The Effects of Oxygen Scavengers on the Colour Stability of Fresh Beef Packaged Under Pure Carbon Dioxide

Modified atmosphere packaging (100% CO₂) and oxygen scavengers were used to achieve a virtually oxygen-free atmosphere and to prevent metmyoglobin formation at the meat surface. Eight retail trays with eye of rump steaks (Gluteus medius) were over-wrapped in a high gas permeability film and packaged under pure CO₂ in a low gas permeability masterpack. An oxygen scavenger was added to each masterpack in order to increase the colour stability of the fresh beef. Two types of commercial O₂ scavenger with different O₂ scavenging capacity were studied. A control system without O₂ scavengers inside the masterpack was also evaluated. The masterpacks were stored at 4±1°C for 7 and 14 days. After the seventh day of storage in MAP and exposure of the retail packs to air, the eye of rump steaks which had been stored in masterpacks with scavengers with a rated capacity for 420mL of O₂ presented a transitory discolouration, but the steaks recovered their typical reddish surface colour during the subsequent storage period. After 14 days, the O₂ concentration inside the masterpacks was reduced to zero and the rump steaks reached their lowest discolouration level ([0-5%], highest intensity of red (8.0) and highest values for redness (a*=-21.18), which showed that steaks packaged with lower rated capacity scavengers (formulated to reduce 300mL of O₂) or the control steaks. Both had comparable brown areas and failed to bloom. Colour differences were most noticeable after 14 days of storage, when the O₂ concentration in the masterpacks with higher capacity scavengers was reduced to zero. After 14 days, all steaks with or without O₂ scavengers had comparable counts (P<0.05) of aerobic and anaerobic bacteria (<10⁵ CFU/g). These results indicated that the masterpack under 100% CO₂ combined with O₂ scavengers could be an appropriate technique to preserve fresh beefsteaks at commercial storage temperatures.

RESUMO

Modificada atmosfera (100% CO₂) e absorvedores de oxigênio foram combinados para atingir uma atmosfera virtualmente livre de oxigênio e prevenir a formação de metamioglobinina (marrom) na superfície da carne bovina fresca. Oito bandejas contendo bifis de miolo de alcatra (Gluteus medius) foram envoltas com um filme com alta permeabilidade a gases e acondicionadas em uma embalagem de transporte constituída por um filme plástico de baixa permeabilidade (masterpack). Imediatamente antes da termosselagem dos sacos, absorvedores de oxigênio foram adicionados no interior de cada masterpack e, em seguida, o ar foi evacuado e o CO₂ puro injetado. Dois tipos comerciais de absorvedores de O₂ com diferentes capacidades de adsorção, foram avaliados. Foram também preparadas embalagens tipo masterpack com CO₂ puro, porém sem absorvedores de O₂, que foram consideradas como sistema controle. Os masterpacks foram estocados a 4±1°C por 7 e 14 dias. Após cada período de estocagem em atmosfera modificada, as embalagens de varejo foram retiradas do masterpack e expostas ao ar. Após 7 dias de estocagem, os bifis de alcatra acondicionados com absorvedores formulados para absorver 420mL de O₂ apresentaram descoloração superficial apenas transitória, uma vez que, após 14 dias de estocagem, os bifis recobriram a cor vermelha superficial típica de carne fresca após re-oxigenação (reblooming). No 14º dia de estocagem, a concentração de O₂ no interior destas embalagens foi reduzida a zero, com os bifis de alcatra atingindo a menor descoloração (0-5%), a maior intensidade de cor vermelha (8.0) e o maior valor de cor vermelha instrumental (a*=-21.18), comparativamente aos demais tratamentos, onde o fenômeno de reblooming não foi observado. No 14º dia de estocagem, os números de bactérias psicrotróficas aeróbias e anaeróbias nos produtos de todos os tratamentos foram comparáveis (<10⁵ UFC/g), independente do uso de absorvedores de oxigênio. Os resultados deste experimento indicaram que a embalagem em masterpack sob 100% CO₂ associada ao uso de absorvedores de O₂ pode ser uma técnica promissora para a conservação de carne fresca bovina, sob temperaturas comerciais de estocagem.
1. INTRODUCTION

The colour of fresh beef depends on the relative amounts of three forms of myoglobin: reduced myoglobin, oxymyoglobin and metmyoglobin. Reduced myoglobin is the predominant muscle pigment in the absence of oxygen. It produces the characteristic purplish colour of vacuum-packaged beef meat. Oxymyoglobin is the oxygenated form of the muscle pigment and is responsible for the bright red colour consumers expect in beef sold in grocery stores. Metmyoglobin is the form showing an undesirable brown colour. Consumers expect in beef sold in grocery stores. Metmyoglobin is the form showing an undesirable brown colour that causes consumers to reject pre-packaged meats [SARANTÔPOULOS, 1991].

