http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 14 (3): 236-240, 2011
ISSN 1028-8880 / DOI: 10.3923/pjbs.2011.236.240

Effect of Ozone and Potassium Lactate on Lipid Oxidation and Survival of *Salmonella typhimurium* on Fresh Pork

T. Piachin and N. Trachoo
Foodborne Pathogens and Biofilm Research Laboratory, Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Muang District, Maha Sarakham, 44000, Thailand

Abstract: The objective of this research was to evaluate the effect of ozone gas and potassium lactate on lipid oxidation and survival of *Salmonella typhimurium* on fresh pork. A total of 144 samples of fresh pork samples were cut into pieces approximately (8×5×0.6) cm in size, then (0, 2 and 4%) potassium lactate (KL), inoculated with *S. typhimurium* was applied by spreading a 0.5 mL cell suspension over each sample. The pork samples were then packed in (6"×8") airtight polyethylene bags with a thickness of 87.5 μm and a volume of 3.5 L with and without ozone. Ozone gas was injected into the plastic bags at a rate of 0, 200, 500 and 1,000 mg h^{-1} and the samples later stored at 8°C. Thio-barbituric Acid Reactive Substance (TBARS) and microbial loads were determined on days 0, 5, 10 and 15. Data was statistically analyzed using SPSS software and differences among means detected at the 0.5% confident level using the Scheffe's test. Samples treated with 2 and 4% KL had significantly ($p<0.01$) lower TBARS value than non-treated samples. Combination of ozone and KL showed inhibitory effects on *S. typhimurium* in samples. *S. typhimurium* was sensitive to 4% KL with and without ozone. At 1,000 mg h^{-1}, ozone improved KL inhibitory effect on *S. typhimurium*. Ozone and KL are potential substances for inhibition of *S. typhimurium*.

Key words: Ozone, potassium lactate, *S. typhimurium*, fresh pork, lipid oxidation

INTRODUCTION

Fresh pork is a source of protein often contaminated with pathogen microorganisms such as *Salmonella* sp. and *Listeria monocytogenes*. Meat and meat products contaminated with these pathogens can create a considerable hazard for public health. *Salmonella* is a common contaminant of raw pork (Escartin *et al*., 2000). *S. typhimurium* was the most frequently isolated serotype (Korsak *et al*., 2003). Survival of *Salmonella* depended on environmental conditions including pH, temperature and relative humidity (Soliman *et al*., 2009). In an attempt to eliminate the pathogens from carcasses, various applications such as washing and sanitizing with chilled water, hot water, chlorinated water, steam, steam vacuuming, organic acids and salt alone and in combination have been developed and used. Another more recently proposed treatment involves the utilization of ozone, either as a washing tool or as a gaseous treatment (Rice, 2002). Ozone is one of the most powerful oxidants that leaves no detectable residues in or on treated produce and is a powerful antimicrobial agent (Mudway and Kelly, 2000). The ozone has since received full U.S. FDA approval as a direct contact food-sanitizing agent (U.S. FDA, 2001). Stivarius *et al.* (2002) reported that 1% ozonated water decreased *S. typhimurium* by 0.78 log CFU g^{-1}. Williams *et al.* (2004) reported that ozone and treatment temperature reduced *S. typhimurium* in apple cider and orange juice. This has raised commercial interests in the deployment of ozone for various applications in the food industry such as food surface hygiene, sanitation of food plant equipment, treatment and lowering BOD and COD of food plant waste. The bactericidal effects of ozone on a wide variety of organisms, including Gram positive and negative bacteria as well as spores and vegetative cells have been documented (Guzel-Seydim *et al*., 2004).

Potassium lactates are already widely used to extend the shelf life and increase the safety of meat and poultry products (Aran, 2001; Knock *et al*., 2006; Quilo *et al.,* 2010). Quilo *et al.* (2010) reported that 200 ppm peroxyacetic acid followed with 3% potassium lactate reduced *Salmonella* on ground beef and Kim *et al.* (2010) reported that lactate with phosphate enhancement had

Corresponding Author: Nathanon Trachoo, Foodborne Pathogen Research Laboratory, Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Muang District, Maha Sarakham, 44000, Thailand

beneficial effects on color and lipid oxidation stability of beef cuts under high oxygen conditions. The US Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) approved use of potassium salts (up to 4.8% w/w) in meat products as an antimicrobial ingredient to control pathogens (USDA FSIS, 2000). The aim of this research was to study the effect of ozone gas and potassium lactate on lipid oxidation and survival of *Salmonella typhimurium* on fresh pork.

