

## Stabilization of Minced Meat Colour by Carbon Monoxide

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### Abstract

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The effect of carbon monoxide was studied on the colour stabilisation of minced meat and on oxidation of lipids. The meat colour was evaluated using reflectance spectrophotometry and video image analysis (software LUCIA 5.20). Minced meat (beef and mixture of beef and pork) was packed under industrial conditions into three different modified atmospheres (MA) consisting of combinations of oxygen, carbon dioxide, carbon monoxide, and nitrogen. Carbon monoxide prevented the oxidation of lipids and haem pigments in minced meat and thus stabilised the red colour of minced meat. The redness  $a^*$  of the meat packed under CO was constant during storage (nearly  $a^* = 20$ ), whereas in MA containing oxygen this value decreased ( $a^* = 5$ ). The thiobarbituric acid reactant substances content rose in the samples packed under MA to 2 mg/kg, whereas in CO atmosphere it did not exceed 0.1 mg/kg.

**Keywords:** carbon monoxide; colour; packaging; minced meat

The colour stability of packaged minced fresh meat is limited, which causes problems during the distribution. Haem pigments myoglobin and oxymyoglobin are oxidised into metmyoglobin and the originally red colour turns into brownish one. Lipid radicals and hydro peroxides, resulting from the oxidation of fatty tissue, accelerate this colour change. In order to prolong the colour stability in packaged meat, different modified atmospheres (MA) are used. The use of high levels of oxygen in MA facilitates the oxymyoglobin formation and the oxidation of haem pigments to met-form is for a certain time inhibited (BEHRENDTS *et al.* 2003).

However, a high oxygen concentration makes the lipid oxidation easier, which promotes the

oxidation of haem pigments. The growth of micro-organism is influenced as well (JOHN *et al.* 2005; MARTINÉZ *et al.* 2005). Oxygen stimulates the growth of aerobic bacteria and inhibits the growth of strictly anaerobic ones (PHILIPS 1996).

Very prospective seems to be the use of carbon monoxide, which in combination with haem pigments produces very stable carboxyderivatives and thus stabilises the redness of meat. Although carbon monoxide is a toxic gas, its amount in the packages is regarded as safe (SØRHEIM *et al.* 1997). However, an objection rises against its use on the ground that it can potentially mask the meat spoilage (STENZEL & FELDHUSEN 2004). It was found that the use of carbon monoxide in

modified atmosphere extends the shelf life of fresh beef and reduces microbiological hazards. Minute levels of carbon monoxide retarded the growth of *E. coli* O157 in minced beef (ANONYMOUS 2007). The question is, whether the colour change is the only significant criterion of the meat spoilage or whether it is advantageous to add oxygen in to MA and risk the lipid oxidation and microbial growth.

## MATERIAL AND METHODS

The aim of this study was to ascertain the effect of the use of carbon monoxide in modified atmosphere for packaged minced meat. The samples of minced beef and of a mixture of beef and pork were prepared under industrial conditions in a local processing plant and then packaged under modified atmosphere. All samples were stored at the temperature of 4°C for 24 days.

The initial compositions of internal atmospheres were:

- (1) 80% oxygen + 20% carbon dioxide
- (2) 25% carbon dioxide + 74% nitrogen + 1% carbon monoxide
- (3) 25% carbon dioxide + 25% oxygen + 49% nitrogen + 1% carbon monoxide.

### Methods

The colour was measured by reflectance spectrophotometry and video image analysis on the surface of meat, in the internal layer (“deep”), and at the bottom. TBA test was used for the evaluation of lipid oxidation.

**Video image analysis (VIA).** The pictures of the meat samples were taken using digital camera Panasonic DMC FZ7 under illumination with two fluorescent tubes (light temperature 3000 K). The pictures were evaluated using the software for video image analysis LUCIA 5.20 (Laboratory Imaging Prague). The results were expressed in RGB system as mean red (R), mean green (G), and mean blue (B), and as brightness (MB) and saturation (MS). From these values, the ratios for red (r), green (g) and blue (b) were calculated as follows:

$$r = R/(R + G + B); g = G/(R + G + B); b = B/(R + G + B)$$

**Reflectance spectrophotometry.** The reflectance spectra were measured using Chroma Meter Minolta CM-2600d spectrophotometer. The light

source D<sub>65</sub> and the standard observer angle 10° were used. The measured data were calculated using the software Spectra Magic Ver. 3.3 (Minolta 2001, Japan) and the results were expressed in terms of lightness *L*<sup>\*</sup>, redness *a*<sup>\*</sup>, yellowness *b*<sup>\*</sup>, chromacity *C*<sup>\*</sup>, and hue angle *h*.

