A COLOR STABILIZING BLEND OF SUCCINIC AND GLUTAMIC ACID WILL ALSO REDUCE MICROBIAL GROWTH

E. Slinde*,[1], P. Berg[2] and B. Egelandsdal[1]

[1] Norwegian University of Life Sciences, P.O.Box 5003, 1432-Aas, Norway

[2] Nortura SA, Postboks 360 Økern, 0513 Oslo, Norway

*Corresponding author email: erik@salei.no

1. Abstract – Glutamic and succinic acid stabilize color of beef meat. Glutamic and succinic acid are specific organic acids that reduce oxygen *post mortem* through the electron transport chain to water. When the oxygen pressure is almost zero, met-myoglobin is reduced to deoxymyoglobin. Glutamic and succinic acids were tested for their effect on microbial growth using vacuum-packed chicken mince and filets. A decrease in total viable counts of poultry was observed when glutamic and succinic acid were used. A brine with 0.2 g succinic acid plus 0.037 g glutamic acid/100g at pH 5.7-5.8, gave the best microbial result for minces. Color change upon chill storage was small for chicken with no changes in L*, a* b* for minces. For surface sprayed filets a low surface pH correlated with reduced a* and increased b*. The increase in b* seemed to be due to surface protein denaturation. The color stabilizing mixture will not lead to increased microbial growth, but this result was, at least partly, due to the reduction in pH induced by the organic acids. Thus if meat pH originally was > 5.7-5.8, both color stabilization and reduced microbial growth will be achieved by the additives.

Key Words – color, shelf life, organic acids

I. INTRODUCTION

Succinic and glutamic acid stimulates mitochondrial respiration by producing NADH and $FADH_2$, and in modified atmosphere (MAP), at reduced oxygen pressure, deoxymyoglobin (Mb) is formed from met-myoglobin [1]. Additives to meat that improve color are in frequent use, but they often exhibit multiple functions. Nitrites indirectly affect flavor, but are also important for color and shelf life. Dilute solutions of organic acids (*e.g* lactic acid, acetic acids) are also used to diminish bacterial growth and thereby increase shelf life of meat and meat products. Chicken filets and mince are typically relevant product to apply organic acids to, in order to reduce bacterial loads. Mountney and O'Malley [2] studied 10 different organic acids to be used on chicken carcasses including acetic and lactic acids, and suggested succinic and adipic acids as the most relevant acids for flavor reasons. Optimal removal of oxygen will, however, take place when succinic acid is combined with glutamic acid [3]. The combination of the two acids acts as an antioxidant, since oxygen is reduced to water. The effect of this blend of succinic and glutamic acid on bacterial growth has not been examined. For lasting use of such a blend it should not accelerate, but reduce microbial growth. The aim of this work was to identify the effect of succinic and glutamic acid on the microbial quality of chicken mince and filet since these are more prone to bacterial growth than beef.

II. MATERIALS AND METHODS

Chemicals: Succinic and glutamic acid were added as food grade chemicals.

Raw materials: To fresh chicken mince (I) with a pH of 6.13 was added 5g brine/100g connective tissue to give: a) 0.25g succinic acid/100g mince; b) 0.20g succinic acid plus 0.037g glutamic acid/100g brine; c) 0.15g succinic acid/100g mince; d) 0.12g succinic acid plus 0.0223g glutamic acid. Fresh chicken filets

(II) were sprayed with either 5 ml brine having a) 0.71% succinic acid; b) 0.65 % succinic acid and 0.14% glutamic acid; c) 0.43% succinic acid and 0.28% glutamic acid or 3 ml brine with a) 1.18 % succinic acid; b) 0.94 % succinic acid and 0.24 % glutamic acid; c) 0.71 % succinic acid and 0.47% glutamic acid. Five samples were measured for each group.
Packaging: The minced samples were vacuum packaged and chill stored at 4 °C for 22 days.
Methods: pH was measured with Kicks Portamess pH portable electrode. Color was measured with Minolta Chroma Meter CR 400 (Konica Minolta Sensing Inc.) The instrument was calibrated with a white plate with value L* = 95; a*=-0.5 and b*=3.10. The samples were measured on the packaging material. Total viable counts (TVC) was measured on 3M™ Petrifilm™ according to the suppliers instructions (3M Norge AS).
Statistics: Minitab version 17 was used for regression and contour plots.

III. RESULTS AND DISCUSSION

Since the compounds were added as acids, pH would decline (Table 1 and 2).

