RELATIONSHIPS OF EXTRACT-RELEASE VOLUME AND REDUCTION OF RESAZURIN AND TETRAZOLIUM DYES TO MICROBIAL CONTAMINATION OF PORK

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ABSTRACT

RELATIONSHIPS OF EXTRACT-RELEASE VOLUME AND REDUCTION OF RESAZURIN AND TETRAZOLIUM DYES TO MICROBIAL CONTAMINATION OF PORK

by Linda Sue Miller

Bacterial spoilage of meat has been studied extensively with regard to physical changes caused in the tissues by contaminating microorganisms but relatively little research has been directed toward the effects of microbial spoilage on the chemical properties of meat. The objective of this study was to determine the influences of pure and mixed bacterial populations on some chemical and physical properties of pork muscle over a 20 day storage period. Some rapid methods for determining the extent of microbial spoilage in meat were evaluated. These procedures necessitated procurement of sterile or relatively bacteria-free samples by special slaughtering and meat handling techniques.

The extract-release volume (ERV) phenomenon, previously reported as a reliable rapid indicator of bacterial spoilage in meat, was observed to be inversely related to bacterial numbers in the pork samples regardless of population type (homogeneous or heterogeneous) of the contaminating microorganisms. However, the correlations between ERV and bacterial numbers were never large enough to justify the use of ERV for prediction of the bacteriological condition of the pork. The correlation between ERV and bacterial numbers was much higher for mixed culture contamination of the samples than for contamination by pure cultures of

bacteria. Minor differences in ERV between control and inoculated samples on day 0 of storage appeared to indicate that ERV responded to growth of bacteria rather than to bacterial numbers per se. The differences observed in day 0 ERV values for differing samples seemed to indicate that there is a wide range of water-holding capacities in pork *longissimus dorsi* muscle samples. This disparity minimizes the significance of the influence of bacterial growth on ERV response for practical applications.

Complete reductions of resazurin and tetrazolium dyes were, in the majority of instances, better indicators of bacterial numbers in the samples regardless of the type of contamination. The correlations observed between bacterial numbers and reduction times of both resazurin and tetrazolium were usually sufficiently high to sanction the use of these tests as reliable indices of the microbial quality of the samples. Significant ($P < .001$) differences in dye reduction times were contributed by inoculation level, storage time, and the interaction of inoculation x time.

The effect of pH of the meat samples on ERV was studied because pH-associated effects on muscle proteins were anticipated to have considerable influence on ERV. Results indicated that the response of ERV to pH was variable. Decreases in ERV with bacterial growth or storage time could not be explained solely as a pH effect, yet pH changes appeared to influence the magnitude of the changes observed in ERV.

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INTRODUCTION

The problem of microbial spoilage of meat has plagued the meat packing industry since its recognized inception in the 1600's and is today one of the major concerns of that industry. Though the practices of salting, drying, and smoking of meat for purposes of preservation were developed centuries ago and were mentioned in literature dated 1,000 B.C. as common practice, it was not until the latter part of the nineteenth century that the application of scientific research for the improvement of meat production and processing was begun in earnest (Armstrong and Schweigert, 1960). With the development of analytic laboratories as functional parts of some packing plants and the growing impetus of research in leading laboratories concerning the safety, composition, and dietary significance of meat, meat science has expanded to include many disciplines such as chemistry, food technology, histology, microbiology, and many more.

It is interesting to note, then, that though many workers have studied the effects which microorganisms have on the physical properties of meat, relatively little research has been done concerning the effects of spoilage microorganisms on the chemical properties of meat. Borton (1966), using aseptic techniques of slaughter and sample procurement, was able to obtain pork samples with low bacterial counts which he compared with intentionally contaminated samples regarding several test criteria. His results indicated that bacteria did, indeed, influence

some chemical characteristics of meat such as emulsifying capacity, protein extractability, and extract release volume, in addition to their well-known effects on such physical properties as flavor, odor, texture, and color.

The need for simple and rapid quality control measures to test raw materials and products is recognized by the meat packer. A rapid method or methods of estimating the microbial quality of the meat he receives from various supply sources would be of great advantage to his operation. Many techniques have been suggested for the estimation of proteolysis or spoilage in meat. Those based directly on bacterial content of the meat include total numbers, most probable numbers, and dye reduction times. Other procedures concern the various biochemical changes (production of ammonia, indole, and ninhydrin-positive substances) and physical changes (pH change, color change, differences in electrical conductivity) which take place during spoilage. Jay (1964a, 1964b) suggested that the extract-release volume (ERV) phenomenon would be a good rapid indicator of the microbial quality of meat. Other workers (Proctor and Greenlie, 1939; Walker et al., 1959; Harmon et al., 1961) have indicated that reduction rates of tetrazolium and resazurin dyes could be used to predict the bacteriological quality of different foods including meat.

This project was undertaken to determine:

1. If bacteria-free meat samples could be obtained without the use of elaborate procedures and expensive equipment.
2. If ERV and dye reduction times were correlated highly enough with bacterial numbers to warrant their use as rapid indicators of the

microbial quality of meat.

3. If pure cultures of different types of bacteria had different effects on the test parameters of ERV, dye reduction time, pH, and bacterial numbers.

LITERATURE REVIEW

Microbial Contamination of Muscle

Heavy loads of bacteria, yeasts, and molds may be carried into packing plants on the hooves, hide, hair, and skin of the animals which are to be slaughtered there (Lawrie, 1966). Empey and Scott (1939) reported that approximately 33 million bacteria, 850 molds, and 580 yeasts were carried into the slaughter house on every square centimeter of beef hide, while Ayres (1955) indicated that the skin of animals slaughtered for meat production carried an average of 3.9 million aerobic bacteria, 100 million anaerobic bacteria, and 100 yeasts and molds per square centimeter. The gastrointestinal tracts of these animals also harbor many microorganisms which may contaminate the carcass during evisceration (Ayres, 1955). Haines (1937) showed that the contents of the large intestine of hogs may contain as many as 33×10^{12} viable bacteria per gram.

Ayres (1955) found that each contact of the carcass with the equipment and water used in the slaughter operation and with the personnel involved served to increase the microbial load of the tissues significantly. Of particular importance (Jenson and Hess, 1941) was the fact that bacteria on the sticking knife and neck area of the animal were plunged into the animal's blood stream during the sticking process and were circulated throughout the tissues of the animal until heart action had stopped. The length of time required for total cessation of

heart beat varied from two to nine minutes. Normally these bacteria would be cleared from the blood stream of the living animal by the filtering action of the lymph nodes and the phagocytic action of the reticulo-endothelial system, but as Zender (1958) showed, these defense mechanisms become inoperative when the animal dies. The bacterial flora of the lymph nodes can then proliferate and spread into the surrounding tissues (Cosnett et al., 1956). Lepovetsky et al. (1953) agreed that since the lymph nodes trap bacteria from the blood stream of the animal they are probably the principal source of bacterial spoilage of the deep tissues.

Reports from Jenson and Hess (1941) and Ayres (1956) showed that the scalding tank used in hog slaughter operations was an important reservoir of some of the spoilage organisms carried on the feet, hair, and skin of the hogs. Should the hog be dropped into the scalding tank before cessation of heart action the contaminated water would be circulated through the animal and deposit potential spoilage organisms or their spores in the tissues as well as on the surfaces of the carcass.

Frazier (1967) suggested that to reduce invasion of the tissues by contaminating microorganisms, (1) the animal should be held 24 hours before slaughter without food to reduce the bacterial load in the gut, (2) the methods of killing and bleeding should be as sanitary and quick as possible, (3) the animal should be well rested to ensure that some glycogen remains in the muscle to be converted to lactic acid by anaerobic glycolysis and thereby lower the pH of the tissues, and (4) that the cooling of the carcass should be as rapid as possible in order to slow the rate of invasion of microorganisms into the tissues.

Microbial Spoilage of Meat

Meat, which differs from muscle because of a series of biochemical and biophysical changes which are initiated in muscle at the death of an animal (Lawrie, 1966), is still a very satisfactory culture medium for a great many types of microorganisms. The elimination of blood-borne oxygen supply as a result of bleeding and the consequent fall in redox potential render the cytochrome system inoperative and make the resynthesis of ATP (adenosine triphosphate) in the tissues impossible. Lactic acid accumulates and renders the proteins of the meat susceptible to denaturation (Lawrie, 1966). The same author indicated that denatured proteins provided a rich medium to support the growth of bacteria. Jay (1966c) reported the existence of eight different rigor mortis associated phenomena which had definite effects on the growth of bacteria in post-rigor meats. These are: (1) reduction of the pH of the meat by accumulation of lactic acid; (2) cessation of the normal phagocytic functions of the tissues and consequent spread of bacteria; (3) lowering of the redox potential as the oxygen supply to the tissues ceases; (4) accumulation of metabolites which bacteria can utilize; (5) formation of actomyosin; (6) breakdown of proteins by the native cathepsins; (7) changes in the hydration capacity of the proteins; (8) leakage of calcium from, and uptake of potassium by, the skeletal proteins.

Frazier (1967) stated that meat is high in moisture, rich in both simple and complex nitrogenous substances, has many minerals and accessory growth factors required by microorganisms, usually contains some fermentable carbohydrate, and is normally at a pH favorable to the growth of most bacteria. The growth of microorganisms in meat depends

upon the amount and kind of contamination of the meat, the physical and chemical properties of the meat, the availability of water and oxygen, and the temperature at which the meat is held.

Spoilage of meat as the result of bacterial action includes lipoxidation, proteolysis, souring, putrefaction, sliminess, and stickiness. Some of the genera of bacteria responsible for the aerobic spoilage of meat are Pseudomonas, Achromobacter, Lactobacillus, Leuconostoc, Streptococcus, Micrococcus, and Pediococcus. Species of Clostridium and Bacillus are the usual causative agents of the putrefaction of meat which is observed under anaerobic conditions (Frazier, 1967).

The common low meat storage temperatures of 2-6 C dictate that the dominant spoilage organisms occurring will be psychrophiles or psychrotrophs (Ayres, 1963). Early studies by Lochhead and Landerkin (1935) reported that the predominant flora of meat stored at low temperatures belonged to the genus Achromobacter. Empey and Scott (1939) agreed with these findings when they indicated that the four principal genera of low-temperature bacteria isolated from fresh meat were Achromobacter (90%), Micrococcus (7%), Flavobacterium (3%), and Pseudomonas (less than 1%). Ayres et al. (1950), Kirsch et al. (1952), and Wolin et al. (1957) presented reports that the Pseudomonas species were definitely responsible for the low-temperature spoilage of meat to a far greater degree than were any other bacterial species. Reassessment of the taxonomy of the psychrophilic organisms isolated by Empey and Scott in 1939 showed that almost all were actually pseudomonads (Brown and Weidemann, 1958). Ayres (1960) indicated that these discrepancies probably stemmed from the fact that the nomenclature of the 5th Edition of Bergey's Manual of

Determinative Bacteriology was different from that of the 3rd Edition which was current when the earlier studies were conducted. The differentiation of these two genera of bacteria was, in the later study, based on the type of flagellation of the organisms rather than on the production of water-soluble pigments which had been used in the earlier work. Sulzbacker (1950) was able to isolate twenty-eight strains of Pseudomonas from frozen pork and six Pseudomonas strains from frozen lamb. Kirsch et al. (1952) isolated 532 pseudomonads from refrigerated hamburger. These findings were substantiated by Ayres (1956) and Halleck et al. (1958).

Ayres (1960) showed that the microbial population of uncooked flesh products immediately after processing could be as much as 80% chromogenic cocci, yeasts, and molds, but that upon storage at 10 C or less, the population tended toward pseudomonads with increasing rapidity until they represented 98% of the ultimate population. Studies by Wolin et al. (1957) earlier had indicated that Gram-positive rods were the predominant initial microbial flora of ground beef but that storage at 20 C changed the population to aerobic, Gram-negative, non-pigmented rods with the polar flagella characteristic of pseudomonads.

Review of Test Organisms

Pseudomonas fluorescens - The ability of this proteolytic organism to grow well at low temperatures makes it an important source of meat spoilage. Peterson and Gunderson (1960) indicated that the degradative action of the proteolytic enzymes of P. fluorescens caused formation of disagreeable flavors and odors in meat. Ayres (1960) reported that since this organism was so widespread and caused such a variety of

changes in foods that it was difficult to store meat at refrigerator temperatures for even short periods of time without observing putrefaction, production of fruity or unclean odors and bitter or rancid flavors, discoloration, and slime formation caused by contamination of the meat with P. fluorescens. This Gram-negative, polarly-flagellated rod-type organism is a normal soil inhabitant (Frazier, 1967) and may be transferred to carcasses in the slaughter house from the hooves, hide, and hair of animals, from water used in the operation, and from the air in chilling rooms. Witter (1961) indicated that this organism is fairly salt-tolerant (as much as 4% sodium chloride), will not survive pasteurization, and is sensitive to pH reduction and the action of most antibiotics.

Frazier (1967) reported that pseudomonads are important in foods for the following reasons: (1) they possess remarkable ability to utilize many different non-carbohydrate compounds for energy production; (2) their ability to produce end-products deleterious to flavor is marked; (3) they are able to use simple nitrogenous substances like ammonia and nitrates; (4) when required they can synthesize their own necessary growth factors; (5) they are proteolytic and lipolytic; (6) they are able to grow rapidly aerobically and produce oxidative products and slime at food surfaces; (7) they grow well at refrigeration temperatures; (8) they are able to produce pigments in foods.

P. fluorescens is, according to Stanier et al. (1963), the most omniverous bacterium known. They report single strains of this organism can oxidize more than 90 different organic substances including acids, alcohols, amino acids, and ring compounds. Such an organism requires a

tremendous array of enzymes for these metabolic activities - more than could be present at any one time in a single cell. Those of P. fluorescens are inducible and produced in response to specific induction by the substrate in which the organism is growing.

Peterson and Gunderson (1960) characterized the extracellular and endocellular enzymes of P. fluorescens. They reported that liberation of the enzymes was greatest at 0 C and smallest at 30 C and that this tendency toward greatly increased enzyme production at 0 C was directly related to the lesser activity of the enzymes at the lower temperature. As the temperature was increased, the greater activity of the enzymes apparently limited the need for as much enzyme to produce a given level of protein attack. These same workers noted that the extracellular enzymes possessed more activity per unit weight than did the endocellular ones; the endocellular enzymes, however, were three times as active at 10 C as they were at 35 C. Earlier work by Camp and Van Der Zant (1957) showed that endocellular enzyme activity was greatest at 35 C and decreased when the temperature was lowered to 25 C. Alford (1960) indicated that P. fluorescens produced the same amount of enzymes at 5 C as it did at 20 C but that enzyme production at 30 C was slight.

Streptococcus faecalis - Hall (1964) reported that two groups of streptococcal organisms were considered common food contaminants and that one group, the enterococci, included S. faecalis. Though this organism has been implicated rather infrequently in outbreaks of foodborne illness, the importance of its occurrence in foods should be emphasized because it is a normal inhabitant of the intestines of man and warm-blooded animals. The presence of S. faecalis in foods, however,

may or may not imply direct fecal contamination because the enterococci have been observed to become established rather easily in food processing plants (Niven, 1963).

S. faecalis is an exceptionally hardy organism (Davis et al., 1968) which grows well in the temperature range of 10-45 C, resists 62 C for 30 minutes, can tolerate 6.5% sodium chloride and 0.05% sodium azide or pH 9.6, is non-hemolytic, and produces appreciable amounts of lactic acid from carbohydrates with a homofermentative type of fermentation. These Gram-negative organisms are frequent but undesirable contaminants of brined meat and bacon, yet they have been successfully employed as the acid-producing agent in several desirable food fermentations. A number of workers have used S. faecalis in experimental production of Cheddar cheese. Dahlberg and Kosikowsky (1949) reported successful production of Cheddar cheese with desirable ripened flavor and good texture in 2.5 months at 16 C storage when S. faecalis was used as the starter culture.

The relationship of S. faecalis to food poisoning outbreaks has been somewhat confusing. Frequently reports of food-borne disease incidents describe any case of gastroenteritis in which salmonellae or staphylococci cannot be demonstrated as "etiology unknown" when streptococci may have been the actual causative agents. Meyer (1953) stated that until more exhaustive bacterial analyses accompany these medical reports the actual prevalence of illness caused by ingestion of S. faecalis can not be estimated with any degree of accuracy or certainty .

Ingestion of large numbers of S. faecalis results in acute gastroenteritis after an incubation period of two to eighteen hours. Fever

and leukocytosis are not evident; severe nausea, emesis, and diarrhea are the clinical manifestations of streptococcal gastroenteritis (Dack, 1962). Meyer (1953) indicated that the illness appeared to be caused by a preformed heat-stable toxin. He observed that tyramine, produced when S. faecalis decarboxylated tyrosine, might be the substance in question even though earlier studies by Dack et al. (1949) showed that human volunteers were not adversely affected by ingestion of large amounts of tyramine. The substance responsible for the symptoms of the illness has not yet been identified.

Pediococcus cerevisiae - The importance of this organism in foods is indicated by its occurrence, either desirable or detrimental, in various food fermentations. P. cerevisiae is a fastidious homofermentative organism capable of producing 0.5-0.9% lactic acid from fermentable carbohydrate and is therefore important in the production of fermented meat items such as Thuringer, Cervelat, and Summer sausages. It also causes cloudiness in beer ("sarcina sickness"), is involved in pickle fermentations. P. cerevisiae is a microaerophilic organism which is salt-tolerant (5.5-9.0% sodium chloride) and is capable of good growth at refrigerator temperatures. These attributes favor its use in the previously mentioned fermented meat products where inoculation with 0.05% of the organism favors the desired lactic acid formation in the product (Frazier, 1967).

Clostridium perfringens - The ubiquity of C. perfringens is conceded by many researchers (McKillop, 1959; Yamamoto et al., 1961; Smith, 1963) to make it a probable contaminant of most foods. Presence of this organism on the hides, hooves, and in the gut of most animals slaughtered

for meat production indicates the possibility of contamination of the carcasses of the animals during and after slaughter. Weadon (1961) reported that 10-35% of retail market meats contain the heat-resistant spores of C. perfringens which have been implicated in food-borne disease outbreaks. Studies by Hall and Angelotti (1965) demonstrated that over half of 161 specimens of various types of raw market meat tested positive for the presence of C. perfringens and that all specimens of ground beef and ground pork examined showed positive results when tested for this organism.

Though this organism's pathogenic importance is generally attributed to the role it plays as the most important etiologic agent of gas gangrene in man, the significance of contamination of foods (especially meat and meat dishes) by C. perfringens has become more and more evident since the first reports of gastroenteritis associated with it were received by the United States Public Health Service (Dauer and Davids, 1959). Reports from the British Isles showed that C. perfringens was one of the principal causes of food-borne illness in that area and that meat was implicated in almost every case (Angelotti et al., 1962).

Hobbs et al. (1953) indicated that gastroenteritis caused by C. perfringens occurred after ingestion of several hundred million living cells, that the incubation period lasted from 8-20 hours, that the symptoms included abdominal upset with marked cramps and diarrhea, and that emesis and fever were uncommon. English workers (Dische and Elek, 1957) noted that the illness was caused by an atypical type A strain of C. perfringens (types A,B,C,D,E, and F have been characterized) which produced heat-resistant spores and elaborated an alpha-toxin along with

small quantities of a theta-toxin. The alpha-toxin is now known to be a lecithinase; it is thought that phosphoryl choline, a product of lecithin degradation, may be the chemical agent involved in this type of illness.

This Gram-negative, nonmotile organism is often difficult to culture in the laboratory because of the difficulties associated with anaerobic cultivation (Angelotti et al., 1962) and thus its role in some outbreaks of food poisoning has gone unrecognized because of basic limitations in methodology for routine detection and identification of anaerobic bacteria. However, reports from Hobbs et al. (1953) and Hall and Angelotti (1965) indicated that whenever meat was cooked, cooled slowly, and rewarmed the germination of the heat-resistant spores found so frequently in the raw meat was a probable event.

Aseptic Sampling Procedures

The problem of obtaining germ-free animals or germ-free samples of tissues and exudates from animals for experimental purposes has been approached in several ways. Gnotobiotics, the science of rearing laboratory animals in a specifically known environment, usually free of microorganisms, has been developed in answer to this problem. Landy et al. (1961) indicated that the bulk of this gnotobiotic work being done was based on the fact that the embryos or fetuses of many animals are free of microorganisms in utero and that it is during and after birth and suckling that microbes are taken into the animal and establish themselves as normal flora, especially in the gastrointestinal tract. Ayres (1955) stated that neonatal pigs, lambs, and calves, after exposure to food and their environment, carry heavy populations of microorganisms in the

rumen and intestines. Although most of these microorganisms are essential to the digestive processes of the animal, once they pass the membranes of the tract and enter the systemic circulation they represent potential sources of disease and serious contamination of the tissues. Zender (1958) found that the normal muscle tissue of healthy living animals was relatively free of microorganisms while great numbers of bacteria could be isolated from the lymph nodes of these same animals. With death, then, the permeability of the gut is altered, the phagocytic action of the reticulo-endothelial system is halted, and the microbes in the gut can penetrate rather easily into the inner tissues of the animal and cause severe contamination; the bacteria trapped in the lymph nodes also proliferate and spread into the tissues after the animal dies.

Waxler and Whitehair (1966) were able to collect piglets from sows just prior to normal parturition by the technique of sterile hysterotomy; these piglets could be maintained free of microorganisms for 5 to 6 weeks in a sterile environment. For this operation a sterile surgical isolator of vinyl film with attached rubber sleeves and gloves was secured to the sow's flank with sterile adhesive. Operating through the floor of the isolator, the surgeon made a single incision in the abdominal wall and exposed the uterus. The piglets were then removed from the uterus, cleaned, and passed through sterile air-locks into adjoining sterile rearing isolators which were then sealed. All materials passing into the isolators for maintainance and testing of the piglets were sterile. Repeated culturing of the animals' skin, feces, urine, and tissues indicated that no microorganisms were established in or on the piglets. Ockerman et al. (1964) had previously slaughtered and

eviscerated germ-free mice in a similar isolator arrangement and were able to store the carcasses for several months at 23 C with no evidence of bacterial decomposition.

Davis (1965) slaughtered beef with sterile sticking knives. The remainder of the slaughtering and eviscerating procedures were normal with the exception that the carcass was left unsplit and the hide over the loin area remained intact. Prior to cooler storage of the carcass, bacteriocidal soap and ethanol were used to rinse the loin. The chilled loin was then placed on a cart and a sterile isolator was attached to the hide. The longissimus dorsi muscle was exposed, excised, and ground within the confines of the isolator with sterile equipment. Samples taken in this manner were stored for 35 days at 2-5 C and showed no evidence of bacterial contamination.

Borton (1966) demonstrated that muscle with very low bacterial loads could be obtained from hogs without the use of the cumbersome and expensive sterile isolators if precautions were taken to prevent contamination of the carcass and meat from the usual sources both during and after slaughter. His procedure involved the use of sterile sticking knives, alcohol rinsing and flaming of the unsplit carcass, and sterile surgical instruments for excision of the loin samples. The samples were ground aseptically and stored in sterile covered containers for 17 days at 4-7 C. Bacterial counts of tissues taken in this manner ranged from 10 organisms per gram on day 0 of storage to 1 million per gram on the 17th day of storage. The initial counts were considered low for any type of meat and the counts on day 17 were lower than the 2-95 million organisms per gram which has been reported for fresh ground beef

purchased in many retail markets (Kirsch et al., 1952).

Infusion of antibiotics such as aureomycin and chlortetracycline has been shown to be effective in reducing the number of microorganisms in muscle tissue when administered through the jugular vein at slaughter or when arterially pumped into cuts of meat. Weisser et al. (1964) noted that chlortetracycline was excellent for prevention of "sour round" in beef (a type of spoilage resulting from growth of mixed types of Gram-positive bacteria which gain admittance at slaughter and spoil the deep tissues of the round). Further studies by these workers indicated that the lymph nodes represented a serious source of bacterial contamination of muscle. They were able to reduce dramatically the number of bacteria in the deep lymph nodes (ischiatic and popliteal) with infusion of aureomycin.

Dye Reduction

The use of dye reduction tests as rapid means for roughly differentiating between satisfactory and unsatisfactory foods according to bacterial content has been a convenient tool for quality control work for some types of food processing (Ayres, 1960). The milk industry, in particular, has employed reduction tests using methylene blue, resazurin, and triphenyltetrazolium chloride (TTC) dyes for estimation of bacterial numbers in dairy products.

Elliker (1949) indicated that the tests involved the addition of a certain amount of a dye to a food sample in a suitable sterile medium followed by observation of the dye for color changes. If bacteria present in the sample lowered the redox potential sufficiently the dye would undergo a color change. Resazurin, slate blue at the pH of normal

milk, was reduced to pink resorufin at a potential of +0.2 to +0.05 volt. Further reduction of the dye resulted in the formation of colorless dehydroresorufin white at a redox potential of +0.15 to 0 volt. TTC was reduced first to a pale pink compound and finally to a red formazan. Methylene blue reduction resulted in the formation of leucomethylene blue (methylene white), a colorless compound.

