

Changes in the colour and sensory properties of beef frozen after seven days of ageing in a modified atmosphere

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The aim of the study was to evaluate the colour, sensory properties and shear force values of meat from ten young bulls produced by crossing Polish Black-and-White Holstein-Friesian cows with Belgian White Blue bulls. The quality of the longissimus lumborum (LL) muscle was determined after seven-day ageing under various modified atmosphere (MA) conditions (vacuum – group B; 40% CO₂ + 60% N₂ – group C; 30% CO₂ + 70% Ar – group D) followed by freezing and frozen storage. The process of seven-day ageing in MA composed of 40% CO₂ + 60% N₂ significantly increased the colour lightness of the beef samples. Eight-month frozen storage increased colour lightness in the meat samples aged in MA composed of 30% CO₂ + 70% Ar. Meat samples aged under various MA conditions had a higher contribution of redness (*a**) and yellowness (*b**) than non-aged beef. Meat samples frozen after ageing in MA containing Ar had less redness than the samples from other groups. After frozen storage, meat samples from all groups had less redness and yellowness. Ageing and frozen storage had no significant effect on the juiciness of the beef. The beef aged in vacuum conditions was the most tender, both before and after frozen storage. Ageing had no significant influence on the shear force of meat samples evaluated before freezing. Meat samples aged in MA composed of 30% CO₂ + 70% Ar evaluated after frozen storage had lower average shear force values than beef that had not been aged prior to freezing.

KEY WORDS: beef / meat quality / ageing / frozen storage / modified atmosphere

Annual consumption of beef in Poland, despite its taste and aroma attributes and numerous health benefits, is less than 1.5 kg per capita [12]. Increased consumption of beef in the future will depend in part on improvement in the sensory quality of this meat, especially its texture [11, 14]. According to consumers, the main barriers to increased beef consumption are its high price and uncertainty about the quality of the product to be purchased. Another important factor shaping consumer behaviour in the Polish meat market is the difference in the prices of meat obtained from different species of animals.

The production of good-quality beef requires an understanding of the factors determining the quality of individual skeletal muscles of the beef carcass, as this is of great importance in choosing the optimal conditions and time of ageing [10]. The ageing process is an important element in the acquisition of beef. It substantially improves quality traits that determine suitability for consumption, such as tenderness and palatability, as well as suitability for processing. The ultimate quality of meat is also determined by the means of packaging [1, 27]. The wrong storage conditions and meat packaging techniques can cause unfavourable changes in meat, leading to a reduction in its quality indicators [5].

The use of a suitable method of meat preservation is essential, because it is a raw material that easily spoils. The susceptibility of meat to spoilage results from its chemical composition, mainly its high content of water, proteins, carbohydrates and fat, which is easily oxidized. The consequences of this include changes in sensory properties. The nature of these changes depends primarily on the raw material and the processing and preservation technology used.

The frozen storage process is indispensable in the handling of raw meat when there is market surplus, and it plays an important role in global meat exports. The most important factors determining the quality of frozen meat include the quality of the raw meat and parameters of freezing (mainly the rate of freezing), storage and thawing. This means that the quality of frozen meat depends on both primary and secondary changes occurring at individual stages of freezing and storage [15]. Spoilage of frozen meat or deterioration of its quality may be the result of the interaction of several factors, e.g. post-slaughter treatment, the degree of microbial contamination, activity of tissue and bacterial enzymes, and the time, temperature and method of storage.

The aim of the study was to evaluate the colour, sensory properties and shear force of meat from young bulls produced by crossing Polish Black-and-White Holstein-Friesian (PFH HO) cows with Belgian White Blue (BBB) bulls. The quality of the longissimus lumborum (LL) muscle was determined after seven-day ageing under various modified atmosphere conditions (vacuum – group B; 40% CO₂ + 60% N₂ – group C; 30% CO₂ + 70% Ar – group D) followed by freezing and frozen storage.

Material and methods

The experimental material consisted of the carcasses of ten young F₁ bulls produced by crossing the breeds Polish Black-and-White Holstein-Friesian (PHF HO) and Belgian White Blue (BBB). The crossbred bulls came from one farm, where they were housed indoors. Their diet was based on on-farm feedstuffs. In the autumn and winter, they received hay (ad libitum), maize silage, and meal from a mixture of cereals (about 2 kg). In the summer, they received green forage (ad libitum), cereal meal, and hay.

