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PENGEMASAN ATMOSFIR TERMODIFIKASI MENGGUNAKAN KARBON MONOKSIDA PADA STUDI PENYIMPANAN DAGING SAPI

CARBON MONOXIDE MODIFIED ATMOSPHERE PACKAGING BEEF SHELF LIFE STUDIES

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ABSTRACT

The combination of O2, CO2, and low concentrations of CO in CO-MAP has repeatedly been shown to increase the shelf-life of red meat products. Concentrations of CO2 of 30% has been shown to slow microbial growth while O2 preserves the natural color of red meat. The addition of low concentrations of CO (<0.4%) preserves the color stability of the meat while allowing for lower levels of O2 which reduces the oxidative spoilage of the product. Shelf-life extension of 5 to 10 days above traditional MAP has been seen with CO-MAP technologies. The addition of active/smart films such as antimicrobial films and/or the combination of irradiation further extends the shelf-life of red meat. Undetectable levels of E. coli at storage temperatures as high as 10oC at 28 days have been shown with CO-MAP and irradiation.

1. INTRODUCTION

The meat industry uses a variety of approaches to extend the shelf-life and improve the customer experience while at the same time balancing nutritional quality and product safety (Yam *et al.*, 2005). Approaches used include chilled storage, modified atmosphere packaging (MAP)/vacuum packaging (VP), canning, freezing, and preserving (Kerry *et al.*, 2006). The increased interest in minimally processed foods has made MAP an attractive technology (Arvanitoyannis and Stratakos, 2012). MAP packaging involves the use of a package tray wrapped with a barrier film, typically PE, containing the meat with the air in the headspace replaced by a mixture of O_2, CO_2, and CO (La Storia *et al.*, 2012). The replacement of the normal atmosphere with a controlled mixture of gases takes advantage of the anti-microbial properties of CO2 while the O2 prevents anaerobic production of microorganisms such as C. botulinum and increases the development and maintenance of the red color the consumer expects (Gómez and Lorenzo, 2012). CO is the third gas commonly used today in MAP for beef (CO-MAP). The history of CO use is one of misunderstandings and difficult regulatory hurdles. CO was used in Norway since the 1980's but has been discontinued in 2004 due to EU regulations prohibiting the use of CO (Cornforth and Hunt, 2008). It was not until 2002 that the FDA approved CO (at 0.4%) in bulk packaging and 2004 in retail packaging for red meats and ground beef in the US. It is now considered 'Generally Recognized As Safe' (GRAS) (Cornforth and Hunt, 2008). CO binds with the meat pigments (myoglobin and hemoglobin) to preserve the red

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color even in low O_2 packaging (Ramamoorthi *et al.*, 2009a). Meats stored with CO in anaerobic conditions (CO_2, N_2 and CO) will see a significant increase in microbial shelf-life (Hunt *et al.*, 2004). The cutting edge of research and technology now involves the use of active and/or smart packaging in combination with MAP technologies to provide an extended shelf-life with increased visual appeal and greater safeguards against hidden spoilage (Luño *et al.*, 1998).

The use of MAP packaging provides a more attractive product at the retail outlet while extending the shelf-life of red meats and ground beef. These technologies are cost effective for the producer and safe for the consumer. No technology by itself can guarantee the safety of any food product, but the combination of practical regulatory rules and oversight, modern reliable technology, and consumer education has resulted in the safest red meat and ground beef products available to date (Painter *et al.*, 2013).

In this critical review I will argue that the methods used by the respective authors to measure the qualitative and microbial traits of red meat and ground beef, while varied, are sound. Valid statistical analysis of the results reported in the literature support the author's conclusions of increase in shelf-life compared to conventional packaging and the general safety of the MAP technologies. The evidence apparent from the decreasing trends in food-borne illness (Painter *et al.*, 2013) from red meat products refutes arguments that MAP masks spoilage (Lambert *et al.*, 1991) when taken in the context of the full range of product safety regulations and procedures (USDA, 2013).

