

# The Effect of Citric Acid, NaCl, and CaCl<sub>2</sub> on Qualitative Changes of Horse Meat in Cold Storage

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## Abstract:

In this study, we aimed to analyze the effect of citric acid, NaCl, and CaCl<sub>2</sub> on the qualitative changes of horse meat during cold storage. The study material was the longest dorsal muscles (M. longissimus dorsi (LM)) obtained from twelve half-carasses of horses. The muscle was cut into five steaks, each of which was about 3 cm thick. One sample was kept as a control sample, and the remaining ones were treated with NaCl, citric acid, and CaCl<sub>2</sub> (0.2 M and 0.3 M). The study material was obtained 24 h after the slaughter of the animals and was marinated in solution (citric acid and 0.2 M and 0.3 M calcium chloride) and by sprinkling and rubbing (sodium chloride). The applied treatments significantly ( $p \leq 0.05$ ) improved the texture parameters of horse meat (univariate analysis of variance). Citric acid caused deterioration of the study material with respect to the binding and retention of intrinsic water. Among the tested material, the lightest color of the meat was obtained for sample marinated in 0.3 M CaCl<sub>2</sub>. However, the darkest color of the meat was obtained after the addition of NaCl.

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## Article

# The Effect of Citric Acid, NaCl, and CaCl<sub>2</sub> on Qualitative Changes of Horse Meat in Cold Storage

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**Abstract:** In this study, we aimed to analyze the effect of citric acid, NaCl, and CaCl<sub>2</sub> on the qualitative changes of horse meat during cold storage. The study material was the longest dorsal muscles (M. longissimus dorsi (LM)) obtained from twelve half-carcasses of horses. The muscle was cut into five steaks, each of which was about 3 cm thick. One sample was kept as a control sample, and the remaining ones were treated with NaCl, citric acid, and CaCl<sub>2</sub> (0.2 M and 0.3 M). The study material was obtained 24 h after the slaughter of the animals and was marinated in solution (citric acid and 0.2 M and 0.3 M calcium chloride) and by sprinkling and rubbing (sodium chloride). The applied treatments significantly ( $p \leq 0.05$ ) improved the texture parameters of horse meat (univariate analysis of variance). Citric acid caused deterioration of the study material with respect to the binding and retention of intrinsic water. Among the tested material, the lightest color of the meat was obtained for sample marinated in 0.3 M CaCl<sub>2</sub>. However, the darkest color of the meat was obtained after the addition of NaCl.

**Keywords:** citric acid; horse meat quality; marinating; sodium chloride; physicochemical properties; sensory analysis

## 1. Introduction

Nowadays, the consumption of horse meat has significantly increased in many European countries [1]. Previous studies have reported the physicochemical properties of horse meat [2–5]. The horse meat is distinct in that it contains low levels of fat and cholesterol in comparison to other kinds of meat (about 20% less) [6]. Due to a high level of unsaturated fatty acids (over 55%) and iron, the consumption of horse meat may be beneficial to human health [7,8]. Another characteristic feature of horse meat is its high protein content [8–10] and dark red color with a light brown shade [11,12]. However, the high content of myoglobin is a less attractive feature of the horse meat [8,13]. Moreover, the meat obtained from older horses is undesirable due to stringiness and hardness, which results, among others, from the greater proportion of connective tissue (collagen) [2,14]. Moreover, there are reports that horse meat has a slightly sweetish aftertaste, which results from the high content of carbohydrates, mainly glycogen [12,15,16].

The literature describes some methods to improve the tenderness of horse meat. One of the most popular methods is the application of calcium salts before and slaughter process. Some studies point out that marinating, injecting, or infusing calcium salt solution into the muscle tissue accelerates

post-mortem changes [17–19]. The use of a high concentration of calcium salts results in a product with changed taste and bitter taste [20]. The original procedure of meat tenderizing suggested using of calcium chloride with 10% (*wt/wt*) of 0.3 M  $\text{CaCl}_2$  solution to infuse carcasses. However, a lot of modifications of this procedure were proposed. For example, injection of 0.2 M  $\text{CaCl}_2$  solution at 5% (*wt/wt*) improves meat tenderness of lamb and beef cattle without other palatability side effects [21].