If the number of bacteria in the sample is high and the reducing capability of the organisms is substantial, the rate of fall of potential and consequent reduction of the dye will be relatively rapid. Conversely, if the bacterial population of the sample is low or if it is composed of weakly reducing organisms, a longer period of time will be required for dye reduction.

Greene and Jamison (1959) noted that since great variations existed in the abilities of different bacterial species to reduce the dyes the tests might be of negligible value in estimating the bacterial quality of a food sample. Psychrophiles, especially, they reported, had long generation times and relatively weak reducing abilities. These factors may restrict the utility of the dye reduction tests when psychrophiles are the potential spoilage organisms in question as may be the case with dairy and meat products. These tests also fail to detect weakly reducing thermoduric bacteria (Elliker, 1949).

Proctor and Greenlie (1939) and Ferguson et al. (1958) reported that the correlation between numbers of bacteria and reduction time of resazurin in fresh and frozen meat and vegetables was relatively poor. Straka and Stokes (1957) were able to classify meat pies as acceptable or nonacceptable according to bacterial population by the use of resazurin

dye reduction tests. Studies by Wolin et al. (1959) showed that a good correlation existed between the time required for resazurin reduction and the number of bacteria per square centimeter on the skin surface of poultry. Saffle (1961) found that the relationship between resazurin reduction time and spoilage of meat was closer than that observed between total numbers of bacteria and spoilage as judged by odor score. Mallmann et al. (1958) used reduction of TTC to predict the shelf life of dressed poultry and found there was a definite relationship between the dye reduction time and the number of psychrophiles on the poultry. Harmon et al. (1961) measured the correlations between reduction times of resazurin, methylene blue, and TTC and the shelf life of cottage cheese. These correlations ranged from $r = 0.74$ to $r = 0.80$. The highest correlation was that reported between the shelf life of the cottage cheese and the reduction of resazurin to resorufin.

Walker et al. (1959) showed that a resazurin reduction time of 8 hours indicated a bacterial population of 10,000 organisms per ml of reaction medium when poultry was the sample tested. Resazurin reduction tests on milk containing P. fluorescens were reported by Greene and Jamison (1959) and showed that a bacterial population of 100 million organisms per ml of reaction medium reduced the dye in 45-90 minutes while a population of 100,000 organisms per ml required 5 hours to reduce the dye.

Bradshaw et al. (1961) found that the reduction rates of different tetrazolium chloride salts varied greatly not only according to different compounds but also according to the same compounds produced by different companies. These authors indicated that 2-(p-iodophenyl)-3-

(p-nitrophenyl)-5-phenyltetrazolium chloride produced the most satisfactory and reproducible results in their dye reduction tests.

Extract-Release Volume (ERV)

The extract-release volume phenomenon, according to Jay (1964a), is based upon the amount of fluid extract a slurry of meat plus diluent will release when filtered through filter paper for a given period of time (usually 15 minutes). Jay and Kontou (1964) indicated that the ERV of ground beef showed a linear reduction in value as spoilage of the meat progressed and bacterial numbers increased. Large volumes of extract (high ERV) corresponded to fresh beef samples of rather low bacterial contamination; lesser volumes of extract (low ERV) were associated with beef undergoing definite microbial spoilage. These same authors suggested that the correlation between bacterial numbers and ERV ($r = -0.808$) was high enough to warrant the use of the ERV phenomenon as a rapid test of the microbial quality of beef.

Jay (1964b) found that ground beef with an ERV of 24.6 ml carried a bacterial load of log 8.5 (320 million organisms per gram) and was considered unacceptable after organoleptic evaluation by a trained panel. Jay and Kontou (1964) showed in another study that a trained panel rejected ground beef when the mean ERV value was 30.4 ml. This ERV corresponded to a bacterial population of log 7.8 (63 million organisms per gram) in the meat sample. Kontou et al. (1966) were in close agreement with Jay's observations when they reported that the average ERV of ground beef at organoleptic rejection by a trained panel was 22.0 ml and that the bacterial population associated with that value was log 8.8 (630 million organisms per gram). Jay and Kontou (1964) suggested that an

ERV of 25.0 ml (approximating a bacterial population of log 7.2 or 16 million organisms per gram) should be a probable cut-off value for ERV to differentiate between organoleptically acceptable and unacceptable ground beef. Studies by Price et al. (1965), however, indicated that the onset of meat spoilage occurred when the ERV was 30-40 ml and that meat with an ERV of less than 30 ml was undergoing frank spoilage. Recent work by Borton (1966) showed that the ERV of ground pork with a bacterial population of log 8.2 (150 million organisms per gram) was 29.0 ml. This same author sampled ground pork over a storage period of 17 days and noted that after the initial rapid rise in bacterial numbers and concurrent fall in ERV, both values exhibited rather steadying trends by day 7 and remained fairly constant for the remainder of the storage period.

Jay (1964a) also observed some other factors which affected the ERV of ground beef. He found that the pH at which the extraction occurred had marked effects on the amount of extract obtained. At pH less than 4.9 or greater than 11.0 the ERV was zero. ERV was maximum at pH 5.0 - 5.8. ERV was not significantly affected by a fat content of the meat of 20% or less (Jay, 1964a); no significant effect of the fat content of pork on ERV was observed by Price et al. (1965). Jay (1964a) did report, however, that ERV values were increased when the fat content of the samples was much higher than 20%. Though ERV was collected at temperatures ranging from 7-37 C by Jay (1964a), Price et al. (1965) observed that the procedure was more controlled and results were more easily duplicated if the extractions were run at the normal cooler temperatures of 2 - 6 C. Addition of proteases to ground beef caused a

decline in ERV similar to that observed as meat spoiled (Jay, 1964a). This author concluded, as a result of this observation, that the proteolytic action of bacteria was responsible for the reduction of ERV of spoiled meat. Jay (1966d) later concluded that the most striking change occurring during meat spoilage was not proteolysis but the significant increase in the hydration capacity of the muscle proteins.

Reidel et al. (1967) evaluated the ERV phenomenon as a means of detecting microbial spoilage in meat and reached the conclusion that the test was not reliable. They observed that: ERV was influenced by the type of microbial flora and associated pH changes in the meat; ERV did not always reflect spoilage in ground beef because ERV increased with spoilage in some instances; meat of different pH (6.2 and 6.7) and similar initial bacterial loads exhibited very different ERVs; ground meat sometimes spoiled without an appreciable change in ERV values.

EXPERIMENTAL PROCEDURES

Slaughtering

The ten 68-127 kg hogs used in this study were obtained from the Michigan State University farms and from a local farmer. Pairs of hogs were slaughtered in the M.S.U. Meat Laboratory after being held without feed overnight. Each animal was electrically stunned, shackled, and hoisted by a rear leg while warm pHisohex bactericidal soap solution (Winthrop Laboratories) was used to scrub the sticking area, forelegs, and ventral side of the front quarters. The soap was rinsed off, but, contrary to the procedure of Davis (1965), the neck area was not shaved. The hog was allowed to exsanguinate by heart action after being stuck with a sterile knife. After five to nine minutes of bleeding the hog was scalded and dehaired and the carcass was washed with pHisohex solution and rinsed prior to dropping of the head and evisceration. After evisceration the body cavity was rinsed with 100% ethanol and flamed. The entire unsplit carcass was then rinsed with 100% ethanol prior to storage in the 2 - 4 C cooler for 24 or 48 hours.

Sample Excision

After 24- or 48-hour storage in the cooler the carcass was again rinsed with 100% ethanol and the shoulder portion was removed at the third rib. The remainder of the carcass was placed belly-down on a previously steamed stainless steel paper-covered table. Excision of the samples was accomplished in a 5 C cooler in which the air flow had been

minimized by shutting off the fan. The operators used sterile equipment, wore clean laboratory coats, and used sterile surgical gloves during the procedure.

Following Borton's procedure (1966), the operator made one long incision down the midline of the loin backfat cover; two cuts (one at each end of the original incision) were made perpendicular to the backline at approximately 3.8 cm behind the anterior end and just above the hookbones (tuber coxae). The backfat cover from one side was rolled back and peeled down as close as possible to, but without cutting into, the longissimus dorsi muscle. All but the extreme anterior and posterior portions of the muscle were exposed. Using a different sterile knife, the operator excised the muscle in approximately 2.5 cm slices; at no time was the knife allowed to penetrate past the ribs into the body cavity. The pieces of loin muscle were transferred with a sterile hemostat into a sterile covered stainless steel container which was labeled "sample for inoculation". The procedure was repeated on the remaining side of the loin and the muscle pieces were stored in another covered sterile container labeled "control sample".

Inocula Selection and Preparation

The organisms used in this study were chosen because they represented a few of the types of bacterial spoilage which may occur in meat. Included were a typical proteolytic psychrophile which causes surface slime and discoloration of meat (Pseudomonas fluorescens), a lactic acid-producing fecal streptococcus which has been implicated in food poisoning outbreaks (Streptococcus faecalis), a homofermentative lactic acid-producing bacterium which is used as a starter culture in the production

of fermented meat products (Pediococcus cerevisiae), and a proteolytic type of clostridial organism which does not grow at refrigeration temperatures but will proliferate in meat held warm for period of time and is an important etiologic agent of food-borne illness (Clostridium perfringens). A culture of unknown mixed organisms taken from ground beef spoiled at refrigeration temperature was also used so that general comparisons might be made between the spoilage patterns established in meat by homogeneous and heterogeneous bacterial populations. This culture consisted mainly of Gram-negative rods some of which were flagellated.

The pure cultures of organisms used in this study were obtained from the American Type Culture Collection (C. perfringens, P. fluorescens) and from the Michigan State University Department of Microbiology and Public Health (P. cerevisiae, S. faecalis). With the exception of the clostridial inoculum, all inocula were prepared in the following manner: the organisms were grown in Bacto All Purpose plus Tween broth (APT), a formulation of the medium described by Evans and Niven (1951), which is distributed by Difco Laboratories. APT agar slants were prepared from the broth cultures in the usual manner. Cells used for inoculation were prepared from 24-hour slant cultures by washing the slants with sterile phosphate-buffered water (pH 7.0). One milliliter of a barely turbid suspension of the washings was transferred aseptically to a 99 ml sterile buffered water blank. The "light" inoculum in each case consisted of 0.1 ml of this suspension in 9.9 ml of sterile buffered water; the heavy inoculum consisted of 1 ml of this suspension in 9 ml of sterile buffered water. The anaerobe (C. perfringens) was cultured in Bacto Fluid Thioglycollate Medium (Difco) and was diluted for inoculation in the same

manner as the aerobically cultured organisms.

Sample Treatment

The samples used for this study were treated according to the following protocol with each control sample and each inoculated sample having subsamples stored at 2 C and at 10 C. In each instance in the chart below, "c" indicates a control sample which was treated with 10 ml of sterile buffered water; "i" indicates that the sample was inoculated with either a light or a heavy inoculum of the particular organism listed. In all cases, animal A inoculated samples received the light inoculum; animal B inoculated samples received the heavy inoculum. Two different animals were used in each trial. The A and B designations refer both to animal and inoculum level differences.

Sample Inoculation

The control sample was ground as aseptically as possible through a sterilized prechilled grinder with a 0.5 cm plate. At the time the meat was extruded through the plate, 10 ml of sterile phosphate-buffered water were added in drops from a sterile pipette in such a manner that the last of the water was added as the last of the meat passed through the plate. The sample was ground aseptically again to facilitate mixing and was transferred aseptically into each of 14 sterile covered glass jars. The procedure was repeated for the inoculated sample with the exception that 10 ml of a selected dilution (light or heavy) of a particular bacterial culture were added to the ground meat with a sterile pipette. The sample groups then consisted of 14 jars of control samples and 14 jars of inoculated samples. Half of each sample group was stored at 2 C and the other half was stored at 10 C.

TRIAL	INOCULUM LEVEL AND ANIMAL	TREATMENT
I	A	c_i - *P. fluorescens*
	B	c_i - *P. fluorescens*
II	A	c_i - *P. cerevisiae*
	B	c_i - *P. cerevisiae*
III	A	c_i - *S. faecalis*
	B	c_i - *P. cerevisiae*
IV	A	c_i - Mixed culture
	B	c_i - Mixed culture
V	A	c_i - *C. perfringens*
	B	c_i - *C. perfringens*

Measurement of bacterial numbers, extract-release volume (ERV), extract pH, and reduction times of resazurin and the tetrazolium salt, TTCS, was accomplished after 0, 2, 4, 8, 12, 16, and 20 days of storage utilizing the contents of a single sample jar for each day and each treatment.

Bacterial Numbers

Quantitation of bacterial numbers in the samples by total plate count was done according to the method described by the American Public

Health Association (1966). Eleven gram samples of the ground pork tissue were blended in sterile blender cups with 99 ml of sterile phosphate-buffered water for 2 minutes. The slurries were then serially diluted into triplicate sterile Petri plates. APT agar was added and the plates were allowed to solidify. Plates containing dilutions of the samples contaminated with C. perfringens were poured with APT agar containing 3% sodium thioglycollate and were incubated both aerobically and anaerobically. Anaerobic incubation was carried out in a nitrogen-flushed incubator. Incubation of all plates for 48-72 hours was at temperatures optimum for the growth of the different organisms used (P. fluorescens, 20 C; S. faecalis and P. cerevisiae, 25 C; C. perfringens, 35 C; mixed culture, 37 C, 25 C and 4 C). Colonies were counted and reported as the logs of the numbers of bacteria per gram of sample. Bacterial numbers reported for samples contaminated with C. perfringens were the sums of the aerobic and anaerobic counts.

Extract-Release Volume (ERV)

The following slightly modified procedure of Jay (1964a) was used in this experiment: duplicate 25 gram samples of each of the 4 sample treatments of pork tissue were tempered for at least 1 hour at 5 C and then were blended in a Waring Blendor for 2 minutes with 100 ml of 0.1M phosphate buffer at pH 5.8. The slurry was filtered through Whatman No. 1 filter paper which had been folded to yield eight sides. The amount of filtrate collected after 15 minutes was reported as the extract-release volume (ERV) and was recorded as the average of the duplicate samples. The pH of the filtrates was determined using a Beckman Zeromatic pH Meter. The entire extraction procedure was performed in a 5 C

cooler.

Dye Reduction

Skim milk was the medium used for the dye reduction tests. Bacteria present in the milk as a result of post-pasteurization contamination would have influenced the reduction rates of the dyes; therefore, it was decided to heat treat the milk in some way prior to its use in the tests. Autoclave sterilization of the milk proved unsuccessful because it caused release of sufficient sulfhydryl groups from the milk proteins to reduce the dyes rapidly. The milk was instead heated to 63 C for 30 minutes in a water bath; the 10 ml aliquots of milk were contained in sterile, screw-capped test tubes. Milk treated in this manner did not reduce either of the dyes used in less than 36 hours.

Resazurin (diazoresorcinol) dye was prepared by aseptic addition of one standard resazurin tablet (National Aniline Division, Allied Chemical Corporation) to 200 ml of sterile water. Preparation of the tetrazolium dye (TTCS) involved addition of 0.1 gram of the salt, 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (Aldrich Chemical Company), to 100 ml of sterile water. These solutions were prepared fresh biweekly and were stored at 5 C in foil-covered containers.

The dye reduction tests involved addition of 1 ml of either dye and 1 gram of ground pork aseptically to duplicate tubes of the prepared milk. All tubes of the reaction mixture were agitated briefly on a Vortex Mixer, incubated at 30 C in a covered water bath, and observed every 30 minutes for color change. Results were recorded as both first and second stage reduction times in hours for each sample treatment using resazurin and TTCS dyes. Colors noted in the reaction mixtures were

compared with Munsell Color Standards (Munsell Color Company, Incorporated). The first stage reduction of blue resazurin to pink resorufin was considered complete when the color of the reaction mixture was similar to Munsell Color 5 RP 8/5. Second stage reduction of the dye was complete when the mixture became white as a result of the formation of dehydroresorufin. The first stage reduction of TTCS involved formation of a pink compound which approximated the Munsell Color 2.5 R 9/3; a red formazan was produced during the second stage reduction of TTCS. When the color of the reaction mixture approached that of Munsell Color 10 RP 6/2 the reduction was considered complete.

Experimental Design and Statistical Analysis

The experiment comparing control and bacterially inoculated pork tissues consisted of five trials (I-V) which each involved two animals given different inoculation levels (light or heavy) of bacterial suspensions. A different bacterial inoculum was used for each trial with the exception of Trial III. This will be explained in the discussion of Trial III following on page 57. Each animal received two treatments (the right half of the loin was a control sample and the left half was inoculated with a suspension of bacteria. Other differences involved storage of each of the treatments at two different temperatures (2 C and 10 C) for seven different storage times (0, 2, 4, 8, 12, 16, and 20 days). Each trial then consisted of 56 subsamples on each of which the following measurements were made: ERV, first and second stage dye reduction times for resazurin and TTCS, pH, and bacterial numbers.

The data were presented to the Michigan State University Computor Laboratory for analysis by Fortran 3600 according to the BASTAT

routine which calculates statistics for individual variables and for pairs of variables. Determined for all variables were sums, sums of squares, means, standard deviations from the means, and standard deviations. Statistics concerning pairs of variables included simple correlations and simple least squares statistics (least squares coefficients, standard errors of the estimates for the simple least squares equations, and sums of squares and cross-products). In some instances regression equations were set up (to show prediction of one variable from another) with standard errors of the estimates to indicate the spread of points about the regression line. The data were also analyzed by the AOV routine. Analyses of variance indicated which sample means and interactions were recognized as significant sources of variation in the experiments.

Effects of *C. perfringens* on ERV, Dye Reduction Times, pH, and Bacterial Numbers

The original experimental conditions were recognized as not being conducive to the growth and enumeration of *C. perfringens* in the pork tissue because the samples were stored aerobically at low temperatures and were not plated in a medium allowing growth of anaerobes. Drastic changes in the procedure would have eliminated Trial V from the statistical analysis; therefore, Trial V was conducted in the same manner as Trials I-IV. An additional experiment was performed in order to study the effects of an actively growing proteolytic anaerobe (*C. perfringens*) on the test criteria. At the same time observations of the effects which high temperature storage of samples had on the test parameters were made. The inoculated samples were expected to show the

effects of both bacterial growth and high temperature storage while the control samples would exhibit only the temperature effects if they were obtained bacteria-free. The experiment was performed according to the original design with the following modifications: The samples, after inoculation with C. perfringens or treatment with sterile water, were stored at 35 C in a nitrogen-flushed desiccator for 0, 2, 4, 6, and 8 days. Subsamples for plating were blended 15 seconds in order to minimize cell injury. Plating of the subsamples was done in both sulfite-polymyxin-sulfadiazine (SPS) agar and APT agar; incubation of the plates was at 35 C both aerobically and anaerobically. The ERV and dye reduction tests were performed as they were in Trials I-IV. Plating of control and inoculated samples in SPS and APT agar both aerobically and anaerobically was done so that initial contamination of the samples by aerobic or anaerobic organisms could be detected in addition to the intentional contamination added. Obviously these conditions of incubation did not allow for growth of typical psychrophilic meat contaminants.

RESULTS AND DISCUSSION

The bacterial data for the samples of pork tissue within individual trials (I-V) were graphed separately according to inoculation levels. All graphs marked A show data of control samples and samples which received a light inoculation of bacteria; all B graphs pertain to control samples and those samples which were inoculated with a heavy suspension of bacteria. An identical procedure was followed for graphical presentation of the ERV (extract-release volume) data. The effects of storage of all samples at two different temperatures (2 C and 10 C) were also indicated on the graphs. For statistical analysis, however, the bacterial level, ERV, and dye reduction data for both inoculation levels and storage temperatures were pooled. Correlation analyses were performed on the pooled data within each trial and analyses of variance within each trial aided in interpretation of the data. The entire array of control data for Trials I-V was pooled and subjected to correlation analysis; a similar procedure was followed for the data for the inoculated samples of Trials I-V.

Small numbers of organisms in the control samples were detected by the plating of very low sample dilutions. When 10.0 ml of a 1:10 dilution of sample were distributed equally among three Petri plates a total of 1.0 gram of sample was plated. It was assumed, then, that even though no colonies appeared on the plates after incubation, the bacterial population of the particular sample was not necessarily 0 per gram but

rather was approximately 0.1 organism per gram. The value -1.0 (the log of 0.1) was used in the statistical analysis in every instance where no organisms were demonstrated in the sample by plate counts. Zero was used as the log of bacterial numbers on the graphs whenever no growth appeared on the plates examined. Each graph which has no growth curve for a particular sample indicates that no organisms were recovered from the sample in question. For example, Figure 4 on page 42 indicates by the absence of growth curves for the control samples that those samples were bacteria-free (contained less than 0.1 organism per gram) for the entire storage period. When bacterial populations in a sample were initially greater than 0 but then decreased to 0 for the remainder of the storage period the growth curve for that sample terminates at the x-axis (Figure 11, page 62). The code listed below was used for all figures, tables, appendices, and in the following discussions;

TTCS = the tetrazolium salt 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride
Res 1 = time (hrs) for reduction of resazurin to resorufin (1st stage)
Res 2 = time (hrs) for reduction of resorufin to dehydroresorufin (2nd stage)
Tet 1 = time (hrs) for reduction of TTCS to a pink compound (1st stage)
Tet 2 = time (hrs) for reduction of TTCS to a red formazan (2nd stage)
Logba = log of bacterial numbers - by aerobic count
Logban = log of bacterial numbers - by anaerobic count
pH = pH of meat extract after filtration
ERV = extract-release volume (ml)
A-2 = lightly inoculated samples stored at 2 C
A-10 = lightly inoculated samples stored at 10 C
(tissue taken from 1st hog ea. trial; 24 hrs post-mortem)

B-2 = heavily inoculated samples stored at 2 C
B-10 = heavily inoculated samples stored at 10 C
(tissue taken from 2nd hog ea. trial; 48 hrs post-mortem)

control = sample treated with 10.0 ml sterile water
inoculated = sample inoculated with 10.0 ml of a bacterial suspension

The results for the individual trials are discussed separately. Following these discussions are a general comparison of the control and inoculated sample data obtained from all trials and a brief summary of the experiment concerning further effects of C. perfringens on the test parameters.

Trial I

The relationships between storage time and ERV and the accompanying storage temperature effects on the control samples and the samples receiving light (A) or heavy (B) inoculations of P. fluorescens are presented in Figures 1 and 2 on pages 38 and 39, respectively. Appendices A and B give complete listings of treatments, storage temperatures, storage times, mean ERV values, average dye reduction times, logs of bacterial numbers, and pH values for all sample observations in Trial I.