The bulls were slaughtered at about 23 months of age. They were transported to meat plants over a distance of about 90 km. The average weight of the bulls after transport was 762 ± 47 kg. They were kept in lairage in individual pens for about 18 hours before slaughter. The average hot carcass weight was 447 ± 42 kg. After weighing, the carcasses were cooled at $2^{\circ}\text{C} (\pm 2^{\circ}\text{C})$ for 48 h. Then the pH_{48} of the LL muscle was measured between the first and second lumbar vertebrae in order to eliminate defective meat. During dissection of the carcasses into primal cuts, lumbar sections of the longissimus dorsi (LD) muscle were collected between the last two thoracic vertebrae and the last lumbar vertebra – the longissimus lumborum (LL), which after weighing were vacuum-packed and transported in isothermal containers to the laboratory. Then the muscles were divided into parts of similar weight (about 1500 g), and these were assigned to four groups (A, B, C and D):

- Some of the samples from group A were immediately subjected to laboratory tests (i.e. about 54 hours after slaughter), and the rest were vacuum-packed and frozen.
- The group B samples were vacuum-packed.
- Samples from group C were packaged in a modified atmosphere (MA) composed of the gas mixture $40\% \text{CO}_2 + 60\% \text{N}_2$.
- Samples from group D were packaged in a modified atmosphere (MA) composed of the gas mixture $30\% \text{CO}_2 + 70\% \text{Ar}$.

After seven days of ageing under refrigeration at $2^{\circ}\text{C} (\pm 2^{\circ}\text{C})$, samples from groups B, C and D were unpacked; some of the samples were used for analyses, and the rest were vacuum-packed and frozen in air at -26°C by means of using quick freezing (5–20 cm/h) and then stored at the same temperature for 8 months. After the frozen storage period, the samples were thawed in air at $2^{\circ}\text{C} (\pm 2^{\circ}\text{C})$ until the geometric centre of the samples reached a temperature of approximately 4°C . After thawing, the samples were tested for quality parameters. The colour of the meat was characterized on the basis of L^* , a^* , b^* , C^* and h° parameters in the CIELAB system [4]. Evaluation of sensory properties was carried out on samples without fat or external connective tissue membranes [16]. The meat was heated in a 0.6% NaCl solution at 96°C applied until

the samples reached an internal temperature of 80°C. In the sensory evaluation of the meat samples, carried out by a five-person panel with proven sensory sensitivity, a five-point scale was used (where 5 was the highest score and 1 was the lowest). The shear force of the meat was measured after heat treatment, in the Warner–Bratzler chamber of an INSTRON 5542 testing machine equipped with a 500 N load cell. Meat samples were heat-treated in an aqueous environment at 75°C for 50 minutes and then cooled down in a water bath for about 40 minutes at a constant temperature of 1–5°C. After being cooled and dried, the samples were stored in aluminium foil at 4°C for 24 hours. After this time, five cylinders with a diameter of 1.27 cm and a height of 2 cm were cut from the samples. During the measurement, the maximum force necessary to cut through each of them across the muscle fibres was recorded.

Statistical analysis of the results was performed in STATISTICA software, version 12.0 [33]. The results presented in the tables include the arithmetic means (\bar{x}) and the standard error of the mean (SEM) for individual features. Student's t-test was used to determine the variation in the quality of the LL muscle of the crossbred bulls (PHF HO x BBB), evaluated before and after frozen storage. One-way analysis of variance was carried out to estimate the effect of seven-day ageing in various MA conditions on the quality of the LL muscles. Statistical significance of differences between means for groups was determined using Duncan's multiple range test.

Results and discussion

Colour is one of the most important sensory characteristics of beef, assessed by the consumer before flavour and tenderness. Owing to visual assessment, the potential buyer can make an initial determination of the quality and freshness of the meat and make a purchase [3].

