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# STUDY ON THE BEEF PIGMENTS

— short communication —

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**Abstract:** Myoglobin, meat pigment is an iron containing protein found in muscle consisting of heme connected to a single proteide chain. Color is the most important factor in meat products influencing the consumer purchase decision and affecting the perception of freshness. This research presents a spectophotometric method tested identify myoglobin in beef, readings being made at several wavelenghts. Oxidation of myoglobin pigment in metmyoglobin, was also analysed using peroxide hydrogene. A considerable decrease of myoglobin compared with the standard sample was observed (approximately 7 times).

**Key words** : myoglobin, metmyoglobin, beef, spectrophotometry, peroxide

## INTRODUCTION

The myoglobin content is dependent to the animal's specie, age, type of muscles and rate of growth (Mancini and Hunt, 2005). When exposed to atmospheric oxygen, oxidation of myoglobin in metmyoglobin takes place. This reaction is highly dependent to $O_2$, to muscle enzymes and NADH reserve (Mancini and Hunt, 2005) (Boles and Pegg, 2011).
The indentification of myoglobin and of metmyoglobin in beef is very important as Carpenter et al., (2001) noticed, while consumers are tempted to

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choose a red instead of a violet or brown meat, associating the pleasant guise with the idea of freshness (Boles and Pegg, 2011).
This subject has been treated by several authors, as Anderson and Robertson (1995), Boles and Pegg (2011), as well as Mancini and Hunt (2005). Meat color is determined by a relative quantity of the three myoglobin derivates: reduced myoglobin Mb, oxymyoglobin $MbO_2$, metmyoglobin MMb (Renerre, 1990).
Immediately after slaughter meat has a purple colour, but after a while (during exposure), the colour intensifies, metmyoglobin being formed. The oxidation of myoglobin to metmyoglobin continuously takes place in the muscles, the maintenance of meat colour requiring conditions in which reduced forms predominate (Boles and Pegg, 2011).
Lactic bacteria, in presence of oxygen, during metabolic processes can produce hydrogen peroxide ($H_2O_2$). The forming of hydrogen peroxide takes place in raw-dry meat. Hydrogen peroxide appears in the process of meat pigments transformation, when the applied bacteria are not positive, therefore the pigments will suffer changes. If meat products are not heat-treated through pasteurization, the risk of the final product to contain viable germs of *Lactobacillus viridescens* exists. These germs produce hydrogen peroxide which reacts with myoglobin and gives the product a green colour. These products have a less attractive colour, but they are still safe to consume (Baron, 2002).
MMb might react to $H_2O_2$, resulting perferrymyoglobin (*MbFe (IV) = O), which rapidly reduces to a more stable composite which is ferryl-myoglobin (MbFe (IV) = O). The latter in time can be transformed into MMb through reduction (Baron, 2002).
The main purpose of this study is the quantification of myoglobin found in beef, using the spectrophotometric method, readings being made at several wavelenghts ($\lambda = 525$, $\lambda = 545$, $\lambda = 565$, $\lambda = 575$). The goal of this analysis is to offer an overall view of meat colour (Suman and Joseph, 2012), the main pigment responsable for this being myoglobin (Perez et al., 2010), myoglobin having the purpose to transport and keep the oxygen in the muscles ( Boles and Pegg, 2011).

## MATERIALS AND METHODS

The studied sample was beef taken from SC Cazan Paraschiva SRL, in the same day with the analysis, kept in the fridge at $4^0$ C.

Reagents: peroxide 0.1% prepared in the lab from peroxide 30 % brought from Chimopar, distilled water necessary for the extraction of myoglobin from beef.

For myoglobin quantification, the method suggested by Krzwicki (1982), (Yin and Chen, 2006), was used. Therefore 10 g of meat from the analysed sample were weight, choped with a blender (Philips 700 Watt); afterwards was brought to a volume of 100 ml with distilled water. The extraction was made by using a magnetic stirrer (IKA-RH basic 2). The so treated sample was filtered and two samples were obtained: the first sample was the filtrate itself and the second sample consisted on 10 ml filtrate treated with 0.2 ml $H_2O_2$ 0.1 %.