Observation of the Trial I data showed that the initial ERVs of the meat taken from two hogs were quite different from each other whereas the ERVs of the meat taken from a single hog were more consistent with each other in value. The ERVs for A and B control samples on day 0 of storage were 36.5 ml and 50.5 ml while the ERVs of the A and B inoculated samples were 35.0 ml and 54.2 ml. The differences in initial ERVs of the meat from different hogs of approximately the same age may have limited the value of the ERV phenomenon for the estimation of the microbial quality of pork in Trial I. When the B inoculated samples exhibited frank spoilage as determined by bacterial count and organoleptic observation the ERVs were 38.0 ml (B-2) and 26.0 ml (B-10). The ERV value of the B-2 inoculated sample at spoilage was not significantly different from the ERV values of the A control samples at freshness which were 36.5 ml (A-2) and 35.0 ml (A-10). The inoculated A samples, however, reached much lower ERV values of 19.5 ml (A-2) and 16.0 ml (A-10) at frank spoilage. The fact that the ERVs of the A samples were similar (control ERV = 36.5 ml and inoculated ERV = 35.0 ml) on day 0 of storage when the control sample contained no viable bacteria and the inoculated

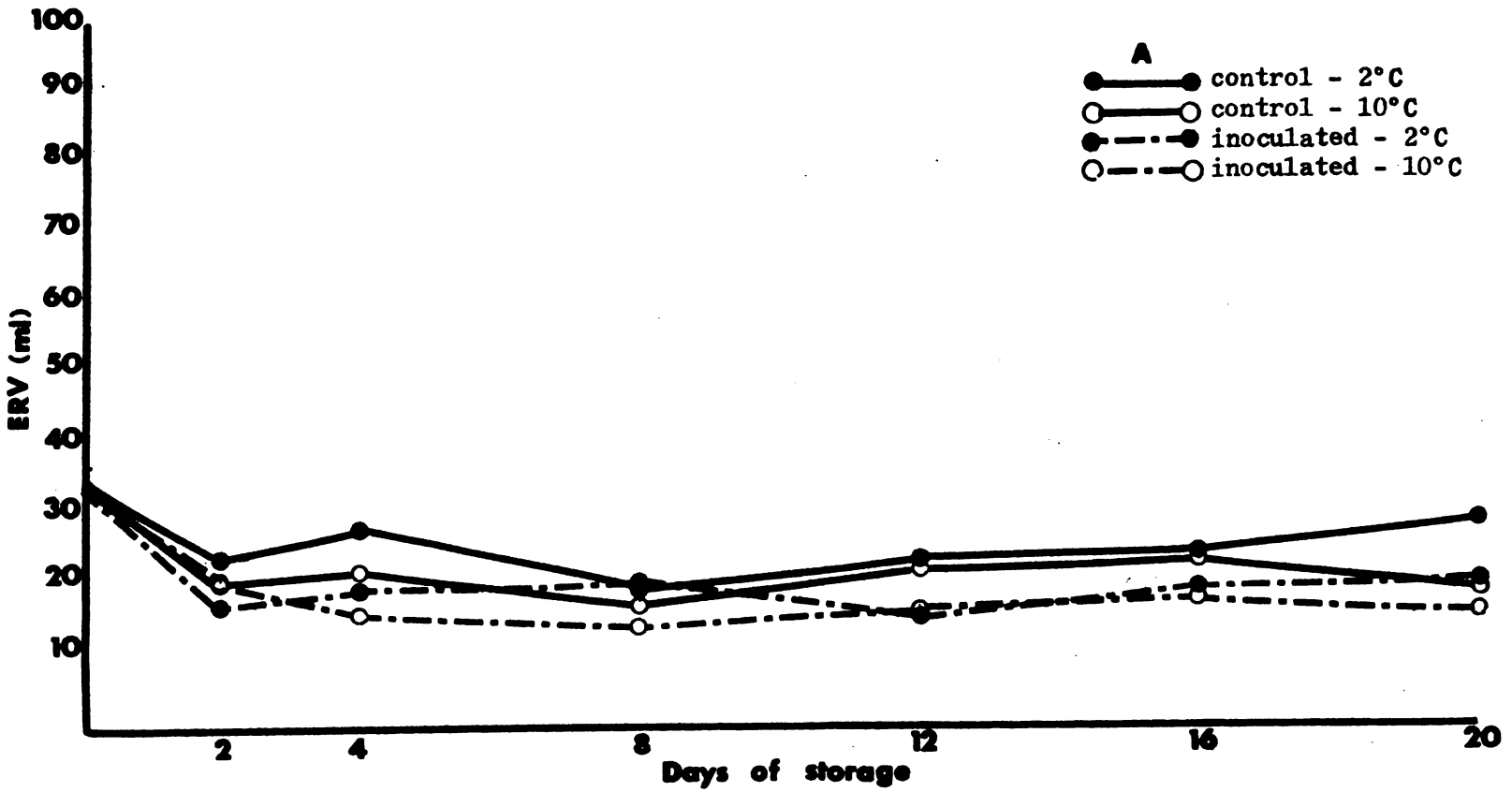


Figure 1. **Relationship of storage time and ERV of control and inoculated samples. (*Pseudomonas fluorescens*)**

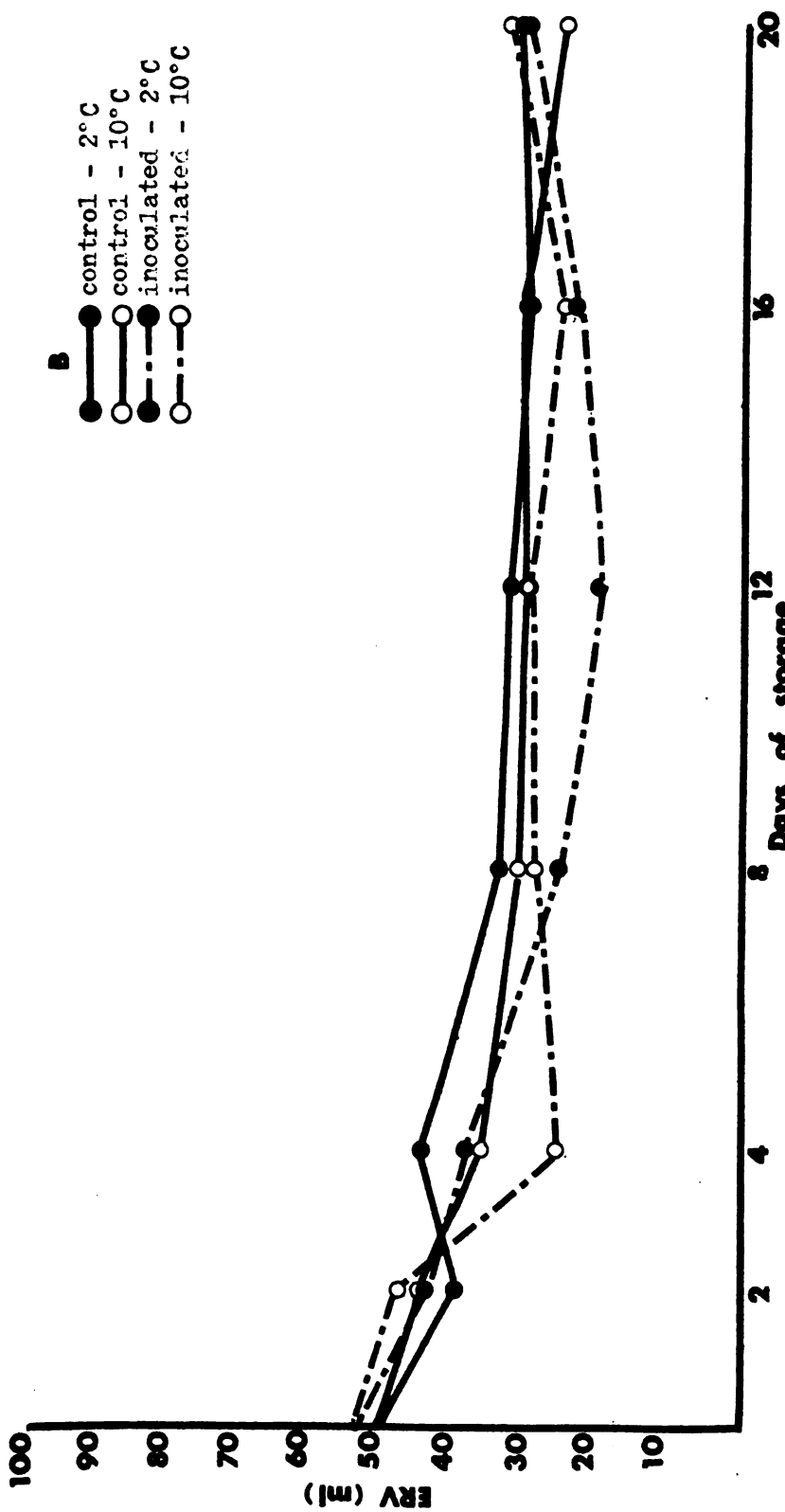


Figure 2. Relationship of storage time and ERV of control and inoculated samples. (Pseudomonas fluorescens)

sample carried a bacterial population of log 5.11 (130,000 organisms per gram) was an indication that the drastic reduction observed in ERV of the inoculated samples during storage was a result of the active growth of the bacteria and was not merely due to presence of microorganisms in the sample. The ERVs of the B samples were also similar on day 0 of storage when the inoculated sample carried a bacterial load of log 6.56 (3.6 million organisms per gram) and the control sample was bacteria-free.

The control A-2 and B-2 samples and the inoculated samples A-2, B-2, and B-10 increased slightly in ERV during the last 4 days of storage; the ERVs of the remaining three samples decreased slightly during the same time period. The general trend of the ERVs observed in Trial I was a fairly rapid initial decrease in value (usually occurring within the first 2 days of storage) which stabilized and was then followed by a scattering of values toward the end of the storage period.

The relationships between storage time and bacterial numbers for A and B control and inoculated samples are presented in Figures 3 and 4 on pages 41 and 42. Maximum bacterial numbers in all Trial I inoculated samples except B-10 were reached by day 16. The B-10 inoculated sample attained its maximum bacterial load by day 12. Though the inoculation levels of the A and B samples were quite different the maximum bacterial populations observed in the samples were similar. The peak populations were log 9.69 (5.0 billion organisms per gram) in sample A-2, log 10.57 (37 billion organisms per gram) in sample A-10, log 10.19 (15 billion organisms per gram) in sample B-2, and log 9.57 (3.7 billion organisms per gram) in sample B-10. Bacterial populations in the heavily

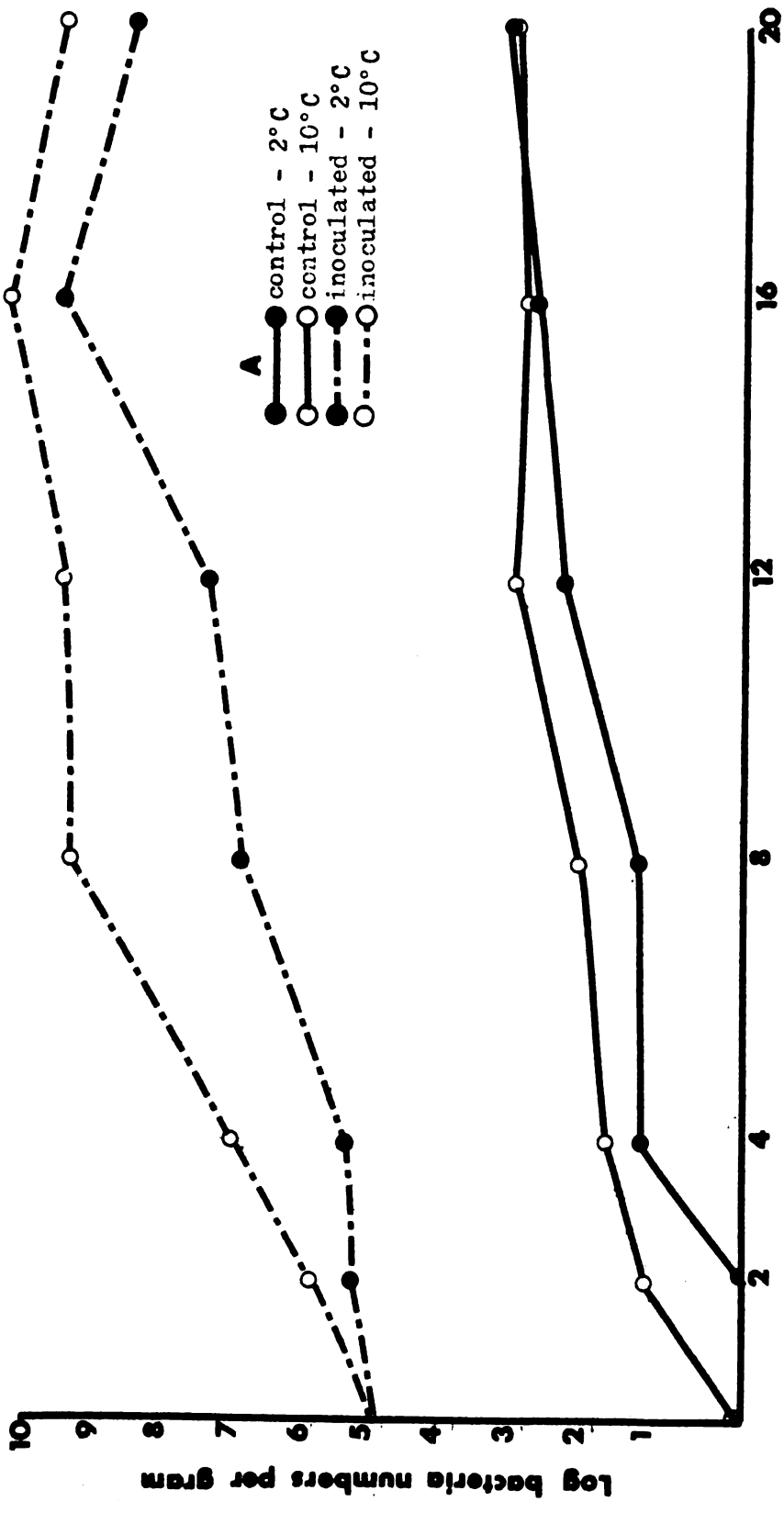


Figure 3. Relationship of storage time and log bacteria numbers of control and inoculated samples. (Pseudomonas fluorescens)

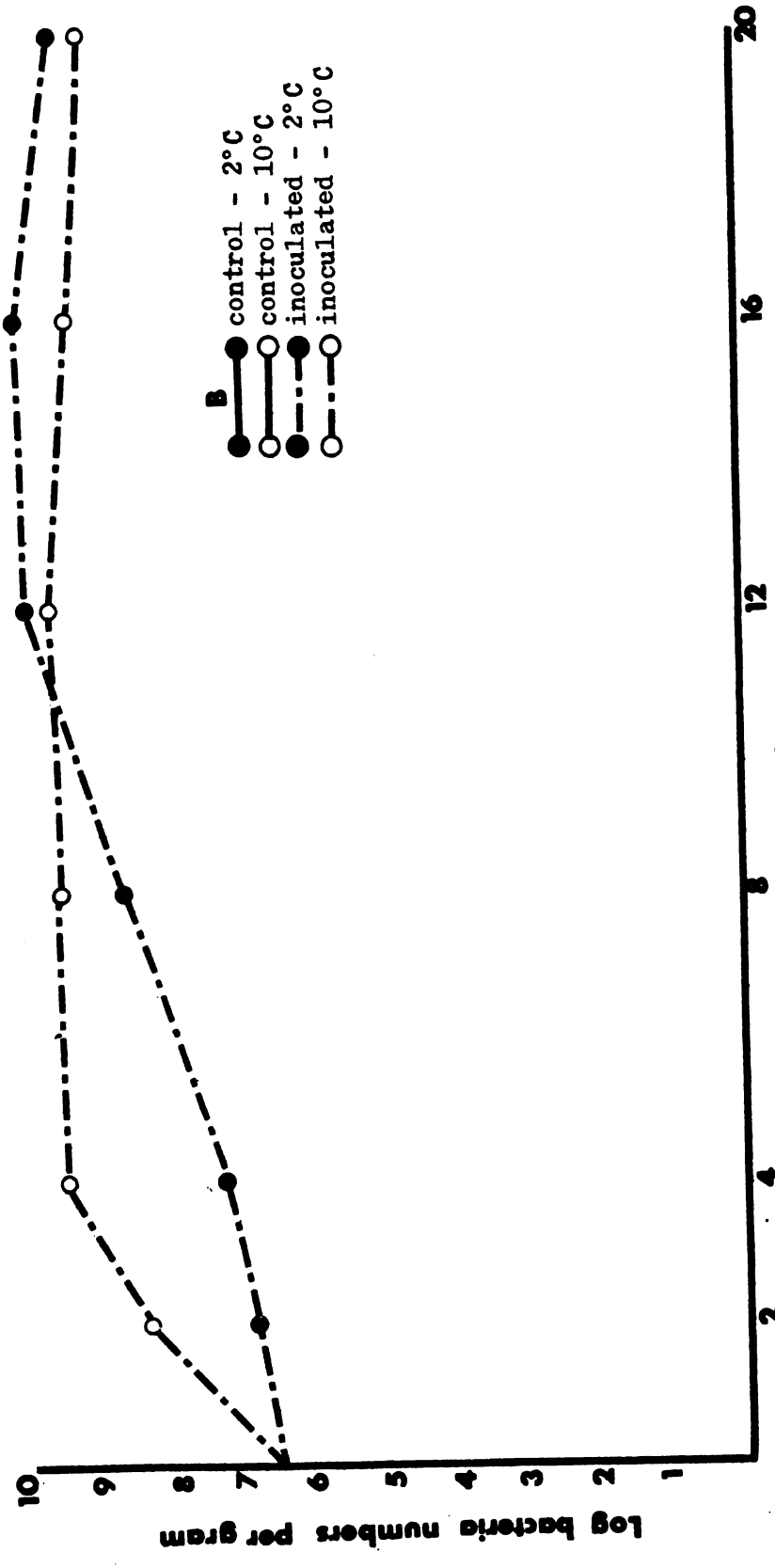


Figure 4. Relationship of storage time and log bacteria numbers of control and inoculated samples. (Pseudomonas fluorescens)

inoculated B samples were higher until day 8 when the population in the A-10 inoculated sample exceeded that of the B-10 inoculated sample. All B inoculated samples were considered organoleptically unacceptable by day 4 of storage because they had developed an off-color, slimy, putrid condition, the bacterial populations at that time were log 7.19 (15 million organisms per gram) in the B-2 sample and log 9.42 (2.6 billion organisms per gram) in the B-10 sample. The A inoculated samples were also considered unacceptable on day 4 when they carried bacterial loads of log 5.64 (440,000 organisms per gram in sample A-2) and log 7.26 (18 million organisms per gram in sample A-10). These increases in bacterial numbers tended to coincide with decreases in ERV values especially between day 2 and day 8 of the storage period.

The procedure for obtaining bacteria-free pork samples was partially successful in Trial I. All B control samples were bacteria-free for the entire storage period of 20 days while the A-10 control sample was only slightly contaminated on day 0 with log 1.30 or 20 organisms per gram. By day 20 the A-10 control sample carried a bacterial population of log 3.34 (2,200 organisms per gram). No organisms were recovered from the A-2 control sample until day 4 when the contamination was log 1.30 (20 organisms per gram). By day 20 the bacterial population in the A-2 control sample was log 3.32 (2,000 organisms per gram). The delayed appearance of a bacterial population in the A-2 control sample could have been an indication that the contaminating organisms were not psychrophilic and were therefore experiencing adverse growth conditions at the storage temperature of 2 C. Borton (1966) was able to procure a pork sample which contained only 10 organisms per gram on day 0 of

storage; that sample, however, attained a total bacterial population of 1 million organisms per gram by the 17th day of storage.

Dye reduction times reported for Trial I (Appendices A and B) for the first and second stage reductions of resazurin and the tetrazolium chloride salt, TTCS, showed some interesting trends. The first stage reduction times, Res 1 and Tet 1, by all samples were never greater than 2.0 hours and were, in 156 of 224 observations (69.6%), 1.0 hour. If Res 1 and Tet 1 reduction times were different, Tet 1, in 220 of 224 observations (98.2%), was the longer of the two. The average second stage reduction times, Res 2 and Tet 2 (Table 1, page 44), showed more variation though only 8 of 224 observations (3.5%) indicated that Tet 2 was less than Res 2.

Table 1. Average second stage dye reduction times (Res 2 and Tet 2) in hours for resazurin and TTCS of A and B control and inoculated samples in Trial I (P. fluorescens inoculation).

Storage time (days)	Control Res 2 (hrs) A	B	Inoculated Res 2 (hrs) A	B	Control Tet 2 (hrs) A	B	Inoculated Tet 2 (hrs) A	B
0	7.0	4.0	5.7	4.5	10.0	5.0	7.0	5.0
2	7.0	6.5	5.0	4.5	9.5	7.5	6.8	4.5
4	7.0	7.5	4.5	3.5	9.0	8.0	6.2	3.8
8	7.2	6.0	2.7	3.0	8.0	7.0	4.0	3.5
12	7.0	5.5	2.7	2.0	7.8	7.5	3.5	2.5
16	7.0	4.5	1.5	1.5	8.0	7.5	1.5	2.0
20	6.5	4.5	2.2	2.0	7.8	6.0	2.0	2.5

The range of Res 2 reduction times for the entire 20-day storage period was 1.0 - 8.0 hours for both A and B groups of samples. Res 2 reduction times longer than 6.0 hours were never reported for any of the inoculated samples. The Res 2 reduction times for the A and B inoculated

samples decreased from 6.0 and 5.0 hours respectively (with bacterial populations of log 5.11 or 120,000 organisms per gram and log 6.56 or 3.7 million organisms per gram) to 1.0 hour with bacterial populations of log 10.57 (37 billion organisms per gram) and log 10.19 (15 billion organisms per gram) respectively as the growth curves peaked at about the 16th day of storage.

The range of Tet 2 reduction times was from 5.0 - 8.0 hours in the bacteria-free control samples of the B group and 7.5 - 10.0 hours in the slightly-contaminated A control samples. The shortest Tet 2 reduction time noted for any of the A control samples was 7.5 hours when the bacterial load of the sample was log 3.32 (2,000 organisms per gram). Table 1 shows the average second stage dye reduction times without reference to inoculation level or storage temperature. As was the case with the Res 2 reductions times the lowest value for Tet 2 reduction was observed for the inoculated sample as the growth curves peaked on day 16 (Figures 3 and 4, pages 41 and 42). The shortest Tet 2 reduction time of 1.0 hour observed for the A-10 inoculated sample on day 16 corresponded to the highest bacterial population of log 10.57 (37 million organisms per gram) observed in Trial I (Appendix A). Tet 2 reduction times of 2.0 hours were noted in several instances where bacterial numbers tended to be high and ranged from log 8.75 (560 million organisms per gram) to log 10.19 (15 billion organisms per gram).

Average second stage reduction times, Res 2 and Tet 2, decreased more rapidly with length of storage time in both inoculated samples than in the control samples (Table 1, page 44). Variations in Res 2 and Tet 2 reduction times among the control samples were not great and were

seemingly unrelated to bacterial populations as might be expected. The largest and most significant differences in second stage reduction times for both dyes were between inoculated and control samples; differences in inoculation level and/or animals (tissue source) significantly influenced Res 2 and Tet 2 reduction times in relation to corresponding changes in total numbers of bacteria (Appendix K, tables a and b). Obviously, variation in storage time accounted for many differences in second stage reduction times particularly in the A and B inoculated sample groups.

The A and B control samples showed pH changes of -0.2 pH unit from the slurry buffer pH of 5.8 while the A and B inoculated samples exhibited pH values ranging from 5.6 to 5.9. The fact that the slurry was buffered may have indicated that the pH of the meat extract did not truly reflect pH changes which P. fluorescens caused in the meat as spoilage progressed. It was expected that P. fluorescens growth might result in pH increases in the meat as the proteolytic enzymes of the organisms released ammonia and amines from the meat proteins. An alkaline reaction was observed in only one sample (B-2 inoculated) on the 12th day of storage.

Table 2 on page 47 shows the simple correlation coefficients and their levels of significance calculated between all variables in Trial I. Jay and Kontou's report (1964) stated that the correlation between ERV and bacterial numbers in ground beef contaminated with normal psychrophilic flora was high enough ($r = -0.808$) to justify the use of the ERV phenomenon as a rapid indicator of the microbial quality of beef. With the use of a pure culture of a proteolytic organism (P. fluorescens) for

inoculation of the samples in Trial I, and recognizing that ground pork was used rather than ground beef, it was expected that correlation values significantly different from that observed by Jay and Kontou would be obtained.

Table 2. Simple correlation coefficients calculated between all variables (Time, Res 1, Res 2, Tet 1, Tet 2, Logba, pH, and ERV) for Trial I (*P. fluorescens* inoculation).

	Time	Res 1	Res 2	Tet 1	Tet 2	Logba	pH	ERV
Time	1.00							
Res 1	-0.41	1.00						
Res 2	-0.38	0.62	1.00					
Tet 1	-0.27	0.26	0.51	1.00				
Tet 2	-0.37	0.62	0.92**	0.55	1.00			
Logba	0.23	-0.57	-0.76*	-0.37	-0.81*	1.00		
pH	-0.47	0.21	0.03	0.15	0.33	-0.17	1.00	
ERV	-0.47	0.30	0.18	0.18	0.13	-0.34	0.56	1.00

** = significant at the 1% level ($P < .01$)
* = significant at the 5% level ($P < .05$)

The observed correlation between ERV and bacterial numbers in Trial I ($r = -0.34$) was not statistically significant and indicated that under the conditions of Trial I ERV did not accurately predict the microbial quality of the pork samples. ERV was positively correlated with pH; the opposite effect would have been expected considering the usual pH effect on water-binding properties of muscle proteins. Ordinarily ERV decreases as pH increases. Tissue characteristics or animal differences had a greater influence on ERV of pork than did bacterial growth. Statistical treatment of the data (Appendix K, tables c and d) indicated that a large portion of the variation in ERV values could be attributed to animal and/or inoculum level differences. Inoculation treatment and

storage time also contributed significantly to differences in ERV. The significant inoculation level x time interaction (Appendix K, table c) would be expected from observations of trends in the ERV data.

The relationship between dye reduction times Res 2 and Tet 2 was highly significant ($P < .01$) with a correlation coefficient of $r = 0.92$. The only other correlations of statistical significance were those observed between Logba and Res 2 ($r = -0.76$, $P < .05$) and between Logba and Tet 2 ($r = -0.81$, $P < .05$). Because the correlation between Res 2 and Tet 2 was so high ($r = 0.92$) the similar relationship between Logba and both second stage dye reduction times was expected. Roughly 58.0% and 66.0% of the variation in bacterial numbers (Logba) was predicted by Res 2 and Tet 2 reduction times, respectively. Regression equations and standard errors of the estimates for these predictions were as follows:

$$\text{Logba} = -1.595 \text{ Res } 2 + 11.783 \pm 2.835$$

$$\text{Logba} = -1.385 \text{ Tet } 2 + 12.248 \pm 2.527$$

The data obtained for Trial I indicated that ERV was not a reliable rapid indicator of the microbial quality of ground pork; second stage reduction times of resazurin and the tetrazolium salt, TTCS, could be used more accurately to predict the bacteriological status of the pork samples.

