



THE TENDERISATION OF WILD BOAR MEAT USING A CALCIUM CHLORIDE, KEFIR, WINE AND PINEAPPLE MARINADE

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ABSTRACT

Changes in textural parameters, thermal drip losses, sensory properties as well as mean fibre cross sectional area (CSA), fibre shape, and *endomysium* and *perimysium* thickness of muscles from wild boars were evaluated. Two muscles: *Biceps femoris* (BF), and *Semimembranosus* (SM) subjected to ageing with calcium chloride, kefir, wine and pineapple juice marinades for 7 days were studied. Muscle texture was determined with the TPA test, performed with an Instron 1140 device. Structural elements were measured in muscle samples using a computer image analysis programme. BF, with its higher CSA, thicker connective tissue as compared with SM muscle, was harder, more cohesive, springy and stringy, and was characterised by the higher connective tissue perceptibility than the second muscle. Muscles ageing, regardless of methods, resulted in a decrease in both the CSA and thickness of the connective tissue, and improve in fibre shape. As a consequence ageing caused a reduction in hardness, cohesiveness, springiness, perceptibility of connective tissue and stringiness as well as in augmentation of tenderness, juiciness and general attractiveness of the muscles studied.

As demonstrated by obtained data, regardless of ageing methods, SM compared to BF, were more susceptible to tenderisation. The highest structural and textural changes, but the worst general attractiveness were found in muscles marinated with pineapple juice addition. Insignificantly lower changes in both quality traits were found in muscles aged with kefir marinade which at the same time were characterised by the high tenderness, the highest juiciness and general attractiveness.

Key words: wild boar muscle, tenderisation, calcium chloride, kefir, wine, pineapple juice marinade, structure, texture

INTRODUCTION

The sensory characteristics (texture, flavour, aroma, and colour) of meat are important quality attributes. The texture is mostly determined by the both connective tissue and myofibrillar protein components of muscle [24, 40]. Since most meat are consumed cooked, texture is related to tenderness which is rated by consumer as the one of the most important facets of the eating quality of meat [27] and is related to mechanical strength which decreases during post-mortem ageing. The tenderisation of meat is mainly due to the weakening of myofibrils, and is chiefly caused by the structural weakening of the endomysium and perimysium connective tissue [39]. Many methods of altering meat tenderness have been evaluated, including the use of marinades [10], organic acids [9,11,25,38], salt and calcium chloride addition [3,34], blade tenderisation or vacuum mixing [3]. Marinades are important in applications involving muscles rich in connective tissue [6] and the tenderising effects offers a commercially important means of upgrading them [11,25]. Recent statistical updates indicate the significantly enhanced consumer interest in the

venison meat, especially wild boar meat [19] because of its high level of proteins, vitamins, mineral salts and lower, compared to meat of domestic slaughter animals, content of fat [35, 36]. At a microstructure level wild boar meat was characterised by the thicker connective tissue compared to pork meat [20]. The unique properties of wild boar meat reflect the physical and histochemical composition of the meat [45]. In meat processing it produced different palatabilities, as judged by tenderness, juiciness, texture, and flavour [43] and it could be modified by e.g. different marinades applying.

The study presented here was aimed at comparing changes of structure, texture and sensory properties of selected muscles from wild boar during post-mortem tenderisation with calcium chloride, kefir, wine and pineapple juice marinade addition.

MATERIAL

Raw materials

A total of 7 animals hunted during spring in an enclosed area in the forest of the Western Pomeranian district, Poland, were used. The carcass weights of the wild boars were 50 ± 4 kg, while their ages were about 3 years. Half-carcasses, kept at 4°C for 48 h after shot were deskined and used to obtain 14 hams of pH 5.7–5.9. Each cut was deboned, and cleaned of external fat. The *Biceps femoris* (BF), and *Semimembranosus* (SM) muscles were dissected out of the hams. After trimming, individual muscle weight ranged from 1050 to 1200 g.

Tenderisation

Six about 4 cm thick slices were cut perpendicularly to the fibres from each muscle. Five slices, after weighing, were individually vacuum packaged in plastic bags and cold stored at $4 \pm 1^\circ\text{C}$ with 10% addition of red dry wine, 0.3 M CaCl_2 , kefir and raw pineapple juice contained the bromelin, respectively, and last sample was packaged with any ingredients accelerating ageing. The samples were taken for assays on the 7th day of *post mortem* (*p.m.*) cold storage. The control sample (non stored) was tested immediately after trimming.

