VOLATILE COMPOUNDS FROM LIPID OXIDATION AND GROWTH OF MICROORGANISMS OF CHILL STORED MINCED MEAT ADDED KREBS CYCLE INGREDIENTS

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Abstract – Minced meat with added Krebs cycle substrates was stored 13 days in low oxygen atmosphere and 8 days in high oxygen atmosphere. Two volatile compounds, hexanal and butanal were headspace analyzed. Glutamate/malate/succinate in low oxygen atmosphere preserved deoxymyoglobin (DMb), reduced butanal as a marker of microbial growth and reduced lipid oxidation measured as hexanal. Glutamate/malate in high oxygen atmosphere increased butanal formation but stabilized oxymyoglobin (OMb), while succinate addition developed oxidative off-flavour, *i.e.* rancidity. Optimal concentration of colour stabilizing additives is needed to minimize microbial growth and lipid oxidation in high oxygen.

Key Words – Antioxidants, Myoglobin, Colour stability, Flavour

I. INTRODUCTION

Meat colour is very important quality attribute for consumer's acceptance. Colour is defined by three dominant states of the myoglobin; oxymyoglobin (OMb), deoxymyoglobin (DMb), and metmyoglobin (MMb). Improved understanding of different biochemical factors on sensory characteristics of the meat represents a great potential for extension of colour stability.

Meat spoilage is associated with changes in flavour, texture and colour. An effective method in extension of shelf life of fresh red meat is modified atmosphere packaging (MAP), both from a microbiological and a sensory point of view. MAP combines oxygen, carbon dioxide and nitrogen in suitable concentrations to maintain meat quality [1]. CO_2 has a bacteriostatic effect on microorganisms, increasing the lag phase of growth and decreasing the growth rate in log phase. CO_2 has an inhibitory effect on the growth of Gram-negative bacteria and Gram-positive lactic acid bacteria become dominant [2]. High O_2 in MAP preserve the myoglobin (Mb) in its oxygenated form but at the same time O_2 promote lipid oxidation and rancid flavour. High CO_2 in MAP in absence of O_2 at low storage temperature inhibits growth of *Brochothrix thermosphacta*. In high O_2 *Pseudomonas spp*. are indicated as organisms that contribute to the odours of minced beef meat [3,4]. Colour changes and off-flavour as product of lipid oxidation and microbial growth might be slowed by antioxidants. *Post mortem* addition of Krebs cycle substrates (KCS) generates FADH and NADH for the mitochondria. O_2 are reduced to H_2O and extra electrons can be used for MMb reduction [5]. High O_2 atmosphere is challenging regarding microbial growth and lipid oxidation, but stimulate transient formation of OMb, a state attractive to consumers.

The aim of the present study was to evaluate the effect of glutamate/malate, succinate and pyruvate on colour stabilization and volatiles as products of lipid oxidation and microbial growth in minced meat stored in high and low oxygen atmosphere at the maximum storage period of 8 and 13 days, respectively.

II. MATERIALS AND METHODS

Sample: Minced meat (14 % beef or pig fat w/w) from 16-19 and 46-81 months old cattle was stored 8 days in a high-oxygen (75% oxygen/25% CO_2); and 13 days in low-oxygen atmosphere (60% CO_2 /40% N_2). Pork and beef fat contained 22% and 2% of polyunsaturated fatty acids, respectively. KCS were added according to a mixture design within a factorial design (totally 156 samples prepared).

KCS concentrations were 0.05 and 0.1 mol/kg mince.

HS-GC/MS: Volatile compounds were analyzed by a dynamic headspace analyzer Teledyne Tekmar HT3 (Teledyne Tekmar, Ohio, USA) coupled to Aglient gas chromatograph 6890N; the ion source 5975 (temperature 230^{0}C) (Aglient Technologies Santa Clara, CA, USA) quadropole mass spectrometer (interface line 250 ^{0}C). The minces were heated to 70^{0}C in the instrument. Compounds of interest were compared with the analytical standards and/or mass spectra of compounds in the NIST 05 Mass spectral Library (Agilient Technologies, Santa Clara, CA, USA).

Statistics and experimental design: The Minitab version 16 (mixture design procedure) was used for graphical presentations while the analysis of variance was according to Phung et al. [6].

III. RESULTS AND DISCUSSION

Combinations of KCS will affect oxy- and deoxy-myoglobin levels as presented in Table 1 and Figure 1 and 2 [5].

