

CONTROLLED MICROBIOLOGICAL AND ENVIRONMENTAL
TECHNIQUES IN MEAT PROCESSING

by

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Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

ANIMAL SCIENCE

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May, 1970

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ACKNOWLEDGMENTS

The author wishes to express his sincere thanks and appreciation to Dr. R. F. Kelly and Dr. P. P. Graham for their guidance, suggestions, and assistance throughout his graduate program and in the preparation of this manuscript.

Special thanks are given to Dr. R. E. Benoit and Dr. E. N. Boyd for their interest, advice, and friendly council in the preparation of this manuscript.

Further appreciation is extended to

and

for their technical assistance.

The author would also like to thank Dr. C. Y. Kramer for consultation and assistance on statistical analysis.

Thanks are also given to Merck and Company, Inc., Rahway, New Jersey, for supplying the ACCEL starter culture.

Deepest gratitude is given to the author's wife, for typing this manuscript and her understanding and encouragement.

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INTRODUCTION

The food microbiologist is concerned with two major processes: (1) those microbial activities which lead to spoilage of a product and (2) those microbial activities which lead to enhancement of a product. However, the microbiological metabolic processes are similar whether the changes in the food are desirable or undesirable, and the distinction between the two may not be clear. Considerable research concerning the influence of microorganisms on meat products has involved primarily mixed cultures and their spoilage of the product. Little work has been done using bacteria in the development of new, safe and appealing meat products which have acceptable keeping qualities.

Niven (1952) stated that employment of bacteria in foods as starter cultures should stimulate our imagination as to possible means of controlling certain types of spoilage as well as food poisoning hazards. In addition, Niven (1952) pointed out that as product development proceeds in the food industry, the actual and potential usefulness of microorganisms as food enhancers greatly overshadows their economic importance as spoilage bacteria. In this connection, Ockerman et al. (1964) and Bothast et al. (1968) have developed surgical and dip techniques respectively, for

obtaining undenatured muscle tissue with a low bacterial count. These techniques furnish the meat scientist with a new tool to study the influence of bacteria (pure or mixed cultures) on meat products.

This research was designed to further develop a technique to study the influence of bacteria on meat.

Pediococcus cerevisiae (Gnotobiotic treatment), an accepted starter culture, was used in the pickle curing of pork loins. In addition, the predominate microorganisms of the Conventional and Reduced Initial Count (RIC) treatments were studied to determine their influence on the product.

REVIEW OF LITERATURE

Microbiology of Meat

Many investigators (Ayres, 1956; Niven, 1957; Zender et al., 1958; Sharp, 1963; Ockerman et al., 1964) have pointed out that the skeletal muscles and edible organ tissue of healthy meat animals are essentially sterile. However, upon dressing and chilling, the meat surface becomes contaminated with a myriad of bacteria. These diverse, contaminating microorganisms may remain dormant, may die slowly, or may grow, depending on their physical and chemical environment. Those species or varieties that can adapt to a particular environment will become the predominant flora and may enhance or spoil the product.

Meat processing or curing was originally designed for preservation of meats by salt without refrigeration, but it also affects meat color and flavor. Therefore, as time passed, processed or cured meats became valued for their organoleptic quality per se and there has been a tendency to lower the concentration of the curing ingredients along with the development of starter cultures (Jensen, 1949, and Lawrie, 1966).

Microorganisms in fermented and cured meat products.--
Early investigators such as Gibbons (1939) and Brooks et al.

(1940) stated that little information could be found on the role of bacteria in meat curing and that bacterial activity in curing brines was not essential to the production of a satisfactory flavor in bacon and ham. However, Tanner (1944) and Jensen (1949, 1954) indicated that there is a relationship between brine microorganisms and flavor production. Microorganisms act on meats in a variety of ways. Their enzymes--proteinasases, peptidases, gelatinase, collagenase, hydrolases, lipases, phosphatases, oxidases, peroxidases, dehydrogenases, catalase, lecithinase, hexosidases, etc.--all produce characteristic entities in meats.

Jensen (1954) reported that thuringer types of sausage are characterized by a pleasant "tangy" flavor formed by many species of lactobacilli and occasionally Leuconostoc. In addition, the author indicated that chance inoculation was responsible for the lactic acid bacteria and that chance inoculation was never economical. Therefore, starter cultures such as Lactobacillus plantarum, Lactobacillus brevis and Lactobacillus fermenti were recommended for the production of a standard product. However, these cultures have not been widely used in the meat industry.

Niven (1952a) showed that the lactic acid bacteria are a group of Gram-positive, non-spore forming microorganisms that characteristically ferment sugars with the production of large quantities of lactic acid. The

homofermentative members of this group (Streptococcus and Lactobacillus) produce nearly two moles of lactic acid per mole of dextrose fermented. The heterofermentative members (Leuconostoc and Lactobacillus) produce one mole each of lactic acid, ethanol, and carbon dioxide per mole of dextrose fermented. Members of this group are peculiarly adapted to growing in various types of foods and have thus found a number of industrial applications in some of the food industries. They are essential in the manufacture of certain types of sausages and other fermented meat foods. Aside from their many useful purposes, they are encountered as spoilage organisms in the meat industry. Various types of undesirable souring, sliming and discoloration of sausages and cured meat products are caused by members of this group. Turbidity development in vinegar pickled meat products is usually due to the growth of acid tolerant varieties of the lactic acid bacteria in the pickle.

Evidence was presented by Niven et al. (1949) which indicated that sausage discoloration results from the production of hydrogen peroxide by microorganisms. Niven (1951) reported that a group of harmless bacteria is capable of changing the color of the cured meat pigment (nitric oxide hemochromogen) to a pale green color on cured meat products. The majority of these greening bacteria fall into the so-called lactic acid group and belong to the genus, Leuconostoc, and the genus, Lactobacillus. The

exact mechanism of this greening is unknown. However, the greening organisms are devoid of catalase, and it is thought that when a sufficient concentration of hydrogen peroxide accumulates, it oxidizes the nitric oxide hemochromogen of the meat to perhaps a series of green, yellow, or colorless porphyrin compounds. Evans and Niven (1951) further reported on the nutrition of the heterofermentative lactobacilli that cause greening of cured meat products. These organisms require the addition of manganese and citrate for optimum growth, even in complex laboratory media. In a casein hydrolysate medium, they also require thiamine, nicotinic acid, riboflavin, pantothenic acid, biotin and asparagine. The lag phase may be shortened for some strains by the addition of "tween 80", folic acid, and pyridoxine.

Wilson et al. (1955-1956) selected Pediococcus cerevisiae as the microorganism for a sausage starter culture. This selection was followed by a number of experiments with summer sausage which gave some further justification for believing that the starter culture could be used in a wide variety of fermented meat products. Based on these experiments and the development of a laboratory test, it has been possible to some extent to predetermine the acidity or pH of a product by adjusting the inoculum strength. This starter culture was approved by the Meat Inspection Division of the USDA for use in the manufacture

of fermented sausage, and is being produced and distributed commercially by Merck and Company, Inc., Rahway, New Jersey.

In September, 1968, Merck and Company, Inc., introduced LACTACEL, an improved form of ACCEL. Everson et al. (1969) reported that LACTACEL shortens processing by eliminating the rehydration period and greatly improves the uniformity of flavor, texture, pH and yield.

Studies concerned with the bacterial flora of commercial sausages by Deibel and Niven (1957) revealed the frequent occurrence of pediococci. Pilot plant experiments and field trials were conducted in which a pure culture of Pediococcus cerevisiae was added to summer sausage mixes. The inoculated sausages possessed a superior texture, a tangy, clean flavor and uniformity from batch to batch. The complete processing schedule was reduced from seven to two days. Niven et al. (1958) claimed that the sausage industry must and will adopt the use of starter cultures in the manufacture of semi-dry and dry sausages in order to minimize the many spoilage hazards inherent in their manufacture and to achieve uniformity in quality from batch to batch. This is not possible with chance inoculation. It was pointed out also that various types of sausage can be made with the A.M.I.F. starter culture (Pediococcus cerevisiae) such as Summer Sausage, Thuringer, Lebanon Bologna, and other meat foods in which an acid fermentation is required for the desired flavor. In addition, good

results have been achieved with pork roll.

Mills and Wilson (1958) developed a formula and processing schedule for pork roll using the Pediococcus starter culture. Under experimental conditions, 0.5 ounce of starter per hundred pounds of emulsion was as effective as 1.5 ounces. However, due to the competitive action of the naturally contaminating bacteria in a processing plant, 1 ounce per hundred pounds was recommended for commercial use. It was concluded that the starter culture should be used within one hour after reconstitution.

