ANALYSIS OF RELATIONSHIP BETWEEN BASIC COMPOSITION, pH, AND PHYSICAL PROPERTIES OF SELECTED BOVINE MUSCLES

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Abstract

The aim of this study was to analyse the correlation between basic meat composition (water, protein, fat, and connective tissue contents), pH value, and physical parameters (texture and colour, drip loss) of selected beef muscles. Five different muscles (LTH, SEM, SET, PSM, TRI) from 20 carcasses were examined. Measurements of the basic meat composition were conducted using a near infrared spectrometry method, colour parameters in L*a*b system, and texture parameters. Drip loss correlated strongly negatively with the pH value. Depending on the muscle type, the correlation ranged $-0.601 \leq r \leq -0.282$, whereas drip loss correlated moderately positively with connective tissue content. The most significant positive correlation ($r \approx 0.67$) between hardness, tenderness, and fat content was noted in the SEM. Colour parameters strongly depended on pH values ($-0.831 \leq r_1 \leq -0.338$; $-0.828 \leq r_a \leq -0.542$; $-0.912 \leq r_b \leq -0.719$) and to a lesser extent on the content of connective tissue.

Key words: beef, pH, basic composition, tenderness, drip loss.

The most important physical characteristics determining bovine meat quality are colour, texture (tenderness), and water holding capacity (WHC) (13, 30). Beef quality is influenced by many factors including: species (*Bos taurus* vs *Bos indicus*), breed, sex, age, and type of muscle. Anatomic characteristics are also affected by the husbandry systems including type of feeding and pre-slaughter transportation, as well as post-mortem factors (conditions during slaughter, chilling of carcasses, and their aging) (23, 18).

Tenderness and other meat texture parameters are directly associated with the structure of myofibrillar proteins, content of connective tissue, and their interaction. All muscle fibres are built from myofibrils, which contain myosin and actin filaments. The structural integrity of myofibrils changes during maturation, causing improvement of meat tenderness. Hardness of meat is caused by e.g. the shortening of sarcomeres by the rapid cooling of the muscles while they are still in the process of rigor-mortis ("cold shortening") (33, 24).

Another factor, which determines the tenderness is the amount of intramuscular fat (IMF) (7, 31). This fat has a more delicate structure than muscle tissue, therefore a high content of intramuscular fat (marbling) increases meat tenderness (34). Moreover, intramuscular fat causes an increase in juiciness and improves the flavour (17).

Physical properties, in particular texture, are dependent on the rate of glycolysis, decrease in temperature after slaughter, and ultimate pH value of muscles, which is in a range between 5.4 and 7.2 (14). However, the relationship between pH and tenderness is controversial. Some authors have shown a linear relationship between these two parameters; however, the majority of others suggest that there is a nonlinear dependence of meat hardness within the limits of pH in the range of 5.8-6.3. This may be due to different proteolytic activity leading to improved tenderness during aging (10). An increase in meat tenderness when pH increases from 6 to 7 is associated with a higher activity of calpain enzyme, which reaches its maximum activity at neutral pH (9).

Meat colour is one of the most important visual features. An appropriate proportion and distribution of oxymyoglobin and methmyoglobin is the main determinant of an acceptable colour of fresh meat. The bright red colour of meat occurs due to the oxygenation of myoglobin during exposure to a mixture of gases, which contains oxygen (6, 11). Post slaughter glycolysis lowers pH causing the development of a brighter colour and wetter surface. At high meat pH values, the physical state of proteins is above their isoelectric point. This causes proteins to absorb water in muscles and therefore the muscles fibres are more tightly packed. A negative

trait of meat is the dark colour, formed due to its reduced ability to light scattering on the surface, which is called DFD (dark, firm, dry). This was not reported at a lower meat pH (1).

Evaluation of beef meat texture properties can be measured by objective methods, using extensive instrumental and sensory tests by an expert panel, or by subjective methods - consumer panels (5). Objective methods include the measurement of shear forces, penetration, compression, or torsional force (29, 16).

The aim of this study was to analyse the relationship between basic composition (water, protein, fat, and connective tissue content), pH value, and physical properties (the texture parameters, colour, drip loss) of selected bovine muscles.

