TEXAS TECH
UNIVERSITY

Department of Animal and Food Sciences

Box 42141
Lubbock, TX 79409-2141
(806) 742-2825
FAX (806) 742-0898

August 23, 2006

Laura M. Tarantino, Ph.D.
Director, Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Subject: Use of Carbon Monoxide in Fresh Meat Packaging:
Docket No. 2005P-0459

Dear Dr. Tarantino,

I offer the following comments to address some of the issues raised regarding the use of carbon monoxide (CO) in modified atmosphere packaging for fresh meat. Specifically, I want to address comments submitted to you on June 14, 2006 regarding the microbiological quality of product packaged in MAP packages (Reply Comments of Kalsec, Inc. Attachment A).

I am an associate professor of food safety microbiology and the director of the International Center for Food Industry Excellence at Texas Tech University. During my professional career I have studied the microbiological aspects of meat and poultry and I have recently completed two large studies (in publication) on the impact of CO, High-OX and traditional overwrap packaging on the microbiological quality and safety of ground beef. Our study on microbiological quality showed that the spoilage characteristics of meat packaged in different atmospheres are similar when factors such as age, temperature, and source are controlled. Pre-publication copies of these studies, which were funded by the National Cattlemen's Beef Association (NCBA), are attached.

In the packet submitted to you it is stated that the "results are limited," but the authors do not point out the limitations to the studies adequately. Instead of conducting numerous, small studies with limited application, they should have focused efforts on one statistically sound and valid study.

2005P-0459 C9

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There are several limitations and key points that should be noted in all of the submitted studies:

1. The numbers of samples are not statistically valid. There are no reports of power tests or statistical methods used in analyzing the study. No standard deviations, variances or other relevant statistical information is presented. None of the studies are replicated. It is irresponsible to report data with small sample sizes and/or without replication. This puts bias into the study and enables the person conducting the study to report results that are potentially misleading. The purpose of following sound scientific principles is to avoid bias in reporting of data.
2. The comparisons between the samples were not valid. In a study, it is imperative that the samples be standardized based on age of sample, storage conditions, handling conditions and all potential variables that might have an impact on the data. It is valid to compare samples in a large retail survey, however, in order to do so, the sample size must be large enough to account for the confounding variables so the final conclusions are valid. In this study the samples were not controlled initially. The meat was not from the same source, the sell by dates were not always the same and the initial microbial loads were not the same. Therefore, it is not appropriate to compare microbial counts between the CO packages and the high-ox packages. This is evident in the fact that the initial counts were not the same at the beginning of the studies comparing the CO packages and the High Oxygen packages.
3. The study makes erroneous conclusions based on an assumption that 10^7 cfu/g of total aerobic bacteria should be considered evidence of spoilage. Scientific evidence in the study and in the literature, however, does not support a conclusion that a microbial population at this level necessarily indicates spoilage. Much more information is needed. Spoilage is based on the type(s) of bacteria present and if the bacteria have had the opportunity to grow in the product. Because the type of bacteria was not determined in the study, the data are essentially meaningless. Spoiled product can have much lower counts than 10^7 cfu/g, while product that is acceptable to consumers can have much higher counts. Thus, ground beef can have a relatively low bacterial count and be spoiled or a relatively high one and not be spoiled. For example, research in our labs has proven that 10^8 cfu/g of lactic acid bacteria can be added to fresh ground beef and not have a significant impact on spoilage. In sum, judging spoilage from simply a microbial count is erroneous. Microbial loads must be taken into consideration with the sensory attributes of the product.
4. The data are not converted to $\log_{10}$ cfu/g. Standard microbiological practices require that data be converted to log numbers in order to account for the limitations of direct plating. It is unclear how valid statistical comparisons were made without converting the data to log numbers. Additionally, lack of conversion to log numbers is somewhat misleading when interpreting the data.
5. As noted previously, we have recently completed two large studies using MAP packaging using CO, High Ox MAP packaging and traditional overwrap packaging. We ground and packaged the product from the same lot of ground

beef. The studies were replicated and temperature controlled. We have provided a summary of the results.

Thank you for considering these comments.

Sincerely,

Mindy Brashears

Mindy Brashears

FINAL REPORT

SUBMITTED TO

NATIONAL CATTLEMAN'S BEEF ASSOCIATION

Effect of Meat Packaging Technologies on the Safety and Spoilage-Indicating Characteristics of Ground Beef – Phase 1: Safety Characteristics

SUBMITTED
BY

THE INTERNATIONAL CENTER FOR FOOD INDUSTRY EXCELLENCE
DEPARTMENT OF ANIMAL AND FOOD SCIENCES
TEXAS TECH UNIVERSITY

PRINCIPLE INVESTIGATORS
Mindy Brashears, PhD
J Chance Brooks, PhD
Mark Miller, PhD

RESEARCH PROJECT SUMMARY OUTLINE - FINAL REPORT
July 1, 2006

I. **Principal Investigator(s):**

Mindy M. Brashears, PhD
J Chance Brooks, PhD
Mark F. Miller, PhD

II. **Institution:**

Texas Tech University
International Center for Food Industry Excellence
Box 42141
Lubbock, TX 79409-2141

III. **Project Title:**

Effect of Meat Packaging Technologies on the Safety and Spoilage-Indicating Characteristics of Ground Beef – Phase 1: Safety Characteristics

IV. **Stated Objectives:**

To determine the safety of ground beef packaged with carbon monoxide gas flush, hi-oxygen gas flush, and rosemary oleoresin in modified atmosphere packaging of ground beef patties by determining the growth or survival of *E. coli* O157:H7 and *Salmonella* over time.

V. **Background information about the need for this research.**

Recent petitions to FDA and USDA have requested the re-evaluation of carbon monoxide gas as an approved packaging component. The Food and Drug Administration (FDA) and USDA FSIS requires that approved processes and/or ingredients do not in any way result in the product becoming adulterated or misbranded including the presence of microbial pathogens (FSIS, 2003). Thus, research was needed to provide the industry and government officials with scientific data regarding the safety characteristics of various packaging systems commonly used in the beef industry.

VI. **Achievement of the specific objectives stated in your proposal.**

All stated objectives were met.

