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BY ELECTRONIC MAIL & FEDERAL EXPRESS

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

Re: Citizen Petition Requesting FDA to Enforce Ban on Carbon Monoxide in Case-Ready Meat Packaging; Docket No. 2005p-0459

Dear Dr. Tarantino:

This submission is made on behalf of Kalsec, Inc. ("Kalsec") in support of the above referenced Citizen Petition filed on November 15, 2005. This submission includes scientific evidence from limited unpublished studies that were sponsored by Kalsec and conducted by S&J Laboratories of Portage, Michigan. These studies were designed to evaluate selected microbial and sensory characteristics of ground beef sold at retail in packaging containing carbon monoxide gas (carbon monoxide modified atmosphere packaging or "CO-MAP"), compared to ground beef sold in high oxygen modified atmosphere packaging ("high oxygen-MAP"). The experiments in this submission were conducted using ground beef samples purchased at retail in CO-MAP and high oxygen-MAP packaging. The study reports from these experiments are provided in Appendices One, Two, and Three.

Although the results from these studies are limited, and involved a relatively small number of ground beef samples purchased in a local region, the findings lend support to the scientific evidence documenting the food safety and consumer deception concerns that have been raised concerning the use of carbon monoxide in fresh meat packaging, as detailed in the Kalsec petition and related submissions to the agency. A summary of our key findings are as follows:

1. On average, the commercially available CO-MAP ground beef samples tested were shown to have a statistically significant higher bacterial count, on the date of purchase or within a day of purchase, than commercially available ground beef packaged in high oxygen modified atmosphere packaging (high oxygen-MAP).

2. Some of the CO-MAP ground beef samples tested within their "use or freeze by" dates were found to have bacterial counts indicative of spoilage (i.e, bacterial counts of $>10^7$ colony forming units (cfu) /gram). In contrast, none of the high oxygen-MAP ground beef samples tested within their "sell by" dates had bacterial counts indicative of spoilage.

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3. The artificial reddish pigment (carboxymyoglobin) formed by the reaction of carbon monoxide in the CO-MAP ground beef samples was found to be stable, giving the ground beef a red appearance after the meat had been temperature abused or become spoiled.

4. The high oxygen-MAP ground beef samples tested showed color loss during temperature abuse and became "discolored" when the bacterial levels reached around $1x10^5$ to $1x10^6$ cfu per gram.

5. There was a significant difference in the odor profiles observed in the CO-MAP ground beef samples and high oxygen-MAP ground beef samples tested as they aged, when the packaging was opened. In the CO-MAP ground beef samples, a sulfury odor was observed, while in the high oxygen-MAP ground beef samples, a rancid odor more commonly associated with the spoilage of meat was observed. No aroma was observed in the CO-MAP ground beef samples or high oxygen-MAP ground beef samples before the packaging was opened.

6. In the early stages of temperature abuse, the rate of growth of microbes was higher in the CO-MAP ground beef samples tested, than in the high oxygen-MAP ground beef samples tested. In the latter stages of temperature abuse, the rate of growth of microbes was higher in the high oxygen-MAP ground beef samples tested, than in the CO-MAP ground beef samples tested.

The purpose of the original studies (see Appendix One, Studies I, II, and III) was to examine changes in microbial growth, color and odor that occurred in CO-MAP ground beef and high oxygen-MAP ground beef when samples were subjected to temperature abuse and to evaluate possible correlations between these changes. The studies were made more difficult by the high initial bacteria levels that were found in some of the CO-MAP ground beef samples that had been purchased from retail stores. Although the samples were tested within the labeled "use or freeze by" dates, some of the CO-MAP ground beef samples had initial aerobic and anaerobic plate counts of $>1x10^6$ to around $1x10^7$ cfu/gram. The studies were conducted in spite of this challenge, but three further studies were conducted to determine whether the high initial bacterial levels observed in the CO-MAP samples purchased directly from retail outlets were an anomaly (Studies IV, V and VI, associated with Appendices One, Two and Three). These studies examined the microbiological status of ground beef just as it was being purchased by consumers. All the studies detailed in this letter lend support to and help document the deception and food safety risks outlined in Kalsec's Citizen Petition.

Study I (Appendix One – Tables 1A and 1B).

Study I Objective:

To examine the effect of radical temperature abuse on bacterial growth in commercially available packages of CO-MAP and high oxygen-MAP ground beef samples.

Study I Method:

Twenty packages of CO-MAP ground beef (a brand containing 8 % fat with an April 18, 2006 "use or freeze by" date) were purchased from a single retail store in the local region. This was the freshest CO-treated meat available in the store on the day of purchase. Twenty packages of high oxygen-MAP ground beef (a brand containing 20% fat with an April 23, 2006 sell by date) were purchased from another retail store in a different city in the local region. The meat samples were transported on ice in coolers to S&J Laboratories Inc. Twelve samples of CO-MAP and twelve samples of high oxygen-MAP ground beef were used in the temperature abuse study and were placed on a table, without stacking, in a 70° F room under normal room fluorescent lighting. Eight control samples for each package type were stored in the dark at 4° C (39° F). Sensory and microbial analysis was conducted at time 0, after 8 hours of temperature abuse at 70° F, then every four hours up to 24 hours of temperature abuse. At the appropriate intervals, packages were visually examined, photographed, and then opened and analyzed for aroma and micro count. Microbial analysis was conducted in duplicate with each sample tray being alcohol swabbed where the tray film was cut with a sterilized scalpel. Tray film was peeled back and an informal aroma sensory panel was conducted with personnel from both Kalsec and S&J Laboratories. Microbial analyses were conducted in duplicate; two trays per treatment per sample period, measuring both aerobic and anaerobic plate counts. Microbial analyses were conducted under aseptic sampling procedures (USDA Microbiological Guide Book, 3rd ed., 1998). Samples were first physically divided into six portions. Half of the sample (1st, 3rd, & 5th, or 2nd, 4th & 6th portions) was collected and mixed thoroughly inside a sterile bag. Eleven (11) grams of mixed sample was withdrawn into a filtered stomacher bag and 99 mL of sterile phosphate buffer was added to provide a 10-fold dilution. A further dilution protocol was followed to dilute up to 1,000,000 times. Petrifilm® was used in this study (AOAC official methods 990.12). The aroma was evaluated by three untrained individuals, carefully comparing the odor to refrigerated control samples. Digital photographs were taken for each sample period using a Nikon cool pix 950 digital camera. See Appendix One for experimental results.

Study I Result Summary:

The initial high bacterial load in the CO-MAP ground beef samples made a true comparison of bacterial growth rates difficult. It was decided to repeat the experiment (Study II).

Study I Discussion:

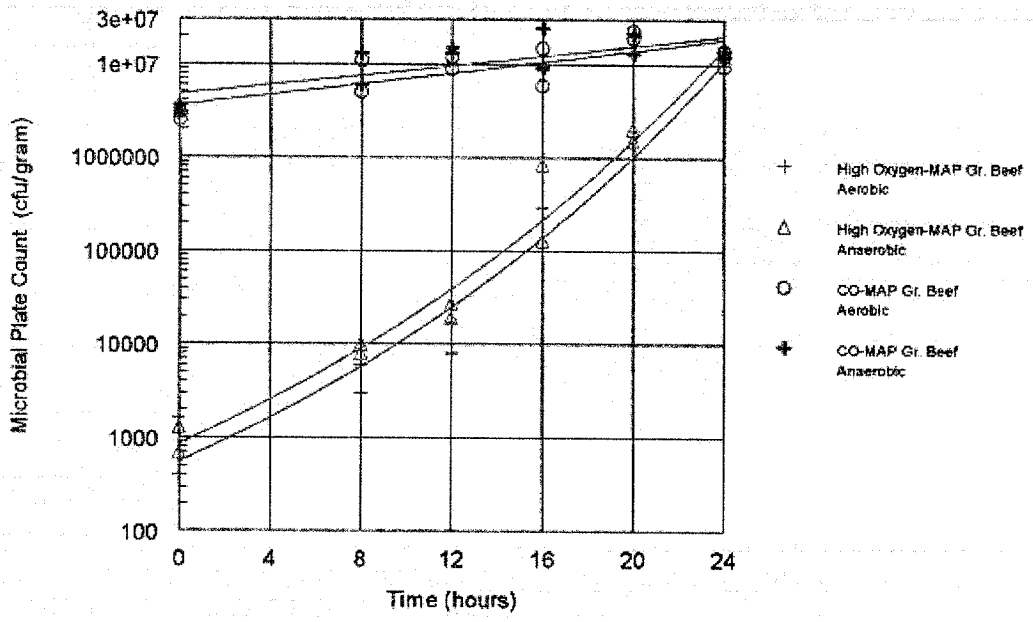
The aerobic and anaerobic plate counts of the samples stored at room temperature are listed in Table 1 and plotted in Figure 1 as a function of time. The plate counts for the CO-MAP ground beef samples, prior to any temperature abuse were very high (Table 1, top two data rows).

Table 1. Aerobic and Anaerobic Plate Counts of High Oxygen – MAP and Carbon Monoxide – MAP Meat Samples. Effect of Temperature Abuse – Study I.

	Hours at 70° F	High Oxygen-MAP Aerobic Plate Count	High Oxygen-MAP Anaerobic Plate Count	CO-MAP Aerobic Plate Count	CO-MAP Anaerobic Plate Count
Sample	0	400	700	2,500,000	3,100,000
Duplicate	0	1,600	1,300	3,000,000	3,600,000
Sample	8	3,000	10,000	5,000,000	6,000,000
Duplicate	8	6,000	8,000	11,000,000	13,000,000
Sample	12	8,000	19,000	12,000,000	13,000,000
Duplicate	12	17,000	27,000	9,000,000	15,000,000
Sample	16	290,000	840,000	15,000,000	24,000,000
Duplicate	16	110,000	130,000	5,900,000	9,100,000
Sample	20	1,700,000	1,500,000	19,000,000	13,000,000
Duplicate	20	1,900,000	2,000,000	23,000,000	21,000,000
Sample	24	10,000,000	13,000,000	9,600,000	12,000,000
Duplicate	24	13,000,000	15,000,000	14,000,000	14,000,000

As seen in Table 1, the anaerobic plate count of the CO-MAP ground beef samples prior to temperature abuse (the time zero samples in the first two data rows of Table 1) were initially over 3000 times higher than that of the high oxygen-MAP ground beef samples tested, with the ratio decreasing to about 1000 times after eight hours of temperature abuse at 70° F, and to about 600 times at twelve hours, when the CO-MAP ground beef samples were still red. Ratios of aerobic plate count were of similar magnitudes in the CO-MAP and high oxygen MAP ground beef samples tested.

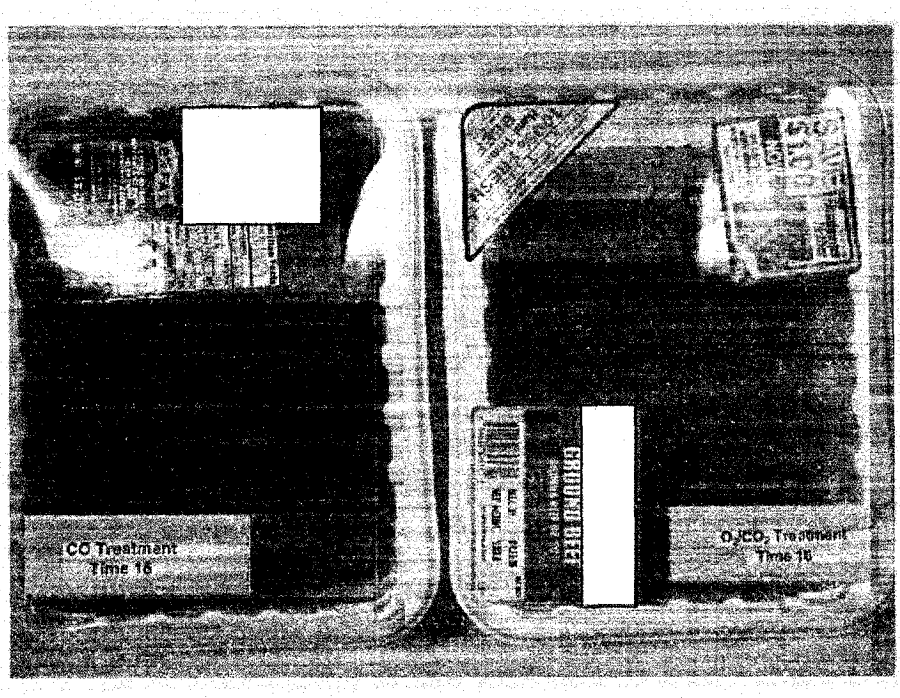
Figure 1. Microbial Plate Count of Ground Beef Stored at 70° F. Study I.

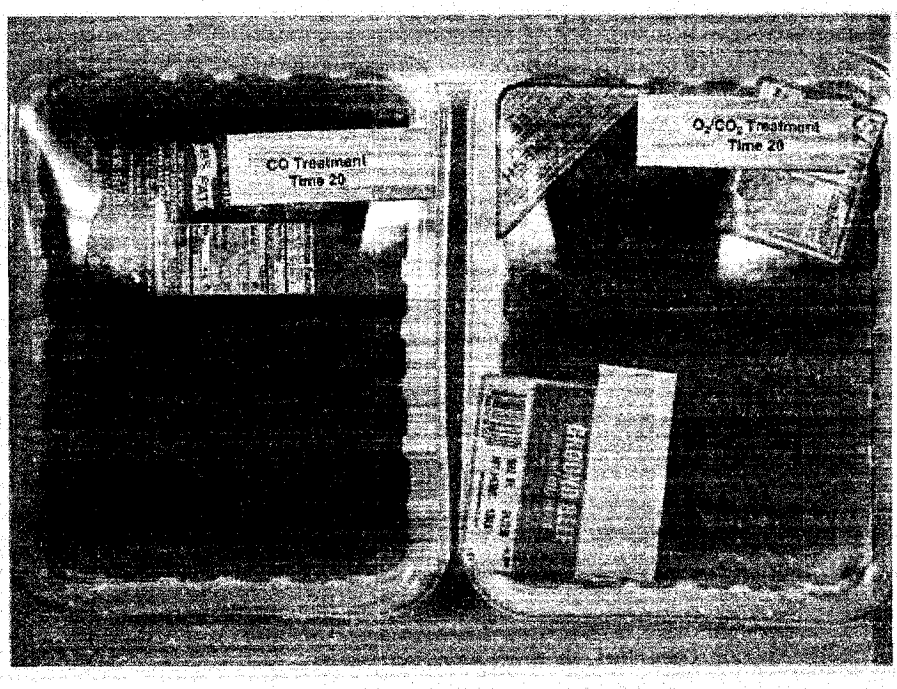


When purchased, the CO-MAP ground beef had 4 days remaining before the "use or freeze by" date. While this was toward the end of the shelf life, this was the freshest meat available at the retail outlet, and meat from this lot was abundantly available, with 20 packages of this lot being purchased. A roughly equal amount of CO-MAP ground beef was available with only 3 days left before the "use or freeze by" date at the same retail outlet. Figure 1 hints at an effect seen more clearly in Study II, namely, that microbial growth in the high oxygen-MAP ground beef samples occurred slowly at first and then built up steadily with time. For high oxygen-MAP ground beef samples, the most rapid increase in microbial count occurred toward the end of the temperature abuse period. In contrast, the rate of microbial growth in the CO-MAP ground beef occured more rapidly at first and then tapered off, seemingly reaching an asymptote somewhere above $1x10^7$ cfu / gram. For CO-MAP ground beef samples, the most rapid increase in microbial count occurred at the beginning of the temperature abuse period.

