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Comparative Effects of Sodium Levulinate and Sodium Lactate on Microbial Growth, Color, and Thiobarbituric Acid (TBA) Values of Fresh Pork and Turkey Sausages During Storage

Mihir N. Vasavada
Utah State University

COMPARATIVE EFFECTS OF SODIUM LEVULINATE AND SODIUM LACTATE ON MICROBIAL GROWTH, COLOR, AND THIOBARBITURIC ACID (TBA) VALUES OF FRESH PORK AND TURKEY SAUSAGES DURING STORAGE

by

Mihir N. Vasavada

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ABSTRACT

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Mihir N. Vasavada, Master of Science

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Major Professor: Dr. Charles E. Carpenter
Department: Nutrition and Food Sciences

This study compared the effects of 1.4 or 2.7% sodium levulinate or sodium lactate on aerobic plate count (APC), color, pH, and TBA value of fresh pork and turkey sausage. Both sodium lactate and levulinate inhibited growth of aerobic microorganisms during storage, compared to controls. Bacteriostatic effects of sodium lactate were dose-dependent, wherein 2.7% lactate was significantly more antimicrobial than 1.4% lactate. This was not the case for sodium levulinate, where 1.4% sodium levulinate was as inhibitory to microbial growth as 2.7% sodium levulinate. Additionally, 1.4% sodium levulinate was as inhibitory to microbial growth as the higher level (2.7%) of sodium lactate. TBA values, color, and pH were not affected by treatment with sodium lactate or levulinate. In conclusion, sodium levulinate may have potential as an antimicrobial agent in fresh sausages if it can be obtained at a reasonable cost on a commercial basis.

(95 pages)

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Mihir N. Vasavada

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CHAPTER I

INTRODUCTION

The relatively short shelf life of fresh meat and poultry items often limits the commercial success of refrigerated processed meats. Extending the shelf life is a commercial necessity. Limiting product contamination and delaying or inhibiting the growth of spoilage organisms in the meat product are the major factors for improving fresh processed meats. A longer shelf life enables processors to develop new processed fresh products, such as battered meats, seasoned fresh pork sausages, and marinated chicken breast or pork loins.

Several recent cases of food poisoning, linked to meat products, have demonstrated the importance of incorporating safety hurdles in these products. Safety of meat products can be improved by reducing contamination with pathogenic microorganisms as well as inhibiting their growth during handling and storage.

The shelf life of meat products is limited either by the microbial spoilage, or by color or flavor deterioration. The addition of non-meat ingredients may prolong shelf life by influencing these different aspects of shelf life. Some of these non-meat ingredients when used in combination may provide synergistic effects for prolonging shelf life. Sodium lactate at levels of 1.5-3.0% of meat weight is widely used in the industry as an antimicrobial additive, and to improve yield and various quality attributes of meat including overall color and color stability (Miller, 1998).

Sodium levulinate may have similar anti-microbial properties and may help in preserving the quality of fresh meat sausages. A couple of plausible explanations can be put forward for explaining the antimicrobial effect of organic acids. The equilibrium concentrations of acid anions can be calculated using the Henderson - Hasselbach equation. For an organic acid with pKa = 4, 100 mM of acid anion outside the cell at pH 6.0 would concentrate to about 1000 mM inside the cell at pH 7. To counterbalance the negative charge of the acid anion, the cell would accumulate an additional 1000 mM of cations. This accumulation of ions inside the bacterial cell alters the ionic strength, which is a critical factor effecting metabolic processes.

Lactic acid bacteria have the capability to expel lactate ions from their interior. However, bacterial cells may not have a mechanism to effectively expel levulinic acid out into the system and hence the levulinate may accumulate to a greater degree than lactate.

Also the high levels of lactate may directly inhibit glycolysis by feedback inhibition and thus impair the ability of the bacteria to produce energy for cell growth. It is not clear if levulinate would have such a specific feedback inhibition mechanism for itself.

This belief prompted us to carry out this research using sodium levulinate at 1.4% or 2.7% and comparing it with sodium lactate at the same levels in fresh pork and turkey sausages upon storage for 14 days at 2°C.

CHAPTER II

LITERATURE REVIEW

SODIUM LACTATE AS AN ANTIMICROBIAL ADDITIVE IN MEAT

The shelf life of fresh sausage is limited as compared to cured sausages due to the absence of anti-microbial substances such as nitrite. The cured product contains salt for flavor, which is enough to produce only a small preservative effect as compared to ground pork. Several recent cases of food poisoning linked to meat products have demonstrated the need to incorporate additional antimicrobial hurdles in fresh meat products.

Lactate at the level of 1.5-3% is extensively used in the industry as an anti-microbial additive and to improve various quality attributes of meat. Addition of 2% or 3% sodium lactate delays microbial deterioration of fresh pork sausage by 7 to 10 days at 4°C (Brewer et al., 1991). *Streptococcus faecalis*, *Staphylococcus aureus* and *Salmonella typhimurium* are inhibited when grown in a medium containing sodium lactate at a constant water activity (De Wit and Rombouts, 1990). Beef/cooked top round roasts containing 3-4% sodium lactate have a 2-log reduction in aerobic plate count (Papadopoulos et al., 1991). Sodium lactate (3% and 4%) decreases growth of *Salmonella typhimurium, Listeria monocytogenes*, *Escherichia coli O157:H7*, and *Clostridium* *perfringens* in cooked beef held at 10°C for 28 days (Papadopoulos et al., 1991). In fresh pork sausage, 3% sodium lactate delays the lag phase of microbial growth and reduces off-odor development (Lamkey et al., 1991). The addition of 1 and 2% sodium lactate extends the shelf life of sliced poultry sausage packaged in air and stored at 6-8°C by 3-fold and 7-fold, respectively (Cegielska and Pikul, 2002). Sodium lactate (pH 7.30 and 5.50) retards the growth of spoilage bacteria and enhances cook yields.

Combinations of various organic acids or anti-microbial agents with organic acids have been shown to reduce microbial growth. A combination of 2.5% sodium lactate and 0.2% sodium diacetate or sodium acetate greatly enhances the shelf life of refrigerated and temperature-abused ready-to-eat meats (Mbandi and Shelef, 2001). Sodium lactate also exhibits an antibotulinal effect which is concentration dependent, and it is the lactate and not the sodium ion that is the principal factor in delaying botulinal toxin formation (Maas et al., 1989). Under optimum growth conditions (pH 6.5, 20°C) Gram-positive bacteria are more sensitive to sodium lactate than Gram-negative bacteria, and strains that grow at water activities ≤ 0.95 in the presence of NaCl (*Staphylococcus aureus*, *Listeria monocytogenes*, *Brochothrix thermosphacta*) are especially inhibited by sodium lactate. Yeasts are resistant to 10% w/v concentration of sodium lactate. Thus, the addition of lactate to food products with a pH near neutrality offers good prospects to prolong shelf life (Houtsma et al., 1993). Sodium diacetate in combination with sodium lactate or pediocin delays the growth of *Listeria monocytogenes* in turkey slurries (Schlyter et al., 1993). *Listeria monocytogenes* is inhibited by sodium lactate and by lower pH. Treatments with 2% sodium lactate, 2% lactate and 0.25% glucono-delta-lactone (GDL) and with 2% lactate and 0.50% GDL suppress the growth of *Listeria monocytogenes* (Qvist et al., 1994). A 1:1 combination of sodium lactate (60%) and nisin (4000-6000 IU/ml) decreases the count of *Listeria monocytogenes* in smoked fish (Nykanen et al., 2000). A combination of DL-lactic acid (2% vol/vol), acetic acid (2% vol/vol) and trisodium phosphate (12% wt/vol) decreases the aerobic plate counts of ground beef (Dorsa et al., 1998). Sodium lactate (2.5-3.3%) inhibits the growth of *L. curvatus* in cooked ham product (Stekelenburg and Kant-Muermans, 2001). Thus, the salts of lactic acid and other organic acids are effective as antimicrobial additives in meat products.

EFFECT OF SODIUM LACTATE ON OTHER PROPERTIES OF MEAT

Lactate positively affects flavor, flavor shelf life, microbial shelf life and safety of pork (Miller, 1998). Injection of beef strip loins with phosphate / lactate (2.5% w/w) / chloride solutions in combination, improves their tenderness, juiciness and flavor profile (Vote et al., 2000).

Sodium lactate reduces the development of off-flavor associated with lipid oxidation. TBA values are an indication of shelf life and off-flavor development. In pork sausage with 0-3% sodium lactate and stored at 4°C from 0-21 days there was no change in TBA values. Sodium lactate indirectly assists in limiting lipid oxidation by decreasing the contribution of microorganisms to lipid oxidation (Maca et al., 1999).

Although the antimicrobial effect of sodium lactate increases with increased concentration, problems in flavor may develop with sodium lactate above 2% levels depending on pH. Tray-packed broiler breast meat was treated either with tap water (pH 7.85) or 2% sodium lactate solutions (pH 7.30, 5.50, 5.00, 4.50 and 4.00) and stored at 2°C for 12 days (Williams and Phillips, 1998). An acidic after taste was detected by about 15% of the panel members in samples with 5.0 pH. Slight sodium or metallic off-flavor was detected by 10% of the panel members in all samples treated with sodium lactate. There was a development of intense acidic off-odors and off-flavors in samples at pH 4.50 and 4.00. Hamburgers containing sodium lactate (1.5% and 3.0% levels) had lower cook losses, were softer in texture and had better sensory properties than controls (Walczycka et al., 1998). Sodium lactate (3%) acted as a bacteriostat, color stabilizer and antioxidant, increasing shelf life and quality of cooked beef rounds, even under temperature abuse conditions (Maca et al., 1999).

A solution of 2% sodium lactate, 0.25% sodium tripolyphosphate and 0.35% NaCl was injected into the semimembranosus muscle from USDA Select cattle carcasses (McGee et al., 2003). Injections increased tenderness determined by both instrumental and sensory measurements, and reduced cooking and reheating losses, as well as lipid oxidation. Using a gelatin-based (2%) system containing various concentrations of sodium lactate (SL) (0, 1, 2 or 3%) and sodium tripolyphosphate (STP) (0, 0.1, 0.2, 0.3 or 0.4%), a 13-member trained sensory panel scored samples for saltiness, bitterness and soapiness (Kim and Brewer, 1996). As SL and STP levels increased, saltiness increased. Bitterness decreased with addition of 1% SL and increased with 2 and 3% SL. Soapiness increased with increasing levels of SL. Bitterness and soapiness were not affected by increasing STP concentration.

Regulations pertaining to use of sodium lactate

FDA affirms natural sodium and potassium lactate as a GRAS substance for use as a direct human food ingredient. The USDA approves sodium and potassium lactate as flavoring agents up to 2% in various meat and poultry products. For all products regulated under the 9 Code of Federal Regulations (CFR) 319.180 (emulsified products as frankfurters, bologna, wieners, etc.) the use up to 2% of actual sodium or potassium lactate (which is 3.3% added by weight, of the 60% commercially available solution) is permitted as a flavoring agent or flavor enhancer.

