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MINIMISING PROTEIN OXIDATION IN RETAIL-PACKED MINCED BEEF USING THREE-GAS MA-PACKAGING

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Abstract – Minced beef is usually packed in high oxygen modified atmosphere packaging (MAP) with a gas mixture consisting of 70-80% oxygen (O_2) and 20-30% carbon dioxide (CO_2). Unfortunately, this results in rubbery and less juicy beef patties with a more rancid flavour compared with fresh or non-oxygen packed beef. To establish whether three-gas MAP (O_2, CO_2 and N_2), instead of two-gas MAP (O_2 and CO_2), would affect sensory attributes, shelf life, protein and lipid oxidation, minced beef was packed in MAP with either 40%, 50% or 80% O_2 and 20% or 40% CO_2 with N_2 as filler gas. When comparing traditional MA-packaging (80% O_2 + 20% CO_2) with a low oxygen packaging atmosphere (40% O_2 + 20% CO_2 + 40% N_2), the latter is seen to increase the meat oxidative stability during storage but decrease acceptability and shelf life. In contrast, high oxygen MAP (80% and 50% O_2) results in more oxidation but a longer shelf life. However, this was not sensorially detectable in the first five days of storage. To maintain shelf life, packaging in 50% O_2 + 40% CO_2 + 10% N_2 or 80% O_2 + 20% CO_2 is preferable, although this gas mixture will not prevent lipid or protein oxidation in the meat.

I. INTRODUCTION

Modified atmosphere packaging is widely used in the packaging of fresh and processed foods. Traditionally, red meat is packed in 70-80% oxygen (O_2) to obtain an attractive bloom colour and with 20-30% carbon dioxide (CO_2) to extend microbiological shelf life (Singh et al. 2011). It has been reported that high oxygen MAP results in less tender and less juicy meat with a more rancid flavour and premature browning (PMB) in beef patties (Clausen 2005). This loss of eating quality is caused by oxidative modifications of lipids and oxidation of structural proteins (Jongberg 2011, Estevez, 2011). However, it is possible to lower the oxygen content in minced beef packaging (50% O_2, 30% CO_2, 20% N_2) and still obtain meat with an acceptable colour and low microbial count as well as a good oxidative stability for up to 14 days at 4°C (Esmer, 2011). However, the impact on protein oxidation has not been investigated in detail. Therefore, the objective of this study was to investigate the effect of low oxygen three-mixture of gas for MAP on the shelf life, protein oxidation markers and eating quality of minced beef.

II. MATERIALS AND METHODS

Experiment 1- Raw meat evaluation:
Experimental design: Bovine shoulder clod (120 kg, approx. 6% fat) was pre-ground two days after slaughter at a Danish deboning plant using nitrogen for cooling. The meat was then transported to a Danish packing plant, where it was weighed out into 500 g trays and sealed with five different gas mixtures, as presented in Table 1 below. The samples were stored for 13 days at 5°C under fluorescent lighting (1200 lux). Samples were taken out on days 0, 6, 8, 11 and 13 after packaging. Samples for shelf life evaluation were immediately assessed whiles samples for protein and lipid oxidation were vacuum-packed and stored in a freezer at -80°C until analysis.

Table 1. Experimental design for experiment 1 - Raw meat evaluation: shelf life, lipid and protein oxidation.

1 x cut	**Minced 2 days after slaughter**				
5 x gas	1	2	3	4	5
	40% O_2 20% CO_2 40% N_2	40% O_2 40% CO_2 20% N_2	50% O_2 20% CO_2 30% N_2	50% O_2 40% CO_2 10% N_2	80% O_2 20% CO_2 -
1 x storage	1200 lux, 5°C, for up to 13 days				

Shelf life
Shelf life was measured based on raw meat odour, colour and overall acceptance of bloomed and degassed meat, 30 minutes after opening of the package, using a 4-point scale, where 1 = no off-odour; 2 = slight off-odour, acceptable; 3 = off-odour, unacceptable; 4 = intense off-odour, unacceptable.

Lipid oxidation
TBARS determination: Meat samples (10 g) were homogenised for 1 min. in 30 mL of TCA solution (7.5% TCA, 0.1% PG and 0.1% EDTA), using an Ultra-Turrax mixer. The mixture was filtered through Whatman Grade 2 filter paper (Sigma-Aldrich), and 5 mL of 0.002M TBA was added to 5 mL of the filtrate. The solution was then incubated for 40 min. at 100°C. Following incubation, absorbance at 532 nm was measured spectrophotometrically against a blank sample and the results were expressed as mg MDA/kg of sample.