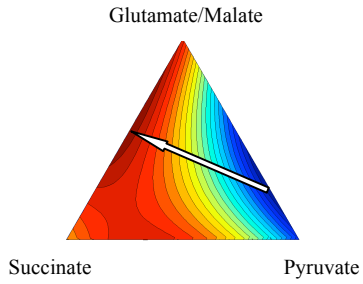

Figure 1. Effect of the KCS on DMb (arrow: from low to high DMb) for minced meat stored for 13 days in low oxygen atmosphere

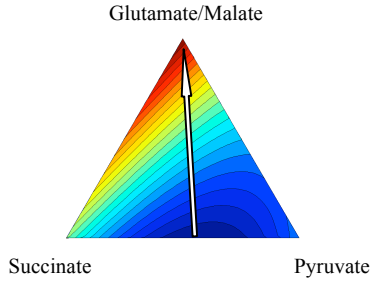

Figure 2. Effect of the KCS on OMb (arrow: from low to high OMb) for minced meat stored for 8 days in high oxygen atmosphere

Desired Mb state was obtained with a combination of succinate and glutamate/malate in a both high and low oxygen atmosphere.

Table 1 Example of KCS additives (in mol/kg) that effect OMb in high oxygen stored 8 days and on DMb in low oxygen stored 13 days

Additives used to adjust deoxymyoglobin level								
Low oxygen	Succinate	Pyruvate	Glutamate	Malate	Glutamate share (%)	Glutamate + Malate	Total KCS	Deoxymyoglobin (%)
	0.025	0	0.019	0.006	75	0.025	0.05	100
	0	0	0	0	0	0	0	97*
	0	0	0	0.1	0	0.1	0.1	9
Additives used to adjust oxymyoglobin level								
High oxygen	Succionate	Pyruvate	Glutamate	Malate	Glutamate share (%)	Glutamate + Malate	Total KCS	Oxymyoglobin (%)
	0	0	0.009	0.029	25	0.038	0.05**	57
	0	0	0	0	0	0	0	38*
	0	0	0.1	0	100	0.1	0.1	25

*only water added; ** with citrate added

Table 2 shows the influence of KCS and other design variables on several identified volatile compounds while Figure 3 and 4 only indicate the effect of KCS on hexanal and butanal.

Increased age of animal increased Mb content; a prooxidant that can contribute to oxidative off-flavours from lipids. Older animals have higher contents of Mb that can contribute to oxidative off-flavours from lipids. Age and fat type were the strongest determinants of volatiles (from lipid oxidation and microbial metabolism) in low and high O_2 atmosphere, respectively (Table 2).The effect of KCS, all volatiles evaluated together, was small and actually depended on the batch (muscle age) used (Table 2).

Table 2 The effect of design variables on the production of volatile compounds* in low oxygen and high oxygen and their significant interactions

Factor	Low oxygen (13 days storage)		High oxygen (8 days storage)	
	Expl. var. (%)	P-value	Expl. var. (%)	P-value
Age of animal	7.1	<0.001	5.9	<0.001
Fat type	2.5	0.01	4.1	0.004
Mixture	1.9	n.s.	2.8	n.s
Age*Fat type	12.7	<0.001	8.3	<0.001
Mix*Age	4.8	0.04	3.3	n.s.
Mix*Fat type	1.8	n.s	3.1	n.s
Mix*Age* Fat type	3.4	n.s.	2.5	n.s

* 9 volatile compounds related to lipid oxidation and microbial metabolism (dimethyl sulfide, acetone, butanal, pentanal, ethyl alcohol, hexanal, 2-penten-1-ol (Z), 2-octen-1-ol (Z), and acetic acid)- modeled with standardized data.

The volatiles hexanal and butanal could be significantly (P<0.05) related to KSC addition (Figure 3 and 4). Hexanal is a marker of lipid oxidation formed when unsaturated fatty acids react with oxygen. Butanal is a product of microbial metabolism and its production seemed stimulated by combinations of added KCS.

Low oxygen:
Hexanal: The status regarding oxidation (hexanal production) was not so obvious, but at least the lower hexanal production seemed to take place when succinate was present in high amounts while higher glutamate/malate addition dominated hexanal production (Figure 3).
Butanal: Succinate/glutamate/malate mixtures preserved DMb (Table 1 and Figure 1) and minimized butanal production in low oxygen atmosphere (Figure 3).
Pyruvate addition was unfavorable for colour and seemed unsuitable due to butanal production. Thus a combination where succinate is kept at fairly high relative to glutamate/malate seemed favorable regarding preserving DMb and preventing lipid oxidation. However, it should be remembered that the effect of the mixture on lipid oxidation is small when lipid unsaturation increased (Table 2).