Meat packed in a modified atmosphere (MA) composed of 40% CO₂ + 60 % N₂, evaluated after a seven-day ageing process (Group C), had a lighter colour ($P \leq 0.05$) than meat samples from the other groups (Table 1). There were no significant differences ($P > 0.05$) in the lightness (L^*) of the samples evaluated after frozen storage. Meat aged in an MA composed of 30% CO₂ + 70% Ar (group D) assessed after frozen storage had a lighter colour ($P \leq 0.05$) than before freezing.

Meat samples that were not aged (group A) had a smaller share ($P \leq 0.01$) of the colour red (a^*) than those stored under various MA conditions (Table 1). The samples from groups A, B and C evaluated after freezing had more ($P \leq 0.05$) red than the samples of meat aged in an MA composed of 30% CO₂ + 70% Ar (group D). In the meat samples of all groups (A, B, C and D) evaluated before freezing, a higher ($P < 0.01$) mean a^* value was found than in the samples evaluated after frozen storage.

Meat not aged before freezing (group A) had a smaller ($P \leq 0.01$) share of yellow (b^*), than the meat samples from the other groups (B, C and D) – Table 1. In meat samples aged for seven days in a vacuum (group B), evaluated after frozen storage, a higher ($P \leq 0.05$) share of yellow (b^*) was found than in frozen samples aged in an atmosphere of 30% CO₂ + 70% Ar (group D). Frozen storage caused a decrease ($P \leq 0.01$) in the share of yellow (b^*) in the meat of all groups.

In the fresh meat (group A), the average chroma value (C^*) was lower ($P \leq 0.01$) than in the samples aged under various conditions, evaluated before freezing (groups B, C and D) – Table 1. Evaluation of samples after frozen storage indicated that samples from group D (aged in an MA composed of 30% CO₂ + 70% Ar) had a lower ($P \leq 0.05$) chroma value than the samples from groups B and C. Frozen storage affected ($P \leq 0.01$) the chroma of the samples in all groups. The value of the C^* parameter evaluated in the samples before frozen storage was higher than in the samples stored in a frozen state.

The analysis of variance regarding hue (h°) showed no differences ($P > 0.05$) in the values of this parameter between groups before frozen storage, i.e. after the ageing process (Table 1). The samples from group A evaluated after freezing had a lower ($P \leq 0.05$) h° value than the frozen samples (group D) aged in an MA of 30% CO₂ + 70% Ar. In addition, the average h° value in the group A samples before freezing was higher ($P \leq 0.01$) than after frozen storage. In the remaining groups, frozen storage caused no ($P > 0.05$) variation in the h° parameter.

During storage of meat, the colour changes both on its surface and under the surface. Therefore, the time of meat storage should be adjusted to the type of meat and muscle, the storage temperature, and the type and method of packaging, because these factors largely determine the scope of changes in meat colour [34]. The desired meat colour can be stabilized by optimizing the storage parameters of the raw meat [18]. Our research showed dynamic changes in the values of the a^* and b^* parameters during the ageing of meat under refrigeration in various MA conditions, as well as during frozen storage (Table 1). Domaradzki et al. [7], in a study on the impact of frozen storage on the physicochemical properties of vacuum-packed beef, showed a significant effect of the freezing process on the colour of the meat. That study showed a decrease in lightness (L^* parameter) and an increase in the colour red (a^*) in meat samples evaluated after frozen storage relative to the average values before freezing. The primary factor determining the colour of meat is muscle pigments – their quantity, their composition and transformations they undergo. The changes in meat colour found in the present study were a natural consequence of these transformations. Chemical transformations of the basic meat pigment, myoglobin, have the greatest influence on changes in meat colour during storage and ageing [18, 20]. The myoglobin level in the skeletal muscles of cattle is influenced by the breed and age of the animals and the type of muscle, which is linked to their physical activity during

Table 1
Values of meat colour parameters (arithmetic average \pm SEM)

