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A COMPARISON OF HISTOLOGICAL AND CHEMICAL ANALYSIS IN MECHANICALLY SEPARATED MEAT

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Abstract

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The aim of this study was evaluation of quality of mechanically separated chicken meat (MSCM) samples obtained by three different separators, by means of a histological (qualitative and quantitative) and chemical examination. Histological examinations used Green Trichrome and Alizarine red staining. The examination was focused on the evaluation of muscle, fat, collagenous connective tissue, bone fragment and calcium content and on the degree of damage to the muscle fibres. Chemical analysis was focused on calcium-, fat- and collagenous connective tissue content. The product obtained by the separator 1 (hard separation) gave similar good results to the separator 3 (soft separation), while separator 2 (hard separation) gave worse results as for being bone fragments and calcium content. As demonstrated, the quality of the product obtained by the separator 1 has improved over the years. The results of the histological analysis were in accordance with the results of chemical analysis. For the quantitative determination of collagen, calcium and bone fragments, image analysis was used. In the present study, good correlation between quantitative histological analysis and chemical analysis was observed (0.673, 0.718 and 0.809, $\alpha = 0.01$).

mechanically separated meat, chicken, image analysis, histological analysis, chemical analysis

In Europe, consumption of processed or minced meat products increases by approximately 30%. But the more processed the product is the more difficult it is for the consumer to identify the nature of the meat raw material it contains. To improve transparency for the consumer, European Union Regulations require declaring the use of mechanically separated meat (MSM) in the list of ingredients on the label of the finished product (Sifre *et al.*, 2009).

MSM is a generic term used to describe residual meat which has been recovered or separated by the application of pressure or shearing forces to animal bones or poultry carcasses from which the bulk of the meat has been removed. This permits the recovery of most of the residual meat which would otherwise be difficult or uneconomical to acquire. The resultant MSM has the appearance of finely comminuted meat. MSM is used in a wide range of meat products either as a binding agent or as an inexpensive source of meat since it has good nutritional and functional properties (Day and Brown, 2001). Use of mechanical recovering systems has increased the utilization of poultry meat in further-processed products (Yuste *et al.*, 1999).

MSM is characterised by a pasty texture. This texture is due to the high proportion of pulverised muscle fibre residue and the presence of a significant quantity of partly destructured muscle fibres. The term used for this loss or modification of muscle fibre structure is "destructuration". Manufacturers specialised in the production of meat raw material and the purchasers of intermediate foodstuffs generally use evaluation of this more or less pasty texture when characterising raw material. Most of them quite naturally proceed by touch and observation of raw material samples when evaluating the degree of destructuration (Sifre *et al.*, 2009).

The recovery machines used to separate the residual meat from the bone can essentially be divided into two main types: those which exert pressure to force the meat to flow from the bones by means of a hydraulically powered piston, *hard separation*, and those which use an auger feed, *soft separation* (Crosland *et al.*, 1995).

The raw material requirements and its using are stated in the Decree of the European Parliament and the Council (ES) No. 853/2004 and in the Commission Decree (ES) No. 2074/2005 (Commission Regulations 853/2004 and 2074/2005).

The aim of the present study is the evaluation and comparison of quality of separated chicken meat given in different conditions. To achieve this aim, histological and chemical examinations for the qualitative and quantitative comparison of samples were used in the present study. This comparison was based on the evaluation of muscle tissue, adipose tissue, collagenous connective tissue, bone fragments and degree of damage to muscle fibres. Chemical analysis focused on calcium-, fat-, and collagenous connective tissue content.

MATERIAL AND METHODS

Samples

The samples used for this study originated from one chicken breed obtained in industrial conditions. The poultry was slaughtered at the operating slaughterhouses by means of a standard method. The samples examined include mechanically separated meat (MSM) obtained utilizing different types of machines (Tab. I). In our experiments, three different types of machines were used. Separator 1 is a machine that generates 3 mm minced meat by using a hydraulic system that removes meat from the bones at gentle pressure (25 bar). Separator 2 is a representative of the hard separation. It is a worm machine that works at pressure 25 bar. Bones are partly grinded and soft tissues are pressed through a sieve and the bone fragments are separated. Separator 3 represents the soft separation; it works at pressure between 5–16 bar. Soft tissue passes through apertures that are set to 2–3 mm.

