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CHANGES IN COLLAGEN SOLUBILITY OF RAW AND ROASTED BOVINE *PSOAS MAJOR* AND *MINOR* AND *SEMITENDINOSUS* MUSCLES DURING COLD STORAGE

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During 12-day post-mortem ageing of *psoas major* and *minor* (PM) and *semitendinosus* (ST) muscles of calves, heifers and cows at 4°C, changes in pH and thermal soluble intramuscular collagen were determined at 3-day intervals. Additionally, on the 1st and 12th day of cold storage, the muscle samples were roasted at 170°C to internal temperature of 78°C and stored for next 12 days at 4°C, then soluble collagen was determined. Raw and heated PM muscle contained less collagen of better solubility in comparison to ST muscle. During post-mortem ageing, pH and collagen solubility increased. The increase in the solubility of intramuscular collagen during cold storage was considerably higher in calves than in older cattle and higher in PM than in ST muscles. During cold storage of heated muscles, a significant increase in collagen solubility was observed, most evident in calf muscles heated after 12 days of cold ageing.

INTRODUCTION

Study on the structure of *psoas major* and *minor* (PM) and *semitendinosus* (ST) muscles of cattle showed that the space between myofibrillar mass of adhered muscle fibres increases during post-mortem ageing [Kołczak *et al.*, 2003]. The increase in this space between muscle fibres during cold ageing of meat may be an effect of weakening the intramuscular connective tissue, which was indicated in the studies of many authors [Stanton & Light, 1990; Liu *et al.*, 1994, 1995; Dransfield *et al.*, 1995; Nishimura *et al.*, 1995, 1996, 1999].

The main protein component of intramuscular connective tissue is collagen, which constitutes 2-6 % of dry matter of muscle and about 40% of dry matter of extracellular matrix [Light, 1987; Greaser, 1997]. About 90% of intramuscular collagen is found in *perimysium* [Sadowska, 1992]. With increasing physiological maturity of animals, the structure of collagen becomes more compact and its solubility decreases [Bailey & Light, 1989]. Changes in the solubility of intramuscular collagen occur at different rate in various muscles [Kołczak *et al.*, 1992]. The reason of this phenomenon is the conversion of heat-labile reductable to heat-stable unreductable cross-links of collagen fibres [Eyre *et al.*, 1984; Reiser *et al.*, 1992]. Mechanisms of stabilisation of intra- and intermolecular links of collagen fibres during growth of animal are not known. It is known, however, that distribution and stabilisation of collagen fibres in extracellular matrix of muscles are influenced by the amount and composition of proteoglycans which are in its colloidal phase. The content of proteoglycans in intramuscular connective tissue decreases with the age of animals [Sadowska & Łagocka, 1997].

For many years there has been an assumption that degradation of the collagenous component of extracellular matrix during post-mortem ageing has a negligible effect on texture after meat is cooked to 60°C or above [Lewis & Purslow, 1989]. The results of recent studies indicate, however, that quantity of free hydroxyproline in beef increases 2-fold during 14-day post-mortem ageing and only collagen can be its source [Feidt *et al.*, 1996]. It is possible that during post-mortem ageing of meat a part of intramuscular collagen turns into soluble form, exo- and endopeptidases of which may degrade to amino acids. This process may probably occur also during cold storage of heated meat [King & Harris, 1982] influencing the properties of meat products. It is an important problem in the context of ready-to-eat meat products, which become more and more popular. It seems reasonable to conduct a study on collagen solubility of cooked meat during cold storage and its effect on the quality of such products.

The aim of this study was to determine changes in the solubility of intramuscular collagen during 12-day post-mortem ageing of PM and ST muscles from calves, heifers and cows, and to observe if the collagen solubility changes during subsequent 12 days of cold storage after roasting. Measurements of dry matter and pH values of meat were performed as an accompanying analysis.

MATERIAL AND METHODS

The PM and ST muscles were taken from left half-carcasses of female calves (3 months old), heifers (18 months old) and cows (around 8 years old) 24 h after slaughter. In each of the examined groups, analyses were carried out on

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muscles taken from three carcasses. Each of the muscles was divided into 5 parts perpendicularly to the direction of muscle fibres. They were vacuum-sealed and stored at 4°C for 12 days. On the 1st, 3rd, 6th, 9th and 12th day of cold storage, the muscle samples were analysed. The following parameters were measured: dry matter, pH, total and soluble intramuscular collagen. Soluble intramuscular collagen was also measured in muscles roasted on the 1st and 12th day post-mortem at 170°C till internal temperature of 78°C immediately after roasting and cooling and after 12-day-storage of roasted meat at 4°C.