Deibel et al. (1961) studied the manufacture of fermented sausages. They noted little or no microbial activity during the generally practiced 2 to 4-day holding period of the sausage mix prior to smoking. Microbial activity occurred mainly during the heating and smoking periods. Reduction of nitrate occurred generally during the first 2 to 16 hours of smoking and heating, while acid production was initiated usually after 8 to 16 hours. Lactobacillus plantarum comprised the predominant flora in commercially produced fermented sausages and a significant number of these bacteria were capable of synthesizing a polysaccharide from sucrose. Also, Pediococcus cerevisiae was observed frequently in sausage samples. However, the ultimate aim of the above study was to employ a starter culture to affect greater control in the production of these meat items. Thus this aim led to the work of Deibel et al. (1961a) in which

a lyophilized starter culture of Pediococcus cerevisiae, capable of reconstitution and direct addition to the sausage emulsion, was considered to be the ideal inoculum. Nevertheless, some difficulty was encountered in the lyophilization of the cells due to the apparent loss of sodium chloride tolerance in the process. However, the loss of salt tolerance could be averted by maintaining the culture in a medium containing 6.5 per cent salt. This starter culture also performed satisfactorily under manufacturing conditions and has proved to be useful in the commercial production of a variety of fermented sausages.

Pohja (1960) listed a selection procedure for starter cultures suitable for the manufacture of dry sausages as follows: (1) ability to reduce nitrate, (2) ability to form acid from glucose in aerobic conditions, (3) ability to grow in media containing sodium chloride, (4) intensity of nitrate reduction. Out of 700 strains of micrococci tested, only three were suitable.

Niinivaara et al. (1964) reported that Micrococcus strain M 53 was suitable for the European type of fermented sausage (Rohwurst). Since 1957, this culture has been available as a lyophilized commercial product and is being produced and distributed by Rudolf Muller Company, Hamburg, Germany, under the name Baktofermente. Niinivaara et al. (1964) also pointed out that continuous selection of new strains from successful fermented meat products or curing

brines is necessary since strains degenerate. Degeneration also was noted by Pohja (1960). Some strains show a great sensitivity to phages while others are altered by the increase in sodium chloride content during curing.

Ostlund and Regner (1968) reported that Isterbrand, a non-heat treated, fermented, smoked sausage product was shown to develop a Lactobacillus-Leuconostoc flora during manufacture, resulting in the low final pH of the product. Slight variations in the raw materials did not affect the final flora. Inocula of Staphylococcus aureus grew and survived well in Isterbrand; Salmonella typhimurium and Bacillus cereus did not survive so well. Thus the developed flora had an antagonistic effect on certain species of Salmonella and Bacillus while Staphylococcus aureus was resistant.

Deibel et al. (1955) and Barbe and Henrickson (1967) studied the role of microorganisms in curing hams. These authors indicated that bacteria play no role in the physical, organoleptic or chemical changes that take place during the curing of hams by the rapid, modern methods that are now generally used commercially. The work of Deibel et al. (1955) was directed toward the development of methods so that certain bacteria might be employed to improve the stability of hams without adversely affecting organoleptic acceptability. Representative strains of lactobacilli and pediococci were isolated from fermented sausages. A number of these

organisms were inoculated into hams with the curing pickle. By controlling the concentration of the various curing ingredients and the time and temperature of curing and smoking, it was possible to control to some extent the growth of these "starter cultures" in the hams. However, it was difficult to accurately control the final pH of the hams, and the palatability was also quite variable. Most of the hams had a sour flavor, but this was not necessarily proportional to the pH. Aging generally resulted in greatly decreased palatability. However, some of the hams with a pH as low as 5.0 to 5.2 were considered to have an acceptable flavor.

McLean and Sulzbacher (1959) questioned whether or not the growth of a bacterium in a meat curing brine would affect flavor of the meat. Special washing procedures were used to obtain sterile hams and 50-gram chunks were removed aseptically from the biceps and placed in a jar of brine. A species of Pseudomonas, strain HB 28, was selected for use in these experiments in flavor production by screening 25 cultures isolated from ham curing brines. They concluded that this species of Pseudomonas added to the curing brine produced a distinctive flavor, and since no bacteria were found within the tissues in the inoculated meat, the change produced by bacteria resulted from the passage of bacterial metabolites from the brine into the meat.

Deibel and Niven (1958) studied the role of microorganisms in the development of characteristic ham flavor

in the modern rapid method of commercial curing. Lactobacilli were found to be the most common bacterial contaminants in the commercial ham curing brines that were examined. The most common Lactobacillus found was a motile, homofermentative, salt-tolerant, low acid producing variety that had characteristics in common with both Lactobacillus casei and Lactobacillus plantarum and yet possessed features which readily distinguished it from either of these two established species. It was concluded that the motile lactobacilli are of no significance in modern streamlined ham curing.

Deibel and Niven (1959) reported another common Lactobacillus, found in all of the brines tested, that was capable of synthesizing large amounts of a polysaccharide from sucrose. The population attained by the polysaccharide synthesizing lactobacilli, in the curing brines as well as in and on the hams as they are cured and smoked, appeared to be too small to be of any significance with regard to flavor and color development. However, it is entirely possible that these microorganisms play an important role in the processing of hams in which a longer cure and lower smoking temperature are employed.

Influence of microorganisms on beef and poultry flavor.-- Ingram (1966) reported on the importance of sodium glutamate in enhancing the flavor of meat and meat products even though it has little flavor itself. With this

in mind, many workers have been interested in the possibility of its synthesis by microorganisms. Recent work by Gardner and Stewart (1966) with stored comminuted beef indicated that the predominant meat flora (Pseudomonas-Achromobacter species) has been shown to possess glutaminase which has high activity at the pH and temperature of stored meats; and for the most part, the changes in glutamic acid and glutamine can be attributed to bacterial activity, with meat glutaminase playing little or no part. Although the amounts of monosodium glutamate normally added to foods are in excess of those shown to be produced in meat by bacteria, this synthesis may be a partial explanation why the process of "aging" enhances the flavor of meat. In this connection, it is interesting to note that Williams (1960) has described a process whereby meat can be tenderized and flavor enhanced by inoculating the surface of meat with Pseudomonas and/ or Achromobacter organisms. Both organisms produced different flavors when used separately, but in combination a "deeper, richer flavor" was obtained.

According to Shrimpton (1966), at least some of the gamy flavor of birds has its origin in the metabolism of the caecal flora. Direct evidence of the extent of this contribution can be obtained most readily from bacteria free birds. With this in mind, Harris et al. (1968) studied the influence of intestinal flora on chicken flavor. Chickens, including those reared under germfree, gnotobiotic (in contact only

with Clostridium perfringens, Escherichia coli, and Streptococcus faecalis) or conventional conditions were compared for flavor. Dark and light meat were evaluated separately. The results of these tests indicated a highly significant difference between the flavor of germfree and conventional chicken meat, a difference of lower significance between gnotobiotic and conventional chicken meat, and no significant difference between the flavor of gnotobiotic and germfree chicken meat. These results indicate that bacteria present in the intestinal tract do affect flavor of the chicken meat. However, it is possible that other organisms in the intestinal tract contribute more to flavor than the ones tested or it also is possible a synergistic effect may occur when the total intestinal flora are present. At any rate, the three organisms tested did not produce the same flavor as the total flora of the intestinal tract of the intact birds.

Effect of bacterial action on meat constituents.--

Evans and Niven (1960) pointed out that most of the bacterial changes produced in meat are the result of their growth and metabolism. Lawrie (1966) states that in satisfying their requirements for nourishment and survival, invading organisms alter meat in a variety of ways. The nature, range and sequence of the changes in meat caused by the biochemical activities of a single species of invading organisms

can be exemplified by the behavior of Clostridium welchii, an anaerobe. First, the meat liquifies because the organism excretes a collagenase which hydrolyses the connective tissue between the muscle fiber bundles, causing them to disintegrate. This hydrolysis is followed by gas production. The free amino acids present are attacked by deaminases with the production of hydrogen, carbon dioxide and ammonia; and glycogen, if present, is fermented to give acetic and butyric acids. These activities cause foul smells and unpleasant tastes.

Haines (1937) reported that the putrid odors are produced mainly by anaerobes through the decomposition of proteins and amino acids yielding indole, methylamine and hydrogen sulfide and sour odors through the decomposition of sugars and other small molecules.

