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# Oxidative Stability and Quality Parameters of Veal During Ageing

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The objective of the present study was to evaluate the effects of the ageing period (1, 7, 14, and 21 days) on the quality parameters, sensory attributes, and oxidative stability of loins (*longissimuss lumborum* muscle) obtained from twelve Simmental calves less than 8 months old and slaughtered in a commercial processing plant. After 21 days, the colour of veal samples became yellower (increase in *b** colour parameter value), and the instrumentally-measured texture improved (Warner-Bratzler shear force decreased from 84.61 N to 56.79 N). Ageing time enhanced sensory-evaluated tenderness, juiciness, aroma, and flavour. The amount of the lipid oxidation products determined as thiobarbituric acid reactive substance content remained unchanged; in contrast, the amount of protein carbonyls increased, without compromising veal quality.

**Key words:** ageing, veal, quality parameters, TBARs, protein carbonyls

## INTRODUCTION

Meat ageing is a common name for many biochemical processes, such as enzymatic degradation of proteins and, to a lesser extent, enzymatic degradation of lipids. In the process of proteolysis, endogenous proteolytic enzymes cause fragmentation of the microstructure of muscle fibres, resulting in improved tenderness of the meat [Franco *et al.*, 2009; Kemp *et al.*, 2010]. During the degradation of myofibrillar proteins, peptides and amino acids are released that act as water-soluble flavour precursors [Koutsidis *et al.*, 2008]. The result is an improvement in the aroma and flavour of the meat, which acquires the desired gastronomic properties, mainly related to the aroma and flavour specific of aged meat [Lee *et al.*, 2021]. The speed and extent of ageing depend on several factors, such as the species, age, diet, and breed of the animal, as well as the type of muscle. The meat of young animals is, in contrast to the meat of older animals, generally considered softer and requires less time to reach the same degree of tenderness. Veal is the meat of cattle up to 8 months old and should be characterised by a tender texture, pale pink colour, high water content, and low-fat content [Government of the Republic of Slovenia Regulation, 2019]. The most important factor determining the acceptability of veal and influencing the consumer's purchase decision is tenderness [Reicks *et al.*, 2011]. Although veal is considered tender meat than beef and is usually not aged, some studies have confirmed the positive influence of ageing on its tenderness, juiciness, and overall acceptability [Baldi *et al.*, 2015; Revilla & Vivar-Quintana, 2006].

Oxidation is one of the main causes of meat deterioration, along with microbial spoilage. Muscle lipids and proteins are susceptible to oxidation as a result of internal and external factors. Meat contains several endogenous initiators of oxidation, such as ferrous heme pigments, transition metal ions, and oxidative enzymes. The ageing of meat can enhance oxidative degradation. Meat is subjected to oxidation of lipids and proteins, resulting in the formation of various oxidation products, including carbonyl groups and lipid oxidation products, such

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as thiobarbituric acid reactive substances (TBARs). Lipid oxidation is often responsible for quality loss *via* rancid flavour development [Domínguez *et al.*, 2019; Popova *et al.*, 2009]. To reduce the unbeneficial effects of oxidation processes on meat quality, the meat industry uses vacuum packaging, which exerts a strong oxidation-inhibiting effect. Bonny *et al.* [2017] showed that TBAR levels were the lowest in vacuum-packed beef samples compared to the modified atmosphere-packed (MAP) samples. Similar results were observed for carbonyl group content, where vacuum packaging presented significantly lower carbonyl content after 10 days than MAP treatments. The effects of lipid oxidation on meat aroma and overall quality have been thoroughly studied, while the effects of protein oxidation are still poorly understood [Lund *et al.*, 2011]. The formation of carbonyl and sulfhydryl groups causes meat to lose functional groups, leading to the formation of intra- and/or inter-protein disulfide cross-links. These factors have a strong influence on the functionality of muscle proteins and, consequently, reduce the water-holding capacity. The chemical changes that occur during protein oxidation are responsible for changes in protein solubility, protein fragmentation, and protein aggregation. Protein oxidation can affect eating quality of meat, including especially its tenderness, juiciness, flavour, and discoloration [Bao & Ertbjerg, 2019].

