Histometric Evaluation of Meat Products – Determination of Size and Number of Objects

BOHUSLAVA TREMLOVÁ[1] and PAVEL ŠTARHA[2]

[1]Faculty of Veterinary Hygiene and Ecology – University of Veterinary and Pharmaceutical Sciences, Brno; [2]Faculty of Mechanical Engineering – Brno University of Technology, Brno, Czech Republic

Abstract

TREMLOVÁ B., ŠTARHA P. (2002): **Histometric evaluation of meat products – determination of size and number of objects**. Czech J. Food Sci., **20**: 175–180.

In the framewort of the quantitative histologic evaluation of poultry products, the size and the number of bone fragments have been determined using the image analysis. Bone fragments were identified by their colour and analysed automatically. The samples contained 135 to 2167 bone particles the length of which varied from 5 to 2088 µm. Comparing products of the same kind, we found differences in the contents of bone fragments; this fact was possibly due to inadequate observance of the technological procedure by some producers.

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

The histological examination of meat products is a specialised analysis enabling direct differentiation and identification of individual components of animal and plant origins as well as providing information on their distribution, size and number. This method of examination of meat products is not common in the Czech Republic. In a number of European countries (Austria, Germany, France, the Netherlands), however, it belongs to the aimed examinations listed in the food-hygiene legislation and included in analytical methods of the foodstuff evaluation. The result of such an analysis can be a decisive factor in the evaluation of the adherence to the technological procedures and of some ways of the foodstuff adulteration.

It is usually a qualitative examination, i.e., the detection of the presence of individual tissues and the assessment of their admissibility or suitability for the product. In some raw materials for meat products (such as mechanically separated meat, plant additives), it is important both to identify and to evaluate quantitatively their proportions. The identification of the individual components depends on the morphological characteristics described, and on the stainability using different staining procedures.

The quantitative examination is based on the determination of the number and of the size of the analysed components, respectively, relative to the area of the sample. If there are not many counted components within the viewing field, it is easy to determine their number throughout the area using only manual procedures. To avoid errors in counting more numerous components it is useful to employ the so-called counting eyepiece with a square-shaped screen enabling to scale the viewing field down or up. In any case, it is necessary to measure the area of the whole viewing field or square using the eyepiece and an objective micrometer, and to compute the area of the section. The above-mentioned aids are also used to measure the size and the area of the individual objects important for the result of the examination. This procedure needs much work because, according to our practical experience, it is necessary to evaluate at least 6 sections of each sample, each section being represented by several viewing fields

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. 162700005), and Project of the CEZ: J 22/98: 261100009.

to obtain objective results. Despite many convincing studies published, the extensive use of such an evaluation in practise is limited by the demanding manual measurement and counting. This procedure is described in the German Official Collection of Analytical Methods (1989). It is, however, not used in practise. The possibility of rationalisation and objectification of histometry using special computer programs of image analysis has been confirmed by HILDEBRANDT *et al*. (1977, 1978).

The image analysis helps to describe quantitatively and to specify the image information obtained by macroscopic or microscopic scanning. It also facilitates detailed comparisons of various samples, a precise processing of information, and different ways of result presentation. The input is via a digital camera; it is, however, possible to process also standard photographs following their transformation to the digital format. A great advantage is the possibility to compare objects scanned currently from the microscopic slide with those obtained previously. Data can be evaluated statistically. The system of image analysis includes a number of procedures for the image processing such as:

– determination of co-ordinates, linear measures, angles and areas
– automated and interactive methods to create overlays to identify the objects
– geometric, physical, and photometric methods of object analysis
– image stacking using algebraic procedures (addition, subtraction, multiplication, ...)
– image stacking to form mosaics
– geometric procedures with the image (rotation, change of size and proportions, ...)
– standard tools to change the contrast, brightness, saturation, and balance
– image sharpening by alteration of frequency characteristics
– help tools (histograms, zoom, crop, rotation, scale, …).

The analysing program has its indisputable advantage in the automatic measurement and counting of all objects which have been pre-selected on the basis of certain parameters such as colour and brightness. The size and the area of the individual selected objects can be naturally determined on the basis of manually performed location. In such a way, the measured objects can be labelled with the data determined.