The CO-MAP ground beef samples remained bright red throughout the temperature abuse period. The color of the high oxygen-MAP ground beef samples changed throughout the course of the test, showing an easily noticeable color change at about 16 hours, when the micro count reached between about 10^5 and 10^6. Figures 2 and 3 show photos of selected meat samples.

Figure 2. Photographs of Meat Samples After 16 and 20 Hours of Temperature Abuse. CO Treatment = CO-MAP Ground Beef; O_2/CO_2 Treatment = High Oxygen-MAP Ground Beef. Study I.





At time zero (no temperature abuse), the CO-MAP ground beef sample had an initial slight sulfur aroma (hydrogen sulfide), that dissipated quickly once the package was opened. Jay reports that vacuum packaged (anaerobic) meat often displays objectionable odors with bacterial levels in the range of 10^6 to 10^7, and that H_2S is a

typical by-product of anaerobic bacterial growth [Jay, J.M., Modern Food Microbiology, Sixth Edition, Aspen Publishers Inc. 2000. Chapter 4, pg. 72.]. After 8 hours of temperature abuse at room temperature the CO-MAP ground beef sample still had a slight sulfur aroma, but it was observed that the aroma in this sample was less than that noted in the refrigerated CO-MAP control sample. There was no detectable difference in aroma or color between the 8 hour and the 12 hour temperature abused CO-MAP ground beef samples. After 16 hours of temperature abuse at room temperature, the aroma of the CO-MAP ground beef samples was noticeably different from the refrigerated CO-MAP ground beef control sample, with panelists describing a slight off aroma. After 20 hours of temperature abuse, the CO-MAP ground beef sample had a very noticeable sulfur aroma. The color remained bright red and fresh looking. After 24 hours of temperature abuse at room temperature, the CO-MAP ground beef had a strong H_2S aroma, but continued looking bright red and fresh.

At time zero (no temperature abuse), the high oxygen-MAP ground beef sample had no initial off aroma, and no significant off-aromas were noted in the high oxygen-MAP ground beef samples that had been temperature abused for 8 or 12 hours. After 16 hours of temperature abuse, the high oxygen-MAP ground beef had a slight buttery aroma and the color was starting to change. After 20 hours of temperature abuse at room temperature, the high oxygen-MAP ground beef sample had an off, spoiled, sour note, and was a very noticeable brownish color compared to the refrigerated high oxygen-MAP control sample. After 24 hours of temperature abuse at room temperature the high oxygen-MAP ground beef had a very noticeable off, spoiled / sour note.

Study II (Appendix One, Tables 2A., 2B., and 2C.)

Study II Objective:

A. To examine the effect of radical temperature abuse on bacterial growth in commercially available packages of CO-MAP and high oxygen-MAP ground beef samples.

B. To compare the aromas that developed during temperature abuse.

Study II Method:

Thirteen packages of CO-MAP ground beef (a brand containing 4 % fat with a May 9, 2006 "use or freeze by" date) were purchased in a single a retail store in the local region. This was the freshest CO-treated ground beef available on the day of purchase. Eighty-four packages of high oxygen-MAP ground beef (a brand containing 4% fat with a May 4, 2006 sell by date) were purchased from another retail store in a different city in the local region. The meat samples were transported on ice in coolers to S&J Laboratories Inc. Thirteen samples of CO-MAP and fourteen samples of high oxygen-MAP meat were used in the temperature abuse study and were placed on a table, without stacking in a 70° F room under normal room fluorescent lighting. Digital photography, sensory analysis, colorimeter readings and microbial analysis were conducted at time 0, after 8 hours of temperature abuse at 70° F, then every four hours up to 28 hours of temperature abuse. At intervals, packages were visually examined, photographed, and then opened and analyzed for aroma and micro count. The aroma was evaluated by three untrained individuals (using both Kalsec and S&J Laboratories personnel). Since the packages were labeled with brand names, the panelists were aware of the sample

identities when the aromas were being evaluated. The photographs taken at each analysis period on one tray from each treatment were made using a Nikon cool pix 950 digital camera by Kalsec personnel. Micro analyses were conducted in duplicate by S&J Laboratories personnel performed as in Study I, except for the time zero point for the CO-MAP ground beef sample, where only one package was analyzed. After sensory analysis, one tray from each treatment was measured for C.I.E. 1976 L*a*b* values using a Minolta CR-300 Chroma meter using the "C" light source and multi measure reading (average of three successive readings). L*a*b* readings were taken by Kalsec personnel directly through the package film with three readings taken per tray.

Study II Result Summary:

The bacterial growth curves for the CO-MAP and high oxygen-MAP ground beef samples were different. For high oxygen-MAP ground beef samples, the most rapid increase in microbial count occurred toward the end of the temperature abuse period. For CO-MAP ground beef samples, the most rapid increase in microbial count occurred at the beginning of the temperature abuse period.

The CO-MAP ground beef samples remained bright red and fresh-looking during the course of the temperature abuse and meat color was independent of bacterial load. The high oxygen-MAP ground beef samples, in contrast, showed color changes after the temperature abuse led to increased bacterial levels.

There was a significant difference in the odor profiles of CO-MAP and high oxygen-MAP ground beef samples as they aged.

Study II Discussion:

Study II was basically a repeat of Study I, using a very similar protocol. It was started on April 27, 2006, using CO-MAP ground beef samples with a "use or freeze by" date of May 9, 2006. The high oxygen-MAP ground beef samples used in this study had a sell by date of May 4, 2006. The CO-MAP ground beef samples used in Study II had lower initial microbial plate counts than the CO-MAP ground beef samples used in Study I. However, other CO-MAP ground beef samples purchased the same day, with "use or freeze by" dates of May 2, 2006 and May 4, 2006 had higher initial plate counts and could not be used for this part of the study (see Study VI, below, for a discussion of the behavior of these samples initially and upon refrigerated storage).

The aerobic and anaerobic plate counts of the CO-MAP and high oxygen-MAP ground beef samples stored at room temperature are listed in Table 2 and plotted in Figure 3 as a function of time. Once again, the microbial growth in the high oxygen-MAP ground beef samples was seen to grow slowly at first and then more rapidly with time. In contrast, the microbial growth in the CO-MAP ground beef samples occurred more rapidly at first and then tapered off, seemingly reaching an asymptote somewhere above $1x10^7$ cfu / gram. Significant differences between the CO-MAP ground beef samples and the high oxygen-MAP ground beef samples were observed after 8 hours of temperature abuse. High oxygen-MAP ground beef samples showed low values ($\sim10^3$) for both aerobic and anaerobic plate counts, whereas CO-MAP ground beef samples jumped to $\sim10^4$ for aerobic and $\sim10^5$ for anaerobic plate counts, respectively. Higher

rates of growth of anaerobic microorganisms in the CO-MAP ground beef samples, as compared to the high oxygen-MAP ground beef samples, was observed after 12 hours at 21°C, during which time the microbial count reached ~10^6 in some testing samples, whereas the high oxygen-MAP ground beef samples remained around ~10^3. The anaerobic environment of the CO-MAP ground beef samples appeared to enhance the growth of anaerobic microorganisms. The high oxygen-MAP ground beef samples did not reach 10^6 plate count until 24 hours of temperature abuse at 70° F, and grew to ~10^7 after another 4 hours. The CO-MAP ground beef samples reached ~10^6 (anaerobic) after 16 hours at 70° F under fluorescence light and reached ~10^7 at around 24 hours.

For CO-MAP ground beef samples, the color was consistent throughout the entire study (Table 2, redness: a* value ~ 26) despite the microbial count increase from ~10^3 to ~10^7. The CO-MAP ground beef samples remained fresh looking during the entire temperature abuse study. In contrast, the high oxygen-MAP ground beef samples lost their fresh red appearance gradually and a* values dropped from ~25 to ~15 over the course of the test (see Figure 4). Photographs of CO-MAP and high oxygen-MAP ground beef samples after 16 hours of temperature abuse are shown in Figure 5.

Both high oxygen-MAP and CO-MAP ground beef samples had similar fresh beef odor initially. However, after 8 hours incubation at 70° F, the high oxygen-MAP ground beef samples began to develop a slightly oxidized, rancid odor and the CO-MAP ground beef samples started to have a slight sulfur compound odor. For high oxygen-MAP ground beef samples, the intensity of the oxidized, rancid odor increased in each of 4-hour evaluation periods. The unique sulfur compound odor in the CO-MAP ground beef samples also increased in strength at each 4-hour evaluation period.

Table 2. Aerobic and Anaerobic Plate Counts of High Oxygen – MAP and Carbon Monoxide – MAP Meat Samples. Effect of Temperature Abuse – Study II.

	Hours at 70° F	High Oxygen-MAP Aerobic Plate Count	High Oxygen-MAP Anaerobic Plate Count	CO-MAP Aerobic Plate Count	CO-MAP Anaerobic Plate Count	a* High Ox.-MAP	a* CO-MAP
Sample	0	1,000	200	4300	6,800	25.5	24.1
Duplicate	0	1,600	800				
Sample	8	1,000	2,000	79,000	91,000	19.4	25.0
Duplicate	8	2,000	2,000	200,000	190,000		
Sample	12	8,000	4,000	510,000	1,400,000	20.0	26.5
Duplicate	12	9,000	4,000	560,000	640,000		
Sample	16	100,000	80,000	1,100,000	2,700,000	17.9	25.9
Duplicate	16	130,000	70,000	1,000,000	4,000,000		
Sample	20	500,000	1,200,000	4,300,000	7,800,000	17.5	25.2
Duplicate	20	190,000	280,000	4,400,000	12,000,000		
Sample	24	3,100,000	4,400,000	7,200,000	11,000,000	14.7	26.1
Duplicate	24	2,000,000	1,700,000	7,200,000	11,000,000		
Sample	28	24,000,000	28,000,000	16,000,000	23,000,000	15.3	26.9
Duplicate	28	30,000,000	26,000,000	12,000,000	28,000,000		

Figure 3. Microbial Plate Count of Ground Beef Stored at 70° F. Study II.

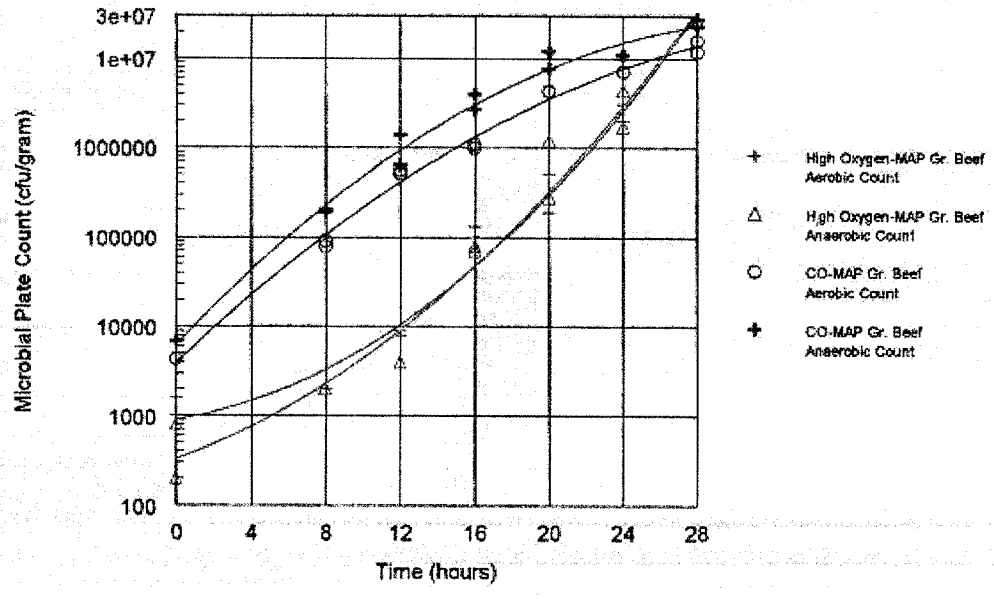


Figure 4. Color (a*) as a Function of Time. CO-MAP vs. high oxygen-MAP ground beef samples. Study II.

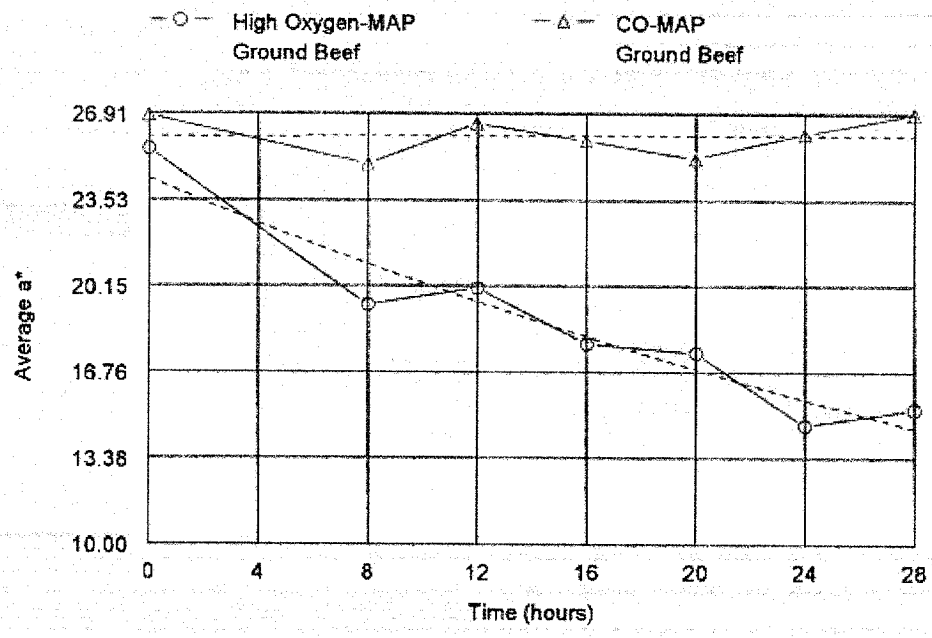
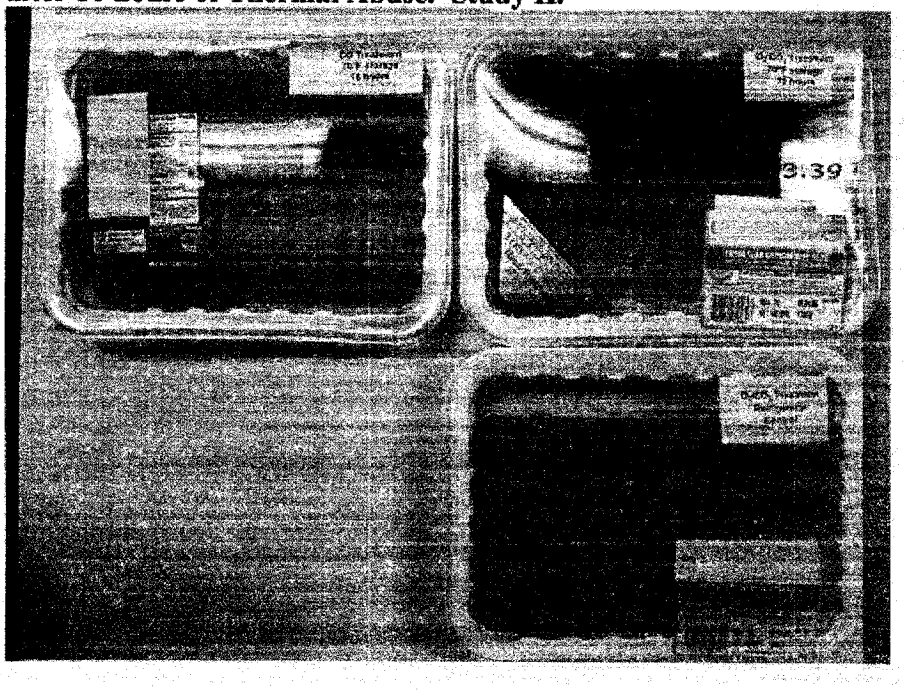


Figure 5. Photographs of CO-MAP and High Oxygen-MAP ground beef samples after 16 hours of Thermal Abuse. Study II.