2. ANALYSIS

A critical review of the papers cited indicate that the use of modified atmosphere packaging is effective at preserving the visual appeal of red meat products while increasing the shelf-life at normal storage temperatures. The addition of CO can further extend the shelf-life and does not mask microbial spoilage. The results from each study reviewed shows a remarkable consistency in the results and the repeatability of the results over an extended period of time.

3. HYPOTHESIS

La Starta (2012) suggests that the combination of anti-microbial films and MAP gases can effectively inhibit the growth of both gram positive (antimicrobial film) and gram negative (MAP) growth as compared to MAP only. Additionally it is asserted that without MAP or antimicrobial films the shelf-life of beef steaks is no more than 4 days. The study looks at samples at 4°C only (La Storia *et al.*, 2012).

D`Agata (2010) asserts that VP and MAP will have different effects on both the qualitative and microbiological characteristics of a loin beef steak. It is claimed that storage times of 7, 14, and 21 days will show significant differences in qualitative and microbiological characteristics of the loin steak as compared to fresh (0 days of storage) steak. The study looks at storage temperatures of 4°C only and develops a mathematical model to predict storage limits and color change characteristics (D'Agata *et al.*, 2010).

Limbo (2010) claims that ground beef (minced beef) is often stored at unsafe temperatures. It is claimed that in southern Europe 30% of refrigerated foods are kept at above 10°C in the retail outlets and home refrigerators. The paper suggests that more than 40 methods have been put forth to measure bacterial spoilage in ground beef but that few studies have measured freshness through a full range of characteristics at varying temperatures (Limbo *et al.*, 2010).

Venturini (2010) asserts that in Brazil the majority of `problems` with beef sold at retail is due to a lack of inspection. It is assumed that the use of CO at concentrations of at least 1% when combined with 24% O^2 and CO^2 at 50% is required to prevent the masking of spoilage. The study aims to look at color, off-odor, and microbiological interrelationships of fresh beef steaks at 21 days storage at 2°C using low levels of CO, 30% CO^2 with and without O^2 (Venturini *et al.*, 2010)

Ramamoorthi (2009) states that E. coli remains a serious threat the health and that a method for the complete control of E. coli remains a goal for the processing and packaging of raw beef. The use of radiation combined with CO-MAP is the most reliable way to eliminate E. coli while preserving the sensory qualities of the meat. The paper claims that radiation is the only reliable way to eliminate bacteria from meat products and that a radiation dose of 1.5 kGy results in a 6-log reduction of E. coli. The maximum allowable radiation dose for uncooked, chilled red meat is 4.5 kGy. The study looks at red meat exposed to 0.5, 1.0, 1.5 and 2.0 kGy of radiation (Cobalt 60) using controls of aerobic storage, CO-MAP compared with irradiated aerobic and irradiated CO-MAP samples (Ramamoorthi *et al.*, 2009a).

Cornforth (2008) assumes that CO-MAP is safe and compares high-oxygen MAP against low-oxygen CO-MAP and explores the environmental

CO exposures under different use cases. No independent testing is done, the paper only sites the work of other researchers. As this is an American Meat Science Association (AMS) founded publication (white paper) and given the assumptions that CO-MAP is safe are not challenged or tested for safety or efficacy the conclusions reached must be considered biased (Cornforth and Hunt, 2008). This is in contrast to other works contributed to by the author (Carpenter *et al.*, 2001; Jayasingh *et al.*, 2001) which are more objective. The fact that the conclusions are for the most part supported by independent research does not justify the publication of an apparently authoritative paper which in reality is a selective review of favorable research supporting AMS goals.

Hunt (2004) asserts that the use of CO-MAP will favorably affect the sensory and microbiological characteristics of beef steaks and ground beef. He states the fact that CO-MAP has been used in Norway successfully in retail packaging but, at the time of publication, was not permitted for retail packaging in the US. The study compares traditional aerobic packaging to CO-MAP at 2 and 6^oC for 35 days before being removed from the CO-MAP packaging and stored at 1^oC (Hunt *et al.*, 2004).