It is equally important to study the effects of NaCl and citric acid under conditions similar to that of  $\text{CaCl}_2$ . According to Vlahova-Vangelova et al. [22], changes that occur in the connective meat tissues are mainly responsible for meat tenderness during meat marinating in acid environment. Tenderizing the meat by acidic marination leads to the weakening of the structures, proteolysis by cathepsins, and an increase in the conversion of collagen to gelatin during meat cooking [23]. Moreover, this process improves the quality of the meat products and reducing the pink color of *sous vide* products. The acceptable level of citric acid in marinades of meat products is below 10% [24]. In the European Union, in accordance with the Commission Regulation (EU) No. 1129/2011, the amount of citric acid in meat is used on a quantum satis basis [25]. However, the citric acid marinade can reduce the pH of the meat product and generate sour flavor [24]. Citric acid is also commonly used as a chelator to control the activity of pro-oxidant metals [26]. The addition of sodium chloride to meat has influence on the color of product [27]. It may decrease the heat stability of myoglobin and hemoglobin. However, excessive addition of sodium chloride can reduce the redness of cooked meat as a result of hasten oxidation of myoglobin. In cured meat, sodium chloride intensifies the conversion of nitrite to nitric oxide, thus, accelerating the formation of the color. Moreover, NaCl is a compound, which is used for taste and texture improvement, extension of shelf life of products, and increased water-holding capacity [27]. Traditionally, salt is used as a preservative. It is added to meats as it imparts sensory, functional, and preservation properties [28]. However, NaCl increases the level of lipid oxidation when in concentrations of up to 2%, and shows little or no pro-oxidant effect when in concentrations higher than 3% [29].

Considering the aforementioned information, in this study, we aimed to analyze the effect of food additives (NaCl, citric acid, and  $\text{CaCl}_2$ ) on horse meat quality under cold storage. We hypothesized that these additives have a significant effect on the physical, chemical, sensory qualities, as well as on texture parameters of horse meat during cold storage.

## 2. Material and Methods

### 2.1. Raw Material

In this study, the study material was samples of the *M. longissimus dorsi* (LM) that were obtained from 12 half-horse carcasses coming from individual farmers in the southeastern part of Poland. The horses were 10 years old ( $10 \pm 0.50$ ) and weighed approximately between 500 and 560 kg ( $530 \pm 30$ ). The horses were grouped according to their gender: 50% were geldings and 50% were mares. In this study, we used Malopolski Horses and Silesian Horses. The horses were in a normal condition and were kept in an extensive system. The transportation of the animals to the same abattoir via a road over short distances (no longer than 4 h) while maintaining animal welfare. To prevent injury during transportation, the horses were transported in separate boxes. After transportation, they were kept separately in warehouses for about 24 h. Then, the horses were slaughtered on the same day in compliance with methodology binding in the meat industry. In this study, we used single-phase cooling by placing half-horse carcasses in a chamber in which they were exposed to cold air (temperature from  $-2$  to  $0$  °C) with high relative humidity (approximately 95%), flowing half-carcasses at a speed of 2–3 m/s. In this study, first-class horse meat was selected for further research. For the purpose of determining the horse meat quality, we collected 20 h postmortem samples from the batch of the *M. longissimus dorsi* (LM) weighing approximately 700 g, at the level of the 13th–14th thoracic vertebra. Then, the external fat, connective tissue, and tendons were removed from the samples during the cleaning process. The muscle was cut up into five, 3 cm thick steaks. The meat pieces were used in the

same order for different procedures. One of the pieces was a control sample (CS) and the rest were treated with one of the following additives:

1. NaCl—a meat sample—batch was sprinkled and rubbed with NaCl (2% concentration in relation to meat weight);
2. citric acid (CA) (analytically pure monohydrate—basic 99.4%;  $C_6H_8O_7 \times H_2O$ ; 2-hydroxypropane-1,2,3-tricarboxylic acid hydrate)—3% water solution in relation to meat weight;
3. 0.2 M and 0.3 M  $CaCl_2$  (pure hexahydrate;  $CaCl_2 \times 6 H_2O$ ; calcium chloride 6-hydrate)—0.2 M and 0.3 M  $CaCl_2$  solutions were prepared. Two different concentrations of  $CaCl_2$  were used to investigate whether a higher concentration does not degrade hydration properties and color parameters of horse meat.

The additives were injected into the muscle tissue 24 h postmortem by means of marination (citric acid and 0.2 M and 0.3 M calcium chloride) and by sprinkling and rubbing (sodium chloride). Intended-for-marinating meat samples were placed in glass containers with 2:1 (salt:meat).

## 2.2. Analytical Methods

For the purpose of determining horse meat quality in the analyzed raw material, there were determined the following: active acidity, basic chemical composition, hydration properties, texture parameters, and color coordinates.

The active acidity (pH) of the cooled meat was determined with the use of OSH 12-01 electrode and CPC-411 pH meter (produced by ELMETRON, Zabrze, Poland) after 1, 48, 72, and 96 h postmortem.