The effectiveness of lactate varies with its concentration. Both spoilage and pathogenic organisms found in meat are relatively sensitive to lactate; even those that are relatively salt tolerant. At a 3.3% use level, the shelf life of a further processed meat product may be extended by 30 to 100%. The use of lactate fits with normal production procedures. Lactate is simply added at the blending stage or is easily mixed with curing pickle. Sodium and potassium lactates are more commonly handled in their liquid forms, which is a 60% concentrate. The recommended use level is 3.3% lactate (60% solution) based on the weight of the total formulation.

Lactate extends product shelf life, controls pathogens and enhances flavor, while not adversely affecting other product characteristics. Because of its hygroscopicity, lactate also has a positive effect on the waterholding capacity, which may result in less purge and a higher yield for cooked products. The use of lactate as an ingredient offers meat manufacturers a natural way to improve the quality of their products.

Other uses of lactate

Organic acids such as lactic acid (1-3%) are used as dips and to wash meat carcasses. They are usually more efficacious as dips than carcass washes because some residual activity remains on the meat. These acid concentrations generally cause no adverse effect on the sensory properties of the meat. *Listeria monocytogenes* and *E.coli*, however, are more resistant to acid treatments than *Yersinia* and *Salmonella* (Greer and Dilts, 1995; Smulders and Greer, 1998). Both lactic acid (1.7%) and acetic acid (2%)

reduced *Listeria monocytogenes* populations on lean beef tissue by 2-3 logs for up to 7 days (Blom et al., 1997). When lean pork tissue and pork fat were artificially inoculated with *Listeria monocytogenes* and then dipped in 3% lactic acid or water for 15 sec., the numbers of *Listeriae* were reduced. A reduction of 1-2 logs for the lean meat portions and up to 7 logs for the fat during 15 days of refrigerated storage was observed (Greer and Dilts, 1995). The more potent effects observed for pork fat were probably due to the fact that acid-treated fat was approximately 2.5 pH units lower than acid-treated lean tissue. A similar effect was observed in pork liver sausage with 22-67% fat treated with propionate or lactate wherein at higher fat levels, the kill was approx. 2-3 times greater (Hu and Shelef, 1996).

The treatment for artificially contaminated raw chicken legs wherein a wash with a 10% lactic acid/ sodium lactate buffer, pH 3.0 followed by packaging in 90% carbon dioxide and 10% oxygen was carried out, extended the shelf life of the chicken from 6 days to 17 days. Chicken treated with the lactate buffer without modified atmosphere packaging had a shelf life of 10 days (Zeitoun and Debevere, 1991). Addition of 1.8% or 2% lactic acid to raw or cooked ground beef did not appreciably affect the survival and growth of *Listeria monocytogenes* (Harmayani et al., 1993; Vignolo et al., 1998). Data from another experiment indicated that lactic acid slightly reduced the thermal tolerance of *Listeria monocytogenes* in ground beef (McMahon et al., 1999). Sodium lactate (4%)

was reported to suppress the growth of *Listeria monocytogenes* in cooked strained beef (Chen and Shelef, 1992) and beef roasts (Miller and Acuff, 1994) although there were viable listeriae left in the meat during refrigeration. *Listeria monocytogenes* inoculated onto cooked chicken that was treated with lactate had a longer lag phase but still grew during storage (Barakat and Harris, 1999). Brines containing monoalurin and lactate pumped in to beef roasts (microwave ready beef roasts) enabled a greater kill of *Listeria monocytogenes* during cooking in bags in water baths than brines with only lactate (Unda et al., 1991).

Mechanism of action of lactate

Sodium and potassium lactates are clear syrupy liquids derived from lactic acid, which is naturally present in the animal tissue. Lactates act as a bacteriostat by increasing the lag phase or dormant phase of microorganisms. Studies on the specific action of lactate indicate mechanisms that interfere with the metabolism of the bacteria, such as intracellular acidification, interference with the proton transfer across the cell membrane and feedback inhibition from lactate anion accumulation (Shelef, 1991). This antimicrobial action suppresses growth for extended periods of time assuring a longer shelf life and an increased product safety.

The addition of sodium lactate at levels greater than 2% increases the lag phase of aerobic spoilage microorganisms in pork sausage. This anti-microbial property is due to the anion portion of sodium lactate, which can enter into the microbial cell where it then inhibits the metabolic process of glycolysis and results in the accumulation of cations to counter the negative charge of lactate (Shelef, 1991). Sodium lactate also prevents growth of food-borne pathogens (Miller, 1998).

It has also been suggested that high levels of lactate ion may shift the pyruvate to lactate reaction closer to its thermodynamic equilibrium, thereby inhibiting a major anaerobic energy production pathway essential for growth (Maas et al., 1989). Also, the addition of sodium lactate has been suggested to lower the water activity of the meat and hence slow down microbial growth (Miller, 1998). However, Shelef (1994) found that small decreases in water activity appeared insufficient to explain the antimicrobial effect, and lactates had no intracellular pH lowering effect. Shelef (1991) also reported that lactate addition did not alter beef or chicken meat pH and no difference was observed between the effect of sodium lactate and sodium chloride on meat pH. This inferred that the lactate ion, and not pH or water activity, was responsible for the delay in listerial growth in their samples. The mechanism by which sodium levulinate inhibits bacteria remains to be determined although it is reasonable to assume that it could have similar modes of action to lactate.

Levulinic acid production and usage

Levulinic acid, or 4-oxopentanoic acid, is the simplest member of the comparatively rare class of organic compounds known as gamma-keto acids. It reacts as a ketone and as a fatty acid since it has both, a ketonic carbonyl group and an acidic carboxyl group. Levulinic acid can be produced by boiling hexoses or other carbohydrates containing hexoses with dilute mineral acid for 20+ hours and then separating by vacuum distillation. Levulinic acid can be produced by high temperature acid hydrolysis of carbohydrates, such as glucose, galactose, sucrose, fructose, chitose and also from biomeric material such as wood, starch and agricultural wastes. Isolation and purification of levulinic acid can be accomplished either by partial neutralization, filtration of humin material and vacuum steam distillation, or by solvent extraction.

This bifunctional chemical intermediate can also be made by hydrolyzing corn - starch with dilute acid in a twin screw extruder (Ghorpade and Hanna, 1999). The present commercial process is a continuous process for preparing levulinic acid from starch in a reactive extrusion process. The extrusion takes place in a twin-screw extruder having several temperature zones wherein the starch slurry is preconditioned, extruded, filter pressed, reboiled, vacuum distilled, condensed and centrifuged, whereby the waste effluent from the centrifugation is reprocessed upstream to the preconditioning stage.

Levulinic acid is a highly versatile chemical with several industrial uses. Applications of levulinic acid include; antifreeze (sodium salt), a fuel additive (esters), a plasticizer, glass-like synthetic resins, and as a constituent of hydraulic brake fluids. Other authors have proposed levulinic acid (sodium salts) as a replacement for ethylene glycol in automobile antifreeze.

FOOD AND PHARMACEUTICAL USAGE

The production of levulinic acid from corn - starch at a low cost would result in its consideration for various food applications. Several reports indicate that levulinic acid has been successfully used as an acidulant in carbonated and fruit juice beverages, jams and jellies. On the basis of chemical structure and pKa it should act similarly to acetic and propionic acids. Ethyl levulinate is used for flavoring (Leonard, 1956). Alkyl metal halide reacts with levulinate esters to yield a series of g-valerolactones and some of them are used as perfumes and flavors. Levulinic acid has been found in many food products. Twenty eight food samples of vegetable protein hydrolysates, pickles and soy sauce were analyzed, and the levulinic acid content was found to be 9-256 mg/100 ml. Also eight samples including chili sauce, tomato sauce, candied fruits, dried soybean curd, dried roast beef and dried shredded pork were analyzed, and the results indicated that all the 8 samples contained levulinic acid at 0.17-18.27 mg/100 g (Youk et al., 1997). The chemical composition and shelf life of 4 commercial shottsuru (Japanese fish sauce) samples produced by different manufacturers were examined for bacterial growth. Bacteria were not detected after storage in the 2 samples containing high levels of levulinic acid and having low pH (4.54 and 5.02) (Fujii et al., 1992). Levulinic acid was also found to be present in apple puree (Opatova et al., 1992) and the levulinic acid content of a soy sauce can be used as an index for VPH (vegetable protein hydrolysate) adulteration (Mei et al., 1999).

Salts of levulinic acid, such as calcium levulinate, are used to treat various medical conditions including tuberculosis. Gordon (1933) reported intravenous injections of calcium levulinate did not produce irritating effects and it was found to be stable for intravenous administration. Calcium levulinate has advantage over calcium gluconate; it contains 40% more calcium, is soluble in water and 30% solution does not form crystals in ampules for indefinite lengths of time (Gordon, 1933). Heterocyclic compounds derived from this acid are used as bacteriostatic and analgesic agents. (Leonard, 1956).

CHAPTER III

HYPOTHESIS AND OBJECTIVES

The hypothesis underlying this study was that sodium levulinate when added to fresh pork and turkey sausages would protect against bacterial growth during refrigerated storage without detrimental effect on quality. To test this hypothesis, two different levels of sodium lactate and sodium levulinate (1.4 and 2. 7%) were added to fresh pork and turkey sausages. The samples were then stored at 2°C and were evaluated for color, TBA values, aerobic plate count and pH at 0, 1, 3, 7 and 14 days of storage and results were noted.

CHAPTER IV

USE OF LEVULINIC ACID IN FRESH SAUSAGE[1]

INTRODUCTION

The growth and proliferation of many types of microbes affect the quality of meat. The efficacy of various anti-microbial treatments such as steam and hot water wash of carcasses, acid wash of carcasses, and addition of anti-microbial substances such as sodium lactate, sodium acetate, sodium diacetate, nisin and others has been extensively studied and is still being investigated. Lactates are commonly added to processed meats because their antimicrobial activity prolongs shelf life (Shelef, 1994). Lactate at the level of 1.5-3.0% of meat weight is extensively used in the industry as an anti-microbial additive and to improve various quality attributes of meat. Lactate has positive effects on flavor, microbial shelf life and safety of pork (Miller, 1998).

Levulinic acid (4-Oxopentanoic acid, $C_5H_8O_3$) is a 5-carbon organic acid commercially available as a by-product of corn extrusion. Very little is known about food uses of levulinic acid. However, it may have antimicrobial activity similar to other short chain organic acids such as sorbic, lactic and propionic acids (Shelef 1994; Chichester and Tanner 1968).

NOTE: The **CHAPTER IV** has been published in the Journal of Muscle Foods.

