Research Article

The effect of packaging method and dietary vitamin D3 supplementation on the quality of beef in rectus femoris, gluteus medius, and adductor femoris beef muscles

Aleksandra LIPIŃSKA*, Andrzej PÓŁTORAK, Agnieszka WIERZBICKA, Jarosław WYRWISZ

Department of Human Nutrition and Consumer Sciences, Division of Engineering in Nutrition,
Warsaw University of Life Sciences, 02-767 Warsaw, Poland

Abstract: The goal of the study was to assess the impact of packaging methods and doses of vitamin D_3 fed to animals (with feed) in the last days of finishing on selected indicators of beef quality. The parameters studied include the measurement of CIE L*, a*, and b* colour spaces and the Warner–Bratzler shear force. The muscles used in the study were the *m. rectus femoris*, *m. gluteus medius*, and *m. adductor femoris*, which were packed in two variants of modified atmosphere (MAP1, 80% O_2:20% CO_2 and MAP2, 60% O_2:40% CO_2) and in skin-pack technology; they were later aged for 10 and 14 days. Before slaughter, the animals were fed a vitamin D_3 supplemented diet in the following doses: 0, 3.5, 7, and 10 M IU day^{-1} animal^{-1}. The study proved that vitamin D_3 dosages of 3.5 and 10 M IU significantly (P ≤ 0.05) increased tenderness of unpackaged beef. Packaging and modified atmosphere resulted in a significant increase of parameters L*, a*, and b*, while skin packing lowered the values. Vitamin D_3 supplementation had a negative impact on selected beef colour spaces by lowering them.

Key words: Beef, colour, modified atmosphere storage, tenderness, vacuum packaging, vitamin D_3

1. Introduction

One of the most important factors in beef quality and a determinant of consumer purchase decisions is colour. Red colour is associated with the freshness and wholesomeness of beef, which is why even minor changes in colour may result in losses in the meat sector (1).

Moreover, the colour of beef depends on the period of beef ageing, type of muscles analysed, and animal sex. The factor playing a key role in the stabilisation of meat is the packaging system. One of the systems used for packaging fresh beef is modified atmosphere packaging (MAP), which mixes in a high oxygen concentration. It results in the development of an intense colour in the beef. Vacuum packaging has a negative impact on red colour retention in beef, which is why it is the most poorly rated by consumers (2). Yet vacuum packaging combined with cold storage is currently the most efficient method of extending meat sell-by dates due to the tiny amounts of oxygen in the packaging (3). The colour of beef is also influenced by the feed composition and supplementation with vitamins and minerals. The following vitamins are used to improve the colour of fresh beef: E, A, C, and D_3 (4).

An important indicator of beef quality, besides colour, is its tenderness as it significantly impacts consumer

appreciation of the taste (5). In recent years, scientists have attempted to devise a method to improve tenderness before slaughter. One of the ways of achieving this is to feed animals a diet containing vitamin D_3 supplemented feed. Vitamin D_3 serves to mobilise the calcium ions that stimulate the activation of calpain enzymes, mostly μ-calpain and m-calpain. Degradation of enzymes, which in the case of muscles, are found in cytoplasm, results in the weakening of muscle fibre structure, which improves the textural properties of beef (6).

This work presents the results of an analysis aimed at determining the impact of modified atmosphere and skin packing on selected quality indicators of beef. The research also covered the impact of a D_3 supplementation regimen on selected parameters to determine optimum dosage and timing of the feeding so that it decreases the hardness of beef and improving its tenderness.

2. Materials and methods

2.1. Animals and feeding strategy

The study material consisted of 48 crossbreeds between Holstein–Friesian (HF) and Limousin, which were slaughtered in the 18th and 19th months of life. The bulls reached a final weight of 680 ± 20 kg. The animals were

* Correspondence: aleksandra_lipinska@sggw.pl

divided into four study groups corresponding to the different vitamin D_3 supplementation regimes: 0, 3.5, 7, and 10 M IU day^{-1} animal^{-1}. The animals from each group were kept in separate stalls. The bulls were divided into 4 groups according to the D_3 supplementation type in the diet (Table 1).

The feed composition is presented in Table 2. Vitamin D_3 was fed as a dry powder stabilised with DL-α-tocopherol (Merck Millipore, Darmstadt, Germany). The appropriate dose of vitamin D_3 was dissolved in water and later mixed with 0.5 kg of ground oats. The mix, prepared as described above, was fed to animals daily before the morning feed.

