Histometric Evaluation of Meat Products – Determination of Area and Comparison of Results Obtained by Histology and Chemistry

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Abstract

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Histological examination of meat products enables direct identification and differentiation of the individual components. Image analysis is described in literature as a method that gives objective and accurate results comparable with chemical assay findings. The aim of this study was, therefore, to establish a procedure for the quantitative evaluation of meat products based on histological examination. For this reason, bone fragments in poultry meat products were detected. The technique used included the preparation of the mounts (staining with alizarin red), the application of digital photography, processing and analysis of the micrographs (ACC program, Image Structure and Object Analyser, version 4.0). The results were confirmed by comparison with chemical analyses determining the calcium levels by atomic absorption spectrometry. The correlation of both methods can be expressed by the coefficient of 0.78. A modification of the procedure of image recording and analysis was necessary to achieve this result.

Keywords: image analysis; histological evaluation; meat products; bone fragments

Microscopy and histology belong to the oldest methods used for the analysis of foodstuffs in order to detect their contamination or intentional adulteration. With meat products they have been used since 1910. The object is usually a qualitative examination, i.e., the detection of the presence of the individual tissues, and the evaluation of their acceptability or suitability for the given product. The development of food-processing histology brought about considerations of its possible use for the evaluation of the individual components in the product on the quantitative basis. The fact that, when examining more numerous histological specimens from different parts of the product, a common image is found was considered to be the basis for the possibility to evaluate the relations between the quantities of the individual components of the product. The quantities of the individual components of the product used to be initially described verbally. Later, however, the contents were expressed in percents and this fact initiated the actual start of the so-called histometry of the meat products. Some authors proposed the use of a grid-divided counting ocular or the projection of the histological image onto a grid area. It was found that 6 histological sections are sufficient for the purpose of the meat product evaluation because a higher number of sections does not

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considerably lower the variance of the sample on the statistical processing. Histometry of the meat products developed through thorough analyses and the proposal of objective procedures of the sample preparation and staining, and the evaluation of the results (Prändl 1961).

A quantitative examination can be performed as:

– a rough study expressing the frequency of occurrence verbally (prevailing, considerable, medium, moderate, negligible, and sporadic) or by graphic symbols such as +, ++, +++, etc.

– a precise expression of the results (obtained by histometry) on the basis of the data on the number of particles and the areas of objects measured and the sections evaluated. It is then presented as the number of objects per one section or 1 mm^2 of the section, the content of one tissue in percents (computed from the ratio of the areas of objects and the total area of the section) or the proportions of the individual objects (components, tissues) in percents.

Studies on the possibility to use histological methods for the quantitative determination of the meat product components as well as on the relations between the histological composition and chemical analyses were published in a number of papers in seventies and eighties of the last century. It was, for example, Koolmees and Bijker (1985) who discussed the advantages and disadvantages of the use of histometric and chemical methods for the determination of the percentage of collagen fibrous tissue in meat products. In the opinion of the above-mentioned authors, both methods were of the same value and had some limitations. Histometry, however, provided a more complete view of the composition of the product. Methods of histometry and planimetry were also used to determine the percentage of emulsion in model samples (Schingnitz & Hildebrandt 1985). There exist specialised books and papers describing the use of image analysis for the evaluation of meat and meat products as a method providing objective and precise results comparable to the data found by chemistry. The above-mentioned references include, for example, the objective evaluation of the marbling of meat (Albrecht *et al.* 1996) or the measurements of a number of parameters of muscle fibres suitable for the meat quality control (Buche & Mauron 1997). Image analysis was used to determine the quantity of soya protein in liver paté (Boutten *et al.* 1997) as well as the contents of collagenous and elastic fibrous tissues and of bones in meat products (Hildebrandt & Hirst 1985), i.e., of tissues which affect the quality of the product.

The presence of bone tissues in products results directly from the use of specific raw materials such as mechanically produced (separated) meat. This raw material in meat products brings about some hygienic risks and deteriorates the quality parameters. The use of separated meat is currently a topical problem. A number of methods are employed to confirm even minute quantities of bone tissues in more or less finely ground material of meat products. These are basically direct and indirect methods. Indirect methods are based on chemical analyses determining the concentration of calcium using, for example, KOH, atomic absorption spectrometry or the conversion of the ash content. Direct methods (histology, radiography) provide information on the quantity of bone tissue as well as the size, shape and location of the bone fragments in the product. The quantity and size of the bone fragments depend, of course, on the quantity of the raw material used and its quality which is influenced by the way of the preparation (source material, type of machine).

Aim

The aim of this study was to establish a procedure for the use of image analysis for the quantitative evaluation of meat products on the basis of histological examination as an alternative to chemical analysis. For the application of image analysis of histological samples, we selected the determination of bone fragments. The presence of bone fragments is in a close association with the use of a specific material (i.e., separated meat); otherwise, they occur only exceptionally. Separated meat is a common raw material for the production of poultry meat products. The bone tissue can be selectively stained. When evaluating the results, it is possible to a certain degree to apply the requirements of the technical norm for the mechanically separated poultry meat as well as the published data.

