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Effects of Modified Atmosphere Storage on Colour and Microbiological Shelf Life of Normal and Pale, Soft and Exudative Pork

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ABSTRACT

Pale, soft and exudative (PSE) and normal pork loins (n=9 of each) were deboned, divided and packaged in modified atmospheres (MA) of 100% carbon dioxide containing 0, 0·5 and 1·0% residual oxygen (O_2). The meat was stored at 3°C, first in MA for 21 days, followed by 5 additional days under retail display conditions with access to air. Before packaging, PSE loins were more light and less red than those of normal meat. The drip loss after MA storage was twice as high from PSE meat as from normal meat. PSE meat was not more discoloured after MA storage than normal meat. The level of residual O_2 in the MA had a significant impact on the colour of the two types of meat. Discoloration was observed on both PSE and normal meat with 0·5% O_2 and even more clearly with 1·0% O_2, as demonstrated by instrumental and visual colour analyses. After MA storage, the microbiological shelf life and flora were not affected by the type of meat, or level of residual O_2. © *1997 Elsevier Science Ltd*

INTRODUCTION

Pale, soft and exudative (PSE) pork is causing considerable economic loss to the meat industry due to its poor colour and low water-holding capacity. The fast glycolysis and rapid pH decline in PSE muscle early *post mortem*, combined with high *pre rigor* temperatures cause partial protein denaturation, in particular of the myosin and sarcoplasmic proteins (Stabursvik *et al.*, 1981; Honikel and Kim, 1986). Causes of the development of PSE pork have been reviewed extensively (Frøystein *et al.*, 1981; Puolanne *et al.*, 1992), focusing on genetics of breeding pigs, pre-slaughter stress and slow chilling of *pre rigor* muscle.

Modified atmosphere packaging (MAP) with high concentrations of carbon dioxide (CO_2) is widely used for extending the shelf life of meat (Gill, 1996). In a CO_2 atmosphere, low levels of residual oxygen (O_2) can lead to formation of metmyoglobin and discolouration of the meat (Ledward, 1970; Cornforth, 1994). Loins of normal pork were discoloured

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after exposure to as low as 1·0% O_2 at 4°C (Sørheim *et al.*, 1995). However, PSE pork seems to be more sensitive to discolouration. Under storage in CO_2 with 1·2% O_2 and at −1·5°C discolouration was not detected on normal pork, only on PSE pork (Jeremiah *et al.*, 1992). Extracted and purified myoglobin from porcine PSE muscles was oxidised to metmyoglobin 1·5 to 2 times faster than from normal muscles (Bembers and Satterlee, 1975).

There are few reports comparing the microbiology of PSE and normal pork. However, under aerobic retail conditions both Rey *et al.* (1976) and Greer and Murray (1988) found less bacterial growth on PSE meat. For determining the overall shelf life of different pork qualities, it is important to consider both the colour and microbiological stability of the meat.

The aim of the present study was to compare PSE and normal pork for colour, microbiological shelf life and drip loss when stored in CO_2 atmospheres containing low levels of residual O_2.

MATERIALS AND METHODS

Meat sampling

Pork loins were obtained from a commercial abattoir 24 hr after slaughter. The carcasses, all of Norwegian Land Race, had initially been blast-chilled and weighed an average of 75 kg. Nine PSE and 9 normal loins were selected at the abattoir using a five-point visual colour and structure standard (Agriculture Canada, 1984) with PSE scores of 2 or less and normal scores of 3. The next day the loins were deboned, trimmed to 5 mm backfat, cut into three sections of approximately 1 kg each and packaged.

Packaging

The loin sections were placed fat side down in polyamide/polyethylene bags of 30×60 cm (Sandvik, Bergen, Norway) with O_2 transmission rates of 25 $cm^3 m^{-2} 24 hr^{-1}$ atm at 23°C and 50% RH. Packaging was performed with an Intevac IN30 chamber machine (Intevac Verpackungsmaschinen, Wallenhorst, Germany). After evacuating air from the bags (to 9 mbar), they were filled with CO_2 to a gas to meat ratio of approximately 3:1 with O_2 concentrations below 0·5% (see gas analyses). Atmospheres free of O_2 in our experimental measurement conditions were obtained by the use of two Ageless® FX 50 O_2 absorbers (Mitsubishi Gas Chem. Co. Inc., Tokyo). To obtain atmospheres with 0·5 and 1·0% O_2, appropriate volumes of air were injected through a selfsealing rubber patch attached to the bag. All samples were stored in the dark for 21 days at 3°C.

