EFFECT OF POST-MORTEM AGEING, METHOD OF HEATING AND REHEATING ON COLLAGEN SOLUBILITY, SHEAR FORCE AND TEXTURE PARAMETERS OF BOVINE MUSCLES

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The muscles *semitendinosus* (ST) and *psoas major* (PM) were removed from young bull carcasses 24 h after slaughter and stored at 4°C. On the 1st, 6th, and 12th day of post-mortem ageing the chemical composition (moisture, fat, protein) and contents of total and soluble collagen were estimated. The muscle steaks were boiled at 100°C, roasted at 170°C or fried in vegetable oil at 160°C to the internal temperature of 75°C in each case. The heated muscles were stored at 4°C for 6 days and reheated in the same heating environment to the internal temperature of 60°C. In heated and reheated muscles determinations of total and soluble collagen, WB shear force measurements and texture profile analysis (TPA) were conducted. The raw PM muscle contained two times less intramuscular collagen than ST muscle. The solubility of PM muscle collagen was higher than the solubility of ST muscle collagen. During post-mortem ageing the collagen solubility of both muscles increased. In the boiled muscles the level of soluble collagen was lower than in the roasted or fried ones. During post-mortem ageing the WB shear force values were the highest for boiled, middle for roasted and the lowest for fried muscles. The roasted muscles had the highest and fried ones the lowest values of TPA hardness, whereas values of TPA chewiness were similar, independently of the heating method used. The reheating of muscles after 6 days of cold storage had no effect on the WB shear force, although their TPA hardness and TPA chewiness values increased.

INTRODUCTION

During post-mortem ageing, muscle structures become looser because of degradation myofibrilar and cytoskeletal proteins. These changes are known as the tenderization or ageing process of meat [Koohmaraie, 1996]. The results of our studies carried out on calf, heifer and cow semitendinosus (ST) and psoas (PM) muscles indicated that during post-mortem ageing changes in ultrastructure of muscle fibres [Kołczak et al., 2003c], in degradation of myofibrillar and cytoskeletal proteins [Kołczak et al., 2003b] as well as in Warner-Bratzler (WB) shear force [Kołczak et al., 2005] are faster and more intensive in muscles of younger animals and in PM muscle than in ST muscle. During post-mortem ageing of meat, changes also occur in the extracellular matrix of meat, which cause an increase in solubility of its main constituent collagen [Stanton & Light, 1990; Kołczak et al., 1992; Nishimura et al., 1995; Palka, 2003]. Many papers confirm that perimysial collagen and structural integrity of intramuscular networks are damaged during ageing [Purslow, 2005]. Enzyme activity which may relate to collagen solubility is probably caused by cathepsins which are active post-mortem [Dutson & Lawrie, 1974; Roncales et al., 1995].

During the heating of meat, sarcoplasmic, myofibrilar and connective proteins undergo denaturation [Cheng & Parrish, 1976; Jones *et al.*, 1977; Bendall & Restall, 1983], muscle fibres contract longitudinally and transversally [Davey & Gil-

bert, 1974; Bendall & Restall, 1983], and perimysial collagen shrinks and partly gelatinizes [Mohr & Bendall, 1969]. The increase in beef tenderness during heating in temperature range of 50°-60°C appears to be due to a reduced breaking strength of perimysial tissue, induced by partial denaturation and shrinkage of collagen fibres [Christensen et al., 2000]. The decrease in tenderness of beef during heating above 60°C is due to denaturation of myofibrillar proteins, disintegration filaments in the I-band and shrinkage of the filaments in the A-band of myofibrils [Leander et al., 1980]. Increased disintegration, and shrinkage of myofibrils occur as meat temperature is increased during cooking [Leander et al., 1980; Pohlman et al., 1997; Christensen et al., 2000]. Meat gets less tender as internal temperature increases. The method of cooking has a significant effect on microscopic morphology, the shear force, and the sensory properties of meat [Pohlman et al., 1997].

There has been an assumption that degradation 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; Lewis *et al.*, 1991]. Our research indicated that during cold storage of heated bovine muscles an increase in collagen solubility is observed, most evident in muscles heated after 12 days of cold ageing [Kołczak *et al.*, 2003a]. It is possible that during post-mortem ageing of meat a part of intramuscular collagen turns into soluble forms which may be degraded by exo- and endopeptidases to the amino acids

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[Spanier *et al.*, 1990]. This process probably also occurs during cold storage of cooked meat [King & Harris, 1982]. It may be an important problem in the context of ready-to-eat meat products.