Protein oxidation
Free thiol determination: Meat samples (0.5 g) were homogenised with a Polytron PT1200E system in 10 mL of buffer solution (Trizma base 50 mM, EDTA 1mM, pH 7.4) and 100 µL of freshly prepared BHT solution (1mg/mL in methanol). The homogenate was placed in Eppendorf tubes and centrifuged at 13800 g for 10 minutes. The supernatant was then filtered through a 0.45 mM wheel filter (Sartorius). The free thiol concentration was determined fluorometrically on 50 µL of the filtrate using the Amplite fluorometric Thiol Quantification kit (ATT Bioquest). Fluorescence at Ex/Em of 490/520 nm was measured with a SpectraMAX Gemini fluorometer (Molecular Devices). The results were expressed as percentage compared to the thiol content of initial samples stored in vacuum, representing 100% of the free thiol group content.

Experiment 2 - Cooked meat evaluation:
Experimental design: Non-specified bovine forequarter (800kg, approx. 7.1% fat) was pre-ground five days after slaughter at a Danish deboning plant and standardised to the target fat content of 10-12% under nitrogen cooling.
The meat was then transported to an industrial packing plant, where it was minced finely (3 mm particle size) and weighed out into 450 g trays.

Table 2. Experimental design for experiment 2 - Cooked meat evaluation: sensory profiling.

1 x cut	**Minced beef 6 days after slaughter**		
3 x gas	1	2	3
	40% O_2	50% O_2	80% O_2
	20% CO_2	40% CO_2	20% CO_2
	40% N_2	10% N_2	-
1 x storage	1200 lux, 5°C, 6 days		

The trays were then transported to DMRI and MA-packed in the three different gas mixtures shown in Table 3. Sensory analysis was performed five days after packaging.

Sensory analysis: For cooked meat evaluation, the meat in each tray was formed into four 110 g beef patties (with a diameter of approx. 9 cm and a thickness of approx. 1 cm), and cooked on a pre-heated pan (170°C) greased with a thin layer of grape seed oil. The patties were turned every two minutes and cooked to a core temperature of 63°C.

Each beef patty was divided between two assessors and served under aluminium covers on pre-heated plates.

The cooked beef patties were evaluated by a professional trained sensory panel consisting of eight assessors at the Danish Meat Research Institute. All of the assessors had participated in two training sessions in accordance with ISO 4121, ASTM-MNL 13, DIN 13299 and were trained in sensory assessment of meat. The beef patties were assessed according to descriptors developed during the training of the panel.

All beef patties were evaluated on the same day as they were collected from storage on a 15-point unstructured scale anchored at the extremes (0 = low intensity and 15 = high intensity). The descriptive attribute for appearance was: internal colour (doneness). The descriptive attributes for taste were: sweet, acid and bitter. The descriptive attributes for flavour were: beef flavour, warmed-over flavour (WOF), metallic flavour, rancid and stale. The descriptive attributes for texture were: juiciness, cohesiveness, rubbery texture, chewing time and crumbliness.

III. RESULTS AND DISCUSSION

Shelf life: The initial psychrotrophic plate count of the raw minced meat, two days after slaughter, was measured at 3.7 log cfu/g. During storage, the appearance and odour of the raw meat were evaluated in relation to acceptability, and, when the average score reached 2.5, it was regarded as predominantly unacceptable. As shown in Table 3, the acceptance limit varies depending on the gas mixture. When using only 40% O_2, the meat deteriorates within 7-8 days, while 50% O_2 extends the shelf life by 2-4 days. The longest shelf life is achieved for samples

packaged in 50% O_2 + 40% CO_2 + 10% N_2 or 80% O_2 + 20% CO_2.

Table 3. Acceptability limit (approx. storage time for score = 2.5) of raw minced beef stored in MAP with different gas mixtures at 5°C (n=25).

	40% O_2	50% O_2	80% O_2
20% CO_2	8 days	10 days	12 days
40% CO_2	7 days	11 days	-

Lipid oxidation: TBARS revealed that raw meat samples containing 40% O_2 were, irrespective of the CO_2 content, more stable than meat samples containing 50% or 80% oxygen (Figure 1). Therefore, gas mixtures with 40% oxygen (40/20/40 or 40/40/20) are preferred in relation to oxidative stability. CO_2 content did not affect the development of lipid oxidation.

