

POTASSIUM SORBATE AS A FUNGISTATIC AGENT

IN COUNTRY HAM PROCESSING

by

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1.0 INTRODUCTION

Country ham has long been a food commodity in Virginia and throughout the Southeastern United States. One of the recurring problems in its production and distribution has been the growth of molds and yeasts on ham surfaces during conditions of high relative humidity. Such conditions are not uncommon in the Southeast.

Mold growth on country ham was at one time thought to be indicative of proper aging and flavor development. However, studies have revealed that the development of flavor in dry cured ham is more the result of enzymatic and chemical changes than the presence of microbial growth (Lillard and Ayres, 1969; Giolitti et al., 1971). Larger processors have also expressed interest in exporting their products to locations outside the Southeastern United States. Consumers outside the "country ham belt", unfamiliar with the commodity, would be inclined to dispose of hams exhibiting significant amounts of mold growth.

A far more ominous problem from fungal growth on country ham is the potential presence of mycotoxins. Studies have revealed that several species of mold isolated from country ham possess the capability of producing mycotoxins. These toxins include ochratoxin, sterigmatocystin, citrinin, and aflatoxin (Strzelecki et al., 1969; Sutic et al., 1972; Escher et al., 1973; Hall and Ayres, 1973; Wu et al., 1974).

Sorbic acid and its potassium salt have been shown to be effective at inhibiting mold growth, including the genera that have

the mycotoxin producing capability. Studies conducted during the past 23 years have also demonstrated sorbate's lack of toxicity when it is administered to rats, mice, and dogs (Duel et al., 1954a, 1954b; Shitenberg et al., 1970; Gaunt et al., 1975).

Many investigations have been made on the use of sorbate in food products; it has already been approved as an antimicrobial agent in many foods including dairy, bakery, and fruit products. Less work, however, has been done on the use of sorbate in meats. The only meat products where its use is presently permitted are dry sausage casings (2.5% dip) and in dog food patties (Chichester and Tanner, 1972).

Therefore, a need exists for an effective fungistatic agent for use on country cured hams. The purpose of this study was to explore the feasibility of utilizing potassium sorbate as a fungal inhibitor on country hams.

The specific objectives of this investigation were:

1. To determine the lowest level of potassium sorbate effective against fungal growth.
2. To determine the concentrations of sorbate deposited on ham surfaces by various methods of application.
3. To determine the longevity of sorbate's effectiveness under environmental conditions favorable to mold growth.
4. To determine if and to what extent sorbate would degrade following application.

5. To determine the optimum time for application of potassium sorbate (60 day old ham vs. 90 day old ham).
6. To develop an effective procedure for application of sorbate which would be commercially feasible under processing practices.

2.0 REVIEW OF LITERATURE

2.1 Production of Country Cured Hams

Country cured hams have long been a favorite food with the people of the Southeastern United States. For many years Virginia has been noted for its country cured hams. In fact, Virginia hams were one of the first agricultural exports from the British colonies in the New World (Kelly *et al.*, 1974).

A country ham is characterized by a production process comprised of three different steps (curing, equalization, and aging). The first step is curing, the procedure where a dry mixture of salt (NaCl) and sodium nitrate and/or sodium nitrite is rubbed on the surface of the ham. Sugar (sucrose) is often, but not always, another ingredient of the curing mixture. The mixture is usually divided into three portions, which are applied over a period of about 15 days at five to ten day intervals (Hunt *et al.*, 1939; Kelly *et al.*, 1974). Just prior to equalization, hams were washed to remove excess salt.

The second step is equalization, which is the period just after curing when the hams are hung at an intermediate temperature (50 to 55°F) to facilitate distribution of the curing ingredients within the muscles of the ham. Equalization is allowed a two to four week time period. The curing time and equalization period will undoubtedly vary among processors and regions of the country, but in all instances the length of salt curing and equalization should be at least 45 days in commercially produced country hams (Mussman, 1977).

The smoking of country hams varies with geographical localities and consumer preferences; therefore this step is optional. If the hams are smoked, it is generally at a fairly low temperature, such as 100°F for 48 hours (Anonymous, 1971). The smoking process always utilizes deciduous or hardwood trees, like hickory, maple, apple, etc., which impart a desirable smoked flavor. Softwoods like pine, cedar, etc. are undesirable because they will create a smoke which will deposit resins on the meat (Kelly et al., 1974).

Aging is the final stage of processing prior to sales and distribution. During this period the product develops its characteristic "country" flavor. The past three or four decades have seen much work done on the physical and chemical changes occurring during aging. Hunt et al., (1939) described the important physical and chemical changes that occur during aging: an increase in fat hydrolysis; a dramatic increase in the total and amino soluble nitrogen, although these reactions would become stationary after about two years of aging; and an increase in redness in the lean and yellowness in the fat. Salt concentration increases from 5.7% after one month of aging to 8.4% after two years of aging. Shrinkage occurs up to 33% during the first year. Acidity increases over the two year period, especially if the hams were obtained from full fed hogs. Moisture content, as would be expected, drops to approximately 40% and below after two years of aging. Current market demands and processing practices do not require a two year ham. Present day commercial hams are aged one to four months, producing hams that are

90 to 180 days old. Economic restraints apparently limit the age of a commercially produced country ham (Baldock, 1977).

Fresh hams are cut in two different styles for dry curing: the long cut and the packer style cut. Kelly *et al.* (1974) noted several advantages of the long cut ham. In the packer cut both the shank and butt are cut short, exposing greater areas of lean surface. The possibility of microbial and insect invasion is increased during aging when large areas of lean are exposed, in addition, there is color and flavor deterioration in these exposed areas during aging. These hazards are increased in the Southeastern United States where high relative humidities and temperatures are commonplace during a significant portion of the year. For these reasons packer style cut hams are aged commercially for shorter periods of time. Long cut hams, though, are cut in a violin shape which gives them greater integrity. These hams are also cut below the knee which gives them a longer shank.

2.2 Microbiological and Chemical Influences on Country Ham

Bacteria are extensively involved in the European curing process described by Leistner (1960). The process he described, though, was a brine curing method. Country style hams are usually produced by a dry cure (salt) method without the use of a brine or pickle and without adding any microorganisms that are not originally present. Probably the one European variety most resembling the country ham is an Italian type raw ham (Parma ham). Curing agents for the Italian

hams consist of salt only, while the country hams of the U.S. use salt, nitrate and/or nitrite, and usually sugar (Giolitti et al., 1971). However, both are dry cured. Giolitti and coworkers have investigated the microbiological changes that occur in Parma hams during the curing step. They found that the number of microflora remained low during both curing and aging, with halotolerant or halophilic microorganisms predominating as would be expected.

Chemical changes also occur during curing and subsequent aging. Lillard and Ayres (1969) studied the flavor of country cured hams as affected by these chemical reactions. Most of the volatiles identified were not unique to hams, but also have been found in other varieties of cooked meat. They found 12 alkanals, six alk-2-enals, and four alk-2,4-dienals in the lipid portion of the hams. The variations in lipid and protein degradation were attributed to variations in lengths and temperatures of aging. The country cured hams utilized in the study were all obtained from processors in Virginia, North Carolina, Kentucky, Missouri, and Georgia. The hams from Virginia and North Carolina had a higher concentration of free fatty acids in the lean than the ones from the other states, which Lillard and Ayres (1969) believed to be the result of either aging the hams at a higher temperature or for a longer time. They concluded that the flavors in these hams were not water soluble; that the unique flavors were developed during aging; and that carbon-yls, free fatty acids, and amino acids all contributed to the flavor.

One of the questions that now arises is what possible influence bacteria have on the aging of country hams. Giolitti et al. (1971) contended that the development of flavor and tenderness was not dependent on the presence of microorganisms in a Parma ham, but on enzymes endogenous to the pork. This finding is contrary to the work of Leistner (1960), which demonstrated that bacteria played an important role in the development of flavor in brine cured hams. Giolitti and his coworkers conceded, however, that the breakdown of fatty acids in the lipid portion may have been due to both microbial and enzymatic activity.

The most significant conclusion that can be drawn from the studies of both Leistner and Giolitti is that brine and dry cured hams seem to differ as to their mechanisms of flavor development. Brine cured hams seem to depend primarily on microbial breakdown while dry cured hams depend more on the action of tissue enzymes. Obviously because country ham's curing process more closely resembles the Italian ham process than the brine cured type, it can be assumed that a country cured ham's flavor is derived through an enzymatic process. Bartholomew and Blumer (1977) noted that hams with lower bacterial counts after aging were, in fact, superior in flavor to those with higher counts. Their study concluded that the lactic acid producer, Pediococcus cerevisiae, may be used eventually as a starter culture in country ham cures to reduce the numbers of any potential pathogens, though country hams are not generally associated with outbreaks of food poisoning (Bryan, 1974).

2.3 The Mycology of Country Cured Hams

While bacteria may not find the cured country ham a suitable environment for growth, fungi might find it satisfactory with the proper relative humidity. The dilemma faced by the country ham producer is the role of mold in the curing and aging process. Ayres et al. (1967) noted that sometimes the growth of mold on a ham is the sign of proper aging. However, too much mold growth is thought to contribute a musty flavor to the product; in addition, consumers not familiar with country hams would be inclined to discard a country ham showing mold growth. Ayres et al. (1967) found mold growth on all 44 ham samples after six to nine months of storage. The amount of growth varied with the relative humidity in the aging or holding room. Thus, at low relative humidities (65% and below) only sparse growth was observed. Of the molds identified, penicillia and aspergilli were the ones most commonly encountered. Aspergillus growth was favored by a decrease in water activity (a_w) and an increase in temperature during the latter stages of aging. An important point made by Ayres and coworkers was that mold growth on ham surfaces seemed to inhibit fat oxidation and rancidity.

Of the two primary genera obtained from cured ham surfaces, penicillia were generally dominant during the early stages of aging. However, as aging proceeded and a_w decreased, the aspergilli became the prevailing genera (Leistner et al., 1965). These two genera were thought to contribute some desirable characteristics in addition to their inhibition of oxidative rancidity. Some Penicillium isolates

were thought to contribute to flavor because of their strong lipolytic characteristics, while some other isolates of both Aspergillus and Penicillium were found to be proteolytic (Leistner et al., 1965). However, Leistner and his coworkers expressed some concern over the effects of mold on the quality of a country cured ham. These effects included the potential danger from mycotoxins.

Studies on the identification of molds isolated from country hams have been very important because of the public health significance of mycotoxins. Sutic et al. (1972) isolated 562 strains of mold from country cured hams. Again the two most representative genera were Penicillium and Aspergillus. Of the two Penicillium had the largest number of isolates, with 403 being identified, while only 121 isolates were identified as aspergilli. Most of the remaining 36 or so isolates were identified as members of the genera Cladosporium and Alternaria.

Aging and moisture content had a definite effect on the types of mold isolated (Sutic et al., 1972). Hams aged for a year or longer tended to yield greater numbers of aspergilli than hams aged between one and three months. However, if these one to three month old hams were aged under dry conditions the number of aspergilli isolated would be higher than if a moist environment prevailed, in which case the counts of penicillia would be greater. Particular attention was directed towards the isolation of Aspergillus flavus due to its ability to produce aflatoxin. Only three of the 121 strains were A. flavus. Out of the 356 hams examined in the study,

only two A. flavus strains were found to produce aflatoxin. Sutic and coworkers concluded, though, that if suitable conditions were provided for the production of toxin, a clear and definite health hazard could have been demonstrated.

Escher et al. (1973) found that ochratoxins A and B were produced on country cured hams by Aspergillus ochraceus. Both ochratoxins are lethal, but B is one-tenth as toxic to day-old chicks as A is (Peckham et al., 1971). Neither A nor B are reported to be carcinogenic.

Penicillium viridicatum produces the toxin citrinin on country cured hams. Many varieties of penicillia and aspergilli produce citrinin, which is both a nephrotoxin and an antibiotic (Wu et al., 1974). Wu and coworkers found that seven strains of P. viridicatum were able to produce citrinin on sterile slices of ham. Production of the toxin was found to be dependent on temperature, with the maximum amounts produced between 25 and 30°C. At lower temperatures quantities detected decreased; at 10°C no citrinin was produced because at that temperature there was a cessation in mold growth. Wu and coworkers recommended that ham storage and aging temperatures be kept lower, although in practice temperatures will fluctuate in aging rooms because of climatic and night and day variations.

The toxin sterigmatocystin can be produced on country cured hams by strains of Aspergillus versicolor. Hall and Ayres (1973) investigated its possible production after Sutic et al. (1972) had noticed it was the second most commonly occurring aspergilli found

on country ham. They grew three isolates on ham slices at 20 and 28°C and after 14 days sterigmatocystin was detected in 11 out of 12 slices. It was thought that aging hams for one or two years under conditions of varying humidity might induce sterigmatocystin production, although most hams are aged one to four months by commercial producers. A review of the literature by Hall and Ayres (1973) revealed that the compound had been shown repeatedly to be toxic and, in some cases, carcinogenic to a wide variety of animals.

Of all the mycotoxins that may be produced on a country cured ham, aflatoxin is the most toxic and carcinogenic. Strzelecki *et al.* (1969) isolated ten different molds from country hams and found that out of that ten, four were *A. flavus* strains capable of producing the toxin. Using Verrett's (1964) method of determining toxicity they found that 0.025 µg of toxin per fertile egg resulted in the embryo's death. One day-old ducklings would develop proliferation of the bile duct cells after being administered an extraction from the mold cultures, a symptom indicative of aflatoxin B₁. Of the external influences that will affect the type of aflatoxin produced (either the B or G variety), pH has been found to be very important. Buchanan and Ayres (1975) found that pH levels below six favored B toxin production, while levels above six favored the G variety.

2.4 Introduction to the Sorbates

Sorbic acid and its salts are substances used as preservatives in commercially prepared foods. Sorbates are presently on the FDA list of Generally Recognized As Safe (GRAS) substances (Sec. 121.101

of the Code of Federal Regulations); but as an approved additive its use is restricted to certain food products. Sorbic acid has a large advantage over many other food additives because of its lack of distinctive flavor and odor in normal food applications (Chichester and Tanner, 1972), and in addition, its very low toxicity (Gaunt et al., 1975). Sorbic acid has a very low solubility in water, i.e. 0.16 g/100 ml at 20°C. Consequently, sorbic acid can not be used with water as a dip or spray, but instead must be dissolved in propylene glycol or in ethanol. It can also be dissolved with hydroxides (i.e. sodium and potassium) for use as a liquid (Chichester and Tanner, 1972). Due to the limited solubility of sorbic acid in water and the large need for aqueous solutions as sprays or dips, the potassium salt of sorbic acid is a more convenient form of the substance, with its water solubility of 139.2 g/100 ml at 20°C.

