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Effect of Time and Temperature in Sous-Vide Heat Treatment on Selected Physicochemical Properties of Horsemeat

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Abstract: The sous-vide technique is most commonly used for meat preparation, offering superior nutritional retention, a delicate texture, and enhanced juiciness in the resulting dishes. The purpose of this study was to analyze the effect of time and temperature in sous-vide heat treatment on selected physicochemical properties of horsemeat. Samples of *m. longissimus thoracis* were heated to 55, 60, 65 °C for 4, 8, 12, and 24 h, and the impact of these heat treatment parameters on chemical composition, physicochemical properties, amount of heme pigments, and color parameters of the tested raw material was determined. Horsemeat subjected to sous-vide heat treatment at 55 °C for 4 h was characterized by a high proportion of red color and oxymyoglobin. Sous-vide treatment carried out at low temperatures and for a short period of time reduces the amount of weight loss and the increase in the TBARS index, allowing the preservation of the red color of the raw material studied. Prolonging the duration or increasing the temperature of sous-vide processing results in a deterioration of the mentioned parameters and also leads to significant changes in the chemical composition of horsemeat, including a significant reduction in the water content and increase in the amount of fat in horsemeat.

Keywords: sous-vide; horsemeat; chemical composition; physicochemical properties; heme pigments; myoglobin

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1. Introduction

Horsemeat is consumed by a well-defined group of consumers whose preferences vary depending on country and region. The main reason for the unsatisfactory quality of this raw material is its insufficient tenderness, which is a consequence of the high content of connective tissue proteins and the high heat resistance of collagen. Consequently, horsemeat, especially from older animals, is characterized by undesirable stringiness and hardness that may not completely disappear even after heat treatment [1]. Other unfavorable characteristics of horsemeat include a dark red color with a bluish sheen, which results from the high content of the pigment myoglobin, and a slightly sweetish aftertaste due to the elevated carbohydrate content [2–4].

The unsatisfactory quality of horsemeat is attributed to the fact that horses are slaughtered at an older age, making it challenging to consistently obtain a raw material with adequate and satisfactory qualities. However, owing to its high dietary value, horsemeat is considered a valuable resource and is in demand, especially in the European Union, where it holds a significant role in many countries, including Spain, Belgium, France, and Italy [5].

As consumers of horsemeat, it is essential to ensure supply of high-quality meat. In the food and catering industry, several methods are employed to enhance the quality of the raw material. Currently, one popular method is sous-vide technology. This method involves a controlled cooking process in which fresh food is vacuum-sealed in heat-stable, high-barrier plastic bags or films and then cooked (pasteurized) under time–temperature conditions selected individually for each product [6]. For tough meat like beef chuck and pork shoulder, it is recommended to cook them 10–12 h at 80 °C or 24–48 h at 55–60 °C, while for intermediate meat like beef sirloin, the recommended cooking time is 6–8 h at 55–60 °C [7]. After the heat treatment, the product should be rapidly cooled to about 0–3 °C and can be stored under these conditions for 3–5 weeks before being reheated and consumed [8,9].

The sous-vide cooking method has found its greatest application in the thermal processing of meat offering numerous advantages, including high nutritional value, tender texture, and enhanced juiciness in the final products [10]. Extended, low-temperature cooking leads to an increased shelf life of products, while the use of vacuum packaging prevents flavor loss, reduces evaporative losses, and eliminates the risk of product recontamination [11]. Furthermore, vacuum packaging facilitates highly efficient heat transfer from water to food, resulting in moist, tender, and flavorful meat. The strict control of sous-vide processing conditions, specifically time and temperature, ensures the reproducibility of product quality and guarantees microbiological safety [7,12,13]. Sous-vide heat treatment also reduces the formation of heterocyclic aromatic amines in meat products compared to pan-frying [14]. Sous-vide cooking has a significant potential for mitigating quality losses in meat due to its low-temperature application, although this positive transformation in physical and chemical properties is highly time-dependent. The primary objective of the sous-vide method is to mitigate lipid oxidation in meat by minimizing its exposure to free oxygen in the surrounding air, thereby preserving the quality of the food. Additionally, sous-vide cooking not only enhances the quality of meat but also extends the meat's shelf life. Moreover, this method preserves the color of the meat [7,14]. Sous-vide is one of the techniques that enhances meat tenderness, while concurrently reducing protein damage, lipid deterioration, and the degradation of other heat-sensitive compounds. Sous-vide selectively denatures specific proteins while leaving others intact, resulting in meat with superior qualities such as improved texture, juiciness, and tenderness [7,10].

To date, research has been conducted on the impact of sous-vide cooking on the quality of various types of meat. However, horsemeat, despite its value as a raw material, has been relatively neglected in such studies, as evidenced by the absence of reports in the literature concerning the influence of sous-vide heat treatment on the quality of this meat. There is a dearth of research that comprehensively examines the effects of both varying time parameters and varying temperature parameters on the quality of this particular raw material. Therefore, it is of significant importance to analyze the effects of various time and temperature parameters on specific quality attributes of horsemeat. Such an investigation will undoubtedly help bridge the existing research gap and advance our knowledge in terms of enhancing the quality of horsemeat, potentially stimulating greater interest from consumers and meat-processing facilities in utilizing this raw material.