**Calculation of metmyoglobin ratio.** The ratios of the myoglobin forms (i.e. met, oxy and red) were calculated from the reflectance spectra according to IZUMIMOTO and OZAWA (1993) method. This method could be used only for minced beef muscle; the evaluation of the samples of the beef/pork mixture was impossible because of a relatively high content of adipose tissue.

**Lipids oxidation.** The thiobarbituric acid (TBA) test after TARLADGIS (1960) in the modification by DAVÍDEK *et al.* (1977) was used for the evalu-

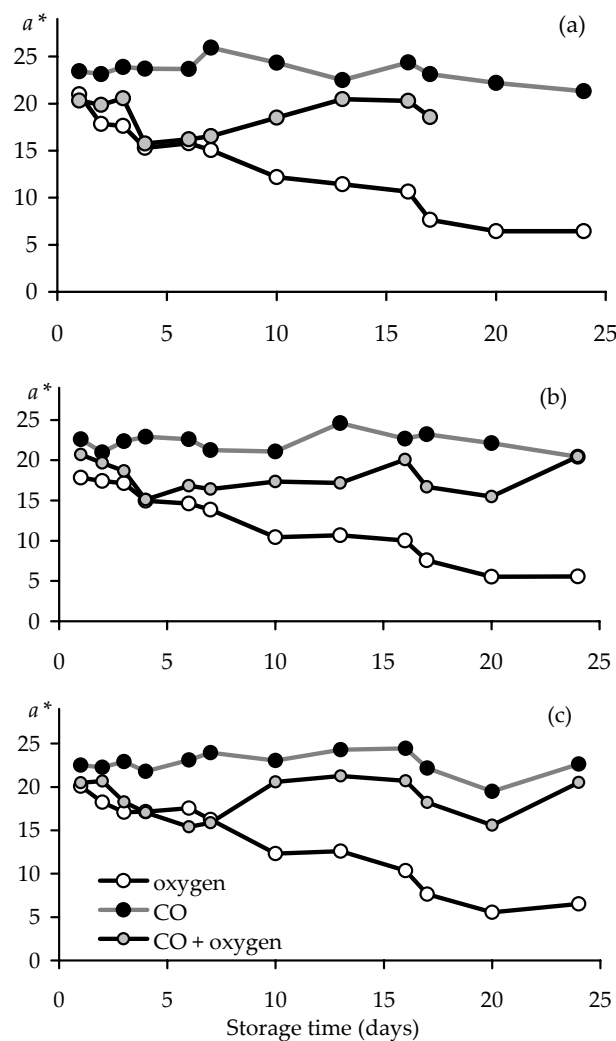


Figure 1. Changes of redness *a*<sup>\*</sup> on the surface (a), in the deep layers (b) and on the bottom (c) of minced beef during storage

ation of lipid oxidation. After distillation and reaction with TBA, the absorbance at 538 nm was measured.

## RESULTS AND DISCUSSION

### Reflectance spectrophotometry

From the values of reflectance spectrophotometry, it could be observed that the red colour of meat decreases in all modified atmospheres; the most apparent are the changes of redness  $a^*$  (Figures 1 and 2). It is obvious that only in the samples packed under the atmosphere with carbon monoxide and without oxygen, this value remained on the same level for the whole time of observation. In the samples where the atmosphere

contained oxygen, the decrease of this value occurred. Although at the beginning of the storage of the packages with oxygen atmosphere (80% O<sub>2</sub>) the red colour seemed to be stable, the reflectance spectrophotometer discovered that the redness  $a^*$  decreased since the beginning and that the conversion of oxymyoglobin to met-form had started immediately. The changes, which were not visually apparent, were detectable instrumentally, i.e. these objectively measured differences were below the limit of visual detection. A similar observation was described also by NICOLADE *et al.* (2005).

It was possible to find negligible differences between different layers of meat (surface, bottom, depth), probably due to a different access of oxygen. In the case of oxygen atmosphere, the coordinate  $a^*$  in the mean layer was lower at the beginning, evidently due to a slower oxygen access into those layers; during storage however, it came up to equalisation.

Carbon monoxide also slowed down the oxidation of the haem pigments and lipids in the bags with oxygen containing atmosphere, but under the modified atmosphere containing 1% CO and 25% O<sub>2</sub> the measured values were inconsistent. To get more information, further measurements are necessary with the use of different oxygen contents. Positive effects are reported in the literature (e.g. LUÑO *et al.* 1998).