All of the sorbates are effective as fungistatic agents. The susceptibility of microorganisms to sorbate is dependent on the concentration of sorbate, the pH of the system, and microbe itself. Bell et al. (1959) found in studies with 66 species of filamentous fungi, 32 species of yeasts, and six species of lactic acid bacteria that all were able to grow in a 0.1% sorbate solution at pH 7. The yeasts and molds, however, were inhibited at pH 4.5 and the bacteria at pH 3.5 at the same 0.1% solute strength. The experiments thereby revealed that the lower the pH, the greater the concentration of undissociated sorbic acid which, in turn, provides the antimicrobial property of the sorbates (Bell et al., 1959).

2.5 Mode of Sorbic Acid Inhibition

There have been many theories put forth to explain the mode of inhibition by sorbic acid and its salts. One of the older ones relating to mold control was devised by Melnick et al. (1954a). The key to an understanding of the theory by Melnick is in the mechanism of beta oxidation of fatty acids. This mechanism exists in practically all living organisms (Conn and Stumpf, 1972). In animal cells this system is membrane bound as opposed to bacterial cells, where it occurs freely soluble in the microbe's cytoplasm (Conn and Stumpf, 1972). Figure 1 illustrates the normal route of degradation for either caproic acid or sorbic acid by both animal and mold. Melnick's experiments seemed to indicate that sorbate's fungistatic properties were due to inhibition of the dehydrogenase enzyme system during beta oxidation of the fatty acids. Mukherjee (1952) established the basis of this theory in the early 1950's. He utilized cyanide as a selective inhibitor of the oxidation of butyric acid to ketones in fungi.

Melnick et al. (1954a) drew upon Mukherjee's finding in proposing their hypothesis of mold inhibition by sorbate. Mukherjee (1952) found that the dehydrogenase enzyme which converts butyric to crotonic acid was not affected by the cyanide. We may assume what he meant by dehydrogenase enzyme was the acyl-CoA dehydrogenase which catalyzes the conversion of butyryl-CoA to crotonyl-CoA. He did find, however, the enzyme system responsible for the conversion of crotonic acid (crotonyl-CoA) to hydroxybutyric acid (hydroxybutyryl-

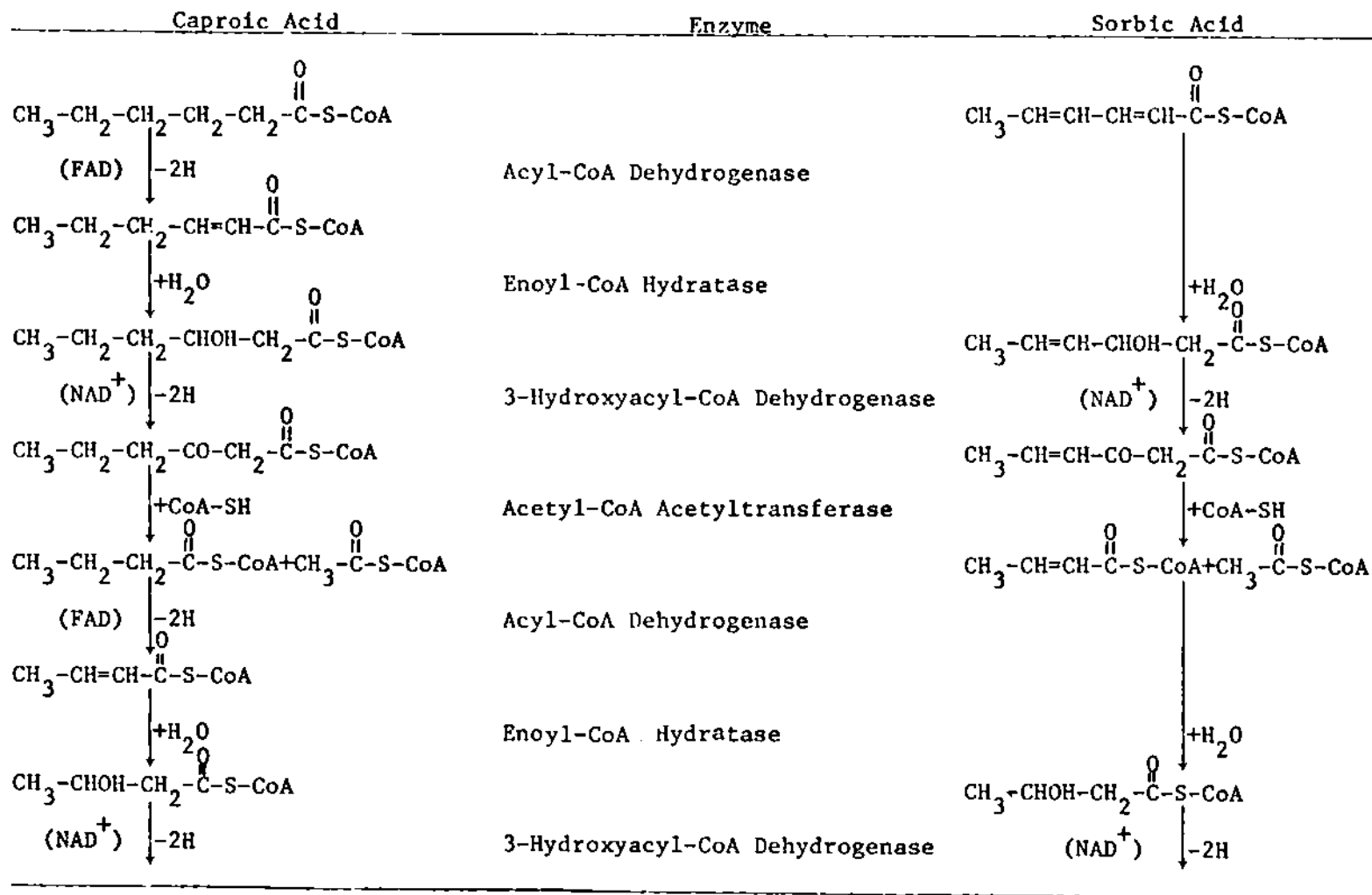


Figure 1. Normal β -oxidation of fatty acids by animal organisms and molds.

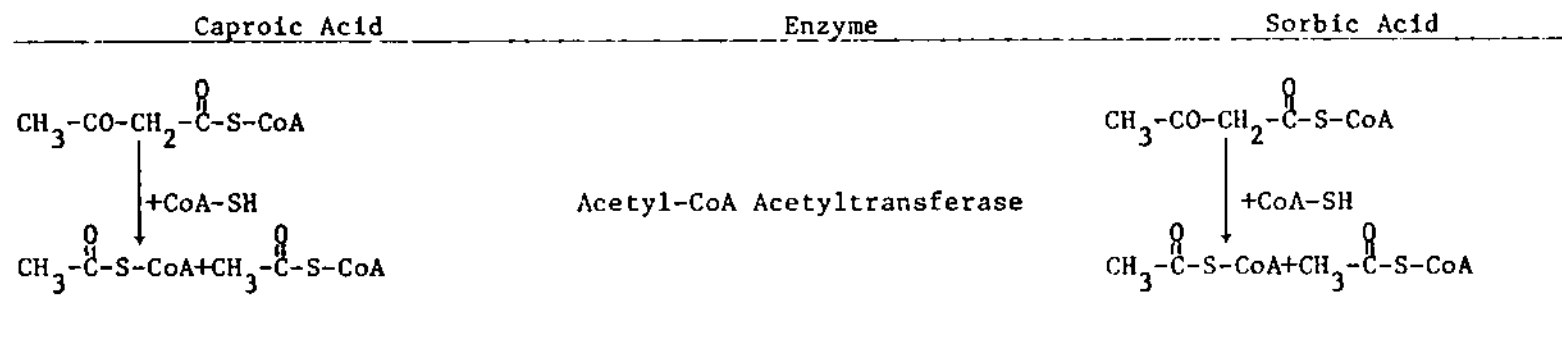


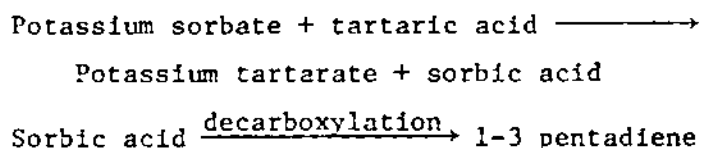
Figure 1. Continued

CoA) to be completely inhibited in the presence of cyanide. Mukherjee's experiments illustrated that cyanide enhanced unsaturated fatty acid production (crotonyl-CoA) during butyric acid oxidation. However, when the maximum level was reached the concentration of these unsaturated products did not change significantly; for the product formation was inhibited by the high concentration of α - β -unsaturated fatty acids (crotonyl-CoA). The high concentration of unsaturated fatty acids caused feedback inhibition of the acyl-CoA dehydrogenase system. Hence, an α - β -unsaturated fatty acid, e.g. sorbic acid, will inhibit the dehydrogenase enzyme system of molds in the same manner as did crotonyl-CoA.

The studies of Melnick et al. (1954a) and Mukherjee (1952) conclusively demonstrated the fungistatic nature of sorbic acid, though its effectiveness is dependent on the amount of mold growth. A heavy mold concentration will therefore overcome the feedback inhibition of sorbic acid and metabolize it as it would any other fatty acid.

The ability of some molds to metabolize potassium sorbate was a subject of an investigation by Marth et al. (1966). As his test organism he used members of the genus Penicillium. In Penicillium cultures where potassium sorbate had been added, a "hydrocarbon-like" odor was observed after 24 to 36 hours of incubation. Volatiles extracted from the cultures were analyzed by gas chromatography and infrared spectroscopy. The analysis indicated the presence of a 1-3 pentadiene. It was thought that this substance could be a

decarboxylation product of sorbate. Marth and his coworkers proposed the following reactions as the possible mechanism:



The fungistatic properties of sorbate would be lost through the decarboxylation reaction.

Troller (1965) investigated the role of the enzyme catalase in the inhibitory properties of sorbate. After noting that previous research had disclosed the inhibitory effects of sorbic acid on catalase production in some bacteria, he proposed an investigation to determine the effect it had on the catalase system of Aspergillus niger. Catalase is the enzyme which breaks down hydrogen peroxide. Troller postulated that if catalase was inhibited, the hydrogen peroxide concentration might increase to toxic levels. Because spores of A. niger contain smaller amounts of catalase than vegetative cells, sorbate would have a more drastic effect on the germinating spores. The amount of inhibition was shown to be dependent upon the following: 1) the concentration of sorbic acid; 2) how long the catalase was exposed to it; and 3) the pH. Troller theorized that sorbate, as it breaks down via auto-oxidative deterioration, goes through a number of oxidative states. One state was shown to be sorbyl peroxide, which possibly inactivated catalase or other enzymes.

More recent work has revealed other metabolites produced by molds in response to the addition of sorbate. Kurogochi et al. (1974) studied the intermediates of sorbic acid metabolism in two molds obtained from soil samples. They were members of the genera Mucor and Geotrichum. By utilizing a medium consisting of 0.1% yeast extract, 500 ppm potassium sorbate, 1.0% polypeptone, and 5.0% glucose, they found that trans-4-hexenol was the main metabolite of the Mucor sp. while trans-4-hexenoic acid and ethyl sorbate were the major ones produced by Geotrichum sp.

The inhibitory properties of sorbic acid and its salts have not been limited only to the molds. Kotyk et al. (1971) found with actidione-treated (protein synthesis inhibited) S. cerevisiae cells that potassium sorbate inhibited the uptake of L-aspartic acid and glycine, while actually enhancing the uptake of L-lysine. The mechanism of sorbic acid inhibition of alcohol fermentation by Saccharomyces cerevisiae was investigated extensively by Azukas et al. (1961). The extent of inhibition was found to be dependent on pH; however, with cell free extracts the inhibition was not pH dependent. It was concluded that the effects of pH were dependent on the permeability of the cell wall. The cell wall had a greater permeability to the undissociated sorbic acid which existed at a lower pH. Sorbate was specifically shown to have an adverse effect on the production of CO₂ by cell free extracts from fructose 1-6 diphosphate, glucose, and 3-phosphoglyceric acid. The site of inhibition was pinpointed between 2-phosphoglyceric acid and phosphoenolpyruvate during

CO₂ production. Enolase was greatly inhibited by sorbate and Azukas and coworkers concluded that it was the primary inhibition site during alcoholic fermentation.

York and Vaughn (1964) examined the mechanism of inhibition of Saccharomyces cerevisiae, Pseudomonas aeruginosa, and Escherichia coli by sorbic acid. At concentrations ranging from 15 to 105 mg sorbate/100 ml, oxidative assimilation of glucose, acetate, succinate, and fumarate was inhibited. Oxidation of these same substrates was inhibited at higher levels of sorbic acid. Fumurate, aspartase, and succinate dehydrogenase, which are all sulfhydryl enzymes, were thought to be inhibited by sorbate through a thiol addition. York and Vaughn reached this conclusion after it was demonstrated that sorbic acid exhibited a loss of activity after reacting with cysteine.

2.6 Stability of the Sorbates

Sorbic acid has two main differences to distinguish it from naturally occurring unsaturated fatty acids. The first is its conjugation of the double bonds and the second is its shorter relative length (Melnick et al., 1954b). Conjugated fatty acids are found in nature in food lipids, though sorbate is not among them (Melnick et al., 1954b). Sorbic acid is present as the lactone parasorbic acid in the mountain ash berry (Woodward and Adams, 1970).

Saturated fatty acids have an advantage over unsaturated ones in that they are more resistant to autoxidation (Melnick et al., 1954b). The greater the number of double bonds in an unsaturated

fatty acid the faster the rate of oxidation; and its rate increases geometrically as the number of double bonds increase. Peroxides are formed from polyunsaturated fatty acid breakdown and they are, in turn, degraded to secondary products like aldehydes and ketones which contribute to off-flavors. Melnick et al. (1954b) worked with sorbic acid applications in cheese to examine its susceptibility to atmospheric oxidation. They concluded that with cheese, atmospheric oxidation of the sorbate is not responsible for its disappearance. The disappearance of sorbic acid is due to its migration into the cheese (Melnick and Luckmann, 1954a). Sorbic acid, when found as a spray dust on cheese wrapping material, is quite stable to oxidation. In packaged cheese sorbate is stable for six weeks at 45°F.

The stability of sorbic acid in storage containers is somewhat different than when it is present in an actual food system. McCarthy et al. (1973) determined the stability of aqueous solutions of sorbic acid by spectrophotometric analysis. They found that the type of container had an effect on the storage stability. Of containers composed of polyvinyl chloride (PVC), polythene (medium), brown glass, or polypropylene, the least amount of sorbate degradation occurred in the polythene container. McCarthy and coworkers also studied the effects of different storage temperatures on the stability of sorbic acid. The general trend was the lower the temperature the greater the retention of sorbate. For example, at 10°C, 94.5% of the sorbate remained after 12 weeks of storage compared to only 54% retention at 75°C. Inhibition of E. coli in each

of the solutions after the period of storage also appeared to follow the same general pattern of the higher the storage temperature the greater the loss of inhibition.

