# 5-P18

## COMBINED EFFECT OF RESIDUAL OXYGEN AND ILLUMINATION ON THE STABILITY OF NITROSYLMYOGLOBIN IN AQUEOUS SOLUTION AND IN CURED HAM PACKAGED IN MODIFIED ATMOSPHERE

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**Background:**
The visual appearance of meat and meat product is of major importance when consumers assess product quality and palatability (Shahidi & Pegg, 1995). For cured meat products the method used for packaging greatly influences the colour stability. The pigment responsible for the characteristic pink colour of cured meat products is nitrosylmyoglobin, and in mildly heat-treated products the predominant form will be the denatured nitrosylmyochrome. Exposure of nitrosylmyoglobin and/or nitrosylmyochrome to light in combination with even very low oxygen levels can promote substantial oxidation to metmyoglobin, which imposes a dull greyness to the meat surface.

Only few studies have investigated the relationship between pigment stability and low oxygen concentrations when exposed to illumination (Walsh & Rose, 1956; Bailey et al., 1964; Andersen & Skibsted, 1992), and further work is clearly needed for conditions relevant to modified atmosphere (MA) packaging of meat products. Some studies have investigated how to improve the colour stability of sliced, pasteurised ham by means of various packaging conditions, including I) different vacuum degree combined with dark storage prior to illumination and blocking of ultra-violet light (Andersen et al., 1988), II) MA with high carbon dioxide content (Andersen et al., 1990), and III) active removal of residual oxygen in the package by absorbers (Andersen & Rasmussen, 1992). However, these approaches all have one or more drawbacks, for instance will dark storage prior to display at the retailers add extra cost, and application of high carbon dioxide atmosphere has negative effects on product appearance due to package deformation.

**Objectives:**
The objective of our current work is to identify critical levels of residual oxygen in combination with monochromatic light of differing wavelengths with respect to stability of the pigment, nitrosylmyoglobin, towards thermal oxidation and photo-induced oxidation in aqueous solution.

The objective is further to use results obtained from the model system to test the colour stability of MA packaged cured ham in order to establish a threshold value for residual oxygen below which no discoloration occurs.

**Methods:**
*Nitrosylmyoglobin in aqueous solution:* Nitrosylmyoglobin was synthesised and purified according to Andersen & Skibsted (1992). Nitrosylmyoglobin was flushed with gas mixtures containing varying levels of oxygen (0.1% - 1.5%) and 20% carbon dioxide. Afterwards the pigment's stability towards thermal oxidation (25°C) and photo-induced oxidation (17°C) was followed spectrophotometrically ($450 < \lambda < 800$ nm) in an air-sealed system. The quantitative changes in nitrosylmyoglobin concentration was monitored and compared for both thermal oxidation and photo-induced oxidation performed with monochromatic light with wavelengths characteristic for fluorescent light, i.e. 366, 436 or 546 nm.

*Cured meat products:* Slices of cured ham cooked to a centre temperature of 65°C was packaged in MA using gas mixtures consisting of 20% carbon dioxide and varying residual oxygen levels (0.02%, 0.1% and 0.5%), all mixtures were balanced with nitrogen. A laminated film with a high oxygen barrier layer of ethylene vinyllindene alcohol was used. Afterwards the samples were stored at 5°C in chill cabinets either in the dark or exposed to illumination (fluorescent tubes giving 1000 lux at the sample surface) for 27 days during which measurements of surface colour (Hunter L), gas composition and volume changes were performed.

**Results and discussions:**
*Nitrosylmyoglobin in aqueous solution:* Figure 1 shows the quantitative changes in nitrosylmyoglobin concentration during spectrophotometrically measurements of thermal and photo-induced oxidation. Contents of oxygen are also shown based on the oxygen solubility at the temperature of gas flushing (0-1°C) in water together with the partial pressure of oxygen in the gas mixture. A minimum of two determinations are included for each experimental value and values for photo-induced oxidation are averages of measurement performed at three different wavelengths of monochromatic light (statistical analysis shows no significant differences depending on wavelength applied). No measurements of photo-induced oxidation are included at 1.5% oxygen.

Thermal oxidation of nitrosylmyoglobin is in very good accordance with the oxygen content present giving a reaction stoichiometry of 1:1 between pigment and oxygen. In the case of photo-induced oxidation this ratio is slightly exceeded because nitrosylmyoglobin is degraded to a higher extent than the oxygen present indicating an alternative reaction mechanism compared to thermally induced oxidation of nitrosylmyoglobin. Walsh & Rose (1956) suggest the ratio 2:1 between pigment and oxygen for photo-induced oxidation of nitrosylmyoglobin. A likely explanation for the higher degree of nitrosylmyoglobin degradation with illumination can be formation of reactive intermediates such as peroxynitrite which has been shown to occur in thermal oxidation (Arnold & Boyle, 1996). In this context, it will be interesting further to elucidate the role of carbon dioxide as a peroxynitrite scavenger (Squadrito & Pryor, 1998).

*Cured meat product:* Figure 2 shows changes in oxygen content in package headspace during storage of sliced ham. A small increase in oxygen levels was observed after 14 hours of storage but during the following storage oxygen levels decreased in all samples. At

day 17 the oxygen levels reach similar levels independent of initial oxygen level. Figure 3 shows the observed changes in a-value (redness) measured on the surface of the top ham slice. The red colour of illuminated samples with 0.5% initial oxygen faded quickly during the first 28-54 hours of light exposure, whereas the colour of all other samples remained stable throughout the whole storage period for both illuminated and dark storage. Statistical analysis shows that average a-values for illuminated samples are significant different ($P<0.001$) from the average values for samples kept in dark after 24 hours of storage while after subsequent 4 hours storage only illuminated samples with 0.5% initial oxygen can be differentiated from the other ($P<0.01$).