Parameter	Time of laboratory analyses	Method of beef processing			
		non-aged beef	beef aged for 7 days in vacuum packages	beef aged for 7 days in MA composed of 40% CO ₂ + 60% N ₂	beef aged for 7 days in MA composed of 30% CO ₂ + 70% Ar
		A	B	C	D
<i>L</i> [*] lightness	before freezing	35.36 ^{bx} \pm 1.08	35.22 ^{bx} \pm 1.06	38.10 ^{ax} \pm 0.82	35.12 ^{bx} \pm 0.66
	after frozen storage	36.59 ^{ax} \pm 0.89	37.60 ^{ax} \pm 1.21	38.33 ^{ax} \pm 0.93	39.14 ^{xy} \pm 0.96
<i>a</i> [*] redness	before freezing	19.26 ^{bx} \pm 0.74	24.97 ^{ax} \pm 1.02	24.10 ^{ax} \pm 1.01	24.74 ^{ax} \pm 0.92
	after frozen storage	13.29 ^{xy} \pm 0.50	13.19 ^{xy} \pm 0.61	13.44 ^{xy} \pm 0.70	11.48 ^{xy} \pm 0.54
<i>b</i> [*] yellowness	before freezing	20.99 ^{bx} \pm 1.03	24.83 ^{ax} \pm 0.69	24.70 ^{ax} \pm 0.67	24.56 ^{ax} \pm 0.57
	after frozen storage	11.83 ^{by} \pm 0.32	12.76 ^{xy} \pm 0.38	12.69 ^{xy} \pm 0.42	11.67 ^{by} \pm 0.18
<i>C</i> [*] chroma	before freezing	28.73 ^{bx} \pm 1.04	34.79 ^{ax} \pm 1.23	34.53 ^{ax} \pm 1.15	34.85 ^{ax} \pm 1.03
	after frozen storage	17.83 ^{by} \pm 0.49	18.40 ^{xy} \pm 0.57	18.52 ^{xy} \pm 0.72	16.41 ^{by} \pm 0.45
<i>h</i> ^o hue	before freezing	46.82 ^{ax} \pm 1.02	44.94 ^{ax} \pm 0.41	45.84 ^{ax} \pm 0.69	44.91 ^{ax} \pm 0.46
	after frozen storage	41.79 ^{by} \pm 1.13	44.21 ^{ax} \pm 1.43	43.56 ^{ax} \pm 1.20	45.72 ^{ax} \pm 1.25

Values in the same row with the same letters indicate homogeneous groups defined by Duncan's test: a, b, c – $P \leq 0.05$; A, B, C – $P \leq 0.01$

Values in the same row with the same letters indicate homogeneous groups defined by Student's t-test: x, y – $P \leq 0.05$; X, Y – $P \leq 0.01$

life [16, 21]. Differences in the proportions between individual types of muscle fibres in different breeds or genotypes of cattle may be a source of variation in meat colour [9, 28]. Under conditions of elevated partial oxygen pressure, myoglobin is converted to a bright red oxidized form – oxymyoglobin, which under low oxygen conditions is oxidized to greyish-brown metmyoglobin, which undergoes reduction in specific conditions. Confirmation of the progressive formation of metmyoglobin in our research was the decrease in the a^* value observed in meat after frozen storage. Factors influencing the rate of these changes, and consequently the colour of the meat, include the concentration of hydrogen ions (pH), O_2 availability, temperature, access to light, tissue structure, activity of reducing enzymes, and fat oxidation processes [19, 22, 24, 28, 31, 32]. Changes in meat colour may be intensified with increased refrigeration and frozen storage time. They are also promoted by severe dehydration of the surface layer of the raw meat, which facilitates the penetration of oxygen molecules into the tissue. As negative colour changes following thawing of meat are only partially reversible, they are highly undesirable. The simplest method of prevention is to protect the meat surface from drying and exposure to atmospheric oxygen, which can be achieved with an appropriate storage method [7].

Samples of meat frozen without ageing (group A) had a less favourable ($P \leq 0.01$) aroma intensity than the meat of this group evaluated before frozen storage (Table 2). The samples from group A also had a less desirable smell after frozen storage than the samples from groups B ($P \leq 0.05$) and D ($P \leq 0.01$). The aroma of meat from group D after frozen storage was more desirable ($P \leq 0.05$) than that of the group C meat. The desirability of the aroma of the group A samples deteriorated ($P \leq 0.01$) after frozen storage relative to the samples from this group evaluated before freezing.

