






Article

Is Prolonged Ageing a Necessity for Improving the Quality of Sous-Vide Cooked Beef?

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Abstract: This study aimed to determine the effect of wet ageing time (4, 14 and 21 days) on the quality of sous-vide cooked beef products. The research material consisted of *longissimus lumborum* muscles obtained from the carcasses of Polish Holstein-Friesian bulls (n = 9, average age at slaughter 22 months). The meat was wet-aged at 4 ± 1 °C for 4, 14 and 21 days post-mortem. The analyses were conducted on uncooked samples (colour and pH) and after the sous-vide cooking (60 °C, 4 h) (colour, cooking loss, Warner–Bratzler shear force, texture profile analysis and sensory assessment). It was found that ageing decreased redness, yellowness and chroma ($p < 0.05$) in the cross-section area of sous-vide cooked beef. The values of shear force, hardness, springiness and chewiness decreased during ageing ($p < 0.05$). The samples were scored similarly in sensory assessment, except for tenderness which was scored higher ($p < 0.01$) in the products obtained from 14 and 21 d aged beef compared to 4 d aged samples. Overall, the findings highlighted that, also in the production of sous-vide cooked beef, *longissimus lumborum* muscles should be aged for at least 14 days.

Keywords: ageing; *longissimus lumborum*; meat quality; sous-vide cooking; storage



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1. Introduction

Globally, beef ranks among the three most popular meat types, following pork and poultry [1]. However, compared to poultry and pork, beef prices are higher, and the preparation of high-quality dishes from beef necessitates proficiency and culinary expertise from consumers [2]. As a result, a noteworthy proportion of consumers prefer to consume beef at restaurants or opt to purchase ready-to-eat beef products [3]. Therefore, it is reasonable to search for the most appropriate and economical methods for the preparation of beef for consumption to thereby deliver products that align with consumer preferences.

It is well documented that the tenderness of beef increases during post-mortem ageing [4–7]. Various beef ageing methods exist, including dry ageing, wet ageing and ageing in semi-permeable bags, with wet ageing being the most frequently employed technique [7,8]. Wet ageing mainly consists of vacuum packaging the entire muscle or its cuts and subsequently storing it under controlled refrigerated conditions [9]. The time of wet ageing might differ depending on factors such as muscle type, the hygienic conditions of the meat and the expected outcome. An inadequate ageing time might result in undesired tenderness whilst prolonged ageing could lead to losses in lipid oxidative stability, colour and palatability of meat [7,10], as well as increased microbial populations [11]. Therefore, it is reasonable to adjust the ageing time for a particular beef product to optimise its quality and production cost.

