EVALUATION OF FAT GRAINS IN GOTHAJ SAUSAGE USING IMAGE ANALYSIS

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ABSTRACT

Fat is an irreplacable ingredient in the production of sausages and it determines the appearance of the resulting cut to a significant extent. When shopping, consumers choose a traditional product mostly according to its appearance, based onwhat they are used to. Chemical analysis is capable to determine the total fat content in the product, but it cannot accurately describe the shape and size of fat grains which the consumer observes when looking at the product. The size of fat grains considered acceptable by consumers can be determined using sensory analysis or image analysis. In recent years, image analysis has become widely used when examining meat and meat products. Compared to the human eye, image analysis using a computer system is highly effective, since a correctly adjusted computer program is able to evaluate results with lower error rate. The most commonly monitored parameter in meat products is the aforementioned fat. The fat is located in the cut surface of the product in the form of dispersed particles which can be fairly reliably identified based on color differences in the individual parts of the product matrix. The size of the fat grains depends on the input raw material used as well as on the production technology. The present article describes the application of image analysis when evaluating fat grains in the appearance of cut of the Gothaj sausage whose sensory requirements are set by Czech legislation, namely by Decree No. 326/2001 Coll., as amended. The paper evaluates the size of fat mosaic grains in Gothaj sausages from different manufacturers. Fat grains were divided into ten size classes according to various size limits; specifically, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 5.0, 8.0 and over 8 mm. The upper limit of up to 8 mm in diameter was chosen based on the limit for the size of individual fat grains set by the legislation. This upper limit was not exceeded by any of the products. On the other side the mosaic had the hightest representation of 0.25 mm fat grains.

Keywords: fat grain; Gothaj sausage; image analysis;

INTRODUCTION

Gothaj is a favourite traditional Czech meat product. It is characteristic primarily by its specific mosaic rich in visible fat grains. It is a meat product which is affordable for the majority of consumers. However, consumers are used to a certain standard even in this cheaper assortment of meat products. When purchasing it, the decisive factors are attractiveness as well as previous experience of the customer. As with many other selected meat products, correct recipes and prescribed sensory, physical and chemical properties set by legislation must be followed.

The characteristic size and appearance of lard grains in the mosaic is closely related to the selection of input ingredients, it's mincing and the method of mixing the product. In their work, **Saláková et al. (2013)** analysed fat content as one of the criteria for assessing quality of Gothaj sausage. The study revealed that unlike water content, the fat content in Gothaj has decreased in the last twenty years. Specifically, it was found that the limit set by Decree No. 326/2001 Coll, i.e. 40%, or the limit set by the original quality standard (ČSN 57 7231), i.e. 42%, was not reached by any of the tested samples. Only one of the studied samples reached 32.7% fat content, with the remaining samples scoring even lower.

The filling of soft salamis, which include Gothaj as well, can be processed in two ways: either as homogenous filling with finely cut and ground binder or non-homogenous, containing large-grained core ingredient in the filling. Gothaj is ranked among sausages with large grains of fat in the filling (**Steinhauser et al., 1995**). The results of sensory evaluation clearly point to the fact that fat in the product has significant influence on its sensory properties. In Gothaj, fat represents one of the core ingredients of the filling and is therefore essential for the appearance of the cut product. Saláková et al. (2013) discovered that currently, the popularity of products with lower fat content is growing among consumers. In a sensory evaluation it was determined that the more visible the lard in the sausage section, the lower the preference of the product.

In terms of quality management of food products, attention is focused on delivering consistent products to the consumer for an affordable price. The ideal method to ensure food safety and quality is regular monitoring food products in all stages of their production and distribution. It is therefore necessary to have effective monitoring systems which can obtain reliable information on the content, composition and safety characteristics of food ingredients and finished products. The processes

associated with quality assurance of food products usually incorporate chemical or mechanical analyses or human senses. Unfortunately, these are time consuming, laborious, costly and destructive methods, or methods requiring specific preparation of the sample. Non-invasive methods have thus been introduced into the process of quality assessment in recent years which are both sufficiently objective and quick. One of these is the use of computer visualisation **(Elmasry and Nakauchi, 2016)**.

