VOLATILE FATTY ACIDS AS PRESERVATIVES FOR SHORT TERM STORAGE OF BOVINE HIDES

By

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VOLATILE FATTY ACIDS AS PRESERVATIVES

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BOVINE HIDES

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CHAPTER I

INTRODUCTION

In the past treating bovine hides with salt (0.75-1 lb. salt/lb. hide) has been the conventional method for preservation. In recent years there has been a growing concern related to this practice. The dumping of the used salt, as waste, is recognized as a pollution problem. Salting the hides can double the weight of material to be transported from a slaughter plant to a tannery. An alternative method of preservation that would reduce these problems is desirable.

Another factor to consider in finding new methods of preservation of bovine hide is the interest in using collagen (the chief protein in hide) from the hides, as an ingredient in foods. Collagen has been suggested as an ingredient which may function as a binder, filler, extender, moisturizer, texturizer, and nutrient enhancer. Since collagen has been proposed for food use, it is important to consider the possibility that a preservative might in some way remain with the collagen through the recovery process and be present in a food ingredient. This may lead to its presence in food products. For this reason it would be advantageous to have a preservative that would be acceptable

to the Food and Drug Administration (FDA) as a food additive.

The objective of this study was to find a method, other than salting, of bovine hide preservation; such that the preservative would inhibit growth of spoilage microorganisms and food borne pathogens on th hides for four to eight days. The preservative should pose less of a pollution problem than the present method of salting, be economical, easy to use, and be acceptable to the FDA as a food additive. Results from this study could have important applications in other areas of hide or food preservation.

CHAPTER II

REVIEW OF LITERATURE

Short term preservation of hides has been defined by Hopkins et al. (1973) as the prevention of spoilage for seven days following removal of the hide from the carcass. The term "preservation" refers to not only the retention of leather-making properties, but also the control, reduction, or elimination of microbial contaminants.

Factors Affecting Hide Preservation

There are many variables affecting hide preservation. Hopkins et al. (1973) mentioned several. These included microbes, enzymes, hide composition, and hide conditions. The types and numbers of bacteria, yeasts, and molds might vary from hide to hide. Enzymes that could alter some characteristics of the hide would be present in the tissues, blood, manure, and microorganisms associated with the hide. Hide composition depends on species and age, and could vary with respect to amount of fat, protein, and hair. Several factors might affect the growth and action of microorganisms. Hide condition after removal from the carcass could be very important; trimming, fleshing, and washing could affect the numbers of microorganisms present. Time would be

another significant factor; the length of time from hide removal, storage to curing is extremely important. Finally, a variable of great influence would be the temperature at which the hide was held at each step.

Generally, a preservative is needed to prevent spoilage of the hide. Hopkins et al. (1973) and Bailey et al. (1976) have been concerned with having an economical preservative. A preservative might create a major effluent or waste problem, Orlita and Navratil (1978), Haffner and Haines (1975), Cooper (1973) and Hopkins et al. (1973). The labor involved, or room needed was suggested as a point of concern by Bailey et al. (1976).

Methods of Preservation of Bovine Hides

In studying various chemicals as preservatives for hides, Hopkins et al. (1973) and Hopkins and Bailey (1975) placed 100 g samples of hide (stored in a freezer until needed) in one quart mason jars. Samples were immersed in designated treatment solutions and the tops were sealed. The jars were placed on a rotary shaker for 15 minutes. The sealed jar, with hide still immersed in treatment solutions, were stored at room temperature and were examined at different time intervals. The chemicals tested included 0.25 to 2.0% (based on weight of hide) sodium bisulfite alone, 0.25 to 2.0% sodium sulfite plus 2.0% sodium bisulfate, and 0.25 to 2.0% sodium sulfite plus 1.0% acetic acid. All

solutions contained 0.03% tergitol 15-S-9. The hide samples were evaluated subjectively by sensory examination, in which an "off odor" produced during storage was taken as a signal that preservation was failing. The time was then noted and a microbial count determined in most cases. All chemicals tested were considered potentially usable as a short term (6 days) preservative. The combinations of chemicals that maintained an acceptable odor for the longest periods of time were: 2.0% sodium bisulfite alone (33 days), 1.5% sodium sulfite plus 2.0% sodium bisulfate (30 days), and 0.5% sodium sulfite plus 1% acetic acid (31 days). This confirmed the work of Bailey et al. (1974).

Hopkins and Bailey (1975) and Bailey and Hopkins (1975) pointed out that acetic acid was a better source of acid than bisulfate to enhance the preservation properties of sulfite. Also, acetic acid seemed to mask the odor of SO_2. Experiments conducted by Bailey and Hopkins (1977) demonstrated again that sulfite/acetic acid treatment was an effective preservative for seven days of storage. They found this treatment had no adverse effect on quality of leather manufactured from treated hides. During Bailey and Hopkins (1977) studies an increase in storage temperature due to summer time (July-August) heat was taken into account. Leather made from the sulfite/acetic acid treated hides stored at the warmer temperatures was also acceptable.

Further experiments of Hopkins and Bailey (1975) involved the use of 1.5% sulfite/2.0% sodium bisulfate and

1.5% sulfite/1.0% acetic acid treatments on hide samples which were not held in closed containers as was done in previous studies. The samples were either wrung or allowed to drip drain prior to storage. They were stored by three different methods: hung over rods, laid flat on stainless steel wire screens, or in polyethylene bags. The results were inconclusive.

Bailey et al. (1976) investigated the effect of various methods of applying the treatment. In method I, the hides were sprayed with sulfite/acetic acid (in an amount equal to 1% of the weight of the sides being treated) then placed in 55 gallon fiber barrels. The barrels were covered with airtight lids and held at ambient temperatures for seven days. In method II, the hide samples were agitated in sulfite/acetic acid solutions (in an amount equal to 1% of the weight of the sides being treated) in 55 gallon fiber barrels. These were then covered with an airtight lid and held at ambient temperatures for seven days. The majority of spray treated (method I) hides were less acceptably preserved than the hides that were submerged (method II).

Haffner and Haines (1975) investigated the effects of biocides applied to hides either as a spray to the hide surface or as a soak liquor for complete immersion of the hide. Their work showed that a 5% solution of sodium chlorite or a mixture of 15% Gloquat C (a quarternary ammonium compound) plus 10% Glokill 77 (linear and cyclic hydroxylamine) sprayed over the flesh surface of a hide retarded

bacterial growth for a period of six days at 26°C. Hides immersed in 0.4% Vantocil IB (polymeric biquianide) plus 0.2% Vantoc CL (lauryl dimethyl benzyl ammonium chloride) remained in a good state of preservation for eight days at 26°C.

Haffner and Haines (1975) also demonstrated that a number of biocides were more effective when used as a soak treatment rather than a spray for preserving hides. These included Vantocil IB, pentachlorophenate, metabisulphate, and sulfite plus acetic acid.

Experiments conducted by Haines (1973) showed that sheepskins were adequately preserved for two days by spraying the flesh surface immediately after flaying with a 10% solution of Vantocil IB. A somewhat longer period of preservation was obtained if the hides were piled flesh to flesh or if they were cooled to 20°C or below.

Of eight different bacteriocide treatments (0.05% B-napthol, 0.2% sodium 2,4,6-trichlorphenate, 0.2% sodium 2,4,5-trichlorphenate, 0.2% sodum pentachlorphenate, 0.2% sodium 2,3,4- and 2,3,6-trichlorphenates, 1% napthalene plus 3% soda ash, 1% napthalene plus 1% boric acid and B-napthol plus the chlorinated phenates) tested by Vivian (1969) only hides cured with 1% napthalene plus 1% boric acid were free of bacterial growth. Two percent soda ash plus one percent nathalene along with salting was effective in preservation of hides (Hausam, 1964). Espie and Manderson (1979) found agitating sheepskins in salt with 1% napthalene, plus a 1% pH modifier (boric acid or oxalic acid), plus a 0.25%

fungiside (Busan 30 or Chloroacetamide or Benlate) to be the most effective biocide they tested. Boric acid (1%) plus napthalene (1%) used as an antiseptic in salt, according to Woods et al. (1970) had both bactericidal and bacteriostatic effects when used for post brining preservation. Use of various concentrations of boric acid alone resulted in preservation of the hides up to six days according to George and Krishnamurthy (1966). Hide samples soaked for 15 minutes in saturated aqueous boric acid were adequately preserved for five days at 30°C according to Hughes (1974). Hides treated in a like manner were considered satisfactory after 29 days storage at 14°C.

Of twenty-five different antiseptics at five different concentrations tested, Hendry, Cooper and Woods (1971) found zinc chloride and zinc silicofluoride (both 0.1% concentration) to be the most effective, while sodium fluoride, BSM-11 (50% 2,4,6-trichlorophenate plus 10% phenyl mercuric acetate), Dowicide (79% sodium pentachlorphenate plus 11% other sodium salts), B-napthol, pentachlorphenol, sodium pentachlorophenate, and sodium silicofluoride were effective at higher concentrations (0.25-0.75%). Sivaparvathi and Nandy (1973) also found that sodium silicofluoride provided adequate preservation, although other preservatives were more effective.

Zinc salts, studied by Margold and Heidemann (1977), were considered effective in controlling growth of bacteria. However, they did not protect the hide from mildew. In

addition they found three especially effective bacteriocides, chloracetamide, p-toluene sulfonamide and an isothiazoline manufactured by Rohm and Haas. Combinations of the more effective agents had a synergistic effect.

Cooper (1973) and Cooper and Galloway (1974) demonstrated effective preservation of hides for 20-21 days at 25°C employing several antiseptics, either separately or in mixtures. The tested antiseptics were sodium chloride, sodium silicofluoride, sodium pentachlorophenate, sodium fluoride, zinc chloride and "stermist" (contains 31% quarternary ammonium compound). Leather manufactured from these treated hides was of slightly inferior quality. Prajs (1967) reported just the opposite. Leather from sheepskins, which had been preserved by the use of sodium silicofluoride did not show any inferior physical or chemical properties compared with leathers from sheepskins on which other preservation chemicals had been used. Woods et al. (1970) reported evidence that sodium silicofluoride improved the quality of the crust leather, but in this case the antiseptic was being used as inhibitors of post brining bacterial activity rather than on fresh hides.

Gaseous chlorine was investigated as a hide preservative by Money (1970). Portions of hide were hung in a sealed container which was filled with chlorine and left for ten to forty minutes. Exposure to chlorine for ten minutes was sufficient to prevent bacterial growth at 30°C for six days and the leather made was acceptable. Leathers made

from the hides treated for 20, 30, or 40 minutes were not acceptable. Howard, Rochwell, and Crist (1929) reported that chlorine dissolved in water was not a reliable preservative unless concentrations were used that resulted in damage to the hide.

Money (1970) found the use of concentrated sodium chlorite sprays or diluted solutions of sodium chlorite plus sodium pentachlorphenate was acceptable for use as short-term preservatives. These treatments allowed the hides to be held for several days without damage. She also stated the length of time the hides could be held depended on storage temperature and concentration of the preservatives in the solution. Since the concentrations could be adjusted, acceptable treatments for winter or summer temperatures could be developed.

Money (1970) warned that sodium chlorite was a strong oxidizing agent and required certain precautions for safe use. It should not come in contact with combustable materials, including rubber, or with reducing agents or mineral acids. Because of the hazards associated with its use, it is considered to be an unsafe preservative. Thus in 1974 alternative methods which included the use of benzalkonium chloride, zinc chloride, sodium dichloroisocyanurate plus sodium pentachlophenate, sodium fluoroide plus sodium pentachlorphenate, (0.1%) Proxel, or calcium hypochlorite plus sodium pentachlorphenate (other fungisides that were investigated were sodium trichlorphenate, phenol, and dichlorophen)

were studied. Preservatives that were not satisfactory under the conditions of the trials were the sodium dichloroisocyanurate solution, sodium fluoride solution, or the 0.1 percent Proxel treatment. Although benzalkonium chloride was effective, it was not as effective as the zinc chloride or calcium hypochlorite treatments. Hendry, Cooper and Woods (1971), Sipos and Vernes (1978), and Espie and Manderson (1979) agreed with Money and found the use of sodium fluorides inadequate. Hendry, Cooper and Woods (1971) and Sivaparvathi and Nandy (1973) found zinc chloride also to be an effective agent. George and Krishnamurthy (1966) and Margold and Heidemann (1977) also found zinc chloride to be an effective agent to inhibit bacteria, but it did not protect the hides from growth of mildew.

While zinc chloride treatments appeared to be the safest and cheapest method, in some cases zinc may create an effluent problem (Money, 1974). She recommended, in these cases, methods using calcium hypochlorite or sodium chlorite even though hazardous as an aqueous solution were the answer, proivded the chemicals were handled with caution.

Cordon et al. (1964), Benrud (1969) and Sivapanvathi and Nandy (1973) reported using benzalkonium chloride (BAC), a quarternary ammonium compound, as a preservative. They all found BAC to be effective as an inhibitor for short term preservation (5-7 days). Cordon et al. (1964) also tested BAC in conjunction with salt. They found if no salt treatment was included in the cure, the leather was not as uniform as desired.

Limited work was done with formaldehyde by Sharphouse and Kimweri (1978), neem oil by Krishnamurthi et al. (1977), N,N,-bis(methoxy)mehtyluron by Weaver et al. (1972), and butyl carbitol by Hopkins et al. (1971). Sharphouse and Kimweri (1978) recognized formaldehyde as an effective preservative and when used in small quantities it caused little effluent problems. The bitter principals, extracted from neem oil (using alcoholic solvents) were used by Krishnamurthi et al. (1977) and were effective for about two days. Weaver et al. (1972) noted hides from freshly slaughtered animals are preserved by treatment with a mixture of water, acetic or propionic acid, and N,N'-bis (methoxy) methyluron. Sodium acid sulfate was also effective when used in place of the acetic of propionic acid.

Twenty-six different preservatives were studied by Sivaparvathi and Nandy (1973), many of which were reported to have considerable preservative efficiency. The inhibitory action of the preservatives was tested against bacterial cultures responsible for spoilage of hides. Mercuric chloride (0.04%) and phenyl mercuric nitrate (0.04%) completely inhibited the growth of the organisms. Sodium silicofluoride (0.04%), benzalkonium chloride (0.04%) and sodium pentachlorophenate (0.04%) were moderately effective. Sodium borofluoride (0.04%) and para-chloro-meta-cresol (0.04%) were less effective than the previous mentioned chemicals, but significantly more effective than nineteen other chemicals tested.

Satisfactory preservative effects for bovine and porcine hides were obtained with propionic acid (10%), sodium pyrosulfite (5%), and sodium chlorite (2%) (Orlita and Navratil, 1978). Combination of chlorite and sodium tetraborate resulted in a higher effectiveness of preservation. The most effective of the tested materials were diethelyamine (1.5%) and Orthosan OV 143 (an antiseptic preparation).