VII. **Materials and Methods**

Experimental Design

The survival of *E. coli* O157 and *Salmonella* was evaluated in ground beef patties exposed to five treatments as follows: **1**) control (foam tray with film over-wrap); **2**) high-oxygen (80% O_2 / 20% CO_2) modified atmosphere package (MAP); **3**) high-oxygen MAP with added rosemary

extract (Kalsec, Inc.); **4**) low-oxygen carbon monoxide (0.4% CO, 30% CO_2, 69.6% N_2) MAP; and **5**) low-oxygen carbon monoxide MAP with added rosemary extract

The project was a split-plot design with meat block serving as the main plot, and blocked by retail display case. Data was analyzed using the GLM procedures of SAS (Cary, NC). Least-square means were computed for each dependent variable, and statistically separated by pair-wise t-test (PDIFF option of SAS) with predetermined $\alpha = 0.05$. MAP samples could not be unpackaged and used for repeated measures, so samples were replicated over time.

Preparation of Patties

Coarse ground beef was obtained from a commercial processor immediately after its release from the company's "test and hold" program, and transported to the Texas Tech University BSL II Pathogen Processing Laboratory for processing. The coarse ground beef was placed in a mixer and blended prior to a final grind. After the final grind, patties were formed (150 g) using a hand operated patty maker. All equipment was cleaned, rinsed and dried between each treatment. Rosemary extract was added to the coarse ground beef and mixed prior to a final grind. Additionally, concentrated cultures of a 3 strains mixture of *Salmonella* and a 4 strain cocktail of *E. coli* O157:H7 were added during grinding to yield populations of approximately 1×10^4 cfu/g. Beef patties were evaluated for changes in microbial loads over time (0,1, 3, 5, 7, 14 and 21 d) under simulated retail display conditions using coffin-style and multi-deck cases under continuous fluorescent lighting (approximately 1900 lux using high-output bulbs with a color temperature rating of 3500°K).

Microbiological Tests

On each day of sampling, packages were aseptically opened and subjected to serial dilutions in peptone dilution water. Samples inoculated with *Salmonella* were plated on XLD agar overlayed with TSA using the thin-layer agar method to allow for recovery of injured cells. Samples containing *E. coli* O157 were plated onto MacConkey Agar overlayed with TSA using the thin-layer agar method as well. Samples were plated using a spiral plating system and counted using a Q count automated counting system. All plates were incubated for 24-28 h at 37 C prior to counting.

VIII. Summary of Results and Discussion

Figure 1 illustrates the changes in the populations of *E. coli* O157 over time in ground beef during storage in various packaging materials at refrigeration temperatures. On day 1, there were no significant differences in the populations of the pathogens in all packages with total initial counts of *E. coli* O57 ranging from 4.83 to 5.20 $\log_{10}$ cfu/g. On days 1, 3, 5, and 7 there were no significant increases or decreases in the populations and no

differences among counts of *E. coli* O157 in the various packages. Significant differences were observed on day 7. In the MAP packages, *E. coli* O157 populations ranged from 4.51-4.73 $\log_{10}$ cfu/g with no differences among the counts of *E. coli* in the ground beef in the various packages. The total *E. coli* O157:H7 in the ground beef in the traditional overwrap packages was at 5.73 $\log_{10}$ cfu/g which was significantly higher than the counts in the ground beef in the MAP packages. After 21 days, the total counts of *E. coli* O157:H7 in ground beef packaged in traditional packaging increased to 5.60 $\log_{10}$ cfu/g and this was significantly higher than all *E. coli* O157:H7 counts in the ground beef in the MAP packages. The *E. coli* O157:H7 counts in the MAP packages on day 21 ranged from 3.94 to 4.34 $\log_{10}$ cfu/g.

Similar results were observed for samples inoculated with *Salmonella*. On day 7, the initial populations ranged from 4.40 to 4.58 $\log_{10}$ cfu/g (Figure 2). There were significant differences observed among the treatments after 3 days of storage. Samples packed in traditional packaging had total *Salmonella* counts of 5.20 $\log_{10}$ cfu/g while all those in MAP packages were significantly lower at 4.50 $\log_{10}$ cfu/g. Similar results were observed on day 5, but after 7 days there were no significant differences among the treatments. After days 14 and 21 the total *Salmonella* in the ground beef subjected to traditional overwrap packaging was at 4.29 and 4.27 $\log_{10}$ cfu/g, respectively which was significantly higher than counts in the MAP packages which were between 4.30 and 3.94 $\log_{10}$ cfu/g on day 14 and between 3,75 and 4.01 $\log_{10}$ cfu/g on day 21.

Over time the reductions in *E. coli* O157:H7 and *Salmonella* could be occurring due to the presence of carbon dioxide in the atmospheres. It has been known for some time that carbon monoxide is inhibitory towards various microorganisms. Further research is needed to determine the inhibitory factors in the packaging gases.

IX. Publications, abstracts, manuscripts in progress, thesis or presentations that resulted from this research.

J.C. Brooks, M.M. Brashears, M.F. Miller, A.R. Hoyle, J.D. Kellermeier, and J.M. Mehaffey. *New Developments and Future Needs in Fresh Meat Packaging: The Spoilage Characteristics of Ground Beef Packaged in High-Oxygen and Low-Oxygen Modified Atmosphere Packages.* Proceedings of the Reciprocal Meat Conference, University of Illinois at Urbana-Champaign, June 18-21, 2006.

J.C. Brooks, M.M. Brashears, M.F. Miller, A.R. Hoyle, J.D. Kellermeier, and J.M. Mehaffey. *New Developments and Future Needs in Fresh Meat Packaging: The Spoilage Characteristics of Ground Beef Packaged in High-Oxygen and Low-Oxygen Modified Atmosphere Packages.* Presented at Reciprocal Meat Conference, University of Illinois at Urbana-

Champaign, June 21, 2006 in Update on Modified Atmosphere Packaging of Fresh Meat Session.

X. Additional funding secured as a result of beef industry support of this project.

To date, no additional funding has been secured as a result of this project.

