

## THE EFFECT OF SALT ON MYOGLOBIN FORMS OF CATTLE AND PIG MUSCLES<sup>1</sup>

Maria Walczycka, Marzena Nowak T. Kolczak<sup>2</sup>

**Abstract:** The aim of research was to estimate the effect of salt addition on myoglobin forms in muscles of cattle with different content of haem pigments and in pig muscles with different quality characteristics.

The experimental material were muscles *psaos major*, *trapezius*, *semitendinosus* and *sternomandibularis* of calves, heifers and cows and *longissimus dorsi* of pigs with PSE, normal and DFD characteristics. The total haem pigments' content and the percentages of reduced myoglobin (Mb), oxymyoglobin (MbO<sub>2</sub>) and metmyoglobin (MetMb) were measured in unsalted and salted with 2% of NaCl muscles.

The amount of haem pigments increased with somatic ageing of cattle and was the lowest in calves, medium in heifers and the highest in cows. The addition of salt was causing the percentage growth of MetMb levels by lowering MbO<sub>2</sub> levels.

The haem pigments content in pork muscles was the lowest in PSE, medium in normal and the highest in DFD muscles. MbO<sub>2</sub> was the main myoglobin form in analyzed pork muscles but its percentage level was lower than in cattle muscles. The NaCl addition increased MetMb levels in PSE and normal meats and did not change the MetMb level in DFD meat.

### Introduction

Meat colour is the most important quality attribute for the consumer at the time of purchase (Abril *et al.*, 2001; Cornforth, 1994; Karlsson and Lundström, 1991). In modern meat trade of culinary cuts, in big retailing centers, the meat colour is the most important way of estimation of quality and freshness (shelf-life) (Stryer, 1997; Palka *et al.*, 1994; Renner and Labas 1987).

The main factor influencing the colour of meat is myoglobin and its forms content. (Abril *et al.* 2001, Giddings, 1977). The pinky-red colour is pointing the meat freshness for the consumer. Brown meat colour is poorly estimated, meat is seen as not fresh and is in lower demand (Philips *et al.*, 2001).

The aim of research was to estimate the effect of salt addition on myoglobin forms in muscles of cattle with different content of haem pigments and in pig muscles with different quality characteristics.

### Material and methods

The experimental materials were muscles *psaos major*, *trapezius*, *semitendinosus* and *sternomandibularis* of calves, heifers and cows as well as muscle *longissimus dorsi* of pigs with PSE, normal and DFD characteristics. Quality of pigs' muscles was estimated on the basis of pH measurements at 1<sup>st</sup> (pH<sub>1</sub>) and 24<sup>th</sup> (pH<sub>24</sub>) hour after slaughter; muscles with pH<sub>1</sub>>6,3 and pH<sub>24</sub> 5,6-5,8 were considered as a normal, with pH<sub>1</sub><6,0 and pH<sub>24</sub><5,6 as a PSE, with pH<sub>1</sub>>6,5 and pH<sub>24</sub>>6,0 as a DFD. Muscle samples were taken at 24 hour after slaughter from all carcasses. The sample of muscle (proximately 150g) was ground through a 0,3 cm plate in a mixer/grinder, mixed thoroughly by hand and divided into two parts. The first part, directly after grounding, was estimated for total haem pigments' content and the percentages of myoglobin forms i.e. reduced myoglobin (Mb), oxymyoglobin (MbO<sub>2</sub>) and metmyoglobin (MetMb). The second part was salted with 2% of sodium chloride, mixed, vacuum packaged and stored for 20 hours in 2°C, then percentages of Mb, MbO<sub>2</sub> and MetMb were measured. The total haem pigments content was estimated on haematin concentration basis (Hornsey, 1956). Percentage of myoglobin forms in muscle extracts of 0,04 M phosphate buffer pH 6,8 (Warris, 1979) was measured using formula of Krzywicki (1982). Muscle samples in each experimental group were taken from three animal carcasses. All variables were analyzed by analysis of variance with Statistcs 5.1.

