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Effects of metabolic substrates on myoglobin redox forms in packaged ground beef

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ABSTRACT

Various direct (citrate, malate and succinate) and indirect (pyruvate and glutamate) Krebs cycle substrates were added to ground beef in order to investigate their effect on the inter-conversion of Mb redox forms in aerobic and anaerobic packaging. Glutamate, malate, succinate, pyruvate, and citrate added (up to totally 0.1 mol/kg) to ground bovine *M. semimembranosus* mixed with either ground porcine or bovine fat, altered the myoglobin redox forms in aerobic and anaerobic packaging systems. In anaerobic packaging, a mixture of succinate and glutamate formed deoxymyoglobin rapidly and it remained in this state for 13 days. In aerobic packaging (75% O_2), the highest oxymyoglobin level occurred with a molar ratio of (glutamate–malate) to citrate of 3:1. In this case, oxymyoglobin was more prevalent after 6–8 days of storage in aerobic condition than without addition of these compounds. Pyruvate induced metmyoglobin formation, acting as a pro-oxidant.

Succinate presumed leading to $FADH_2$; was most effective at converting metmyoglobin to deoxymyoglobin in anaerobic packaging. In aerobic packaging, NADH presumed formed by the oxidation of glutamate may maintain oxymyoglobin levels, but adding citrate as well is recommended. Overall, a combination of substrates relevant to mitochondrial oxygen consumption, improved meat color stability.

1. Introduction

Consumers prefer meat with red color, which is associated with freshness. However, an attractive red color in meat is difficult to maintain in post mortem packaging. The shelf life of meat on display is, therefore, limited by color defects caused by the oxidation of surface myoglobin (Fraqueza et al., 2008).

Muscle is normally purple under anaerobic conditions due to deoxymyoglobin (DMb). In atmospheric oxygen, myoglobin (Mb) in meat turns the muscle transiently red as oxymyoglobin (OMb) forms. In low-oxygen packaging, muscle usually starts with a high percentage of OMb, which then forms metmyoglobin (MMb), transiently changing the color of meat to brownish, depending on whether the mitochondrial or cytosolic MMb reducing systems are intact (Ledward, 1985; Madhavi & Carpenter, 1993; Slinde, Phung, & Egelandsdal, 2011).

Prolonged storage demands anaerobic conditions to avoid oxidation (Resconi et al., 2012). Ground beef, however, has lower color stability than steaks, because of tissue disruption and the incorporation of oxygen. Oxygen removal involves packaging with either CO_2, N_2, or a combination of both, providing good color stability and extended shelf life (Tewari, Jayas, & Holley, 1999). Packaging in low-oxygen environments results in abundant surface MMb caused by residual O_2 (Sørheim, Westad, Larsen, & Alvseike, 2008). To get DMb on the meat surface, the packaging atmosphere needs to contain less than 0.1% O_2 (Sørheim et al., 2008), so residual oxygen must be removed quickly and completely to avoid accumulation of MMb and create an environment conducive to reducing MMb. The absence of oxygen allows the DMb to form (Mancini & Hunt, 2005). Thus, the mitochondria function as oxygen scavengers and as an MMb reducing system (Tang et al., 2005; Slinde et al., 2012).

Substrates that produce reducing equivalents like nicotinamide adenine dinucleotide (NADH) and flavine adenine dinucleotide ($FADH_2$) increase the rate of MMb reduction (Mancini, Ramanathan, Suman, Dady, & Joseph, 2011; Mohan, Hunt, Barstow, Houser,

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& Muthukrishnan, 2010; Ramanathan, Mancini, Van Buiten, Suman, & Beach, 2012). The reducing equivalents are oxidized by the electron transport system (ETS) at complex I (for NADH) and complex II (for $FADH_2$) in the inner mitochondrial membrane. The ETS must be sufficiently active to keep MMb low. The ETS remains partly active even after 3 weeks of storage at low post mortem pH (Phung, Saelid, Egelandsdal, Volden, & Slinde, 2011; Phung et al., 2013). However, the level of activity decreases with time, and some muscles do not have sufficient activity to complete these color conversions. Some intermediate metabolites from glycolysis and the Krebs cycle could help fuel the metabolic pathways for oxygen consumption and ferric heme reduction. Succinate improves color stability if added at levels higher than 0.02 mol/kg (Zhu, Liu, Li, & Dai, 2009), and Mancini et al. (2011) found it was a better substrate for color stabilization than pyruvate. Malate also stabilized color, but Mohan et al. (2010) suggested that the degree of color stabilization was muscle-dependent. With the exception of Mohan et al. (2010), who used malate, lactate, and pyruvate, most previous studies have used only one substrate, added at a fixed level. Information is limited, however, on glutamate, as well as the combined effects of added substrates on Mb stabilization, partly because we have lacked a suitable methodology to monitor redox states when meat remains in packages throughout the measurement period.