We are aware that there are many studies on meat ageing, but there are few on the topic of veal ageing and practically none addressing longer ageing periods. Although veal is considered to be more tender than beef, based on promising results of limited similar studies, we believe that ageing will result in additional improvement in its tenderness, aroma, and flavour. Therefore, the objective of the present study was to determine the effects of 21-day ageing of veal on its quality parameters and sensory attributes. Since the oxidation process in meat is related to its quality and consumer acceptance, the extent of lipid and protein oxidation of veal was determined as well.

## MATERIALS AND METHODS

### Experimental design

In the experimental part of the study, twelve calves (Simmental breed) were randomly selected and purchased at a local butcher (Ljubljana, Slovenia). The calves were from Slovenian breeding and rearing and slaughtered on the same day in a commercial slaughterhouse at less than 8 months of age. The average cold carcass weight was 104±8 kg. The procedures of the preslaughter period, slaughter and primary processing of carcasses were conducted according to standard technology [Council Regulation, 2009; Government of the Republic of Slovenia Regulation, 2018]. Carcasses were chilled at 4±1°C for 24 h, and the *longissimus lumborum* (LL) section of the *longissimus thoracis et lumborum* muscle was removed from both the left and the right side of the carcass. The muscles were separated from the bone and divided, perpendicular to the muscle fibres, into four approximately equal parts. Samples were weighed, vacuum-packed in polyvinylidene chloride (PVDC) laminate bags (Cryovac, Elmwood Park, NJ, USA), and randomly selected for different ageing periods (7, 14, and 21 days *postmortem*, *pm*). Samples in group 1 (24 h *pm*) were immediately analysed for meat quality parameters.

The ageing period was selected based on the results of sensory analysis of the preliminary study, which included four calves (Simmental breed) from Slovenian breeding and rearing. The experimental design was similar to that of the main experiment, with the only difference being the duration of ageing. After 28 days of ageing, the panelists evaluating sensory attributes of meat perceived its off-aroma and off-flavour (aroma/flavour associated with meat at the end of shelf life), thus the ageing period in the main experiment was 21 days instead of 28 days.

Ageing took place in a cooling chamber at a constant temperature of 2±1°C. After each ageing period, one part of the sample was immediately analysed for meat quality parameters, and another part of the sample was vacuum-packed and frozen at −21±1°C until contents of protein carbonyl and TBARs were determined. Before analysis, the meat samples were thawed at 4±1°C for 24 h.

### Analysis of meat quality parameters

After each ageing period, weep loss (expressed as a percentage of excreted meat juice during ageing) was determined. Then, a 3–4 cm thick slice with core temperature of 2±1°C was cut from the corresponding sample, in which the pH value was measured directly using a combined glass-gel spear electrode (Type 03, Testo Pty Ltd, Croydon South, Australia) with a thermometer (Type T, Testo Pty Ltd) connected to a pH metre (Testo 230, Testo Pty Ltd). The reading accuracy was 0.01 pH units.

Colour parameters of the meat samples were measured using a CR-400 colorimeter (Konica Minolta Optics, Inc., Osaka, Japan) with standard illuminant C and 0° viewing angle. The CIE $L^*$ (lightness), $a^*$ (redness), and $b^*$ (yellowness) values were determined at four different points on the freshly-cut surface after 30 min of bloom time at 4±1°C. The surface of meat sample was drained of excreted meat juice before measurement. The remaining piece of the raw sample was weighed, and colour and marbling were visually evaluated by a sensory panel.

The samples were then vacuum-packed and heat-treated in a combi oven (Rational FRIMA SCC61, Landsberg am Lech, Germany) at 75°C for 1 h using the *sous-vide* (vacuum cooking) method. After the heat treatment, cooking loss was determined and expressed as a percentage of excreted meat juice during heat treatment. The remaining sensory properties (juiciness, tenderness, aroma and flavour) were evaluated on the warm part of the sample.