Display

After MA storage, a 2 cm thick centre chop from each section was placed on a polystyrene tray and wrapped in a polyvinylchloride film with O_2 transmission rate > 10 000 $cm^3 m^{-2} 24 hr^{-1}$ atm at 23°C and 0% RH. The chops were displayed for five days at 3°C in a room under continuous 1000 lux light.

Gas analyses

Oxygen was analysed with a Toray LC 700-F instrument with a zirkonium oxide sensor and CO_2 with a Toray PG-100 instrument with an infrared sensor (both Toray Eng., Japan).

Headspace samples of 10 ml were taken with a gastight syringe through patches on the bags. Analytical threshold levels used in this experiment were 0·05% for O_2 and 0·5% for CO_2. The MA packages were tested immediately after packaging, and at 3, 7, 14 and 21 days of storage.

pH

The pH measurements were made directly in the meat with an Ingold Xerolyt electrode (Mettler-Toledo A.G., Greifensee, Switzerland) before packaging, after MA storage and after display.

Colour

A Minolta Chroma Meter CR-300 (Minolta Camera Co., Osaka, Japan) with 8 mm viewing port and illuminant D_{65} was used for measuring the *m. longissimus lumborum et thoracis* of the loin sections for CIE (1976) L^* (lightness), a^* (redness) and b^* (yellowness) (Hunter and Harold, 1987). The measurements were made through the packaging film at days 0, 1, 3, 7, 14 and 21 of MA storage. The $L^*a^*b^*$ values of the meat were modified for those of the packaging film by subtraction.

A six-member experienced panel evaluated *m. longissimus lumborum et thoracis* of the loin sections at the end of MA storage. The two evaluated characteristics were: colour (scale 1 = very pink for normal meat and very light pink for PSE meat, 2 = slightly pink for normal meat and slightly light pink for PSE meat, 3 = slightly grey/green, 4 = moderately grey/green, and 5 = extremely grey/green) and discoloration (scale 1 = none, 2 = 1–10%, 3 = 11–20%, 4 = 21–60%, 5 = 61–100% of the muscle area) (National Live Stock and Meat Board, 1991).

Drip loss

Loin sections and chops were weighed before and after MA storage and display, respectively, to calculate percentage drip losses (Honikel and Kim, 1986).

Microbiology

Analyses were performed on all loins before packaging, on all loin sections after MA storage and on five chops from each treatment after display. A 25 cm^2 meat surface area was marked with a sterile aluminium template and a 2–3 mm thick layer removed with a scalpel. The sample was placed in physiological saline solution of pH 7·0, homogenized for 1 min. and spread in duplicate on five different media as follows:

- PCA (Plate count agar; Difco) for total viable counts;
- MRS agar (Oxoid) adjusted to pH 5·7 for lactic acid bacteria (Man *et al.*, 1960);
- STAA (agar base, type CM881 with a selective supplement SR 151; Oxoid) for *Brochothrix thermosphacta*;
- CFC (Pseudomonads agar base type CM 559 with selective supplement SR 103; Oxoid) for pseudomonads;
- VRBA (Violet red bile agar; Difco) for coliforms.

Plates with PCA, MRS, STAA and CFC were incubated at 20°C for four days, and with VRBA at 30°C for two days, all aerobically. Counts were expressed as colony forming units (CFU) per cm^2.

Statistics

One-way (initial instrumental colour, pH, drip loss and microbiology) and two-way (visual and other instrumental colour) analyses of variance with Tukey's multiple comparisons test was carried out by using the SAS programme (SAS Institute, Inc., 1986).

RESULTS AND DISCUSSION

Characterisation of PSE meat

Measurements confirming the initial grouping of the PSE and normal meat at the abattoir are shown in Table 1. Before packaging, the PSE loins were lighter and less red than normal loins ($p < 0.01$), but did not differ in yellowness ($p > 0.05$). The pH of the unpackaged loins was 0·16 units lower in the PSE than the normal group ($p < 0.01$). The pH in *post rigor* PSE pork is commonly 0·1–0·2 units lower than in normal pork (Rey *et al.*, 1976; Greer and Murray, 1988; Laack *et al.*, 1994). However, in the present experiment no difference was found in the pH of the two meat types after MA storage or subsequent display, ($p > 0.05$). The drip loss of the loin sections after MA storage was twice as high in PSE as normal meat, although the drip loss from displayed chops was not different ($p > 0.05$). In agreement with the description of PSE meat (Honikel and Kim, 1986), the PSE meat chosen for our experiment was paler, less red in colour and had a low water-holding capacity.