2.7 Food Applications of Sorbic Acid

Martin et al. (1972) investigated the effectiveness of potassium sorbate against Alcaligenes viscolactis, a common cause of ropiness in milk. It was found to be one of the most effective inhibitors examined, with growth almost completely halted at concentrations ranging from 2.0 to 3.0 mg/ml. Sodium benzoate was found to be effective at the same concentrations, however, nitrofurazone, ethylenediaminetetraacetic acid (EDTA), and propyl-p-hydroxybenzoate were not as effective as potassium sorbate against A. viscolactis.

Potassium sorbate has also been studied for other applications in dairy products. Levels of 0.05, 0.075, and 0.10% have been found to inhibit the growth of psychrophilic yeasts and molds in cottage cheese (Bradley et al., 1963). Extensions in lag time were noted in the growth curves of these microorganisms that correlated with the concentration of the sorbate salt and the microorganism's sensitivity to the chemical. In an investigation by Smith and Rollins (1954), sorbic acid was effective at inhibiting fungal growth in natural American cheese at percentages ranging from 0.042 to 0.084. Sorbate was found to be stable in storage either alone or as an additive in thermoplastic-coated cellophane. Taste panels demonstrated a four- to ten-fold difference between the concentration where mold growth was inhibited and where it was perceived

organoleptically. Smith and Rollins concluded that sorbate was applicable to cheeses, especially those wrapped in a moisture-impermeable film. Concurrently Melnick and Luckmann (1954a) established the migration pattern of sorbate in cheese. They found that it would penetrate through five slices (each slice being 0.32 cm thick) in approximately two weeks.

Bonner and Harman (1957) investigated 12 bacteria, three molds, and two yeasts that cause spoilage in cottage cheese. They studied a number of variables, including the natural resistance of some microorganisms to sorbic acid. Very few were inhibited at 0.05, 0.10, and 0.25% levels at pH 5.2, but when the pH was lowered to 5.0 and 4.8 inhibition increased considerably at the same concentrations.

Sorbate as an additive to fish and fishery products is not recommended to exceed 0.1% (W/W) (Chichester and Tanner, 1972). Fish, in contrast to cheese and other dairy products, is sprayed or dipped in solutions of sorbic acid; but like cheese it can also have the sorbate applied in powder form.

Boyd and Tarr (1955) added various levels of sorbic acid to a 20% (W/V) sodium chloride solution. Dips of one hour duration, containing 0.5 and 1.0% sorbic acid, incorporated an average of 0.052 and 0.1025% sorbate respectively in sable-fish. These fish were then smoked and stored at 4°C. Mold growth was inhibited for 40 days. Pieces of sable-fish and halibut were also treated with powdered sorbic acid at 1 g/800 g ratios (Boyd and Tarr, 1955). Inhibition of mold lasted for 50 days. The amount of sorbate that

was found in the treated fish did not decrease appreciably for periods up to 42 days.

Studies investigating the use of sorbic acid in dry salt curing mixtures have also been made with lingcod (Boyd and Tarr, 1955). Sorbate added to the salt at 0.1 and 0.2% concentrations inhibited the growth of mold for up to 60 days at 25°C and 75% relative humidity. Residues of sorbic acid in the fish itself ranged from 0.030 to 0.037% at the 0.2% application level and from 0.033 to 0.036% at the 0.4% application level. Again sorbic acid concentrations did not decrease with the length of time in storage.

Smoked fish studies have also been conducted with the potassium salt of sorbate (Geminder, 1959). Instantaneous pre-smoke sprays or dips of 5% (W/V) aqueous solutions deposited from 0.03 to 0.05% (W/W) on the fish. Post-smoke 10% sprays resulted in 0.05 to 0.15% sorbate residues on the fish. In the case of pre-smoke applications the heat of the smoke apparently had no effect on changing the sorbic acid concentrations. Both the 5 and 10% application levels were successful at inhibiting mold growth on refrigerated, smoked fish.

Sorbate has been experimentally applied to poultry parts by Perry et al. (1964). A hot spray (71.1°C) was administered at a 7.5% sorbate concentration to the cold chicken, which left approximately 0.1 to 1.0 mg sorbate per square centimeter of surface. The treated parts did not spoil for 18 days, as compared to the control samples which spoiled after five days of storage at 7.2°C. Flavor

was not adversely affected by treatment and the microorganisms were not able to become tolerant to the sorbate after repeated exposure to it. Following long term storage of the sorbate treated chicken parts there was no detectable difference in the general character of the microflora as compared to the controls.

Tompkin et al. (1974) found that potassium sorbate was effective against salmonellae and Clostridium botulinum in cooked, uncured sausages. In particular, C. botulinum toxin did not appear in inoculated samples containing sorbate until after ten days of storage at 27°C. Control samples developed toxin after only four days of incubation. Salmonellae growth was also inhibited in sausages containing sorbate.

Investigations on the effectiveness of sorbic acid in combination with other preservatives have been made on meat and fish. Amano et al. (1968) reported that a combination of sorbic acid and tylosin were most effective for firm textured fish sausages, with concentrations of 40 ppm tylosin and 0.01% sorbate described as the optimum levels.

Potassium sorbate might be used in the foreseeable future in combination with sodium nitrite. While current regulations limit 156 ppm of sodium nitrite in bacon, combinations with potassium sorbate would enable the nitrite concentration to be dropped to 40 ppm (Anonymous, 1976). These concentrations have been found to be approximately as effective in cured meats as the present 156 ppm sodium nitrite (Anonymous, 1976). The major advantage of the use of

lower nitrite concentrations would be the reduced potential of nitrosamine formation. Additional advantages of utilizing potassium sorbate would include: the retention of color and flavor in cured meats; increased shelf life; no change required of processing methods; and sorbate's ability to be metabolized as any other fatty acid (Anonymous, 1976).

Potassium sorbate and sorbic acid are used in bakery products that are not yeast-raised, such as toppings, fillings, cakes, pies, and icings (Barrett, 1970). Because sorbate is effective up to pH 6.5, it is particularly suited for bakery products. In addition to its properties of being essentially tasteless and odorless, it is also four times more effective against mold than the propionates (Barrett, 1970). The inhibition of yeast by sorbic acid, however, does make it unsuitable for use in breads and other yeast leavened products.

The sorbates have a particular advantage in foods where fermentation is desirable, as in curing cucumbers, because normal fermentation is permitted while undesirable mold and yeast growth is inhibited (Phillips and Mundt, 1950). When compared to sodium benzoate, Duel et al. (1954a) found that sodium sorbate was superior to sodium benzoate in smaller concentrations. Effective levels of sorbate will vary from product to product; often its effectiveness will depend on the natural pH of the food. A 0.075% concentration of sorbate inhibited fungal growth in strawberry puree or tomato juice at their normal pH levels (Beneke and Fabian, 1955). The more

important spoilage fungi, Botrytis and Rhizopus, were inhibited at even lower levels (0.025%) because of low pH levels.

As mentioned earlier, sorbate is not entirely non-selective in its microbial inhibition powers. It lacks effectiveness against lactic acid bacteria and Clostridium species (Sauer, 1977). However, Robach (1977) has found 2% potassium sorbate effective against out-growth and toxin production of spores of C. botulinum 10755A and 213B for up to seven days at pH 5.5 and 7.0. While sorbate can be used in foods of a higher pH range (e.g. bakery products), its greatest value is in low acid foods like cheese and cheese products. Sauer (1977) has also found that sorbic acid contributes less of an off-flavor to acid foods than does sodium benzoate.

Mold studies with synthetic media probably give the best indication of the optimum levels at which sorbic acid is effective. One investigation utilized a medium containing 10 g of peptone and 40 g of glucose per liter of water (Beneke and Fabian, 1955). Sorbate was added to the medium at the following levels: 0.010, 0.025, 0.050, 0.075, and 0.10% at pH 3, 5, and 7. Once again pH appears to have been the decisive factor in determining growth. The two fungi that were tested, Collectotrichum phomoides and Fursarium sp., were completely inhibited at pH 3 and completely uninhibited at pH 7 at all five levels of sorbate. At pH 5 both fungi would grow only at 0.010 and 0.025% concentrations. With a strawberry media adjusted to pH 3.3, three genera that commonly occur on country ham (Penicillium, Aspergillus, and Alternaria)

grew only at the 0.025% sorbate level. At pH 4 some isolates of Penicillium and Aspergillus were able to grow at the 0.050% level but not at the 0.075% level.

2.8 Toxicity of Sorbic Acid

The lack of toxicity is probably one of the strongest arguments favoring the use of sorbic acid as a food preservative. Dietary levels up to 10% have been shown to be non-carcinogenic in rats (Gaunt et al., 1975). Even after two years of feeding experiments Gaunt and coworkers found no detrimental changes in rats. These conclusions were made following hematological, serum, and histopathological examinations. The thyroid gland weights increased at the 10% level in male rats, although the rats that exhibited the thyroid weight gains also exhibited changes in the renal system. The renal changes were not attributed to the sorbic acid. Kidney and liver weight increases were also noticed at the 10% level, however these gains were described as quite slight. The experiments conducted at the 1.5% dietary level revealed no physiological changes in rats after two years of feeding experiments.

The Joint FAO/WHO Expert Committee on Food Additives (1967) recommends 12.5 mg sorbate/kg body weight per day as the maximum intake. If this guideline is followed there is no chance of approaching even a 1.5% level of intake.

Sorbate has also proven its safety in comparisons to other well known preservatives. Short term studies with cat food have demonstrated that no toxic effects were evident at the 2% sorbate level

while benzoic acid exhibited a definite hazard in cat food preparations (Bedford and Clarke, 1973). Studies in the Soviet Union have been probably the most enlightening at illustrating the safety of sorbic acid. Shitenberg et al. (1970) compared the toxicity of four preservatives: nisin (antibiotic), sorbic acid, benzoic acid, sodium bisulphite; and the combinations of sorbic acid/nisin and benzoic acid/sodium bisulphite. Growth, survival, hematology, blood chemistry, kidney functions, reproduction, and stress factors (dietary restriction, low temperature, and carbon tetrachloride administration) were all investigated. Long and short term studies were made on both mice and rats. Sorbic acid again appeared to be the least toxic. In fact, Shitenberg believed that sorbic acid had a positive benefit as a nutritional additive. Recommendations were made and accepted by the Soviet Ministry of Health to restrict the use of benzoate and sodium bisulphite in foods and replace them with sorbic acid.

One of the few studies to reveal any adverse effects from feeding quantities of potassium sorbate was conducted by Singh and Bucher (1971). They investigated the effectiveness of certain food additives against microorganisms found in a synthetic diet of an insect, Agria affinis auct. nec. Fallén. While potassium sorbate was found to inhibit yeasts, bacteria, and Penicillium growth, it also had a detrimental effect on the insect's survival.

However, other studies on animals possessing a closer resemblance to human physiology did not reveal similar adverse

effects. As mentioned previously, some of the more recent studies by Gaunt et al. (1975) did not demonstrate any sorbate toxicity on rats after two years of testing. Earlier, Duel et al. (1954b) studied the metabolism of sorbic acid. They were of the belief that sorbic acid followed the same metabolic pathway as did the naturally occurring hexanoic acid, caproic acid. Their hypothesis was proved correct by in vivo experiments on rats. Duel and coworkers concluded that α - β -unsaturated fatty acids are intermediates in saturated fatty acid oxidation and under normal conditions of digestion sorbate is oxidized to H_2O and CO_2 . Conversely, benzoic acid is a strong inhibitor of liver enzyme oxidation of fatty acids and as a result must be detoxified in the liver (Witter et al., 1950).

Duel et al. (1954b) conducted toxicity studies on dogs as well as rats and found sorbate to be harmless to both species when fed at the 5% dietary level. Their research also demonstrated the decisive advantage of sodium sorbate over sodium benzoate in terms of toxicity. The LD_{50} of sodium sorbate was found to be 5.94 g/kg in rats as compared to 3.45 g/kg for sodium benzoate when the animals had access to food. In fasting rats sodium sorbate had a LD_{50} of 4.3 g/kg in males and 3.65 g/kg in females as contrasted to 2.1 g/kg for sodium benzoate. The calculated ratio on free acid basis of the LD_{50} was found to be 1.72 in one laboratory and 1.90 in another (LD_{50} of sodium sorbate: LD_{50} of sodium benzoate). While sodium sorbate was not toxic at the 8% dietary level, sodium benzoate adversely affected both growth and survival in rats. Duel and

coworkers also noted kidney and liver histopathological alterations when sodium benzoate was administered to rats.

2.9 Federal Regulations Governing Sorbate

Sorbic acid and its salts are classified as Generally Recognized As Safe (GRAS) substances by the Food and Drug Administration. Tolerance levels are not set except under Standards of Identity (Chichester and Tanner, 1972). Pasteurized process cheese spreads may contain sorbic acid, potassium sorbate, sodium sorbate, or any combination of the two in amounts up to 0.2% by weight sorbic acid. Cold pack cheese, club cheese, or comminuted cheese may contain the same ingredients up to 0.3% sorbic acid by weight (Fine, 1973).

The following foods may also contain sorbic acid or one of its salts: baked goods (except yeast raised products); beverages and beverage syrups; fruit juices, wines (levels up to 0.1% need not be listed on the label); artificially sweetened jams, jellies, and preserves in addition to sugar-sweetened fruit butters, fruit cocktails and salads; dried fruits; pickles and pickled products; margarine (potassium sorbate only); and fish, dry sausage surfaces, and dog food patties (Chichester and Tanner, 1972).

2.10 Methods of Analysis

There are a number of methods for analyzing sorbate residuals. Titrimetric, colorimetric, and spectrophotometric techniques are among the most common. Melnick and Luckmann (1954b) utilized a

spectrophotometric method for their investigations on cheese. The principle of this technique is in the conjugated double bonds of sorbic acid, which will absorb light in the UV portion of the spectrum. Concisely, the sorbate yields a characteristic curve when exposed to ultraviolet light; at the maxima of the curve the concentration of sorbate can be determined. Spectrophotometric techniques have also been developed for detecting sorbic acid in fresh dairy products (Wilamowski, 1974).

The titrimetric method, as described by Spanyol and Sándor (1958), does not require a spectrophotometer. Basically, the sorbate is neutralized quantitatively in an alcoholic solution by standardized sodium hydroxide. Colorimetry is another method of analysis that is used with dried fruits (Nury and Bolin, 1962). The sample of fruit is blended in water and the filtrate has potassium permanganate added to it. The sorbate is thereby converted to malonaldehyde. By adding thiobarbituric acid a color is formed which can then be read on the spectrophotometer.

2.11 Sorbate Manufacture

The sorbates are presently used in many food products in the United States; however, no sorbate production plants are located in the U.S. (Crocco, 1977). Up until now all sorbates utilized were imported. Crocco notes in June 1977 the Monsanto Company, Inc. is scheduled to open a 10 million pounds per year production capacity plant. The production scheme, called the "stream-lined, scaled-up"

ketene/crotonaldehyde process, was obtained from the Nippon Synthetic Chemical Industry Company (Crocco, 1977). The U.S., being the largest consumer of sorbates in the world, has a demand for 7 to 8 million pounds per year. Canada consumes a little less than a million pounds per year. Hence, this plant should satisfy the North American demands for sorbate in the foreseeable future (Crocco, 1977).