Trial II

Borton (1966) suggested that acid-producing bacteria growing in meat would influence the ERV phenomenon in a manner unlike the typical proteolytic meat spoilage organisms because of pH-associated changes in the meat proteins. As bacteria produce acid which lowers the pH of the meat denaturation of the proteins occurs. Proteins in the denatured

state tend to lose their ability to bind water (Lawrie, 1966). Therefore, ERV would tend to increase with reductions in pH. The production of alkaline substances by proteolytic bacteria increases the pH of meat and the proteins appear to retain more fluid at alkaline pHs. ERV would then be expected to be quite low in meat spoiled by the typical low temperature proteolytic spoilage organisms. This phenomenon was demonstrated in Trial IV. The purpose for performing Trial II was the determination of how the ERV test and reduction of resazurin and TTCS would be affected by populations of P. cerevisiae in the pork samples because of the lactic acid produced by the organisms. However, some difficulties were encountered in obtaining outgrowth of P. cerevisiae from the inoculated samples (Figures 7 and 8, pages 53 and 54) so the data obtained from Trial II must be considered of questionable value.

Inoculation of pork samples with P. cerevisiae produced no recoverable bacterial populations in the A or B inoculated samples stored at either 2 C or 10 C until day 8 of the storage period. Viable bacteria were recovered on day 8 from the A-10 inoculated sample (log 6.58 or 3.8 million organisms per gram) and the B-10 inoculated sample (log 7.28 or 19 million organisms per gram). No organisms were recovered from any of the inoculated samples after day 8 though changes in the samples (pH reduction, souring, and off-color) were noted from day 4 until the end of the storage period.

The APT agar used for plating was considered a good growth medium for lactic organisms such as P. cerevisiae. Some homofermentative lactic acid bacteria are fairly fastidious in their requirements for thiamine but because pork is high in thiamine the organism's requirements

for it should have been met. Often these organisms produce enough acid to inhibit themselves; this fact did not seem an applicable explanation for the lack of outgrowth in Trial II because no organisms were recovered from the inoculated samples on days 0 and 2 when, theoretically, acid production would not yet have been limiting. Production of excessive amounts of acid in the pork samples also seemed unlikely because of the lack of sufficient fermentable carbohydrate in the meat. The two hogs slaughtered for Trial II were found to have PSE (pale, soft, exudative) musculature but whether or not the tissue was bacteriostatic is not known. There was also the possibility that the hogs received antibiotic treatment prior to slaughter. If this were true, residual antibiotic in the meat could have inhibited the growth of P. cerevisiae. This explanation dictated that the organisms recovered on the same day of storage at similar levels from the A-10 and B-10 inoculated samples would have been chance contaminants; however, all inoculated samples were sour-smelling during storage and the possibility of the bacterial populations being chance contaminants seemed unlikely. Explanation of the outgrowth failure in Trial II was not possible under the circumstances.

The relationships between storage time and ERV of the A and B control and inoculated samples appear in Figures 5 and 6 on pages 51 and 52. The initial ERVs of all samples in Trial II were much higher than the initial ERVs observed for Trial I samples. Higher initial values may have been a direct result of the PSE musculature of both hogs. Animal variation in ERV was again evident in the pork samples. Initial ERVs for control A (71.0 ml) and inoculated A (65.0 ml) samples were different than the initial ERVs of the control B (60.5 ml) and inoculated B

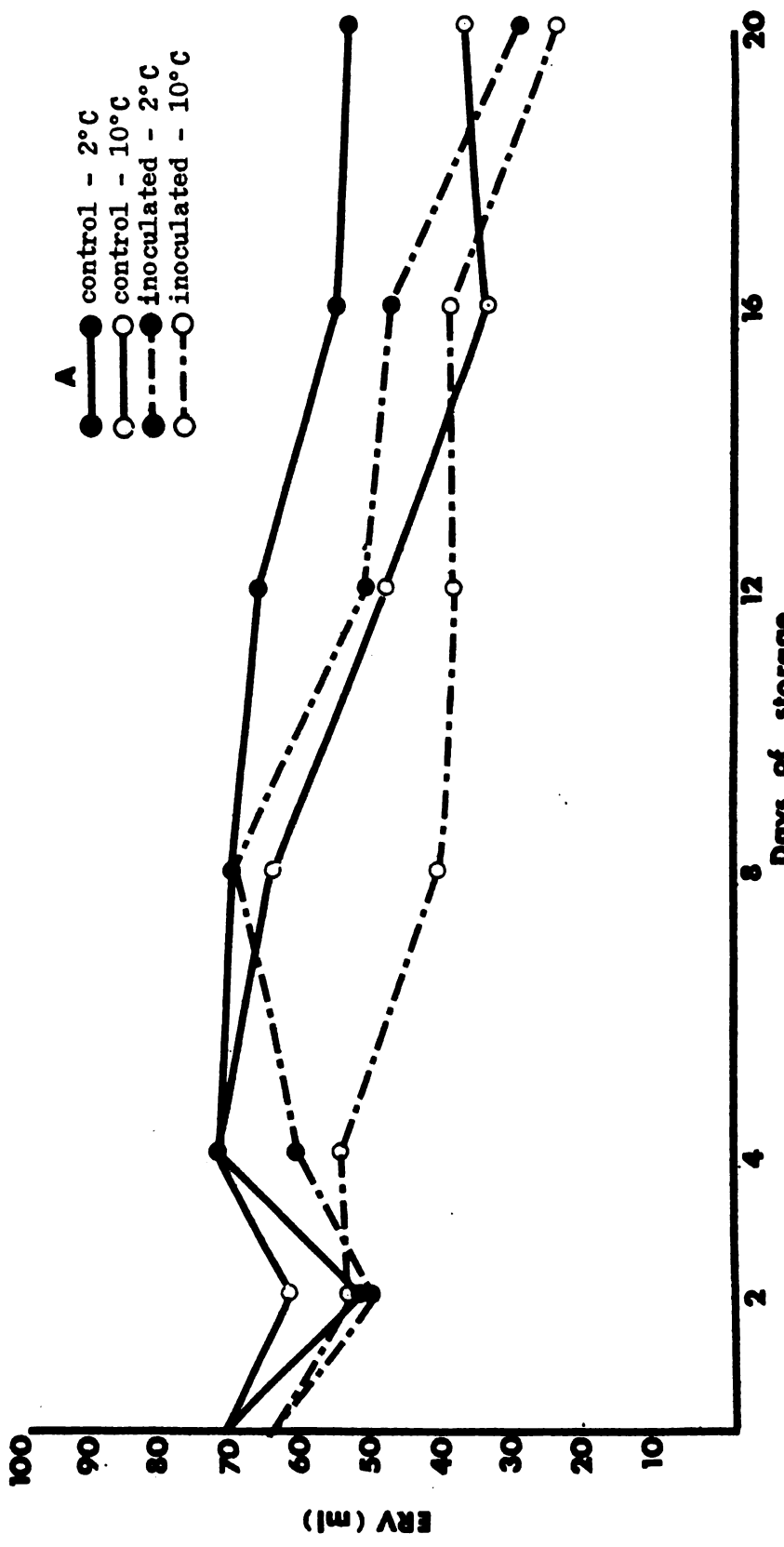


Figure 5. Relationship of storage time and ERV of control and inoculated samples. (Pediococcus cerevisiae)

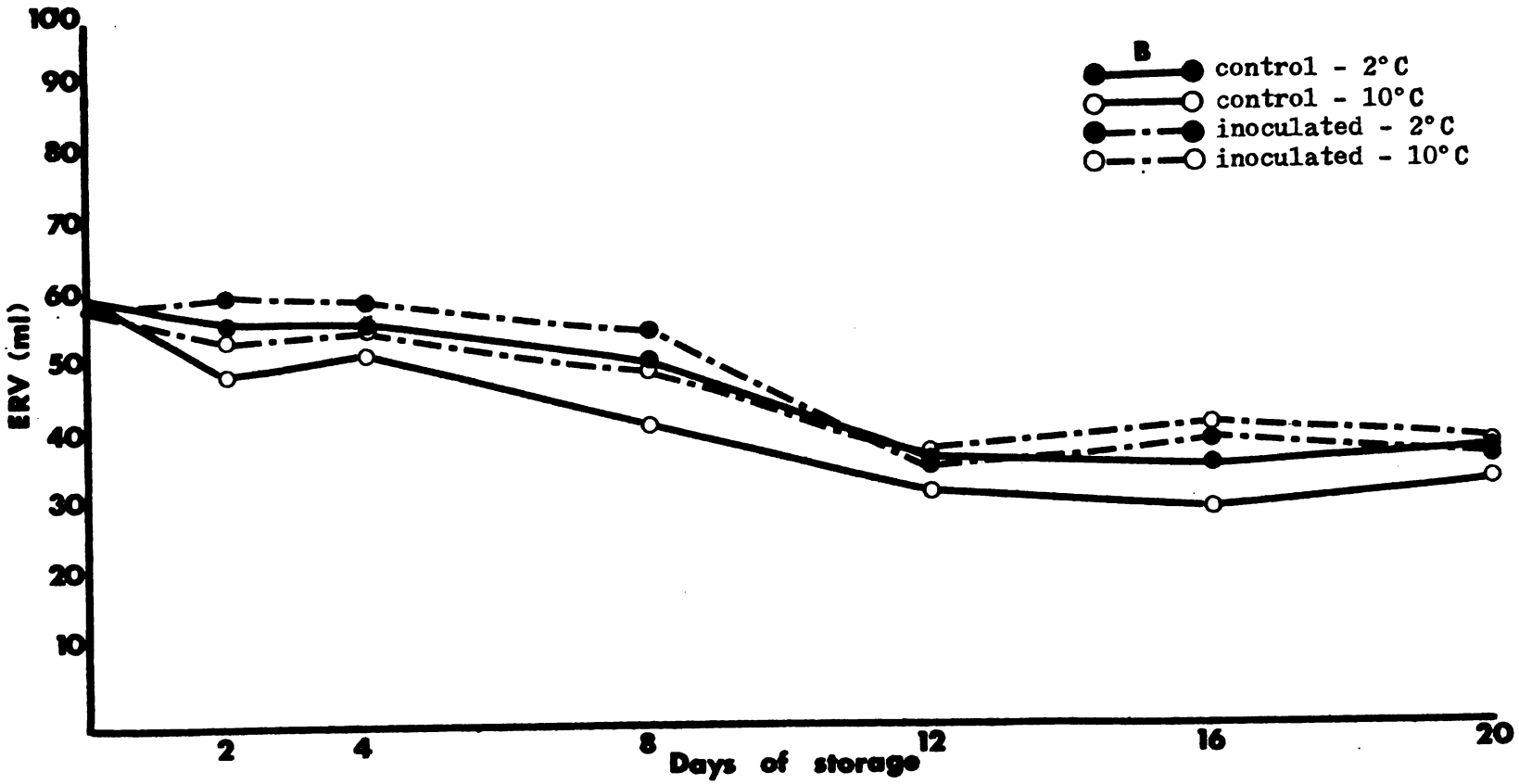


Figure 6. Relationship of storage time and ERV of control and inoculated samples. (*Pediococcus cerevisiae*)

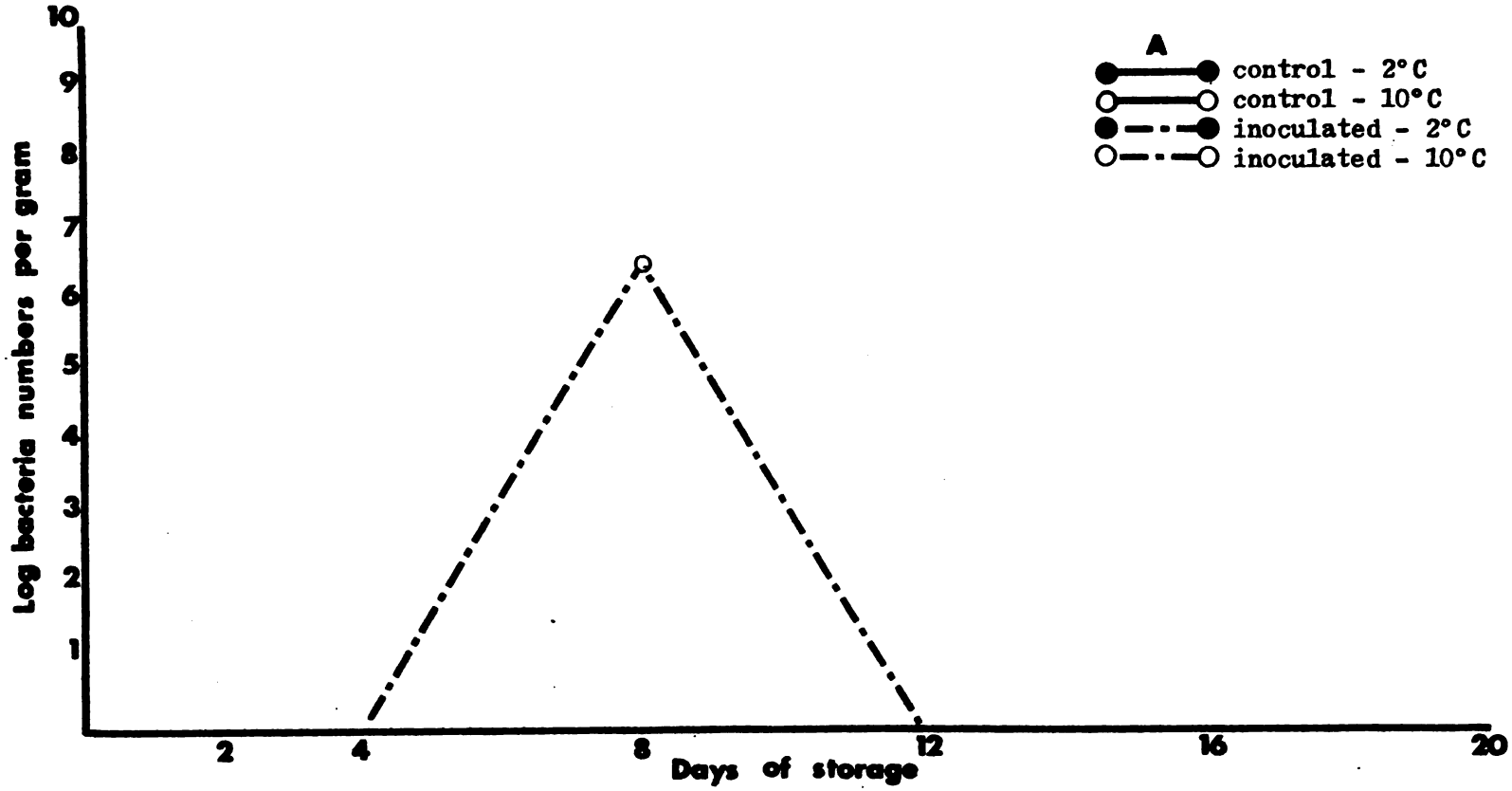


Figure 7. **Relationship of storage time and log bacteria numbers of control and inoculated samples. (*Pediococcus cerevisiae*)**

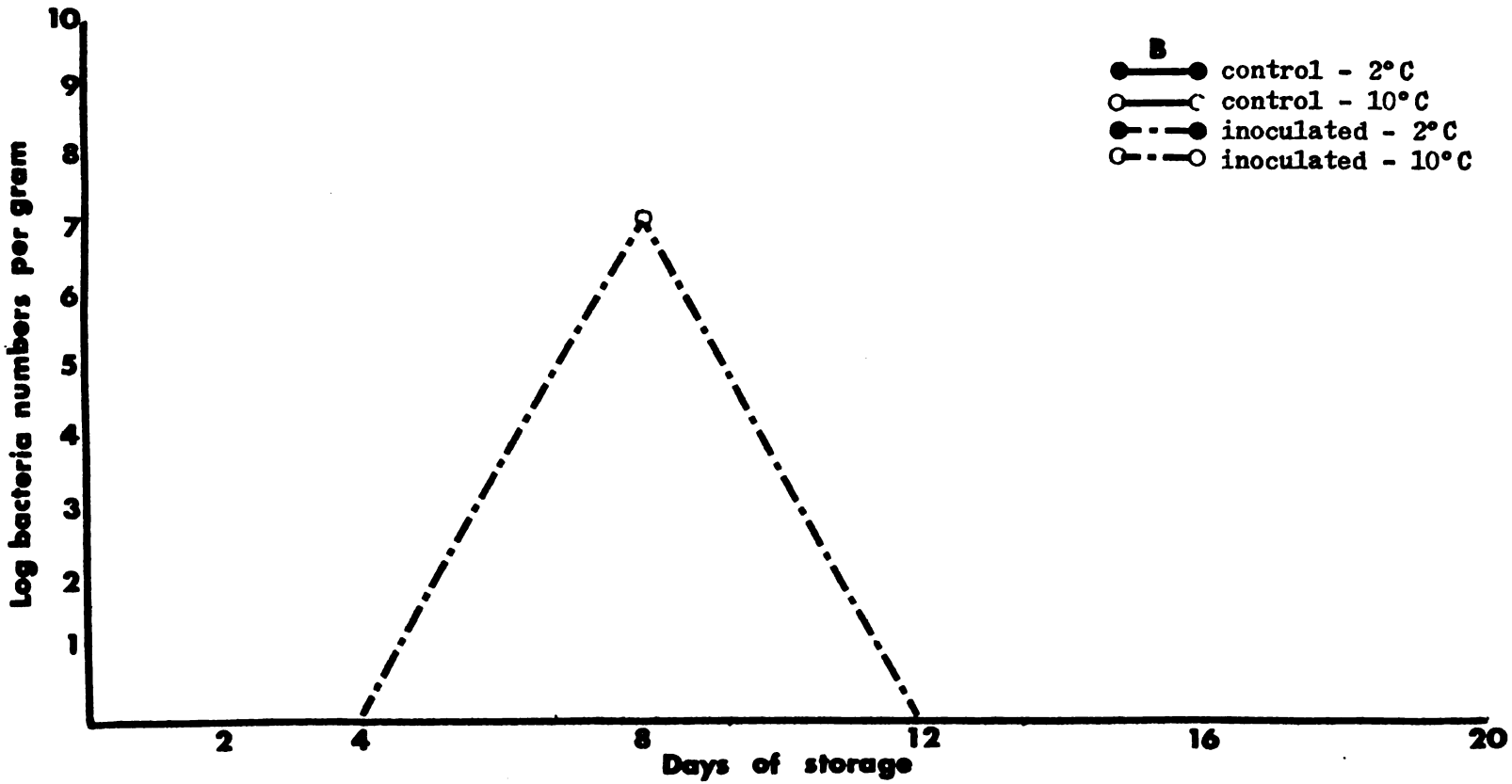


Figure 8. Relationship of storage time and log bacteria numbers of control and inoculated samples. (*Pediococcus cerevisiae*)

(59.0 ml) samples. There was no reason to expect that differences in ERV between control and inoculated samples from the same source should occur on the initial day of storage. Initial day ERV values appeared to be higher for the A samples than for the B samples.

The ERV values for the A samples appeared erratic over the storage period. Following a decline in ERV between day 0 and day 2, values for the control A samples were high on day 4 and slowly declined over the remainder of the storage period. The ERV values for the inoculated A samples held at 2 C followed a similar pattern. However, the ERVs of the inoculated A samples stored at 10 C declined during storage.

The ERV values reported for B samples were more consistent than the ERVs observed for the A sample groups. The B-2 and B-10 control samples had ERVs which dropped steadily from the maximum of 60.5 ml to minimums of 37.5 ml (B-2) and 31.0 ml (B-10) on day 16 and rose slightly on day 20. This trend of ERV for Trial II was similar to that observed for Trial I. The pattern of ERV for both inoculated B samples was quite similar to the control B-2 ERV pattern and no striking differences among the 4 sample groups were evident; there were no plausible explanations for the minor differences shown other than inherent variation in the meat.

All of the data collected for Trial II appear in Appendices C and D. First stage dye reduction times, Res 1 and Tet 1, for both A and B samples were never more than 2.0 hours. First stage reduction times exhibited a tendency to decrease with storage time for the inoculated A-2 and A-10 samples but this tendency was not evident for the B samples. The trend of shorter reduction time with lengthening storage for the

inoculated A samples is also evident in the average second stage dye reduction data for Res 2 and Tet 2 which appear in Table 3 below.

Table 3. Average second stage dye reduction times (Res 2 and Tet 2) in hours for resazurin and TTCS for A and B control and inoculated samples in Trial II (P. cerevisiae inoculation).

Storage time (days)	Res 2 (hrs) Control A	Res 2 (hrs) Control B	Res 2 (hrs) Inoculated A	Res 2 (hrs) Inoculated B	Tet 2 (hrs) Control A	Tet 2 (hrs) Control B	Tet 2 (hrs) Inoculated A	Tet 2 (hrs) Inoculated B
0	6.0	4.0	6.0	4.0	8.0	5.0	8.0	4.0
2	8.0	6.0	7.0	5.0	9.2	7.2	7.5	6.0
4	9.5	7.0	6.5	6.0	10.0	7.5	7.0	6.0
8	9.0	8.5	5.0	5.5	9.0	8.0	6.0	6.5
12	9.0	9.5	4.0	5.5	8.0	8.5	4.5	6.0
16	6.0	9.7	2.0	5.5	7.5	10.0	1.5	6.5
20	5.0	10.0	1.5	5.0	6.0	10.5	1.5	5.0

The reduction times were not compared with bacterial populations because of the previously mentioned outgrowth failures.

The pH of any of the meat-buffer slurries from the ERV procedure was always equal to or less than that of the original buffer (pH 5.8). The pH decrease of 0.5 pH unit with storage time was most evident in the inoculated A and B samples held at 10 C. Changes in pH appeared to parallel the decline in Res 2 and Tet 2 reduction times with the A inoculated samples. These observations seemed to indicate that the organisms added were growing and producing enough lactic acid to sour the samples; pH changes of the order of 0.5 pH unit must be considered fairly important in the well-buffered medium of the extract.

The failure to recover bacteria from a majority of the samples, even though acid was definitely being formed, made it useless to speculate upon any relationships between bacterial numbers and ERV, pH, or

dye reduction times. However, as in all other trials, simple correlation coefficients were obtained between all variables (Table 4, page 57) with pooled data from Trial II. Although there were few significant relationships, pH was again unexpectedly positively related to ERV; ERV was negatively related to storage time ($r = -0.73$, $P < .05$) as in Trial I. A high degree of interrelationship between the two second stage reduction times, Res 2 and Tet 2, was again evident ($r = -0.90$, $P < .01$).

Table 4. Simple correlation coefficients calculated between all variables (Time, Res 1, Res 2, Tet 1, Tet 2, Logba, pH, and ERV) for Trial II (*P. cerevisiae* inoculation).

	Time	Res 1	Res 2	Tet 1	Tet 2	Logba	pH	ERV
Time	1.00							
Res 1	0.13	1.00						
Res 2	-0.06	0.69	1.00					
Tet 1	-0.09	0.41	0.62	1.00				
Tet 2	-0.17	0.64	0.90**	0.61	1.00			
Logba	-0.05	-0.01	-0.10	-0.03	-0.14	1.00		
pH	-0.42	0.12	0.34	0.21	0.49	-0.10	1.00	
ERV	-0.73*	0.02	0.19	0.09	0.30	-0.05	0.57	1.00

* = significant at the 5% level ($P < .05$)
** = significant at the 1% level ($P < .01$)

Statistical analysis of the results from Trial II must be considered of questionable value because of the lack of bacterial data. No conclusions concerning the reliability of the ERV or dye reduction tests as rapid indicators of the microbial condition of the pork samples could be made.

Trial III

Because inconclusive data concerning effects of acid-producing bacteria on the test parameters were obtained from Trial II another

experiment involving lactic-acid organisms was performed. P. cerevisiae was again used. However, because of the possibility that outgrowth of this particular organism again would not be obtained, only half of the inoculated samples were treated with P. cerevisiae. The other half of the samples were treated with S. faecalis, a lactic-acid organism with temperature requirements similar to those of P. cerevisiae. The A group of inoculated samples received a light inoculum of S. faecalis; B inoculated samples were inoculated heavily with P. cerevisiae.

