HISTOLOGICAL ANALYSIS FOR QUALITY EVALUATION OF CURED MEAT SAUSAGES

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Abstract. The aim of this study was to evaluate the quality of cured meat sausages of two Lithuanian producers.

Histological analysis of meat products enables assessment of animal tissue content and can give information on the quality of the used raw material and the quality of the processing steps. The cured meat sausage samples were collected at retail within a one month period. These sausages consisted of extra and first category quality sausages. Sections of these sausages were stained with Calleja methods and examined histologically for the presence of various animal tissue types. To quantify the amount of individual tissues, an image analysis software was used.

The histological evaluation of the cured meat sausage samples demonstrated presence of skeletal muscles, adipose tissue, connective tissues, and blood vessels. Glandular and nerve tissues were found only in 3 and 2 samples, respectively. No cartilage and bone tissues were found in any of the examined samples. The amount of skeletal muscles, adipose and connective tissues differed between the sausage samples of the two producers. The much higher amount of skeletal muscles ($P<0.05$) was found in sausages of extra quality in comparison to the first category sausages of producer. However the amount of adipose tissues was higher ($P<0.05$) in the first category sausages, respectively. For collagen tissues, these differences were significant between first quality category sausage samples of two producers ($P<0.05$).

In conclusion, automated image analysis is able to quantitatively evaluate tissue contents of meat products, however it is recommended to verify such results by an alternative methods.

Keywords: Cured Meat Sausages, Quality, Animal Tissues, Histology

Introduction. Cured sausages are popular processed meat products that compromise a preparation of raw materials, including spices, filled either in natural or artificial casings, ripened and dried. There is a large market for cured sausages, in many countries such as the Mediterraneans, Middle East countries, EU countries and other countries (Font-i-Furnols and Guerrero, 2014; Paulos et al., 2015).

The quality of such meat products is very closely related to the ratio of skeletal muscle and connective tissue, the latter reducing the quality of such sausages (Ghisleni et. al., 2010). The meat products producers should have reliable information on product quality to guarantee the quality of meat products for consumers as adulteration of meat products concerns diet of consumers, health and religious issues along with unjustified economic benefit (Damez and Clerjon, 2008; Dosti et al., 2014). Substantial problem might be the consumption of pork meat or horse meat for people based on religious or ethical concerns (Bargen et al., 2014). In European Union the requirements for meat products are described in Regulation (EC) No.853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

To evaluate the composition of sausages, histological analysis can be used. That method is able to precisely evaluate the quality parameters of meat products by detecting (and quantifying) specific tissues of animal organs, extracellular connective tissues, fat content, bone tissues and others (Damez and Clerjon, 2008; Messia et al., 2008; Ghisleni et al 2003). There are some advantages and disadvantages of using histological analysis and chemical methods for quantification of tissues in meat products. However, histological analysis can reveal more comprehensive view of the composition of the meat products (Tremlova and Starna, 2003).

The aim of this work was to define the quality of cured meat sausages by quantification of the amount of skeletal muscles, adipose tissue, connective tissues and blood vessels by histological analysis and automated image analysis.

Material and methods

Sample collection

Altogether, sausages from two producers (A and B) were collected within a one month period at retail shops in Kaunas, Lithuania. These included sausages of different quality categories as indicated by the producer on the label: first and extra categories (Table 1). In total, 20 cured sausage samples were bought and transferred to

the laboratory at 4-6 °C temperature. Histological analysis was started within 3 hours after delivering samples.

Histological analysis

Samples of each cured sausage were fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding (Anderson and Bancroft, 2002). Three histological sections (5 µm thickness) of each cured sausage sample were stained with Calleja method (Exbrayat, 2013). The histological sections were examined with a BX43 light microscope with digital camera DP73 (Olympus, Tokyo, Japan) at a 40x magnification to identify the presence of various types of animal tissues (skeletal muscles, connective tissue, nerves and others). The 100x magnification was used to generate figures 1, 2 and 3 displaying different animal tissues found in the examined cured sausage samples.

Image analysis

Further one of three prepared sections was used for image analysis to quantify the amount of individual animal tissue content. Different tissues were detected and scored with automated and manual colour threshold image analysis by use of an Olympus BX43 light microscope and image analysis program "Olympus Stream Essentials" version 1.9.1 (Olympus). For each Calleja-stained section of cured sausage samples, three areas of 3.62 mm^2 were examined and the percentage area densities for each type of animal tissues were quantitatively determined.