To evaluate the sensory qualities, a panel of six panellists qualified and experienced in the field of meat products was appointed. The panel was trained in sensory profiling of veal from the Slovenian market in five training sessions. In the first session, the panellists were trained on samples of unaged veal (24 h *pm*). In the four following sessions, samples of aged veal (with different ageing times: 7, 14, 21, and 28 days) were served. The panellists evaluated the sensory characteristics of the veal and agreed on descriptors and their definitions. The sensory evaluation of veal was conducted under defined, precisely prescribed, controlled

and reproducible operating conditions. These included: arrangement of laboratory, samples, accessories and organization of assessment [ISO, 2007; ISO, 2012]. Assessment of the coded samples took place in a standard sensory laboratory. Regarding sensory evaluation, the thermally-treated samples with discarded edges were cut into 1 $cm^3$ cubes, and two cubes per panellist were wrapped in aluminium foil and randomly presented to the panellists for evaluation. To neutralise the taste, the panel used the middle dough of white bread and water.

The analytical-descriptive test [Gašperlin *et al.*, 2014] was carried out by scoring the sensory attributes according to a non-structured scale from 1 to 7 points, whereby a higher score indicated a stronger expression of a particular attribute. The definitions of the descriptors are listed in Table 1.

After each ageing period, marbling and colour were visually assessed on raw samples. After thermal treatment, the aroma and flavour of the samples were assessed immediately after un-wrapping the aluminium foil. The juiciness and tenderness were assessed by tasting the samples. The samples were served and scored in four sessions (after each ageing period), and their attributes were recorded individually.

The Warner-Bratzler shear force (WBSF) was measured according to the procedure of Campo *et al.* [2000], with some modifications. The analysis was performed using the TAXT plus texture analyser (Stable Micro Systems Ltd., Godalming, United Kingdom) with a 50 kg load cell. The shear force (N) was measured perpendicular to the muscle slices (10×10×40 mm) cooled to room temperature (20±1°C) in four parallels as the resistance of the sample to cutting by blade set consists of guillotine edge and a Warner-Bratzler blade (HDP/BS) with the operating conditions: blade speed 50 mm/min, penetration into the muscle 10.5 mm.

### TBAR and protein carbonyl content determination

The extent of lipid oxidation of veal samples was monitored by measuring TBAR content as described by Penko *et al.* [2015]. Briefly, to 0.100±0.001 g of a homogenised sample, 1 mL of 35% trichloroacetic acid, and 2 mL of 0.36% thiobarbituric acid (in 0.1 M $Na_2SO_3$) were added and mixed intensively for 5 min on an orbital shaker (KS260, IKA, Staufen, Germany), followed by the addition of 1 mL of 0.9% butylated hydroxytoluene in *n*-hexane. The samples were heated at 90°C for 30 min in a thermoblock (VLM EC1, Bielefeld, Germany). After cooling to room temperature, 1 mL of trichloroacetic acid and 2 of mL chloroform were added and then centrifuged at 1,500×*g*, 6 min (5810 centrifuge, Eppendorf, Darmstadt, Germany). After centrifugation, the absorbance in the supernatant was measured at 532 nm with a Cary 8454 UV-Vis spectrophotometer (Agilent Technologies, Waldbronn, Germany). 1,1,3,3-Tetraethoxypropane was used as a standard for the determination of TBARs. Results were expressed in mg of malondialdehyde equivalents per kg of meat.