A spectrum for wavelenghts between 350-450 nm (T 80 UV/VIS Spectrometers PG instruments 1td) was measured. The obtained samples, using the above principles were analised by using the spectrometric method at different wavelenghts ( $\lambda = 525$, $\lambda = 545$, $\lambda = 565$, $\lambda = 572$).

The estimations were made using the formula of Yin and Chen (2006):

$Mb= 0.369R_1 + 1.140R_2 – 0.941R_3 + 0.015$(nmol/l);

$MMb= - 2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098$ (nmol/l).

Several readings have been made at the above mentioned wavelenghts, an the resulted absorbents were reported in the following way :

$R_1=A_{572}/A_{525}$;

$R_2= A_{565}/A_{525}$;

$R_3= A_{545}/A_{525}$.

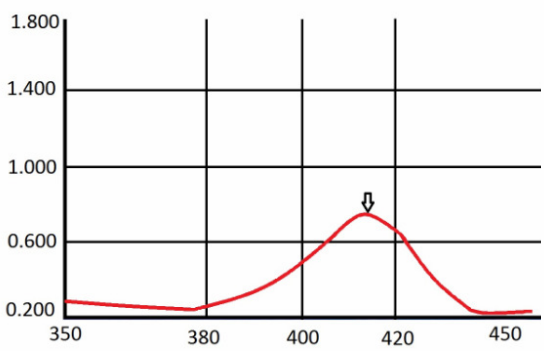
## RESULTS AND DISCUSSIONS

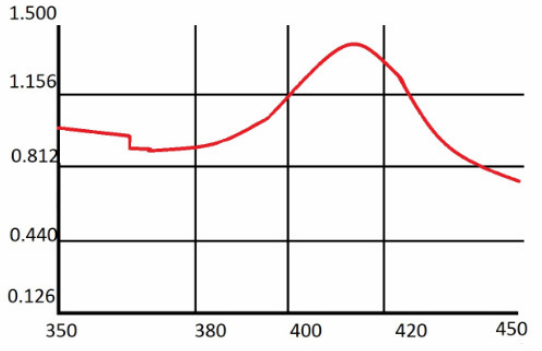
After the spectrophotometrical readings, a difference between the two spectra were obtained. The differences refer to the wavelenghts at which the maximum absorption take place, that is myoglobin turned into metmyoglobin.

As one can see in the Figures 1a and 1b and in accordance to what Govindarajan (1979) says, a good absorption of myoglobin and of all its derivates was registered in the interval $\lambda= 350 - 450$ nm.

Yin and Chen (2006) discovered that the wavelenghts at which the maximum absorption for myoglobin is 418 nm, and for metmyoglobin 410 nm. In our case the wavelenght for myoglobin is $\lambda= 414$ nm, with an absorption of 0.728 (Figure 1a). Under the action of atmospheric oxygen, the transforming of myoglobin into metmyoglobin takes longer. In the present study, to accelerate the reaction, 0.2 ml $H_2O_2$ 1%, at 10 ml filtrate, were added. In

Figure 1b a growth of the absorption from the initial sample to 0.918, is observed, maximum wavelenght being λ = 416.5 nm, wavelenght different




the first sample.

Figure 1a. The spectrum of the extract without $H_2O_2$

Figure 1b. The spectrum with $H_2O_2$

In order to determine myoglobin and metmyoglobin in the analised samples, these were analysed spectrophotometrical at different wavelenghts. The resulted absorbants are summarised in Table 1.

Table 1.The absorbants of the analised samples at different wavelenghts

| Sample | Wavelenghts | | | |
|---|---|---|---|---|
| | λ = 525 | λ = 545 | λ = 565 | λ = 572 |
| Extract without peroxide | 0.221 | 0.142 | 0.089 | 0.089 |
| Extract with peroxide | 1.312 | 1.336 | 1.276 | 1.276 |

The estimates have been calculated after the principles of (Krzywicki, 1982) (Yin and Chen, 2006).