Table 1- pH of brined minces

	Samples			
Mince	a	b	c	d
pH	5.74 ±0.16	5.79 ±0.03	5.93 ±0.04	5.97 ±0.04

For details about a)-d), see Materials and Methods

As can be seen from Table 1 and 2, the brined samples had the target final pH values of approximately 5.8, while the filets had a lower pH value that desired. This may be due to the fact that the pH was measured in the drip of the filets, and the drip may not be in equilibrium with the meat sample. The target value was calculated based on the titration curve of the meat.

Table 2- pH of sprayed filets

	Samples			
Filet	a-5 ml	b-5ml	c-3 ml	d-3ml
pH	5.43 ±0.08	5.48 ±0.09	5.50 ±0.09	5.55 ±0.04

For details about a)-d), see Materials and Methods

Figure 1 shows that increasing amount of succinic and glutamic acid reduced the amount of bacteria. Of the combinations used in the mince 0.2 % succinic acid combined with 0.037 % glutamic acid gave the lowest amount of bacteria in the mince. Stepwise regression indicated that succinic acid had the highest contribution (P<0.001) to reduce TVC, but also glutamic acid contributed (P = 0.022), while adding water tended to increase the amount of bacteria, TVC (P=0.078). The reason why succinic acid is a more effective organic acid is partly due to its low pKa.

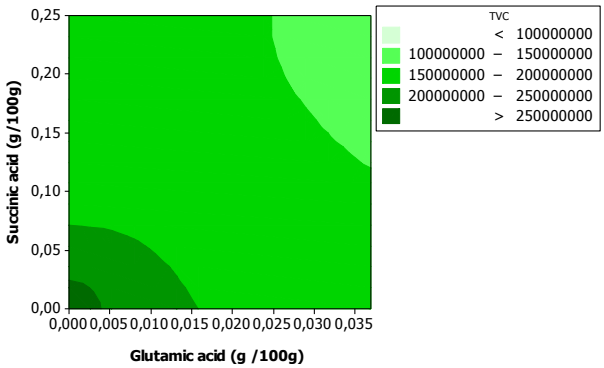

Figure 1. Contour plot of total viable counts in minced meat as a function of succinate and glutamic acid addition. (R^2= 0.81)

The reduction in pH accounted partly for the reduction in TVC, but the correlation between amount of succinic acid and TVC was higher than between pH and TVC. As seen from Table 1, the addition of the two acids reduced pH from 6.13 to 5.79. The filets were intentionally added the same amount (in moles) even though either 3 or 5 ml was used. The approach was to detect differences in the effect of added amount of spray *i.e.* 5 ml or 3 ml to the surface (to 140 g filet). Figure 2 shows that there was an effect (P=0.004) of adding the acids, but in this case the pH reduction explained equally well the reduction in TVC.

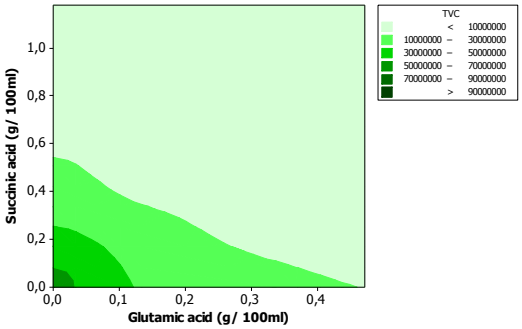

Figure 2. Contour plot of total viable counts on filet meat as a function of succinate and glutamate acid addition ($R^2 = 0.48$).

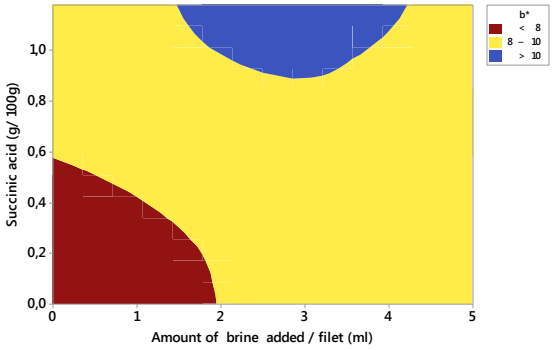

Figure 3. Contour plots of b* of filet as a function of succinic acid and brine volume added ($R^2 = 0.30$).

The effect on color was also measured for mince and filets. Regarding the mince there were no significant changes in L*, a*, b* upon adding the optimal brine with 20 % molar replacement of succinic acid with glutamic acid. This means that the samples were all well reduced as expected for fresh samples and after 22 days chill-storage [1]. For less fresh meat, we would observe more favorable effects of the additives.

