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Research Article

Quality, Microstructure, and Technological Properties of *Sheep* Meat Marinated in Three Different Ways

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The objective of this study was to explore the effect of 24 and 48 h alkaline (2% pentasodium tripolyphosphate), acid (2% sodium lactate), and water-oil marinating (water : sun flavor oil = 1 : 1 and 2% salt) as well as brine soaking (2% salt) on microstructure, changes in protein, and lipid fractions and technological properties of sheep (*m. Longissimus dorsi*). Strong myofibrillar fragmentation after 48 h alkaline marinating was observed. Significant swelling and increasing of spaces between myofibrils were found after 24 h brine soaking. Marinating in water-oil emulsions did not show a significant impact on the muscle microstructure. Alkaline and acid marinating as well as salt soaking promote the myofibrillar protein solubility and increased the free amino nitrogen content. After 24 h acid and 48 h alkaline marinating SDS-PAGE electrophoresis showed increasing of 25–30 kDa protein bands. The results obtained for the lipid and protein oxidation confirm prooxidant action of the sodium lactate (2%) and antioxidant effect of polyphosphates (2%) in marinated sheep.

1. Introduction

Marinating is a simple technological treatment used to improve the functional and sensory properties of meat by soaking, injecting, or tumbling with aqueous solutions, composed of different ingredients [1].

While alkaline marinade solutions contain salt-phosphate mixture, acid solutions contain organic acids or their salts. The third type is water-oil emulsions which contain salt, sugar, vinegar or citric acid, and other supplements.

Sheep is traditional and preferred meat for consumers in Kazakhstan. In Bulgaria significant part from livestock belongs to sheep population. Meat from these animals contains larger amount of cross-linked collagen and is usually tough [2]. It is known that the meat tenderness is one of the most important factors affecting the meat consumers satisfaction [3]. In the postmortem period meat tenderness is influenced by the proteolysis of myofibrillar and connective tissue proteins. Breaks in the sarcomeres occur and meat becomes tendered. Probably calcium-dependent enzymes play the most important role in the proteolytic changes in meat [4].

Injection of marinade solutions had a positive effect on meat tenderness [5]. The addition of phosphates such us sodium tripolyphosphate increases water holding capacity due to protein extraction and shifting of the pH from the muscle proteins isoelectric point [6]. Sodium carbonate and bicarbonate are known to be superior marinating agents, which reduce drip loss and improved yield [7]. Marinating with alkaline phosphates decreases the shear force and consequently increases meat tenderness [8].

The use of organic acids during marinating had the strong effect on muscle fibers and connective tissue and enhances tenderness. Citric acid, a food acidulant, not only is often used in acid marinating to improve the water holding capacity and tenderness of beef muscle but is also commonly used as a chelator to control the activity of prooxidant metals [9]. Lactic acid is often used in the meat industry as an antimicrobial agent [10]. But marinade solutions with lactic acid did not improve meat juiciness probably due to the high loss of meat juice during cooking [11].

Spices and herbs added in marinades significantly enhance meat safety [12].

The influence of different types of marination on the meat microstructure and quality was discussed about poultry [1, 13, 14], turkey [5, 15], pork [16], and beef [17–20]. Only few studies focus on marinating effects on microstructural, proteolytical, and oxidative changes in sheep.

Considering all these aspects, the aim of this study was to investigate the effects of alkaline, acid, and water-oil marination, as well as brine soaking, on sheep quality by monitoring the changes of microstructure, hydrolytic and oxidative processes, and some technological properties.

2. Materials and Methods

2.1. Materials. The fresh (1 h postmortem) sheep *m. Longissimus dorsi* with pH 6.95 was supplied by the company Unitemp Ltd., Voyvodinovo Village, District Plovdiv, Bulgaria. Sheep *m. Longissimus dorsi* was cut into thirty pieces (100 × 60 × 20 mm, each weighting approx. 50 g). Sodium lactate (60% aqueous solution) was purchased by "Teokom" Ltd. (Sofia, Bulgaria). Sodium tripolyphosphate (E451) was supplied by the company "FILLAB" Ltd. (Sofia, Bulgaria). Sodium chloride (salt), sugar, and sunflower oil were bought from the local market.

2.2. Sample Preparation. Four types of solutions were used. The alkaline marinating solution (ALS) was prepared with 2% polyphosphates (E 451) and 2% salt added in water. The pH of alkaline marinating solution (ALS) was 8.0. The acid marinating solution (ACS) was prepared with 2% sodium lactate and 2% salt added in water. The pH of acid marinating solution (ACS) was 6.75. The water-oil marinating solution (WOS) was made as mixture of water and sunflower oil (1 : 1) with 2% salt addition. The pH of water-oil marinating solution (WOS) was 6.9. The brine soaking solution (BSS) represents 2% salt water solution with pH 6.85. All solutions (ALS, ACS, WOS, and BSS) were cooled to the temperature of 4°C and used for meat marinating. The meat samples (*m. Longissimus dorsi*) were added to marinating solutions in ratio 1 : 2 and separately stored in closed plastic boxes at 0–4°C for 24 h and 48 h. The test samples were as follows: AL24: alkaline marinated sheep for 24 h, AC24: acid marinated sheep for 24 h, WO24: water-oil marinated sheep for 24 h, BS24: soaked in salt solution sheep for 24 h, AL48: alkaline marinated sheep for 48 h, AC48: acid marinated sheep for 48 h, WO48: water-oil marinated sheep for 48 h, and BS48: soaked in salt solution sheep for 48 h. Individual measurements were defined for marinade solutions (ALS, ACS, WOS, and BSS, resp.). The control samples (*m. Longissimus dorsi*) at 4 h postmortem (C0) were measured immediately. The other control samples were stored separately in plastic bags for 2 days in air conditions at 0–4°C and were measured at 24 h postmortem (C24) as well as at 48 h postmortem (C48).

