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Effect of sex, muscle, and processing temperature on heme iron content in lamb meat*

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The objectives of this study were to assess the effect of sex and a muscle type on heme iron content in meat of Uhruska lambs, and to determine the effect of different processing temperatures (60, 70, 80, and 90°C) on colour attributes, cooking loss and heme iron concentration. Mutton and lamb, just like beef and veal is abundant in iron while iron deficiency is one of the most common causes of nutritional diseases worldwide. The lamb's sex did not significantly influence heme iron content in skeletal muscle with average heme iron level of 14.3µg/g. Concentration of heme iron appeared to be significantly (P≤0.01) muscle type-dependent. Lower heme iron content was found in the *semitendinosus* muscle compared to *semimembranosus*, *biceps femoris*, and *gluteus medius* muscles. Increasing of the processing temperature significantly affected meat colour by increasing lightness (L*), and hue (h°) and reducing redness (a*), yellowness (b*), and saturation (C*). Rising the temperature of treatment from 60 to 90°C significantly increased the cooking loss from 9.1 g/100 g to 36.2 g/100 g and heme iron content from 1.58 mg/100 g to 2.23 mg/100 g. From nutritional point of view, the cooked lamb can be a significant source of heme iron in human diet.

KEY WORDS: colour / lamb / heat treatment / heme iron

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Iron deficiency is one of the most common causes of nutritional diseases worldwide [Clark 2008]. In the human diet, meat is the major source of iron. This chemical element is present as non-heme iron (poorly absorbed form) and heme iron (easily absorbed form) – built into the porphyrin ring, eg. myoglobin or haemoglobin [Samman 2007]. In the raw bird muscle heme iron accounts for 38-42% whereas in mammals 56-90% of total iron [Lombardi-Boccia *et al*. 2002]. The bioavailability of heme iron is high and ranges from 15 to 35%, while non-heme iron absorption is lower oscillating between 2 and 20% [Monson 1988] and depends on many promoters and inhibitors [Samman 2007]. The meat from sheep, horse (2.2 mg/100 g) just like beef and veal (0.9-2.1 mg/100 g) is abundant in iron, while lower of iron is detected in poultry meat (0.59-0.79 mg/100 g) [Lombardi-Boccia *et al*. 2002] (except for ostrich meat (2.32-3.04) [Sales and Horbańczuk, Poławska *et al*. 2011]) and pork (0.36-0.49 mg/100 g) [Lombardi-Boccia *et al*. 2002]. High quantities of iron are also found in ruminant offal [Abdullah 2008, Florek *et al*. 2012, Seong *et al.* 2014]. For this reason, the nutritional value of sheep meat may make it an important competitor on the meat market [Pannier *et al*. 2014].

The colour of meat is one of the most important quality attributes evaluated by consumers upon purchase. The colour of meat is determined by two major pigments i.e. myoglobin and haemoglobin, by cytochrome C, cobalamin, and adipose tissue. The intensity of colour depends on the pigment concentration (indirectly – on heme content) and the species, breed, sex, age of animals as well as on type of muscle or muscle fibre [Mancini and Hunt 2005, Pannier *et al*. 2014]. Meat colour after the heat treatment relies on pH, species, fat content, packaging conditions, freezing process, added substances and fixing treatment, such as ionization or high pressure [Domaradzki *et al*. 2011, King and Whyte 2006].

The heat treatment of the food is carried out to ensure product appropriate eating qualities including taste, flavour and texture, extend the product shelf life through heat inactivation of enzymes and microorganisms and finally, to increase the nutrient bioavailability, particularly proteins [Tornberg 2005, King and Whyte 2006].

The objectives of this study were 1) to determine the effect of sex and a muscle type on heme iron content in lamb meat, and 2) to determine the effect of heat processing on colour attributes, cooking loss and heme iron concentration.