The relationships between storage time, storage temperature, and ERV of the A and B control and inoculated samples are presented in Figures 9 and 10 on pages 59 and 60. Complete listings of Trial III sample data appear in Appendices E and F. Initial ERVs of the A and B samples showed slight animal variation. The A group had ERVs of 60.0 ml (A-2 and A-10 control samples) and 61.5 ml (A-2 and A-10 inoculated samples) while B samples had initial ERVs of 56.5 ml (B-2 and B-10 control samples) and 63.0 ml (B-2 and B-10 inoculated samples). ERV recorded for the A-2 control samples decreased from 60.0 ml to 44.2 ml by day 8 and increased during the remainder of the storage period to 57.0 ml on day 20. The A-10 control sample decreased in ERV from 60.0 ml to 39.5 ml on day 12 and then increased to 50.0 ml by day 20. Initial ERV of the B-2 control sample (56.5 ml) increased steadily over the entire storage period to a maximum value of 70.0 ml on day 20. The B-10 control sample ERV decreased from 56.5 ml to 44.7 ml on day 12 but then the ERV increased to 63.0 ml by day 20 of storage.

Inoculated samples were expected to behave rather dissimilarly because different numbers of two bacterial species were used for

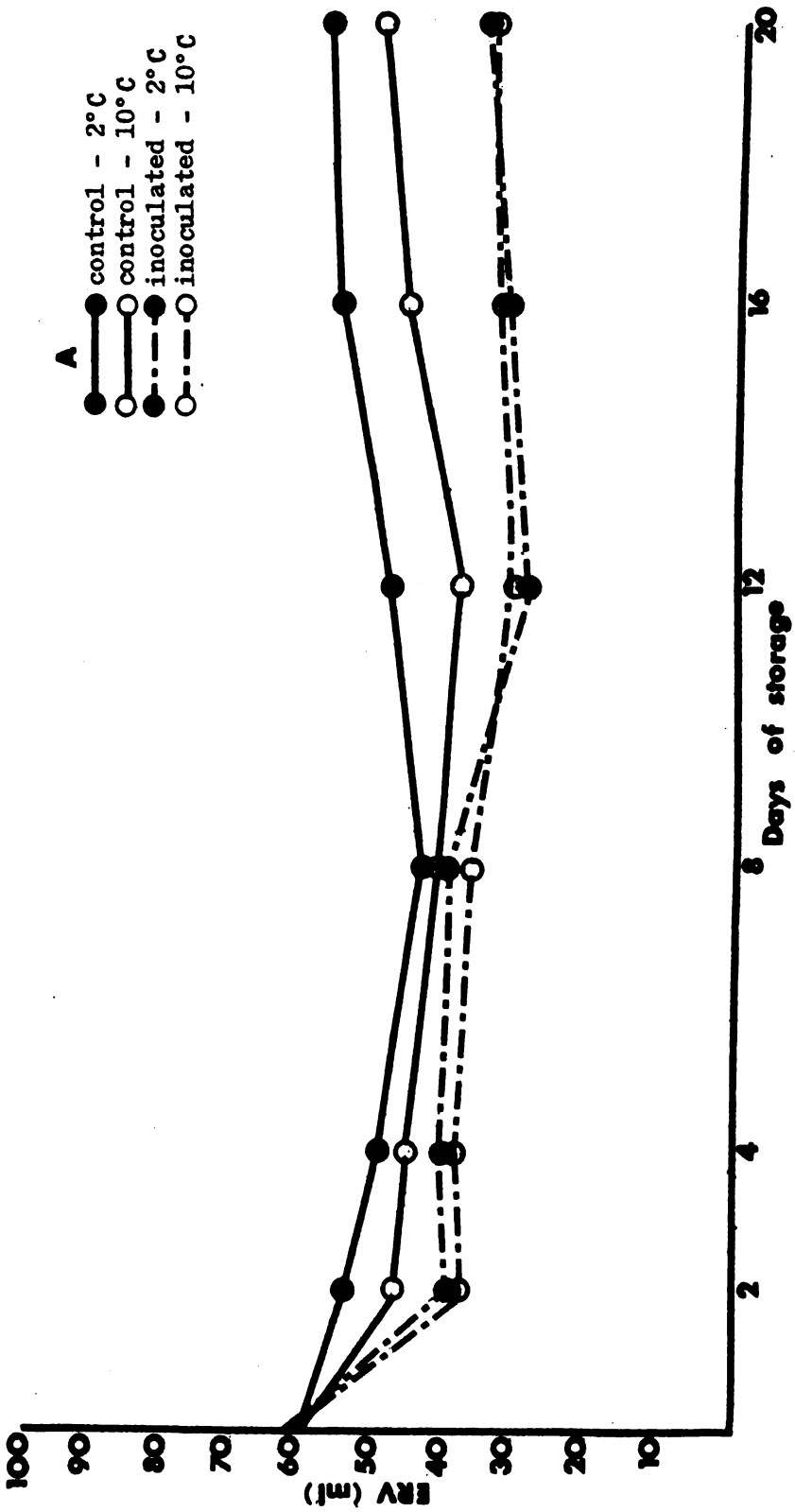


Figure 9. Relationship of storage time and ERV of control and inoculated samples. (Streptococcus faecalis)

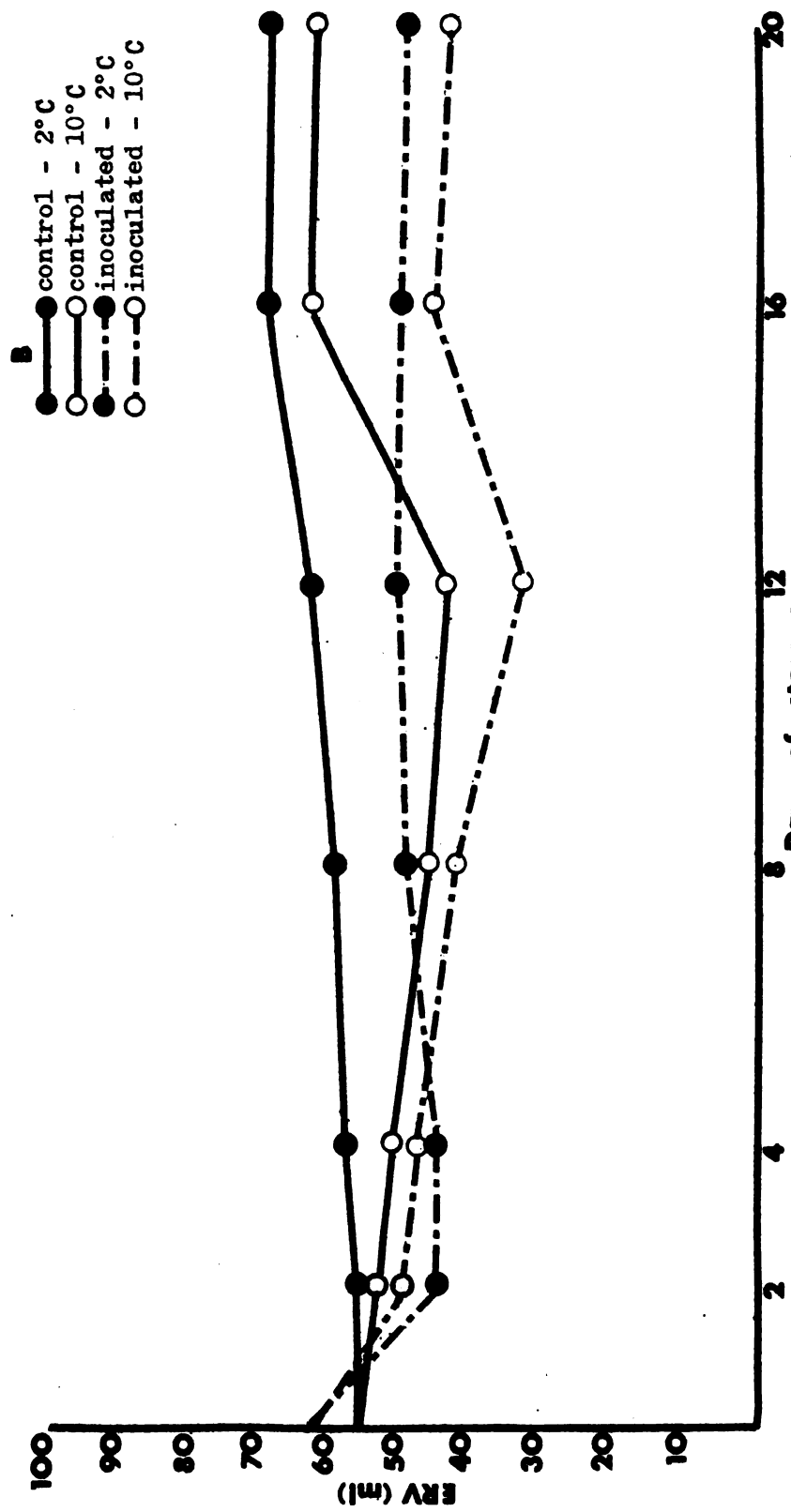
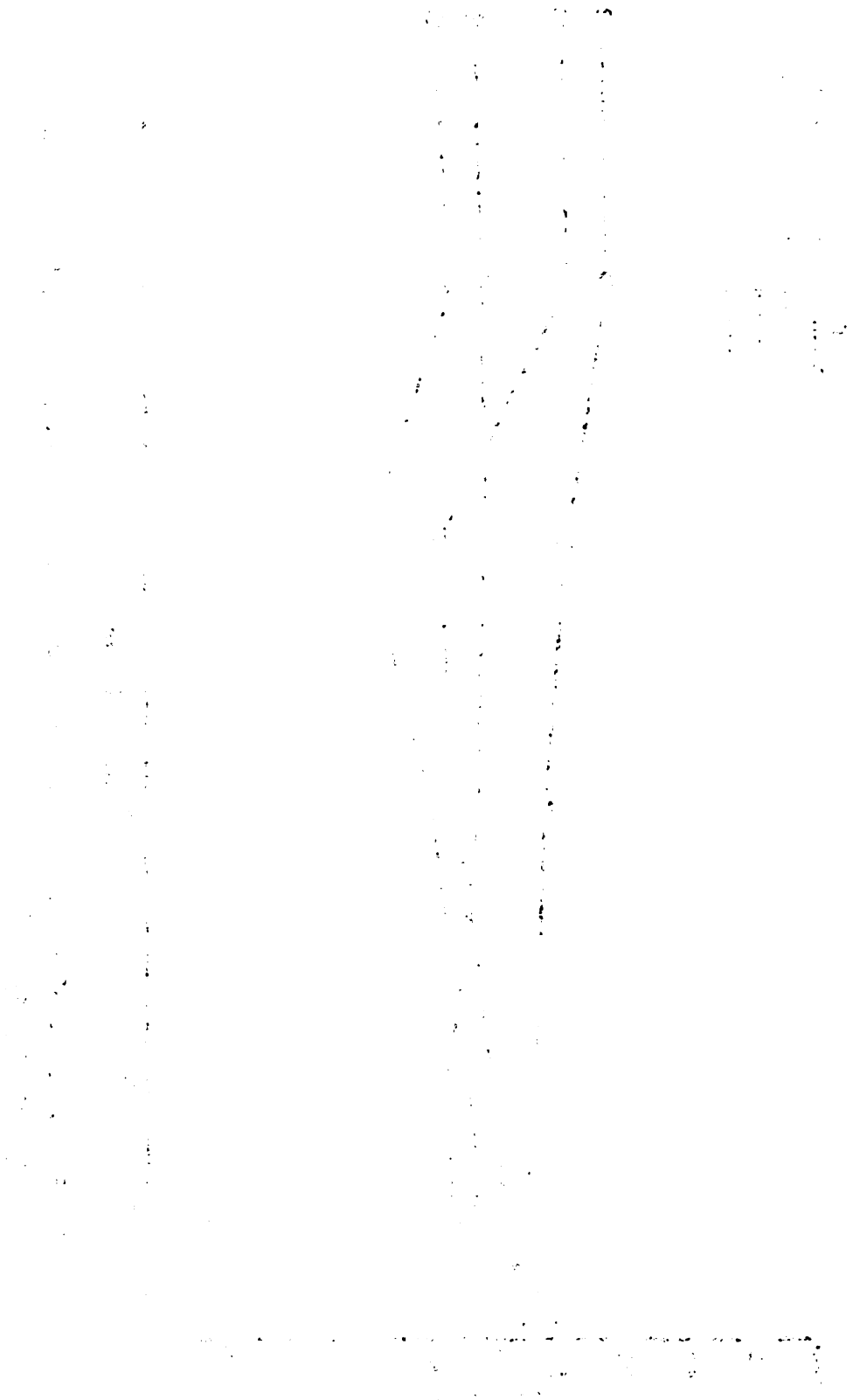


Figure 10. Relationship of storage time and ERV of control and inoculated samples. (Pediococcus cerevisiae)



inoculation of the samples. The ERVs of the A-2 and A-10 samples, initially 61.5 ml, dropped to 38.5 and 39.5 ml at day 2, exhibited a steadying trend followed by a slight decline on day 12, and then tended to increase toward the end of the storage period. Inoculated sample B-2 had an initial ERV value of 63.0 ml which dropped to 44.5 ml on day 2 and then rose steadily and slowly to 50.0 ml on day 20. Following the initial drop in ERV a steady increase was noted in the ERV of sample B-10 through day 12. After that time the ERV increased.

The relationships between storage time and bacterial numbers of the A and B control and inoculated samples of Trial III appear in Figures 11 and 12 on pages 62 and 63 respectively. The A inoculated samples treated with a light inoculum of S. faecalis registered little variation in bacterial numbers at either 2 C or 10 C storage over the entire 20 day storage period. Bacterial populations recovered from the A sample on day 0 were log 4.34 (22,000 organisms per gram). Maximum bacterial numbers in the A-2 and A-10 inoculated samples were recovered on the 12th and 8th days of storage, respectively, when the ERV of either sample was approaching its lowest point. Evidence of chance nonuniform contamination of the A control samples was shown by the presence of bacteria in the A-10 samples initially with outgrowth appearing in the A-2 samples only after day 8 of storage. This sample reached maximum bacterial numbers of log 3.49 (18,000 organisms per gram) on day 16.

The B inoculated sample group which was contaminated with a heavy population of P. cerevisiae attained higher bacterial numbers than did the A samples treated with S. faecalis. Bacterial numbers in the B samples on day 0 were log 4.41 (26,000 organisms per gram). The B-2

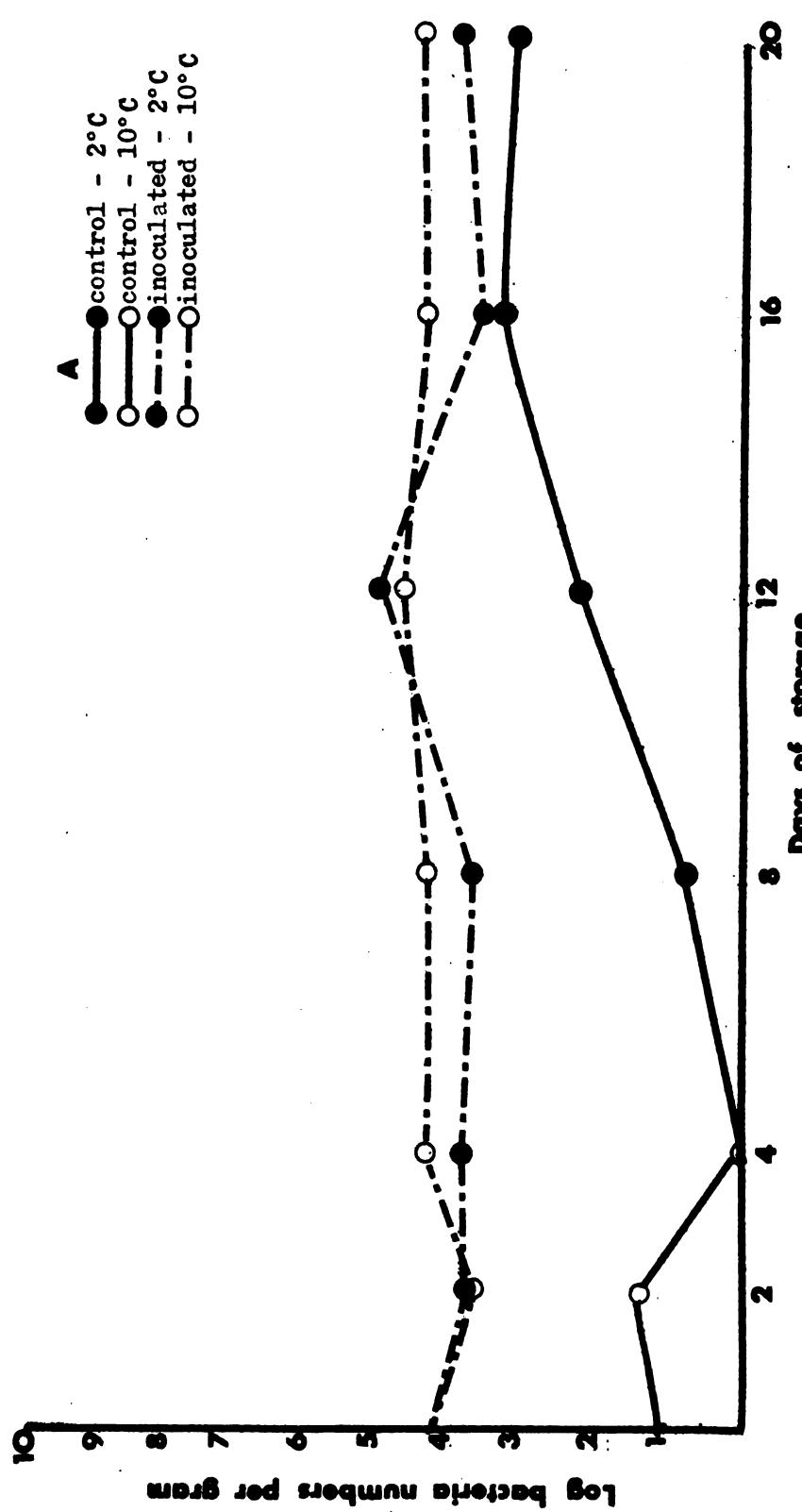


Figure 11. Relationship of storage time and log bacteria numbers of control and inoculated samples. (Streptococcus faecalis)

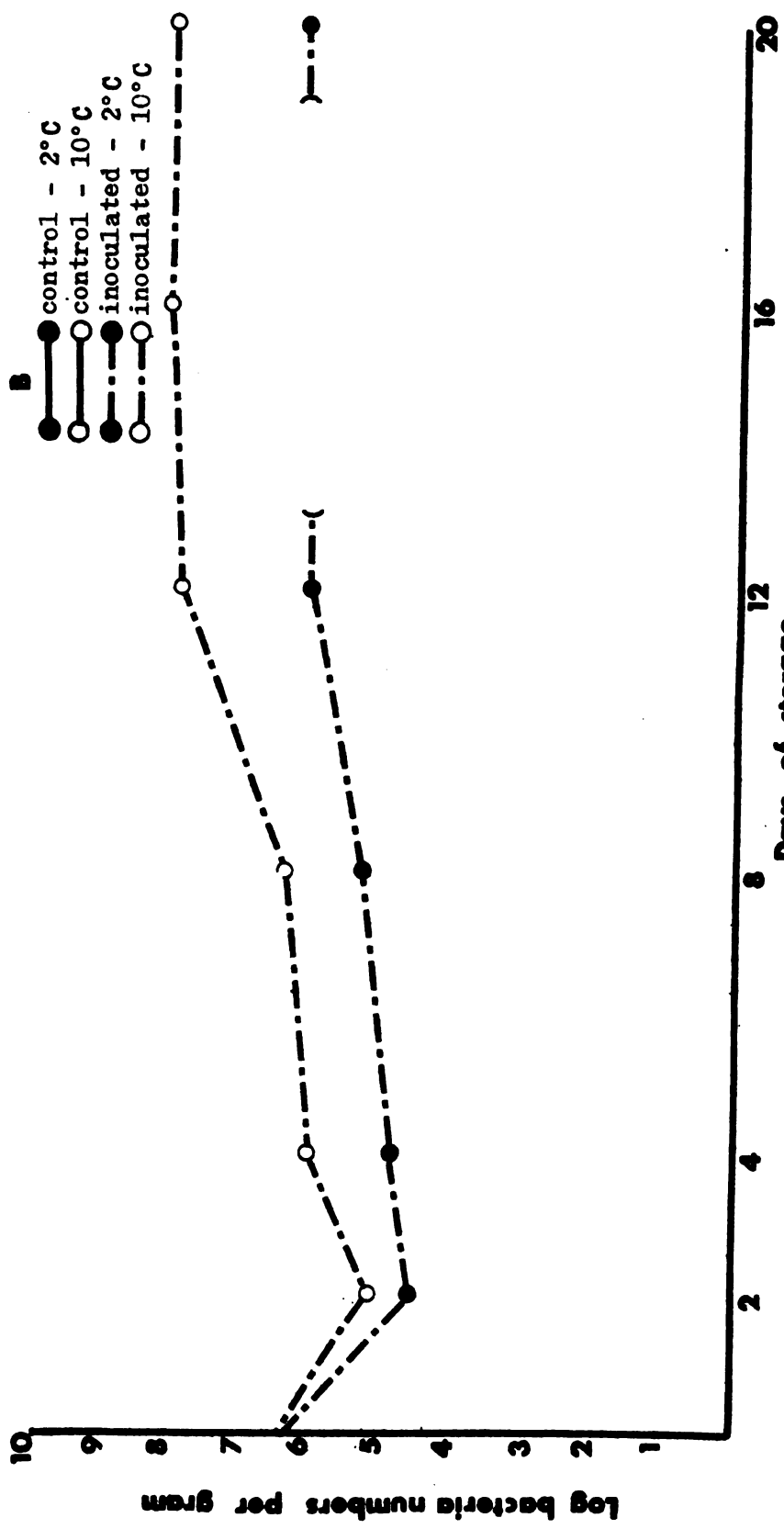
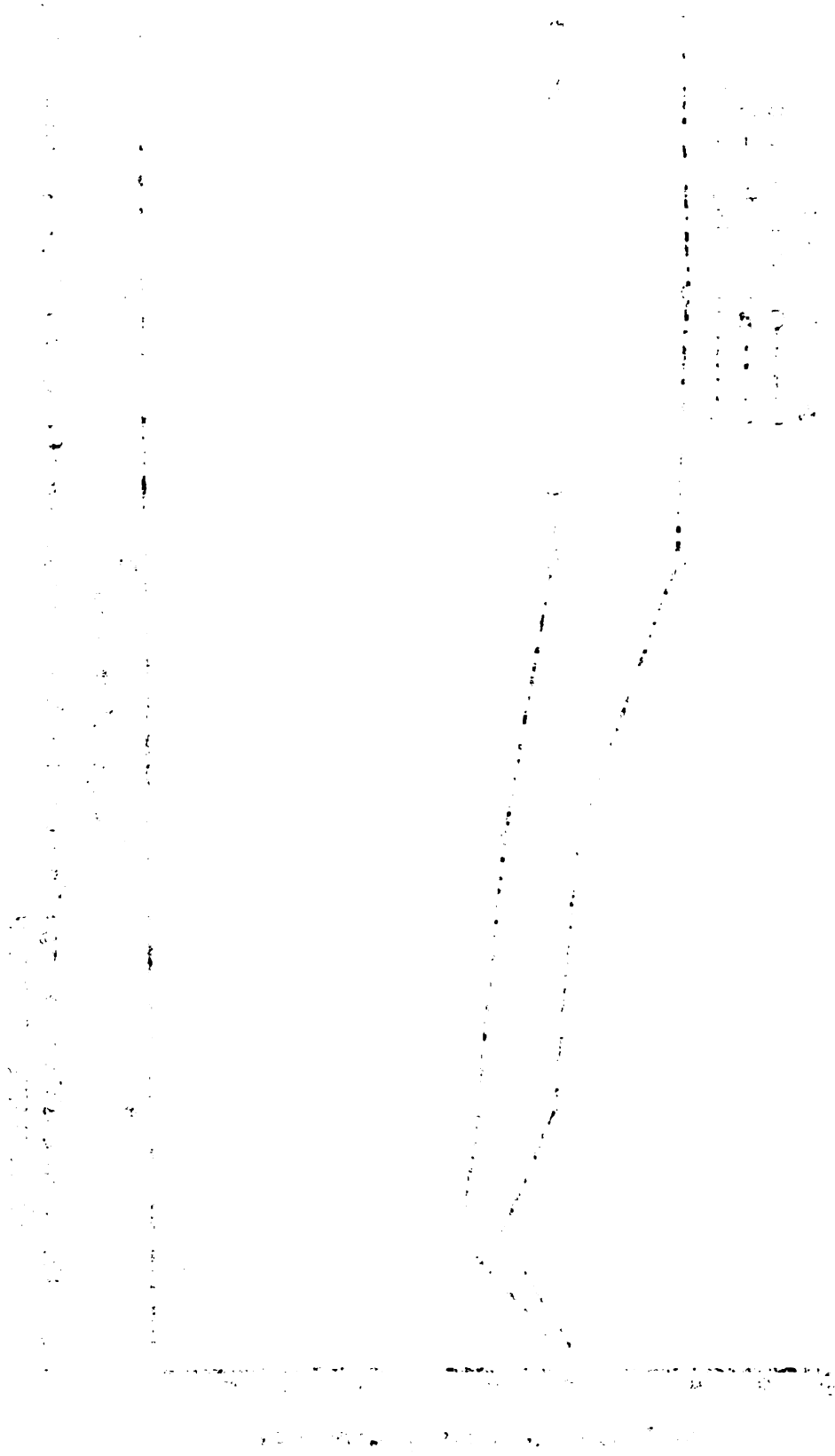


Figure 12. Relationship of storage time and log bacteria numbers of control and inoculated samples. (Pediococcus cerevisiae)



sample population increased steadily but slowly to log 6.27 (1.9 million organisms per gram) on day 20 of the storage period while maximum bacterial levels in sample B-10 were reached on day 16 when the population was recorded as log 8.08 (120 million organisms per gram). The B control samples were obtained bacteria-free.