Jensen (1954) states that discoloration may be due to alteration or destruction of meat pigments. Myoglobin may be oxidized to brown metmyoglobin: it may combine with hydrogen sulfide, produced by bacteria, to form sulphmyoglobin, or be broken down to form yellow or green bile pigments by microbially produced hydrogen peroxide.

Evans and Niven (1960) indicated bacteria carry out two types of enzymatic attack on fats--hydrolysis by a lipase, and oxidation of the fatty acids by oxidases. Some of the products of these reactions give rise to the odors and flavors that we generally recognize as rancidity. However,

most rancidity problems are not of microbial origin.

Lawrie (1966) suggested the use of objective chemical tests to determine the degree of microbial spoilage in meat. Consequently, Fields and Richmond (1968) have suggested the following compounds as chemical indicators of low quality in high-protein foods: ammonia, indole, trimethylamine, volatile bases, volatile fatty acids, acetylmethylcarbinol, diacetyl, lactic and succinic acids, hydrogen sulfide, and histamine.

Niinivaara et al. (1964) showed the chemical composition of dry sausage is changed during the ripening process because of the evaporation of water. Organic acids, among which lactic acid may be the most important, are formed from the glycogen of the meat and from the sugars added during preparation of fermented meat products. In addition, solubility of proteins decreased during fermentation. The tissue enzymes, instead of bacteria, appeared to be responsible for the proteolytic changes of the proteins during the first phase of ripening. Also the significance of fats and carbonyls in the flavor of dry sausage was indicated. Preliminary gas chromatographic studies on the carbonyl fraction extracted from dry sausage showed how the amounts of carbonyls changed during processing.

Meat Curing

On a historical basis, meat curing may be defined as

the addition of salt (sodium chloride) to meat for the purpose of preservation. The origin of curing meats by salting is unknown and may have been initiated quite by chance. Nevertheless, as the art progressed, additional substances were added to meat for curing purposes. As a result, the term "meat curing" eventually came to be understood as the addition of salt, nitrate, nitrite, sugar, or in some instances, other ingredients for the purpose of preserving and flavoring meat (Wilson, 1960, and Lawrie, 1966).

Greenberg et al. (1943) reported that the attractive red color of cured meats is nitrosomyoglobin; the mechanism of its formation is controversial. Normally, nitrite is reduced through a series of reductions to nitric oxide, which enters directly into the curing or "color fixation" reaction with myoglobin. Eddy et al. (1960) indicated that brine microorganisms can reduce nitrite to nitric oxide and as a result aid curing. Walters and Taylor (1963, 1964) and Taylor and Walters (1967) reported that the reduction of both metmyoglobin and myoglobin and of nitrite to nitric oxide was probably brought about by surviving activity of enzyme systems of the muscle itself. Fox and Thomson (1963) showed that nitric oxide can react directly with metmyoglobin and that the complex can then be reduced to nitrosomyoglobin. Below pH 5.4, the rate of formation of nitrosomyoglobin is much faster than the rate of reduction of metmyoglobin. Therefore, the latter cannot be an essential

step in the process.

Greenwood et al. (1940) reported that dextrose, levulose, mannose, galactose, xylose, sucrose, maltose, and lactose produce significant changes in color and other characteristics of the blood pigments in the presence of microorganisms. The color changes of the pigments could be correlated with the growth of microorganisms which utilize the sugars, yielding acid products, and which also aid in establishing reducing conditions. However, Mills et al. (1960) showed that sugars have no effect upon color.

Lawrie (1966) indicated reduction of nitrite to nitric oxide can be affected by either muscle enzymes or bacteria; the reduction of nitrate can be affected only by the latter. Hence there is a need for careful control of curing brines, when nitrate is employed, to insure the necessary microbial reduction of nitrate. Moreover, Leistner (1960) pointed out that various contaminating organisms in the brine can exert a deleterious effect on the cured meat, i.e., souring, putrefaction and excessive sweetening caused respectively, by species of Lactobacillus, Vibrio and Bacillus. In addition, Leistner (1960) related a rather thorough study of bacterial families and genera found in ham curing brines.

Castellani and Niven (1955) studied the bacteriostatic action of sodium nitrite and reported that tolerance toward nitrite varied widely among different groups of bacteria. Nitrite appeared to influence the availability of pyruvate

and sulfhydryl substances in the nutrition of some microorganisms.

Callow (1947) stated only a limited range of microorganisms can grow on cured products due to the selective action of salt. Bulman and Ayres (1952) showed varying degrees of bacteriostasis by levels of 3.5 to 4.4 per cent sodium chloride, while no spoilage was observed at concentrations exceeding 4.4 per cent.

Dunker et al. (1953) reported that the majority of bacteria in cured hams were nonpathogenic Micrococcaceae. In this regard, Lechowich et al. (1956) showed that staphylococci, including the food poisoning variety, are not inhibited by any palatable salt concentration. If pH of the meat is lowered to 4.8 to 5.0, anaerobic growth of staphylococci was prevented even in the absence of nitrite. In a broth medium, anaerobic growth of staphylococci was inhibited at pH 5.6. Aerobic growth in broth was diminished at pH 5.6 and prevented at pH 4.8.

According to Johnson and Bull (1952), the rate of cure diffusion can be increased by hot curing of bacon and pig feet. Mullins et al. (1958) obtained slightly higher panel scores on uniformity of color in ham injected while hot than ham injected with the same solution after being chilled. Cure penetration was also better but not enough to be significant. Also, in this regard, Arganosa and Hendrickson (1969) found that the nitrosopigment content of muscle

cured prior to chilling was significantly ($P < .01$) greater than muscles cured after chilling. Cure diffusion distance, moisture, sodium chloride and pH values were significantly increased ($P < .01$) by the pre-chill treatment.

Some Palatability Characteristics of Meat

The ultimate consumer acceptance of any meat product is dependent upon its palatability characteristics such as flavor and tenderness.

Flavor.-- Flavor is a complex sensation. It involves odor, taste, texture, temperature and pH. Of these, odor is the most important. Without it, one or the other of the four primary taste sensations--bitter, sweet, sour or saline--predominates (Lawrie, 1966). Odor and taste are most difficult to define objectively; nevertheless, their evaluation still depends on the taste panel (Blumer, 1954; Caul, 1957; Meyer, 1960).

The odor and taste of cooked meat arise from water or fat soluble precursors and by the liberation of volatile substances pre-existent in the meat. Crocker (1948) concluded that meat fibers on heating produced typical meat flavor and that expressed meat juices produced a nontypical low intensity flavor.

Batzer et al. (1960, 1962) attempted to isolate from raw beef the precursors which eventually produce the distinctive odor and taste of the cooked commodity. A

dialysate of the water-soluble extract of uncooked beef contained inosinic acid and a glycoprotein, the carbohydrate portion of which is glucose, and the following amino acids: serine, glutamic, glycine, alanine, isoleucine, leucine, B-alanine and proline. It was concluded that only some of the amino acids are essential precursors of meat flavor. Bender et al. (1958) analyzed commercial beef muscle extract and fresh beef muscle for nonprotein constituents. The major differences between the two preparations were in the free amino acids and reducing sugar concentrations. Amino acids totaled 11 per cent of the fresh extract and only 2.4 per cent of the commercial material; the reducing sugars totaled 2 per cent of the fresh extract but were absent in the commercial extract. Presumably, the loss of amino acids and reducing sugars was due to a browning reaction. Wood (1961) concluded that the development of the brown color and meaty flavor characteristic of these extracts is a result of the Maillard reaction.

Howe and Barbella (1937) considered both lean and fat important and related the time and temperature of heating to the quality of the flavor. However, Hornstein and Crowe (1960, 1963) stated that species' differences in taste and odor are determined by volatile fat soluble carbonyls which are characteristic and present in the meat before cooking. Later, Hornstein (1965) suggested that a basic meaty flavor is common to the lean portion of all meats regardless of

species, since the composition of free amino acids and reducing sugars are similar in pork, beef, and lamb--since organoleptic qualities obtained from water extracts of these meats are similar.

McCain et al. (1968) studied the changes in free amino acids of dry-cured hams during aging to determine whether these changes were associated with organoleptic characteristics of the ham. Significant ($P < .05$) increases were observed for serine, glutamic acid, threonine, leucine, valine, phenylalanine, proline, tyrosine, alanine, glycine, and histidine during successive aging periods. It was concluded that amino acids are important as precursors of meat flavor.

Tenderness.-- Of all the palatability factors, tenderness is rated most important by the average consumer. The overall impression of tenderness includes texture and consists of at least three components. The first is the ease with which the teeth sink into the meat when chewing begins, the second is the ease with which the meat breaks into fragments, and the third is the amount of residue remaining after chewing (Weir, 1960).

