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Lipid oxidation in minced beef meat with added Krebs cycle substrates to stabilise colour

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ABSTRACT

Krebs cycle substrates (KCS) can stabilise the colour of packaged meat by oxygen reduction. This study tested whether this reduction releases reactive oxygen species that may lead to lipid oxidation in minced meat under two different storage conditions.

KCS combinations of succinate and glutamate increased peroxide forming potential (PFP, 1.18–1.32 mmol peroxides/kg mince) and thiobarbituric acid reactive substances (TBARS, 0.30–0.38 mg malondialdehyde (MDA) equivalents/kg mince) under low oxygen storage conditions. Both succinate and glutamate were metabolised. Moreover, under high oxygen (75%) storage conditions, KCS combinations of glutamate, citrate and malate increased PFP (from 1.22 to 1.29 mmol peroxides/kg) and TBARS (from 0.37 to 0.40 mg MDA equivalents/kg mince). Only glutamate was metabolised.

The KCS combinations that were added to stabilise colour were metabolised during storage, and acted as pro-oxidants that promoted lipid oxidation in both high and low oxygen conditions.

1. Introduction

Beef meat has been demonstrated to contain the strongest peroxide forming potential (PFP) among the most commonly consumed fresh meats in western population. The dominant reason for its high PFP is likely due to the meat's high myoglobin or haem levels (Yi, Haug, Nyquist, & Egelandsdal, 2013). All forms of myoglobin are reactive, and the highest iron oxidation levels are always the most reactive. Cycling of haem in myoglobin between different oxidation states is undesirable as it keeps oxidation in progress. This can lead to protein crosslinking (reduced tenderness) and degradation of lipids, resulting in an unwanted rancid flavour (Campo et al., 2006; Lepetit, 2008).

The Krebs cycle is an important metabolic pathway that oxidises Krebs cycle substrates (KCS) like succinate for the production of nicotinamide adenine dinucleotide (NADH) and flavine adenine dinucleotide ($FADH_2$) that feed into the electron transport system (ETS). In *vivo* the ETS produces adenosine triphosphate (ATP) and removes oxygen. The latter reaction is crucial for colour stability in meat. Specific combinations of Krebs cycle substrates (KCS) will stabilise and maintain myoglobin in the deoxymyoglobin (DMb) state in modified atmosphere (low oxygen) or in the desired full oxymyoglobin (OMb) state (high oxygen packaging) (Slinde et al., 2012). However, a certain amount of metmyoglobin (MMb) will always be present, in both low and high oxygen packaging, due to oxidation. It is the oxidation of KCS such as succinate and glutamate, that produces reducing equivalents that are transported from the mitochondrial membrane to reduce MMb (Phung et al., 2012, 2013; Tang et al., 2005). However, the mitochondrial membrane is a major source of reactive oxygen species (ROS) and this is especially true in meat due to the deterioration of the electron transport chain (ETC) (Barksdale, Perez-Costas, Melendez-Ferro, Roberts, & Bijur, 2010; Lenaz, 2001; Werner, Natter, & Wicke, 2010). This may suggest that some combinations of KCS could also act as pro-oxidants and promote lipid oxidation by feeding into the disintegrating ETC.

So far only KCS have been identified that have an effect on stabilisation of myoglobin states. Little is known about their effect on PFP or lipid degradation (Liu, Fiskum, & Schubert, 2002). However, the mitochondrial ETC as one of the major cellular generators of ROS, produces superoxide, hydrogen peroxide and hydroxyl free radicals which can lead to lipid degradation (Boveris, Oshino, & Chance, 1972; Loschen, Flohé, & Chance, 1971).

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Substrates of the ETC that can lead to ROS production are glutamate/malate through NADH to the flavine mononucleotide group (FMN) of complex I (Liu et al., 2002). Pyruvate/malate may act similarly (Chen, Vazquez, Moghaddas, & Hoppel, 2003). Some recent studies suggest that there are substantial changes in complexes I and III-V of the ETC in beef 4 h *post mortem*. Although oxygen consumption remains, sometimes for several weeks, it is reduced in intensity (Barksdale et al., 2010; Phung et al., 2012, 2013; Werner et al., 2010). It seems therefore relevant to identify if there are any indications of enhanced lipid oxidation when KCS are added *post mortem* to secure oxygen removal and myoglobin stability.

The aim of the present study was to explore how different combinations of KCS used to stabilise colour in minced meat may affect (lipid) oxidation using three different measurements: (1) volatile lipid degradation compounds; (2) peroxide; (3) thiobarbituric acid reactive substances (TBARS). We also aimed to document whether KCS were metabolised during storage, which would support involvement of ETC-ROS formation.