I: *Identification and characteristics of the analysed samples*

Group No.	Product	Kind of raw material	Type of machine (pressure)
1	MSM	breast bone	SEPARATOR 1 (25 bar)
2	MSM	front back	
3	MSM	bottom back	
4	MSM	wings	
5	MSM	necks	
6	MSM	breast bone	SEPARATOR 2 (25 bar)
7	MSM	front back	
8	MSM	bottom back	
9	MSM	wings	
10	MSM	necks	
11	MSM	cut breast slices	SEPARATOR 3 (5–16 bar)

Chemical analyses

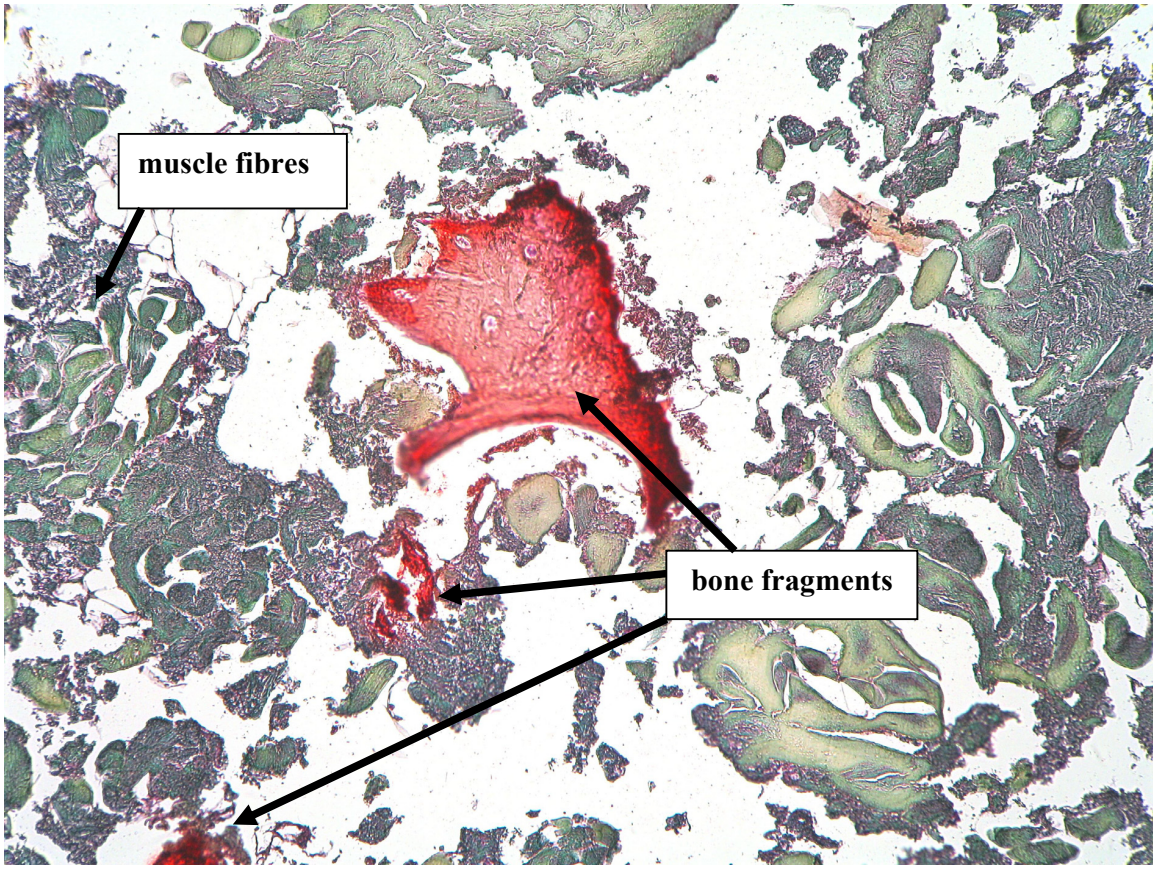
Chemical analyses of samples included determination of selected basic parameters. Net muscle protein content was obtained by subtraction of the collagen from the net protein content; collagen content - spectrophotometrically after hydrolysis of proteins to amino acids by recounting from the hydroxyproline content; fat – CSN, ISO 1443; calcium – the Atomic absorption spectroscopy (AAS); clean muscle protein – photometrically after the mineralisation. These chemical analyses were carried out in a certified laboratory (Accredited Laboratory with the Czech Institute for Accreditation under No. 1051).