Gas composition

Monitoring gas concentrations in MA storage experiments is important in the evaluation of pigment oxidation and microbiological growth. In the present experiment, the bags with O_2 absorbers had a maximum O_2 concentration of 0·5% at the time of packaging, and no O_2 was detected in these bags from day 3 to day 21 of MA storage, due to the O_2 depletion by the absorbers. In bags initially containing 0·5 and 1·0% O_2 the O_2 concentration did not change during storage (results not shown). CO_2 concentrations were reduced from about 96% to 91% during storage in all bags (results not shown), probably due to CO_2 absorption by the meat (McMullen and Stiles, 1991; Gill, 1996). The remaining gas in the MA's, except CO_2 and O_2, is likely to be predominantly N_2.

TABLE 1
Instrumental Colour Values, pH and Drip Loss of Pale, Soft and Exudative (PSE) and Normal Pork Before and After Modified Atmosphere Storage and After Display at 3°C

Analysis	*Day*	*Sample*	*PSE*	*Normal*	*p*
L^* (lightness)			56·4	54·2	< 0·01
a^* (redness)	0	Loins	7·3	8·3	< 0·01
b^* (yellowness)			6·0	6·2	n.s.
	0	Loins	5·51	5·67	< 0·01
pH	21	Loin sections	5·51	5·53	n.s.
	26	Chops	5·61	5·60	n.s.
Drip loss (%)	21	Loin sections	2·6	1·3	< 0·05
	26	Chops	1·5	1·3	n.s.

n.s. is not significant, $p > 0.05$.

Colour

The surface colour of both PSE and normal meat was highly affected by the concentration of residual O_2 in the atmosphere. Figures 1 (a), (b) and (c) show the L^* (lightness), a^* (redness) and b^* (yellowness) values of the loin sections during 21 days of MA storage. The L^* values generally increased from the time of packaging to seven days storage and thereafter stabilized. At 21 days storage, the L^* values were higher ($p < 0.05$) in 1·0% O_2 than in 0·5 and 0% O_2 for both PSE and normal meat. Following a slight initial increase, the a^* values generally decreased after exposing the meat to atmospheres with residual O_2. After seven days and more of MA storage, PSE meat in 0% O_2 had higher a^* values than meat in 0·5 and 1·0% O_2 ($p < 0.05$). A similar difference in a^* values was observed for normal meat after 14 days storage. After a slight initial drop, the b^* values increased during storage, more so on meat packaged in atmospheres with residual O_2. From day 3 and throughout storage, the b^* values were significantly higher ($p < 0.05$) in 0·5 and 1·0% O_2 than in 0% O_2, for both PSE and normal meat.

The visual colour evaluation supported the data from the $L^*a^*b^*$ measurements as shown in Fig. 2. Because the scales used for the PSE and normal meat were not identical, comparisons can only be made within, not between, the two meat groups. Both for the colour and discolouration scores, significant differences were found between the three gas treatments, with most greying/greening and greatest area of discolouration for meat in 1·0% O_2, followed by 0·5 and 0% O_2 ($p < 0.05$).

The instrumental measurements of redness in Fig. 1 (b) did not support previous studies concerning the low stability of porcine PSE myoglobin. The lower a^* values on PSE compared to normal meat in our experiment, seemed to be due to the initial colour of the meat, not to the effects of MA storage. Bembers and Satterlee (1975) found larger auto-oxidation rates for extracted and purified myoglobin from porcine PSE muscle, when compared to similar preparations from normal muscle. The conversion of PSE oxymyoglobin to metmyoglobin was 1·5 to 2 times faster than for that of normal oxymyoglobin. Jeremiah *et al.* (1992) stored PSE and normal pork loins in CO_2 atmospheres containing 1·2% O_2 for four weeks at −1·5°C, and found a^* values to decrease substantially on extreme PSE and to a lesser extent on PSE samples. Contrary to our findings, no decrease in a^* values was observed on normal meat under these storage conditions.

Our experiments revealed that discolouration ultimately occurred on pork with residual O_2 concentrations down to 0·5% in the MA, as demonstrated by the a^* values and the visual scores. In addition, the discolouration of the pork was greater in 1·0 than 0·5% O_2, as shown by the visual scores. Previously, the limit for discolouration of pork was reported to be above or below 1·0% O_2, respectively (Penney and Bell, 1993; Sørheim *et al.*, 1995). The first of these experiments was performed with a storage temperature of −1·5°C compared to 3°C in our experiment, which could decrease the rate of myoglobin oxidation and discoloration (Cornforth, 1994; Gill, 1996).