The color of the meat was determined using a cross-section of the meat in the CIE  $L^*a^*b^*$  system using the HunterLabUltraScan PRO (HunterLab, Reston, VA, United States) electronic spectrophotometer (D65 light source, 8 mm measuring the head opening, white reference standard calibration:  $L^*—99.18$ ,  $a^*—0.07$ ,  $b^*—0.05$ ) 24, 48, and 96 h postmortem. Using  $L^*$ ,  $a^*$ , and  $b^*$  values, the absolute color differences were calculated (between the color of the control sample and the color of the other samples of meat subjected to the treatments with the applied substances). For this purpose, the following equation was used:

$$\Delta E = \sqrt{(L_0^* - L_1^*)^2 + (a_0^* - a_1^*)^2 + (b_0^* - b_1^*)^2}, \quad (1)$$

where  $\Delta E$  is the absolute color difference;  $L_0^*$ ,  $a_0^*$ , and  $b_0^*$  are the color components of the control sample; and  $L_1^*$ ,  $a_1^*$ , and  $b_1^*$  are the color components of the other samples of meat subjected to marination.

Meat samples were analyzed for water content (PN-ISO, 1442, 2000) [30], protein content (based on nitrogen) (PN-A-04018: 1975/Az3, 2000) [31], and fat content (PN-ISO, 1444, 2000) [32].

We analyzed the chemical composition of the raw horse meat samples 96 h postmortem.

For the analysis of physicochemical parameters, such as thermal and forced drips, meat samples were minced twice in a device, which is called laboratory wolf (HENDI, Warsaw, Poland) using sieves (4 mm diameter holes). The meat samples was thoroughly mixed in order to homogenize the sample.

Thermal drip was determined according to Janicki and Walczak [33]. The size of thermal drip (meat samples were subjected to heating in a water bath at a temperature of 85 °C for 10 min) was calculated according to the equation:

$$Td (\%) = \frac{WI - WII}{WI} \cdot 100\% \quad (2)$$

where Td refers to the thermal drip rate (%), WI refers to the weight of the sample before heat treatment (g), and WII refers to the weight of the sample after cooling down (g).

To determine the forced drip of meat, we applied Grau and Hamm's method [34] by placing a milled sample (300 mg) on Whatman paper no. 1, according to the procedure described by Stanisławczyk et al. [12].

We determined the hydration properties of horse meat in 96 h postmortem samples.

For the purpose of determining the texture parameters of the analyzed raw meat, from each batch of meat there were cut out samples in the shape of cubes with sides of 20 mm. Texture parameters of the analyzed meat samples were determined with the use of Texture Profile Analysis (TPA) made with Texture Analyzer-CT3-25 (Brookfield, WI, United States). The device was equipped with a cylindrical attachment of 38.1 mm in diameter and 20 mm in length. Each sample was compressed twice and samples were is compressed to a 50% of its height with test the roll travel speed 2 mm/s, and with the interval between compressions equal 2 s. Through software Texture Pro CT (V.1.9 Build 39, Brookfield, WI, United States), the following texture parameters were determined: hardness, adhesiveness, cohesiveness, springiness, chewiness, and resilience. During serial measurements, all texture parameters were counted automatically.

The 96 h postmortem sample was used in the estimation of texture parameters.

### 2.3. Sensory Evaluations

In this study, we used the method proposed by Baryłko-Pikielna and Matuszewska to scale the sensory qualities of the marinated horse meat [35]. The 96 h postmortem sample (100 g) was steamed at 95 °C in order to obtain an internal temperature of 80 °C ± 2 °C. The temperature was measured by using a digital thermometer with a needle probe (Sous Vide Thermapen, MERA, Warsaw, Poland). In order to carry out the sensory evaluation, the heat-treated samples were cooled to 20 °C ± 2 °C and cut into 1.5 cm thick slices, perpendicular to the run of meat fibers. The samples were put in disposable plastic boxes covered with lids. All samples for the assessment were coded individually and given in random order. The panel assessed each sample thrice. The sensory analysis was made by an evaluation team consisting of 16 individuals who were tested in terms of sensitivity and sensory fitness according to ISO 8586-2:2008 [36] and ISO 8587:2006 [37]. The assessment was conducted by a permanent trained laboratory team, who tested for sensory sensitivity in three independent assessment sessions. Ethical approval was obtained for the sensory session and the participants signed the consent form. The panel consisted of 16 researchers from the Institute of Food and Nutrition Technology of the University of Rzeszow. The assessors were experienced in evaluating meat and meat products. There was applied a five-point scale with a defined value limit, including the following parameters: aroma intensity (from very negative (1) to very strong (5)), taste intensity (from very negative (1) to very desirable (5)), aroma desirability (from not desirable (1) to highly desirable (5)), taste desirability (from not desirable (1) to highly desirable (5)), juiciness (from very dry (1) to very juicy (5)), and tenderness (from very hard (1) to very tender (5)). The triangular method was used to check the sensory sensitivity and performance of the candidates for the evaluation team, and the evaluators had to indicate the direction of the difference of two different samples. The assessments were made at a sensory laboratory conforming to all the requirements of the relevant standard [38]. After each test, the assessors had a 30-s break during which, they rinsed their mouths with mineral water. In total, 5 sessions with 12 samples per session were performed.

