Title: Use of Carbon Monoxide Packaging for Improving the Shelf-Life of Pork  
NPB # 00-153

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Date Received: 1/2/2002

Abstract:
Injected and uninjected pork chops were packaged with either aerobic overwrap, 
vacuum barrier bags, modified atmosphere (MAP) with 20% carbon dioxide/80% 
nitrogen or MAP with 0.5% carbon monoxide 70% carbon dioxide 29.5% nitrogen.  
Color, purge, rancidity, and microbial counts were monitored during refrigerated  
storage.

Redness of pork chops was dramatically increased by the carbon monoxide treatment  
over the other packaging systems. The redness by carbon monoxide was retained over  
the full 36 days of refrigerated storage, well after color decline in the other package  
environments. Rancidity development was also suppressed by the MAP system with  
carbon monoxide. Visual acceptability was similar for the MAP with carbon monoxide  
and the overwrapped (aerobic) chops prior to color losses in the aerobic packages.  
Both the MAP/carbon monoxide and aerobic packages were superior to vacuum or  
MAP with carbon dioxide for color and visual acceptability. Microbial counts suggested  
shelf life of 7 days for overwrapped chops, 23-24 days for vacuum, 28 days for  
MAP/carbon dioxide and 36+ days for MAP/carbon monoxide. Purge, however, was  
increased significantly in uninjected chops by the MAP/carbon monoxide treatment.  
Injected chops did not show a significant increase in purge for the carbon monoxide  
treatment.

The results of this study show that carbon monoxide packaging has numerous potential  
shelf-life advantages (color, rancidity, microbial control) for fresh pork. The  
observations on purge, however, need to be studied in more detail to determine the  
differential effects on purge between uninjected and injected chops.
Introduction

It has been clearly shown in both previous research and current industry practice that modified atmosphere packaging (MAP) increases the shelf life of fresh pork over that of conventionally overwrapped (aerobic) packages (Farber, 1993; Sorheim, 1996). The increased shelf life of MAP systems is achieved by carbon dioxide in the package headspace gases. Carbon dioxide is well recognized as inhibitory for microbial growth (Blickstad and Molin, 1983). The MAP systems have advantages over vacuum packages because the greater headspace allows use of greater amounts of carbon dioxide and usually results in less purge (free water). If, however, carbon dioxide levels are too high (over about 40%), surface discoloration/browning of fresh meat can occur (Silliker, et al., 1977; Sebranek, 1985). Consequently, MAP systems typically utilize 20% to 30% carbon dioxide. It is likely that shelf-life could be improved over that of current MAP systems if carbon dioxide could be used at higher levels.

It has also been clearly shown in previous research that carbon monoxide gas will produce a very stable, bright-red meat color, identical to the red color achieved by oxygen “bloom”. Concerns for safety have limited the use of carbon monoxide in the past. However, recent research has demonstrated that very low levels (0.5%) of carbon monoxide are sufficient for meat color development. Current gas mixing technology and availability of pre-mixed gases has virtually eliminated risks for human exposure to carbon monoxide from packaging systems. Further, a few European meat processors, particularly in Norway, have begun utilizing carbon monoxide packaging (0.5% CO or less) in commercial applications (Sorheim et al., 1999). Therefore, the hypothesis for this research was that carbon monoxide packaging would improve color and color stability in fresh pork while reducing purge and microbial growth during refrigerated storage. Further, if demonstration of the effectiveness of this technology is clear, then regulatory approval for use will be facilitated.

References


Project Objectives:
1. Investigate the potential for a low concentration of carbon monoxide combined with a high concentration of carbon dioxide to increase shelf life of fresh pork and injected fresh pork over that of current packaging systems.
2. Evaluate the impact of carbon monoxide on fresh pork color stability and acceptability during extended storage.
3. Assess the potential advantages of a carbon monoxide – MAP system for reducing purge from fresh pork and injected fresh pork.