[1]VASAVADA, M.N., CARPENTER, C.E., CORNFORTH, D.P. and GHORPADE, V. 2003. Sodium levulinate and sodium lactate effects on microbial growth and stability of fresh pork and turkey sausages. J. of Muscle Foods 14(2), 119-129.

Levulinic acid is known to have pro-oxidative activity that could limit shelf life (Yi and Kim, 1982). This study was conducted to evaluate possible antimicrobial and other effects of levulinic acid in fresh meat systems. Specifically, this study compared the effects of sodium levulinate and sodium lactate on aerobic plate count, color, TBA values and pH of fresh pork and turkey sausages.

MATERIALS AND METHODS

Apparatus

We used: Hobart grinder model 4152 (Hobart Mfg. Co., Troy, OH); Hunter lab Miniscan portable colorimeter (Reston, VA); Pressure cooker (101; Mirromatic, Mirro Corp., Manitowoc, WI); pH-meter (Fisher Accumet, model 610A, Houston, TX), pH-meter electrode (Corning G-P combo electrode, Corning, NY); Wrapping and Sealing equipment (Heat Sealing Manufacturing Company, Cleveland, OH).

Experimental Design and Statistics

Water or syrups (15% and 30% w/w) of sodium lactate and sodium levulinate were added to fresh pork sausage at 10% of the meat weight. The sausage mix was divided into 5 portions, then placed on styrofoam trays, over-wrapped with PVC film and stored at 2°C. A single package from each treatment was removed at days 0, 1, 3, 7 and

14 and color, aerobic plate count, pH and TBA values were monitored, and fat content was measured on the 0 day samples. The experiments were replicated with three separate batches of pork sausage, and on 3 separate batches of turkey sausage.

The data was analyzed using STATISTICA (Statsoft Inc., Tulsa, OK) software. The effect of replication was blocked in order to avoid the variations due to the different meat batches used for each replicate. While blocking for replication, we can estimate the main effect of replication but we cannot estimate the interaction terms.

The data was analyzed by MANOVA as a complete factorial [(2+2+1) * 5] in a split plot model. The whole plot factors were salt type (levulinic or lactic), level of salt in sausage (1.4 or 2.7%), or water control. Whole plot factors had $n = 3$ reflecting independent observations from three separate batches of sausage. Storage time in days (0, 1, 3, 7 and 14) was the subplot factor. To compare means, LSD value was calculated when $p < 0.05$.

Preparation of Sodium Lactate and Sodium Levulinate

A 30% sodium lactate solution was prepared using a 60% sodium lactate solution (Sigma Chemicals, DL-Lactic acid sodium salt, 60% (w/w) syrup) and diluting it 1:1 with distilled water. Sodium levulinate solution was prepared by adding 20% NaOH to a 98+% levulinic acid solution (Sigma Chemicals, St. Louis, MO) to obtain a pH of 6.59.

Because the pKa value of levulinic acid is 4.59, the 2-pH unit difference due to titration with NaOH ensures that 99% of the levulinic acid was in the form of its sodium salt. The resulting levulinate was diluted with enough distilled water to give a 30% solution. The 30% solutions of sodium lactate and sodium levulinate were diluted 1:1 with distilled water to give 15% solutions. The 15% or 30% solutions were then added to the meat samples at the rate of 10% w/w of meat weight to obtain 1.5% or 3.0% solutions based on meat weight equivalents or to obtain 1.4% or 2.7% solutions expressed as % of final product weight.

Sample Preparations

Approximately 6 kg of fresh pork (80:20 trim) or 6 kg fresh turkey breast meat was coarsely ground once through a grinder (Hobart Mfg. Co. Troy, OH). Pre-mixed spices (Heller JD's country pork sausage seasoning GD-5076, Modesto, CA) were added to the sausage at the rate of 0.02 kg per kg meat and it was passed again through the coarse grinder plate (0.64 cm diameter pore size). The mixture was divided into 5 portions of 1 kg and each portion was mixed with 10% of either water (control), 15% lactate, 30% lactate, 15% levulinate or 30% levulinate. The coarsely ground mixes were separately passed through the fine grinder plate (0.32 cm diameter pore size). Each treatment mix was further divided into 5 samples of 200 g each, packaged on a styrofoam tray and covered with an oxygen-permeable polyvinyl chloride film (SWM select wrap, Anchor Packaging, Marmaduke, AR) and stored for 0, 1, 3, 7, and 14 days at 2°C.

Color Measurement

The color was measured on day 0, 1, 3, 7 and 14 using the Hunter Lab Digital Color Difference Meter D25D2A (Hunter Associates Laboratories, Inc., Reston, VA). The instrument was standardized using a white and black standard plate and then the L*, a* and b* values were measured. The hue angle = $\tan^{-1}(b^*/a^*)$ was calculated. Larger hue-angle values are associated with less red color (Van Laack et al., 1996), where hue-angle 0 = red and hue-angle 90 = yellow. The saturation index was also measured $(a^{*2} + b^{*2})^{1/2}$ and it corresponds to color intensity.

Aerobic Plate Count

The aerobic plate count of the meat samples was determined by placing 10 g from each sample into bottles containing 90 ml of 0.1% sterile bacto-peptone solution (Difco, Detroit, MI). Serial dilutions and pour-plate counts were implemented following standard procedures (Messer et al., 1978). Standard plate count agar (Difco, Detroit, MI) was used as growth media. Pork samples were incubated at 37°C for 48 hours. Turkey samples were incubated for 60 hours at 37°C to obtain larger colonies that could be distinguished from meat particles at 10^{-1} dilution.

TBA values

Thiobarbituric acid-reactive substances (TBARS) were measured as described by Buege and Aust (1978). Duplicate samples (0.5 g) for each treatment were placed in tubes and mixed with 2.5 ml stock solution containing 0.375% TBA (Sigma Chemical Co., St. Louis, MO), 15% trichloroacetic acid (Mallinckrodt Baker Inc, Paris, KY) and 0.25 N HCl. The samples were then heated for 10 min in a boiling water bath (100°C) to develop pink color and then cooled under tap water. Samples were centrifuged (Sorvall Instruments, Model RC 5C, Dupont, Wilmington, DE) at 12,465 X g for 10 min rather than 1000 X g as specified by Buege and Aust (1978), to reduce sample turbidity. Absorbance at 532 nm was measured (Spectronic 21D, Milton Roy, Rochester, NY) against a blank sample having all the reagents except the meat. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (Sinnhuber & Yu, 1958). The MDA concentration was converted to TBA number (mg MDA/kg meat sample) as follows:

1). TBA number (mg / kg) = sample A_{532} X (1M MDA / 1.56X10^5) X [(1mole / L) / M] X (0.003L / 0.5 g meat) X (72.07g MDA / mole MDA) X (1000 mg / g) X (1000 g / kg)

Or

2). TBA number (ppm) = sample A_{532} X 2.77

pH Values

The pH values for each sample were measured by adding 90 ml deionized water to 10 g sample. The samples were then thoroughly mixed and passed through Whatman filter paper no. 2 (Fisher Scientific, Salt Lake City, UT). The pH of the filtrate was measured using a pH meter calibrated at pH 4.0 and 7.0 (Fisher Chemicals, Fair Lawn, NJ).

Fat Content

The fat content of the samples was measured using the AOAC Goldfish fat extraction method (AOAC, 1990).

RESULTS

Microbial load of both pork and turkey sausages were significantly ($p < 0.05$) affected by treatments, storage time and the interaction of treatment and storage time (Table 1). By day 14 of storage of pork sausages, both control and sausages with 1.4% lactate had a high log_{10}APC (6.23 and 5.90, respectively, Fig. 1). In comparison, pork sausage containing sodium lactate at 2.7% level, and both levels of sodium levulinate (1.4% and 2.7%) had significantly less bacterial load after 14 days storage with log_{10}APC of 4.78, 4.42 and 4.29 respectively ($LSD_{0.05} = 0.59$, Fig. 1). These numbers were similar to those reported by Lamkey et al. (1991) for fresh pork sausage, who reported $\log_{10}$APC of 7.5 for control sausages and 4.0 for sausages with 3.0% sodium lactate after 15 days storage at 2°C. Brewer et al. (1991) have also reported that the addition of sodium lactate at 2% or 3% levels delayed microbial fresh sausage deterioration by 7 to 10 days at 4°C. In this study, 1.4% sodium levulinate was a more effective anti-microbial agent than 1.4% sodium lactate in fresh pork sausages after 14 days storage with $\log_{10}$APC of 4.42 and 5.90 respectively ($LSD_{0.05}$ = 0.59, Fig. 1).

Table 1. Summary of significance ($p < 0.05$) as determined by analysis of variance (ANOVA).

Meat Type	Effect	$\log_{10}$ APC[1]	TBA[1]	L*[1]	a*[1]	b*[1]	Hue angle[1]	Saturation index[1]	pH
Pork	Treatment[2]	S[4]	NS[4]	NS	NS	NS	NS	NS	NS
	Storage time[3]	S	S	NS	S	NS	S	S	NS
	Treatment * time	S	NS	NS	NS	NS	NS	NS	NS
Turkey	Treatment	S	NS	S	NS	NS	NS	NS	S
	Storage time	S	S	S	S	S	S	S	S
	Treatment * time	S	NS	NS	NS	NS	NS	NS	NS

[1]APC = $\log_{10}$ aerobic plate count / gm sample, TBA = Thiobarbituric acid number, L* = Lightness; a* = redness; b* = yellowness; Hue angle = $\tan^{-1}$ (b* / a*), where lower values indicate more redness; Saturation index = $(a^{*2} + b^{*2})^{1/2}$

[2]Treatments were control (water), 1.5% or 3.0% sodium lactate or sodium levulinate

[3]Storage times were 0, 1, 3, 7 and 14 days at 2°C.

[4]S = significant at $p < 0.05$; NS = not significant at $p < 0.05$.