Hence, the present research endeavors to scrutinize the impact of sous-vide heat treatment duration and temperature on selected physicochemical properties of horsemeat. It is anticipated that through the manipulation of these factors, certain physicochemical characteristics of the investigated raw material can be enhanced.

2. Materials and Methods

2.1. Experimental Design

The study material comprised samples from the longest thoracis muscle (*m. longissimus thoracis*) obtained from 16 half-carcasses of horses sourced from individual farmers in the southeastern region of Poland. The animals had an average age ranging from 21 to 24 years and pre-slaughter weights between 510 and 560 kg (mean weight of 530 ± 30). The age of

the horses was determined from the purchase documentation. Among the animals, 50% were geldings and 50% were mares. The study utilized horses from the Malopolski and Silesian breeds, all of which were healthy and raised in an extensive system. Following transportation, the horses were housed in separate enclosures within livestock facilities for approximately 24 h, ensuring their welfare and under the supervision of appropriate veterinary services. All horses were slaughtered on the same day, using a captive bolt pistol, following the standard methodology employed in the meat industry. The selection of half-carcasses was made based on purchase documentation and without any direct contact with live animals. To investigate the impact of sous-vide heat treatment's time and temperature on the quality of horsemeat, samples were extracted from the longest thoracic muscle at the level of the 13th–14th thoracic vertebrae. The collected muscle samples were subsequently refrigerated at 4 °C ± 0.5 °C for a period of 10 days.

2.2. Cooking Procedure

Subsequent to the refrigerated transport of the muscle specimens (3 °C ± 0.5 °C) to the laboratory, they were portioned into samples weighing approximately 300 ± 30 g each. These samples were vacuum-sealed using a vacuum packer (Inauen, Schwanden, Switzerland) within mesh bags designed for sous-vide heat treatment. The meat samples underwent heat treatment in a water bath (Hendi, Gądki, Poland) at temperatures of 55 °C, 60 °C, and 65 °C, each for varying durations of 4, 8, 12, and 24 h (16 half-carcasses × 3 temperature parameters × 4 time parameters = 192 meat samples). In each device, distinct temperatures (55 °C, 60 °C, and 65 °C) were set, and samples were retrieved for laboratory analysis after 4, 8, 12, and 24 h. The heat treatment was performed in eight batches (comprising 2 half carcasses × 4 temperature variations = 8 samples) within a single apparatus. Following the heat treatment, the meat samples were promptly cooled to 4 °C ± 0.5 °C.

2.3. Analytical Methods

The water content of the samples was determined following the PN-ISO, 1442:2000 standard [15].

The protein content of the samples was ascertained using the Kjeldahl method. In this method, the nitrogen content measured in the samples was converted into protein content in accordance with the PN-75/A-04018 standard [16].

The fat content of the samples was determined using the Soxhlet method, conforming to the PN-ISO, 1444:2000 standard [17].

The methodology for assessing the color of the meat samples was previously described in a prior publication [18]. The blooming process occurred under controlled conditions in darkness and at a temperature of 4 °C, and it was conducted using fresh meat. Meat color was evaluated based on the L^*, a^*, b^* values in the CIE LAB color system, employing the reflectance method with an NR20XE camera (3nh Technology Co., Ltd., Shenzen, China). Instrumental color was recorded at three locations of each sample of meat after sous-vide and after cooling on a cross-section of the sample after 60 min storage at 4 °C.

The percentage of heme pigments in the meat samples was determined as outlined by Krzywicki [19].

Weight loss was calculated based on the difference in the meat's weight before and after cooking. Each sample was meticulously dried and weighed with a precision of 0.01 g. The loss was calculated using the following formula [20]:

$$\text{Weight loss } (\%) = \frac{\text{weight of raw meat} - \text{weight of cooked meat}}{\text{weight of raw meat}} \cdot 100 \quad (1)$$

The oxidation–reduction potential (ORP) was assessed using an ERPt-13 Hydromet No. 235-type combination electrode in conjunction with a CPC-501 digital pH/conductivity meter (produced by Elmetron, Zabrze, Poland). The recorded results were taken after achieving ORP stabilization. The acquired measurements were converted to redox potential

values referenced to a standard EH hydrogen electrode (W mV). To achieve this, the known potential value of the reference electrode (Em = 211 mV at 20 °C) was added to the measured Em potential value.

For each sample weighing 10 g, which had been ground in a laboratory mill with a mesh diameter of 3 mm, homogenization was carried out with 50 cm^3 of distilled water at 20 °C using an ULTRA TURAX T25 homogenizer (IKA, Staufen, Germany) operating at a spindle speed of 15,000 rpm. The potential of the resulting suspension was measured at 20 °C, and the reading was recorded with a precision of 0.01 [21].

Water activity was determined using a Novasina AG LabMaster—aw neo water activity measuring apparatus (Lachen, Switzerland), following the method described by Duma-Kocan et al. [22].

The assessment of the TBARS (2-thiobarbituric acid-reactive substances) index was conducted as detailed by Pikul et al. [23].

2.4. Statistical Analysis

All analyses were conducted in triplicate, and the results were subjected to statistical analysis after grouping. Various physical and chemical properties, color parameters, and pigment levels in the meat were evaluated using a two-way analysis of variance (ANOVA) via the GLM procedure in Statistica (STATISTICA v. 10; StatSoft, Krakow, Poland). In cases where significant effects were observed ($p < 0.05$), post hoc comparisons were performed using post hoc Tukey's honestly significant difference test (ANOVA).