As reported by **Brosnan and Sun (2004)**, image analysis is a suitable alternative for the evaluation of meat and meat products. Image analysis can be applied from the early stages of the production process through to the finished products intended for consumers.

Various sensory and physical-chemical parameters as well as the composition and more specific chemical composition are generally assessed not only in meat products, but in other food products as well. Selected quality characteristics, such as color, smell, taste and mincing can be determined in meat and meat products relatively quickly and without complex laboratory analyses. Since determining other, primarily chemical parameters which influence the quality of the food requires special laboratory equipment and work with chemicals, the development of monitoring methods is heading towards an innovative form of instrumental methods for quality management in food businesses. These methods also include digital image analyses **(Zapotoczny, Szczypiński, Daszkiewicz, 2016)**. Also **Pospiech et al. (2009)** confirms that by using image analysis for quantitatively evaluate of meat products are obtained objective and accurate results that are comparable to chemical analysis.

Pork fat does not contain caroteneoids, which makes it usually white **(Steinhauser et al., 1995)**. The fat particles provide sufficiently intense contrast to other ingredients and are thus easily detectable through image analysis. The evaluation of visible fat using image analysis on a section of dry sausage was studied by **Girolami et al. (2014)**. The reasearch assessed the ratio of fat in the analysed portion of the product based on evaluation of the color and fat content of the cut product. The study demonstrated that image analysis is suitable even for meat products such as sausages.

The use of a computer visualisation system for the evaluation of fat in the product was also studied by **Chmiel et al. (2011)**. They specifically focused on the assessment of fat content in chicken and turkey breast and thigh muscles. The analysis was based on assessing color and saturation. When compared to the Soxhlet method, the results showed that image analysis is not suitable for this category of food products. Determination using image analysis was problematic due to the contrast between fat and muscle mass being insufficient.

Image analysis appears to be a suitable method for evaluating quality for food products with sufficient color contrast between the individual components of the product. Such meat products include e.g. salamis and sausages. Evaluation of fat grains using image analysis methods was examined by **Čáslavková et al. (2014)**. The author analysed a cut of Poličan fermented sausage. The suitability of image analysis for the detection of fat in fermented meat products was also confirmed by **Pospiech et al. (2013)**.

The aim of the study was to compare fat grains in Gothaj type products from various manufacturers and evaluate the consistency of their product lots.

MATERIAL AND METHODOLOGY

The object of examination were Gothaj sausages from seven different manufacturers, always in three lots. Samples were taken in the period of October 2014 – January 2015. From each sample, 3 sections were prepared for image analysis at a thickness of 0.5 each. Thus, a total of 63 sections were prepared for the examination.

Constant lighting conditions were established for the taking of images using EASY LIGHT lamps – 3.3 x 28 W and 5000 – 5500 K. The stability of light intensity was monitored using a lux meter (Voltcraft LX – 1108), with light intensity level set to 7290 ±50 lux. Images were captured using EOS 600D camera (Canon, JPN). The images were captured in manual mode with the following settings: exposure time 1/100, aperture F8, image size L, sensitivity ISO 100, data format JPEG, maximum zoom 55 mm, with the additional use of the white balance function. For the contrast between the sample and the background to be sufficient, the pictures were taken on a deep blue background.

The analysis itself was performed in NIS – Elements BR 4.13.00 software (Laboratory imaging, CZE). The program is commonly used to study, scan and archive image structures. The samples can be measured manually or automatically. A scale was attached to each sample when the picture was taken. The scale was calibrated for all the studied images before the analysis itself. For each lots of samples, the size of 1 pixel was determined.

The measuring of the analysed objects in NIS – Elements BR was performed using the fully automated macro function. The macro was defined for the opening of the file, delimiting the region of interest (ROI) and identification of fat grains. Image and binary adjustment, such as double smoothing, hole separation and hole filling, were also included.

Binary transposition of fat grains was performed in HSI color system. The system allows to define a threshold for hue, saturation and intensity of color corresponding to the fat grain contained in the sample matrix. Based on these, the program is capable of automatically marking the boundaries of fat grains. The next step was binarisation, which ensured effective evaluation of fat grains and other components, including the image background (see Fig. 1). The subject of the analysis was the fat grains of all sizes, which were subsequently categorised in size classes: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 5, 8 and above 8 mm; see the chapter Results and Discussion.