Study III (Appendix One, Tables 3A., 3B. and 3C.)

Study III Objectives:

A. To examine the effect a period of temperature abuse followed by refrigerated storage on bacterial growth in high oxygen MAP and CO-MAP ground beef samples.
B. To compare the aromas that developed during temperature abuse.

Study III Method:

Twenty-four packages of CO-MAP ground beef (containing 7 % fat with a May 9, 2006 "use or freeze by" by date) was purchased in a single retail store in the local region. This was the freshest CO-MAP ground beef available in the store on the day of purchase. Some of the packages of high oxygen-MAP ground beef (a brand containing 4% fat with a May 4, 2006 sell by date) purchased as described in Study II were used in this study. The meat samples were transported on ice in coolers to S&J Laboratories Inc., the same day they were purchased (April 27, 2006). Thirteen packages of CO-MAP ground beef and fourteen packages of high oxygen-MAP ground beef were used in the temperature abuse study and were placed on a table, without stacking, in a 70° F room under normal room fluorescent lighting. After 8 hours of temperature abuse at 70° F, all the samples were moved to a temperature controlled 4° C refrigerator in the dark. Digital photography, sensory analysis, colorimeter readings and microbial analysis were conducted at time 0, after 8 hours of temperature abuse, then once a day for seven days. A photographic record was taken at each analysis period on one tray from each treatment using a Nikon cool pix 950 digital camera. Sensory, micro analyses and colorimetry analyses were conducted as in Study II.

Study III Result Summary:

The bacterial growth curves for the CO-MAP and high oxygen-MAP ground beef samples were different. Bacterial levels in the CO-MAP ground beef samples increased more rapidly during temperature abuse than did the high oxygen-MAP ground beef samples.

CO-MAP ground beef samples remained bright red and fresh-looking during the course of the entire study and meat color was independent of bacterial load. High oxygen-MAP ground beef samples, in contrast, showed color changes as measured by a* values after the temperature abuse led to increased bacterial levels.

There was a significant difference in the odor profiles of CO-MAP and high oxygen-MAP ground beef samples as they aged.

Study III Discussion:

In the third study, CO-MAP and high oxygen-MAP ground beef samples were subjected to temperature abuse at 21° C (70° F) for 8 hours and then returned to proper refrigerated storage (4° C, 39.2° F). Microbial counts were measured when the samples were newly-purchased, prior to temperature abuse, immediately after 8 hours storage at room temperature (21° C, 70° F) and daily during seven days of post-temperature abuse refrigerated storage. The study was started on April 27, 2006, using CO-MAP ground beef samples that had a "Use or Freeze By" date of May 9, 2006, and high oxygen-MAP

ground beef samples that had a "Sell By" date of May 4, 2006. The microbial growth curves are shown graphically in Figure 6. Once again, the CO-MAP ground beef samples initially had a higher plate count ($>10^5$) than the high oxygen-MAP ground beef samples ($\sim10^2$ to 10^3). When thermally abused, the CO-MAP ground beef samples showed a rapid rise in micro count to about $4x10^6$. The increase in micro count continued at a much higher level in the CO-MAP ground beef samples during subsequent refrigerated storage, reaching a plateau at about $3x10^7$ cfu/gram. In contrast, the high oxygen-MAP ground beef samples showed a smaller initial jump in plate count during thermal abuse and had a much more gradual rate of increase, reaching only $\sim10^5$ at the close of the test. The CO-MAP ground beef samples exceeded the spoilage limit of 10^7 colony forming units (cfu) per gram on day 1 or 2 of refrigerated storage. At this point, the samples were still within their "Use or Freeze By" dates. At day seven, the "Sell By" date for the high oxygen-MAP ground beef samples was reached, and even the temperature abused samples had relatively low plate counts.

Once again, the CO-MAP ground beef samples remained bright red and fresh looking in appearance throughout the test, even though microbial levels were above 10^7 cfu / gram. The color behavior for both CO-MAP and high oxygen-MAP ground beef samples is shown in Figure 7, where a* values are plotted as a function of time. Data can be found in Appendix 1, Table 3B.

The high oxygen-MAP ground beef samples developed a slight oxidized odor immediately after temperature abuse. This odor remained about the same quality and intensity until just before the end of the refrigerated period. The CO-MAP ground beef samples developed a slight sulfur odor immediately after the temperature abuse period. Two days after being returned to the refrigerator, the sulfur odor became more noticeable. Four days after being returned to the refrigerator, the sulfur odor was described as being strong. At the end of the study, the sulfur odor was described as very strong. Aroma data can be found in Appendix One, Table 3C.

Figure 6. Plate Count During Refrigerated Storage After Temperature Abuse. CO-MAP vs. High Oxygen-MAP. Study III.

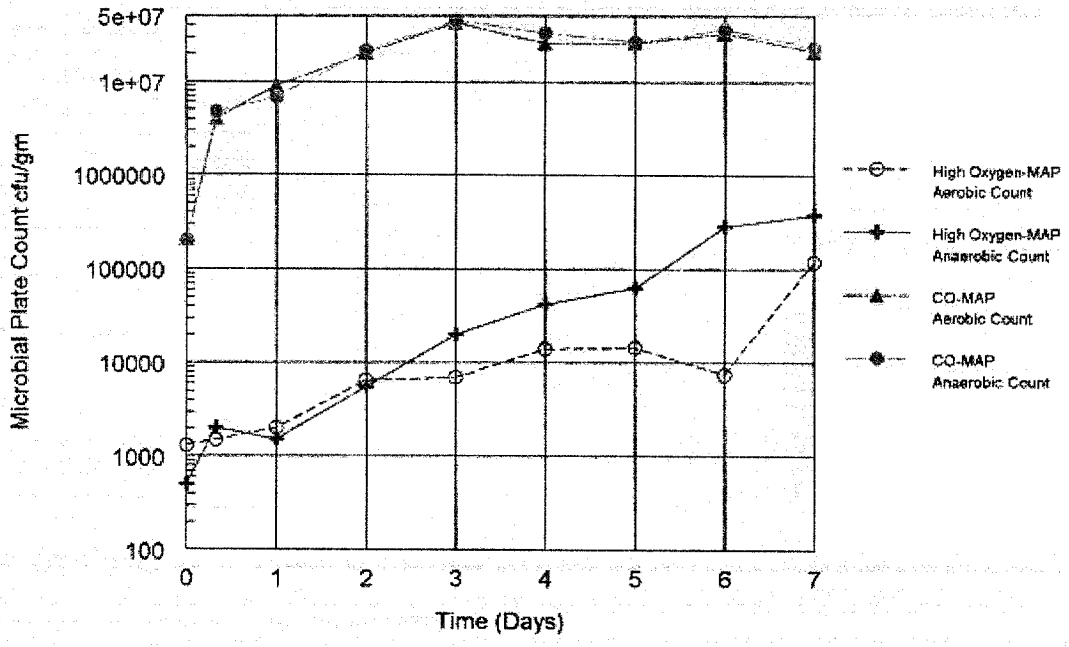
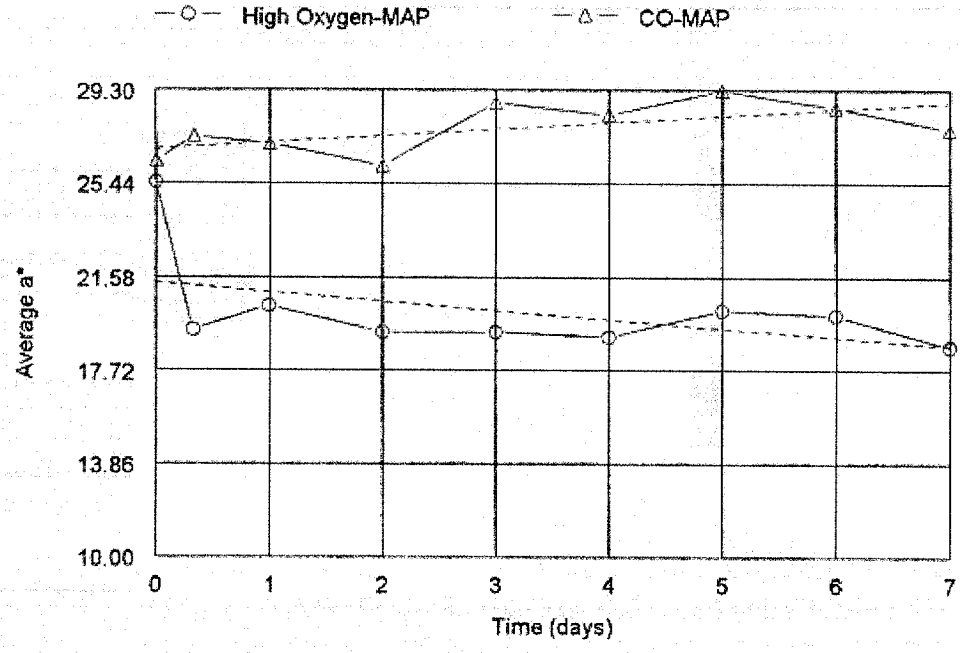


Figure 7. Effect of Temperature Abuse and Subsequent Refrigerated Storage on Color in Ground Beef. a* vs. time. Study III.



Study IV – A Survey of Commercial Ground Beef (Appendix One, Table 4)

Study IV Objective:

To measure and compare the bacterial counts in freshly-purchased ground beef packages, comparing CO-MAP, high oxygen-MAP and traditional plastic overwrap packaging. A chub pack and a paper wrapped sample were also examined.

Study IV Method:

Twenty-three samples of ground beef were obtained from seven different retail stores. Seven of these samples were purchased on May 8, 2006, immediately packed on ice and then refrigerated overnight. Sixteen samples were purchased on May 9, 2006 and transported on ice in coolers to S&J laboratories Inc. for microbial analysis. Prior to microbial analysis the package headspace was measured for oxygen and carbon dDioxide in the MAP packages using a Dansensor Checkmate 9900. This headspace analysis was done by Kalsec personnel. Micro analyses were conducted using the same protocol as described earlier by S&J Laboratories personnel, except that measurements were made on single packages, with no replicates. See Appendix One.

Seven samples were obtained from a retail store in the local region on 5-8-06.

1. 4% fat, CO-MAP with a 5-16-06 "use or freeze by" by date.
2. 75% lean, CO-MAP ground beef with a 5-11-06 "use or freeze by" by date.
3. 75& lean, CO-MAP ground beef with a 5-9-06 "use or freeze by" by date.

4. 75% lean, CO-MAP ground beef with a 5-9-06 "use or freeze by" by date.
5. 8% fat ground beef patties, CO-MAP with a 5-16-06 "use or freeze by" by date.
6. 8% fat, CO-MAP ground beef with a 5-17-06 "use or freeze by" by date.
7. 85% lean, CO-MAP ground round with a 5-16-06 "use or freeze by" by date.

Three samples were obtained from a second retail store in the local region on 5-9-06.

1. 4% fat, high oxygen-MAP ground beef with a 5-13-06 sell by date.
2. 83% lean, high oxygen-MAP ground chuck with a 5-15-06 sell by date.
3. 26% fat, Certified Angus Beef over-wrap package with a 5-10-06 sell by date.

Two samples were obtained from a third retail store in the local region on 5-9-06.

1. 86% lean, certified ground round over-wrap package with a 5-11-06 sell by date.
2. 90% lean, certified ground sirloin over-wrap package with a 5-11-06 sell by date.

One sample was obtained from the meat counter at a fourth retail store in the local region on 5-9-06. This was ground hamburger with 27% fat.

Two samples were obtained from a fifth retail store in the local region on 5-9-06.

1. 90% lean, CO-MAP ground sirloin with a 5-20-06 "use or freeze by" by date.
2. 80% lean, CO-MAP ground beef with a 5-18-06 "use or freeze by" by date.

One sample was obtained from a sixth retail store in the local region on 5-9-06. It was an over-wrap ground hamburger with a 5-11-06 sell by date.

Seven samples were obtained from a seventh retail store in the local region on 5-9-06.

1. 96% lean, high oxygen-MAP extra lean ground beef with a 5-10-06 sell by date.
2. 96% lean, high oxygen-MAP extra lean ground beef with a 5-16-06 sell by date.
3. 85% lean, high oxygen-MAP ground round with a 5-16-06 sell by date.
4. 85% lean, high oxygen-MAP ground round with a 5-10-06 sell by date.
5. 73% lean, high oxygen-MAP ground beef with a 5-15-06 sell by date.
6. 73% lean, high oxygen-MAP ground beef with a 5-13-06 sell by date.
7. 93% lean, ground beef chub with a 5-16-06 sell by date.

Study IV Result Summary:

The levels of bacteria in the CO-MAP ground beef samples were, on average, higher than those in the high oxygen-MAP ground beef samples, in a statistically significant manner.

Neither the overwrap packages, the chub package, nor the paper-wrapped ground beef sample were modified atmosphere packaged. None of these samples had been treated with carbon monoxide. They were included in this study in a very limited fashion simply to get a feel for the microbial plate counts for commercial examples of ground beef samples packaged in these ways.

Study IV Discussion:

Since so many packages of CO-MAP ground beef used in Studies I, II, and III were found to have very high micro counts immediately after purchase, and well within the "Use or Freeze By" date, a preliminary survey of commercial ground beef samples was performed. Several samples of ground beef in various types of MAP and non-MAP

packaging were purchased from seven different retail stores in the local region, and transported to S&J Laboratory, Inc. on ice and subjected to aerobic and anaerobic plate count measurements. The raw data is shown in Table 3. Also included in Table 3 are the results of the initial (time zero) measurements from Studies II and III. Statistical analysis of this non-normal data, using the one-sided t-test shows that with greater than 95% confidence, microbial levels for CO-MAP samples are greater than for all other samples not containing CO ($p = 0.029$ for anaerobic counts and $p = 0.027$ for aerobic counts). In a direct comparison between CO-MAP and high oxygen-MAP ground beef samples (eliminating the overwrap, paper wrapped and chub samples), and using the one-sided t-test, the CO-MAP ground beef samples are shown to have higher microbial levels than the high oxygen-MAP ground beef samples with greater than 96% confidence. The averages and ranges for aerobic and anaerobic plate counts for these MAP products are shown in Table 4.

Table 3. Preliminary Survey of Plate Counts of Commercial Ground Beef Samples. Study IV.