Material and methods

Animals, slaughter procedures and sample collection

The research material comprised different skeletal muscles of 10 slaughter lambs of Uhruska breed; 5 rams and 5 ewes. The animals were slaughtered at the age of 120-135 days, and weight of 34.5±3.9 kg for the rams and 32.7±3.2 kg for the ewes. Stunning, slaughter of animals, and dissection of carcass were performed in accordance with the standard procedures of the meat industry under constant

supervision of veterinary inspection. During leg dissection, 4 skeletal muscles were collected i.e. *semimembranosus* – SM, *semitendinosus* – ST, *biceps femoris* – BF and *gluteus medius* – GM which were weighed and vacuum packed in PA/PE foil bags and stored at 4°C until assayed. A total of 40 skeletal muscle samples were collected.

Total pigment and heme iron

Total heme pigment content was determined in SM, ST, BF and GM muscles according to the Hornsey's method [1956]. The homogenised muscle samples (raw or heated) were extracted with an acetone and HCl solution, stored sealed for 2 h in the dark at the room temperature (with repeated manual mixing), and then filtered (Whatman No 1). The absorbance was measured using a Varian Cary 300 Bio spectrophotometer (Varian Australia PTY, Ltd.) at 640 nm wavelength against a blank sample. Using the ratio of 680 and the weight of the test portion, the overall concentration of heme pigments (hematin) in μg/g muscle tissue was calculated. Heme iron content was estimated by multiplying the hematin quantity by factor 0.0882 μg iron/μg hematin [Merck 1989].

Cooking loss measurement

The *gluteus medius* muscle samples of approx. 50 g weight were put into foil bags and heat-treated in water bath (LaboPlay, W615, Poland) for 45 min. at 60, 70, 80 or 90°C, and then cooled with running water for 30 minutes and stored at 4°C until assayed. The cooking loss following the heat treatment was estimated by the difference in the muscle sample weight before and after the thermal treatment.

Colour measurement

The colour attributes of the *gluteus medius* muscle were evaluated before and after cooking using chroma meter Minolta CR-310 (Minolta Camera Co. Ltd., Osaka, Japan), illumination D65, 0° projection angle and 50 mm measurement area. The colour measurement was performed on the exposed surface of the muscle cross-sectional area and the results in colour space CIE (CIE 2004) are given for the following attributes: L* – lightness; a* – redness; b* – yellowness, h° – hue and C* – saturation. Additionally a*/b* proportion was calculated and the total colour difference (ΔE) between fresh meat and heat-treated meat at four temperatures was defined by the formula:

$$\Delta E = [(L^* - L_0)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{0,5}$$

where:

ΔE – total colour difference;

L^*_0, a^*_0, b^*_0 – fresh meat colour parameters;

L^*, a^*, b^* – values of colour parameters after heat treatment (60, 70, 80 and 90°C).

Statistical analysis

Statistical analyses were performed using SAS software (Enterprise Guide 6.1 2013). Two-way analysis of variance was used to verify the impact of sex (♂♂ vs. ♀♀) and a muscle (SM, ST, BF i GM) on heme iron content. A univariate analysis of variance evaluated the impact of temperature (60, 70, 80 or 90°C) on colour attributes, cooking loss and heme iron content. The significance of differences between the groups was determined by Tukey's test ($P \leq 0.05$ and $P \leq 0.01$). In order to determine the relationship between the treatment temperature and cooking loss, colour traits and heme concentration, Spearman's rank correlations were calculated.

Results and discussion

Table 1 presents average weights of the investigated skeletal lamb muscles. No significant impact of lamb sex on muscle weight was recorded. The differences observed in the skeletal muscle weight, irrespective of lamb sex, were due to the obvious anatomical differences.

Table 1. Lamb skeletal muscle weight (g) in relation to sex

Muscle	Sex				Average	
	ewe		ram			
	LSM	SD	LSM	SD	LSM	SD
SM	379	69	411	43	392.6	55
BF	262	43	278	25	269.8	35
ST	97	23	104	17	99.9	20
GM	163	38	177	27	170.8	32

SM – *semimembranosus*; GM – *gluteus medius*; BF – *biceps femoris*; ST – *semitendinosus*.