The sample without $H_2O_2$:

Mb = 0.369R1 + 1.140R2 – 0.941R3 + 0.015 = 0.518 (nmol/l)

MMb = -2.514 R1 + 0.777R2 + 0.800R3 + 1.098= 0.22 (nmol/l)

The sample with $H_2O_2$:

Mb = 0.369R1 + 1.140R2 – 0.941R3 + 0.015 = 0.079 (nmol/l)

MMb = -2.514R1 + 0.777R2 + 0.800R3 + 1.098 = 0.241 (nmol/l)

As can be observed in Figure 2, by adding hydrogen peroxide to the extract, a remarcable decrease of myoglobin took place, from 0.518 (nmol/l) to 0.079

(nmol/l). One can also see an increase of myoglobin concentration, but not as remarcable as in the other case (from 0.22 nmol/l to 0.24 nmol/l).

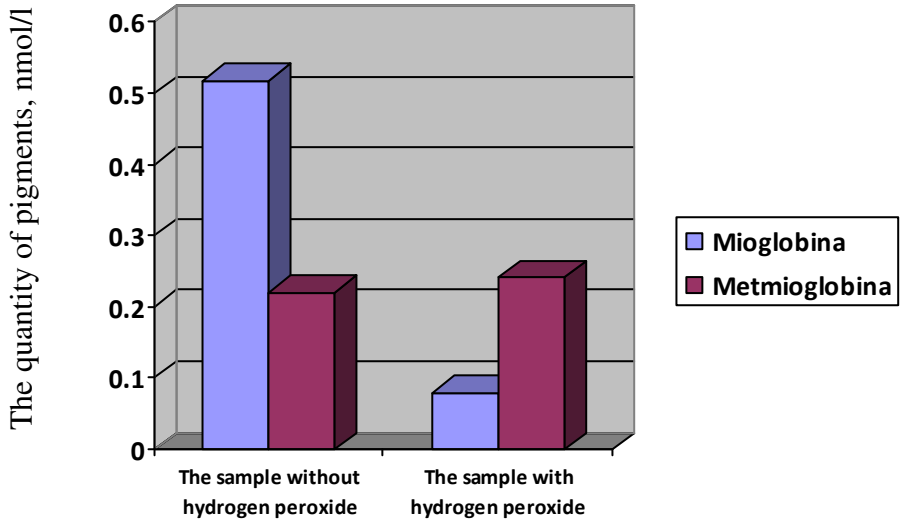



Figure 2. The quantity of myoglobin and metmyoglobin in beef

The results show that peroxide has an oxidating effect. This leads to changing spectrum Soret region, in the event that a spectral absorption maximum can be noticed, the π-π * transitions of the aromatic rings of the ligand porferrinic.. "The Soret Peak" is frequently used in the study of meat pigments or oder compounds which contain the heminic group, for example citocroms. Oxidative processes that occurred were also observed during two forms of quantitative analysis can be found in the myoglobin (Mb and MMB. It is evident that there is a form that is quite unstable, perferrymyoglobin (* MbFe (IV) = O), and fairly stable form-ferrylmyoglobin (MbFe (IV) = O).

## CONCLUSIONS

The spectrophotometric method show that preexisting myoglobin has been affected after adding peroxide. The quantity of myoglobin in the sample with peroxide decreased aproximately 7 times in comparison to the simple sample, and the quantity of metmyoglobin increased with 0.02 nmol/l. This is due to the fact that myoglobin interracts with the oxygen in the reactive environment, forming metmyoglobin. Kept in contact with peroxide, the meat pigment will migrate to a brown shade, which shows the forming of

metmyoglobin. Reaction is explained by the fact that the oxidation of the atom took place from $Fe^{2+}$ to $Fe^{3+}$.
The absorption is related to the level of myoglobin oxidation, and taking into consideration the obtained results, in which the absorbants of $H_2O_2$ sample are clearly higher in comparison to the simple sample, we can conclude that a higher oxidation of myoglobin took place.

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