XI. Brief Lay Interpretation of Results suitable for public release (maximum 200 words as a separate report).

The impact of various packaging methods on the survival and growth of *E. coli* O157:H7 and *Salmonella* in ground beef was evaluated in this study. Product was stored in retail display cases under refrigeration temperatures. After 14 and 21 days of storage, the numbers of both *Salmonella* and *E. coli* O157 increased in product subjected to traditional overwrap packaging. For product packaged in modified atmospheres (high-oxygen (80% O_2 / 20% CO_2) and low-oxygen carbon monoxide (0.4% CO, 30% CO_2, 69.6% N_2) both with and without rosemary oleoresin) the total numbers of *Salmonella* and *E. coli* O157:H7 decreased over time. Modified atmospheres may be inhibitory to food-borne pathogens and may provide added safety to ground beef products.

Figure 1. Survival of *E. coli* O157:H7in Ground Beef Packaged under Various Conditions

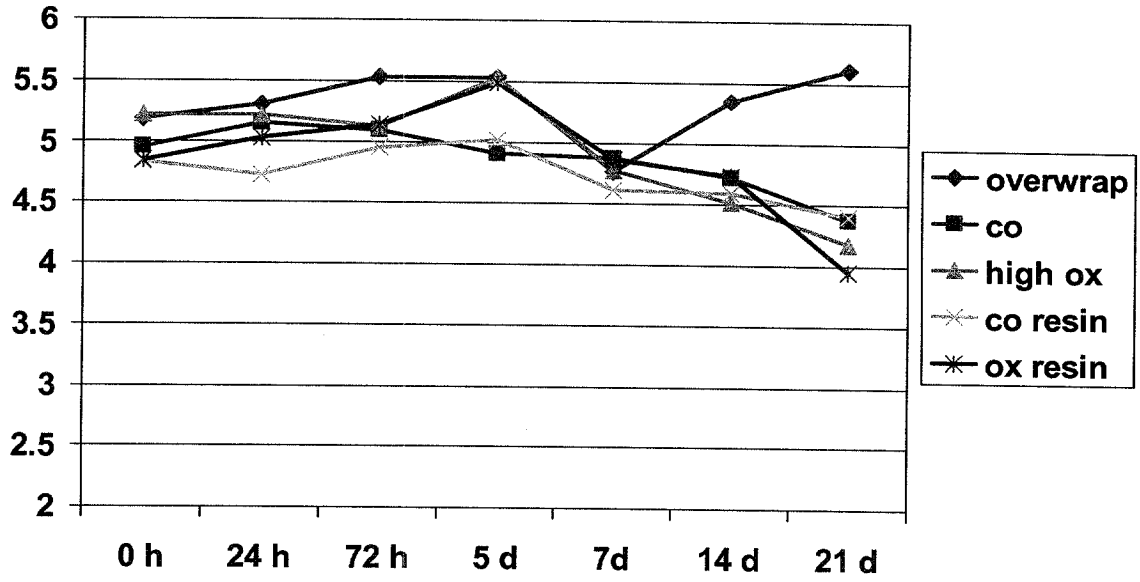
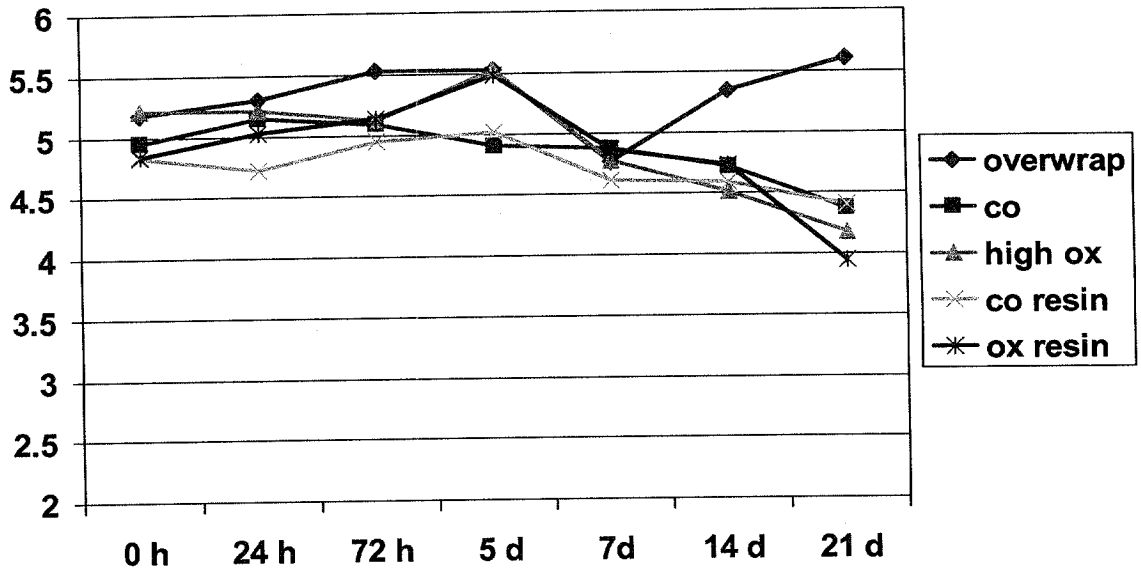


Figure 2. Survival of *Salmonella* in Ground Beef Packaged under Various Conditions



RESEARCH PROJECT SUMMARY OUTLINE - FINAL REPORT
June 27, 2006

I. Principal Investigator(s):

J Chance Brooks, PhD
Mindy M. Brashears, PhD
Mark F. Miller, PhD

II. Institution:

Texas Tech University
International Center for Food Industry Excellence
Box 42141
Lubbock, TX 79409-2141

III. Project Title:

Effect of Meat Packaging Technologies on the Safety and Spoilage-Indicating Characteristics of Ground Beef – Phase 2: Spoilage Characteristics

IV. Stated Objectives:

To determine the spoilage characteristics of carbon monoxide gas flush, hi-oxygen gas flush, and rosemary oleoresin in modified atmosphere packaging of ground beef patties through affective and analytical sensory (color and odor) analysis, and microbial analysis

V. Background information about the need for this research.

Recent petitions to FDA and USDA have requested the re-evaluation of carbon monoxide gas as an approved packaging component. The Food and Drug Administration (FDA) and USDA FSIS requires that approved processes and/or ingredients do not in any way result in the product becoming adulterated or misbranded, which includes making the meat product look better or of greater value than untreated products and the normal spoilage indicators can not be masked (FSIS, 2003). Thus, research was needed to provide the industry and government officials with scientific data regarding the spoilage characteristics of various packaging systems commonly used in the beef industry.