Procedures:
Boneless pork loins were selected for normal quality and purchased from a local supplier. A total of 48 loins were used for each of two replications of this experiment. Twenty-four loins in each replication were injected with 12% of the green weight using a brine composed of 9.3% potassium lactate, 3.7% sodium phosphate and 2.8% sodium chloride to result in 1% lactate, 0.4% phosphate and 0.3% salt in the injected products. The other 24 loins in each replication were not injected. All loins, injected and uninjected, were then cut into 1¼ inch thick chops prior to packaging.

Four packaging environments were compared in this study. These included traditional overwrap (aerobic – high oxygen permeable film), vacuum (high barrier film), gas flush/modified atmosphere of 20% carbon dioxide/80% nitrogen, and gas flush/modified atmosphere of 0.5% carbon monoxide, 70% carbon dioxide and 29.5% nitrogen. Each of the modified atmospheres were enclosed in high barrier bags.

Products were stored at 1º C to 3º C in lighted display and evaluated at 3-day intervals until quality deterioration occurred. The quality evaluations included instrumental color by Hunter L* a* b* reflectance, sensory panel assessment of color, odor and appearance, package purge, thiobarbituric acid values for rancidity and microbial counts (total plate counts and lactic acid organisms).

Results and Discussion

Instrumental Color
All measurements (n=16) on the pork chops were taken first on day 1 after packaging and subsequently on days 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 29, 32, 34, and 36. Surface color of the pork chops was measured with the Hunter Lab instrument. L* (lightness), a* (redness), and b* (yellowness) measurements were taken on two random locations for each chop. The results are reported in Table 1.
Table 1. The effect of packaging atmosphere on the Least Square Means of L* (lightness), a* (redness), and b* (yellowness) values of pork chop measurements.

<table>
<thead>
<tr>
<th>Item</th>
<th>OW</th>
<th>Vacuum</th>
<th>MAP</th>
<th>MAP-CO</th>
<th>OW</th>
<th>Vacuum</th>
<th>MAP</th>
<th>MAP-CO</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>49.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>51.32&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>48.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>48.79&lt;sup&gt;d&lt;/sup&gt;</td>
<td>48.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>52.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34</td>
</tr>
<tr>
<td>a*</td>
<td>6.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.80&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.30&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17</td>
</tr>
<tr>
<td>b*</td>
<td>12.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.07&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>12.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.79&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard error of means.

<sup>b</sup>-<sup>e</sup>Means within same row with different letters are significantly different at P<0.001

MAP-packaged chops resulted in the lowest L* values for both the control and injected chops, while the MAP-CO chops showed the highest L* values (p<0.001). The L* values for MAP packages tended to be the lowest over the entire storage period for both the control and the injected treatments. The L* values generally remained steady over the entire storage period and neither the control (uninjected) nor the injected treatment significantly (p>0.001) affected the L* values (Figures 1 and 2). The L* values, however, remained higher at the end of the storage period for the injected chops relative to the other packaging treatments (Figure 2).

**Figure 1.** Least square means for control L* values of pork chops as related to storage time for OW, Vacuum, MAP and MAP-CO (S.E. = 1.32).
The a* values were greatly affected by each packaging atmosphere (Table 1). After day 4, the MAP-CO treatments produced significantly (p<0.001) higher redness (a*) values than all other treatments for the entire storage period (Figure 3 and 4). Color development (redness) in the MAP-CO treatments typically occurred within 24 hours. However, this is not reflected in figures 3 and 4 because of an equipment breakdown for the first replication in this experiment. Chops were repackaged on day 4, after which the a* (redness) clearly developed. The delayed color development in figures 3 and 4 does not reflect the actual rate of color development for carbon monoxide packaging. In the second replication, measurements taken on day 1 showed that a* values were at 10.07 and 10.32 for the control and injected groups, respectively. This confirms that even a low level of carbon monoxide will result in a bright red color for pork chops overnight. The overwrap (OW) treatments were also significantly (p<0.001) higher in a* values than either the vacuum or the MAP, but at the same time were still significantly (p<0.001) lower than the MAP-CO treatments throughout the entire storage period (Table 1). The overwrap packages clearly declined in redness with time as the color losses typical of spoilage occurred (Figures 3 and 4). In both the control and injected treatments, the MAP-CO packaged chops maintained their high a* values for the complete storage period. Color remained highly attractive even after 36 days (See photos – appendix).
The $b^*$ ( yellowness) values between each packaging treatment did not change as greatly as the $L^*$ or $a^*$ differences. The overwrap packages in both treatments were significantly ($p<0.001$) higher than all other treatments and the MAP treatments are significantly ($p<0.001$) lower than all other treatments (Table 1). Figures 5 and 6 also show that overwrap treatments were greater throughout the entire storage period. Vacuum and MAP-CO in the control group showed no significant ($p>0.001$) difference as evidenced by Figures 5 and 6. The $b^*$ values are not as clearly related to visual color of fresh meat as the $L^*$ and $a^*$ values.
Purge