Luño (2000) claims that it is well known that CO_2 in low concentrations extends the shelf-life of beef and that CO concentrations up to 0.5% is safe. The study aims to determine the critical level of CO needed to maintain the desired red color while also evaluating the micro flora at differing CO levels (Luño *et al.*, 2000).

Nissen (2000) states that the use of CO is controversial because of its ability to maintain the expected red coloration of the meat product beyond the microbial shelf-life of the product. It is further stated that the shelf-life based on odor at storage temperatures of 4^oC only is significantly longer. At what is termed abuse temperatures (>8^oC) E. coli and Salmonella spp. is claimed to grow to unsafe levels. The test looks at ground beef stored at 4^oC and 10^oC to verify the assertions (Nissen *et al.*, 2000).

Franco-Abuin (1997) asserts that MAP can increase the shelf-life of raw ground beef from 50-400%. His study compares pure CO_2, 65% CO_2 + O_2 and N_2 and CO_2 20% + O2 for Listeria growth from 0 to 18 days storage (Franco-Abuin *et al.*, 1997).

4. METHODS

Comparing the Materials and Methods used by each of the authors, with the exception of Cornforth whose work I dismiss, to prepare the meat samples, prepare the inoculant (if used), package, store, and test the samples I find no obvious deficiencies. Each author documents the Materials and Methods in detail as summarized:

(D'Agata *et al.*, 2010), (Venturini *et al.*, 2010), (Ramamoorthi *et al.*, 2009a), (Luño *et al.*, 2000) and (Nissen *et al.*, 2000) all prepared there meat samples from purpose slaughtered cattle under controlled conditions. (La Storia *et al.*, 2012), (Limbo *et al.*, 2010), (Hunt *et al.*, 2004) and (Franco-Abuin *et al.*, 1997) obtained their meat from a commercial supplier and then prepared their samples from those products. (Franco-Abuin *et al.*, 1997) tested the samples for listeria prior to inoculation with listeria cultures. None of the other authors pre-tested there samples, they all used control samples to account for any unexpected contamination.

Preparation of the CO-MAP or MAP packaging was done with commercial grade equipment by each of the authors and the gas mixtures measured with standard laboratory equipment.

Preparation of inoculants used by (Nissen *et al.*, 2000) and (Franco-Abuin *et al.*, 1997) were performed independently using cultures produced by each researcher. Each culture was tested before the meat was inoculated. Testing in each paper is done using modern testing equipment.

Each of the authors use valid statistical methods to analysis their results. In each case where the p-value is greater than 0.05 or 0.01 (values falling outside a 90% or 98% confidence interval) the data is marked as such.

5. INNOVATION AND CONTRIBUTION TO THE BODY OF KNOWLEDGE

(Luño *et al.*, 1998, 2000) are cited by the other authors as pioneering research. Each author builds on existing work reinforcing the repeatability of the results and the conclusions that can be drawn from the test results.

(La Storia *et al.*, 2012) and many other un-cited researchers continue to innovate by combining the still emerging active/smart film science with the well proven MAP/CO-MAP packaging (Ramamoorthi *et al.,* 2009b) combine irradiation and CO-MAP so as to produce an extremely extended shelf-life with the natural color preserved.

6. LIMITATIONS

Each of the papers presents results obtained under well controlled laboratory conditions. This approach serves the researcher well when results are compared to carefully chosen control samples. In the real world of food processing, distribution, retailing, and consumer use there are many uncontrolled variables. Inspections can only do so much to ensure adherence to the regulations concerning meat products. Mistakes can be made and greed can outweigh concern for public safety. The packaging technologies and the conclusions drawn from these studies are one tool in a chain of tools that provide a safe food product to the consumer.