The animals were slaughtered after 10 days of feeding appropriate dosages of vitamin D_3. The supplementation was delivered in January, at the time of lowest light intensity and minimal exposure to the sun.

2.2. Sampling and sample packaging

Sampling for testing purposes was conducted in the 48 h after slaughter. Three muscles from each test animal were taken for comparison: *m. adductor femoris* (ADD), *m. rectus femoris* (REF), and *m. gluteus medius* (GLU). The isolated muscles were cut into 20-mm steaks that were later packaged in two varieties of modified atmosphere: MAP 1 (80% O_2:20% CO_2) and MAP 2 (60% O_2:40% CO_2) and vacuum skin packing (VSP).

A Cryovac VS26 packager (Sealed Air Corporation, New Jersey, USA) was used for the vacuum skin packing of meat. The packaging and sealing of the film was performed at 200 °C. The beef was packed on PET/PE support trays (135 × 250 × 20 mm, Cryovac RSB03X56; Sealed Air Corporation). The film used was 100 μm polyethylene with a maximum O_2 transmission rate of 2 cm^3/m^2/24 h/bar at 23 °C and relative humidity of 0% (Cryovac TS201; Sealed Air Corporation).

For modified atmosphere packaging, an M3 packaging machine (Sealpack, Oldenburg, Germany) was used. The meat was packaged on PET/PE film trays (187 × 137 × 50

mm; Pomona Company, Żyrardów, Poland), and the foil used was 44 μm PET/CPP+AF laminate with a maximum O_2 transmission rate not exceeding 10 cm^3/m^2/24 h/bar at 23 °C and relative humidity of 0% (EC04; Corenso, Helsinki, Finland).

The packed muscles were stored cold (refrigerated) for 10 (10d) and 14 (14d) days at 2 °C (±1 °C). The control group was fresh unpackaged meat without D_3 supplementation (CONTROL).

2.3. CIE L*, a*, and b* evaluation

The measurement of CIE colour spaces, L* (light reflection), a* (redness), and b* (yellowness), was performed on the surface of the steaks made from each muscle included in the study immediately after opening the packaging. The measurements were performed with a colour measuring instrument (Minolta CR400; Konica Minolta Sensing Inc., Osaka, Japan) with the following parameters: observation, angle 10°, 8-mm aperture size. The device used for testing the samples had previously been properly calibrated with a calibration plate (Y: 93.5; x: 0.3162; y: 0.3330). The final result was the arithmetic mean of 10 measurements performed on the surface of every steak.

2.4. Warner–Bratzler shear force (WBSF)

The shear force test was performed with the use of a Warner–Bratzler shear device in a Universal Testing Machine (Instron 5965; Instron Corp., Norwood, USA). Samples weighing approximately 150 g were heat-treated in a bath (WNE 14; Memmert, Schwabach, Germany) until the temperature at their geometrical centre reached 74 °C, and later chilled to 2 °C (±1 °C). Samples in the form of 6 cylinders with a diameter of 8 mm were cut from the cooked steaks parallel to the muscle fibres. Thus, the selected samples were cut perpendicular to the muscle fibres.

2.5. Statistical analysis

The results obtained were subjected to statistical analysis with Statistica 10.0 and IBM SPSS 21 software. The impact of the selected packaging systems and effect of various doses of vitamin D_3 on component colours L*, a*, and b* and shear force (WBSF) were tested with one-way

Table 1. Supplementation parameters.

Diet parameters	Experimental groups			
	I*	II*	III*	IV*
D_3 dose [a]	0.0	3.5	7	10
Supplementation time [b]	0	6	3	3
Duration of feeding without supplementation [b]	10	4	7	7
Total feeding time [b]	10	10	10	10

*Experimental groups, where I is the control group;
[a]expressed in M IU day^{-1} animal^{-1};
[b]expressed in days.

Table 2. Diet components in the final period of feeding.

Ingredient	Percent
Ground oats	70.0
Rye silage	20.0
Beet decoction	7.0
Fodder chalk	2.2
Primasan	0.3
Vitamin D_3	0.00035–0.001

ANOVA. Homogeneous groups of levels of the factor tested were defined with Tukey's HSD test at the level of confidence P ≤ 0.05.

3. Results

It was determined through one-way variance analysis (P ≤ 0.05) that the L* colour component differed significantly depending on the dose of vitamin D_3 and the packaging system applied, as presented in Table 3.

