KREBS CYCLE SUBSTRATES ADDED TO GROUND MEAT WILL MAKE FROZEN AND THAWED MEAT BLOOM AS IF FRESH

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Abstract–Mitochondrial reduction of met-myoglobin was achieved by addition of the Krebs cycle substrates glutamic acid and succinic acid that produced the reducing equivalents NADH and $FADH_2$ in ground meat packaged in modified atmosphere. The purpose was to stabilize the color of ground meat when NADH and $FADH_2$ were exhausted due to storage/freeze storage, and therefore the production of reducing equivalents had ceased. These additives were added as brine and spray and the addition was compared with no addition and adding 10 g NaCl/ kg ground meat as a brine and 1 g NaCl/ kg ground meat spray. Spraying 0.003 mol/kg ground meat of glutamic acid and succinic acid on the surface of 50% frozen and 50% fresh (by weight) beef made the product appear redder and bloom better than fresh meat. This was due to the increased availability of mitochondrial substrates on the meat surface that reduced metmyoglobin. Consumers would not detect this low concentration of GS.

Key Words –color stability, mitochondria, glutamic acid, succinic acid, NaCl

I. INTRODUCTION

Freezing of meat is a necessity in order to regulate the meat market and secure that fluctuating seasonal demands for specific meat cuts or trimmings are maintained. Frozen (at - 18°C) and thawed meat has reduced color display life and easily turns brown, *i.e.* to metmyoglobin. Investigations aimed to stabilize the color of frozen and thawed meat are largely missing. In the Norwegian ground meat market two types of ground meat are displayed: ground meat without additives and ground meat added 10 g NaCl/kg ground meat in order to prepare a more ready-made ground meat in terms of binding properties. Also blends of fresh and frozen ground meat are used to supply the market with more ground meat when there are seasonal greater demands and lack of available fresh meat. Freezing and salting stress the metmyoglobin reducing capacity of meat.

We describe the use of glutamic acid and succinic acid as means to supply reducing equivalents and thereby stabilize color of frozen ground meat.

II. MATERIALS AND METHODS

Fresh (4 *days post mortem*) and frozen and thawed ground beef were split into three raw material groups: 1) fresh; 2) 70 % fresh and 30 % (w/w) frozen; 3) 50 % fresh and 50 % (w/w) frozen ground meat. These groups were added glutamic and succinic acid brine (GS-B) (molar ratio 1:1) having pH 5.8, to stabilize color (Slinde et al., 2012). This was done by adding 5% (by weight) of GS brine to give an average molar concentration in the ground meat of 0.03 mol GS/kg ground meat and by spraying the ground meat surface to give an average GS concentration of 0.003 mol/kg ground meat. In addition, ground meat with no additives, with 10g NaCl/kg added as 5% brine and with 1g NaCl/kg obtained by spraying the surface of ground meat, were prepared. This gave 5 samples with or without additives within the 3 raw material groups. Ten replicates were made for the 15 different samples. The samples were packed in MAP (60 % CO_2 and 40 % N_2) and kept in a dark cold room (4°C), but removed for surface color measurements (L*a*b* using Minolta Chromameter CR-410) at 15 degrees.

Color measurements were on day 1, 2, 7 and day 13. On day 13 the packages were opened and a blooming test was carried out. Sensory analysis was carried out on the 10th day by 6 trained sensory panelists. Multiple triangle tests were done in order to identify if added compounds gave detectable flavour changes. The statistical calculations were carried out in Minitab version 16.

III. RESULTS AND DISCUSSION

Table 1 shows the L* a* b* values measured for the 5 different samples (no additive=NO, salted brine=S-B, salted spray=S-S, glutamine succinic acid brine=GS-B and glutamine succinic acid spray=GS-S) with or without additives were studied for the 3 different raw material systems. The measurements were made after chill storage for 7 days. The largest colour difference on day 7, using fresh sample, was between sample NO and S-S. The sample brined with 10 g NaCl/ kg ground meat had lower a* and higher b*, suggesting more metmyoglobin. A tendency for higher a* compared to NO was found for GS-B. The results measured for the system with 30 % frozen meat and 70 % fresh meat were similar to the fresh system. Using 50 % frozen ground meat gave a higher a* and a nominally lower b* for NO compared to both S-B and S-S (Table 1). Spraying seemed to be less effective in promoting metmyoglobin than brining as a* for S-B was lower than a* for S-S. However, spraying the surface with NaCl did not have the same function as brining.

On the other hand GS-B and GS-S both had higher a* than NO, and GS-B had a lower b* than NO (50% frozen). This suggested that both GS-B and GS-S had more deoxymyoglobin than NO.

Figure 1 shows the changes in a* from day 1 to day 13. It is obvious that the difference between samples emerged after the 7th day and became even larger after 13 days chill storage. On day 13 (Figure 1) the GS-B samples had the highest a*.