### Results and discussion

The haem pigment levels in beef muscles are shown in table 1. The contents of haem pigments depended significantly on cattle age and anatomical muscle origin. The haem pigment content was the lowest in calves, medium in heifers and the highest in cow's muscles. The amounts of myoglobine in analysed muscles are in agreement with other data (Abril *et al.* 2001, Lawrie 1985). During development and ageing of animal the affinity of haem pigments to oxygen diminishes (Cornforth, 1994; Maclean, 1978). The higher

<sup>1</sup> Original scientific paper – Originalni naučni rad

<sup>2</sup> Maria Walczycka PhD., Marzena Nowak MSc., Tadeusz Kolczak PhD. Full Professor: Animal Products Processing Dept., Food Technology Faculty, University of Agriculture, Krakow, Poland.

synthesis of myoglobin, the higher growth of its concentration in cells and change in muscles colour. These are caused by assuring enough metabolic efficiency of muscles during growth and development of animal.

Table 1. Haem pigments (haematin content) in cattle muscles (mean  $\pm$  standard error).

Cattle age	Muscle	Haematin content [mg/100g of muscle]
Calves	<i>m. psoas major</i>	12,12 $\pm$ 0,35
	<i>m. semitendinosus</i>	7,23 $\pm$ 0,31
	<i>m. sternomandibularis</i>	9,27 $\pm$ 0,89
	<i>m. trapezius</i>	9,84 $\pm$ 0,56
Heifers	<i>m. psoas major</i>	10,48 $\pm$ 1,08
	<i>m. semitendinosus</i>	11,45 $\pm$ 0,95
	<i>m. sternomandibularis</i>	15,57 $\pm$ 1,49
	<i>m. trapezius</i>	16,23 $\pm$ 0,60
Cows	<i>m. psoas major</i>	17,39 $\pm$ 1,48
	<i>m. semitendinosus</i>	15,31 $\pm$ 1,27
	<i>m. sternomandibularis</i>	21,39 $\pm$ 1,17
	<i>m. trapezius</i>	20,62 $\pm$ 1,43

The haem pigment content was higher in *sternomandibularis* and *trapezius* muscles than in *psoas major* and *semitendinosus* muscles. All these muscles differentiate in physiological activity and muscle fibres composition. *Psoas major* and *semitendinosus* muscles are less active, contain more of white fibres with less sarcoplasm and have weaker circulatory system in comparison to *sternomandibularis* and *trapezius* muscles (Cornforth, 1994; Rickansrud and Henrickson, 1967). Those data were confirmed in this assessment. The relative high level of haem pigments in *psoas major* muscle of calves was rather surprising and there should be further investigation.

Table 2. The percentage content of reduced myoglobin (Mb), oxymyoglobin (MbO<sub>2</sub>), metmyoglobin (MetMb) of calves, heifers and cows' muscles not salted and salted (mean  $\pm$  standard error)