The measurement of different parameters using the image analysis is in the instance of foodstuff samples often in relation to important sensory and technological characteristics of foodstuffs and food raw materials. For example, the morphology of fat crystals plays an important role for the properties such as spreading, stiffness and fineness of final products (margarine, butter). Parameters such as the size and the shape of crystals were measured using the semi-automated process of analysis of the image obtained by electron microscopy (HEERTJE & LEUNIS 1997). Changes in the diameter and the length of sarcomeres during heating can be responsible for altered textural characteristics of beef (PALKA & DAUN 1999). Similar parameters were determined in relation to the ability of meat to bind water under different conditions of pH (RAO *et al*. 1989). Bone fragments in final meat products were evaluated using the image analysis by, e.g. HILDEBRANDT and HIRST (1985). BIJKER *et al*. (1983) evaluated the size of components and their numbers in mechanically separated meat in relation to the separator and raw materials used.

We selected the bone fragments with the aim of applying the image analysis in the histological examination of meat products (of poultry meat). Their presence is associated with the use of specific production of raw materials (mechanically separated meat), otherwise they are only occasionally and sporadically present. In the products from poultry meat, the separated meat is a common raw material and, therefore, the bone fragments are supposed to be present. This tissue can be rendered more distinct by selective staining procedures.

It was the aim of the present study to use the image analysis in the quantitative histologic evaluation of meat products. The paper includes the determination of the size and the number of objects (components), and the use of such data for the computation of the contents of the individual components of the meat products.

MATERIAL AND METHODS

Samples of a total of 26 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 by alizarin red (i.e., the selective staining for the bone tissue). Image scanning and the subsequent analysis were made using 4 selected slides (one of each sample) of a corresponding staining quality (Table 1).

The image analysis was performed using the ACC program (Image Structure and Object Analyser 4.0, by SOFO firm, CZ). It is necessary to calibrate photometrically and to transform the values of pixels in order to ensure the correct identification of objects; the process being automatic and employing the pre-selected parameters of colour and brightness. The preparation of our own templates for the selection of objects within the image is thus a prerequisite for the image evaluation. It is preferable that minute objects be removed because of no importance existing for the final evaluation, prolongation of the analysis and questions about their classification into the category of bone fragments. The proper analysis included the measurement of the size of the selected objects, the determination of the number of objects, and the area of the section and

Table 1. Procedure of image processing and analysis

Image recording	a digital camera Olympus C-2500 (resolution of 1600 × 1200 pixels) automatic program, highest image quality Jenaval 250 – CF microscope, objective 3.2 (32× magnification)
Image correction	photometric calibration, transformation of values of pixels determination of the scale with regard to the resolution used
Analysis	→ identification of objects (according to the given parameters of colour and brightness) → removal of objects smaller than 30 pixels → analysis total number of objects size of objects area of the section and mass, respectively
Computations	→ division into classes according to the size → total area of the section (mass, respectively) → number of particles per mm^2 of the area of the section (mass, respectively)

mass, respectively, in the sample (i.e., the area without empty places within the section). Each image can be labelled with its text documentation, while the operations performed by the image analyser are automatically recorded. A total of 854 images were processed. The results of the study are documented on compact discs.

RESULTS AND DISCUSSION

Experienced evaluating persons are able to distinguish bone fragments in histological specimens stained routinely with hematoxylin and eosin. The image analysis, however, needs selective staining procedures differentiating distinctly the bone fragments from other components because the identification is based on the selection of objects according to colour and brightness. This requirement was met by staining the specimens using alizarin red. The stain forms chelates with calcium ions. Bone fragments stain bright red while other components show shades of blue to green colour. Some components of the plant origin do not stain at all. Other procedures were also mentioned in literature, e.g., modified staining according to Kossa by HILDEBRANDT and HIRST (1985) or staining by Azan according to KÖNIGSMANN *et al.* (1980). In processing the images automatically by the image analyser, it is very important to use perfectly stained specimens because any deviation from the colour scheme standard leads to incorrect results.

For the identification of objects, we prepared an overlay using a selected standard of a bone fragment stained and defined as to the shade and the intensity of colour. Using this overlay, it was possible to determine automatically the objects of the same parameters within the whole image.

The image analyser identified in individual meat product specimens bone fragments amounting to 135 to 2167 particles. The amount of bone fragments in the product depends on the proportion of the separated meat. High numbers of minute particles can also be found in specimens differing from the standard staining. It is possible in these cases to select the objects individually. This is, however, a change in the conditions which should be kept identical in all measurements.

For a rough examination, the results can be expressed as the number of particles or the mean number of fragments per one section (under the conditions of examination of not equal numbers of sections from each sample). There are, however, differences in the total area and, therefore, a more precise procedure needs to be used for its measuring and for the expressing of the results as the number of objects per mm^2 of the area of the section or mass of the sample, respectively. All these results then have their informative value only when compared to a standard sample. Because the standard sample, was not available, we selected and compared products of the same kind from the collection in which the same composition (Tables 2 and 3) was to be expected.