Antimicrobial Action of Volatile Fatty Acids

Formic and acetic acid have been shown to be inhibitory to several organisms. Hentges (1967b) noted that shigellae were inhibited when grown with Klebsiella sp. Formic and acetic acid, metabolic products produced by Klebsiella sp., were responsible for the inhibition of shigellae. Goepfert and Hicks (1969) noted the effects of several volatile fatty acids (formic, acetic, propionic, and butyric) on Salmonella typhimurium. They stated that the sensitivity of S. typhimurium to volatile fatty acids depended in part on chain length of the acid. Although not of a profound nature, a general trend of decreasing bactericidal effect with increasing chain length of fatty acid was noted. Kham and Katamay (1969) suggested that shorter chain fatty acids exerted a bacteriostatic and/or a bactericidal effect on Salmonella species.

The inhibitory action of acetic acid is due to its undissociated molecule, not to hydrogen ion concentration alone (Levine and Fellers, 1940). Hentges (1967a) reported the toxicity of formic and acetic acids for Shigella was greatly influenced by the pH of the medium. At low pH (pH 6.0, pH 5.5), the toxicity of the acids was enhanced, however this was not due to a hydrogen ion effect alone. This evidence supports the conclusion that undissociated formic and acetic acid molecules are responsible for inhibition of Shigella growth (Hentges, 1967a). Goepfert and Hicks (1969) also agreed with the premise that the undissociated acid molecule is the bactericidal moiety.

Levine and Fellers (1940) compared acetic, lactic and hydrochloric acids for their bacteriostatic and bactericidal effects on a typical yeast (Saccharomyces cereviseae), mold (Aspergillus niger) and bacterium (Salmonella aertrycke). They found in the comparative studies the acetic acid was more toxic than either lactic or hydrochloric acid to S. aertrycke, S. cereviseae, and A. niger. These organisms were inhibited or destroyed at a higher pH value with acetic acid than with lactic or hydrochloric acids.

Acetic and formic acid have been investigated to be used as a santizer or preservative for many foods. Mountney and O'Malley (1965) studied the effect of ten organic acids on the general flora of poultry carcasses and found acetic acid effective for reducing bacterial numbers. Khan and Katamay (1967) concluded shorter chain fatty acids exert

inhibitory, and in some cases, bactericidal effects against Salmonella in meat and bone meal. Acetic acid alone and a mixture of acetic acid and propionic acid (60:40 w/w) were examined for the preservative effect on pork carcasses. Both were effective, but the use of propionic acid with acetic acid resulted in a reduction of microorganisms at higher pH than when only acetic acid was used (Reynolds and Carpenter, 1974). Numbers of bacteria were significantly reduced on lamb carcasses, refrigerated for twelve days, by spraying with a solution of acetic or lactic acid (Ockerman et al. 1974). Anderson et al. (1977a), Anderson et al. (1977b) and Anderson et al. (1979) demonstrated that a 3-4% solution of acetic acid was a highly effective sanitizer for beef. Quartey-Papafio et al. (1980) found 2% formic acid and 1% formic acid plus 1% acetic acid were the most effective combinations of acids that they had screened for antimicrobial effect on beef. Experiments by Hayashi et al. (1979) used acetic acid to retard growth of contaminating bacteria during shoyu-koji (Japanese fermented soy sauce) manufacturing process. The acetic acid showed a pronounced retarding effect on the growth of some tested strains of contaminating bacteria belonging to Micrococcus and Bacillus sp. The acid also effectively retarded the growth of some strains of bacteria belonging to Staphylococcus sp., Gram negative aerobes, and Enterobacteria, which were artificially added to the koji-substrate.

CHAPTER III

EXPERIMENTAL PROCEDURE

Source and Preparation of Hide Samples

A portion of bovine hide (approximately 12 X 24 cm) was obtained from the Oklahoma State University meat laboratory, immediately after slaughter. The section of hide was rinsed in cold tap water, placed in a sterile 18 ounce Whirl-Pak bag (Nasco; Atkinson, Wisconsin), packed in ice, and transported to the microbiology laboratory. It was held in the ice until utilized (no longer than one-half hour).

On a few occasions, when hide samples were not available from the meat laboratory, samples were obtained from Ralph's Meat Market of Perkins, Oklahoma. The hide section, with one exception, was handled as previously mentioned. The exception was the sample was not washed until it reached the microbiology laboratory.

Treatment Procedure

Assignment to Treatment

Sequentially numbered segments (approximately 3 x 3 cm) cut from the hide sample, were assigned at random to each of three treatments. Numbered (1-14) chips, uniform in size

and shape were drawn randomly from a container to determine order of treatment assignment. The first six numbers drawn were assigned as controls. The next four drawn were assigned to the 0.33M acid treatment and the final four were assigned to the 0.67M acid treatment. The set of six numbered chips (representing controls) were drawn randomly to determine storage time. The same procedure was used for the two other sets of number chips, for each treatment. Each sample was designated with an appropriate code number prior to treatment. An example of the assignment to treatments is shown in Table I.

TABLE I

EXAMPLE OF RANDOMIZATION AND CODING OF TREATMENT PORTIONS OF THE HIDE SAMPLE

TREATMENT	SEG. NO.	SAMPLE DAY	CODE
	5	0	AO-5
	9		AO-9
(A)	7	2	A2-7
CONTROL	1		A2-1
	4	4	A4-4
	12		A4-12
(B)	8	2	B2-8
0.33M	2		B2-2
ACID SOLUTION	11	4	B4-11
	6		B4-6
(C)	3	2	C2-3
0.67M	13		C2-13
ACID SOLUTION	10	4	C4-10
	14		C4-14

Preparation of Hide Segments

The iced hide sample (approximately 12 x 24 cm) was removed from the Whirl-Pak bag and aseptically cut into 14 (approximately 3 x 3 cm) segments (Figure 1). This was done on a sterile plastic cutting board, with a sterile single-edged razor blade for cutting. Sterile latex surgical gloves (Pharmaseal; Bendale, California) were worn in order to prevent further contamination of the hide. Further handling of the segments was done with the sterile forceps.

8	9	10	11	12	13	14
1	2	3	4	5	6	7

Figure 1. Segments

Treatment Solutions

Treatment solutions were prepared in wide-mouth plastic (polymethylpentene) bottles of 125 ml capacity (Nalgene 2117; Scientific Products). Six were prepared containing 100 ml of distilled water, four with 98 ml of distilled, and four more with 96 ml of distilled water. All were autoclaved at 121°C for 15 minutes. Prior to treatment of the hide segments, 2 ml of glacial acetic acid (Fisher Scientific; Fairlawn, New Jersey) Reagent A.C.S., was added to each of

the four jars containing 98 ml of sterile distilled water and 4 ml of glacial acetic acid was added to the jars containing 96 ml of sterile distilled water. This provided four jars containing 100 ml of 0.33M acetic acid and four with 0.67M acetic acid. The six jars containing 100 ml sterile distilled water were used for treating the control samples. Thus a single jar was provided for treating each individual hide segment.

When formic acid, 88% (Fisher Scientific Co.; Fairlawn, New Jersey) certified A.C.S., was to be used, six jars were prepared that contained a 100 ml of distilled water, four with 98.3 ml of distilled water, and four with 96.5 ml of distilled water. To obtain four jars of 0.33M formic acid and four more of 0.67 M formic acid, 1.7 ml and 3.5 ml of formic acid (88%) were added to the 98.3 ml and 96.5 ml of distilled water, respectively.

Treatment solutions of potassium sorbate were prepared in a similar manner. Once again, six jars contained 100 ml of distilled water. To obtain four jars of approximately 0.33M Sorbate and four jars of 0.67M sorbate, 5 g and 10 g of Monitor$^{k}_{TM}$ Granular or potassium sorbate (Monsanto; Saint Louis, Missouri), were added to 95.0 ml and 90.0 ml of distilled water, respectively.

Treatment Procedure

Designated hide segments (approximately 3 x 3 cm) were dipped into the 100 ml. of indicated treatment solutions

(see Table I) using sterile forceps for 30 seconds. The excess liquid was removed by shaking the segment after removal from the treatment solution.

Storage of Treated Samples

The dipped hide samples were stored in properly labeled, sterile plastic petri dishes (hair side down). All petri dishes, except the two containing hide segments, designated to be analyzed on day zero, were stored at 21°C in a jar with a loose fitting cover. Segments were removed for microbiological analyses on designated days.

Microbiological Examination

All segments of hide were analyzed for total numbers of aerobic microorganisms, Gram negative bacteria, Clostridium perfrigens, coagulase positive staphylococci, and yeasts and molds. Counts obtained from these experiments were calculated on a per gram basis.

Diluents and Dilution

Sterile 0.1% peptone (Difco) was used as the diluent. Dilution blanks (99 ml) were prepared according to procedures described in Compendium of Methods for the Microbiological Examination of Foods (Speck, 1976). The initial dilution (1:10) was prepared by weighing the hide segment into a sterile, empty, wide-mouthed dilution bottle. An amount of sterile diluent equal to nine times the weight of

the hide segment was added to the bottle. The dilution was shaken and subsequent dilutions prepared (using 99 ml dilution blanks), in accordance with procedures in Compendium of Methods for the Microbiological Examination of Foods (Speck, 1976). The required dilutions were placed into sterile petri plates and poured with the appropriate agar media. However, for the Baird-Parker medium it was necessary to spread the required dilutions onto the surface of the prepoured plates containing the solidified medium.

Media for Enumeration of Microbial Groups

Trypticase soy agar (Baltimore Biological Laboratories (BBL); Cokeysville, Maryland) prepared according to the manufacturer's direction was employed for the enumeration of total numbers of aerobic microorganisms. The plates were incubated at 32°C for 48 hours. After which, all colonies visible with the aid of a Quebec colony counter were counted.

Yeast and mold counts were determined by plating the samples on acidified potatoe dextrose agar (Difco). It was prepared and used according to the manufacturer's directions. Plates were incubated at room tempterature for five days. After which, both yeast and mold colonies visible with the aid of a Quebec colony counter were counted.

Gram negative bacteria were enumerated by plating appropriate dilutions of the samples on crystal violet tetrazolium (CVT) agar (Speck, 1976). CVT agar is Plate Count agar (Difco), to which 1 ppm of crystal violet has

been added. The dissolved medium was dispensed in 100 ml aliquots, in screw cap bottles, and autoclaved for 15 minutes at 121°C. Prior to use, the medium was melted, tempered to 45°C, and 0.5 ml of a 1.0% solution of TTC (2, 3, 5, triphenyl tetrazolium chloride; J.T. Baker Chemical Co.; Phillipsburg, New Jersey), which had been "filter sterilized" using a sterile 0.45 um membrane filter (Millipore Corporation; Bedford, Massachusetts), was added to give the final concentration of 50 ppm. Plates were incubated at 32°C for 48 hours. After which, only red colonies visible with the aid of a Quebec colony counter were counted.

Numbers of Clostridium perfringens were enumerated using Egg Yolk free Tryptose sulfite cycloserine agar (TSC-D; Speck, 1976) SFP (Shahi di Ferguson perfringens) base (Difco) was prepared, dispensed in 100 ml aliquots per screw cap bottle, and autoclaved for 10 minutes at 121°C. Prior to use the medium was melted, tempered to 45°C, and 1 ml of a 4% D-cycloserine (Sigma; St. Louis, Missouri) solution, which had been "filter sterilized" using a 0.45 um membrane filter (Millipore Corporation; Bedford, Massachusetts), was added to give the final concentration of 400 ug per ml. Once the agar had solidified an overlay of the same media was poured. Plates were incubated anaerobically in Gas Pak jars (BBL) for 48 hours at 37°C. After which, plates containing black colonies were selected to be counted with the aid of a Quebec colony counter.

Baird-Parker agar (Difco) was used to enumerate coagulase positive staphylococci. The media was prepared according to manufacturer's directions, dispensed in 95 ml aliquots per screw cap bottle, and autoclaved for 15 minutes at 121°C. To prepare "spread" plates, the medium was melted, tempered to 45°C, and 5 ml of tempered (45°C) Bacto EY Tellurite Enrichment - Egg yolk solution (Difco) was added. The medium was mixed by inverting the bottle six times, carefully, to avoid formation of bubbles. The resulting medium was aseptically dispensed (8 to 10 ml) into sterile (15 x 100 mm) petri dishes. Plates were placed on the laboratory bench, 16 to 18 hours, at room temperature, to permit partial drying of the agar medium surface. The plates were then placed in plastic bags and stored at 5°C until needed. After spreading the appropriate dilutions onto the surface of the agar medium, the plates were incubated at 37°C for 48 hours, after which shiney black colonies surrounded by a halo were counted with the aid of a Quebec colony counter.

Direct Comparison of Acetic and Formic Acid as Preservatives

Further experiments were conducted examining the effects of acetic acid and formic acid on hide segments from the same hide sample. Everything was handled similarly as previously mentioned except for a few changes. One change was the storage time was altered from 0, 2, and 4 days to 0,

4, and 8 days. Another difference was that instead of comparing two molar concentrations of the same acid, two acids of the same molar concentration (0.33M) were compared.

Treatment Assignments for Comparisons

As before, fourteen plastic jars, six containing 100 ml of distilled, four with 98 ml of distilled water, and four with 98.3 ml of distilled water, were autoclaved for 15 mm at 121°C. Prior to treatment of the hide segments 2 ml of glacial acetic acid was added to each of the four jars containg 98 ml of sterile distilled water, and 1.7 ml of 88% formic acid was added to the jars containing 98.3 ml of sterile distilled water. This provided four jars containing a 100 ml of 0.33M glacial acetic acid and four jars with 0.33M formic acid. Again, a single jar was used for treating only one hide segment. (See Table II).

Statistical Analysis

The computations of an analysis of variance for a randomized block with subsampling, two observation per cell (Animals-block; treatment number-treatments; observations-subsamples), were made using the Statistical Analysis System (SAS). Further analysis was done using Duncan's Multiple Range test within the SAS system. (Appendix II)

TABLE II

EXAMPLE OF RANDOMIZATIONS AND CODING OF TREATMENT PORTIONS OF THE HIDE SAMPLE FOR DIRECT COMPARISONS OF ACETIC VS FORMIC ACIDS

TREATMENT	SEG. NO.	SAMPLE DAY	CODE
	3	0	AO-3
(A)	1		AO-1
CONTROL	7	4	A4-7
DISTILLED	14		A4-14
WATER	2	8	A8-2
	5		A8-5
(B)	8	4	B4-8
0.33M	6		B4-6
GLACIAL	10	8	B8-10
ACETIC ACID	12		B8-12
(C)	13	4	C4-13
0.33M	4		C4-4
FORMIC	9	8	C8-9
ACID	11		C8-11

CHAPTER IV

RESULTS

The data obtained from each trial showing the counts for individual groups of microorganisms are presented in summary tables in Appendix I. The counts are expressed as $\log_{10}$/g of hide.