The average heme iron contents in the raw skeletal muscles of lambs are summarized in Table 2. Statistical analysis did not confirm any interaction of sex x muscle or a significant effect of sex on heme iron level in lamb's meat. Lower iron contents (of about 1.6 μg/g) were found in ewe meat compared to that of rams. The heme iron contents in lamb reported in the present study are congruent with the study results of Carpenter and Clark [1995] (14 μg/g) and Lombardi-Boccia *et al*. [2002] (16.2 μg/g). Purchas *et al.* [2003] found significant effect of lamb sex on iron content in three skeletal muscles (*longissimus lumborum*, *semitendinosus* and *triceps brachii*). Contrary to the current study, the aforementioned authors found higher iron level (ca. 17%) in ewes (15.6 μg/g) compared to rams (13.6 μg/g).The authors, however, could not provide any good justification for that as the lambs were at the same age and raised contemporarily. In subsequent studies, Purchas *et al*. [2004] confirmed also lack of significant effect of cattle and sheep sex on iron content in beef and sheep meat, a higher level of this element was detected in lamb (approx. 17%).

Table 2. Heme iron content (μg/g) in raw muscles depending on sex and lamb muscle

Sex	SM		BF		ST		GM		Average	
	LSM	SD	LSM	SD	LSM	SD	LSM	SD	LSM	SD
♂♂	17.3^{c}	2.2	15.8^{bc}	2.3	10.7^{a}	1.0	16.5^{bc}	2.2	15.1	2.9
♀♀	15.2^{bc}	1.4	14.0^{b}	0.6	10.3^{a}	1.0	14.4^{b}	1.4	13.5	1.1
Total	16.2^{b}	1.7	15.5^{b}	1.5	10.5^{a}	1.0	14.9^{b}	1.8	14.3	2.8

SM – *semimembranosus*; GM – *gluteus medius*; BF – *biceps femoris*; ST – *semitendinosus*.
^{abc}Within rows means with different superscripts differ significantly at P≤0.05.

A significant (P≤0.05) differentiation in the iron level between the assessed muscles (Tab. 2) was found in our study. Lower heme iron content was recorded in the ST muscle as compared to the BF muscle (about 4.39 μg/g), GM (about 4.96 μg/g) and SM (about 5.73 μg/g).

Significantly (P≤0.05) highest heme iron level was found in the SM muscle of rams, whereas the lowest in the ST muscle, regardless sex (Tab. 2). In the other muscles, the heme iron content did not differ significantly. Differences in iron content in various lamb skeletal muscles were demonstrated by Purchas *et al*. [2003]. The *semitendinosus* contained 10.5 μg/g heme iron, *triceps brachii* 15.8 μg/g, whereas *longissimus lumborum* 16.9 μg/g. Similar variability was also shown by Carpenter and Clark [1995] for beef and poultry meat samples obtained from different body parts. The heme iron content ranged from 20 μg/g in minced meat, 21 μg/g in the leg, up to 26 μg/g in roast beef. The poultry breast meat contained 1.4 μg/g of heme iron on average, whereas the meat from legs contained 3.6 μg/g heme iron. Lombardi-Boccia *et al*. [2002] indicated a similar heme iron content in muscles of animals from the above mentioned species, that is: poultry 1.2-2.9 μg/g; beef 16.8-21.1 μg/g, and lamb, horsemeat and pork, 16.8; 17.5 and 2.6 μg/g, respectively.

The results of the instrumental colour measurement of the GM muscle before and after the heat treatment according to CIE are shown in Table 3. The heat treatment causing the muscle proteins denaturation, including myoglobin, has significantly

Table 3. Results of colour measurement and the total difference according to CIE for L*a*b* of *gluteus medius* muscle in different temperatures of treatment