3.0 MATERIALS AND METHODS

3.1 Experimental Design

The study consisted of three different phases of study. The first phase utilized 60 and 90 day old long cut hams to conduct the following evaluations: mold and yeast colony counts; sorbic acid residuals; and organoleptic preferences. The second phase utilized ham slices to evaluate for visible mold growth and sorbic acid residuals. The third phase utilized 70 day old packer style cut hams to conduct the following evaluations: mold and yeast colony counts; sorbic acid residuals; and amount of visible mold growth.

3.2 Sampling Treatment of 60 and 90 Day Old Hams

Twenty-four 60 day old long cut hams (ITT Gwaltney) were subjected to either a 0, 2.5, or 5% aqueous potassium sorbate solution (Table 1). One half of the hams were sprayed until wet and the other half were subjected to an instantaneous dip (71.1°C). One day prior to treatment each ham had been scrubbed to remove the heavy coating of pepper applied by the commercial producer. Hams were hung on a rack to dry overnight. Following sorbate treatment the hams were aged at $22\pm 5^{\circ}\text{C}$ (less than 65% relative humidity) for 60 days. Hams were evaluated periodically for sorbate residuals and mold and yeast colony counts. Sixteen of the hams were placed in refrigerated storage (7.2°C and approximately 74% relative humidity) after the 60 day aging period. These refrigerated hams were evaluated for visible mold growth after 45 days of storage.

Table 1. Allotment of 60 and 90 day old long cut hams to treatment

Day of Analysis	Potassium sorbate concentration (% aqueous solution W/V)					
	0		2.5		5	
	Spray ^a	Dip	Spray	Dip	Spray	Dip
0	2 ^b	2	2	2	2	2
60	2	2	2	2	2	2

^aMethod of application

^bNumber of hams analyzed

Twelve 90 day old long cut hams (ITT Gwaltney) were treated in an identical fashion to the 60 day old hams. All were sampled at 0 days and evaluated for sorbic acid residuals, organoleptic preferences, and number of mold and yeast colony forming units.

3.3 Sampling Treatment of Ham Slices

3.31 120 Day Study

The ham slice phase was divided into two sections. The first section was placed in an incubator at 7°C following sorbate treatment and the second section was incubated at 22°C.

The slices were all obtained from a long cut ham (ITT Gwaltney) that had been cured 30 days, equalized 30 days, and aged 150 days. Thirty-six ham squares of 5.1 x 5.1 x 1.3 cm dimensions were obtained from the interior of the shank half of the ham. The ham squares were then dried overnight in an incubator at 7°C so they would more closely resemble the dry outer surface of a whole ham. All squares were obtained from the interior of the ham in order that they would all have approximately the same moisture content at the initiation of the experiment. Ham squares that exhibited any degree of case hardening at the time they were cut were discarded. Each square was subjected to an ambient dip (24.5°C) at the concentrations illustrated in Table 2. Following treatment one half of the ham squares were inoculated with a mixed culture of mold that had been growing on Czapek agar (Difco) for five days. The other group was not inoculated. Each of these treatment groups were further divided into the two storage temperature sections (7°C and 22°C). These ham squares were

Table 2. Allotment of ham slices to treatment in 120 day study

Treatment (% Potassium sorbate (W/V))	Uninoculated ^a		Inoculated	
	22°C ^b	7°C	22°C	7°C
0	1 ^c	1	1	1
2.5	2	2	2	2
5	2	2	2	2
10 ^d	2	2	2	2
10	2	2	2	2

^aUninoculated indicates normal flora

^bStorage temperature

^cNumber of ham slices (squares)

^dInstantaneous dip; all others are one minute dips

then observed for fungal growth over a period of 120 days. Representative samples were analyzed for sorbate residuals at the end of the 120 day test period.

3.32 60 Day Study

The 60 day study was conducted on ham slices obtained from four long cut hams (ITT Gwaltney) that had been cured 30 days, equalized 30 days, and aged 205 days. Each ham was cut in half and the butt half was discarded. The shank half was then sliced into ten 1.3 cm thick slices, numbered one to ten starting with the center cut slice. The slices were individually suspended from smoke sticks and placed in a refrigerated room overnight prior to treatment (7°C). The following day ten slices from each of the four hams were subjected to ambient dips (24°C) as illustrated in Table 3. One half of the slices were then observed for fungal growth for 60 days at $22\pm 5^{\circ}\text{C}$ and $70\pm 10\%$ relative humidity; one fourth were immediately analyzed for sorbate residuals; and the final one quarter were tested for sorbate residuals after 60 days.

3.4 Sampling Treatment of 70 Day Old Hams

Twenty-eight 70 day old packer style cut hams (V. W. Joyner and Co.) were obtained for the purpose of studying the inhibitory properties of 2.5, 5, and 10% one minute sprays of aqueous potassium sorbate. Again, the hams were washed and scrubbed to remove excess pepper and hung on racks to dry overnight. The hams were bagged in stockinettes and hung shank end down. The hams were then sprayed with appropriate concentrations of potassium sorbate

Table 3. Allotment of ham slices to treatment in 60 day study

% Potassium Sorbate W/V	Observed for fungal growth		0 day sorbate analysis		60 day sorbate analysis	
	Slice #	Ham #	Slice #	Ham #	Slice #	Ham #
0	1	46,40,37,52 ^a	6	46,40	6	37,52
2.5	2	46,40,37,52	7	46,40	7	37,52
5	3	46,40,37,52	8	46,40	8	37,52
10 ^b	4	46,40,37,52	9	46,40	9	37,52
10	5	46,40,37,52	10	46,40	10	37,52

^aNumber of slices

^bInstantaneous dip; all others are one minute dips

in a procedure outlined in Sect. 3.751. The hams were aged for 60 days in a room held at $21.1 \pm 0.5^{\circ}\text{C}$ and $70 \pm 5\%$ relative humidity. Hams were removed for periodic testing as indicated in Table 4.

3.5 Apparatus

3.51 Spectrophotometer

Spectrophotometric determinations for sorbate residuals were made with a Perkin-Elmer, Coleman Model 124 double beam, grating spectrophotometer equipped with a Perkin-Elmer Model 56 recorder.

3.52 Humidifier

Constant humidity was maintained in the aging rooms with the aid of an Arvin Model 50H52-01 humidifier.

3.53 Heater

Constant temperature was maintained in the aging rooms with the aid of a Manning Bowman Model 325016 electric heater.

3.54 Sprayer

Whole hams subjected to spraying were sprayed with a Chapin Model 168 compressed air, stainless steel sprayer. Its total capacity was 3 gallons, useable capacity 2.25 gallons.

3.55 Psychrometer

Relative humidity in the aging rooms was measured with a Bendix Model 566-2 electrically aspirated psychrometer.

3.6 Reagents, Solutions, and Media

3.61 1:1 Petroleum Ether-Ethyl Ether Solution

A 1:1 petroleum ether-ethyl ether solution was prepared by

Table 4. Allotment of 70 day old packer cut hams to treatment

Treatment (% Potassium Sorbate W/V)	Day of Analysis ^a		
	0	30	60
0	1 ^b	3	3
2.5	1	3	3
5	1	3	3
10	1	3	3

^aAnalysis includes sorbate residual, mold and yeast colony count, and visible mold evaluation

^bNumber of hams analyzed

mixing 1 liter certified ACS grade petroleum ether (Fisher) with 1 liter certified ACS grade diethyl ether (Fisher).

3.62 Metaphosphoric Acid Solution

Metaphosphoric acid solution was prepared by dissolving 5 g of metaphosphoric acid pellets (Baker) in a 1 liter volumetric flask with 250 ml of distilled, deionized water. The solution was then diluted to volume with 95% ethyl alcohol (USI). The solution was stable for one week under refrigerated conditions.

3.63 Potassium Sorbate Solutions

Aqueous solutions of potassium sorbate were prepared by dissolving 25, 50, or 100 g of powdered potassium sorbate in distilled, deionized water. The concentrated solutions would be poured into 1 liter volumetric flasks and diluted to volume (with additional water) to yield 2.5, 5, and 10% solutions of potassium sorbate respectively.

3.64 Saline Solution

A 0.85% (W/V) saline solution was prepared by placing 8.5 g of sodium chloride (Baker) in a 1 liter volumetric flask and diluting to volume with distilled, deionized water. It was then dispensed in 10 ml aliquots in 150 x 15 mm test tubes and autoclaved at 121°C for 15 minutes.

3.65 Czapek Agar

Czapek agar (Difco) was utilized for mold and yeast colony counts obtained from the surface of the hams. It was acidified to pH 4.5 with 10% tartaric acid (0.1 ml/200 ml agar) to inhibit

bacterial growth.

3.66 Tartaric Acid

The 10% tartaric acid solution was prepared by dissolving 10 g of certified granular tartaric acid (Fisher) per 100 ml of distilled, deionized water. The solution was then autoclaved for 15 minutes at 121°C.

3.67 Reference Solution

The reference solution was prepared in the same manner as the samples prepared in Sect. 3.733. Ham scrapings, though, were obtained from control hams rather than ones treated with potassium sorbate. In the event where there was three or more controls, all three would be analyzed spectrophotometrically at 250 nm versus the metaphosphate-ether blank described in Sect. 3.731. The sample that most closely approximated the average absorbance of all the samples was chosen as the reference solution.

3.7 Procedures

3.71 Enumeration of Mold and Yeast Colonies

Prior to testing, aluminum foil templates (25.8 cm² area) were constructed and autoclaved for 15 minutes at 121°C. The sterile template would then be placed on to a relatively flat, lean surface of the ham. The swab method was utilized for sampling the surface of the ham (Baldock, 1974). A sterile cotton swab would be moistened in one of the test tubes containing 10 ml sterile saline solution (Sect. 3.64). The swab would then be rubbed over the area outlined by the aluminum foil template. After the swab had been placed in

the test tube containing the saline solution, a second dry sterile swab would be rubbed over the same area. It would then be placed in the test tube containing the first swab. This procedure would be repeated for each ham tested.

Estimations of the total number of mold and yeast colonies were made with the pour plate technique. One ml of each saline dilution was placed in duplicate petri plates. One tenth ml of the same saline dilution was also placed in duplicate plates. Approximately 15-20 ml of the liquid acidified Czapek agar were poured into the petri plates containing the saline dilutions. The agar and dilutions were evenly mixed by swirling the plates on the lab bench. Once the plates had solidified they were incubated at 22°C for five days (FDA, 1976). The plates were checked after three days for possible mold overgrowth (FDA, 1976). The plates were then counted for mold and yeast colonies with the aid of a Quebec Colony Counter. Periodic microscopic observations of the colonies were made to assure that they were molds and yeasts and not bacteria. Molds were identified as members of the genera Penicillium and Aspergillus (Bielecka, 1976) from plates made of the 90 day old long cut hams.

3.72 Visible Mold Evaluations

Visible mold evaluations were made at 0, 30, 60, and 120 days on whole hams and ham slice studies, depending on duration. Intensity of mold growth was rated on a subjective scale of 0 to 5, described as follows: 0=no growth; 1=very slight growth; 2=slight growth; 3=moderate growth; 4=marked growth; and 5=intense growth.

Photographs were made at 30 and 60 days on the 70 day old packer cut hams and 60 days on the 60 day ham slice study.

3.73 Spectrophotometric Analysis of Sorbate Residuals

3.731 Preparation of the Standard Curve

The standard curve was prepared by dissolving 0.134 g of potassium sorbate (equivalent to 0.1 g sorbic acid) in 100 ml of distilled, deionized water. One, two, four, and six ml of this solution was pipetted to separate 100 ml volumetric flasks. One additional flask did not have any of the potassium sorbate solution added to it. Each flask was then filled to volume with metaphosphoric acid solution. Five ml from each flask were transferred to a second set of 100 ml volumetric flasks and diluted to volume with the 1:1 petroleum ether-ethyl ether (Sect. 3.61). Five g of anhydrous sodium sulfate (Baker) were added to each flask. Four ml of the ether extract from each flask were transferred to individual spectrophotometric absorption cells (cuvets). Absorbance of each cuvet was determined at 250 nm versus the plain metaphosphoric acid-ether blank. Absorbance was plotted against mg sorbic acid/100 ml ether (Wilamowski, 1974).

3.732 Scraping Technique

The hams were scraped over the entire area of lean at a depth of 2-5 mm until 15 g were collected. The scrapings were weighed on a triple beam balance (Ohaus) to the nearest 0.1 g and placed in Whirl-Pak bags. The bags were refrigerated at 7°C until the actual extraction could begin.

3.733 Extraction of Sorbic Acid

The method for extracting sorbic acid was developed from the methods of Bokus (1968) and Wilamowski (1974). All 15 g of the ham scrapings were placed in separate blender jars and blended for 15-30 seconds (Waring blender). Ten grams of the ground ham were weighed in a 50 ml beaker to the nearest 0.1 g. The 10 g of the ground ham were then transferred to a second blender jar. One hundred ml of the metaphosphoric acid were measured in a volumetric flask and added to the blender jar. The mixture was blended for one minute and allowed to stand for ten more minutes (Bokus, 1968). The contents of the jar were then vacuum filtered through Whatman No. 3 filter paper (Wilamowski, 1974) into a 500 ml filter flask. Five ml of the filtrate were transferred to a 250 ml separatory funnel and 100 ml of the 1:1 petroleum ether-ethyl ether were added. The mixture was shaken for one minute (Wilamowski, 1974) and the aqueous-ether phases were allowed to separate. The aqueous layer was discarded and the ether layer was transferred to a 100 ml volumetric flask. The ether extraction was dried with 5 g anhydrous sodium sulfate (Bokus, 1968; Wilamowski, 1974). Four ml of the dried ether extract were transferred to a spectrophotometric cell (cuvet). The absorbance of each cuvet was determined at 250 nm versus the reference solution (Sect. 3.67); and a scan from 300-220 nm was conducted to insure a peak occurred at 250 nm, which is the peak absorbance of sorbic acid. The spectrophotometric analysis had to be conducted as quickly as possible due to the rapid evaporation of the ether

extraction. Cuvets were rinsed with distilled water, acetone, and petroleum ether respectively between each sample reading. Concentration of sorbic acid was determined from the standard curve (Sect. 3.731).