Histological analyses

The histological examination was carried out at the Microscopic Laboratory of Food (Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences). Each sample (No. 1 to 11) is represented by a group of 21 samples examined by histological analyses. The aims of the qualitative and semiquantitative histological examinations were to determine the nature of sample, to diagnose the presence of different types of tissue and to assess the amount and the degree of the impairment in the original structure of each type of tissue. For this purpose, microscopic slides stained with Alizarine red (Fig. 1; Manual, 1994) and Trichrome green were prepared. For each stain, 10 sections of each sample (prepared from 4 paraffin blocks) were examined by a microscope. Individual parts of the samples were identified according to known morphological criteria and coloration. The qualitative evaluation is expressed verbally using the words expressing occurrence or contingency character of individual parts, in some cases is the evaluation semiquantitative.

Semiquantitative evaluation is expressed verbally using the following scale: prevailing, considerable amount, medium amount, moderate amount, negligible amount, and sporadic occurrence (Official Standard, 1989).

The quantitative histological determination of the collagen, bone and calcium content was performed by image analyses (Druckmüller and Starha, 2007), using slides stained with Trichrome green and Alizarine red. For each sample 10 sections were examined. After scanning the sections the results were obtained as follows: a photometric calibration of the slide was carried out to adjust contrast, brightness and colour. Then the area of the whole slide and image segmentation were calculated based on the stain used to identify the analysed objects, i.e. the area of collagen stained green and bone fragments stained red were measured. This method was previously used to determine bone tissue as described by Tremlova and Starha (2003). Not only area of the



1: *Microscopic slides of samples stained with Alizarine red and Trichrome green*

red coloured bone tissue but also the coloration intensity was taken into account for determination of the bone tissue. Value Collagen-IA presents the rate between substances identified as collagenous connective tissue to the total substance. Value Bones-IA presents the rate of the substance identified as bone fragments to the total substance. Value Ca-IA presents the rate of the values, where the substance is identified to be bone fragments with multiplicity of colouring to the total substance.

Statistical analyses

The statistical analyses included calculations of the relative amount of analysed objects and the weighted relative amount of analysed objects where weight is proportionate to the colour intensity of the analysed object. The results were compared with the results of the chemical analysis and correlations were calculated.

RESULTS AND DISCUSSION

Histological evaluation

Tab. II shows the results of histological analysis evaluated with respect to the type of the separator used. The way of recovery (separation principle, machine alignment, pressure) influences mainly the amount of bone tissue in the resulting material, the size of fragments of different tissue types, and the degree of damage to the muscle fibres. The amount of collagenous, muscle and fat tissue is also associated with the kind of raw material (bones, trimmings).

Even though separator 1 is a hard separator and separator 3 a soft separator, and they process different kind of raw material, their products gave (surprisingly) similar results. On the other hand, the machines working at similar pressure and with the same raw material (separator 1 and separator 2) gave different products. Stiebing (2002) reported that separator 2 does less damage to muscle tissue, but in the present study separator 1 gave better results than separator 2.