Measured parameters such as length, surface, equivalent diameter and width of fat grains were statistically processed further using UNISTAT software version 6.0. Based on a normality test, the data were evaluated as abnormal.

Lots analysis was performed using a multiple comparisons ANOVA test, Dunnet. Analysis of differences between individual manufacturers was carried out via a non-parametric Kruskal-Wallis One-Way Anova test, Dunnet (UNISTAT software version 6.0, UNISTAT Ltd.).

For the purposes of the analysis, we used the measured length of the objects as the average determined by the Decree, as it is the longest quantity which passes through the object. The length values were then statistically evaluated. Further, the quantity of objects for individual size classes was calculated. Histograms were then created for transparency. We focussed primarily on the length, area, equivalent diameter and width of fat grains (Figure 3).

The representation of ideal fat grains was calculated using the methodology in **Čáslavková et al. (2014)**, called the CNSFT coefficient.

RESULTS AND DISCUSSION

The Gothaj sausage is a meat product of a darker meat-pink color at the cut. The filling is finely processed, the fat mosaic irregularly distributed. Occasional fine-grained collagen particles and small air cavities are acceptable. The size of individual fat grains must be up to 8 mm in diameter; particles of spice may be visible (**Decree, 2001**). The present study analysed fat grains, i.e. the fatty tissue from the morphological point of view. Manufacturers of Gothaj sausages most commonly use back fat as the core ingredient for their product. Based on the traditional method of producing Gothaj sausages, the core ingredient is mixed into the filing at grain size of 8 mm (**Šedivý, 1998**). The study also included the determination of the size profile of fat grains and the monitoring of whether the limits for fat grain size were exceeded.

The production technology for the majority of meat products is based on mincing and mixing individual components of the product. Due to the constant development of better and more advanced production machinery, the technological procedure of production changes. Mincing leads to reduction in the size of larger meat and fat particles. To create the desired appearance of the mosaic, excessive heating of the product must be avoided, as it could result in softening the fat tissue and smearing of fat particles (Pipek, 1998).

The results of analysis of the quantity of fat grains show that the limit of 8 mm set for the size of fat grains was not exceeded by any of the manufacturers. For the analysis of the size of fat grains, fat tissue was divided into 10 classes; see Table 1.

As shown in Table 1, the size class 5.1 – 8 was represented by one sample only. It is therefore rather an accidental occurrence than the intention of the manufacturer. On the other hand, based on the calculation

Table 1 Distribution of fat grain sizes.

Sample	Class										CNSFT
	0 – 0.25	0.26 – 0.5	0.26 – 0.75	0.76 – 1.0	1.1 – 1.5	1.6 – 2.0	2.1 – 2.5	2.6 – 5.0	5.1 – 8.0	>8.0	
B2	3020	1720	522	175	65	4	-	-	-	-	0.73×10^{-3}
B5	2097	801	227	95	61	21	4	-	-	-	7.51×10^{-3}
B6	3461	1628	558	229	195	44	10	5	-	-	9.62×10^{-3}
B7	1841	938	289	114	53	13	3	3	1	-	5.84×10^{-3}
B11	3548	1548	310	65	17	-	-	-	-	-	0
B12	3142	1036	329	128	58	12	1	2	-	-	3.19×10^{-3}
B13	3925	984	242	72	21	4	1	-	-	-	0.95×10^{-3}
SUM	**21034**	**8655**	**2477**	**878**	**470**	**98**	**19**	**10**	**1**	-	

Note: The size of fat grains is measured in cm.

$$CNSFT = \frac{\sum fat\ grain\ length\ of\ category\ (1.6 - 2.0 + 2.1 - 2.50 + 2.6 - 5.0)}{\sum fat\ grain\ length\ of\ all\ categories}$$

Table 2 Lot comparison.

Sample	LOT 1	LOT 2	LOT 3
B2	a	ab	b
B5		a	a
B6	a	a	
B7	a	a	
B11		a	a
B12	a	ab	b
B13	a	ab	b

Note: letters indicate statistically significant difference ($p < 0.05$).

Table 3 Differences between manufacturers.