3.734 Calculations: Spectrophotometry

The concentration of sorbic acid in the 10 g sample of ham was calculated by the following formula:

$$\frac{x \text{ mg sorbic acid}/100 \text{ ml } 1:1 \text{ ether mixture}}{500 \text{ mg meat}/100 \text{ ml } 1:1 \text{ ether mixture}}$$

or

$$\text{mg sorbic acid} \times 2000$$

The above formula is derived as follows:

10 g of ham is placed in 100 ml metaphosphoric acid =

10 g ham/100 ml metaphosphoric acid

Removing 5 ml of the above solution =

0.5 g ham/5 ml metaphosphoric acid

Diluting 0.5 g ham/5 ml metaphosphoric acid with 100 ml 1:1 ether mixture =

0.5 g ham/100 ml 1:1 ether mixture (since the aqueous metaphosphoric acid and ether mixture are immiscible)

Above dilution is analyzed by spectrophotometer and from standard curve determine that 0.5 g ham/100 ml 1:1 ether mixture =

x mg sorbic acid/100 ml ether

Thus: $\frac{x \text{ mg sorbic acid}/100 \text{ ml } 1:1 \text{ ether mixture}}{0.5 \text{ g (500mg)ham}/100 \text{ ml } 1:1 \text{ ether mixture}} = \text{ppm}$

3.74 Organoleptic Evaluation of 90 Day Old Hams

Ninety day old long cut hams (ITT Gwaltney) that had been aged for 30 days, then treated with potassium sorbate, were organoleptically evaluated by 12 taste panel members. The members of the panel were selected on their stated preference for country cured ham. Each center cut slice was evaluated by three members of the panel to find if sorbate affected the organoleptic properties of country ham. Slices 1.27 cm in thickness were broiled two at a time in a conventional oven for approximately 13 minutes at a distance of 15 cm from the heating element. The hams were broiled for seven minutes on one side and six on the other. The slices were so arranged that the one that had been in the forward section of the oven for the first seven minutes was switched with the slice in the rear for the final six minutes. Each slice was further divided into three 3.8 x 3.8 cm sections after cooking. The sections came from one of the three areas directly beneath what had been the face of the ham: knuckle, semimembranosus, or the area adjacent to the femur. Each panel member received three analogous areas to sample. Evaluations for aroma (cooked), flavor, saltiness, and overall satisfaction were made on a 1 to 9 hedonic scale as described by Peryam et al. (1952).

3.75 Spraying Procedure for 70 Day Old Hams

3.751 Spraying Operation

Seventy day old packer-style cut hams (V. W. Joyner and Co.) utilized in the final study were sprayed to demonstrate a feasible method for commercial application of potassium sorbate.

Potassium sorbate solutions of 2.5, 5, and 10% concentrations were prepared in the manner previously described (Sect. 3.63) in 5 liter quantities. Each ham, bagged in stockinette material, was suspended from a wooden smoke house stick and placed in a trolley containing a fully expanded 58.42 x 43.18 x 121.92 cm trash can liner (Mobile Chemical Packaging Co.). This liner completely enclosed the ham on all sides except for the top. Prior to unfolding the trash can liner it was weighed in a 3 liter beaker on a direct reading balance (Fisher) to the nearest gram. Once the ham was placed within the can liner 500 ml of one of the solutions presented in Table 4 was poured into the sprayer (Sect. 3.54). The sprayer was then pumped 25 times. The discharge nozzle was situated 8 to 22 cm from the ham surface; at that time the spray trigger was fully depressed, thereby initiating the spraying operation. The nozzle, being set at the fine spray setting, was continuously moving across the surface of the ham for one minute. The ham, after becoming completely saturated, dripped any excess solution into the can liner. The can liner captured any spray that did not fall on the ham surface. At the completion of one minute the spray trigger was released. The ham was allowed to drip for an additional minute to allow excess solution to run off. The ham was then removed from the trolley. The can liner was removed and weighed again in the beaker on the direct reading balance. The solution that remained in the sprayer from the original 500 ml was poured into a 100 ml graduated cylinder and measured. The used can liner was then discarded and a new one

attached to the trolley for the next ham. From these measurements the amount of potassium sorbate remaining on the ham could be calculated.

3.752 Calculations: Spraying Operation

Concentration of potassium sorbate on each ham was calculated from the following formula:

$$\mu\text{g potassium sorbate/g ham} = \frac{[V_a - (V_b + W_a)] C}{(W_b)}$$

where " V_a " is the volume of solution in ml placed in the sprayer (500 ml); " W_a " is the weight of the potassium sorbate solution in g remaining in the can liner after spraying; " V_b " is the volume of solution in ml remaining in the sprayer after spraying each ham; " C " is the concentration of potassium sorbate in $\mu\text{g/ml}$ in the aqueous solution; and " W_b " is the original ham weight in grams.

4.0 RESULTS AND DISCUSSION

4.1 60 and 90 Day Old Long Cut Ham Studies

4.11 60 Day Old Long Cut Ham Study

4.111 Mold and Yeast Colony Counts

The statistical summary of the mold and yeast counts for the 60 day ham study is presented in Tables 5 and 6. While no visible mold growth was noted during the 60 days of ambient storage, the mold and yeast counts did offer some interesting comparisons. The F tests at 0 and 60 days indicated that both mold and yeast counts differed significantly at the $P = .01$ level. Factorial analysis was performed to locate the differences. These results are presented in Table 7. The level of potassium sorbate administered to each ham exerted a strong effect on the number of mold and yeast colony forming units. The 2.5% level alone reduced the mold counts 66% and the yeast counts by approximately 50% as compared to the control. The decrease between the 2.5 and 5% levels was not as dramatic, though the mean of the yeast counts decreased again by 50%. The spray method of application also produced a strong reduction in mold colony forming units. The number of days following treatment illustrated a highly significant effect on the number of yeasts, for the mean of the yeast counts quadrupled after 60 days.

The effect of the interaction of potassium sorbate level, method of application, and days following treatment is presented in Table 8. The 2.5 and 5% treatments significantly reduced the number of mold colony forming units as compared to the analogous 0% applications.

Table 5. Statistical summary of mold colony counts on 60 day old long cut hams^a

F = 6.52^{**b}

Potassium Sorbate Concentration (% Aqueous Solution W/V)

	0		2.5		5	
	Spray ^c	Dip	Spray	Dip	Spray	Dip
	0 Days					
Mean	18.36 ^d	20.63	14.05	3.06	9.49	2.38
Range	1.16-54.25	2.33-62.0	0-38.75	0-11.62	0-23.25	0-4.6
S.D. ^e	21.45	21.58	15.20	3.92	8.62	1.78
	60 Days					
Mean	12.42	58.99	1.46	10.56	4.99	11.58
Range	0-37.2	34.87-100.74	0-7.75	1.16-58.12	0-14.72	0.39-81.37
S.D.	23.89	21.69	2.88	19.32	5.87	28.25

^aAll values expressed as colony forming units/cm²

^b** denotes significance at 1% level between treatments

^cMethod of application

^dMean of 8 observations

^eStandard deviation

Table 6. Statistical summary of yeast colony counts on 60 day old long cut hams^a

$$F = 6.97^{**b}$$

	Potassium Sorbate Concentration (% Aqueous Solution W/V)					
	0		2.5		5	
	Spray ^c	Dip	Spray	Dip	Spray	Dip
	0 Days					
Mean	793.41 ^d	943.84	132.03	193.08	858.83	191.19
Range	13.56- 2243.49	23.25- 3383.37	3.88- 147.23	67.62- 387.48	2.71- 3778.83	26.74- 448.19
S.D. ^e	946.24	1194.44	147.23	142.04	1308.38	177.30
	60 Days					
Mean	3852.29	2584.82	1902.20	3330.46	116.70	1656.54
Range	2095.88- 6019.76 ^f	112.27- 24122.98	0.78- 29659.41	782.13- TNC ^g	0- 313.86	0- 4657.63
S.D.	1115.55	2582.17	2039.88	1865.23	131.51	1866.15

^aAll values expressed as colony forming units/cm²

^b** denotes significance at 1% level between treatments

^cMethod of application

^dMean of 8 observations

^eStandard deviation

^fAll samples >5000 statistically analyzed as 5000

^gToo numerous to count

Table 7. Factorial analysis of the effect of potassium sorbate level (L), method of application (M), and days following treatment (D) on mold and yeast colony counts on 60 day old long cut hams

<u>Potassium Sorbate Level (L)</u>	Mean ^a		F=6.12**
	<u>Molds</u>	<u>Yeasts</u>	
0%	27.60	2043.59	F=15.19**b
2.5%	7.28	1389.44	
5%	7.11	705.82	
<u>Method of Application (M)</u>			
Spray	10.13	1275.91	F=4.84*c
Dip	17.87	1483.32	
<u>Days Following Treatment (D)</u>			
0 Days	11.33	518.73	F—
60 Days	16.66	2240.50	

^aAll values expressed as colony forming units/cm²

^b** denotes significance at 1% level

^c* denotes significance at 5% level

^dF— denotes insignificance

Table 8. Effect of the interactions of potassium sorbate level (L), method of application (M), and days following treatment (D) on mold and yeast colony counts of 60 day old long cut hams

Interaction	Mean ^a		
	Molds	Yeasts	
<u>(L x M)</u>			
0% - Spray	15.38	2322.85	
0% - Dip	39.81	1764.33	
2.5% - Spray	7.76	1017.11	F = 5.72 ^{**b} F— ^c
2.5% - Dip	6.81	1761.77	
5% - Spray	7.24	487.76	
5% - Dip	6.98	923.86	
<u>(L x D)</u>			
0% - 0 Days	19.50	868.62	F — F = 5.76 ^{**}
0% - 60 Days	35.70	3218.56	
2.5% - 0 Days	8.56	162.55	
2.5% - 60 Days	6.01	2616.33	
5% - 0 Days	5.94	525.01	
5% - 60 Days	8.28	886.62	
<u>(M x D)</u>			
Spray - 0 Days	13.97	594.76	F = 13.91 ^{**} F —
Spray - 60 Days	6.29	1957.06	
Dip - 0 Days	8.69	442.70	
Dip - 60 Days	27.04	2523.94	

^aAll values expressed as colony forming units/cm²

^b** denotes significant at 1% level

^cF— denotes insignificance

The yeasts, though, were more dramatically affected by the interaction of potassium sorbate level and days following treatment. The mold colony counts also illustrated a significant difference from the interaction of method of application and days following treatment, although the means did not indicate a definite trend.

Overall it can be concluded that the level of potassium sorbate and method of application exerted the greatest effect on mold colony forming units while the potassium sorbate level and days following treatment were the most dramatic influences on the number of yeasts counted.

4.112 Sorbic Acid Residuals

The statistical summary of the sorbic acid residuals detected in the hams is presented in Table 9. The F test at 0 and 60 days indicated significant differences between treatments at the $P = .01$ level. Wide variations were noted between hams subjected to the same treatment. The variations, though, can be attributed to the inherent irregularities of the hams. Geminder (1959) found that 10% post-smoke sprays (W/V) of aqueous potassium sorbate deposited 500 to 1500 ppm sorbic acid on fish. Both fish and ham are comparable products that will absorb potassium sorbate unevenly, unlike a formulated product such as cottage cheese, which can have the sorbate directly incorporated into the ingredients. Even when 1000 ppm potassium sorbate was added to creamed cottage cheese, the amount recovered ranged from 850 to 1170 ppm (Bradley et al., 1963).

Factorial analysis was performed to locate the differences

Table 9. Statistical summary of sorbic acid residuals in 60 day old long cut hams^a

	<u>Potassium Sorbate Concentration (% Aqueous Solution W/V)</u>			
	2.5		5	
	Spray ^b	Dip	Spray	Dip
	0 Days			
Mean	220 ^c	690	553	1400
Range	190-250	690	510-596	1000-1800
S.D. ^d	42.43	0.00	60.81	565.68
	60 Days			
Mean	160	255	215	410
Range	150-170	240-270	180-250	340-480
S.D.	14.14	21.21	49.50	99.00
	F = 8.04 ^{**e}			

^aAll values expressed as ppm sorbic acid

^bMethod of application

^cMean of two observations

^dStandard deviation

^e** denotes significance at the 1% level (between treatments)

between treatments. These results are presented in Table 10. The level of potassium sorbate, method of application, and days following treatment all exerted strong influences on the sorbate residuals detected in each ham. However, the method of application and the days following treatment were the most important factors, as indicated by the 1% level of significance in the F tests. The effect of the interaction of potassium sorbate level, method of application, and days following treatment is reported in Table 11. The interactions between any two factors were not significant except for the one between method of application and days following treatment. The logical conclusion is that the sorbate residual decreased by approximately 60% over the 60 day duration. Though later experiments on ham slices revealed that different storage temperatures do not produce significant differences in residual values, the decrease could be partially explained by sorbate migrating into the ham. An investigation of migration was outside the scope of this study, however, Melnick and Luckmann (1954a) found that migration did account for sorbate's disappearance in cheese. Other studies had discounted oxidative deterioration as a probable cause for its disappearance in cheese, though the incubation temperature was 7.2°C and the cheese was sealed in an air-impermeable cellophane wrapper (Melnick *et al.*, 1954b).

4.113 Refrigerated Storage

The hams were placed in refrigerated storage (7.2°C and 74% relative humidity) after the 60 days of ambient storage. After 45

Table 10. Factorial analysis of the effect of potassium sorbate level (L), method of application (M), and days following treatment (D) on sorbic acid residuals in 60 day old long cut hams

<u>Potassium Sorbate Level</u>	<u>Residuals</u> ^a	
2.5%	331.25	F=9.28 ^{*b}
5%	664.50	
<u>Method of Application (M)</u>		
Spray	287.00	F=15.26 ^{**c}
Dip	688.75	
<u>Days Following Treatment (D)</u>		
0 Days	715.75	F=19.64 ^{**}
60 Days	260.00	

^aAll values expressed as ppm sorbic acid

^b* denotes significance at 5% level

^c** denotes significance at 1% level

Table 11. Effect of interaction of potassium sorbate level (L), method of application (M), and days following treatment (D) on sorbic acid residuals in 60 day old long cut hams

<u>Interaction</u>	<u>Residuals^a</u>	
<u>(L x M)</u>		
2.5% - Spray	190.00	
2.5% - Dip	472.50	F— ^b
5% - Spray	384.80	
5% - Dip	905.00	
<u>(L x D)</u>		
2.5% - 0 Days	455.00	F—
2.5% - 60 Days	207.50	
5% - 0 Days	976.50	
5% - 60 Days	312.50	
<u>(M x D)</u>		
Spray - 0 Days	386.50	F = 6.23 ^{**c}
Spray - 60 Days	187.50	
Dip - 0 Days	1045.00	
Dip - 60 Days	332.50	

^aAll values expressed as ppm sorbic acid

^bF— denotes insignificance

^c** denotes significance at 1% level

days of refrigerated storage slight mold growth was noted on four of the eight controls. No growth was observed on the eight 2.5 or 5% treated hams. These preliminary observations seemed to indicate that lower levels of sorbate might prove effective at inhibiting psychrophilic mold growth.