After 14 days of storage, the control turkey sausages had a mean $\log_{10}$APC of 5.45, which was significantly higher than all other treatments (Fig. 2). Turkey sausages containing 2.7% lactate, and both levels of levulinate, had lower APC after 14 days than

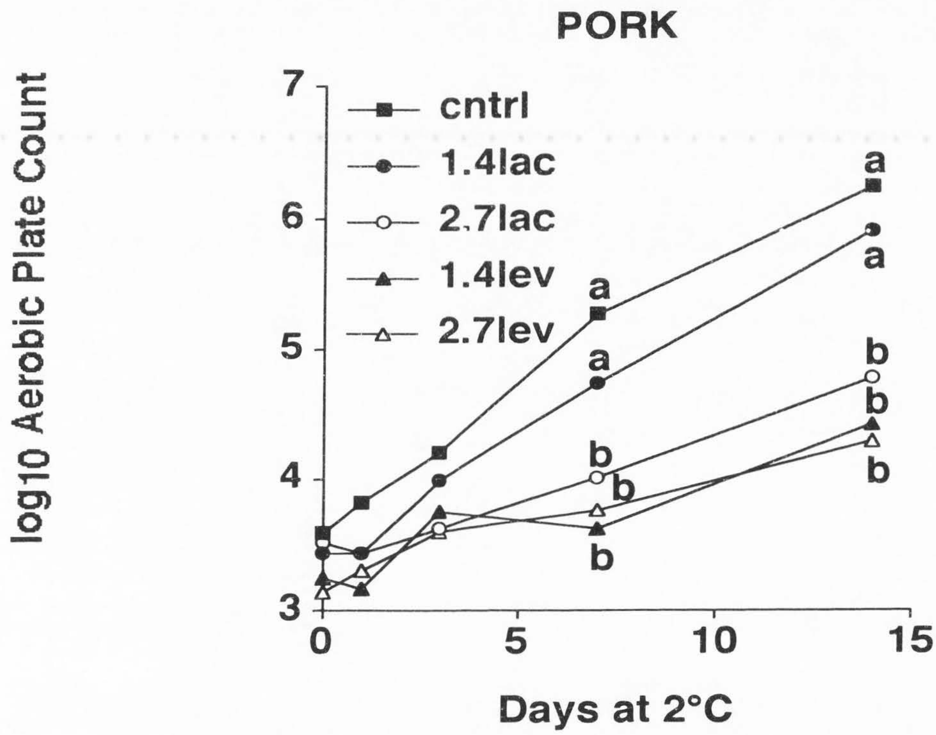

Fig 1. Log_{10} aerobic plate counts for fresh pork sausage during storage at 2°C. Data points at a given day not sharing the same letter are different ($p < 0.05$). Fishers' least significant difference was 0.59.

cntrl = control, 1.4 lac = sodium lactate at 1.4% level based on total product weight, 2.7 lac = sodium lactate at 2.7% level based on total product weight, 1.4 lev = sodium levulinate at 1.4% level based on total product weight, 2.7 lev = sodium levulinate at 2.7% based on total product weight.

1.4% lactate (Fig. 2). Maas et al. (1989) have previously demonstrated the antimicrobial effect of sodium lactate in cook-in-bag turkey products at various levels. Note that 1.4% sodium levulinate was as effective as 2.7% sodium levulinate for inhibition of aerobic microbial growth, as was the case for pork sausage.

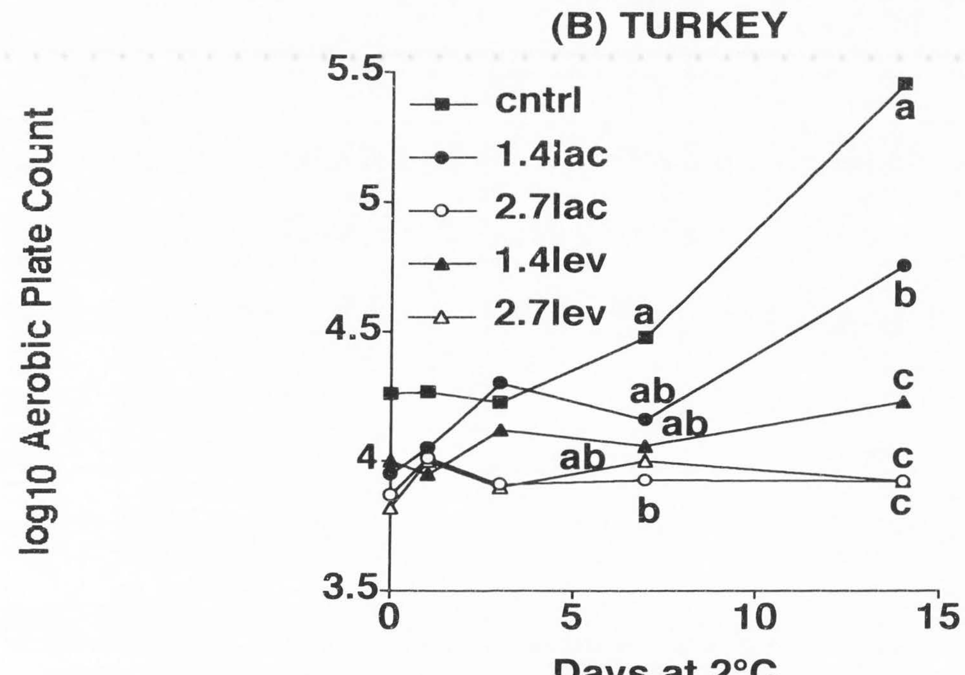

Fig 2. Log_{10} aerobic plate counts for fresh turkey sausage during storage at 2°C. Data points at a given day not sharing a letter are different ($p < 0.05$). Fishers' least significant difference was 0.48.

cntrl = control, 1.4 lac = sodium lactate at 1.4% level based on total product weight, 2.7 lac = sodium lactate at 2.7% level based on total product weight, 1.4 lev = sodium levulinate at 1.4% level based on total product weight, 2.7 lev = sodium levulinate at 2.7% based on total product weight.

TBA number of uncooked pork or turkey sausages was not affected by treatment with sodium lactate or sodium levulinate (Table 1). All TBA values for pork sausages were low ranging from 0.20 to 0.32 (Table 2). Tarladgis et al. (1960) found that the TBA number at which rancid odor was first perceived was between 0.5 and 1.0. This threshold has served as a guide for interpreting TBA test results. Most turkey sausage samples had TBA values less than 0.5 (Table 2). TBA numbers of uncooked pork and turkey sausages decreased slightly, but significantly, during storage (Tables 1 & 2). No explanation for this slight decrease in TBA values during storage is immediately apparent.

Storage time significantly affected Hunter color redness (a*), hue angle and saturation index of pork sausages (Table 1). Redness (a*) values decreased with storage time from 6.1 in day 0 controls to 4.9 after 14 days storage (Table 2). Hue angle of pork sausages increased from 65.8 to 69.7, indicating loss of redness during storage (Table 2).

Storage time significantly affected all Hunter color parameters of turkey sausages (Table 1). L*, a* and b* and saturation index values decreased with storage time, while the hue angle values increased indicating loss of redness during storage (Table 2). Lightness (L*) values were significantly affected by treatment (Table 1). Samples treated with 2.7% sodium levulinate had significantly higher L* values at day 0 than control treatment (L* = 44.3 and 41.3 respectively; $LSD_{0.05}$ = 2.38; Data not shown in Table form).

Table 2. Pooled means by storage time for TBA values, pH, and Hunter color parameters of fresh pork and turkey sausages (treatment effects were pooled).

Meat type and storage time	TBA[1]	L*[1]	a*[1]	b*[1]	Hue angle[1]	Saturation index[1]	pH
Pork							
0 (days)	0.32±0.14[2]	46.6±8.2	6.1±0.6	13.6±2.0	65.8±2.2	14.9±2.0	6.14±0.15
1	0.32±0.09	47.7±11.6	6.6±0.9	14.0±2.6	64.6±3.0	15.5±2.6	6.14±0.25
3	0.30±0.11	46.6±8.0	6.1±0.5	13.7±1.5	65.8±2.8	15.0±1.3	6.23±0.10
7	0.24±0.09	48.3±8.6	5.6±1.1	13.5±1.9	67.5±4.4	14.6±1.9	6.19±0.09
14	0.20±0.09	47.6±8.6	4.9±1.3	13.2±2.3	69.7±4.7	14.1±2.4	6.21±0.10
$LSD_{0.05}$	0.07	NS	0.67	NS	2.58	NS	NS
Turkey							
0	0.52±0.17	42.8±2.2	2.2±0.2	10.6±0.6	78.1±0.9	10.8±0.6	5.87±0.06
1	0.65±0.18	40.3±1.5	2.2±0.2	10.3±0.7	77.8±0.8	10.5±0.7	5.88±0.05
3	0.52±0.13	39.4±0.9	2.0±0.1	9.9±0.6	78.8±0.7	10.1±0.6	5.80±0.09
7	0.43±0.09	37.4±1.4	2.0±0.1	9.3±0.8	77.3±4.3	9.6±0.8	5.83±0.05
14	0.49±0.09	36.9±1.2	1.5±0.4	8.8±0.6	80.4±2.1	9.0±0.6	5.84±0.06
$LSD_{0.05}$	0.1	1.12	0.15	0.50	1.65	0.50	0.05

[1]TBA = Thiobarbituric acid number, L* = Lightness; a* = redness; b* = yellowness; Hue angle = $\tan^{-1}$ (b* / a*), where lower values indicate more redness; Saturation index = $(a^{*2} + b^{*2})^{1/2}$

[2] = Values were expressed as mean ± standard deviation for pooled treatment effects.

NS = not significant at $p < 0.05$.

$LSD_{0.05}$ = Fisher's least significance difference at $p < 0.05$.

Sausage pH was slightly but significantly increased from 5.8 for controls to 5.9 with 2.7% levulinate in turkey sausages (data not shown). Sensory evaluation was not formally done. However, informal panels indicated no flavor changes associated with use of levulinate compared to controls.

DISCUSSION

In this study, 1.4% sodium levulinate was as antimicrobial as 2.7% sodium lactate for both pork and turkey sausages. In both sausages, no difference was found between 1.4% and 2.7% sodium levulinate on aerobic plate counts during storage. Thus, there is no advantage to addition of the higher level of sodium levulinate since the low level of 1.4% was quite effective. This was not the case with sodium lactate. The higher level (2.7%) of sodium lactate was more antimicrobial than the lower level (1.4%) for inhibition of bacterial growth in both pork and turkey sausages. This is in agreement with previous work by Debevere (1989) who showed that increasing sodium lactate levels from 0% to 2% gave lower microbial counts in pork liver samples. Ghorpade et al. (1992) have also previously reported lower aerobic and anaerobic plate counts with increasing concentrations of sodium lactate.

The mechanism of sodium lactate inhibition is still debated. It has been suggested that high levels of lactate ion may shift the pyruvate to lactate reaction closer to its thermodynamic equilibrium, thereby inhibiting a major anaerobic energy production pathway essential for growth (Maas et al., 1989). Also, the addition of sodium lactate has been suggested to lower the water activity of the meat and hence slow down microbial growth (Miller, 1998). However, Shelef (1994) found that small decreases in water activity appeared insufficient to explain the antimicrobial effect, and lactates had no intracellular pH lowering effect. Shelef and Yang (1991) also reported that lactate addition did not alter beef or chicken meat pH and no difference was observed between the effect of sodium lactate and sodium chloride on meat pH. This implied that the lactate ion and not pH or water activity was responsible for the delay in listerial growth in their samples. The mechanism by which sodium levulinate inhibits bacteria remains to be determined. To the best of our knowledge, sodium levulinate has not previously been investigated for use as an antimicrobial agent in meat products. Thus, there are currently no USDA regulations for sodium levulinate use in meat products. Although it has been previously shown that levulinic acid has prooxidant properties (Yi and Kim, 1982), this was not the case for fresh sausages with added sodium levulinate in this study. Sausages made with sodium levulinate did not have noticeably different flavor than control sausages but this observation needs conformation using formal taste panel procedures. It appears that sodium levulinate has commercial potential as an antimicrobial agent for fresh pork or turkey sausages if it can be obtained at a reasonable price.