ANALYSIS DATE	WHERE PURCHASED	PURCHASE DATE	SELL BY DATE	DAYS OF SHELF LIFE REMAINING	DANSENSOR O2	DANSENSOR CO2	PERCENT FAT	AEROBIC	ANAEROBIC	PACKAGE	CARBON MONOXIDE
5/9/2006	A	5/8/2006	5/16/2006	7	0.03	17.20	4	25,000	35,000	MAP	YES
5/9/2006	A	5/8/2006	5/16/2006	7	0	19.70	8	18,000	57,000	MAP	YES
5/9/2006	A	5/8/2006	5/17/2006	8	0.001	20.70	8	170,000	540,000	MAP	YES
5/9/2006	A	5/8/2006	5/9/2006	0	0.00	20.30	25	10,000	110,000	MAP	YES
5/9/2006	A	5/8/2006	5/9/2006	0	0.00	20.3	25	10,000	150,000	MAP	YES
5/9/2006	A	5/8/2006	5/11/2006	2	0.00	20.4	25	1,500,000	3,000,000	MAP	YES
5/9/2006	A	5/8/2006	5/16/2006	7	0.44	15.8	15	2,100,000	4,600,000	MAP	YES
5/9/2006	B	5/9/2006	5/18/2006	9	0.00	20.3	20	15,000	32,000	MAP	YES
5/9/2006	B	5/9/2006	5/20/2006	11	0.00	19.0	10	69,000	170,000	MAP	YES
5/9/2006	C	5/9/2006	5/13/2006	4	71.10	19.7	4	1,700	100	MAP	NO
5/9/2006	C	5/9/2006	5/15/2006	6	69.30	18.6	17	1,500	2,000	MAP	NO
5/9/2006	D	5/9/2006	5/10/2006	1	76.80	13.5	4	6,000	15,000	MAP	NO
5/9/2006	D	5/9/2006	5/10/2006	1	76.60	11.8	15	14,000	41,000	MAP	NO
5/9/2006	D	5/9/2006	5/13/2006	4	78.20	11.6	27	1,200,000	1,700,000	MAP	NO
5/9/2006	D	5/9/2006	5/15/2006	6	79.50	11.1	27	7,700	12,000	MAP	NO
5/9/2006	D	5/9/2006	5/16/2006	7	76.70	13.7	4	500	600	MAP	NO
5/9/2006	D	5/9/2006	5/16/2006	7	80.00	11.4	15	200	400	MAP	NO
5/9/2006	C	5/9/2006	5/10/2006	1	NA	NA	26	7,000	17,000	Overwrap	NO
5/9/2006	E	5/9/2006	5/11/2006	2	NA	NA	10	17,000	19,000	Overwrap	NO
5/9/2006	E	5/9/2006	5/11/2006	2	NA	NA	14	6,000	21,000	Overwrap	NO
5/9/2006	F	5/9/2006	5/11/2006	2	NA	NA		34,000	10,000	Overwrap	NO
5/9/2006	G	5/9/2006	5/9/2006	0	NA	NA	27	54,000	51,000	Paper Wrap	NO
5/9/2006	D	5/9/2006	5/16/2006	7	NA	NA	7	3,900	3,700	Chub	NO
4/27/2006	C	4/27/2006	5/4/2006	7	NA	NA	4	1,300	500	MAP	NO
4/27/2006	A	4/27/2006	5/9/2006	12	NA	NA	4	4,300	6,800	MAP	YES
4/27/2006	A	4/27/2006	5/9/2006	12	NA	NA	7	210,000	200,000	MAP	YES
4/27/2006	A	4/27/2006	5/4/2006	7	NA	NA	7	530,000	3,400,000	MAP	YES
4/27/2006	A	4/27/2006	5/2/2006	5	NA	NA	7	3,900,000	12,000,000	MAP	YES
4/17/2006	A	4/15/2006	4/18/2006	1	NA	NA	8	2,750,000	3,350,000	MAP	YES
4/17/2006	C	4/16/2006	4/23/2006	6	NA	NA	20	1,000	1,000	MAP	NO

* Reference: Official methods of Analysis of AOAC International, Ch.17, 17th edition, 2000. Bacteriological Analytical Manual By AOAC, 8th edition. 1998 Revision A

Table 4. Averages and Ranges for Micro Data From the Ground Beef Survey (Study IV).

Samples	Aerobic Count Average	Anaerobic Count Average	Low Aerobic Count	High Aerobic Count	Low Anaerobic Count	High Anaerobic Count
CO-MAP	808,000	1,975,000	4,300	3,900,000	6,800	12,000,000
High Oxygen-MAP	123,000	177,000	200	1,200,000	100	1,700,000

Figures 8 and 9 show plots of anaerobic and aerobic plate counts, respectively, vs. the attributes of MAP, overwrap, fat level and the presence or absence of carbon monoxide. It shows that the main driver for high microbial levels in this system is the use of the CO packaging atmosphere.

Figure 8. Anaerobic Plate Count vs. Attributes (Study IV).

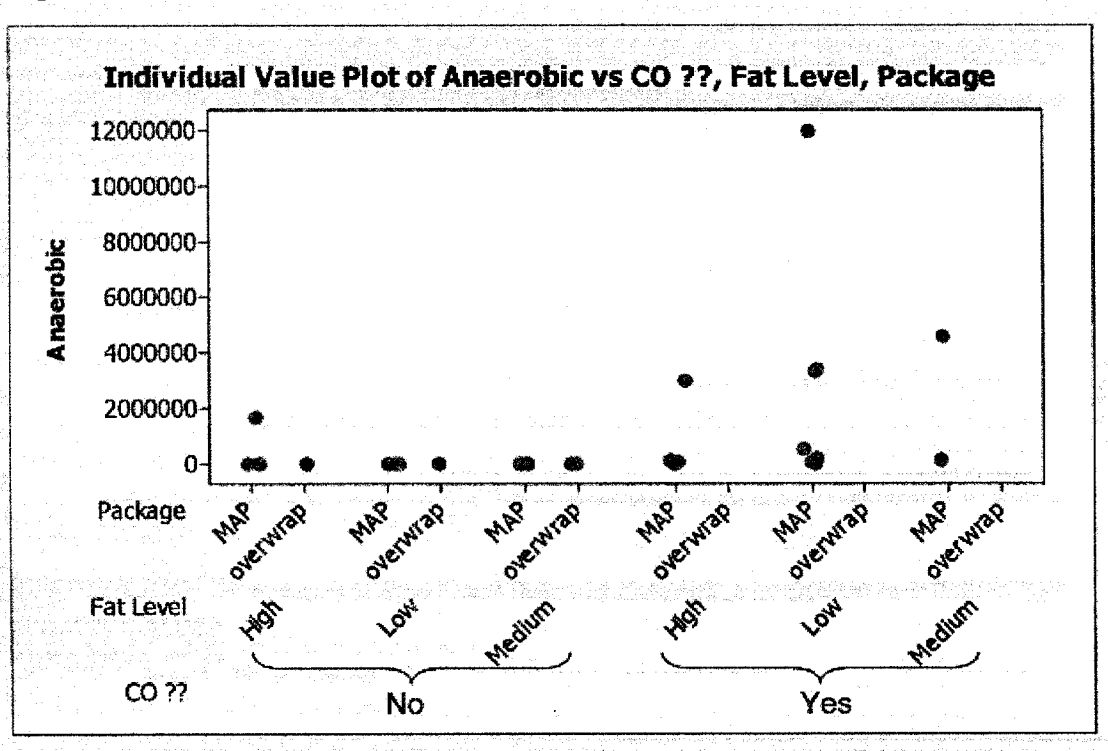
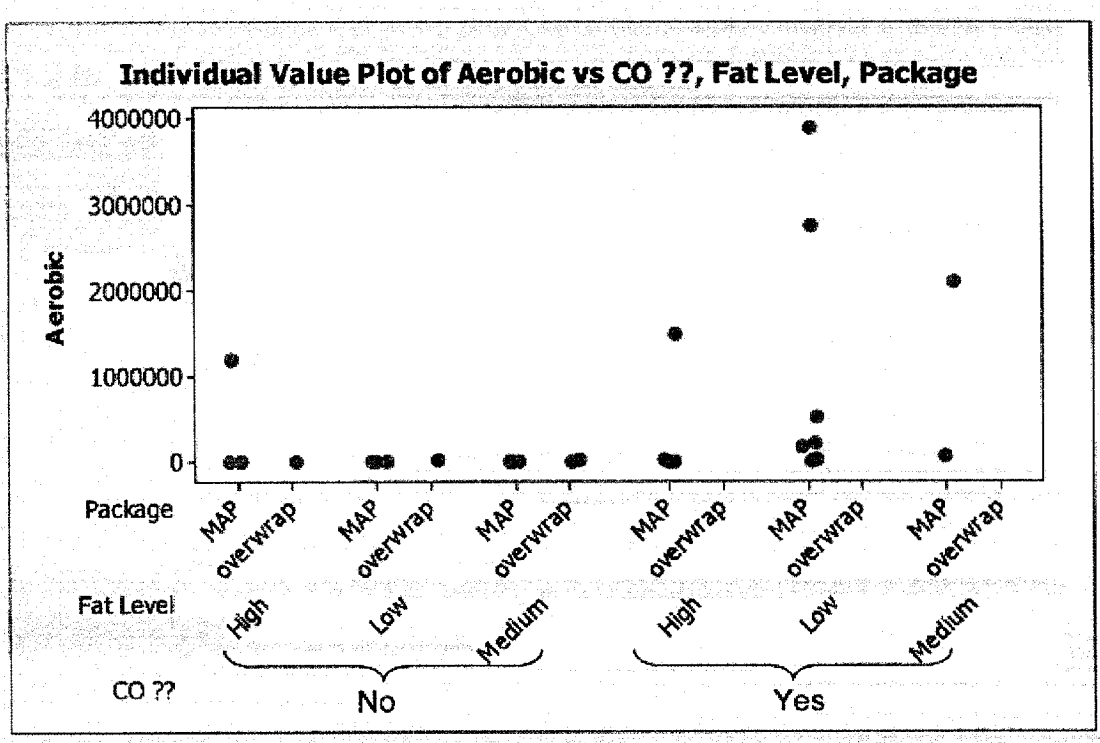


Figure 9. Aerobic Plate Count vs. Attributes. Study IV.



Study V – A Survey of Commercial Ground Beef – Using Replicate Analyses (Appendix Two).

Study V Objective:

To measure and compare the bacterial counts in freshly-purchased ground beef packages in CO-MAP and high oxygen-MAP, using multiple sample replicates.

Study V Method:

Thirty packages of CO-MAP ground beef and thirty packages of high oxygen-MAP ground beef were obtained from four different retail stores in the local region, as outlined in Table 6. Samples were transported to S&J laboratories Inc. on ice in coolers for microbial analysis. Prior to microbial analysis the package headspace was measured for Oxygen and Carbon Dioxide in the MAP packages using a Dansensor Checkmate 9900 by Kalsec personnel. Micro analysis was conducted using the same protocol as described earlier by S&J Laboratories personnel. Data can be found in Appendix Two.

Study V Result Summary:

The levels of bacteria in the CO-MAP ground beef samples were higher than in the high oxygen-MAP ground beef samples, in a statistically significant manner.

Study V Discussion:

In Study IV, individual packages of ground beef representing a number of packaging formats were purchased, transported on ice to S&J Laboratories and analyzed for microbial plate count. While statistically significant differences were observed in the levels of bacterial contamination between the CO-MAP ground beef samples and the other commercially available packaging varieties, it was thought that another study involving a number of replicate analyses would be helpful in confirming the quality differences that were seen in Study IV. In Study V, the type of meat packaging examined was limited to high oxygen-MAP ground beef and CO-MAP ground beef, with more than one brand for each type being examined. Where available, five packages of meat, with the same "use or freeze by" or "sell by" date, were analyzed for each brand examined. The raw data are shown in Table 5. Statistical analysis of this non-normal data, using the one-sided t-test shows that with greater than 99% and 95% confidence, respectively, the aerobic and anaerobic microbial levels for CO-MAP samples are greater than those packaged in the high oxygen modified atmosphere (p-Value = 0.005 for aerobic and 0.042 for anaerobic). The averages and ranges for aerobic and anaerobic plate counts for these MAP products are shown in Table 6.

Table 5. Survey of Plate Counts of Commercially Available CO and High Oxygen MAP Ground Beef. Study V.

Sample #	Purchase Date	Analysis Date	Sell By Date	Days Remaining	% Fat	Location Purchased	CO?	Head Space %O2/%CO2	Aerobic Plate Count (cfu/g)	Anaerobic Plate Count (cfu/g)
1	5/22/2006	5/22/2006	5/24/2006	2	10	A	No	80.7/11.4	1,000	2,000
2	5/22/2006	5/22/2006	5/24/2006	2	10	A	No	81.5/11.6	6,000	13,000
3	5/22/2006	5/22/2006	5/24/2006	2	10	A	No	67.7/10.6	3,000	3,000
4	5/22/2006	5/22/2006	5/24/2006	2	10	A	No	82.4/11.7	10,000	3,000
5	5/22/2006	5/22/2006	5/24/2006	2	10	A	No	80.4/11.7	6,000	6,000
6	5/22/2006	5/22/2006	5/29/2006	7	20	A	No	84.3/10.3	500	600
7	5/22/2006	5/22/2006	5/29/2006	7	20	A	No	83.8/10.5	700	800
8	5/22/2006	5/22/2006	5/29/2006	7	20	A	No	84.1/10.6	900	1,200
9	5/22/2006	5/22/2006	5/29/2006	7	20	A	No	82.6/10.1	700	300
10	5/22/2006	5/22/2006	5/29/2006	7	20	A	No	82.7/10.3	900	700
11	5/22/2006	5/22/2006	5/28/2006	6	4	A	No	84.6/12.3	2,100	700
12	5/22/2006	5/22/2006	5/28/2006	6	4	A	No	83.2/12.5	3,100	2,600
13	5/22/2006	5/22/2006	5/28/2006	6	4	A	No	80.9/12.4	2,000	1,400
14	5/22/2006	5/22/2006	5/28/2006	6	4	A	No	82.9/13.0	1,100	1,800
15	5/22/2006	5/22/2006	5/28/2006	6	4	A	No	82.8/12.7	2,600	2,700
16	5/23/2006	5/23/2006	6/1/2006	9	7	B	Yes	0.024/17.4	160,000	290,000
17	5/23/2006	5/23/2006	6/1/2006	9	7	B	Yes	0.003/18.0	39,000	120,000
18	5/23/2006	5/23/2006	6/1/2006	9	7	B	Yes	0.001/17.7	220,000	290,000
19	5/23/2006	5/23/2006	6/1/2006	9	7	B	Yes	0.000/18.0	99,000	180,000
20	5/23/2006	5/23/2006	6/1/2006	9	7	B	Yes	0.000/18.6	61,000	120,000
21	5/23/2006	5/23/2006	5/31/2006	8	15	B	Yes	0.000/18.1	12,000	58,000
22	5/23/2006	5/23/2006	5/31/2006	8	15	B	Yes	0.000/18.5	19,000	28,000
23	5/23/2006	5/23/2006	5/31/2006	8	15	B	Yes	0.000/19.2	9,000	40,000
24	5/23/2006	5/23/2006	5/31/2006	8	15	B	Yes	0.000/18.4	11,000	78,000
25	5/23/2006	5/23/2006	5/31/2006	8	15	B	Yes	0.000/19.0	35,000	48,000
26	5/23/2006	5/23/2006	5/31/2006	8	4	B	Yes	0.000/19.1	120,000	210,000
27	5/23/2006	5/23/2006	5/31/2006	8	4	B	Yes	0.000/18.6	130,000	330,000
28	5/23/2006	5/23/2006	5/31/2006	8	4	B	Yes	0.000/18.8	120,000	330,000
29	5/23/2006	5/23/2006	5/31/2006	8	4	B	Yes	0.000/18.4	140,000	380,000

Table 5. (Continued) Survey of Plate Counts of Commercially Available CO and High Oxygen MAP Ground Beef. Study V.