4.12 90 Day Old Long Cut Ham Study

4.121 Mold and Yeast Colony Counts

The statistical summary of the mold and yeast colony counts for the 90 day old hams at 0 days is presented in Table 12. The statistical analysis indicated significant differences between treatments, however, the results were quite erratic. For example, both the 0 and 2.5% sprays seemed to lower the number of mold colony forming units to a greater degree than the corresponding dips, while the opposite was true at the 5% level. The yeast counts were lowered at all three levels by the dip method of application. These results for molds and yeasts are somewhat suspect though, because a large number of the plates were too numerous to count. Consequently, the finding of the factorial analysis of variance is not presented. One preliminary conclusion can be made, however. When the means of the mold and yeast colony counts are compared with those of the 60 day hams at 0 days (Tables 5 and 6), it appears that the potassium sorbate application had a greater fungal reducing effect on the 60 day hams.

4.122 Sorbic Acid Residuals

The statistical summary of the sorbic acid residuals detected in the hams is presented in Table 13. The F test did not indicate any significant differences between treatments. Factorial

Table 12. Statistical summary of mold and yeast colony counts on 90 day old long cut ham at 0 days^a

	Spray ^b	Dip	Spray	Dip	Spray	Dip
			Molds F = 3.70 ^{**c}			
Mean	4.80 ^d	2506	32.40	11288.69	2501	1.41
Range	0-11.62	8.52-TNC ^e	0-104.62	1.55-TNC	3.49-TNC	0-4.65
S.D. ^f	3.89	3383.64	37.93	3291.56	2670	1.89
			Yeasts F = 14.03 ^{**}			
Mean	4797.48	3383.64	4026.10	2418.19	TNC	37.12
Range	4069.35- 28473.03 ^g	1581.84- TNC	292.64- 28077.57	154.99- 4262.17	TNC	0- 114.24
S.D.	380.49	1732.02	1722.32	2361.17	-	48.77

^aAll values expressed as colony forming units/cm²

^bMethod of application

^c** denotes significance at 1% level

^dMean of 8 observations

^eToo numerous to count

^fStandard deviation

^gAll samples >5000 statistically analyzed as 5000

Table 13. Statistical summary of sorbic acid residuals in 90 day old long cut hams at 0 days^a

	<u>Potassium Sorbate Concentration (% Aqueous Solution W/V)</u>			
	2.5		5	
	Spray ^b	Dip	Spray	Dip
Mean	505 ^c	550	585	455
Range	420-590	500-600	500-670	420-490
S.D. ^d	120.00	70.71	120.21	49.50
				F— ^e

^aAll values expressed as ppm sorbic acid

^bMethod of application

^cMean of two observations

^dStandard deviation

^eF— denotes insignificance between treatments

analysis (Table 14) confirmed the lack of difference among methods of application and levels of potassium sorbate. Conversely, there were significant differences between treatments in the sorbate residuals of the 60 day old hams (Table 9). Again, as in the mold and yeast colony counts, the findings appear to indicate that the younger hams (60 days) are more "responsive" to treatment. These results could indicate that younger hams exhibit a lesser degree of case hardening, thereby allowing a larger amount of potassium sorbate to penetrate the surface.

4.123 Organoleptic Evaluation

The statistical summary of the organoleptic evaluation is presented in Table 15. The F values were insignificant in all four categories: aroma, flavor, saltiness, and overall satisfaction; in fact, in several areas the 2.5 and 5% treated ham samples were preferred over the controls. Sorbate residuals ranged up to 670 ppm in the surface, inferring that at least at these levels the sorbate could not be detected organoleptically.

4.2 Ham Slice Studies

4.21 120 Day Ham Slice Study

4.211 Sorbic Acid Residuals

The statistical summary of the sorbic acid residuals detected in the 120 day old ham slices is reported in Table 16. The F test indicated significant differences between treatments at the $P = .01$ level. Again wide variations were noted among samples subjected to the same treatment. Factorial analysis was performed to

Table 14. Factorial analysis of the effect of potassium sorbate level (L) and method of application (M) on sorbic acid residuals in 90 day old long cut hams

<u>Potassium Sorbate Level (L)</u>	<u>Residuals</u> ^a	
2.5%	527.50	
5%	520.00	F — ^b
 <u>Method of Application (M)</u>		
Spray	545.00	
Dip	502.5	F —

^aAll values expressed as ppm sorbic acid

^bF — denotes insignificance between treatments

Table 15. Statistical summary of organoleptic evaluation of 90 day long cut hams at 0 days^a

	<u>Potassium Sorbate Concentration (% Aqueous Solution W/V)</u>					
	0		2.5		5	
	<u>Spray</u> ^b	<u>Dip</u>	<u>Spray</u>	<u>Dip</u>	<u>Spray</u>	<u>Dip</u>
			Aroma F— ^c			
Mean	6.17 ^d	6.83	7.17	6.5	7.17	7.17
Range	3-9	5-9	6-8	4-8	6-9	5-9
S.D.	2.04	1.60	0.75	1.52	0.98	1.47
			Flavor F—			
Mean	6.67	7.5	6.5	7.0	6.83	7.83
Range	4-8	7-9	3-8	5-9	5-9	6-9
S.D.	1.75	0.84	1.76	1.41	1.47	1.17
			Saltiness F—			
Mean	5.83	7.33	6.33	7.17	6.67	7.5
Range	2-8	6-9	2-8	5-9	3-9	7-8
S.D.	2.32	1.21	2.25	1.47	1.97	0.55
			Overall Satisfaction F—			
Mean	6.67	7.67	6.83	7.17	7.0	7.67
Range	5-8	7-9	5-8	6-9	6-9	6-8
S.D.	1.21	1.03	0.98	1.17	1.26	0.82

^aHedonic scale from dislike extremely to like extremely, 1-9, respectively

^bMethod of application

^cF— denotes insignificance in all categories

^dMean of six samples

Table 16. Statistical summary of sorbic acid residuals in 120 day old ham slices^a

$$F = 22.61^{**b}$$

Potassium Sorbate Concentration (% Aqueous Solution W/V)

	12.5		5		10 ^c		10	
	22°C ^d	7°C	22°C	7°C	22°C	7°C	22°C	7°C
Mean	410 ^e	415	1650	1315	775	1200	2475	2650
Range	290-530	380-450	1600-1700	1030-1600	700-850	1100-1300	2150-2800	2450-3850
S.D. ^f	169.71	49.50	70.71	403.05	75.00	141.42	459.62	282.84

^aAll values expressed as ppm

^b** denotes significance at 1% level

^cInstantaneous dip; all others are one minute dips

^dTemperature of storage

^eMean of two observations

^fStandard deviation

locate the differences between treatments and storage temperatures. Results are presented in Table 17. One of the most obvious differences was between the 10% instantaneous dip and the 10% one minute dip. This established a definite relationship between the length of dip and the amount of sorbate retained in the sample. The other obvious result was the lack of significant difference between samples stored at 22°C and 7°C.

When the sorbate residuals of the 120 day old ham slices were compared to the ones of the 60 and 90 day old whole ham studies, it was observed that the means were much higher in the ham slices. Careful appraisal, however, can offer several explanations for these apparent contradictions. First, the samples were 5.1 x 5.1 x 1.3 cm ham squares rather than whole hams, which afforded a greater relative surface area for each sample. Second, the dips in all cases except one were of one minute duration in the ham slice study, as compared to a spray or instantaneous dip in the whole ham study. Finally, the 10% dips contained twice as much potassium sorbate as the highest concentration (5%) the whole hams were exposed to.

4.212 Visible Mold Evaluation

Results of the visible mold evaluation are reported in Table 18. After 120 days fungal growth was observed at varying degrees on all inoculated samples at the 0 and 2.5% levels. Growth was detected on only the 0 and 2.5% uninoculated samples that were incubated at 22°C. Half of the samples were inoculated with mold to encourage fungal growth in the low humidity environment (less than

Table 17. Factorial analysis of the effect of potassium sorbate level (L) and temperature of storage (T) on sorbic acid residuals in 120 day old ham slices

<u>Potassium Sorbate Level (L)</u>	<u>Residuals^a</u>	
2.5%	412.50	
5%	1482.50	F=51.11 ^{**b}
10% ^c	987.50	
10%	2562.50	
 <u>Storage Temperature (T)</u>		
22°C	1327.50	
7°C	1395.00	F— ^d

^aAll values expressed as ppm sorbic acid

^b** denotes significance at 1% level

^cInstantaneous dip; all others are one minute dips

^dF— denotes insignificance

Table 18. Visible mold evaluation of 120 day old ham slices^a

Treatment (% Potassium Sorbate W/V)	Uninoculated		Inoculated	
	22°C ^a	7°C	22°C	7°C
0 ^b	4	0	4	5
2.5	2.5	0	1.5	2.5
5	0	0	0	0
10 ^c	0	0	0	0
10	0	0	0	0

^aGrowth expressed on scale of 0 to 5: 0 = no growth; 1 = very slight; 2 = slight; 3 = moderate; 4 = marked; 5 = intense

^bOne observation; all others are average of 2 observations

^cInstantaneous dip; all others are one minute dips

65%) of the incubators. Consequently, it was probably the synergistic effect of sorbic acid and low relative humidity that inhibited growth at the 5 and 10% potassium sorbate levels. Low temperature probably contributed to the inhibition of mold growth in the uninoculated samples stored at 7°C.

4.22 60 Day Ham Slice Study

4.221 Sorbic Acid Residuals

The statistical summary of the sorbic acid residuals detected in the 60 day ham slice study is presented in Table 19. The F test indicated significant differences between treatments at the P = .05 level. Wide variations were again detected on samples subjected to identical treatments. Factorial analysis was performed to locate the differences between treatments and storage times. Results are presented in Table 20. There were significant differences in sorbate residuals in ham slices dipped in varying concentrations of potassium sorbate; these differences were not as dramatic as in the 120 day old ham slices (Table 17). Length of storage also influenced residual concentrations. Sorbic acid concentrations decreased by 50% during the 60 day study (583.5 to 250.0 ppm). These results were not unlike the decrease from 715 to 260 ppm sorbate in the 60 day long cut ham study (Table 10). The results, though, seem to contradict those of Boyd and Tarr (1955). They found that sorbic acid residuals in smoked fish did not decrease appreciably over a period of 60 days.

Table 19. Statistical summary of sorbic acid residuals from 60 day ham slice study^a

F=4.53^{*b}

	Potassium Sorbate Concentration (% Aqueous Solution W/V)			
	2.5	5	10 ^c	10
	0 Days			
Mean	247 ^d	422	625	1040
Range	224-270	440-444	590-660	680-1400
S.D. ^e	32.53	31.11	49.50	590.12
	60 Days			
Mean	135	276	268	323
Range	130-140	178-374	200-336	270-376
S.D.	7.07	138.59	96.17	74.95

^aAll values expressed as ppm sorbic acid

^b* denotes significance at 5% level

^cInstantaneous dip; all others are one minute dip

^dMean of 2 observations

^eStandard deviation

Table 20. Factorial analysis of the effect of potassium sorbate level (L) and days following application (D) on sorbic acid residuals of 60 day ham slice study

<u>Potassium Sorbate Level (L)</u>	<u>Residuals^a</u>	
2.5%	191.00	
5%	349.00	F = 4.53 ^{*c}
10% ^b	446.50	
10%	681.50	
 <u>Days Following Treatment (D)</u>		
0 Days	583.50	F = 11.91 ^{**d}
60 Days	250.00	

^aAll values expressed as ppm sorbic acid

^bInstantaneous dip; all others are one minute dips

^c* denotes significance at 5% level

^d** denotes significance at 1% level

4.222 Visible Mold Evaluation

Findings of the visible mold evaluation at 60 days are presented in Table 21. The results of the factorial analysis of variance made on all the days of mold evaluation (8, 16, 24, 36, and 60 days) are presented in Table 22. F values were significant at the $P = .01$ level for all three parameters: potassium sorbate level; days following treatment; and source of the ham slice. Generally, the longer the duration of storage the greater the amount of observed fungal growth. The same generalization applies to decreasing concentrations of potassium sorbate. However, the largest F value (67.85) was related to the source of the ham slice. The slices that exhibited the most prolific fungal growth were obtained from ham no. 52. The arithmetic mean of its visible evaluation (2.32) was almost twice as great as its nearest competitor, suggesting that the slices from ham no. 52 were more heavily contaminated with mold. As observed by Smith and Rollins (1954), a high concentration of mold could metabolize sorbate and thereby eliminate its fungistatic properties. Representative 60 day old ham slices are shown in Figure 2.

Comparisons of visible mold evaluations from the 120 and 60 day old ham slices (Tables 18 and 21) show more extensive growth on the 60 day samples. A careful appraisal can offer an explanation for the seemingly contradictory findings. The 120 day ham slices were incubated in an atmosphere of low relative humidity (less than 65%), which discouraged fungal growth. Conversely, the 60 day ham slices

Table 21. Visible mold evaluation of 60 day old ham slices^a

<u>Ham no.</u>	Potassium Sorbate Concentration (% Aqueous Solution W/V)				
	<u>0</u>	<u>2.5</u>	<u>5</u>	<u>10^b</u>	<u>10</u>
37	3	1	1	0	0
40	4	1	1	1	1
46	4	2	2	2	1
52	5	3	3	2	2

^aGrowth expressed on scale of 0 to 5: 0 = no growth; 1 = very slight; 2 = slight; 3 = moderate; 4 = marked; 5 = intense

^bInstantaneous dip; all others are one minute dips

Table 22. Factorial analysis of the effect of potassium sorbate level (L), days following treatment (D), and source of ham slices (S) on visible mold evaluation of 60 day ham slice study

	<u>Mean^a</u>	
<u>Potassium Sorbate Level (L)</u>		
0%	2.55	
2.5%	1.10	
5.0% ^b	1.00	F=53.23 ^{**c}
10.0% ^b	0.76	
10.0%	0.36	
<u>Days Following Treatment (D)</u>		
8 Days	0.26	
16 Days	0.46	
24 Days	1.45	F=42.92 ^{**}
36 Days	1.70	
60 Days	1.90	
<u>Source of Ham Slice (S)</u>		
Ham 37	0.45	
Ham 40	0.65	
Ham 46	1.20	F=67.85 ^{**}
Ham 52	2.32	

^aGrowth expressed on scale of 0 to 5: 0 = no growth; 1 = very slight; 2 = slight; 3 = moderate; 4 = marked; 5 = intense

^bInstantaneous dip; all others are one minute dips

^c** denotes significance at 1% level



Figure 2. Comparisons of mold growth on 60 day old ham slices obtained from hams 46 (top) and 37 (bottom). Potassium sorbate treatments, left to right, 0-10%. See Table 21 for details.

were stored at approximately 70% relative humidity, where mold growth was encouraged. Both Melnick et al. (1954a) and Marth et al. (1966) have demonstrated that certain molds can metabolize sorbate. If the mold population is high enough the antifungal benefit is lost. Although the fungi were restricted to all levels of sorbic acid application in the 60 day slice study, some growth was observed even at the 10% level. This limited growth could account for at least a partial reduction in sorbate by mold metabolism.