Cattle group	Muscles	Salt additive [%]	Mb [%]	MbO <sub>2</sub> [%]	MetMb [%]
Calves	<i>m.psoas major</i>	0	25,70 $\pm$ 3,08	51,27 $\pm$ 5,62	4,92 $\pm$ 0,80
		2	17,42 $\pm$ 1,07	55,57 $\pm$ 3,03	24,04 $\pm$ 1,86
	<i>m. semitendinosus</i>	0	29,73 $\pm$ 1,36	28,34 $\pm$ 0,34	46,30 $\pm$ 4,50
		2	29,57 $\pm$ 1,75	<b>36,55 <math>\pm</math> 1,32</b>	<b>23,37 <math>\pm</math> 1,74</b>
	<i>m. sternomandibularis</i>	0	28,96 $\pm$ 0,65	<b>39,25 <math>\pm</math> 1,99</b>	<b>27,33 <math>\pm</math> 2,30</b>
		2	26,33 $\pm$ 1,88	32,06 $\pm$ 2,21	<b>34,65 <math>\pm</math> 0,56</b>
	<i>m. trapezius</i>	0	28,11 $\pm$ 1,26	37,74 $\pm$ 0,90	26,78 $\pm$ 4,35
		2	26,05 $\pm$ 1,72	27,85 $\pm$ 1,80	33,63 $\pm$ 4,59
Heifers	<i>m. psoas major</i>	0	17,85 $\pm$ 1,82	64,18 $\pm$ 4,01	6,97 $\pm$ 0,60
		2	15,01 $\pm$ 1,00	58,31 $\pm$ 2,54	15,74 $\pm$ 3,13
	<i>m. semitendinosus</i>	0	16,54 $\pm$ 1,66	66,50 $\pm$ 3,36	5,49 $\pm$ 0,72
		2	14,26 $\pm$ 0,76	54,38 $\pm$ 2,91	21,95 $\pm$ 1,03
	<i>m. sternomandibularis</i>	0	20,08 $\pm$ 1,67	59,60 $\pm$ 1,86	12,23 $\pm$ 1,20
		2	14,99 $\pm$ 0,92	58,82 $\pm$ 2,94	12,48 $\pm$ 1,35
	<i>m. trapezius</i>	0	19,77 $\pm$ 1,11	77,78 $\pm$ 3,32	12,59 $\pm$ 1,06
		2	14,49 $\pm$ 0,52	44,91 $\pm$ 1,17	33,88 $\pm$ 1,18
Cows	<i>m. psoas major</i>	0	13,32 $\pm$ 0,90	74,98 $\pm$ 2,12	14,36 $\pm$ 1,64
		2	15,28 $\pm$ 2,45	54,29 $\pm$ 1,06	20,09 $\pm$ 2,06
	<i>m. semitendinosus</i>	0	15,42 $\pm$ 2,17	66,12 $\pm$ 3,09	8,21 $\pm$ 0,99
		2	14,44 $\pm$ 1,15	63,92 $\pm$ 0,91	9,74 $\pm$ 0,89
	<i>m. sternomandibularis</i>	0	14,22 $\pm$ 2,35	69,22 $\pm$ 4,34	10,59 $\pm$ 0,94
		2	17,14 $\pm$ 0,83	56,96 $\pm$ 3,43	19,01 $\pm$ 1,96
	<i>m. trapezius</i>	0	9,51 $\pm$ 0,39	75,16 $\pm$ 3,20	7,30 $\pm$ 0,89
		2	11,15 $\pm$ 1,36	70,41 $\pm$ 4,90	20,71 $\pm$ 0,53

In Table 2 the percentage content of three forms of myoglobin of calves, heifers and cows' unsalted fresh muscles and salted ones is shown. All analysed factors i.e. cattle age, muscle kind and salt additive influenced the content of three myoglobin forms. The main form of myoglobin pigment in all cattle muscles was MbO<sub>2</sub>. In cows and heifers muscles MbO<sub>2</sub> stated about 65% haem pigments, however in calves muscles percentage content MbO<sub>2</sub> was significantly lower. Whereas the percentage of MetMb in calves muscle was significantly higher than in heifers and cows muscles. There were slight but meaningful differences in percentage composition of analyzed form of myoglobine in assessed muscles. However there was no their dependence on cattle age observed. It seems that the reductive ability of muscles is stronger in older cattle than in calves. That is why the durability of calves muscles colour during chilled storage and distribution can be lower than older cattle muscles.

The addition of salt caused the growth of MetMb levels by lowering the MbO<sub>2</sub> in muscles. According to many researches salt (NaCl) addition causes the change of haem pigments into oxygenated forms (Gimeno *et al.*, 2001; Barbut and Findlay, 1991; Trout, 1989). Sodium chloride delays activity of reductive enzymes in raw meat (Chu *et al.*, 1988, Govindarajan *et al.*, 1977). Probably reductases of calves muscles are more resistant for salt inhibition.

Table 3. The haematin content in pigs *m. longissimus dorsi* with different quality characteristics (mean  $\pm$  standard error)

Haem pigment	Muscle quality		
	PSE	Normal	DFD
Haematin content, mg/100g	3,91 $\pm$ 0,35	7,94 $\pm$ 0,37	10,59 $\pm$ 0,15

The haem pigment content in three different quality types of pork muscles (normal, PSE, DFD) is shown in table 3. The differences in haem pigments content were statistically significant for all pork muscles. The pigments content of PSE muscle was twice lower than for normal muscles. The DFD muscles contained higher amount of pigments than muscles with normal quality. Similar differences were found by other authors (Feldhusen *et al.*, 1995, Fox 1987, Govindarajan 1973).

Table 4. The percentage composition of three myoglobin forms in not salted and salted pigs muscle with different quality characteristics (mean  $\pm$  standard error).