2.3. Methods. Microstructural analysis was carried out as described by Lawrence et al. [21]. Marinated and control samples (10 mm × 2 mm × 2 mm) were soaked for 24 h at 4°C in a 0.2 M glutaraldehyde solution with 0.2 M sodium cacodylate buffer (pH 7.4), rinsed 5 times in 0.2 M sodium cacodylate buffer, and fixed in 4% osmium tetroxide in 0.2 M sodium cacodylate buffer for 2 h at 4°C. The samples were dehydrated with 50%, 70%, 96%, and 100% ethanol for 30 min at each concentration with constant agitation and then stored overnight in 100% ethanol at 4°C. The samples were then soaked to propylene oxide for 30 min, mixed in epoxy with propylene oxide for 30 min, and coated in fresh epoxy and hardened by heating at 56°C for 48 h. Samples were sliced to a thickness of 60 nm and the sections were mounted on bare 200-mesh hex copper grids. The grids were stained in a saturated solution of uranyl acetate in 50% ethanol for 30 min, followed by staining in alkaline lead citrate solution for 3 min. The samples were observed on a JEM-1200EX/ASID transmission electron microscope (JEOL, Japan) at 12000x and photographed on film that was scanned at 600 dpi on a flatbed transparency scanner.

Extraction of myofibrillar proteins was carried out with PBS buffer (49 mM $Na_2HPO_4 \cdot 7H_2O$ and 4.5 mM $NaH_2PO_4 \cdot H_2O$ and KCl, ionic strength 0.55, and pH = 7.3), according to the procedure described by Khan [22] with some modifications. The chopped muscle tissue (2.5 g) was homogenized with 48.5 cm^3 PBS buffer and stored at 0–4°C for 24 h. The homogenate was centrifugated at 3000 ×g for 15 min. SDS-PAGE was carried out using 10% gels at a constant voltage mode (200 V) in an Omni PAGA Electrophoresis System (Cleaver Scientific Ltd.). Protein markers were obtained from Precision Plus Protein™ Standards (Bio-Rad Laboratories, Inc., Hercules, USA).

The myofibrillar proteins concentration was determined spectrophotometrically at 750 nm [23] with double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd., Cambridge, UK).

Free amino nitrogen (FAN) was determined by modified titration method of Sørensen [24]. The absorption was measured with double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd., Cambridge, UK) at 570 nm.

Protein oxidation was measured by estimation of formed carbonyl groups [25]. Protein concentration was calculated at 280 nm in the HCl control using BSA in 6 M guanidine as standard. Carbonyl concentration was measured on the treated sample by measuring DNPH incorporated on the basis of absorption of 21.0 $mM^{-1}\,cm^{-1}$ at 370 nm for protein hydrazones. The results were expressed as nanomoles of DNPH fixed per milligram of protein.

2.4. Extraction of Total Lipids. The extraction of total lipids was carried out following Bligh and Dyer method [26]. After homogenization, a hundred g of sample was mixed with

Table 1: Changes of the soluble proteins and the free amino nitrogen content of marinated sheep and marinating solutions.

Sample	Soluble proteins ($mg \cdot ml^{-1}$)	Free amino nitrogen, ($mgLeu \cdot g^{-1}$)	Solutions	Soluble proteins ($mg \cdot ml^{-1}$)	Free amino nitrogen ($mgLeu \cdot g^{-1}$)
	Marinated meat			Marinating solutions	
C24	$1.33^{b} \pm 0.14$	$13.33^{b,c} \pm 0.21$		—	—
AL24	$3.48^{d} \pm 0.19$	$19.50^{g} \pm 0.32$	*ALS24*	$3.20^{e} \pm 0.20$	$2.81^{b} \pm 0.08$
AC24	$3.36^{d} \pm 0.22$	$15.80^{f} \pm 0.18$	*ACS24*	$1.88^{a,b} \pm 0.12$	$3.42^{d} \pm 0.12$
WO24	$2.90^{c} \pm 0.15$	$12.94^{b} \pm 0.15$	*WOS24*	$2.25^{c} \pm 0.14$	$2.90^{b} \pm 0.15$
BS24	$3.60^{d} \pm 0.18$	$20.09^{g} \pm 0.17$	*BSS24*	$3.90^{f} \pm 0.12$	$2.64^{a,b} \pm 0.09$
C48	$0.79^{a} \pm 0.08$	$9.91^{a} \pm 0.20$		—	—
AL48	$1.36^{b} \pm 0.15$	$15.00^{e} \pm 0.15$	*ALS48*	$2.56^{d} \pm 0.05$	$2.61^{a} \pm 0.10$
AC48	$1.32^{b} \pm 0.12$	$13.55^{c} \pm 0.18$	*ACS48*	$1.78^{a} \pm 0.07$	$3.17^{c} \pm 0.12$
WO48	$1.30^{b} \pm 0.10$	$14.20^{d} \pm 0.25$	*WOS48*	$2.00^{b,c} \pm 0.11$	$2.77^{a,b} \pm 0.09$
BS48	$5.10^{e} \pm 0.08$	$25.03^{h} \pm 0.22$	*BSS48*	$4.40^{g} \pm 0.18$	$3.50^{d} \pm 0.17$