MATERIALS AND METHODS

Samples of the total of 29 different poultry products were examined (e.g., sausage, salami, minced meat). In all, 4 samples from different areas of each product were collected and processed using the paraffin embedding technique. Slides were stained with alizarin red (i.e., the selective staining for the bone tissue in which calcium reacts with the dye to give a bright red colour; other components are stained to various shades of blue to green colour or not at all).

Image scanning and the subsequent analyses were made using 4 selected slides (one of each sample). Images were primarily obtained by the method of the consecutive recording of the slide (procedure A). Using the lowest possible magnification of 32×, the documentation consisted of an average number of 36 images (in the dependence on the size of the slide). Images were processed separately and the results were summarised. Such a procedure was rather time-consuming. We therefore made a modification (procedure B) and used a scanning device enabling to record the whole slide at the same time and to work only with 4 images per one sample. The proper image analysis was also modified. The results of both procedures were then compared with those ones obtained by chemical analysis. Image preparations, image analyses, and computations were made according to the following scheme (Table 1).

Image analysis was performed using the ACC program (Image Structure and Object Analyser 4.0, by SOFO firm, CZ).

Procedure A. It is necessary to calibrate photometrically and transform the values of pixels in order to ensure the correct identification of the objects; the process being automatic and employing the pre-selected parameters of colour and brightness. It is preferable that minute objects be removed because of no importance for the final evaluation, the prolongation of the analysis, and the question concerning their classification into the category of bone fragments. Minute objects are similar to bone fragments in colour. They, however, originate in faults of the optical system at the borders of the mass and the empty spaces of the section. The proper analysis included the measurement of the areas of the selected objects and of the areas of the mass of the section without empty places within the section.

Table 1. Procedures of sample processing

	A	B
Image recording	1. a digital camera Olympus C-2500 (resolution of 1600 × 1200 pixels) 2. automatic program, highest image quality 3. Jenaval 250 – CF microscope, objective 3.2 (32× magnification)	Film scanner Nikon LS-30 resolution of 2700 dpi
Image correction	Photometric calibration Transformation of values of pixels Determination of the scale with regards to the resolution used	Photometric calibration Transformation of values of pixels Transformation from colour to a black-and-white image showing areas of red colour
Analysis	Identification of objects (according to the given parameters of colour and brightness) Removal of objects smaller than 30 pixels (183 μm^2) Analysis – area of objects – area of mass	Identification of objects (using only the brightness representing the intensity of red colour) Determination of the area of all tissues and the coefficient comprising the total area and intensity of staining of bone tissues without evaluating parameters of individual objects
Computations	Division into classes according to the size Total area of the bone tissue, section and mass, respectively Ratio of the area of the bone tissue and mass of the section = bone tissue content in %	Ratio of the area of bone tissues and the coefficient determining the content of bone tissues = level of calcium

Procedure B. It differs in the method of digital image recording which results in a lesser image degradation due to a simpler optical system and the principle of object identification employing the brightness of a black-and-white image. It does not work with colour images all the time as the procedure A because images are transformed to black-and-white pictures in which the areas of calcium are marked by the intensity of the white. The content of calcium can be evaluated even by measuring the intensity of the object staining. The method was proposed to determine the content of calcium and not to evaluate the bone fragments quantitatively.

The values of the bone tissue content in % in the mass of the section obtained by procedure A and the calcium content in % in the mass of the section obtained by procedure B were compared with the results of chemical analysis using the coefficient of correlation *r*.

$$r = \frac{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

where: x_i – value of the content of calcium determined by chemical analysis

y_i – content of bone tissue and calcium determined by histology using procedures A and B, respectively

i = 1…n

n – number of samples examined

Chemical analyses were based on the determination of calcium using the method of atomic absorption spectrometry.

RESULTS AND DISCUSSION

The data on the area of the individual bone fragments found by procedure A are a prerequisite of further computations. On an individual basis, they can be used to classify and evaluate the samples according to the number of bone fragments in the individual classes. Comprehensive histograms can be drawn using the results (Figure 1).

The contents of bone tissues found by procedure A varied from 0.28 to 5.32% in the individual samples re-computed for the mass of the sample. These values are relatively high because, e.g., Koolmees *et al.* (1983) found the content of bones to vary from 0.8 to 3.8% when performing histometry of separated poultry meat in the dependence on the raw material used (whole bodies or only some parts) and the type of the separating machine.