Material and Methods

Sample origin. Five muscles from 20 carcasses were collected from a local slaughterhouse. The slaughtered animals were between 20 and 24 months of age. Carcass weights ranged from 270 to 310 kg, and were grade as being in the R conformation class with 1+ and 2- fat cover degree (according to the EUROP classification system). After rigor mortis (48 h, $2 \pm 1^\circ\text{C}$), the following muscles were separated from the carcasses: *M. longissimus thoracis et lumborum* – LTH, *M. semimembranosus* – SEM, *M. semitendinosus* – SET, *M. psoas major* – PSM and *M. triceps branchii* – TRI. Then, these muscles were vacuum packed in high-barrier bags and stored at $2 \pm 1^\circ\text{C}$ for 5 d. After aging (7 d), the muscles were frozen within 2 h using Küppersbusch blast-freezer and stored at -18°C until later analysis. Thawing of samples was conducted at $2 \pm 1^\circ\text{C}$ for 24 h. After the thawing process, the muscles were removed from their packages and 2.54 cm thick steaks were sliced.

Drip loss determination. Muscle weight loss during 7-d aging was determined on the basis of the difference in weight before storage (M_0) and after storage (M_1) (allowing for the weight of package). Each measurement was performed in five replications, taking the mean value as the assay result. Drip loss (D_L) was calculated using the equation (1):

$$D_L = \frac{(M_0 - M_1)}{M_0} \cdot 100\% \quad (1).$$

where M_0 – weight before storage, M_1 – weight after storage.

pH value evaluation. pH value of the muscles was measured according to the PN-ISO 2917:2001/Apl:2002 standard. pH-metric results were obtained using a Testo 205 series pH-meter equipped with a glass electrode, which was placed directly into the samples (2 cm deep into the steaks). Each measurement was performed in five replications, taking the mean value as the assay result. The temperature of samples during measurements was $2 \pm 1^\circ\text{C}$.

Instrumental colour measurement in $L^*a^*b^*$ system. Instrumental colour analysis of beef was determined using a Minolta CR-400 chromo meter

calibrated against a white plate ($L^* = 98.45$, $a^* = -0.10$, $b^* = -0.13$), using an 8 mm aperture, Illuminate D65 (6500 K colour temperature) at a standard observation of 2° . L^* (lightness ranged from 0 to 100%), a^* (colour axis ranged from greenness ($-a^*$) to redness ($+a^*$)) and b^* (colour axis ranged from blueness ($-b^*$) to yellowness ($+b^*$)). Measurements of the sample were taken from five locations including every quarter and the centre surfaces. Data was collected after a 30 min blooming period under refrigerated conditions.

Spectrophotometric quantification of basic meat composition. Beef composition (water, fat, protein and connective tissue content (22, 27)) was determined using a near-infrared spectrometer NIRFlex N-500 (Büchi). Measurements were conducted using a NIRFlex Solids module of spectral range $12,500\text{--}400\text{ cm}^{-1}$ in reflectant mode. Meat portions of 100 g were homogenised and placed on a Petri dish covering the surface with a 0.5 cm layer. Three measurements of each sample were conducted at a 32 scanning rate.

Warner-Bretzler shear force determination. Instrumental measurement of hardness (N), tenderness (N), and shear energy (J) was conducted using a universal testing machine Instron 5965 with a Warner-Bratzler shear attachment consisting of a V-notch blade. Six cores (1.27 cm in diameter and 2.5 ± 0.2 cm in length) were obtained from each steak parallel to the muscle fibre's orientation. A 500 N load cell was used and the crosshead speed was set at 200 mm/min.

Statistical analysis. Data was analysed using StatSoft's Statistica 9.0. Verification of significance of investigated parameters for each muscle type (LSD Fisher test) was performed with the significance level set at $P \leq 0.05$. Correlation between basic composition of meat, pH value, and obtained physical parameters was determined using Pearson's linear correlation, and their significances were set at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$.

Results and Discussion

Analysis of differences in the basic composition, pH values, and selected physical properties depending on the type of muscle. Table 1 presents average values of discriminant of the basic meat composition (water, fat, protein, connective tissue content) and the pH values of the five different beef muscles.