2. Materials and methods

2.1. Chemicals

All chemicals used in the study were of analytical grade. Butanedioic acid disodium salt (succinate hexahydrate disodium salt), butanedioic acid (succinic acid), and 2-oxopropanoic acid (pyruvic acid) were purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), whereas 2-oxopropanoic acid disodium salt (pyruvic acid sodium salt), 2-aminopentanedioic acid (glutamic acid), 2-hydroxybutanedioic acid (L-(−)-malic acid disodium salt) and DL-hydroxybutanedioic acid (DL-malic acid disodium salt) were from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Sodium 2-aminopentanedioate (sodium glutamate) was obtained from VWR International BVBA (Leuven, Belgium). 2-Hydroxypropane-1,2,3-tricarboxylic acid monohydrate (citric acid monohydrate) and 2-hydroxypropane-1,2,3-tricarboxylate (trisodium citrate) were purchased from Merck KGaA (Darmstadt, Germany).

2.2. Animal tissues

Beef *M. semimembranosus* and beef fat tissue were collected four days *post mortem* (Fatland A/S, Oslo, Norway). The packaging day was defined as day zero, *i.e.*, four days *post mortem*. Pork fat tissues (from HKScan, Ruokatalo, Finland) were obtained from pigs fed rapeseed oil and vitamin E to obtain and preserve a high content of polyunsaturated fatty acids.

2.3. Preparation of minced meat

Minces (lean *M. semimembranosus*) were prepared as described by Slinde et al. (2012), and contained 14% w/w fat from beef or pork fat tissues. The ground meat was blended manually with solutions (all at pH 5.8) containing various KCS. The experiment used four combinations of raw materials: meat from young (1.5 years) cattle with pork or bovine fat; meat from old (4–5 years) cattle and pork or bovine fat.

2.4. Preparation of solutions

The solutions added to the minced meat were prepared using succinate, pyruvate, glutamate, malate and citrate in different combinations, either as pure sodium/acid based chemicals or as mixtures of 2, 3, 4 and 5 chemicals of varying concentrations. In order to maintain pH at 5.8 the solutions were prepared by mixing the acid form and the corresponding sodium salt of these chemicals. Forty grams of 0.5 M or 1 M solutions were added to ground 360 g minced meat. The solution was stirred into the mince and subsequently packed. The final concentration of KCS were 0.05 mol/kg succinate, 0.025 mol/kg citrate, 0.0125 mol/kg malate and 0.05 mol/kg glutamate. Eight control samples were included which comprised of 360 g mince with 40 mL of distilled water added.

2.5. Modified atmosphere packaging (MAP) and storage

The minced meat with solutions were packed as described previously by Bjelanovic et al. (2013). Briefly, packaging was carried out within 1 h after grinding. The samples were stored in darkness at 4 °C for 8 days in 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). A shorter storage period in high-oxygen was chosen to reduce the influence of bacterial growth. Low oxygen is used as a concept because often approximately 1% oxygen prevails after MAP packaging. The food grade gas mixtures used for packaging were supplied by AGA (Oslo, Norway). The gas-to-meat ratio in a package was approximately 2:1. The thickness of the minced meat layer in the packages was approximately 3 cm.

2.6. Fatty acid composition analysis

Fatty acids of the selected fat tissues were transformed into methyl esters and then analysed by gas chromatography–mass spectrometry GC–MS (7890A GC, Agilent Technologies, Palo Alto, USA). Transesterification of lipids to fatty acid methyl esters (FAME) was performed by using method described previously with minor modifications (Devle, Rukke, Naess-Andresen, & Ekeberg, 2009). Briefly, 0.01 g fat was dissolved in 2.0 mL hexane and 1.5 mL of 3.33 mg/mL sodium methanolate solution was added. The mixture was then placed on a shaker for 30 min, left to settle for 10 min and 200 μL of the top layer was transferred into a new vial. Fatty acid analysis was performed by auto injection of 1 μL at a split ratio of 80/1; constant flow mode; velocity 20.4 cm/s; two replicates. To identify FAMEs, their retention times were compared to those of a known 37-component standard FAME mix, and the mass spectra were compared with spectra available from the NIST (National Institute of Standards and Technology) database.

2.7. Headspace analysis

The headspace analyses were performed according to a modified method by Volden et al. (2011). Each sample was randomly collected three times from inner and surface parts, blended and mixed. Two gram of this mixture was placed in a 20 mL tightly sealed headspace vial and used for volatile compound measurements. The headspace volatile compounds were analysed by a dynamic headspace analyser (Teledyne Tekmar HT3, Teledyne Tekmar, Ohio, USA) coupled to a gas chromatograph (Agilent 6890N, Agilent Technologies Santa Clara, CA, USA). The GC column was connected to the ion source (at 230 °C) of a quadruple mass spectrometer (Agilent 5975, Agilent Technologies, Santa, CA, USA, interface line 250 °C). The carrier gas was helium at a flow of 1.0 mL/min. The oven temperature programmed as 35 °C for 10 min, heating rate 1.5 °C/min up to 40 °C, 4.0 °C/min up to 70 °C, 7.5 °C/min up to 230 °C and 1 min at 230 °C. The retention times of the components of interest were compared with the retention times of the analytical standards and/or mass spectra of compounds in the NIST 05 Mass spectral Library (Agilent Technologies, Santa Clara, CA, USA). Minitab version 16 (mixture design procedure) was used for graphical representation of the