Statistical analysis

Statistical analysis was performed using SPSS v 14.0 for Windows (IBM, Armonk, U.S.) and mean values, standard deviation and statistical significance at $P<0,05$ were calculated.

Results

The histological evaluation of the cured sausage samples revealed that skeletal muscles, adipose, connective (collagene and elastine) tissues, blood vessels, nerves and glandular tissues were found (Tab. 1).

Table 1. **Percentage area density of skeletal muscles, adipose and connective tissues and blood vessels of two different quality category cured meat sausages based on analysis of three digitised images of each histological section**

Producer		Quality category	Sample No.	Detected tissues					
				Skeletal muscles (%)	Adipose tissue (%)	Connective tissue			Blood vessels (%)
						collagen	elastic	in total	
	A	Extra	1	79.00	18.16	2.84			
			2	53.57	28.42	17.54			0.47
			3	87.90	5.54	6.56			
			4	70.22	26.32	3.45			0.01
			5	83.32	12.13	4.45			0.11
		Mean (%); SD		**74,80[1x]±13,541**	**18.11[2]±9.587**	**6.97±6.076**			**0.12±0.241**
		First	1	59.18	32.41	7.23	1.18	8.41	
			2[B]	57.50	28.71	13.78			
			3[B]	43.25	48.68	8.07			
			4[A]	35.90	47.72	16.05			0.33
			5[B]	48.64	46.59	4.76			
		Mean (%); SD		**48,90[1]±9,757**	**40.82[2]±9.489**	**9.98[3]±4.737**			
	B	Extra	1	44.75	25.46	28.93	0.86	29.79	
			2	43.16	50.09	5.81	0.31	6.12	0.62
			3	49.63	42.53	7.52	0.32	7.84	
			4	91.13	5.67	2.02	1.18	3.2	
			5	44.98	49.70	4.88	0.44	5.32	
		Mean (%); SD		**54,73±20,490**	**34.69±19.048**	**9.83±10.859**	**0.62±0.383**	**10.45**	
		First	1[C]	57.33	38.57	3.20	0.68	3.88	0.23
			2	48.65	46.56	4.78			
			3	53.16	40.56	6.28			
			4	56.83	37.57	5.02			0.48
			5	78.11	14.81	6.80	0.24	7.04	
		Mean (%); SD		**58,85±11.344**	**35.61±12.143**	**5.221[3]±1.409**	**0.18±0.308**		**0.14±0.178**

[x] – the same superscript numbers indicate statistically significant differences between two parameters at $P<0,05$;
[A] – Nerve and glandular tissues were found in the sample; [B] – Glandular tissues were found in the samples; [C] – Nerve tissues were found in the sample

Also the study results revealed differences in the tissue composition of cured sausages between two producers and between sausages of the quality categories: first and extra quality. The highest mean values of skeletal muscles were found in extra quality cured sausages of producer A (74.80%), first quality cured sausages of producer B (58.85%), extra quality of producer B (54.73%) and first quality of producer A (48.90%), respectively. Correspondingly, the content of adipose tissue varied between 18.11% (extra quality, producer A) and 40.82% (first quality, producer A) and for connective tissue observed values varied between 9.98% (first quality, producer A) and 5.22% (first quality, producer B).

In single samples, glandular tissues, nerve fibres and blood vessels were detectable. No cartilage and bone tissues were found in any of the examined samples. Figures 1 to 3 exemplarily show Calleja stainings of the cured sausages samples.

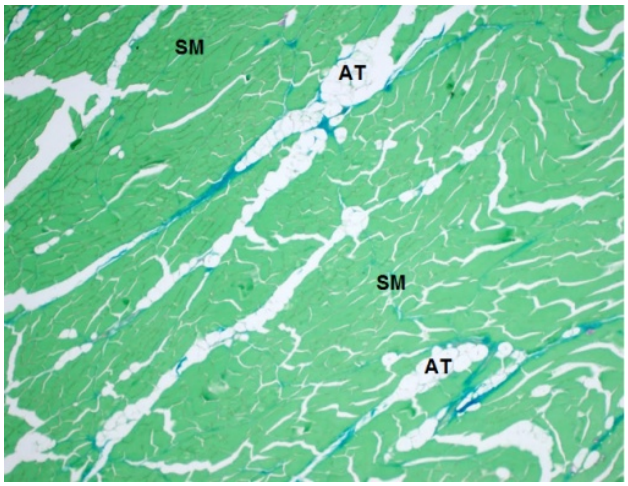

Figure 1. **Skeletal muscle (SM) and adipose tissue (AT) in examined cured meat sausage (Calleja staining 100x)**