Sous-vide cooking is a modern culinary technique that has become increasingly prevalent for the heat treatment of meat and meat products in catering services and food processing [12]. The principle of sous-vide cooking is to vacuum pack the meat and heat it in a moist environment (water bath or steam) under controlled temperature and time [13]. This technique is specifically recommended for tougher meat categories such as beef, lamb, white-stripping chicken breast and spent duck meat since it enhances meat quality in terms of organoleptic characteristics and safety [14,15]. The most frequently recommended combinations of temperature and time by chefs for beef are around 58–63 °C for up to 48 h. Using such low heat treatment temperatures prevents excessive changes in meat proteins and produces tender and juicy meat products [16]. Generally, meat heating causes the denaturation of myofibrillar proteins and initial denaturation, and then the solubilisation of connective tissue [17]. Since the extent of those changes is temperature and time dependent, the meat texture changes during heating. At the initial phase of heating, meat changes from tender (typical for uncooked meat) to tougher, whereas further heating to temperatures from 55 to 60 °C reduces this initial toughness [18]. The myofibrillar and connective tissue proteins denature at different rates, which affects the texture of the different meat cuts as well as changes related to cooking temperature. In the range from 40 to 65 °C, increasing aggregation of the denatured myofibrillar proteins is noted, which is accompanied by a loss of meat juice and shrinkage of the muscle fibres. At approximately 65–67 °C, intramuscular connective tissue fibres shrink, which increases meat toughness because of the denaturation of collagen in the endomysium and perimysium. It also results in squeezing out the water from meat [18]. Up to 80 °C, the increase in meat toughness occurs, however with further increases in temperature above 80 °C, and prolonged heating (as in stewing or casseroles), a tenderisation is noted caused by collagen solubilisation. Collagen can be solubilised also at lower temperatures (such as used in sous-vide cooking), but this requires considerably longer cooking times than used in stewing or casseroles [18]. Moreover, at temperatures of 55–60 °C, the activity of proteolytic enzymes that degrade myofibrillar proteins including calpain, cathepsin B and cathepsin L is partially maintained [19]. There are also changes in connective tissue related to the action of the collagenase [20]. Bearing in mind all the changes occurring during the heating of meat, in the present study, sous-vide cooking was conducted at 60 °C. As a result of sous-vide cooking, changes in meat microstructure occur. As shown by Chotigavin et al. [21], sous-vide cooking proceeded at 60 °C increased the percentage of space between muscle fibres compared with uncooked or boiled beef. This increase resulted from a reduction in the diameter and cross-sectional area of muscle fibres and gradual dissolution of the collagen being part of the intramuscular connective tissue, and was accompanied by a reduction in shear force compared to boiled beef. In the study, the sous-vide cooking of beef steaks from *longissimus lumborum* was proceeded at 60 °C for 4 h. This combination of a sous-vide time and temperature falls within the range indicated in many culinary guidelines, such as the Culinary Pro website [22], and ensures a pasteurisation effect [13]. Patil et al. [23] reported that beef steaks sous-vide processed at a similar temperature to that used in the study (62.5 °C) just after 120 min of cooking met the standards specified for this kind of product (a reduction in bacterial counts for more than five log cycles). Sous-vide cooking at 60 °C produces lower cooking losses in meat than higher temperatures (i.e., 70 °C and 80 °C) [24]. Increasing the processing time also leads to increased cooking loss [24], which in turn might decrease products' juiciness [25]. Therefore, a proposed combination reflected issues associated with food safety, tenderness development, cooking loss and the juiciness of products. The sous-vide temperature and time combination (60 °C 4 h) was also used in our earlier studies [26–28]. In a preliminary study, the microbial safety of meat products after sous-vide cooking at 60 °C for 4 h was confirmed (not published data, total viable counts $2 \log_{10}$ cfu/g, psychrotrophs $1 \log_{10}$ cfu/g, *Salmonella* sp. and *L. monocytogenes* were not detected in 25 g of meat samples).

In many countries, including Poland, Hungary, Czech Republic, Germany and Austria, where the proportion of beef breeds is low [29], beef is produced from dairy breeds. In dairy herds, male cattle offspring are sold to beef producers for further fattening. It is estimated

that in Poland, over 50% of beef is produced from young dairy (mainly Holstein-Friesian) bulls [30]. Therefore, beef obtained from young Holstein-Friesian bulls was used in the study. Our previous study showed that prolonging the ageing time of *longissimus lumborum* muscle obtained from Polish Holstein-Friesian young bulls from 9 to 14 days did not affect tenderness and shear force after sous-vide cooking [26]. However, no information is available on the tenderness of beef sous-vide cooked products obtained from Holstein-Friesian beef aged for wider periods. Based on the assumption that sous-vide cooking enables obtaining tender meat products even from tough meat pieces [14,15] and the fact that ageing meat before its placing on the market requires time and energy, the hypothesis was set that the ageing time of beef subjected to sous-vide cooking might be shortened to 4 days with no quality deterioration. Therefore, in the present study, the quality of sous-vide cooked *longissimus lumborum* muscle after 4, 14 and 21 days of wet ageing was determined.

2. Materials and Methods

2.1. Animals

The study was performed on *longissimus lumborum* muscles obtained from 9 Polish Holstein-Friesian bulls reared under controlled conditions at the Agricultural Experiment Station in Bałcyny, Poland. The protocol of animal research was approved by the Ethics Committee of the University of Warmia and Mazury in Olsztyn (decision no. 8/2020). Animals born on a farm in Bałcyny reared in a traditional way (milk, milk replacer, meadow hay and concentrate feed) were subjected to semi-intensive fattening at the age of 5 months. The diet included corn and grass silage with concentrate and 150 g of premix. The composition of the feed used and their share in the ration in the final 6-month fattening period are presented in Table 1. The animals were slaughtered in a commercial slaughterhouse at the age of 682 ± 14 days with a body weight of 756 ± 12 kg. All slaughter and post-slaughter processes were carried out in accordance with EU regulations No. 1099/2009 of 24 September 2009 on the protection of animals at the time of killing [31]. Electrical stimulation was not applied.

Table 1. Chemical composition (g/kg dry matter (DM)) of the experimental feed and diet.