Table 1- Colour of the different ground meat systems after chill storage (in 40 % Nitrogen and 60% CO_2) for 7 days.

Sample\System	NO			S-B			S-S			GS-B			GS-S		
Ground beef	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
Fresh	50.9 ±1.1	13.5 ±0.6	4.1^{a} ±0.6	49.0 ±1.8	12.0 ±1.0	5.2^{b} ±1.0	50.1 ±1.4	13.2 ±1.5	4.1^{a} ±1.1	50.4 ±1.0	14.0 ±0.7	4.3^{a} ±0.4	51.4 ±2.1	13.5 ±1.2	4.8^{a,b} ±1.2
30 % frozen	50.9 ±2.1	14.7^{a} ±1.4	4.7 ±1.4	50.9 ±2.1	10.4^{b} ±1,4	5.1 ±1.4	51.1 ±1.5	12.5^{c} ±1,4	5.0 ±1.4	50.8 ±1.2	14.2^{a} ±1.4	4.2 ±1.4	51.0 ±1.6	14.2^{a} ±1.4	4.1 ±1.4
50 % frozen	50.5 ±2.2	12.7^{a} ±1.0	4.7^{a} ±0.6	51.2 ±1.3	8.5^{b} ±0.7	6.0^{a} ±0.7	51.8 ±1.6	11.6^{c} ±0.7	5.0^{a} ±0.5	51.4 ±0.9	14.1^{d} ±0.7	4.1^{b} ±0.6	51.2 ±1.7	13.9^{d} ±0.7	4.7^{a} ±0.6

NO=no addition; S-B= salted brine; S-S= salted brine-spray; GS-B =glutamate-succinic acid-brine; GS-S= glutamate-succinic acid-spray.
Different superscript in a row indicates significantly (P<0.05) different samples.

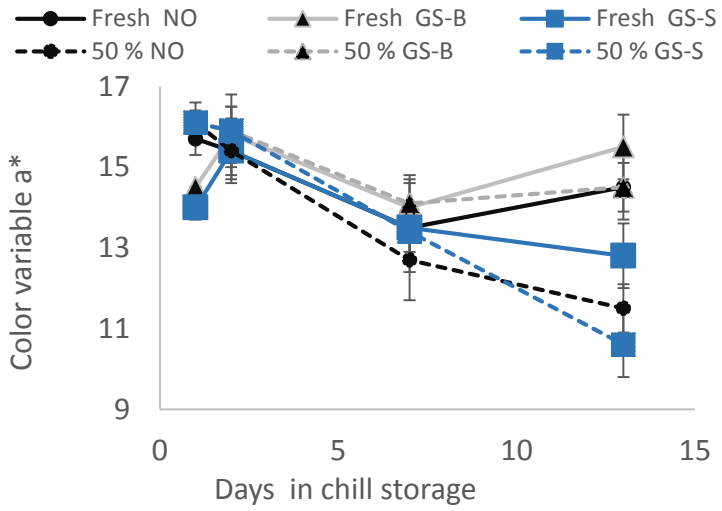

Figure 1. The colour variable a* measured as a function of chill storage for the fresh and the 50 % fresh and 50 % frozen system added glutamic and succinic acid brine. Error bars indicate standard deviation.

Figure 2- The visual difference between the glutamic and succinic acid brined sample (left) and the sample without additives (50 % frozen raw material, right) chill stored for 7 days.

Sample GS-S was more close to NO for the 50 % frozen system (Table 1). Figure 2 shows the difference between NO and GS-B on the 7th day.

A blooming test was made on the 13th day. Blooming is regarded as important to consumers and will give them a sense of meat freshness. Figure 3 shows that the blooming test highly favored GS-B and GS-S samples relative to the other three samples.

The increase in a* with time (Figure 3) may indicate that the oxymyoglobin layer became thicker with time for GS-B and GS-S. This is not the case for the samples with salt. The result supports the above suggestion that for the samples with added GS-B and GS-S there were a maximally reduction to deoxymyoglobin during packaging and this resulted in very efficient blooming when the packaging film was removed. Figure 3 suggests that there is limited difference between using spray and brine as shown in Table 1. However, as observed in Table 1, if the meat is fresh (here 4 days) there is not so much difference between NO (control, no additives) and SG-B and SG-S. The larger difference was found for the 50 % frozen and 50 % fresh system. The interpretation is that for fresh meat there is still endogenous substrates plus active enzymes that will efficiently reduce metmyoglobin and change oxymyoglobin to deoxymyoglobin. The reason that 50 % frozen ground meat is in need of Krebs cycle substrates is due to exhaustion of endogenous substrates before freezing plus the fact that freezing reduced enzyme activity. By adding specific compounds to the meat in adequate quantities, oxygen will gradually be reduced, albeit at a slower speed since the enzyme activity is reduced by freezing (Vinh et al, 2011). However, the slower speed may not have any practical relevance as the samples will be stored for some time after packaging before they are in retail display and in need of being reduced to deoxymyoglobin. Figure 4 and 5 show that compared to the practice of using salted round meat, the GS-B sample looked quite different and had a more red colour at day 13, indicating the presence of oxymyoglobin. To consumers GS-B would appear as a more appealing product since it quickly obtained a red colour (oxymyoglobin colour).