The method of handling meat before freezing and its storage in a frozen state had no significant effect on its taste intensity (Table 2). It was only found that meat samples aged in an MA with 40% CO_2 + 60% N_2 had better ($P \leq 0.05$) flavour intensity before freezing than after frozen storage. The meat evaluated before frozen storage, aged for seven days in an atmosphere of 30% CO_2 + 70% Ar (group D), had a more desirable flavour ($P \leq 0.05$) than the meat aged in a vacuum (group B). Fresh, unaged meat (group A) had a more desirable flavour than frozen-stored meat.

Brewer and Novakofski [2], in a study on the sensory quality of beef stored in a vacuum for periods of 0, 7 and 14 days, found that the storage time had no significant effect on the flavour and aroma attributes of meat. Niedźwiedź et al. [25], in their research on the influence of wet ageing on the muscle texture of crosses of meat breeds of cattle, found no significant changes in the taste, smell or juiciness of beef during seven-day ageing. Only a 14-day ageing period had a significant impact on meat quality, which was evidenced by differences in the assessment of the organoleptic qualities of meat samples. In that study, the desirability of these features increased significantly after 14 days of storage.

Table 2
Aroma and taste (points) of meat (arithmetic average \pm SEM)

Trait	Time of laboratory analyses	Beef processing			
		non-aged beef	beef aged for 7 days in vacuum packages	beef aged for 7 days in MA composed of 40% CO ₂ + 60% N ₂	beef aged for 7 days in MA composed of 30% CO ₂ + 70% Ar
		A	B	C	D
Aroma intensity	before freezing	4.75 ^{ax} \pm 0.08	4.60 ^{ax} \pm 0.12	4.65 ^{ax} \pm 0.11	4.65 ^{ax} \pm 0.11
	after frozen storage	4.30 ^w \pm 0.08	4.55 ^{ax} \pm 0.12	4.45 ^{ax} \pm 0.12	4.60 ^{ax} \pm 0.10
Aroma desirability	before freezing	4.80 ^{ax} \pm 0.08	4.75 ^{ax} \pm 0.08	4.75 ^{ax} \pm 0.08	4.75 ^{ax} \pm 0.08
	after frozen storage	4.30 ^{xy} \pm 0.11	4.65 ^{ax} \pm 0.11	4.50 ^{ax} \pm 0.13	4.90 ^{ax} \pm 0.07
Taste intensity	before freezing	4.75 ^{ax} \pm 0.08	4.70 ^{ax} \pm 0.08	4.75 ^{ax} \pm 0.08	4.85 ^{ax} \pm 0.08
	after frozen storage	4.50 ^{ax} \pm 0.10	4.65 ^{ax} \pm 0.11	4.40 ^w \pm 0.10	4.70 ^{ax} \pm 0.11
Taste desirability	before freezing	4.85 ^{ax} \pm 0.08	4.65 ^{ax} \pm 0.08	4.85 ^{ax} \pm 0.08	4.90 ^{ax} \pm 0.07
	after frozen storage	4.45 ^w \pm 0.12	4.60 ^{xy} \pm 0.10	4.61 ^{ax} \pm 0.12	4.70 ^{ax} \pm 0.11

Values in the same row with the same letters indicate homogeneous groups defined by Duncan's test: a, b, c – $P \leq 0.05$; A, B, C – $P \leq 0.01$
 Values in the same row with the same letters indicate homogeneous groups defined by Student's t-test: x, y – $P \leq 0.05$; X, Y – $P \leq 0.01$

The results obtained in our research show that neither the ageing method in various MA conditions nor the frozen storage process affected the juiciness of the beef (Table 3). Analysis of the tenderness scores showed that meat samples not subjected to the ageing process before freezing (group A) were less tender ($P \leq 0.05$) than meat samples aged in a vacuum (group B). Meat evaluated before freezing after the seven-day ageing process (group B) was more tender ($P \leq 0.05$) than unfrozen meat aged in a modified atmosphere composed of 40% CO₂ + 60% N₂ (group C). Fresh, unaged meat (group A) after frozen storage was less tender ($P \leq 0.05$) than meat aged for seven days in a vacuum (group B) or in an MA composed of 30% CO₂ + 70% Ar (group D). In addition, meat after seven days of ageing in an MA with a composition of 40% CO₂ + 60% N₂ (group C) was more ($P \leq 0.05$) tender after frozen storage than after the ageing process.