Specification	Diet's Constituents				
	Grass Silage	Maize Silage	Triticale	Rapeseed Meal	Diet
Proportion in the diet (% in DM)	35	35	20	10	100
DM (g/kg)	291 ± 1	325 ± 2	881 ± 4	874 ± 4	479 ± 2
On DM basis (g/kg)					
Organic matter	902 ± 2	954 ± 3	956 ± 3	932 ± 3	934 ± 3
Crude protein	118 ± 1	87 ± 1	119 ± 1	385 ± 1	134 ± 1
NDF	539 ± 2	325 ± 2	158 ± 2	286 ± 2	363 ± 2
ADF	316 ± 2	189 ± 2	39 ± 0.3	210 ± 2	205 ± 2
ADL	26.2 ± 0.2	12.4 ± 0.2	-	-	13.5 ± 0.2
NFC	198 ± 1	511 ± 3	624 ± 3	235 ± 2	396 ± 2
UFV	0.85 ± 0.03	0.87 ± 0.02	1.18 ± 0.04	1.03 ± 0.03	0.94 ± 0.03
PDIN	84 ± 0.2	52 ± 0.3	89 ± 0.4	251 ± 1	91 ± 0.5
PDIE	73 ± 0.2	66 ± 0.4	98 ± 0.3	163 ± 0.9	85 ± 0.4

Data are presented as the mean ± standard error of the mean (SEM). NDF—Neutral Detergent Fibre; ADF—acid detergent fibre; ADL—acid detergent lignin; NFC—non-fibre carbohydrate; UFV—feed units for meat production; PDIN—protein digested in the small intestine depending on rumen degraded protein; PDIE—protein digested in the small intestine depending on rumen fermented organic matter.

2.2. Sample Preparation

In the study, 48 h post-mortem ($\text{pH}_{\text{u}} < 5.8$) *longissimus lumborum* muscles ($n = 9$) cut from the left half carcass of each animal were used. The muscles were transported to the laboratory maintaining the cold chain (delivery time of approx. 1 h), and they were then kept at 4 ± 1 °C for 24 h. The next day, each muscle (approx. 1000 g) was divided into four subsamples: one portion for chemical determinations (in total $n = 9$, one from each

muscle) and three steaks (2.51 cm thick) which were subjected to different ageing periods (up to 4, 14 and 21 post-mortem days, in total 27 samples from all muscles). The samples were weighed and individually packaged in vacuum PA/PE bags (thickness 70 μm , Inter Arma Ltd., Rudawa, Poland; total transmission rates not exceeding 10 mg/dm^2 for model liquids, 3% acetic acid, 50% ethyl alcohol 10 days, 40 $^{\circ}\text{C}$ and isooctane 2 days, 20 $^{\circ}\text{C}$). After that, the packed samples were kept at 4 ± 1 $^{\circ}\text{C}$ in a climate chamber (Memmert GmbH, Schwabach, Germany) until the 4th ($n = 9$, each one from a different bull carcass), 14th ($n = 9$, each one from a different bull carcass) and 21st post-mortem days ($n = 9$, each one from a different bull carcass) [32]. They were then frozen and stored at -20 $^{\circ}\text{C}$ until analyses (approx. 4 months). The samples for chemical composition determination were vacuum packed and aged for the 14th day post-slaughter and then frozen. Before analyses, the samples were thawed at 4 ± 1 $^{\circ}\text{C}$ for 48 h.

2.3. Assessment of Uncooked Meat Quality

2.3.1. Thawing Loss and pH

After thawing the samples, the vacuum packages were opened, and the surface of the samples was dried with a paper towel. The samples were weighed, and thaw loss was determined based on the weight of the individual steaks before vacuum packaging (on day 4 post-mortem) and after thawing. On the same occasion, the pH value was measured in triplicate using a Testo 205 pH meter with the automatic temperature compensation function (Testo SE & Co. KGaA, Lenzkirch, Germany) penetrated directly into the meat tissue. Before measurements, the pH meter was calibrated using pH 7 and pH 4 buffers.

2.3.2. Colour

The colour attributes were measured on the surface of steaks and the freshly cut surface after 25 min blooming [33] with a Konica Minolta CR-400 (Sensing Inc., Osaka, Japan) colourimeter (D65 illuminant, 2° view angle, measurement/illumination area φ 8 mm/ φ 11 mm, pulsed xenon lamp as a default light source). Three measurements at randomly selected points were recorded per sample to determine the values of lightness (L^*), redness (a^*) and yellowness (b^*). In addition, the chroma angle (C^*) and hue angle (h°) were calculated according to King et al. [34] using Equations (1) and (2) as follows:

$$C^* = \sqrt{(a^{*2} + b^{*2})} \quad (1)$$

$$h^{\circ} = \arctangent\left(\frac{b^*}{a^*}\right) \quad (2)$$

2.3.3. Chemical Composition

The chemical composition of uncooked beef was determined using a near-infrared spectrophotometer FoodScan (FOSS FoodScanTM 2 Lab/Pro; FOSS Analytical A/S Hillerød, Denmark) [35] where a portion of approx. 200 g of minced beef (mesh size 3 mm) from every muscle was used. Independent readings ($n = 16$) were taken from each sample and averaged to obtain the final reported values. The contents of total moisture, fat, ash and protein including collagen were reported.

