STABILIZING COLOR OF GROUND BEEF IN HIGH AND LOW OXYGEN ATMOSPHERES USING ADDED KREBS CYCLE INGREDIENTS

E. Slinde[1,2], Bjelanovic[1], M. B., Langsrud[3], Ø., Isaksson[1], T., Sørheim[4], O., Phung, V.T.[1], and B. Egelandsdal[1].

[1]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003; N-1432 Ås, Norway; [2]Institute of Marine Research P.O. Box 1870, Nordnes, N-5817 Bergen, Norway; [3]Øyvind Langsrud's Comp., Vardeveien 2, N-1430 Ås, Norway; [4] Nofima, P.O. Box 210 , N-1431 Ås, Norway.

Abstract - Ground beef added Krebs cycle ingredients was packed in low and high oxygen for 13 and 8 days, respectively. Percentages of deoxymyoglobin, oxymyoglobin and metmyoglobin were determined. In low oxygen, the additives succinate and glutamate rapidly increased deoxymyoglobin levels and maintained the level at 100% dexoymyoglobin for 13 days. In high oxygen glutamate/malate supported stabilization of oxymyoglobin, but now citrate addition was important to stabilize oxymyoglobin for longer storage times. Optimal ratios of Krebs cycle ingredients were time dependent in high oxygen packing. Correct additions of Krebs cycle ingredients were superior to adding only water.

Key Words – respiration, mitochondria, substrates

I. INTRODUCTION

When meat is displayed, it is important that the meat looks as red as possible to facilitate the preference of the consumer. An attractive red color of oxymyoglobin (OMb) in meat is difficult to maintain in *post mortem* muscle since myoglobin (Mb) has a tendency to be oxidized to metmyoglobin (MMb). Meat packed in an anaerobic environment has a purple color due to reduced deoxymyoglobin (DMb). The shelf life of meat during display is unfortunately often limited by defects in color due to formation of MMb.

Mitochondria have a strong reducing power as long as there are substrates available. These are substrates from glycolysis, fat degradation and amino acids that are able to enter the Krebs cycle (TCA cycle) that give reducing substrates like NADH and FADH that are oxidized at complex I and II of the respiratory chain. The electrons travel from complex I and II through the Coenzyme Q junction and are supposed to be shuttled by cytochrome c to the outer mitochondrial membrane where MMb is reduced to form DMb. The color of the meat is either a bright red OMb or a purple DMb depending on the presence of oxygen or not. The *post mortem* availability of substrates for oxidation depends on the status of the animal, and addition of NADH or FADH generating substrates might be useful. DMb in meat turns transiently red upon exposure to oxygen from air and becomes OMb. *Post mortem* oxygen consumption by beef heart and pork muscle mitochondria has previously been related to redox behavior and stability of myoglobin [1, 2]. The aim of the present study was to evaluate the effect of glutamate/malate, succinate, pyruvate and citrate on meat color in packages at high and low oxygen tensions. Furthermore, the amounts of the relevant color forms of Mb have been calculated.

II. MATERIALS AND METHODS

Sample: Beef *M. semimembranosus* (collected 4 days *post-mortem)* and pork/beef fat were ground through a plate with 3 mm openings. To 360 g of ground beef was added 40 ml of respiratory substrates. The ground beef was packaged in trays and covered by film (Wipak Multipet and Biaxer) where oxygen transmission rates for tray and top film were 7 and 5 $cm^3/m^2/24$ h at 23 °C, respectively at 50 % relative humidity. The samples were stored in the dark at 4°C for 8 days in a high-oxygen atmosphere (75% oxygen and 25% CO_2) and for

13 days in a low-oxygen atmosphere (60% CO_2 and 40% N_2). The respiratory substrates were succinate, pyruvate, glutamate, malate and citrate in different combinations. The concentrations were 0.05M and 0.1 M, pH 5.8, and all chemicals were of analytical grade.

Reflectance measurements: Samples were scanned, 400 – 1100 nm, with a Foss NIRSystems OptiProbe ™ 6500 Analyzer (Foss NIRSystems Inc., Maryland, USA). All measurements were performed at room temperature (approx. 20^{o}C) according to Kathri er al., [3]. These spectra were used to predict OMb, MMb and DMb according to a principle that has been reported by Kathri et al., [3] and Isaksson et al. [4].