### 2.4. Statistical Analysis

All tests were performed in triplicate (4 selected additives × 12 batches). The results were averaged for all 12 batches and used for further statistical analysis. The total N was 12. Arithmetic averages ( $\bar{x}$ ) for each analyzed characteristic and the values of standard deviation (SD) are presented. In calculation, univariate analysis of variance was employed, and the significance of differences between averages ( $p \leq 0.05$ ) was determined through Tukey's honestly significant difference (HSD). In order to determine the correlation between the individual meat quality characteristics, there were calculated Pearson's simple correlation coefficients. The coefficients were calculated separately for the control sample and for the four groups of meat subjected to individual substances. Pearson's coefficients were calculated at the significance level of  $p \leq 0.05$ . All statistical calculations were performed using STATISTICA v. 10 software (StatSoft, Krakow, Poland, 2012).

### 3. Results and Discussion

Table 1 shows the results of changes in the pH of horse meat under cold storage conditions. According to the results, the most significant drop in pH was recorded for the 48-h postmortem sample that was treated with CA solution. Furthermore, the lowest pH was recorded for the 96-h postmortem sample that was subjected to CA (statistically insignificant differences). The highest pH at that time was recorded for the meat in  $\text{CaCl}_2$  marinade (statistically significant differences in comparison with the control sample and sample marinated in citric acid). After 96 h, the pH level was similar in all samples which maybe because of the accumulation of ammonia, which neutralizes the acidic compounds. The pH of samples marinated using NaCl did not differ significantly from the other groups. Similar tendencies were found by Klinhom et al. [39], who marinated beef in citric acid and  $\text{CaCl}_2$  and found that the sample marinated in citric acid showed the lowest pH value (4.5), whereas those marinated in  $\text{CaCl}_2$  showed similar values to that of the control sample.

**Table 1.** Changes in pH value of horse meat during cold storage ( $\bar{x} \pm S$ ).

pH	CS	NaCl	CA	0.2 M $\text{CaCl}_2$	0.3 M $\text{CaCl}_2$
pH <sub>1</sub>	5.93 <sup>a</sup> $\pm$ 0.58	5.98 <sup>a</sup> $\pm$ 0.50	5.83 <sup>a</sup> $\pm$ 0.48	5.90 <sup>a</sup> $\pm$ 0.44	5.91 <sup>a</sup> $\pm$ 0.39
pH <sub>48</sub>	5.5 <sup>a</sup> $\pm$ 0.01	5.49 <sup>a</sup> $\pm$ 0.03	5.39 <sup>b</sup> $\pm$ 0.03	5.48 <sup>a,b</sup> $\pm$ 0.05	5.50 <sup>a,b</sup> $\pm$ 0.01
pH <sub>72</sub>	5.45 <sup>a</sup> $\pm$ 0.01	5.40 <sup>a</sup> $\pm$ 0.09	5.45 <sup>a</sup> $\pm$ 0.06	5.49 <sup>a</sup> $\pm$ 0.08	5.53 <sup>a</sup> $\pm$ 0.02
pH <sub>96</sub>	5.44 <sup>a</sup> $\pm$ 0.02	5.46 <sup>a,b</sup> $\pm$ 0.04	5.40 <sup>a</sup> $\pm$ 0.01	5.51 <sup>b</sup> $\pm$ 0.04	5.53 <sup>b</sup> $\pm$ 0.01

<sup>a,b</sup>—means with different superscript letters differ significantly in the row at  $p \leq 0.05$ .

Table 2 shows data regarding the chemical composition of horse meat after 96 h of cold storage. According to our results, protein, fat, and water content showed differences between the samples but were statistically insignificant. The additives used in marination did not affect the content of protein, fat, and water in a statistically significant way. However, we recorded slightly higher protein and water content and lower fat content in samples marinated in solutions of CA and 0.2 M and 0.3 M calcium chloride (statistically insignificant differences). This effect might be the result of the duration of cold storage and not by the effects of the substances used.

**Table 2.** Chemical composition of horse meat after 96 h of cold storage (%;  $\bar{x} \pm S$ ).

Component	CS	NaCl	CA	0.2 M $\text{CaCl}_2$	0.3 M $\text{CaCl}_2$
Fat (%)	2.10 <sup>a</sup> $\pm$ 0.10	2.80 <sup>a</sup> $\pm$ 1.65	1.96 <sup>a</sup> $\pm$ 0.20	1.98 <sup>a</sup> $\pm$ 0.05	1.76 <sup>a</sup> $\pm$ 0.05
Protein (%)	20.86 <sup>a</sup> $\pm$ 0.11	20.93 <sup>a</sup> $\pm$ 0.65	21.20 <sup>a</sup> $\pm$ 0.01	21.16 <sup>a</sup> $\pm$ 0.05	21.23 <sup>a</sup> $\pm$ 0.05
Water (%)	75.43 <sup>a</sup> $\pm$ 0.63	74.03 <sup>a</sup> $\pm$ 1.84	76.70 <sup>a</sup> $\pm$ 0.26	76.70 <sup>a</sup> $\pm$ 0.10	76.83 <sup>a</sup> $\pm$ 0.05

<sup>a</sup>—means in the row with superscript the same letters do not differ between significantly.