2.4. Sous-Vide Cooking

The samples (2.5 cm thick, approx. 9 cm \times 12 cm) were individually vacuum packed in plastic pouches suitable for cooking (PA/PE sous vide bags, 70 μm , Inter Arma sp. z o.o., Rudawa, Poland) and heated at 60 $^{\circ}\text{C}$ for 4 h (Sous-vide GN 2/3, HENDI Food Service Equipment, Rheden, the Netherlands). The processing temperature was monitored continuously with a digital thermometer integrated into the device. On the same occasion, 9 samples (3 per each ageing time) were heated (in total, 3 cooking batches were performed). Samples were weighed before and after heating and based on the weight difference, and

cooking loss was calculated and expressed in the percentage of the sample weight before cooking. After the termination of sous-vide cooking, samples for sensory analyses were cut. Sensory assessment was carried out on warm sous-vide cooked beef samples.

2.5. Assessment of Sous-Vide Cooked Meat Quality

2.5.1. Colour

The colour of the sous-vide cooked meat was measured on the surface and cross-section of the steaks, as described in Section 2.3.2.

2.5.2. Texture

To evaluate the impact of ageing on the texture of sous-vide cooked beef, WBSF and texture profile analysis (TPA) were conducted. WBSF values (N) were measured according to the procedure described in Tkacz et al. [27]. For each steak, 5 independent samples were analysed. TPA was performed by a twofold compression method using an Instron 5942 universal testing machine (Instron, Norwood, MA, USA) connected to a PC equipped with Bluehill 3 software. Cubic samples ($n = 3$ from each sample, $1\text{ cm} \times 1\text{ cm} \times 1\text{ cm}$) were cut from sous-vide cooked samples after cooling for 12 h at $4\text{ }^{\circ}\text{C}$. Before measurements, samples were allowed to equilibrate to room temperature, and then they were compressed to 50% of their height, using the head velocity of 5 mm/s. A time of 5 s was allowed to elapse between the two compression cycles.

2.5.3. Sensory Assessment

Immediately after the termination of the sous-vide cooking, the sensory quality of the beef was determined, following Standard PN-ISO 4121 [36]. The evaluation was conducted by a team of 7 panellists (5 women and 2 men, aged 22–55 years old, non-smokers) trained and experienced in sensory analyses of meat products in a way described in detail in Modzelewska-Kapituła et al. [37]. The samples (approximately 2 mm thick, total number of 27) were served randomly and coded with 3 digit numbers on a white plate. A total of five sessions were performed, and a maximum of six meat samples were assessed per session where the same panellists participated in all sessions. Water and bread were provided to clean the palate between samples. The evaluation was conducted under fluorescent light. A scale from 1 to 10 was used in the sensory evaluation. The panellists scored each sample for meat aroma and taste intensity (1, imperceptible; 10, extremely intense), juiciness (1, extremely dry; 10, extremely juicy) and tenderness (1, extremely tough; 10, extremely tender).

2.6. Statistical Analysis

Data were statistically analysed by the Statistica 13.3 program (TIBCO Software Inc., Palo Alto, CA, USA). The results were presented as the mean \pm SEM. The influence of ageing on uncooked meat colour and thaw loss was analysed using the variance components module with ageing as a fixed effect (3 levels: 4, 14 and 21 days) and beef carcass as a random effect (9 repetitions). The influence of ageing on cooked meat colour, cooking loss and texture parameters was analysed using the variance components module with ageing as a fixed effect and beef carcass (9 repetitions) and cooking batch (3 levels) as random effects. The normal distribution of the data (Shapiro–Wilk test) and the homogeneity of variance (Leven's test) were examined. They revealed the normal distribution of data and variance homogeneity; therefore, the significance of differences between the obtained mean values was determined using the Analysis of Variance and Tukey's Multiple Comparison Test at the significance level of $p < 0.05$. When analysing the results of the sensory assessment, ageing was set as a fixed effect, whereas beef carcass, panellist, cooking batch and the number of sessions were assigned as random effects. The non-parametric Kruskal–Wallis test was used to compare the mean values obtained in the sensory assessment.