Cooking

Samples collected from both the cold stored and the control meat were sealed in heat-resistant bags, and subjected to cooking in water heated to 85°C, until the core temperature reached 68°C. The temperature was measured with a PT215 thermometer. Subsequently, the samples were cooled down to about 20°C and stored at 10°C for about 12 h until the analysis were made.

METHODS

Structure

Mean fibre CSA, H and V diameters, *endomysium* and *perimysium* thickness measurements were made on raw samples, both stored and non-stored (control), cut from the BF and SM muscles, three cuts being taken from each muscle. The samples about 6 x 6 x 10 mm were dehydrated in alcohol, fixed in Sannomiya solution, and embedded in paraffin blocks. The blocks were sectioned into 12 µm slices with a microtome. The sections were placed on glass slides, contrast-stained with hematoxylin and eosin, and sealed with Canada balsam [5]. The Multi Scan Base v.13 computer image analysis software was used to measure fibre CSA, *endomysium* and *perimysium* thickness and fibre shape (as a ratio of H and V diameters) per muscle fibre bundle, and 10 primary muscle fibre bundles per each muscle were analysed, and more than 200 muscle fibres and connective tissue thickness/ sample were analysed. A microscope magnification of 100 x was used.

Texture measurement

Muscle texture were assayed following the Texture Profile Analysis (TPA) procedures [4], with an Instron 1140. The test involved driving a 0.61 cm diameter shaft twice, in parallel to muscle fibre into a sample down to 80% of their original height (16 mm), using a crosshead speed of 50 mm min⁻¹ and a load cell of 50 N. The force-deformation curve obtained during the TPA test served to calculate meat hardness, cohesiveness and springiness [4]. The TPA test was repeated 5 times on each sample of muscle.

Sensory texture evaluation

The sensory evaluation of the meat samples was assessed by a trained expert panel of 4 members with, in general, a minimum of four years experience in texture analysis of meat and meat products. The meat tenderness, juiciness, connective tissue perceptibility, stringiness and general attractiveness were assessed using a 5-points scale as following: 1 point: the toughest, the most dry and samples with the low perceptible of connective tissue and fibres and low general attractiveness; 5 points: the most tender, juicy and with the high perceptible of connective tissue and fibres and the highest general attractiveness.

Statistical analyses

Statistical analyses of the data involved the calculation of the mean values and standard deviations (SD) for each muscle and each group of muscles. The differences in texture, structure, and sensory properties between the muscles within a group of wild boars as well as between the methods of tenderisation for each muscle were studied using the analysis of covariance. Treatment differences were tested for significance at the 5% level. All the calculations were performed with Statistica® v.5.0 PL software.

RESULTS AND DISCUSSION

As shown in Table 1, among the non-ageing (control) samples BF compared to SM consisted of fibres with a higher (by about 6%) cross sectional area (CSA) and less regular shape, and of thickest *peri-* and *endomysium* (by about 11 and 6%, respectively). Also numerous authors compared different muscles of wild boar [37,44,45] and have reported smaller fibre and thinner connective tissue of SM than BF muscle.

Table 1. Mean value of structural elements of BF and SM wild boar muscles according to ageing factor

Ageing factor	muscle	Muscle fibre		Connective tissue	
		cross sectional area [μm^2]	H/V	perimysium thickness [μm]	endomysium thickness [μm]
Control sample	BF	1307.21 ^a ₁	0.88 ^b ₁	23.14 ^a ₁	1.98 ^a ₁
	SM	1231.71 ^a ₁	0.91 ^b ₁	20.57 ^b ₁	1.86 ^b ₁
Samples after 7 days of ageing					
Without any ageing agent	BF	1269.47 ^a _{1,2}	0.90 ^b ₁	22.98 ^a ₁	1.92 ^a ₂
	SM	1121.44 ^b ₂	0.94 ^a ₁	20.01 ^b ₁	1.86 ^a ₁
With wine addition	BF	1271.71 ^a ₂	0.94 ^a _{1,2}	20.31 ^a ₂	1.87 ^a _{2,3}
	SM	1143.00 ^b ₂	0.95 ^a _{1,2}	18.71 ^a ₂	1.80 ^b ₂
With kefir addition	BF	1341.55 ^a ₁	0.95 ^a ₂	22.97 ^a ₁	1.90 ^a ₂
	SM	1222.71 ^b ₁	0.97 ^b ₂	19.07 ^b _{1,2}	1.80 ^b ₂
With CaCl ₂ addition	BF	1197.24 ^a ₂	0.93 ^a ₂	20.96 ^a _{2,3}	1.82 ^a ₃
	SM	1107.51 ^a ₂	0.95 ^a ₂	18.98 ^a _{2,3}	1.74 ^b ₂
With pineapple juice addition	BF	1108.67 ^a ₃	0.95 ^a ₂	18.71 ^a ₃	1.70 ^a ₄
	SM	1050.74 ^b ₃	0.96 ^a ₂	17.06 ^a ₃	1.62 ^b ₃