Tab. III shows the comparison of results of histological and chemical analyses. These are always mean values of 3 examinations per sample (chemical analysis). Separated meat obtained by using separator 1 (sample No. 1) showed net muscle protein, fat, collagen and calcium content similar to the trimmings obtained by using separator 3 (sample No. 11). Separated meat obtained by using separator 2 (sample No. 6) showed the highest content of collagen, fat and calcium but on the other hand, the lowest content of net muscle protein. The calcium contents ranged from 155 to 830 mg/kg and were beneath the set limit of 1 000 mg/kg (Regulation, 2005). This value was exceeded only in sample No. 6

II: *Comparison of different kinds of mechanically separated meat by histological examination*

Raw material (machine type) (sample No.)	Evaluated parameters				
	Muscle tissue	**Collagenous connective tissue**	**Adipose tissue**	**Degree of damage to the muscle fibres**	**Bone fragments**
Poultry bones (SEPARATOR 1) (1,2,3,4,5)	Skeletal muscle prevailing	Washy collagenous tissue moderate and cartilage sporadic	Clumps of adipose cells negligible	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses and fibres	More than 3 fragments in 3 samples (from 21 examined samples)
	Skeletal muscle prevailing	Washy collagenous tissue moderate and cartilage negligible	Clumps of adipose cells negligible	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses and fibres	More than 3 fragments in 1 sample (from 21 examined samples)
	Skeletal muscle prevailing	Washy collagenous tissue moderate	Clumps of adipose cells moderate	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses and fibres	Less than 3 fragments (from 21 examined samples)
	Skeletal muscle prevailing	Washy collagenous tissue moderate	Clumps of adipose cells negligible	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses and fibres	Less than 3 fragments (from 21 examined samples)
	Skeletal muscle prevailing	Washy collagenous tissue moderate	Clumps of adipose cells negligible	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses	More than 3 fragments in 1 sample (from 21 examined samples)
Overall evaluation	Skeletal muscle prevailing	Collagenous tissue moderate and cartilage sporadic	Clumps of adipose cells negligible	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses and fibres	3 samples contained bone fragments in negligible amount
Poultry bones (SEPARATOR 2) (6,7,8,9,10)	Skeletal muscle prevailing	Washy collagenous tissue and cartilage considerable	Clumps of adipose cells moderate	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses	More than 3 fragments in 16 samples (from 21 examined samples)
	Skeletal muscle prevailing	Washy collagenous tissue moderate and cartilage negligible	Clumps of adipose cells moderate	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses	More than 3 fragments in 9 samples (from 21 examined samples)
	Skeletal muscle prevailing	Washy collagenous tissue considerable, cartilage moderate	Clumps of adipose cells moderate	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses	More than 3 fragments in 8 samples (from 21 examined samples)
	Skeletal muscle prevailing	Washy collagenous tissue and cartilage moderate	Clumps of adipose cells moderate	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses	More than 3 fragments in 6 samples (from 21 examined samples)
	Sceletal muscle prevailing	Washy collagenous tissue and cartilage moderate	Clumps of adipose cells moderate	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses	More than 3 fragments in 18 samples (from 21 examined samples)
Overall evaluation	Skeletal muscle prevailing	Washy collagenous tissue moderate and cartilage sporadic	Clumps of adipose cells moderate	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses	All samples contained boned fragments

Raw material (machine type) (sample No.)	Evaluated parameters				
	Muscle tissue	Collagenous connective tissue	Adipose tissue	Degree of damage to the muscle fibres	Bone fragments
Poultry trimmings (SEPARATOR 3) (11)	Prevailing, Muscle fibres moderately damaged	Moderate amount, sporadic occurrence of cartilage	Clumps of adipose cells negligible	tissue structure is well noticeable	Less than 3 fragments (from 21 examined samples)
Overall evaluation	Skeletal muscle prevailing	Collagenous tissue negligible and cartilage sporadic	Clumps of adipose cells negligible	Moderate failure, primary structure is well noticeable, obvious muscle lemniscuses and fibres	Less than 3 fragments in every examined samples