3. Results and Discussion

3.1. Proximate Composition of Beef

The proximate composition of *longissimus lumborum* steaks was determined for the characterisation of the beef. Accordingly, the beef contained $71.0 \pm 0.6\%$ moisture, $21.6 \pm 0.17\%$ protein including $1.9 \pm 0.07\%$ collagen, $5.4 \pm 0.7\%$ fat and $1.1 \pm 0.04\%$ ash. The meat used in the present research was characterised by a high protein content, which is typical for beef, as also reported in other studies [30,38].

3.2. The Influence of Ageing on Uncooked Beef

The impact of wet ageing on the physico-chemical parameters of the steaks is presented in Table 2. Ageing time did not affect meat pH and thaw loss ($p > 0.05$). The L^* values of the beef surface increased during ageing, where 4 d aged samples were darker than 21 d aged ones ($p < 0.05$). Significant differences in the colour of the cross-section area between samples aged 4, 14 and 21 days were also noted in terms of lightness and redness. The values of L^* measured on the cross-section area of the beef samples aged for 4 days were lower than those aged for 21 days ($p < 0.05$), and those samples showed lower a^* values than 14 d and 21 d aged samples ($p < 0.05$). Values of b^* , C^* and h° were not affected ($p > 0.05$) by the ageing time in either the surface or cross-section area colour parameters of the steaks.

Table 2. The effect of wet ageing on the quality of uncooked *longissimus lumborum* steaks.

Attribute	Ageing Time (days)			Significance
	4	14	21	
pH	5.55 ± 0.01	5.55 ± 0.01	5.56 ± 0.02	NS
Thaw loss (%)	8.3 ± 0.5	8.0 ± 0.5	8.2 ± 0.5	NS
Surface colour				
L^*	$30.0^b \pm 0.5$	$31.2^{ab} \pm 0.4$	$32.3^a \pm 0.5$	***
a^*	14.2 ± 0.4	14.6 ± 0.4	15.1 ± 0.4	NS
b^*	4.85 ± 0.15	5.23 ± 0.18	5.35 ± 0.20	NS
C^*	15.0 ± 0.4	15.5 ± 0.4	16.0 ± 0.5	NS
h°	18.9 ± 0.3	19.8 ± 0.5	19.5 ± 0.5	NS
Cross-section colour				
L^*	$30.7^b \pm 0.4$	$31.9^{ab} \pm 0.5$	$32.9^a \pm 0.4$	*
a^*	$15.5^b \pm 0.4$	$17.4^a \pm 0.5$	$17.29^a \pm 0.28$	*
b^*	4.36 ± 0.17	5.39 ± 0.27	5.10 ± 0.16	NS
C^*	16.1 ± 0.4	18.2 ± 0.5	18.0 ± 0.3	NS
h°	15.6 ± 0.3	16.8 ± 0.7	16.3 ± 0.3	NS

Data are presented as the mean \pm standard error of the mean (SEM). ^{a,b} mean values in rows with different letters differ significantly at $p < 0.05$, NS—non-significant differences ($p > 0.05$), * differences significant at $p < 0.05$; *** differences significant at $p < 0.001$; L^* —lightness, a^* —redness, b^* —yellowness, C^* —chroma, h° —hue angle.

As reported by Holman et al. [4], the pH of meat might increase during wet ageing, however, the most pronounced increase was noted after 8 weeks of ageing. When meat ageing is conducted for a shorter period, the changes might not be so obvious. Yu et al. [7] noted similar pH values in beef aged 3, 7 and 14 days; moreover, the samples aged 7, 14 and 21 days did not differ in pH either. Generally, the pH increase might be explained by the accumulation of protein degradation products. On the other hand, in vacuum packages, lactic acid bacteria can grow and produce acids, which affect meat pH. This might alleviate the effect of the alkalisation caused by proteolytic breakdown, which explains the lack of changes in beef pH during ageing in the present study.