Statistics and experimental design: To each sample in a factorial design (2^2 design in animal age and fatty acid composition) was added a 3 component mixture (succinate, pyruvate and glutamate/malate) according to a simplex lattice mixture design. Each of the experimental mixture points had 4 factors (glutamate/malate ratio, total added mixture levels, citrate concentrations, oxygen concentrations) at 2-levels; *i.e.* a 2^4 design. The design was later fractioned to a quarter and then some extra mixture points were added to check for reproducibility. Finally, we added 8 samples consisting of only added water. Totally, we had 164 samples. Four measurements were made on each batch.

III. RESULTS AND DISCUSSION

Figure 1 and 2 show the effect of the Krebs cycle ingredients in the mixture design at low and high oxygen concentration, respectively. Both the responses (Figure 1 and 2) in Mb states to mixture ingredients are depicted at the average citrate concentration (0.02 mol/kg) for the design. The optimal mixture for high DMb ratio in low oxygen was an almost 1: 1 molar mixture of succinate and glutamate/malate. The condition giving low DMb concentration was always a pyruvate-malate-gluatamate mixture and the worst mixture in terms of low DMb did not change much with time (Figure 1). OMb and MMb, both present at the surface upon packing decreased with time in low oxygen packaging (not shown). The first measurements taken 4-7 hours after packing had a DMb content between 11-92 %. The latter high percentage was obtained for a system with the ratio 1:1 of succinate and glutamate/malate (0.05 mol/ kg of total Krebs cycle ingredients). This value suggested that the surface reduction was fast with the right mixture, and myoglobin remained reduced as long as we monitored the minces. A high DMb value was obtained after 8 days of chill storage when only water was added. Due to mitochondrial respiration, DMb increased from start and throughout the experimental period. The optimal mixture for maximizing OMb in high oxygen changes with time in chill storage. At start, a mixture of succinate and pyruvate gave the most oxidizing conditions, while pure glutamate/malate supported OMb. On day 6 a trend towards pyruvate becoming a stronger pro-oxidant was observed. This trend continued until day 8. However, on day 8 a small amount of succinate seemed advantageous in order to keep OMb stable. In high oxygen thus a combination of glutamate/malate with a small amount of succinate seemed the combination that maintained OMb highest on the final observation day. In the high oxygen packages there was hardly any DMb. OMb decreased throughout the storage period while MMb increased. This indicated either a direct turnover from ferrous myoglobin with bound oxygen to ferric myoglobin or that the transition through DMb was not rate limiting.

Effect of citrate: Figure 1 and 2 represent both the same, rather low average citrate concentration. The effect of citrate on OMb (Fig 2) became significant from day 1, but from day 6 and onwards citrate was the most influential ($P < 0.05$) Krebs cycle additive. The effect of citrate was to stabilize OMb. Since glutamate/malate stabilized OMb at earlier times, it could actually be suggested that a combination of citrate/glutamate/malate will lead to optimal stabilization of OMb. The effect of citrate in low oxygen packing was significant from day 3. However, this additive effect was of lower importance than that of other Krebs cycle ingredients. In addition citrate stimulated formation of the OMb state and should therefore not be added to low oxygen packing where the DMb state is favored. Table 1 and 2 give the maximum and minimum values measured for

DMb and OMb in low and high oxygen packing, respectively. In low oxygen, at day 13, many systems were pure DMb systems. At this time the reference system also had a high DMb level, but as reported above, systems with high DMb levels were formed just after packing in succinate-glutamate/malate systems. Surprisingly, it was possible to obtain low DMb levels. Such conditions were characterized by the presence of pyruvate, but also malate and citrate seemed negative. The conditions giving low DMb levels were surprisingly diverse and this actually suggested that stabilizing DMb through additives would need a specific mixture. Pyruvate provoked oxidation and MMb formation in both low and high oxygen packaging. Pyruvate is a product of glycolysis and has to be converted to acetyl-coenzyme A (acetyl-CoA) to cross the mitochondrial membrane and enter the TCA cycle. However, for this to happen, sufficient coenzyme A (CoA) has to be available.