VI. Achievement of the specific objectives stated in your proposal.

The researchers were able to achieve the stated objectives in the research proposal. The spoilage characteristics of all packaging treatments were documented through affective and analytical sensory analysis, as well as analytical laboratory testing.

VII. Materials and Methods

Ground beef patties were evaluated because of the ability to obtain spoilage characteristics in an accelerated time frame. Coarse ground beef was

obtained from a commercial processor immediately after its release from the company's "test and hold" program, and transported to the Gordon W. Davis Meat Science Laboratory for processing. The coarse ground beef was placed in a mixer and blended prior to a final grind. After the final grind, patties were formed (150 g) using a patty forming machine (Hollymatic, Inc.). All equipment was cleaned, rinsed and dried between each treatment. Five packaging treatments were evaluated: **1**) control (foam tray with film over-wrap); **2**) high-oxygen (80% O_2 / 20% CO_2) modified atmosphere package (MAP); **3**) high-oxygen MAP with added rosemary extract (Type HT-25 at 0.2%, Kalsec, Inc.); **4**) low-oxygen carbon monoxide (0.4% CO, 30% CO_2, 69.6% N_2) MAP; and **5**) low-oxygen carbon monoxide MAP with added rosemary extract. Rosemary extract was added to the coarse ground beef and mixed prior to a final grind. Beef patties were evaluated for changes in color and odor (trained and consumer panelists) over time (0,1, 3, 5, 7, 14 and 21 d) under simulated retail display conditions using coffin-style and multi-deck cases under continuous fluorescent lighting (approximately 1900 lux using high-output bulbs with a color temperature rating of 3500°K).

Both trained and consumer panelists were used to detect differences in color and odor. Panelists were trained by meat science faculty in multiple sessions using representative samples prior to the start of the project. Trained panelists evaluated the color of ground beef patties using a five-point, verbally anchored scale (1 = very bright red; 5 = very dark red or brown) and surface discoloration (1 = no discoloration; 5 = severe discoloration, 61-100%) according to AMSA color guidelines. Consumer panelists were recruited from the surrounding area and asked to determine if the ground beef patties had good color (1 = very strongly agree; 7 = very strongly disagree) and how likely they would be to purchase (1 = definitely would purchase; 5 = definitely would not purchase) the package based on the color (AMSA, 1991).

Odor panels were conducted on packages removed from the cases at each sampling interval. Odor samples were presented to trained and consumer panelists under red lighting. Trained panelists were asked to determine if an off-odor is present (1 = no off-odor; 5 = extreme off-odor) and to characterize the off-odor if present (1 = rancid; 2 = arid; 3 = sweet; 4 = sour; 5 = acid; and 6 = putrid). Consumer panelists were asked if the meat in the package smelled fresh (1 = very strongly agree, 7 = very strongly disagree) and how likely they would be to consume the meat (1 = definitely would consume; 5 = definitely would not consume) based upon the odor.

Microbial loads were determined using standardized methods. Total Aerobic Plate Counts (APC) were determined by plating on total plate count agar, Lactobacilli were determined by plating on LBS agar and total coliforms were determined by plating on VRBA agar. The plates were incubated at 37 °C for 48 h. Total psychrophilic aerobic bacteria were determined by plating onto APC agar and incubating at 7 °C for 7 d.

The project was a split-plot design with meat block serving as the main plot, and blocked by retail display case. Data was analyzed using the GLM procedures of SAS (Cary, NC). Least-square means were computed for each

dependent variable, and statistically separated by pair-wise t-test (PDIFF option of SAS) with predetermined α = 0.05. MAP samples could not be unpackaged and used for repeated measures, so samples were replicated over time.

VIII. Summary of Results and Discussion.

Trained panelists scores for lean color and percentage of surface discoloration are presented in Figures 1 and 2, respectively. There was a treatment by day of retail display interaction for trained panel color scores and percent surface discoloration scores. Results indicate the low oxygen carbon monoxide gas flush had a stabilizing effect on meat color after the formation of carboxymyoglobin. The carbon monoxide gas mixture was also effective at preventing the development of surface discoloration during retail display.

Consumer responses to the statement "The meat in this package has good color" and purchase intent scores based on the color of the meat in the package are shown in Figures 3 and 4, respectively. There was a significant treatment by day of retail display interaction for both consumer responses to color. Consumer panel scores followed the same pattern as trained panel scores for meat color. Consumers in this study also ranked high-oxygen packages containing meat with rosemary extract to be more desirable at days 5 and 7 of display than high oxygen packages containing meat without rosemary extract. After the development of carboxymyoglobin in packages containing carbon monoxide, consumers indicated they would purchase meat in these packages that had been displayed 21 days at 0-2 °C.

Figure 5 depicts the percentage of trained panelists detecting no off-odors among treatments during retail display. These results indicate that off-odors will develop in traditional and modified atmosphere packages over time. Traditional and high-oxygen packages develop off-odors earlier in the display period than packages containing carbon monoxide gas.

Consumer responses to the statement "The meat is this package smells fresh" and their likeliness to consume the product based upon its odor are presented in Figures 6 and 7, respectively. Consumer indicated that beef patties packaged in an atmosphere containing carbon monoxide were more likely to smell fresh at 7, 14, and 21 days of display, but they would probably not consume these product after 14 days of display based upon the odor.

There was a significant packaging treatment by day of retail display interaction for psychrophilic aerobic bacteria counts (Table 1) and total coliforms (Table 2). These results indicate that modified atmosphere packaging suppresses the growth of psychrophilic aerobic bacteria when compared to traditional packages exposed to air. Packaging treatment main effects for total aerobic plate counts and lactobacillus are present in Table 3. Generally, traditional packages had significantly higher total plate counts and lactobacillus bacteria counts than modified atmosphere packages. Changes in total aerobic plate counts and lactobacillus during retail display are shown in Table 4. These results indicate that total plate counts and lactobacillus bacteria increased as day of retail display increased for all packaging treatments.

