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MICROSCOPIC DETERMINATION OF BAMBOO FIBER IN MEAT PRODUCTS

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ABSTRACT

Fiber, a suitable additive to meat products with water-holding capacity, reduces curing losses and maintains juiciness of the meat. The risk is the use of excessive amounts of flour or other ingredients of vegetable origin, in which the fiber is contained. In some cases, sensory characteristics of products can be affected. Detection of fiber may be prevention of adulteration in some meat products. It is therefore very important to regularly detect the amount of fiber in meat products and check its contents. Fiber in meat products can be detected by various methods, applied are for example gravimetric, spectroscopic, histochemical, and microscopic methods. For this reason, a model meat product (Vysočina salami) was prepared in our experiment with the addition of bamboo fiber of selected concentrations of 0%, 2%, and 3%. Subsequently, a series of microscopic sections was made on different days of curing (day no. 7, 14 of the drying phase and 28, 42 of storage). Individual sections were examined and captured using a polarization microscope, the amounts of fiber in individual sections were analyzed by means of image analysis software and the values obtained were compared with each other. Also the influence of drying on the measured area of fiber in sections was monitored. The results indicate a noticeable reduction in the area of fiber until the seventh day of ripening, which is caused by the rapid loss of water in the product. In contrast, sections of products from the following days of drying contained mildly increased concentrations of fiber, which was caused by gradual drying of the products, while the area of fiber refrained form becoming smaller. Between the individual days of drying, a difference that was statistically significant was demonstrated from the 14th day of (storage or drying). Correlation was observed between the date of (storage or drying) and amount of added fiber. Among the tested mean values for the sample with the addition of fiber concentration of 2 % an insignificant difference was found. The difference between test values (day/fiber) in the sample with addition of 3% fiber was, however, statistically significant.

Keywords: fiber; polarization microscopy; image analysis; vysočina salami

A healthy lifestyle is currently very frequently debated issue, which is also associated with the increasing consumer demands for high quality food and thus the raw materials used. Nutritional characteristics of meat and meat products, in particular their quantity in one's diet, are also often discussed. Although their increased consumption can cause significant health problems, their popularity has not declined. In this area, an important role is played by fiber, which is a mixture of substances of plant origin and which can has a positive impact on the consumer's health. Thanks to its properties, it is a good addition to meat products. Disproportionate amount of flour and similar materials of vegetable origin affects the sensory properties of products and detection of fiber may also be an evidence of consumer deception. It is therefore important to regularly detect the amount of fiber in meat products and check its contents. There are many methods used to detect and control the composition of meat products, they are mainly used to test the quality of the product, i.e. to detect individual ingredients and assess their suitability for the product according to Czech regulation **(Decree, 2001)**.

The aim of this paper was to verify the possibility to detect fiber in a meat product and to describe the impact of drying on the area of fiber identified by image analysis.

Fiber is a vegetable part of one's diet resistant to enzymatic degradation in the gastrointestinal tract. The individual components of fiber can be divided on the basis of their solubility/fermentability to insoluble (less fermentable) and soluble (well fermentable) substances. The former group includes cellulose, hemicellulose, and lignin. The latter group contains pectin, plant gums, and mucilages.

Dietary fiber is a complex mixture of polysaccharides with various functions in the digestive tract, where these functions are given by their physico-chemical properties, such as particle size and volume, surface characteristics and moisturizing properties **(Dhingra et al., 2012)**.

Due to technological reasons and because of its positive impact on consumer's health, addition of fiber in meat products is on the increase. Fiber is a suitable additive to meat products because of its water-holding capacity, reduction of curing losses and maintaining juiciness of the meat.

Suitable is the use of oat bran fiber as fat replacer in ground beef and pork sausages. Other advantages include the possibility to use oat bran instead of fat in meat balls.

Dietary fiber is of great importance in food industry in terms of nutrition and technology. The popular saying "the more the better" does not apply here. Addition of 6% of fiber in the product causes a clear deterioration of sensory properties. White dots are visible in the section and consistency is too stiff and dry. Addition of fiber can reduce or even completely prevent soft center of the salami, and moreover, the time required to dry the product can be shortened. In combination with phosphate, we obtain better texture of the product, which is evident already after five days of ripening **(Kameník, 2012)**.