From both tables it is clear that there is no difference in results expressed in the relation both to the area of the section and the mass without free spaces. From the prac-

Table 2. Histometric evaluation of samples (spa sausage)

Sample	Number of particles			
	total	per section	per mm^2 of section	per mm^2 of mass
05	525	131.25	1.21	1.96
11	672	168.00	2.21	5.00
33	497	124.25	2.09	2.79
53	779	194.75	2.92	6.42

Table 3. Histometric evaluation of samples (Bavarian backed meat loaf)

Sample	Number of particles			
	total	per section	per mm^2 of section	per mm^2 of mass
05	525	131.25	1.21	1.96
07	781	195.25	2.93	4.17
40	586	146.50	1.84	3.13
55	270	67.50	1.29	1.72

tical point of view, it is easier to use the automatic measurement of the mass using the overlay than to mark the whole section manually. The variability of the results of the comparison of the products of the same kind may be due to the characteristics of the raw material (i.e., the mechanically separated meat). It is, however, the effect of the observance of the production procedure (i.e., the proportion of the mechanically separated meat). There are, however, not sufficient data for the purpose of the evaluation of the importance of differences and for the determination of the acceptable range of values.

The size of the objects (length) varied from 5 to 2088 µm. Similarly to other authors (BIJKER *et al*. 1983), we found that the majority of the objects had the size of up to 1000 µm. In our case, it was up to 500 µm (Table 4). The size of the bone fragments depends on the raw material used for the production of the separated meat as well as on the type of the separating machine and, above all, on the adjustment and correct function thereof. According to the Technical regulations PN 27/2000 – Mechanically separated poultry meat, poultry oven-ready product,

Table 4. Length of bone fragments

Sample	Number of particles				
	up to 100 µm	up to 500 µm	up to 1000 µm	up to 2000 µm	up to 3000 µm
01	649	49	2	0	0
02	837	58	0	0	0
03	1067	95	0	0	0
04	1098	136	11	3	1
05	346	171	6	1	0
06	100	22	3	1	0
07	679	102	0	0	0
08	118	17	0	0	0
10	1180	136	7	1	1
11	526	147	1	0	0
13	1108	77	3	0	0
16	1923	226	14	4	0
21	379	65	5	0	0
26	374	62	0	0	0
28	142	31	1	0	0
32	850	73	2	0	0
33	492	5	0	0	0
40	531	52	3	0	0
42	575	78	3	1	0
43	413	38	6	0	0
53	700	77	2	0	0
54	333	68	2	0	0
55	236	33	1	0	0
58	315	80	0	0	0
59	338	36	2	0	0
64	225	28	2	0	0

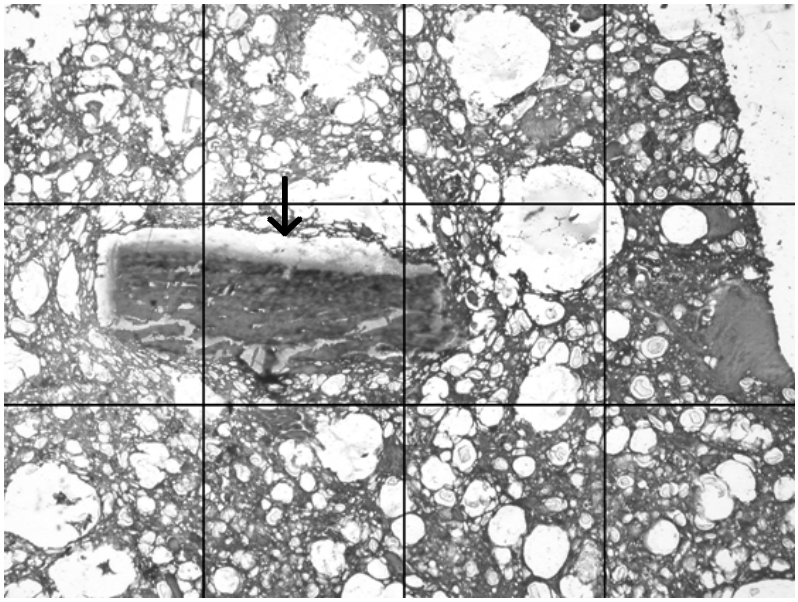

Fig. 1. Overlay for magnification 32×, sample 10 (arrow – bone fragment)

Xaverov holding, 90% of all particles must be smaller than 1 mm and no one may be over 2 mm. The above mentioned requirements apply also to the meat products from this raw material. With regard to this, two samples failed. From the practical point of view, a quick and rough examination using an overlay such as a net of 1 × 1 mm (Fig. 1) would be sufficient.