Generally, meat colour is the first and the most important visual attribute based on which consumers evaluate the quality of meat [39]. Redness (a^*) values higher than 14.5 were proposed as an indicator of consumer acceptance of beef [40]. In the present study, 14 d and 21 d aged uncooked beef steaks showed a^* values (measured on the beef surface) above this threshold, whereas a^* values of 4 d aged samples were slightly below

the threshold. However, a^* values measured on the cross-section after 20 min of blooming exceeded the threshold value in all samples. The course of colour changes in the cross-section area of beef during wet ageing (the increase in lightness and redness) stays in agreement with the results of our previous study [33] and partially with the findings of Holman et al. [41], who noted an increase in lightness as a result of the chilled storage of *longissimus lumborum* muscles. Changes in meat colour primarily arise from modifications in myoglobin forms. Naves Aroeira et al. [42] who investigated changes in myoglobin forms during beef ageing in vacuum packages reported that between day 0 (24 h post-mortem) and day 7, the proportion of oxymyoglobin increased, whereas deoxymyoglobin decreased; then, both forms remained unchanged during further ageing until day 21. In turn, no differences in the metmyoglobin proportion were noted. Therefore, the results of the present study might be explained by changes in the proportions of different myoglobin forms in the samples aged for different times.

3.3. The Influence of Ageing on Sous-Vide Cooked Beef

Sous-vide cooking changed the colour of beef (Tables 2 and 3). The colour of the surface of sous-vide steaks, regardless of the ageing time, was darker, less red and less yellow compared to the cross-section colour, which results in less saturated and vivid (lower chroma) colour (Table 3).

Table 3. The effect of wet ageing on the colour of sous-vide cooked *longissimus lumborum* steaks.

Attribute	Ageing Time (days)			Significance
	4	14	21	
Surface colour				
L^*	32.7 ^b ± 0.6	32.3 ^b ± 0.4	35.2 ^a ± 0.5	*
a^*	8.22 ± 0.23	7.40 ± 0.17	7.84 ± 0.21	NS
b^*	6.69 ^{ab} ± 0.16	6.37 ^b ± 0.11	7.05 ^a ± 0.12	*
C^*	10.68 ± 0.27	9.84 ± 0.22	10.65 ± 0.18	NS
h°	39.1 ^b ± 0.7	40.6 ^{ab} ± 0.6	41.9 ^a ± 1.0	*
Cross-section colour				
L^*	49.0 ± 0.7	48.8 ± 0.6	49.4 ± 0.6	NS
a^*	18.4 ^a ± 0.4	16.6 ^b ± 0.3	16.1 ^b ± 0.3	***
b^*	8.4 ^a ± 0.1	7.73 ^b ± 0.12	7.90 ^b ± 0.08	**
C^*	20.2 ^a ± 0.4	18.3 ^b ± 0.3	17.98 ^b ± 0.29	***
h°	24.8 ± 0.5	25.1 ± 0.4	26.2 ± 0.4	NS

Data are presented as the mean ± standard error of the mean (SEM). ^{a,b} mean values in rows with different letters differ significantly at $p < 0.05$; NS—non-significant differences ($p > 0.05$), * differences significant at $p < 0.05$; ** differences significant at $p < 0.01$; *** differences significant at $p < 0.001$; L^* —lightness, a^* —redness, b^* —yellowness, C^* —chroma, h° —hue angle.

The colour of cooked meat, described as dull brown, has been associated with heat-induced myoglobin denaturation [43]. Myoglobin denaturation starts around 55–65 °C [21]; therefore, it occurs at the temperature of 60 °C, which was used in the present study in sous-vide cooking. According to Ishiwatari et al. [44], heat treatment during cooking induces a non-uniform distribution of protein denaturation in macro meat systems, which might explain the differences in colour between the surface and cross-section area. Moreover, the outer surface of sous-vide cooked products was subjected to longer contact with the temperature of 60 °C, which provoked more intense myoglobin denaturation. Moreover, there is a strong relationship between the chemistry of myoglobin (its redox form) in uncooked meat and cooked products, which affects the colour of cooked meat [43]. The thermal stability of myoglobin depends on its redox state—deoxymyoglobin is more resistant to heat-induced denaturation than oxymyoglobin, whereas oxymyoglobin is more resistant than metmyoglobin [43]. Probably, on the outer surface of beef steaks, there was a higher proportion of less resistance to heat-induced denaturation myoglobin forms (oxymyoglobin and metmyoglobin) than in the centre of beef steaks.

After the sous-vide cooking process, significant differences in the colour of the surface and cross-section area of the beef steaks subjected to different ageing times were recorded (Table 3, Figure 1).



Figure 1. The appearance of sous-vide cooked beef steaks produced from *longissimus lumborum* aged for 4, 14 and 21 days.