3. Results and Discussion

3.1. Chemical Composition

Table 1 presents data on the chemical composition of horsemeat in relation to the time and temperature of sous-vide heat treatment. The parameters that exhibited statistically significant effects on the fat and water content in the studied raw material were the temperature used, the duration of sous-vide heat treatment, and the interaction between time and temperature. Increasing the duration of sous-vide heat treatment from 4 to 24 h at 55 °C and 60 °C led to a significant reduction in water content and a significant increase in the fat content of horsemeat ($p < 0.05$). Considering the influence of temperature on the content of fundamental chemical components in the studied raw material, it was observed that increasing the temperature from 55 °C to 65 °C led to a statistically significant reduction in water content and an increase in fat content after 4 and 8 h of sous-vide heat treatment. A two-factor ANOVA analysis demonstrated no significant effect of the applied temperature and duration of sous-vide heat treatment on the protein content of horsemeat.

Table 1. Chemical composition of horsemeat (%) in relation to time and temperature of sous-vide heat treatment (means ± SE).

Specification	Temp. (°C)	Time of Sous-Vide Heat Treatment (h)				
		4	8	12	24	ANOVA
Fat	55	$7.90 \pm 0.10^{x,a}$	$8.55 \pm 0.35^{a,b,x}$	$9.35 \pm 1.06^{a,b}$	10.85 ± 0.64^{b}	T *; S *; T × S *
	60	$8.75 \pm 0.07^{x,y,a}$	$9.45 \pm 0.07^{x,y,a,b}$	$9.85 \pm 0.77^{a,b}$	11.66 ± 0.07^{b}	
	65	10.15 ± 0.21^{y}	10.85 ± 0.49^{y}	11.25 ± 0.21	11.95 ± 0.07	
Water	55	$70.60 \pm 0.14^{x,a}$	$70.20 \pm 0.35^{x,a,b}$	$69.40 \pm 0.42^{a,b}$	68.65 ± 0.98^{b}	T *; S *; T × S *
	60	$70.35 \pm 0.07^{x,y,a}$	$69.65 \pm 0.10^{x,y,a,b}$	$69.65 \pm 0.63^{a,b}$	68.20 ± 0.56^{b}	
	65	69.10 ± 0.21^{y}	68.70 ± 0.42^{y}	68.25 ± 0.14	67.63 ± 0.07	
Protein	55	19.10 ± 0.06	19.20 ± 0.11	19.45 ± 0.14	19.83 ± 0.15	
	60	19.20 ± 0.12	19.40 ± 0.10	19.40 ± 0.14	19.65 ± 0.21	
	65	19.90 ± 0.15	19.30 ± 0.14	19.30 ± 0.10	19.90 ± 0.09	

^{a,b}—values marked with different letters in the same lines are statistically significantly different between heat treatment times—$p < 0.05$. ^{x,y}—values marked with different letters in the columns are statistically significantly different between treatment temperatures—$p < 0.05$. ANOVA: two-way analysis of variance among temperatures of sous-vide heat treatment, T; duration of sous-vide heat treatment, S. * $p < 0.05$.

The obtained results from this study are consistent with the findings of other researchers [24,25]. Ismail et al. [24] investigated the impact of different combinations of temperature and time on the quality of cooked goat gluteus medius and biceps femoris. They observed a significant dependence of water content on both temperature ($p < 0.001$) and time ($p < 0.001$) in both muscle types. Samples subjected to high temperatures and extended cooking (up to 12 h) showed a significant reduction in water content, regardless of the treatment employed. Similarly, in a study by Głuchowski et al. [25], chicken breast (musculus pectoralis major) samples subjected to higher sous-vide process temperatures (i.e., 66 °C for 80 min and 75 °C for 35 min), exhibited lower water content compared to samples treated at lower temperatures (64 °C for 60 min). These authors noted that the sous-vide heat treatment methods reduced water content while increasing protein and fat content. Protein content increased as water content decreased ($r = -0.88$, $p \leq 0.05$), and a similar trend was observed for fat content. Bıyıklıa et al. [26] demonstrated changes in the protein and fat content of turkey cutlet samples changed during sous-vide cooking. Increasing the cooking time from 20 min to 40 and 60 min resulted in statistically significant alterations in the chemical composition's basic components ($p < 0.05$). However, the interaction between the cooking parameters and the temperature alone had no remarkable effect on the protein and fat content of the turkey cutlets. The reduction in water content in meat exposed to high temperatures is attributed to the release of cellular juice under the influence of elevated temperatures. Furthermore, myofibrillar protein shrinkage, which initiates at 40 °C, leads to a subsequent reduction in interfibrillar volume, diminishing the myofibrils' capacity to retain water. Additionally, the compression of myofibril bundles due to contraction of perimuscular connective tissue at 56–62 °C also contributes to water release from the meat.