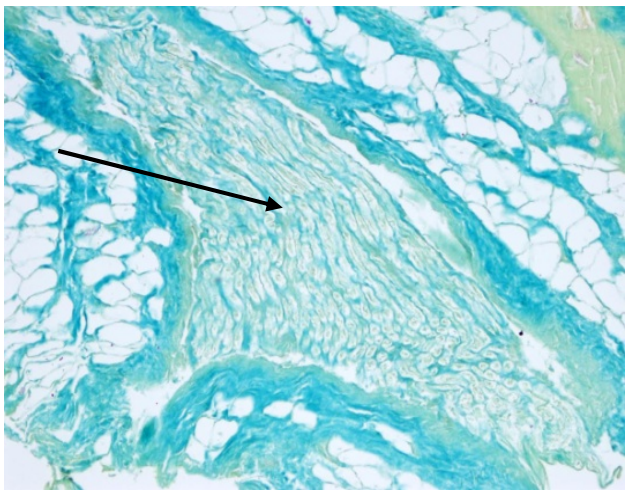

Figure 2. **Nerve tissue (indicated with black arrow) in examined cured meat sausage (Calleja staining 100x)**

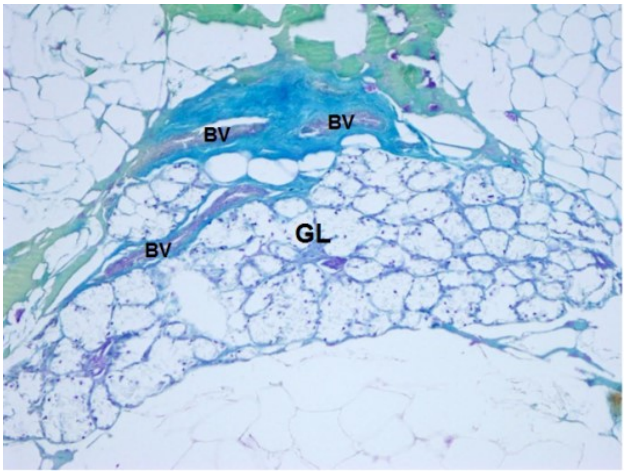

Figure 3. **Glandular tissue (GL) and blood vessels (BV) in examined cured meat sausage (Calleja staining 100x)**

Discussion

Within the last decades a significant increase of food fraud has been observed including false labelling and undeclared use of food additives or fillers to replace skeletal muscles in the product to achieve economical profit (Everstine et al. 2013; Ordunaa et. al., 2015). Such situation increased the awareness of consumers and food fraud has gained a lot of attention. It is the producer's responsibility to ensure that the quality of meat products is in compliance with the requirements of regulation (EC) No. 853/2004. The histological examination methods could serve as one of the quality control options as several studies revealed capability of these methods to detect food fraud and specifically adulterations in meat products (Damez et al, 2008; Ghisleni et al, 2010; Latorre et al., 2015; Sezer et al., 2013).

Our study showed large differences in the tissue constitution of cured sausages of two quality categories and from two producers. Interestingly, there was no significant differences of the amount of skeletal muscles, adipose tissues and collagen tissues between extra and first quality categories sausage samples of producer B. However the amount of skeletal muscles and adipose tissues differed significantly between extra and first quality categories sausage samples of producer A ($P<0.05$). It should be noted that all examined sausage samples passed the requirements specified in the Regulation (EC) No.853/2004 of the European Parliament. According to this technical regulation, non-heat-treated meat products are classified into Extra and I quality categories (Technical Regulation on Meat Products in Lithuania; N0. 3D-78; 2015). However only the content of the non-collagen meat protein, moisture and meat-substitution protein are specified for cured meat sausages as non-heat-treated meat products.

Occasionally, glandular tissues, nerve fibres and blood vessels were detectable in the examined sausage samples. These occasional findings were within the "technologically unavoidable" frame. No cartilage and bone tissues were found in any of the examined samples.

In the U.S. quality evaluation of eight different brands of hamburgers revealed that peripheral nerve fibres, plant material, cartilage and bone tissues could be found as well (Prayson et al., 2008).

This study shows that histological analysis can be used to describe the composition of sausages and quantify tissue content. Automated image analysis is able to quantitatively evaluate tissue contents of meat products, however it is recommended to verify such results by an alternative methods.

Conflicts of interest

There are no conflicts of interest in this study.

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