There have been many attempts to devise objective physical and chemical methods of measuring tenderness which would compare with subjective assessments by taste panels (Lawrie, 1966). Warner (1928) briefly reported to the American Society of Animal Production on a shearing device

that was being developed and showed promise for measuring tenderness. In 1932, the shear device was modified and improved by Bratzler (1932) and has since been known as the Warner-Bratzler shear. Kramer (1957) also developed a physical method for measuring tenderness--the shear press. Chemical methods have involved determination of connective tissue (Lowry et al., 1941; Neuman and Logan, 1950).

Weir (1960) indicated that species is the most general factor affecting tenderness. Most emphasis on tenderness is given to beef. Although tenderness is important for pork, lamb and veal, the variations between animals and between cuts is not as great. Also, pork, lamb and veal are usually younger and therefore more tender than beef. Nevertheless, Ginger and Weir (1958) pointed out that tenderness measured by shear values and panel scores within a given muscle may vary significantly.

The relationship of aging to tenderness is illustrated by Lawrie (1966) and Wilson (1960). Aging--the holding of unprocessed meat above the freezing point in the absence of microbial spoilage to increase tenderness and flavor--is largely the result of proteolytic activities of meat enzymes. Sharp (1963) has shown these enzymes to operate much more readily at 37°C than at 5°C and that, in general, higher temperatures produce a given degree of tenderizing in a considerably shorter time than do lower temperatures. In addition, Radouco-Thomas et al. (1959) and Sharp (1963)

have shown that at higher pH's the degree of proteolysis is less. In rabbit longissimus dorsi, after storage for 16 days at 37°C, 17 per cent of the total tyrosine was soluble when the ultimate pH was 5.8. However, the corresponding value was only about 9 per cent when the ultimate pH was 6.8.

Commercial application of aging is the Tenderay process used by the Kroger Company, in which beef is aged at 15°C for 3 days using ultraviolet lights to control surface spoilage. Research application lies in the area of controlled microbiological and environmental techniques.

Germfree and Gnotobiotic Meat

Undenatured muscle tissue, with a low bacterial count or germfree, furnishes the meat scientist with a new research tool that allows separation of deterioration of stored tissue into that caused by bacteria and that resulting from other reactions (Bothast et al., 1968). In this connection, Ockerman and Cahill (1967) described two procedures used to obtain germfree meat samples. The first procedure is based on the fact that the fetus is normally sterile but is usually contaminated during birth. Thus, by sterile cesarian surgery of mice, rabbits, etc., followed by introduction into a germfree isolator--germfree meat samples may be obtained. Similarly, Harris et al. (1968) have produced germfree chickens by sterilizing the external surface of eggs and then introducing the eggs into a

germfree isolator and allowing them to hatch. The second procedure is based on the premise that before an animal is slaughtered, its healthy muscle tissue is sterile if the lymph system is avoided. Therefore, special sanitary and surgical techniques are used to obtain the muscle which is then transferred aseptically to a sterile isolator.

Ockerman et al. (1964) obtained sterile muscle tissue working with gnotobiotic mice. The animals were slaughtered, eviscerated, cleaned, and packaged under several gaseous environments (air, nitrogen, oxygen, and vacuum) without bacterial contamination. Physical appearance and odor were maintained for several months in muscle tissue stored at 23°C under vacuum and nitrogen. Greater deterioration was observed when the storage temperature was increased and when larger quantities of oxygen were added to the atmosphere.

Davis (1965) and Ockerman et al. (1969) used a combination of an aseptic slaughter technique and a sterile surgical isolator to remove longissimus dorsi muscle tissue from beef animals without contamination. The excised tissue was aseptically transferred into a sterile, plastic isolator, where the sample was ground and placed into sterile jars. These authors then studied transformations occurring during storage of aseptic and contaminated bovine muscle tissue at refrigeration temperatures. Davis (1965) found that the emulsifying capacity of the tissue decreased during the first five to ten days, with a subsequent increase after

the initial period. This increase was more pronounced in the case of the contaminated samples. Ockerman et al. (1969) showed that the emulsifying power increased from the tenth day through the remainder of storage, in the case of contaminated samples. Also, protein fractionation revealed no significant treatment differences in sarcoplasmic or myofibrillar fractions. Borton et al. (1968) used a similar procedure to obtain porcine muscle samples that were relatively free of microbial contamination. After rinsing the chilled carcass with alcohol, the longissimus dorsi muscle was excised with the aid of sterile knives. The tissue was ground through a sterilized, pre-chilled grinder into sterilized jars. Thus muscle samples relatively free of bacteria were obtained. Then a paired sample was inoculated with a bacterial culture (organism not identified) to compare with a control. The extent of bacterial growth reduced the extract release volume significantly and effectively lowered the emulsifying capacity of porcine muscle. The inoculated sample had a lower emulsifying capacity than the control throughout the 17 day storage period.

Bothast et al. (1968) and Graner (1969) have studied another procedure for the preparation of animal tissue samples with a low level of contamination. Essentially, this technique consists of submerging the samples (rabbit carcasses) in hot water for 90 seconds and then introducing them, aseptically, into a sterile, plastic isolator for

sectioning, and placing into sterile jars. The first author stored dip treated rabbit muscle at 3°C for 35 days and found that the surface psychrophilic bacterial load was reduced and that only slight visual and odor deteriorations occurred. Graner (1969) also showed that the dip procedure was effective in removing surface contaminants and that samples with a higher degree of microbial contamination have a greater emulsifying capacity.

The influence of microorganisms on meat products is considerable. Therefore, if the meat scientist is going to control and use bacteria as food enhancers, the effects of pure and mixed cultures must be studied. Development of isolator techniques should provide the researcher with the ideal tool to study these microorganisms and their effects. Also, these techniques may alter meat processing as we know it today. They may impart a real advantage in increased shelf life and improved quality of meat products.

OBJECTIVES

The objectives of this study were:

1. To confirm that the isolator dip technique will reduce the surface bacterial load.
2. To further develop a technique for studying the influence of pure or mixed cultures on meat products.
3. To study the feasibility of controlled environmental processing.
4. To compare and cure pork loins under three different environments or treatments; Reduced Initial Count, Gnotobiotic, and Conventional, and to take advantage of beneficial bacteria in the Gnotobiotic system.
5. To determine the viable bacterial load of each curing solution at the end of the curing process and to identify the predominant microorganism(s) in each treatment.
6. To determine the treatment effects on the product via sensory and objective evaluations.

EXPERIMENTAL PROCEDURE

Porcine longissimus dorsi was selected as the muscle tissue for this experiment. Two one-year-old sows were slaughtered in the Meat Laboratory, V.P.I., according to the procedure outlined by Cole (1951). A third sow--similar size and age--was slaughtered commercially at Valleydale Packers, Salem, Virginia. The loins from the first sow carcass were removed according to the methods outlined at the Fourth Reciprocal Meat Conference (Cole, 1951), and the longissimus dorsi muscles were cut to yield three 20-centimeter sections for Trial I. Each section was then randomly assigned to one of the three treatments--Gnotobiotic (N), Reduced Initial Count (RIC), and Conventional (C). Trials II and III were conducted similarly, using loins from carcass #2 in Trial II and loins from carcass #3 in Trial III. Trials II and III followed Trial I by three and six weeks, respectively.

The Gnotobiotic treatment was designed to serve as an indicator of the success of the dip technique being developed, i.e., does the technique allow an inoculated organism to predominate and its influence on a meat product to be studied? In this particular research, Pediococcus cerevisiae was inoculated into the curing solution to take

advantage of any beneficial properties it might impart to the product. The RIC treatment was designed to produce low bacterial loads on meat so that a direct comparison could be made between this treatment and the Gnotobiotic treatment. The Conventional treatment functioned as the control. This treatment was "conventional" in the sense that loins were cured by a process (temperature, time, technique, etc.) similar to the one used by meat processors today. However, the salt concentration used in this treatment was considerably lower than that used by industry to avoid differences due to salt concentrations.