Table 3 shows the results of selected hydration properties and color parameters of horse meat in cold storage. According to the results, thermal drip was the highest for meat, which was marinated in CA, whereas thermal drip was significantly lower for others samples. In the case of forced drip, the highest data was obtained for meat that was treated with CA (statistically significant differences). The lowest values for forced drip were obtained in the case of meat marinated using NaCl and the highest values were obtained for samples marinated in 0.2 M and 0.3 M calcium chloride solutions (statistically significant differences in comparison with the CS). NaCl increases water retention capacity, which explains the data obtained in this study. Pérez-Chabela et al. [40] also reported high water retention in horse meat that was subjected to the process of marination using  $\text{CaCl}_2$ .



**Table 3.** Selected hydration properties and parameters of horse meat color during cold storage ( $\bar{x} \pm S$ ).

Specification	CS	NaCl	CA	0.2 M CaCl <sub>2</sub>	0.3 M CaCl <sub>2</sub>
Thermal drip <sub>96</sub> (%)	22.85 <sup>a</sup> $\pm$ 1.12	23.20 <sup>a</sup> $\pm$ 4.12	37.47 <sup>b</sup> $\pm$ 3.53	26.50 <sup>a</sup> $\pm$ 4.47	26.03 <sup>a</sup> $\pm$ 1.89
Forced drip <sub>96</sub> (cm <sup>2</sup> )	4.80 <sup>a</sup> $\pm$ 0.60	2.46 <sup>b</sup> $\pm$ 0.68	13.66 <sup>c</sup> $\pm$ 0.83	5.34 <sup>b</sup> $\pm$ 1.10	6.01 <sup>b</sup> $\pm$ 0.76
<i>L</i> <sup>*</sup> <sub>24</sub>	39.35 <sup>a,c</sup> $\pm$ 1.05	31.21 <sup>a</sup> $\pm$ 1.11	41.00 <sup>d,c</sup> $\pm$ 3.06	48.46 <sup>b,d</sup> $\pm$ 1.09	51.79 <sup>b</sup> $\pm$ 5.86
<i>a</i> <sup>*</sup> <sub>24</sub>	18.77 <sup>a</sup> $\pm$ 3.57	14.15 <sup>a,c</sup> $\pm$ 1.84	6.64 <sup>b</sup> $\pm$ 0.50	11.21 <sup>c</sup> $\pm$ 1.02	9.28 <sup>c</sup> $\pm$ 1.28
<i>b</i> <sup>*</sup> <sub>24</sub>	10.02 <sup>a</sup> $\pm$ 1.00	4.95 <sup>b</sup> $\pm$ 0.86	5.49 <sup>b</sup> $\pm$ 1.55	5.97 <sup>b</sup> $\pm$ 1.07	4.74 <sup>b</sup> $\pm$ 1.96
$\Delta E$		10.64	13.05	12.51	16.51
<i>L</i> <sup>*</sup> <sub>48</sub>	38.82 <sup>a,c</sup> $\pm$ 1.48	30.90 <sup>b,d</sup> $\pm$ 1.66	42.23 <sup>c</sup> $\pm$ 3.51	52.07 <sup>b</sup> $\pm$ 1.37	52.81 <sup>b</sup> $\pm$ 4.83
<i>a</i> <sup>*</sup> <sub>48</sub>	18.23 <sup>a</sup> $\pm$ 4.08	13.16 <sup>a,b</sup> $\pm$ 3.70	6.65 <sup>b</sup> $\pm$ 0.73	9.65 <sup>b</sup> $\pm$ 0.11	9.51 <sup>b</sup> $\pm$ 1.60
<i>b</i> <sup>*</sup> <sub>48</sub>	9.69 <sup>a</sup> $\pm$ 0.58	5.50 <sup>b</sup> $\pm$ 0.85	5.65 <sup>b</sup> $\pm$ 1.96	6.98 <sup>a,b</sup> $\pm$ 0.65	5.74 <sup>b</sup> $\pm$ 1.03
$\Delta E$		10.29	12.72	16.01	16.95
<i>L</i> <sup>*</sup> <sub>72</sub>	38.35 <sup>a</sup> $\pm$ 1.82	29.34 <sup>b</sup> $\pm$ 2.79	41.73 <sup>a</sup> $\pm$ 2.73	40.73 <sup>a</sup> $\pm$ 3.59	41.73 <sup>a</sup> $\pm$ 2.73
<i>a</i> <sup>*</sup> <sub>72</sub>	17.42 <sup>a</sup> $\pm$ 1.17	13.70 <sup>b</sup> $\pm$ 1.26	6.33 <sup>c</sup> $\pm$ 1.11	6.92 <sup>c</sup> $\pm$ 0.69	6.83 <sup>c</sup> $\pm$ 1.11
<i>b</i> <sup>*</sup> <sub>72</sub>	10.28 <sup>a</sup> $\pm$ 0.33	4.32 <sup>b</sup> $\pm$ 0.41	4.83 <sup>b</sup> $\pm$ 0.85	6.00 <sup>b</sup> $\pm$ 0.63	4.83 <sup>b</sup> $\pm$ 0.85
$\Delta E$		11.42	12.81	11.58	12.37
<i>L</i> <sup>*</sup> <sub>96</sub>	36.81 <sup>a</sup> $\pm$ 3.20	28.05 <sup>c</sup> $\pm$ 1.48	42.76 <sup>a</sup> $\pm$ 1.29	53.02 <sup>b</sup> $\pm$ 3.65	54.85 <sup>b</sup> $\pm$ 1.22
<i>a</i> <sup>*</sup> <sub>96</sub>	16.69 <sup>a</sup> $\pm$ 1.05	13.30 <sup>a</sup> $\pm$ 1.51	5.24 <sup>b</sup> $\pm$ 0.63	8.92 <sup>b</sup> $\pm$ 2.87	6.27 <sup>b</sup> $\pm$ 0.79
<i>b</i> <sup>*</sup> <sub>96</sub>	10.51 <sup>a</sup> $\pm$ 0.74	4.70 <sup>b,c</sup> $\pm$ 0.98	4.88 <sup>b</sup> $\pm$ 0.15	7.75 <sup>c</sup> $\pm$ 1.08	7.10 <sup>b</sup> $\pm$ 0.67
$\Delta E$		11.04	14.07	18.18	21.11