Many studies indicated a quality problem of meat products reheated after cold storage, connected with accumulation of warmed-over flavor (WOF) components which are products of the oxidation of unsaturated fatty acids, mainly phospholipids fraction [Ang & Lyon, 1990]. The lower scores in sensory evaluation were observed when meat products were reheated after 7 days of cold storage [White *et al.*, 1988]. Also, microwave reheating of roasted meat resulted in worse tenderness [Lin & Keeton, 1998]. In addition, changes in the texture of beef steaks after reheating were observed but were small when the internal temperature during reheating of steaks was not higher than 60°C [Cooksey *et al.*, 1993].

The aim of this study was to determine changes in the solubility of intramuscular collagen, WB shear force and some texture parameters of bovine ST and PM muscles subjected to heating during 12-day post-mortem ageing and then reheated after 6 days of cold storage. The effects of boiling meat in water, roasting in hot air and deep-frying in rapeseed oil were compared.

MATERIAL AND METHODS

Young Lowland Black and White bulls were slaughtered at their commercial weight (~550 kg) following standard handling procedures. Stunning was performed with a captive bolt. Carcasses were suspended from the Achilles tendon, 40 min on average after stunning, and chilled under commercial conditions at 2°C. The ST and PM muscles were removed from chilled carcasses 24 h after slaughter; pH of the muscles was in the range of 5.5-5.7. Each muscle was divided into 3 parts, which were vacuum-sealed and stored at 4°C. On the 1st, 6th and 12th day of ageing samples were taken and the chemical composition (moisture, protein, fat) as well as the total and soluble collagen content were estimated. Samples were divided into 6 steaks of about 20 mm in thickness. Two of them were boiled in water bath at 100°C, the second two were roasted at 170°C, and the third two were fried in a rapeseed oil bath at 160°C. In each case heating was conducted to the internal temperature 75°C. After the chilling of the meat to a room temperature, the total and soluble collagen content, Warner-Bratzler (WB) shear force, and texture profile analysis (TPA) parameters were evaluated. Three steaks from each muscle, heated by different method, were vacuum sealed, stored at 4°C for 6 days, reheated in the same heating environment to the internal temperature of 60°C and chilled to the room temperature. In the reheated muscles the total and soluble collagen, WB shear force and TPA parameters were measured. Three series of experiments were conducted.

The moisture was determined by drying the sample at 105°C [PN-ISO 1442:2000]. Protein content was determined by the Kjeldahl method [PN-75/A-04018]. Fat was evaluated by ethyl ether extraction [PN-ISO 1444:2000]. Collagen content was measured by hydroxyproline determination [PN-ISO 3496:2000]. Soluble collagen was measured after heating the homogenized meat slurry prepared with diluted Ringer solution at 77°C for 70 min [Palka, 1999].

The WB shear force was determined using seven cylindrical samples of 14 mm in diameter and 15 mm in length cuts. The measurements were carried out for samples cut perpendicular to the fibre direction using a texturometer TA-XT2 (Stable Micro Systems, UK) and a Warner-Bratzler knife with a triangular cut-out.

The TPA analysis was conducted as described by Breene [1975] using a TA-XT2 texture analyzer with a 50 mm diameter cylindrical probe. Muscle samples 14 mm in diameter and 10 mm in length, cut lengthwise to the fibres, were compressed twice, parallel to the fibre direction to 70% of their original height at the probe travel rate before testing 5 mm/s, as well as during and after testing 2 mm/s, with a 3-sec interval between the first and second stroke. Each measurement was repeated seven times. The TPA parameters of hardness and chewiness were chosen.

The data were evaluated statistically using the STATIS-TICA program for Windows, version 5.1.

RESULTS AND DISCUSSION

The chemical composition of raw ST and PM muscles during 12-day post-mortem ageing is presented in Table 1. The PM muscle contained approximately the same amounts of water, protein and fat as the ST muscle but almost two times less intramuscular collagen. A larger amount of intramuscular collagen in ST bovine muscle than in PM bovine muscle was also observed in other experiments [McKeith *et al.*, 1985;

TABLE 1. Chemical composition of bovine muscles during post-mortem ageing (mean \pm standard error, n = 3 for each group).