The surface of the samples aged for 4 days showed lower L^* and h° values when compared with that of samples aged for 21 days ($p < 0.05$). A reduction in a^* , b^* and C^* values was also noted on the cross-section of the steaks after 14 days ($p < 0.05$), which indicated that the meat aged for longer periods was less red and yellow. This finding might indicate that myoglobin was more resistant to heat in 4 d aged samples compared to 14 d and 21 d aged samples. It might be explained by differences in the redox status of myoglobin in uncooked meat. As mentioned above, deoxymyoglobin content decreases during ageing [42]. Therefore, it might be assumed that in 4 d aged beef, there was a higher proportion of deoxymyoglobin, which is more resistant to heat-induced denaturation than other myoglobin forms [43], which contributed to the lower denaturation of myoglobin after sous-vide cooking compared with 14 d and 21 d aged beef.

Generally, the colour of the heat-treated beef surface might also result from the Maillard reaction, which is associated with brown colour formation. However, in sous-vide cooked meat products, the Maillard reaction and its impact on cooked meat colour is low [45]. Additionally, it should be pointed out that Maillard reaction products being created on the external surfaces of meats during cooking do not affect the colour of the cross-section area of cooked meat [43].

Cooking loss and the textural attributes of the beef steaks are shown in Table 4. The ageing time did not affect cooking loss ($p > 0.05$), which was also reported in earlier studies [4,7,11]. During heating (even at 60 °C), meat proteins undergo denaturation, which results in shrinkage in the sarcomere length and transverse shrinkage, creating greater gaps between fibres and reducing the ability of the proteins to hold water [17,46].

Table 4. The effect of wet ageing on cooking loss and texture of sous-vide cooked *longissimus lumborum* steaks.

Attribute	Ageing Time (days)			Significance
	4	14	21	
Cooking loss (%)	22.0 ± 0.8	24.0 ± 1.0	23.8 ± 0.9	NS
WBSF (N)	48.1 ^a ± 1.8	30.0 ^b ± 1.2	22.9 ^c ± 0.8	***
Hardness-1 (N)	80.0 ^a ± 5.0	63.0 ^b ± 3.0	51.0 ^b ± 3.0	*
Hardness-2 (N)	68.0 ^a ± 4.0	52.9 ^b ± 2.6	43.7 ^b ± 2.4	*
Springiness (mm)	2.03 ^a ± 0.05	1.96 ^{ab} ± 0.05	1.80 ^b ± 0.04	*
Adhesiveness (N·mm)	−2.32 ± 0.04	−2.36 ± 0.08	−2.37 ± 0.06	NS
Cohesiveness (-)	2.49 ± 0.06	2.41 ± 0.03	2.48 ± 0.06	NS
Chewiness (N·mm)	408 ^a ± 28	299 ^b ± 18	231 ^b ± 21	*

Data are presented as the mean ± standard error of the mean (SEM). ^{a,b} mean values in the same rows with different letters differ significantly at $p < 0.05$; NS—non-significant differences ($p > 0.05$), * differences significant at $p < 0.05$; *** differences significant at $p < 0.001$.

WBSF decreased along with the ageing time, and the lowest values were noted after 21 days of ageing compared to 14 d and 4 d aged samples ($p < 0.05$). The results obtained from the TPA test proved a significant improvement in sous-vide beef texture during ageing in terms of hardness, springiness and chewiness. The changes in these texture attributes were specifically noted between 4 and 14 days, except for springiness, which significantly decreased after 21 days compared to 4 days ($p < 0.05$). The changes in the texture parameters (WBSF and TPA attributes) noted in this study reflect proteolytic changes that occur during meat ageing [18]. Post-mortem proteolysis conducted by the endogenous enzymatic system during the ageing of meat is used widely in the meat industry to improve meat tenderness; however, it is associated with increased production costs [47]. During ageing structural proteins, including the C-protein, M-protein and cytoskeletal proteins such as titin, nebulin, desmin, dystrophin and vinculin, are broken down by endogenous enzymes (calpains), whereas actin and myosin (being the major contractile proteins) are minimally affected (but they might be regarded as tenderisation markers). During long-term ageing, lysosomal enzymes, cathepsins, and other enzyme groups are also involved in tenderisation [18,48]. During ageing, changes in collagen structure also occur as a result of the degradation of proteoglycans which stabilise the bonds between collagen fibrils, which is demonstrated by increasing the proportion of total soluble collagen and acetic-acid soluble collagen, as well as a decrease in insoluble collagen concentration in cooked meat [49]. The extent of post-mortem proteolysis affects meat tenderness, but it is also independently shaped during the heat treatment of meat [18]. Moreover, these proteolytic changes continue during sous-vide cooking. In the present study, beef tenderisation was achieved by the combined effect of ageing and sous-vide cooking. Prolonged ageing up to 14 and 21 days increased proteolysis extent, whereas conducting heating at a relatively low temperature (60 °C) for a long time (4 h) resulted in the limited denaturation of myofibrillar and connective tissue proteins as well as the weakening of connective tissue via collagen solubilisation [50].