Regarding the filet, only b* increased (P = 0.007) with addition of the lower volume of brine (see Figure 3 and 4) where the concentration of succinic acid was at its highest. This seemed due to extensive pH reduction where surface protein denaturation took place upon adding the more concentrated acid solution in 3 ml. (Table 3; Fig. 3 and Fig. 4). This means that there should be a balance between amount and concentration of added brine to prevent denaturation of myoglobin. Succinic acid is a dicarboxylic acid and succinic acid dissolved directly in water gives pH 2.5.

Table 3- b* of sprayed filets

	Samples			
Filet	a-5ml	b-5ml	c-3ml	d-3 ml
b*	9.2 ±0.50	8.7 ±0.79	11.2 ±0.25	10.0 ±0.74

*only 5 ml water = 8.6; For details about a)-d), see Materials and Methods

Figure 4. A1 is added 3 ml of 1.18% succinic acid (sample c in Table 3). To the right is a filet without spray.

Our results suggest that that the concentration of organic acid needs to be reduced in order to eliminate protein surface denaturation. This will, however, reduce the effect on bacterial growth. The reduction here was 1 log unit for filet and 0.5 log unit for the mince. The use of only acids is favourable as no sodium is added

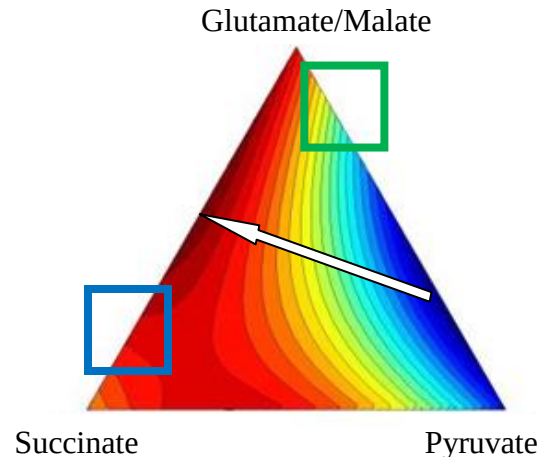

Figure 5. Optimal formation of deoxymyoglobin, (white arrow) and optimal reduction of TVC (blue square) and minimal lipid oxidation (green square). Data from references [1] and [4].

In this experiment we used a mixture of only succinic acid, and with 20 % and 40 % molar replacement with glutamic acid. There is a limitation as to how much of glutamic acid that can be added due to its solubility. As Figure 5 shows, 20 % glutamic acid is not the optimum to preserve myoglobin in the deoxymyoglobin state. We have previously shown that direct and indirect Krebs cycle ingredients may also be pro-oxidative [4]. The oxidation is in particular reduced by reduction in the amount of succinate (Figure 5) giving a minimum of lipid oxidation stability (green square). The observation that respiration is still active in beef meat at low pH i.e. 5.6 [5] due to generation of $FADH_2$ and NADH indicate that more thorough studies have to be preformed on metabolism connected to the Krebs cycle as well as the intermediates of the Krebs cycle. Such studies must also include studies of change in TVC.

IV. CONCLUSION

Succinic and glutamic acid (0.2% and 0.03%) added to minced chicken meat reduced bacterial load with 0.5 log unit. This is largely due to a reduction in pH. The color was not changed.
Added to the surface of filet, the reduction was about 1 log unit but the most concentrated solution of succinic acid (1.18%) gave denaturation of proteins at the surface.

REFERENCES

1. Bjelanovic, M., Egelandsdal, B., Phung, V.T., Langsrud, Ø., Sørheim, O., Hunt, M. & Slinde, E. (2016) Effects of metabolic substrates on myoglobin redox forms in packed ground beef. *Food Pack and Shelf Life*. 8: 24-32.
2. Moutney, G.J. & O'Malley, J.(1965). Acids as Poultry Meat Preservation. *Poultry Science* 44 (2): 582-586.
3. 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. In Proceeding 57th International Congress of Meat Science and Technology (pp. 679-683), 7-12th August. Ghent, Belgium.
4. Yi, G. , Grabež, V. , Bjelanovic, M. , Slinde, E., Olsen, K., Langsrud, O., Phung, V. T., Haug, A., Oostindjer, M. & Egelandsdal. B. (2015). B Lipid oxidation in minced beef meat with added Krebs cycle substrates to stabilise colour. *Food Chemistry* 187:563-571.
5. Phung, V.T., Sælid, E., Egelandsdal, B., Volden, J. & Slinde E. (2011). Oxygen consumption rate of permeabilized cells and isolated mitochondria from muscle and liver tissues examined at different pH values fresh and after freeze-thawing. *J. Food Science*. 76, 929-936.