30	5/23/2006	5/23/2006	5/31/2006	8	4	B	Yes	0.000/18.6	180,000	300,000
31	5/23/2006	5/23/2006	5/30/2006	7	8	B	Yes	0.000/18.4	110,000	220,000
32	5/23/2006	5/23/2006	5/30/2006	7	8	B	Yes	0.000/19.0	79,000	220,000
33	5/23/2006	5/23/2006	5/30/2006	7	8	B	Yes	0.000/18.5	70,000	160,000
34	5/23/2006	5/23/2006	5/30/2006	7	8	B	Yes	0.000/19.4	100,000	190,000
35	5/23/2006	5/23/2006	5/30/2006	7	8	B	Yes	0.000/20.2	14,000	54,000
36	5/24/2006	5/24/2006	5/29/2006	5	4	C	No	73.2/17.6	100	200
37	5/24/2006	5/24/2006	5/29/2006	5	4	C	No	70.0/17.2	100	300
38	5/24/2006	5/24/2006	5/29/2006	5	4	C	No	71.5/17.3	100	200
39	5/24/2006	5/24/2006	5/29/2006	5	4	C	No	74.8/17.6	100	200
40	5/24/2006	5/24/2006	5/29/2006	5	4	C	No	73.9/17.3	300	200
41	5/24/2006	5/24/2006	5/29/2006	5	20	C	No	78.9/12.0	1,100	1,000
42	5/24/2006	5/24/2006	5/29/2006	5	20	C	No	77.5/13.1	900	1,000
43	5/24/2006	5/24/2006	5/29/2006	5	20	C	No	79.1/12.4	500	700
44	5/24/2006	5/24/2006	5/29/2006	5	20	C	No	80.1/12.5	600	900
45	5/24/2006	5/24/2006	5/29/2006	5	20	C	No	81.5/12.3	1,200	800
46	5/24/2006	5/24/2006	5/29/2006	5	17	C	No	79.7/12.6	800	100
47	5/24/2006	5/24/2006	5/29/2006	5	17	C	No	79.4/12.9	1,000	500
48	5/24/2006	5/24/2006	5/29/2006	5	17	C	No	80.3/12.9	600	500
49	5/24/2006	5/24/2006	5/29/2006	5	17	C	No	79.3/13.0	900	800
50	5/24/2006	5/24/2006	5/29/2006	5	17	C	No	81.0/12.4	500	300
51	5/25/2006	5/25/2006	5/28/2006	3	10	D	Yes	0.002/26.9	2,900,000	27,000,000
52	5/25/2006	5/25/2006	5/28/2006	3	10	D	Yes	0.000/22.0	1,400,000	4,600,000
53	5/25/2006	5/25/2006	5/28/2006	3	10	D	Yes	0.000/21.9	940,000	3,700,000
54	5/25/2006	5/25/2006	5/28/2006	3	10	D	Yes	0.000/22.6	1,800,000	9,200,000
55	5/25/2006	5/25/2006	6/3/2006	9	10	D	Yes	0.000/24.1	1,000	1,000
56	5/25/2006	5/25/2006	6/5/2006	11	20	D	Yes	0.000/22.4	170,000	440,000
57	5/25/2006	5/25/2006	6/5/2006	11	20	D	Yes	0.000/22.4	72,000	310,000
58	5/25/2006	5/25/2006	6/5/2006	11	20	D	Yes	0.000/23.2	260,000	400,000
59	5/25/2006	5/25/2006	6/5/2006	11	20	D	Yes	0.000/22.0	180,000	720,000
60	5/25/2006	5/25/2006	6/5/2006	11	20	D	Yes	0.000/23.1	120,000	300,000

Table 6. Averages and Ranges for Micro Data From the Ground Beef Survey (Study V).

Samples	Aerobic Count Average	Anaerobic Count Average	Low Aerobic Count	High Aerobic Count	Low Anaerobic Count	High Anaerobic Count
CO-MAP	319,000	1,677,000	1,000	2,900,000	1,000	27,000,000
High Oxygen-MAP	1,650	1,580	100	10,000	200	13,000

Figures 10 and 11 show plots of anaerobic and aerobic plate counts, respectively, vs. the attributes of fat level and the presence or absence of carbon monoxide.

Figure 10. Anaerobic Plate Count vs. Attributes (Study V).

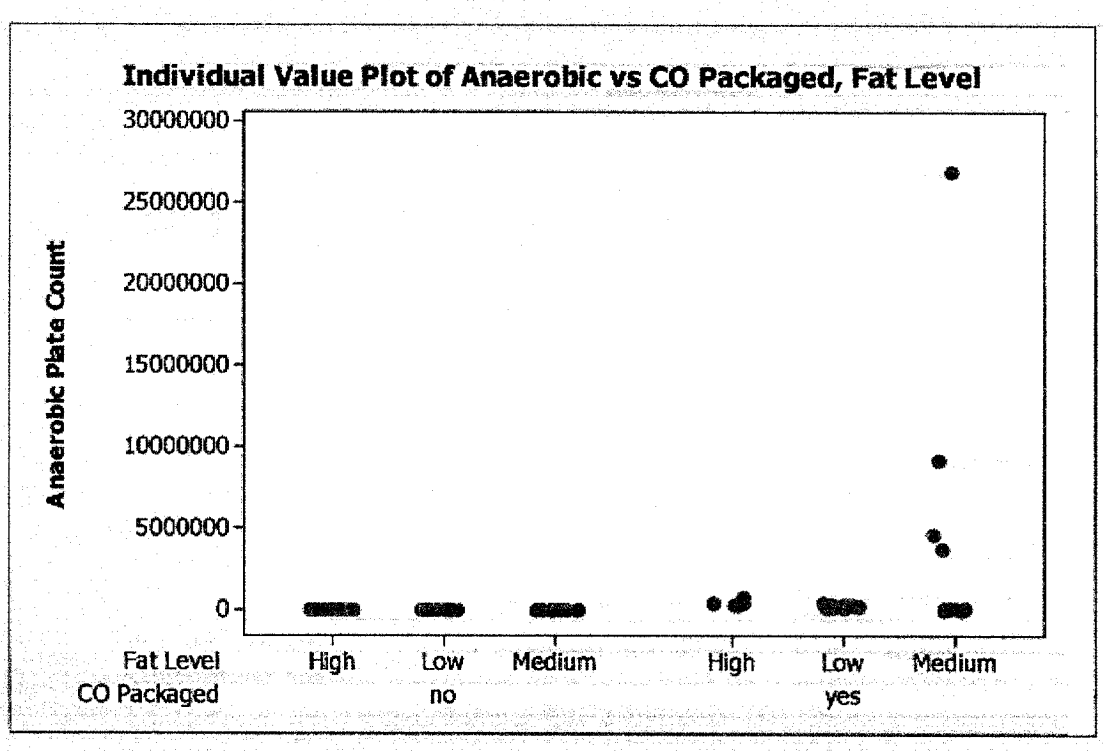
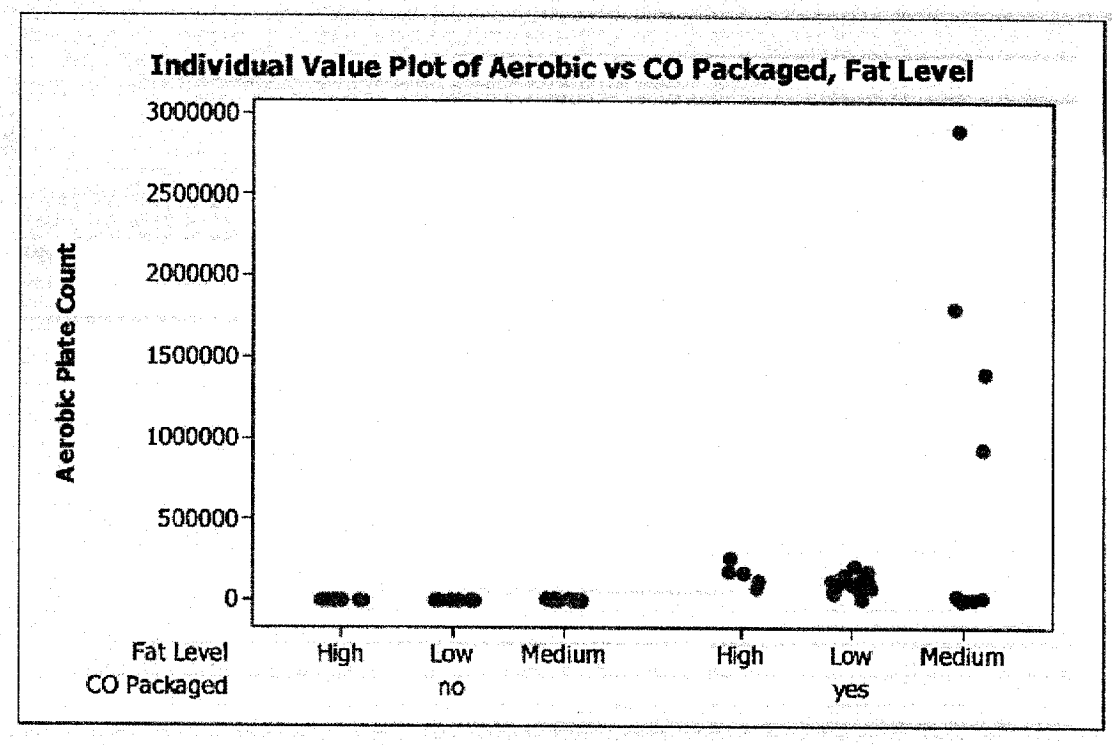


Figure 11. Aerobic Plate Count vs. Attributes (Study V).



Study VI (Appendix Three).

Study VI Objective:

To measure the anaerobic and aerobic plate counts of commercial meat samples immediately after purchase and at time intervals under proper temperature storage (4° C) until the end of the shelf life as determined by the printed "Use or Freeze by" date.

Study VI Method:

Thirteen packages of CO-MAP ground beef (containing 7 % fat with "use or freeze by" dates of May 2, 2006, and twenty-four packages of CO-MAP ground beef (containing 7% fat with a "use or freeze by" date of May 4, 2006) were purchased in a single retail store in the local region. Five packages of CO-MAP ground beef with the May 2, 2006 "use or freeze by" date and five packages of the CO-MAP ground beef with the May 4, 2006 "use or freeze by" date were used in this study. Ten packages of the high oxygen-MAP ground beef described in Study II (a brand containing 4% fat with a May 4, 2006 sell by date) were also used in this study. Samples were transported on ice in coolers to S&J Laboratories Inc. where they were stored in the dark in a controlled, 4° C refrigerator. Microbial analyses were conducted at time 0, and days 2, 4, 6, and 7, by methods described above. For the CO-MAP ground beef, one tray from each of the two sell by dates was analyzed for sensory and micro for each sample period. For the high oxygen MAP samples, micro analyses were run in duplicate. See Appendix Three for experimental results.

Study VI Results Summary

High levels of bacteria indicative of spoilage were found in samples of CO-MAP ground beef that had been purchased and then stored in the dark at 4° C (39.2° F) and analyzed periodically until the "Use or Freeze By" date had been reached. The counts were much higher than for the high oxygen-MAP ground beef samples treated in the same way.

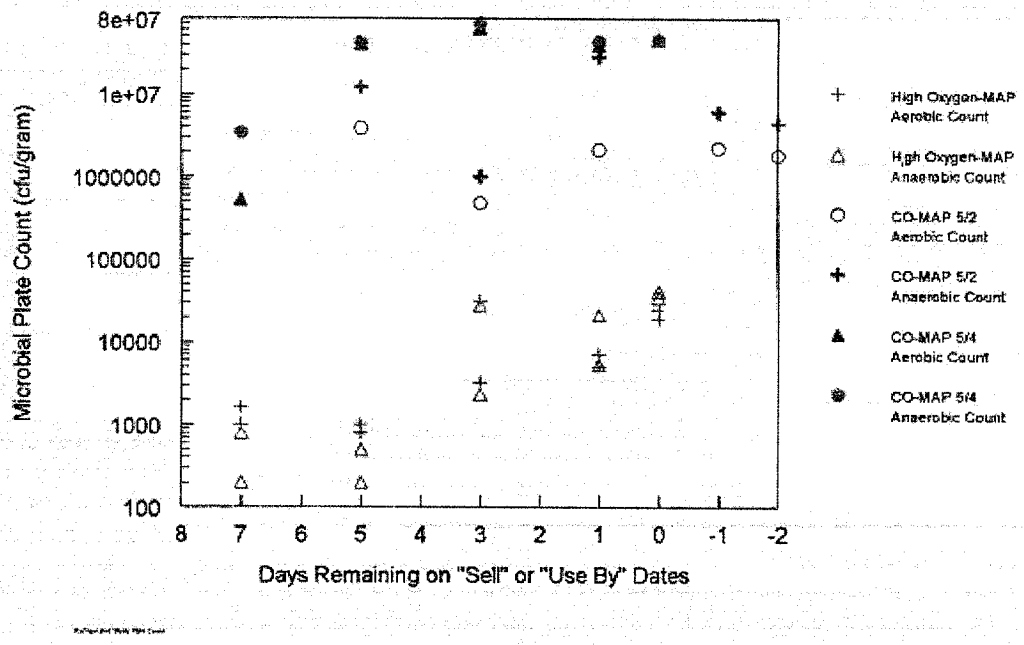
Study VI Discussion

Of the three lots of meat purchased for Study 2, only one could be used for the temperature abuse study, because the other two, with "Use or Freeze By" dates of May 2, 2006 and May 4, 2006, had very high plate counts. These meat samples were stored in the dark at 4° C and, periodically, a package of each lot was removed from the refrigerator and analyzed for aerobic and anaerobic plate count. Samples of high oxygen-MAP with a "Sell By" date of May 4, 2006 were also refrigerated and analyzed similarly. The results, presented in Table 7, and graphically in Figure 12 show two distinct populations. The CO-MAP meat exhibited bacterial counts of greater than 10^7, indicative of spoilage, up to five days before the "Use or Freeze By" date, even though they had not been subjected to any temperature abuse and were stored under refrigeration at 4° C. These meat samples appeared bright red and fresh looking. In contrast, the meat packaged in the high oxygen-MAP system showed much lower plate counts.

Table 7. Bacterial Counts on Commercial Meat Samples as a Function of Shelf Life (Time until "Sell By" or "Use or Freeze By" Dates are Reached). Study VI.