Dye reduction data (Appendices E and F) indicated that Res 1 and Tet 1 reduction times were never more than 2.0 hours for any of the A or B sample groups. Trends in first stage dye reduction of either dye by A or B samples appeared non-consistent. Average second stage dye reduction data are illustrated by Table 5 on page 65. The A control samples showed a great range in Res 2 reduction times for individual observations. Res 2 values were not particularly logical though the decrease in the A-2 samples after day 8 did correspond to an increase in bacterial numbers by unknown contaminating species. Res 2 reduction times for A inoculated samples were always 3.0, 4.0, or 5.0 hours while those for B inoculated samples were 2.0 - 9.0 hours. Longer Res 2 reduction times for either set of inoculated samples did not always occur at the same times when the smaller bacterial populations were noted. Tet 2 reduction by A control samples varied from 3.0 to 6.0 hours, did not exhibit the extreme fluctuations observed for the Res 2 reductions, and were not much different from the Tet 2 reduction times for the A inoculated samples. Tet 2 reduction times by B control samples were 4.0 - 9.0 hours. B inoculated samples showed Tet 2 reduction times of 1.0 - 9.0 hours. The shorter reduction times did not coincide with the observance of the larger bacterial populations.

Table 5. Average second stage dye reduction times (Res 2 and Tet 2) in hours for resazurin and TTCS of A and B control and inoculated samples in Trial III (S. faecalis and P. cerevisiae inoculation).

Storage time (days)	Control Res 2 (hrs)		Inoculated Res 2 (hrs)		Control Tet 2 (hrs)		Inoculated Tet 2 (hrs)	
	A	B	A	B	A	B	A	B
0	3.0	1.8	3.0	4.0	3.0	4.0	2.0	1.8
2	2.5	7.0	4.0	5.0	3.0	6.0	3.0	1.0
4	8.0	10.0	4.0	9.0	3.5	8.0	3.0	8.0
8	8.0	13.0	4.5	8.0	4.0	4.0	3.5	4.0
12	8.5	12.5	5.0	7.5	6.0	6.5	5.0	2.5
16	13.5	11.0	3.5	8.0	5.0	9.0	5.0	9.0
20	--	10.0	4.0	2.0	4.0	6.0	4.0	4.0

The pH changes in the A control samples were as much as -0.3 pH unit even in the bacteria-free samples; the changes observed in A samples inoculated with S. faecalis were also -0.3 or less pH unit. B samples heavily inoculated with P. cerevisiae and incubated at 2 C showed pH changes of -0.4 pH unit; those B samples stored at 10 C exhibited pH changes of as much as -0.5 pH unit. The bacteria-free B control samples showed pH decreases of -0.2 pH unit. In most cases the larger pH reduction with time occurred in the inoculated samples. Apparently the storage temperature of 10 C tended to favor acid production by the organisms.

Table 6 (page 66) shows the correlation coefficients calculated between all variables in Trial III. ERV was not significantly related to bacterial numbers and was again correlated positively with pH. Also, the two second stage dye reduction times (Res 2 and Tet 2) were not significantly correlated with bacterial numbers in Trial III. Random fluctuations in ERV, Res 2, and Tet 2, and the lack of a significant increase in bacterial numbers over the initial added levels in the inoculated samples precluded any possibility for meaningful relationships among a

majority of the parameters under consideration as rapid indices of the microbial condition of pork. For this reason regression equations were not reported.

Table 6. Simple correlation coefficients calculated between all variables (Time, Res 1, Res 2, Tet 1, Tet 2, Logba, pH, and ERV) for Trial III (S. faecalis and P. cerevisiae inoculation).

	Time	Res 1	Res 2	Tet 1	Tet 2	Logba	pH	ERV
Time	1.00							
Res 1	0.44	1.00						
Res 2	0.19	0.04	1.00					
Tet 1	0.66	0.82*	0.07	1.00				
Tet 2	0.39	0.21	0.47	0.29	1.00			
Logba	0.08	0.01	-0.39	-0.04	-0.32	1.00		
pH	-0.68	-0.21	-0.33	-0.33	-0.30	-0.51	1.00	
ERV	-0.16	0.11	0.06	0.06	0.15	-0.45	0.35	1.00

* = significant at the 5% level ($P < .05$)

Analysis of variance tables (Appendix L, tables a - d) show that important variations in Res 2, Tet 2, ERV, and pH stemmed from various sources at different significance levels.

Under the conditions of Trial III neither ERV nor the dye reduction tests proved to be reliable indices of the microbial quality of the pork sampled. ERV did not respond to pH changes in the meat in the manner expected. Instead of decreasing as pH increased, ERV tended to increase as pH increased.

Trial IV

The bulk of the reported ERV work has been concerned with the spontaneous refrigerator spoilage of ground beef by unknown mixed low-temperature microbial flora. ERV was usually observed to decrease linearly as bacterial numbers in ground beef increased. The objective of

Trial IV was that of determining if ERV showed a similar type of relationship to the mixed culture spoilage of refrigerated ground pork. Data obtained for Trial IV were also expected to illustrate, very generally, how a heterogeneous population of bacteria would affect the test parameters differently than the homogeneous populations of bacteria used in Trials I-III and V.

The relationships between storage time and ERV of the A and B samples and the effects of sample storage at different temperatures are shown in Figures 13 and 14 on pages 68 and 69. The ERVs of A-2 and A-10 control samples showed similar decreases from 61.5 ml to 47.5 ml and 45.5 ml, respectively, over the entire storage period. More variation in minimum ERV values achieved was demonstrated by B-2 and B-10 control samples which declined from a maximum of 68.5 ml to 49.0 ml and 35.0 ml, respectively. If the A-10 control had shown a minimum ERV similar to the minimum ERV of the B-10 control sample the reduction in ERVs could have been attributed to the effect of storage of the samples at the higher temperature of 10 C. Because this did not occur the achievement of a much lower ERV by the B-10 control sample could not be explained as a temperature effect.

Figures 15 and 16 on pages 70 and 71 show relationships between storage time and bacterial numbers of the A and B control and inoculated samples of Trial IV. The initial ERVs of the A-2 and A-10 inoculated samples fell tremendously from 64.5 ml to 12.0 ml and 9.0 ml, respectively. A large increase in bacterial numbers took place in these samples between the 4th and 12th days of storage (Figure 15, page 70) and coincided with the dramatic reductions observed in ERVs of the A

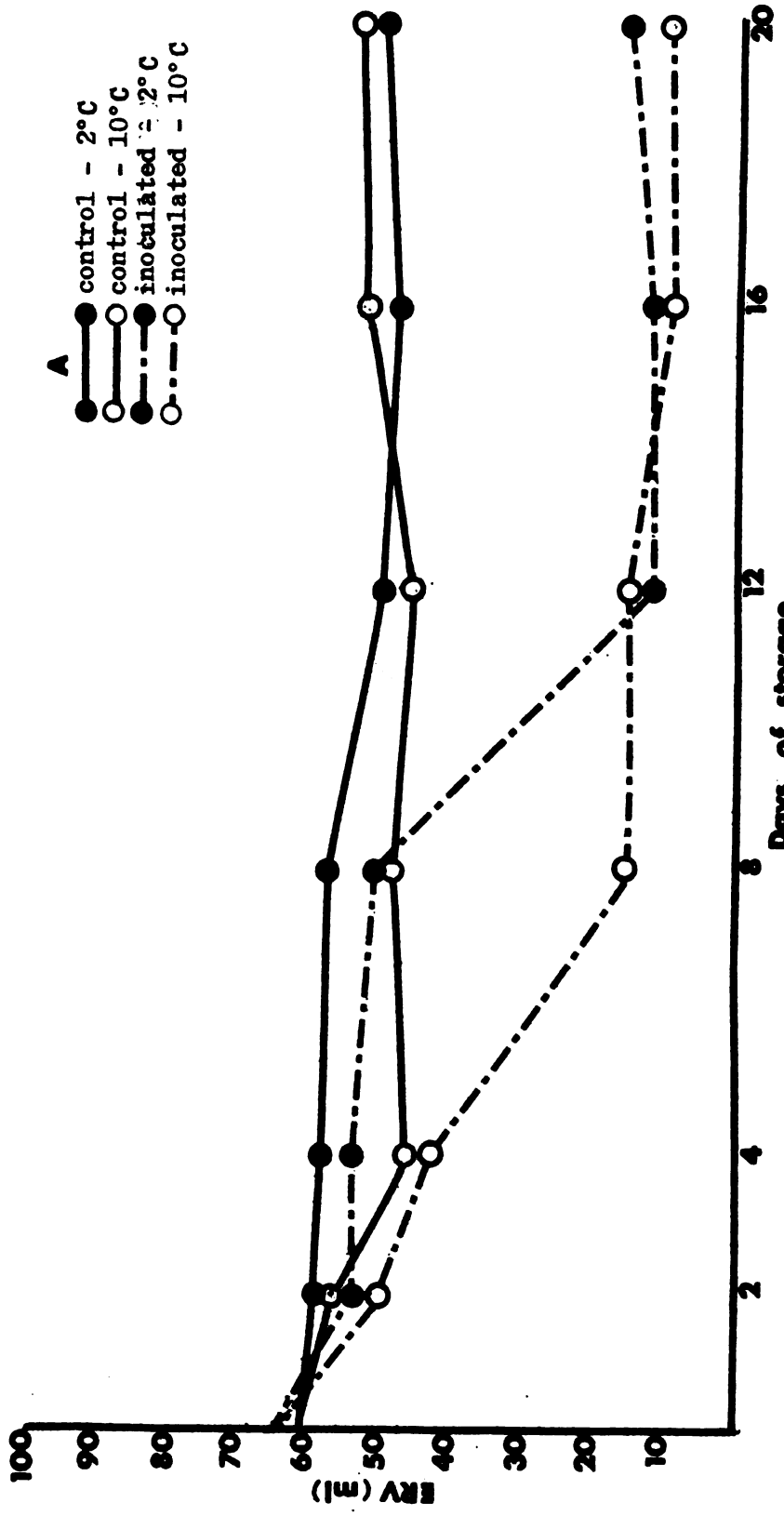
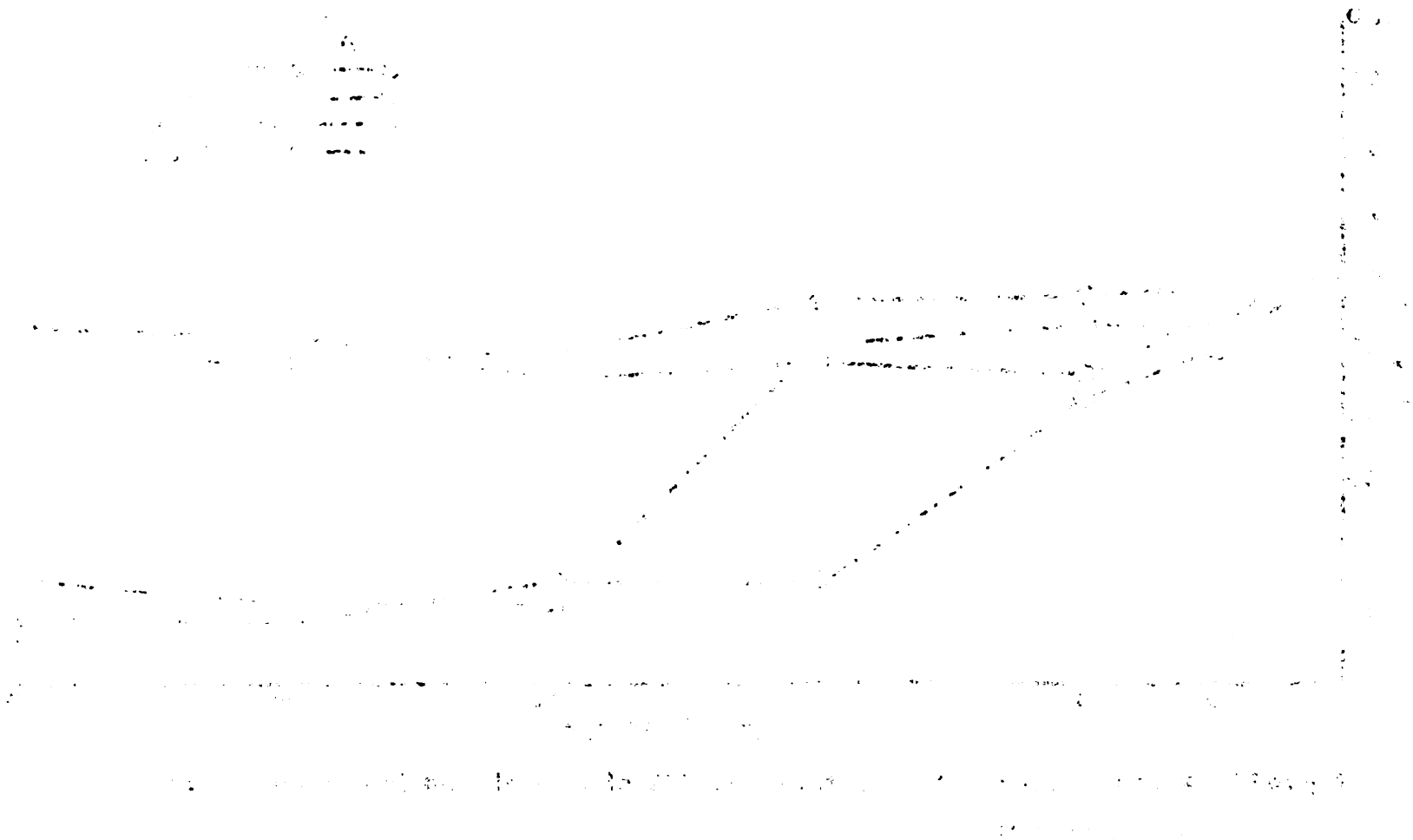


Figure 13. Relationship of storage time and ERV of control and inoculated samples. (Mixed culture)



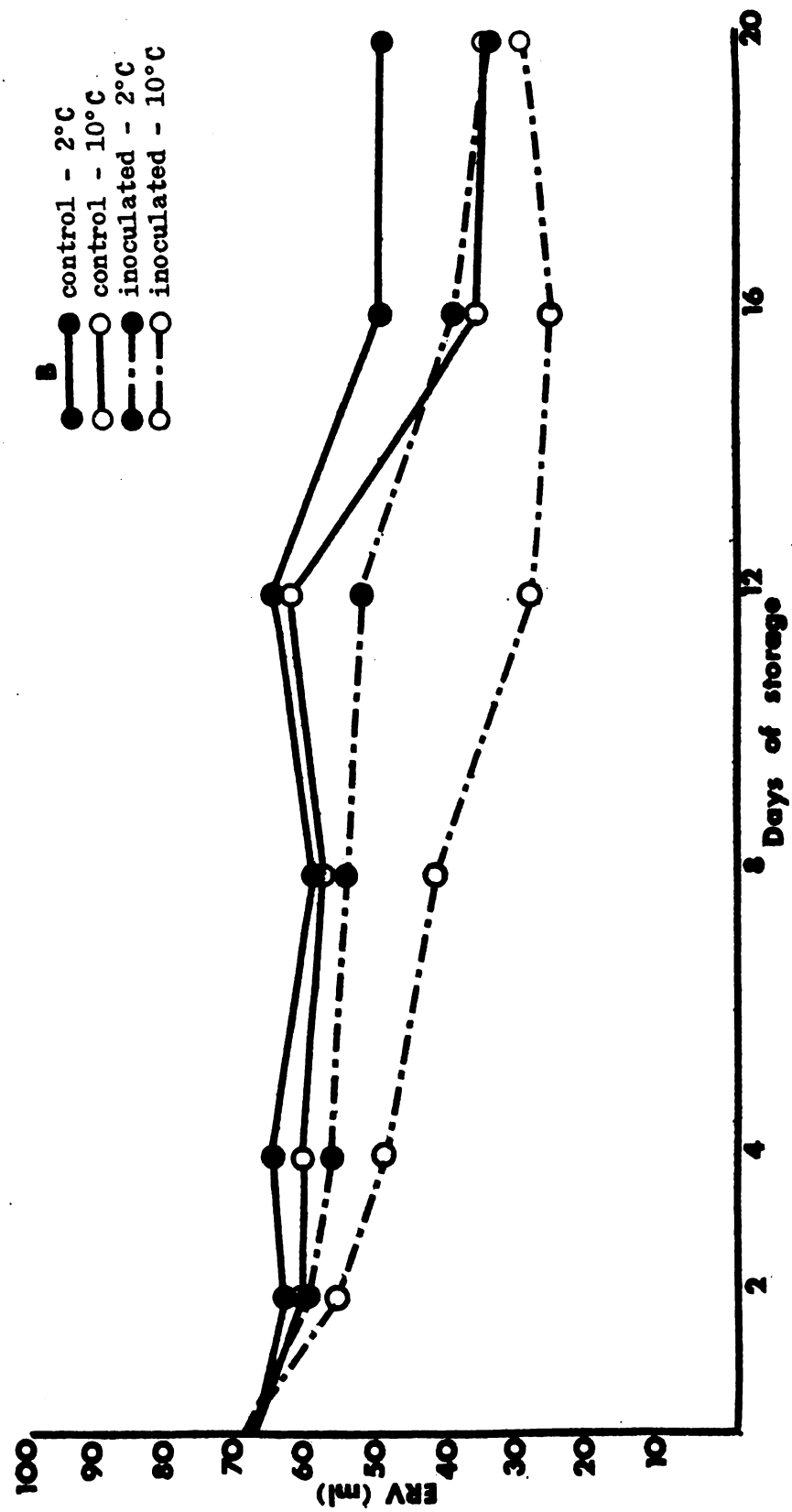


Figure 14. Relationship of storage time and ERV of control and inoculated samples. (Mixed culture)

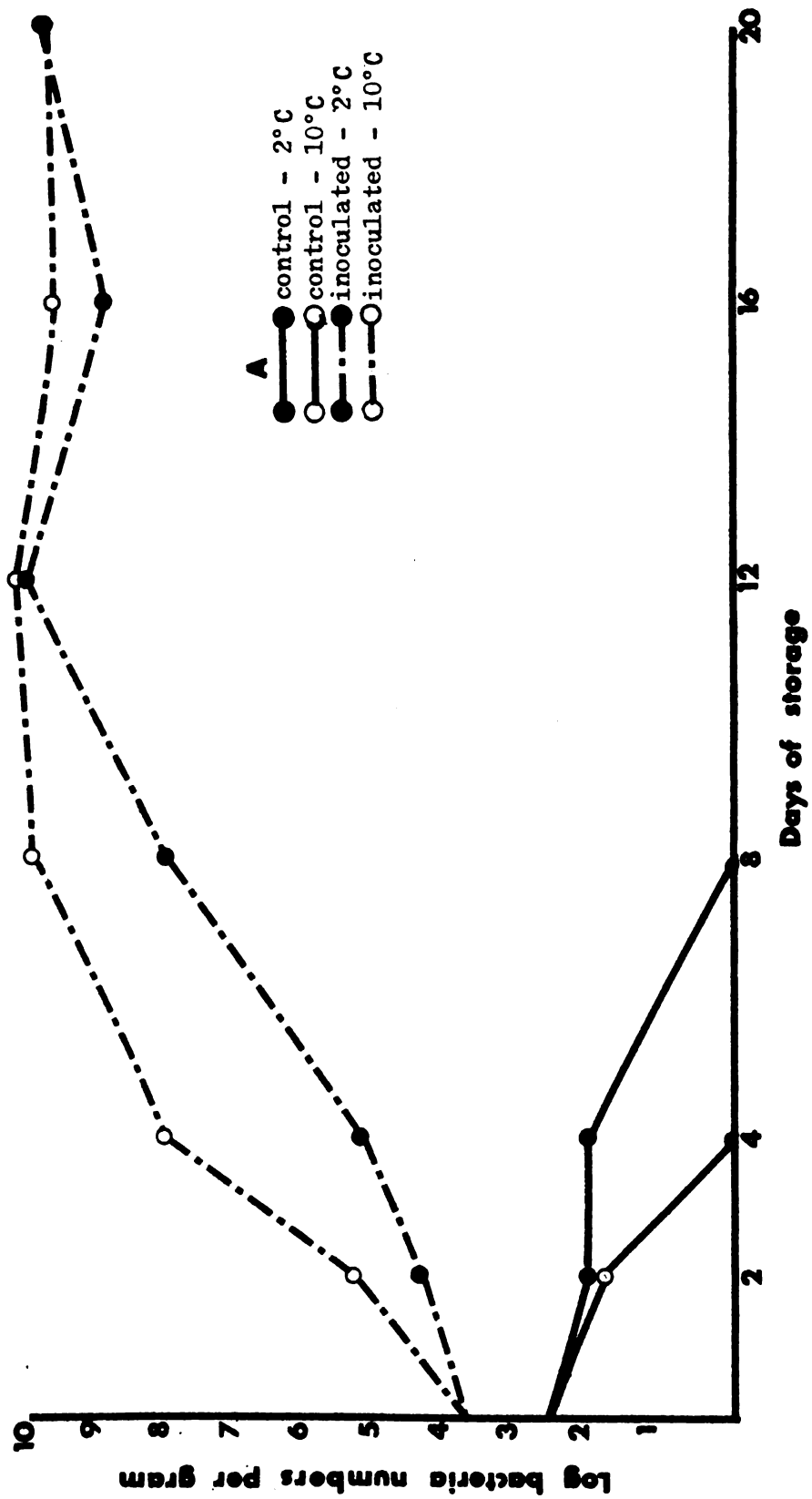


Figure 15. Relationship of storage time and log bacteria numbers of control and inoculated samples. (Mixed culture)

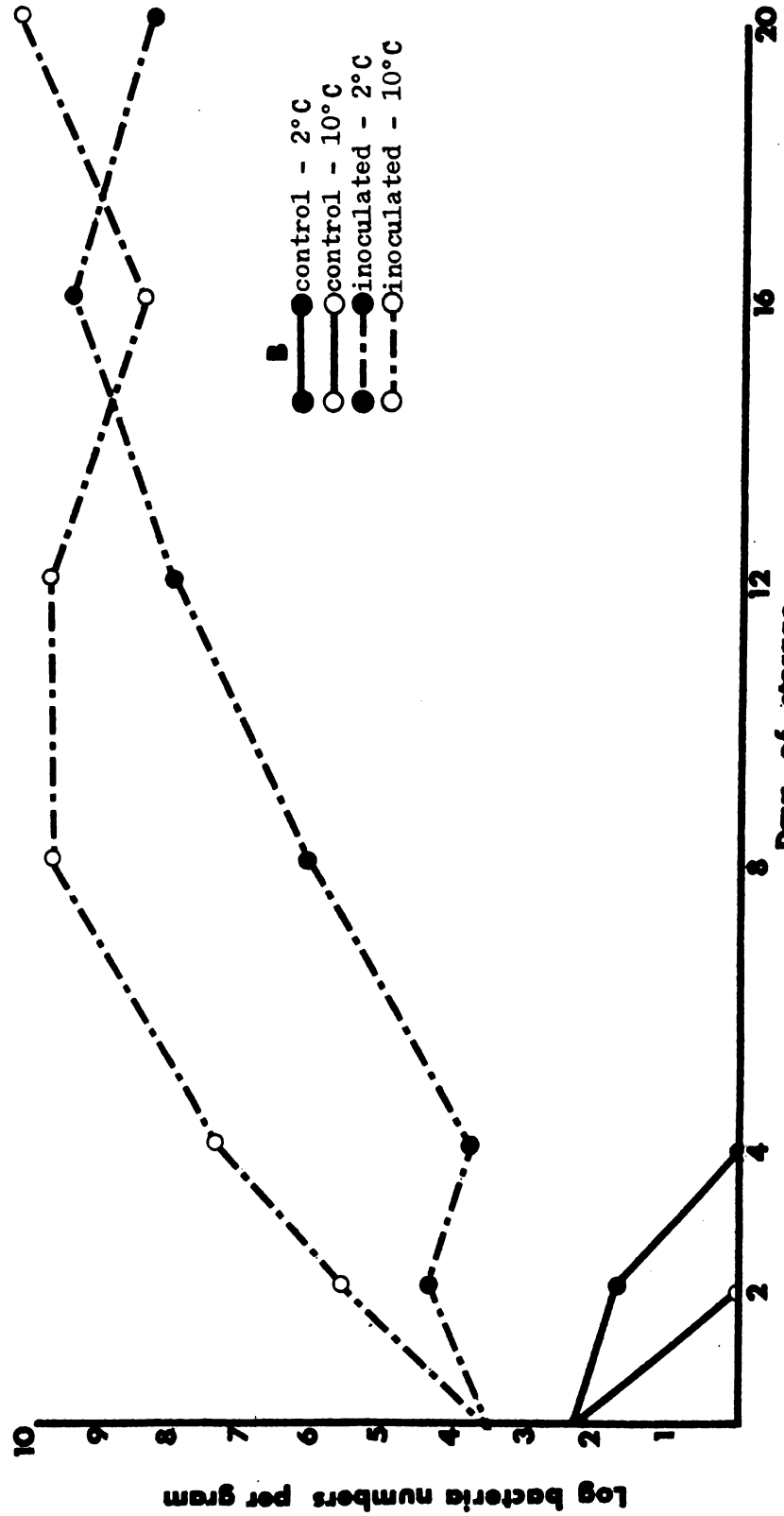


Figure 16. Relationship of storage time and log bacteria numbers of control and inoculated samples. (Mixed culture)

inoculated samples. By the 8th day of storage the bacterial population of the A-2 inoculated sample was log 8.07 (120 million organisms per gram) and the ERV was 51.0 ml. However, on day 12 of storage this sample carried a bacterial load of log 10.18 (15 billion organisms per gram) while the ERV was only 12.0 ml. The A-10 inoculated sample had a bacterial population of log 8.06 (110 million organisms per gram) and an ERV of 43.0 ml on day 4 of storage. Bacterial numbers in the A-10 sample increased to log 10.35 (22 billion organisms per gram) by day 12 when the ERV was recorded as 15.5 ml.

