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CHANGES IN THE INTRAMUSCULAR COLLAGEN CONTENT AND DEGREE OF ITS THERMOHYDROLYSIS IN BOVINE MUSCLES UNDER THE INFLUENCE OF CURING AND PASTEURISATION*

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Key words: bovine muscle, curing, thermal hydrolysis of collagen, WBSF

The aim of the study was to assess the effect of the introduction of curing brine, massaging and pasteurisation on the dynamics of changes in collagen content and its thermohydrolysis rate in two bovine muscles with different topographic positions in the carcass: the semimembranosus muscle *(m. semimembranosus)* and the lumbar part of the longissimus dorsi muscle *(m. longissimus lumborum)*. Brine contained, among other things, NaCl, phosphates and collagen protein. In the study the pH value of muscles, contents of crude protein and dry matter were determined together with the amount of collagen and its susceptibility to thermohydrolysis; moreover, shear force value was determined using the WBSF test. On the basis of the conducted tests it was found that the semimembranosus muscle contained more collagen than the lumbar part of the longissimus dorsi muscle. The introduction of brine, massaging and pasteurisation resulted in changes not only in its amount, but also in its susceptibility to thermohydrolysis. Curing resulted in a decreased rate of collagen thermohydrolysis in both analysed muscles in comparison to raw muscles: from several times in *m. semimembranosus* to around fifteen times in *m. longissimus lumborum* in terms of crude protein and over twenty times in relation to the total collagen content. The rate of collagen thermohydrolysis in both muscles varied: it was higher in *m. semimembranosus* than in *m. longissimus lumborum*. The most profound effect on changes in tenderness (test WBSF) was found for massaging, which in the semimembranosus muscle resulted in its decrease, while in the lumbar part of the longissimus dorsi muscle it caused an increase in comparison to the value obtained after the introduction of brine. It was found that after pasteurisation the dorsal muscle was more tender in comparison to the semimembranosus muscle in spite of the fact that it was characterised by a higher share of collagen in the total amount of protein.

INTRODUCTION

The amount of collagen, its level in muscles and the rate of thermohydrolysis are important factors determining the quality of meat products subjected to thermal processing [Pezacki, 1981; Boutten *et al*., 2000]. Sarcoplasmic proteins, which are dissolved in water and in weak salt solutions, have a slight effect on meat tenderness, whereas they play a significant role in the modification of the structure of comminuted products [Van Ecnacme *et al.,* 1994]. However Bouton *et al*. [1978] claimed that the mechanical properties of meat are a function of the mechanical properties of myofibrils and connective tissue. The content, spatial distribution and composition of connective tissue, linked with *e.g.* the location of the muscle in the animal carcass, have a significant effect on the tenderness of cooked meat [Sadowska *et al*., 2003]. Connective tissue protein constitutes from 2 to 6% of the total protein amount in meat [Leight, 1987; Greaser, 1997; Pospiech *et al*., 2003; Torrescano *et al*., 2003]. The basic component of the muscle connective tissue is collagen [Listrat & Hocquette, 2004]. The amount of collagen, which is contained in the connective tissue protein of muscle, depends *e.g.* on the type of muscle and its location in the animal carcass [Belew *et al*., 2003]. According to Kovanen, skeletal muscles undergoing rigor more slowly contain more collagen than those shrinking faster [Nakamura *et al*., 2003]. During ageing changes occur in the *endomysium* and *perimysium* which are connective tissue structures surrounding single muscle fibers and their bundles, respectively [Ueno *et al*., 1999]. Changes in intramuscular connective tissue also occur which cause an increase in the solubility of its main constituent collagen [Kołczak *et al*., 2003]. However, changes in the solubility of intramuscular collagen occur at different rate in various muscles [Kołczak *et al*., 1992]. Some researchers indicate that extended meat ageing affects the degradation of the above-mentioned structures and is correlated with meat tenderness [Nishimura *et al*., 1996; Swatland & Findlay, 1999]. However, no clear-cut hypothesis may be proposed on the interdependence between the varied ripening of muscle fibers and collagen content in the muscle [Listrat *et al*., 1999]. Tenderness in beef depends on many factors, *e.g.* growth rate of live animals, age, thermal conditions of post-slaughter storage of meat, its pH value and the connective tissue contents, and on the temperature during thermal processing of the raw material [Van Ecnacme *et al*., 1994; Palka, 2003; Honikel, 2004; Kołczak *et al*., 2005]. The value of this parameter depends on the location of the muscle in the animal carcass. Belew *et al*. [2003] divided bovine muscles into groups, using the results