High oxygen:
Hexanal: Pure succinate should not be added in high oxygen as it seemed to increase lipid oxidation.
Butanal: In high oxygen (Figure 2) conditions that preserved OMb were glutamate/malate with small amounts of succinate added, but this was not optimal regarding low butanal production (Figure 4). Figure 4 suggests that adding slightly more succinate than what seemed optimal from Figure 2, may be a solution worth testing.
The effect of pyruvate addition was unclear in high oxygen.

Thus, it seemed that the most preservative combination of KCS for OMb maintenance would stimulate microbial growth and oxidation. Good hygiene is therefore a prerequisite to prevent meat spoilage.

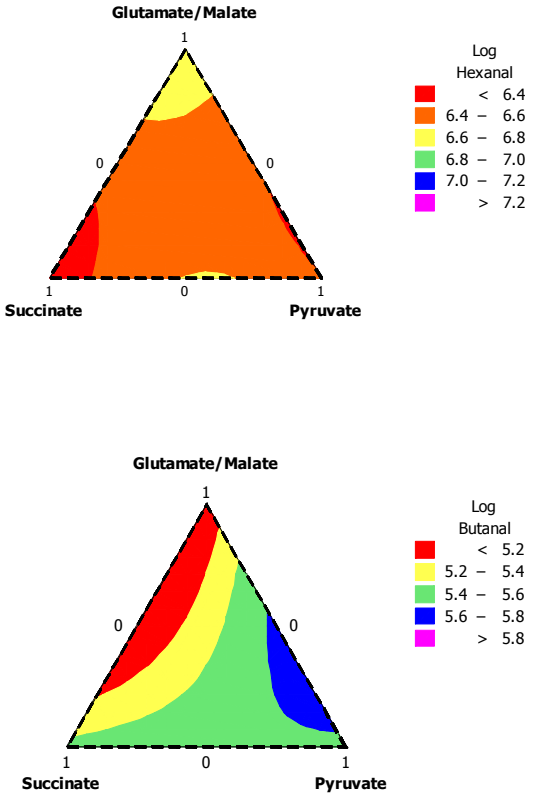

Figure 3. Hexanal and butanal as a function of: succinate, pyruvate and glutamate/malate mixtures for minced meat stored for 13 days in MAP. The corners of triangles give the pure components.

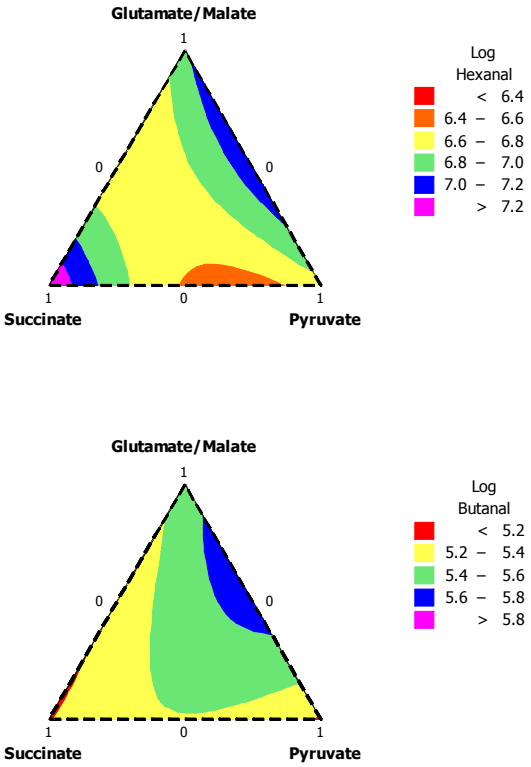

Figure 4. Hexanal and butanal as a function of: succinate, pyruvate and glutamate/malate mixtures for minced meat stored for 8 days in high O_2. The corners of triangles give the pure components.

Optimization of the glutamate/malate/succinate mixture in modified atmosphere will be necessary when colour stabilization is the aim, since succinate promotes rancidity through hexanal production especially in high oxygen.

IV. CONCLUSION

Krebs cycle substrates added to minced meat will only affect formation of volatile compounds from lipid degradation and microbial metabolism to a small extent. Optimal substrate addition stabilize colour of minced beef meat. A mixture of succinate/glutamate/malate will keep DMb concentration high in low oxygen, reduce lipid oxidation and stabilize microbial growth as assessed from hexanal and butanal production. In high oxygen glutamate/malate mixture had positive effect on stabilization of OMb, but there is a need for identifying better the tolerance towards succinate additions in terms of lipid oxidation, colour stability and microbial growth.

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