Purge loss was measured and calculated for each treatment and the results are presented in Table 2.

Table 2. The effect of packaging atmosphere on the Least Square Means of purge values of pork chops.

<table>
<thead>
<tr>
<th>Packaging Atmosphere</th>
<th>Control</th>
<th>Injected</th>
<th>S.E.(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OW</td>
<td>1.25(^e)</td>
<td>0.94(^e)</td>
<td>0.24</td>
</tr>
<tr>
<td>Vacuum</td>
<td>2.63(^{cd})</td>
<td>1.19(^e)</td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>3.53(^{bc})</td>
<td>2.18(^d)</td>
<td></td>
</tr>
<tr>
<td>MAP-CO</td>
<td>4.53(^b)</td>
<td>1.46(^{de})</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Standard error of means.
\(^{b-e}\)Means within same row with different letters are significantly different at P<0.001

Injection, as expected, had a significant (p<0.001) effect on the purge loss of the vacuum, MAP and MAP-CO treatments. Although the overwrap treatments were not significantly (p>0.001) different for purge, the injected chops still had a slightly lower purge percentage. The uninjected (Control) MAP-CO was significantly (p<0.001) higher for purge than the other three control treatments. The results show nearly a 2% increase in purge by the MAP-CO treatment compared to vacuum. However, in the injected chops, the percentage purge loss was much less than the uninjected control. In this case, the MAP-CO treatment was not significantly (p<0.001) higher than the overwrap or the vacuum packaged samples.

The reasons for increased purge with the MAP systems is not clear. Other researchers have reported a decreased surface pH for meat products packaged in carbon dioxide atmosphere, and attributed color changes to surface pH alterations. We conducted separate experiments with additional MAP-carbon monoxide packages and measured surface pH values as well as purge. The increased purge in MAP-CO was repeatable but there was no difference in surface pH. Other researchers have also reported
increased cooking losses for products packaged in MAP-CO which also implies a decreased retention of moisture. It will be important to determine the cause of these purge observations for MAP-CO packaging. It should be noted that the increased purge occurred only with uninjected chops. Injected chops showed slightly larger purge values for the MAP-CO packages compared with the overwrapped chops but the difference was not statistically significant.

TBA values

The TBA (2-thiobarbituric acid) measurements are presented in Table 3.

<table>
<thead>
<tr>
<th>Packaging Atmosphere</th>
<th>Control</th>
<th>Injected</th>
<th>S.E.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>OW</td>
<td>Vacuum</td>
<td>MAP</td>
<td>MAP-CO</td>
<td></td>
</tr>
<tr>
<td>TBA Values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.365&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.096&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.111&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.118&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.198&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>a Standard error of means.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b-c Means within same row with different letters are significantly different at P&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results showed a significant (p<0.001) difference between control (uninjected) overwrapped (OW), injected OW, injected MAP and the remaining five treatments. The control overwrap treatment was the highest for TBA values. This was expected due to the oxygen exposure of the overwrapped product and the more rapid development of rancidity. The injected product in the overwrap package is lower probably due to the antioxidant contributions of lactate and phosphate. The overwrap treatments were eliminated from the study on day 25 due to their very obvious spoilage, indicated by color and sensory observations. Other than the overwrap treatments, there were no major differences in TBA values between the other treatments; all were equally effective for suppressing rancid flavor development.