IX. Publications, abstracts, manuscripts in progress, thesis or presentations that resulted from this research.

J.C. Brooks, M.M. Brashears, M.F. Miller, A.R. Hoyle, J.D. Kellermeier, and J.M. Mehaffey. *New Developments and Future Needs in Fresh Meat Packaging: The Spoilage Characteristics of Ground Beef Packaged in High-Oxygen and Low-Oxygen Modified Atmosphere Packages.* Proceedings of the Reciprocal Meat Conference, University of Illinois at Urbana-Champaign, June 18-21, 2006.

J.C. Brooks, M.M. Brashears, M.F. Miller, A.R. Hoyle, J.D. Kellermeier, and J.M. Mehaffey. *New Developments and Future Needs in Fresh Meat Packaging: The Spoilage Characteristics of Ground Beef Packaged in High-Oxygen and Low-Oxygen Modified Atmosphere Packages.* Presented at Reciprocal Meat Conference, University of Illinois at Urbana-Champaign, June 21, 2006 in Update on Modified Atmosphere Packaging of Fresh Meat Session.

X. Additional funding secured as a result of beef industry support of this project.

To date, no additional funding has been secured as a result of this project.

XI. Brief Lay Interpretation of Results suitable for public release (maximum 200 words as a separate report).

Recent petitions to FDA and USDA have requested the re-evaluation of carbon monoxide gas as an approved packaging component. Researchers at Texas Tech University have partnered with the National Cattlemen's Beef Association to investigate the spoilage characteristics of ground beef packaged in high-oxygen modified atmosphere packages and low-oxygen packages containing carbon monoxide gas. Results from the study show that modified atmosphere packaging suppressed the growth of aerobic bacteria that grow at cold temperatures when compared to traditional retail packages. Beef patties exposed to carbon monoxide gas maintained a bright red lean color throughout the study, but were observed to have an off-odor by 83% of trained panelists after 14 days of display. High oxygen modified atmosphere packages containing beef with added natural rosemary extract had a more desirable lean color and odor than patties without the rosemary extract after 5 days of retail display. The study also showed that meat color and package odor were very good indicators of spoilage in all packaging systems. Therefore, consumers can use color and odor as indicators of meat spoilage in traditional packages, high-oxygen modified atmosphere packages, and low-oxygen modified atmosphere packages containing carbon monoxide gas.

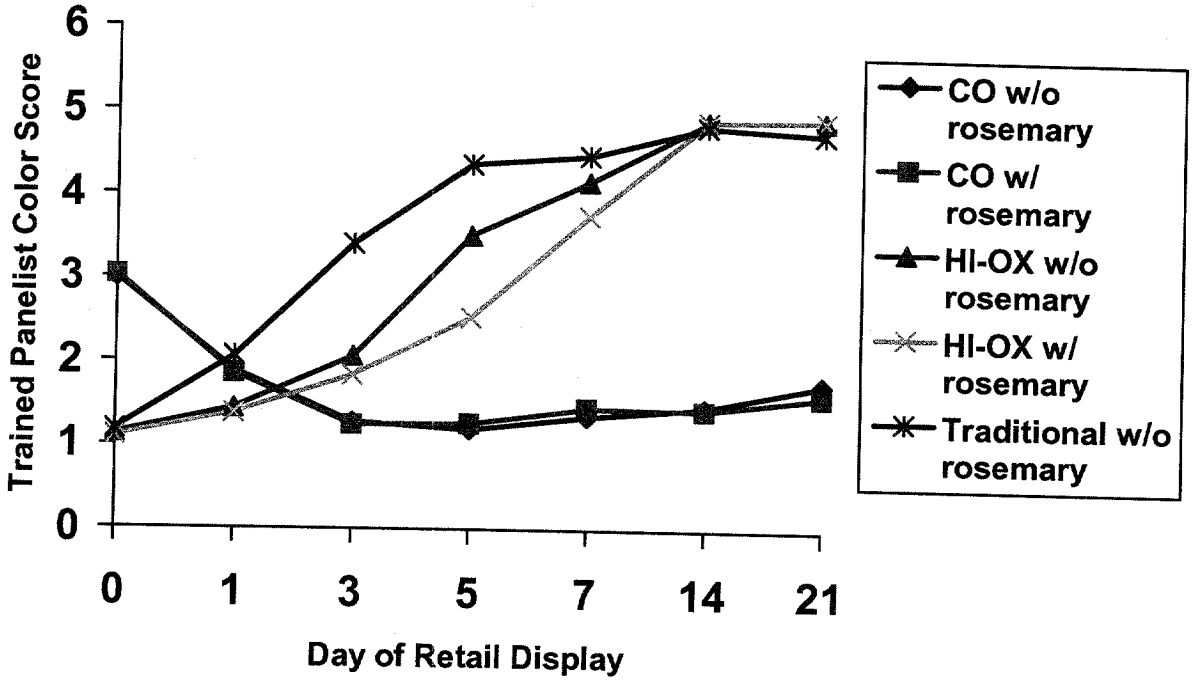


Figure 1 – Trained Panelists Color Scores for Each Packaging Treatment Over Retail Display Time. Color Scores: 1 = very bright red; 5 = very dark red or brown (AMSA color guidelines).