The pH values recorded reached a similar level ($P > 0.05$) and ranged between 5.62 and 5.78 indicating no meat defect DFD (dark, firm, dry) (8). The water content ranged between 74.5 and 75.7%. Statistical analysis carried by LSD Fisher test indicated statistically significant differences between water content in LTH and PSM and TRI, SEM, and SET ($P \leq 0.01$). Similar results were also obtained by Jeremiah *et al.* (12) and Li *et al.* (18), who reported diverse water content in different beef muscles.

Table 1
Mean $\pm$ standard deviation of the basic meat composition and pH of beef muscles

	LTH (n=20)	TRI (n=20)	SEM (n=20)	SET (n=20)	PSM (n=20)
Water (%)	74.64 ^{Aa} $\pm$ 1.83	75.72 ^{Bb} $\pm$ 0.99	75.50 ^{ABb} $\pm$ 0.97	75.46 ^{ABb} $\pm$ 1.08	74.51 ^{Aa} $\pm$ 1.31
Fat (%)	2.17 ^{Bb} $\pm$ 1.49	1.62 ^{ABa} $\pm$ 0.75	1.34 ^{Aa} $\pm$ 0.56	1.53 ^{ABa} $\pm$ 0.76	1.09 ^{Aa} $\pm$ 0.19
Protein (%)	22.47 ^{Bb} $\pm$ 0.44	21.90 ^{Aa} $\pm$ 0.47	22.62 ^{Bb} $\pm$ 0.47	22.39 ^{Bb} $\pm$ 0.60	21.80 ^{Aa} $\pm$ 0.44
Connective tissue (%)	1.43 ^{BCb} $\pm$ 0.35	1.62 ^{Cc} $\pm$ 0.27	1.37 ^{Bb} $\pm$ 0.20	1.27 ^{Bb} $\pm$ 0.27	0.97 ^{Aa} $\pm$ 0.25
pH	5.78 $\pm$ 0.44	5.69 $\pm$ 0.28	5.75 $\pm$ 0.42	5.77 $\pm$ 0.50	5.62 $\pm$ 0.23

Means with different letters (a,b; A,B) in lines differ significantly (LSD test: $P \leq 0.05$; $P \leq 0.01$).

Table 2
Mean $\pm$ standard deviation of texture parameters, colour, and drip loss of beef muscles.

	LTH (n=20)	TRI (n=20)	SEM (n=20)	SET (n=20)	PSM (n=20)
Hardness	24.63 ^{Aa} $\pm$ 7.62	44.55 ^{Bb} $\pm$ 4.24	20.86 ^{Aa} $\pm$ 6.13	52.08 ^{Bb} $\pm$ 12.57	21.92 ^{Aa} $\pm$ 5.82
Shear energy	0.099 ^{Aa} $\pm$ 0.031	0.168 ^{Bb} $\pm$ 0.043	0.091 ^{Aa} $\pm$ 0.025	0.189 ^{Bb} $\pm$ 0.074	0.106 ^{Aa} $\pm$ 0.034
Tenderness	11.90 ^{Aa} $\pm$ 2.88	23.16 ^{Bb} $\pm$ 6.40	10.71 ^{Aa} $\pm$ 2.78	25.94 ^{Bb} $\pm$ 6.68	11.70 ^{Aa} $\pm$ 2.76
L* value (%)	36.84 ^{Aa} $\pm$ 3.58	37.40 ^{Aa} $\pm$ 2.70	35.90 ^{Aa} $\pm$ 4.59	41.19 ^{Bb} $\pm$ 4.74	36.25 ^{Aa} $\pm$ 2.81
a* value	19.37 ^{Aa} $\pm$ 2.85	23.11 ^{BCcd} $\pm$ 2.28	20.83 ^{ABab} $\pm$ 3.99	21.69 ^{ABCbc} $\pm$ 4.08	23.78 ^{Cd} $\pm$ 2.78
b* value	8.53 ^{Aa} $\pm$ 2.17	10.28 ^{ABbc} $\pm$ 1.61	9.50 ^{ABac} $\pm$ 2.81	11.44 ^{Bb} $\pm$ 3.18	10.93 ^{Bbc} $\pm$ 1.97
Drip loss (%)	2.24 $\pm$ 1.33	1.95 $\pm$ 0.64	3.00 $\pm$ 1.20	3.04 $\pm$ 1.13	2.32 $\pm$ 1.39

Means with different letters (a,b; A,B) in lines differ significantly (LSD test: $P \leq 0.05$; $P \leq 0.01$).