A modified spectrophotometric method of Soglia *et al.* [2016] was used to determine protein carbonyl content of meat. In brief, 1,000 g of muscle sample was homogenised in 10 mL of ice-cold 0.15 M KCl solution. To 100 µL of the homogenate, 1 mL of 10% trichloroacetic acid was added. A blank was prepared by adding 1 mL of 10% trichloroacetic acid to 100 µL of distilled water. After centrifugation at 4,000×*g* for 6 min (Eppendorf 5810 centrifuge), the supernatant was removed, and 400 µL sodium dodecyl sulfate was added to the residue. After heating (10 min, 90°C) and sonication (30 min, 40°C) (3510 ultrasonic cleaner, Branson, Danbury, CT, USA), the samples were treated with 0.8 mL of a 0.3% (*w/v*) solution of 2,4-dinitrophenylhydrazine (DNPH) in 3 M HCl. To the blank, 0.8 mL of 3 M HCl was added. After incubation (30 min), 400 µL of 40% trichloroacetic acid was added and centrifuged (4,000×*g*, 6 min). The supernatant was removed, and 1 mL ethanol-ethyl acetate mixture (1:1, *v:v*) was added to the residue. After centrifugation (4,000×*g*, 10 min), the supernatant was again removed. This procedure was repeated three times. To the dried precipitate, 1.5 mL of 6 M guanidine hydrochloride in 20 mM sodium phosphate buffer (pH 6.5) was added. After overnight incubation (4°C), the samples were filtered by 0.45 µm Phenex regenerated cellulose (RC) syringe filters (Phenomenex, Torrance, CA, USA), and carbonyl groups were detected, by reactivity of carbonyl groups with DNPH to form protein-bound 2,4-dinitrophenylhydrazones, at wavelengths of 370 nm and 280 nm with a Cary 8454 UV-Vis spectrophotometer (Agilent Technologies). The protein carbonyl

**Table 1.** Definitions of descriptors for the sensory evaluation of veal.

| Descriptor | Definition | Scale |
|---|---|---|
| Colour | Colour typical for veal (visual evaluation) | 1 – too light<br>4 – pale pink (optimal)<br>7 – too dark |
| Marbling | The proportion of intramuscular fat (visual evaluation) | 1 – absence of marbling<br>7 – extreme marbling |
| Juiciness | The degree of juiciness perceived after the first three chews between the molar teeth | 1 – extremely dry<br>7 – extremely juicy |
| Tenderness | Perception of tenderness after the first three chews between the molar teeth | 1 – extremely tough<br>7 – extremely tender |
| Aroma | Aroma associated with cooked veal loin | 1 – absence of veal aroma<br>7 – fully expressed veal aroma |
| Flavour | Flavour associated with cooked veal loin | 1 – absence of veal flavour<br>7 – fully expressed veal flavour |

content, expressed as nmol/mg of protein, was calculated according to the following equation:

$$\text{Content of protein carbonyls} = \frac{A_{370}}{22{,}000 \times [A_{280} - (A_{370} \times 0.43)]} \times 10^6 \quad (1)$$

where: 22,000 – absorption coefficient of protein-bound 2,4-dinitrophenylhydrazones at 370 nm; $A_{370}$ – absorbance at 370 nm; $A_{280}$ – absorbance at 280 nm; and 0.43 – correction factor.

### Statistical analysis

To determine the effects of variability and the characteristics of its influence on some physical, chemical, and instrumental parameters of veal, the univariate UNIANOVA test (IBM SPSS Statistics version 22.0, Chicago, IL, USA) was used, where the model included fixed effects of ageing time ($A_i$; 1, 7, 14, 21 days) and repetition (animal) ($R_j$; 1–12). The model was represented as equation (2).

$$y_{ijk} = \mu + Ai + Rj + e_{ijk} \quad (2)$$

where: $y$ was the observed parameter, μ – the general mean, and $e$ – a residual random term with variance $\sigma_e^2$. The effect of calf sex was not observed. The Shapiro-Wilk test for the sensory parameters did not show a normal distribution of the data ($p<0.05$), so the Kruskal-Wallis test was used to evaluate the influence of ageing time on the above traits. Since this non-parametric test does not allow us to evaluate the differences between ageing days, the UNIANOVA test was also performed and was upgraded with a post-hoc multiple comparison least significant difference (LSD) test. Sensory trait values were compared at each day with a significance level of 0.05.

## RESULTS AND DISCUSSION

The results referring to weep loss during ageing, cooking loss during heat treatment, pH values, instrumentally-measured colour parameters and texture are summarised in Table 2. The pH values of the veal samples varied from 5.66 (24 h *pm*) to 5.72 (after 21 days). The pH value measured after 24 h *pm* indicated normal muscle quality. The pH values in the present study are similar to those reported by other authors [Revilla & Vivar-Quintana, 2006].