Mean value ± SE. [a,b,c,d,e,f,g,h]Different letters (in columns) on the means with statistical differences at 24 h or 48 h marinated samples ($P \leq 0.05$). ALS24: alkaline marinating solution (2% polyphosphates (E 451) and 2% salt) after 24 h treatment; ACS24: acid marinating solution (2% sodium lactate and 2% salt) after 24 h treatment; WOS24: water-oil marinating solution (water and sunflower oil (1 : 1) with 2% salt) after 24 h treatment; BSS24: brine solution (2% salt water solution) after 24 h treatment; ALS48: alkaline marinating solution (2% polyphosphates (E 451) and 2% salt) after 48 h treatment; ACS48: acid marinating solution (2% sodium lactate and 2% salt) after 48 h treatment; WOS48: water-oil marinating solution (water and sunflower oil (1 : 1) with 2% salt) after 48 h treatment; BSS48: brine solution (2% salt water solution) after 48 h treatment; C0: control samples at 4 h postmortem; C24: control samples at 24 h postmortem; C48: control samples at 48 h postmortem; AL24: alkaline marinated sheep for 24 h; AC24: acid marinated sheep for 24 h; WO24: water-oil marinated sheep for 24 h; BS24: soaked in salt solution sheep for 24 h; AL48: alkaline marinated sheep for 48 h; AC48: acid marinated sheep for 48 h; WO48: water-oil marinated sheep for 48 h; BS48: soaked in salt solution sheep for 48 h.

300 mL chloroform and 200 mL methanol. After filtration the final biphasic system was separated and the chloroform phase was collected. Lipid content was determined after evaporating of chloroform phase to dryness under nitrogen.

Acid value (AV) of the extracted lipids was measured following EN ISO 660:2001 procedure based on the acid-base titration techniques in nonaqueous solvents [27]. One gram of fat was digested with 20 mL neutral alcohol-ether mixture (1 : 2) with few drops of phenolphthalein addition. The mixture was titrated with 0,01 N KOH to pale pink colorisation. Acid value (AV) was expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids contained in 1 g of oil.

Peroxide value (POV) of the tested samples was measured by the oxidation of Fe^{2+} to Fe^{3+} in the presence of hydroperoxides [28]. The absorption was measured with double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd., Cambridge, UK) at 507 nm.

TBARS were determined by the method described by Botsoglou et al. [29]. The double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd., Cambridge, UK) was used.

The pH of the marinating solutions was measured directly [30] with pH-meter Microsyst MS 2004 (Microsyst, Plovdiv, Bulgaria), equipped by combined pH electrode Sensorex combination recorder S 450 CD (Sensorex pH Electrode Station, Garden Grove, CA, USA).

The water holding capacity (WHC) was measured according to Modzelewska-Kapitula and Cierach procedure [31]. One meat cut (0,3 g) was placed on filter paper between two glass plates and pressed with a 1 kg weight for 10 min. The water holding capacity was determined as difference between the areas of the two spots by planimeter measuring.

Calculations for percentage marinade uptake and cooking loss were as follows: % marinade uptake = marinated weight − raw weight/raw weight × 100 [18]; % cooking loss = 100 × (raw weight − cooked weight)/raw weight [5].

2.5. Statistical Analysis. All the analyses were replicated nine times ($n = 9$). Results were expressed as means ± standard error (SE). Statistical analyses were conducted using SPSS 11.0 software (SPSS Inc., Chicago, Illinois, USA). Data were analyzed independently by ANOVA software (Excel 5.0). The Duncan multiple comparison test was used to determine the differences between the mean values. If P values for the differences between the means were less than 0.05, they were considered statistically significant.

3. Results and Discussion

3.1. Proteolytical Changes in Marinated Sheep and Marinating Solutions

3.1.1. Changes in Myofibrillar Protein Solubility. Compared to the control samples (C24, Table 1), alkaline and acid marinating (AL24, AC24) as well as salt soaking (BS24) increased up to four times the solubility of myofibrillar protein fraction. This data correspond to [7] study for poultry marinating.

After 24 h treatment, the greatest amount of soluble proteins was extracted in salt solution (BS24) and in alkaline solution (AL24).

Table 2: Changes of the total protein carbonyls, acid value, peroxide value, and TBARS of the marinated sheep.

Sample	Protein carbonyls, $nmol \cdot mg^{-1}$ proteins	Acid value, $mg\ KOH \cdot g^{-1}$	Peroxide value, $\mu eqO_2 \cdot kg^{-1}$	TBARS, $mg\ MDA \cdot kg^{-1}$
C24	$0.14^{b} \pm 0.008$	$0.33^{b,c} \pm 0.04$	$0.30^{d} \pm 0.012$	$0.45^{d} \pm 0.02$
AL24	$0.11^{a} \pm 0.008$	$0.34^{c} \pm 0.03$	$0.23^{b} \pm 0.009$	$0.20^{a} \pm 0.01$
AC24	$0.16^{c,d} \pm 0.009$	$0.46^{d} \pm 0.05$	$0.18^{a} \pm 0.007$	$0.65^{e} \pm 0.02$
WO24	$0.15^{b,c} \pm 0.005$	$0.46^{d} \pm 0.03$	$0.26^{c} \pm 0.008$	$0.24^{b} \pm 0.01$
BS24	$0.15^{b,c} \pm 0.007$	$0.38^{c} \pm 0.03$	$0.29^{d} \pm 0.010$	$0.35^{c} \pm 0.01$
C48	$0.16^{c,d} \pm 0.008$	$0.37^{c} \pm 0.04$	$0.31^{d} \pm 0.015$	$0.47^{d} \pm 0.02$
AL48	$0.12^{a} \pm 0.005$	$0.25^{b} \pm 0.05$	$0.22^{b} \pm 0.008$	$0.22^{a,b} \pm 0.01$
AC48	$0.20^{e} \pm 0.006$	$0.12^{a} \pm 0.04$	$0.38^{f} \pm 0.013$	$0.37^{c} \pm 0.01$
WO48	$0.16^{c,d} \pm 0.008$	$0.35^{b,c} \pm 0.06$	$0.34^{e} \pm 0.011$	$0.26^{b} \pm 0.01$
BS48	$0.17^{d} \pm 0.009$	$0.30^{b} \pm 0.02$	$0.42^{g} \pm 0.013$	$0.37^{c} \pm 0.02$