The LTH was characterised by the highest fat content (2.17%, $P \leq 0.05$), whereas the lowest content was reported for the PSM (1.09%), though at a comparable level to the SEM (1.34%, $P > 0.05$). The LTH, SEM, and SET contained the highest protein level (22.5%) and the results differed statistically ($P < 0.01$) from those obtained for the TRI (21.9%) and PSM (21.8%). With regard to the connective tissue content, the highest value was obtained in the TRI (1.61%), but at a comparable level to the LTH. The lowest content was reported in the PSM (0.97%).

Jeremiah *et al.* (12), Prieto *et al.* (28), and Chang *et al.* (4) had previously indicated a difference between the basic meat composition of various kinds of beef muscles. These researchers identified many factors, which may influence the results, such as the intensity of muscle work, a calorific value of the animal feed, methods and duration of aging, *etc.*

The instrumental measurement of texture parameters by shear force test with Warner-Bratzler attachment allowed for the determination of differences between various muscles in regard to hardness (N) (maximum shear force), shear energy (J) (area below curve), and tenderness (N) (shear force at first peak on the curve) (Table 2). Values of hardness, shear energy, and tenderness were significantly lower for SEM, PSM, and LTH than in SET and TRI ($P \leq 0.01$). Lu *et al.* (19) suggested that the sequence of tenderness of raw beef muscles might be as follows: SET > SEM > LD (*m. longissimus dorsi*). Differences between the texture parameters of individual muscles may be a consequence of various factors: the proportion of isotropic (bright) to anisotropic fibers (dark), thickness perimysium (3), pH (20), structure and composition of connective tissue (29), intramuscular fat (IMF) (17), and sarcomere length (32).

The highest value of lightness was recorded for SET ($L^* = 41.19\%$); the values of lightness of other muscles were significantly ($P \leq 0.01$) lower ($L^* \approx 36\%$). The highest redness was recorded for PSM ($a^* = 23.78$) and TRI ($a^* = 23.11$), whereas the lowest was for LTH ($a^* = 19.37$) ($P \leq 0.01$). Yellowness ($b^* = 8.53$) of LTH differed statistically ($P \leq 0.01$) from values recorded for this parameter for SEM and PSM ($b^* = 11.44$ and $b^* = 10.93$, respectively). Page *et al.* (25) obtained 25.05 as a value of redness (a^*) for LTH and 10.94 as a value of yellowness (b^*). Differences in colour of individual muscles can be caused by myoglobin content, connective tissue, as well as the colour of intramuscular fat (11, 21).

The values of drip loss ranged from 1.95 to 3.04. The difference between results might have been influenced by the protein and collagen content of meat. Furthermore, aging may affect water binding ability as well. Some scientists also claim that the increasing ability to bind water is related to the degradation of cytoskeletal proteins (15).

Correlation between basic meat compositions, pH value, and selected physical properties of bovine muscles. Correlation analysis can be used to estimate and predict many quality parameters of beef. The values of correlation coefficients are presented in Table 3. Low correlation was found between the intramuscular fat content and hardness (N) ($-0.078 \leq r \leq 0.261$), shear energy (J) ($-0.148 \leq r \leq 0.314$), and tenderness (N) ($0.068 \leq r \leq 0.312$) in all muscles, with the exception of the SEM. A significantly high positive correlation was observed between fat content and hardness ($r = 0.673$), shear energy ($r = 0.648$), and tenderness ($r = 0.666$) in the SET. Change of colour parameters L^* , a^* , b^* were slightly dependent on changing intramuscular fat contents.

Fig. 1. Relationship between tenderness (N) and connective tissue content (%) of various bovine muscles: TRI – *M. triceps brachii*; SEM – *M. semimembranosus*; SET – *M. semitendinosus*.