3.2. Physical and Chemical Properties

Table 2 presents data regarding the physical and chemical properties of horsemeat concerning the duration and temperature of sous-vide heat treatment. The measurement of substances undergoing a color reaction with 2-thiobarbituric acid (TBARS) is a fundamental method for assessing lipid oxidation processes. The TBARS value indicates the presence of secondary products of lipid oxidation, including alcohols, acids, aldehydes, and ketones, which can impart undesirable flavors or aromas to meat [27]. Meat, particularly rich in unsaturated fatty acids, is highly susceptible to oxidation processes during heat treatment [11,28]. The variation in the fatty acid composition of triglyceride molecules among different livestock species indicates that horse fat and pig fat are the most susceptible to oxidative decomposition, with sheep tallow slightly less susceptible and cattle tallow demonstrating the highest resistance [29]. Horse muscle exhibits a high content of unsaturated fatty acids (60.49–63.04%), with monounsaturated acids—such as oleic (38–55%) and palmitoleic (3–10%)—being the most abundant, together constituting as much as 45.16% of all fatty acids. Notable polyunsaturated fatty acids (PUFAs) in horse muscle include linoleic (9.14%) and linolenic (8.02%) acids [30–32]. Research by Berruga et al. [33] establishes a critical concentration of malondialdehyde (MDA) at which oxidative changes may become noticeable to consumers, set at 2 mg MDA kg^{-1}. According to Zakrys et al. [34], a TBARS value of 1.0 mg MDA kg^{-1} for cooked beef is considered a threshold level, beyond which an increase in rancid taste and odor is observed [35]. Consumers are unlikely to detect changes in meat products at TBARS values below a threshold of approximately 0.5 mg MDA kg^{-1} [36]. The influence of sous-vide heat treatment on TBARS values in horsemeat is shown in Table 2. The parameters that exhibited statistically significant effects on TBARS values in the studied raw material were the temperature used, the duration of sous-vide heat treatment, and the interaction between time and temperature. All experimental samples displayed the presence of secondary products of fat oxidation, forming colored complexes with thiobarbituric acid; however, none of the samples exceeded the 2 mg MDA kg^{-1} threshold. Notably, an increase in sous-vide heat treatment duration from 4 to 24 h resulted in a significant rise in TBARS values at higher temperatures, specifically at

60 °C and 65 °C. Conversely, elevating the heat treatment temperature from 55 °C to 65 °C resulted in a significant increase in the analyzed parameter after 8, 12, and 24 h. The highest TBARS index value, reaching 1.35 mg MDA kg^{-1}, was observed in horsemeat samples subjected to 24 h of sous-vide heat treatment at 65 °C. Analyzing the outcomes of this study leads to the conclusion that the use of higher temperatures and longer duration of sous-vide heat treatment amplifies lipid oxidation and, consequently, elevates TBARS values. Moreover, Özcan and Bozkurt [37] reported higher TBARS values in beef samples subjected to elevated temperatures (120 °C). The authors attribute the increase in the TBARS index to the degradation of the lipoprotein complex, leading to the release of the lipid fraction and making it more susceptible to oxidation. In contrast, Sánchez del Pulgar et al. [38], who examined the effects of various factors on pork cheeks cooked by the sous-vide method, found higher TBARS values for samples cooked for 12 h at 60 °C (4.0 mg/kg) and lower values for samples cooked for 12 h at 80 °C (2.4 mg/kg). A significant interaction effect (p = 0.007) between time and temperature was observed, while temperature and time tended to have a significant effect (p = 0.072 and p = 0.084, respectively). According to the authors of this study, higher cooking temperatures and longer durations would result in increased lipid oxidation. Haghighi et al. [39] demonstrated that the lowest TBARS value of 0.29 mg MDA kg^{-1} was achieved in processed chicken breast fillets when employing a lower sous-vide processing temperature of 60 °C and a shorter processing time of 60 min, as opposed to 70–150 min and 80–150 min. Increasing the duration of sous-vide heat treatment from 60 min to 150 min led to an escalation in TBARS from 0.29 to 0.94 mg MDA $kg^{-1.}$, even at a constant temperature of 60 °C. In a study by Shin et al. [40], the TBARS values in the case of duck breast meat increased with the increase in cooking temperature at constant cooking time ($p < 0.05$). Similarly, the TBARS values increased with the increase in cooking time at a constant cooking temperature. The duck breast meat cooked using the sous-vide method at 80 °C for 180 min exhibited the highest TBARS value. Bıyıklı et al. [26] reported the lowest TBARS value (0.078 ± 0.01 mg/kg sample) when employing lower cooking parameters (65 °C–40 min). Increasing the duration of sous-vide heat treatment from 20 to 40 min at 65 °C resulted in a significant ($p < 0.05$) reduction in TBARS values. Although the authors noted that temperature had a significant impact on the observed changes in the TBARS values ($p < 0.05$), neither the cooking time alone nor the temperature–time combinations exhibited a discernible effect on TBA ($p > 0.05$).

Table 2. Physical and chemical properties of horsemeat as a function of time and temperature of sous-vide heat treatment (means ± SE).