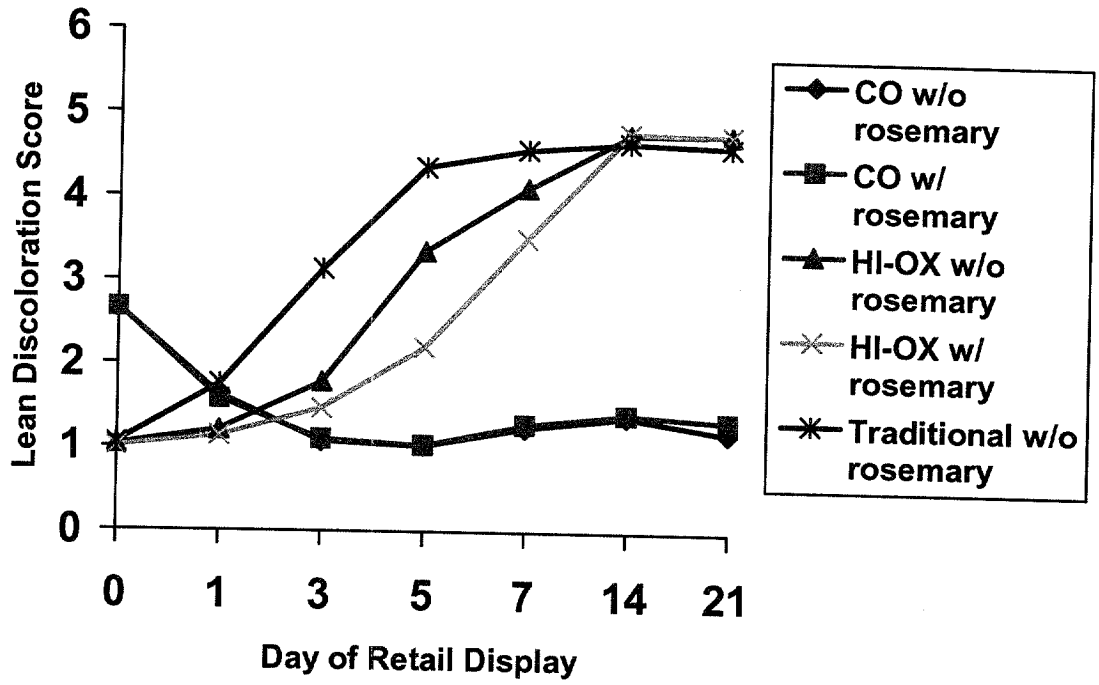


Figure 2 – Trained Panelists Percent Surface Discoloration Scores for Each Packaging Treatment over Retail Display Time. Surface discoloration 1 = no discoloration; 5 = severe discoloration, 61-100% (AMSA color guidelines).

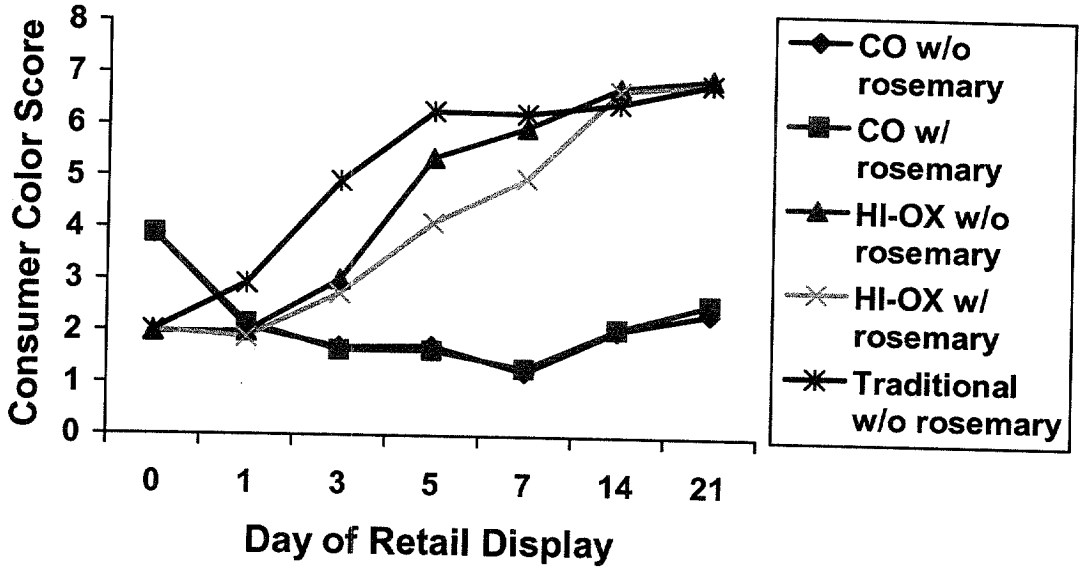


Figure 3 – Consumer Responses to the Statement "The meat in this package has good color". Color Scores: 1 = very strongly agree; 7 = very strongly disagree.

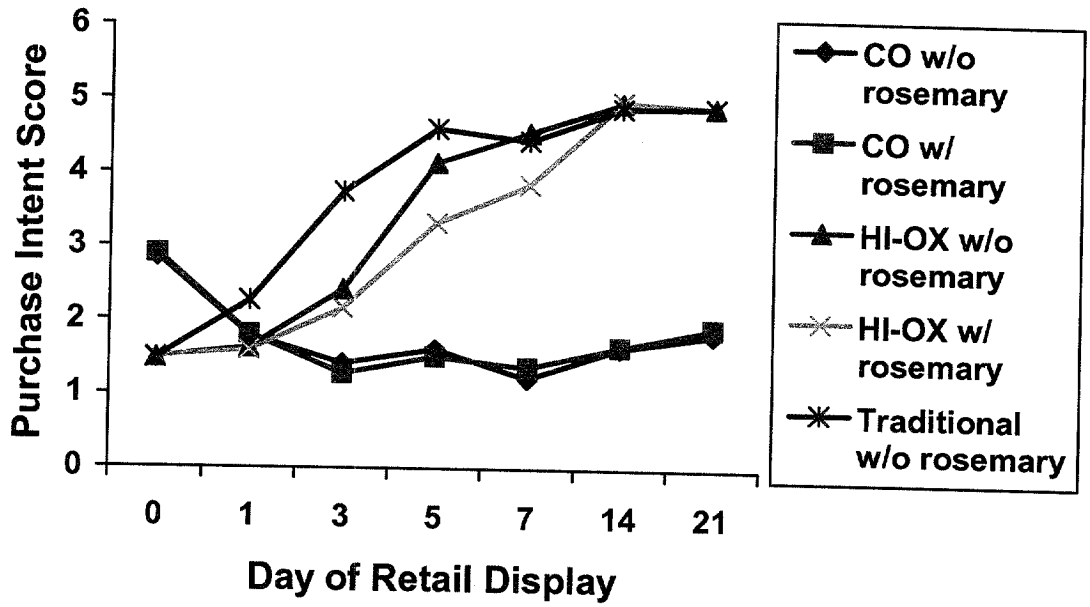


Figure 4 - Consumer Scores for Likeliness to Purchase Based on the Color of the Meat in the Package. Intent scores: 1 = definitely would purchase; 5 = definitely would not purchase the package based on the color of the meat.