Comparison of Numbers of Microorganisms Among Hide Samples

Significant variations from animal to animal with respect to numbers of total aerobic microorganisms ($p<0.0107$), Gram negative bacteria ($p<0.0039$), Clostridium perfringens ($p<0.0148$), coagulase positive staphylococci ($p<0.0169$), and yeasts and molds ($p<0.0145$) were noted during trials in which acetic acid was tested as a preservative. In trials in which formic acid was tested, significant animal to animal variation was observed only for numbers of C. perfringens ($p<0.0418$). Numbers of Gram negative bacteria varied ($p<0.0366$) from animal to animal in the experiments involving potassium sorbate; otherwise no significant variations were noted. In the experiments comparing the preservative action of acetic acid to that of formic acid variations were noted for numbers of total aerobic microorganisms

($p<0.0004$), Gram negative bacteria ($p<0.0026$), numbers of *C. perfringens* ($p<0.0010$) and yeasts and molds ($p<0.0462$). The numbers of coagulase positive staphylococci ($p>0.0793$) from animal to animal were not significantly different.

Evaluation of Acetic Acid as a Preservative

Total Aerobic Microorganisms

The numbers of total aerobic microorganisms on the samples treated with both 0.33M and 0.67M acetic acid were significantly lower than the control samples ($p<0.005$), after two and four days of storage (Figure 2). The mean $\log_{10}$ counts per gram for the control samples were 9.08 and 9.61 on days two and four respectively. The mean $\log_{10}$ counts for the samples treated with 0.33M acetic acid were 4.80 and 4.72 on days two and four. The 0.67M solution was more effective ($p<0.005$) than the 0.33M solution. The numbers actually decreased from day zero to day two on samples treated with 0.67M acetic acid. This was followed by a slight increase on day four.

Gram Negative Bacteria

After two and four days of storage, numbers of Gram negative bacteria on the samples treated with both 0.33M and 0.67M acetic acid were significantly lower ($p<0.005$) than on the control samples (Figure 3). The control samples attained mean $\log_{10}$ counts per gram of 8.64 and 9.35 on days

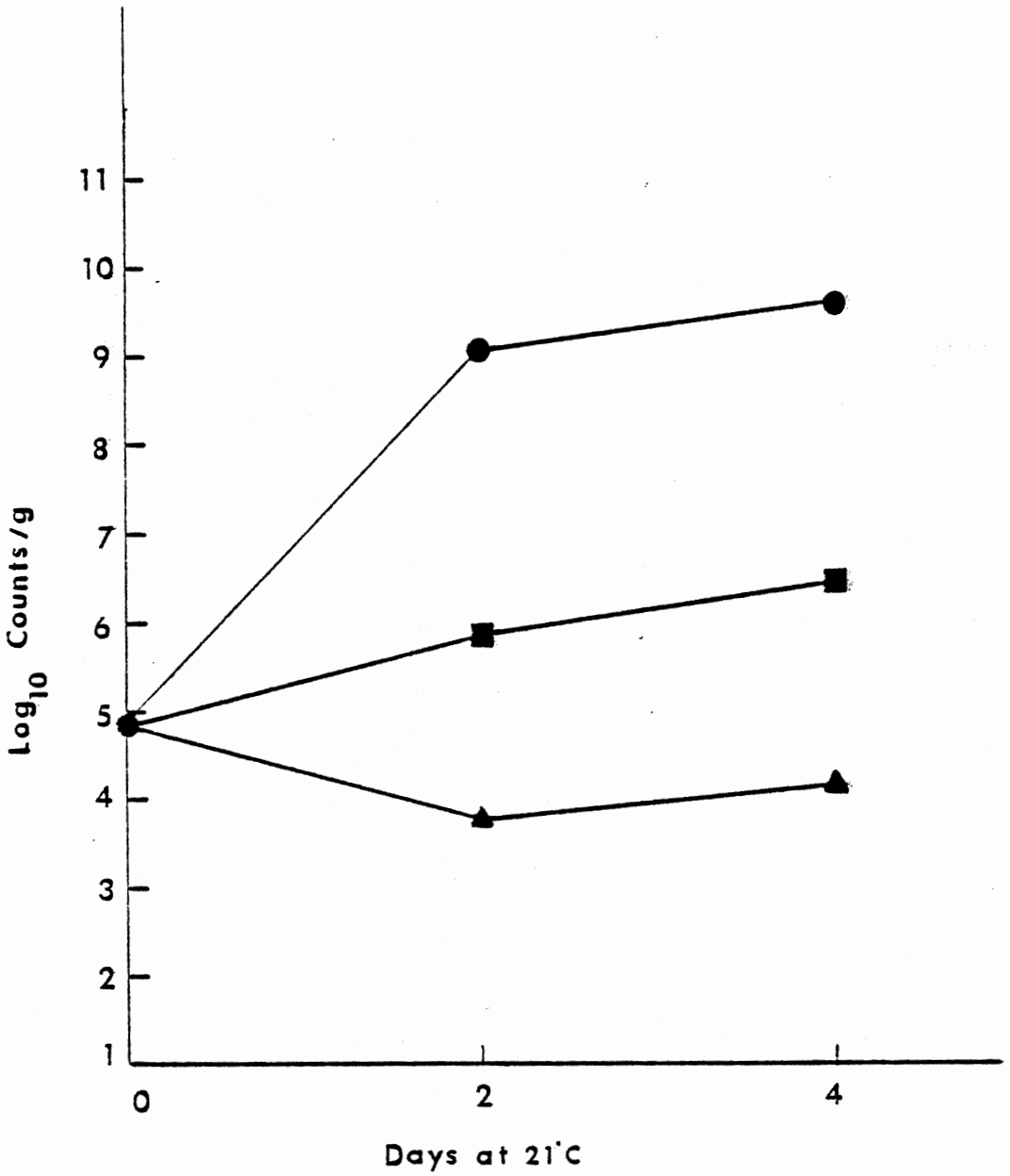

Figure 2. Influence of Acetic Acid on Growth of Total Aerobic Microorganisms on Bovine Hides. ● control, ■ 0.33M acetic acid, ▲ 0.67M acetic acid (Each point represents an average value from seven trials.)

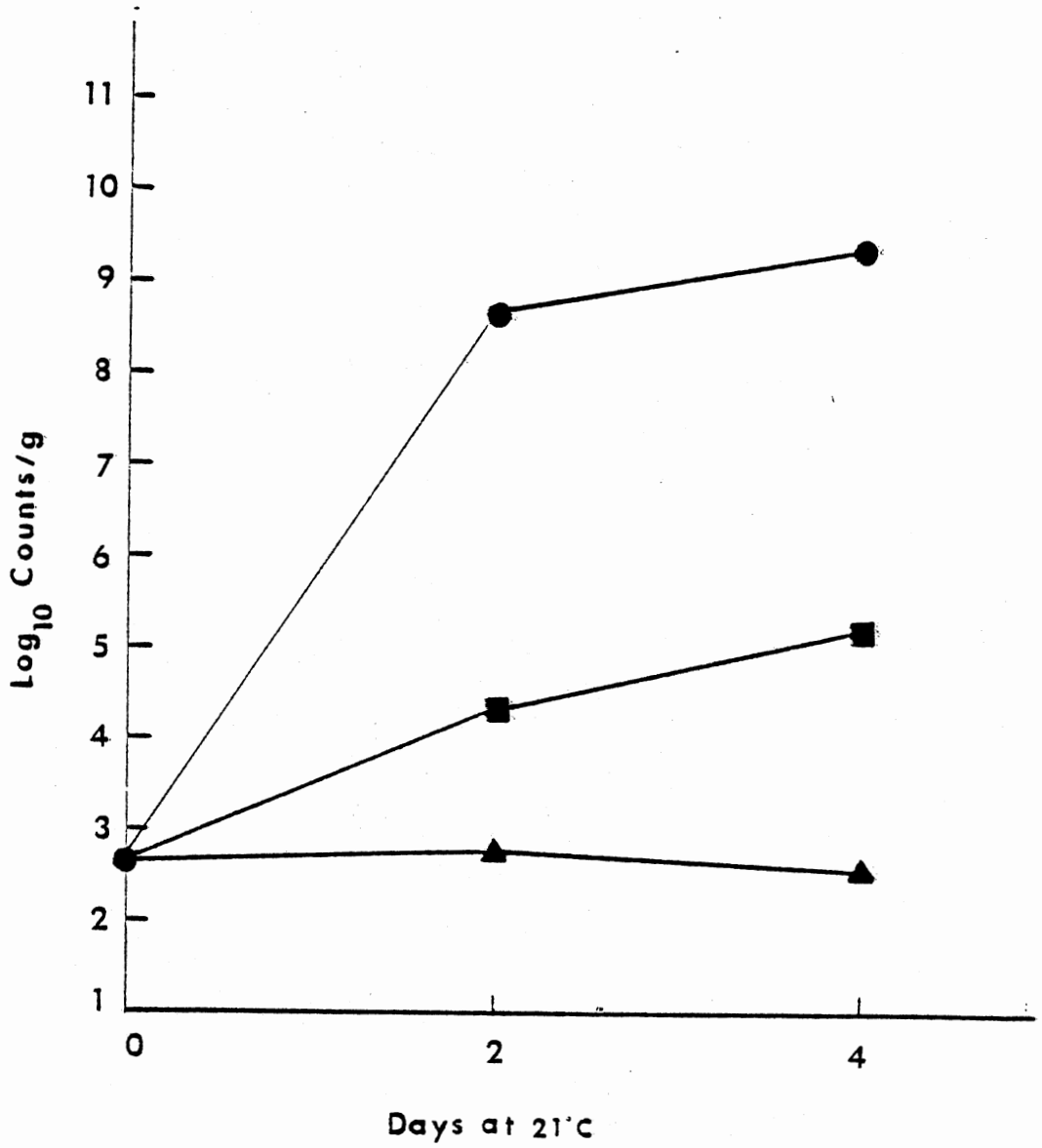

Figure 3. Influence of Acetic Acid on Growth of Gram Negative Bacteria on Bovine Hides. ● control, ■ 0.33M acetic acid, ▲ 0.67M acetic acid (Each point represents an average value from seven trials.)

two and four respectively. This was a greater increase from day zero to days two and four than was observed for the total counts (compare Figure 2 and Figure 3). The 0.67M solution was more effective ($p<0.005$) than the 0.33M solution of acetic acid. The mean $\log_{10}$ counts for samples treated with 0.33M acetic acid increased from 2.70 on day zero to 4.31 and 5.14 on days two and four. The numbers did not increase during storage on the samples treated with 0.67M acetic acid.

Clostridium perfringens

Both concentrations of acetic acid were effective ($p< 0.005$) in inhibiting growth of C. perfringens during the four day storage period (Table III). There appeared to be a slight decline in numbers for samples treated with both concentrations of acid. The samples treated exhibited slight increases from day two to day four. However, they were not significant ($p>0.05$). Thus the 0.33M concentration was just as effective as the 0.67M concentration in controlling growth of these organisms.

Coagulase Positive Staphylococci

Neither concentration of acetic acid significantly ($p> 0.5$) inhibited growth of coagulase positive staphylococci (Table III). Detectable numbers of these organisms on day zero were sporadic (Appendix I, Table X). However, when coagulase positive staphylococci were present the acetic

TABLE III

INFLUENCE OF ACETIC ACID ON NUMBERS OF *CLOSTRIDIUM PERFRINGENS*[a], COAGULASE POSITIVE STAPHYLOCOCCI[b], AND YEASTS AND MOLDS[c] ON BOVINE HIDES DURING STORAGE AT 21°C

	Average Means of Log_{10} Counts/g[d]								
	Clostridium perfringens			Coagulase (+) Staphylococci			Yeasts and Molds		
Day	Control	0.33M	0.67M	Control	0.33M	0.67M	Control	0.33M	0.67M
0	2.15	---	---	2.00	---	---	2.13	---	---
2	4.55	2.00	2.00	2.37	2.13	2.00	3.40	4.38	2.55
4	6.34	2.77	2.14	2.57	2.31	2.00	3.60	5.11	2.94

[a] *Clostridium perfringens*: Tryptose sulfite cycloserine agar.
[b] Coagulase positive staphylococci: Baird-Parker agar.
[c] Yeasts and Molds: Acidified potatoe dextrose agar.
[d] Averages based on 7 trials.

acid apparently was not effective ($p > 0.05$) in controlling their growth.

Yeasts and Molds

The acetic acid treatments had inhibitory effects ($p < 0.0001$) on the growth of yeasts and molds (Table III). The numbers on the samples treated with 0.33M acetic acid increased more than on the control samples ($p < 0.005$), during the two and four days of storage. On the other hand, treatment of samples with the 0.67M acid inhibited the growth of yeasts and molds.

Evaluation of Formic Acid as a Preservative

Total Aerobic Microorganisms

The numbers of total aerobic microorganisms on the samples tested with both 0.33M and 0.67M formic acid were significantly lower than the control samples ($p < 0.005$) after two and four days of storage (Figure 4). The control samples attained mean $\log_{10}$ populations of 9.36 and 9.86 per gram on days two and four respectively. The numbers of total flora actually decreased from day zero to day two and continued to decrease on day four, on samples treated with 0.33M formic acid. The mean $\log_{10}$ count per gram on the samples at day zero was 5.65. The $\log_{10}$ of counts for samples treated with 0.33M formic acid were 4.80 and 4.72 on days two and four respectively. The control samples attained