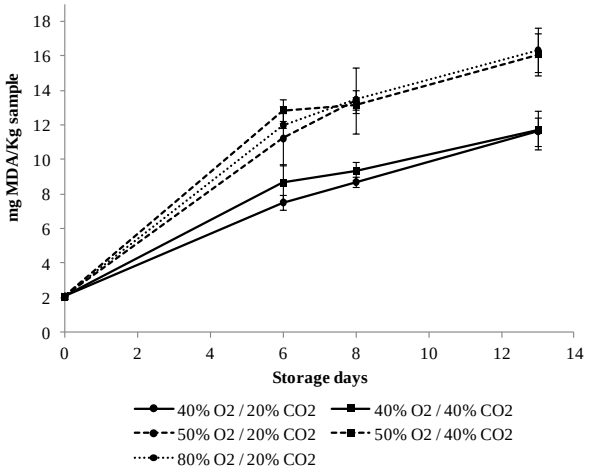

Figure 1. TBARS in raw meat samples from minced beef stored in different MAP gas mixtures for 6, 8 and 13 days.

Protein oxidation: No impact of the gas mixture was observed on free thiols at six, eight or ten days after packaging. Irrespective of the gas composition, a time-dependent decrease in free thiol groups was observed during storage (Figure 2). By day 11, there was a 30-40% decrease in the fluorescence intensity compared with day 0, irrespective of the gas mixture. Protein oxidation occurred, although it was not clearly associated with the MAP gas composition, in contrast to what has previously been reported (Jongberg, 2011), and this might be linked to the method used in our experiment. Alternatively, the O_2 level might need to be lower than 40% to observe any difference between samples.

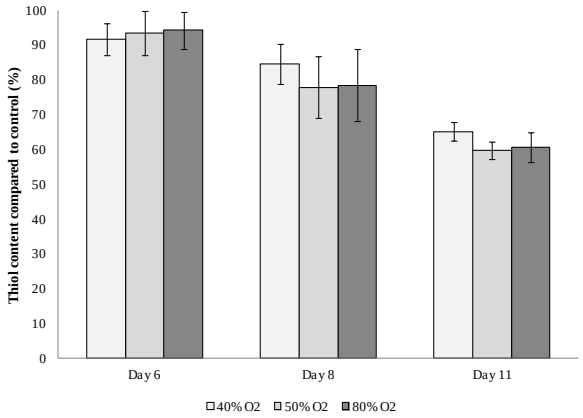

Figure 2. Percentage of free thiol groups in raw meat samples from minced beef stored in different MAP gas mixtures for 6, 8 and 11 days.

Sensory analysis: Sensory profiling of cooked beef patties packed in 40% O_2 + 20% CO_2 + 40% N_2, 50% O_2 + 40% CO_2 + 10% N_2 or 80% O_2 + 20% CO_2 after five days of storage showed no statistically significant differences in taste, flavour or texture between the three different gas mixtures (Table 4).

If the evaluation had been carried out later in the storage period, attributes related to oxidative changes in texture or flavour might have been significantly different when taking the onset of the oxidative changes into account. Three-gas MAP must be muscle-specific, as reported in an earlier study by Tørngren et al. (2013), which recommended packaging fresh pork chops in three-gas MAP with 40% O_2 + 20% CO_2 + 40% N_2, since eating quality was increased.

Table 4. Sensory attributes of cooked beef patties from minced meat stored for 5 days in MAP with different gas mixtures (O_2/CO_2/N_2) at 5°C (n=48).

	MAP 40/20/40	MAP 50/40/10	MAP 80/20	p-value
Cooked colour	10.0	10.7	10.4	0.095
Cooked beef	4.8	5.1	5.0	0.396
WOF	3.8	4.3	3.5	0.309
Rancid	3.2	2.2	2.3	0.159
Cohesiveness	5.5	5.0	5.3	0.101
Juiciness	6.2	6.3	6.5	0.232
Rubbery	4.5	4.9	4.7	0.506
Crumbliness	7.2	7.2	6.8	0.209

IV. CONCLUSION

- Packaging of minced raw beef in three-gas MAP with 50% O_2 + 40% CO_2 + 10% N_2 will result in the same shelf life as traditional high oxygen MAP with 80% O_2 + 20% CO_2. In contrast, using a three-gas mixture with 40% O_2 + 20-40% CO_2 will reduce the shelf life of raw meat at 5°C by 2-4 days compared with 50% O_2 + 20-40% CO_2.

- Reducing the oxygen content in-package from 50% to 40% O_2 delayed lipid oxidation in raw meat. Whereas, protein oxidation was not found to be affected by gas composition, only by storage time.

- Protein and lipid oxidation in raw meat remained unaffected when decreasing the carbon dioxide concentration from 40% to 20% CO_2.

- Sensory profiling of cooked beef patties showed no benefit related to flavour and texture when using three-gas mixtures with 40-50% O_2 instead of a two-gas mixture with 80% O_2.

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