Parameter	Temperature (°C)									
	Raw		60		70		80		90	
	LSM	SD	LSM	SD	LSM	SD	LSM	SD	LSM	SD
L*	44.27	1.85	53.09^{A}	0.91	57.54^{B}	1.07	56.66^{B}	1.00	57.69^{B}	1.02
a*	25.01	0.76	20.73^{D}	0.78	17.58^{C}	0.86	13.85^{B}	0.27	12.45^{A}	0.17
b*	1.30	0.61	5.39^{B}	0.31	4.58^{A}	0.06	4.57^{A}	0.41	4.56^{A}	0.12
C*	25.04	0.78	21.42^{D}	0.69	18.17^{C}	0.84	14.58^{B}	0.36	13.26^{A}	0.17
h°	2.90	1.34	14.52^{A}	1.27	14.52^{A}	0.56	18.12^{B}	1.29	20.06^{B}	0.48
a*/b*	25.00	0.76	3.86^{B}	0.37	3.84^{B}	0.15	3.05^{A}	0.25	2.73^{A}	0.08
ΔE S-60,70,80,90	–	–	10.72^{A}	2.43	15.62^{AB}	2.83	17.08^{B}	2.44	18.73^{B}	2.08

$^{AB...}$In rows means bearing different superscripts differ significantly at P≤0.01.

($P \leq 0.01$) increased lightness L* and hue h°, decreased redness a* and yellowness b* and declined saturation C*.

The temperature and time of heat treatment have significant impact on the physical properties of meat, eating quality and nutritional value. The temperature-dependent colour change relies not only on the total amount of myoglobin and its derivatives in muscles but on the chemical status of the denaturated muscle proteins (i.e. myosin and actin) as well [Tornberg 2005]. The higher thermal treatment temperature, the lighter meat surface becomes because of increased reflectance and scattering of light by denaturated proteins [Young and West 2001]. Lightness of the GM (L*=43.2) similar to that obtained in the present study was also reported by Tschirhart-Hoelscher *et al*. [2006], while for the chromatic parameters a* and b*, lower values (16.5 and 4.3) were estimated.

It is noteworthy that the proportion of redness (a*) and saturation (C*) decreased progressively with increasing temperature. In the case of lightness (L*) and yellowness (b*), no significant differences were found within the range of 70-90°C, whereas hue (h°) within the 80-90°C range. A significant decrease of redness during the thermal processing (from 60 to 90°C – over some 8 units) while yellowness remaining at the same level (70-90°C), significantly ($P \leq 0.01$) reduced the proportion of a*/b*. However, significant differences were observed between the 60-70°C and 80-90°C range. As for total colour difference (ΔE), the temperature 70°C proved to be the limit value. Similar results were found earlier by Liu *et al.* [2013] who subjected the goat SM to heat treatment within the range of 50-90°C. Obuz and Dikeman [2003] evaluating the properties of bovine *longissimus lumborum,* heated the samples up to the final temperature 70°C and obtained values for L*=56.77, and for a*=19.49. Gašperlin *et al.* [2000] heated beef *longissimus dorsi* to 75°C, and reported the following CIE values; L*=56.9, a*=12.9 and b*=12.0. The lower L* and a* values (54.6 and 8.7 respectively) and slightly higher b* (12.7) were recorded for the heated muscle after 60 minutes of exposure to oxygen (blooming).

It was evidenced that the heme pigment contents in pork [Lindahl *et al*. 2001] and beef [Florek *et al*. 2009] were correlated with the a* value (at approx. 0.50). Besides, based on the redness loss (decrease in a*), the degree of meat pigment oxidation can be evaluated [Mancini and Hunt 2005]. Higher a*/b* value indicates higher concentration of both (deoxy)myoglobin (Mb) and oxymyoglobin (MbO_2) on the meat surface, whereas low value shows high concentration of metmyoglobin (MetMb) [Strange *et al.* 1974]. The present study highlighted two significantly different limit ranges of temperature for this trait, i.e 60-70°C and 80-90°C. The total colour difference ΔE (as a function of changes in L*, a* and b*), can be regarded as an indicator describing the effect of heating on the overall colour change.