a, b, c, d—means with different superscript letters differ significantly in the row at  $p \leq 0.05$ .

Pérez et al. [19] reported a decrease in the water-retaining capacity in meat subjected to the process of marination in CaCl<sub>2</sub>, which they attributed to the occurrence of proteolysis of myofibrillar proteins and the lowering of pH in the tested meat samples. In contrast, low pH (<4.5) causes irreversible changes in the functional activity and the capacity of meat to retain water [18]. Klinhom et al. [39] arrived at similar conclusions. They subjected beef to the process of marination in solutions of CA and calcium chloride and showed a statistically significant increase in the value of thermal drip in the case of CA and a small, statistically insignificant increase in the case of CaCl<sub>2</sub> in comparison to the CS. In addition, Aktaş et al. [41] subjected beef to the process of marination in calcium chloride and sodium chloride solutions and showed a statistically significant increase in the thermal drip value.

In this study, we analyzed the color parameters of horse meat after marination with the selected additives. According to our results, the meat marinated in 0.3 M CaCl<sub>2</sub> solution was the lightest in color ( $p \leq 0.05$ ). However, the darkest meat was recorded after the addition of NaCl. Most likely, NaCl caused the displacement of water molecules from muscle tissue, which resulted in the dark color of the meat. After 24, 48, 72, and 96 h of cold storage, meat subjected to solutions of CA, and calcium chloride demonstrated a light color. However, this difference was not statistically significant in every case, which was partly due to the proportion of water added to make the marinade. The lowest values of red color (*a*<sup>\*</sup>) were found in the meat treated with citric acid at all the analyzed time points. In this study, the additives decreased in the amount of red color during the cold storage of the horse meat. In terms of yellowness values (*b*<sup>\*</sup>), it was found that the lowest values of this parameter were found in salted meat. Similar to the *a*<sup>\*</sup> values, the additives decreased the value yellowness parameter (*b*<sup>\*</sup>) of horse meat in cold storage. Based on the *L*<sup>\*</sup>, *a*<sup>\*</sup>, and *b*<sup>\*</sup> color components, the absolute color differences ( $\Delta E$ ) between the control sample and the marinated sample were above 10, which means that the color difference between the study groups was clear. Klinhom et al. [39] reported an increase brightness and a decrease of redness and yellowness of beef marinated in solutions of CA and calcium chloride in comparison with the CS.

According to previous studies [20,39,42,43], CaCl<sub>2</sub> contributes to the reduction in the cutting strength of meat compared to control samples. Table 4 presents the texture parameters of horse meat texture after 96 h of cold storage. According to the results, the additives reduced the values of hardness and stiffness of horse meat in a statistically significant way. Marinated horse meat in CA resulted the

lowest hardness of horse meat. In the opinion of Hosseini and Esfahani Mehr [23], the organic acids decrease the pH value, which causes solubilization of collagen tissue and increases tenderness of the raw material. Citric acid reduced the hardness of the meat more than other additives. In addition, compared to the CS, NaCl and citric acid caused a statistically significant reduction in springiness. In the case of  $\text{CaCl}_2$  and NaCl solutions, the values of gumminess and chewiness of horse meat increased, which might have been caused by an increase in cohesiveness. Perez-Chabela et al. [44] showed significantly low values of hardness of horse meat marinated in calcium chloride solution in comparison with CS.