	Muscles						
Components	M. semitendinosus			M. psoas major			
	Time of post-mortem ageing (days)						
	1	6	12	1	6	12	
Water (%)	76.42 ± 1.22 ^a	76.31 ± 1.09 ^a	76.14 ± 1.11 ^a	77.34 ± 1.24 ^a	77.56 ± 1.10^{a}	77.18 ± 1.20^{a}	
Protein (N x 6.25) (%)	20.97 ± 0.87 ^a	20.81 ± 0.91 ^a	20.87 ± 1.02 ^a	20.25 ± 0.82 ^a	19.83 ± 0.79 ^a	20.11 ± 0.69 ^a	
Fat (%)	2.35 ± 0.15 ^a	2.24 ± 0.18 ^a	2.29 ± 0.12 ^a	1.91 ± 0.13 ^a	2.11 ± 0.15 ^a	2.07 ± 0.19 ^a	
Total collagen (%)	0.85 ± 0.08 $^{\rm a}$	0.84 ± 0.09 ^a	$0.86 \pm 0.10^{\text{ a}}$	0.46 \pm 0.07 $^{\rm b}$	0.43 ± 0.05 ^b	0.45 ± 0.06 ^b	
Soluble collagen (% of total collagen)	3.74 ± 0.18 ^a	4.22 ± 0.21 ^a	17.49 ± 0.25 ^b	7.25 ± 0.29 °	$8.45^{\circ} \pm 0.35^{\circ}$ d	14.59 ± 0.51 °	

a, b, c, d - all values in a row with different superscripts are different at p<0.05.

		Muscles							
	Multiplicity	M. semitendinosus			M. psoas major				
	of heating *	Time of post-mortem ageing (days)							
		1	6	12	1	6	12		
Boiling	А	10.86 ± 0.74	11.81 ± 0.89	17.99 ± 0.81	14.21 ± 0.75	18.14 ± 0.56	19.55 ± 0.44		
	В	17.94 ± 0.88	18.72 ± 0.87	26.83 ± 0.68	13.83 ± 0.78	14.89 ± 0.73	15.78 ± 0.82		
Roasting	А	16.51 ± 0.58	17.65 ± 0.74	28.76 ± 0.66	19.85 ± 0.40	22.17 ± 0.79	23.90 ± 0.66		
	В	17.35 ± 0.74	16.83 ± 0.78	29.45 ± 0.54	21.79 ± 0.84	22.85 ± 0.76	23.50 ± 0.96		
Frying	А	12.50 ± 0.40	13.29 ± 0.65	19.86 ± 0.74	22.72 ± 0.87	23.53 ± 0.58	25.20 ± 0.40		
	В	15.17 ± 0.89	15.55 ± 0.42	18.67 ± 0.72	24.50 ± 0.90	25.01 ± 0.85	25.42 ± 0.48		

TABLE 2. Influence of heating of bovine muscles by different methods on soluble collagen content (% of total collagen) in relation to time of post--mortem ageing (mean \pm standard error, n = 3 for each group).

*A - heating of raw muscles to 75°C; B - reheating of cooked muscles to 60°C after 6 days of cold storage

Kołczak *et al.*, 1992]. The solubility PM muscle collagen was higher in comparison with ST muscle collagen. During post-mortem ageing the solubility of intramuscular collagen of both muscles increased. This increase was particularly visible on the 12th day of post-mortem ageing. Similar results were obtained in our previous study on PM and ST cattle muscles of different maturity [Kołczak *et al.*, 2003a]. The increase in solubility of intramuscular collagen during longer-term post-

-mortem ageing was also noticed by other authors [Stanton & Light, 1988; Mills *et al.*, 1989].

The amounts of soluble collagen in heated and reheated muscles in relation to the length of post-mortem ageing and method of heating are presented in Table 2 and the results of four-way analysis of variance collagen solubility in heated muscles in Table 4. Irrespective of the length of post-mortem ageing and method of cooking, the heated PM muscle con-

TABLE 3. Influence of heating of bovine muscles by different methods on WB shear force, TPA hardness and TPA chewiness in relation to time of post-mortem ageing (mean \pm standard error, n = 3 for each group).