Jay (1964b) indicated that a bacterial load of log 8.5 (320 million organisms per gram) in ground beef was observed when an ERV of 24.0 ml was reported. Jay and Kontou (1964) showed that ground beef with an ERV of 30.4 ml carried a bacterial population of log 7.8 (63 million organisms per gram). None of the literature reviewed reported such high ERVs (51.0 ml and 43.0 ml) to be associated with bacterial numbers of the magnitude of hundreds of millions per gram as reported for Trial IV.

Inoculated B samples showed less drastic reductions in ERV at the times when bacterial populations were terrifically high. Again, a bacterial load of log 8.05 (110 million organisms per gram) was observed when the ERV was relatively high (53.0 ml) in the B-2 inoculated sample. The B-10 inoculated sample showed, on day 8, that its bacterial load was log 9.71 (5.1 billion organisms per gram) and the ERV was 43.5 ml and yet on day 12 the ERV was only 28.5 ml while the bacterial population was log 9.76 (5.8 billion organisms per gram). All A and B control samples held at 2 C or 10 C were free of viable bacteria after day 4 of the

storage period. Initial contamination levels were of the order of hundreds of bacteria per gram of sample. The relationship between ERV and bacterial numbers in the inoculated samples appeared to be negative and somewhat linear. In all cases the samples stored at 10 C reached highest microbial population levels.

Dye reduction data (Appendices G and H) showed that Res 1 and Tet 1 reduction times were never more than 2.0 hours. In all but 4 of the 224 observations (1.8 %) the Tet 1 reduction time, if different from the Res 1 reduction time, was the longer of the two. A-2 and A-10 inoculated samples had Res 1 times ranging from 0.2 to 1.0 hour as did the inoculated B-2 samples. Tet 1 reduction times for A-2 and A-10 control samples were 0.2 to 1.0 hour and were 0.5 to 1.0 hour for the B-2 and B-10 control samples.

Table 7 (page 74) shows the average second stage dye reduction times, Res 2 and Tet 2, for control and inoculated A and B samples in Trial IV. The A inoculated samples showed gradual decreases in dye reduction times over the 20 day storage period with the reduction of resazurin being somewhat more rapid than the reduction of TTCS. The B inoculated samples exhibited very little fluctuation in the average reduction times for Res 2 while more variation was evident in the reduction time of Tet 2 by these same samples. Reduction of Res 2 by A control samples was rather consistent after the 2nd day of storage but Res 2 reduction by B control samples showed more fluctuation over the storage period. However, the Tet 2 reduction by the A control samples was erratic while the Tet 2 reductions by B control samples showed little variation during the storage period. No suitable explanations for these

random fluctuations could be made.

Table 7. Average second stage dye reduction times (Res 2 and Tet 2) in hours for resazurin and TTCS for A and B control and inoculated samples in Trial IV (Mixed culture inoculation).

Storage time (days)	Control Res 2 (hrs)		Inoculated Res 2 (hrs)		Control Tet 2 (hrs)		Inoculated Tet 2 (hrs)	
	A	B	A	B	A	B	A	B
0	3.0	3.0	2.5	3.0	7.5	8.0	6.0	7.0
2	4.5	8.0	2.0	4.0	7.5	8.5	4.0	5.5
4	8.0	7.7	1.2	3.5	8.5	9.0	2.5	4.5
8	9.5	10.0	1.0	3.5	11.0	9.5	1.2	3.7
12	8.0	9.0	0.5	2.7	9.5	9.5	1.2	3.2
16	8.0	2.5	1.0	3.5	16.0	9.0	2.0	3.5
20	8.0	6.0	0.6	3.5	17.0	8.0	1.7	2.5

The A control sample group exhibited pH changes of -0.2 pH unit while the A inoculated samples showed marked changes in pH ranging from -0.3 to +0.8 pH unit. Alkaline reactions of this order would appear to indicate that spoilage of the inoculated samples was due to proteolysis caused by typical low-temperature meat spoilage organisms of the mixed culture inoculum. The B control samples varied only -0.1 pH unit from the buffer pH of 5.8 and the inoculated B samples showed pH changes of ±0.2 pH unit. Since similar bacterial populations were recovered from both A and B inoculated samples it was expected that the pH changes in the meat would be similar. This, however, was not the case.

Simple correlation coefficients calculated between all variables of Trial IV are presented in Table 8 on page 75. More correlations of statistical significance were observed for Trial IV than in any of the other trials. ERV was correlated more highly with bacterial numbers ($r = -0.64$) than it was in Trials I-III and V but the relationship was

not statistically significant. This correlation more closely approached Jay's value (r = -0.808) than did any correlation observed between ERV and Logba for any other trial. That this relationship was a result of contaminating the meat with a bacterial population more similar to those used by Jay seemed likely. The expected negative correlation of ERV with pH was finally observed (r = -0.71, P <.05). Bacterial numbers showed correlations of significance at the 1% level with Res 2 (r = -0.85) and Tet 2 (r = -0.95) and with Res 1 at the 5% level of significance (r = -0.83). The relationship observed between Res 2 and Tet 2 reduction times was statistically significant (r = 0.85, P <.01) with a correlation coefficient similar to those observed for the same relationship in Trials I (r = 0.92, P <.01) and II (r = 0.90, P <.01). Reduction time Res 1 showed identical correlations (r = 0.80, P <.05) with both Res 2 and Tet 2 reduction times.

Table 8. Simple correlation coefficients calculated between all variables (Time, Res 1, Tes 2, Tet 1, Tet 2, Logba, pH, and ERV) for Trial IV (Mixed culture inoculation).

	Time	Res 1	Res 2	Tet 1	Tet 2	Logba	pH	ERV
Time	1.00							
Res 1	-0.05	1.00						
Res 2	-0.10	0.80*	1.00					
Tet 1	-0.30	0.53	0.56	1.00				
Tet 2	-0.17	0.80*	0.85**	0.62	1.00			
Logba	0.12	-0.83*	-0.85**	-0.62	-0.95**	1.00		
pH	0.30	-0.55	-0.55	-0.53	-0.60	0.61	1.00	
ERV	-0.65	0.56	0.46	0.65	0.67	-0.64	-0.71*	1.00

* = significant at the 5% level (P <.05)
** = significant at the 1% level (P <.01)

Regression equations calculated for prediction of bacterial numbers from the second stage dye reduction times, Res 2 and Tet 2, and the

standard errors of the estimates illustrating the spread of points about the regression line were as follows:

$$\text{Logba} = -0.038\ \text{Res}\ 2 + 9.351 \pm 2.311$$

$$\text{Logba} = -0.041\ \text{Tet}\ 2 + 11.782 \pm 1.391$$

Analysis of variance statistics (Appendix M, table d) showed that although the variations in bacterial numbers were largely accounted for by time and the interactions of treatment x temperature and treatment x time, variations in ERV, Res 2, and Tet 2 were effected tremendously by inoculation level, treatment, temperature, time, and the interaction of treatment x time (Appendix M, tables a - c).

Trial IV data indicated that though ERV was again not a satisfactory rapid indicator of the microbial quality of pork it was correlated more highly with bacterial numbers than in any other trial. The complete reductions of resazurin and the tetrazolium salt, TTCS, were considered to be more valid predictors of the bacteriological status of the meat. Apparently the previously reported linear negative response of ERV to bacterial numbers (Jay and Kontou, 1964) was more closely approached by the heterogeneous populations of bacteria used in Trial IV than by the homogeneous bacterial populations used in Trials I-III and V.

Trial V

Trial V was not expected to provide much meaningful data concerning the effects of C. perfringens on the test parameters because the test conditions dictated that growth of the organism in the samples would probably be minimal or nonexistent.

Figures 17 and 18 (pages 77 and 78) illustrate the relationships of storage time, storage temperature, and ERV of the A and B control and

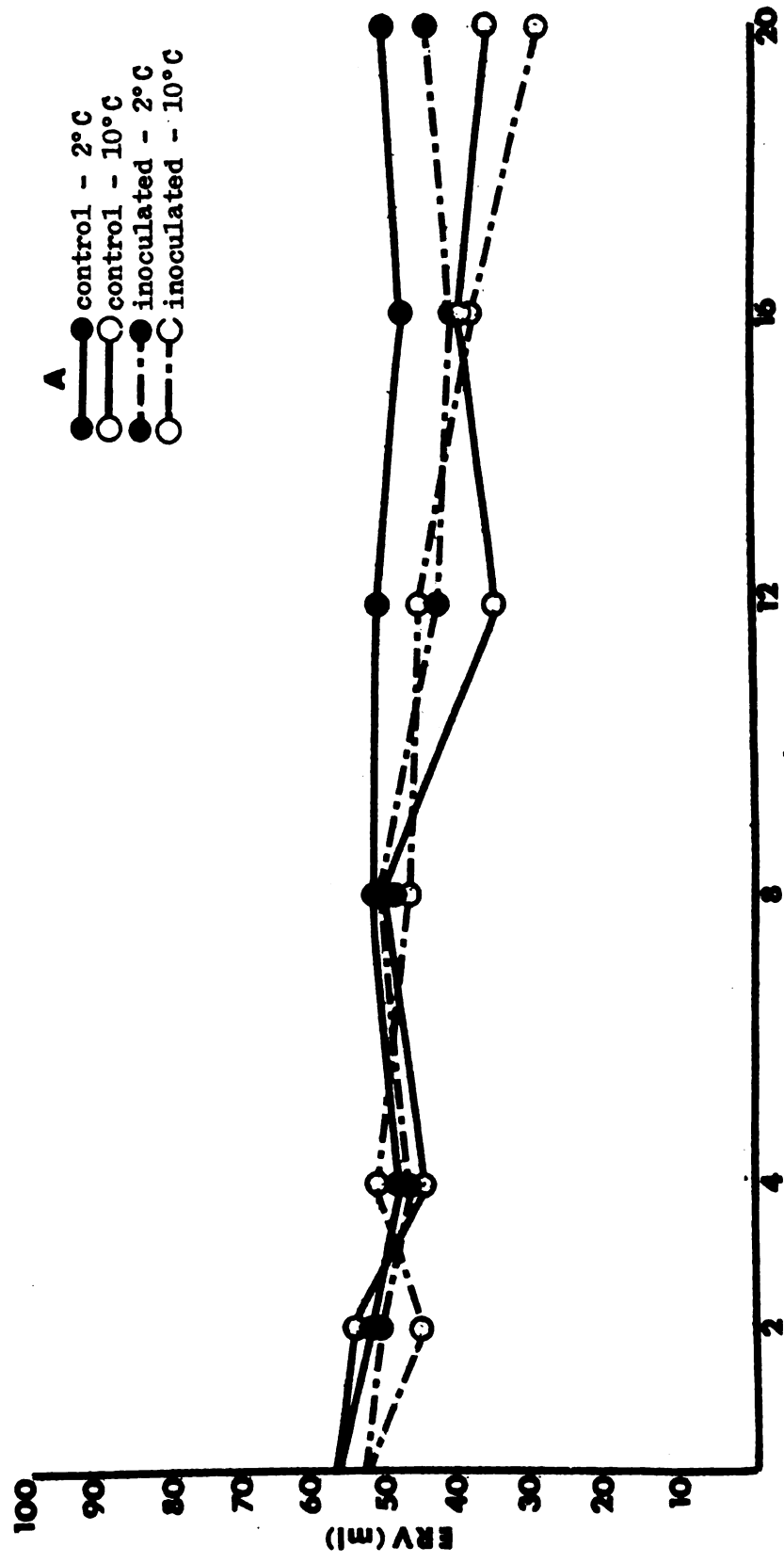


Figure 17. Relationship of storage time and ERV of control and inoculated samples. (*Clostridium perfringens*)

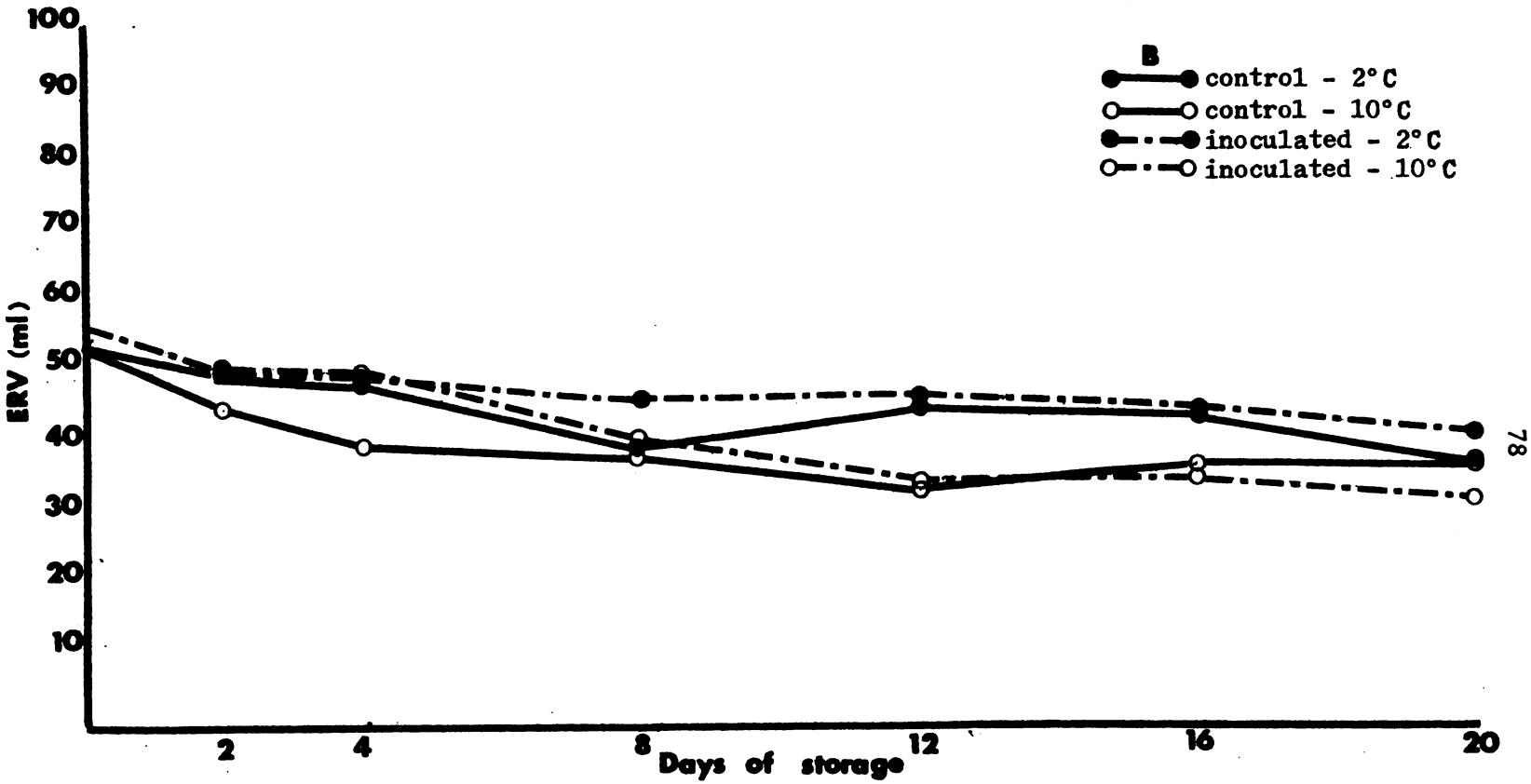


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Figure 18. Relationship of storage time and ERV of control and inoculated samples. (Clostridium perfringens)

inoculated samples. Initial ERVs of A control and inoculated samples were, respectively, 57.5 and 54.5 ml. The B control and inoculated samples had initial ERVs of 53.0 and 54.0 ml. The animal variation in initial ERVs was small in Trial V. Control samples A-2 and B-2 showed decreasing ERV values until about the middle of the storage period; subsequent increases were noted in ERV until the last of the storage period when slight decreases were again reported. The control A-10 sample showed alternately decreasing and increasing ERVs after day 2 while the control B-10 sample exhibited steadily decreasing ERV values until day 12; after that time the ERVs increased. Inoculated sample A-2 behaved similarly to the control A-2 sample but the inoculated A-10 sample showed decreased ERVs after day 4. The ERVs of the B-2 inoculated sample decreased steadily (with the exception of a minor increase on day 12) over the entire storage period. This same trend was noted for the B-10 inoculated sample. Because of the lack of consistently recoverable bacterial populations from the samples the ERV values were not compared with bacterial population levels.

Bacterial data for Trial V (Figures 19 and 20, pages 80 and 81; Appendices I and J) were rather difficult to interpret. Some samples showed aerobic and/or anaerobic contamination alternately appearing and disappearing on consecutive sampling days. All A control samples were free from aerobic contaminating organisms but showed low levels of anaerobic contaminants on no more than 3 of the sampling days. The B control samples were slightly contaminated with anaerobic organisms on 2 of the sampling days while low aerobic contamination was evident only on day 8. This was probably chance contamination with aerobes.

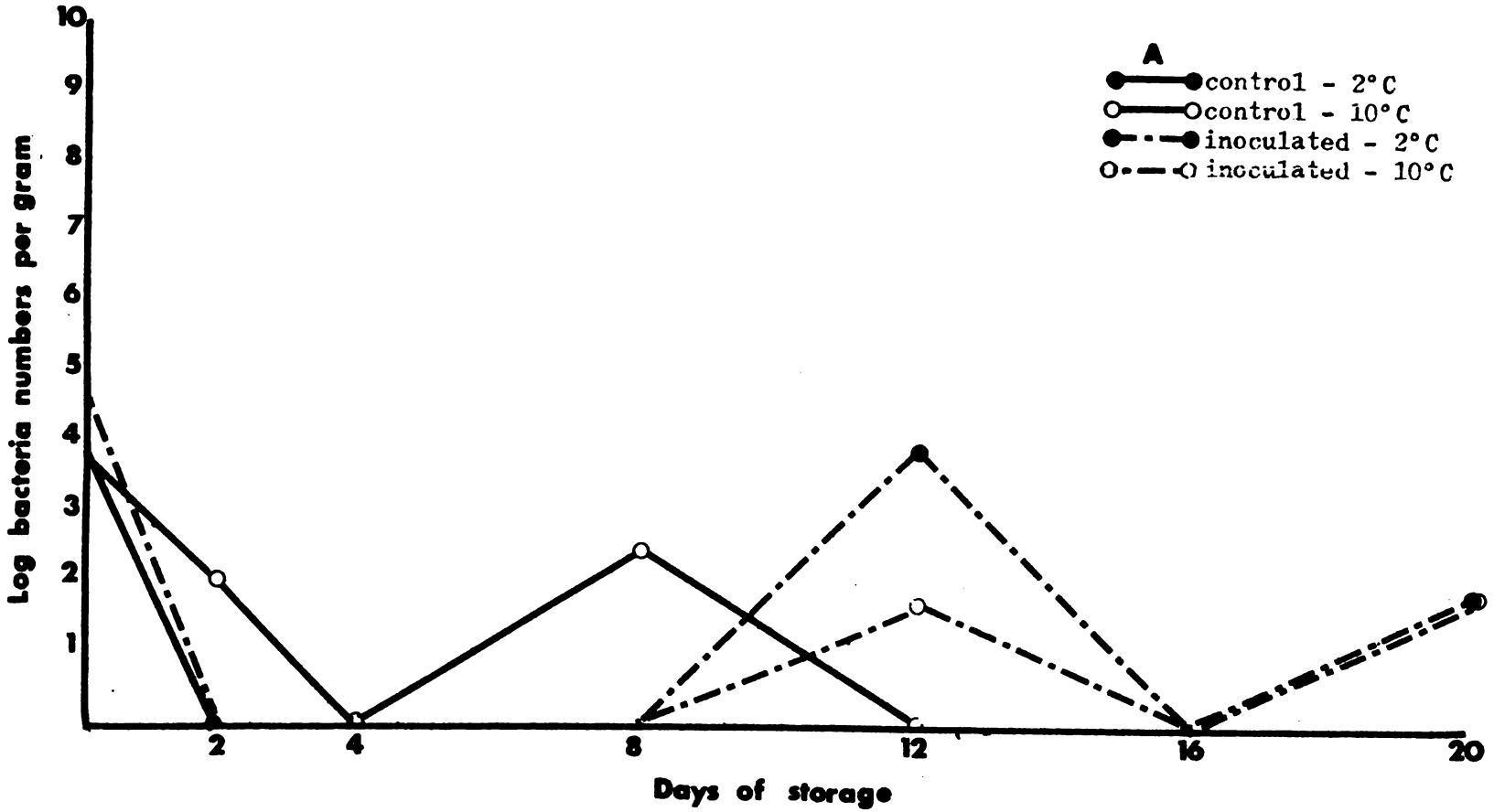
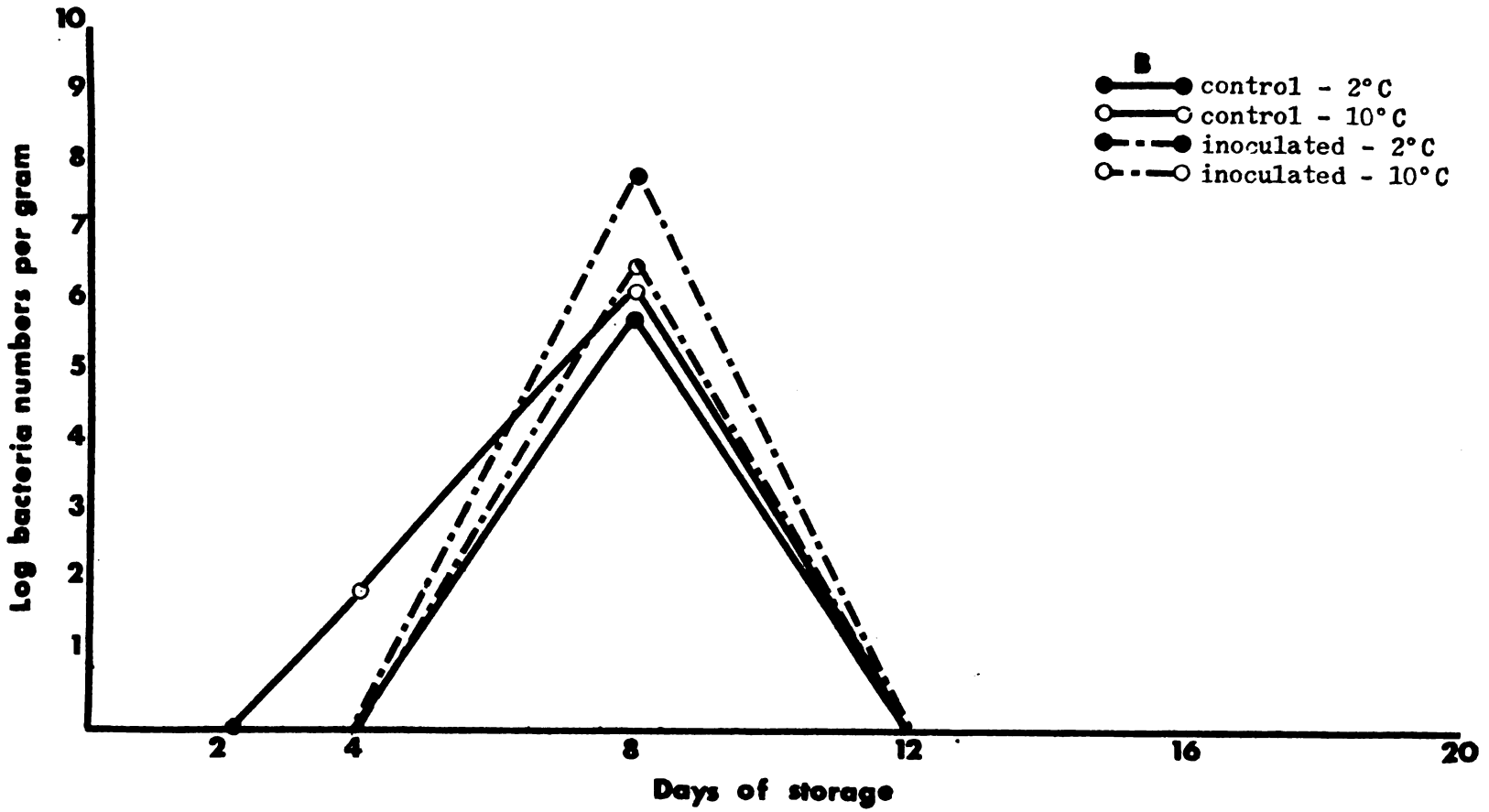


Figure 19. Relationship of storage time and log bacteria numbers of control and inoculated samples. (Clostridium perfringens)



Fiqure 20. Relationship of storage time and log bacteria numbers of control and inoculated samples. (Clostridium perfringens)

Bacterial populations in the A inoculated samples never exceeded log 4.51 (32,000 organisms per gram). Relatively higher peak populations were observed in the more heavily inoculated B samples (log 7.57 or 37 million organisms per gram). It was expected that C. perfringens populations, if any, would be recovered from the samples only on day 0 or day 2 because of the adverse storage conditions. However, organisms were recovered at later times during storage. Stains of organisms obtained from the inoculated meat samples showed Gram-variable short bacilli; some few of the organisms observed had swollen terminal ends indicative of spore formation. The milk used in the dye reduction tests for the inoculated samples displayed the stormy fermentation characteristic of C. perfringens. Some of the control samples showing anaerobic contaminants also exhibited this type of reaction in the milk mixture. These observations indicated that some of the organisms had survived the storage conditions. It is possible that the high reducing activity of the fresh meat may have rendered the storage conditions more conducive to survival of the bacteria.