### Video image analysis

Very similar results were achieved also by video image analysis (only with beef). Apparent changes were observed in the red colour ratio (Figure 3). This value was nearly identical for all modified atmospheres at the beginning of the storage period. But although the oxygen atmosphere seemingly stabilised the colour forming oxymyoglobin, during the storage this r-ratio decreased due to the haem pigment oxidation. It is evident that the changes under oxygen atmosphere are most visible on the surface of meat.

### Calculation of individual myoglobin form ratio

The oxidation of haem pigment (only in the beef samples under oxygen atmosphere 1) was confirmed using the calculations of the ratios of different forms of haem pigments after IZUMIMOTO and OZAWA (1993) (Figure 4). It is obvious that the

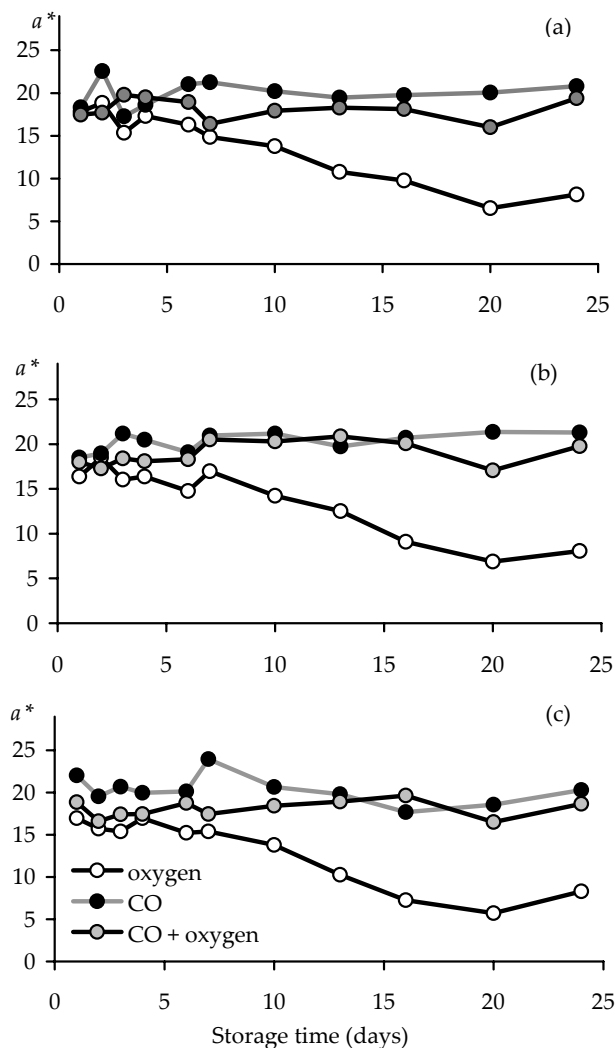


Figure 2. Changes of redness  $a^*$  on the surface (a), in the deep layers (b) and on the bottom (c) of minced mixture of pork and beef during storage