During the 21 days, the veal samples released a noticeable amount of meat juice (Table 2). Weep loss during ageing is caused by denaturation of muscle proteins and decreased ability to bind water [Wiklund *et al.*, 2010]. The highest percentage weep loss was observed after 21 days (6.97%), but without statistical differences ($p>0.05$) compared to day 7 (1.17%) and day 14 (3.43%). One of the possible explanations is that vacuum packaging protects against high water losses. Shi *et al.* [2020], who investigated different packaging methods, reported that the lowest weep losses occurred in wet-aged beef samples due to vacuum packing. Vacuum packaging inhibited evaporation of water, so weep losses decreased. In contrast, dry-aged beef samples had the highest weep losses compared to beef samples aged under various moisture-permeable packages [Laster *et al.*, 2008].

The ageing process affected the cooking loss ($p<0.001$) of veal during heat treatment (Table 2). The highest cooking loss was determined for meat aged for 14 days (22.63%) and similarly maintained at 21 days *pm* (21.78%). Mungure *et al.* [2016] found a significant increase in cooking loss in beef samples after 21 days of ageing (37.51%) compared to 3-day aged beef. The mentioned value was higher than the cooking loss observed in our experiment at day 21. Weight loss during heat treatment depends on the method, temperature and duration of heat treatment, so comparison with other studies is difficult. Longer heat treatment and higher temperature affect the increase in cooking loss, which could be due to increased myosin denaturation and possible weakening of myofibril structure [Zielbauer *et al.*, 2016].

Colour plays an important role in the appearance, presentation, and acceptability of veal [Klont *et al.*, 2000]. Colour parameters of meat 24 h *pm* in our study are shown in Table 2. The values of $L^*$, $a^*$ and $b^*$ were similar to those reported by Hulsegge *et al.* [2001], who performed measurements on *rectus abdominis* muscle. Instrumental colour measurements on the *rectus abdominis* and *pectoralis superficialis* muscle are

**Table 2.** pH value, weep and cooking losses, as well as instrumentally-measured colour parameters and texture of veal after different ageing periods.

| Parameter | | Ageing period (day) | | | | $p_A$ | $p_R$ | SE | Mean across days *pm* |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 | | | | |
| pH | | 5.66$^b$ | 5.71$^a$ | 5.65$^b$ | 5.72$^a$ | 0.065 | 0.653 | 0.05 | 5.79 |
| Weep loss (%) | | 0.0 | 1.17 | 3.43 | 6.97 | 0.153 | - | 1.3 | 2.9 |
| Cooking loss (%) | | 16.02$^b$ | 17.40$^b$ | 22.63$^a$ | 21.78$^a$ | <0.001 | - | 2.8 | 19.5 |
| Colour parameter | $L^*$ | 48.36 | 49.93 | 50.30 | 49.12 | 0.318 | 0.001 | 3.33 | 49.43 |
| | a* | 11.79$^b$ | 13.32$^a$ | 12.41$^{ab}$ | 13.40$^a$ | 0.054 | <0.001 | 2.67 | 12.79 |
| | b* | 2.80$^c$ | 5.60$^a$ | 3.29$^{bc}$ | 3.77$^b$ | <0.001 | 0.025 | 1.12 | 3.86 |
| Texture parameter, WBSF (N) | | 84.61$^a$ | 53.48$^b$ | 63.47$^b$ | 56.79$^b$ | <0.001 | 0.501 | 24.14 | 64.59 |

*pm*, *postmortem*; WBSF, Warner-Bratzler shear force; $p_A$, statistical probability of ageing effect; $p_R$, statistical probability of repetition (animal) effect; SE, standard error of mean. Data with different superscript letters within a row (differences between days of ageing) differ significantly ($p\leq0.05$).