CONCLUSION

In this study, 1.4% sodium levulinate was as antimicrobial as 2.7% sodium lactate for both pork and turkey sausages. In both sausages, there was no significant difference between 1.4% and 2.7% sodium levulinate on aerobic plate counts during storage. TBA values of uncooked sausages were low and generally not affected by treatment with sodium lactate or sodium levulinate. Sausage color was affected by storage time, but generally not affected by treatment. In conclusion, sodium levulinate may have potential as an antimicrobial agent in fresh sausages if it can be obtained at a reasonable cost on a commercial basis.

CHAPTER V

SUMMARY AND CONCLUSIONS

In this study, 1.4 or 2.7% sodium levulinate was as antimicrobial as 2.7% sodium lactate for both pork and turkey sausages. There was no significant difference between 1.4% and 2.7% sodium levulinate on aerobic plate counts during storage in both pork and turkey sausages. There was no increase in the TBA values of uncooked sausages and the TBA values were low and generally not affected by treatment with sodium lactate or sodium levulinate. Sausage color was affected by storage time, but generally not affected by treatment.

Thus, sodium levulinate has greater antimicrobial properties as compared to sodium lactate without adversely effecting the oxidative rancidity or color parameters of fresh pork and turkey sausage. This shows that sodium levulinate has a great potential for future use as an antimicrobial ingredient in fresh sausages and possibly as carcass wash.

Further research needs to be done in a variety of areas related to use of sodium levulinate in the meat industry.

Research needs to be done to evaluate the effect of sodium levulinate on sensory properties of meat samples, by using trained panels to verify that it does not adversely effect the sensory properties.

Research also needs to be done to evaluate the effect of sodium levulinate on specific pathogens found in meat, which may vary by meat species, and to verify if it reduces contamination by pathogens in meat samples.

Further research with the use of sodium levulinate in different species of meat such as chicken and beef would prove that it works very well in preventing spoilage of fresh sausage with different types of meat species.

The use of sodium levulinate as an antimicrobial in cooked meat products and other modified meat products and processed meats would be a good topic for future research.

It would be interesting to determine the effect of sodium levulinate in a combination with other antimicrobials such as sodium lactate, potassium lactate, nisin etc. and to use sodium levulinate as a hurdle in preventing pathogen growth.

Sodium levulinate seems to be a promising antimicrobial in fresh sausages and further research might prove it to be a viable antimicrobial in different types of meats.

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APPENDICES

APPENDIX A

Data from Pork Sausage Experiment

LIST OF ABBREVIATIONS

TRTMT = Treatment

REP = Replicate

SATUR = Saturation

CNTRL = Control

LAC 15 = Sodium lactate at 1.5% of meat weight equivalent

LAC 30 = Sodium lactate at 3.0% of meat weight equivalent

LEV 15 = Sodium levulinate at 1.5% of meat weight equivalent

LEV 30 = Sodium levulinate at 3.0% of meat weight equivalent

Table 3. Pork color data (average of three readings combined for a given day).

TRTMT	REP	DAY	L	A	B	HUE	SATUR
CNTRL	1	0	37.28	5.91	11.65	63.72	13.06
LAC15	1	0	39.82	5.96	12.59	64.72	13.94
LAC30	1	0	39.03	6.52	12.59	62.83	14.19
LEV15	1	0	37.99	6.61	13.09	63.22	14.67
LEV30	1	0	39.06	5.49	11.07	63.58	12.37
CNTRL	1	1	40.31	5.45	11.02	63.49	12.29
LAC15	1	1	36.53	6.17	11.74	62.59	13.28
LAC30	1	1	39.35	6.25	11.65	61.61	13.25
LEV15	1	1	38.68	5.05	11.32	66.17	12.41
LEV30	1	1	38.64	5.58	11.57	64.51	12.86
CNTRL	1	3	39.47	5.39	12.39	66.44	13.52
LAC15	1	3	39.02	6.03	12.28	63.81	13.68
LAC30	1	3	42.35	5.33	12.74	67.27	13.81
LEV15	1	3	38.88	6.47	13.07	64.17	14.62
LEV30	1	3	43.91	5.80	13.11	66.30	14.36
CNTRL	1	7	38.89	4.22	10.96	69.01	11.74
LAC15	1	7	41.19	4.77	12.34	68.82	13.23
LAC30	1	7	41.59	5.88	10.84	61.34	12.34
LEV15	1	7	42.64	4.22	12.21	70.99	12.94
LEV30	1	7	40.31	6.33	12.48	62.96	14.01
CNTRL	1	14	42.19	2.69	10.77	76.26	11.13
LAC15	1	14	39.69	4.61	12.22	69.31	13.07
LAC30	1	14	41.55	3.44	11.36	73.19	11.88
LEV15	1	14	42.13	3.38	9.83	71.11	10.40
LEV30	1	14	40.49	5.17	11.17	65.38	12.32
CNTRL	2	0	56.60	6.79	15.77	67.11	17.18
LAC15	2	0	57.26	5.88	14.31	67.62	15.48
LAC30	2	0	54.42	6.07	14.40	67.10	15.65

LEV15	2	0	58.45	6.50	16.75	68.77	17.97
LEV30	2	0	59.05	6.47	16.52	68.64	17.76
CNTRL	2	1	65.13	7.21	18.38	68.61	19.75
LAC15	2	1	63.23	6.36	16.83	69.24	18.00
LAC30	2	1	60.69	7.60	16.98	65.85	18.61
LEV15	2	1	62.98	7.30	17.77	67.64	19.23
LEV30	2	1	63.70	6.72	16.87	68.24	18.18
CNTRL	2	3	57.19	6.15	15.04	67.85	16.26
LAC15	2	3	56.40	6.21	15.56	68.25	16.76
LAC30	2	3	54.35	5.74	15.16	69.28	16.21
LEV15	2	3	60.54	6.18	16.52	69.49	17.65
LEV30	2	3	57.56	5.70	15.22	69.43	16.26
CNTRL	2	7	59.01	3.52	14.48	76.40	14.91
LAC15	2	7	58.78	5.40	15.54	70.83	16.45
LAC30	2	7	62.16	5.34	15.84	71.40	16.72
LEV15	2	7	60.24	6.46	16.77	68.94	17.98
LEV30	2	7	57.05	5.67	15.39	69.80	16.41
CNTRL	2	14	57.82	3.88	17.00	77.13	17.44
LAC15	2	14	58.77	3.30	14.49	77.15	14.86
LAC30	2	14	57.99	5.40	15.50	70.93	16.43
LEV15	2	14	61.02	6.15	15.95	68.97	17.10
LEV30	2	14	60.34	6.24	16.57	69.38	17.71
CNTRL	3	0	47.43	5.65	14.48	68.84	15.56
LAC15	3	0	41.52	6.88	14.70	65.30	16.26
LAC30	3	0	44.67	4.87	10.06	64.44	11.19
LEV15	3	0	44.01	5.16	12.24	67.07	13.30
LEV30	3	0	41.81	6.45	13.16	64.09	14.67
CNTRL	3	1	45.25	6.81	13.27	63.22	14.96
LAC15	3	1	37.95	8.17	13.51	58.85	15.80
LAC30	3	1	43.60	6.55	13.76	64.92	15.28
LEV15	3	1	37.82	7.33	13.56	61.63	15.42
LEV30	3	1	41.29	6.03	11.68	63.14	13.19
CNTRL	3	3	42.79	6.65	12.43	62.28	14.11

LAC15	3	3	40.87	7.06	13.28	62.31	15.06
LAC30	3	3	44.24	5.87	13.52	66.37	14.78
LEV15	3	3	41.47	6.46	12.88	63.38	14.41
LEV30	3	3	40.08	6.54	11.72	60.88	13.43
CNTRL	3	7	43.79	6.09	13.42	65.40	14.75
LAC15	3	7	47.79	5.45	11.34	64.65	12.64
LAC30	3	7	47.31	6.13	14.25	66.85	15.53
LEV15	3	7	40.33	8.17	13.89	59.60	16.12
LEV30	3	7	43.53	5.70	12.41	65.50	13.66
CNTRL	3	14	43.33	4.67	11.60	68.11	12.52
LAC15	3	14	42.33	5.43	11.80	65.03	13.00
LAC30	3	14	44.07	6.61	14.71	66.46	16.16
LEV15	3	14	40.70	6.57	12.28	61.73	13.95
LEV30	3	14	41.44	5.54	12.03	65.30	13.24

Table 4. Table for TBA and $\log_{10}$APC values for pork (average of two readings for TBA as well as $\log_{10}$APC combined for the given day).

TRTMT	REP	DAY	TBA	$\log_{10}$APC
CNTRL	1	0	0.69	3.10
CNTRL	1	1		3.17
CNTRL	1	3		3.81
CNTRL	1	7	0.24	4.55
CNTRL	1	14	0.29	5.34
CNTRL	2	0	0.32	3.42
CNTRL	2	1	0.34	3.21
CNTRL	2	3	0.19	4.16
CNTRL	2	7	0.23	5.82
CNTRL	2	14	0.15	7.30
CNTRL	3	0	0.26	4.27
CNTRL	3	1	0.26	4.45
CNTRL	3	3	0.24	4.26
CNTRL	3	7	0.10	5.44
CNTRL	3	14	0.12	6.05
LAC15	1	0	0.45	2.90
LAC15	1	1	0.26	2.65
LAC15	1	3	0.34	3.67
LAC15	1	7	0.28	4.21
LAC15	1	14	0.24	5.29
LAC15	2	0	0.24	3.31
LAC15	2	1	0.40	3.23
LAC15	2	3	0.18	3.75
LAC15	2	7	0.34	4.74
LAC15	2	14	0.10	6.29
LAC15	3	0	0.24	4.12
LAC15	3	1	0.29	4.42

LAC15	3	3	0.26	4.54
LAC15	3	7	0.06	5.26
LAC15	3	14	0.06	6.15
LAC30	1	0	0.34	3.04
LAC30	1	1	0.49	2.81
LAC30	1	3	0.60	3.29
LAC30	1	7	0.29	3.91
LAC30	1	14	0.34	4.33
LAC30	2	0	0.24	3.35
LAC30	2	1	0.21	3.25
LAC30	2	3	0.24	3.69
LAC30	2	7	0.29	3.85
LAC30	2	14	0.27	4.46
LAC30	3	0	0.21	4.15
LAC30	3	1	0.25	4.27
LAC30	3	3	0.29	3.89
LAC30	3	7	0.14	4.29
LAC30	3	14	0.12	5.55
LEV15	1	0		2.77
LEV15	1	1	0.46	2.71
LEV15	1	3	0.29	3.25
LEV15	1	7	0.27	3.60
LEV15	1	14	0.36	3.92
LEV15	2	0	0.30	3.28
LEV15	2	1	0.27	3.18
LEV15	2	3	0.29	3.77
LEV15	2	7	0.30	3.83
LEV15	2	14	0.14	4.53
LEV15	3	0	0.24	3.21
LEV15	3	1	0.27	3.63
LEV15	3	3	0.31	4.25
LEV15	3	7	0.17	3.44

LEV15	3	14	0.24	4.80
LEV30	1	0	0.48	3.00
LEV30	1	1	0.44	2.56
LEV30	1	3	0.41	3.35
LEV30	1	7	0.24	3.73
LEV30	1	14	0.29	4.07
LEV30	2	0	0.25	3.18
LEV30	2	1	0.31	3.19
LEV30	2	3	0.21	3.66
LEV30	2	7	0.34	3.92
LEV30	2	14	0.17	4.48
LEV30	3	0	0.19	3.23
LEV30	3	1	0.29	4.17
LEV30	3	3	0.29	3.78
LEV30	3	7	0.29	3.67
LEV30	3	14	0.14	4.33

Table 5. Table with pH values for pork sausage for a given day.