Mean value ± SE. [a,b,c,d,e,f,g]Different letters (in columns) on the means with statistical differences at 24 h or 48 h marinated samples ($P \leq 0.05$). C24: control samples at 24 h postmortem; C48: control samples at 48 h postmortem; AL24: alkaline marinated sheep for 24 h; AC24: acid marinated sheep for 24 h; WO24: water-oil marinated sheep for 24 h; BS24: soaked in salt solution sheep for 24 h; AL48: alkaline marinated sheep for 48 h; AC48: acid marinated sheep for 48 h; WO48: water-oil marinated sheep for 48 h; BS48: soaked in salt solution sheep for 48 h.

Meat soaking with 2% sodium chloride for 48 h showed the greatest impact on the solubility of myofibrillar protein fraction (BS48, Table 1). These results were expected due to proven action of Cl^{-} ions in salt soaking [7] and phosphate effect in alkaline marinating [32]. The known effect of phosphate addition on myofibrillar solubility was not observed.

3.1.2. Changes in Free Amino Nitrogen (FAN) Content. After 24 h marinating proteolytical change associated with an increase in free amino nitrogen (FAN) was established in all studied samples (Table 1). Compared to the control samples (C24), the FAN content increased 2.7 times in salt soaked sheep (BS24, $P \leq 0.05$) and 2.62 times in alkaline marinated sheep (AL24, $P \leq 0.05$). After 24 h acid marinating (AC24) the FAN content increased 2.53 times ($P \leq 0.05$). The slight increase in FAN content was found in water-oil marinated sheep (WO24, $P \leq 0.05$). Other researches showed that with increasing salt or acid concentration the amount of total nitrogen compound in fish was lower [33].

With the extending of marinating time to 48 h statistically significant decreasing in FAN content ($P \leq 0.05$) in all studied samples was obtained (Table 1).

3.1.3. SDS-PAGE Electrophoresis. After 24 h marinating the 200 KDa polypeptides (heavy meromyosin) were identified in all samples (Figure 3). In control samples, polypeptides with this molecular weight were identified only at 4 h postmortem (C0, Figure 2). This finding confirms that the marinating processes leads to proteolytical changes in muscle tissue (Figure 3). In the 48 h marinating sheep 200 KDa protein bands were not extracted from muscle tissue possibly due to proteolysis and passing of these fractions into marinating solutions. Water-oil marinating was not enough effective for extraction of heavy meromyosin chains (WO24, WO48, Figure 3).

The postmortem degradation of muscle proteins is important factor for developing of meat tenderness [33]. As is known the presence of 28 and 30 kDa polypeptides is associated with the degradation of troponin-T and is a key indicator of the meat aging [34]. The 25–30 kDa protein bands were increasing in muscle tissue after 24 h acid marinating (AL24) and 48 h alkaline marinating (AL48). The largest amount of these fractions was identified in all marinating solutions (Figure 4).

After 48 h marinating the protein fractions in marinating solutions increased due to the passage of protein fractions in the marinade solutions (Figure 4). The accumulation of low molecular weight protein fractions in the studied samples (Figure 3) and marinating solutions (Figure 4) shows that the extension of marinating time to 48 h increased the proteolytic changes in marinated meat.

The largest numbers of protein bands were identified in 48 h soaked sheep (BS48, Figure 3). In this sample the greatest amount of heavy meromyosin (MHC) was extracted.

The results obtained (Figure 4) show that the 2% brine solutions as well as 2% sodium tripolyphosphate solutions influenced to the largest extent proteolytical changes in sheep. The significant proteolytical changes in marinated as well as in soaked sheep were confirmed by the data observed for protein solubility and FAN content (Table 1).

3.1.4. Protein Oxidation. It was found that brine soaking (BS) and water-oil (WO) marinating slightly influenced ($P > 0.05$) protein oxidation in sheep (Table 2). The same trend was found after 48 h marinating (control 48 h, AC48, and BS48). The increasing of carbonyl groups and decreasing of pH established after acid marinating confirmed previous researches [35].

In comparison to the control samples (C24, C48, Table 2), after 24 h and 48 h alkaline marinating, the protein carbonyls content decreased by 30% ($P \leq 0.05$).

The opposite trend was established after acid marinating (AC24, AC48, Table 2). Compared to control samples C24, the carbonyl content in AC24 increases by 20% ($P \leq 0.05$). The extension of marinating time to 48 h, increased by

Table 3: Changes of the pH, water holding capacity, weight gain, and cooking loss of marinated sheep.