Various forms of myoglobin in beef exhibit different sensitivity to heat. The least susceptible to the heat treatment proved to be deoxymyoglobin, oxymyoglobin and metmyoglobin, wherein the sensitivity of two forms was very similar [Hunt *et al.* 1999]. The denaturation of myoglobin starts between 55°C and 65°C, and the process

is most intense between 75°C and 80°C. However with increasing temperature, the denaturation rate of myoglobin is reduced [Hunt *et al*. 1999] which is confirmed by the results of studies conducted on lamb stored in vacuum conditions. Seyfert *et al*. [2004] reported that vacuum-packed beef before heat treatment contained primarily deoxymyoglobin, hence heated at 71.1°C it developed light pink colour. Gašperlin *et al.* [2000] showed that in beef heated up to 75°C, myoglobin changes virtually do not occur because of small amount of pigments in the native state. The final meat colour also depends on a muscle type, which may be associated with variable concentration and thermal stability of reacting proteins in the muscle groups. It is in agreement with the results obtained by Lytras *et al*. [1999], who reported higher myoglobin denaturation rate (at temp. <70°C) in loin samples compared to shoulder and leg samples.

Table 4 presents the cooking loss and heme iron content in the GM muscle depending on the heat treatment temperature. Significant increase ($P \leq 0.01$) of cooking loss with the increasing thermal treatment temperature was observed and, notably, at 90°C threefold higher cooking loss was detected as compared to 60°C. It was also found that the increasing temperature caused significant ($P \leq 0.01$) rise of heme iron concentration. During the thermal treatment, collagen and myofibrillar and sarcoplasmic proteins undergo denaturation, shrinking, and their solubility change as well. As a result, the mechanical properties of meat change and cooking loss after the heat treatment occurs [García-Segovia *et al*. 2007]. In the initial phase of heat treatment, the cooking loss increase is caused mainly by sarcoplasmic proteins denaturation in the muscle (initiating at 40°C, and finishing at 65°C) [Tornberg, 2005]. The cooking loss tends to rise with the increasing temperature in the following stage of the process, which can be attributed to thermal denaturation of myosin (40-60°C) and actin (66-73°C), contraction and partial solubility of collagen (56-62°C) [Palka and Daun 1999]. This process causes further intense muscle fibre shortening (along the long axis of muscle) and in turn, thermal loss occurrence at elevating temperature (80-90°C) [Tornberg 2005]. Purchas *et al*. [2004] assessed the impact of cooking temperature (60, 65, 70, 75, 80 and 85°C) on the bovine ST muscle parameters and demonstrated steady and significant cooking loss increase from 27.2% to 38.7%. Liu *et al.* [2013] analyzed the goat SM muscle and arrived at similar results for cooking

Table 4. Cooking loss and heme iron concentration in *gluteus medius* muscle depending on final temperature of thermal treatment

Item	Temperature (°C)							
	60		70		80		90	
	LSM	SD	LSM	SD	LSM	SD	LSM	SD
Cooking loss (g/100 g)	10.9^{A}	0.8	17.6^{B}	1.7	25.3^{C}	2.4	32.6^{D}	3.1
Heme iron (mg/100 g)	1.58^{A}	0.1	1.79^{AB}	0.2	2.17^{BC}	0.1	2.23^{C}	0.4

$^{AB...}$ In rows means bearing different superscripts differ significantly at $P \leq 0.01$.

loss within the temperature range of 60 and 70°C (8,71% and 15,38%) as compared to the present study, yet higher in the 80 and 90°C range (33.08% and 41.25%).

The present research has indicated a significant (P≤0,01) increase of heme iron content (insoluble fraction) after the thermal processing at higher temperature ranges and associated it with significantly higher cooking loss causing elevated concentration of dry matter in the samples [Purchas *et al*. 2004]. However, as the other authors evidenced [Purchas *et al*. 2003, 2004; Lombardi-Boccia *et al.* 2002], generally the content of heme iron in total iron declines under the heat treatment. This is due to the porphyrin ring oxidation and iron release (heme and nonheme) in soluble form [Kristensen and Purslow 2001]. Purchas *et al.* [2004] showed, that such a change could determine iron bioavailability in the ST muscle of young slaughter cattle. Lombardi-Boccia *et al*. [2002] reported 1.68 mg/100 g heme iron in raw lamb meat, while as much as 2.25 mg/100 g after the heat treatment (unknown temperature); its content in total iron decreased by 7%. The authors also indicated a possibility for controlling (to reduce losses) heme iron during thermal treatment through the use of milder processing conditions which enhance the heme molecule stability.