In all muscles studied, the L* parameters of meat packed in MAP1d10 and MAP1d14 and MAP2d10 and MAP2d14 were higher than in the control group (CONTROL). The increase in the parameter was statistically significant. In turn, the meat packed in VSPd10 and VSPd14 had lower L* values, as compared to the control batch (CONTROL). As these differences were not statistically significant in all the muscles in question, the impact of vacuum packing on lowering the L* value cannot be proved. Lower values of the L* parameter, compared to MAP1- and MAP2-packed beef, were observed in all VSP-packed meat. A statistically significant lowering of the L* value was present on the 10th and 14th days of ageing, independent of the supplementation applied. In the case of the REF

muscle, a statistically significant drop in L* value was demonstrated in the case of MAP1- and MAP2-packed meat on the 10th and 14th days of ageing (MAP1d10, MAP1d14, MAP2d10, and MAP2d14) as compared to the control batch (CONTROL) in the case of 3.5 and 10 M IU vitamin D_3 dosage. In the case of the ADD muscle, a statistically significant drop in the L* value was observed in meat packed in MAP1d10 and MAP1d14 with vitamin D_3 supplementation of 10 M IU, as compared to control (CONTROL). Characteristic of the REF muscle were the highest values of L* parameter, compared to other muscles and independent of packing system and vitamin D_3 dose applied.

The values of a* depended on the packaging system applied and the dose of vitamin D_3 (Table 4).

In the REF and GLU muscles, the value of the a* parameter increased in meat packed in MAP1 on the 10th day of storage, as compared to the control batch (CONTROL). These differences were statistically significant for vitamin D_3 supplemented meat. On the 14th day of ageing in MAP1-packed meat, the value of a* parameter dropped, as compared to the beef packed in the same system and aged for 10 days. The differences were

Table 3. Mean values of the L* colour indicator broken down by packaging systems and days from slaughter vs. vitamin D_3 dose, with homogeneous groups denoted in rows and columns of the study performed and a confidence level of P ≤ 0.05.

Vit. D_3[†]	Packaging system and ageing times							
	Control	MAP1 d10	MAP2 d10	VSP d10	MAP1 d14	MAP2 d14	VSP d14	SEM
M. rectus femoris								
0	41.8[bcB]	44.1[dB]	46.5[dC]	39.8[abA]	46.9[dB]	46.9[dC]	37.9[aA]	0.8
3.5	41.2[aAB]	42.0[bcA]	41.4[bcA]	40.9[abA]	43.4[cA]	41.8[bA]	39.9[aB]	0.4
7	40.3[aA]	40.8[aA]	44.7[bBC]	39.1[aA]	44.4[bA]	45.0[bBC]	40.2[aB]	0.5
10	40.7[aAB]	44.3[bB]	44.3[bB]	39.0[aA]	43.2[bA]	44.1[bB]	39.4[aAB]	0.5
M. gluteus medius								
0	35.7[aA]	40.6[cdB]	38.5[bA]	35.4[aA]	42.0[dC]	39.4[bcA]	35.2[aA]	0.6
3.5	36.5[abA]	40.2[cAB]	39.4[cA]	35.3[aA]	40.3[cB]	39.3[cA]	35.1[bA]	0.3
7	35.8[aA]	39.1[bA]	40.0[bA]	35.3[aA]	40.1[bB]	39.4[bA]	35.3[aA]	0.3
10	36.1[aA]	39.3[bAB]	39.2[bA]	36.0[aA]	38.7[bA]	38.0[bA]	35.5[aA]	0.4
M. adductor femoris								
0	34.9[aA]	43.9[dC]	43.4[cdC]	37.4[abB]	44.0[cdB]	40.6[bcB]	37.0[bcC]	0.8
3.5	38.5[bC]	38.6[bA]	41.4[cBC]	35.4[aA]	40.9[cAB]	39.8[bcAB]	35.5[aA]	0.4
7	39.8[cdeC]	40.7[deB]	38.3[bcA]	37.3[aB]	41.5[eAB]	38.8[bcdA]	36.1[aAB]	0.5
10	37.7[aB]	40.3[cAB]	39.4[bcAB]	37.3[aB]	39.7[bcA]	40.8[cB]	37.0[aB]	0.5

[†]Doses of vitamin D_3 were expressed in M IU day^{-1} animal^{-1}; MAP1 = modified atmosphere packaging, 80% O_2 + 20% CO_2; MAP2 = modified atmosphere packaging, 60% O_2 + 40% CO_2; d10 = 10th day of storage, d14 = 14th day of storage; VSP = vacuum skin packaging; [a...e] –used to denote groups that are homogeneous in respect to packaging system on individual days (in rows) with P ≤ 0.05; [A...C] –used to denote groups that are homogeneous in respect to dose of vitamin D_3 (in columns) with P ≤ 0.05.