Dry matter was calculated after controlled drying at 105°C. The pH value was measured in water homogenate of meat using a pH-meter CP-215 (Elmetron) and electrodes ESAgP-360 (Eurosensor). The total and soluble collagen contents in muscle were determined according to the method reported by Palka [1999]. Soluble collagen was measured after heating the homogenised meat slurry prepared with Ringer solution at 77°C for 70 min. The data were evaluated statistically using the STATISTICA program for Windows, version 5.

RESULTS AND DISCUSSION

Results of measurements of dry matter and total intramuscular collagen in PM and ST of calf, heifer and cow muscles are presented in Table 1. The dry matter amount in muscles was similar. The level of total collagen in PM muscle was lower than in ST muscle in each age group of cattle. The quantity of intramuscular collagen decreased with increasing somatic maturity of the cattle. After roasting, dry matter and collagen content in meat increased, probably because of water evaporation and displacement of some meat components to the cooking leak. The ratio showing the relation between the collagen content in raw to roasted muscle was always significantly higher in the PM muscle than in the ST muscle, however, the proportion of dry matter content in the roasted meat to raw meat for both muscles was similar. It indicates that more of solubilized collagen was exuded during roasting from PM than ST muscles and from calf muscles than cow muscles.

Changes in the pH value and solubility of intramuscular collagen during 12-day post-mortem ageing of PM and ST muscles of calves, heifers and cows are presented in Figure 1. The results of statistical evaluation are shown in Table 2. Initial pH of both muscles was similar. During 12 days of ageing, pH of muscles increased, and the lowest increase occurred in calf muscles.

Thermal solubility of intramuscular collagen depended on the age of cattle. Independently on the type of muscle and time of ageing, the highest amount of soluble intramuscular collagen was found in calf and the lowest in cow muscles. On the 1st day after slaughter, soluble collagen in muscles of calves and cows constituted 27.8–31.4% and 7.4–8.9% of total intramuscular collagen, respectively. Decrease in solubility of intramuscular collagen of different muscles with increasing maturity of animals was reported also by others authors [Shimokomaki *et al.*, 1972; Light