The sections assigned to the RIC treatment were processed in an apparatus similar to the one described by Bothast et al. (1968) and is shown in Figure 1. The samples were submerged for 90 seconds in 90°C water contained in a stainless steel "V" tube. The "V" tube was sprayed with 2 per cent peracetic acid 3 hours prior to heating with an electric element. Sterile water was added 2 hours prior to heating. An air chamber was isolated between the door cap of the processing isolator and the water level in the metal tube. This chamber was sprayed with 2 per cent peracetic acid 2 hours before the door cap was removed and the sample was introduced. Fastened opposite to the open-end of the "V" tube was a germfree plastic isolator which contained equipment and supplies necessary for curing. The equipment and the curing solution in a gallon container

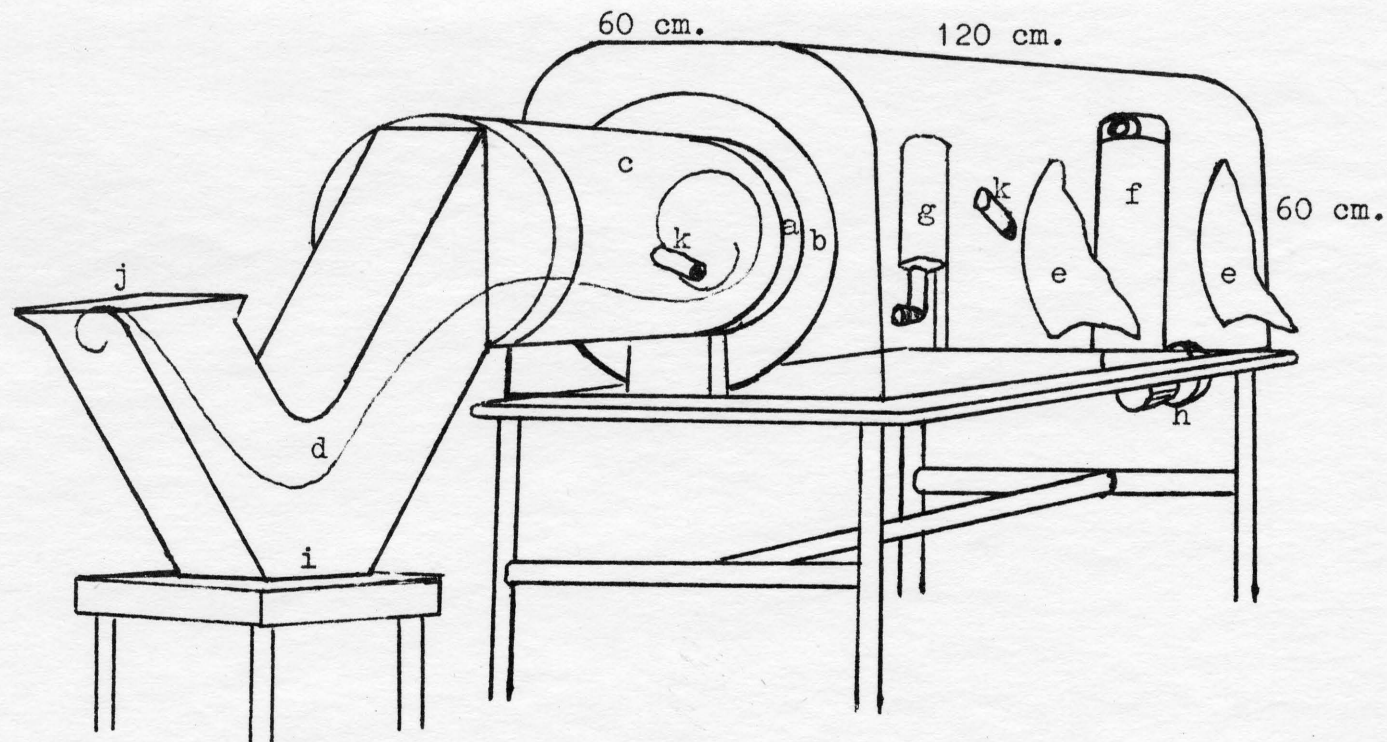


Figure 1. Processing isolator with attached stainless steel "V" tube. This isolator was a transparent, flexible-wall, plastic film isolator, manufactured by Labco, Inc., and sterilized with 2 per cent peracetic acid 48 hours prior to use.

a - door chamber, b - inside door cap, c - transfer sleeve, d - copper wire, e - rubber gloves, f - air inlet filter, g - air outlet filter, h - air blower, i - sterilized water, j - aluminum foil cap, and k - nipple.

were steam sterilized after being wrapped with two layers of Kraft paper and aseptically air-locked into the isolator via a transfer sleeve. A copper wire running from inside the isolator to the outside allowed passage of the samples from the normal environment into the hot water dip; and then, after 90 seconds, into the isolator. After the samples were placed in the isolator, the flexible plastic door cap was put in place to seal the isolator from the "V" tube and its hot water trap.

Once the loin sections were in the isolator, they were put into a large beaker and 2.2 liters of curing solution were added. The curing solution contained 4 per cent sodium chloride, 5 per cent dextrose, and 0.3 per cent of a 12/1 (dry weight) nitrate to nitrite mixture per 2.2 liters. The meat was allowed to cure in the isolator for 7 days at 28°C. Germfree air circulated in the isolator at all times over the solution covered sample.

The Gnotobiotic treatment was essentially the same as the RIC treatment except that 1.12 grams of ACCEL (Pedio-coccus cerevisiae starter culture, produced by Merck and Company, Inc., at the recommended level) were included per 2.2 liters of curing solution. The lypholyzed culture was reconstituted in 10 milliliter of sterile trypticase soy broth (Baltimore Biological Laboratory). The test tube containing the reconstituted culture was next placed in the door chamber of the plastic isolator (after introduction of

the loin) and then the surface of the tube was sterilized with 2 per cent peracetic acid. The air chamber between the door cap of the processing isolator and the water level in the "V" tube were also sterilized at this same time. Four hours later, the culture was aseptically introduced into the isolator and mixed with the curing solution and sample. Curing proceeded under the same conditions as the RIC treatment.

The Conventional treatment consisted of a 7-day cure at 4°C without any special sanitary or inoculative treatment. The loin sections assigned to this treatment were submerged into a large beaker containing 2.2 liters of brine. This solution consisted of the same ingredients as the other two treatments but was mixed by hand and not autoclaved.

When the curing was completed for the three treatments, the curing containers and their contents were removed from their respective environments and evaluated for total bacteria; pH; oxidation of fat; taste differences; tenderness; protein, fat, and moisture content; and color.

Uniform 10-milliliter curing solution samples were obtained aseptically from each treatment. A standard plate count was made, according to the procedure recommended by the American Public Health Association (1966), on 1 milliliter aliquot of these 10-milliliter samples, using tryptone glucose extract (TGE) agar (Difco) incubated at 30°C for 48 hours. The viable bacterial load was determined. Three

representative colonies for each treatment were picked from TGE plates that were countable (between 30 and 300 colonies) and inoculated on brain heart infusion agar (Difco) and APT broth (Difco). Isolation procedures according to Breed et al. (1957) were then used to identify the respective organisms.

Additional uniform 25-milliliter curing solution samples were obtained from each treatment and pH was determined using a Corning Model 12 Research pH meter.

The loin sections were removed from their respective solutions and approximately 200-gram samples were obtained from the end of each section. These samples were wrapped in aluminum foil and refrigerated over night. The next day 10-gram subcutaneous fat samples were obtained from the 200-gram samples and oxidation was measured by TBA (2-thio-barbituric acid) values, according to the procedure of Tarladgis et al. (1960). The remainder of the 200-gram samples were rewrapped and frozen for a later determination of fat, protein and moisture.

The remaining loin sections (those left after removal of a 200-gram sample) were immediately cooked in a 177°C oven to an internal temperature of 73°C. Sections from each treatment were sliced (3.1 millimeter thickness) and 25-millimeter diameter discs were made. These discs were then administered in a triangle taste test (Amerine et al., 1965) to a panel of 5 members. The taste panel members were

trained by several practice sessions prior to the actual test. The meat was warm when served. The panel was offered all possible triangle comparisons--with the three treatments --in duplicate at each testing session. In addition, 2.5-centimeter diameter cores were obtained from the cooked sections and evaluated for tenderness by the Warner-Bratzler shear. Also, sections were subjectively evaluated for quality of color prior to cooking.

When all three trials were completed, the frozen samples of each trial and treatment were thawed at room temperature and evaluated for moisture, fat and protein according to the procedure described by the Association of Official Agricultural Chemists (A.O.A.C., 1965).

Statistical analysis of all data was conducted in accordance with Li (1964) and Kramer (1969).

RESULTS AND DISCUSSION

Preliminary studies confirmed that the technique of Bothast et al. (1968) and Graner (1969) yielded animal tissue with a low level of contamination. No measurable number of bacteria was found in the curing solution of RIC treated samples if a 4°C temperature was maintained. Thus the procedure was effective in reducing the surface psychrophilic bacterial load. However, mesophilic and thermophilic bacteria were not completely destroyed by the process. In addition, Conventionally handled samples cured at room temperature were shown to spoil during a 7-day cure.