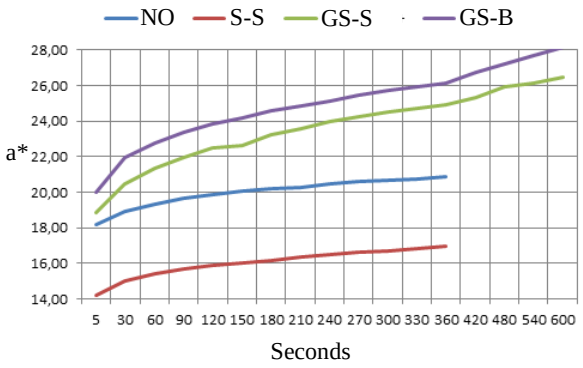

Figure 3- Blooming (a*) of fresh samples, and then stored for 13 days, as a function of time. The figure gives a* measured every 30 seconds after opening the package formerly stored in modified atmosphere

It is possible that GS-S will be an adequate approach to have a nice surface colour. Figure 1 suggests that GS-S (50% frozen) cannot reach the same level of a* as GS-B. However, it is possible that the surface may form cracks under the Minolta instrument, and thus what is measured is a mixture of the surface colour and the colour of the layer below the surface. Unfortunately, we did not cut over the mince to identify if there was a gradient in colour after the sample bloomed. Visually, there was little difference between GS-B and GS-S on day 7 (Fig 4 and 5).

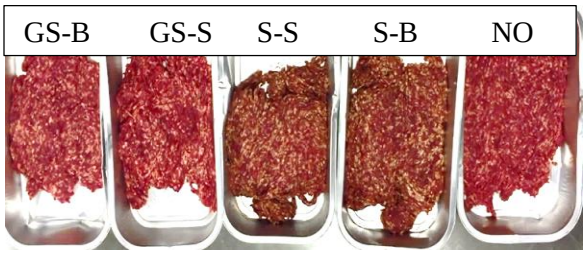

Figure 4- A 10 min blooming test performed on day 13 (30 % frozen meat)

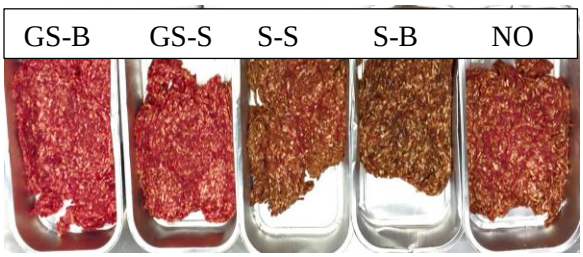

Figure 5- A 10 min blooming test performed on day 13 (50 % frozen meat)

Sensory test: The judged just detected ($P< 0.05$) GS-B versus NO (in one triangle test they did and in another they did not). The judges detected ($P< 0.05$) S-B when compared with NO, but did not detect ($P> 0.05$) GS-B when compared with S-B. The GS-S sample was not tested.

This suggested that brining a ground meat sample with 0.03 mol glutamic acid + succinic acid/kg will be detected by some consumers, in particular if their reference is fresh meat without additives like NaCl.

We do not know the exact minimum amount to be added to obtain reduction to 100% deoxymyoglobin. This will depend on the amount of oxygen in the package and the oxygen transmission rate of the packaging material and chill storage time. If glutamic acid + succinic acid are only added to the surface, we do not foresee that the taste of the product changes.

The additives used here have E-number E-363 (succinic acid)/E-624(disodium succinate) and E620 (L-glutamic acid)/E-621(Monosodium glutamate). Only succinic acid's upper limit is relevant here. Presently we can add up to 0.025 mol/kg (or Liter) to foods. In this experiment we used 0.015 mol /kg ground meat and the same molar amount of glutamic acid in the GS-B samples. Succinic acid is a strong acid regulator and can adjust the pH of ground meat to lower values, if relevant for shelf-life reasons.

IV. CONCLUSION

Glutamic and succinic acid addition can lead to higher redness (a*) after blooming of 50 % frozen and 50% minced raw meat stored for 13 days, than obtainable for fresh minced meat chill stored for the same number of days.

V. REFERENCES

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