a – numbers in columns, marked with identical superscripts are not significantly different between muscles within an ageing method at the 0.05 level of probability.

1– numbers in columns, marked with identical subscripts are not significantly different between ageing methods at the 0.05 level of probability.

Regardless of tenderisation methods and muscle type, meat ageing was found to induce in general the decrease in the fibre CSA and decrease in the *endomysium* and *perimysium* thickness as well as an improve in fibre shape (Table 1). Regardless of tenderisation methods, insignificant extensive changes in the structural elements were observed in SM, lower changes were recorded at the same moments in time in BF. The absence of diversity in fibre CSA between the muscles could be the reason for small differences in their susceptibility to ageing induces changes. Ageing without any ingredients induced a 3–9% decrease in fibre CSA and 1v3% decrease in connective tissue thickness.

Higher changes in structural elements were observed during ageing with CaCl₂ and pineapple juice addition. For example, due to the 7 days of ageing, the CSA of muscles with calcium and fruit marinade addition had decreased about 24–47%, respectively depending on muscle, relative to the control. In the same time changes in the *endomysium* and *perimysium* thickness were about 8–9 and 7–8% when the CaCl₂ was supplemented and 17–19% and 13–14% with pineapple marinade addition.

Probably, through activating m- and μ -calpain while hydrolyzing calpastatin, calcium addition caused an accelerated and more extensive degradation of the susceptible myofibril components [18], leading to i.e. the marked changes of fibre shape. The ratio of H/V diameter were change from 0.88–0.91 to 0.93–0.95 after ageing with CaCl₂ addition.

Also vegetable enzymes such as bromelin addition have a significant effect on structural elements changes because of large spectrum of action being involved in degradation of the main proteins of myofibrillar muscles such as myofibrillar proteins and collagen [28]. According to Ashie et al. [1], the bromelin exhibits a more accentuated hydrolytic action on collagen than on myofibrillar proteins. The significant effect of pineapple juice on the connective tissue was also noticed in our results which showed the highest decrease in *perimysium* and *endomysium* thickness during ageing with pineapple juice addition. The connective tissue of wild boar typically accounts more collagen than meat from commercial pigs [32] and is therefore regarded as a key structure determining collagen toughness. The mechanism of weakening is unclear but it has been suggested that marination may reduce the thermal stability of perimysia and endomysia connective tissue [25].

When the acid marinades (kefir and dry wine) were applying only the insignificant decrease or the slight increase in fibre CSA and decrease in connective tissue thickness were observed. For example, due to the 7 days of ageing, the CSA of muscles with wine and kefir marinade had a decreased about 3–7% and an increased about 1–3%, respectively depending on muscle, relative to the control. In the same time changes in the *endomysium* and *perimysium* thickness were about and 9–12 and 3–5% when the wine was supplemented and 1–7 and 3–4% with kefir addition. The mechanism of the tenderisation action of acidic marinades is believed to involve several factors including weakening of structures due to swelling of meat, increased proteolysis by cathepsins and increased conversion of collagen to gelatin at low pH during thermal treatment [2, 30]. At a microstructure level Oreskowich et al. [31] observed both an increase in bound water and a disruption of the sarcomere structure. In our study it was visible as a higher fibre CSA compared to fibre CSA of muscles aged without any additional factors. Also Karlsson et al. [13] observed an increase in the cross sectional area of muscle fibres with a decrease in pH to pH3.

Differences in muscle structure could be connected with the differences in the textural and sensory parameters of marinated muscles observed in this study (Table 2 and 3). Of all the muscles tested, higher values of hardness, cohesiveness, springiness, higher perceptibility of connective tissue were recorded in the BF – muscle with the coarser structure. Lower values of this parameters and at the same time more delicate structure typically being in the SM muscle and it is in accordance with results of Lachowicz et al. [20] and Żochowska et al. [44], who observed the BF to be tougher than other muscle tested. According to Koohmaraie al. [18], sarcomere length, connective tissue content, and proteolysis of myofibrillar proteins account for most of the explainable variation observed in tenderness of aged meat, after post-mortem storage.