It is common to determine the contents of bone tissues and thus the use of separated meat on the basis of calcium determination. The content of calcium is also related to the way of obtaining separated meat and the type of the raw material used. In the products examined, the levels of calcium found by atomic absorption spectrometry varied from 0.025 to 0.108%. Using procedure B, calcium levels of 0.014 to 0.142% were found. There are still no requirements given in our legislation on the meat products. According to the

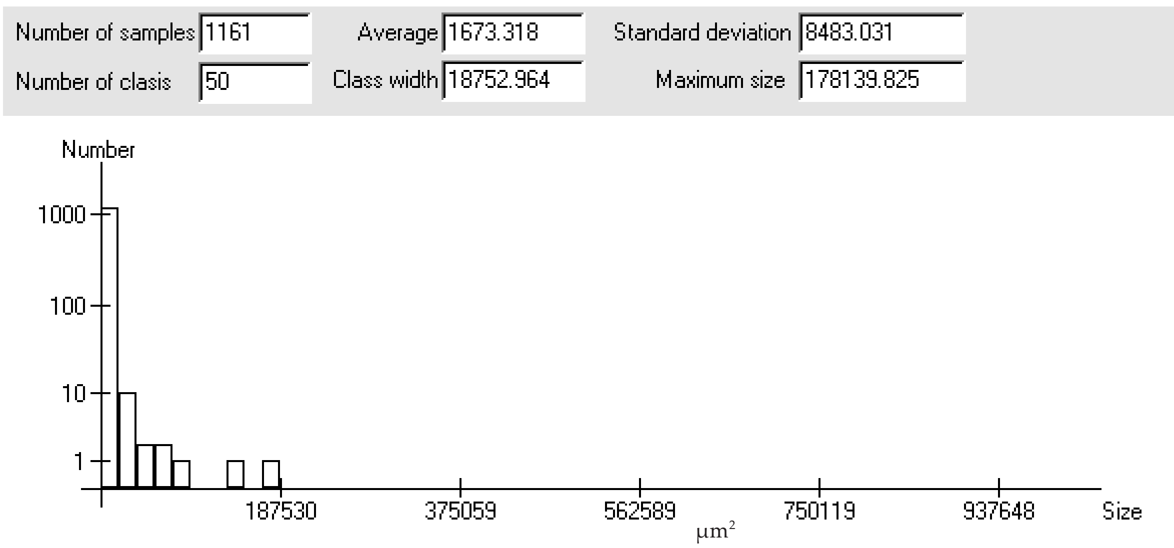

Figure 1. Histogram – sample 03

Table 2. Correlation of histological and chemical analyses

			Coefficient of correlation	
Histology	Procedure A	Content of bone tissues (in mass of the section)	0.41	Chemistry
	Procedure B	Content of calcium (in mass of the section)	0.78	

Technical regulation on separated meat (2000), the maximum level of calcium is 0.3%, i.e., a much higher level than the published data amounting to, for example, 0.07 to 0.025% (Koolmees *et al.* 1983). Austrian legislation (1993) gives the limit for the suspected use of separated meat as 200 mg/kg of calcium, i.e., 0.02%.

The modification of the procedure lowered considerably the time consumption of the whole examination and also proved to be a positive factor increasing the correlation between the histological and the chemical analyses (Table 2). The coefficient of correlation between the results of procedure A and the chemical analysis data was 0.41. The value of 0.78 of the coefficient of correlation (cf. procedure B) in 24 samples examined can be considered as a statistically significant ($\alpha = 0.05$). Hildebrandt and Hirst (1985) examined 51 samples and compared results of image analysis with those of chemical methods and found the image analysis to be an adequate alternative to chemistry (coefficient of correlation higher than 0.9).

Conclusion

We established a procedure for the histological determination of the bone tissue content in meat products using image analysis. The results were confirmed by comparisons with the calcium levels determined by chemical analyses using atomic absorption spectrometry. The correlation between both methods can be expressed by the coefficient of 0.78. A modification of the procedure of the image recording and analysis was necessary to obtain this result. This result, however, makes it possible to obtain more objective results because the correctness of histological examination depends on the number of slides examined. Automatic image analyses are only possible when examining slides of standard staining. Following the processing of a larger collection of samples and eventual further modifications of the method, we intend to evaluate the results again. We suppose that these procedures may also be used for the evaluation of other components of the meat products.

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Souhrn

Tremlová B., Štarha P. (2003): **Histometrické hodnocení masných výrobků – stanovení plochy objektů pomocí obrazové analýzy a srovnání výsledků histologického a chemického vyšetření**. Czech J. Food Sci., **21**: 101–106.

Histologické vyšetření masných výrobků umožňuje přímou identifikaci a diferenciaci jednotlivých součástí. Obrazová analýza je v literatuře popsána jako metoda dávající objektivní a přesné výsledky, které jsou srovnatelné s chemickým vyšetřením. Cílem práce je vytvoření postupu pro využití obrazové analýzy ke kvantitativnímu hodnocení masných výrobků na základě histologického vyšetření jako alternativního postupu k chemickým analýzám. Proto byly v masných výrobcích detekovány kostní úlomky. Postup zahrnoval cílené barvení (alizarinová červeň), zhotovení digitálních snímků a jejich zpracování a analýzu pomocí programu ACC – Image Structure and Object Analyser, verze 4.0. Výsledky jsme porovnali s chemickým stanovením vápníku atomovou absorpční spektrometrií. Korelace obou metod je vyjádřena koeficientem 0,78. Pro získání tohoto výsledku byla nutná modifikace postupu ve fázi snímání obrazu a analýzy obrazu.

Klíčová slova: analýza obrazu; histologické vyšetření; masné výrobky; kostní úlomky

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