Enhancing Crispiness of Pork Belly by Tri-gas MAP

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Abstract – Pan-fried pork belly with parsley sauce is the number one national dish in Denmark. The main quality parameter is crispiness of both the meat and the rind, and methods to optimize crispiness could therefore be of importance to the meat industry and the food service sector. To establish how MAP affects sensory attributes, lipid and protein oxidation, pork belly was packed in MAP with 0%, 40%, 50% or 80% O_2 and 20% or 40% CO_2. A slice of pork belly contains muscles of different fibre type compositions. The eating quality and oxidative stability were compared after 2 and 6-7 days of refrigerated storage. In general, gas composition influenced crispiness of the meat and rind. Using 50% O_2 + 40% CO_2 enhanced crispiness compared to traditional MAP in 80% O_2 + 20% CO_2. Furthermore, secondary lipid oxidation product generation differed between the gas mixtures and different muscles. The light/glycolytic muscle *Cutaneous trunci* showed considerably different levels of carbonylation compared to the surrounding dark/oxidative muscle. The results of the present study demonstrate that the quality of pork belly can be optimized by using tri-gas MAP. Furthermore, the proportion of different muscles may be important to the oxidative stability of pork belly slices.

Key Words – lipid oxidation, protein oxidation, retail packaging

I. INTRODUCTION

The use of modified atmosphere packaging (MAP) for storage of retail pork is common, despite the association of high oxygen levels in the package gas composition (typically 70-80% O_2 and 20-30% CO_2) with protein and lipid oxidation, which affects product quality. This deterioration of quality parameters of pork stored using modified atmosphere packaging with high oxygen levels has been well characterised for pork loin chops [1]. However, recent work has demonstrated that the oxidation markers of different fresh pork products respond differently to the same oxidative conditions and could benefit from individual optimisation of the MAP gas composition [2, 3]. Furthermore, characteristics related to fibre type composition and hence muscle physiology, such as myoglobin content and myosin heavy chain (MyHC) isoform expression were identified as of potential importance to oxidative stability. The aim of the present study was to determine the influence of muscle fibre composition and MAP on eating quality and the oxidative stability of retail-packed pork belly during refrigerated storage.

II. MATERIALS AND METHODS

Pork belly is characterised by the co-existence of a light/glycolytic muscle (*Cutaneous trunci*) and red/oxidative muscles (primarily *Latissimus dorsi*), which makes it ideal for the examination of muscle specificity in relation to oxidative deterioration. The different muscles of the cut were excised separately. Lipid secondary oxidation markers (thiobarbituric acid reactive substances – TBARS) were determined for different MAP gas mixtures and compared between fractions. Furthermore, protein from each fraction was electrophoretically separated, and immunoblot stained for the detection of carbonylated residues. A sensory profile was carried out on day two and six after packaging. For all analysis, all MAP treatments were compared within each pig with 6 replicates per storage day for sensory profiling, 3 replicates for lipid oxidation and 1 replicate for protein oxidation.

Sliced pork belly from 12 female pigs (79-83 kg) slaughtered at the same date were MA-packed in five different gas compositions, stored at 5°C and analysed at different points of time during storage. Day 1 after slaughter, pH_{24}, cutting, deboning, shell freezing and slicing were performed at the slaughterhouse. The meat was then transported to DMRI's pilot plant and retail-packed in MAP day 2 after slaughter. Samples were analysed at day 0, 2 and 6/7 after retail packaging in MAP.

Table1. Design of trial

cut	Pork belly				
5 x gas	0/20	40/20	50/20	50/40	80/20
	-	40% O_2	50% O_2	50% O_2	80% O_2
	20% CO_2	20% CO_2	20% CO_2	40% CO_2	20% CO_2
	80% N_2	40% N_2	30% N_2	10% N_2	-
Storage	2, 6/7 days (5°C)				

Packing: Eight slices of pork belly (9 mm) were MA-packed in five gas mixtures: 1. 20% CO_2 + 80% N_2 (0/20); 2. 40% O_2 + 20% CO_2 + 40% N_2, (40/20); 3. 50% O_2 + 20% CO_2 + 30% N_2 (50/20); 4. 50% O_2 + 40% CO_2 + 10% N_2 (50/40); 5. 80% O_2 + 20% CO_2 (80/20) on a tray sealer (Multivac, T200, Denmark). Samples were stored for 2 and 6-7 days at 5°C.