Table 5 summarizes the Spearman's correlation coefficients which indicate significant positive or negative correlations between the heat treatment temperature and colour parameters (except ΔE), cooking loss and heme iron content in the GM muscle. Positive correlation was found between temperature and: lightness (L*), hue (h°), heme iron content, and cooking loss, while negative for the other colour traits (a*, b*, C* and a*/b*).

Table 5. Spearman's correlation coefficients between heat treatment temperature and colour traits, cooking loss and heme iron content in *gluteus medius*

Trait	Temperature
L*	$0.60^{\#\#}$
a*	$-0.97^{\#\#\#}$
b*	$-0.58^{\#\#}$
C*	$-0.97^{\#\#\#}$
h°	$0.87^{\#\#\#}$
ΔE R-60 70 80 90	0.41
a*/b*	$-0.86^{\#\#\#}$
Heme iron (mg/100 g)	$0.76^{\#\#\#}$
Cooking loss (g/100 g)	$0.95^{\#\#\#}$

$^{\#\#}$P≤0.01; $^{\#\#\#}$P≤0.001.

Buchowski *et al.* [1988] assessed the temperature effect on content of total and heme heme iron and found that meat heated to 97°C retained 85.3% of iron, compared to meat heated up to 60°C (81.6%) or 77°C (78.2%). To retain the highest amounts of iron heme in meat, it should be cooked at a temperature high enough to coagulate proteins readily which in turn, inhibit iron release. It was shown that iron retention

depending on the treatment time increased significantly with rising temperature, that is 80.3% and 81.2% at 60°C to 91.0% and 90.5% at 90°C [Purchas *et al*. 2003]. Buchowski *et al*. [1988] demonstrated that slow heating causes a greater loss of heme iron. In heated meat, hemoproteins do not release their heme residues and most heme is still bound to the globin. For this reason, enhanced release of iron from heme occurs only when the temperature rises from 85°C to 100°C [Han *et al.* 1993].

Purchas *et al.* [2004] showed that the increasing of heat treatment temperature (from 60 to 85°C) of the bovine ST contributed to elevation of heme iron concentration from 2.15 mg/100 g to 2.53 mg/100 g. In the earlier studies, these authors found similar relationships for lamb when considering different heat treatment parameters (temperature=60/80/98°C and time=30/90 min) [Purchas *et al*. 2003]. In their opinion, optimum heat treatment parameters which retain iron in heme form is heating meat in the water bath at 80°C for 30 min.

The present research results indicate that average heme iron content in lamb after the heat treatment (temp. ≥80°C) was ca. 2.2 mg/100 g (Tab. 4). Taking into account only heme iron content and not its total amount, lamb can be regarded as a significant source of iron for adults i.e. product containing at least 15% of the reference intake value of his element (14 mg) in 100 g of meat after heat treatment [Regulation (UE) No 1169/2011 on October, 15 2011, OJ L 304 on November, 22 2001, p. 18]. According to the Polish nutrition standards [Jarosz *et al.* 2012] lamb meet is recognized as a significant source of iron for children, youth, men and women (excluding groups aged up to 50 years) for which the recommended dietary allowances range from 10 to 15 mg.

In conclusion, results of the present research showed that the concentration of heme iron in lamb muscles was similar in ewes and rams, but was significantly muscle type-dependent. Lower heme iron content was determined in the *semitendinosus* muscle as compared to *semimembranosus*, *biceps femoris* and *gluteus medius* muscles. Higher treatment temperature (from 60 to 90°C) significantly increased the cooking loss (from 10.9 g to 36.2 g/100 g) and heme iron concentration (from 1.58 mg to 2.23 mg/100 g). The temperature affected also meat colour by increasing lightness L* and hue h°, and reducing redness a*, yellowness b*, and saturation C*. From nutritional point of view, the lamb meat after cooking could be a significant source of heme iron in human diet.