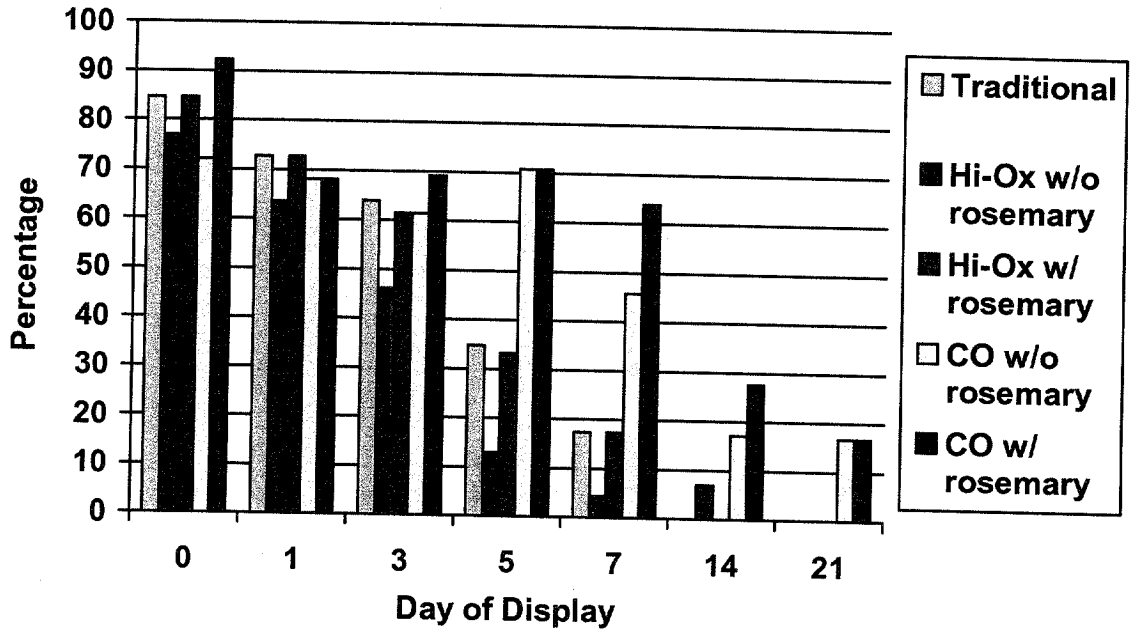


Figure 5 – Percentage of Trained Panelists Detecting No Off-Odor in Packages Over Retail Display.

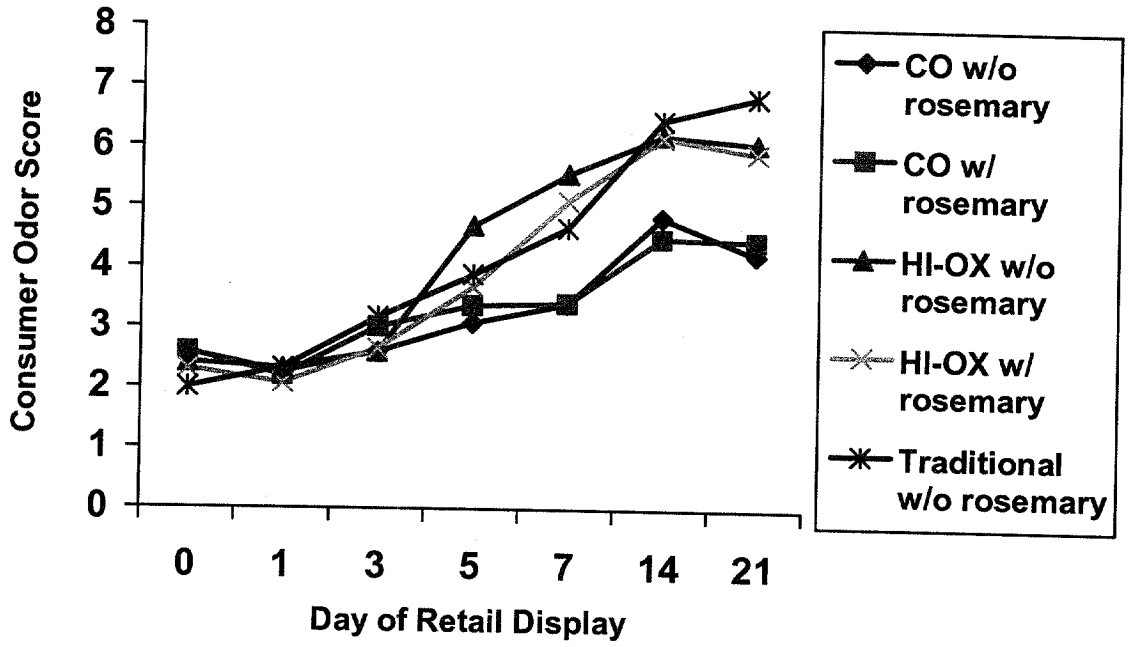


Figure 6 – Consumer Responses to the Statement "The meat is this package smells fresh". Consumer Odor Scores: 1 = very strongly agree, 7 = very strongly disagree.

