EVIDENCE FOR THE PRESENCE OF METMYOGLOBIN REDUCING ACTIVITY IN THE MYOFIBRILLAR FRACTION OF BEEF.

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Background:

The importance of meat colour for its marketability has been emphasized by many investigators[1,2]. Meat colour depends on the amount and state of myoglobin (Mb) in meat. In living animals, there is an equilibrium between the reduced form of Mb, which is purple in colour, and the oxygenated form oxymyoglobin (OxyMb), which is bright red. The heme iron in both forms is in the reduced state (ferrous). Upon exposure of fresh cut meat to air, the conversion of Mb to OxyMb is caused by covalent binding of molecular oxygen to the free binding sites of heme. Metmyoglobin (MetMb), which is tan-brown in colour, is formed by the oxidation of heme iron from the ferrous to ferric state. This redox can be reversible under specific conditions depending on MetMb reducing activity, cofactors and oxygen availability. The accumulation of MetMb at the meat surface during storage is the major factor leading to fresh meat discolouration[3]. MetMb can be reduced to Mb by MetMb reducing enzymes which, in turn, can be oxygenated to bright red OxyMb. However, the role of MetMb reducing activity in the maintenance of fresh meat colour is a matter of debate. Some investigators[4,5,6] have reported that the MetMb reducing activity is the controlling factor retarding the accumulation of MetMb. Others[7,8,9,10] have found no evidence to support the theory.
The standard method to determine the MetMb reducing activity consists of using the supernatant of muscle homogenates after centrifugation at 35,000 xg [9,10,11] or 2000 xg[7] and to discard the resultant pellet (which contains the myofibrillar fraction). There are no reports, to our knowledge, on whether MetMb reducing activity resides in the myofibrillar fraction

Objectives:

To investigate the presence of MetMb reducing activity in the myofibrillar fraction of muscle as a possible reason for the increase of MetMb reducing activity found with aging and to compare this activity to MetMb reducing activity in the sarcoplasmic fraction.

Materials and methods:

Fractionation of reducing activity in beef Longissimus dorsi (LD).

Meat (48 h postmortem) was purchased from a local processing plant and MetMb reductase extracts were obtained using a modification of standard techniques [9,11]. A 6 g sample of knife minced beef LD muscle in 20 ml cold extraction buffer, 2.0 mM phosphate buffer (pH 7.0) contained 1.0 mM EDTA and 0.1 mM dithiothreitol (DTT), were homogenized for 30 s with a Polytron PT 3100 (Polytron, Littau, Switzerland) at 13500 rpm. The homogenate was centrifuged at 35000 xg for 30 min at 4°C and the supernatant filtered through Whatman No.1 filter paper. The pellet was re-homogenized with 20 ml of extraction buffer and the homogenate treated twice as before. The three supernatant extracts were used as the source for sarcoplasmic metmyoglobin reducing activity (SMRA). The resultant pellet, after 3 extractions, was suspended in 100 ml of the extraction buffer and used as the source for particulate metmyoglobin reducing activity (PMRA).
Another fractionation protocol was used[7] to verify that the new measured reducing activity resided in the myofibrillar fraction. A 2 g sample in 20 ml cold extraction buffer were homogenized as described above and the resultant homogenate centrifuged at 2000xg 20 min at 4°C. The supernatant was filtered as before and the pellet re-homogenized with 20 ml of extraction buffer and the process repeated twice. The three supernatant extracts and the resulted pellet suspension were used as the sources for SMRA and myofibrillar MetMb reducing activity (MMRA) as mentioned above.

Metmyoglobin reducing activity.

Metmyoglobin reductase activity was determined as described[11] using an Unicam UV4 spectrometer (Unicam Ltd, UK). The standard assay mixture contained 0.1 ml 5 mM EDTA; 0.1 ml 50 mM phosphate buffer (pH 7.0); 0.1 mL 3.0 mM K_4 Fe $(CN)_6$; 0.2 ml 0.75 mM Mb Fe(III) in 2.0 mM phosphate buffer (pH7.0); 0.1 ml 2.0 mM NADH; muscle extract (0.3 ml for SMRA and 50 µL for MMRA) and water to a final volume of 1 ml assay mixture. The assay mixture pH was 6.4 and the assay was carried out at 25 °C. The reaction was initiated by adding NADH and followed by the change in absorbance at 580 nm. Blanks contained all the additions except NADH, which was replaced by water. The activity was calculated as the mean of three replicates and expressed as nanomoles of MetMb reduced per min per gram of meat.