Sample	B13	B12	B11	B7	B6	B5	B2
B13	-	**	**	**	**	**	**
B12	**	-	-	**	**	-	**
B11	**	-	-	**	**	-	**
B7	**	**	**	-	-	**	-
B6	**	**	**	-	-	**	-
B5	**	-	-	**	**	-	**
B2	**	**	**	-	-	**	-

Note: ** indicate statistically significant difference ($p < 0.05$).

of the sum of occurence frequency for all manufacturers, the greatest representation can be found for fat grains in the 0 – 0.25 category, followed by the 0.26 – 0.5 category, whose frequency is also quite high. Fat grains in the 0 – 0.25 category are at the margin of visibility; therefore, the category plays no part in terms of sensory perception. The high amount of fat grains in this category, however, is indicative of the fat smearing during the incorporation into the product.

Consumers are easily influenced by modern principles which have become rampant in recent years. Therefore, many consumers prefer products with lowest possible fat content. Unfortunately, this attitude is also reflected in purchasing traditional products which are typical by higher fat content. For this reason, the present study evaluates the optimal size of fat grains in the matrix, which for consumers means fat visible macroscopically, i.e. by their naked eye. The resolution capability of the human eye is approximately 0.25 mm. Therefor we chose the value of above 2 mm for the size of fat grains.

To determine which product meets the requirements in terms of the distribution of fat grains best, we used a modified CNSFT ratio coefficient **(Čáslavková, 2014)**. On the basis of the calculation of CNSFT, the best results were achieved by the product from manufacturer B6 (CNSFT = 9.26E-03) which had the largest amount of fat grains approaching the ideal size classes. From among the other tested samples, high CNSFT values were achieved by products from manufacturers B5, B7 and B12. Low CNSFT values in manufacturers B2, B11 and B13 show that their products contained a low number of ideally sized fat grains and a large number of small fat grains **(Table 1)**.

Girolami et al. (2014) evaluated visible fat in a section of salamis and sausages using the image binarisation function, where the color image is converted into a black and white image. The authors also confirm the connection between production process technologies and variability in the appearance of the final product. Similarly, **Saláková et al. (2013)**, based on sensory evaluation the authors came to the conclusion that consumers generally prefer products with lower representation of fat grains.

The size of fat grains naturally has a close relationship to the technology of production of the Gothaj sausage. In our study, we therefore also assessed differences in the representation of fat grain sizes in individual lots of products. The differences between lots are shown in Table 2.

In aggregate, manufacturers B2, B12 and B13 differed in two lots. In other manufacturers, only one out of three lots was different ($p > 0.05$). However, homogeneity of lots which can be evaluated sensorially plays a key role in the consumers' choice of product. Every consumer prefers certain product characteristics; if the products appearance deviates, the consumer is likely to confuse the product with something else, or consider the product poorly made. It is therefore advisable that the manufacturer of standard products monitors the homogeneity of individual lots. An ideal manufacturer should have no differences between lots. Such a case was not found in any of the manufacturers.