The aim of this study was to investigate the effect of substrates and their combinations at various concentrations on desirable myoglobin redox states of ground beef in both aerobic and anaerobic packaging conditions.

2. Materials and methods

Because formation of myoglobin redox forms is impacted by many factors, this investigation utilized a relatively new experimental design with accompanying multivariate analysis to help identify the major Krebs cycle substrates affecting myoglobin color stability.

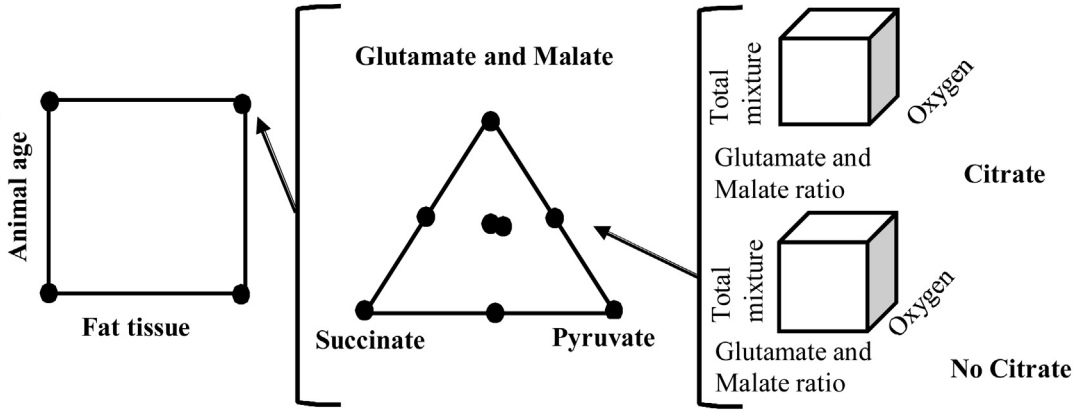
2.1. Chemicals

The following chemicals were purchased from Alfa Aesar GmbH & Co., KG (Karlsruhe, Germany): Sodium salts of succinate hexahydrate, succinic acid, and pyruvic acid. From Sigma–Aldrich Chemie GmbH (Steinheim, Germany), we purchased sodium salts of pyruvate, glutamate, L-malic acid, and DL-malic acid disodium salts. Sodium salts of glutamate monohydrate were purchased from VWR International BVBA (Leuven, Belgium). Sodium salts of citric acid monohydrate and trisodium citrate were purchased from Merck KGaA (Darmstadt, Germany). All chemicals were analytical grade.

2.2. Animal tissues in the ground beef

Semimembranosus muscles and subcutaneous fat were removed from four 16–19 month-old bulls and from four 46–81 month-old cows on day 4 post mortem at a commercial plant (Fatland A/S, Oslo, Norway). Fresh, vacuum-packed subcutaneous fat from pigs fed rapeseed and vitamin E to enhance the content of

Table 1
The experimental design ($2^2 \times 2^3 \times 2^4$) design giving 512 samples which was reduced to 128 samples (2^{9-2}) according to fractional factorial methodology (see text).

Part	A	B	C
Design	2^2	Mixture or an apparent 2^3	2^4
Design point			
Variables	Type of fat tissue: Beef or pork Age of animal: 16-19 or 41-81 months	Succinate: 0-0.1 Molar Pyruvate: 0-0.1 Molar Glutamate Malate: 0-0.1 Molar	Total mixture: 0.05 or 0.1 Molar* Glutamate and Malate ratio: 1:3 or 3:1 Oxygen: 0 or 75 volume % Citrate: 0 or 25 Molar % of total mixture

*When one of the mixture compounds is zero, one or more of the other compounds defining the mixture add up to a total amount of either 0.05 mol or 0.1 mol/kg of mixture compounds.