recommended for objective evaluation of veal colour [Horcada *et al.*, 2013]. For the results obtained after different ageing period (Table 2), there were no significant differences ($p>0.05$) in the $L^*$ values, while significant differences were in parameter $b^*$ with a maximum value observed after 7 days of ageing (5.60, $p<0.001$). Differences observed in parameter $a^*$ were mainly due to the repetition (animal) effect. A possible explanation for increase in parameter $b^*$ is in oxidation of myoglobin to metmyoglobin as a result of dissolved oxygen in the meat [Henriott *et al.*, 2020]. An increase in brightness may have a positive effect on the appearance of veal, while an increase in redness may be a negative factor [Baldi *et al.*, 2015]. Similarly to our results, Vieira *et al.* [2006] found no significant effect of a 14-day ageing period on the $a^*$ values of beef *longissimus thoracis* muscle. In contrast, Florek *et al.* [2015] found significantly higher values of $L^*$, $a^*$, and $b^*$ parameters in LL muscle after 12 days of ageing, regardless of the calf slaughter age. Similar findings were observed by Vitale *et al.* [2014], who reported higher baseline values of $L^*$, $a^*$ and $b^*$ in aged beef (3, 6, 8, 14, and 21 days) than in unaged beef. These results are consistent with Bruce *et al.* [2004], who demonstrated an increase in $L^*$, $a^*$, and $b^*$ values of *longissimus toracis* for 14 days of vacuum-packaged meat compared to unaged meat (24 h *pm*). The differences in $L^*$, $a^*$, and $b^*$ values between aged and unaged meat could be explained by a higher blooming ability of vacuum-aged meat [Oliete *et al.*, 2006].

Ageing had a significant ($p<0.001$) effect on instrumentally-measured texture of heat-treated meat (Table 2). After 21 days of ageing, the WBSF decreased by approximately 33% (from 84.61 N to 56.79 N), and the lowest value was observed after 7 days *pm* (53.48 N), without significant differences ($p>0.05$) compared to day 14 and day 21. A slight increase was observed after 14 days, which could be related to a higher cooking loss. At this point, it should be noted that the values of measured WBSF obtained in our study are very difficult to compare with other studies, because different blade sets (*e.g.*, standard blade, "V" slot blade, rectangular slot blade) and different dimensions and shapes (rectangular or oval) of the sample were used for force measurement. For this reason, comparison with other studies is only possible in relative terms. In this case, our results are in agreement with the results of the study by Baldi *et al.* [2015], who reported a decrease in WBSF for *longissimus dorsi* muscle of veal within 8 days, without further improvement in tenderness. Also similarly to our findings, Franco *et al.* [2009] found that beef aged for 21 days did not differ in WBSF from beef aged for 8 or 14 days. Another difference between our study and some other studies was in the storage of the aged veal samples. In our case, we performed the WBSF measurements immediately after each ageing period. Some other authors reported that the veal samples were frozen before measurements [Baldi *et al.*, 2015; Campo *et al.*, 2000; Revilla *et al.*, 2006]. The data in the literature indicate a positive effect of freezing on the tenderness of the meat, attributed to mechanical damage of the myofibrillar structure of the meat by the ice crystals [Cho *et al.*, 2017; Dang *et al.*, 2021; Lagerstedt *et al.*, 2008; Vieira *et al.*, 2006].

The ageing period affected several sensory attributes of veal (Table 3). After 21 days, the juiciness (by 0.46 points), tenderness (by 1.33 points), aroma (by 0.32 points), and flavour (by 0.41 points) of veal improved significantly ($p\leq0.05$). Similar results were obtained in a preliminary study, in which tenderness, aroma, and flavour improved (increase in scores) up to 21 days; later, at day 28, no further improvement in tenderness, but off-aroma and off-flavour were noted (data not shown). Kaniou *et al.* [2001] reported an increase in the content of biogenic amines during the ageing of vacuum-packed beef. The highest levels of putrescine and cadaverine were found after 26 days. High levels of putrescine and cadaverine are known to enhance histamine or tyramine toxicity. This is another reason why we decided to use a 21-day ageing period.