Microbial counts

Microbial counts were conducted at each sample time. Samples were measured for total aerobic plate counts and for lactic acid bacteria (anaerobic). The results indicated no significant (p>0.001) difference for any of the treatments.

Because of high variability for the microbial measurements, statistical differences could not be determined. Variability was increased due to loins of the second replication which started with relatively high microbial numbers. The high initial numbers probably masked treatment differences in the second replication. However, review of the first replication suggests differential effects on microbial counts by the packaging treatments. In figure 7, for example, the number of days required to reach a 10<sup>6</sup> count is 7 days for overwrapped packages, 23-28 days for vacuum and MAP, and 36+ days for MAP-CO. Figures 7-14 show the logarithmic growth for the microbial counts and are separated into replication 1 and replication 2 to distinguish the differences in the two replications.
Samples with microbial counts of less than $\log_{10} 1.0$ CFU/g of 1.0 are not plotted because small values below 1.0 are automatically deleted from the statistical analysis for microbial counts. The microbial data were also expressed in graph form to measure the slopes and utilize a regression model. The data was transformed to logs to account for the exponential growth rates of bacteria. The least square means of the regressions are presented in Table 4.

**Table 4. The effect of packaging atmosphere on the Least Square Means of microbial growth (CFU/g) regression values of pork chops.**

<table>
<thead>
<tr>
<th>Item</th>
<th>Packaging Atmosphere</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>OW</td>
<td>Vacuum</td>
<td>MAP</td>
<td>MAP-CO</td>
<td>OW</td>
<td>Vacuum</td>
</tr>
<tr>
<td>Aerobic</td>
<td>.281</td>
<td>.093</td>
<td>.160</td>
<td>.099</td>
<td>.157</td>
<td>.109</td>
<td>.119</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>.321</td>
<td>.184</td>
<td>.187</td>
<td>.098</td>
<td>.242</td>
<td>.161</td>
<td>.188</td>
</tr>
</tbody>
</table>

The more rapid growth rate in the overwrapped packages is obvious in Table 4. There is no difference among the other package treatments. The days where microbial values ($\log_{10}$ CFU/g) were less than 1.0 are not plotted in the figures to account for censoring (small values below 1) in the statistical analysis.

**Figure 7.** Aerobic plate count values ($\log_{10}$ CFU/g) on pork chops from control OW, Vacuum, MAP and MAP-CO packages during rep #1.
Figure 8. Aerobic plate count values ($\log_{10}$ CFU/g) on pork chops from inject OW, Vacuum, MAP and MAP-CO packages during rep #1.

Figure 9. Aerobic plate count values ($\log_{10}$ CFU/g) on pork chops from control OW, Vacuum, MAP and MAP-CO packages during rep #2.
Figure 10. Aerobic plate count values (Log$_{10}$ CFU/g) on pork chops from inject OW, Vacuum, MAP and MAP-CO packages during rep #2.

Figure 11. Lactic Acid Bacteria values (Log$_{10}$ CFU/g) on pork chops from control OW, Vacuum, MAP and MAP-CO packages during rep #1.
Figure 12. Lactic Acid Bacteria values ($\log_{10}$ CFU/g) on pork chops from inject OW, Vacuum, MAP and MAP-CO packages during rep #1.

Figure 13. Lactic Acid Bacteria values ($\log_{10}$ CFU/g) on pork chops from control OW, Vacuum, MAP and MAP-CO packages during rep #2.
Sensory Evaluation
Sensory characteristics were measured using a 12-member trained panel. Samples were evaluated on all 16 sampling days. The characteristics measured included color (desirable-undesirable), appearance (desirable-undesirable) and odor (no off odor-extreme off odor) each utilizing a 100 point scale. The sensory data is shown in Table 5.