Specification	Tem. (°C)	Time of Sous-Vide Heat Treatment (h)				
		4	8	12	24	ANOVA
TBARS (mg MDA kg^{-1})	55	0.73 ± 0.01	0.78 ± 0.03 ^{x}	0.88 ± 0.01 ^{x}	0.94 ± 0.01 ^{x}	T *; S *; T × S *
	60	0.84 ± 0.05 ^{a}	1.09 ± 0.07 ^{x,y,a,b}	1.17 ± 0.06 ^{x,y,a,b}	1.23 ± 0.05 ^{x,y,b}	
	65	0.98 ± 0.07 ^{a}	1.13 ± 0.09 ^{y,a,b}	1.26 ± 0.01 ^{y,a,b}	1.35 ± 0.07 ^{b,y}	
ORP (mV)	55	376.30 ± 8.66	372.20 ± 8.98	346.50 ± 7.66	350.10 ± 8.34	
	60	370.40 ± 7.87	384.00 ± 8.94	392.40 ± 9.45	342.30 ± 7.56	
	65	373.50 ± 7.56	384.30 ± 9.01	368.50 ± 6.99	349.60 ± 6.88	
Water activity	55	0.982 ± 0.01	0.985 ± 0.03	0.978 ± 0.01	0.982 ± 0.01	
	60	0.979 ± 0.01	0.982 ± 0.03	0.989 ± 0.02	0.994 ± 0.03	
	65	0.995 ± 0.02	0.995 ± 0.02	0.995 ± 0.01	0.979 ± 0.01	
Weight loss (%)	55	11.33 ± 0.96 ^{x}	11.72 ± 0.86 ^{x}	11.85 ± 0.65 ^{x}	13.62 ± 0.65 ^{x}	T *; S *; T × S *
	60	12.62 ± 1.01 ^{a,x,y}	12.81 ± 0.76 ^{a,b,x,y}	15.57 ± 0.76 ^{a,b,x,y}	16.46 ± 0.67 ^{b,x,y}	
	65	17.60 ± 0.94 ^{a,y}	20.58 ± 1.23 ^{a,b,y}	23.04 ± 1.02 ^{b,y}	23.55 ± 1.03 ^{b,y}	

^{a,b}—values marked with different letters in the same lines are statistically significantly different between heat treatment times—$p < 0.05$. ^{x,y}—values marked with different letters in the columns are statistically significantly different between treatment temperatures—$p < 0.05$. ANOVA: two-way analysis of variance among temperatures of sous-vide heat treatment, T; duration of sous-vide heat treatment, S. * $p < 0.05$.

Cooking time alone and combinations of temperature and cooking time had no effect on TBARS values ($p < 0.05$). The heat treatment process induces protein denaturation and the release of iron ions, which act as catalysts in fat oxidation. Elevated temperatures play

a significant role in oxidation by increasing protein denaturation and relative amounts of phospholipids containing PUFAs. The observed variations in TBARS values were attributed to reactive oxidation products that interacted with the proteins and amino acids present in the meat. According to Domínguez et al. [41], the concentration of free iron in meat is positively correlated with the TBARS value. Therefore, extended heating can enhance the release of free iron, accelerating meat lipid oxidation. Hence, it is essential to perform sous-vide heat treatment at low temperatures and for shorter durations, which can help minimize TBARS values.

Low water activity, in conjunction with pH, serves as a limiting factor for microbial growth and is thus a critical determinant of food quality and shelf life. Water activity values in sous-vide heat-treated products closely resemble those of the raw ingredients [42]. In the case of raw meat, the water activity measures at 0.996 [43]. The study revealed that water activity in sous-vide heat-treated horsemeat samples ranged from 0.978 to 0.995 (Table 2). These values remained consistent across various temperature and process time parameters ($p > 0.05$). Kurp et al. [43] reported that pork loin, cooked using sous-vide technology, exhibited water activity levels ranging from 0.991 to 0.995. Díaz et al. [36] found lower water activity values (0.92–0.94) in pork loin prepared sous-vide.

The ORP in meat is contingent upon the concentrations of oxidants and reductants present in the meat. Elevated levels of oxidants result in higher ORP values, whereas when reductants predominate, ORP values are lower. A high redox potential in the system indicates a prevailing tendency toward oxidation reactions. The study conducted demonstrated that the obtained parameter values were consistent, irrespective of temperature and process time ($p > 0.05$). ORP values in horsemeat samples subjected to sous-vide cooking ranged from 342.30 to 392.40.

When analyzing numerical data concerning the weight loss of horsemeat prepared using sous-vide technology, it was observed that several parameters significantly influenced the weight loss in the studied raw material. These influential factors were the temperature applied, the duration of sous-vide heat treatment, and the interaction between time and temperature. Within the scope of this study, it was determined that a higher processing temperature had a more substantial impact on the weight loss of the raw material compared to the duration of the process itself. Increasing the duration of sous-vide heat treatment from 4 to 24 h resulted in a noteworthy rise in the weight loss parameter at 60 °C. At 65 °C, significant increases in weight loss were observed as the processing time increased from 4 to 12 and 24 h. The most substantial weight losses, ranging from 17.60% to 23.55%, were recorded for meat subjected to the higher temperature of 65 °C. In terms of temperature, a rise from 55 °C to 65 °C led to a statistically significant increase in horsemeat weight loss within all analyzed time intervals. Consequently, it is advisable to conduct the sous-vide heat treatment process for horsemeat at lower temperatures and for shorter durations, as this approach significantly influences the economic efficiency of the process. These findings align with the work of other researchers [38], who similarly demonstrated that cooking pork cheek samples at higher temperatures (80 °C) resulted in significantly greater weight loss compared to lower temperatures (60 °C). In the context of sous-vide technology, it becomes evident that variable processing temperatures exert a more pronounced impact on meat weight loss than the duration of the process. Also, in the study by Shin et al. [40], in the case of duck breast meat, cooking loss in the samples cooked at 80 °C for 180 min was significantly higher than that in the samples cooked at other temperatures (50°, 60 °C, and 70 °C) for 60 or 180 min ($p < 0.05$). This trend is corroborated by studies by Oz and Zikirov [14] as well as Jeong et al. [20], who investigated the effects of sous-vide cooking conditions on pork ham and beef chops. Zhang and Wang [44] conducted experiments on vacuum-packed chicken breast samples, subjecting them to a 30-min heat treatment at varying temperatures. Their findings revealed that heat losses increased as the temperature rose. The most significant cooking losses were observed in the temperature range of 75–85 °C. In parallel studies [45,46] involving chicken muscle, the most substantial weight losses occurred at temperatures ranging from 80 to 100 °C. These weight losses