Table 4. Mean values of a* indicator broken down by the packaging systems on individual days vs. vitamin D_3 dose, with homogeneous groups denoted in rows and columns of the study performed and a confidence level of $P \leq 0.05$.

Vit. D_3[††]	Packaging system and ageing times							
	Control	MAP1 d10	MAP2 d10	VSP d10	MAP1 d14	MAP2 d14	VSP d14	SEM
M. rectus femoris								
0	23.1[bcB]	27.7[dD]	24.4[cB]	21.7[bA]	24.7[bcA]	20.3[aA]	22.9[bcB]	0.5
3.5	19.8[aA]	24.5[dB]	23.3[cAB]	22.1[bA]	21.8[bA]	20.2[aA]	20.4[aA]	0.3
7	20.4[aA]	23.3[dA]	22.8[cA]	21.8[bA]	21.7[bcA]	21.6[abcB]	20.6[abA]	0.3
10	20.6[aA]	25.9[dC]	24.0[cAB]	21.2[abA]	22.1[bA]	21.7[abB]	21.6[abAB]	0.3
M. gluteus medius								
0	27.1[eB]	27.6[eB]	26.0[deC]	23.3[aA]	24.6[cdB]	22.2[abB]	23.7[bcB]	0.6
3.5	22.9[cA]	24.5[dA]	22.3[bcA]	21.2[bB]	21.3[bA]	18.5[aA]	21.8[bcA]	0.3
7	23.8[cA]	24.4[dA]	24.0[dB]	22.3[bcAB]	21.4[abA]	19.8[aA]	22.0[bA]	0.4
10	21.6[cA]	25.9[dA]	23.0[cAB]	22.8[cB]	20.1[bA]	18.3[aA]	23.0[cA]	0.4
M. adductor femoris								
0	24.1[bA]	24.3[bA]	24.1[bB]	22.5[aA]	22.0[aA]	20.8[aB]	23.4[abAB]	0.7
3.5	25.3[dAB]	25.8[dAB]	23.1[cA]	23.1[cA]	21.0[bA]	18.2[aA]	22.8[cAB]	0.3
7	24.8[dB]	24.2[cA]	23.7[cAB]	22.8[bA]	20.9[bA]	16.4[aA]	22.4[bcA]	0.4
10	26.5[dAB]	26.7[dB]	22.2[bA]	21.5[cB]	20.8[bA]	17.4[aA]	24.0[cB]	0.4

[††]Doses of vitamin D_3 were expressed in M IU day⁻¹ animal⁻¹; MAP1 = modified atmosphere packaging, 80% O_2 + 20% CO_2; MAP2 = modified atmosphere packaging, 60% O_2 + 40% CO_2; d10 = 10th day of storage, d14 = 14th day of storage; VSP = vacuum skin packaging; [a...e] –used to denote groups that are homogeneous with respect to packaging system on individual days (in rows) and a confidence level of $P \leq 0.05$; [A...C] –used to denote groups that are homogeneous in respect to dose of vitamin D_3 (in columns) with a confidence level of $P \leq 0.05$.

statistically significant. In the case of the REF and GLU muscles, the values of the a* parameter were lower for MAP2-packed meat on the 10th and 14th days compared to MAP1 d10 and d14. The differences were statistically significant for selected muscles on the 10th day of storage and for the batches with vitamin D_3 supplementation of 3.5 and 10 M IU. On the 10th day, the VSP-packed meat had lower values of a* parameter compared to MAP1 and MAP2 on d10. The differences were statistically significant in all muscles only on the 10th day of storage for all levels of supplementation. In the GLU muscle a decrease in a* value in meat with D_3 supplementation was observed, as compared to the control batch (CONTROL) which contained no vitamins in all packing systems. The negative differences were statistically significant.

The value of b* depended on the dose of vitamin D_3 and the packaging system applied (Table 5).