Various methods are utilized to detect fiber. These can include gravimetric, spectroscopic, histochemical, and microscopic methods **(Mlček et al., 2010; Mongeau, 2003; Nielsen, 2010)**. Gravimetric methods employ enzyme and/or a chemical to remove material, which is digestible in the small intestine; imitation of digestive processes in the colon is considered to be ideal **(Mongeau, 2003)**. We can meet enzymatic-gravimetric methods and nonenzymatic-gravimetric methods **(Davídek, 1981)**. NIRS (near infrared spectroscopy), ranked among the spectroscopic methods, is based on the absorption or reflection of different wavelengths of incident radiation that is affected by the chemical composition of the sample analyzed **(Mlček et al., 2010)**. **Tremlová (2000)** describes histochemical methods as methods suitable for identification and localization of various chemical substances in cells and tissues. Whereas these methods are most commonly used as qualitative methods. **Nielsen (2010)** reports the possibility to use modern microscopy methods for a qualitative as well as quantitative examination of the products. These methods provide reliable information on the location and physical condition of all components of the sample examined. Image analysis are often use as qualitive methods for meat products **(Čáslavková et al., 2014)**. Light microscopy is a fundamental histological method and in case of determination of fiber, it is appropriate to use one of its modifications, such as polarization microscopy. This method is based on the ability of the optically active compounds to rotate the polarized light in the polarization microscopy **(Brychtová and Hlobílková, 2008)**. Polarization microscopy is used for the detection of vegetable origin additions in meat products, mostly soy, starch, and spices. Another of the methods used to describe the microscopic characteristics, is fluorescence or electron microscopy **(Tremlová, 2000)**.

MATERIAL AND METHODOLOGY

A durable cured model meat product called Vysočina was manufactured for the experiment. 3 model products were produced, where one was a control sample (Vysočina with no added fiber – control) and the remaining products contained fiber in concentrations of 2 and 3% (sample 1 and sample 2 respectively). The experiment also included monitoring changes in relation to time, therefore, sampling was performed on the 7^{th}, 14^{th} of the drying phase and 28^{th}, 42^{nd} day of storage.

Sample processing and preparation of microscopic slides included the following steps: sample fixation in 10% formaldehyde, dewatering samples, embedding them in paraffin, cutting paraffin sections, targeted staining (PAS – Calejja), and mounting the sections.

First, the most suitable microscopic method for the detection of fiber in model meat products was selected. The most suitable methods include polarization and fluorescence microscopy. After verifying both of the methods, polarization microscopy was selected for the experiment, because it gave clearer results and was less demanding in terms of sample preparation.

All the samples were photographically documented in natural as well as polarized light microscopy (Jenaval, Karl Zeiss, Jena, DDR). Images were obtained using the DSLR Remote Pro software for Microsoft Windows (USA). The very analysis of fiber in the images from polarization microscope was performed in the Adaptive Contrast Control – Image Structure and Object Analyser program, ver. 6.1 (ACC, company Sofo, Druckmüller, Štarha).

Image segmentation was preceded by decomposition of images into individual components of RGB (red, green, blue). G layer was used for the analysis since it provided

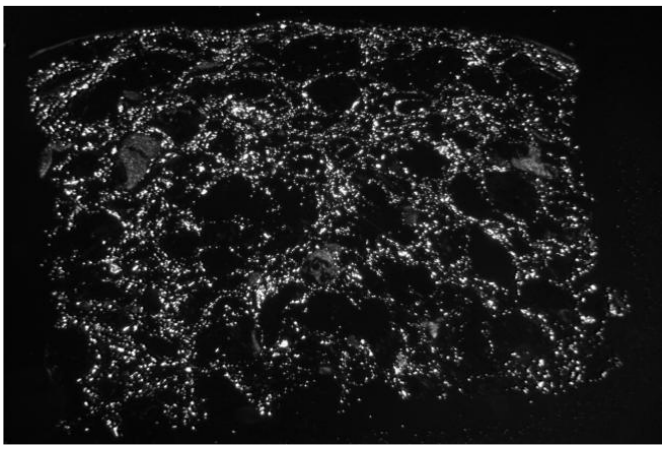

Figure 1 Decomposition into RGB – R layer.

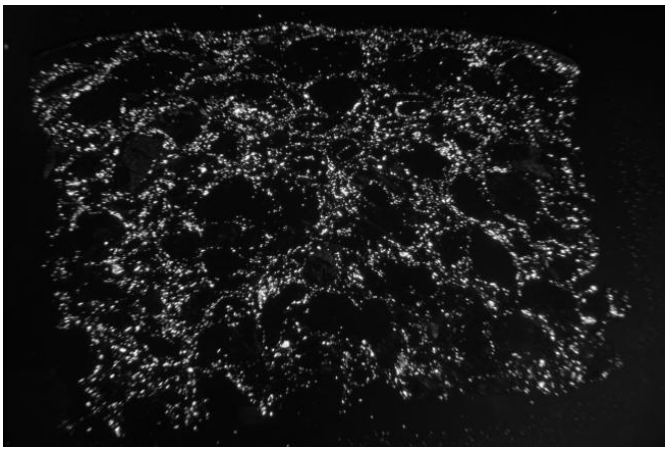

Figure 2 Decomposition into RGB – G layer.

the lowest blur and the highest contrast, thus it was selected as the most appropriate for the analysis. Fig. 1, 2, and 3 show decomposition into individual RGB layers.

