstant	–	–	+	+	+	+
Sample A7	inconstant	+	–	–	+	+	+

- not observed; + present

Table 2. Morphological Characteristics of the different tissue types detected in the category of boiled and smoked sausages, by histological methods

Product type	Integral muscle fibers, delimited by the sarco-lemma	Presence of muscle fiber interrup-tions	The presence of striations	The presence of nuclei	Connect ive tissue	Presence of anhistic material	The presence of spices
Sample A8	+	+	-	-	+	+	+
Sample A9	+	+			+	+	+
Sample A10	+	+	+	+	+	+	+
Sample A 11	+	+			+	+	+
Sample A 12	-	+		inconstant	+	+	+
Sample A 13	Incon-stant	+	_	+	+	+	+

- not observed; + present

Table 3. Morphological Characteristics of the different tissue types detected in the category of cold smoked sausages, by histological methods

Product type	Integral muscle fibers, delimited by the sarcolemma	Presence of muscle fiber interrup-tions	The presence of striations	The presence of nuclei	Conn ective tissue	Presenc e of anhistic material	The presence of spices
Sample A 14	+				+	+	+
Sample A 15	+	+		+	+	+	+
Sample A 16	+	+	+	+	+	+	+
Sample A 17	+	-			+	+	+
Sample A18	+	+			+	+	+
Sample A 19	+	inconstant			+	+	+
Sample A 20	+	+			+	+	+
Sample A 21	inconstant	+	+	+	+	+	+
Sample A 22	-	+	_	Incon-stant	+	+	+

- not observed; + present

CONCLUSIONS

Histological evaluation of sausages subjected to various preservation techniques revealed some non-compliant tissues (glandular tissue, lymphoid tissue, vessels, and nerves) and intracellular parasitic forms (*Sarcocystis* spp.).

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