		Muscles						
	Multiplicity	M. semitendinosus			M. psoas major			
	of heating *	Time of post-mortem ageing (days)						
		1	6	12	1	6	12	
			WB shea	r force (kG/cm ²)				
Boiling	А	7.32 ± 0.17	7.03 ± 0.27	6.80 ± 0.20	6.46 ± 0.22	6.35 ± 0.19	5.69 ± 0.26	
	В	7.40 ± 0.25	7.26 ± 0.19	6.20 ± 0.26	6.46 ± 0.21	6.68 ± 0.18	5.87 ± 0.32	
D (А	6.79 ± 0.20	6.59 ± 0.17	6.58 ± 0.26	5.76 ± 0.26	5.60 ± 0.29	5.14 ± 0.25	
Roasting	В	6.95 ± 0.20	6.78 ± 0.21	6.12 ± 0.23	6.55 ± 0.34	6.23 ± 0.23	6.05 ± 0.19	
г ·	А	6.73 ± 0.16	6.17 ± 0.32	5.78 ± 0.28	5.55 ± 0.32	5.39 ± 0.24	5.06 ± 0.22	
Frying	В	6.51 ± 0.34	6.10 ± 0.16	5.84 ± 0.19	5.68 ± 0.30	5.68 ± 0.19	5.21 ± 0.18	
			TPA	hardness (N)				
	А	54.09 ± 2.82	48.04 ± 1.98	42.02 ± 2.54	41.34 ± 2.98	38.72 ± 2.35	37.75 ± 1.30	
Boiling	В	57.72 ± 1.36	57.52 ± 1.35	54.34 ± 2.21	45.29 ± 1.75	42.15 ± 1.64	39.45 ± 2.61	
D (А	56.10 ± 2.62	53.95 ± 1.75	46.03 ± 1.68	45.15 ± 2.86	43.95 ± 1.83	42.76 ± 2.61	
Roasting	В	62.34 ± 2.21	60.84 ± 2.52	46.71 ± 3.24	46.71 ± 2.21	45.51 ± 2.47	44.59 ± 2.48	
-	А	44.24 ± 2.33	43.96 ± 2.75	38.58 ± 2.79	38.25 ± 1.72	35.19 ± 1.74	32.91 ± 2.55	
Frying	В	54.74 ± 2.34	53.02 ± 2.71	44.52 ± 2.49	38.91 ± 1.96	39.01 ± 2.24	35.14 ± 1.87	
			TPA c	hewiness (N)				
D '1'	А	17.18 ± 1.54	16.07 ± 1.58	13.98 ± 1.89	9.49 ± 0.54	9.36 ± 0.36	9.23 ± 0.45	
Boiling	В	19.94 ± 1.84	16.52 ± 1.69	15.87 ± 2.04	9.64 ± 0.42	9.89 ± 0.84	9.31 ± 0.47	
Roasting	А	18.89 ± 1.45	17.59 ± 1.65	14.37 ± 1.84	9.45 ± 0.45	9.55 ± 0.42	9.35 ± 0.49	
	В	19.71 ± 1.75	18.71 ± 1.62	15.74 ± 1.84	9.99 ± 0.48	9.91 ± 0.62	9.62 ± 0.64	
Frying	А	17.25 ± 2.04	16.26 ± 2.18	15.31 ± 1.91	9.38 ± 0.62	9.48 ± 0.56	9.24 ± 0.65	
	В	18.26 ± 1.89	17.67 ± 2.05	16.59 ± 1.74	9.95 ± 0.61	10.04 ± 0.48	9.73 ± 0.71	

* A - cooking of raw muscles to 75°C, B - reheating of cooked muscles to 60°C after 6 days of cold storage

Source of variance	Degrees of freedom	Soluble collagen	WB shear force	TPA hardness	TPA chewiness
Kind of muscle – A	1	217.43**	15.32**	31.08**	13.87**
Time of ageing – B	2	321.76**	4.04**	4.06**	0.35**
Method of heating - C	2	235.14**	6.04**	5.90**	0.01
Multiplicity of heating - D	1	54.52**	0.65*	6.81**	0.15**
Interactions:					
AxB	2	121.78**	0.10	1.12**	0.28**
AxC	2	235.42**	0.01	0.27**	0.01
AxD	1	70.03**	1.35**	1.66**	0.03**
BxC	4	15.14**	0.12	0.26**	0.04**
BxD	2	5.99**	0.12	0.13	0.01
CxD	2	10.09**	0.32	0.03	0.01
A x B x C	4	11.16**	0.04	0.36**	0.04**
AxBxD	2	1.60**	0.14	0.02	0.01
A x C x D	2	79.54**	0.22	0.06	0.02*
BxCxD	4	2.05***	0.06	0.01	0.01
A x B x C x D	4	3.03**	0.10	0.14*	0.01
Error	72	0.35	0.12	0.04	0.01