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of the WBSF (Warner Bratzler Shear Force) test, *i.e.* the value of force required to cut samples with specific dimensions, recorded for each of 40 analysed muscles. Muscles with similar tenderness were classified into individual groups. They were denoted as "very tender", "tender", "intermediate" and "tough". Muscles were assessed after thermal processing and cooling. A similar division had been proposed earlier by Shackelford *et al*. [1991], assuming slightly different threshold values for the assessed groups of muscles.

An increase in collagen solubility rate is closely connected with an increase in meat tenderness [Listrat & Hocquette, 2004]. A positive correlation was found between collagen content and WBSF testing results, as well as insoluble collagen content in raw muscles [Purslow, 2005]. An important factor affecting collagen solubility and parameters of meat microstructure is the time of its aging after slaughter, as well as the temperature of thermal processing [Listrat *et al*., 1999; Nakamura *et al*., 2003]. The temperature of hydrothermal degradation of collagen and its solubility are determined by structure cross-linking [Torrescano *et al*., 2003], while the dynamics of thermohydrolysis for this protein is directly proportional to the rate of destruction of its structure [Buckenhüskes, 2000]. A special role in the thermohydrolysis of collagen is played *e.g.* by salts of inorganic acids, such as sodium chloride or neutral phosphate, introduced with curing brine. The application of a specific and admissible amount of phosphates in curing brines results among other things in the production of juicy and more tender products. However, the best technological effects are obtained at the combined action of both types of salts [Ueno *et al*., 1999; Pospiech *et al*., 2003]. Sodium chloride and phosphates act synergistically [Offer & Trinick, 1983], causing the swelling of muscle proteins and their extraction from cells and affecting the dynamics of transition of collagen into gelatin [Nishimura *et al*., 1995; Grześ *et al*., 1998; Nakamura *et al*., 2003].

The aim of the study was to compare the dynamics of changes in collagen content and rate of thermohydrolysis in two bovine muscles with different topographic positions in the carcass under the influence of curing and pasteurisation, and to assess the effect of these changes on the tenderness of the raw material analysed.

MATERIALS AND METHODS

MATERIAL

The experimental material consisted of two bovine muscles: the semimembranosus muscle *(m. semimembranosus)* and the lumbar section of the longissimus dorsi muscle *(m. longissimus lumborum)*. The raw material was collected from carcasses of 5-year old cows 72 h after slaughter. After trimming each muscle was divided into 4 parts. The first part, after the removal of the *epimysium,* constituted the control, while the other three were injected with curing brine at +1.3°C and pH 7.3. Brine contained among other things 7.8% sodium chloride and 0.4% phosphates in terms of P_2O_5. It also contained 0.62% collagen. Injection was performed until an increase of muscle weight by 30%. After the introduction of brine the second part of each muscle was collected for analysis, while the other two were placed in airtight plastic bags and massaged in a vacuum massaging machine (95% vacuum). Effective massaging time was 6 h 20 min. Out of two plasticized samples one was collected for analysis, while the last one, *i.e.* the fourth, sealed in a steel tin, was pasteurised at 72°C. Temperature was controlled using a thermocouple at the least heated site. In this way 4 basic samples were obtained for individual muscles, being the result of successive experimental stages: control (R), injected with brine (I), massaged (M) and pasteurised (P). Each muscle was analysed in three replications. Samples for physicochemical analyses were prepared by double comminution of the raw material in a laboratory grinder with mesh size of 3 mm.