Table 2. Mean value of textural elements of BF and SM wild boar muscles according to ageing factor

Ageing factor	Muscle	Hardness (N)	Cohesiveness (-)	Springiness (cm)
	BF	97.87 ^a ₁	0.586 ^a ₁	1.13 ^a ₁
	SM	85.51 ^b ₁	0.494 ^b ₁	1.08 ^a ₁
Samples after 7 days of ageing				
Without any ageing agent	BF	79.09 ^a ₂	0.452 ^a ₂	1.00 ^a ₂
	SM	56.22 ^b ₂	0.390 ^b ₂	0.97 ^a ₂
With wine addition	BF	68.11 ^a ₃	0.463 ^a ₂	1.00 ^a ₂
	SM	44.22 ^b ₃	0.388 ^b ₂	0.95 ^b _{2,3}
With kefir addition	BF	63.70 ^a _{3,4}	0.398 ^a ₃	0.94 ^a ₃
	SM	43.29 ^b _{3,4}	0.350 ^a ₃	0.87 ^b ₃
With CaCl ₂ addition	BF	56.47 ^a _{4,5}	0.407 ^a ₃	0.95 ^a ₃
	SM	47.53 ^b ₃	0.388 ^a ₂	0.90 ^b _{2,3}
With pineapple juice addition	BF	51.98 ^a ₅	0.350 ^a ₄	0.85 ^a ₄
	SM	37.50 ^b ₄	0.333 ^a ₃	0.81 ^a ₄

a – numbers in columns, marked with identical superscripts are not significantly different between muscles within an ageing method at the 0.05 level of probability.

1– numbers in columns, marked with identical subscripts are not significantly different between ageing methods at the 0.05 level of probability.

Ageing and marinating, regardless of the method applying, resulted in a reduction of muscle hardness, springiness, cohesiveness, connective tissue perceptibility and stringiness as well as an increase of tenderness and juiciness. A similar effect of ageing on muscle texture was reported by i.a. Koohmaraie et al. [18], Kolczak et al. [15], Palka [33], Brooks and Savell [7], Maher et al. [26], Monson et al. [29]. The data obtained show the rate and dimension of tenderization were dependent on muscle type and ageing method.

Table 3. Sensory parameters of wild boar muscles according to ageing factor

Ageing factor	Muscle	Tenderness	Juiciness	Connective tissue perceptibility	Stringiness	General attractiveness
	BF	3.00 ^a ₁	3.25 ^a ₁	4.00 ^a ₁	3.75 ^a ₁	3.00 ^a ₁
	SM	3.50 ^a ₁	4.00 ^b ₁	3.50 ^a ₁	3.00 ^b ₁	3.25 ^a ₁
Samples after 7 days of ageing						
Without any ageing agent	BF	3.50 ^a ₁	3.50 ^a ₁	4.00 ^a ₁	3.25 ^a _{1,2}	3.25 ^a ₁
	SM	4.00 ^a _{1,2}	4.25 ^b ₁	3.00 ^b _{1,2}	2.75 ^a ₁	3.75 ^a _{1,2}
With wine addition	BF	3.75 ^a _{1,2}	3.75 ^a _{1,2}	3.75 ^a ₁	3.25 ^a _{1,2}	3.25 ^a ₁
	SM	4.00 ^a _{1,2}	4.25 ^a ₁	3.00 ^b _{1,2}	2.50 ^a _{1,2}	4.00 ^b _{2,3}
With kefir addition	BF	4.00 ^a ₂	4.25 ^a ₂	3.75 ^a ₁	2.75 ^a ₂	4.25 ^a ₂
	SM	4.50 ^a _{2,3}	4.75 ^a ₂	2.75 ^b ₂	2.25 ^a ₂	4.50 ^a ₃
With CaCl ₂ addition	BF	4.00 ^a ₂	3.75 ^a _{1,2}	3.50 ^a _{1,2}	3.00 ^a _{1,2}	3.50 ^a _{1,2}
	SM	4.25 ^a ₂	4.25 ^a ₁	3.00 ^a _{1,2}	2.75 ^a _{1,2}	3.75 ^a _{1,2}
With pineapple juice addition	BF	4.25 ^a ₂	3.75 ^a _{1,2}	3.25 ^a ₂	1.75 ^a ₃	1.00 ^a ₃
	SM	4.75 ^a ₃	4.50 ^b _{1,2}	2.50 ^a ₂	1.25 ^a ₃	0.50 ^a ₄

a – numbers in columns, marked with identical superscripts are not significantly different between muscles within an ageing method at the 0.05 level of probability.