TABLE 4. Mean squares of deviations from analysis of variance of soluble collagen content, WB shear force and TPA parameters (hardness, chewiness) values of heated bovine muscles in relation to kind of muscle, time of post-mortem ageing, method and multiplicity of heating.

*, ** F values significant at p<0.05 and p<0.01 respectively.

tained more of soluble collagen than the heated ST muscle. The amount of soluble collagen was higher in muscles heated after longer-term post-mortem ageing. This relationship was more visible in the case of ST muscle than PM muscle. Independently of the length of post-mortem ageing in both boiled muscles the level of soluble collagen was lower than in roasted or fried ones. It seems that during boiling more intramuscular soluble collagen penetrates to the heating environment than during roasting or frying. The roasted ST muscles contained more soluble collagen in comparison with fried ST muscles. However, in fried PM muscles the amount of soluble collagen was slightly higher than in the roasted ones.

The amount of soluble collagen in reheated muscles (with the exception of boiled PM) was usually higher than in heated muscles. It was most visible in boiled ST muscle. However, in PM muscle lower levels of soluble collagen were observed in twice-boiled samples. Probably a part of the thermal soluble collagen of PM muscle penetrated to the heating environment during the first boiling. The differences in quantity of soluble collagen of both muscles roasted or fried two times in comparison with muscles after first heating were lower than in boiled muscles. In previous research [Kołczak et al., 2003a], a significant increase in soluble collagen was observed in ST and PM muscles of calves, heifers and cows roasted twice after 12 days of cold storage. It is possible that 6-day cold storage of roasted or fried bovine muscles is too short to evoke significant changes in the thermal solubility of intramuscular collagen.

The effect of the analysed factors on WB shear force and TPA hardness and TPA chewiness values of heated muscles is shown in Table 3, and results of four-way analysis of variance are presented in Table 4. Irrespective of post-mortem ageing and method of heating, WB shear force as well as TPA hardness and TPA chewiness values were lower for PM muscle than ST muscle. These results confirm the thesis of many authors that intramuscular collagen content plays a key role in toughness of cooked meat [Purslow, 2005]. During post-mortem ageing the WB shear force, TPA hardness and TPA chewiness values of both muscles decreased. The decrease of WB shear force and TPA parameters during post-mortem ageing of muscles is a result of degradation myofibrilar and cytoskeletal proteins of muscle fibres, proteoglycans and collagen of intramuscular connective tissue [Ouali, 1990; Koohmaraie, 1996].

At the same time of post-mortem ageing the WB shear force values were the highest for boiled, middle for roasted and the lowest for fried muscles (Table 4). Muscles heated by each of these three methods were heated to the same internal temperature of 75°C. When meat is heated to this temperature, changes in its tenderness mostly result from structural changes that are caused by the denaturation of myofibrilar proteins and the contraction of muscle fibres [Pohlman et al., 1997; Leander et al., 1980; Christensen et al., 2000]. In each of the analysed muscles, the decrease in muscle fibre diameter caused by boiling was larger than by roasting or frying (unpresented data). The results obtained by other authors indicate that quantity and distribution of intramuscular water play an important role in tenderness of meat [Curie & Wolfe, 1980; Offer et al., 1984]. The passing of intramuscular collagen into the water during boiling as well as the lower level of immobilized water in boiled muscles than in roasted or fried muscles [Kołczak et al., 2007] might also be a reason of higher WB shear force values of boiled muscles in comparison with roasted or fried ones.