associated with high temperatures are linked to the contraction of myofibrillar proteins at 40–60 °C, collagen shrinkage at 60–70 °C, and actin denaturation at 70–80 °C [39]. Furthermore, Karpińska-Tymoszczyk et al. [47] emphasized that the water and fat content of the meat also impacts the extent of weight loss. Raising the temperature during heat treatment from 40 °C to 80 °C results in the denaturation of myofibrillar proteins and the actomyosin complex. This denaturation process leads to muscle fiber contraction, structural changes, the release of fluid from the muscle fiber, and increased losses during heat treatment [39]. Zielbauer et al. [10] attribute the rise in weight loss during heat treatment above 60 °C to the denaturation of collagen and actin. Consequently, lower temperatures are associated with reduced meat losses during heat treatment. In contrast, studies by Polak and Markowska [48] and Kurp et al. [43] indicate that the weight loss in turkey breast and pork loin is influenced by both the time and temperature of the process. As the sous-vide cooking temperature and duration increased, the percentage of weight loss in the tested meat also increased. Polak and Markowska [48] demonstrated that the smallest loss (approximately 3%) was observed in samples subjected to 60 °C for 150 min. With increasing temperature, weight loss grew, reaching 5.55% for meat samples cooked sous-vide at 64 °C for 150 min. At 60 °C and 68 °C, the percentage of meat weight loss also increased with longer durations.

The cooking loss that occurs during sous-vide thermal processing is primarily attributed to the loss of water from the meat and other constituents, including sarcoplasmic and myofibrillar proteins, lipids, collagen, flavor compounds, minerals, and vitamins. These losses tend to increase with higher temperatures and longer cooking times [43,49]. In the sous-vide method, vacuum-packing meat samples is essential as it provides an excellent barrier against moisture loss. However, as the temperature and cooking duration in sous-vide processing increase, cooking losses also escalate due to the pressure differential between the meat and its environment [49]. Consequently, the use of low temperatures in sous-vide heat treatment, which results in lower temperatures at the geometric center of the meat at the end point, appears to promote the meat's water retention. This is attributed to reduced fiber shrinkage within the meat [43].

3.3. Color Parameters and Pigment Levels

Table 3 presents data regarding the color parameters and pigment levels of horsemeat, which are influenced by the duration and temperature of sous-vide heat treatment. Color changes in muscle tissue can result from various factors, including protein denaturation and oxidation, the Maillard reaction, and the production of different color compounds. Among these factors mentioned, the Maillard reaction is the least impactful, as sous-vide heat treatment employs low temperatures, leading to fewer Maillard products. Based on the findings in Table 3, it can be concluded that the temperature and the duration of sous-vide heat treatment had a statistically significant impact on the brightness (L^*) of horsemeat. Increasing the duration of sous-vide heat treatment from 4 to 24 h at 65 °C significantly decreased the brightness in the color ($p < 0.05$). Conversely, raising the heat treatment temperature from 55 °C to 65 °C significantly increased the value of the analyzed parameter ($p < 0.05$). The lightness of cooked meat is influenced by myofibrillar protein denaturation and aggregation, as well as the water content on the meat surface. The observed reduction in the L^* component in this study may be attributed to the decrease in water content with the prolonged duration of sous-vide heat treatment at each of the temperatures used.

Table 3. Color parameters and pigment levels of horsemeat as a function of time and temperature of sous-vide heat treatment (means ± SE).