Days Remaining until Sell or Use by Dates Reached	Aerobic Plate Count High Oxygen-MAP 5/4/06 Sell by	Anaerobic Plate Count High Oxygen-MAP 5/4/06 Sell by	Aerobic Plate Count CO-MAP 5/2/06 Use by	Anaerobic Plate Count CO-MAP 5/2/06 Use by	Aerobic Plate Count CO-MAP 5/4/06 Use by	Anaerobic Plate Count CO-MAP 5/4/06 Use by
7	1,000	200			530,000	3,400,000
7	1,600	800				
5	1,000	200	3,900,000	12,000,000	41,000,000	42,000,000
5	800	500				
3	3,200	2,300	480,000	1,000,000	62,000,000	71,000,000
3	31,000	28,000				
1	5,000	5,300				
1	7,000	21,000	2,100,000	28,000,000	39,000,000	43,000,000
0	19,000	40,000				
0	24,000	34,000			46,000,000	44,000,000
-1			2,200,000	6,000,000		
-2			1,800,000	4,400,000		

Figure 12. Anaerobic and Aerobic Plate Counts of Commercial Meat Samples as a Function of Days Remaining on Package Date. Study VI.

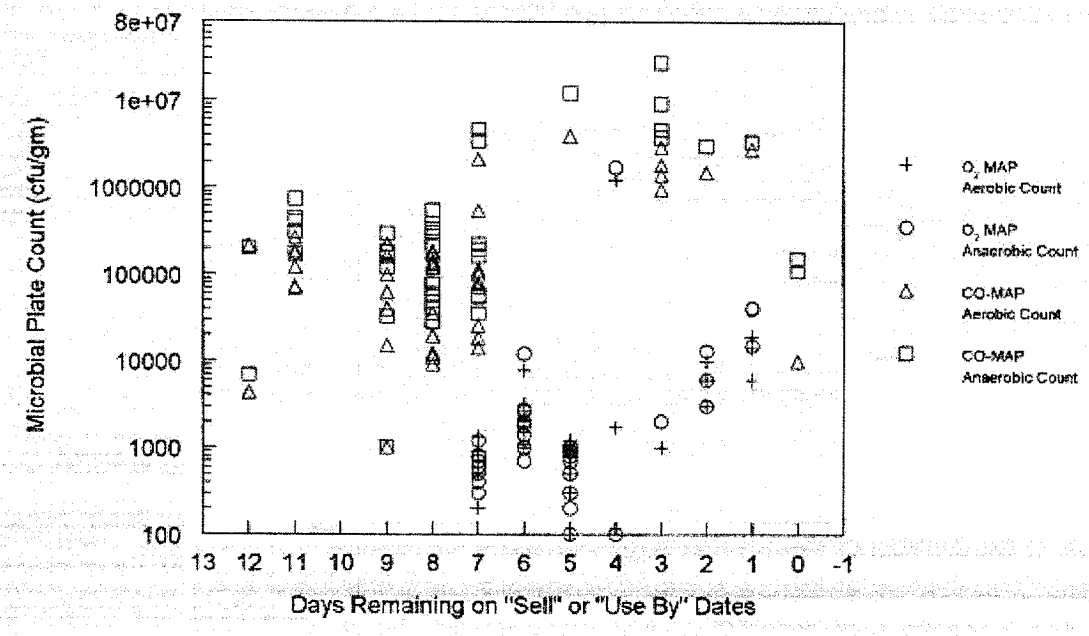


Combined Results from Studies IV and V.

Both Studies IV and V show significant differences in the bacterial counts between samples of CO-MAP and high oxygen-MAP ground beef samples. The average plate counts for the CO-MAP ground beef samples were found to be much higher than those for the high oxygen-MAP ground beef samples.

Figure 13 shows a graph of the anaerobic and aerobic plate count of the CO-MAP and high oxygen-MAP ground beef samples from Study IV and Study V plotted as a function of the number of days remaining on the "Sell By" or "Use or Freeze By" dates. The graph shows a clustering of the data into two distinct regions, with the CO-MAP ground beef samples generally showing much higher microbial levels. Some of the samples of CO-MAP ground beef had bacterial levels greater than 10^7 at the time of purchase and within the "Use or Freeze By" date printed on the packages.

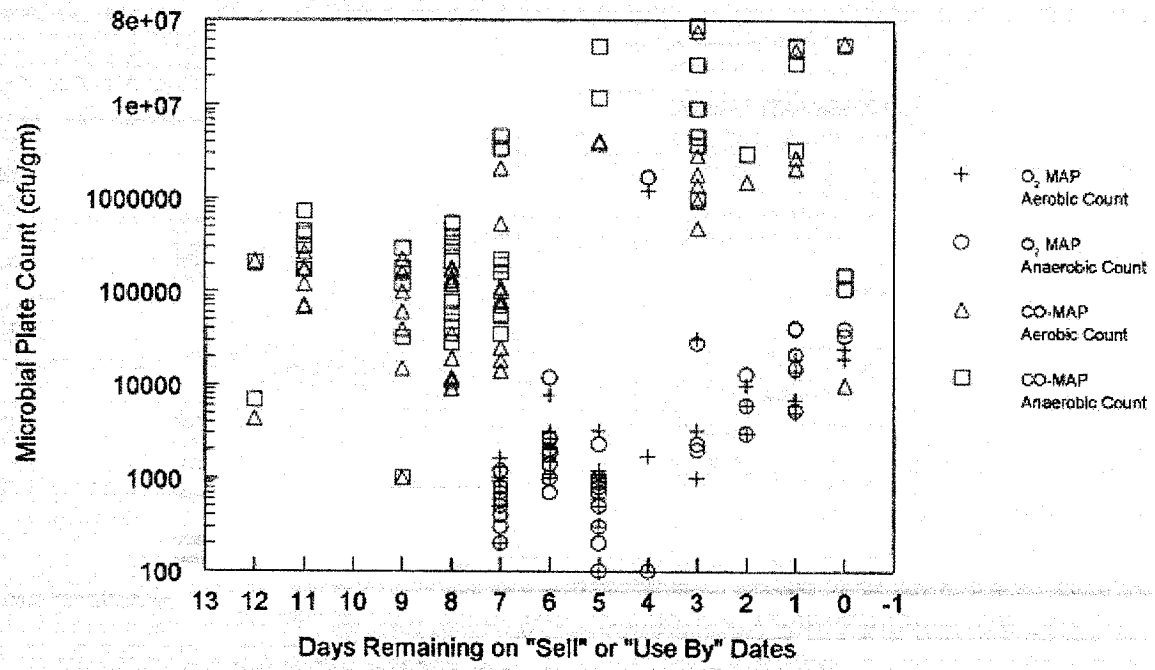
Figure 13. A Comparison of Bacterial Contamination Levels in Commercial Ground Beef. A combination of Data from Studies IV and V.



Combined Results from Studies IV, V and VI.

If one includes the data from Study VI, where both CO-MAP and high oxygen-MAP ground beef packages were purchased, stored under good refrigeration conditions and analyzed up until the "Sell By" or "Use or Freeze By" dates, the resulting graph is that shown in Figure 14. The data shows distinct differences in microbial contamination levels between CO-MAP and high oxygen-MAP ground beef samples.

Figure 14. A Comparison of Bacterial Contamination Levels in Commercial Ground Beef. A combination of Data from Studies IV and V and Data on Refrigerated Storage from Study VI.



The data in this report lend further support to the food safety and consumer deception concerns set forth in the Kalsec citizen petition and related submissions to the agency concerning the use of carbon monoxide in fresh meat packaging. These findings indicate that CO-MAP ground beef sold at retail can remain red and fresh in appearance under conditions in which the meat has been temperature abused or contain bacteria levels indicative of spoilage. In addition, these findings raise concerns regarding the microbiological quality of CO-MAP ground beef that is sold in retail outlets. (See also results from limited study published in *Consumer Reports* at 51 (July 2006)).

Respectfully submitted,

Donald R. Berdahl, Ph.D.
Executive Vice President
Kalsec, Inc.

Appendix One

S & J Laboratories, Inc.

Report To: Dr. Don Berdahl/Mr. Greg Reynhout (Kalsec®, Inc.)

From: James C. Lin, Ph.D. (S & J Laboratories, Inc.)

Date: May 15, 2006

Title: Microbial profiles, color, and sensory evaluation of commercial packaged ground beef under pre-determined storage conditions.

SUMMARY

Commercially available MAP ground beef packages including Hi-Ox samples (82% oxygen/18% carbon dioxide) and CO-pk (0.4% CO) were used to study the temperature/time effect on microbiological, color and sensory profile. The microbial data of each study group were presented; the effects of temperatures/time on the beef color & sensory impact were measured. A preliminary survey of commercially available ground beef in a variety of packaging was conducted to determine whether the high plate counts observed in many of the CO-pk samples was an anomaly. Tentative conclusions, based on current experimental design and data, were drawn.

MATERIALS & METHODS

The analyses on the ground beef packages purchased from local supermarkets were separated into (4) studies. Each study group contained both Hi-Ox samples (82% oxygen/18% carbon dioxide) and CO-pk (0.4% CO).

Study I. Samples were kept at 21°C (70°F) for up to 24 hours under fluorescence light. Control samples were stored in the dark at refrigeration temperature (4°C, or 39°F). Initial very high plate counts in the CO-pk samples led to this study being repeated.

Study II. Study one was repeated using a different, lower plate count CO-pk meat sample. Samples were kept at 21°C (70°F) for up to 28 hours under fluorescence light.

Study III. Samples were abused at 21°C (70°F) for 8 hours under fluorescence light, and then kept at refrigeration temp (4°C, or 39°F) until the end of their labeled use by date.

Study IV. Samples representing a variety of packaging forms were purchased and immediately analyzed for plate count.

All investigations were carried out on the premises of S&J Laboratories, Inc., Portage, MI. Microbiological analyses, including aerobic plate count and anaerobic plate count, were performed by S & J Laboratories personnel. Color measurements (generating L, a*, and b* values), using a Minolta CR-300 hand held Colorimeter were conducted by Kalsec® personnel. The sensory evaluation was conducted by sniffing the odor right after sample packages were opened, with both S&J Lab and Kalsec® personnel being involved in the evaluation. Kalsec® personnel also photographed all meat packages at each study stage. Oxygen and carbon dioxide levels in the atmospheres of certain packages were measured by Kalsec® personnel using a Dansensor Checkmate 9900.

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RESULTS AND DISCUSSION

Study I. Temperature Abuse 21°C (70°F)

Table 1A presents all the microbial data for this study. Initial aerobic and anaerobic plate counts showed significant ($p<0.05$) difference between Hi-Ox and CO-pk samples. The CO-pk samples were about 3 log units higher in microbial count than the High-Ox samples. The anaerobic and aerobic plate counts for the CO-pk samples trended upward reaching a level above $1x10^7$ cfu/g. Anaerobic and aerobic counts for the Hi-Ox samples trended up over the course of the study, reaching $1x10^7$ cfu/g at the end of the study. Given the high initial plate count of the CO-pk samples, it was decided to repeat this study with different meat samples.

No colorimetry was done on this study, but by visual inspection, the CO-pk samples remained bright red and fresh-looking throughout the temperature abuse interval. The red color of the High-Ox samples dropped throughout the test, reaching what was deemed as unacceptable at about 16 hours.

In Table 1B, both Hi-Ox & CO-pk samples had similar fresh beef odor, except CO-pk sample #2 had slight sulfur compound odor. Hi-Ox samples showed slight buttery, oxidized odor after 16 hours and the intensity of this aroma grew throughout the rest of the test. The sulfur aroma became more noticeable at 16 hours into the test.

Study II. 21°C (70°F) under fluorescent light for 28 hours

In Table 2A, the initial bacteria count showed little difference between Hi-Ox and CO-pk ground beef, Hi-Ox was at 10^2~10^3 and CO-pk was at ~10^3. Significant differences were observed right after 8 hours temperature abuse. Hi-Ox samples kept at low plate count, ~10^3, for both aerobic and anaerobic plate counts, whereas CO-pk samples jumped to ~10^4 for aerobic and ~10^5 for anaerobic. The more acceleration of the growth of anaerobic microorganisms in CO-pk were observed after 12 hours at 21°C, which the microbial count reached ~10^6 in some testing samples, whereas Hi-Ox samples were maintained around ~10^3. The CO environment enhanced the growth of anaerobic microorganisms. Hi-Ox samples also reached ~10^6 after 24 hours, and further grew to ~10^7 after another 4 hours. CO-pk reached ~10^6 (anaerobic) after 16 hours at 21°C under fluorescence light & eventually reached ~10^7.

For CO-pk samples, the color was consistent throughout the entire study (Table 2B, redness: a* value ~ 26) despite the microbial count increased from ~10^3 to ~10^7, whereas the Hi-Ox samples lost its fresh redness gradually from ~25 to ~15 in 28 hours. Hi-Ox samples not only lost its redness, the decrease of its brightness also observed ("L" values, Kalsec® data) during 28 hours study. This color fading was mainly due to the metabolites generated during the microbial growth that reduced oxygen concentration, lowered pH value. If the study persisted, the depleted oxygen in conjunction with the lowered pH will eventually denatured meat protein and altered the color to dark brown, a scenario known as met-myoglobin . On the other hand, CO-pk samples always preserve its bright redness despite the microbial load, pH value, even the PI (iso-electric point, ~5.4) was reached. However, the lost of water holding capacity was noticeable by observing running meat juice in some CO-pk samples after 12 hours at 21°C. This exudation may be due to the PI point reached hence the myoglobin denatured.

Both Hi-Ox & CO-pk samples had similar fresh beef odor initially (Table 2C). However, after 8 hours incubation at 21°C, Hi-Ox samples began to show slightly oxidized, rancid odor & CO-pk samples start

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to have slightly sulfur compound odor. For Hi-Ox samples, the intensity of the oxidized, rancid odor increased in each of 4-hour evaluation, whereas CO-pk samples also increased the strength of the unique, unpleasant sulfur compound odor at each 4-hour evaluation period. It may be that CO-pk creates a favorable environment for anaerobic & facultative anaerobes to grow & generates sulfur-containing compounds in the head space of the meat package. This sulfur aroma was not detectable in any of the Hi-Ox samples.

Study III. 21°C (70°F) 8 hours under fluorescent light & stored at 4°C (39.2°F) refrigeration

Initial bacteria count showed difference between Hi-Ox and CO-pk ground beef, Hi-Ox was at 10^2~10^3 and CO-pk was at ~10^5 (Table 3A). After 8 hours abuse at 21°C, Hi-Ox samples remained at low levels (~10^3) for both aerobic and anaerobic plate counts, whereas CO-pk samples increased 1 log cycle to ~10^6 for both aerobic and anaerobic. Refrigeration after first 8 hours temp abuse (21°C) did slow the subsequent rate of microbial growth for both samples. Microbial counts after 20 hours refrigeration showed Hi-Ox samples remaining at low levels (~10^3), whereas the CO-pk samples were between the mid and high end of ~10^5. From the data on the third day (4/29/06), it appears that CO-pk had reached its stationary phase with microbial counts at ~10^7 and kept at similar log values throughout the rest of the study. The microbial counts of Hi-Ox samples increased much slower & reached ~10^5 on May 3rd, 2006.

For CO-pk samples, the color was in a narrow range throughout the study (Table 3B, redness: a* value: 26~ 29) despite the microbial count increased from ~10^5 to ~10^7. Hi-Ox samples lost their bright redness right after the first 8 hours at 70°F (from ~25 to ~19), and remained at a somewhat similar value thereafter. Since Hi-Ox samples were stored at 4°C after the 8 hours of temp abuse, the final microbial counts did not exceed 10^6 hence the colors were maintained. The CO-pk maintained almost the same color throughout the study.