MATERIALS AND METHODS

Raw pork samples were purchased from a local vendor in Maha Sarakham, Thailand during January 2009 to March 2010. Ozone gas was generated from a laboratory corona discharge ozone generator (HGOZ-1000, Enaly M and E, China) using oxygen gas as a substrate, with a working voltage of 220 volt, 50 Hz. *Salmonella typhimurium* was obtained from a stock culture of the Foodborne Pathogens and Biofilm Research Laboratory (FBRL), Mahasarakham University, Thailand.

Bacterial strain: *S. typhimurium* was activated in a tryptic soy broth (Criterion, Santa Maria, USA) and grown in a tryptic soy agar at 37°C for 24 h. Cell suspension was prepared by adding 0.1% sterile peptone water onto culture plates, which was further diluted with 0.1% sterile peptone water to obtain a cell density of 9 Log CFU mL^{-1}.

Inoculation and treatments: Samples of fresh pork were cut into pieces approximately (8×5×0.6) cm in size, then treated with 0, 2 and 4% potassium lactate (KL), (Fluka, Netherlands) by dropping 0.5 mL on each side and inoculated with 0.5 mL *S. typhimurium* suspension on both side by spreading with sterile brush. The samples were then packed in customized airtight polyethylene plastic bags (6"×8") in size with a thickness of 87.5 μm. Ozone gas was injected into the plastic bags at a rate of 0, 200, 500 and 1,000 mg h^{-1}. Controls were uninoculated samples. Total of 144 samples (48 pieces/replicates) were stored at 8°C. The analyses for survival of *S. typhimurium* and Thiobarbituric Acid Reactive Substance (TBARS) were determined on days 0, 5, 10 and 15.

Survival study: Twenty five grams of fresh pork samples were aseptically placed in a sterile stomacher bag containing 225 mL of sterile 0.1% peptone water. Samples were stomached for 2 min. Serial dilution was prepared using 0.1% peptone water and plated in duplicate on Xylose Lysine Desoxycholate agar (XLD) (Criterion, U.S.A). The numbers of colonies were counted after incubation at 37°C for 24 h.

Evaluation of lipid oxidation Secondary lipid oxidation products were evaluated using 2-thiobarbituric Acid (TBA) as described by Vyncke (1970, 1975) and with modifications by Sorensen and Jorgensen (1995). Briefly, 5 g of meat was stomached in 15 mL of 7.5% Trichloroacetic Acid (TCA) with 0.1% propylgallate and 0.1% ethylenediaminetetraacetic acid (EDTA) for 5 min at room temperature and filtered. Filtrate 5 mL was mixed with 5 mL of 0.02 M Thiobarbituric Acid (TBA) and incubated at 100°C in a water bath for 40 min. Absorbance was measured at 532 nm (PU8625 UV/VIS Spectrophotometer, Phillips, Cambridge and England). Results are expressed as 2-thiobarbituric Reactive Substances (TBARS), namely malonaldehyde (mg kg^{-1} dry matter) using a standard curve prepared from 1,1,3,3-tetraethoxypropane (TEP). Mean values of two independent determinations were obtained.

Statistical analysis: A 4×3 factorial design in a randomized completed block design with three replications was used. The same culture, chemical stock and equipment were used in all replicates. The main effects were ozone injection rate (0, 200, 500 and 1,000 mg h^{-1}) and KL concentration (0, 2 and 4%). Data was analyzed with SPSS for windows software (Version 12.0, SPSS Inc, U.S.A.). Significant differences between means were determined using the Scheffe's method (Norusis, 1990). Significance was determined by least square means at $p = 0.05$.