Conclusion

Using the image analysis, we examined 26 samples of poultry meat products with the aim to determine the number and the size of bone fragments and thus to evaluate the products. Bone fragments were identified by their colour and analysed automatically. The samples contained 135 to 2167 bone particles the length of which varied from 5 to 2088 μm. In two samples, we found fragments larger than acceptable according to the Technical regulations on mechanically separated meat. Comparing products of the same kind, we found differences in the contents of bone fragments; this was possibly due to an inadequate observance of the technological procedure.

The use of the image analysis, as far as the histometric examination of the meat products is concerned, speeds up the work. Objective results, however, can be obtained only under conditions of preparing and staining slides of high quality and standards. Manual corrections of the image and re-classification of some incorrectly identified objects are possible. This results, however, in a longer time necessary to process the sample and can bring about further errors. Results can be more objective if higher number of sections of the sample are examined. When it is possible to record a larger area of the slide or, in particular, the whole of it, we can employ a smaller number of slides.

From the practical point of view, the use of histometric examinations of meat products lacks a legislation basis. Controlling, however, the adherence to the norm on mechanically separated meat (i.e., by determining the size and shape of bone fragments), it is possible to obtain precise results using the image analysis. Rough examinations can be made using a simple overlay.

References

ALBRECHT E., WEGNER J., ENDER K. (1996): Eine neue Methode zur objektiven Bewertung der Marmorierung von Rindfleisch. Fleischwirtchaft, **76**: 95–98.

Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG, Bestimmung der geweblichen Zusammensetzung von Fleisch, Fleischerzeugnissen und Wurstwaren L 06.00.13, Beuth Verlag Berlin und Köln, 1989.

BIJKER P.G.H., KOOLMEES P.A., VAN LOGTESTIJN J.G. (1983): Tissue composition of mechanically deboned pork (MDP). Meat Sci., **14**: 257–269.

HEERTJE I., LEUNIS M. (1997): Measurement of shape and size crystals by electron microscopy. Lebensm. Wiss. u. Technol., **30**: 141–146.

HILDEBRANDT G., HIRST L. (1985): Determination of the collagen, elastin and bone content in meat products using television image analyse. J. Food Sci., **50**: 568–570.

HILDEBRANDT G., KÖNIGSMANN R., KRETSCHMER F.-J., RENNER H. (1977): Die Eignung der Fernsehbild – Computer – Analyse zur histometrischen Kollagenbestimmung. Fleischwirtschaft, **57**: 689–697.

HILDEBRANDT G., KÖNIGSMANN G., BERNER H. (1978): Quantitative Fernsehbildanalyse wertmindernder Gewebe in Fleischerzeugnissen. Fleischwirtschaft, **58**: 1985–1986.

KÖNIGSMANN R., HILDEBRANDT G., SINELL S.-J. (1980): Quantitativer Knochenanweis in Fleischerzeugnissen mittels

Fernsehbildanalyse. I. Mitt. Arch. Lebensm. Hyg., **31**: 6–12.

PALKA K., DAUN H. (1999): Changes in texture, cooking losses and myofibrilar structure of bovine *M. semitendinosus* during heating. Meat Sci., **51**: 237–243.

RAO M.V., GAULT N.F.S., KENNEDY S. (1989): Variations in water-holding capacity due to changes in fibre diameter, sarcomere lenght and connective tissue morphology of some beef muscles under acidic conditions below the ultimate pH. Meat Sci., **26**: 19–37.

Received for publication September 19, 2002
Accepted after corrections October 14, 2002

Souhrn

TREMLOVÁ B., ŠTARHA P. (2002): **Histometrické vyšetření masných výrobků – stanovení velikosti a počtu objektů.** Czech J. Food Sci., **20**: 175–180.

Při kvantitativním histologickém vyšetření drůbežích výrobků jsme pomocí programu pro analýzu obrazu stanovili velikosti a počty kostních úlomků. Kostní úlomky byly identifikovány podle zbarvení a automaticky analyzovány. Vzorky obsahovaly 135 až 2167 kostních částic, délka částic se pohybovala od 5 do 2088 μm. Při porovnání výsledků u vzorků stejného názvu byly zjištěny rozdíly, které mohou být důsledkem nedodržení výrobního postupu.

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

Corresponding author:

MVDr. BOHUSLAVA TREMLOVÁ, Ph.D., Veterinární a farmaceutická univerzita Brno, Fakulta veterinární hygieny a ekologie, Ústav hygieny a technologie masa, Palackého 1–3, 612 42 Brno, Česká republika

tel.: + 420 541 562 637, fax: + 420 541 321 230, e-mail: tremlovab@vfu.cz