Data were statistically processed by the Kruskal–Wallis one-way analysis of variance, a modification of the Tukey's test known as the Tukey's HSD was used **(Litschmannová, 2011)**.

RESULTS AND DISCUSSION

The results of the experiment were focused on assessing the suitability of determination of fiber using microscopic methods. The choice to detect fiber as an anisotropic (birefringent) structure by a polarization microscope was found to be more appropriate than using a light microscope where it is necessary to identify fiber on the basis of pink color. **Vorlíčková (2010)** also reports staining by hematoxylin-eosin as suitable for polarization microscopy.

The selected method was applied to examine samples with different amounts of added bamboo fiber. These results were compared with a control product in which no fiber was used. In the control sample, as well as generally in meat products, anisotropic structures were also detected. Their specific occurrence was expected with regard to the use of native spices, the source of which are in particular cell walls of plant cells. Between the control sample and samples 1 and 2 there was demonstrated a statistically significant difference ($p < 0.01$).

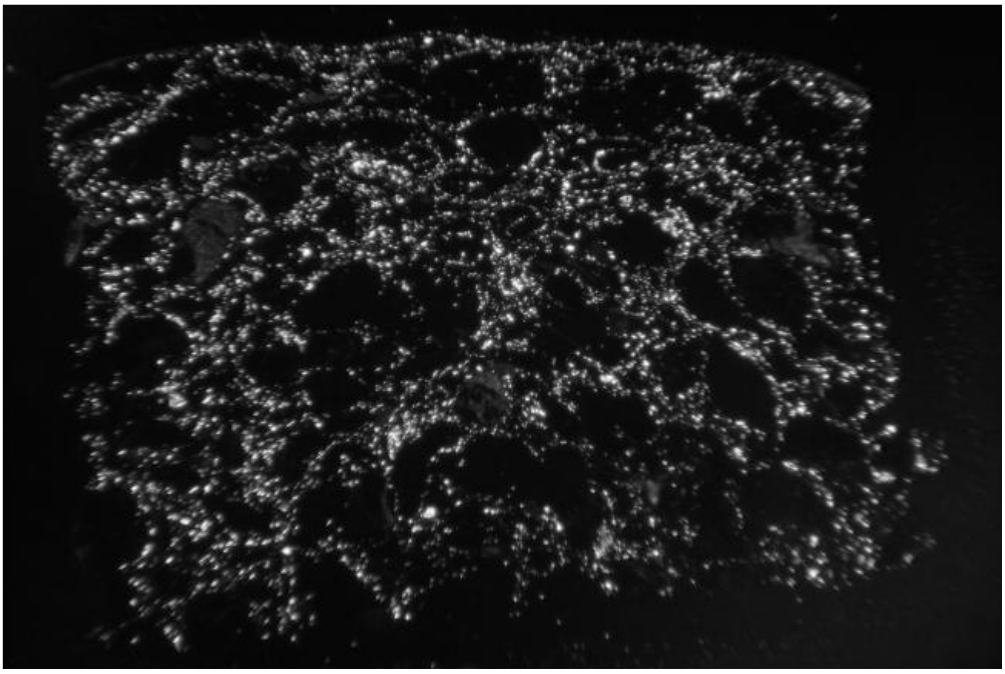

Figure 3 Decomposition into RGB – B layer.