METHODS

In muscles at each stage of the experiment pH value was determined using an Accumet-15 pH-meter, with measurements taken in relation to three automatically introduced buffers with pH values of 4, 7 and 10. Dry matter content was determined by drying to a constant weight at 105°C. Total protein amount was assessed according to Kjeldahl [AOAC, 1990]. Collagen was assayed in raw muscles and muscles subjected to thermohydrolysis for 90 min in a water bath at 100°C. The method of collagen determination was based on hydrolysis of the analysed raw material in the medium of a strong acid using a catalyser, $SnCl_2$ and the determination of hydroxyproline content in the hydrolysate, constituting approx. 13% of all amino acids of this connective tissue protein [Zajdes & Michajlow, 1964]. Due to the fact that meat tenderness is connected with its collagen content and its thermohydrolysis rate, and that sodium chloride and phosphates contained in brine significantly affect its value [Nishimura *et al*., 1995; Grześ *et al.*, 1998; Nakamura *et al*., 2003; Listrat & Hocquette, 2004], also force required for cutting muscle samples with the cross-section area of 1 cm^2 perpendicular to the muscle fibers (WBSF test) was analysed at each stage of the experiment. For this purpose a single-knife Warner-Bratzler device was used, mounted in an Intron 1140 Universal Testing Machine [Girolami *et al*., 2003; Mc Gee *et al*., 2003].

Results were subjected to a two-way analysis of variance to determine differences significant at $p \leq 0.05$ using Statistica 6.0 PL software.

RESULTS AND DISCUSSION

Based on the results, statistically significant differences were found between pH values of raw muscles, with the semimembranosus muscle exhibiting a higher value of this index, on average amounting to 5.74 units in comparison to the other analysed muscle, pH of which was 5.57 (Table 1). The introduction of brine with an alkaline reaction resulted in an increase of the value of the analysed index. Muscles, subjected to massaging and pasteurisation, underwent a further gradual increase of their pH value [Young *et al*., 2005]. The value of pH increased most in both muscles as a result of pasteurisation. In spite of the varied effect of successive stages of the experiment on changes in concentrations of hydrogen ions in the analysed muscles it was found that during the entire experiment the pH value in both muscles increased identically, on average by 0.67 units in comparison to raw muscles.

Both muscles differed in terms of their collagen content. In both compared muscles the lowest amount of collagen was recorded in raw muscles, while higher values were obtained

TABLE 1. Effect of the injection treatment with brine (I), massaging (M) and pasteurisation (P) on pH value, collagen content (%) and degree of its thermohydrolysis (%) in the examined muscles (R – raw muscles).

Muscles	Experimental phase	pH	Content of collagen in muscles (%) in relation to:		Amount of dissolved collagen (%) in relation to:	
			Dry matter	Total proteins	Tissue	Total collagen
M. longissimus lumborum	R	5.57^{a}±0.03	8.08^{a}±0.38	9.07^{a}±0.43	0.36^{a}±0.04	17.55^{a}±2.38
	I	5.76^{b}±0.02	9.87^{b}±0.53	11.88^{b}±0.36	0.02^{b}±0.01	0.73^{b}±0.44
	M	5.93^{c}±0.02	11.83^{c}±0.93	15.39^{c}±0.91	0.02^{b}±0.01	0.84^{b}±0.39
	P	6.24^{d}±0.02	8.96^{b}±0.64	13.49^{b}±0.90	0.01^{c}±0.0	0.23^{c}±0.16
M.semimembranosus	R	5.74^{e}±0.05	8.83^{d}±0.59	9.82^{d}±0.61	0.44^{d}±0.06	20.21^{a}±3.94
	I	5.90^{f}±0.05	11.66^{e}±0.75	15.52^{e}±0.66	0.06^{e}±0.02	2.21^{d}±0.71
	M	6.11^{g}±0.08	12.80^{f}±0.52	16.85^{e}±0.65	0.07^{e}±0.01	2.32^{d}±0.43
	P	6.41^{h}±0.09	9.34^{db}±0.48	12.17^{db}±0.52	0.02^{c}±0.01	0.66^{c}±0.51