Based on the comparison of results, we were also able to confirm the assumption that the finished products also differ from each other. See Table 3 and Figure 2.
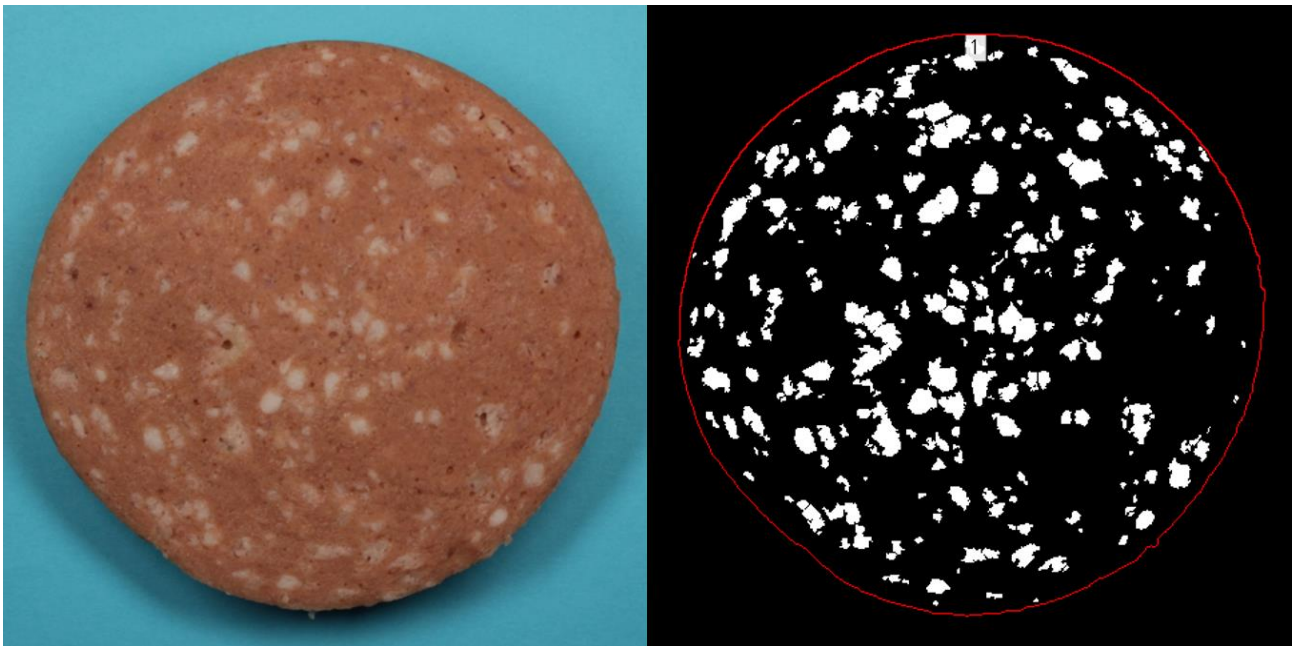
Figure 1 Automatic detection of fat grains in the cut appearance using the binarisation function.
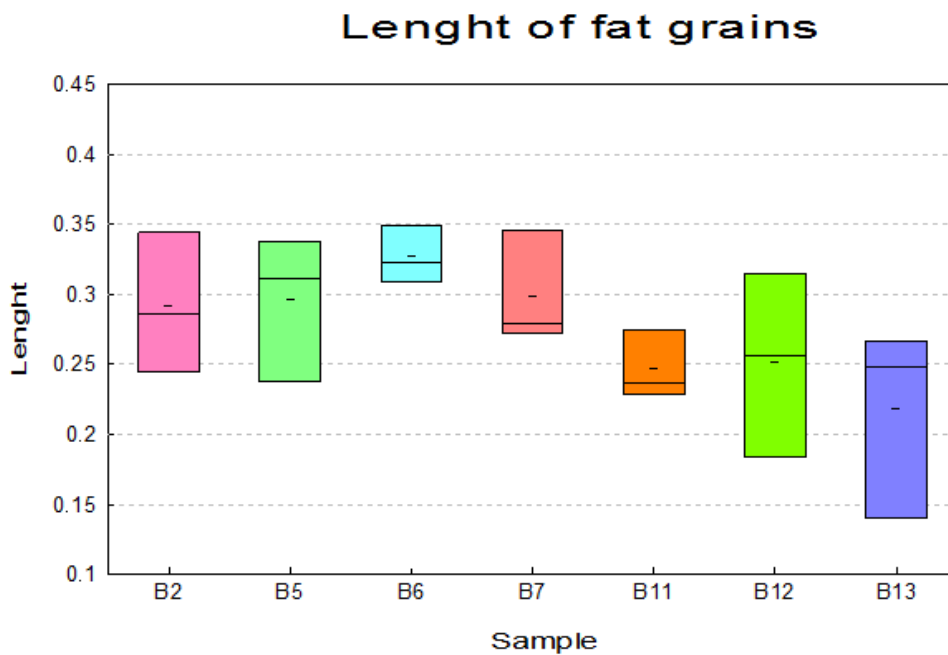

Figure 2 Box diagram for the comparison of similarities between manufacturers based on the length of fat grains.

Product B13 differs the most. The reasons for the differences between manufacturers and lots could be linked to different handling of the raw material added into the cutter as well as heat conditions in the plant or heating caused by intense mincing **(Pipek, 1998)**.