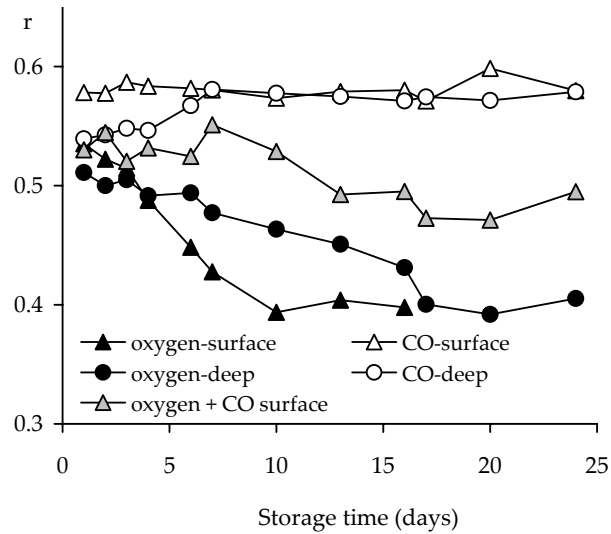


Figure 3. Changes of red colour ratio during storage of minced beef

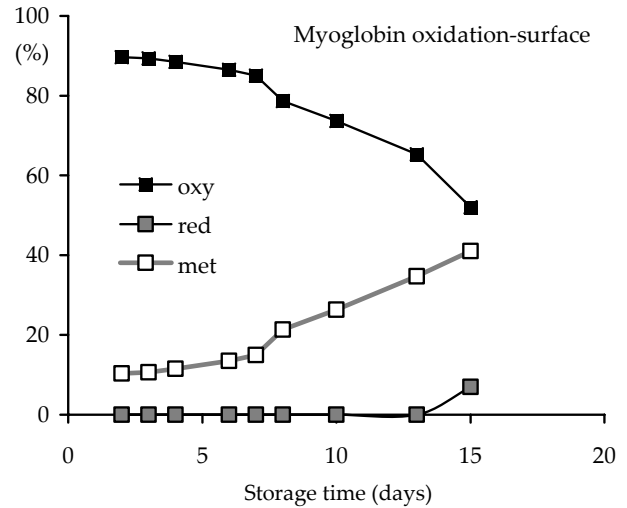


Figure 4. Oxidation of haem pigments on the surface during storage of minced beef

formation of metmyoglobin was similar in all layers at the beginning of the storage, but after a few days the metmyoglobin accumulation in deeper layers accelerated. Probably, in this stage the surface was still protected by a sufficient amount of oxygen bound in the form of oxymyoglobin. Oxymyoglobin ratio decreased on the surface more slowly than in other layers (Figure 5).

Similarly, during the storage of pork loins under modified atmosphere, the decrease of oxygen concentration inside the package was noticed.

Longer periods of storage under lower oxygen concentrations might cause discoloration; increasing discoloration is created by the exhaustion of the muscle reduction ability; this reaches metmyoglobin accumulation (WARREN *et al.* 1992).

### Lipid oxidation

The oxidation of lipids was measured in the same samples. It is evident that, whereas under anaerobic atmosphere with carbon monoxide almost no