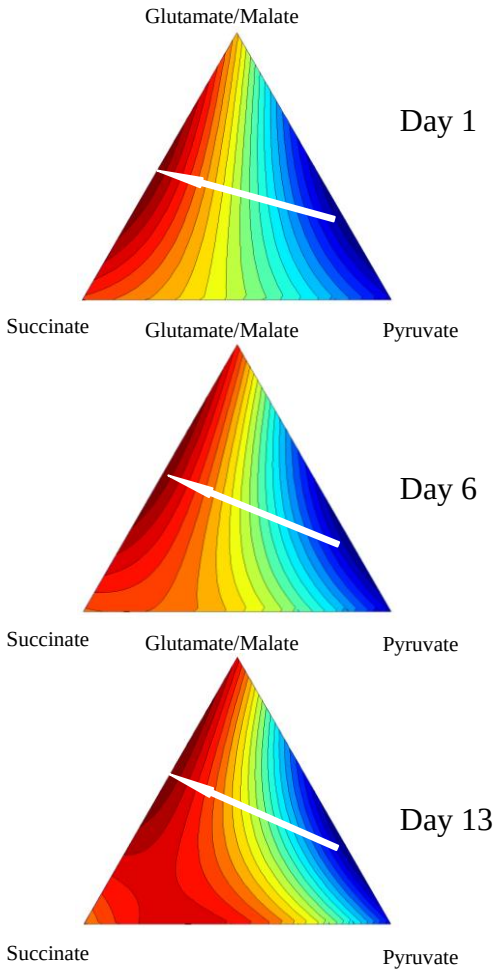

Figure 1. DMb (white arrow, direction dark blue to dark red is from low to high % DMb) at different combinations of succinate, malate/glutamate and pyruvate and packed in low oxygen (~0% O_2). Range of DMb; Day 1:0-100%; Day 6:9-100%; Day 13:9-100 % .

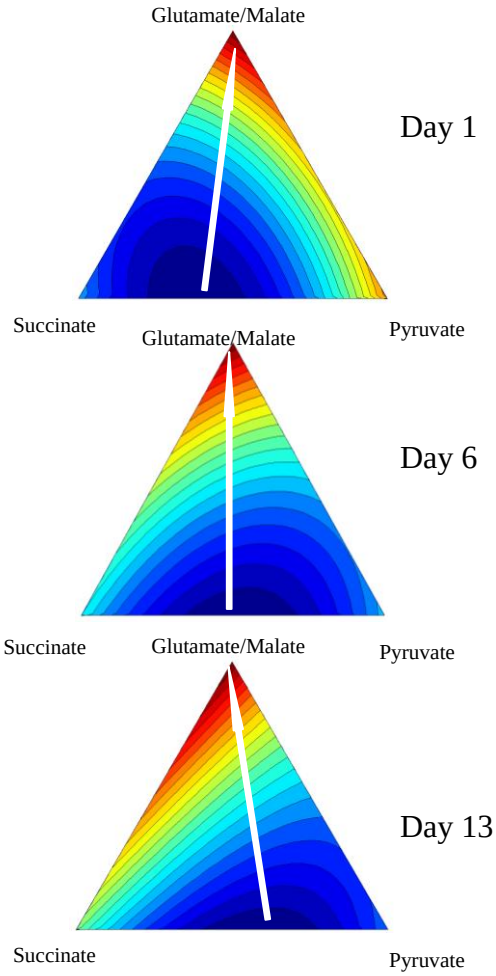

Figure 2. OMb (white arrow, direction dark blue to dark red is from low to high % OMb) at different combinations of malate/glutamate, pyruvate packed in high oxygen (75% O_2). Range of OMb; Day 1: 66-90%; Day 6: 37-65%; Day 8: 25-61%.

Table 1 Minced meat packed in low oxygen (~0% v/v) for 13 days. The five highest and lowest percentages of DMb* measured with added Krebs cycle ingredients (mol/kg) compared to adding water.