* the same letters are used to designate mean values which do not differ significantly at the level of p≤0.05

after the introduction of curing brine and after massaging. This resulted probably from the swelling of muscle cells as a result of absorption of water and from the gradual extraction of intracellular protein outside muscles by brine under the influence of the solution of phosphate salts and NaCl and mechanical processing in a rotating massaging drum. When comparing raw muscles, less collagen was recorded in the lumbar section of the longissimus dorsi muscle, on average 2.07%, than in the semimembranosus muscle, containing 2.22% collagen (Figure 1). The introduction of brine containing collagen resulted in its increased content in muscles. More collagen was recorded in the semimembranosus muscle than in the lumbar section of the longissimus dorsi muscle. In the former muscle this increase was 0.48%, while in the latter it was 0.26%. In terms of dry matter differences between raw muscles and muscles injected with brine amounted to 2.83% and 1.79%, respectively. Differences between the amounts of this protein in both analysed muscles were statistically significant. This fact could have resulted from the varied amounts of brine drip from muscles directly after injection: 4.42% from the lumbar section of the longissimus dorsi muscle and 4.20% from the semimembranosus muscle, which was probably connected with different directions of changes in the histological structure of both muscles after the application of brine. In simultaneous studies [Krzywdzińska-Bartkowiak & Gajewska-Szczerbal, 2006] it was found that brine injection caused an increase in the volume of cells in the semimembranosus muscle, while it caused shrinkage of cells in *m. longissimus lumborum*. In turn, after massaging the cell cross-section area in the lumbar section of the longissimus dorsi muscle increased in comparison to the semimembranous muscle. In the study the total collagen content in the tissue of massaged dorsal muscle increased by 0.40% in comparison to the injected muscle, while it was by only 0.23% in the semimembranosus muscle. In terms of dry matter these values were 1.96% and 1.14% (Table 1). Also after converting collagen content in relation to crude protein for both muscles it was found that there were statistically significant differences between the values obtained. After massaging in *m. longissimus lumborum* there was by 3.51% more collagen protein in relation to the total amount of muscle protein, while in the semimembranosus muscle it was by only 1.33% in comparison to the phase after brine injection. These results would suggest a bigger extraction of muscle proteins from *m. longissimus lumborum* than from *m. semimembranosus* during massaging. As it results from Table 1, the lowest amounts of collagen were contained in muscle subjected to pasteurisation, which resulted from a transformation of this protein into gelatin. No statistically significant differences were found between muscles. After pasteurisation of the lumbar section of the longissimus dorsi muscle collagen constituted 13.49% of the total amount of its proteins, while its content in the semimembranosus muscle was 12.17%, even though this muscle before curing contained higher amounts of this protein. This could have been caused by the varied rate of the transition of collagen into glutin during pasteurisation of both muscles [Nishimura *et al*., 1995; Grześ *et al*., 1998; Listrat *et al*., 1999; Nakamura *et al*., 2003]. This effect could have also been due to the different rate of tissue structure destruction in both muscles [Listrat *et al*., 1999; Nakamura *et al*., 2003] and differences in the rate of cross-linking of intramuscular collagen [Buckenhüskes, 2000; Torrescano *et al*., 2003]. After conversion into total protein it was found that the strongest effect on changes in the amounts of collagen in the lumbar section of the longissimus dorsi muscle was recorded for massaging, while in the semimembranosus muscle it was the introduction of curing brine.