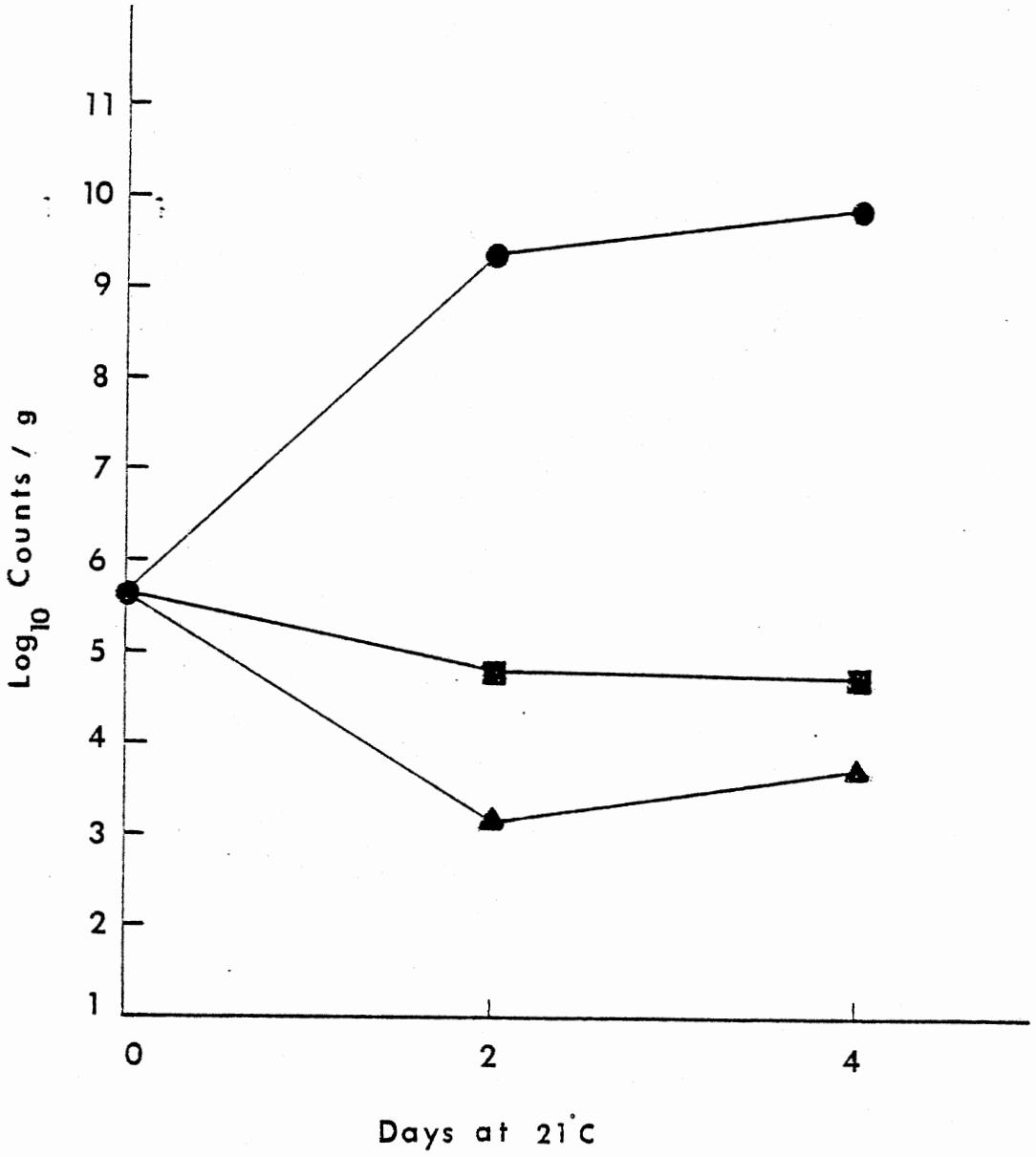

Figure 4. Influence of Formic Acid on Growth of Total Aerobic Microorganisms on Bovine Hides. ● control, ■ 0.33M formic acid, ▲ 0.67M formic acid (Each point represents an average value of 6 trials.)

mean $\log_{10}$ populations of 9.36 and 9.86 per gram on days two and four respectively. The 0.67M solution of formic acid was even more effective ($p<0.005$) than the 0.33M solution. There was a greater decrease from day zero to day two on samples treated with 0.67M formic acid. This was followed by a slight increase on day four.

Gram Negative Bacteria

After two and four days of storage numbers of Gram negative bacteria on the samples treated with both 0.33M and 0.67M formic acid were significantly lower ($p<0.005$) than the control samples (Figure 5). The control samples attained mean $\log_{10}$ populations of 8.72 and 9.71 on days two and four respectively. This was a greater increase from day zero to days two and four than occurred for the total counts (compare Figure 6 and Figure 7). Treatment of the hide samples with 0.67M formic acid resulted in lower counts on days two and four than that observed on day zero. This treatment was significantly more effective than the 0.33M solution ($p<0.025$).

Clostridium perfringens

Use of formic acid as a preservative significantly reduced numbers of C. perfringens during two and four days of storage (Table IV). There appeared to be a decline in numbers for both treated samples from day zero to day two. The treated samples then exhibited a slight increase from day two to day four. However, there was not a significant

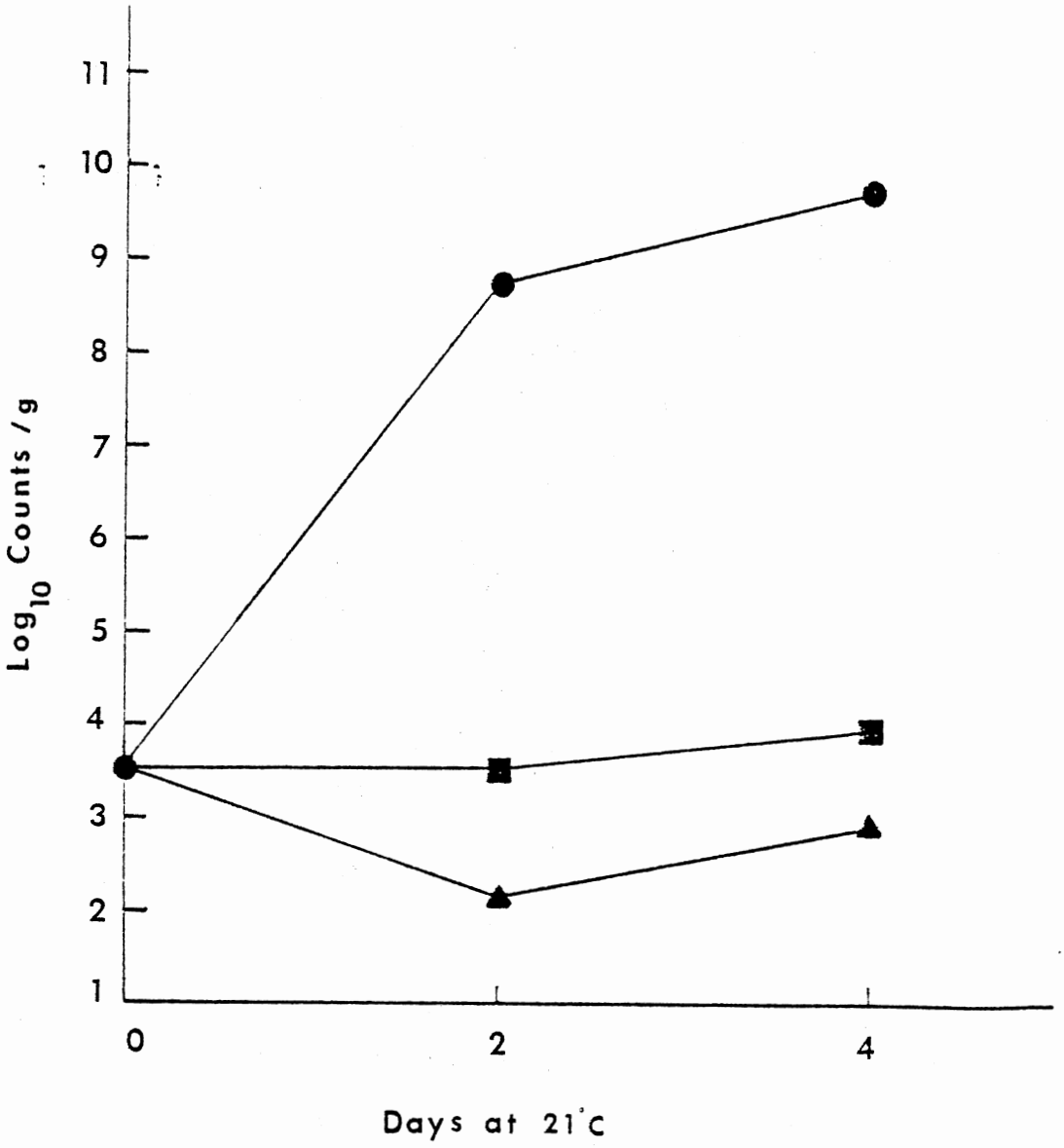

Figure 5. Influence of Formic Acid on Growth of Gram Negative Bacteria on Bovine Hides. ● control, ■ 0.33M formic acid, ▲ 0.67M formic acid. (Each point represents an average value from 6 trials.)

difference ($p > 0.05$) between the 0.33M treated samples or 0.67M treated samples.

Coagulase Positive Staphylococci

While the occurrence of coagulase positive staphylococci was sporadic on the samples (Appendix I, Table XV), both concentrations of formic acid had statistically significant ($p < 0.005$) inhibitory actions toward them (Table IV). There was no significant difference ($p > 0.05$) between the effectiveness of 0.33M solution of formic acid or 0.67M solution.

Yeasts and Molds

Formic acid used as a preservative was moderately effective ($p < 0.025$) in inhibiting growth of yeasts and molds (Table IV). The 0.67M solution of acid was slightly more effective ($p < 0.025$) in reducing growth of these organisms than the 0.33M solution at day two. At day four the samples treated with 0.33M formic acid exhibited an increase in numbers of yeasts and molds to almost the equivalent of counts on the control of day four. Whereas, the 0.67 M formic acid samples decreased from day two to day four.

Evaluation of Potassium Sorbate as a Preservative

Total Aerobic Microorganisms

The numbers of total aerobic microorganisms on the

TABLE IV

INFLUENCE OF FORMIC ACID ON NUMBERS OF *CLOSTRIDIUM PERFRINGENS*[a], COAGULASE POSITIVE STAPHYLOCOCCI[b], AND YEASTS AND MOLDS[c] ON BOVINE HIDES DURING STORAGE AT 21°C

	Average Means of Log_{10} Counts/g[d]								
	Clostridium perfringens			Coagulase (+) Staphylococci			Yeasts and Molds		
Day	Control	0.33M	0.67M	Control	0.33M	0.67M	Control	0.33M	0.67M
0	2.68	---	---	2.20	---	---	2.11	---	---
2	6.27	2.03	2.0	3.55	2.20	2.0	3.64	2.54	2.27
4	7.12	2.61	2.21	4.90	2.0	2.0	3.90	3.62	2.22

[a] *Clostridium perfringens*: Tryptose sulfite cycloserine agar.
[b] Coagulase positive staphylococci: Baird-Parker agar.
[c] Yeasts and Molds: Acidified potatoe dextrose agar.
[d] Averages based on 6 trials.

samples tested with both 0.33M and 0.67M potassium sorbate were lower than on the control samples ($p<0.025$) after two and four days of storage (Figure 6). However, the inhibition was not as great as observed when either formic or acetic acids were used. There were essentially no difference ($p>0.05$) between the 0.33M solution or the 0.67M solution of potassium sorbate.

Gram Negative Bacteria

After two and four days of storage (Figure 7), the numbers of Gram negative bacteria were lower than the control samples ($p<0.025$). Essentially no significant difference ($p>0.05$) was observed between the effectiveness of the 0.33M solution or the 0.67M solution of potassium sorbate. The potassium sorbate was somewhat more effective against the Gram negative bacteria than the total flora (compare Figure 10 and Figure 11).

Clostridium perfringens

The mean numbers of C. perfringens on the samples treated with both concentrations of potassium sorbate were significantly lower on the control samples ($p<0.025$) after two days of storage (Table V). The 0.67M solution was not significantly more effective than the 0.33M solution ($p>0.05$). From day two to day four of storage there was an increase in growth on samples treated with either 0.33M or 0.67M potassium sorbate; the 0.33M treated samples exhibited

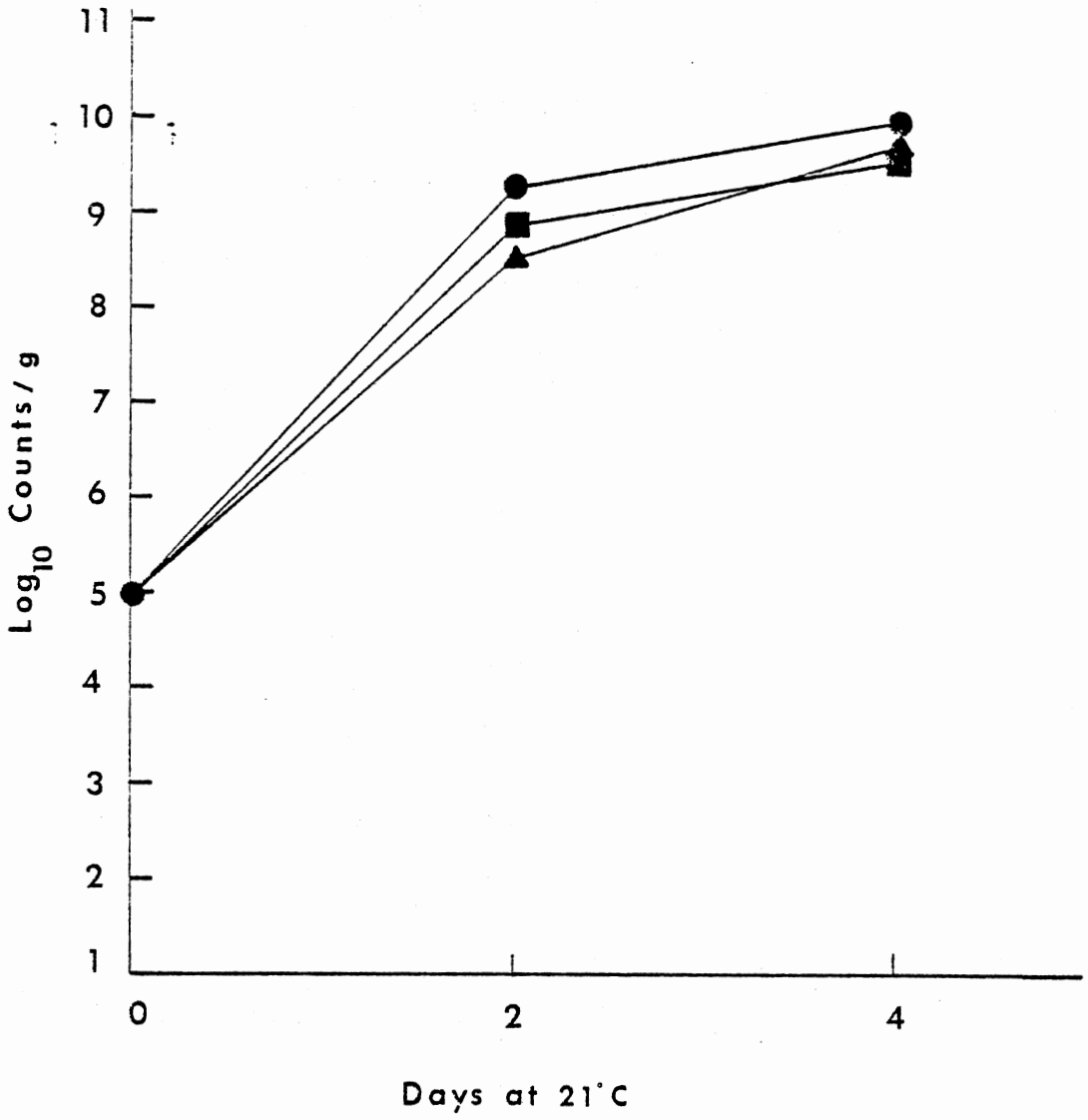

Figure 6. Influence of Potassium Sorbate on Growth of Total Aerobic Microorganisms on Bovine Hides. ● control, ■ 0.33M potassium sorbate, ▲0.67M potassium sorbate (Each point represents an average value from 2 trials.)