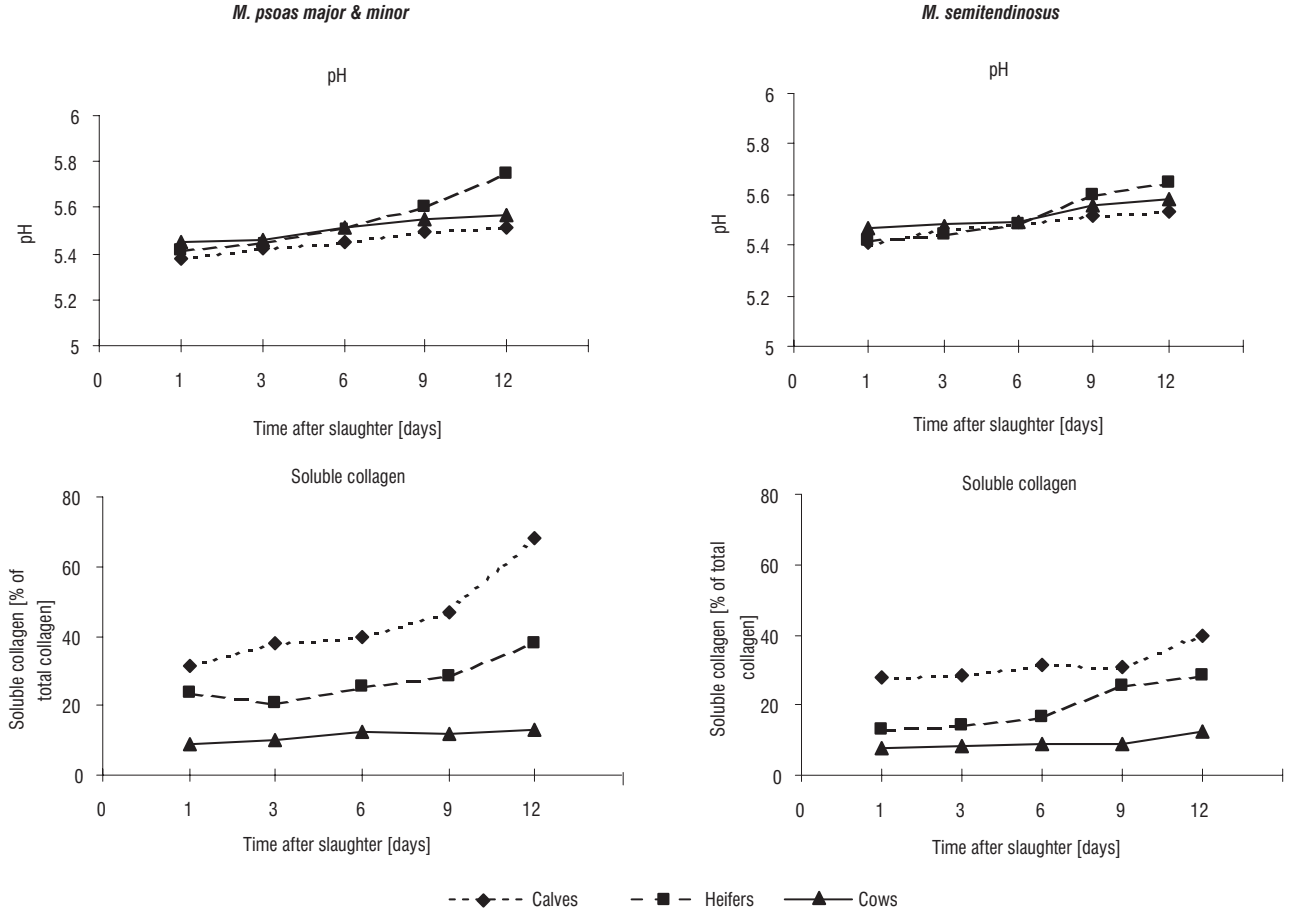

FIGURE 1. Changes in pH and collagen solubility of *psoas major & minor* and *semitendinosus* muscles of calves, heifers and cows stored 12 days after slaughter at 4°C (mean values).

TABLE 1. Dry matter and intramuscular collagen in raw and roasted *psoas major & minor* (PM) and *semitendinosus* (ST) muscles of calves, heifers and cows 1 day after slaugter. Mean values and standard errors.

Components	Muscle	Cattle group					
		Calves		Heifers		Cows	
		Muscle					
		PM	ST	PM	ST	PM	ST
Dry matter [%]	raw	24.60±0.59	23.74±0.44	25.14±0.52	24.28±0.25	24.80±0.39	25.33±0.26
	roasted	35.86±1.09	35.50±0.91	36.70±0.44	36.70±0.43	36.46±0.72	38.24±0.51
Collagen [%]	raw	0.47±0.02	0.73±0.02	0.42±0.02	0.71±0.01	0.41±0.03	0.58±0.01
	roasted	0.60±0.02	1.00±0.03	0.55±0.03	0.97±0.01	0.58±0.05	0.84±0.02

TABLE 2. Mean squares of deviations from analysis of variance of pH values, and quantity of soluble collagen in cattle muscles.

Source of variance	Degrees of freedom	pH	Soluble collagen
A – Type of muscle	1	0.0003	1296.4100^{xx}
B – Animal age	2	0.0330^{xx}	5885.7690^{xx}
C – Time of cold storage	4	0.0949^{xx}	608.9280^{xx}
Interactions:			
A x B	2	0.0058^{xx}	236.7250^{xx}
A x C	4	0.0016	38.0780^{xx}
B x C	8	0.0096^{xx}	105.7400^{xx}
A x B x C	8	0.0009	57.2070^{xx}
Error	60	0.0011	9.5024

x – $p < 0.05$; xx – $p < 0.01$

et al., 1985; Kołczak *et al.*, 1992]. Intramuscular collagen of the PM muscle in all cattle groups had better solubility than collagen of the ST muscle. Similar relations between these muscles have been reported by Kołczak *et al.* [1992].

During post-mortem ageing of muscles, the solubility of intramuscular collagen increased. Changes in collagen solubility as well as interactions of analysed variables (age of animal, kind of muscle, time of cold storage) were statistically highly significant. Particularly intensive increase in collagen solubility occurred in calf muscles. On the 12th day of ageing, soluble collagen in the PM calf muscle amounted to 68% of total intramuscular collagen. The increase in collagen solubility during ageing was higher in the PM muscle than in the ST muscle of all cattle groups.

These results indicate that during 12 days of ageing significant changes occur in properties of intramuscular collagen, causing an increase in its solubility. Similar results were obtained by many authors [Bernal & Stanley, 1986; Etherington, 1987; Stanton & Light, 1988; Mills *et al.*, 1989; Palka, 2000]. Changes in the properties of intramuscular collagen may be responsible for an increase in spaces between adhere muscle fibres [Kołczak *et al.*, 2003] and weakening of intramuscular connective tissue [Nishimura *et al.*, 1995; 1996] during post-mortem ageing, first of all in the muscles of young animals. In collagen of matured animals they are much lower and their importance in meat tenderization process is rather small.

After roasting, more of soluble collagen was in the PM muscle than in the ST muscle, especially in heifer and cow meat (Table 3). In muscles roasted 1 day and 12 days post--mortem and then stored for 12 days at 4°C, a considerable increase in soluble collagen was observed, especially in both calf muscles and in PM muscles in all groups of cattle. The quantity of soluble collagen in muscles roasted on the 12th day of ageing and stored for next 12 days in cold was 2-3--fold higher in comparison with muscles roasted 1 day after slaughter. These changes depended on the type of muscle and age of animals and were larger in the case of calf than heifer and cow muscles. In calf meat roasted on the 12th day of ageing and stored for 12 days at 4°C, soluble collagen in PM and ST muscles amounted in average nearly 49% and 53% of total intramuscular collagen, respectively.