Table 5. The effect of packaging atmosphere on the Least Square Means of sensory characteristic values (100-point scale) of pork chops.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Injected</th>
<th>S.E.(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW</td>
<td>Vacuum</td>
<td>MAP</td>
</tr>
<tr>
<td>Color</td>
<td>62.47(^c)</td>
<td>44.52(^d)</td>
<td>42.42(^d)</td>
</tr>
<tr>
<td>Appearance</td>
<td>62.73(^b)</td>
<td>50.17(^bc)</td>
<td>46.47(^c)</td>
</tr>
<tr>
<td>Odor</td>
<td>35.37(^b)</td>
<td>34.19(^b)</td>
<td>30.28(^b)</td>
</tr>
</tbody>
</table>

\(^a\)Standard error of means.
\(^bc\)Means within same row with different letters are significantly different at \(P<0.001\)

Results during the first sample evaluations indicated that in both the control and injected groups, the MAP-CO treatments received very significantly (\(p<0.001\)) higher scores for color. The high sensory color score also is supported by the high a* values discussed earlier for the MAP-CO treatments. The results also indicated that the panel scored the appearance of the MAP-CO chops and the overwrap chops higher in both the control and injected groups. The odor scores showed no significant (\(p>0.001\)) differences over the entire storage period. Samples were removed from the study when color...
deterioration was obvious thus avoiding the exposure of panelists to extreme off-odors generated by spoilage.

The color scores for each test day over the entire storage period are shown in Figure 15 and 16. As evidenced by the figures, MAP-CO received the highest scores from the panel in both the control and injected chops. The overwrap treatment decreased rapidly in color score compared to the other treatments and was not tested after day 25 due to obvious spoilage. MAP and vacuum treatments were recognized as the least desirable in terms of color, but the scores remained relatively steady for the entire storage period.

![Figure 15. Least square means for control color values of pork chops as related to storage time for OW, Vacuum, MAP and MAP-CO (S.E. = 8.84).](image)

![Figure 16. Least square means for inject color values of pork chops as related to storage time for OW, Vacuum, MAP and MAP-CO (S.E. = 8.84).](image)

Appearance scores were more similar between the treatments than color scores, according to sensory data. This probably reflects a more generalized evaluation of “appearance” by the panel as opposed to a more specific “color” characteristic. The overwrap treatment generally received higher scores for appearance than the other treatments during the first two weeks. The relatively high appearance score for overwrapped chops may reflect the panelists assessment of a traditional expected
appearance (overwrapped tray similar to current retail packages) as opposed to the other packaging systems. The MAP chops earned significantly (p<0.001) lower scores, in both the control and injected groups, than any other treatments. Figures 17 and 18 show the relative similarity of all appearance values over the entire storage period. The deterioration over time for the overwrapped chops is obvious and clearly results from the change in color noted previously.

Figure 17. Least square means for control appearance values of pork chops as related to storage time for OW, Vacuum, MAP and MAP-CO (S.E. = 7.99).

Figure 18. Least square means for inject appearance values of pork chops as related to storage time for OW, Vacuum, MAP and MAP-CO (S.E. = 7.99).

Conclusions
The results of this study show that low levels of carbon monoxide (0.5%) in a modified atmosphere package achieved a dramatically stable, bright-red color over an extended storage period. The carbon monoxide treatment also suppressed lipid oxidation when compared to overwrap-package treatments. Microbial counts were suppressed by the carbon monoxide treatment at least as affectivity as for vacuum. Therefore, the overall shelf life of fresh pork can be increased with the carbon monoxide packaging system. The results also, however, indicated that modified atmosphere packages containing low carbon monoxide and high carbon dioxide increased purge loss of uninjected pork chops. On the other hand, injected/marinated pork chops demonstrated less purge loss.
than non-injected chops, and the MAP-CO did not result in significant changes in purge for injected chops. It appears that the injection treatment prevented the purge effects observed for carbon monoxide packaging on uninjected chops.

Therefore, carbon monoxide packaging showed significant overall advantages as a packaging system for injected pork products. For uninjected pork products, the carbon monoxide packaging system has shelf life advantages but the effects on purge need further investigation to determine means for controlling and minimizing the changes in purge losses.
Photos of chops sampled after 6 days of storage. Vacuum packaged samples are not included due to rapid bloom after opening package.
Photos of chops sampled after 11 days of storage.
Photos of chops after 18 days of storage.
Photos of chops after 25 days of storage.
Photo of chops after 36 days of storage.