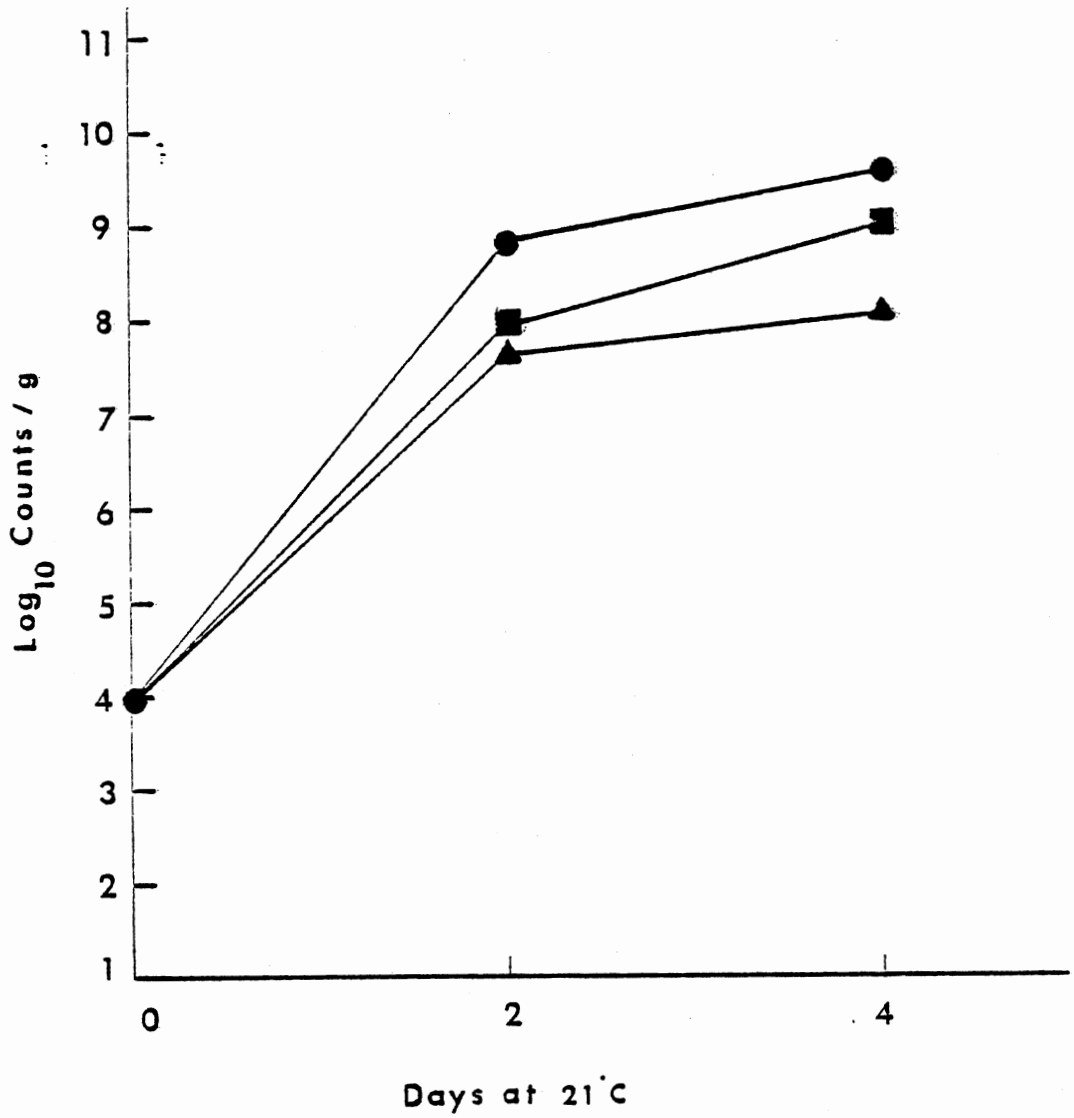

Figure 7. Influence of Potassium Sorbate on Growth of Gram Negative Bacteria on Bovine Hides. ● control, ■ 0.33M potassium sorbate, ▲ 0.67M potassium sorbate (Each point represents an average value from 2 trials.)

TABLE V

INFLUENCE OF POTASSIUM SORBATE ON NUMBERS OF *CLOSTRIDIUM PERFRINGENS*[a], COAGULASE POSITIVE STAPHYLOCOCCI[b], AND YEASTS AND MOLDS[c] ON BOVINE HIDES DURING STORAGE AT 21°C

	Average Means of Log_{10} Counts/g[d]								
	Clostridium perfringens			Coagulase (+) Staphylococci			Yeasts and Molds		
Day	Control	0.33M	0.67M	Control	0.33M	0.67M	Control	0.33M	0.67M
0	2.0	---	---	2.0	---	---	2.69	---	---
2	6.18	3.70	3.83	3.87	4.00	3.45	3.81	4.01	3.33
4	6.66	6.02	4.48	3.61	4.68	5.12	4.40	5.51	5.09

[a] *Clostridium perfringens*: Tryptose sulfite cycloserine agar.
[b] Coagulase positive staphylococci: Baird-Parker agar.
[c] Yeasts and Molds: Acidified potatoe dextrose agar.
[d] Averages based on 2 trials.

the greater increase in numbers.

Coagulase Positive Staphylococci

Neither concentration of potassium sorbate significantly reduced ($p>0.4524$) the growth of coagulase positive staphylococci (Table V). The mean numbers of coagulase positive staphylococci were greater on samples treated with both concentrations of sorbate, rather than on the control samples after four days of storage.

Yeasts and Molds

Potassium sorbate did not significantly reduce ($p>0.1469$) the growth of yeasts and molds (Table V). It seemed rather to increase the growth of these organisms. By day four the counts on both 0.33M and 0.67M potassium sorbate treated samples exceeded the control counts.

Comparison of Acetic and Formic Acids as Preservatives

Total Aerobic Microorganisms

The numbers of total aerobic microorganisms on the samples treated with 0.33M formic acid were significantly lower than on samples treated with 0.33M acetic acid ($p<0.005$) after four and eight days of storage (Figure 8). The control samples attained mean $\log_{10}$ populations of 9.75 and

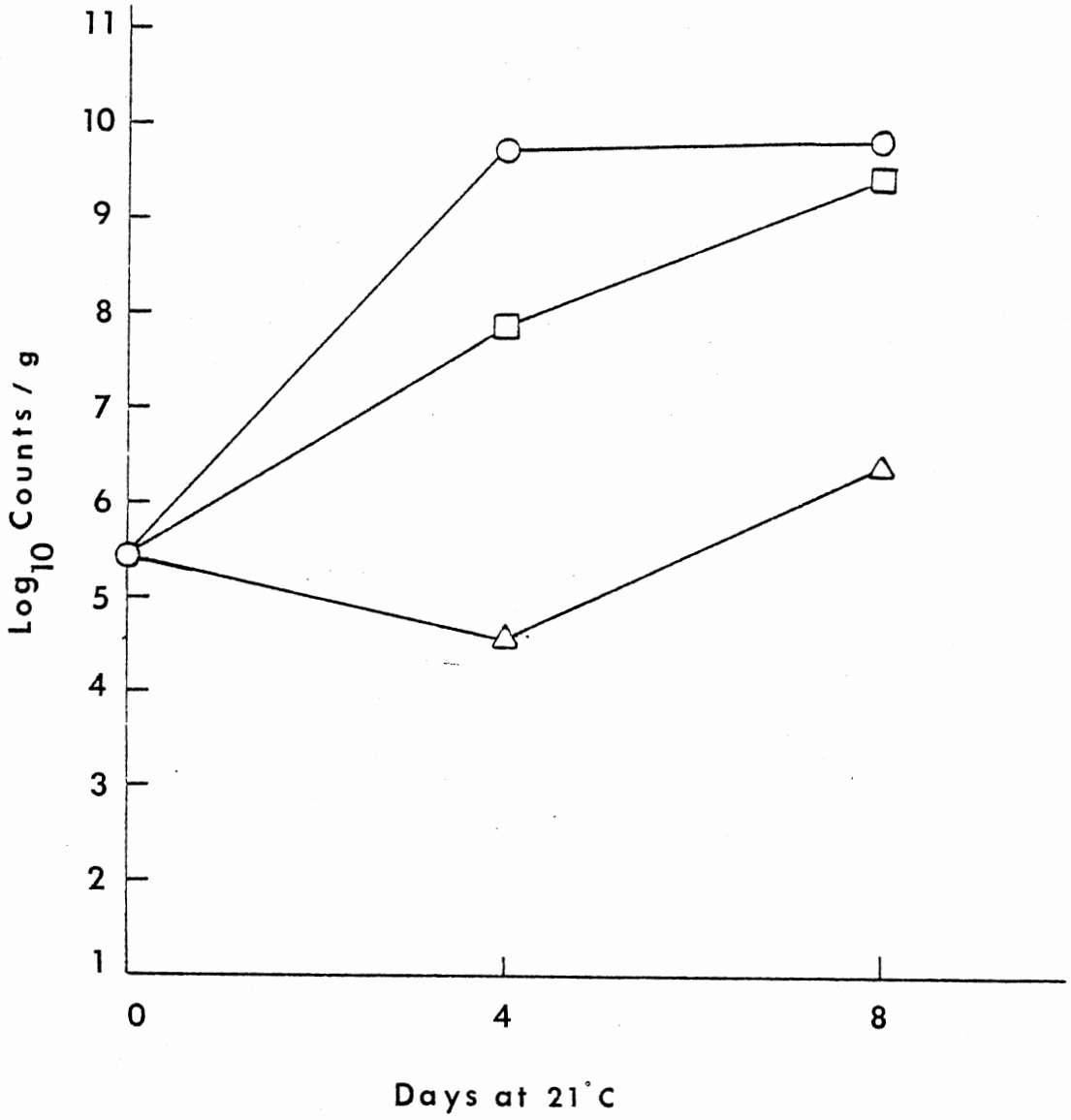

Figure 8. Comparison of the Influence of 0.33M Acetic Acid and 0.33M Formic Acid on Growth of Total Aerobic Microorganisms on Bovine Hides. ○ control, □ 0.33M acetic acid, △ 0.33M formic acid (Each point represents an average value from 7 trials.)

9.83 on days four and eight respectively. The samples treated with the acetic acid exhibited increased counts from day zero to day four and day eight reaching mean $\log_{10}$ counts of 7.87 and 9.45 respectively. The numbers declined from day zero to day four on the samples treated with formic acid, but increased to a mean $\log_{10}$ count of 6.41 per gram by day eight.

Gram Negative Bacteria

After four and eight days of storage, numbers of Gram negative bacteria on the samples treated with 0.33M formic acid were significantly lower ($p<0.005$) than the samples treated with 0.33M acetic acid (Figure 9). In fact, the numbers of Gram negative bacteria decreased from the day zero counts throughout the eight days of storage. By the eighth day of storage the mean count on the samples treated with acetic acid was almost equal to that for the control samples.

Clostridium perfringens

The treatment of hide samples with 0.33M formic acid significantly ($p<0.005$) inhibited growth of C. perfringens (Table VI). The mean $\log_{10}$ counts for these samples decreased from 2.45 on day zero to 2.25 on day four, followed by a slight increase to 3.52 on day eight. Treatment with 0.33M acetic also significantly inhibited the growth of C. perfringens ($p<0.005$). However, the inhibition was not as

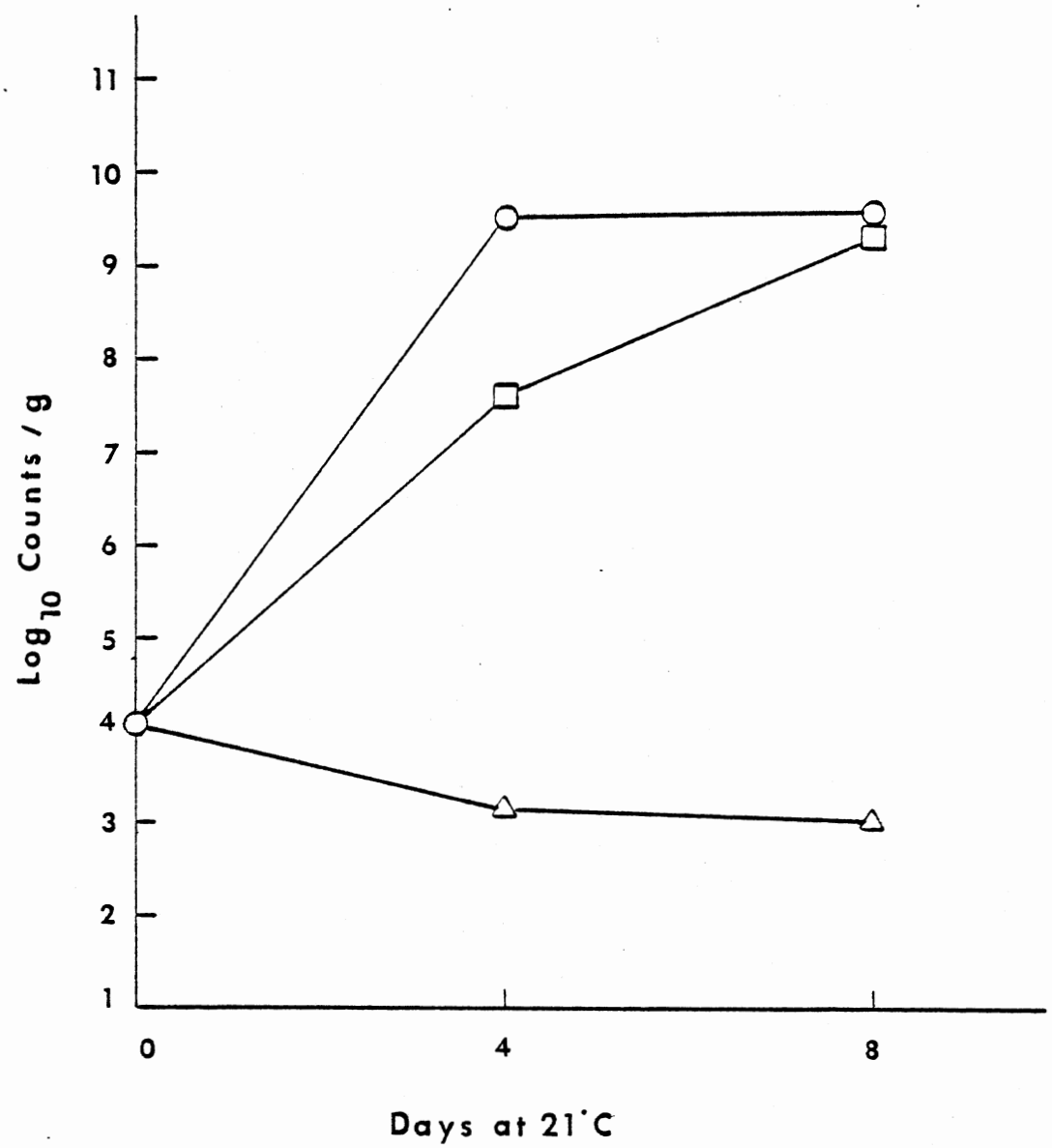

Figure 9. Comparison of the Influence of 0.33M Acetic Acid and 0.33M Formic Acid on Growth of Gram Negative Bacteria on Bovine Hides. ○ control □ 0.33M acetic acid, △ 0.33M formic acid (Each point represents an average value from 7 trials.)