Factorial analysis and the effect of the interactions of potassium sorbate level, days following treatment, and source of the ham slice are presented in Tables 22 and 23. Of the three factors, the interaction between potassium sorbate level and days following treatment was the most significant. The association between the source of the slice and the days following treatment was another important influence on perceptible mold growth. However, an insignificant F value was calculated for the interaction of potassium sorbate level and ham slice source. Overall, it appears that all three of the aforementioned factors have an influence on the extent of mold growth. The most significant observation, though, may be the variation in the fungistatic effectiveness of sorbic acid on different ham slices, indicating a more heavily contaminated ham or ham slice will show less response to treatment.

Table 23. Effect of interactions of potassium sorbate level (L), days following treatment (D), and source of ham slices (S) on visible mold evaluation of 60 day ham slice study^a

F = 2.24 ^{**b}		F --		F = 2.37 ^{*c}	
Interaction (L x D)	Mean	Interaction (L x S)	Mean	Interaction (D x S)	Mean
0%	8 Days	0%	Ham 37	8 Days	Ham 37
	16 Days		Ham 40		Ham 40
	24 Days		Ham 46		Ham 46
	36 Days		Ham 52		Ham 52
2.5%	8 Days	2.5%	Ham 37	16 Days	Ham 37
	16 Days		Ham 40		Ham 40
	24 Days		Ham 46		Ham 46
	36 Days		Ham 52		Ham 52
	60 Days				
5%	8 Days	5%	Ham 37	24 Days	Ham 37
	16 Days		Ham 40		Ham 40
	24 Days		Ham 46		Ham 46
	36 Days		Ham 52		Ham 52
	60 Days				
10% ^d	8 Days	10% ^d	Ham 37	36 Days	Ham 37
	16 Days		Ham 40		Ham 40
	24 Days		Ham 46		Ham 46
	36 Days		Ham 52		Ham 52
	60 Days				
10%	8 Days	10%	Ham 37	60 Days	Ham 37
	16 Days		Ham 40		Ham 40
	24 Days		Ham 46		Ham 46
	36 Days		Ham 52		Ham 52
	60 Days				

^aGrowth expressed on scale of 0 to 5: 0 = no growth; 1 = very slight; 2 = slight; 3 = moderate; 4 = marked; 5 = intense

^b** denotes significance at 1% level

^c* denotes significance at 5% level

^dInstantaneous dip; all others are one minute dips

4.3 70 Day Old Packer Style Cut Ham Study

4.31 Mold and Yeast Colony Counts

The statistical summary of the mold and yeast colony counts at 0 days is reported in Table 24. The F test was insignificant for molds and significant at the $P = .01$ level for yeasts. The most obvious effect, however, was the tremendous reduction in both mold and yeast colony forming units as compared to 0 day counts for the 60 and 90 day long cut hams (Tables 5, 6, and 12). This radical difference might have been due to a change of application methods in the two experiments. The 60 and 90 day hams were either sprayed until wet or instantaneously dipped; the packer cut hams were sprayed for one minute. The one minute spray was chosen as the method of application because it was thought it would be more feasible in commercial country ham production. Similar studies have also revealed that chlorine sprays significantly lowered bacterial populations on beef carcasses (Kotula *et al.*, 1973). The one minute spray alone, without the addition of potassium sorbate, appeared to exert a cleansing effect on the hams by washing off many of the mold and yeast spores initially present. The spray administered to the 60 and 90 day long cut hams probably was not long enough to reduce the spore population on the ham surface. The instantaneous dips that were applied to the 60 and 90 day hams probably did not reduce the number of spores for a different reason. Each ham subjected to the same concentration of potassium sorbate was dipped in the same solution. Therefore each ham that was dipped could have been reinoculated

Table 24. Statistical summary of mold and yeast colony counts on 70 day old packer cut hams at 0 days^a

	Potassium Sorbate Concentration (% Aqueous Solution W/V)			
	0	2.5	5	10
	Molds			
	F — ^b			
Mean	1.26 ^c	0.68	0.58	0.00
Range	0-3.88	0-1.55	0-1.55	0
S.D. ^d	1.77	0.79	0.66	0.00
	Yeasts			
	F=29.26 ^{**e}			
Mean	1090.00	0.29	0.66	0.10
Range	496.00-1362	0-0.78	0.16-1.16	0-0.39
S.D.	403.17	0.37	0.58	0.19

^aAll values expressed as colony forming units/cm²

^bF— denotes insignificance between treatment

^cMean of 4 observations

^dStandard deviation

^e** denotes significance at 1% level

with the fungal spores washed off the previously treated hams.

The effect of adding potassium sorbate to the sprays can be illustrated by comparing yeast colony counts of hams subjected to different concentrations of potassium sorbate. The arithmetic mean for the hams sprayed for one minute with distilled, deionized water was 1090 yeast colony forming units/cm². Hams sprayed with potassium sorbate (2.5, 5.0, or 10%) had less than 1 colony forming unit/cm².

The results of the 30 day mold and yeast colony counts are reported in Table 25. These results could not be statistically analyzed because an overwhelming number of plates were too numerous to count. When compared to the colony counts on the 60 day long cut hams at day 60 (Tables 5 and 6), it seems that the sorbate was less effective at reducing the numbers of colony forming units in 70 day packer cut hams. This apparent contradiction can be explained, however, by differences in relative humidity. The 60 day hams were stored in an atmosphere of low relative humidity (less than 65%) where mold growth was inhibited (Cecil and Woodroof, 1954). The packer cut hams were stored at a relative humidity permitting the growth of mold, i.e. 70±5%. The presence of mold growth on the hams, therefore, accounts for the increase in colony forming units. The only level where counts could even be made was the 10% concentration, which, at least, gives an indication of mold inhibition.

Mold and yeast colony counts were not conducted at 60 days after observing that each ham was covered with mold spores. This observation alone seemed to indicate that the sorbate treatments had

Table 25. Mold and yeast colony counts on 70 day old packer cut hams at 30 days^a

% Potassium Sorbate W/V)	Ham	Molds		Yeasts	
		Average	Range	Average	Range
0	2	TNC ^b	TNC	7339.12 ^c	441.72- 14236.52
	3	TNC	TNC	13247.87	10677.39- 15818.35
	4	86.83	46.50- 140.61	14493.56	2530.94- 38754.96
2.5	9	TNC	TNC	TNC	35986.75- TNC
	10	TNC	TNC	TNC	TNC
	11	TNC	TNC	TNC	1146.30- TNC
5	16	TNC	TNC	TNC	464.97- TNC
	17	TNC	TNC	TNC	TNC
	18	2.52	0.39- 3.88	TNC	TNC
10	23	32.88	15.50- 52.73	TNC	30054.24- TNC
	24	193.94	135.62- 241.67	267.50	147.24- 364.70
	25	19.86	15.50- 26.35	309.86	147.23- 492.13

^aAll values expressed as colony forming units/cm²

^bToo numerous to count

^cAverage of four observations

ceased to be effective at all levels after 60 days of storage.

4.32 Sorbic Acid Residuals

The concentration of potassium sorbate sprayed on each ham was calculated utilizing the weight-volume procedure described in Sect. 3.752. The statistical summary of the concentrations applied is presented in Table 26. The F test indicated that spray treatments differed significantly at the 1% level. A Duncan's multiple range test was performed to locate the differences. It showed that significant differences existed between all three levels. The Duncan's test also demonstrated that the differences within treatments were insignificant. Therefore, this test illustrated that spray treatments at any one level would yield repeatable results.

Sorbate residuals were also analyzed at 0 days with the standard spectrophotometric method. Results are shown on Table 27. The residuals found by the spectrophotometric method appear at first glance to contradict those found by the method of Sect. 3.752. The residuals found by spectrophotometric analysis were expressed on a ppm basis of the upper surface of each ham. The concentrations found by the weight-volume calculations, however, were expressed on a μg potassium sorbate/g ham basis.

The residuals detected on the packer style cut hams also seem indicative of the efficiency of the one minute spray. Concentrations at 0 days are much greater than any detected on the 60 or 90 day old long cut hams (Tables 9 and 13). The high sorbate retention and significant differences between treatments reinforces the concept

Table 26. Statistical summary of concentration of potassium sorbate on 70 day old packer cut hams^a

	F=67.38 ^{**b} For Treatment		F— ^c For Repetitions
	Potassium Sorbate Concentration (% Aqueous Solution W/V)		
	2.5	5	10
	180.66	360.37	537.22
	158.02	265.94	555.12
	209.51	359.65	491.68
Sample ^d	157.74	335.86	484.70
	153.33	293.95	636.71
	192.07	374.37	434.37
	146.58	291.68	620.73
	<u>171.13</u>	<u>325.97</u>	<u>537.22</u>
Range	146.58-209.51	265.94-374.37	434.37-636.71
S.D. ^e	23.36	41.96	73.71

^aAll values expressed as μg potassium sorbate/g ham

^b** denotes significance at 1% level

^cF— denotes insignificance

^dEach sample datum represents one ham

^eStandard deviation

Table 27. Statistical summary of sorbic acid residual on 70 day old packer cut hams^a

	<u>Potassium Sorbate Concentration (% Aqueous Solution W/V)</u>		
	2.5	5	10
	<u>0 Days</u>		
	700.00 ^b	2100.000	2400.00
	<u>30 Days</u>		
	F=13.82 ^{*c} For Treatment		F— ^d For Repetition
Mean	<u>82.67^e</u>	<u>172.60</u>	<u>432.00</u>
Range	30-148	142-228	348-568
S.D. ^f	60.01	48.54	118.86
	<u>60 Days</u>		
	F— For Treatment		F— For Repetition
Mean	<u>32.01</u>	<u>50.00</u>	<u>84.67</u>
Range	0-96	0-100	0-194
S.D.	55.42	50.00	99.32

^aAll values expressed as ppm sorbic acid

^bOne observation only

^c* denotes significance at 5% level

^dF— denotes insignificance

^eMean of 3 observations

^fStandard deviation

that younger (70 day old) hams are more responsive to treatment, as previously discussed in Sect. 4.122.

Stockinettes were utilized for the first time in the packer cut ham study. They too could have been an aid in sorbate retention by absorbing a greater amount of the solution.

Thirty days after application a drastic reduction in the concentration of sorbic acid is evident. Results are presented in Table 27. The differences between treatments were less after 30 days of storage, in fact, a Duncan's multiple range test showed no significant difference between the 2.5 and 5% levels. These decreases can be attributed at least partially to the presence of mold growth on all but one ham (Table 28). As previously illustrated in Sect. 4.222, molds in sufficiently high concentrations could metabolize the sorbate, thereby reducing the fungistatic effect.

After 60 days of storage the sorbate was almost completely degraded in every ham (Table 27). Mold growth was observed on every ham, illustrating the loss of the fungistatic properties of sorbic acid. A Duncan's multiple range test showed there was no significant differences in sorbate residuals between any of the treatment levels. Therefore, if packer cut hams are stored for 60 days in a high humidity environment, it appears that the fungistatic properties of sorbic acid are reduced to insignificant levels.

4.33 Visible Mold Evaluation

Representative hams after 30 days of storage are shown in Figures 3 and 4. The statistical summary for the visible mold

Table 28. Statistical summary of visible mold evaluation of 70 day old packer cut hams^a

	<u>Potassium Sorbate Concentration (% Aqueous Solution W/V)</u>			
	0	2.5	5	10
	<u>30 Days</u>			
	F=35.07 ^{**b}	For Treatment	F— ^c	For Repetitions
	4	4	2	1
Ham	5	3	1	1
	4	3	1	0
Mean	<u>4.33</u>	<u>3.33</u>	<u>1.33</u>	<u>0.67</u>
Range	4-5	3-4	1-2	0-1
S.D. ^d	0.58	0.58	0.58	0.58
	<u>60 Days</u>			
	F—	For Treatment	F—	For Repetitions
	5	4	4	3
Ham	4	4	4	4
	5	4	4	5
Mean	<u>4.67</u>	<u>4.0</u>	<u>4.0</u>	<u>4.0</u>
Range	4-5	-	-	3-5
S.D.	0.58	0	0	1.0

^aGrowth expressed on scale of 0 to 5: 0 = no growth; 1 = very slight; 2 = slight; 3 = moderate; 4 = marked; 5 = intense

^b** denotes significance at 1% level

^cF— denotes insignificance

^dStandard deviation

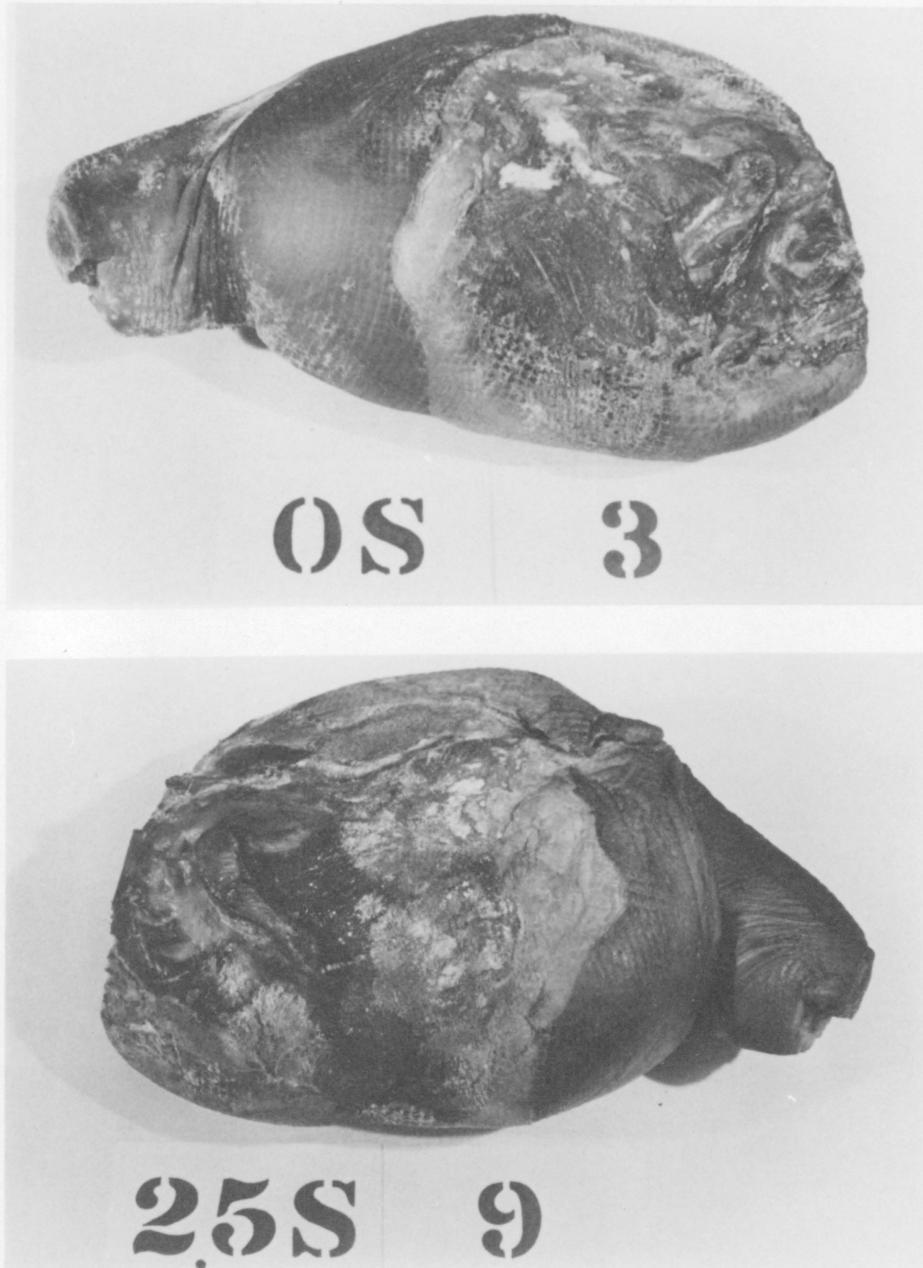


Figure 3. Cured country hams (packer style cut) 30 days after treatment with 0% (top) and 2.5% (bottom) potassium sorbate.