7. AUTHORS CONCLUSIONS

La Storia (2012) shows that for the samples in Table 1 the results for related microbial groups are as in Table 2. As can be seen, the total viable counts and the totals for each group at each storage interval is lowest with the combination of the antimicrobial film and 60% O_2 + 40% CO_2 MAP (La Storia *et al*., 2012).

D`Agata (2010) shows results in Table 3. It can be seen that the Vacuum packaging results in the lowest bacteria colony counts but given the positive effect of the MAP packaging on the color (remains stable and similar to fresh meat) the MAP packaging has its advantages (D'Agata *et al*., 2010).

Table 1. Packaging conditions for beef stored at 4 oC for 12 days.

Samples	Condition	
	Initial Atmosphere	Inter-slice film
A	Air	NA
AF	Air	HDPE
AAF	Air	Antimicrobial HDPE
MAP	60% O_2 . 40% CO_2	NA
MAPF	60% O_2 - 40% CO_2	HDPE
MAPAF	60% O_2 - 40% CO_2	Antimicrobial HDPE

HDPE film had the following characteristics:
0.95 g/cm^3 density, PO_2 2650 cm^3/m^2/day/atm at 23°C

Table 2. Viable counts of different spoilage-associated microbial groups in beefsteak during storage at 4°C for 12 days

Log CFU/g±SD at 1 7 and 12 days of storage

nple	Total viable counts (PCA)			B. thermosphacta (STAA)			LAB (MRS agar)			Enterobacteriaceae (VRBGA)			Pseudomonas spp. (CFC)		
	1	7	12	1	7	12	1	7	12	1	7	12	1	7	12
	2.51±1.08a	8.16±0.71ab	7.68±1.07a	1.07±0.41a	6.19±0.72a	6.39±1.37a	1.49±0.60a	4.95±0.29a	4.19±1.01a	<1.00	<1.00	1.89±0.55a	2.16±1.00a	7.85±0.93a	7.21±1.22a
	2.81±0.41a	7.25±0.95a	7.06±0.90a	1.60±0.91a	6.49±0.48a	5.47±1.74a	1.37±0.74a	4.72±0.34a	4.69±1.36a	<1.00	<1.00	2.03±1.11a	1.80±0.80a	6.30±1.92a	7.14±0.60a
F	1.81±0 97a	6.28±1.94b	6.71±0.52a	< 1.00b	3.85±1.06b	4.36±2.79b	<1.00b	4.19±0.22a	4.00±0.36a	<1.00	<1.00	1.77±0.77a	1.72±0.76a	6.33±1.83a	7.77±0.54a
P	2.08±0.76A	4.04±0.79a	4.17±1.22A	1.14±0.13A	3.68±0.31A	3.03±1.51A	1.58±0.76A	3.22±0.92A	4.70±1.89A	<1.00	<1.00	<1.00	1.79±0.91A	3.23±1.57A	1.58±0.47A
PF	2.43±0.24A	4.45±1.42A	4.83±1.99A	1.85±0.90A	3.05±1.01A	3.64±2.93A	1.37±0.39A	3.51±1.38A	4.02±1.89B	<1.00	<1.00	<1.00	1.36±0.63A	2.27±0.46AB	3.05±1.14A
PAF	2.73±0.18A	2.69±0.93A	3.67±1.38B	< 1.00b	< 1.00b	1.11±1.19B	< 1.00b	1.00±0.00B	1.82±0.49B	<1.00	<1.00	<1.00	1.53±0.66A	1.69±0.80B	1.77±0.62A

ne microbial loads at time zero were: PCA: 3.22±0.49; STAA: 1.64±0.61; MRS: 1.83±1.01; VRBGA: b1.00; PSA: 1.66±0.79. Values with different superscripts corresponding to the same time of storage differ significantly 0.05).