Fig. 2. Relationship between drip loss (%) and pH value of various bovine muscles: LTH – *M. longissimus thoracis et lumborum*; TRI – *M. triceps brachii*; SEM – *M. semimembranosus*; SET – *M. semitendinosus*; PSM – *M. psoas major*.

Only in the case of the SEM, the correlation was moderate, but statistically significant ($r_{L^*}=0.514$, $P\leq 0.05$; $r_{a^*}=0.444$, $P\leq 0.05$; $r_{b^*}=0.573$, $P\leq 0.01$). Correlation coefficient between drip loss and the intramuscular fat content was positive and moderate for the TRI ($r=0.442$), SEM ($r=0.465$, $P\leq 0.05$), and high for the SET ($r=0.605$, $P\leq 0.01$).

No significant correlations were found between protein content and any other physical parameters except for lightness (L^*). A moderate negative correlation, statistically significant ($P\leq 0.05$), was noted for the PSM,

SET, and TRI. Connective tissue content positively correlated with texture parameters (hardness, shear energy, tenderness (Fig. 1)). However, a high correlation was found only for the SET ($r_{\text{hardness}}=0.673$, $r_{\text{shear energy}}=0.605$, $r_{\text{tenderness}}=0.653$; $P\leq 0.01$).

High positive correlation was recorded between connective tissue content and drip loss size for the TRI ($r=0.635$; $P\leq 0.01$) and a moderate correlation for the SEM ($r=0.556$; $P\leq 0.05$).

Table 3
Correlation analysis between the basic meat composition, pH, and physical characteristics of beef muscles

	Content			pH
	Fat	Protein	Connective tissue	
LTH				
Hardness (N)	-0.078	0.001	0.064	0.039
Shear energy (J)	-0.148	0.009	0.120	0.01
Tenderness (N)	-0.083	0.260	0.117	-0.139
Drip loss (%)	0.166	0.021	0.391	-0.501*
L* value (%)	-0.163	-0.120	0.127	-0.689***
a* value	0.267	-0.122	0.463*	-0.542*
b* value	0.273	0.018	0.453*	-0.719***
TRI				
Hardness (N)	0.023	-0.106	0.372	-0.629**
Shear energy (J)	0.092	-0.087	0.379	-0.644**
Tenderness (N)	-0.079	-0.283	0.297	-0.566**
Drip loss (%)	0.442	0.016	0.635**	-0.529*
L* value (%)	0.070	-0.485*	0.228	-0.338
a* value	0.281	-0.080	0.510*	-0.828***
b* value	0.325	0.018	0.434	-0.912***
SEM				
Hardness (N)	0.235	0.158	0.326	-0.522*
Shear energy (J)	0.314	0.349	0.350	-0.625**
Tenderness (N)	0.272	0.223	0.219	-0.554*
Drip loss (%)	0.465*	0.087	0.556*	-0.601**
L* value (%)	0.514*	-0.194	0.320	-0.756***
a* value	0.444*	0.059	0.517*	-0.742***
b* value	0.573**	0.168	0.542*	-0.837***
SET				
Hardness (N)	0.673**	0.125	0.673**	-0.184
Shear energy (J)	0.648**	0.222	0.605**	-0.258
Tenderness (N)	0.666**	-0.013	0.653**	-0.188
Drip loss (%)	0.605**	0.118	0.333	-0.556*
L* value (%)	0.417	-0.518*	-0.022	-0.831***
a* value	0.282	0.096	0.133	-0.593**
b* value	0.361	0.378	0.128	-0.779***
PSM				
Hardness (N)	0.261	-0.422	-0.491*	-0.432
Shear energy (J)	0.117	-0.227	0.383	-0.370
Tenderness (N)	0.261	-0.343	-0.462*	-0.495*
Drip loss (%)	-0.082	-0.052	-0.021	-0.282
L* value (%)	-0.084	-0.553*	-0.201	-0.745***
a* value	-0.072	-0.417	-0.502*	-0.811***
b* value	-0.043	-0.388	-0.523*	-0.820***