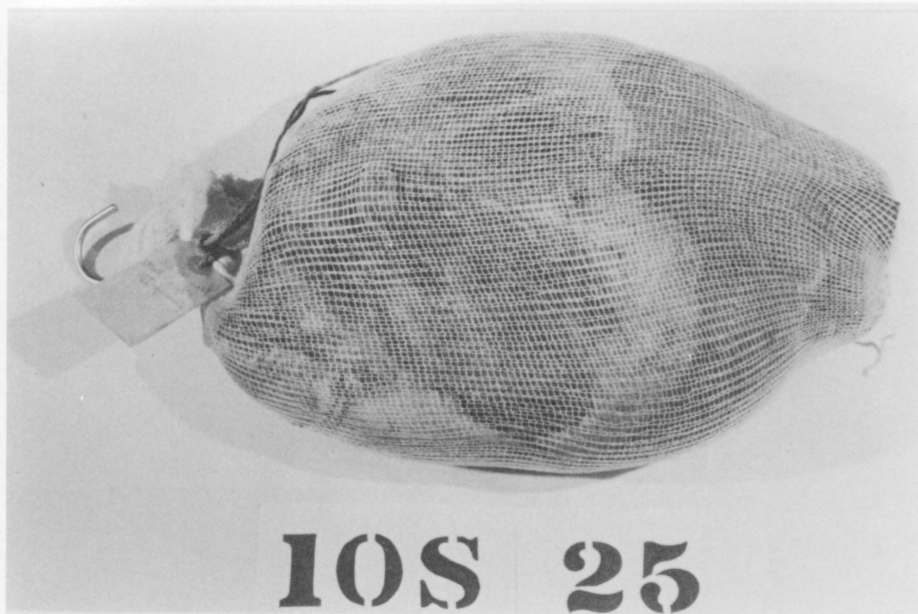
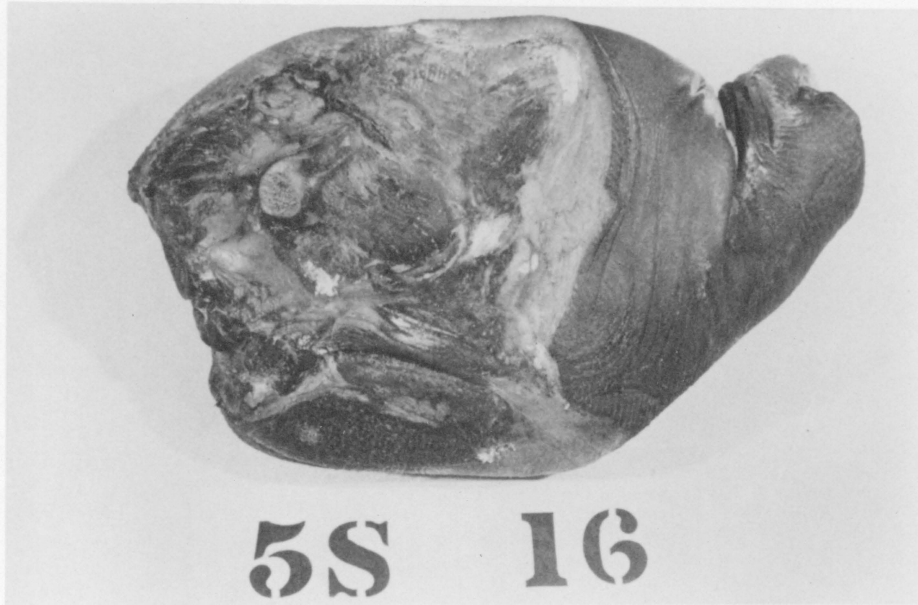


Figure 4. Cured country hams (packer style cut) 30 days after treatment with 5% (top) and 10% (bottom/ with stockinette) potassium sorbate.

evaluation at 30 days is presented in Table 28. The F value indicated that the extent of mold growth was significantly different between treatments at the $P = .01$ level. The F value for within treatments, however, was insignificant, indicating that hams subjected to identical treatments exhibited approximately the same amount of mold growth. A Duncan's multiple range test was conducted to locate the differences between potassium sorbate levels. It showed that the extent of fungal growth was significantly different between the 0 and 2.5% levels, however, mold growth at the 2.5% concentration was still above the "moderate" level. The 5% one minute spray, though, provided an acceptable measure of inhibition. Apparently both the 5 and 10% sprays were quite effective at inhibiting mold growth for at least 30 days in an ambient, humid environment ($21.5 \pm 0.5^{\circ}\text{C}$ and $70 \pm 5\%$ relative humidity).

Results of the 60 day evaluation are also presented in Table 28. The amount of mold growth on all hams ranged from moderate to intense. A Duncan's multiple range test indicated no significant differences between treatment levels. These evaluations, along with the 60 day sorbic acid residuals, seem to reinforce the conclusion that the ability of sorbic acid to inhibit mold growth on a country ham is lost after 60 days of storage in an ambient, humid environment.

4.4 Effect of Variables Not Studied On Sorbate's Fungistatic Properties in Country Ham

Many variables may determine why a certain level of potassium sorbate is effective on one ham and not on another. One variable is

pH. Beneke and Fabian (1955) found that Penicillium, Aspergillus, and Alternaria, three genera commonly found growing on country ham, grew in the presence of 250 ppm sorbic acid at pH 3.3 on a strawberry medium. At pH 4, though, some isolates of Penicillium and Aspergillus were able to grow at the 500 ppm level.

Country hams have pH levels that range from 5.4-6.8 after one month of aging (Graham, 1970). Some of the lower sorbate residuals detected in these studies would not inhibit mold (see Table 27), considering the pH values of country ham. The hams, however, possess other properties that would tend to discourage microbial growth. These properties include lower moisture, lower a_w , and higher salt content. Country hams have been found to have a_w values that range from .92-.99. Haas et al. (1975) found that an a_w of .83 and 40% moisture inhibited Aspergillus glaucus growing on a synthetic medium for 24 days at the 1000 ppm sorbic acid level and 34 days at the 3000 ppm level. Average moisture for a country ham after one month of aging ranges from 36.71-69.78% (Graham, 1970). Considering the pH, a_w , and percent moisture of a country ham, mold could still occur because the highest level of sorbate detected in this study was 2850 ppm.

Other inhibitory properties inherent to country ham cannot be disregarded. Salt can range from 5.45-8.10% after one month of aging (Graham, 1970). Smoke too possesses some antimicrobial properties (e.g. creosols, phenolic compounds etc.), but they are more often described as bacteriostatic and bacteriocidal than

fungistatic (Urbain, 1971). Still there is a definite antifungal nature to smoke (Baldock, 1977).

5.0 SUMMARY AND CONCLUSIONS

A series of experiments were conducted to explore the potential of potassium sorbate as a fungistatic agent in country ham processing. The experiments were categorized into three phases: the first utilized 60 and 90 day old long cut hams; the second used country ham slices; and the third phase utilized 70 day old packer style cut hams. Whole hams were evaluated on three different criteria: 1) number of mold and yeast colony forming units; 2) sorbic acid residuals; and 3) visible mold where applicable. The 90 day hams were also evaluated organoleptically to determine if sorbate affected aroma, flavor, saltiness, or overall satisfaction. Ham slices were examined only for visible mold and sorbic acid residuals.

The findings of the experiments indicated that initiation of fungal outgrowth on hams was delayed by the addition of potassium sorbate. Of the different levels of potassium sorbate that were tested, the 5% concentration was the lowest level to effectively inhibit mold growth. The 5% one minute spray utilized in the packer cut ham study deposited 2100 ppm sorbic acid on the ham surface or 325.97 μg potassium sorbate/g whole ham.

Sorbic acid was retained on ham surfaces, although the residuals fluctuated widely among hams that were identically treated. These fluctuations were attributed to the inherent irregularities in treating a product like ham and the nonuniformity of scrapings from the ham surfaces used for residual samples. Sorbic acid cannot be directly incorporated into a ham as it can into a formulated product

such as cottage cheese or cake batter. Even when incorporated into liquid-based foods variations in recovery of sorbate residuals are known to exist.

The residual level of sorbic acid appeared to decrease during the periods of storage in each study. Its disappearance could be attributed to three possible occurrences: 1) the metabolism of the sorbate by high initial inoculums of mold; 2) its migration towards the interior of the ham; or 3) oxidative deterioration. The rate of oxidative deterioration of a fatty acid is temperature dependent according to Lee (1975); therefore one might conclude that sorbic acid might behave in a similar manner, to be retained in higher concentrations at lower temperatures of storage. Contrary to our expectations, the ham slices, incubated at 7 and 22°C for 120 days, did not show statistical differences in their sorbate residuals. However, the temperature differential may not have been large enough to affect the rate of sorbate oxidation. Therefore, either the sorbate deteriorated at approximately the same rate at both storage temperatures, or oxidation was not responsible for the decrease in sorbate residuals in country ham.

Although the mechanism of sorbate deterioration bears further investigation, there is no doubt that fungal outgrowth on hams is delayed by the addition of potassium sorbate. Both colony and visible mold evaluations demonstrated less growth on treated hams than on controls. The packer cut ham study illustrated that even under demanding conditions of high humidity sorbate inhibited

fungus growth at the 5 and 10% levels for between 30 and 60 days.

Relative humidity and temperature influenced the effectiveness of sorbic acid. Low humidity alone (less than 65% relative humidity) was shown to inhibit mold growth on the 120 day ham slices incubated at 22°C. Low temperature contributed to mold inhibition on slices incubated at 7°C. Thus, atmospheric and climatic conditions will dictate the effectiveness and the amount of potassium sorbate that a commercial producer of country ham will need to achieve fungal inhibition.

The age of the hams when treated was shown to have an influence on the potency of potassium sorbate treatments. Mold and yeast colony counts tended to be lower on the 60 day long cut hams than on the 90 day ones. There was also a significant difference in sorbate residuals between treatments on the 60 day hams while none existed between treatments on the 90 day ones. These results could be an indication that a younger ham is more "responsive" to sorbate treatment, perhaps because younger hams exhibit a lesser degree of case hardening.

The 60 day slice study illustrated how the initial level of contamination can influence sorbate's fungistatic prowess. Visible mold evaluations showed that slices obtained from a particular ham had a statistically higher amount of fungus growth at every potassium sorbate level. Therefore, a sorbate level that may be very effective on one ham might prove to be less so on another ham processed under unsanitary conditions.

Several methods of potassium sorbate application were tested during the entire study. The one minute spray was decided as the best for the following reasons: 1) it is feasible for commercial application; 2) it drastically reduces initial mold and yeast colony counts; and 3) it does not present the potential for recontamination as a dip does.

The stockinette proved to be an asset during treatment, not only by giving a measure of physical protection, but also by absorbing some of the excess potassium sorbate solution that would otherwise run off the hams. The stockinette is already used in many commercial operations, for it is often the only way to hang a ham with a short shank.

Evaluations were made on potassium sorbate's influence on the organoleptic properties of country ham. Taste panel members were unable to detect differences among spray and dip applications of 0, 2.5, and 5% aqueous potassium sorbate. Residuals ranged up to 670 ppm and among the four categories; aroma, flavor, saltiness, and overall satisfaction; the 2.5 and 5% treated hams were preferred in some cases over the controls.

One of the most difficult observations to resolve in this study is the lack of correlation between sorbate residuals and mold and yeast colony counts. Simply stated, the sorbic acid residuals are not inversely proportional to the mold and yeast colony counts. As can be seen by referring to the appropriate tables, it is not possible to predict that x ppm sorbic acid will inhibit y colony

forming units. It is not possible because a country ham is not an ideal system with controlled conditions. Many variables may determine why certain levels of potassium sorbate are effective on one ham and less so on another (e.g. pH, a_w , percent moisture, salt, and smoke).

Sorbic acid, then, has been shown to be effective at inhibiting fungal growth. But its effectiveness is limited by environmental conditions (high relative humidity); and by the inherent physical and chemical characteristics of each ham. With regards to tolerance levels, the highest residual found in this study (2850 ppm) did not exceed the 0.3% limit placed on cold pack cheese products. During this era of resource conservation sorbate should prove to be an invaluable aid for preserving the quality of country hams with or without refrigeration or humidity controls. Although processing under controlled environmental conditions will restrict mold growth, large expenditures of energy are required to maintain these controls. When the product leaves the commercial processor it may be without environmental controls for several weeks; therefore, a need for potassium sorbate goes beyond the processing plant.

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POTASSIUM SORBATE AS A FUNGISTATIC AGENT
IN COUNTRY HAM PROCESSING

by

Philip R. Frank

(ABSTRACT)

A study was conducted to determine the feasibility of utilizing potassium sorbate as a fungistatic agent in country ham processing. The study was divided into three phases: the first utilized 60 and 90 day old cured country (long cut) hams; the second used country ham slices; and the third phase utilized 70 day old country cured (packer style cut) hams. Samples were incubated at various temperatures and relative humidities to determine the extent of protection offered by sorbate against fungal growth.

Intensity of fungal growth was determined subjectively through periodic visible evaluations and were quantitated by culture plating methods. A UV spectrophotometric technique was utilized to determine the concentrations of sorbic acid deposited on ham surfaces or slices by various methods of application.

Of the different methods of application that were tested, the 5% (W/V) 1 minute spray offered the lowest effective level for inhibition of fungal growth. The 5% 1 minute spray significantly lowered initial mold and yeast colony counts and protected the hams for 30 to 60 days under conditions conducive to fungal outgrowth ($21.1 \pm 0.5^{\circ}\text{C}$ and $70 \pm 5\%$ relative humidity). An analogous 10% spray provided a slightly greater measure of mold inhibition than the 5% treatment under identical conditions.