Lamb and beef seem to have lower tolerance levels for discolouration of residual O_2 than pork. Discoloration of lamb occurred with 0·15% O_2 in the MA (Penney and Bell, 1993). Beef was discoloured with 0·01 to 0·1% of O_2 and had more discolouration at temperatures above than slightly below 0°C (Gill and McGinnis, 1995).

Microbiology

Before packaging the bacterial counts were somewhat higher on PSE than normal meat, especially for pseudomonads and *Brochothrix thermosphacta* ($p < 0.05$) (Table 2), perhaps due to a higher release of water onto the surface of the PSE meat.

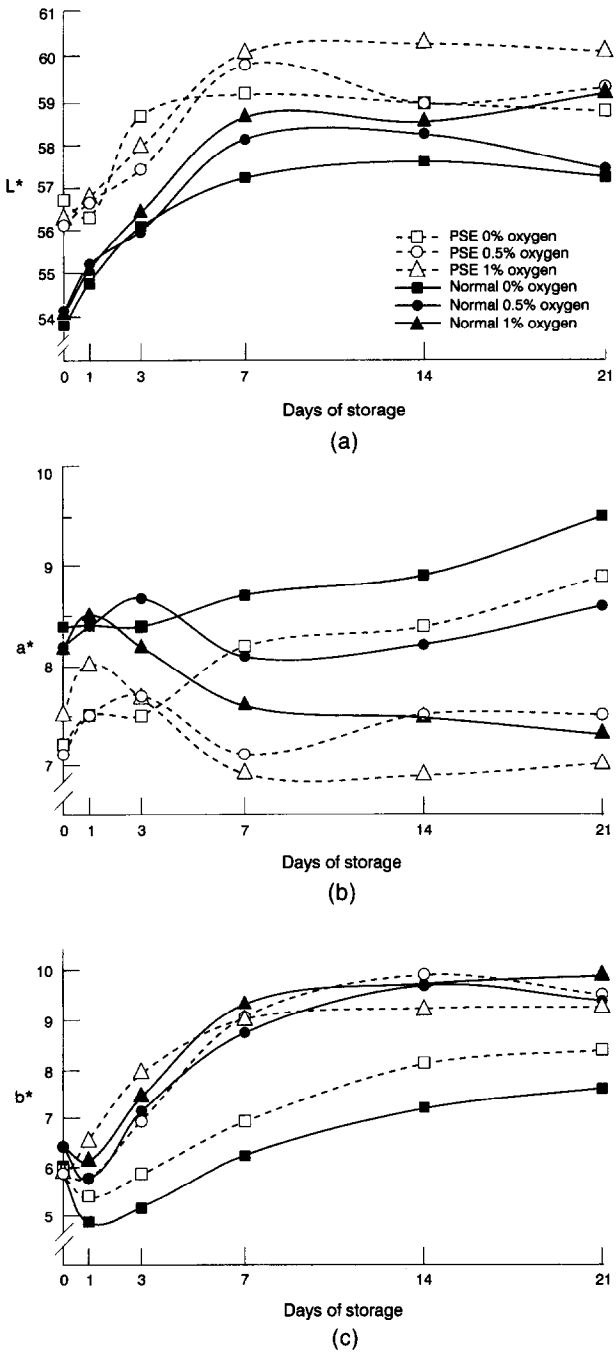

Fig. 1. (a), (b) and (c). L^* (lightness), a^* (redness) and b^* (yellowness) values of PSE and normal pork loin sections during storage in CO_2 with 0, 0·5 and 1·0% residual O_2 at 3°C for 21 days. Legend in (a) also applies to (b) and (c).

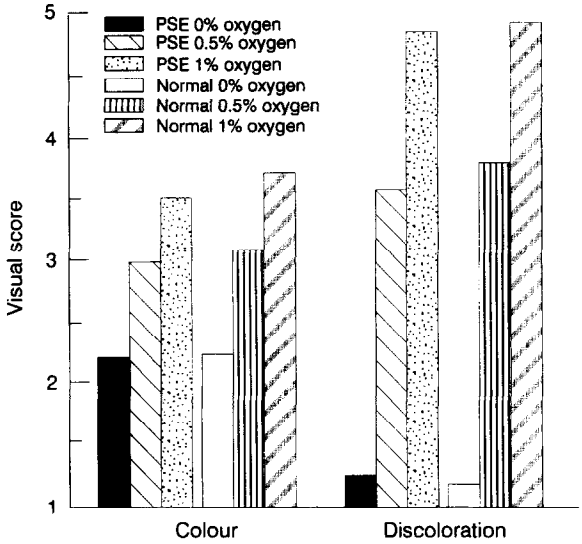

Fig. 2. Visual colour and discoloration scores of PSE and normal pork loin sections stored in CO_2 with 0, 0·5 and 1·0% O_2 at 3°C for 21 days. Colour scale: 1 = very light pink (PSE) or very pink (normal), 5 = extremely grey/green (PSE and normal). Discoloration scale: 1 = none, 5 = 61–100%.