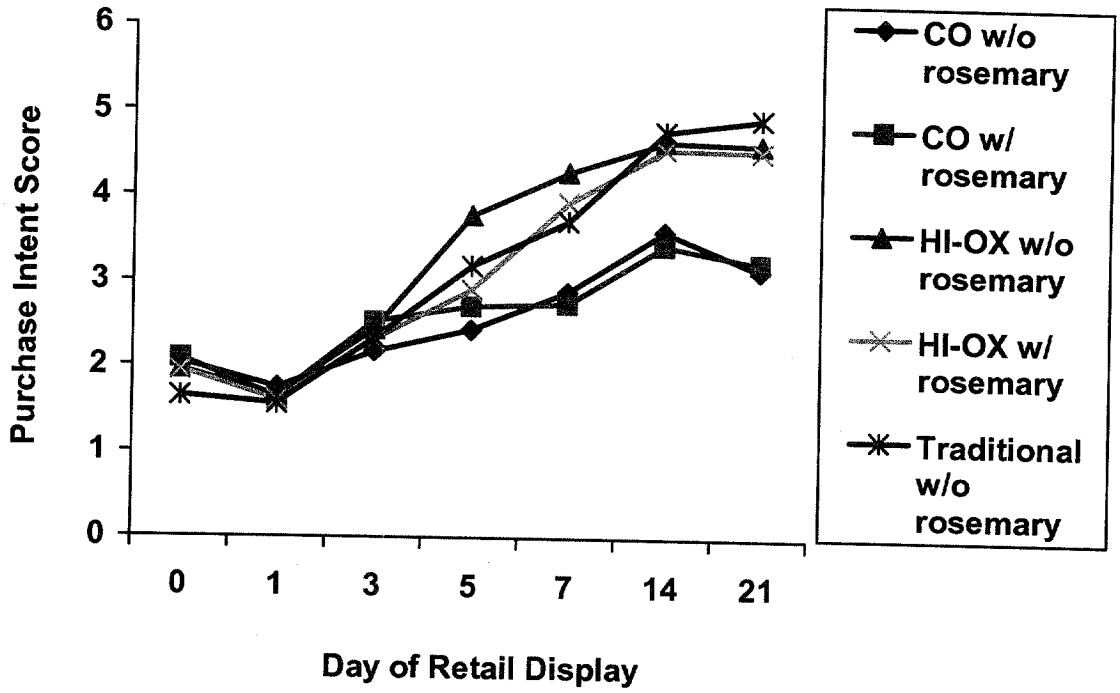


Figure 7 - Consumer Scores for Likeliness to Consume Meat Based On the Odor of the Meat in the Package. Intent scores: 1 = definitely would consume; 5 = definitely would not consume based upon the odor.

Table 1. Least squares means for psychrophilic aerobic bacteria counts (log cfu/g) for the treatment by day of display interaction (P = 0.0507) means.

Treatment	Retail Display, d						
	0	1	3	5	7	14	21
Traditional over-wrap package	$2.33^{a,x}$	$2.09^{a,x}$	$4.08^{b,y}$	$5.89^{c,y}$	$5.91^{c,y}$	$8.21^{d,z}$	$8.49^{d,y}$
High-oxygen without rosemary	$2.16^{a,x}$	$2.14^{a,x}$	$2.87^{ab,x}$	$3.34^{ab,x}$	$3.88^{b,x}$	$6.31^{c,xy}$	$6.72^{c,x}$
High-oxygen with rosemary	$2.69^{a,x}$	$2.69^{a,x}$	$3.07^{a,x}$	$3.59^{ab,x}$	$4.55^{b,x}$	$6.57^{c,y}$	$6.41^{c,x}$
Low oxygen CO without rosemary	$2.33^{a,x}$	$1.98^{a,x}$	$2.61^{ab,x}$	$2.98^{ab,x}$	$3.70^{b,x}$	$5.25^{c,x}$	$5.92^{c,x}$
Low oxygen CO with rosemary	$2.80^{a,x}$	$2.65^{a,x}$	$3.16^{a,x}$	$3.68^{a,x}$	$3.82^{a,x}$	$5.38^{b,xy}$	$6.13^{b,x}$

^{a,b,c}Least squares means within a row lacking a common superscript letter differ ($P < 0.05$).
^{x,y,z}Least squares means within a column lacking a common superscript letter differ ($P < 0.05$).

Table 2. Least squares means for total coliform counts (log cfu/g) for the treatment by day of retail display interaction ($P = 0.0013$) means.

Treatment	Retail Display, d						
	0	1	3	5	7	14	21
Traditional over-wrap package	$1.22^{a,x}$	$1.80^{ab,x}$	$2.65^{b,x}$	$5.60^{c,y}$	$4.83^{c,z}$	$7.35^{d,z}$	$7.62^{d,z}$
High-oxygen without rosemary	$1.18^{a,x}$	$1.83^{a,x}$	$2.81^{ab,x}$	$1.95^{a,x}$	$1.99^{a,xy}$	$5.62^{c,y}$	$3.65^{b,xy}$
High-oxygen with rosemary	$1.59^{a,x}$	$1.91^{ab,x}$	$2.82^{ab,x}$	$2.18^{ab,x}$	$3.28^{b,y}$	$5.79^{c,y}$	$4.51^{c,y}$
Low oxygen CO without rosemary	$1.08^{a,x}$	$1.72^{ab,x}$	$2.98^{bc,x}$	$1.30^{a,x}$	$1.44^{ab,x}$	$3.61^{c,x}$	$2.67^{abc,x}$
Low oxygen CO with rosemary	$2.00^{ab,x}$	$2.17^{ab,x}$	$3.31^{bc,x}$	$1.98^{ab,x}$	$1.86^{a,x}$	$3.66^{c,x}$	$3.83^{c,xy}$

^{a,b,c,d}Least squares means within a row lacking a common superscript letter differ ($P < 0.05$).

^{x,y,z}Least squares means within a column lacking a common superscript letter differ ($P < 0.05$).

Table 3. Least squares means for treatment effect on Total Aerobic Plate Counts (APC) and Lactobacillus counts (log cfu/g).

	Traditional	High oxygen without rosemary	High oxygen with rosemary	Low oxygen CO without rosemary	Low oxygen CO with rosemary
APC	5.60^{c}	4.72^{ab}	4.97^{b}	4.15^{a}	4.52^{ab}
Lactobacillus	4.14^{b}	3.68^{a}	3.51^{a}	3.33^{a}	3.61^{a}

[a,b]Least squares means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 4. Least squares means for Total Aerobic Plate Counts (APC) and Lactobacillus counts (log cfu/g) over display time (days).

	Retail Display, day						
	0	1	3	5	7	14	21
APC	2.97[a]	2.92[a]	4.09[b]	4.53[b]	4.69[b]	6.79[c]	7.59[d]
Lactobacillus	1.97[a]	2.41[ab]	2.57[b]	3.34[c]	4.25[d]	5.78[e]	5.30[e]

[a,b,c,d,e]Least squares means within a row lacking a common superscript letter differ ($P < 0.05$).