The packaging atmosphere can affect the colour of meat. For more extensive trading of retail meat, a modified atmosphere must be used to extend the storage life of the product YOUNG et al. 1988). Unfortunately, a ratio of about 1:3 for the meat to gas volume is required to maintain an adequately preservative composition of the atmospheres [GILL; JONES, 1996] and oversized packages are generally viewed unfavourably by consumers. Retail problems due to oversized display packs can be avoided by sealing a number of display trays into a masterpack, which contains the anoxic atmosphere [GILL, 1996]. Under pure CO2 stored beef is maintained in the purple reduced form of myoglobin [ISDELL et al. 1999]. As the product is exposed to air after the removal from the masterpack, a desirable bright red meat colour can bloom [SARANTÔPOULOS, 1991].

However optimum conditions for metmyoglobin formation occur at low partial pressures of oxygen [GILL, 1996]. Inserting sachets of oxygen-scavenging chemicals during packing might prevent discoloration of beefsteaks in the masterpack. Existing O2 scavenging technologies are based on oxidation of one or more substances such as iron powder, ascorbic acid, photosensitive dyes, enzymes, unsaturated fatty acids and others [SUPPAKUL et al., 2003]. Commercial O2 scavengers based on iron powders (‘activated iron oxide’) are mixed with acids and/or salts and a humectant to promote oxidation of the iron [VERMEIREN et al., 1999; GILL; MCGINNIS, 1995; LABUZA; BREENE, 1989]. The humectant may be dry or pre-wetted according to the type of chemical reaction. The first type is moisture-dependent and requires humidity for activation, which prevents O2 scavengers from functioning during handling in air, whereas the other one is self-activating as soon as it is exposed to air. SMITH et al. (1995) reported the relationship between the types of O2 scavenger, reaction speeds and their specific use.

TEWARI et al. (2001), reported that the prevention of metmyoglobin formation might be affected by the type and capacity of the O2 scavenger employed, the storage temperature, pack atmosphere [air/N2/CO2] and initial O2 concentration.

The objective of this study was to evaluate the effects of a pure CO2 atmosphere in association with oxygen scavengers on the colour of steaks from muscles with inherent low stability, such as gluteus medius.