The most important quality characteristic of meat is tenderness, a highly variable attribute that depends on many intrinsic and extrinsic factors and their interactions. According to the Government of Republic of Slovenia Regulation [2019], calves are slaughtered at less than 8 months of age; consequently, consumers expect tender meat. In our study, tenderness was rated at 6.51 points (Table 3), which is almost the maximum value for this attribute. Both, sensorial and instrumental methods confirmed improvement in tenderness of veal, although some differences between methods were observed. The lowest WBSF

**Table 3.** Sensory attributes of veal after different ageing periods.

| Sensory attribute | Ageing period (day) | | | | $X^2$ | $p_A$ (Kruskal Wallis test) | SE | Mean across days *pm* |
|---|---|---|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | | | | |
| Colour (1–4–7) | 4.08$^a$ | 4.08$^a$ | 3.69$^b$ | 3.82$^b$ | 15.90 | 0.001 | 0.47 | 3.9 |
| Marbling (1–7) | 1.65$^c$ | 1.65$^c$ | 1.85$^b$ | 2.06$^a$ | 8.31 | 0.040 | 0.82 | 1.8 |
| Juiciness (1–7) | 5.43$^b$ | 5.58$^b$ | 5.53$^b$ | 5.89$^a$ | 22.68 | <0.001 | 0.40 | 5.6 |
| Tenderness (1–7) | 5.18$^d$ | 5.71$^c$ | 6.18$^b$ | 6.51$^a$ | 89.50 | <0.001 | 0.42 | 5.9 |
| Aroma (1–7) | 6.00$^c$ | 6.00$^c$ | 6.13$^b$ | 6.32$^a$ | 53.20 | <0.001 | 0.17 | 6.1 |
| Flavour (1–7) | 6.08$^c$ | 6.15$^c$ | 6.28$^b$ | 6.49$^a$ | 47.95 | <0.001 | 0.22 | 6.3 |

*pm*, *postmortem*, (1–7); scale from 1 to 7 points (a higher score indicates a stronger expression of a particular attribute); $p_A$, statistical probability of ageing effect; SE, standard error of mean. Data with different superscript letters within a row (differences between days of ageing) differ significantly ($p\leq0.05$, least significant difference test).

was measured on day 7, while from a sensory point of view, the highest score for tenderness was achieved after 21 days of ageing. The reason for this deviation may be in the sensory perception of tenderness, which is indirectly related to juiciness [Choe *et al.*, 2016]. This coincides with the increase in juiciness (the highest value for juiciness was observed after 21 days of ageing). The increase in juiciness is the result of the activation of calpains during *postmortem* aging period, which influences the increase in the amount of free water in meat [Jaspal *et al.*, 2021]. Sensory-rated colour scores for the fresh cut veal sample decreased significantly during ageing, from 4.08 on the first day to 3.69 after 14 days (Table 3). The colour was described as brighter, which is desirable for veal and in accordance with an increase in parameter *b** (yellowness) ($p<0.001$). Marbling varied from 1.65 points to 2.06 points ($p=0.040$), the differences were minimal and probably due to the influence of the individual animal in the experiment. It is difficult to compare our results directly with other studies because no study has been conducted with the same ageing conditions. Revilla & Vivar-Quintana [2006] reported that the quality of veal improved during ageing; tenderness, colour, and odour were the main attributes affected by ageing, and a 7-day ageing period seemed to be sufficient to obtain veal of high quality. Baldi *et al.* [2015] found that during a 4-day ageing period, the tenderness and juiciness of *longissimus dorsi* muscle significantly improved ($p<0.001$), while ageing had no effect on aroma and flavour.

The results regarding the development of oxidation in vacuum-packed veal samples are presented in Table 4. The ageing process had no effect on TBAR formation ($p=0.589$), indicating that the selected ageing conditions were appropriate. Campo *et al.* [2006] related TBAR values to the sensory attributes of beef. Higher TBAR values indicate a higher degree of oxidation, but not always a change in sensory attributes [Penko *et al.*, 2015]. The cut-off point for consumer detection of rancidity is between 1.0 and 3.0 mg malondialdehyde/kg, according to Campo *et al.* [2006]. In the current study, TBAR values ranged from 0.24 to 0.27 mg malondialdehyde/kg, well below the critical level at which rancidity is detected; hence, the sensory attributes of veal were not affected. One of the possible explanations for the minimal formation of TBARs in our experiment could be in the vacuum packaging of the samples. Both lipid and protein oxidation increased during storage due to the high oxygen content in the packaging atmosphere [Lund *et al.*, 2007]. Clausen *et al.* [2009] reported very low TBAR values for vacuum-stored samples (23 days *pm*), while a sharp increase in lipid oxidation was observed for samples stored 6 days after vacuum packaging in MAP. In contrast, Popova *et al.* [2009] reported that 14 days of vacuum storage affected lipid oxidation in beef samples and it was higher than that in nonvacuum-packed meat, with a significant difference observed in the content of TBARs on the first and sixth day of storage. According to Insausti *et al.* [2021], lipid oxidation is directly related to the formation of metmyoglobin during meat display; meat with a higher pigment content is more oxidized and therefore less stable in colour. Veal is considered a younger animal meat and has less available myoglobin for the oxidation process.