After 12-day storage of meat roasted on the 1st day post slaughter, the changes in collagen solubility were higher in the PM than in the ST muscles. In meat roasted on the 12th day of cold ageing, however, relatively higher changes were found in the ST muscles. The reason of this phenomenon was probably more intensive changes in collagen properties in raw PM than in ST muscles during ageing.

The increase in collagen solubility observed during cold storage of roasted meat was a little lower than changes during ageing of raw meat for the same period of time. Irrespective of the extent of these changes, the results

TABLE 3. Soluble collagen (as % of total collagen) in roasted *psoas major & minor* (PM) and *semitendinosus* (ST) muscles of calves, heifers and cows as related to different time post-mortem and different time of cold storage of roasted meat. Mean values and standard errors.

Time post-mortem [days]	Cold storage of heated muscle [days]	Muscle	Cattle group		
			Calves	Heifers	Cows
1	0	PM	7.67±1.16	6.20±0.63	5.23±0.58
		ST	7.97±0.84	4.59±0.50	3.67±0.78
	12	PM	14.23±2.58	13.96±1.16	10.51±0.53
		ST	10.14±1.86	9.38±1.30	6.46±1.62
12	0	PM	22.33±1.81	16.78±1.23	16.29±0.83
		ST	17.31±0.77	11.69±0.80	6.43±1.12
	12	PM	53.40±0.92	27.89±0.80	24.54±2.12
		ST	48.96±4.07	23.80±0.93	17.39±1.86

obtained indicate that processes which result in higher solubility of intramuscullar collagen do not stop when meat is cooked up to 78°C. These processes occur also during cold storage of heated meat and may have a significant influence on textural properties of meat products and their culinary quality.

Thermally-denatured collagen is more susceptible to proteolytic digestion in the human alimentary tract. Causes of changes in thermally-denatured intramuscular collagen which occur during cold storage may be various. One of them may be proteolytic damages connected with activity of endogenous carboxyproteases and other endogenous muscle proteinases, *e.g.* cathepsins D and B+L [King & Harris, 1982; Spanier *et al.*, 1990]. It was found that these enzymes may retain more than 20% of their activity at the end-point cooking temperatures of meat above 70°C [Spanier *et al.*, 1990]. The explanation of reasons of increased solubility of thermally-denatured intramuscular collagen occurring during cold storage, except for theoretical aspects, is also of practical importance. It is associated with the production of convenient food, which is designed for consumption after re-heating, and the recognition of factors which determine its quality.

CONCLUSIONS

1. Thermal solubility of intramuscular collagen of cattle muscles increases during post-mortem ageing. The changes in collagen solubility during ageing are more intensive in the muscles of young animals and in *psoas major* and *minor* muscle than in *semitendinosus* muscle.

2. Thermal solubility of intramuscular collagen of roasted cattle meat increases during cold storage. The increments in collagen solubility are more intensive in meat from younger animals and in meat roasted after longer time of post-mortem ageing.

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ZMIANY ROZPUSZCZALNOŚCI KOLAGENU MIĘŚNI BYDLĘCYCH *PSOAS MAJOR* I *MINOR* ORAZ *SEMITENDINOSUS* SUROWYCH I OGRZEWANYCH PODCZAS PRZECHOWYWANIA CHŁODNICZEGO

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Określono zmiany rozpuszczalności cieplnej kolagenu śródmięśniowego mięśni *psoas major* i *minor* (PM) oraz *semitendinosus* (ST) cieląt, jałówek i krów podczas 12-dniowego dojrzewania poubojowego w 4°C. Ponadto próbki mięśni w 1 i 12 dniu dojrzewania pieczono w 170°C do temperatury wewnętrznej 78°C, składowano po ogrzaniu przez 12 dni w 4°C oraz oznaczano w nich ilość rozpuszczalnego kolagenu śródmięśniowego. Zarówno nieogrzewane jak i ogrzewane mięśnie PM zawierały mniej kolagenu o większej rozpuszczalności niż mięśnie ST (tab. 1). Podczas poubojowego dojrzewania chłodniczego mięśni wartość pH i rozpuszczalność kolagenu wzrastały. Wzrost rozpuszczalności kolagenu był większy w mięśniach cieląt niż w mięśniach jałówek i krów oraz bardziej intensywny w mięśniach PM niż w ST (rys. 1). Rozpuszczalność kolagenu mięśni ogrzewanych wzrastała podczas ich przechowywania chłodniczego (tab. 3), szczególnie intensywnie w mięśniach cieląt ogrzewanych po 12 dniach dojrzewania poubojowego.