* - $P \leq 0.05$; ** - $P \leq 0.01$; *** - $P \leq 0.001$

Positive, moderate correlation was observed for the LTH, TRI, and SEM between connective tissue content and coordinates a^* and b^* ($0.463 \leq r_{a^*} \leq 0.517$; $P \leq 0.05$ and $0.434 \leq r_{b^*} \leq 0.542$; $P \leq 0.05$). For the TRI, SEM, and PSM, the relation between muscle pH value and texture parameters obtained in the shear test was negative, moderate, or high. Statistically significant ($P \leq 0.01$) correlations were obtained for the TRI ($r_{\text{hardness}} = -0.629$, $r_{\text{shear energy}} = -0.644$, $r_{\text{tenderness}} = -0.566$). Strong correlations between pH value and hardness ($r = -0.522$; $P \leq 0.05$), pH value and shear energy ($r = -0.625$; $P \leq 0.01$) were recorded for SEM as well.

Braghieri *et al.* (2) indicated a linear and positive correlation between the pH value measured 1, 24, and 48 h after slaughter and the tenderness of beef meat (*M. longissimus dorsi*) after 2 and 7 d of aging process (tenderness evaluated by a sensory panel). However, no significant correlation was found between pH and tenderness determined by instruments. Furthermore, the research showed that the initial value of pH (1 h after slaughter) and the final value (48 h after slaughter) were both good indicators of meat tenderness. The same authors implied that meat with a higher final pH value was initially more tender than meat with a normal pH value due to rapid meat tenderisation. Nevertheless, these differences decreased when prolonging the aging process. Moreover, the correlation between pH value and tenderness was less significant in relation to values obtained during 2nd d of aging.

Negative moderate correlation was reported between drip loss size and pH values ($-0.601 \leq r \leq -0.501$), with the exception of the PSM ($r = -0.282$) (Fig. 2).

Based on the analysis of pH values of muscles and parameters L^* , a^* , b^* , significant Pearson correlation coefficients were negative. Lightness (L^*) strongly correlated with the pH value of the SET ($r = -0.831$; $P \leq 0.001$) and considerably correlated in case of the SEM and PSM ($r = -0.756$ and $r = -0.745$, respectively; $P \leq 0.001$).

Very strong relationship was recorded between pH values and redness (a^*) for the PSM ($r = -0.811$; $P \leq 0.001$) and the TRI ($r = -0.828$; $P \leq 0.001$), and a strong correlation was found in case of the SEM ($r = -0.742$; $P \leq 0.001$). Parameters for yellowness (b^*) indicated a very high correlation with pH value ($-0.912 \leq r \leq -0.719$; $P \leq 0.001$) for all examined muscles. Page *et al.* (25) also found a negative correlation between pH value and L^* ($r = -0.40$), a^* ($r = -0.58$), b^* ($r = -0.56$) due to increasing pH values of meat. Values of colour parameters tended to decrease. The strong negative correlation between pH values and muscle colour might be explained by the colour of muscle tissue being conditioned by reflectance light of free water and a degree of oxidation of myoglobin (1).

Proteins have a higher ability to bind water in muscles with higher pH values. This leads to a lower level of free water and results in limiting free space between swelling muscle fibres. Meat with higher pH value is darker because there is less free water, which reflects light. In muscles with a higher pH, enzymes using oxygen are more active, which leads to lower oxygenation of myoglobin and a darker colour (25).

In conclusion, raw beef meat characteristics depend on many factors such as: genotype, age, sex, type of feeding, transport conditions, slaughter, duration, and temperature of aging process. These variables influence quality of beef (colour, tenderness, flavour, juiciness). pH values, connective tissue, and intramuscular fat content are indicators used to determine the colour, tenderness, hardness and level of drip loss. Basic meat composition (water, protein, fat, and connective tissue content) depends on the muscle type, its location, and muscle work intensity during animal's life. Muscles, which work less (*e.g.* LTH) contain more fat and less connective tissue in comparison with TRI and SEM. The drip loss correlates strongly negatively with the pH value of beef. Drip loss correlates moderately positively with connective tissue content in beef muscles. Hardness and tenderness correlate strongly positively with fat content in SEM. Colour parameters are most dependent on pH values and in to a lesser degree on the content of connective tissue.

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