RESULTS AND DISCUSSION

Survival of *S. typhimurium*: With the initial microbial load of 8.0 Log $CFUg^{-1}$ on day zero, *S. typhimurium* decreased by 1 Log CFU g^{-1} ($p<0.01$) after treated with 2% KL or more with and without ozone (Table 1). Stivarius *et al.* (2002) observed similar results, reporting beef trimmings inoculated with *S. typhimurium* then treated with either 1% ozonated water for 7 min (7O) or 15 min (15O). The 15O treatment reduced *S. typhimurium* 0.78 Log CFU g^{-1} ($p<0.05$), respectively in ground beef. However, Castillo *et al.* (2003) reported that the aqueous ozone treatment had no significant improvement over a water wash in reducing *S. typhimurium* and *Escherichia coli* O157:H7 on beef carcass surfaces. In this study, ozone and KL showed inhibitory effects on *S. typhimurium* in samples. *S. typhimurium* was sensitive to 4% KL with and without ozone. At 1,000 mg h^{-1}, ozone improved KL inhibitory effect on *S. typhimurium*. It is postulated that the primary attack of ozone was on the cell wall or membrane of the bacteria, probably by reaction with the double bonds of lipids and

Table 1: Survival of *S. typhimurium* at 8°C during 15 days of storage after treatment of ozone and potassium lactate

		S. typhimurium population (log cfu g^{-1})			
Ozone (mg h^{-1} r)	KL[1] (%)	Day 0	Day 5	Day 10	Day 15
0	0	8.0 ± 0.06^{bB}	7.3 ± 0.18^{eA}	7.4 ± 0.05^{eA}	7.6 ± 0.07^{dA}
0	2	7.3 ± 0.01^{aB}	7.0 ± 0.07^{bcA}	7.1 ± 0.01^{cdA}	7.1 ± 0.02^{bcA}
0	4	7.0 ± 0.03^{aC}	6.6 ± 0.03^{aA}	6.8 ± 0.05^{aB}	6.9 ± 0.02^{aBC}
200	0	8.1 ± 0.02^{bC}	7.2 ± 0.04^{cA}	7.3 ± 0.01^{deA}	7.4 ± 0.02^{dB}
200	2	7.2 ± 0.03^{aB}	7.0 ± 0.06^{cA}	7.1 ± 0.03^{cdAB}	7.1 ± 0.03^{cAB}
200	4	7.0 ± 0.03^{aB}	6.6 ± 0.05^{aA}	6.9 ± 0.03^{abB}	6.9 ± 0.06^{abB}
500	0	8.0 ± 0.08^{bB}	7.0 ± 0.08^{cA}	7.1 ± 0.01^{cdA}	7.1 ± 0.02^{cA}
500	2	7.2 ± 0.05^{aA}	7.0 ± 0.07^{cA}	7.1 ± 0.05^{bcA}	7.0 ± 0.04^{abcA}
500	4	7.0 ± 0.06^{aB}	6.6 ± 0.04^{aA}	6.8 ± 0.06^{aAB}	6.9 ± 0.06^{aB}
1.000	0	7.1 ± 0.07^{aC}	6.7 ± 0.04^{abA}	6.8 ± 0.03^{aAB}	6.9 ± 0.05^{aB}
1.000	2	7.1 ± 0.10^{aC}	6.5 ± 0.06^{aA}	6.9 ± 0.02^{aBC}	6.8 ± 0.02^{aB}
1.000	4	7.0 ± 0.15^{aB}	6.5 ± 0.05^{aA}	6.8 ± 0.04^{aAB}	6.8 ± 0.04^{aAB1}

KL: Potassium lactate $^{a-c}$Means with common superscript in the same column are not significantly different ($p>0.01$); $^{A-C}$Means with common superscript in the same row are not significantly different ($p>0.01$)

Table 2: Lipid oxidation as determined by malonaldehyde at 8°C during 15 days of storage after treatment of ozone and potassium lactate