At the same stage of ageing of muscles, TPA hardness values were the highest for roasted, middle for boiled and the lowest for fried samples. The values of TPA chewiness of heated muscles at the same time of post-mortem ageing were similar irrespective of the method of heating and were not correlated with WB shear force values. TPA hardness is the force necessary to obtain a given deformation of product [Szcześniak, 1963]. TPA chewiness is the energy needed to crumble a product of solid consistency [Breene, 1975]. The reasons of differences in TPA hardness and lack of correlation

between WB shear force and TPA chewiness of meat heated by three methods commonly used in practice need explanation. In fried muscles the level of immobilized water is higher than in roasted or boiled muscles [Kołczak *et al.*, 2007]. This fact (and probably oil penetration into intramuscular spaces during frying) is a reason of lower TPA hardness for fried muscles in comparison with roasted or boiled ones. Differences in TPA hardness of roasted and boiled muscles are more difficult to explain. It is likely that greater water evaporation during the roasting of meat is a reason of its higher TPA hardness.

The reheating of muscles after 6 days of cold storage had no significant effect on the WB shear force, although their TPA hardness and TPA chewiness values increased independently of the time of ageing and method of heating. The deterioration of these texture parameters in reheated beef muscles were also observed by Cooksey *et al.* [1993]. The increase of TPA hardness and TPA chewiness after reheating of meat may be a result of water losses during the second heating (unpresented data).

CONCLUSIONS

1. Bovine ST and PM muscles contain more soluble collagen after a longer period of post-mortem ageing. The amount of soluble collagen is lower in boiled muscles in comparison with the roasted and fried ones.

2. The WB shear force values are the highest for boiled muscles, middle for roasted, and the lowest for fried ones. The fried muscles are also characterised by the lowest TPA hardness. Taking into account the tenderness and texture of meat, boiling is not the best method of heating.

3. Reheating of muscles after 6 day of cold storage results in an increase of TPA parameters of hardness and chewiness.

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WPŁYW DOJRZEWANIA POUBOJOWEGO ORAZ METODY DWUKROTNEGO OGRZEWANIA NA ROZPUSZCZALNOŚĆ KOLAGENU, SIŁĘ CIĘCIA I PARAMETRY TEKSTURY MIĘŚNI BYDLĘCYCH

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Z tusz młodych buhajków pobrano w 24 h po uboju mięśnie *semitendinosus* (ST) i *psoas major* (PM) i przechowywano je w 4°C. W 1, 6 i 12 dniu poubojowego dojrzewania określano skład chemiczny mięśni (woda, tłuszcz, białko), zawartość kolagenu ogólnego i rozpuszczalnego oraz poddawano je ogrzewaniu trzema metodami. Plastry mięśni ogrzewano do temperatury wewnętrznej 75°C stosując gotowanie w wodzie w 100°C, pieczenie w powietrzu w 170°C oraz smażenie w oleju roślinnym w 160°C. Ogrzewane kawałki mięśni przechowywano w 4°C przez 6 dni i poddawano je powtórnemu ogrzewaniu do temperatury wewnętrznej 60°C w tych samych środowiskach grzejnych jak przy pierwszym ogrzewaniu. W mięśniach jednokrotnie i dwukrotnie ogrzewanych oznaczano zawartość kolagenu ogólnego i rozpuszczalnego oraz dokonywano pomiarów siły cięcia i parametrów profilu tekstury (TPA). Mięsień PM zawierał 2-krotnie mniej kolagenu śródmięśniowego o większej rozpuszczalności niż mięsień ST (tab. 1). Podczas poubojowego dojrzewania rozpuszczalność kolagenu obu mięśni wzrastała. Niezależnie od okresu dojrzewania poziom kolagenu rozpuszczalnego w mięśniach gotowanych był niższy niż w mięśniach pieczonych i smażonych (tab. 2). Podczas dojrzewania chłodniczego mięśni wartość siły cięcia oraz wartości parametrów TPA (twardość i żujność) zmniejsząły się (tab. 3). W tym samym okresie dojrzewania chłodniczego wartości siły cięcia mięśni gotowanych były najwyższe, mięśni pieczonych pośrednie, a mięśni smażonych najniższe (tab. 3). Mięśnie pieczone charakteryzowały się największą twardością TPA, a mięśnie smażone najmniejszą (tab. 3). Żujność TPA mięśni ogrzewanie mięśni po składowaniu chłodniczym nie miało wpływu na wartość siły cięcia, chociaż podwyższyło wartości obu parametrów TPA mięśni (tab. 3).