Table 3. Viable counts of different spoilage-related microbial groups detected on beef during storage

Log cfu/g ± standard deviation

Storage time (days)		Mesophilic bacteria	Psychrotrophic bacteria	Lactic acid bacteria	Pseudomonas spp	B. thermosphacta	Enterobacteriaceae
resh	0	3 66 ± 0 24e	3 38 ± 0 65d	2 02 ± 0 60f	2 56 ± 0 55f	2 05 ± 0 59d	2 55 ± 0 52d
'P	7	7 88 ± 0 39b	7 97 ± 0 37b	4 16 ± 0 72e	7 67 ± 0 67b	5 86 ± 0 90b	5 26 ± 0 74b
	14	9 24 ± 0 46a	8 46 ± 0 44ab	4 82 ± 0 54cde	9 31 ± 0 78a	7 73 ± 0 97a	6 15 ± 0 74a
	21	9 64 ± 0 38a	9 19 ± 0 40a	5 00 ± 0 41cde	9 93 ± 0 62a	7 97 ± 0 83a	6 86 ± 0 51a
'P	7	5 62 ± 0 85d	5 60 ± 0 88c	4 40 ± 1 15de	4 87 ± 0 74e	4 02 ± 0 80c	4 08 ± 0 44c
	14	6 66 ± 0 88c	6 41 ± 0 96c	5 78 ± 0 71bc	5 79 ± 0 77de	5 66 ± 0 90b	4 75 ± 0 52bc
	21	7 49 ± 0 49bc	6 29 ± 1 13c	7 29 ± 0 61a	6 03 ± 1 12d	5 73 ± 0 26b	4 88 ± 0 41bc
IAP	7	6 63 ± 1 24c	6 39 ± 1 10c	4 05 ± 1 19e	6 17 ± 1 06cd	4 31 ± 0 47c	5 23 ± 0 76b
	14	7 50 ± 0 99bc	7 54 ± 1 10b	5 27 ± 0 78cd	7 29 ± 1 20bc	7 12 ± 0 99a	6 14 ± 0 74a
	21	8 26 ± 0 61b	8 30 ± 0 48ab	6 37 ± 0 35ab	8 03 ± 0 67b	7 59 ± 0 72a	6 24 ± 0 69a

leans with different letters in a column differ significantly (P < 0.05).

'P, polyvinyl chloride packaging; VP, under vacuum packaging; MAP, modified atmosphere packaging.

Ramamoorthi (2004) gets the results in Table 5 and concludes that the combination of irradiation at 1.5 and 2.0 kGy in combination can keep total coliforms below detectable levels for 28 days and preserve the natural red color for 21 days at the storage temperatures of 2-4^{o}C (Ramamoorthi *et al*., 2009a)

Hunt (2004) concludes that although MAP and CO-MAP produce extended color stability beyond the microbial shelf-life of the product that the color stability is not sufficient to mask the spoilage indicated by off-odor (Hunt et al., 2004).

Luño (2000) concludes that CO-MAP containing 50% CO_2 and 0.5 to 0.75% CO in the presence of O2 at 24% can extend the shelf-life of beef steaks by 5 to 10 days at 1 +/- 1^{o}C when compared to the shelf-life of MAP at 70% O_2 and 20% CO_2 (Luño et al., 2000).

Nissin (2000) concludes that at high storage temperatures E. coli growth was almost entirely inhibited by high CO_2/low CO mixtures. The results for other pathogens such as listeria (as inoculated) is mixed and the author emphasizes the requirement for proper control of storage temperatures (Nissen *et al*., 2000).

Franco-Abuin (1997) concludes that a CO2 concentration was required to inhibit the growth of L. monocytogenes and L. innocua (Franco-Abuin *et al*., 1997).