TBARS: Secondary lipid oxidation products were analysed according to the method described by [3] and modified by [4]. In short, pork samples (5 g) stored for 2 days (0/20 + 80/20) and 7 days (all gas compositions) were homogenized for 1 min. at approximately 12,000 rpm in 30 mL of trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% PG and 0.1% Ethylenediaminetetraacetic acid (EDTA)), using an Ultra-Turrax mixer (Janke & Kunkel IKA Labortechnik, Staufen, Germany). The mixture was filtrated through Whatman Grade-2 filter paper (Sigma-Aldrich, USA) and 5 mL of 20 mM thiobarbituric acid were added to 5 mL of the filtrate. The solution was then incubated for 40 min. at 100°C in closed test tubes. Following incubation, absorbance at 532 nm was measured against a blank sample with a Shimadzu UV-1800 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, USA) and was quantified using a standard curve. Results were expressed as µmol malondialdehyde (MDA) equivalent/kg of tissue.

Carbonyls: Pork belly samples (1 g) stored for 2 (0/20 + 80/20) and 7 (all gas compositions) days were homogenized with a Polytron PT1200E system in 10 mL of Trizma buffer solution, and the protein content was adjusted to 5 mg protein/mL and reacted in the dark at room temperature with 2.4-Dinitrophenylhydrazine (DNPH) solution (10% in THF). After 15 min., the reaction was stopped, samples were loaded unto the gel and the derivatised proteins were electrophoretically separated under non-reducing conditions, and chemiluminescence detected using a DSLR camera. The blue channel of the RGB output was extracted and used for interpretation. The membrane was incubated with rabbit anti-DNP primary monoclonal antibody (Sigma) at a dilution of 1:7500 and HRP-conjugated goat anti-rabbit secondary polyclonal (DAKO Denmark A/S, Denmark) at a dilution of 1:3000.

Cooking loss was measured as: (raw meat weight – cooked meat weight) * 100/raw meat weight.

Sensory analysis: The pork belly slices were tempered at room temperature to 10-12°C and then cooked in an oven at 200°C for approx. 20 min until they were brown and crispy. Samples were evaluated by eight trained assessors using a 15 point unstructured line scale anchored at the extremes (0 = low intensity and 15 = high intensity). The descriptive attributes were developed during training, with focus on flavour, texture, juiciness and appearance.

Statistical analysis: Data were analysed using mixed models (SAS, 9.2, 2002-2008). The model included gas mixture, storage time and interaction as fixed effects, and assessors and pig (storage time) as random effects. Non-significant interactions were deleted from the model. Least squares (LSmeans) were calculated and separated using probability of difference. Levels of significance: $p > 0.05$ = non-significant (ns), $0.05 > p > 0.01$ = *, $0.01 > p > 0.001$ = **, $p < 0.0001$ = ***.

III. RESULTS AND DISCUSSION

Lipid oxidation

In general, TBARS values were very low for all samples. However, for both muscle fractions, TBARS levels were significantly lower for samples stored in anoxic (0/20) MAP compared with samples in high-oxygen (80/20) MAP (Table 2). At day 7, samples were divided into three groups 0/20 < 40/20, 50/20, 50/40 < 80/20; showing that tri-gas MAP with intermediate oxygen levels is able to reduce lipid oxidation in retail packed pork belly.

Table 2. TBARS (µmol MDA equivalent/kg of tissue) in two different muscle fractions (dark/light). Effect of five gas compositions and storage time (2 days/7 days).

		0/20	40/20	50/20	50/40	80/20
$TBARS^{dark}$	2	0.7^{ax}	-	-	-	1.3^{bx}
	7	0.8^{ax}	1.6^{b}	1.5^{b}	1.4^{b}	1.9^{cy}
$TBARS^{light}$	2	0.8^{ax}	-	-	-	1.1^{bx}
	7	0.8^{ax}	1.1^{b}	1.2^{b}	1.1^{b}	1.7^{cy}

a, b, c: denotes that means (gas mixtures), within the row, with different superscripts are significantly ($P < 0.05$) different.

x, y: denotes that means (storage), within the column, with different superscripts are significantly ($P < 0.05$) different.