Sample	pH	Water holding capacity (%)	Weight gain (%)	Cooking loss (%)
C24	$6.20^{b} \pm 0.02$	$9.75^{a} \pm 0.72$	—	$40.20^{a} \pm 1.05$
AL24	$6.92^{e,f} \pm 0.09$	$20.31^{b} \pm 0.85$	$14.82^{b} \pm 2.67$	$41.40^{a} \pm 1.69$
AC24	$6.60^{c} \pm 0.02$	$19.86^{b} \pm 1.05$	$14.15^{b} \pm 3.99$	$41.10^{a} \pm 2.45$
WO24	$6.70^{d} \pm 0.02$	$9.75^{a} \pm 0.90$	$7.23^{a} \pm 2.07$	$40.05^{a} \pm 2.14$
BS24	$6.85^{e} \pm 0.08$	$21.22^{b} \pm 1.23$	$13.99^{b} \pm 5.32$	$43.45^{a} \pm 2.48$
C48	$6.00^{a} \pm 0.02$	$9.75^{a} \pm 0.72$	—	$40.40^{a} \pm 1.00$
AL48	$7.07^{g} \pm 0.05$	$24.27^{c} \pm 0.85$	$22.53^{c} \pm 3.91$	$41.90^{a} \pm 1.15$
AC48	$6.97^{f} \pm 0.01$	$21.16^{b} \pm 1.05$	$21.99^{c} \pm 3.11$	$41.75^{a} \pm 1.17$
WO48	$6.87^{e} \pm 0.07$	$9.86^{a} \pm 0.90$	$13.02^{b} \pm 1.15$	$41.85^{a} \pm 1.36$
BS48	$7.00^{f} \pm 0.01$	$25.03^{c} \pm 1.23$	$22.86^{c} \pm 2.69$	$46.15^{b} \pm 1.17$

Mean value ± SE. [a,b,c,d,e,f]Different letters (in columns) on the means with statistical differences at 24 h or 48 h marinated samples ($P \leq 0.05$). C24: control samples at 24 h postmortem; C48: control samples at 48 h postmortem; AL24: alkaline marinated sheep for 24 h; AC24: acid marinated sheep for 24 h; WO24: water-oil marinated sheep for 24 h; BS24: soaked in salt solution sheep for 24 h; AL48: alkaline marinated sheep for 48 h; AC48: acid marinated sheep for 48 h; WO48: water-oil marinated sheep for 48 h; BS48: soaked in salt solution sheep for 48 h.

28.20% protein oxidation in acid marinated sheep (AC48). Sharedeh et al. [35] explain that free amino groups (NH or NH2) are very sensitive to the free oxygenated radicals generated by the pH decrease. Decrease of carbonyl content in alkaline marinated sheep was in agreement with the known protective effect of phosphates on protein oxidation [32].

3.2. Lipolytic Changes in Marinated Sheep

3.2.1. Acid Value (AV). After 48 h the free fatty acids content in water-oil marinating sheep (WO48) increased two times ($P \leq 0.05$), compared to alkaline marinated meat. The observed AV was not surprising because these samples had oil addition and as a result the lipolytic processes in water-oil marinated sheep were found higher. The results for AV were in accordance with our other studies about water-oil marinating of horse meat evaluated by tested committee with lower scores due to negative rancid odor and taste [36].

3.2.2. Peroxide Value (POV). A similar tendency was found in primary products of lipid oxidation (Table 2). Compared to the control samples C48, the peroxide value in water-oil marinated sheep (WO48) increases by 9.7% and corresponds with the higher AV levels in the samples. The most significant increase by 20% ($P \leq 0.05$) in primary products of lipid oxidation was found after 48 h acid marination (AC48). In all other samples (AL48, BS48) the peroxide value was lower compared to control samples (C48). These results confirm the existence of induction period (Table 2) and formation and accumulation of lipid hydroperoxides which were converted into secondary products [37].

3.2.3. TBARS. The levels of TBARS of all studies samples vary in the range 0.22–0.65 mg malondialdehyde$\cdot$kg^{-1} lipids, which is lower than the limit for fresh meat of 1.00 mg malondialdehyde$\cdot$kg^{-1} lipids [37].

Acid marinating (AC24) increases by 44.44% ($P \leq 0.05$) the secondary products of lipid oxidation (Table 2). In samples AL24, WO24, BS24, AL48, WO48, and BS48 the TBARS values were lower than those obtained for the control samples C24 and C48, respectively.

The results for alkaline marinated sheep confirmed many previous researches about antioxidant effect of phosphates [13, 32]. Oppositely, the results obtained for lactate marinated meat (AC48) are difficult to explain. On the one hand many researches prove the antioxidant effect of sodium lactate [9–11, 36]. On the other hand the data for POV and TBARS determination showed that lipid oxidation significantly increases after marinating with 2% sodium lactate. Our results confirm prooxidant action of the sodium lactate added as 2% marinating solution with 2% salt and are in accordance with previous studies about prooxidant action of the organic acids in beef loin steaks [17, 38].

Marinating with polyphosphates was the most effective method for inhibition of primary and secondary lipid oxidation products in sheep and confirms the previous researches for alkaline marinated beef [39].

3.2.4. Changes in Physicochemical Characteristics of Marinated Sheep. After 24 h treatment (Table 3), a pH in the alkaline marinated meat (AL24) increases by 6.5% ($P \leq 0.05$), followed by the meat soaked in a brine solution (BS24, 5.4%, $P \leq 0.05$) and water-oil marinated meat (3.1%, $P \leq 0.05$).

After 24 h treatment, small but statistically significant ($P \leq 0.05$) increase in pH was established in 2% acid marinated meat (AC24) (Table 3). Obviously, sodium lactate as salt of weak organic acid had a slight effect on meat pH.