Preliminary studies with various levels of salt in the curing solution showed 4 per cent sodium chloride to be the maximum concentration for good growth of the ACCEL starter culture. Higher levels of sodium chloride strongly inhibited the growth of the microorganism. Ten preliminary tasters indicated that the level of salt (65° salimeter) recommended by Ziegler (1966) yielded an unpalatable product in a 7-day cure. Pediococcus cerevisiae, the microorganism in the ACCEL starter culture, readily ferments dextrose to lactic acid and since this is considered one of the major processes involved in the beneficial use of bacteria, 5 per cent dextrose was used in the curing solution. Therefore,

Ziegler's recipe was modified to the one presented in the materials and methods, and to avoid added variation, the same curing solution was used in all three treatments.

Viable bacteria.-- Table 1 shows the results of standard plate counts on the curing solution. These data agree with counts determined by the Petroff-Hausser counting chamber. The curing solution of the Conventional treatment was significantly ($P < .01$) lower in bacteria than solutions of the other two treatments. This lower level of the Conventional treatment can be attributed to the 4°C curing temperature. At 4°C the metabolic processes of most microorganisms are restricted and in seven days very little microbial growth will occur.

No significant difference existed in the bacterial level of the RIC and Gnotobiotic solutions. It was surprising that they were so similar since the Gnotobiotic solution was inoculated and the RIC solution was not. Possibly the microorganisms of the Gnotobiotic solution reached and were well into the stationary phase of growth. The levels indicated (10^7 organisms/milliliter) are the maximum which can be supported per milliliter of curing brine. Similarly, very few microorganisms survived the hot water dip of the RIC treatment. These organisms might have been at a maximum level but somewhat earlier in the stationary phase. Nevertheless, the physical (28°C , oxygen level, etc.) and chemical (sodium chloride, dextrose, etc.) environment of the RIC and

TABLE 1
STANDARD PLATE COUNT PER MILLILITER
OF CURING SOLUTION^a

Trial	Treatment		
	Reduced Initial Count	Gnotobiotic	Conventional
I	7.68	7.36	3.20
II	7.72	7.48	4.08
III	8.08	6.64	3.56
	—	—	—
\bar{X}	7.83	7.16	3.61**

^aCounts are expressed in logarithms.

**Means significantly different ($P < .01$).

Gnotobiotic treatments appeared to be excellent for certain microbial growth and development.

Visual evaluation of the curing solutions also reflected the results of the total counts. The RIC and Gnotobiotic solutions were extremely turbid while the Conventional was clear. A slight difference in color also occurred between the RIC and Gnotobiotic solutions. The former showed a tinge of pink while the latter exhibited a pale yellow color.

Isolation and identification of the predominant bacteria.-- Table 2 shows the predominant microorganism found in the curing solutions for each treatment and trial. Also the morphological and physiological properties, upon which identification is based, are presented for each organism. Representative colonies from all three trials for the RIC treatment yielded Lactobacillus spp. The colonies were small, white, and pin head size. They exhibited poor surface growth and were embedded within the agar (micro-aerophilic). The organisms were Gram-positive rods with rounded ends and occurred singly, but primarily in chains. They were catalase negative, non-motile, produced no pigments and did not liquify gelatin. Acid was produced in dextrose, lactose, mannitol and litmus milk. Nitrates were not reduced. No differentiation of the species in the genus Lactobacillus was made due to the inadequacy of comparative information.

Many investigators (Niven, 1951, 1952; Jensen, 1954; Deibel and Niven, 1958, 1959; Frazier, 1958) have reported

TABLE 2

ISOLATION AND IDENTIFICATION OF THE PREDOMINANT BACTERIA FOUND IN THE CURING SOLUTION

Morphological and Physiological Properties

Treatment	Trials		Growth at 30° on TGE	Growth at 37°	APT broth	Brain Heart Infusion	Gram stain	Morphology	Pigment	Motility	Catalase	Growth on TPEY	Dextrose (acid)	Lactose (acid)	Mannitol (acid)	Gelatin hydrolysis	Litmus milk (acid)	Nitrate reduction	Indole	Urease	Coagulase
Reduced Initial Count	(I,II,III)	<u>Lactobacillus spp.</u>	E	+	+	⊕	+	Rc	-	-	-	+	+	+	-	+	-				
Gnotobiotic	(I), (II,III)	<u>Pediococcus cerevisiae</u>	E	+	+	⊕	+	C	-	-	-	+	+	-	-	-	-				
Conventional	(I,II)	<u>Staphylococcus epidermidis</u>	S	+	+	+	+	Cc	-	-	+	+	+		-		+				-
	(III)	<u>Flavobacterium diffusum</u>	S, Sp	-	+	+	-	R	V	+	+	-	(+)	-	-	-	-	+	-	-	-

E = embedded colonies
S = surface colonies
Sp = spreading growth

(+) = restricted
R = rods
Rc = rods in chains

C = cocci in pairs, tetrads and single
Cc = cocci in clusters
V = variable

that lactobacilli are the most common bacterial contaminants in fermented foods and commercial curing solutions. The importance of these organisms has been stressed in the development of a pleasant "tangy" flavor, establishment of reducing conditions for proper color formation, and in green discoloration. Leistner (1960) studied the microbiology of ham curing and found Lactobacillus in 70 per cent of the pumping brines and 95 per cent of the cover brines examined. Therefore, it was not surprising that Lactobacillus spp. predominated in the RIC brine. Certain species of this genus are heat tolerant; thus, this property may account for their survival of the hot water dip and subsequent perpetuation in the curing solution.

Pediococcus cerevisiae was the predominant organism isolated from representative colonies of Gnotobiotic solutions for all trials. This was the organism added to the curing solution in the form of the ACCEL starter culture. Therefore, it was possible to inoculate with a given microorganism and have it predominate. The colonies were small, white, and pin head size, just as the Lactobacillus spp. They showed poor surface growth and were embedded within the agar (microaerophilic). The organisms were Gram-positive cocci and occurred singly, in pairs, and in tetrads. They were catalase negative and non-motile. Acid was produced in dextrose and lactose but not in mannitol and litmus milk. Nitrates were not reduced. This organism has also been

studied by many investigators (Jensen, 1954; Wilson et al., 1955-1956; Mills and Wilson, 1958; Deibel et al., 1961) in connection with production of fermented meats and curing brines. Leistner (1960) found that 10 per cent of pumping brines and 15 per cent of ham cover brines contained Pediococcus.

In addition to Pediococcus cerevisiae being found in Gnotobiotic brine, a species of Lactobacillus was also isolated from the Gnotobiotic brine in Trial I, and only in Trial I. This organism appeared to be the same as the one found in the RIC brine for all three trials. Therefore, we can conclude that Pediococcus cerevisiae predominated in the Gnotobiotic treatment but a species of Lactobacillus was also present. The presence of Lactobacillus spp. in the Gnotobiotic brine seemed likely, since the RIC and Gnotobiotic treatments are so similar.

Isolates from the representative colonies of the Conventional treatment for Trials I and II yielded Staphylococcus epidermidis while Flavobacterium diffusum was isolated from the representative colonies of Trial III. Staphylococcus epidermidis colonies were circular and white; they grew well on the surface and the cells were spherical. They occurred in clusters, singly, and in pairs. They were Gram-positive, non-motile, and catalase positive. Acid was produced from dextrose and litmus milk but not mannitol. The organism was coagulase negative and reduced tellurite

on a TPEY (Baltimore Biological Laboratory) selective media. Leistner (1960) found Staphylococcus in 5 per cent of ham pumping brines and 10 per cent of ham cover brines. Graham (1970) found coagulase negative Staphylococcus aureus prevalent in dry-cured hams. Breed et al. (1957) reported that Staphylococcus epidermidis are very salt tolerant, growing vigorously in media containing 10 per cent sodium chloride and are aerobic to facultatively anaerobic. They are normally found on the skin and mucous membranes of man and other animals and in a variety of food products. Therefore, since the ingredients were mixed by hand, it was not unusual to find Staphylococcus epidermidis in the Conventional solution.