		TBARS[1] as malonaldehyde (mg Kg^{-1})			
Ozone (mg h^{-1} r)	KL (%)	Day 0	Day 5	Day 10	Day 15
0	0	0.34 ± 0.01^{nsA}	0.42 ± 0.01^{bB}	0.67 ± 0.01^{cdC}	1.05 ± 0.01^{bD}
0	2	0.34 ± 0.01^{A}	0.38 ± 0.01^{aB}	0.62 ± 0.01^{abC}	0.98 ± 0.01^{aD}
0	4	0.33 ± 0.01^{A}	0.38 ± 0.00^{aA}	0.60 ± 0.04^{aB}	0.98 ± 0.01^{aC}
200	0	0.36 ± 0.00^{A}	0.44 ± 0.01^{bB}	0.73 ± 0.00^{dC}	1.14 ± 0.01^{cD}
200	2	0.34 ± 0.01^{A}	0.39 ± 0.01^{aB}	0.62 ± 0.01^{abcC}	0.98 ± 0.01^{aD}
200	4	0.33 ± 0.01^{A}	0.38 ± 0.01^{aB}	0.62 ± 0.00^{abcC}	0.98 ± 0.01^{aD}
500	0	0.35 ± 0.01^{A}	0.43 ± 0.01^{bB}	0.66 ± 0.00^{bcC}	1.03 ± 0.01^{bD}
500	2	0.34 ± 0.01^{A}	0.38 ± 0.01^{aB}	0.62 ± 0.00^{abcC}	0.98 ± 0.01^{aD}
500	4	0.34 ± 0.00^{A}	0.37 ± 0.00^{aB}	0.62 ± 0.00^{abcC}	0.98 ± 0.01^{aD}
1.000	0	0.34 ± 0.01^{A}	0.38 ± 0.01^{aB}	0.62 ± 0.00^{abcC}	0.99 ± 0.01^{aD}
1.000	2	0.33 ± 0.01^{A}	0.37 ± 0.01^{aB}	0.62 ± 0.00^{abC}	0.98 ± 0.01^{aD}
1.000	4	0.331 ± 0.01^{A}	0.37 ± 0.01^{aB}	0.62 ± 0.01^{abC}	0.98 ± 0.01^{aD1}

TBARS: 2-thiobarbituric reactive substances; $^{a-c}$Means with no common superscript in the same column differ at ($p<0.01$); $^{A-D}$Means with no common superscript in the same row differ at ($p<0.01$); ^{ns} Means in this column values are not significantly different at ($p>0.01$)

that leakage orlysis of the cells depended on the extent of that reaction (Scott and Lesher, 1963). It is possible that the antimicrobial effect of KL may be due to the lactate anions attack on cell wall or membrane leading to malfunction of enzyme activity (Reddy *et al*., 2007). Reddy *et al.* (2007) observed that in addition of 1% calcium lactate or 2% of potassium and sodium lactate to injected pork was able to control *Clostridium perfringens* germination and outgrowth to <1 log CFU g^{-1}. Shelef and Potluri (1995) studied the effect of different lactates on the listeriostatic activity in cooked liver sausage and found that Calcium Lactate (CaL) was consistently more inhibitory than sodium lactate (NaL). They also observed that growth of *S. typhimurium* was abated in sausage by 3% sodium or calcium lactate and indicated that the antibacterial activity could be due to lactate rather than Ca or Na ions. Maas *et al.* (1989) studied the effect of lactate and sodium ion on botulinum toxin formation and noted that lactate concentration, rather than sodium ion concentration was the antibotulinal factor.

Lipid oxidation: Resultant thiobarbituric acid reactive substance (TBARS) value (Table 2) was not significant on day 0 but decreased ($p<0.01$) significantly on day 5 in samples using 2% KL or more with and without ozone and the same on days 10-15. Sample treated with 200 mg h^{-1} ozone without KL had the highest TBARS value on every day of storage. Samples treated with 2 and 4% KL had significantly ($p<0.01$) lower TBARS value than untreated samples. Similar results were observed by Quilo *et al.* (2009) who reported the effect of antimicrobial treatment on pH and lipid oxidation on ground beef. In that study, the control had the highest ($p<0.05$) lipid oxidation compared to samples treated with 3% potassium lactate, sodium metasilicate, 200 ppm peroxyacetic acid, 1000 ppm acidified sodium chlorite on all days of display. Mancini *et al.* (2010) observed the effects of lactate and modified atmospheric packaging on premature browning in cooked ground beef patties. The data showed lactate decreased lipid oxidation in ground beef patties in all packaging types. Nnanna *et al.* (1994) reported similar results where sodium lactate was effective in controlling lipid oxidation in ground pork and in a model system containing methyl linoleate, Tween-20, ascorbate and ferric chloride. In addition, the antioxidant activity of lactate also has been reported in pork sausage, chicken

sausage and chicken patties (Bloukas *et al*., 1997; Choi and Chin, 2003; Naveena *et al*., 2006; Cegielska-Radziejewska and Pikul, 2004). Lactate's role in minimizing lipid oxidation may be due to its ability to scavenge superoxide (O_2^-) and Hydroxyl radicals (OH^-) (Groussard *et al*., 2000). Ozonated samples were higher in TBARS than control and lactate treated samples because ozonation increased oxygen on samples. Similarly, further high-oxygen packaging systems provide favorable environments for oxidation to occur (Jackson *et al*., 1992; Taylor *et al*., 1990). Seyfert *et al*. (2005) reported that high-oxygen atmospheres increased TBARS values throughout storage and display.