An increase in the b* parameter in MAP1- and MAP2-packed meat on the 10th and 14th days of ageing was observed in all the muscles in the study, as compared to the control batch (CONTROL). The differences were statistically significant in vitamin D_3 supplemented

meat. The b* parameter was lower for VSPd10- and VSPd14-packed meat, as compared to the control batch (CONTROL). The differences were statistically significant for GLU and ADD muscles at all levels of supplementation. In all the muscles covered by the study, a decrease in the value of b* parameter was observed in the case of the VSPd10- and VSPd14-packed meat, as compared to the MAP1 and MAP2 on both the 10th and 14th days of ageing. The drops in these values were statistically significant at all levels of supplementation. Moreover, lower values of the b* parameter were observed in meat supplemented with vitamin D_3, as compared to nonsupplemented meat. The differences were statistically significant for MAP1d10-packed meat in all muscles in the study. In the case of MAP1- and MAP2-packed meat, a drop in the value of parameter b*, as compared to the control batch (CONTROL), was demonstrated on the 14th day of ageing, and—in the case of REF and GLU muscles—also on the 10th day of ageing. However, these differences were not statistically significant. Therefore, the impact of ageing time on the value of parameter b* cannot be proved for meat packed in modified atmosphere.

Table 5. Mean values of b* indicator broken down by the packaging systems on individual days vs. vitamin D_3 dose, with homogeneous groups denoted in rows and columns of the study performed with a confidence level of $P \leq 0.05$.

Vit. D_3[‡]	Packaging system and ageing times							
	Control	MAP1 d10	MAP2 d10	VSP d10	MAP1 d14	MAP2 d14	VSP d14	SEM
M. rectus femoris								
0	8.9[bB]	13.6[dC]	12.3[cdB]	4.6[aA]	12.3[cdB]	12.0[cB]	5.5[aA]	0.3
3.5	5.1[abA]	11.6[cA]	11.3[cA]	5.5[bA]	11.4[cA]	10.9[cA]	4.5[aA]	0.2
7	5.4[aA]	12.0[bA]	11.7[bAB]	5.0[aA]	11.9[bAB]	11.9[bB]	4.8[aA]	0.2
10	6.1[bA]	12.5[cB]	12.1[cB]	4.9[aA]	11.5[cAB]	11.6[cAB]	5.5[abA]	0.2
M. gluteus medius								
0	11.8[bcB]	13.1[cB]	12.7[bBC]	4.8[aA]	12.5[bcC]	12.4[bB]	6.0[aB]	0.3
3.5	7.5[bA]	11.7[dA]	10.6[cA]	5.2[aA]	10.8[cdAB]	10.0[cA]	5.1[aA]	0.2
7	8.2[bA]	11.4[cdA]	11.9[dC]	5.5[aA]	11.2[cdB]	10.3[cA]	5.6[aAB]	0.2
10	6.9[bA]	12.1[dA]	11.0[aC]	5.2[aA]	10.3[cA]	10.4[cA]	5.7[aAB]	0.2
M. adductor femoris								
0	10.9[bA]	14.3[dB]	12.5[dB]	5.1[aA]	12.1[cB]	11.5[cB]	6.5[aA]	0.4
3.5	9.9[bA]	12.5[eA]	12.1[deAB]	5.6[aA]	11.2[cdA]	10.8[cAB]	5.8[aA]	0.2
7	10.1[dA]	12.5[cA]	11.5[bcA]	5.9[aA]	11.7[bA]	10.6[bA]	6.0[aA]	0.2
10	10.1[bA]	13.1[dA]	11.9[cdAB]	7.1[aB]	11.7[cA]	11.3[cAB]	6.9[aA]	0.2

[‡] Doses of vitamin D_3 were expressed in M IU day^{-1} animal^{-1}; MAP1 = modified atmosphere packaging, 80% O_2 + 20% CO_2; MAP2 = modified atmosphere packaging, 60% O_2 + 40% CO_2; d10 = 10th day of storage, d14 = 14th day of storage; VSP = vacuum skin packaging; [a...e] –used to denote groups that are homogeneous in respect to packaging system on individual days (in rows) with a confidence level of $P \leq 0.05$; [A...C] –used to denote groups that are homogeneous with respect to dose of vitamin D_3 (in columns) with a confidence level of $P \leq 0.05$.

Variant analysis ($P \leq 0.05$) demonstrated the presence of statistically significant interactions between the packaging system used, dosage of vitamin D_3, and shear force (Table 6).