There were no significant differences ($P > 0.05$) between the mean shear force values of the meat in groups A, B, C and D before frozen storage (Table 3). It was only found that samples of meat aged for seven days in an MA composed of 30% CO₂ + 70% Ar (group D) evaluated after freezing had a lower ($P \leq 0.05$) average shear force than frozen meat that was not aged. A decrease in the shear force of meat after frozen storage has also been observed by Domaradzki et al. [7]. Niedźwiedz et al. [26] reported that ten-day ageing resulted in a reduction in the shear force of muscles by over 10 N relative to the baseline values. Niedźwiedz et al. [25] also found that ageing of meat samples under vacuum conditions caused a significant reduction in the shear force, from about 78 N (maximum shear force two days after slaughter) to about 33 N (after 14 days of ageing). After seven days of ageing the shear force had decreased by about 40%. According to Destefanis et al. [6], 'tender' beef cannot have an instrumental shear force greater than 42.9 N. Meat becomes tender during endogenous proteolysis of muscle proteins during ageing [2, 36]. The increase in meat tenderness during ageing is also the effect of the weakening of titin and nebulin connections with Z-disc proteins and the degradation of desmin and troponin T [23, 30]. Many researchers indicate that the proteolytic non-lysosomal enzymes μ - and m-calpain play a fundamental role in proteolytic changes in proteins responsible for the weakening of meat structure and fragmentation of myofibrils [8, 13]. Both forms of calpain are inactivated by calpastatin, acting as their specific inhibitor [35, 37], which is believed to play a large role in meat tenderization during ageing [17].

Summing up, both ageing in MA and frozen storage of meat after seven days of ageing under various MA conditions influenced changes in meat colour parameters, especially the share of red (a^*) and yellow (b^*) and the chroma value. Taste and smell after frozen storage deteriorated mainly in meat that was not subjected to the ageing process. Ageing and frozen storage did not affect the juiciness of the meat, but influenced its tenderness, which was best in meat aged in a vacuum, assessed

Table 3
Juiciness, tenderness (points) and shear force (N) of meat (arithmetic average \pm SEM)

Trait	Time of laboratory analyses	Beef processing			
		non-aged beef	beef aged for 7 days in vacuum packages	beef aged for 7 days in MA composed of 40% CO ₂ + 60% N ₂	beef aged for 7 days in MA composed of 30% CO ₂ + 70% Ar
		A	B	C	D
Juiciness	before freezing	4.40 ^{ax} \pm 0.07	4.70 ^{ax} \pm 0.08	4.50 ^{ax} \pm 0.11	4.50 ^{ax} \pm 0.13
	after frozen storage	4.50 ^{ax} \pm 0.10	4.55 ^{ax} \pm 0.12	4.20 ^{ax} \pm 0.08	4.25 ^{ax} \pm 0.19
Tenderness	before freezing	3.85 ^{bx} \pm 0.21	4.65 ^{bx} \pm 0.08	4.05 ^{bx} \pm 0.20	4.20 ^{bx} \pm 0.19
	after frozen storage	4.25 ^{bx} \pm 0.11	4.70 ^{bx} \pm 0.13	4.55 ^{cy} \pm 0.05	4.60 ^{bx} \pm 0.12
Shear force	before freezing	33.68 ^{ax} \pm 2.86	30.99 ^{ax} \pm 2.71	35.93 ^{ax} \pm 3.19	32.01 ^{ax} \pm 3.34
	after frozen storage	39.36 ^{ax} \pm 3.11	34.29 ^{bx} \pm 3.69	34.01 ^{bx} \pm 1.85	27.10 ^{bx} \pm 3.35

Values in the same row with the same letters indicate homogeneous groups defined by Duncan's test: a, b, c – $P \leq 0.05$; A, B, C – $P \leq 0.01$
 Values in the same row with the same letters indicate homogeneous groups defined by Student's t-test: x, y – $P \leq 0.05$; X, Y – $P \leq 0.01$

both before and after frozen storage. Ageing had no significant effect on the shear force of the meat samples evaluated before freezing, possibly because the process was too short.

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