**Table 4.** Texture parameters of horse meat after 96 h of cold storage ( $\bar{x} \pm S$ ).

Specification	CS	NaCl	CA	0.2 M $\text{CaCl}_2$	0.3 M $\text{CaCl}_2$
Hardness 1 (N)	139.62 <sup>a</sup> $\pm$ 18.73	101.83 <sup>b</sup> $\pm$ 26.05	87.04 <sup>c</sup> $\pm$ 22.59	105.93 <sup>b</sup> $\pm$ 27.05	100.50 <sup>b</sup> $\pm$ 25.89
Hardness 2 (N)	73.89 <sup>a</sup> $\pm$ 16.33	67.87 <sup>c</sup> $\pm$ 3.58	40.40 <sup>b</sup> $\pm$ 6.83	46.22 <sup>b</sup> $\pm$ 11.10	42.09 <sup>b</sup> $\pm$ 10.76
Stiffness up to 5 mm (N)	30.75 <sup>a</sup> $\pm$ 15.39	19.36 <sup>b</sup> $\pm$ 5.11	12.37 <sup>c</sup> $\pm$ 3.06	20.83 <sup>b</sup> $\pm$ 5.84	16.58 <sup>b</sup> $\pm$ 2.83
Stiffness up to 8 mm (N)	98.28 <sup>a</sup> $\pm$ 3.57	68.57 <sup>b</sup> $\pm$ 5.34	40.83 <sup>c</sup> $\pm$ 9.50	57.77 <sup>b</sup> $\pm$ 8.02	59.18 <sup>b</sup> $\pm$ 11.28
Adhesiveness (mJ)	1.26 <sup>a</sup> $\pm$ 1.00	2.10 <sup>a</sup> $\pm$ 1.76	0.93 <sup>a</sup> $\pm$ 0.05	0.70 <sup>a</sup> $\pm$ 0.07	1.13 <sup>a</sup> $\pm$ 0.09
Resilience	0.05 <sup>a</sup> $\pm$ 0.32	0.10 <sup>a</sup> $\pm$ 0.04	0.05 <sup>a</sup> $\pm$ 0.01	0.14 <sup>a</sup> $\pm$ 0.03	0.14 <sup>a</sup> $\pm$ 0.07
Cohesiveness	0.07 <sup>a</sup> $\pm$ 0.02	0.19 <sup>a</sup> $\pm$ 0.03	0.10 <sup>a</sup> $\pm$ 0.01	0.20 <sup>a</sup> $\pm$ 0.02	0.17 <sup>a</sup> $\pm$ 0.07
Springiness(mm)	4.57 <sup>a</sup> $\pm$ 0.27	3.01 <sup>b</sup> $\pm$ 0.85	2.96 <sup>b</sup> $\pm$ 0.16	3.54 <sup>a,b</sup> $\pm$ 0.19	3.67 <sup>a,b</sup> $\pm$ 0.41
Gumminess (mm)	9.77 <sup>a</sup> $\pm$ 2.82	17.59 <sup>a</sup> $\pm$ 3.07	8.70 <sup>a</sup> $\pm$ 1.73	20.74 <sup>a</sup> $\pm$ 2.76	17.08 <sup>a</sup> $\pm$ 2.73
Chewiness (mJ)	44.66 <sup>a</sup> $\pm$ 6.17	55.50 <sup>a</sup> $\pm$ 9.26	25.76 <sup>b</sup> $\pm$ 1.11	73.33 <sup>c</sup> $\pm$ 5.69	62.70 <sup>c</sup> $\pm$ 7.11

a, b, c—means with different superscript letters differ significantly in the row at  $p \leq 0.05$ .