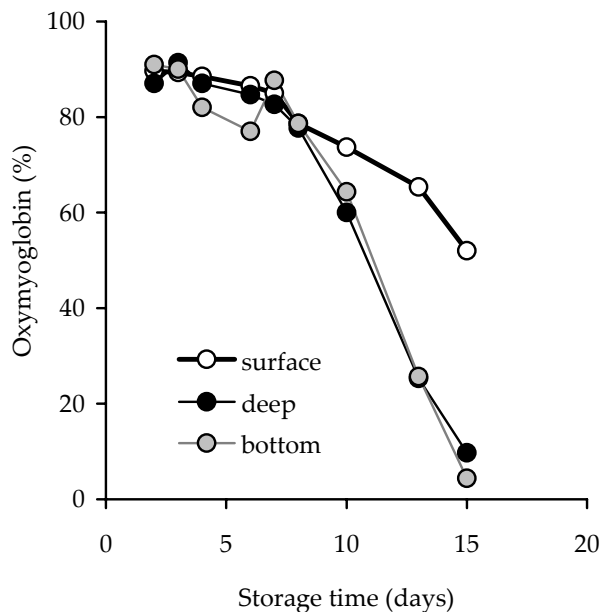


Figure 5. Oxy myoglobin ratio in different layers during storage of minced beef

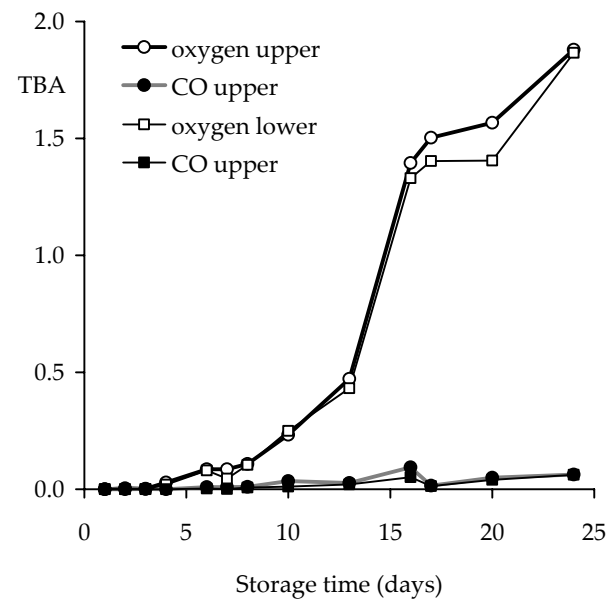


Figure 6. TBA during storage of mixture of minced pork and beef

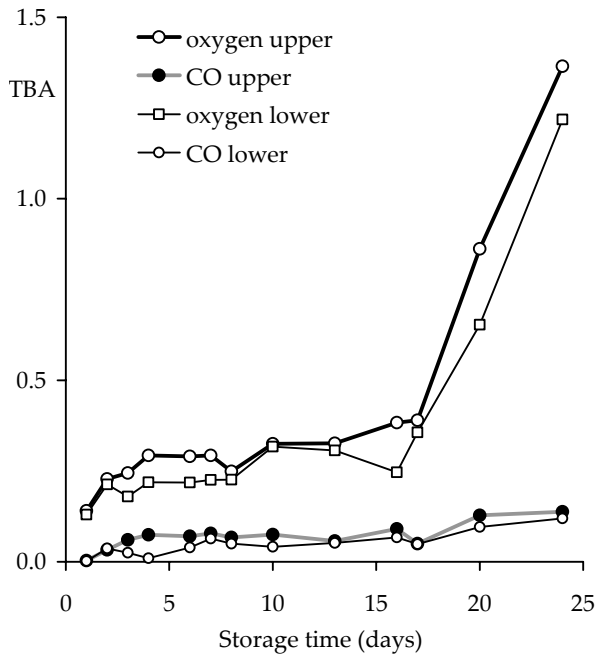


Figure 7. TBA during storage of minced beef

oxidation was detected, in the oxygen atmosphere a steep increase of TBA number occurred (Figures 6 and 7). It can be assumed that this oxidation is delayed under CO atmosphere not only because of the absence of oxygen, but also because of the direct effect of the carboxymyoglobin formation. Carboxymyoglobin is not oxidised and thus a lower amount of  $\text{Fe}^{\text{III}+}$ , which catalyses the lipid oxidation, is available. However, the oxidation of lipids inside the meat and on the surface differs just slightly.

The extent of the lipid oxidation under the atmosphere containing both oxygen and carbon monoxide was lower than in oxygen but higher as compared to carbon monoxide (Figure 8).

Regardless of the atmosphere used, the oxidation of lipids and haem colours occurred in all samples, which was evident by the decrease of  $a^*$  value. However, significantly different were the speed and range of those changes. The use of carbon monoxide and the elimination of oxygen suppressed the oxidation of the haem pigments and lipids and stabilised the colour of minced meat. From the comparison of three different mixtures in the modified atmospheres it was found out that samples under the atmosphere containing CO and no oxygen are more stable than those packaged under common atmosphere containing oxygen. Such results were similar both with minced beef and the mixture of pork

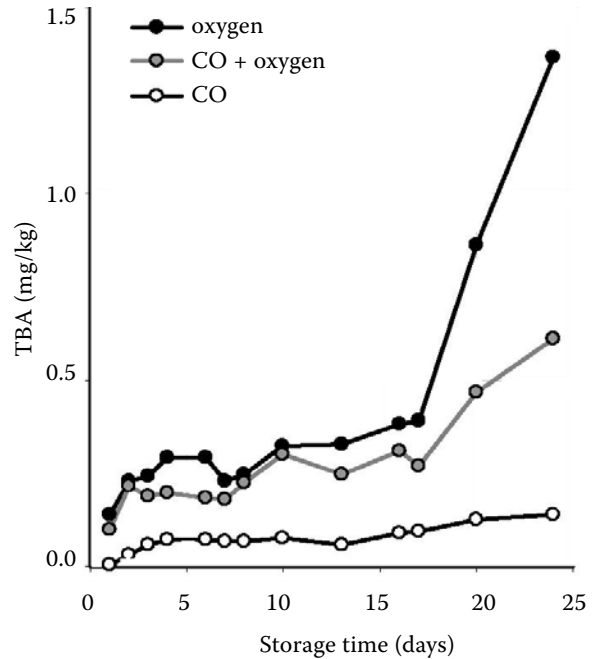


Figure 8. Comparison of the influence of all atmospheres on the TBA increase during storage of beef

and beef. This observation can be documented using two methods for the evaluation of the meat colour: reflectance spectrophotometry and video image analysis. It was possible to observe a distinct run of the individual layers of the meat (on the surface, at the bottom of the package, and in the deep layers of meat).

## CONCLUSIONS

Carbon monoxide prevents the oxidation of lipids and haem pigments in minced meat and thus stabilises the red colour of minced meat. Commonly used atmosphere formed by oxygen and carbon dioxide can not prevent this oxidation, it only masks, for a certain time, the colour changes by creating red oxymyoglobin. The colour changes and the lipid oxidation were most intensive on the surface, where the oxygen level was the highest.

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