TRTMT	REP	DAY	pH
CNTRL	1	0	
LAC15	1	0	
LAC30	1	0	
LEV15	1	0	
LEV30	1	0	
CNTRL	1	1	
LAC15	1	1	
LAC30	1	1	
LEV15	1	1	
LEV30	1	1	
CNTRL	1	3	
LAC15	1	3	
LAC30	1	3	
LEV15	1	3	
LEV30	1	3	
CNTRL	1	7	
LAC15	1	7	
LAC30	1	7	
LEV15	1	7	
LEV30	1	7	
CNTRL	1	14	
LAC15	1	14	
LAC30	1	14	
LEV15	1	14	
LEV30	1	14	
CNTRL	2	0	5.98
LAC15	2	0	5.99
LAC30	2	0	5.98

LEV15	2	0	6.01
LEV30	2	0	6.06
CNTRL	2	1	5.57
LAC15	2	1	6.08
LAC30	2	1	6.03
LEV15	2	1	6.13
LEV30	2	1	5.96
CNTRL	2	3	6.11
LAC15	2	3	6.10
LAC30	2	3	6.25
LEV15	2	3	6.15
LEV30	2	3	6.09
CNTRL	2	7	6.04
LAC15	2	7	6.09
LAC30	2	7	6.08
LEV15	2	7	6.17
LEV30	2	7	6.19
CNTRL	2	14	6.35
LAC15	2	14	6.17
LAC30	2	14	6.10
LEV15	2	14	6.13
LEV30	2	14	6.16
CNTRL	3	0	6.25
LAC15	3	0	6.27
LAC30	3	0	6.24
LEV15	3	0	6.34
LEV30	3	0	6.32
CNTRL	3	1	6.35
LAC15	3	1	6.34
LAC30	3	1	6.28
LEV15	3	1	6.34
LEV30	3	1	6.31

CNTRL	3	3	6.33
LAC15	3	3	6.32
LAC30	3	3	6.27
LEV15	3	3	6.32
LEV30	3	3	6.31
CNTRL	3	7	6.23
LAC15	3	7	6.23
LAC30	3	7	6.27
LEV15	3	7	6.31
LEV30	3	7	6.28
CNTRL	3	14	6.04
LAC15	3	14	6.22
LAC30	3	14	6.28
LEV15	3	14	6.33
LEV30	3	14	6.29

Table 6. Effect of sodium lactate and sodium levulinate levels on mean aerobic plate count[1], thiobarbituric acid number[2] and hunter color values[3] of fresh pork sausage stored at 2°C.

Treatment	Storage days	APC	TBA	L^*	a^*	b^*	Hue	Saturation
Control	0	3.59±0.61	0.42±0.23	47.1±9.7	6.1±0.6	14.0±2.1	66.6±2.6	15.3±2.1
Control	1	3.83±0.87	0.30±0.06	50.2±13.1	6.5±0.9	14.2±3.8	65.1±3.0	15.7±3.8
Control	3	4.21±0.08	0.22±0.04	46.5±9.4	6.1±0.6	13.3±1.5	65.5±2.9	14.6±1.4
Control	7	5.27±0.65	0.19±0.08	47.2±10.5	4.6±1.3	13.0±1.8	70.3±5.6	13.8±1.8
Control	14	6.23±0.99	0.19±0.09	47.8±8.7	3.7±1.0	13.1±3.4	73.8±5.0	13.7±3.3
1.5Lactate	0	3.44±0.62	0.31±0.12	46.2±9.6	6.2±0.6	13.9±1.1	65.9±1.5	15.2±1.2
1.5Lactate	1	3.44±0.91	0.32±0.07	45.9±15.0	6.9±1.1	14.0±2.6	63.6±5.3	15.7±2.4
1.5Lactate	3	3.99±0.48	0.26±0.08	45.4±9.5	6.4±0.5	13.7±1.7	64.8±3.1	15.2±1.5
1.5Lactate	7	4.74±0.53	0.23±0.15	49.3±8.9	5.2±0.4	13.1±2.2	68.1±3.2	14.1±2.1
1.5Lactate	14	5.90±0.54	0.13±0.10	46.9±10.3	4.4±1.1	12.8±1.4	70.5±6.1	13.6±1.1
3.0Lactate	0	3.52±0.57	0.26±0.07	46.0±7.8	5.8±0.9	12.4±2.2	64.8±2.2	13.7±2.3
3.0Lactate	1	3.44±0.75	0.32±0.15	47.9±11.3	6.8±0.7	14.1±2.7	64.1±2.2	15.7±2.7
3.0Lactate	3	3.63±0.30	0.38±0.19	47.0±6.5	5.6±0.3	13.8±1.2	67.6±1.5	14.9±1.2
3.0Lactate	7	4.02±0.24	0.24±0.08	50.4±10.6	5.8±0.4	13.6±2.6	66.5±5.0	14.9±2.3
3.0Lactate	14	4.78±0.67	0.24±0.11	47.9±8.9	5.1±1.6	13.9±2.2	70.2±3.4	14.8±2.6
1.5Levulinate	0	3.25±0.05	0.27±0.05	46.8±10.5	6.1±0.8	14.0±2.4	66.4±2.8	15.3±2.4
1.5Levulinate	1	3.17±0.46	0.34±0.11	46.5±14.3	6.6±1.3	14.2±3.3	65.1±3.1	15.7±3.4

1.5Levulinate	3	3.76± 0.50	0.30±0.01	47.0±11.8	6.4±0.2	14.2±2.1	65.7±3.3	15.6±1.8
1.5Levulinate	7	3.63± 0.20	0.25±0.07	47.7±10.9	6.3±2.0	14.3±2.3	66.5±6.1	15.7±2.5
1.5Levulinate	14	4.42± 0.45	0.25±0.11	47.9±11.3	5.4±1.7	12.7±3.1	67.3±4.9	13.8±3.4
3.0Levulinate	0	3.14± 0.13	0.31±0.15	46.6±10.8	6.1±0.6	13.6±2.7	65.4±2.8	14.9±2.7
3.0Levulinate	1	3.31± 0.81	0.35±0.08	47.9±13.8	6.1±0.6	13.4±3.0	65.3±2.6	14.7±3.0
3.0Levulinate	3	3.60± 0.22	0.30±0.10	47.2±9.2	6.0±0.5	13.4±1.8	65.5±4.3	14.7±1.4
3.0Levulinate	7	3.77± 0.13	0.29±0.05	47.0±8.9	5.9±0.4	13.4±1.7	66.1±3.5	14.7±1.5
3.0Levulinate	14	4.29± 0.21	0.20±0.08	47.4±11.2	5.6±0.5	13.3±2.9	66.7±2.3	14.4±2.9
$LSD_{0.05}$		0.59	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

[1]APC = $\log_{10}$ aerobic plate count/gm sample, [2]TBA = Thiobarbituric acid number, [3]L^* = Lightness; a^* = redness; b^* = yellowness; Hue angle = $\tan^{-1}(b^*/a^*)$, where lower values indicate more redness; Saturation index = $(a^{*2} + b^{*2})^{1/2}$

Table 7. Effect of treatment, day, and treatment * day interaction on L* value of pork sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	12.1490	4	3.037241	.4533317	.7694618
Error	321.5913	48	6.699819		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	33.8363	4	8.459076	1.262583	.2977013
Error	321.5913	48	6.699819		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	56.8310	16	3.551938	.5301542	.9173360
Error	321.5913	48	6.699819		

Table 8. Effect of treatment, day, and treatment * day interaction on a* value of pork sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	4.35223	4	1.088057	1.615959	.1855524
Error	32.31932	48	0.673319		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	25.25634	4	6.314086	9.377551	.0000110
Error	32.31932	48	0.673319		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	10.52920	16	.6580749	.9773594	.4948473
Error	32.31932	48	.6733193		

Table 9. Effect of treatment, day, and treatment * day interaction on b* value of pork sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	1.98213	4	0.495533	.4147891	.7971259
Error	57.34379	48	1.194662		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	5.58763	4	1.396908	1.169291	.3361454
Error	57.34379	48	1.194662		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	12.76440	16	0.797775	.6677828	.8100078
Error	57.34379	48	1.194662		

Table 10. Effect of treatment, day, and treatment * day interaction on hue angle of pork sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	52.4721	4	13.11803	1.981626	.1123493
Error	317.7521	48	6.61983		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	230.8069	4	57.70172	8.716489	.0000226
Error	317.7521	48	6.61983		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	108.9753	16	6.810958	1.028871	.4452519
Error	317.7521	48	6.619835		

Table 11. Effect of treatment, day, and treatment * day interaction on saturation index of pork sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	3.22054	4	0.805135	.5479661	.7013508
Error	70.52716	48	1.469316		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	16.23759	4	4.059398	2.762781	.0380192
Error	70.52716	48	1.469316		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	16.02830	16	1.001769	.6817928	.7970017
Error	70.52716	48	1.469316		

Table 12. Effect of treatment, day, and treatment * day interaction on $\log_{10}$APC value of pork sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	10.63918	4	2.659796	20.33876	.0000000
Error	6.27719	48	0.130775		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	32.56093	4	8.140233	62.24622	.0000000
Error	6.27719	48	0.130775		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	6.126938	16	.3829336	2.928193	.0020560
Error	6.277187	48	.1307747		

Table 13. Effect of treatment, day, and treatment * day interaction on TBA Values of pork sausage after blocking for effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	.0188295	4	.0047074	.7815823	.5431140
Error	.2710294	45	.0060229		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	.1630146	4	.0407536	6.766476	.0002365
Error	.2710294	45	.0060229		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	.1245959	16	.0077872	1.292944	.2431819
Error	.2710294	45	.0060229		

Table 14. Effect of treatment, day, and treatment * day interaction on pH value of pork sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	.0516480	4	.0129120	.3622082	.8331055
Error	.8912000	25	.0356480		

Day effect

Case name	Sums of squares	DF	MEAN	F	p - level
Effect	.0580880	4	.0145220	.4073721	.8015530
Error	.8912000	25	.0356480		

Treatment * Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	.0766320	16	.0047895	.1343554	.9999343
Error	.8912000	25	.0356480		

Appendix B

Data from Turkey Sausage Experiment

LIST OF ABBREVIATIONS

TRTMT = Treatment

REP = Replicate

SATUR = Saturation

CNTRL = Control

LAC 15 = Sodium lactate at 1.5% of meat weight equivalent

LAC 30 = Sodium lactate at 3.0% of meat weight equivalent

LEV 15 = Sodium levulinate at 1.5% of meat weight equivalent

LEV 30 = Sodium levulinate at 3.0% of meat weight equivalent

Table 15. Turkey color data (average of three readings combined for a given day).