TABLE VI

COMPARISON OF THE INFLUENCE OF 0.33M ACETIC ACID AND 0.33M FORMIC ACID ON *CLOSTRIDIUM PERFRIGENS*[a], COAGULASE POSITIVE STAPHYLOCOCCI[b], AND YEASTS AND MOLDS[c] ON BOVINE HIDES DURING STORAGE AT 21°C

	Average Means of Log_{10} Counts/g[d]								
	Clostridium perfringens			Coagulase (+) Staphylococci			Yeasts and Molds		
Day	Control	0.33MA[e]	0.33M	Control	0.33MA	0.33M	Control	0.33MA	0.33M
0	2.45	---	---	2.00	---	---	2.69	---	---
2	7.01	4.05	2.25	4.16	2.68	2.00	4.88	7.00	4.32
4	7.26	6.46	3.52	2.71	2.22	2.0	4.71	7.17	5.68

a *Clostridium perfringens*: Tryptose sulfite cycloserine agar.
b Coagulase positive staphylococci: Baird-Parker agar.
c Yeasts and Molds: Acidified potatoe dextrose agar.
d Averages based on 7 trials.
e A: acetic; F: formic.

great as that produced by the formic acid. The mean $\log_{10}$ count for samples treated with acetic acid was 6.46 at day eight, compared to 7.26 for the controls and 3.52 for the samples treated with formic acid.

Coagulase Positive Staphylococci

Statistical analysis of the data indicated that both 0.33M acetic acid and 0.33M formic acid significantly inhibited ($p < 0.0014$) growth of coagulase positive staphylococci on hide samples (Table VI). However, the occurrence of coagulase positive staphylococci on the samples was sporadic (Appendix I, Table XV).

Yeasts and Molds

Following with the trend previously shown, 0.33M acetic acid significantly increased ($p < 0.005$) growth of yeasts and molds (Table VI). Samples treated with 0.33M formic acid also exhibited increased growth of yeast and mold. The formic acid mean $\log_{10}$ counts for the treated samples exceeded that of the control counts by day eight.

CHAPTER V

DISCUSSION

The variations in microbial counts among hide samples may have been due to several factors. The weather conditions could have affected how much dust, dirt, manure, etc. was accumulated on the hides. This in turn might affect the numbers and kinds of bacteria and fungi present on the hide. The adequacy of trimming, fleshing, and washing the hides could also influence the microbial flora of the samples. Hopkins et al. (1973) listed these as factors that may affect preservation, along with hide composition and the presence of enzymes.

One group of microorganisms chosen to be monitored, which occurred in sporadic numbers from sample to sample was the coagulase positive staphylococci. The sporadic occurrence made it difficult to draw definite conclusions about control of this group. It is possible that coagulase positive staphylococci can produce enterotoxins that cause staphylococcal food poisoning. This enterotoxin is unique among bacterial toxins in that it is more difficult to destroy than others. It is conceivable that the enterotoxin, if produced during hide storage, could survive through the recovery of the collagen from the hides. Thus,

it could be present in foods prepared using the collagen as an ingredient. Therefore, it was deemed important to have a preservative to inhibit growth of the staphylococci.

Although the results demonstrated that the presence of coagulase positive staphylococci was sporadic; when they were present on untreated hides, they grew during storage. While both 0.33M acetic acid and the 0.33M formic acid significantly inhibited growth of coagulase positive staphylococci on hide samples, the formic acid treatment was the most effective. In fact, formic acid actually lowered numbers of coagulase positive staphylococci during storage.

Clostridium perfringens, another food borne pathogen, was monitored in these studies. The reason being it is a spore-forming bacteria which might be expected to grow on material such as the hides during storage at ambient temperatures. If spores of this organism were produced during storage of the hides, it is conceivable that they would not be destroyed or completely removed during recovery and processing of the collagen. Thus, they could be present in foods which included the collagen as an ingredient.

The treatment of hide samples with either 0.33M acetic acid or 0.33M formic acid showed adequate inhibition of growth of C. perfringens. However, the inhibition of the 0.33M acetic acid treatment was not as great as that produced by the 0.33M formic acid treatment. The formic acid treatment actually lowered numbers of C. perfringens from control counts.

Total aerobic microorganisms, Gram negative bacteria and yeast and mold numbers were also monitored because of their capability of causing spoilage of the hides. In each case formic acid was a more effective preservative than the acetic acid. The numbers of total aerobic microorganisms and Gram negative bacteria were actually reduced when 0.67 molar formic acid was used; meaning there was a bactericidal effect from the formic acid. The greatest effect was seen on the Gram negative bacteria.

Although the formic acid did do a better job of inhibiting growth of yeasts and molds than the acetic acid, both permitted some growth of these organisms. This result could be due to the fact that yeasts and molds are acid tolerant. It may be possible to use some other preservative, such as sorbate in conjunction with formic acid, that would inhibit the growth of yeasts and molds. The control of these organisms is necessary since they can produce many types of enzymes that might spoil the hides. There also is concern that certain of the molds might produce mycotoxins during growth on the hides. It would not be desirable to have collagen contaminated with mycotoxins.

Results obtained from trials conducted to observe the effectiveness of potassium sorbate as a preservative indicated that at the concentrations tested, the sorbate was not an effective preservative. It did not adequately inhibit any of the groups of microorganisms monitored. Sorbic acid is most effective in its undissociated form and the amount

of this effective form increases at lower pH values (York and Vaughn, 1954; Raevuor, 1976; and Sotos et al., 1980). In other words, the microbial activity of sorbic acid is pH dependent. Thus, the lack of effectiveness of potassium sorbate could be due to the lack of a low pH environment on the bovine hides. Perhaps a combination of acetic or formic acid with sorbate should be tested. Both formic and acetic acid treatments were more effective than sorbate treatments.

Goepfert and Hicks (1969) stated the sensitivity of Salmonella typhimurium to volatile fatty acids depended not only on the pH of the medium, but also on the chain length and concentration of the acid. In general, the shorter the fatty acid chain, the greater the inhibitory action. Results from the present study are in agreement with this. Both concentrations of acetic and formic acid displayed significant inhibitory actions. The formic acid was more inhibitory than the acetic acid. Overall, the more concentrated treatments of acetic or formic acid were more effective inhibitors of all groups of microorganisms monitored (based on relative counts).

Gram negative bacteria in each case grew more rapidly than the total aerobic microorganisms. Initially, they appeared in low numbers in relation to total counts, however, by the end of the storage periods they were the predominant flora. Wadd et al. (1975) indicated that bacteria on skins after storage were predominantly gram negative rods. Thus, control of this group of bacteria is needed

since it is likely that the most active spoilage organisms would be in this group. Control of this group of bacteria was achieved with treatments of acetic acid or formic acid, with the formic acid treatments being best.

Even though the more concentrated treatments of volatile fatty acids were more effective, other factors need to be considered. For instance, the cost of using the higher concentrations of the acid. Certainly, use of the lower concentration (0.33M) would be expected to be half that of using the higher concentration (0.67M) with respect to cost of the preservative. However, at a higher concentration the treatment solution could perhaps be used several times more compared to the lower concentration treatment. Also, the pungent odor associated with both acids would increase with increasing concentration of the acid. A lower concentration of the volatile fatty acid may be effective enough as a preservative. The volatile fatty acids would also, presumably be less of an effluent problem than salt, which has been used as the conventional preservative of cattle hides.

Bailey and Hopkins (1975), Hopkins and Bailey (1975), Bailey et al. (1976), and Bailey and Hopkins (1977) all reported using sulfite/acetic acid treatments as preservative for cattle hides. They found this combination to be an effective preservative based on subjective evaluations. In all these studies, sulfite was named the active ingredient. Bailey and Hopkins (1977) stated, "Sulfite, the effective material in this preservative method ...." Bailey et al.

(1976) stated, "Sulfur dioxide is the active material in the elimination of the microbial activity." In only one paper was acetic acid even mentioned as a preservative. Bailey (Hopkins and Bailey, 1975) in answer to a question during the discussion, mentioned "acetic acid alone will hold the hide very well for three days, but almost predictably on the fourth day mold growth will be seen. We think that five percent acetic acid alone might be very effective for a short three-day preservation." Results from the present study show that either formic or acetic acid could be used as an effective preservative for bovine hides for at least 8 days at 21°C.

No effort in this study was made to determine if combining acetic and formic acids would improve the effectiveness of preservation on bovine hides. The combination may work better. At this time though, formic acid appears to be the most effective preservative tested.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Acetic acid, formic acid, and potassium sorbate, at 0.33M and 0.67M concentrations, were evaluated for their effectiveness as a preservative of bovine hides. Portions of hide were submerged for 30 seconds in the desired preservative or sterile distilled water (control), drained and stored at 21°C. Numbers of microorganisms were determined at selected storage intervals. Groups monitored were total numbers of aerobic microorganisms, Gram negative bacteria, Clostridium perfringens, coagulase positive staplylococci, and yeasts and molds.

Potassuim sorbate at any concentration tested was not an adequate preservative of the bovine hides. The lack of preservation may have been due to the fact that the potassium sorbate is most inhibitory as the undissociated molecules. A low pH is needed to increase numbers of undissociated molecules. Most likely, the bovine hide does not provide a low enough pH environment for the potassium sorbate to be effective as a preservative, at the concentrations tested.

Both acetic and formic acid, at all concentrations tested, significantly inhibited growth of all groups of microorganisms. The 0.67M concentration of both acids were

significantly more effective than the 0.33M solutions. Even though the 0.67M concentration was more effective, other practical and economic factors need to be considered. For example, the cost of using the 0.33M concentration would be one-half of that using the 0.67M concentration. Also, at the lower concentrations less problems due to the characteristic pungent odors of these acids would be expected.

At the 0.67M concentration of formic acid, not only was there an inhibitory effect, but there was also a reduction in numbers of total aerobic microorganisms, Gram negative bacteria, Clostridium perfringens, and coalgulase positive staphylococci. At the 0.33M level formic acid was again better than the acetic acid as an effective preservative for all organisms tested. The 0.33M formic acid also had a bactericidal effect on total aerobic microorganisms and Gram negative bacteria.

Although formic acid was more effective than acetic acid in controling yeasts and molds, neither inhibited growth of these organisms as well as they inhibited growth of the other groups of microorganisms monitored. This could be due to the fact yeasts and molds are acid tolerant. Perhaps potassium sorbate in conjunction with formic acid would be able to inhibit this group of microorganisms adequately.

Overall, results from the present study indicate formic acid to be the best short term preservative of those tested. Further studies are needed to determine if a lower concentration

would be adequate in preserving bovine hides. Also, it might be interesting to determine if combinations of acetic acid, formic acid, and potassium sorbate would be more effective perservatives. Results from this study and future studies could have important applications in other areas of hide or food preservation.

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APPENDIXES

APPENDIX A

DATA OBTAINED FROM EACH TRIAL SHOWING THE COUNTS FOR INDIVIDUAL GROUPS OF MICROORGANISMS

TABLE VII

INFLUENCE OF ACETIC ACID ON TOTAL AEROBIC COUNTS[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g							
DAYS	TREATMENT	DSN[b]	I[c]	II	III	IV	V	VI	VII	Avg.
0	Control	1	5.04	3.78	5.53	3.95	4.97	4.59	5.94	4.88
		2	5.64	4.08	5.52	4.15	4.82	4.48	5.79	
2	Control	1	9.15	8.59	8.99	9.51	8.77	9.30	9.48	9.08
		2	9.04	8.48	8.79	9.77	8.86	9.11	9.34	
	0.33M	1	6.56	<4.00	7.15	4.65	7.28	3.65	6.11	5.86
		2	5.64	4.00	6.66	5.30	9.41	4.15	7.42	
	0.67M	1	5.34	<4.00	3.79	3.60	2.60	3.30	5.30	3.79
		2	4.00	<4.00	3.63	3.00	2.60	2.85	5.04	
4	Control	1	9.52	8.85	9.79	10.08	9.51	9.85	9.82	9.61
		2	10.04	8.62	9.72	10.18	9.30	9.54	9.74	
	0.33M	1	5.51	7.81	7.95	7.45	2.70	2.48	9.32	6.44
		2	8.08	6.48	8.08	7.49	6.53	2.00	8.28	
	0.67M	1	4.30	3.60	7.15	2.95	5.26	2.90	6.23	4.18
		2	4.00	3.00	3.63	5.61	2.30	3.91	3.70	

[a] Total aerobic counts: Trypticase Soy Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE VIII

INFLUENCE OF ACETIC ACID ON GRAM NEGATIVE BACTERIA[a] ON BOVINE HIDES DURING STORAGE AT 21°C

DAYS	TREATMENT	DSN[b]	Log_{10} Counts/g I[c]	II	III	IV	V	VI	VII	Avg.
0	Control	1	<2.00	2.30	2.48	2.48	<2.00	3.26	4.04	2.68
		2	<2.00	2.48	2.85	2.30	3.00	2.30	4.08	
2	Control	1	8.49	8.26	8.52	9.23	8.11	9.00	9.11	8.64
		2	8.18	8.11	8.49	8.97	8.40	8.94	9.15	
	0.33M	1	5.71	<2.00	6.28	2.30	7.15	<2.00	4.40	4.31
		2	4.08	<2.00	5.76	3.20	6.28	<2.00	7.11	
	0.67M	1	<3.00	<2.00	<2.00	<2.00	<2.00	<2.00	>3.00	2.79
		2	<3.00	2.00	<2.00	<2.00	5.00	<2.00	7.11	
4	Control	1	9.77	7.70	9.34	10.00	8.74	9.85	9.76	9.35
		2	9.71	8.63	9.18	10.20	8.87	9.23	9.94	
	0.33M	1	<3.00	4.20	7.32	4.79	<2.00	<2.00	9.08	5.14
		2	8.18	<2.00	7.85	3.77	7.49	<2.00	8.32	
	0.67M	1	<3.00	<2.00	4.49	<2.00	5.04	<2.00	<2.00	2.54
		2	<3.00	2.00	<2.00	<2.00	<2.00	<2.00	<2.00	

[a] Gram negative bacteria: Crystal Violet Tetrazolium Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE IX

INFLUENCE OF ACETIC ACID ON NUMBERS OF CLOSTRIDIUM PERFRINGENS[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g							
DAYS	TREATMENT	DSN[b]	I[c]	II	III	IV	V	VI	VII	Avg.
0	Control	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	3.00	2.15
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	3.15	
2	Control	1	LA[d]	5.15	6.36	3.78	3.52	<2.00	6.26	4.55
		2	LA	4.64	5.18	LA	3.60	3.28	6.28	
	0.33M	1	LA	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	LA	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	0.67M	1	LA	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	LA	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
4	Control	1	<6.08	6.38	6.79	>7.48[e]	4.84	6.68	6.95	6.34
		2	7.26	6.20	6.28	6.08	5.18	6.04	6.52	
	0.33M	1	<3.00	<2.00	<2.00	<2.00	<2.00	<2.00	5.51	2.77
		2	3.48	<2.00	2.00	<2.00	3.08	<2.00	5.67	
	0.67M	1	<3.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.14
		2	<3.00	<2.00	<2.00	2.00	<2.00	<2.00	<2.00	

[a] Enumeration of C. perfingens: Tryptose Sulfite Cyclo-serine Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.
[d] LA: Lab accident.
[e] < or > sign were ignored and the indicated number used for determining Avg. since this was necessary for statistical analysis.