Oppositely after 48 h marinating the most significant increase in the meat pH was found in samples AL48 (with 8.77%) and BS48 (with 7.69%, $P \leq 0.05$, Table 3). The observed pH effect after alkaline marinating is expected and confirmed many other studies in poultry [13, 14] and beef [17].

After 24 h marinating the water holding capacity of samples AL24, AC24, and BS24 was not significantly different ($P > 0.05$ (Table 3)).

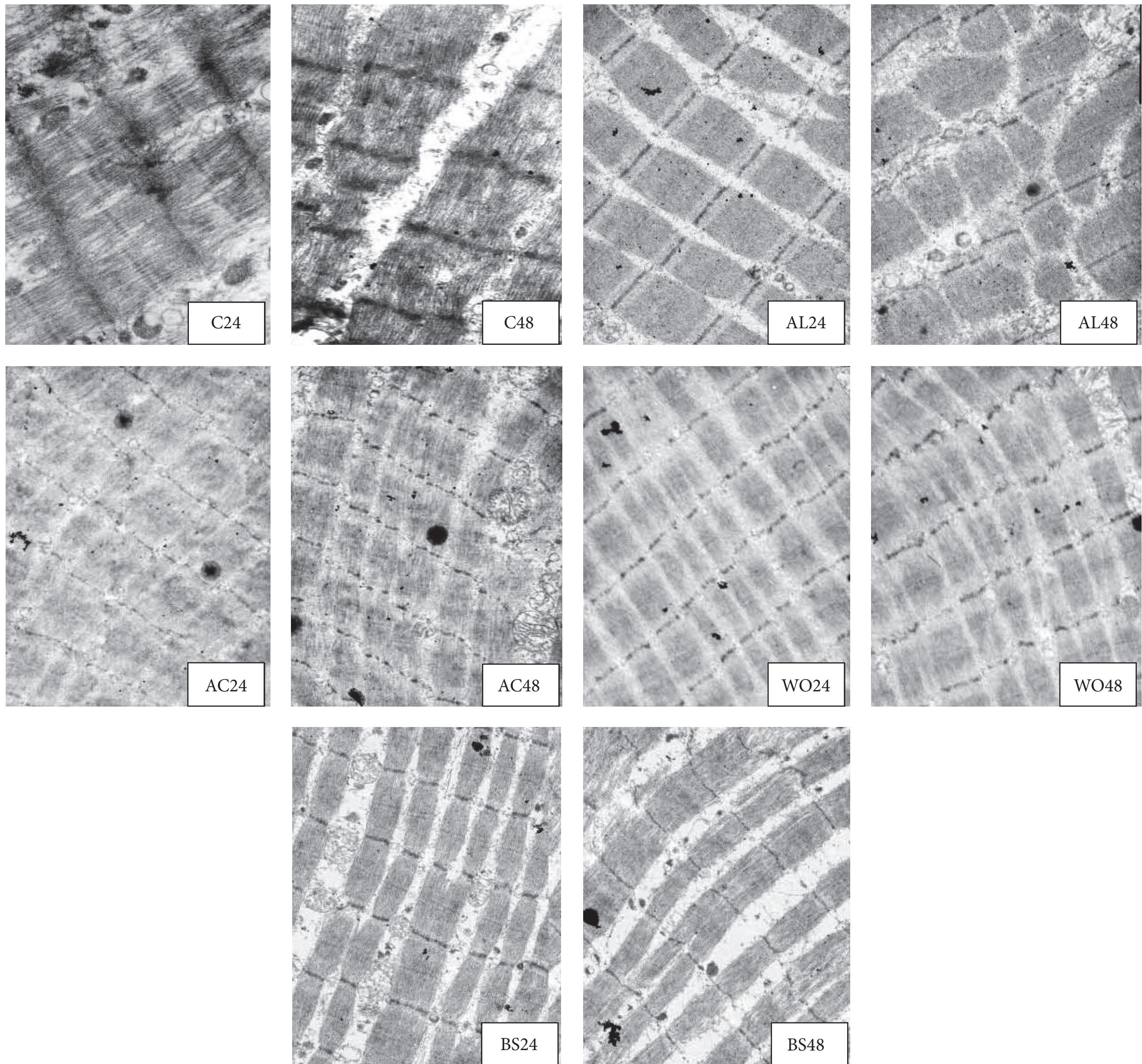

Figure 1: Electron micrographs of longitudinal section of marinated sheep (*m. Longissimus dorsi*), 12000x. C24: control sample stored at 0–4°C in air conditions for 24 h; C48: control sample stored at 0–4°C for 48 h; AL24: test sample after 24 h alkaline marinating; AL48: test sample after 48 h alkaline marinating; AC24: test sample after 24 h acid marinating; AC48: test sample after 48 h acid marinating; WO24: test sample after 24 h water-oil marinating; WO48: test sample after 48 h water-oil marinating; BS24: test sample after 24 h brine soaking; BS48: test sample after 48 h brine soaking.

It was surprising that after 48 h marinating the water holding capacity was highest in brine treated meat (BS48) followed by alkaline marinated meat (AL48) ($P > 0.05$) (Table 3).

It is known that the comparatively high pH during alkaline marinating increases water holding capacity [6]. The expected effect from phosphate marinating on WHC was not observed. One possible reason was that the high pH in meat for marinating (6.95) leads to strong effect after soaking in brine solution.