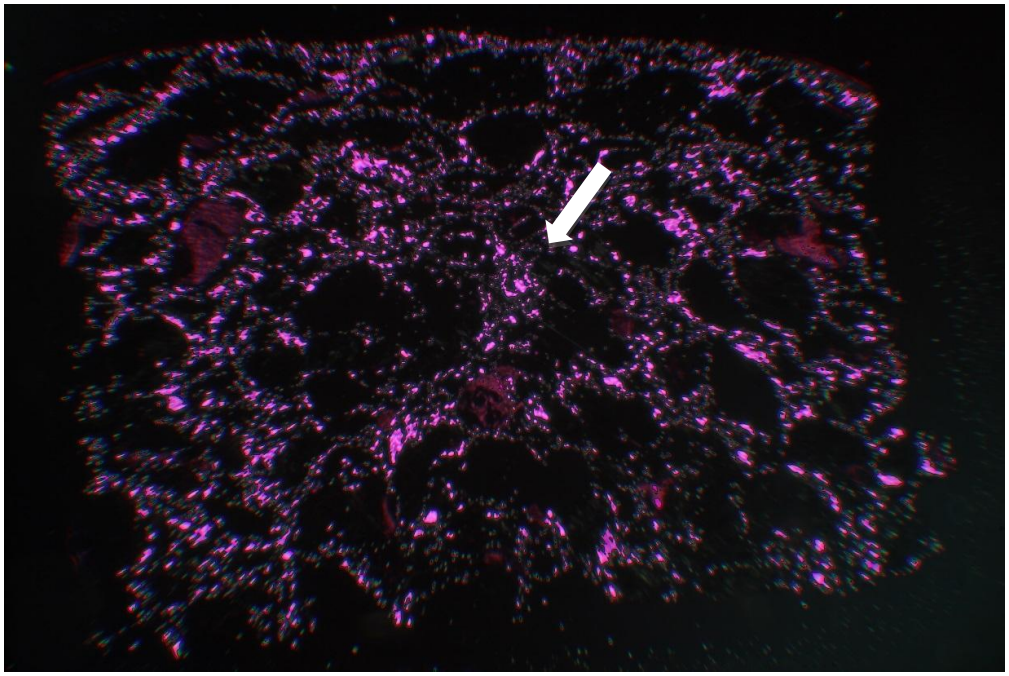

Figure 4 Analysis of fiber in the ACC Image Analyser 6.1.

Table 1 Results of measurement of fiber area [%] versus time after drying.

Day	Sample					
	Control	SD	Sample 1	SD	Sample 2	SD
1	0.086^{abcd}	0.04	13.99^{abcd}	1.66	19.90^{abcd}	3.70
7	0.013^{af}	0.01	8.63^{a}	1.30	10.21^{ae}	1.22
14	0.019^{bg}	0.01	7.10^{b}	1.45	12.49^{b}	2.45
28	0.012^{ce}	0.01	8.22^{c}	2.27	14.17^{ce}	2.38
42	0.050^{defg}	0.03	8.97^{d}	1.56	12.76^{d}	2.34

$^{a, b, c, d, e, f, g}$ – within columns, similar superscript letters indicate significant differences (p <0.01)

A difference at the significance level of p <0.01 was also between the samples 1 and 2. The used method can demonstrate the presence, absence, and quantity of fiber in the sample, regardless of use of spice in the product. The study further evaluated the area of fiber depending on the duration of drying.

Table 1 shows the values of fiber area for individual days of drying. On the first day of curing of the model meat product, the samples contained the highest amount of water. For this reason, the percentage of fiber measured in the section was high. This corresponds to the ability of fiber to hold water and swell, as reported by **Dhingra et al. (2012)**. From the seventh day on, however, due to release of water from the model products, rapid reduction in the area of fiber occurred, which can also be expected to be accompanied by fiber volume reduction. The fact that meat products release water and shrink in volume at the beginning of the drying phase is also confirmed by **Heinz and Hautzinger (2007)**. These authors also state that, depending on the type of meat and the size of meat pieces, the weight to can drop to 45 – 35% of its original weight just after one day, and to 30 – 20% of its original weight after two days.

From the seventh day, on the contrary, a slight increase in the area of fiber began to occur. This was caused by persistent drying of the samples, however, the fiber "held" the water bound, thus the volume of fiber was not being reduced significantly any more, while the area of other ingredients in the meat product in the section was still decreasing and thus the area of fiber in the section increased in proportion to the rest. In the control sample, no significant changes in the area of anisotropic structures occurred during drying and storage. This result points out that there is no change in volume of spices and other present anisotropic structures, possibly because they are not involved in the drainage system. Between samples analyzed on the 7th day of product drying there was no statistically significant difference found between the model product 1 and 2. Up to this day area of fiber was also being reduced. The reduction in sample 2 in contrast to sample 1 could occur due to the fact that fiber creates a three-dimensional network. The network acts as a drainage system through which the water gets better from the product to its surface where it evaporates **(Kameník, 2012)**. It can be stated that the addition higher by 1% resulted in 10% higher water loss. Water bound in the fiber evaporated more easily and the total area of fiber decreased, which can be used to shorten the drying time. Between individual concentrations sampled on the 14th, 28th, and 42nd day there was a statistically significant difference detected. From the 14th day on, the sample with higher concentration achieved higher resulting values of fiber area with statistically demonstrable difference (p <0.01). This points to the continuing ability of fiber to hold water in the product.

CONCLUSION

Based on the results obtained, we cannot doubt the possibility of using polarization microscopy for detection of fiber. Between the control sample and samples with added fiber, there were statistically significant differences in the content of anisotropic structures. Detection of fiber was possible already from the addition of 2%.

Drying also affected the total area of fiber. Until the seventh day after the product was cured, there was a significant loss of water, namely 38% from the sample with 2% bamboo fiber and 49% from the sample with addition of 3%, thus the reduction of area of fiber in the sample decreased as well. From the 14th day of storage, a small difference in the area of fiber was recorded. After a rapid release of water from the sample at the beginning of ripening, gradual drying occurs. During the drying phase, the total area of the meat product was decreasing and the analyzed area of fiber was slightly increasing because of the water-holding capacity of fiber. This can be used to enhance the economic profitability of meat products.

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