Table 5 presents the data of sensory evaluation of horse meat marinated with additives. According to the results, citric acid significantly reduced the sensory quality of horse meat. In this case, individual distinguishing features of the qualitative sensory evaluation of horse meat obtained statistically significant low scores in comparison to the control sample and other samples of marinated meat. It should be noted that the largest numerical value characterizing the individual sensory features was found for meat marinated with  $\text{CaCl}_2$ . In this case, they resulted in statistically significant and better juiciness and tenderness qualities in comparison to the CS and other samples of marinated horse meat. In addition, subjecting of horse meat to NaCl caused in an improvement in the sensory quality of the meat when compared to the control sample. In comparison to samples marinated in  $\text{CaCl}_2$  solutions, NaCl demonstrated better, statistically significant, juiciness, and tenderness of the analyzed sample ( $p \leq 0.05$ ). Klinhom et al. [39] marinated beef with 0.05 M CA solution and obtained a statistically significant reduction in the taste and acceptability of the raw material in comparison with the control sample. Furthermore, they showed that the use of this acid solution reduced the score of other quality parameters based on sensory evaluation, such as juiciness, tenderness, and smell in comparison with the control sample. They also marinated beef in a 0.2 M solution and showed a significantly high score of tenderness of the analyzed raw material in comparison with the control sample and meat marinated with 0.05 M citric acid solution. They demonstrated that samples marinated in 0.2 M  $\text{CaCl}_2$  solution had better juiciness and acceptability compared to the control sample and had significantly high scores for juiciness, tenderness, taste, and acceptability in comparison to meat marinated in 0.05 M citric acid solution. Pérez et al. [19] showed that all distinguishing features of the qualitative sensory evaluation of horse meat marinated with  $\text{CaCl}_2$  solution were high in scores in comparison with the CS.

The analysis of Pearson's correlation coefficients shows that there are no statistically significant ( $p \leq 0.05$ ) correlations between the examined meat quality characteristics. Moreover, the values of these coefficients were below 0.400, which means the strength of the correlation at a medium or low level. Similarly, the substances used did not have a statistically significant influence ( $p \leq 0.05$ ) on the change in the strength and direction of the analyzed interdependencies.



**Table 5.** Sensory properties of horse meat during cold storage ( $\bar{x} \pm S$ ).

Specification	CS	NaCl	CA	0.2 M CaCl <sub>2</sub>	0.3 M CaCl <sub>2</sub>
Aroma: Intensity	3.20 <sup>a</sup> $\pm$ 0.25	3.75 <sup>b</sup> $\pm$ 0.15	2.50 <sup>c</sup> $\pm$ 0.21	4.20 <sup>b</sup> $\pm$ 0.16	4.05 <sup>b</sup> $\pm$ 0.20
Aroma: Desirability	3.17 <sup>a</sup> $\pm$ 0.11	3.83 <sup>b</sup> $\pm$ 0.10	2.89 <sup>c</sup> $\pm$ 0.16	4.31 <sup>b</sup> $\pm$ 0.12	4.26 <sup>b</sup> $\pm$ 0.14
Juiciness	2.55 <sup>a</sup> $\pm$ 0.31	3.05 <sup>a</sup> $\pm$ 0.18	1.54 <sup>c</sup> $\pm$ 0.09	4.36 <sup>b</sup> $\pm$ 0.09	4.42 <sup>b</sup> $\pm$ 0.12
Tenderness	2.82 <sup>a</sup> $\pm$ 0.12	3.18 <sup>a</sup> $\pm$ 0.12	1.12 <sup>c</sup> $\pm$ 0.10	4.01 <sup>b</sup> $\pm$ 0.05	4.10 <sup>b</sup> $\pm$ 0.08
Taste: Intensity	2.85 <sup>a</sup> $\pm$ 0.15	4.15 <sup>b</sup> $\pm$ 0.17	1.05 <sup>c</sup> $\pm$ 0.05	4.45 <sup>b</sup> $\pm$ 0.10	4.50 <sup>b</sup> $\pm$ 0.12
Taste: Desirability	2.99 <sup>a</sup> $\pm$ 0.17	4.28 <sup>b</sup> $\pm$ 0.21	1.16 <sup>c</sup> $\pm$ 0.08	4.38 <sup>b</sup> $\pm$ 0.12	4.56 <sup>b</sup> $\pm$ 0.09
General Acceptability	2.93 $\pm$ 0.18	3.70 $\pm$ 0.15	1.71 $\pm$ 0.11	4.28 $\pm$ 0.10	4.28 $\pm$ 0.12

a, b, c—means with different superscript letters differ significantly in the row at  $p \leq 0.05$ .

#### 4. Conclusions

In this study, among the additives tested, meat with the lightest color at all individual timepoints of cold storage was obtained for 0.3 M CaCl<sub>2</sub> marinade. In turn, meat with the darkest color was obtained for NaCl. After 24, 48, 72, and 96 h of cold storage, meat marinated with citric acid, and CaCl<sub>2</sub> exhibited light color. The application of citric acid to horse meat caused a significant deterioration in the hydration properties of the analyzed raw material. Marinating the raw material contributed, in a statistically significant way, to an improvement in textural parameters, such as hardness, stiffness, and springiness. The lowest hardness was found for the sample marinated with citric acid. The use of this solution for marinating significantly reduced the sensory quality of the meat, whereas the highest values for individual sensory qualities were obtained for horse meat marinated with CaCl<sub>2</sub> in comparison with the control sample. The most beneficial effect on the quality of horse meat was found in the case of marinating horse meat in CaCl<sub>2</sub> solutions.

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