Flavobacterium diffusum produced various colored colonies: yellow, white, red and brown. The colonies spread over the surface of TGE agar plates. The cells were Gram-negative rods, motile, and catalase positive. Slight acid was produced from dextrose but non from lactose, mannitol, or litmus milk. Nitrates were reduced but indole was not produced. Urease was not present and gelatin was not hydrolyzed. The organism grew well at 30°C but not at all at 37°C. Leistner (1960) found Flavobacterium in 10 per cent of ham pumping brines and in 15 per cent of ham curing brines. Breed et al. (1957) stated that this organism was aerobic to facultatively anaerobic and normally found in soil, fresh and sea waters. Thus, again it was not surprising to find this organism in the Conventional solution. However, it was unusual that

Flavobacterium diffusum did not show up in the first two trials. Slaughtering was conducted at a different location in Trial III and this may account for the different predominating flora.

Final pH of the curing solutions.-- Acidity of the curing brine was affected by treatment as shown in Table 3. The pH of the Conventional solution was significantly higher ($P < .01$) than the pH of either RIC or Gnotobiotic solutions. However, no difference in pH existed between the RIC or Gnotobiotic solutions. These results are generally correlated with the total counts given in Table 1, i.e., as the number of organisms increase so does acid production; consequently, the pH was lower for both the RIC and Gnotobiotic treatments. Also the predominate organisms (Lactobacillus and Pediococcus) in these treatments are extremely active acid formers. From previous work (Niven, 1952), it is assumed that the major acid formed was lactic but no determination of acid products was made. Nevertheless, the organisms of the RIC and Gnotobiotic treatments were at a similar stage of acid production which confirmed a similar phase of growth as pointed out in the discussion on total counts. The pH range (4.6 to 4.9) attained in the RIC and Gnotobiotic solutions agreed with the values of 4.5 to 4.8 reported by Everson et al. (1969) and Mills and Wilson (1958). The pH range of the Conventional solutions (5.6 to 5.8) appeared to be similar to the ultimate pH (5.5 reported

TABLE 3
FINAL pH OF THE CURING SOLUTIONS

Trial	Treatment		
	Reduced Initial Count	Gnotobiotic	Conventional
I	4.6	4.6	5.8
II	4.9	4.7	5.6
III	4.9	4.9	5.7
	—	—	—
\bar{X}	4.8	4.7	5.7**

** Means significantly different ($P < .01$).

by Lawrie, 1966) attained in porcine muscle. Bacteria did not appear to influence, measurably, the pH of the Conventional solution due to both the level and type of organism present.

Oxidation of Fat.--- Oxidation of fat was not significantly affected by any of the experimental treatments as measured by 2-thiobarbituric acid (TBA) values shown in Table 4. Optical density as a measure of oxidation ranged from .010 to .028 for the Conventional treatment, .012 to .045 and .022 to .101 for the RIC and Gnotobiotic treatments, respectively. The peracetic acid (used to sterilize the isolator), the 28°C curing temperature, and the hot water dip did not appear to increase oxidation. This is contrary to earlier work reported by Bothast et al. (1968) where the peracetic acid and hot water dip increased oxidation. Perhaps the lower fat oxidation observed in this research was the result of the loin sections being submerged throughout the curing process and by the short duration (7 days) of the process. Bothast et al. (1968) reported values from .280 to .780 in rabbit fat for the isolator and dip technique. It appears that the microorganisms involved in this study were not increasing oxidation--at least not in seven days; but on the contrary may be establishing reducing conditions (Leistner, 1960) and thereby inhibiting oxidation.

Composition of longissimus dorsi.--- Treatments did not significantly affect the content of protein, fat and

TABLE 4
TBA VALUES OF FAT^a

Trial	Treatment		
	Reduced Initial Count	Gnotobiotic	Conventional
I	.045	.031	.028
II	.012	.022	.014
III	.022	.101	.010
\bar{X}	.026	.051	.017

^aValues are expressed as optical density.

moisture in porcine longissimus dorsi as shown in Table 5. Moisture was slightly lower; consequently, fat was slightly higher in the RIC and Gnotobiotic treatments as compared to the Conventional. Perhaps the warmer curing temperature and fermentation of the first two treatments accounted for this slightly drier product. Protein remained fairly constant throughout the treatments; thus, no proteolytic changes were indicated. These results are contrary to those of McCain et al. (1968) who reported enzymatic degradation of protein during aging of dry-cured hams. Perhaps the short duration of the curing process and the technique used to determine protein content account for the results shown here. Amino acid analysis, fatty acid analysis, etc. would be much more useful in determining subtle bacterial effects. Lawrie (1966) stated that in a broad sense the composition of meat can be approximated to be 75 per cent water, 18 per cent protein, 3 per cent fat, and 3.5 per cent soluble non-protein substances. The values reported here of 17 to 21 per cent protein, 2 to 15 per cent fat and 60 to 74 per cent moisture are near the reported values considering the nature and variability of meat.

Tenderness.-- Table 6 shows the individual Warner-Bratzler shear values for each treatment and trial. In Trial I, cores differed significantly ($P < .05$) within treatments. This difference can be accounted for by variation within a given muscle as reported by Ginger and Weir

TABLE 5
COMPOSITION OF LONGISSIMUS DORSI^a

Trial	Treatment		
	Reduced Initial Count	Gnotobiotic	Conventional
<u>PROTEIN CONTENT</u>			
I	20.83	21.36	17.44
II	18.78	19.92	20.46
III	17.50	21.61	21.30
	—	—	—
\bar{X}	19.04	20.96	19.73
<u>FAT CONTENT</u>			
I	3.85	4.04	2.69
II	13.29	6.91	5.10
III	15.14	7.66	4.21
	—	—	—
\bar{X}	10.76	6.20	4.00
<u>MOISTURE CONTENT</u>			
I	73.14	74.10	73.73
II	70.44	71.45	71.74
III	60.82	69.56	72.59
	—	—	—
\bar{X}	68.13	71.70	72.68

^aValues expressed as per cent.

TABLE 6

WARNER-BRATZLER SHEAR VALUES^a

	Reduced Initial Count		Gnotobiotic		Conventional	
	Core 1	Core 2	Core 1	Core 2	Core 1	Core 2
	Trial I	4.3	8.6	9.6	8.4	11.8
	7.2	10.1	13.4	9.0	11.4	13.6
	8.3	13.1	13.7	9.6	7.3	14.5
	—	—	—	—	—	—
\bar{X}_c	6.6*	10.6	12.2	9.0*	10.2*	13.8
\bar{X}_t	8.6		10.6		12.0	
Trial II	5.0	4.3	6.6	5.0	10.0	10.4
	7.1	3.7	5.1	4.4	10.6	7.5
	7.2	5.5	5.6	4.6	9.8	11.8
	—	—	—	—	—	—
\bar{X}_c	6.4	4.5	5.8	4.7	10.1	9.9
\bar{X}_t	5.5		5.2		10.0*	
Trial III	4.5	7.5	5.2	4.7	10.5	13.3
	5.6	6.9	4.9	5.7	9.7	14.5
	5.5	9.6	4.6	4.6	8.0	9.8
	—	—	—	—	—	—
\bar{X}_c	5.2*	8.0	4.9*	5.0	9.4*	12.5
\bar{X}_t	6.6		5.0		11.0	

^aValues are expressed as pounds necessary to shear a 2.5 centimeter diameter core.

\bar{X}_c -- Core mean.

\bar{X}_t -- Treatment mean.

*Means significantly different ($P < .05$).

(1958). There was no significant treatment effect in this trial but the RIC and Gnotobiotic samples averaged 3.4 and 1.4 pounds, respectively, more tender than the Conventional samples. In Trial II, there was a significant ($P < .05$) treatment effect and no significant variation of cores within treatments. RIC and Gnotobiotic samples were 4.5 and 5.2 pounds, respectively, more tender than Conventional samples. Trial III yielded similar results as Trial I with a significant difference ($P < .05$) between cores within treatments while the RIC and Gnotobiotic samples were more tender than the Conventional samples. Therefore, it was not surprising that when the results were combined over trials (Table 7), a significant difference ($P < .01$) existed between cores within treatments and between the treatments. RIC and Gnotobiotic treated samples required significantly less force to shear a 2.5 centimeter diameter core than Conventional samples. A significant difference ($P < .05$) was found between trials. Trial I was less tender than either Trial II or III. This difference may be explained by animal variation.