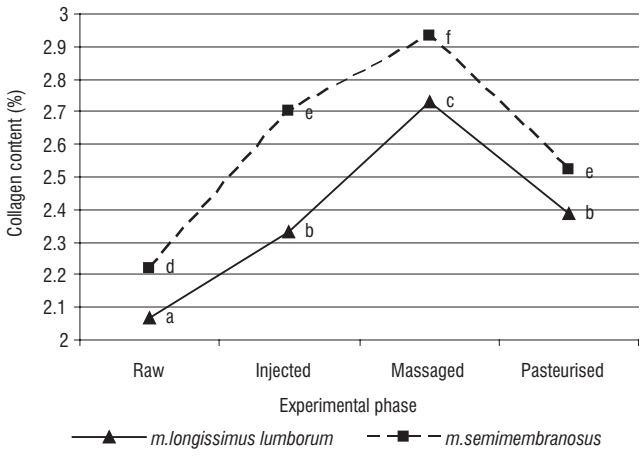

FIGURE 1. Changes in collagen content in the examined muscles (%).

Collagen of each muscle was characterised by a different rate of thermohydrolysis and the assayed amounts dif-

ferent statistically significantly (Table 1). In the tissue of the lumbar section of the longissimus dorsi muscle 0.36% collagen was transformed into glutin, which constituted 17.55% of the total amount of muscle protein, in comparison to 0.44% and 20.21%, respectively, in the semimembranosus muscle. The addition of curing brine significantly decreased the rate of collagen thermohydrolysis in both analysed muscles: 18 times in the former and 7 times in the latter. The lowest amount of soluble collagen was recorded in muscles after pasteurisation. The introduction of curing brine resulted in a decrease of the susceptibility to thermohydrolysis by over 20 times in the dorsal muscle and approx. 9 times in the semimembranosus muscle in relation to its total amount. Massaging and pasteurisation had a much less pronounced effect on this phenomenon.

Based on the comparison of WBSF testing results for raw muscles it was found (Figure 2) that a greater tenderness was shown for the semimembranosus muscle than for the lumbar section of the longissimus dorsi muscle. Force required for cutting samples of the first muscle was on average 20.71±1.8 N, while for the other muscle it was 29.49±4.59 N. The greatest effect on the variation in muscle tenderness was found for massaging, which caused its distinct increase for the dorsal muscle (16.36±2.09 N), whereas it caused an increase in hardness of the other analysed muscle (29.15 ± 7.08 N). *Musculus longissimus lumborum* after pasteurisation was less tender than after massaging and the difference in shear force was on average 7.66 N. In spite of that fact, the value of the WBSF testing result for pasteurised samples of this muscle was lower than for the semimembranosus muscle, amounting on average to 24.02±1.93 N in comparison to 31.64±5.82 N for the other analysed muscle. It was found that the value of the WBSF test for pasteurised muscles is connected with the amount of collagen. This parameter adopted a lower value for the dorsal muscle, containing less collagen in relation to the tissue and dry matter than for the semimembranosus muscle.

Pasteurisation resulted in an almost four-fold reduction of the amount of collagen, susceptible to thermohydrolysis in both muscles in comparison to massaged muscles. When assessing the rate of collagen thermohydrolysis in individual muscles at each stage of the experiment it was found that less collagen in relation to its total amount underwent thermohydrolysis in *m. longissimus lumborum.* Despite the fact that in the dorsal muscle collagen after pasteurisation constituted a bigger part of the total amount of protein than in the semimembranosus muscle, *i.e.* 13.49% and 12.17%, respectively, its smaller percentage was susceptible to thermohydrolysis than in the other analysed muscle. Thus it may be concluded that proteins other than collagen, probably myofibrillar proteins, affect muscle tenderness [Bouton *et al*., 1978; Kołczak *et al*., 1992;Ueno *et al*., 1999; Torrescano *et al*., 2003].

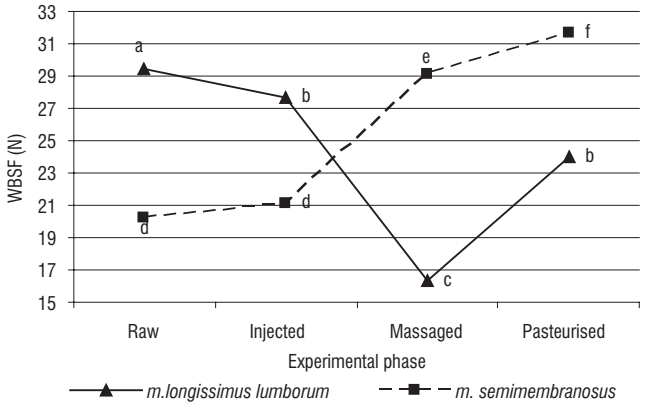

FIGURE 2. Results of the WBSF test in the examined muscles (N).