Characteristic of meat packed in MAP1 and MAP2 and aged for 10 and 14 days was a drop in the shear force, as compared to the control batch (CONTROL). The differences were statistically significant for all muscles at all levels of supplementation. A drop in the value of shear force was observed in VSPd10- and VSPd14-packed meat, as compared to the control batch (CONTROL). Statistically significant drops were demonstrated in all muscles at all levels of supplementation. In the case of the GLU and ADD muscles, lowering of shear force was observed in MAP2d10-packed meat with 10 M IU vitamin D_3 supplementation. The drop in value was statistically significant. Similarly, a drop in the shear force was demonstrated for the ADD muscle, in the case of unpackaged meat (CONTROL). The drops were statistically significant for the doses 3.5 and 10 M IU. In the case of REF and ADD muscles, a drop in the shear force value was recorded for VSP-packed meat aged for 14 days with vitamin D_3 supplementation of 10 M IU. The demonstrated differences were statistically significant.

4. Discussion

This study demonstrated that modified atmosphere packaging (MAP1, MAP2) had a bearing on increases in parameter L*, which is responsible for the lightness of meat, on the 10th and 14th days of ageing. This is concurrent with results obtained by Insausti et al. (7). They discovered that parameter L* grows parallel to the duration of storage, which results from progressing denaturation. These processes result in the degradation of proteins and, consequently, increased lightness of meat. Vacuum packing resulted in greater lowering of the L* value than modified atmosphere packaging (MAP1, MAP2) on the 10th and 14th days of storage, regardless of vitamin D_3 dosage. These results are consistent with those obtained by Insausti et al. (8), who demonstrated higher values of parameter L* after a 15-day period of meat storage, compared to vacuum packed meat. The results obtained validated that vacuum packing (VSP) resulted in

Table 6. Mean values of WBSF shear force [N], broken down by the packaging systems on individual days vs. vitamin D_3 dose, with homogeneous groups denoted in rows and columns of the study performed with a confidence level of $P \leq 0.05$.

Vit. D_3[‡‡]	Packaging system and ageing times							
	Control	MAP1 d10	MAP2 d10	VSP d10	MAP1 d14	MAP2 d14	VSP d14	SEM
M. rectus femoris								
0	37.5[bcA]	31.5[abA]	36.5[cB]	34.3[abA]	31.1[abA]	29.7[aA]	36.2[abcB]	1.2
3.5	41.8[bAB]	35.2[aA]	33.5[aA]	30.9[aA]	31.7[aA]	32.6[aAB]	32.5[aAB]	0.7
7	43.1[bAB]	35.4[aA]	36.3[aA]	33.2[aA]	32.4[aA]	33.3[aAB]	31.0[aAB]	0.6
10	47.7[bB]	34.7[aA]	32.6[aA]	35.3[aA]	30.0[aA]	35.4[aB]	30.8[aA]	0.6
M. gluteus medius								
0	53.5[eA]	32.1[abA]	42.8[dB]	36.3[bcAB]	27.0[aA]	38.0[cdA]	31.9[abA]	0.9
3.5	53.7[dA]	47.8[cB]	42.1[bcB]	34.6[aA]	36.8[abB]	37.9[abA]	39.4[abcB]	0.5
7	48.7[dA]	34.0[abA]	40.0[bcAB]	43.2[cdB]	37.7[abcB]	35.9[abA]	31.9[aA]	0.6
10	51.4[cA]	36.0[abA]	33.1[aA]	34.8[abA]	37.9[abB]	39.7[bA]	33.1[aAB]	0.6
M. adductor femoris								
0	52.5[dC]	43.8[cB]	42.7[bcB]	36.9[bA]	37.6[bA]	28.6[aA]	38.2[bcA]	0.6
3.5	50.1[cB]	35.2[aA]	36.9[abAB]	38.8[abA]	34.7[aA]	34.1[aAB]	41.8[bA]	0.4
7	45.3[cAB]	39.5[bA]	35.9[abAB]	36.9[abA]	37.6[abA]	33.5[aAB]	36.0[abA]	0.5
10	38.5[cA]	31.9[aA]	35.0[aA]	41.8[bA]	33.5[aA]	36.7[abB]	35.3[abB]	0.5