Both Hi-Ox & CO-pk samples had similar fresh beef odor initially (Table 3C). However, after 8 hours incubation at 21°C (70°F), Hi-Ox samples began to show a slight oxidized, rancid odor & CO-pk samples started to exhibit a slight sulfur compound odor. For Hi-Ox samples, the intensity of the oxidized, rancid odor remained at a very similar level throughout the study. On the other hand, the sulfur compound note developed faster in CO-pk samples. The 8-hour temp abuse may accelerate the odor development.

Study IV. Preliminary Survey of Plate Counts of Commercial Ground Beef Samples

Various samples of ground beef were purchased by Kalsec® personnel and transported to S&J laboratories in coolers. The bacteria counts of freshly-purchased ground beef samples in various types of packaging are shown in Table 4. Additional instances of high plate counts in CO-pk meat samples have been found – within the use or sell by dates. Only one sample with over 10^6 plate count in a High-Ox sample was found. The bright red fresh-looking color of all the CO-pk samples

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CONCLUSION

Hi-Ox and CO-pk ground beef packages developed two different odor and color patterns that are very easy to differentiate. The traditional visual evaluation to predict ground beef quality that was associated with meat freshness and microbial load can no longer be used as an indicator where CO-pk ground beef was involved.

A preliminary survey of meat available to consumers indicates that very high microbial plate counts are observed more often in CO-treated meat than in the other forms available. We recommend investigation of additional samples to verify this.

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Table 1A. The microbial profile of ground beef samples abused at 21 °C, (70 °F) under fluorescent light for 24 hours.

TESTING TIME	ANALYSIS	High Ox G.B. Sample # 1 (80% Lean, sell by 4/23/06)	High Ox G.B. Sample # 2 (80% Lean, sell by 4/23/06)	CO Packed G.B. Sample # 1 (92% Lean, use or freeze by 4/18/06)	CO Packed G.B. Sample # 2 (92% Lean, use or freeze by 4/18/06)
4/17/06 12 A.M.	Aerobic Plate Count/gm	400	1,600	2,500,000	3,000,000
4/17/06 12 A.M.	Anaerobic Plate Count/gm	700	1,300	3,100,000	3,600,000
4/17/06 8 A.M.	Aerobic Plate Count/gm	3,000	6,000	5,000,000	11,000,000
4/17/06 8 A.M.	Anaerobic Plate Count/gm	10,000	8,000	6,000,000	13,000,000
4/17/06 12 P.M.	Aerobic Plate Count/gm	8,000	17,000	12,000,000	9,000,000
4/17/06 12 P.M.	Anaerobic Plate Count/gm	19,000	27,000	13,000,000	15,000,000
4/17/06 4 P.M.	Aerobic Plate Count/gm	290,000	110,000	15,000,000	5,900,000
4/17/06 4 P.M.	Anaerobic Plate Count/gm	840,000	130,000	24,000,000	9,100,000
4/17/06 8 P.M.	Aerobic Plate Count/gm	1,700,000	1,900,000	19,000,000	23,000,000
4/17/06 8 P.M.	Anaerobic Plate Count/gm	1,500,000	2,000,000	13,000,000	21,000,000
4/18/06 12 A.M.	Aerobic Plate Count/gm	10,000,000	13,000,000	9,600,000	14,000,000
4/18/06 12 A.M.	Anaerobic Plate Count/gm	13,000,000	15,000,000	12,000,000	14,000,000

* Reference: Official Methods of Analysis of AOAC International, Ch.17, 17th edition, 2000. Bacteriological Analytical Manual By AOAC, 8th edition, 1998 Revision A.

Table 1B. The sensory evaluation of ground beef samples abused at 21 °C, (70 °F) under fluorescent light for 24 hours.

TESTING TIME	**High Ox G.B. Sample # 1&2** **(80% Lean, sell by 4/23/06)**	**CO Packed G.B. Sample # 1&2** **(92% Lean, use or freeze by 4/18/06)**
4/17/06 12 A.M.	Fresh beef odor	Very slight sulfur comp odor
4/17/06 8 A.M.	No off odor detected	Very slight sulfur comp odor
4/17/06 12 P.M.	No off odor detected	Slight sulfur comp odor
4/17/06 4 P.M.	Slight butter /oxidized odor	Noticeable sulfur comp odor
4/17/06 8 P.M.	Off spoiled / sour odor	Noticeable sulfur comp odor
4/18/06 12 A.M.	Off spoiled / sour odor	Strong sulfur comp odor

Table 2A. The microbial profile of ground beef samples abused at 21°C (70°F) under fluorescent light for 28 hours.

TESTING TIME	ANALYSIS	High Ox G.B. Sample # 1 (96% Lean, sell by 5/4/06)	High Ox G.B. Sample # 2 (96% Lean, sell by 5/4/06)	CO Packed G.B. Sample # 1 (96% Lean, use or freeze by 5/9/06)	CO Packed G.B. Sample # 2 (96% Lean, use or freeze by 5/9/06)
4/27/06 12 P.M.	Acrobic Plate Count/gm	1,000	1,600	4,300	N/A
4/27/06 12 P.M.	Anaerobic Plate Count/gm	200	800	6,800	N/A
4/27/06 8 P.M.	Aerobic Plate Count/gm	1,000	2,000	79,000	91,000
4/27/06 8 P.M.	Anaerobic Plate Count/gm	2,000	2,000	200,000	190,000
4/28/06 12 A.M.	Aerobic Plate Count/gm	8,000	9,000	510,000	560,000
4/28/06 12 A.M.	Anaerobic Plate Count/gm	4,000	4,000	1,400,000	640,000
4/28/06 4 A.M.	Aerobic Plate Count/gm	100,000	130,000	1,100,000	1,000,000
4/28/06 4 A.M.	Anaerobic Plate Count/gm	80,000	70,000	2,700,000	4,000,000
4/28/06 8 A.M.	Aerobic Plate Count/gm	500,000	190,000	4,300,000	4,400,000
4/28/06 8 A.M.	Anaerobic Plate Count/gm	1,200,000	280,000	7,800,000	12,000,000
4/28/06 12 P.M.	Aerobic Plate Count/gm	3,100,000	2,000,000	7,200,000	7,200,000
4/28/06 12 P.M.	Anaerobic Plate Count/gm	4,400,000	1,700,000	11,000,000	11,000,000
4/28/06 4 P.M.	Aerobic Plate Count/gm	24,000,000	30,000,000	16,000,000	12,000,000
4/28/06 4 P.M.	Anaerobic Plate Count/gm	28,000,000	26,000,000	23,000,000	28,000,000

* Reference: Official Methods of Analysis of AOAC International, Ch.17, 17th edition, 2000. Bacteriological Analytical Manual By AOAC, 8th edition, 1998 Revision A.

Table 2B. The color (a* value) profile of ground beef samples abused at 21°C (70°F) under fluorescent light for 28 hours.

TESTING TIME	**High Ox G.B. Sample # 1&2** (96% Lean, sell by 5/4/06)	**CO Packed G.B. Sample # 1&2** (96% Lean, use or freeze by 5/9/06)
4/27/06 12 P.M.	25.53±1.76	26.11±2.38
4/27/06 8 P.M.	19.41±3.34	24.96±0.84
4/28/06 12 A.M.	20.07±1.13	26.54±0.58
4/28/06 4 A.M.	17.88±0.60	25.86±2.53
4/28/06 8. A.M.	17.52±0.18	25.17±1.37
4/28/06 12 P.M.	14.69±1.89	26.14±1.22
4/28/06 4 P.M.	15.32±1.13	26.91±0.42

Table 2C. The sensory evaluation of ground beef samples abused at 21°C (70°F) under fluorescent light for 28 hours.

TESTING TIME	**High Ox G.B.** **Sample # 1&2** **(96% Lean, sell by 5/4/06)**	**CO Packed G.B.** **Sample # 1&2** **(96% Lean, use or freeze by 5/9/06)**
4/27/06 12 P.M.	Fresh beef aroma	Fresh beef aroma
4/27/06 8 P.M.	Slight oxidized odor	Slight off, unpleasant odor
4/28/06 12 A.M.	Slight oxidized odor	Off odor, sulfur comp odor
4/28/06 4 A.M.	Oxidized, acidic or sour odor	Strong sulfur comp odor
4/28/06 8. A.M.	Oxidized, acidic or sour odor	Stronger sulfur comp odor
4/28/06 12 P.M.	Strong oxidized odor	Very strong sulfur comp odor
4/28/06 4 P.M.	Strong oxidized odor	Very strong sulfur comp odor

Table 3A. The microbial profile of ground beef samples abused at 21°C (70°F) 8 hours under fluorescent light & stored at refrigeration temp (4°C, 39.2°F).

TESTING TIME	ANALYSIS	High Ox G.B. Sample # 1 (96% Lean, sell by 5/4/06)	High Ox G.B. Sample # 2 (96% Lean, sell by 5/4/06)	CO Packed G.B. Sample # 1 (93% Lean, use or freeze by 5/9/06)	CO Packed G.B. Sample # 2 (93% Lean, use or freeze by 5/9/06)
4/27/06 12 P.M.	Aerobic Plate Count/gm	1,000	1,600	210,000	N/A
4/27/06 12 P.M.	Anaerobic Plate Count/gm	200	800	200,000	N/A
4/27/06 8 P.M.	Aerobic Plate Count/gm	1,000	2,000	4,000,000	4,000,000
4/27/06 8 P.M.	Anaerobic Plate Count/gm	2,000	2,000	5,600,000	3,800,000
4/28/06 4 P.M.	Aerobic Plate Count/gm	1,000	3,000	9,600,000	6,200,000
4/28/06 4 P.M.	Anaerobic Plate Count/gm	1,000	2,000	8,500,000	7,400,000
4/29/06 4 P.M.	Aerobic Plate Count/gm	4,000	9,000	21,000,000	19,000,000
4/29/06 4 P.M.	Anaerobic Plate Count/gm	1,000	10,000	17,000,000	26,000,000
4/30/06 4 P.M.	Aerobic Plate Count/gm	6,000	8,000	38,000,000	48,000,000
4/30/06 4 P.M.	Anaerobic Plate Count/gm	14,000	26,000	32,000,000	57,000,000
5/1/06 4 P.M.	Aerobic Plate Count/gm	22,000	6,000	32,000,000	19,000,000
5/1/06 4 P.M.	Anaerobic Plate Count/gm	64,000	20,000	34,000,000	32,000,000
5/2/06 4 P.M.	Aerobic Plate Count/gm	11,000	18,000	38,000,000	14,000,000
5/2/06 4 P.M.	Anaerobic Plate Count/gm	69,000	56,000	15,000,000	38,000,000
5/3/06 4 P.M.	Aerobic Plate Count/gm	8,000	7,000	28,000,000	38,000,000
5/3/06 4 P.M.	Anaerobic Plate Count/gm	260,000	310,000	27,000,000	45,000,000
5/4/06 4 P.M.	Aerobic Plate Count/gm	120,000	120,000	21,000,000	21,000,000
5/4/06 4 P.M.	Anaerobic Plate Count/gm	270,000	490,000	23,000,000	23,000,000

* Reference: Official Methods of Analysis of AOAC International, Ch.17, 17th edition, 2000. Bacteriological Analytical Manual By AOAC, 8th edition, 1998 Revision A.

Table 3B. The color (a* value) profile of ground beef samples abused at 21°C (70°F) 8 hours under fluorescent light & stored at refrigeration temp (4°C, 39.2°F).

TESTING TIME	High Ox G.B. Sample # 1&2 (96% Lean, sell by 5/4/06)	CO Packed G.B. Sample # 1&2 (93% Lean, use or freeze by 5/9/06)
4/27/06 12 P.M.	25.53±1.76	26.44±1.04
4/27/06 8 P.M.	19.41±3.34	27.40±2.21
4/28/06 4 P.M.	20.37±0.57	27.09±1.06
4/29/06 4 P.M.	19.32±0.54	26.18±2.27
4/30/06 4 P.M.	19.26±1.77	28.84±0.83
5/1/06 4 P.M.	19.11±3.03	28.32±1.44
5/2/06 4 P.M.	20.18±0.79	29.27±0.54
5/3/06 4 P.M.	19.98±0.59	28.63±0.74
5/4/06 4 P.M.	18.698±1.30	27.734±1.48

Table 3C. The sensory evaluation of ground beef samples abused at 21°C (70°F) 8 hours under fluorescent light & stored at refrigeration temp (4°C, 39.2°F).

TESTING TIME	**High Ox G.B. Sample # 1&2** (96% Lean, sell by 5/4/06)	**CO Packed G.B. Sample # 1&2** (93% Lean, use or freeze by 5/9/06)
4/27/06 12 P.M.	Fresh beef aroma	Fresh beef aroma
4/27/06 8 P.M.	Slight oxidized odor	Slight off odor, very slight sulfur odor
4/28/06 4 P.M.	Slight oxidized odor	Very slight sulfur comp odor
4/29/06 4 P.M.	Slight oxidized odor	Sulfur comp odor
4/30/06 4 P.M.	Slight oxidized odor	Noticeable sulfur comp odor
5/1/06 4 P.M.	Slight oxidized odor	Strong sulfur comp odor
5/2/06 4 P.M.	Slight oxidized odor	Strong sulfur comp odor
5/3/06 4 P.M.	Slight sour, oxidized odor	Very strong sulfur comp odor
5/4/06 4 P.M.	Oxidized odor	Very strong sulfur comp odor

Table 4. Preliminary Survey of Plate Counts of Commercial Ground Beef Samples.