REFERENCES

1. ABDULLAH B.M., 2008 – Composition, chemical and microbiological properties of Jordanian ovine organ meats. International ***Journal of Food Science and Technology*** 43(4), 746-751.
2. BUCHOWSKI M.S., MAHONEY A.W., CARPENTER C.E, CORNFORTH D.P., 1988 – Heating and distribution of total and heme iron between meat and broth. ***Journal of Food Science*** 53, 43-45.
3. CARPENTER C.E., CLARK E., 1995 – Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. ***Journal of Agricultural Food and Chemistry*** 43, 1824-1827.
4. CIE, 2004 – Commission International de l'Eclairage. Colourimetry. (3rd ed.). Commission International de l'Eclairage. Vienne, Austria (pp. 16-20).
5. CLARK S.F., 2008 – Iron deficiency anemia. ***Nutrition in Clinical Practice*** 23, 128-141.

6. DOMARADZKI P., SKAŁECKI P., FLOREK M., LITWIŃCZUK A., 2011 – Wpływ przechowywania zamrażalniczego na właściwości fizykochemiczne mięsa wołowego pakowanego próżniowo (Effect of freezing storage on physicochemical properties of vacuum-packed beef). In Polish, summary in English. ***ŻYWNOŚĆ. Nauka, Technologia, Jakość*** 3(76), 117-126.
7. FLOREK M., LITWIŃCZUK Z., SKAŁECKI P., GRODZICKI T., 2009 – Colour of carcass and meat of calves slaughtered at different body weights. ***Fleischwirtschaft International***, 24(4), 59-62.
8. FLOREK M., LITWIŃCZUK Z., SKAŁECKI P., KĘDZIERSKA-MATYSEK M., GRODZICKI T., 2012 – Chemical composition and inherent properties of offal from calves maintained under two production systems. ***Meat Science*** 90, 402-409.
9. GARCÍA-SEGOVIA P., ANDRÉS-BELLO A., MARTÍNEZ-MONZÓ J., 2007 – Effect of cooking method on mechanical properties, colour and structure of beef muscle (M. *pectoralis*). ***Journal of Food Engineering*** 80, 813-821.
10. GAŠPERLIN L., ŽLENDER B., ABRAM V., 2000 – Colour of normal and high pH beef heated to different temperatures as related to oxygenation. ***Meat Science*** 54, 391-398.
11. HAN D., MCMILLIS K.W., GODBER J.S., BIDNER T.D., YOUNATHAN M.T., MARSHALL D.L., HART L.T., 1993 – Iron distribution in heated beef and chicken muscles. ***Journal of Food Science*** 58, 697-700.
12. HORNSEY H.C., 1956 – The colour of cooked cured pork I. Estimation of the nitrioxidehaem pigments. ***Journal of Science Food and Agriculture*** 7, 534-540.
13. HUNT M.C., SŘRHEIM O., SLINDE E., 1999 – Colour and heat denaturation of myoglobin forms in ground beef. ***Journal of Food Science*** 64, 847-851.
14. JAROSZ M. (Ed.), 2012 – Normy żywienia dla populacji polskiej – nowelizacja (Detary references for the Polish population - the amendment.). In Polish. Instytut Żywności i Żywienia, Warszawa.
15. KING N.J., WHYTE R., 2006 – Does it look cooked? A review of factors that influence cooked meat colour. ***Journal of Food Science*** 71, 4, R31-R40.
16. KRISTENSEN L., PURSLOW P.P., 2001 – The effect of processing temperature and addition of mono- and di-valent salts on the heme-nonheme-iron ratio in meat. ***Food Chemistry*** 73, 433-439.
17. LINDAHL G., LUNDSTROM K., TORNBERG E., 2001 – Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. ***Meat Science*** 59(2), 141-151.
18. LIU F., MENG L., GAO X., LI X., LUO H., DAI A., 2013 – Effect of end point temperature on cooking losses, shear force, colour, protein solubility and microstructure of goat meat. ***Journal of Food Processing and Preservation*** 37(3), 275-283.
19. LOMBARDI-BOCCIA G., MARTINEZ-DOMINGUEZ B., AGUZZI A., 2002 – Total Heme and Non-heme Iron in Raw and Cooked Meats. ***Journal of Food Science*** 67, 1738-1741.
20. LYTRAS G.N., GEILESKEY A., KING R.D., LEDWARD D.A., 1999 – Effect of muscle type, salt and pH on cooked meat haemoprotein formation in lamb and beef. ***Meat Science*** 52, 189-94.
21. MANCINI R.A., HUNT M.C., 2005 – Current research in meat colour. ***Meat Science*** 71, 100-121.
22. MERCK, 1989 – Merck Index: An encyclopedia of chemicals, drugs, and biologicals. (11th ed.). Merck and Company. Rahway, N.J., pp. 1605.
23. MONSON E.R., 1988 – Iron nutrition and absorption: dietary factors which impact iron bioavailability. ***Journal of American Dietetic Association*** 88, 786-90.
24. OBUZ E., DIKEMAN M.E., 2003 – Effects of cooking beef muscles from frozen or thawed states on cooking traits and palatability. ***Meat Science*** 65, 993-997.
25. PALKA K., DAUN H., 1999 – Changes in texture, cooking losses, and myofibrillar structure of bovine m. semitendinosus during heating. ***Meat Science*** 51, 237-243.