Table 4. Plate count (log CFU/g) from gluteus medius (GM) and longissimus dorsi (LD) before and after MAP storage at 2±1°C

atment	T0	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
e (days)	0	7				14				21			
S. aureus	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.3	2.8	<2.0	2.5	2.4	2.3	2.9
Enterobacteria	<1.0	2.2	1.5	2.8	2.2	3.3	4.4	2.9	4.8	4.6	4.9	5.3	7.3
B. thermosphacta	<2.0	<2.0	<2.0	2.8	2.3	4.1	4.4	4.9	5.0	4.5	5.0	5.1	7.2
Lactic acid bacteria	<1.0	1.4	1.5	1.7	<1.0	3.8	2.5	3.6	3.8	4.7	4.5	3.9	5.2
Psy aerobic	3.8	3.7	4.3	4.4	4.2	6.2	5.4	7.0	5.7	6.2	6.3	6.5	9.7
Psy anaerobic	3.4	3.5	3.3	3.1	3.2	5.5	5.5	7.0	5.8	6.2	6.6	6.5	9.9
S. aureus	<2.0	2.5	2.4	<2.0	<2.0	3.3	<2.0	<2.0	3.0	<2.0	2.3	<2.0	2.5
Enterobacteria	<1.0	2.2	3.2	1.7	2.9	5.2	4.8	4.0	5.2	5.0	5.7	5.2	5.7
B. thermosphacta	<2.0	1.3	1.4	2.4	3.1	4.6	6.6	4.3	3.8	5.4	4.8	5.3	3.9
Lactic acid bacteria	<1.0	2.2	2.1	1.3	<1.0	4.0	4.2	3.5	3.6	5.4	4.8	4.2	4.9
Psy aerobic	3.8	5.6	4.3	5.2	4.2	5.8	6.7	6.8	4.9	8.3	6.3	7.9	6.5
Psy anaerobic	3.3	3.5	4.4	4.2	4.3	5.8	6.7	6.9	4.9	8.2	6.4	7.9	6.6

Table 5. Effect of gas atmosphere and irradiation on total coliforms (log10 CFU/g) of fresh beef during storage

ge	Gas Packaging									
	CO-MAP Irradiation dose (kGy)					Aerobic Irradiation dose (kGy)				
	0	0 5	1 0	1 5	2 0	0	0 5	1 0	1 5	2 0
	5 20 ± 0.23a	3 51 ± 0.85ab	1 55 ± 1.09c	nd	nd	5 56 ± 0.35a	3 97 ± 0.22ab	2 02 ± 0.02b	nd	nd
	5 30 ± 0.30a	3 93 ± 0.14a	2 10 ± 0.47b	nd	nd	5 12 ± 0.35a	3 72 ± 0.28ab	2 20 ± 0.84b	nd	nd
	5 02 ± 0.05a	3 04 ± 0.33ab	2 56 ± 0.23ab	nd	nd	4 39 ± 0.28a	3 56 ± 0.20ab	2 62 ± 0.22b	nd	nd
	4 45 ± 0.24a	3 36 ± 0.25ab	2 00 ± 0.36b	nd	nd	4 16 ± 0.17a	3 28 ± 0.31ab	2 08 ± 0.68b	nd	nd
	4 35 ± 0.07a	3 04 ± 0.29ab	1 43 ± 0.50c	nd	nd	4 11 ± 0.22a	3 06 ± 0.17ab	1 67 ± 0.93c	nd	nd

8. CONCLUSIONS

The consistency of the results for all of the studies reviewed and the repeatability of the results over time (1997-2012) reinforces the safety and the effectiveness of MAP. The use of CO-MAP is also shown to have a positive effect on the color of the meat while not masking spoilage at extended storage times or high temperatures.

The importance of these findings cannot be understated. MAP and CO-MAP in combination with practical regulations and sensible handling of the meat products by the consumer add up to a reduced incidence of food borne illness from red meat products. The authoritative data provided by the CDC confirm that red meat products have never been safer and the trend of contamination continues to decrease (Painter *et al*., 2013)

The encouraging findings related to the combination of MAP/CO-MAP and irradiation and/or active/smart films shows exciting promise for even safer products in the near future.

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