III: *Comparison of results of histological and chemical analyses*

Sample No.	Raw material / conditions		
	Breast bones/ SEPARATOR 1 1	Breast bones/ SEPARATOR 2 6	Trimmings/ SEPARATOR 3 11
Muscle tissue	Skeletal muscle prevailing, primary structure is well noticeable, obvious muscle lemniscuses and fibres	Skeletal muscle prevailing, primary structure is well noticeable, obvious muscle fibres	Skeletal muscle prevailing, primary structure is well noticeable, obvious muscle lemniscuses and fibres
Net muscle protein g.kg^{-1}	190.0	157.0	199.2
Collagenous tissue	Washy collagenous tissue moderate and cartilage sporadic	Washy collagenous tissue and cartilage considerable	Moderate amount, sporadic occurrence of cartilage
Collagen g.kg^{-1}	4.3	8.8	3.5
Adipose tissue	Clumps of adipose cells negligible	Clumps of adipose cells moderate	Clumps of adipose cells negligible
Fat g.kg^{-1}	80.8	99.8	80.0
Bone fragments	More than 3 fragments in 3 samples (from 21 examined samples)	More than 3 fragments in 16 samples (from 21 examined samples)	Less than 3 fragments in all 21 samples
Calcium mg.kg^{-1}	155.0	1790.0	160.0

IV: *Results of chemical and quantitative histological analyses (IA-image analysis)*

Group No.	Separator No.	Chemical analyses		Histological analyses		
		Collagen [%]	Ca [mg/kg]	Collagen-IA	Bones-IA	Ca-IA
1	1	0.43	155	0.133710	0.001565178	0.0000648438
2	1	0.79	191	0.147244	0.001091097	0.0000444596
3	1	0.67	165	0.146615	0.000813349	0.0000324437
4	1	0.72	102	0.155603	0.000207620	0.0000065836
5	1	0.65	196	0.153260	0.000902452	0.0000299869
6	2	0.88	1790	0.169411	0.010496062	0.0009967100
7	2	1.08	398	0.138927	0.004508897	0.0003842840
8	2	0.73	489	0.156330	0.002840239	0.0002090930
9	2	1.55	472	0.214683	0.002922856	0.0001467990
10	2	0.96	830	0.195248	0.007118623	0.0005525680
11	3	0.35	160	0.27023	0.003335127	0.0001313650

(1 790 mg/kg). This extreme value was a result of the pressure used and the type of raw material.

Values established by chemical and histological analyses are shown in Tab. IV. The quantitative histological examination was carried out according to the method reported in the study of Tremlova and Starha (2003). In the present study statistically significant correlations of results were found. The cor-

relation coefficient of collagen results was 0.673 (α = 0.05) and Tremlova et al. (2006) found it 0.78 (α = 0.05) and Koolmees and Bijker (1985) 0.88. As for the correlation of chemical determination of the calcium content and histological determination of the bone tissue, it is possible to say that there is statistically significant dependence (correlation coefficient 0.718, α = 0.05). Tremlova and Starha (2003) achieved in their work similar result, 0.78 (α = 0.05). Hildebrandt and Hirst (1985) also compared results obtained by image analyses with chemical methods. They concluded, that the image analyses are adequate alternative to the chemical analyses (correlation coefficient more than 0.9). The histological and chemical analyses give similar results, but they are not identical. They should be used in combination with each other and not independently. In this way histological analysis should be able to obtain a more complete picture of the composition and/or quality of meat than that obtained via chemical analysis only (Koolmees and Bijker, 1985).

There was a strong correlation between the calcium content determined both chemically and via the image analyses (0.809, α = 0.01). Values enter into the correlation relationship in dependence on the intensity of the coloration of the identified bone fragments.

SUMMARY

MSM is a commodity of good quality that enables the poultry industry to utilize almost all the accessible material. It is a better substitute in the meat products of big animals than vegetable proteins because of its animal origin. In consequence of review of this material in the past, technological procedures were regulated and technical equipment innovated. We can say that it is possible to get high-quality material for meat products manufacture by using suitable device.

Results of qualitative or semiquantitative histological and chemical examinations confirmed in all parameters better material made by separator 1. Histological examination gave furthermore information about the degree of damage to the muscle fibres. Next result of this work is another proof of possible using of histological examination also for potential quantification of the food material components or foodstuff. Results of both types of quantitative analyses correlate well, which shows that chosen method used in the picture analyses is right.

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