The results of the sensory analysis are shown in Table 5. In general, the sensory scores of the beef steaks aged at different times fell within acceptable ranges. The duration of ageing exhibited negligible impact on both the aroma and taste intensity of the meat, as evidenced by comparable scores across all treatments, with consistently high values. The high scores noted in this study for aroma and taste indicate that all sous-vide cooked products showed an intensive meat flavour.

Table 5. The effect of wet ageing on sensory attributes of sous-vide cooked *longissimus lumborum* steaks.

Attribute	Ageing Time (days)			Significance
	4	14	21	
Meat aroma intensity	8.47 ± 0.09	8.30 ± 0.13	8.24 ± 0.11	NS
Meat taste intensity	8.32 ± 0.12	8.26 ± 0.13	8.20 ± 0.19	NS
Juiciness	8.15 ± 0.15	7.51 ± 0.17	7.75 ± 0.20	NS
Tenderness	6.42 ^b ± 0.22	7.51 ^a ± 0.22	8.10 ^a ± 0.22	**

Data are presented as the mean ± standard error of the mean (SEM). ^{a,b} mean values in rows with different letters differ significantly at $p < 0.05$, NS—non-significant differences ($p > 0.05$), ** differences significant at $p < 0.01$; points in the scale from 1 to 10.

Generally, the flavour characteristics of cooked meat are associated with volatile compounds, which are created during meat heating as a result of Maillard reactions and the thermal degradation of lipids. When cooking proceeds at a low temperature (as used in the present study, 60 °C), the Maillard reaction and thiamine degradation products do not play a significant role in shaping the flavour of meat [12]. In contrast, volatile compounds derived from fatty acid degradation, such as linear saturated aldehydes (from butanal to nonanal), unsaturated aldehydes, a ketones, furans and furanones, are detected in sous-vide meat, which indicates their role in shaping the flavour of sous-vide meat [12]. The changes in lipids proceed in both high and moderate (or low) temperatures as a result of thermal lipid degradation and lipid autoxidation, respectively [12]. Lipid oxidation is noted during ageing, even if it proceeds in vacuum packages [51]. As it might be assumed from the results of the present study, ageing which was conducted in vacuum packages up to 21 days did not change the fatty acid composition to the extent that could reflect the flavour profile of sous-vide cooked beef determined by the sensory panel.

No significant differences were recorded in the juiciness scores of the samples, which corresponds with the lack of differences in cooking loss. Sous-vide products obtained from beef aged for 14 days and 21 days were more tender than those aged for 4 days ($p < 0.05$), but no differences were noted between the 14 d and 21 d aged samples. The result indicated also that there is no difference in sensory-assessed tenderness between beef with WBSF values of 30 N and 23 N. The tough structure of beef aged for a short time might be a factor which limits its consumption for some individuals, especially for elderly populations with chewing problems [52]. Hence, ongoing efforts are directed towards the development of alternative techniques and processing strategies aimed at enhancing the tenderness of beef, such as combining sous-vide with modest pressure conditions [21]. In the present study, significant decreases in WBSF and hardness-1 and hardness-2, as well as in springiness and chewiness, were noted as a result of ageing. Furthermore, beef samples aged for 14 and 21 days were scored higher in terms of tenderness by panellists. These results indicate the need for applying the ageing process to beef intended for sous-vide products. According to Liang et al. [53], consumers prefer beef steaks with a shear force value below 41.4 N. In the present study, the values obtained by 14 d and 21 d aged beef were much lower than this threshold, whereas 4 d aged beef had values slightly above it. To increase the tenderness of the beef subjected to short ageing (4 days), additional technological processes increasing its tenderness might be implemented, such as marinating [28].

4. Conclusions

Although sous-vide cooking is regarded as a method for achieving tenderness across various meat cuts, the current work demonstrated the necessity of ageing in beef before sous-vide cooking. Based on the results of the present study, it might be concluded that 4 d ageing is insufficient for obtaining high-quality beef sous-vide products and longer ageing is required (i.e., 14 days). Nonetheless, ageing prolonged to 21 days did not improve the overall quality of sous-vide products. Consequently, the findings of the present work highlighted that to obtain a tender sous-vide product from 4 d aged

longissimus lumborum muscle, a modification of the standard sous-vide cooking procedure is needed (i.e., margination, prolonging heating, high-pressure processing combined with sous-vide).

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