Pigment form	Salt additive [%]	Muscle quality		
		PSE	Normal	DFD
MbO <sub>2</sub>	0	34,01 $\pm$ 1,58	26,45 $\pm$ 0,50	31,69 $\pm$ 0,10
	2	22,23 $\pm$ 1,19	17,83 $\pm$ 1,08	31,36 $\pm$ 0,47
Mb	0	31,58 $\pm$ 0,87	36,33 $\pm$ 1,01	34,11 $\pm$ 0,10
	2	29,72 $\pm$ 1,32	37,66 $\pm$ 2,80	34,94 $\pm$ 0,72
MetMb	0	20,40 $\pm$ 1,43	22,83 $\pm$ 0,68	17,44 $\pm$ 0,27
	2	35,28 $\pm$ 1,18	31,13 $\pm$ 1,76	16,86 $\pm$ 0,22

Table 4 contains the percentage composition of three myoglobin forms unsalted and salted pork muscles with different meat quality. Quality of muscles and salt addition influenced significantly composition of myoglobin forms. In unsalted muscles with normal quality characteristics the prevailing form of myoglobine was Mb and the share of MbO<sub>2</sub> was slightly higher than MetMb. In PSE muscles MbO<sub>2</sub> occurred in the highest concentration, slightly lower was Mb share and the lowest was MetMb concentration. In unsalted DFD muscles the contribution of Mb and MbO<sub>2</sub> was twice higher than MetMb share. The differences in composition of analysed myoglobine forms in pork muscles with different quality characteristics probably were result of different reductive components' levels and activity of reductive muscles' structures.

The salt additive to grounded pork muscles caused significant changes in proportions of examined myoglobin forms with normal quality characteristics and in PSE muscles whereas did not change pigments composition in DFD muscles.

The salt additive to normal and PSE muscles lowered the MbO<sub>2</sub> share and increased MetMb share with no changes in Mb share. The low level of MetMb in DFD muscles and absence of significant influence of salt additive on that pigment points the higher oxy-reductive potential of those muscles type.

#### Conclusions

1. The haem pigments content in muscles grows with somatic ageing of cattle and is higher in muscles with their greater *ante mortem* activity.
2. The haem pigments content is the lowest in PSE, medium in normal and the highest in DFD pork muscles.
3. The main myoglobin form in grounded beef muscles is oxymyoglobin. The percentage share of MbO<sub>2</sub> in pig muscles, despite the muscle quality characteristics, is lower than in cattle muscles.
4. The lower level of MetMb in grounded cattle muscles points that reductive activity of older cattle may be higher than of calves. It suggests that the calf meat colour durability during chilled storage is smaller than cow's meat colour.
5. The sodium chloride addition to grounded meats increases the MetMb share profiting on MbO<sub>2</sub> share. The changes of pigment forms shares may significantly influence salted meat colour.

## UTICAJ SOLI NA FORME MIOGLOBINA U MIŠIĆIMA GOVEDA I SVINJA

Maria Walczycka, Marzena Nowak T. Kolczak

#### Rezime

Cilj istraživanja je bio ocean uticaja dodavanja soli na oblike mioglobina u mišićima goveda sa različitim količinama pigmentata i u mišićima svinja sa različitim karakteristikama kvaliteta.

Ogledni material su bili mišići *psaos major*, *trapezius*, *semitendinosus* i *sternomandibularis* kod teladi, junica i krava odnosno *longissimus dorsi* kod svinja sa PSE, normalnim i karakteristikama DFD mesa. Ukupni sadržaj haem pigmentata i procenat smanjenog mioglobina (Mb), oksimiooglobina (MbO<sub>2</sub>) i metmiooglobina (MetMb) su mereni kod nesoljenih i mišića soljenih sa 2% NaCl.

Količina haem pigmentata se povećavala sa somatskim starenjem goveda i bila je najniža kod teladi, srednja kod junica i najveća kod krava. Dodavanje soli je prouzrokovalo veći procenat MetMb nivoa smanjivanjem nivoa MbO<sub>2</sub>.

Sadržaj haem pigmentata u mišićima svinja je bio najniži kod PSE mišića, srednji kod normalnog i najveći kod DFD mišića. MbO<sub>2</sub> je bio osnovni oblik mioglobina u analiziranim svinjskim mišićima ali njegov procenat odnosno nivo je bio niži nego kod mišića goveda. Dodavanje NaCl je povećalo nivo MetMb kod PSE i normalnog mesa i nije uticalo na promene nivoa MetMb u DFD mesu.