The least impact on meat water holding capacity (Table 3) and brine absorption (Table 3) was found after water-oil marinating (WO24, WO48). Probable reason for this finding was the high salt concentration in the aqueous phase of the water-oil marinade solution. The water-oil emulsion was prepared from water and plant oil (1 : 1). Therefore the salt concentration in the aqueous phase in water-oil emulsion reaches 4%. As is known the brine absorption in meat during the soaking increases up to salt concentrations of 0.34 mol·L^{-1} (approximately 3% NaCl in brine) and then decreased [40].

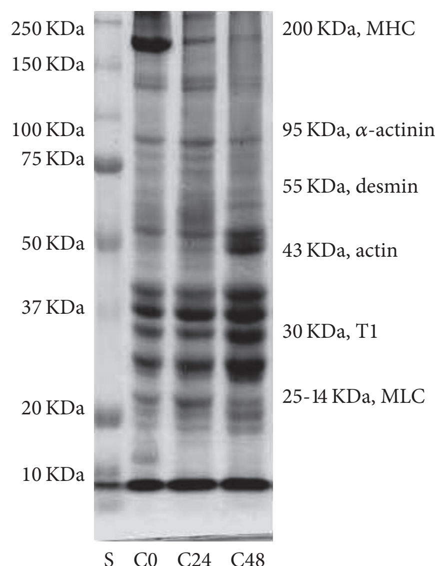

Figure 2: SDS-PAGE electrophoresis of control samples sheep (*m. Longissimus dorsi*), stored in air conditions at 0–4°C. S: standard; C0: control samples at 4 h postmortem; C24: control samples stored in air conditions at 0–4°C for 24 h; C48: control samples stored in air conditions at 0–4°C for 48 h.

We can conclude that due to 4% salt concentration in aqueous phase the estimated lower WHC was normal.

The weight gain increased after alkaline (AL) and acid (AC) marinating, as well as brine soaking (BS) and were in accordance with the data for WHC and pH.

It was interesting that the cooking loss was minimal after water-oil marinating (WO24, WO48). The lowest brine absorption in water-oil marinated sheep can explain this finding (Table 3).

The opposite trend was established in samples BS24 and BS48 where brine absorption was greatest, but in these samples the loss was biggest ($P \leq 0.05$) after cooking. Despite higher WHC and brine absorption, only the use of 2% salt solution does not have effect on marinade retention after roasting (Table 3).

The alkaline marinating with 2% polyphosphates and acid marinating with 2% sodium lactate contribute to penetration and retention of marinade solutions in muscle tissue. The results confirm the triple action of phosphates on muscle tissue: increasing of pH, effect on ionic strength, and myosin extraction from myofibrillar structures in meat [32].

The least impact on the technological properties was established after water-oil marinating (Table 3).

3.2.5. Microstructural Changes in Marinated Sheep. After 24 h alkaline marinating (*m. Longissimus dorsi*) due to the penetration of the marinade solution the myofibrils had "garland" form (AL24, Figure 1). The native structure of myofibrils in sample was preserved. The myofibrils were similar to the control with clearly defined A- and I-bands, H-zones, and M-lines. Our results confirm Komoltri and Pakdeechanuan [41] hypothesis that the use of phosphates opened the protein grid and increased the amount of retained water in myofibrils. As a result the space between myofibrils was increasing.

Wavy myofibril with longitudinal cuts was observed after 48 h alkaline marinating (AL48, Figure 1). Z-disks fragmentation and large gaps formation were an indicator for deep destructive processes in the muscle fibers. This observation was confirmed by lower soluble proteins in AL48 than those in AL24 suggesting no more destructuration of myofibrils (Table 1). TEM observation was in accordance with higher WHC and brine absorption after alkaline marinating (Table 3).

After 24 h acid marinating (AC24, Figure 1) A-, I-, and H-zones and the M-lines were difficultly identifiable (AC48, Figure 1). Fragmentation in the sarcomeres and Z-lines destructions showed deeper destructive changes with the prolonging of acid marination to 48 h. However, the microstructural changes after 48 h acid marination were slighter than changes observed in 48 h alkaline marinated sheep (AL48, Figure 1). This result confirmed SDS-PAGE electrophoresis of marinated (Figure 3) meat and the data established for protein solubility and FAN content (Table 1). But, opposite to phosphates, acid marinating solutions affect native structure of connective tissue due to proteases activation [42, 43].

Slight impact on myofibrillar structure was found after water-oil marinating. Individual sarcomeres were undamaged. A- and I-zones were clearly distinguished. Because of the slight penetration of marinate solution the impact on myofibrils was minimal (WO24, Figure 1).

Marinating in water-oil emulsions for 48 h had little impact on the muscle microstructure. Z-disks and A- and I-zones were distinguished, and the native structure of myofibrils was preserved (WO48).

Significant increasing of space between myofibrils was observed after 24 h soaking in 2% sodium chloride (BS24). Z-lines are undamaged, and A-, I-, H-zones and M-lines are clearly visible.

Penetration of the brine (BS24) in myofibrillar spaces produces longitudinal fissures in the sarcomeres. H-zones were dispersed, but myofibrillar structure was undamaged. Identified microstructural changes of soaked sheep confirmed the data obtained for water absorption (Table 3).

Despite significant swelling and brine penetration after soaking, the reported cooking losses in these samples increased significantly (Table 3).

Our results confirm that soaking had the most significant impact on the solubility of the protein fraction (Table 1) and increased brine penetration and water holding capacity (Table 3) to the maximal extent but does not contribute to retention of the brine (Table 3) in marinated sheep.