TRTMT	REP	DAY	L	A	B	HUE	SATUR
CNTRL	1	0	42.95	2.13	10.35	78.37	10.56
CNTRL	1	1	39.79	1.96	9.32	78.21	9.53
CNTRL	1	3	39.61	1.86	9.22	78.59	9.41
CNTRL	1	7	37.39	1.90	8.72	77.66	8.92
CNTRL	1	14	34.95	1.81	8.76	78.32	8.94
CNTRL	2	0	41.06	1.85	9.33	78.76	9.51
CNTRL	2	1	37.98	2.14	9.20	76.92	9.45
CNTRL	2	3	38.80	2.05	9.47	77.73	9.69
CNTRL	2	7	37.96	1.91	9.96	79.17	10.14
CNTRL	2	14	37.88	1.56	8.43	79.66	8.58
CNTRL	3	0	39.98	2.14	10.20	78.09	10.42
CNTRL	3	1	39.38	2.38	9.63	76.10	9.92
CNTRL	3	3	41.49	2.01	10.96	79.66	11.15
CNTRL	3	7	37.54	2.10	9.92	78.14	10.14
CNTRL	3	14					
LAC15	1	0	47.19	2.11	11.01	79.15	11.21
LAC15	1	1	43.89	2.39	11.48	78.24	11.73
LAC15	1	3	39.04	1.80	9.40	79.18	9.57
LAC15	1	7	36.92	2.13	9.37	77.31	9.62
LAC15	1	14	35.19	1.38	8.23	80.49	8.34
LAC15	2	0	43.99	2.26	11.31	78.71	11.54
LAC15	2	1	40.49	2.17	10.41	78.23	10.63
LAC15	2	3	40.11	1.79	9.68	79.49	9.85
LAC15	2	7	38.25	2.04	10.24	78.73	10.43
LAC15	2	14	36.94	1.17	8.55	82.23	8.63
LAC15	3	0	39.46	2.20	9.61	77.09	9.87
LAC15	3	1	39.41	2.20	10.23	77.72	10.48
LAC15	3	3	39.30	1.98	10.47	79.31	10.65

LAC15	3	7	37.85	1.91	8.80	77.76	9.00
LAC15	3	14					
LAC30	1	0	42.64	2.37	10.27	77.02	10.54
LAC30	1	1	41.71	2.46	10.68	77.08	10.97
LAC30	1	3	38.25	2.09	9.51	77.58	9.74
LAC30	1	7	37.04	1.89	9.01	78.13	9.21
LAC30	1	14	36.03	2.15	9.85	77.62	10.09
LAC30	2	0	41.17	2.14	10.22	78.19	10.44
LAC30	2	1	38.14	2.12	9.50	77.38	9.73
LAC30	2	3	38.93	1.92	9.23	78.29	9.43
LAC30	2	7	34.82	1.77	9.22	79.13	9.39
LAC30	2	14	38.00	1.15	8.26	82.17	8.34
LAC30	3	0	41.14	2.47	10.24	76.46	10.54
LAC30	3	1	39.34	2.07	9.85	78.20	10.07
LAC30	3	3	38.94	2.30	10.94	78.12	11.18
LAC30	3	7	35.34	2.07	9.34	77.51	9.57
LAC30	3	14					
LEV15	1	0	44.66	2.25	10.89	78.33	11.12
LEV15	1	1	41.51	2.57	11.22	77.13	11.51
LEV15	1	3	39.87	2.17	10.33	78.19	10.56
LEV15	1	7	39.09	2.01	6.90	61.78	7.44
LEV15	1	14	38.40	1.78	8.72	78.22	8.91
LEV15	2	0	43.25	1.95	10.63	79.56	10.80
LEV15	2	1	39.44	2.13	9.94	77.90	10.16
LEV15	2	3	39.52	1.86	9.75	79.22	9.93
LEV15	2	7	39.16	2.11	10.07	78.14	10.29
LEV15	2	14	37.57	1.04	8.69	83.17	8.75
LEV15	3	0	41.87	2.52	11.05	77.10	11.33
LEV15	3	1	41.17	2.12	10.74	78.79	10.95
LEV15	3	3	39.23	1.95	9.73	78.67	9.92
LEV15	3	7	37.22	2.14	9.56	77.41	9.81
LEV15	3	14					
LEV30	1	0	45.38	2.32	11.22	78.34	11.46

LEV30	1	1	40.85	2.49	11.45	77.79	11.72
LEV30	1	3	39.69	2.02	9.77	78.35	9.98
LEV30	1	7	39.94	1.82	10.21	79.86	10.37
LEV30	1	14	36.53	1.74	9.30	79.34	9.46
LEV30	2	0	41.77	2.11	10.34	78.46	10.56
LEV30	2	1	40.26	1.99	10.29	79.07	10.48
LEV30	2	3	38.12	1.93	9.92	78.94	10.11
LEV30	2	7	36.86	1.84	9.45	79.01	9.63
LEV30	2	14	37.09	1.14	9.55	83.21	9.62
LEV30	3	0	45.62	2.55	11.64	77.61	11.92
LEV30	3	1	41.86	2.07	10.28	78.59	10.48
LEV30	3	3	40.82	1.87	10.69	80.08	10.85
LEV30	3	7	36.34	1.82	9.45	79.08	9.62
LEV30	3	14					

Table 16. Table for TBA and $\log_{10}$APC values for turkey sausage (average of two readings for TBA as well as $\log_{10}$APC combined for the given day).

TRTMT	REP	DAY	TBA	$\log_{10}$APC
CNTRL	1	0	0.29	4.70
CNTRL	1	1	0.65	4.52
CNTRL	1	3	0.62	4.54
CNTRL	1	7	0.49	4.96
CNTRL	1	14	0.35	5.71
CNTRL	2	0	0.70	4.47
CNTRL	2	1	0.58	4.55
CNTRL	2	3	0.52	4.55
CNTRL	2	7	0.41	4.51
CNTRL	2	14	0.51	5.94
CNTRL	3	0	0.45	3.61
CNTRL	3	1	0.85	3.74
CNTRL	3	3	0.39	3.58
CNTRL	3	7	0.38	3.98
CNTRL	3	14	0.43	4.72
LAC15	1	0	0.35	4.07
LAC15	1	1	0.90	4.20
LAC15	1	3	0.86	4.61
LAC15	1	7	0.49	4.26
LAC15	1	14	0.36	5.37
LAC15	2	0	0.74	4.37
LAC15	2	1	0.54	4.29
LAC15	2	3	0.55	4.46
LAC15	2	7	0.35	4.58
LAC15	2	14	0.60	4.52
LAC15	3	0	0.54	3.41
LAC15	3	1	0.36	3.64

LAC15	3	3	0.39	3.82
LAC15	3	7	0.41	3.62
LAC15	3	14	0.57	4.36
LAC30	1	0	0.39	3.45
LAC30	1	1	0.79	3.71
LAC30	1	3	0.48	3.52
LAC30	1	7	0.39	3.68
LAC30	1	14	0.40	3.81
LAC30	2	0	0.72	4.69
LAC30	2	1	0.77	4.63
LAC30	2	3	0.46	4.61
LAC30	2	7	0.37	4.50
LAC30	2	14	0.61	4.47
LAC30	3	0	0.41	3.48
LAC30	3	1	0.41	3.70
LAC30	3	3	0.35	3.62
LAC30	3	7	0.46	3.60
LAC30	3	14	0.52	3.48
LEV15	1	0	0.36	4.02
LEV15	1	1	0.77	3.84
LEV15	1	3	0.57	4.00
LEV15	1	7	0.70	4.18
LEV15	1	14	0.39	4.71
LEV15	2	0	0.78	4.38
LEV15	2	1	0.82	4.33
LEV15	2	3	0.66	4.56
LEV15	2	7	0.38	4.49
LEV15	2	14	0.60	4.54
LEV15	3	0	0.43	3.62
LEV15	3	1	0.37	3.67
LEV15	3	3	0.48	3.81
LEV15	3	7	0.39	3.50

LEV15	3	14	0.56	3.44
LEV30	1	0	0.48	3.52
LEV30	1	1	0.80	3.62
LEV30	1	3	0.54	3.63
LEV30	1	7	0.44	3.71
LEV30	1	14	0.41	3.76
LEV30	2	0	0.72	4.39
LEV30	2	1	0.61	4.53
LEV30	2	3	0.60	4.52
LEV30	2	7	0.46	4.53
LEV30	2	14	0.54	4.47
LEV30	3	0	0.42	3.55
LEV30	3	1	0.49	3.85
LEV30	3	3	0.41	3.55
LEV30	3	7	0.40	3.77
LEV30	3	14	0.54	3.53

Table 17. Table with pH values for turkey sausage for the given day.