For gas compositions other than anoxic (0/20) or high-oxygen (80/20) MAP, TBARS was significantly higher in the dark muscle type compared to the white *Cutaneous trunci*, which could be attributed to the more prominent presence of the pro-oxidative myoglobin. The above indicates that different muscle types will respond in a different manner to an increasing oxidative environment, although at high oxygen MAP and after sufficient storage time a similar maximal value might be attained.

Protein oxidation

The protein oxidation of the light and dark muscle fractions was evaluated in a qualitative manner by blotting against derivative carbonylated groups, using an anti-DNP antibody (Figures 1-2). In general, the signal was located primarily on protein with a MW more than 171 kDa, for both fractions. For the dark muscle fraction, storage time had an impact under anoxic conditions (0/20), as an increase in signal intensity and number of bands was visible at 7 days compared to 2 days of storage (Figure 1, D-0-2 vs. D-0-7). However, the same was not apparent for samples stored in high-oxygen MAP (80/20).

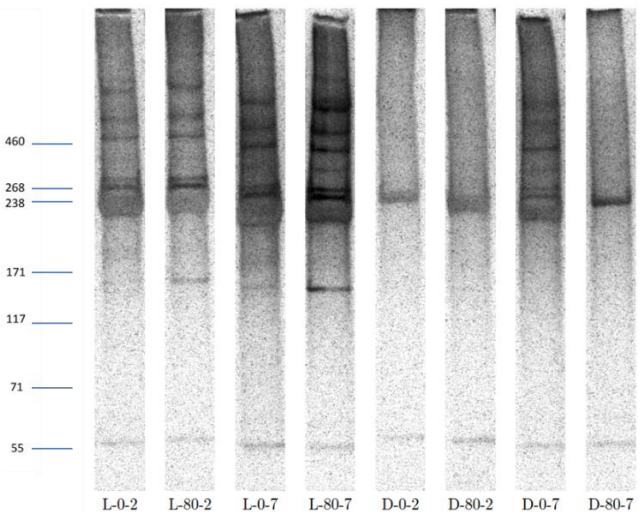

Figure 1. Anti-DNP blot for the light and dark muscles stored in anoxic and high-oxygen MAP for 2 and 7 days. Molecular weights are noted in kDa. The succeeding lanes are described by the following abbreviation: Muscle (D: dark muscle, L: light muscle) – Oxygen concentration (%) – Storage time (days).

Increase of O_2 levels appeared to result in a gradual increase of total signal intensity at 7 days of storage (Figure 2). Storage in high-CO_2 MAP (50/40) resulted in increased oxidation levels of the light muscle fraction compared with samples stored in intermediate MAP (50/20), with additional bands appearing between 117 kDa and the MyHC band. For both the light and dark muscle fractions, an additional band was visible at approximately 117 kDa.

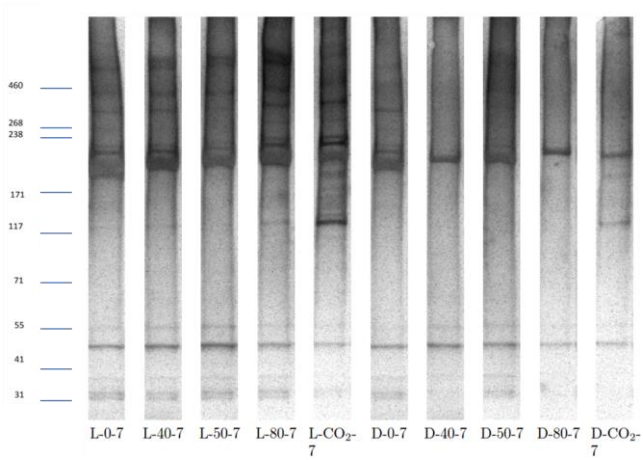

Figure 2. Anti-DNP blot for the light and dark muscles stored for 7 days under all MAP. The lanes marked as L-CO_2 and D-CO_2 correspond to the light and dark muscles of samples packed in high-CO_2 MAP, respectively.