Specification	Temp. (°C)	Time of Sous-Vide Heat Treatment (h)				
		4	8	12	24	ANOVA
*L**	55	60.6 ± 0.23 ^{x}	60.3 ± 0.19 ^{x}	60.1 ± 0.23 ^{x}	60.0 ± 0.34 ^{x}	T *; S *
	60	60.7 ± 0.31 ^{x,y}	60.4 ± 0.14 ^{x,y}	60.3 ± 0.28 ^{x,y}	60.2 ± 0.21 ^{x,y}	
	65	62.1 ± 0.33 ^{a,y}	62.0 ± 0.33 ^{a,b,y}	61.4 ± 2.24 ^{a,b,y}	61.0 ± 0.32 ^{b,y}	
*a**	55	24.1 ± 0.85 ^{a,x}	20.8 ± 1.00 ^{b,x}	20.4 ± 0.65 ^{c,b,x}	14.7 ± 0.95 ^{d}	T *; S *; T × S *
	60	19.8 ± 0.75 ^{a,y}	16.5 ± 0.63 ^{b,y}	14.7 ± 1.97 ^{c,b,y,z}	13.3 ± 0.64 ^{d,c}	
	65	16.9 ± 1.56 ^{a,z}	13.2 ± 0.98 ^{b,z}	12.7 ± 0.27 ^{c,b,z}	12.1 ± 0.07 ^{d,b,c}	
*b**	55	12.9 ± 0.15	13.1 ± 0.17	13.2 ± 0.11	13.3 ± 0.06	
	60	13.1 ± 0.18	13.4 ± 0.11	13.5 ± 0.11	13.6 ± 0.11	
	65	13.3 ± 0.13	13.6 ± 0.16	13.6 ± 0.07	13.9 ± 0.06	
Mb (%)	55	23.2 ± 0.23 ^{a,x}	28.8 ± 1.13 ^{a,x}	50.3 ± 6.31 ^{b}	49.0 ± 0.30 ^{b,x,y}	T *; S *; T × S *
	60	39.4 ± 0.52 ^{a,y}	49.0 ± 0.32 ^{b,c,y,z}	53.7 ± 0.75 ^{b}	46.7 ± 1.13 ^{c,x}	
	65	49.1 ± 0.94 ^{z}	49.9 ± 0.07 ^{z}	53.9 ± 0.32	53.1 ± 0.53 ^{y}	
MMb (%)	55	39.5 ± 1.18 ^{a,x,y}	43.1 ± 0.68 ^{a,x}	38.0 ± 3.53 ^{a,x}	34.0 ± 0.68 ^{b,x}	T *; S *; T × S *
	60	35.5 ± 0.36 ^{a,y}	27.4 ± 0.77 ^{b,y,z}	23.2 ± 4.36 ^{b,y,z}	28.4 ± 2.33 ^{a,b,x,y}	
	65	25.9 ± 1.55 ^{z}	24.5 ± 0.58 ^{z}	20.6 ± 0.17 ^{z}	24.3 ± 4.65 ^{y}	
$Mb{\cdot}O_2$ (%)	55	37.1 ± 1.37 ^{a,x}	27.9 ± 0.97 ^{c}	11.6 ± 9.82 ^{b,x}	22.9 ± 0.58 ^{c}	T *; S *; T × S *
	60	25.0 ± 0.41 ^{y,z}	26.4 ± 1.03	25.8 ± 3.91 ^{y,z}	18.7 ± 1.24	
	65	24.9 ± 0.63 ^{z}	22.5 ± 0.55	22.5 ± 0.49 ^{z}	22.4 ± 4.14	
OZB (mg/g)	55	158.1 ± 3.32 ^{a,x}	188.7 ± 9.62 ^{b,x,z}	179.7 ± 19.15 ^{c,b,x}	263.9 ± 1.07 ^{d,x}	T *; S *; T × S *
	60	259.2 ± 1.07 ^{a,y}	300.7 ± 0.50 ^{b,y}	213.3 ± 0.86 ^{c,y}	296.8 ± 1.59 ^{d,b,y}	
	65	194.8 ± 3.53 ^{a,z}	189.4 ± 0.31 ^{a,z}	146.6 ± 0.99 ^{b,z}	233.5 ± 1.68 ^{c,z}	

^{a,b,c,d}—values marked with different letters in the same lines are statistically significantly different between heat treatment times—$p < 0.05$. ^{x,y,z}—values marked with different letters in the columns are statistically significantly different between treatment temperatures—$p < 0.05$. ANOVA: two-way analysis of variance among temperatures of sous-vide heat treatment, T; duration of sous-vide heat treatment. S. * $p < 0.05$. Mb—myoglobin; MMb—metmyoglobin; Mb-O_2—oxymyoglobin; OZB—total heme pigment content.

Changes in the proportion of red meat color (*a**) during sous-vide heat treatment are associated with myoglobin content and the extent of denaturation, which takes place between 55 °C and 65 °C, though it extends until 75 °C or 80 °C. The temperature applied, the duration of sous-vide heat treatment, and the interaction between time and temperature all had statistically significant effects on the color parameter *a**. Meat samples treated at lower temperatures exhibited the highest proportion of red color (*a**). An increase in the temperature during sous-vide heat treatment led to a statistically significant reduction in this parameter, owing to more pronounced pigment denaturation. Furthermore, prolonging the sous-vide heat treatment duration significantly decreased the average values of the proportion of red color (*a**) across all temperature ranges.

When analyzing the proportion of yellow color (*b**) in sous-vide-treated horsemeat samples, it was observed that the values of this parameter increased with rising temperature and duration of heat treatment; however, this correlation was not statistically significant. The elevation in the analyzed parameter suggests the formation of a brown color, which is a consequence of the heat denaturation of metmyoglobin.

In a study conducted by Sánchez del Pulgar et al. [38] on pork cheeks cooked using the sous-vide method, it was determined that the brightness *L**, as well as color parameters *a** and *b**, were influenced by cooking temperature. Meat samples cooked at 60 °C displayed higher *L** values compared to those cooked at 80 °C. Additionally, pork cheek samples cooked at 60 °C exhibited a more pronounced red color (higher *a** values) in contrast to those cooked at 80 °C. In contrast, Ismail et al. [24] demonstrated that for beef semitendinosus, the values of the *L**, *a**, and *b** parameters were significantly influenced by both cooking temperature ($p < 0.001$ for all color parameters) and cooking time (*L**, $p < 0.001$; *a**, $p = 0.008$; *b**, $p < 0.001$). Steaks cooked sous-vide at a lower temperature (60 °C) exhibited a lighter color compared to steaks cooked at a higher temperature (75 °C), possibly due to the retained water impregnating the cut surface just before color measurement. Furthermore, the cited authors found that steaks cooked at a lower temperature and for a shorter time displayed a more pronounced redness. This observation can be attributed to the fact that

an increase in cooking temperature and duration leads to a greater degree of myoglobin denaturation, a phenomenon most pronounced with temperatures exceeding 75 °C. The b^* value appeared to increase with temperature, although the effect of time on b^* values was inconsistent, yet statistically significant at $p < 0.001$. In the study by Bıyıklıa et al. [26], it was determined that the temperature and duration of sous-vide heat treatment had no effect on the a^* and b^* color component values ($p > 0.05$). However, these factors significantly influenced the L^* value of turkey cutlet samples ($p < 0.05$).