CONCLUSIONS

On the basis of the experiments it was found that the semimembranosus muscle contained more collagen than the lumbar section of the longissimus dorsi muscle. The introduction of brine, massaging and pasteurisation triggered changes not only in its amount, but also in its susceptibility to thermohydrolysis. Curing resulted in a decrease of the rate of collagen thermohydrolysis in both analysed muscles in comparison to raw muscles: from several times in *m. semimembranosus* to around fifteen times in *m. longissimus lumborum* in terms of crude protein and over twenty times in relation to the total collagen content. The rate of collagen thermohydrolysis in both muscles varied: it was higher in *m. semimembranosus* than in *m. longissimus lumborum*. The greatest effect on changes in tenderness (WBSF test) was recorded for massaging, which in the semimembranosus muscle resulted in its decrease, while in the lumbar section of the longissimus dorsi muscle it caused its increase in relation to the values obtained after the introduction of brine. It was found that after pasteurisation the dorsal muscle was more tender in comparison to the semimembranosus muscle in spite of the fact that it had a higher share of collagen in the total protein content.

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ZMIANY ZAWARTOŚCI KOLAGENU ŚRÓDMIĘŚNIOWEGO I STOPNIA JEGO TERMOHYDROLIZY POD WPŁYWEM PEKLOWANIA I PASTERYZACJI MIĘŚNI BYDLĘCYCH

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Przedmiotem pracy była ocena wpływu wprowadzenia solanki peklującej, masowania i pasteryzacji na dynamikę zmian zawartości i stopnia termohydrolizy kolagenu w dwóch mięśniach bydlęcych o zróżnicowanym położeniu topograficznym w tuszy: mięśnia półbłoniastego *(m. semimembranosus)* i części lędźwiowej mięśnia najdłuższego grzbietu *(m. longissimus lumborum)*. Solanka zawierała m.in.: NaCl, fosforany oraz białko kolagenowe. W pracy oznaczano wartość pH mięśni, zawartość białka ogólnego, suchej masy, ilość kolagenu i jego podatność na termohydrolizę oraz określono wartość siły cięcia (test WBSF). Na podstawie przeprowadzonych badań stwierdzono, że miesień półbłoniasty zawierał więcej kolegenu, niż część lędźwiowa mięśnia najdłuższego grzbietu. Wprowadzenie solanki, masowanie i pasteryzacja spowodowały zmiany nie tylko jego ilości, ale także podatności na termohydrolizę. Peklowanie spowodowało zmniejszenie stopnia termohydrolizy kolagenu w obu badanych mięśniach w porównaniu do mięsni surowych: od kilkukrotnego w *m. semimembranosus* do kilkunastokrotnego w *m. longissimus lumborum* w przeliczeniu na białko ogólne i przeszło dwudziestokrotnego w stosunku do ogólnej zawartości kolagenu (tab. 1, rys. 1). Stopień termohydrolizy kolagenu w obu mięśniach był zróżnicowany: większy w *m. semimembranosus* niż w *m. longissimus lumborum*. Największy wpływ na zmiany kruchości (test WBSF) miało masowanie, które w mięśniu półbłoniastym wpłynęło na jej zmniejszenie, natomiast w części lędźwiowej mięśnia najdłuższego grzbietu spowodowało jej zwiększenie w porównaniu do wartości, uzyskanych po wprowadzeniu solanki (rys. 2). Stwierdzono, że po pasteryzacji bardziej kruchy był mięsień grzbietowy w porównaniu do półbłoniastego mimo to, że charakteryzował się większym udziałem kolagenu w ogólnej ilości białka.