Eating quality & cooking loss

Flavour and crispiness of pork belly varied with gas compositions. Packaging in 50/40 resulted in a more crispy bite of the meat and the rind compared with the other gasses (Table 3). For pork belly packed in 80/20, the crispiness decreased during storage and had the least crispy bite.

The isolated effect of CO_2 was limited to crispiness of the rind only. Measurements on lipid oxidation, cooking loss and stale flavour revealed that the high-CO_2 MAP did not affect these quality markers, whereas crispiness of the rind was found crispier when packed in 50/40 compared to 50/20 after 6 days of storage.

Table 3. Sensory attributes of sliced pork belly in MAP with different gas mixtures (day 6).

Attribute	Days	0/20	40/20	50/20	50/40	80/20
Crispiness meat	2	5.2^{abX}	5.2^{abX}	5.4^{abX}	6.0^{bX}	4.7^{aX}
	6	4.9^{bX}	6.5^{cX}	6.4^{cX}	7.1^{cX}	3.2^{aY}
Crispiness skin	2	7.7^{abX}	6.9^{aX}	6.7^{aX}	8.5^{bX}	6.4^{aX}
	6	6.8^{bX}	8.0^{bX}	7.9^{bX}	9.7^{cX}	4.2^{aY}
Stale flavour	2	2.2^{abX}	2.2^{abX}	1.5^{aX}	1.9^{abX}	2.7^{bX}
	6	4.0^{bcY}	4.0^{bcY}	3.2^{abY}	2.7^{aX}	4.6^{cY}
Cooking loss	2	57.1^{bX}	54.3^{abX}	57.8^{bX}	57.1^{bX}	52.4^{aX}
	6	59.9^{bX}	62.3^{bcY}	64.0^{cY}	65.8^{cY}	51.8^{aX}

a, b, c: within each row (gas mixture), means with different superscripts are significantly ($P < 0.05$) different.
X, Y: within each column (storage time), means with different superscripts are significantly ($P < 0.05$) different.

TBARS and stale flavour were highest for samples packed in high O_2 MAP (80/20) as expected, but lowest for samples packed in high CO_2 MAP (50/40). Nevertheless, crispiness and cooking loss was highest in 50/40 and lowest in 80/20. This indicates that lipid oxidation and protein oxidation might be affected differently.

IV. CONCLUSION

Lipid and protein oxidation markers were affected by storage time and MAP gas mixture in both light and dark muscles of pork belly. Eating quality was not affected by a single gas but by the combination of O_2 and CO_2 in the gas mixture.

High CO_2 levels in combination with intermediate O_2 levels (50/40) increased the crispiness of pork belly. Cooking loss also increased in this tri-gas MAP combination, suggesting that the water-holding capacity (WHC) of pork belly is affected by CO_2 in the MAP. These quality changes may relate to increased protein oxidation at high CO_2 levels. More work is required to elucidate this effect and establish correlations between protein oxidation and WHC.

To summarize, the meat industry should MA-pack in a tri-gas MAP with a gas mixture of 50% O_2 + 40% CO_2 + 10% N_2 to obtain optimal quality of pork belly.

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REFERENCES

1. Lund, M. N., Lametsch, R., Hviid, M. S., Jensen, O. N., and Skibsted, L. H. (2007). High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissimus dorsi during chill storage. Meat Science 77:295-303.
2. Spanos, D., Tørngren, M. A., Christensen, M., & Baron, C. P. (2016). Effect of oxygen level on the oxidative stability of two different retail pork products stored using modified atmosphere packaging (MAP). Meat science, 113, 162-169
3. Tørngren, M. A., Darré, M. T., & Kristensen, L. (2013). Optimising the eating quality and shelf life of retail pork cuts using muscle specific three-gas MA packaging. In 59th International Congress of Meat Science and Technology, Izmir, Turkey.
4. Vyncke, W. (1970). Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. Fette, Seifen, Anstrichmittel, 72(12), 1084-1087.
5. Sørensen, G., & Jørgensen, S. S. (1996). A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. Zeitschrift für Lebensmittel-Untersuchung und Forschung, 202(3), 205-210.