26. PANNIER L., PETHICK D.W., BOYCE M.D., BALL A.J., JACOB R.H., GARDNER G.E., 2014 – Associations of genetic and non-genetic factors with concentrations of iron and zinc in the *longissimus* muscle of lamb. ***Meat Science*** 96, 1111-1119.
27. POŁAWSKA E, MARCHEWKA J, COOPER R.G, SARTOWSKA K, POMIANOWSKI J, JÓŹWIK A, STRZAŁKOWSKA N, HORBAŃCZUK J.O., 2011 – The ostrich meat – an updated review. II. Nutritive value. Animal Science Papers and Reports, 29, 89-97.
28. PURCHAS R.W., RUTHERFORD S.M., PEARCE P.D., VATHER R., WILKINSON B.H.P., 2004 – Cooking temperature effects on the forms of iron levels of several other compounds in beef *semitendinosus muscle*. ***Meat Science*** 68, 201-207.
29. PURCHAS R.W., SIMCOCK D.C., KNIGHT T.W., WILKINSON B.H.P., 2003 – Variation in the form of iron in beef and lamb meat and losses of iron during cooking and storage. ***International Journal of Food Science and Technology*** 38, 827-837.
30. SALES J., HORBAŃCZUK J., 1998 – Ratite meat. ***World's Poultry Science Journal*** 54, 59- 67.
31. SAMMAN S., 2007 – Iron. ***Nutrition & Dietetics*** 64(Suppl 4), 5126-5130.
32. SAS, 2013 – Enterprise Guide 6.1. SAS Institute Inc., Cary, NC, USA.
33. SEONG P.N., KANG G.H., PARK K.M., CHO S.H., KANG S.M., PARK B.Y., MOON S.S., BA H.V., 2014 – Characterization of Hanwoo Bovine By-products be Means of Yield, Physicochemical and Nutritional Compositions. ***Korean Journal for Food Science of Animal Resources*** 34(4), 434-447
34. SEYFERT M., MANCINI R.A., HUNT M.C., 2004 – Internal premature browning in cooked ground beef patties from high oxygen modified-atmosphere packaging. ***Journal of Food Science*** 69(9), 721-725.
35. STRANGE E.D., BENEDICT R.C., GUGGER R.E., METZGER V.G., SWIFT C.E., 1974 – Simplified methodology for measuring meat colour. ***Journal of Food Science*** 39(5), 988-992.
36. TORNBERG E., 2005 – Effects of heat on meat proteins - Implications on structure and quality of meat products. ***Meat Science*** 70, 493-508.
37. TSCHIRHART-HOELSCHER T.E., BAIRD B.E., KING D.A., MCKENNA D.R., SAVELL J.W., 2006 – Physical, chemical, and histological characteristics of 18 lamb muscles. Meat Science 73, 48-54.
38. YOUNG O.A., WEST J., 2001 – Meat colour. In Meat Science and Applications (Y.H. Hui, W.-K. Nip, R.W. Rogers and O.A. Young, eds.) pp. 39–69, Marcel Dekker Inc., NewYork, NY.