TABLE X

INFLUENCE OF ACETIC ACID ON NUMBERS OF COAGULASE POSITIVE STAPHYLOCOCCI[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g							
DAYS	TREATMENT	DSN[b]	I[c]	II	III	IV	V	VI	VII	Avg.
0	Control	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
2	Control	1	4.48	<2.00	2.00	<2.00	<2.00	<2.00	<2.00	2.37
		2	4.73	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	0.33M	1	3.76	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.13
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	0.67M	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
4	Control	1	<6.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.57
		2	<6.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	0.33M	1	<2.00	2.48	4.98	<2.00	<2.00	<2.00	<2.00	2.31
		2	2.85	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	0.67M	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	

[a] Enumeration of coagulase positive staphylococci; Baird Parker Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE XI

INFLUENCE OF ACETIC ACID ON YEAST-MOLD COUNTS[a]
ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g							
DAYS	TREATMENT	DSN[b]	I[c]	II	III	IV	V	VI	VII	Avg.
0	Control	1	LA[d]	<2.00	<2.00	<2.00	2.00	<2.00	2.60	2.13
		2	LA	<2.00	<2.00	<2.00	<2.00	<2.00	2.95	
2	Control	1	LA	3.45	3.41	4.15	3.56	3.08	4.67	3.40
		2	LA	<2.00	2.48	3.26	4.49	2.48	3.71	
	0.33M	1	LA	<2.00	5.26	3.26	6.00	2.48	6.32	4.38
		2	LA	<2.00	3.80	4.79	7.11	3.72	5.74	
	0.67M	1	LA	<2.00	2.00	<2.00	2.48	<2.00	5.11	2.55
		2	LA	<2.00	<2.00	<2.00	3.83	<2.00	3.15	
4	Control	1	<2.00	<2.00	3.70	3.48	4.32	5.18	4.49	3.60
		2	3.30	<2.00	2.48	4.00	4.52	4.28	4.60	
	0.33M	1	<2.00	5.66	6.60	>7.48	3.78	<2.00	6.70	5.11
		2	7.67	6.48	2.00	5.95	6.18	<2.00	7.00	
	0.67M	1	<2.00	<2.00	7.23	<2.00	5.34	<2.00	2.60	2.94
		2	<6.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	

[a] Yeast and mold counts: Acidified Potatoe Dextrose Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.
[d] LA: Lab accident.

TABLE XII

INFLUENCE OF FORMIC ACID ON TOTAL AEROBIC COUNTS[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Count/g						
DAYS	TREATMENT	DSN[b]	I[c]	II	III	IV	V	VI	Avg.
0	Control	1	7.34	4.57	8.23	5.11	4.83	4.40	5.65
		2	6.26	4.23	8.20	5.20	4.97	4.40	
2	Control	1	9.15	9.18	9.08	9.28	9.43	9.76	9.36
		2	9.43	9.11	9.26	9.30	9.60	9.68	
	0.33M	1	5.04	4.51	3.20	5.88	5.88	4.52	4.80
		2	6.65	4.32	2.95	3.63	4.81	6.15	
	0.67M	1	4.56	3.97	2.15	3.28	3.08	3.51	3.19
		2	3.45	3.95	2.00	2.70	2.70	2.95	
4	Control	1	9.83	10.11	9.98	9.64	9.84	9.73	9.86
		2	9.96	9.92	9.79	9.91	9.96	9.70	
	0.33M	1	5.74	3.52	7.43	2.85	4.45	3.58	4.72
		2	5.71	4.90	7.11	3.00	LA[d]	3.62	
	0.67M	1	3.00	5.72	5.43	3.00	LA	2.48	3.74
		2	3.00	3.68	5.48	2.95	LA	2.70	

[a] Total aerobic counts: Trypticase Soy Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.
[d] LA: Lab accident.

TABLE XIII

INFLUENCE OF FORMIC ACID ON GRAM NEGATIVE BACTERIA[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g						
DAYS	TREATMENT	DSN[b]	I[c]	II	III	IV	V	VI	Avg.
0	Control	1	4.83	2.90	4.04	4.15	2.78	3.52	3.54
		2	4.04	2.30	3.68	4.00	3.00	3.18	
2	Control	1	8.53	8.42	8.49	8.95	9.04	9.04	8.72
		2	8.32	8.49	8.58	8.97	9.04	8.81	
	0.33M	1	4.00	3.26	2.30	4.52	4.74	4.52	3.53
		2	4.67	3.20	<2.00	2.60	4.52	<2.00	
	0.67M	1	2.30	2.90	<2.00	2.30	2.70	2.00	2.18
		2	<2.00	2.00	<2.00	<2.00	2.00	<2.00	
4	Control	1	9.20	9.92	9.51	9.76	10.08	9.71	9.71
		2	9.49	9.83	9.30	9.95	10.00	9.79	
	0.33M	1	<2.00	<2.00	7.40	2.00	3.42	3.34	3.97
		2	<2.00	<2.00	7.04	7.46	5.64	3.34	
	0.67M	1	<2.00	<2.00	4.83	6.15	<2.00	<2.00	2.90
		2	<2.00	<2.00	5.52	<2.00	2.30	<2.00	

[a] Gram negative bacteria: Crystal Violet Tetrazolium Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE XIV

INFLUENCE OF FORMIC ACID ON NUMBERS OF *CLOSTRIDIUM PERFRINGENS*[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g						
DAYS	TREATMENT	DSN[b]	I[c]	II	III	IV	V	VI	Avg.
0	Control	1	4.45	2.48	3.00	2.90	2.48	<2.00	2.68
		2	3.30	<2.00	2.00	2.90	2.60	2.00	
2	Control	1	5.72	5.54	6.38	7.20	6.66	6.51	6.27
		2	5.83	5.15	6.73	6.51	6.69	6.32	
	0.33M	1	<2.00	<2.00	<2.00	2.00	2.30	2.00	2.03
		2	<2.00	<2.00	<2.00	<2.00	2.00	<2.00	
	0.67M	1	<2.00	<2.00	<2.00	2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	2.00	<2.00	
4	Control	1	6.59	6.28	8.26	6.79	7.45	7.04	7.12
		2	6.58	6.98	7.79	7.08	7.58	7.00	
	0.33M	1	<2.00	<2.00	5.49	<2.00	2.30	<2.00	2.61
		2	<2.00	<2.00	5.52	<2.00	<2.00	<2.00	
	0.67M	1	<2.00	<2.00	3.58	<2.00	<2.00	<2.00	2.21
		2	<2.00	<2.00	LA[d]	2.30	2.48	<2.00	

[a] Enumeration of *C. perfringens*: Typtose Sulfite Cyclo-serine Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.
[d] LA: Lab accident.

TABLE XV

INFLUENCE OF FORMIC ACID ON NUMBERS OF COAGULASE POSITIVE STAPHYLOCOCCI[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g						
DAYS	TREATMENT	DSN[b]	I[c]	II	III	IV	V	VI	Avg.
0	Control	1	<2.00	2.30	2.00	<2.00	<2.00	2.48	2.20
		2	<2.00	3.30	2.30	<2.00	<2.00	2.00	
2	Control	1	<2.00	3.20	<2.00	5.73	<2.00	4.66	3.55
		2	5.30	2.60	<2.00	5.62	<2.00	5.51	
	0.33M	1	3.18	<2.00	<2.00	<2.00	<2.00	<2.00	2.20
		2	3.18	<2.00	<2.00	<2.00	<2.00	<2.00	
	0.67M	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
4	Control	1	6.96	<2.00	<2.00	>7.48	>7.48	<2.00	4.90
		2	6.88	4.28	<2.00	>7.48	>7.48	2.70	
	0.33M	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	0.67M	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	

[a] Enumeration of coagulase positive staphylocci; Baird Parker Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE XVI

INFLUENCE OF FORMIC ACID ON YEAST-MOLD COUNTS[a] ON BOVINE HIDES DURING STORAGE AT 21°C

DAYS	TREATMENT	DSN[b]	Log_{10} Counts/g I[c]	II	III	IV	V	VI	Avg.
0	Control	1	2.00	<2.00	<2.00	2.30	<2.00	2.48	2.11
		2	<2.00	2.00	2.00	2.48	<2.00	<2.00	
2	Control	1	3.00	3.92	4.11	4.04	4.04	3.79	3.64
		2	2.90	3.00	3.32	3.96	3.96	3.64	
	0.33M	1	2.85	<2.00	<2.00	<2.00	3.61	<2.00	2.54
		2	<2.00	4.23	<2.00	<2.00	3.83	<2.00	
	0.67M	1	<2.00	3.51	2.30	<2.00	<2.00	<2.00	2.27
		2	<2.00	3.40	<2.00	<2.00	<2.00	<2.00	
4	Control	1	2.90	3.18	4.51	5.56	4.86	3.36	3.90
		2	3.20	4.15	4.20	3.68	3.92	3.45	
	0.33M	1	6.26	<2.00	3.26	<2.00	4.38	3.30	3.62
		2	4.23	2.00	5.00	<2.00	5.65	3.36	
	0.67M	1	3.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.22
		2	2.95	<2.00	2.70	2.00	<2.00	<2.00	

[a] Yeast and mold counts; Acidified Potatoe Dextrose Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE XVII

INFLUENCE OF POTASSIUM SORBATE ON TOTAL AEROBIC COUNTS[a] ON BOVINE HIDES DURING STORAGE AT 21°C

DAYS	TREATMENT	DSN[b]	Log_{10} Counts/g I[c]	II	Avg.
0	Control	1	4.70	5.08	4.98
		2	4.61	5.51	
	Control	1	9.26	9.04	9.27
		2	9.57	9.20	
2	0.33M	1	9.23	8.48	8.82
		2	9.20	8.38	
	0.67M	1	8.97	7.89	8.50
		2	9.11	8.04	
	Control	1	9.73	10.57	9.92
		2	9.71	9.65	
4	0.33M	1	9.80	9.30	9.51
		2	9.75	9.20	
	0.67M	1	9.74	10.96	9.69
		2	9.25	8.81	

[a] Total aerobic counts: Trypticase Soy Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE XVIII

INFLUENCE OF POTASSIUM SORBATE ON GRAM NEGATIVE COUNTS[a] ON BOVINE HIDES DURING STORAGE AT 21°C

DAYS	TREATMENT	DSN[b]	Log_{10} Counts/g I[c]	II	Avg.
0	Control	1	3.88	3.96	3.92
		2	3.57	4.28	
	Control	1	9.00	8.62	8.81
		2	9.11	8.51	
2	0.33M	1	8.62	7.08	7.93
		2	8.63	7.40	
	0.67M	1	8.18	7.23	7.65
		2	8.11	7.08	
	Control	1	9.73	9.43	9.59
		2	9.60	9.60	
4	0.33M	1	9.63	8.79	9.01
		2	9.11	8.51	
	0.67M	1	8.76	7.61	8.04
		2	8.08	7.70	

[a] Gram negative counts: Crystal Violet Tetrazolium Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE XIX

INFLUENCE OF POTASSIUM SORBATE ON NUMBERS OF OF *CLOSTRIDIUM PERFRINGENS*[a] ON BOVINE HIDES DURING STORAGE AT 21°C

DAYS	TREATMENT	DSN[b]	Log_{10} Counts/g I[c]	II	Avg.
0	Control	1	<2.00	<2.00	<2.00
		2	<2.00	<2.00	
	Control	1	6.00	6.15	6.18
		2	6.40	6.18	
2	0.33M	1	5.52	2.0	<3.70
		2	4.96	2.30	
	0.67M	1	5.04	2.00	3.83
		2	5.18	3.11	
	Control	1	6.45	6.53	6.66
		2	6.83	6.84	
4	0.33M	1	6.36	5.99	6.02
		2	5.81	5.90	
	0.67M	1	6.04	<3.00	<4.48
		2	5.90	<3.00	

[a] Enumeration of *C. perfringens*: Tryptose Sulfate Cyclo-servine Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE XX

INFLUENCE OF POTASSIUM SORBATE ON NUMBERS OF COAGULASE POSITIVE STAPHYLOCOCCI[a] ON BOVINE HIDES DURING STORAGE AT 21°C

DAYS	TREATMENT	DSN[b]	Log_{10} Counts/g I[c]	II	Avg.
0	Control	1	<2.00	2.00	2.00
		2	<2.00	2.00	
	Control	1	<2.00	5.87	3.87
		2	<2.00	5.59	
2	0.33M	1	<2.00	4.70	4.00
		2	3.57	5.71	
	0.67M	1	3.91	3.93	3.45
		2	3.97	<2.00	
	Control	1	2.70	2.78	3.61
		2	2.78	6.18	
4	0.33M	1	3.95	6.08	4.68
		2	6.67	<2.00	
	0.67M	1	4.61	4.30	5.12
		2	5.98	5.59	

[a] Enumeration of coagulase positive staphylococci: Baird Parker Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE XXI

INFLUENCE OF POTASSIUM SORBATE ON YEAST-MOLD COUNTS[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g		
DAYS	TREATMENT	DSN[b]	I[c]	II	Avg.
0	Control	1	2.60	3.00	2.69
		2	2.00	3.15	
	Control	1	3.68	4.04	3.81
		2	3.53	3.99	
2	0.33M	1	4.51	3.48	4.01
		2	4.53	3.52	
	0.67M	1	4.15	2.60	< 3.33
		2	4.58	<2.00	
	Control	1	4.11	5.57	4.40
		2	3.85	4.08	
4	0.33M	1	5.28	5.71	5.51
		2	4.85	6.18	
	0.67M	1	5.97	4.49	5.09
		2	5.62	4.26	

[a] Yeast and mold counts: Acidified Potatoe Dextrose Agar.
[b] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[c] Roman numeral refers to trial number.

TABLE XXII

COMPARISON OF THE INFLUENCE OF 0.33M ACETIC ACID AND 0.33M FORMIC ACID ON TOTAL AEROBIC COUNTS[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g							
DAYS	TREATMENT	DSN[c]	I[d]	II	III	IV	V	VI	VII	Avg.
0	Control	1	5.36	6.45	5.00	4.63	6.11	4.86	5.54	5.45
		2	5.69	6.79	4.96	4.15	5.97	5.49	5.32	
4	Control	1	9.40	9.86	9.48	10.00	9.82	9.92	9.71	9.75
		2	9.46	9.90	9.79	9.90	9.81	9.88	9.57	
	A[b] 0.33M	1	7.85	9.04	7.49	7.08	9.18	7.04	8.87	7.87
		2	6.88	7.40	6.79	5.83	9.30	9.48	7.96	
	F[b] 0.33M	1	3.72	3.18	2.60	4.23	6.65	5.69	5.89	4.55
		2	7.20	4.65	3.86	3.08	5.15	4.81	3.04	
8	Control	1	9.60	9.75	10.11	9.82	10.18	9.68	9.61	9.83
		2	9.73	9.90	9.93	9.75	9.86	10.04	9.72	
	A 0.33M	1	8.36	10.00	9.23	9.08	10.23	9.54	9.63	9.45
		2	10.04	10.18	7.38	8.72	10.18	9.83	9.86	
	F 0.33M	1	4.57	6.64	3.08	6.18	>7.48	>7.48	>7.48	6.41
		2	5.34	5.94	>7.48	5.65	>7.48	>7.48	>7.48	

[a] Total aerobic counts: Trypticase Soy Agar.
[b] A: acetic acid treatment; F: formic acid treatment.
[c] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[d] Roman numeral refers to trial number.