ANALYSIS DATE	WHERE PURCHASED	PURCHASE DATE	SELL BY DATE	DAYS OF SHELF LIFE REMAINING	DANSENSOR O2	DANSENSOR CO2	PERCENT FAT	AEROBIC	ANAEROBIC	PACKAGE	CARBON MONOXIDE
5/9/2006	A	5/8/2006	5/16/2006	7	0.03	17.2	4	25,000	35,000	MAP	YES
5/9/2006	A	5/8/2006	5/16/2006	7	0	19.7	8	18,000	57,000	MAP	YES
5/9/2006	A	5/8/2006	5/17/2006	8	0.001	20.7	8	170,000	540,000	MAP	YES
5/9/2006	A	5/8/2006	5/9/2006	0	0	20.3	25	10,000	110,000	MAP	YES
5/9/2006	A	5/8/2006	5/9/2006	0	0	20.3	25	10,000	150,000	MAP	YES
5/9/2006	A	5/8/2006	5/11/2006	2	0	20.4	25	1,500,000	3,000,000	MAP	YES
5/9/2006	A	5/8/2006	5/16/2006	7	0.44	15.8	15	2,100,000	4,600,000	MAP	YES
5/9/2006	B	5/9/2006	5/18/2006	9	0	20.3	20	15,000	32,000	MAP	YES
5/9/2006	B	5/9/2006	5/20/2006	11	0	19	10	69,000	170,000	MAP	YES
5/9/2006	C	5/9/2006	5/13/2006	4	71.1	19.7	4	1,700	100	MAP	NO
5/9/2006	C	5/9/2006	5/15/2006	6	69.3	18.6	17	1,500	2,000	MAP	NO
5/9/2006	D	5/9/2006	5/10/2006	1	76.8	13.5	4	6,000	15,000	MAP	NO
5/9/2006	D	5/9/2006	5/10/2006	1	76.6	11.8	15	14,000	41,000	MAP	NO
5/9/2006	D	5/9/2006	5/13/2006	4	78.2	11.6	27	1,200,000	1,700,000	MAP	NO
5/9/2006	D	5/9/2006	5/15/2006	6	79.5	11.1	27	7,700	12,000	MAP	NO
5/9/2006	D	5/9/2006	5/16/2006	7	76.7	13.7	4	500	600	MAP	NO
5/9/2006	D	5/9/2006	5/16/2006	7	80	11.4	15	200	400	MAP	NO
5/9/2006	C	5/9/2006	5/10/2006	1	NA	NA	26	7,000	17,000	Overwrap	NO
5/9/2006	E	5/9/2006	5/11/2006	2	NA	NA	10	17,000	19,000	Overwrap	NO
5/9/2006	E	5/9/2006	5/11/2006	2	NA	NA	14	6,000	21,000	Overwrap	NO
5/9/2006	F	5/9/2006	5/11/2006	2	NA	NA		34,000	10,000	Overwrap	NO
5/9/2006	G	5/9/2006	5/9/2006	0	NA	NA	27	54,000	51,000	Paper Wrap	NO
5/9/2006	D	5/9/2006	5/16/2006	7	NA	NA	7	3,900	3,700	Chub	NO
4/27/2006	C	4/27/2006	5/4/2006	7	NA	NA	4	1,300	500	MAP	NO
4/27/2006	A	4/27/2006	5/9/2006	12	NA	NA	4	4,300	6,800	MAP	YES
4/27/2006	A	4/27/2006	5/9/2006	12	NA	NA	7	210,000	200,000	MAP	YES
4/27/2006	A	4/27/2006	5/4/2006	7	NA	NA	7	530,000	3,400,000	MAP	YES
4/27/2006	A	4/27/2006	5/2/2006	5	NA	NA	7	3,900,000	12,000,000	MAP	YES
4/17/2006	A	4/15/2006	4/18/2006	1	NA	NA	8	2,750,000	3,350,000	MAP	YES
4/17/2006	C	4/16/2006	4/23/2006	6	NA	NA	20	1,000	1,000	MAP	NO

* Reference: Official methods of Analysis of AOAC International, Ch 17, 17th edition, 2000. Bacteriological Analytical Manual By AOAC, 8th edition. 1998 Revision

Appendix Two

S & J Laboratories, Inc.

Report To: Dr. Don Berdahl/Mr. Greg Reynhout (Kalsec®, Inc.)

From: James C. Lin, Ph.D. (S & J Laboratories, Inc.)

Date: June 1, 2006

Title: Microbial profiles of commercial packaged ground beef – a comparison of different MAP packaging technologies using replicates.

SUMMARY

Commercially available MAP ground beef packages including high oxygen (Hi-Ox) samples and low oxygen / carbon monoxide (CO-pk) samples were purchased by Kalsec personnel and transported to S&J Labs in coolers and analyzed for aerobic and anaerobic plate count using standard methods. Where possible 5 replicate samples with the same "use or freeze by" or "sell by" date were tested for each commercial sample. The results confirm an earlier study for Kalsec (dated May 15, 2006) which showed high plate counts in commercial CO-pk samples relative to Hi-Ox samples.

MATERIALS & METHODS

Samples representing Hi-Ox and CO-pk packaging forms were purchased and immediately analyzed for plate count. All investigations were carried out on the premises of S&J Laboratories, Inc., Portage, MI. Microbiological analyses, including aerobic plate count and anaerobic plate count, were performed by S & J Laboratories personnel. Oxygen and carbon dioxide levels in the atmospheres of certain packages were measured by Kalsec® personnel using a Dansensor Checkmate 9900.

Micro analysis was conducted under aseptic sampling procedures (USDA Microbiological Guide Book, 3rd ed., 1998). Samples were first physically divided into six portions. Half of the sample (1st, 3rd, & 5th, or 2nd, 4th & 6th portions) was collected and mixed thoroughly inside a sterile bag. Eleven (11) grams of mixed sample was withdrawn into a filtered stomacher bag and 99 mL of sterile phosphate buffer was added to provide a 10-fold dilution. A further dilution protocol was followed to dilute up to 1,000,000 times. Petrifilm® was used in this study (AOAC official methods 990.12).

RESULTS AND DISCUSSION

Various samples of ground beef were purchased by Kalsec® personnel and transported to S&J laboratories in coolers. The samples were analyzed for microbial count the same day they were purchased. The coded identity of the samples and the associated bacteria counts of freshly-purchased ground beef samples in various types of packaging are shown in the attached table. In all cases but one (Samples 51-55), five replicates with the same "use or freeze by" or sell by" dates were purchased and analyzed. Additional instances of very high plate counts in CO-pk meat samples have been found – within the "use or freeze by" dates. The highest plate count seen for the CO-pk samples was 27,000,000 cfu/g, with many samples exceeding 10^5 cfu/g. . No Hi-Ox samples exceeded 13,000 cfu/g, and most were much lower than that. All the samples had a bright red and fresh appearance.

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CONCLUSION

Freshly purchased Hi-Ox and CO-pk ground beef packages show different levels of bacterial contamination. This study supports the conclusion of a preliminary survey of meat available to consumers, that very high microbial plate counts are observed more often in CO-treated meat than in the other forms available.

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Table 1. Plate Counts for Commercially Available Ground Beef Samples

Sample #	Purchase Date	Analysis Date	Sell By Date	% Fat	Location Purchased	CO ?	Head Space %O2/%CO2	Aerobic Plate Count (cfu/g)	Anaerobic Plate Count (cfu/g)
1	5/22/2006	5/22/2006	5/24/2006	10	A	No	80.7/11.4	1,000	2,000
2	5/22/2006	5/22/2006	5/24/2006	10	A	No	81.5/11.6	6,000	13,000
3	5/22/2006	5/22/2006	5/24/2006	10	A	No	67.7/10.6	3,000	3,000
4	5/22/2006	5/22/2006	5/24/2006	10	A	No	82.4/11.7	10,000	3,000
5	5/22/2006	5/22/2006	5/24/2006	10	A	No	80.4/11.7	6,000	6,000
6	5/22/2006	5/22/2006	5/29/2006	20	A	No	84.3/10.3	500	600
7	5/22/2006	5/22/2006	5/29/2006	20	A	No	83.8/10.5	700	800
8	5/22/2006	5/22/2006	5/29/2006	20	A	No	84.1/10.6	900	1,200
9	5/22/2006	5/22/2006	5/29/2006	20	A	No	82.6/10.1	700	300
10	5/22/2006	5/22/2006	5/29/2006	20	A	No	82.7/10.3	900	700
11	5/22/2006	5/22/2006	5/28/2006	4	A	No	84.6/12.3	2,100	700
12	5/22/2006	5/22/2006	5/28/2006	4	A	No	83.2/12.5	3,100	2,600
13	5/22/2006	5/22/2006	5/28/2006	4	A	No	80.9/12.4	2,000	1,400
14	5/22/2006	5/22/2006	5/28/2006	4	A	No	82.9/13.0	1,100	1,800
15	5/22/2006	5/22/2006	5/28/2006	4	A	No	82.8/12.7	2,600	2,700
16	5/23/2006	5/23/2006	6/1/2006	7	B	Yes	0.024/17.4	160,000	290,000
17	5/23/2006	5/23/2006	6/1/2006	7	B	Yes	0.003/18.0	39,000	120,000
18	5/23/2006	5/23/2006	6/1/2006	7	B	Yes	0.001/17.7	220,000	290,000
19	5/23/2006	5/23/2006	6/1/2006	7	B	Yes	0.000/18.0	99,000	180,000
20	5/23/2006	5/23/2006	6/1/2006	7	B	Yes	0.000/18.6	61,000	120,000
21	5/23/2006	5/23/2006	5/31/2006	15	B	Yes	0.000/18.1	12,000	58,000
22	5/23/2006	5/23/2006	5/31/2006	15	B	Yes	0.000/18.5	19,000	28,000
23	5/23/2006	5/23/2006	5/31/2006	15	B	Yes	0.000/19.2	9,000	40,000
24	5/23/2006	5/23/2006	5/31/2006	15	B	Yes	0.000/18.4	11,000	78,000
25	5/23/2006	5/23/2006	5/31/2006	15	B	Yes	0.000/19.0	35,000	48,000
26	5/23/2006	5/23/2006	5/31/2006	4	B	Yes	0.000/19.1	120,000	210,000
27	5/23/2006	5/23/2006	5/31/2006	4	B	Yes	0.000/18.6	130,000	330,000
28	5/23/2006	5/23/2006	5/31/2006	4	B	Yes	0.000/18.8	120,000	330,000
29	5/23/2006	5/23/2006	5/31/2006	4	B	Yes	0.000/18.4	140,000	380,000
30	5/23/2006	5/23/2006	5/31/2006	4	B	Yes	0.000/18.6	180,000	300,000
31	5/23/2006	5/23/2006	5/30/2006	8	B	Yes	0.000/18.4	110,000	220,000
32	5/23/2006	5/23/2006	5/30/2006	8	B	Yes	0.000/19.0	79,000	220,000
33	5/23/2006	5/23/2006	5/30/2006	8	B	Yes	0.000/18.5	70,000	160,000
34	5/23/2006	5/23/2006	5/30/2006	8	B	Yes	0.000/19.4	100,000	190,000
35	5/23/2006	5/23/2006	5/30/2006	8	B	Yes	0.000/20.2	14,000	54,000
36	5/24/2006	5/24/2006	5/29/2006	4	C	No	73.2/17.6	100	200

37	5/24/2006	5/24/2006	5/29/2006	4	C	No	70.0/17.2	100	300
38	5/24/2006	5/24/2006	5/29/2006	4	C	No	71.5/17.3	100	200
39	5/24/2006	5/24/2006	5/29/2006	4	C	No	74.8/17.6	100	200
40	5/24/2006	5/24/2006	5/29/2006	4	C	No	73.9/17.3	300	200
41	5/24/2006	5/24/2006	5/29/2006	20	C	No	78.9/12.0	1,100	1,000
42	5/24/2006	5/24/2006	5/29/2006	20	C	No	77.5/13.1	900	1,000
43	5/24/2006	5/24/2006	5/29/2006	20	C	No	79.1/12.4	500	700
44	5/24/2006	5/24/2006	5/29/2006	20	C	No	80.1/12.5	600	900
45	5/24/2006	5/24/2006	5/29/2006	20	C	No	81.5/12.3	1,200	800
46	5/24/2006	5/24/2006	5/29/2006	17	C	No	79.7/12.6	800	100
47	5/24/2006	5/24/2006	5/29/2006	17	C	No	79.4/12.9	1,000	500
48	5/24/2006	5/24/2006	5/29/2006	17	C	No	80.3/12.9	600	500
49	5/24/2006	5/24/2006	5/29/2006	17	C	No	79.3/13.0	900	800
50	5/24/2006	5/24/2006	5/29/2006	17	C	No	81.0/12.4	500	300
51	5/25/2006	5/25/2006	5/28/2006	10	D	Yes	0.002/26.9	2,900,000	27,000,000
52	5/25/2006	5/25/2006	5/28/2006	10	D	Yes	0.000/22.0	1,400,000	4,600,000
53	5/25/2006	5/25/2006	5/28/2006	10	D	Yes	0.000/21.9	940,000	3,700,000
54	5/25/2006	5/25/2006	5/28/2006	10	D	Yes	0.000/22.6	1,800,000	9,200,000
55	5/25/2006	5/25/2006	6/3/2006	10	D	Yes	0.000/24.1	1,000	1,000
56	5/25/2006	5/25/2006	6/5/2006	20	D	Yes	0.000/22.4	170,000	440,000
57	5/25/2006	5/25/2006	6/5/2006	20	D	Yes	0.000/22.4	72,000	310,000
58	5/25/2006	5/25/2006	6/5/2006	20	D	Yes	0.000/23.2	260,000	400,000
59	5/25/2006	5/25/2006	6/5/2006	20	D	Yes	0.000/22.0	180,000	720,000
60	5/25/2006	5/25/2006	6/5/2006	20	D	Yes	0.000/23.1	120,000	300,000

Appendix Three

S & J
Laboratories, Inc.

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REPORT TO: Mr. Greg Reynhout
Kalsec, Inc.
P.O. Box 50511
Kalamazoo, MI 49005-0511

MICROBIAL PROFILE OF GROUND BEEF: 4°C STORAGE

REPORT DATE: May 8, 2006

SAMPLE RECEIVED: April 27, 2006

P.O. NUMBER: TBD

INVOICE NUMBER: TBD

ANALYSES	Testing Date/Time: April 27, 2006/12:00 p.m.			
	High Ox G.B. Sample # 1	High Ox G.B. Sample # 2	CO Packed G.B. Sample # 1	CO Packed G.B. Sample # 2
Aerobic Plate Count/gm	1,000	1,600	530,000	3,900,000
Anaerobic Plate Count/gm	200	800	3,400,000	12,000,000

ANALYSES	Testing Date/Time: April 29, 2006/4:00 p.m.			
	High Ox G.B. Sample # 1	High Ox G.B. Sample # 2	CO Packed G.B. Sample # 1	CO Packed G.B. Sample # 2
Aerobic Plate Count/gm	1,100	800	41,000,000	480,000
Anaerobic Plate Count/gm	200	500	42,000,000	1,000,000

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MICROBIAL PROFILE OF GROUND BEEF: 4°C STORAGE

REPORT DATE: May 8, 2006

SAMPLE RECEIVED: April 27, 2006

P.O. NUMBER: TBD

INVOICE NUMBER: TBD

ANALYSES	Testing Date/Time: May 1, 2006/4:00 p.m.			
	High Ox G.B. Sample # 1	High Ox G.B. Sample # 2	CO Packed G.B. Sample # 1	CO Packed G.B. Sample # 2
Aerobic Plate Count/gm	3,200	31,000	62,000,000	2,100,000
Anaerobic Plate Count/gm	2,300	28,000	71,000,000	28,000,000

ANALYSES	Testing Date/Time: May 3, 2006/4:00 p.m.			
	High Ox G.B. Sample # 1	High Ox G.B. Sample # 2	CO Packed G.B. Sample # 1	CO Packed G.B. Sample # 2
Aerobic Plate Count/gm	5,000	7,000	39,000,000	2,200,000
Anaerobic Plate Count/gm	5,300	21,000	43,000,000	6,000,000

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Kalamazoo, MI 49005-0511

MICROBIAL PROFILE OF GROUND BEEF: 4°C STORAGE

REPORT DATE: May 8, 2006 **SAMPLE RECEIVED:** April 27, 2006

P.O. NUMBER: TBD **INVOICE NUMBER:** TBD

ANALYSES	Testing Date/Time: May 4, 2006/4:00 p.m.			
	High Ox G.B. Sample # 1	High Ox G.B. Sample # 2	CO Packed G.B. Sample # 1	CO Packed G.B. Sample # 2
Aerobic Plate Count/gm	19,000	24,000	46,000,000	1,800,000
Anaerobic Plate Count/gm	40,000	34,000	44,000,000	4,400,000

* High Ox G.B.= (96% Lean, Sell by 05.04.06); CO Packed G.B. Sample # 1= (93% Lean, Use or Freeze by 05.04.06); CO Packed G.B. Sample # 2= (93% Lean, Use or Freeze by 05.02.06).
* Reference: Official Methods of Analysis of AOAC International, Ch.17, 17th edition, 2000.
Bacteriological Analytical Manual By AOAC, 8th edition, 1998 Revision A.