An explanation of why the RIC and Gnotobiotic samples were more tender than Conventional samples probably lies in the activity of proteolytic enzymes within the muscle. Sharp (1963) has shown that these enzymes are more active at higher temperatures and lower pH's. Therefore, since these treatments were conducted at 28°C and a pH of 4.7

TABLE 7

WARNER-BRATZLER SHEAR VALUES COMBINED OVER TRIALS^a

Trial	Reduced Initial Count		Gnotobiotic		Conventional		\bar{X}_R
	Core 1	Core 2	Core 1	Core 2	Core 1	Core 2	
I	6.6	10.6	12.2	9.0	10.2	13.8	10.4*
II	6.4	4.5	5.8	4.7	10.1	9.9	6.9
III	5.2	8.0	4.9	5.0	9.4	12.5	7.5
\bar{X}_C	6.1**	7.7	7.6	6.2**	9.9**	12.1	
\bar{X}_t	6.9		6.9		11.0**		

^aValues are expressed as pounds necessary to shear a 2.5 centimeter diameter core.

\bar{X}_C -- Core mean.

\bar{X}_R -- Trial mean.

\bar{X}_t -- Treatment mean.

*Means significantly different ($P < .05$).

**Means significantly different ($P < .01$).

was attained, it is reasonable to suspect meat proteolytic enzymes as the cause of this increased tenderness. However, bacterial effects can not be ruled out completely. Certain bacteria (Proteus, Clostridium, Pseudomonas, etc.) manufacture and excrete proteolytic enzymes that might also tenderize the muscle. Consequently, tenderization may result from combined bacterial-proteolytic enzyme action. Nevertheless, the predominant organisms identified in these treatments are not known for their proteolytic activity. Also the samples did not have any rank odors, which are an indication of proteolysis.

Triangle taste test for acceptability.-- Triangle taste panel results are presented in Table 8 for all trials. The number of correct judgments (two possible) for a particular comparison are listed for each individual. By examination of the data, one can see that there is extreme variation between and within individuals from trial to trial and within trials. Therefore no statistical analysis was made on these data but some overall comparisons were made as shown in Table 9. When the number of correct judgments for a particular triangle test were summed for all individuals over trials, consistent results were obtained only for Conventional samples. Overall comparisons showed that the panel did a better job distinguishing Conventional samples than RIC or Gnotobiotic samples. They differentiated RIC and Conventional samples 61 per cent of the time, Gnotobiotic

TABLE 8

DIFFERENCE BETWEEN PORK LOINS CURED BY THE REDUCED
INITIAL COUNT, GNOTOBIOTIC AND CONVENTIONAL
TREATMENTS AS DETERMINED BY TRIANGLE TESTS^a

Individual	R-R-N ^b	N-N-R	R-R-C	C-C-R	N-N-C	C-C-N
<u>Trial I</u>						
1	2 ^c	2	1	2	2	1
2	2	0	0	1	1	2
3	2	1	2	0	1	2
4	2	1	1	1	2	0
5	1	0	0	0	1	2
x ^d	9	4	4	4	7	7
<u>Trial II</u>						
1	1	0	2	1	1	2
2	2	2	1	2	1	2
3	1	2	1	2	1	1
4	0	2	2	2	1	2
5	2	0	2	1	1	0
x ^d	6	6	8	8	5	7
<u>Trial III</u>						
1	0	0	2	2	1	1
2	0	0	2	1	1	1
3	1	2	0	2	0	2
4	1	1	1	0	2	1
5	1	0	2	1	2	1
x ^d	3	3	7	6	6	6

^aR, N, and C represent the three treatments--Reduced Initial Count, Gnotobiotic, and Conventional respectively.

^bEach letter represents the treatment of the sample in the same triangular test.

^cThis number designated (x) represents the number correct out of the indicated duplicate comparisons for each individual.

^dx^d represents the correct judgments--10 possible--made by all individuals on a particular comparison.

TABLE 9

DIFFERENCE BETWEEN PORK LOINS CURED BY THE REDUCED INITIAL COUNT, GNOTOBIOTIC AND CONVENTIONAL TREATMENTS AS DETERMINED BY TRIANGLE TESTS^a

Trials Combined						
	R-R-N ^b	N-N-R	R-R-C	C-C-R	N-N-C	C-C-N
X ^c	18	13	19	18	18	20
Overall Comparison						
	R - N		C - R		N - C	
K ^d	31		37		38	
% accuracy	51.66		61.66		63.33	
Preference of Correct Judgments	R = 18		R = 19		N = 14	
	N = 9		C = 10		C = 18	
	O ^e = 4		O = 8		O = 6	

^aR, N, and C represent the three treatments--Reduced Initial Count, Gnotobiotic, and Conventional respectively.

^bEach letter represents the treatment of the sample in the triangular test.

X^c = $\sum (x^d)$ for each particular comparison and represents the correct judgments of thirty possible.

K^d = $\sum X^c$ for the designated comparison and represents the correct judgments of sixty possible.

^eIndicates no preference.

and Conventional 63 per cent, while they exercised only 51 per cent accuracy between RIC and Gnotobiotic samples. Generally it can be concluded that the taste panel results agree with the tenderness and acidity results previously presented. The RIC and Gnotobiotic samples are similar, while the Conventional samples differ. Preferences of the panel on correct judgments showed that RIC samples were favored over Gnotobiotic and Conventional samples, and Conventional samples were slightly preferred over Gnotobiotic samples. Nevertheless, sizeable preference for or dissatisfaction with any of the treatments was now shown.

Cured color.-- A subjective evaluation for color development of all loin sections showed the cured red color to be more uniform, deeper, brighter and more desirable after 7 days in the RIC and Gnotobiotic loins than in the Conventional loins. Perhaps the higher curing temperature of the first two treatments accounted for this, but it is also likely that bacteria, via establishing reducing conditions (Greenwood et al., 1940, and Lawrie, 1966) are responsible for this enhanced color.

SUMMARY

A previously developed technique was adapted to study the influence of certain microbiological populations and their effects on processed meat. This technique consisted of an initial reduction of surface bacteria on conventionally handled muscle tissue via a hot water dip, followed by processing at room temperature in a sterile plastic isolator where Pediococcus cerevisiae (ACCEL starter culture approved by the USDA) was inoculated into the curing solution and allowed to grow (Gnotobiotic treatment). This treatment was compared to samples initially reduced of surface bacteria (RIC treatment) and samples conventionally cured (Conventional treatment). The curing solution of the Conventional treatment was significantly ($P < .01$) lower in bacteria than the solutions of the other two treatments. A species of Lactobacillus was the predominant organism in the RIC treatment and Pediococcus cerevisiae (inoculated) was predominant in the Gnotobiotic treatment, i.e., the inoculation yielded a dominant flora. Staphylococcus epidermidis and Flavobacterium diffusum were predominant in the Conventional treatment depending upon the trial. The pH of the Conventional solution was significantly higher ($P < .01$) than pH of either of the RIC or Gnotobiotic

solutions. No difference in pH existed between the RIC or Gnotobiotic solutions. Oxidation of fat was not significantly affected by any of the experimental treatments. Also, treatments did not affect the protein, fat or moisture content of the samples. However, tenderness was significantly ($P < .01$) affected by treatment since 2.5-centimeter cores from RIC and Gnotobiotic samples required significantly less shear force than cores from Conventional samples. Taste panel results supported tenderness and acidity data. The RIC and Gnotobiotic samples were similar, while the Conventional samples differed. All samples were acceptable organoleptically. Subjective evaluations indicated that cured color was more desirable in RIC and Gnotobiotic loins than in Conventional loins. Pediococcus cerevisiae (used as a beneficial organism) did not yield a more palatable product than the naturally occurring Lactobacillus spp. isolated in the RIC treatment. The microbial effects on the product are modest for the technique employed in this study.

This work demonstrates that meat can be processed at room temperature with the production of a wholesome product. However, this technique may be too involved for present commercial use. The major impact of this work is that it introduces new alternatives for studying the microbial influences (beneficial or harmful) on processed meats.

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CONTROLLED MICROBIOLOGICAL AND ENVIRONMENTAL
TECHNIQUES IN MEAT PROCESSING

Rodney J. Bothast

Abstract

A previously developed technique was adapted to study the influence of certain microbiological populations and their effects on processed meat. The technique consisted of an initial reduction of surface bacteria on conventionally handled muscle tissue via a hot water dip, followed by processing at 28°C in a sterile plastic isolator where Pediococcus cerevisiae was introduced into the curing solution. This treatment was compared to Reduced Initial Count and Conventional samples. Identification of the bacteria in the curing solution of each treatment indicated that a Lactobacillus spp. was predominant in the Reduced Initial Count treatment. The inoculated Pediococcus cerevisiae was predominant in the Gnotobiotic treatment, while Staphylococcus epidermidis and Flavobacterium diffusum were predominant in the Conventional treatment depending upon the trial. Tenderness, pH, and bacterial load were significantly affected by treatment. Oxidation and muscle composition were not affected by treatment. Samples from all treatments were acceptable organoleptically.