After 21 days of MA storage and 5 additional days of display, no differences were found in bacterial counts between PSE and normal meat ($p > 0{\cdot}05$) (Table 2). At this time of MA storage, the total counts of bacteria had increased to more than $\log_{10} 6\,cm^{-2}$ and the flora was dominated by lactic acid bacteria, as previously reported (Gill and Harrison, 1989, Sørheim *et al.*, 1995). The lactic acid bacteria seemed to inhibit growth of pseudomonads and *B. thermosphacta*. This inhibition lasted even after display in air, as previously shown both for pork and beef (Sørheim *et al.*, 1996; Nissen *et al.*, 1996). The number of coliforms increased somewhat after the exposure to air, but not sufficiently to cause spoilage.

In the present experiment, O_2 levels of 0·5 and 1·0% in the CO_2 atmospheres did not affect microbiological counts either after MA storage or display, compared to CO_2 without residual O_2 ($p > 0{\cdot}05$) (Table 2). Sheridan *et al.* (1997) describe a spoilage pattern for lamb packaged in 100% CO_2 and stored at 5°C. The meat had a high growth of lactic acid bacteria and low growth of other microorganisms, similar to the results of our study on pork with CO_2 and up to 1% residual O_2 at 3°C. However, other studies have shown that higher concentrations of residual O_2 may stimulate the growth of the spoilage bacteria *B. thermosphacta* and pseudomonads. Sørheim *et al.* (1995) found a shortening of the microbiological shelf life of pork loins from six to four weeks at 4°C, when the CO_2 atmosphere contained 4% instead of 0% O_2, possibly due to growth of *B. thermosphacta*. In a comparison between atmospheres of 30/70% CO_2/N_2 and 30/68/2% $CO_2/N_2/O_2$, the latter atmosphere gave a 100-fold increase in psychrotrophic counts on pork after 20 or 40 days storage at 2°C (Spahl *et al.*, 1981). Residual O_2 levels of 2 to 3% also resulted in an increase in pseudomonads and *B. thermosphacta* on pork during four weeks storage in a mixture of 40% CO_2 in N_2 at 4·4°C (McMullen and Stiles, 1991). Based on these studies, it seems that concentrations of O_2 in MA's have to be 2% or higher to increase growth of spoilage bacteria on pork. However, a higher CO_2 concentration in the MA will also interact with the O_2 level and influence the microbiological growth.

TABLE 2
Microbiological Counts ($\log_{10}$ CFU cm^{-2}) of Pale, Soft and Exudative (PSE) and Normal (N) Pork Before and After Modified Atmosphere (MA) Storage in CO_2 with 0, 0·5 or 1·0% O_2 and After Display at 3°C

	Before packaging (loins) n=9		*% O_2*	*21 days MA storage (loin sections) n=9*		*Additional five days display (chops) n=5*	
	PSE	*N*		*PSE*	*N*	*PSE*	*N*
Total viable counts	3·5*	2·8*					
			0	6·8	6·6	6·5	6·7
			0·5	7·1	6·8	6·4	6·4
			1·0	6·9	6·9	7·0	6·4
Lactic acid bacteria	1·0	1·0					
			0	6·7	6·6	6·4	6·6
			0·5	6·7	6·5	6·2	6·1
			1·0	6·9	6·6	6·7	6·3
Brochothrix thermosphacta	1·6*	1·2*					
			0	1·5	1·5	2·6	3·0
			0·5	1·7	2·0	2·7	2·1
			1·0	2·1	1·7	3·1	2·4
Pseudomonads	3·3*	2·5*					
			0	2·5	2·3	2·3	3·1
			0·5	2·6	2·4	2·9	2·8
			1·0	2·3	2·4	3·3	2·7
Coliforms	1·2	1·1					
			0	1·0	1·2	2·8	3·0
			0·5	1·7	1·2	2·6	3·0
			1·0	1·7	1·5	3·0	3·0

*Means in a line are significantly different, $p < 0·05$.

CONCLUSION

After exposure to MA with CO_2 and low levels of O_2, PSE and normal pork were equally susceptible to grey/green discoloration. The discoloration occurred with as low as 0·5% residual O_2 for both meat types. After MA storage, the microbiological flora did not differ on PSE and normal pork or by the level of O_2 up to 1%. The drip loss during MA storage was higher on PSE than normal meat.

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