Pork lard is an indispensable material in the production of traditional food products. The production of Gothaj sausage, in which pork lard is a core ingredient, requires the raw lard to be solid and hearty. If that is the case, the lard will be minced properly. It is therefore necessary that the lard is easy to cut, does not smear and does not melt at low temperatures. The viscosity of lard has deteriorated in the last few decades. Fat is rather soft and causes fairly significant problems in the technology of producing sausages **(Pipek et al., 2012)**. Fat consistency depends on the content of saturated and unsaturated fatty acids. With the growing content of unsaturated fatty acids, the consistency of the fat changes as well, causing the fat to become softer. Softer fat is suitable for the production of minced meat products and unsuitable for the production of sausages, where solid fat is required **(Feiner, 2006)**. The influence of technology used on the standard nature of the finished product was also confirmed by **Čáslavková et al., (2014)**.

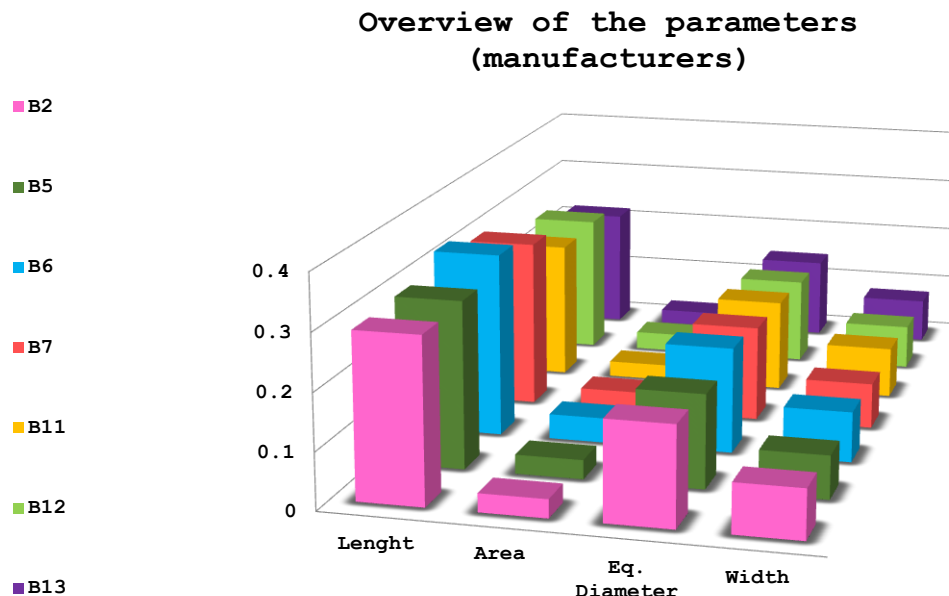## Overview of the parameters (manufacturers)

Figure 3 3D chart shows a summary of the parameters for the size and shape of contained fat grains for each manufacturer.

The subject of the examination was the dry fermented sausage Poličan. Image analysis was used as the evaluation method. The results showed a statistically significant difference ($p < 0.05$) between manufacturers using different technologies (preparation of the product only in a bowl cutter, or in a combination of a bowl cutter and a vaccum filler with a sausage grinder). The results indicate that the desirable grain size of 1.5 – 3 mm is typical for technology using a vaccum filler with a sausage grinder.

CONCLUSION

The studied method of image analysis appears to be a suitable method for the detection of fat grains in soft sausages. Using a properly programmed visualization system, it is possible to obtain information regarding the size, shape and many other geometric parameters of the fat particles contained in the product in a very short span of time. The data obtained in the image analysis are then very easy to be processed statistically. By our results, we determined that the present study has a high representation of fat grains in size classes 0 – 0.25 and 0.26 – 0.5 mm. Based on gained data, we derived an assumption that grains within these categories formed due to the smearing of the raw material during technological processing. In comparison, only a very small number representation was detected for fat grains in size classes of 2.6 – 5.0, 5.1 – 8.0 and above 8 mm. From this point of view, it is worth pondering why there is a fat grain size limit of 8 mm for the Gothaj sausage set by legislation.

The comparison of the results obtained also showed that the structure of the cut product differs between individual manufacturers. In addition, there is a noticeable difference between individual lots by the same manufacturer.

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