1– numbers in columns, marked with identical subscripts are not significantly different between ageing methods at the 0.05 level of probability.

A comparison between the dimensions of tenderisation of two wild boar muscles showed the tender muscles (SM) compared to BF was the most susceptible to texture and sensory parameters changes. For example, due to the 7 days of ageing, regardless of marinating methods, the hardness of SM muscle had decreased about 34–56%, relative to the control. In the same time changes in the BF hardness were about 19–47% (Table 2). Also numerous authors compared different beef muscles [8,14,17,42] and have reported similar effects of tenderisation.

When the marinating methods were compared, the lowest decrease in texture and sensory parameters was observed in samples subjected to cold storage without any ingredients. The significant higher decrease in those parameters showed muscle after ageing with CaCl₂, kefir and wine addition and the most significant effects on texture parameters had a marinating with pineapple juice addition. For example, due to the 7 days of ageing, the hardness of muscles with calcium and kefir marinade addition had decreased by about 42–44 and 35–49%, respectively depending on muscle, relative to the control. In the same time changes in hardness were about 19–34% in meat ageing without any ingredients (Table 2).

Injection enhancement of meat decreased shear force values when the enhancement solutions contained sodium or calcium lactate and salt [21,41]. The calcium in calcium lactate probably acts as a postmortem activator of calpain enzymes in decreasing shear force [16]. Addition of calcium-contained ingredients (such as calcium lactate or kefir) in enhanced wild boar meat has been shown to increase proteolysis via calpain enzymes more than simple increase in tenderness due to an increase in ionic strength which is in agreement with results obtained for beef by Lawrence et al. [23]. Also Lawrence et al. [21,22] found injection enhancement with several solutions increased sensory-panel scores for tenderness and juiciness while decreasing the perception of connective tissue, like calcium solutions in this study.

The other possibility for increase in tenderness is the optimal pH for activity of cathepsins is in range 3.5–5.0 and hence the lowering of meat pH in marinades may well enhance proteolytic attack by these enzymes [6], what is visible in samples of wild boar meat marinated with kefir and wine addition. As showed in Table 2, wine addition affected on decrease in hardness by about 35–49%, relative to the control, as well as an increase in tenderness and juiciness. Also Gault [9,10,11] and Offer and Knight [30] found that meat which had been soaked in acidic marinades, and consequently had a pH below 5.0, took up water and became less tough compared with controls.

The enzymatic ageing of wild boar meat with bromelain, also led to the improvement of the hydrophilic characteristic of meat protein and to the decrease in hardness (by about 47–56%) compared to the samples without exogenous enzymes. The results of our study showed also that wild boar meat tenderised with a pine apple juice had a higher tenderness, juiciness; strongly lower perceptibility of connective tissue and stringiness but the worst general attractiveness which was connected with a appearance and slimy texture. According to Ionescu et al. [12] bromelain showed hydrolytic activity on the connective tissue, leading to the better tenderisation of the tough meat but sometimes lead to over – tenderisation and to a product with a pasty texture [28].

CONCLUSIONS

The result obtained show a significant effects of marinating on reduction of wild boar muscle hardness and springiness, and an increase of tenderness and juiciness as a consequence of structural changes such as an increase in fibre CSA, an improve in fibre shape as well as decrease in connective tissue thickness. The rate and dimension of tenderisation were dependent on muscle type and marinating methods. Of the muscles tested, the SM compared to BF was the most susceptible to tenderisation. The addition of calcium ions (as calcium chloride or kefir), decrease in pH value (wine and kefir marinades), and exogenous enzyme (pineapple juice) was found to be an efficient means of improving the quality of wild boar meat. However, the effects of those ingredients diversified between the methods. The highest structural and textural changes, but the worst general attractiveness were found in muscles ageing with pineapple juice addition. Insignificantly lower changes in both quality traits were found in muscles marinated with wine, calcium chloride and kefir, and the latter at the same time were characterised by the high tenderness, the highest juiciness and general attractiveness. Thus, the combined addition of wine, kefir or pineapple juice or calcium may be considered as a possible alternative for organoleptic quality when wild boar meat is marinated.

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