Additives used to adjust deoxymyoglobin level								Deoxy myo-globin (%)
Succinate	Pyruvate	Glutamate	Malate	Citrate	Glutamate share (%)	Glutamate + Malate	Total Krebs cycle ingred.	
0.025	0	0.019	0.006	0	75	0.025	0.05	100
0	0	0.05	0	0	100	0.05	0.05	100
0	0	0.025	0.075	0	25	0.1	0.1	100
0.1	0	0	0	0	Na**	0	0.1	100
0.033	0	0	0	0.017	Na	0	0.05	100
0	0	0	0	0	0	0	0	97***
0	0.038	0	0	0.012	Na	0	0.05	27
0.075	0	0	0	0.025	Na	0	0.1	24
0.017	0.017	0.006	0.009	0	25	0.017	0.05	13
0	0.1	0	0	0	Na	0	0.1	9
0	0	0	0.1	0	0	0.1	0.1	9

* normalized data;**Na=No glutamate or malate added; *** only water added

Table 2 Minced meat packed in high oxygen (75% v/v) for 8 days. The five highest and lowest percentages of OMb* measured with added Krebs cycle ingredients (mol/kg) compared to adding water.

Additives used to adjust oxymyoglobin level								Oxy myo-globin (%)
Succinate	Pyruvate	Glutamate	Malate	Citrate	Glutamate share (%)	Glutamate + Malate	Total Krebs ccycle ingred.	
0	0	0	0	0.05	Na**	0	0.05	61
0	0	0.057	0.018	0.025	75	0.075	0.1	58
0	0	0.009	0.029	0.012	25	0.038	0.05	57
0	0	0	0	0.1	Na	0	0.1	56
0.019	0	0.005	0.014	0.012	25	0.019	0.05	56
0	0	0	0	0	0	0	0	38***
0.05	0.05	0	0	0	Na	0	0.1	35
0.033	0.033	0.025	0.008	0	75	0.033	0.1	34
0	0.1	0	0	0	Na	0	0.1	34
0.033	0.033	0.025	0.008	0	75	0.033	0.1	30
0	0	0.1	0	0	100	0.1	0.1	25

* normalized data;**Na=No glutamate or malate added; *** only water added

Glutamate generated more reducing equivalents than malate. Malate alone doesn't support oxygen consumption as oxaloacetate cannot be metabolized if acetyl-CoA (or CoA) is absent. However, glutamate can be oxidized, when acetyl-CoA is absent, by glutamate dehydrogenase, which ultimately leads to the production of two reducing equivalents. The effect of citrate could be due to its ability to chelate free iron. Addition of compounds like glutamate and succinate, even in small amounts, may give flavour to the minced meat. Thus optimal amounts and combinations of respiratory additives have to be found for different products.

IV. CONCLUSION

Addition of small amounts of mitochondrial substrates, especially succinate and glutamate in packages without oxygen reduce the concentration of oxygen to zero avoiding discoloration. Furthermore, at zero oxygen concentration MMb is reduced by mitochondrial respiration to DMb. This favor OMb formation when the packages are opened.

ACKNOWLEDGEMENTS

NFR184846/I10 from the Research Council of Norway. We also thank TINE SA and Nortura SA for support.

REFERENCES

1. Tang, J., Faustmann, C., Hoagland, T.A., Mancini, R.A., Seyfrat, M., & Hunt. M.C. (2005). Postmortem oxygen consumption by mitochondria and ist effects on myoglobin form and stability. J.Agric. Food Chem., 53:1223-1230.
2. Slinde E., Phung V.T. & Egelandsdal, B. (2011) Conversion of met-myoglobin directly to oxy-myoglobin by mitochondria from pork muscle (*M. Masseter)* and liver. 57th International Congress of Meat Science and Technology, 7-12 August 2011, Ghent-Belgium
3. Khatri, M., Phung,V.T, Isaksson, T. Sørheim, O., Slinde E. &B. Egelandsdal (2012). A comparison of two sample preparation methods and two methods of calculating the different myoglobin states present beef M. semimembranosus surfaces. Meat Science. In press.
4. Isaksson, T., Kathri, M. Bjelanovic, M. Sørheim, O., Slinde, E & Egelandsdal, B. (2012) Determination of the myoglobin states using reflectance spectra of whole and ground beef and multivariate regression. In Proceedings 58th International Congress of Meat Science and Technology, 12-17 August 2012, Montreal, Canada