[‡‡] Doses of vitamin D_3 were expressed in M IU day^{-1} animal^{-1}; MAP1 = modified atmosphere packaging, 80% O_2 + 20% CO_2; MAP2 = modified atmosphere packaging, 60% O_2 + 40% CO_2; d10 = 10th day of storage, d14 = 14th day of storage; VSP = vacuum skin packaging; [a…e] –used to denote groups that are homogeneous with respect to packaging system on individual days (in rows) with a confidence level of $P \leq 0.05$; [A…C] –used to denote groups that are homogeneous with respect to dose of vitamin D_3 (in columns) with a confidence level of $P \leq 0.05$.

the lowering of parameter L* on the 10th and 14th days of meat ageing, as compared to unpackaged meat. Similar results were obtained by Farouk and Swan (9), who claimed that vacuum system packing combined with cold storage has an impact on lowering the L* parameter. Vacuum packing helps to decrease the denaturation of proteins, which is responsible for decreasing the diffusion of light. Furthermore, it slows down lipid oxidisation, which may result in lower values of the parameter in question (10). A modified atmosphere with an appropriate concentration of gases (MAP1) combined with the appropriate dosage of vitamin D_3 (10 M IU) had an impact on lowering the L* parameter in beef. This was similar to the results obtained by Wilborn et al. (11), who demonstrated that increased vitamin D_3 supplementation in pig feed resulted in lowering the L* parameter. Montgomery et al. (12) and Reiling and Johnson (13) demonstrated a lack of vitamin D_3 impact on the value of the L* parameter.

The red colour of meat is a basic sensory feature and the most important among the independently assessed distinctive factors related to appearance; it is an indication of freshness and quality (3). The studies conducted

demonstrated that modified atmosphere packaging (MAP1), supplementation with vitamin D_3, and the 10-day period of meat ageing had a bearing on the increase of parameter a*, which is responsible for the red colour of meat. These results are partially consistent with those reported by Lindahl et al. (14), who also demonstrated an increase in parameter a* for meat packed in modified atmosphere and aged for 8 days. The MAP1-packed meat aged for 14 days deteriorated in redness, due to the lowering of the value of parameter a*, as compared to meat packed in the same system but aged for 10 days. The colour of beef obtained after 10 days was of greater value. This agrees with the results obtained by O'Sullivan et al. (15), who demonstrated that a drop in parameter a* is linked to the duration of storage. Similar results were obtained by John et al. (16), who concluded that the browning of meat related to the drop in a* is observed after a 14-day period of storage and results from the development of metmyoglobin on the surface of the beef. The studies conducted demonstrated that a gas mixture with a higher oxygen concentration (MAP2) had a bearing on lowering of a* parameter value on the 10th and 14th days

of meat ageing, when compared to the mix with a lower proportion of oxygen (MAP1). The results obtained are in line with Zakrys et al. (17), who demonstrated that high oxygen concentration in the gas mix results in red colour deterioration due to an increase in the oxidisation of lipids and proteins, which has an impact on metmyoglobin level. Skin-pack packing technology and a 10-day ageing period resulted in the lowering of parameter a* in beef when compared to meat packed in modified atmospheres (MAP1, MAP2). Similar results were presented by Lagerstedt et al. (18), who stated that the value of the parameter in question is decidedly higher in meat packed in modified atmosphere than in vacuum packed meat, as a result of the high concentration of oxygen in the gas mix. Packed beef had a dark, violet-red colour, which resulted from the removal of oxygen and is consistent with the results reported by Boles and Pegg (4). The results obtained confirmed that vitamin D_3 had a bearing on the lowering of the a* value and, consequently, had a negative impact on the red colour of meat in the case of GLU muscle. Meat without vitamin supplementation was redder, regardless of the system of packing or meat ageing period. This is contrary to results obtained by Lawrence et al. (19), who demonstrated that vitamin D_3 supplementation of meat does not have a significant impact on changes in the value of a*.