CONCLUSIONS

Ozone and KL showed inhibitory effects on *S. typhimurium* in samples. *S. typhimurium* was sensitive to 4% KL with and without ozone. At 1,000 mg h^{-1}, ozone can improve KL inhibitory effect on *S. typhimurium*. Potassium lactate retarded lipid oxidation. Ozone and KL are potential substances for inhibition of *S. typhimurium*.

ACKNOWLEDGMENTS

This research was financially supported by Thailand Research Fund Master Research Grants (Grant No. MRG-WI515S093).

REFERENCES

Aran, N., 2001. The effect of calcium and sodium lactates on growth from spores of *Bacillus cereus* and *Clostridium perfringens* in a sous-vide beef goulash under temperature abuse. Int. J. Food Microbiol., 63: 117-123.

Bloukas, J.G., E.D. Paneras and G.C. Fournitzis, 1997. Sodium lactate and protective culture effects on quality characteristics and shelf-life of low-fat frankfurters produced with olive oil. Meat Sci., 45: 223-238.

Castillo, A., K.S. McKenzie, L.M. Lucia and G.R. Acuff, 2003. Ozone treatment for reduction of *Escherichia coli* O157:H7 and *Salmonella* serotype typhimurium on beef carcass surfaces. J. Food Protect., 66: 775-779.

Cegielska-Radziejewska, R. and J. Pikul, 2004. Sodium lactate addition on the quality and shelf life of refrigerated sliced poultry sausage packaged in air or nitrogen atmosphere. J. Food Prot., 67: 601-606.

Choi, S.H. and K.B. Chin, 2003. Evaluation of sodium lactate as a replacement for conventional chemical preservatives in comminuted sausages inoculated with *Listeria monocytogenes*. Meat Sci., 65: 531-537.

Escartin, E.F., J.S. Lozano and O.R. Garcia, 2000. Quantitative survival of native *Salmonella serovars* during storage of frozen raw pork. Int. J. Food Microbiol., 54: 19-25.

Groussard, C., I. Morel, M. Chevanne, M. Monnier, J. Cillard and A. Delamarche, 2000. Free radical scavenging and antioxidant effects of lactate ion: An *in vitro* study. J. Applied Physiol., 89: 169-175.

Guzel-Seydim, Z.B., A.K. Greeneb and A.C. Seydim, 2004. Use of ozone in the food industry. Lebensm. Wiss. Technol., 37: 453-460.

Jackson, T.C., G.R. Acuff, C. Vanderzant, T.R. Sharp and J.W. Savell, 1992. Identification and evaluation of the volatile compounds of vacuum and modified atmosphere packaged beef striploins. Meat Sci., 31: 175-190.

Kim, Y.H., E. Huff-Lonergan, J.G. Sebranek and S.M. Lonergan, 2010. Effects of lactate/phosphate injection enhancement on oxidation stability and protein degradation in early postmortem beef cuts packaged in high oxygen modified atmosphere. Meat Sci., 86: 852-858.

Knock, R.C., M. Seyfert, M.C. Hunt, M.E. Dikeman and R.A. Mancini *et al*., 2006. Effects of potassium lactate, sodium chloride and sodium acetate on surface shininess/gloss and sensory properties of injection-enhanced beef strip-loin steaks. Meat Sci., 74: 319-326.

Korsak, N., B. Jacob, B. Groven, G. Etienne, B. China, Y. Ghafir and G. Daube, 2003. *Salmonella* contamination of pigs and pork in an integrated pig production system. J. Food Prot., 66: 1126-1133.

Maas, M.R., K.A. Glass and M.P. Doyle, 1989. Sodium lactate delays toxin production by *Clostridium botulinum* in cook-in-bag Turkey products. Applied Environ. Microbial., 55: 2226-2229.

Mancini, R.A., R. Ramanathan, S.P. Suman, M.K.R. Konda and P. Joseph *et al*., 2010. Effects of lactate and modified atmospheric packaging on premature browning in cooked ground beef patties. Meat Sci., 85: 339-346.