Results and discussion:

The presence of MetMb reducing activity in the soluble fraction of muscle extracts is well documented for bovine[9]; ovine[10] and porcine[11] muscles. However, previous studies[7,10] indicated that an increase in muscle MetMb reducing activity occurred during post-mortem storage. One possible source for the increase in activity is the existence of MetMb reducing activity in the myofibrillar fraction of the muscle. To check this, the LD muscle samples were fractionated into sarcoplasmic and particulate fractions and the level of MetMb reducing activity determined in these two fractions.
The spectral scan of the assay mixture for the myofibrillar fraction is in fig. 1. MetMb reducing activity was present in the particulate fraction as shown by the decrease in 505 and 630 nm peaks, which characterize metmyoglobin, and an increase in 540 and 580 nm peaks, which characterize oxymyoglobin. No reduction was found in the absence of the particulates fraction or NADH, indicating that the reduction is enzymatic and requires NADH as a source of electrons (reducing equivalents).
The effect of different amounts of the particulate fraction as the source of the reducing activity is shown in fig. 2. The rate of change in the absorbance was proportional to the amount of added myofibrillar fraction. Preliminary work on the particulate fraction showed that following the change in absorbance at 580 nm gave consistent results. Measuring the reaction product, after a fixed period of incubation then centrifugation, gave relatively high variations in the measurements. This was due to the variation in lag time between addition of the reactants and the start of the reduction reaction. The MetMb reducing activities for sarcoplasmic and particulate fractions of LD muscle are in fig. 3. The sarcoplasmic MetMb reducing activity of 367 nmole min^{-1} g^{-1} was similar to previous reports.[10,12] Particulate and sarcoplasmic MetMb reducing activities had a ratio of 5.8:1. From the results it is apparent that 85% of available muscle MetMb reducing activity was unaccounted for in previous studies.[9,10,11,12] An earlier report[7] employed a different fractionation protocol and obtained a 2000 xg supernatant from a beef homogenate which, after centrifugation at 100000 xg, yielded an insoluble fraction. This fraction had 4-7 times MetMb reducing specific activity than the original 2000 xg supernatant. Given the differences in the fractionation protocol it is reasonable that the activity in the 35000 xg particulate in the present study was similar to the reported[7] activity in the 100000 xg sediment. If this is correct then the 2000 xg sediment

should contain little or no MetMb reducing activity. To test this, we prepared a 2000 xg sediment after 3 washes with extraction buffer as described in the material and methods section, and measured the MetMb reducing activity. The 2000 xg sediment (which contains the myofibrillar fraction) contained 63% of the total MetMb reducing activity. This result indicates that MMRA is different from that previously reported activity.[7]The results confirm the presence of myofibrillar MetMb reducing activity which represents 63% the total activity. The difference between the two fractionation protocols reflect the MetMb reducing activity in the mitochondrial fraction.
The increase in sarcoplasmic MetMb reducing activity in aged meat which resulted in a negative correlation with colour stability[7,10] may be explained by the presence of myofibrillar MetMb reducing activity. Release of MetMb reducing activity from the myofibrillar into the sarcoplasmic fraction during aging may maintain or increase the SMRA.

Conclusions:

It has been shown that a large amount of MetMb reducing activity is present in the myofibrillar muscle fraction. This activity may explain some of the contradictory results on the relationship between MetMb reducing activity and colour stability of fresh meat. However, the effect of myofibrillar MetMb reducing activity on colour and colour stability still needs to be determined.

Pertinent literature:

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Figure 1. Spectral changes during MetMb reduction using the myofibrillar muscle fraction

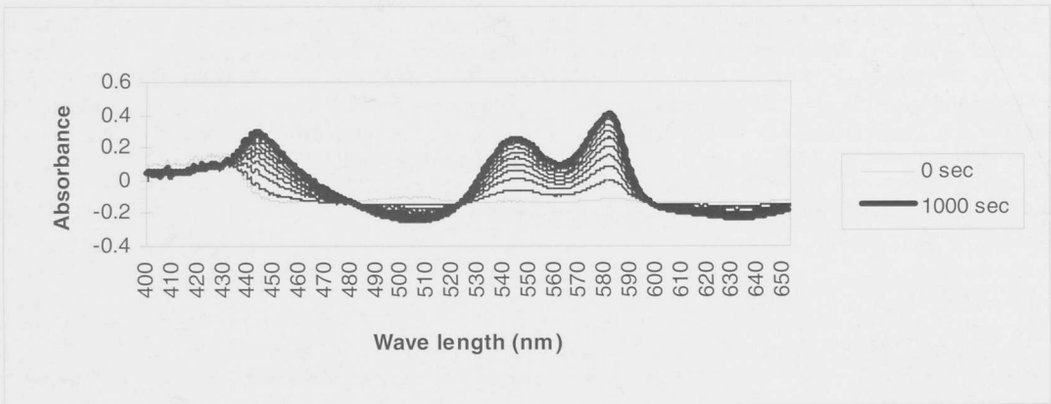

Figure 2. Effect of using various amount of myofibrillar extract on the changes in absorbance at 580 nm.

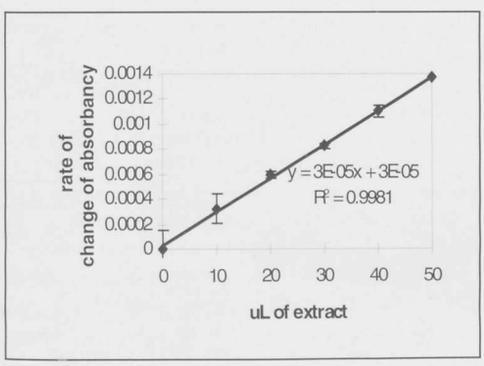

Figure 3. MetMb reducing activity in the sarcoplasmic and myofibrillar fraction of LD muscle