TRTMT	REP	DAY	pH
CNTRL	1	0	5.79
LAC15	1	0	5.81
LAC30	1	0	5.81
LEV15	1	0	5.93
LEV30	1	0	5.95
CNTRL	1	1	5.82
LAC15	1	1	5.82
LAC30	1	1	5.85
LEV15	1	1	5.89
LEV30	1	1	5.92
CNTRL	1	3	5.73
LAC15	1	3	5.79
LAC30	1	3	5.78
LEV15	1	3	5.85
LEV30	1	3	5.88
CNTRL	1	7	5.88
LAC15	1	7	5.83
LAC30	1	7	5.82
LEV15	1	7	5.88
LEV30	1	7	5.91
CNTRL	1	14	5.83
LAC15	1	14	5.78
LAC30	1	14	5.79
LEV15	1	14	5.88
LEV30	1	14	5.90
CNTRL	2	0	5.84
LAC15	2	0	5.90
LAC30	2	0	5.89

LEV15	2	0	5.94
LEV30	2	0	5.92
CNTRL	2	1	5.87
LAC15	2	1	5.90
LAC30	2	1	5.91
LEV15	2	1	5.94
LEV30	2	1	5.98
CNTRL	2	3	5.86
LAC15	2	3	5.88
LAC30	2	3	5.87
LEV15	2	3	5.92
LEV30	2	3	5.95
CNTRL	2	7	5.82
LAC15	2	7	5.88
LAC30	2	7	5.81
LEV15	2	7	5.81
LEV30	2	7	5.89
CNTRL	2	14	5.90
LAC15	2	14	5.81
LAC30	2	14	5.77
LEV15	2	14	5.90
LEV30	2	14	5.95
CNTRL	3	0	5.82
LAC15	3	0	5.83
LAC30	3	0	5.85
LEV15	3	0	5.89
LEV30	3	0	5.95
CNTRL	3	1	5.89
LAC15	3	1	5.83
LAC30	3	1	5.82
LEV15	3	1	5.89
LEV30	3	1	5.92

CNTRL	3	3	5.69
LAC15	3	3	5.67
LAC30	3	3	5.67
LEV15	3	3	5.74
LEV30	3	3	5.75
CNTRL	3	7	5.78
LAC15	3	7	5.76
LAC30	3	7	5.73
LEV15	3	7	5.83
LEV30	3	7	5.87
CNTRL	3	14	5.82
LAC15	3	14	5.76
LAC30	3	14	5.81
LEV15	3	14	5.86
LEV30	3	14	5.87

Table 18. Effect of sodium lactate and sodium levulinate levels on mean aerobic plate count[1], thiobarbituric acid number[2] and hunter color values[3] of fresh turkey sausage stored at 2°C.

Treatment	Storage days	APC	TBA	L^*	a^*	b^*	Hue	Saturation
Control	0	4.26±0.57	0.48±0.21	41.3±1.5	2.0±0.2	10.0±0.6	78.4±0.3	10.2±0.6
Control	1	4.27±0.46	0.70±0.14	39.1±0.9	2.2±0.2	9.4±0.2	77.1±1.1	9.6±0.3
Control	3	4.23±0.56	0.51±0.12	40.0±1.4	2.0±0.1	9.9±0.9	78.7±1.0	10.1±0.9
Control	7	4.48±0.49	0.43±0.06	37.6±0.3	2.0±0.1	9.5±0.7	78.3±0.8	9.7±0.7
Control	14	5.45±0.65	0.43±0.08	36.4±2.1	1.7±0.2	8.6±0.2	79.0±0.9	8.8±0.3
1.5Lactate	0	3.95±0.49	0.54±0.20	43.5±3.9	2.2±0.1	10.6±0.9	78.3±1.1	10.9±0.9
1.5Lactate	1	4.05±0.35	0.60±0.27	41.3±2.3	2.3±0.1	10.7±0.7	78.1±0.3	10.9±0.7
1.5Lactate	3	4.30±0.42	0.60±0.24	39.5±0.6	1.9±0.1	9.8±0.6	79.3±0.2	10.0±0.6
1.5Lactate	7	4.16±0.49	0.42±0.07	37.7±0.7	2.0±0.2	9.5±0.7	78.0±0.7	9.7±0.7
1.5Lactate	14	4.75±0.54	0.51±0.13	36.1±1.2	1.3±0.2	8.4±0.2	81.4±1.2	8.5±0.2
3.0Lactate	0	3.87±0.71	0.51±0.19	41.7±0.9	2.3±0.2	10.2±0.0	77.2±0.9	10.5±0.1
3.0Lactate	1	4.01±0.54	0.65±0.22	39.7±1.8	2.2±0.2	10.0±0.6	77.6±0.6	10.3±0.6
3.0Lactate	3	3.91±0.61	0.43±0.07	38.7±0.4	2.1±0.2	9.9±0.9	78.0±0.4	10.1±0.9
3.0Lactate	7	3.93±0.50	0.41±0.05	35.7±1.2	1.9±0.2	9.2±0.2	78.3±0.8	9.4±0.2
3.0Lactate	14	3.92±0.51	0.51±0.11	37.0±1.4	1.6±0.7	9.1±1.1	79.9±3.2	9.2±1.2
1.5Levulinate	0	4.00±0.38	0.52±0.22	43.3±1.4	2.2±0.3	10.9±0.2	78.3±1.2	11.1±0.3
1.5Levulinate	1	3.95±0.35	0.65±0.25	40.7±1.1	2.3±0.3	10.6±0.6	77.9±0.8	10.9±0.7

1.5Levuli-nate	3	4.12±0.39	0.57± 0.09	39.5±0.3	2.0±0.2	9.9±0.3	78.7±0.5	10.1±0.4
1.5Levuli-nate	7	4.06±0.50	0.49± 0.18	38.5±1.1	2.1±0.1	8.8±1.7	72.4±9.2	9.2±1.5
1.5Levuli-nate	14	4.23±0.69	0.51± 0.11	38.0±0.6	1.4±0.5	8.7±0.0	80.7±3.5	8.8±0.1
3.0Levuli-nate	0	3.82±0.50	0.54± 0.16	44.3±2.2	2.3±0.2	11.1±0.7	78.1±0.5	11.3±0.7
3.0Levuli-nate	1	4.00±0.47	0.64± 0.15	41.0±0.8	2.2±0.3	10.7±0.7	78.5±0.6	10.9±0.7
3.0Levuli-nate	3	3.90±0.54	0.52± 0.10	39.5±1.4	1.9±0.1	10.1±0.5	79.1±0.9	10.3±0.5
3.0Levuli-nate	7	4.00±0.46	0.43± 0.03	37.7±1.9	1.8±0.0	9.7±0.4	79.3±0.5	9.9±0.4
3.0Levuli-nate	14	3.92±0.49	0.50± 0.07	36.8±0.4	1.4±0.4	9.4±0.2	81.3±2.7	9.5±0.1
LSD $_{0.05}$		0.48	N.S.	2.38	N.S.	N.S.	N.S.	N.S.

[1]APC = $\log_{10}$ aerobic plate count/gm sample, [2]TBA = Thiobarbituric acid number,

[3]L^* = Lightness; a^* = redness; b^* = yellowness; Hue angle = $\tan^{-1}(b^* / a^*)$, where lower values indicate more redness; Saturation index = $(a^{*2} + b^{*2})^{1/2}$

Table 19. Effect of treatment, day, and treatment * day interaction on L* value of turkey sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	24.22779	4	6.056947	2.851271	.0350144
Error	91.34479	43	2.124297		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	307.7342	4	76.93355	36.21600	.0000000
Error	91.3448	43	2.12430		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	27.29407	16	1.705879	.8030322	.6744322
Error	91.34479	43	2.124297		

Table 20. Effect of treatment, day, and treatment * day interaction on a* value of turkey sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	0.113278	4	.0283194	1.149756	.3462487
Error	1.059126	43	.0246308		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	4.079892	4	1.019973	41.41042	.0000000
Error	1.059126	43	0.024631		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	0.525134	16	.0328209	1.332512	.2222890
Error	1.059126	43	.0246308		

Table 21. Effect of treatment, day, and treatment * day interaction on b* value of turkey sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	3.97701	4	.9942520	2.353485	.0689035
Error	18.16575	43	.4224594		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	24.46637	4	6.116591	14.47853	.0000001
Error	18.16575	43	0.422459		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	5.42631	16	.3391443	.8027854	.6746878
Error	18.16575	43	.4224594		

Table 22. Effect of treatment, day, and treatment * day interaction on hue angle of turkey sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	25.5188	4	6.379694	1.466945	.2289093
Error	187.0056	43	4.348968		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	69.5301	4	17.38251	3.996928	.0076286
Error	187.0056	43	4.34897		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p – level
Effect	83.7229	16	5.232680	1.203200	.3043079
Error	187.0056	43	4.348968		

Table 23. Effect of treatment, day, and treatment * day interaction on saturation index of turkey sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - .level
Effect	3.76817	4	.9420415	2.353301	.0689208
Error	17.21318	43	.4003065		

Day effect

Case name	Sums of Squares	DF	MEAN	F	P - level
Effect	26.75299	4	6.688249	16.70782	.0000000
Error	17.21318	43	0.400306		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	5.24709	16	.3279429	.8192297	.6576276
Error	17.21318	43	.4003065		

Table 24. Effect of treatment, day, and treatment * day interaction on $log_{10}APC$ value of turkey sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	3.930411	4	.9826028	11.34247	.0000015
Error	4.158261	48	.0866304		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	2.019531	4	.5048828	5.828007	.0006616
Error	4.158261	48	.0866304		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	2.665232	16	.1665770	1.922846	.0414561
Error	4.158261	48	.0866304		

Table 25. Effect of treatment, day, and treatment * day interaction on TBA Values of turkey sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	0.023383	4	.0058458	.2791181	.8900612
Error	1.005309	48	.0209439		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	0.364657	4	.0911642	4.352769	.0043924
Error	1.005309	48	.0209439		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	0.078477	16	.0049048	.2341866	.9986882
Error	1.005309	48	.0209439		

Table 26. Effect of treatment, day, and treatment * day interaction on pH value of turkey sausage after blocking the effect of replication.

Treatment effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	.1088613	4	.0272153	15.26950	.0000000
Error	.0855520	48	.0017823		

Day effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	.0648347	4	.0162087	9.094073	.0000150
Error	.0855520	48	.0017823		

Treatment * Day interaction effect

Case name	Sums of Squares	DF	MEAN	F	p - level
Effect	.0173920	16	.0010870	.6098748	.8601329
Error	.0855520	48	.0017823		

APPENDIX C

Permission letter to reprint journal article

JOHN O'NEIL

From: mihir vasavada <mihirvasavada_2000@yahoo.com>
To: <msbrewer@uiuc.edu>
Sent: Monday, March 01, 2004 5:19 PM
Subject: Permission-to-reprint letter

Hi Dr. Brewer,

This is Mihir Vasavada from Utah State University, Logan, Utah. I have a publication in the Journal of Muscle Foods Volume 14, Number 2, May 2003 Issue. (Page no. 119-129).

I have included this publication as a Chapter (Chapter 4) of my Masters Thesis here at Utah State University. As per the requirements of the Graduate School, they require a Permission-to-reprint letter from the copyright holder to accompany all the published chapters in the thesis.

I request you to please send me a forwarding FAX letter for the same at the FAX No. 435-797-2379 and if possible a paper copy of the letter to :

C/o Mihir Vasavada (Utah State Univ.
Department of Nutrition and Food Sciences
750 N 1200 E, Logan, UT-84322.

I would appreciate if you could send the FAX letter at the earliest, and the paper copy (whenever time permits) so that I can go forward towards the submission of the Thesis to the Graduate School. It will help me a lot.

Hoping for a favorable and prompt response.

Thanking you.

Sincerely,
Mihir Vasavada.

=====

mihir.n.vasavada
930n 700e, appt. no. 5
logan, ut 84321
phone no. (home): 435-787-2933
(lab.) : 435-797-2114

Dear Mihar, March 2, 2004

Permission granted,

Sincerely,

John J. O'Neil
Publisher

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