2. MATERIALS AND METHODS

The Gluteus medius muscles from chilled carcasses of Nelore breed (Bos indicus) cattle were excised one day after slaughter and vacuum packaged. After 3 days, two steaks (390±40g) sliced into approximately 10-15mm pieces, were placed in an expanded polystyrene tray (3RR, Cryovac) over a soak pad (DryLock, Cryovac). Each tray was overwrapped with clear polyolephinic shrink film presenting an O2 transmission rate of about 12.232ml(STP)/(m2·day) at 25°C, 75% R.H. and 1atm. The film was perforated along the two largest sides of the tray to allow for free exchange of atmospheres during the gas flushing and reblooming. Eight retail packs were distributed in masterpacks (3.1±0.1kg) with an O2 transmission rate of 19 mL(STP)/(m2·day) at 25°C and 75% R.H. and 1atm. Two different types of commercial oxygen scavenger based on iron powders were used in this study [S, and S]. Both types were self-activated as soon as exposed to air. Considering that the rate of oxygen uptake by commercial scavengers declines exponentially with the decrease in oxygen concentrations to below 1% [GILL, 1996], the scavenging capacity of both types was overestimated by approximately 20%. In the first treatment, the scavenger put into the masterpack was formulated to reduce 300ml of O2 [S] and in the second treatment, another commercial scavenger with a rated capacity of 420ml of O2 [S] was added. The masterpacks were evacuated by a ‘double vacuum-flush’ cycle, filled with 3.5L of pure CO2/kg of meat, and sealed using a gas-flushing machine (A300, CVP Systems Ltd., USA). The control masterpacks were evacuated, filled with pure CO2 and sealed with no O2 scavengers. The O2 and CO2 concentrations in the headspace were analysed by a gas analyzer (Dansensor), via a hypodermic needle inserted through a stick-on septum. Immediately after the needle was removed, the punctured area was sealed. After gas analysis, all packs were stored in a cold chamber in the dark for up to 14 days at 4 ± 1°C. Two masterpacks for each type of scavenger tested and one having no O2 scavenger were opened on the 7th and 14th days of storage after the gas analysis, and all the retail trays were placed in an illuminated (incandescent light) display case in air at 5±1°C. The steaks on display were assessed for colour and discolouration 1h after opening of the masterpacks, through the transparent shrink film. The purple, bright red and brown colours were evaluated using a non-structured scale of 9cm where 0 cm = no colour and 9 cm = intense colour. Discolouration was scored on a six-point scale where 1 = no discolouration, 2 = 5% discolouration, 3 = 5-15% discolouration, 4 = 15-25% discolouration, 5 = 25-35% discolouration, 6 = 35-100% discolouration. The overall steak quality was scored on a seven-point scale where 1 = very poor and 7 = excellent. Colour evaluation was also carried out with a portable spectrophotometer (Minolta Co., Ltd. – CMS508d). Reflectance measurements were expressed as lightness (L*), redness (a*) and yellowness (b*) values. The average of eight readings was recorded for each steak. For each treatment, four trays were randomly removed from masterpacks. The illuminant was C, the observer angle was 10° and the specular component was excluded. After retail pack opening, the pH of the meat surface was measured.
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directly by a combined glass electrode. All muscle tissue surfaces from each Gluteus medius sample were randomly sampled for analysis of the initial flora immediately after withdrawing from its vacuum pack. Samples (25g) were removed with a sterile scalpel and ground with 225mL of 0.1% peptone saline water. Two trays were removed randomly for each treatment immediately after opening the masterpacks and the steaks were similarly sampled. Aliquots (0.1mL) of appropriate dilutions were then spread on duplicate plates of Plate Count Agar (Merck). The plates were incubated for 2 days at 35°C for the bacterial mesophiles count and for 3 days at 20°C for growth of aerobic and anaerobic psychrotrophic bacteria (VANDERZANT; SPLITTOESSER, 1992). The results were analysed using the analysis of variance (ANOVA). Scheffe’s test was used to compare the means at a level of 0.05%.

3. RESULTS AND DISCUSSION

The O₂ concentration increased from 0.16% to 1.82% on the 1st day of storage in the masterpack with no O₂ scavenger, whereas in masterpacks with S₁ or S₂ scavenger types, O₂ increased to 2.30 and 1.55, respectively (Table 1). The oxygen concentration decreased continuously until day 14 in masterpacks without oxygen scavengers (1.59%) and with S₁ scavengers (1.04%), whereas masterpacks containing S₂ scavengers reached 0.01%. The increase in O₂ concentration in the masterpacks after packaging is inevitable (TEWARI et al., 2001). Such an increase may be attributed to the entrapment of O₂ in the absorbent pad, the corners of the tray, inside the expanded polystyrene tray, underneath the steaks, or under the over-wrap film, during evacuation.

As shown in Table 1, the presence of traces of O₂ and other atmospheric gases in the evacuated pouch is a result of the almost impossibility of establishing an atmosphere that is completely free of air at the time of sealing a masterpack with available commercial equipment designed for oxygen-depleted packaging.

Variations in pH after each storage period were measured directly on the steaks (Table 2). On the first day, the pH of the beefsteaks was 5.69 ± 0.12. After 14 days, the steaks packaged with or without O₂ scavengers showed small pH variations (<0.03) and all steaks had a normal pH (<5.80) independent of storage time and packaging system.

### TABLE 1. Modified atmosphere inside masterpacks during 14 days of storage at 4±1°C.

<table>
<thead>
<tr>
<th>Storage time</th>
<th>Control</th>
<th>S₁</th>
<th>S₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂</td>
<td>CO₂</td>
<td>O₂</td>
<td>CO₂</td>
</tr>
<tr>
<td>0h</td>
<td>0.16</td>
<td>99.9</td>
<td>0.16</td>
</tr>
<tr>
<td>2.5h</td>
<td>0.53</td>
<td>99.9</td>
<td>1.33</td>
</tr>
<tr>
<td>22h</td>
<td>1.74</td>
<td>99.9</td>
<td>2.26</td>
</tr>
<tr>
<td>1 day</td>
<td>1.82</td>
<td>99.9</td>
<td>2.30</td>
</tr>
<tr>
<td>2 days</td>
<td>1.84</td>
<td>99.0</td>
<td>2.31</td>
</tr>
<tr>
<td>5 days</td>
<td>1.60</td>
<td>97.5</td>
<td>1.67</td>
</tr>
<tr>
<td>7 days</td>
<td>1.49</td>
<td>92.3</td>
<td>1.67</td>
</tr>
<tr>
<td>14 days</td>
<td>1.59</td>
<td>94.6</td>
<td>1.04</td>
</tr>
</tbody>
</table>

S₁: masterpacks contained scavengers with rated capacity for 300mL of O₂.
S₂: masterpacks contained scavengers with rated capacity for 420mL of O₂.