#### References

1. ABRIL M., CAMPO M.M., ONENC A., SANUDO C., ALBERTI P., NEGUERUELA A.I. (2001) Beef colour evolution as a function of ultimate pH. *Meat Science* 58:69-78.
2. BARBUT S., FINDLAYC. J. (1991) Influence of Sodium, Potassium and Magnesium Chloride on Thermal Properties of Beef Muscle. *Journal of Food Science*, 56, 180-182.
3. CHU Y.H., HUFFMAN D.L., EGBERT W.R., TROUT G.R. (1987) Color and color stability of frozen restructured beef steaks. Effect of processing under gas atmospheres with differing oxygen concentration. *Journal of Food Science*, 53:3:705-710.
4. CORNFORTH D. (1994) Ch. 2: Color: its basis and importance. In: Pearson A.M. & Dutson T.R., Quality attributes and their measurements in meat, poultry and fish products. *Adv. Meat Research*. Blackie Academic & Professional, Glasgow, UK, 35-78.
5. FELDHUSEN F., WARNATZ A., ERDMANN R., WENZEL S. (1995) Influence of storage time on colour stability of beef. *Meat Science*, 40:2: 235-243.

6. FOX J.B.jr. (1987) The pigments of meat. *Science of Meat Products* 139-216.
7. GIDDINGS G.G. (1977) The basis of color in muscle foods. *Critical Reviews of Food Science and Nutrition*, 8:1: 81-114.
8. GIMENO O., ASTIASARAN I., BELLO J. (2001) Calcium ascorbate as a potential partial substitute for NaCl in dry fermented sausages: effect on colour, texture and hygienic quality at different concentrations. *Meat Science*, 57: 23-29.
9. GOVINDARAJAN S., HULTIN H.O., KOTULA A.W. (1977) Myoglobin oxidation in ground beef: mechanistic studies. *Journal of Food Science*, 42:3: 571-577.
10. HORNSEY H.C. (1956) The colour of cooked cured pork. Estimation of the nitric oxide-haem pigments. *Journal of Science of Food and Agriculture*, 7:534-540.
11. HUTCHINGS J.B. (1994) *Food colour and appearance*. Ed. Chapman & Hall. Blackie Academic & Professional, Glasgow, UK.
12. KARLSSON A., LUNDSTROM K. (1991) Meat pigment determination by simple and non-toxic alkaline haematin method – an alternative to the Hornsey and the cyanomet-myoglobin methods. *Meat Science*, 29: 17-24.
13. KRZYWICKI K. (1982) The determination of haem pigments in meat. *Meat Science*, 16: 29-36.
14. LAWRIE R.A. (1985) *Meat Science*. 6th ed. Pergamon Press, Oxford UK.
15. MACLEAN N. (1978) *Haemoglobin*. Bulletin Studies in Biology no. 93. Arnold E. Publishers Ltd. London UK.
16. PALKA K., KUPIEC B., KOŁCZAK T. (1994) Wpływ pH i poziomu barwników hemowych na stopień denaturacji i barwę mięsa podczas ogrzewania. *Zeszyty Naukowe., AR, Kraków; seria Technologia Żywności, z.6*.
17. PHILLIPS A.L., FAUSTMAN C., LYNCH M.P., GOVONI K.E., HOAGLAND T.A., ZINN S.A. (2001) Effect of dietary  $\alpha$  – tocopherol supplementation on color and lipid stability in pork. *Meat Science*, 58: 389-393.
18. PRICE J.F., SCHWEIGERT B.S. (1971) *The science of meat and meat products*. W.H. Friman & Co., San Francisco, USA.
19. RENERRE M., LABAS R. (1987) Biochemical factors influencing metmyoglobin formation in beef muscles. *Meat Science*, 19: 151-165
20. RICKANSRUD D.A., HENRICKSON R.L. (1967) Total pigments and myoglobin concentration four bovine muscles. *Journal of Food Sciences*, 32:57-61.
21. STRYER L. (1997) *Biochemia*. PWN, wyd.III; Warszawa.
22. TROUT G.R. (1989) Variation in myoglobin denaturation and color of cooked beef, pork and turkey meat as influenced by pH, sodium chloride, sodium triphosphate, and cooking temperature. *Journal of Food Science*, 54: 3:536 – 540.
23. WARRIS P.D. (1979) The extraction of haem pigments from fresh meat. *Journal of Food Technology*, 14, 75-80.