The total amount of protein carbonyls in the veal samples increased significantly during ageing (3.98 nmol/mg protein at day 1 *vs.* 6.13 nmol/mg protein at day 21) (Table 4). The highest increase was observed after 14 days of ageing (6.22 nmol/mg protein). Popova *et al.* [2009] reported that a significantly higher amount of carbonyls was formed (20 nmol/mg proteins) after 6 days of ageing of beef in a vacuum package at a temperature of 4°C compared to our results. According to the authors, during a 10-day storage of bovine LL muscle and *diaphragma pedialis*, the total protein carbonyl content increased from 3.1 to 5.1 nmol/mg protein and from 4.8 to 6.9 nmol/mg protein. Large content variability could be explained by differing ageing conditions, especially temperature, muscle type, packaging method, and duration [Estévez, 2011]. Oxidation of myofibrillar proteins during storage can have a significant effect on muscle condition. Under highly oxidative conditions, intermolecular cross-links can be formed, affecting proteins that are less susceptible to enzymatic proteolysis and reducing the development of tenderness due to proteolysis in meat [Davies, 2005]. In our study, a relationship between meat tenderness and the protein oxidation process was not found; in contrast, meat tenderness improved during ageing. The selected ageing conditions in our experiment were suitable to obtain the protein carbonyl content at a low level. The process of protein oxidation during storage of meat in the refrigerator is probably slower than the process of oxidation under conditions where radicals are formed more rapidly, such as heat treatment, freezing and thawing, irradiation and packaging of meat in an oxygen-rich atmosphere. This is one of the current research areas that can help us better understand the formation of protein carbonyls related to oxidative changes in meat [Estévez *et al.*, 2019].

## CONCLUSIONS

According to the current situation of veal on the Slovenian market and promising results of limited studies considered ageing

**Table 4.** Oxidation parameters of veal after different ageing periods.

| Parameter | Ageing period (day) | | | | $p_A$ | $p_R$ | SE | Mean across days *pm* |
|---|---|---|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | | | | |
| TBARs (mg malondialdehyde/kg) | 0.24 | 0.23 | 0.23 | 0.27 | 0.589 | 0.630 | 0.05 | 0.24 |
| Protein carbonyls (nmol/mg proteins) | 3.98$^b$ | 4.03$^b$ | 6.22$^a$ | 6.13$^a$ | <0.001 | 0.436 | 0.99 | 5.09 |

*pm*, *postmortem*; TBARs, thiobarbituric acid reactive substances; $p_A$, statistical probability of ageing effect; $p_R$, statistical probability of repetition (animal) effect; SE, standard error of mean. Data with different superscript letters within a row (differences between days of ageing) differ significantly ($p\leq0.05$).

as a routine processing of veal to improve meat quality and to find the best compromise between the improvement in sensory properties and minimising loss of colour, lipid, and protein stability, the results obtained in the present study fully confirm that 21-day *pm* ageing under vacuum conditions at a temperature of 2±1°C reflects improvements of some sensory characteristics, especially tenderness, juiciness, aroma, and flavour. The intensity of lipid oxidation was low, as the content of TBARs during ageing remained below the threshold for the detection of rancid odour. In contrast, the duration of ageing affected the formation of protein carbonyls as their content increased with time without compromising meat quality. These results are promising for the use of veal ageing in the meat industry and gastronomy.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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