After 17 days of illuminated storage the discoloration of samples with 0.5% initial oxygen stopped and colour measurements could not detect further changes in redness during the remaining 10 days of storage. Stable colour coincides with oxygen levels reaching the same level in all packages. It is evident from our results that for the present packaging conditions (packaging size and product amount), a threshold value for residual oxygen in packages right after closure will be in the range between 0.1 – 0.5%.

**Conclusions:**

Model system experiments, in which thermal and photo-induced oxidation of nitrosylmyoglobin was compared have shown that illumination causes extensive nitrosylmyoglobin degradation beyond what is expected from the actual quantity of oxygen present. This was further supported by a storage experiment where sliced, cured ham regardless of residual oxygen content were found to have stable colour when kept in the dark during 27 days of storage. On the other hand, illuminated samples exhibit fast discoloration at 0.5% residual oxygen whereas samples with 0.1% residual oxygen in headspace show no changes in colour.

**Pertinent literature:**


Andersen, H.J.; G. Bertelsen; L. Boegh-Soerensen, C.K. Shek; L.H. Skibsted (1988). Effect of light and packaging conditions on the colour stability of sliced ham. Meat Science **22**, 283-292

Andersen, H.J.; G. Bertelsen; A. Ohlen; L.H. Skibsted (1990). Modified packaging as protection against photodegradation of the colour of pasteurised, sliced ham. Meat Science **28**, 77-83

Andersen, H.J.; M.A. Rasmussen (1992). Interactive packaging as protection against photodegradation of the colour of pasteurised, sliced ham. Int. J. Food Science Tech. **27**, 1-8

Andersen, H.J. & L.H. Skibsted (1992). Kinetics and mechanism of thermal oxidation and photooxidation of nitrosylmyoglobin in aqueous solution. J. Agric. Food Chem. **40**, 1741-1750

Arnold, E.V. & D.S. Bohle (1996). Isolation and Oxygenation Reactions of Nitrosylmyoglobins. Methods in Enzymology **269**, 41-55

Bailey, M.E.; R.W. Frame, H.D. Neumann (1964). Studies of photooxidation of nitrosomyoglobin. J. Agric. Food Chem. **12**(1), 89-93

Shahidi, F.; R.B. Pegg (1995). Nitrite alternatives for processed meats. In Food Flavors, Analysis and Process Influence. Editor: G. Charalambous. Elsevier Science. Amsterdam. pp. 1223-1241

Squarito, G.L. & W.A. Pryor (1998). Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide. Free Radical Biology & Medicine **25**(4/5), 392-403

Walsh, K.A. & D. Rose (1956). Factors affecting the oxidation of nitric oxide myoglobin. J. Agric. Food Chem. 4(4), 352-355


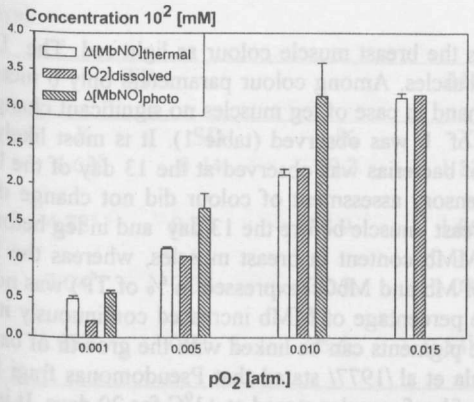


**Figure 1.** Quantitative changes in nitrosylmyoglobin concentration for thermal (25°C for 7 hours) and photo-induced oxidation (17°C for 14 hours with an average light intensity of $1.9 \cdot 10^{20}$ einstein $l^{-1}$ $hour^{-1}$) in comparison to oxygen dissolved in the aqueous solution.

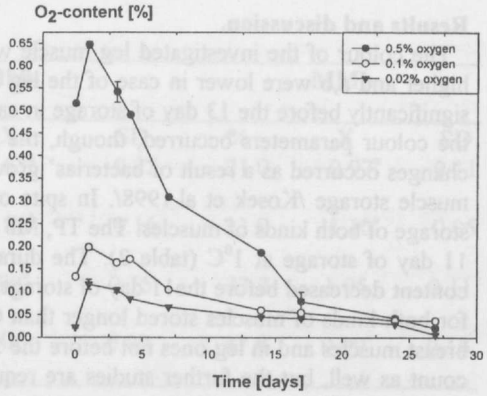


**Figure 2.** Oxygen content in headspace of modified atmosphere packages of cured ham during 27 days of storage at 5°C.

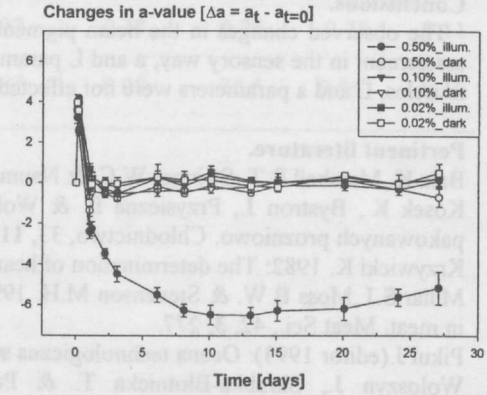


**Figure 3.** Changes in surface redness (a-value) of sliced, cured ham packaged in modified atmosphere during 27 days of storage at 5°C.