Mudway, I.S. and F.J. Kelly, 2000. Ozone and the lung: A sensitive issue. Mol. Aspects Med., 21: 1-48.

Naveena, B.M., A.R. Sen, M. Muthukumar, S. Vaithiyanathan and Y. Babji, 2006. The effect of lactates on the quality of microwave-cooked chicken patties during storage. J. Food Sci., 71: S603-S608.

Nnanna, I.A., D.O. Ukuku, K.B. McVann and L.A. Shelef, 1994. Antioxidant activity of sodium lactate in meat and model systems. Lebensm. Wiss. Technol., 27: 78-85.

Norusis, M.J., 1990. SPSS Guide to Data Analysis. 4th Edn., SPSS Inc., Chicago.

Quilo, S.A., F.W. Pohlman, A.H. Brown, P.G. Crandall, P.N. Dias-Morse, R.T. Baublits and J.L. Aparicio, 2009. Effects of potassium lactate, sodium metasilicate, peroxyacetic acid and acidified sodium chlorite on physical, chemical and sensory properties of ground beef patties. Meat Sci., 82: 44-52.

Quilo, S.A., F.W. Pohlman, P.N. Dias-Morse, A.H. Jr. Brown, P.G. Crandall and R.P. Story, 2010. Microbial, instrumental color and sensory characteristics of inoculated ground beef produced using potassium lactate, sodium metasilicate or peroxyacetic acid as multiple antimicrobial interventions. Meat Sci., 84: 470-476.

Reddy, V.P., L. Rajagopal, V. Juneja and H. Thippareddi, 2007. Use of calcium, potassium and sodium lactates to control germination and outgrowth of *Clostridium perfringens* spores during chilling of injected pork. Food Microbiol., 24: 687-694.

Rice, R.G., 2002. Century 21-Pregnant with ozone. Ozone Sci. Eng., 24: 1-15.

Scott, D.B.M.N. and E.C. Lesher, 1963. Effect of ozone on survival and permeability of *Escherichia coli*. J. Bacteriol., 85: 567-576.

Seyfert, M., M.C. Hunt, R.A. Mancini, K.A. Hachmeister, D.H. Kropf, J.A. Unruh and T.M. Loughin, 2005. Beef quadriceps hot boning and modified-atmosphere packaging influence properties of injection-enhanced beef round muscles. J. Anim. Sci., 83: 686-693.

Shelef, L.A. and V. Potluri, 1995. Behavior of food borne pathogens in cooked liver sausage containing lactates. Food Microbiol., 12: 221-227.

Soliman, E.S., E.G. Taha, M.A.A. Sobieh and P.G. Reddy, 2009. The Influence of ambient environmental conditions on the survival of *Salmonella enteric* serovar typhimurium in poultry litter. Int. J. Poult. Sci., 8: 848-852.

Sorensen, G. and S.S. Jorgensen, 1995. A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. Zeitschrift Lebensmittel Untersuchung Forschung A, 202: 205-210.

Stivarius, M.R., F.W. Pohlman, K.S. McElyea and J.K. Apple, 2002. Microbial instrumental color and sensory color and odor characteristics of ground beef produced from beef trimmings treated with ozone or chlorine dioxide. Meat Sci., 60: 299-305.

Taylor, A.A., N.F. Down and B.G. Shaw, 1990. A comparison of modified atmosphere and vacuum skin packing for the storage of red meats. Int. J. Food Sci. Technol., 25: 98-109.

U.S. FDA, 2001. Secondary direct food additives permitted in food for human consumption. Federal Register, 66: 33829-33830.

USDA FSIS, 2000. Food additives for use in meat and poultry products: sodium diacetate, sodium acetate, sodium lactate and potassium lactate: Direct final rule. Federal Register, 65: 3121-3123.

Vyncke, W., 1970. Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. Fette Seifen Anstrichmittel, 72: 1084-1087.

Vyncke, W., 1975. Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in Mackerel. Fette Seifen Anstrichmittel, 77: 239-240.

Williams, R.C., S.S. Sumner and D.A. Golden, 2004. Survival of *Escherichia coli* O157:H7 and *Salmonella* in apple cider and orange juice as affected by ozone and treatment temperature. J. Food Prot., 67: 2381-2386.