The changes in meat coloration that occur during heat treatment depend on both the temperature of the process and its duration. Regardless of the temperature used, the primary factor responsible for color changes during heat treatment is denaturation of the protein component of heme pigments. Denaturation of the protein portion of heme dyes initiates above 50 °C, intensifies at 65 °C, and reaches its peak in the temperature range of 75–80 °C. On the other hand, denaturation of myoglobin itself occurs at approximately 62 °C. The various redox forms of myoglobin present in unsealed meat exhibit varying resistance to heating. The most heat-resistant form is native myoglobin, while the least heat-resistant is metmyoglobin, which denatures most rapidly. Heat-denatured metmyoglobin forms metmyochromogen (Ch·MMb) Fe^{3+} which is an undesirable hemin dye with a brownish tint, resulting in a brownish-gray color. The specific characteristics of this coloration vary and are largely influenced by the presence of different initial forms of heme dye in the environment. This is because native myoglobin (Mb) and oxymyoglobin ($Mb{\cdot}O_2$)Fe^{2+} denature into myochromogen (Ch·Mb) Fe^{2+}, which is a red heme dye. However, in practice, this dye rapidly oxidizes to become brown metmyochromogen (Fe^{3+}) during and after processing and remains in its initial form as myochromogen (Fe^{2+}) only under certain conditions. The red local coloration of meat following heat treatment is primarily influenced by the high initial content of myoglobin and hemoglobin in the meat, which are the most thermally resistant pigments and lead to the formation of red myochromogen and hemochromogen. The disappearance of the red color starts in the temperature range of 65–70 °C during thermal processing and is mostly completed when meat is heated to 75–80 °C. Effective denaturation of hemoglobin requires temperatures as high as about 85 °C [50].

In the study conducted by the authors of the publication, the temperature applied, the duration of sous-vide heat treatment, and the interaction between time and temperature exhibited statistically significant effects on the levels of pigments present in horsemeat samples (refer to Table 3). The myoglobin content in horsemeat samples prepared using sous-vide technology was at its highest after 12 h of treatment across all temperature ranges tested. Extending the duration of sous-vide heat treatment to 12 h led to an increase in myoglobin content in horsemeat samples at all temperature ranges, whereas the opposite effect was observed after 24 h of treatment. A noteworthy increase in this parameter ($p < 0.05$) was observed when the temperature was raised from 55 °C to 65 °C for meat samples treated sous-vide for 4 and 8 h and from 55 °C to 60 °C for meat samples subjected to sous-vide for 24 h.

Based on the study findings, it can be deduced that an increase in the duration of heat treatment from 4 to 24 h resulted in a significant increase in the levels of total heme pigments across all temperature ranges employed. Conversely, there was a notable reduction in the level of oxymyoglobin and metmyoglobin at 55 °C ($p < 0.05$). Considering the temperature aspect, it can be inferred that elevating the sous-vide heat treatment temperature from 55 °C to 60 °C significantly raised the levels of total heme pigments., However, a further increase in temperature to 65 °C led to a significant decrease in total heme dyes in the horsemeat samples at all time ranges studied. Regarding meat pigments $Mb{\cdot}O_2$ and MMb, raising the temperature from 55 °C to 60 °C and to 65 °C resulted in a significant decrease in the tested horsemeat pigments after 4 h of sous-vide heat treatment ($p < 0.05$). Furthermore, increasing the temperature from 55 °C to 65 °C resulted in a significant decrease in MMb for all other durations of heat treatment and in $Mb{\cdot}O_2$ only after 12 h of sous-vide heat treatment.

Color is the primary sensory characteristic of horsemeat, serving as a visible indicator of meat quality freshness. Horsemeat typically exhibits a dirty red color with a subtle bluish tinge. However, when exposed to air, horsemeat darkens rapidly, acquiring a blackish-brown hue, which is generally considered an undesirable characteristic of this raw material. The majority of surveyed consumers strongly prefer meat with a light pink color. The study demonstrated that horsemeat subjected to sous-vide heat treatment at lower temperatures and for shorter durations was distinguished by a heightened redness, a feature that is favorable from the consumer's perspective.

4. Conclusions

The present study suggests that it is challenging to definitively determine which specific experimental factors comprehensively influence selected physicochemical properties of horsemeat. When considering the impact of sous-vide heat treatment on the quality of horsemeat, it becomes evident that the alterations in this raw material are not markedly distinct from those observed in other types of meat. The majority of properties assessed in this study, which play a role in determining the quality of horsemeat, were statistically significantly affected by the temperature employed, the duration of sous-vide heat treatment, and the interaction between time and temperature. Considering the preservation of the red color in horse meat, which is crucial for maintaining the quality of this raw material, as well as taking into account weight loss and the TBARS index, it is advisable to conduct sous-vide thermal processing of horse meat at a temperature of 55 °C for a brief duration (4 h).

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