The studies conducted demonstrated the impact of meat packing in modified atmospheres (MAP1, MAP2) on increases in parameter b* on the 10th and 14th days of ageing when compared to the control. Modified atmospheres (MAP1, MAP2) make it possible to maintain higher values of parameter b*, as compared to vacuum packed meat, regardless of vitamin D_3 dose. This is confirmed by Xin et al. (20), who proved that parameter b* was decidedly higher for MAP-packed meat compared to the control and vacuum packed meat. In their studies, Lorenzo and Gómez (21) demonstrated a significant increase in parameter b* in the case of meat packed in modified atmospheres. VSP resulted in a drop in parameter b* value on the 10th and 14th days of storage, as compared to the control batch. These results are not in agreement with those reported by Bingol and Ergun (22), who demonstrated a drop in parameter b* parallel to the period of ageing. Different results were obtained by Bruce et al. (23), who demonstrated an increase in parameter b* for meat stored in vacuum for 14 days. Vitamin D_3 in doses of 3.5, 7, and 10 M IU combined with modified atmosphere packaging (MAP1) and a 10-day period of storage resulted in the lowering of the b* parameter value. The above is not in agreement with Reiling and Johnson (13), who demonstrated that there is no correlation between vitamin D_3 supplementation of meat and changes in the colour spaces.

Supplementation with vitamin D_3 improves beef tenderness (6). Studies conducted demonstrated that modified atmosphere packing (MAP1, MAP2) results in increased beef tenderness on the 10th and 14th days of ageing, as compared to unpackaged meat. Similar results were also obtained by Tørngren (24), who claimed that modified atmosphere that contains oxygen and carbon dioxide results in an increase in beef tenderness. Clausen et al. (25) stated that meat packed in modified atmospheres is less tender. They explained the mechanism as a decrease in proteolysis through inactivation of proteolytic enzymes in an aerobic environment. The experiment proved that vacuum packaging also had a positive impact on increasing beef tenderness in the case of 10- and 14-day storage periods, when compared to the control. Similar results were obtained by Lagerstedt et al. (18), who demonstrated an increase in beef tenderness after 14 days of storage. The value of shear force is reduced with the extended period of beef storage, as documented by Monsón et al. (26). Vitamin D_3 supplementation of 3.5 and 10 M IU has a bearing on the increased tenderness of unpacked beef. Differing results were obtained by Gonzalez et al. (27), who demonstrated that supplementation of vitamin D_3 at 7.5 M IU resulted in a reduction in WBSF. In their studies, Montgomery et al. (12) used doses ranging from 0.5 to 7.5 M IU and various ageing times. They ascertained that the use of vitamin D_3 has a significant impact on improving meat tenderness, which is manifested by a drop in shear force for vitamin doses of 0.5 and 7 M IU, in the case of meat aged for 14 days. An increase in the tenderness of meat through supplementation with vitamin D_3 results from the activity of intracellular enzymes, notably calpains. They are responsible for protein proteolysis, a process that loosens up the muscle structure (28). A dose of 10 M IU combined with vacuum packing (VSP) and a 14-day storage period has an impact on beef tenderness. Studies by Hansen et al. (29) demonstrated the impact of vitamin supplementation (in a dose of 7 M IU) on improving the tenderness of the vacuum packed beef on all days of the ageing process. Vitamin dosed at 10 M IU combined with modified atmosphere packing (MAP2) and a 10-day ageing period resulted in increased beef tenderness. Zakrys et al. (17) demonstrated a tendency of shear force to grow in parallel to the increase in oxygen content in the packing, which is discordant with the results obtained in the previous study. One of theories proposed to explain the phenomenon is oxidisation of the enzymes involved in the process of tenderisation, which leads to a slower process of meat tenderisation or to its complete termination (30).

The studies conducted proved a significant impact of modified atmosphere packaging on decreases in parameters L*, a*, and b*. For meat packed in a gas mixture, more favourable results were obtained after

a 10-day ageing period. On the other hand, skin-pack technology packing and vitamin D_3 supplementation resulted in a drop in the parameters responsible for the colour of beef on both the 10th and 14th days of ageing. The meat ageing process, both 10 and 14 days, and the packing systems applied had a bearing on beef tenderness. Moreover, the studies demonstrated a significant impact of vitamin D_3 dosage (3.5 and 10 M IU) on increases in unpackaged beef tenderness, which is why such supplementation to improve meat tenderness is justified. Fourteen-day ageing of vacuum packed meat and 10-day storage of meat packed in gas mixture containing 60% O_2 and 40% CO_2 is a way of improving meat tenderness with vitamin D_3 supplementation at M IU. Supplementation makes it possible to obtain higher tenderness values for beef, yet this is linked to the lowering of colour parameters; colour in turn, is the most significant distinctive factor from the point of view of the consumer. To increase beef tenderness, in addition to vitamin D_3 supplementation, the meat packing systems discussed above can be applied in combination with suitable ageing times.

Acknowledgements

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