4. Conclusions

We can conclude that the alkaline marinating with 2% polyphosphates and acid marination with 2% sodium lactate improve the water holding capacity, solubility of the protein fraction, and marinade uptake.

The brine solution greatly enhances the solubility of the protein fraction but does not contribute to the stable brine retention after heating.

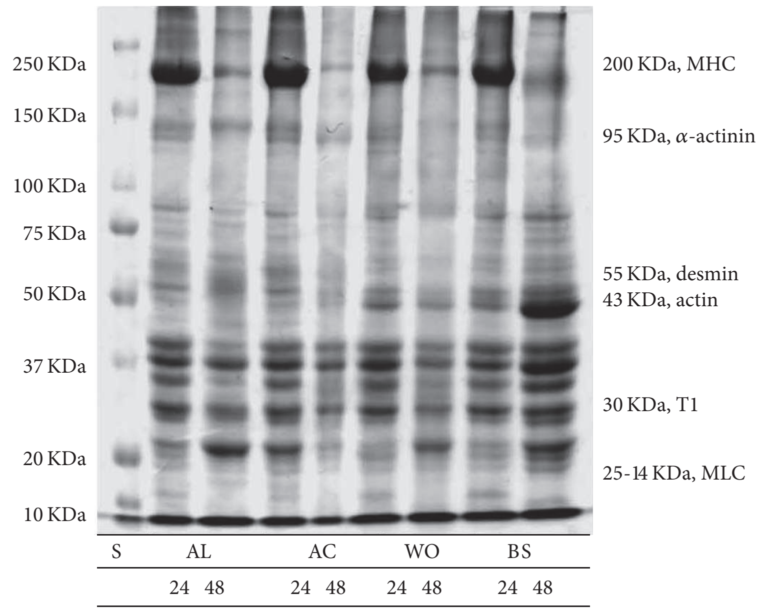

Figure 3: SDS-PAGE electrophoresis of marinated sheep. S: standard; AL24: test sample after 24 h alkaline marinating; AL48: test sample after 48 h alkaline marinating; AC24: test sample after 24 h acid marinating; AC48: test sample after 48 h acid marinating; WO24: test sample after 24 h water-oil marinating; WO48: test sample after 48 h water-oil marinating; BS24: test sample after 24 h brine soaking; BS48: test sample after 48 h brine soaking.

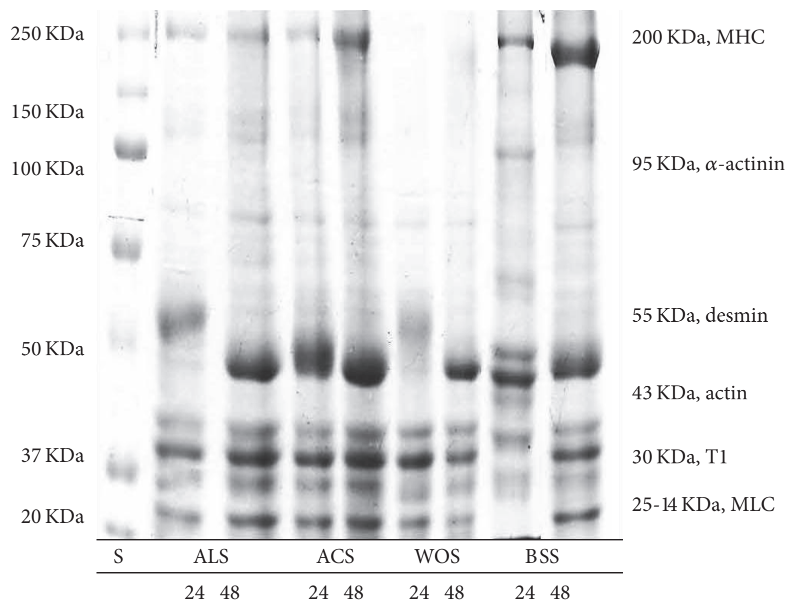

Figure 4: SDS-PAGE electrophoresis of marinating solutions. S: standard; ALS24: alkaline marinating solution after 24 h treatment; ALS48: alkaline marinating solution after 48 h treatment; ACS24: acid marinating solution after 24 h treatment; ACS48: acid marinating solution after 48 h treatment; WOS24: water-oil emulsion after 24 h treatment; WOS48: water-oil emulsion after 48 h treatment; BSS24: brine solution after 24 h treatment; BSS48: brine solution after 48 h treatment.

The water-oil marinating increases the hydrolysis processes in lipid fraction from sheep.

The alkaline marinating with 2% of sodium tripolyphosphate inhibits protein and lipid oxidation, while the acid marinating with 2% sodium lactate had the prooxidant effect and increased the protein and lipid oxidation in sheep.

The most significant destructive changes in the sheep muscle structure were established after 48 h marinating.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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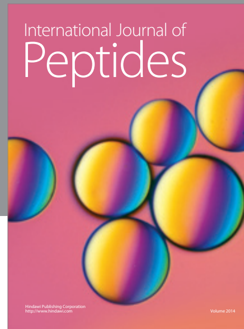

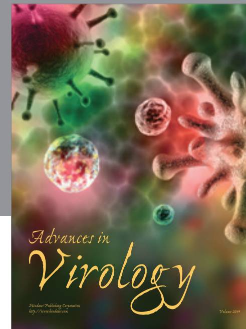

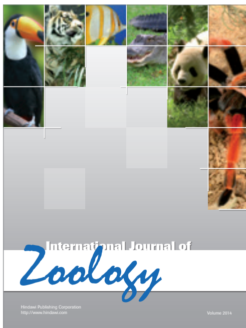

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