TABLE XXIII

COMPARISON OF THE INFLUENCE OF 0.33M ACETIC ACID AND 0.33M FORMIC ACID ON GRAM NEGATIVE COUNTS[a] BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g							
DAYS	TREATMENT	DSN[c]	I[d]	II	III	IV	V	VI	VII	Avg.
0	Control	1	4.36	4.54	2.85	4.04	4.58	4.61	4.53	4.08
		2	4.68	4.73	3.00	2.00	4.08	4.88	4.26	
	Control	1	9.20	9.65	9.20	9.86	10.04	9.79	9.61	9.57
		2	9.18	9.52	9.72	9.64	9.77	9.46	9.32	
4	A[b] 0.33M	1	7.91	8.96	6.77	7.08	9.26	6.11	8.94	7.63
		2	6.77	7.15	6.51	5.49	9.30	8.48	8.04	
	F[b] 0.33M	1	<2.00	<2.00	<2.00	3.76	2.90	5.23	4.51	3.14
		2	5.30	<2.00	<2.00	<2.00	3.81	4.49	<2.00	
	Control	1	9.32	9.56	LA[e]	9.56	10.28	9.32	9.30	9.61
		2	9.51	9.85	LA	9.67	9.81	9.69	9.40	
8	A 0.33M	1	8.23	9.95	9.42	9.18	10.00	9.26	9.60	9.36
		2	10.08	10.04	7.08	8.75	10.26	9.76	9.49	
	F 0.33M	1	<2.00	<2.00	<2.00	5.08	4.38	3.32	2.00	3.05
		2	<2.00	4.23	<2.00	3.76	4.84	<2.00	3.04	

[a] Gram negative counts: Crystal Violet Tetrazolium Agar.
[b] A: acetic acid treatment; F: formic acid treatment.
[c] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[d] Roman numeral refers to trial number.
[e] LA: Lab accident.

TABLE XXIV

COMPARISON OF THE INFLUENCE OF 0.33M ACETIC ACID AND 0.33 M FORMIC ACID ON NUMBERS OF *CLOSTRIDIUM PERFRINGENS*[a] ON BOVINE CATTLE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g							
DAYS	TREATMENT	DSN[c]	I[d]	II	III	IV	V	VI	VII	Avg.
0	Control	1	2.70	2.00	<2.00	<2.00	2.00	3.42	2.78	2.45
		2	2.78	2.00	2.00	<2.00	<2.00	3.85	2.70	
	Control	1	7.46	6.99	7.92	6.28	7.95	8.46	6.20	7.01
		2	7.42	6.20	7.72	6.11	5.23	8.34	5.90	
4	A[b] 0.33M	1	3.54	5.30	2.60	<2.00	5.53	4.88	5.28	4.05
		2	4.45	2.30	<2.00	<2.00	5.86	5.49	5.52	
	F[b] 0.33M	1	2.48	<2.00	2.00	<2.00	4.15	<2.00	<2.00	2.25
		2	2.85	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	Control	1	6.04	7.00	6.97	6.51	8.82	6.28	6.64	7.26
		2	6.97	7.90	7.82	7.04	8.51	7.89	7.28	
8	A 0.33M	1	6.94	6.54	6.04	4.93	8.18	7.54	6.99	6.46
		2	6.38	7.08	<3.00	3.95	8.46	6.99	7.46	
	F 0.33M	1	2.00	2.00	2.48	<2.00	5.66	5.08	5.48	3.52
		2	2.30	2.30	<2.00	<2.00	5.00	5.30	5.70	

[a] Enumeration of *C. perfringens*: Tryptose Sulfite Cyclo-serine Agar.
[b] A: acidic acid treatment; F: formic acid treatment.
[c] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[d] Roman numeral refers to trial number.

TABLE XXV

COMPARISON OF THE INFLUENCE OF 0.33M ACETIC ACID AND FORMIC ACID ON NUMBERS OF COAGULASE POSITIVE STAPHYLOCOCCI[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g							
DAYS	TREATMENT	DSN[c]	I[d]	II	III	IV	V	VI	VII	Avg.
0	Control	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	Control	1	<2.00	5.52	<2.00	<2.00	<2.00	7.08	4.98	4.16
		2	<2.00	6.81	>7.48	<2.00	<2.00	6.58	5.76	
4	A[b] 0.33M	1	<2.00	2.90	<2.00	<2.00	3.76	<2.00	<2.00	2.68
		2	<2.00	<2.00	<2.00	<2.00	3.81	7.11	<2.00	
	F[b] 0.33M	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	Control	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.71
		2	<2.00	7.30	<2.00	<2.00	<2.00	6.65	<2.00	
8	A 0.33M	1	<2.00	<2.00	5.04	<2.00	<2.00	<2.00	<2.00	2.22
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
	F 0.33M	1	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	2.00
		2	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	

[a] Enumeration of Coagulase Positive Staphylococci; Baird Parker Agar.
[b] A: acidic acid treatment; F: formic acid treatment.
[c] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[d] Roman numeral refers to trial number.

TABLE XXVI

COMPARISON OF THE INFLUENCE OF 0.33M ACETIC ACID AND 0.33M FORMIC ACID ON YEAST-MOLD COUNTS[a] ON BOVINE HIDES DURING STORAGE AT 21°C

			Log_{10} Counts/g							
DAYS	TREATMENT	DSN[c]	I[d]	II	III	IV	V	VI	VII	Avg.
0	Control	1	2.00	3.36	2.30	2.60	3.08	2.00	3.00	2.69
		2	2.70	3.60	2.00	2.30	2.70	3.30	2.78	
	Control	1	5.72	5.11	5.61	3.49	4.81	4.79	4.79	4.88
		2	5.79	5.15	4.96	3.75	3.81	5.00	5.49	
4	A[b] 0.33M	1	6.52	>7.48	6.46	6.85	7.38	7.48	7.11	7.00
		2	6.99	7.26	6.72	6.65	7.34	6.89	6.93	
	F[b] 0.33M	1	6.54	2.60	2.00	4.11	6.59	6.28	6.88	4.32
		2	<2.00	4.83	4.00	<2.0	5.11	5.49	<2.0	
	Control	1	4.74	6.00	4.83	4.26	3.72	4.65	5.57	4.71
		2	4.88	5.72	3.95	3.28	3.92	5.94	4.52	
8	A 0.33M	1	7.28	7.48	>7.48	7.08	>7.48	7.08	7.00	7.17
		2	7.28	7.00	7.26	7.23	7.30	6.72	6.72	
	F 0.33M	1	4.60	4.04	5.59	6.15	6.00	>7.48	6.04	5.68
		2	5.49	5.86	3.00	5.00	6.58	7.56	6.15	

[a] Yeast and mold counts: Acidified Potatoe Dextrose Agar.
[b] A: acetic acid treatment; F: formic acid treatment.
[c] DSN: duplicate sample number; two 3 x 3 cm portions of each hide analyzed at each sampling time for each treatment.
[d] Roman numeral refers to trial number.

APPENDIX B

REPRESENTATIVE SAMPLES OF STATISTICAL PROCEDURES

TABLE XXVII

LIST OF SYMBOLS USED IN STATISTICAL ANALYSIS

a= animal
t= treatment
s= sample
tot= total
an= animal
trt= treatment
err= error
Y_{ijk}= observation on animal number i, treatment number j, and sample number k

$$CF = \frac{(\sum Y_{ijk})^2}{ats} = \text{correction factor}$$

A_i = total of t x s = number of observations for animal number i

T_j = total of a x s = number of observations for treatment number j

S_{ij} = total number of s = number of observations for animal number i and treatment number j

$$L_i = \sum_{j=1} C_o T_o \text{ where } \sum C_o = 0(\sum \text{Coef}^2)$$

$$SS\ (L_i) = \frac{NL_i^2}{\sum C_j^2} \qquad n = (s)(t)$$

$$F = \frac{SS\ (L_i)}{mse}$$

TABLE XXVIII

EQUATIONS USED IN THE ANALYSIS OF VARIANCE

Source	df	SS	MS = $\frac{ss}{df}$	F = $\frac{MS}{MSE}$
Total	ats - 1	$\lesssim Y^2_{ijk}$ - C.F.	$\frac{SS(tot)}{(ats-1)}$	
Animal	a - 1	$\lesssim \frac{Ai^2}{ts}$ - C.F.	$\frac{SS(an)}{a-1}$	F = $\frac{SS(Li)}{MSE}$
Treatment	t - 1	$\lesssim \frac{Tj^2}{as}$ - C.F.	$\frac{SS(trt)}{t-1}$	F = $\frac{SS(Li)}{MSE}$
an x trt	(a-1) (t-1)	$\left(\lesssim \frac{S^2ij}{S} - C.F.\right)$ -SS(an)-(trt)	$\frac{SS(an \times trt)}{(a-1)\ (t-1)}$	F = $\frac{SS(Li)}{MSE}$
Error	at (s-1)	SS(tot)-SS(an)-SS(trt)-SS(an x trt)	MSE = $\frac{SS(err)}{at(S-1)}$	

TABLE XXIX

EXAMPLE OF THE ANALYSIS OF VARIANCE; INFLUENCE OF ACETIC ACID ON NUMBERS OF TOTAL AEROBIC MICROORGANISMS ON BOVINE HIDES DURING STORAGE AT 21°C

	Day 0		Day 2					
	TREATMENT							
Animal #	1 Control		2 Control		3 0.33M		4 0.67M	
1	5.04	5.64	9.15	9.05	6.56	5.64	5.34	4.00
2	3.78	4.08	8.59	8.48	4.00	4.00	4.00	4.00
3	5.53	5.52	8.99	8.79	7.15	6.66	3.79	3.63
4	3.95	4.15	9.51	9.77	4.65	5.30	3.60	3.00
5	4.97	4.82	8.77	8.86	7.28	9.41	2.60	2.60
6	4.59	4.48	9.30	9.11	3.65	4.15	3.30	2.85
7	5.94	5.79	9.48	9.34	6.11	7.42	5.30	5.04
ΣT_j	33.80	34.48	63.79	63.40	39.40	42.58	27.93	25.12
$\bar{T}_j$	4.83	4.93	9.11	9.06	5.63	6.08	3.99	3.59
ΣT_j^2	166.93	172.96	582.04	575.26	235.43	281.08	117.58	94.46
$\frac{\Sigma(T_j)^2}{n}$	163.21	169.84	581.31	574.22	221.77	259.01	111.44	90.14

	Day 4								
Animal #	5 Control		6 0.33M		7 0.67M		ΣA_j	ΣA_j^2	$\frac{\Sigma(A_j)^2}{n}$
1	9.52	10.04	5.51	8.08	4.30	4.00	91.87	663.76	602.86
2	8.85	8.62	7.81	6.48	3.60	3.00	79.29	518.21	449.06
3	9.79	9.72	7.95	8.08	7.15	3.63	96.38	725.27	663.51
4	10.08	10.18	7.45	7.49	2.95	5.61	87.69	647.41	549.25
5	9.51	9.30	2.70	6.53	5.26	2.30	84.91	618.23	514.98
6	9.85	9.54	2.48	2.00	2.90	3.91	72.11	482.06	371.42
7	9.82	9.74	9.32	8.28	6.23	3.70	101.51	791.02	736.02
ΣT_j	67.42	67.14	43.22	46.94	32.39	26.15	613.76	4445.96	3887.10
$\bar{T}_j$	9.63	9.59	6.17	6.71	4.63	3.74		↓	↑
ΣT_j^2	650.30	645.59	310.36	343.86	166.17	103.92	4445.94	← ΣY_{ijk}^2	$\frac{\Sigma A_i^2}{tn}$
$\frac{\Sigma(T_j)^2}{n}$	649.35	643.97	266.85	314.77	149.87	97.69	4293.44	← $\frac{\Sigma T_j}{an}$	

TABLE XXX

FURTHER COMPUTATIONS FOR ANALYSIS OF VARIANCE

To obtain: $\sum \frac{S_{ij}^2}{S}$

Add together two sample numbers of animal #i, treatment #j; then $\sum \frac{S_{ij}^2}{S}$

i.e. $\frac{(5.04 + 5.64)^2}{2} = \frac{(10.68)^2}{2} = 57.03$

Animal #	TREATMENTS 1	2	3	4	5	6	7
1	10.68	18.19	12.20	9.34	19.56	13.59	8.30
2	7.86	17.07	8.00	8.00	17.47	14.29	6.60
3	11.05	17.78	13.81	7.42	19.51	16.03	10.78
4	8.10	19.28	9.95	6.60	20.26	14.94	8.56
5	9.79	17.63	16.69	5.20	18.81	9.23	7.46
6	9.07	18.41	7.80	6.15	19.39	4.48	6.81
7	11.73	18.82	13.53	10.34	19.56	17.60	9.93
$\sum \frac{S_{ij}^2}{S}$	339.63	1157.02	512.51	210.82	1295.64	642.04	252.03

Total = 4409.69 $\sum \frac{ES^2_{ij}}{2}$

TABLE XXXI

ANALYSIS OF VARIANCE TABLE FOR DATA[a]: INFLUENCE OF[b] ACETIC ACID ON NUMBERS OF TOTAL AEROBIC MICROORGANISMS[b] ON BOVINE HIDES DURING STORAGE AT 21°C

Source	df	SS	MS	F
Total	97	602.05	6.21	
Animal	6	43.21	7.20	9.73
Treatment	6	449.55	74.925	101.28
an x trt	36	73.04	2.03	2.74
Error	49	36.25	0.7398	

[a] Data: see Table XXVIV (Appendix B)
[b] Total aerobic microorganisms: enummerated on Trypticase soy agar.

For further computations, treatment was broken down into orthog and contrasts, i.e.,

Is there a difference between no acid and acid

$$L_g = 4 \times T_1 + 4T_2 + 4T_5 - 3T_3 + 3T_4 + 3T_6 + 3T_7$$

Where T: mean of treatment number j

(* means obtained from Duncan's multiple range test)

TABLE XXXII

DUNCAN'S MULTIPLE RANGE[a]
(FOR ALL POSSIBLE TREATMENT PAIRS)

- U_{ti} differs from the U_{tj} if $/\bar{T}_i - \bar{T}_j/ \geq q_{.05}$, p, d_{fE} x S
- The observed $\bar{T}$ values are ordered (smallest to largest):
    1. p is the "number of means" from i to j in the order
    2. df_E is degrees of freedom from error row of ADV table
    3. $S = \sqrt{MSE/(a \times s)}$
    4. $q_{.05}$, p, df_e is on page 442-443 of Steel and Torrie (1960)

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[a] Source: Steel and Torrie (1960, p. 107).

VITA

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