### TABLE 2. Effect of storage time and packaging system on the mean pH values of beef (GM) steaks stored in masterpacks in a CO₂ atmosphere for up to 14d at 4±1°C.

<table>
<thead>
<tr>
<th>Storage Time (days)</th>
<th>Control</th>
<th>S₁</th>
<th>S₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>7</td>
<td>5.59</td>
<td>0.05</td>
<td>5.60</td>
</tr>
<tr>
<td>14</td>
<td>5.45</td>
<td>0.07</td>
<td>5.42</td>
</tr>
</tbody>
</table>

S₁: masterpacks contained scavengers with rated capacity for 300mL of O₂.
S₂: masterpacks contained scavengers with rated capacity for 420mL of O₂.
SD: standard deviation.
The initial microflora was composed of approximately 10^2 CFU/g of mesophiles and 10^3 CFU/g of aerobic and anaerobic psychrotrophic bacteria. After 14 days, all steaks with or without O₂ scavengers had comparable counts (<0.05) of aerobic and anaerobic bacteria (<10^3 CFU/g).

Immediately after packaging of the steaks into the masterpacks, the average values of the coordinates of the CIE L*a*b* system were L*=35.02±3.98; a*=22.03±4.52 and b*=1.42±2.70. Since redness (a*) was considered as a good index of steak discoloration during storage, it was verified at all storage times and 1h after opening of the masterpacks. The values for a* on the surface of steaks from the S₂ treatment, increased from the 7th (a*=16.15) to the 14th days (a*=21.18) of storage (Table 3).

After 7 days in MAP steak reblooming was not verified in any of the treatments 1h after opening the masterpacks. However, after 14 days of storage, when the O₂ concentration in the higher capacity scavenger (S₂) masterpacks was reduced to nearly zero, the steaks rebloomed and the values for a* increased. At this point of storage, the S₂ steaks redness was significantly higher than in the Control and S₁ treatments. The substantial metmyoglobin formed in both the Control and S₁ treatments was not sufficiently reduced to myoglobin to allow for the development of a desirable colour when exposed to air. These results are supported by the observations that the metmyoglobin formed on the surfaces of steaks can be reduced to deoxymyoglobin as result of metmyoglobin reducing activity (MRA) within the muscle tissue, when the O₂ residue is not excessive (TEWARI et al., 2001; GILL; MCGINNIS, 1995; O’KEEFFE; HOOD, 1981).

The overall steak quality for the product stored with the S₂ scavengers was 4 (neither good nor poor) or 5 (good) after 7 days, and increased to 5 (good) or 6 (very good) for all steaks after 14 days. Scores of 1 (very poor) or 2 (poor) were recorded for overall steak quality for all steaks from the Control and S₁ treatments, after 7 days or longer. These steaks had considerable brown areas and failed to rebloom. The steaks packaged with S₂ showed the least discolouration (0-5%) and the highest red intensity (8.0) when compared to the other systems. In summary, the steaks stored using the S₂ treatment were the most attractive.

Thus the capacity and the absorption rate of O₂ scavengers determine the shelf life of fresh beef steaks packaged in masterpack in an atmosphere of pure carbon dioxide with the inevitable residual O₂ in the headspace after packaging.

**TABLE 3.** Mean values for lightness (L*), redness (a*) and yellowness (b*) during storage at 4±1°C, measured on the steak surfaces (GM) 1h after opening the masterpacks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>7 days</th>
<th>Storage time</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a*</td>
<td>b*</td>
</tr>
<tr>
<td>Control</td>
<td>36.98</td>
<td>11.80</td>
<td>3.10</td>
</tr>
<tr>
<td>S₁</td>
<td>38.02</td>
<td>11.00</td>
<td>5.02</td>
</tr>
<tr>
<td>S₂</td>
<td>34.34</td>
<td>16.15</td>
<td>3.84</td>
</tr>
</tbody>
</table>

*Means in the same column with no common superscript are significantly different (P < 0.05)

Control: masterpacks with no O₂ scavenger
S₁: masterpacks contained scavengers with rated capacity for 300mL of O₂
S₂: masterpacks contained scavengers with rated capacity for 420mL of O₂

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