Changes in pH of the meat-buffer extract for A samples were -0.1 to +0.2 pH unit for the control samples and -0.4 to +0.3 pH unit for the inoculated samples. B control samples exhibited minor pH fluctuations (±0.1 pH unit) Changes in the pH of the B inoculated samples were from -0.1 to +0.2 pH unit. Observance of both acid and alkaline reactions in the samples requires explanation of two phenomena which seem unlikely to be operative at the same time. The proteolytic enzymes elaborated by C. perfringens digest muscle proteins with consequent liberation of amines and other nitrogenous compounds which would increase the pH of

the meat. Because C. perfringens is the most saccharolytic of the clostridia it would ferment any residual sugars in the meat to lactic or butyric acid and thereby lower the pH of the meat. It seems unlikely that pork would contain enough fermentable sugar for the organism to produce enough acid to change the pH of a buffered system 0.4 pH unit.

First stage dye reduction times of Res 1 by both A and B control and inoculated samples ranged from 0.2 to 2.0 hours. The Tet 1 reductions by both A and B sample groups were 0.5 to 2.0 hours. Table 9 below shows the average second stage reduction times of resazurin and TTCS by all control and inoculated samples without regard to inoculation level or storage temperature.

Table 9. Average second stage dye reduction times (Res 2 and Tet 2) in hours for resazurin and TTCS of A and B control and inoculated samples in Trial V (C. perfringens inoculation).

Storage time (days)	Control Res 2 (hrs)		Inoculated Res 2 (hrs)		Control Tet 2 (hrs)		Inoculated Tet 2 (hrs)	
	A	B	A	B	A	B	A	B
0	10.0	9.0	7.5	8.5	7.0	7.5	6.0	9.5
2	9.0	10.0	5.0	8.0	9.0	9.5	5.0	8.0
4	10.0	8.5	4.0	9.0	10.0	9.0	2.0	8.0
8	9.5	9.0	7.0	7.5	9.0	7.5	6.5	7.0
12	9.0	8.0	6.5	5.0	9.5	6.5	6.0	5.0
16	8.5	8.5	6.5	6.5	10.0	9.5	9.0	6.5
20	8.0	9.5	5.0	5.5	9.5	10.0	7.0	8.0

Reduction of Tet 2 by A and B control samples were quite consistent and were always between 8.0 and 10.0 hours. Variations were evident in Res 2 reduction by A and B inoculated samples; these times ranged from 4.0 - 7.0 hours and from 5.0 - 9.0 hours, respectively. Reduction of Tet 2 by A control samples was, except in one case, always 9.0 - 10.0 hours. B control sample Tet 2 reduction times were more variable. The reduction

times for inoculated A and B samples were not much shorter than the control sample reduction times and showed unexplainable alternatively decreasing and increasing trends.

Table 10 (page 84) presents the correlation analysis of Trial V data. Only two relationships of statistical significance were noted. Again, Tet 2 was positively related to Res 2 at the 5% level of significance. The relationship between ERV and storage time was negative as it was in Trials I-IV. The expected negative relationship of ERV with pH was noted, but it was statistically insignificant.

Table 10. Simple correlation coefficients calculated between all variables (Time, Res 1, Res 2, Tet 1, Tet 2, Logba, pH, Logban, and ERV) for Trial V (C. perfringens inoculation)

	Time	Res 1	Res 2	Tet 1	Tet 2	Logba	pH	ERV	Logban
Time	1.00								
Res 1	0.10	1.00							
Res 2	-0.30	0.10	1.00						
Tet 1	0.31	0.08	0.09	1.00					
Tet 2	0.18	0.13	0.67*	0.19	1.00				
Logba	0.07	-0.16	-0.09	-0.13	-0.11	1.00			
pH	0.32	-0.01	-0.01	0.05	0.22	0.18	1.00		
Logban	-0.34	-0.47	0.19	-0.21	-0.20	0.62	0.12	1.00	
ERV	-0.70*	-0.26	0.28	-0.08	-0.03	-0.22	-0.19	0.22	1.00

* = significant at the 5% level ($P < .05$)

Results of Trial V indicated again that ERV was not correlated highly enough with bacterial numbers to be considered an index of the microbial quality of pork. Neither of the dye reduction times, Tet 2 and Res 2, could be used to predict bacterial numbers in the meat.

Comparison of Control and Inoculated Sample Data

The entire array of control sample data obtained from Trials

I-V was pooled for correlation analysis; this procedure was repeated for the inoculated sample data.

Correlation coefficients calculated between all experimental variables for the control data showed only one relationship of statistical significance. Logba was positively correlated with Logban at the 5% level of significance. The lack of significant relationships indicates the great variability observed in the test parameters between trials.

Correlation analysis of inoculated sample data revealed but one relationship of statistical significance. Tet 2 was correlated positively with Res 2 ($r = 0.739$, $P < .05$) over all trials. This relationship was expected because these two parameters were usually correlated quite highly with one another within trials.

Effects of C. perfringens on ERV, pH, Dye Reduction Times, and Bacterial Numbers

Inoculation of ground pork with C. perfringens followed by high temperature storage (32 C) and subsequent plating of the samples in SPS agar produced fairly large viable populations of the organism when incubation was accomplished in a nitrogen-flushed evacuated chamber. Table 11 (page 86) shows the experimental data obtained for this short study.

Both control and inoculated samples exhibited very noxious odors after the second day of storage. It appeared that the odor of the inoculated samples was due, in part, to the proteolytic action of C. perfringens on the meat proteins. That the effect of the high storage temperature contributed to the odor development was evidenced by the fact that the bacteria-free control samples also developed disagreeable

off-odors. This condition was thought to be due to accelerated enzymatic autolysis of the control samples since they did not contain viable bacteria.

Table 11. ERV, dye reduction times (Res 1, Res 2, Tet 1, Tet 2), Logba, Logban, and pH for control and inoculated pork samples (C. perfringens inoculation).

	Storage time (days)	Mean ERV (mls)	Logba	Logban	Res 1 (hrs)	Res 2 (hrs)	Tet 1 (hrs)	Tet 2 (hrs)	pH
Control	0	56.5	-1.00	-1.00	0.25	0.50	0.25	0.50	5.8
	2	50.0	-1.00	-1.00	0.50	1.00	0.50	1.00	5.8
	4	47.5	-1.00	-1.00	1.00	4.00	1.50	3.00	5.7
	6	53.5	-1.00	-1.00	1.00	4.00	2.00	5.00	5.7
	8	55.5	-1.00	-1.00	1.00	5.00	2.00	5.00	5.7
Inoc.	0	52.0	-1.00	+4.32	0.25	0.50	0.25	0.50	5.8
	2	63.0	-1.00	+5.67	0.50	1.00	0.50	1.00	5.9
	4	72.0	-1.00	+7.69	0.50	1.00	1.00	2.00	6.2
	6	68.5	-1.00	+6.08	1.00	3.00	1.00	4.00	6.1
	8	58.5	-1.00	+6.17	1.00	2.00	1.00	4.00	6.1

The meat-buffer slurries of the control samples did not appear physically different from control sample slurries observed in Trials I-V but the slurries of the inoculated samples behaved rather differently than was expected. In Trials I-V the larger populations of bacteria in the meat generally seemed to be associated with slurries of increasingly thick and gummy character which released progressively decreasing amounts of extract. This effect could have been the result of bacterially-caused alterations in the hydration capacity of the muscle proteins or the result of water-holding by-products of the metabolism of the organisms added. However, the slurries of the pork containing C. perfringens were thin and serous and released larger amounts of

extract as bacterial numbers increased. If the slurries of the bacteria-free control samples had also tended to become watery the high temperature storage of the samples could have explained the different character of the slurries. But, this was not observed, so the proteolytic activity of C. perfringens was considered the probable reason for the odd behavior of the slurries. This effect was in direct contrast to the proteolytic action of the mixed culture used in Trial IV. Usually proteolysis will bring about reductions in ERV values; however, the extent of proteolysis occurring might dictate whether ERV would show an increase or reduction in value.

The ERV of the inoculated samples increased as bacterial numbers increased until day 4; decreases in bacterial numbers until day 8 were followed by concommitant decreases in ERV. Changes in the pH of the meat-buffer extract also closely paralleled the changes in ERV and bacterial numbers. Increases in pH occurred when bacterial numbers and ERV were increasing; when ERV and bacterial populations were decreasing the pH of the extracts also decreased. Dye reduction times did not follow these trends. The second stage reduction times for the inoculated samples increased slightly as bacterial numbers increased. No explanations could be offered for this observation.

The results of this experiment indicated that ERV of meat spoiled by C. perfringens under conditions optimal for survival of the organism increased as spoilage progressed. This phenomenon might be indicative of the action of the collagenase and hyaluronidase, produced by the organism, which could digest the meat proteins sufficiently to destroy most of their water-binding capacity.

SUMMARY AND CONCLUSIONS

The procedure for obtaining pork muscle samples relatively free of microbial contamination through the use of special slaughtering and handling techniques was considered successful. Ten of twenty control sample groups remained bacteria-free for the entire 20 day storage period. The remainder of the control samples were contaminated with very low populations of bacteria. The maximum bacterial load of any control sample after 20 days of storage was log 3.44 (2,700 organisms per gram) which was considered to be a very low level of contamination for any ground meat sample.

The control pork samples were compared (regarding ERV, dye reduction times, pH, and bacterial numbers) with duplicate samples which had been inoculated with a particular bacterial culture. Responses of these tests were somewhat dependent upon the type and numbers of bacteria used.

ERV values for control and inoculated samples from the same animal were similar on day 0 of storage; therefore, the subsequent reduction in ERV of the inoculated samples as storage progressed seemed to be, in part, dependent upon the active growth of the microorganisms added and appeared not to be due to their presence per se. The correlations observed between ERV and bacterial numbers were never high enough to indicate that the ERV phenomenon could be used successfully to estimate microbial quality of pork samples. However, the response of the test to the heterogeneous bacterial populations of the mixed culture

spoilage of pork more closely approached statistical significance than did its response to homogeneous bacterial populations in the meat. ERVs were always correlated negatively with time of storage of the samples and with bacterial numbers. The relationship observed between ERV and pH was variable and statistically significant in only one trial. The expected inverse relationship between pH and ERV was observed in two trials; statistical significance was indicated in Trial IV.

The dye reduction tests were considered to be of value as rapid indicators of the microbial spoilage of ground pork only for Trials I and IV where the bacterial populations were typical psychrophilic meat spoilage organisms. Lack of bacterial data in Trial II and random fluctuations in the dye reduction times in Trial III precluded any assumptions about the value of the test under those experimental conditions. Although the reduction times Res 2 and Tet 2 were not always correlated highly with bacterial numbers they were correlated quite highly with each other in Trials I, II, IV, and V.

The response of the ERV phenomenon to the action of C. perfringens in the pork samples was totally in opposition to the reported usual linear negative relationship.

It is the contention of the author that the ERV test, though reported to be a reliable rapid indicator of microbial quality of ground beef, is of little value in estimating pure culture spoilage in ground pork and is of questionable value for prediction of bacterial numbers in ground pork contaminated with mixed psychrophilic microorganisms. However, under normal circumstances, the second stage reduction times, Res 2 and Tet 2, appeared to be valid indices of the potential for spoilage in

pork because short reduction times occurred even in freshly inoculated samples before spoilage was evident.

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APPENDIX

Appendix A. Treatments, storage temperatures, storage times, mean ERV values, dye reduction times, logs of bacterial numbers, and pH values for Trial I - A (light inoculation of *P. fluorescens*).

Treatment	Storage temperature	Storage time - days	Mean ERV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH
control	2 C	0	36.5	01.00	06.00	01.00	10.00	-01.00	5.7
control	2 C	2	23.5	02.00	07.00	01.00	10.00	-01.00	5.7
control	2 C	4	27.5	01.00	07.00	01.50	09.00	+01.30	5.7
control	2 C	8	19.0	01.00	08.00	01.50	08.00	+01.41	5.7
control	2 C	12	23.5	01.00	06.00	01.00	08.00	+02.55	5.6
control	2 C	16	25.0	01.00	06.00	01.00	08.00	+02.94	5.6
control	2 C	20	28.5	00.50	05.00	02.00	08.00	+03.32	5.6
control	10 C	0	36.5	01.00	08.00	01.00	10.00	-01.00	5.7
control	10 C	2	20.0	01.00	07.00	01.00	09.00	+01.30	5.7
control	10 C	4	22.0	01.00	07.00	01.00	09.00	+01.90	5.6
control	10 C	8	16.5	01.00	06.50	01.00	08.00	+02.32	5.6
control	10 C	12	22.0	01.00	08.00	01.00	07.50	+03.27	5.6
control	10 C	16	23.0	01.00	08.00	01.00	08.00	+03.17	5.6
control	10 C	20	18.0	01.00	07.00	01.00	07.50	+03.34	5.6
inoculated	2 C	0	35.0	00.50	05.50	01.00	07.00	+05.11	5.7
inoculated	2 C	2	17.5	01.00	05.00	01.00	06.50	+05.51	5.7
inoculated	2 C	4	19.5	01.00	05.00	01.00	06.50	+05.64	5.7
inoculated	2 C	8	20.5	01.00	04.00	01.50	06.00	+07.10	5.6
inoculated	2 C	12	16.0	00.50	04.00	01.00	05.00	+07.63	5.6
inoculated	2 C	16	19.0	00.50	02.00	01.00	02.00	+09.69	5.6
inoculated	2 C	20	21.0	00.50	03.00	01.00	02.00	+08.75	5.7
inoculated	10 C	0	35.0	00.50	06.00	02.00	07.00	+05.11	5.8
inoculated	10 C	2	21.0	01.00	05.00	01.00	06.00	+06.10	5.8
inoculated	10 C	4	16.0	01.00	04.00	01.00	06.00	+07.26	5.7
inoculated	10 C	8	14.5	00.25	01.50	01.00	02.00	+09.56	5.7
inoculated	10 C	12	16.0	00.25	01.50	00.50	02.00	+09.74	5.6
inoculated	10 C	16	18.0	00.25	01.00	00.50	01.00	+10.57	5.6
inoculated	10 C	20	15.5	00.25	01.50	00.50	02.00	+09.70	5.6

Appendix B. Treatments, storage temperatures, storage times, mean ERV values, dye reduction times, logs of bacterial numbers. and pH values for Trial I - B (heavy inoculation of P. fluorescens).

Treatment	Storage temperature	Storage time - days	Mean ERV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH
control	2 C	0	50.5	01.00	04.00	01.00	05.00	-01.00	5.8
control	2 C	2	40.0	01.00	07.00	01.00	08.00	-01.00	5.8
control	2 C	4	45.0	01.00	08.00	01.00	08.00	-01.00	5.8
control	2 C	8	34.5	01.00	06.00	01.00	07.00	-01.00	5.7
control	2 C	12	33.5	01.00	06.00	01.00	07.00	-01.00	5.7
control	2 C	16	30.0	01.00	05.00	01.00	07.00	-01.00	5.7
control	2 C	20	32.0	01.00	05.00	01.00	06.00	-01.00	5.7
control	10 C	0	50.5	01.00	04.00	01.00	05.00	-01.00	5.8
control	10 C	2	45.5	01.00	06.00	01.00	07.00	-01.00	5.8
control	10 C	4	36.5	01.00	07.00	01.00	08.00	-01.00	5.7
control	10 C	8	31.5	01.00	06.00	01.00	07.00	-01.00	5.7
control	10 C	12	30.5	01.00	05.00	01.00	08.00	-01.00	5.7
control	10 C	16	30.5	01.00	04.00	01.00	06.00	-01.00	5.7
control	10 C	20	25.0	01.00	04.00	01.00	06.00	-01.00	5.7
inoculated	2 C	0	54.2	00.50	04.00	01.00	05.00	+06.56	5.8
inoculated	2 C	2	44.0	01.00	05.00	01.00	05.00	+06.83	5.7
inoculated	2 C	4	38.0	01.00	04.00	01.00	05.00	+07.19	5.7
inoculated	2 C	8	26.5	01.00	03.00	01.00	04.50	+08.56	5.7
inoculated	2 C	12	20.0	00.50	02.00	01.00	03.00	+09.96	5.9
inoculated	2 C	16	24.0	00.50	01.00	00.50	02.00	+10.19	5.8
inoculated	2 C	20	31.0	00.50	02.00	00.50	03.00	+09.45	5.8
inoculated	10 C	0	54.2	02.00	05.00	01.00	05.00	+06.56	5.8
inoculated	10 C	2	48.0	01.00	04.00	01.00	04.00	+08.31	5.8
inoculated	10 C	4	26.0	01.00	03.00	01.00	02.50	+09.42	5.7
inoculated	10 C	8	29.0	00.50	03.00	00.50	02.50	+09.45	5.6
inoculated	10 C	12	30.5	00.50	02.00	00.50	02.00	+09.57	5.6
inoculated	10 C	16	25.0	00.50	02.00	00.50	02.00	+09.38	5.6
inoculated	10 C	20	32.0	00.50	02.00	00.50	02.00	+09.10	5.6

Appendix C. Treatments, storage temperatures, storage times, mean ERV values, dye reduction times, logs of bacterial numbers, and pH values for Trial II - A (light inoculation of *P. cerevisiae*).

Treatment	Storage temperature	Storage time - days	Mean ERV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH
control	2 C	0	71.0	01.00	06.00	01.00	08.00	-01.00	5.8
control	2 C	2	53.0	01.00	08.00	01.00	09.50	-01.00	5.8
control	2 C	4	73.0	01.00	10.00	01.00	10.00	-01.00	5.7
control	2 C	8	71.5	02.00	12.00	02.00	10.00	-01.00	5.7
control	2 C	12	67.0	01.00	09.00	01.00	08.00	-01.00	5.7
control	2 C	16	56.5	01.00	06.00	01.00	08.00	-01.00	5.7
control	2 C	20	54.5	01.00	05.00	01.00	06.00	-01.00	5.7
control	10 C	0	71.0	01.00	06.00	01.00	08.00	-01.00	5.8
control	10 C	2	63.0	01.00	08.00	01.00	09.00	-01.00	5.8
control	10 C	4	71.5	02.00	09.00	01.00	10.00	-01.00	5.7
control	10 C	8	65.5	02.00	06.00	01.00	08.00	-01.00	5.7
control	10 C	12	49.0	01.00	09.00	01.00	08.00	-01.00	5.7
control	10 C	16	34.5	01.00	06.00	01.00	07.00	-01.00	5.7
control	10 C	20	38.5	01.00	05.00	01.00	06.00	-01.00	5.7
inoculated	2 C	0	65.0	01.00	06.00	01.00	08.00	-01.00	5.6
inoculated	2 C	2	51.0	01.00	07.00	01.00	08.00	-01.00	5.6
inoculated	2 C	4	62.0	01.00	08.00	01.50	08.00	-01.00	5.6
inoculated	2 C	8	71.0	01.00	06.00	01.00	07.00	-01.00	5.5
inoculated	2 C	12	53.0	00.50	05.00	01.00	05.00	-01.00	5.5
inoculated	2 C	16	48.5	00.50	03.00	00.50	02.00	-01.00	5.4
inoculated	2 C	20	30.0	00.50	02.00	00.50	02.00	-01.00	5.5
inoculated	10 C	0	65.0	01.00	06.00	01.00	08.00	-01.00	5.6
inoculated	10 C	2	54.0	01.00	07.00	01.00	07.00	-01.00	5.5
inoculated	10 C	4	55.5	01.00	05.00	01.00	06.00	-01.00	5.5
inoculated	10 C	8	42.0	01.00	04.00	01.00	05.00	-01.00	5.5
inoculated	10 C	12	40.0	00.50	03.00	00.50	04.00	-01.00	5.5
inoculated	10 C	16	40.0	00.50	01.00	00.25	01.00	-01.00	5.3
inoculated	10 C	20	25.5	00.25	01.00	00.25	01.00	-01.00	5.3

Appendix D. Treatments, storage temperatures, storage times, mean ERV values, dye reductiom times, logs of bacterial numbers, and pH values for Trial II - B (heavy inoculation of P. cerevisiae).

Treatment	Storage temperature	Storage time - days	Mean ERV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH
control	2 C	0	60.5	00.50	04.00	01.00	05.00	-01.00	5.6
control	2 C	2	57.5	00.50	06.00	01.00	07.00	-01.00	5.6
control	2 C	4	57.5	01.00	07.00	01.50	08.00	-01.00	5.5
control	2 C	8	52.5	01.00	08.00	02.00	09.00	-01.00	5.5
control	2 C	12	38.5	02.00	09.00	01.50	07.00	-01.00	5.5
control	2 C	16	37.5	02.00	10.00	01.00	10.00	-01.00	5.5
control	2 C	20	39.0	01.00	10.00	02.00	11.00	-01.00	5.5
control	10 C	0	60.5	00.50	04.00	01.00	05.00	-01.00	5.6
control	10 C	2	50.0	00.50	06.00	01.00	07.50	-01.00	5.7
control	10 C	4	53.5	01.00	07.00	01.00	07.00	-01.00	5.5
control	10 C	8	43.5	01.00	09.00	01.00	07.00	-01.00	5.5
control	10 C	12	33.5	02.00	10.00	02.00	10.00	-01.00	5.5
control	10 C	16	31.0	02.00	09.50	01.00	10.00	-01.00	5.5
control	10 C	20	35.0	01.00	10.00	01.00	10.00	-01.00	5.5
inoculated	2 C	0	59.0	00.50	04.00	01.00	04.00	-01.00	5.6
inoculated	2 C	2	61.5	01.00	06.00	01.00	07.00	-01.00	5.6
inoculated	2 C	4	60.0	01.00	07.00	01.00	07.00	-01.00	5.4
inoculated	2 C	8	56.0	01.00	05.00	01.00	08.00	-01.00	5.5
inoculated	2 C	12	38.0	01.00	05.00	02.00	06.00	-01.00	5.5
inoculated	2 C	16	41.5	01.00	05.00	01.00	07.00	-01.00	5.4
inoculated	2 C	20	38.0	01.00	05.00	01.00	06.00	-01.00	5.4
inoculated	10 C	0	59.0	00.50	04.00	01.50	04.00	-01.00	5.6
inoculated	10 C	2	54.5	00.50	04.00	01.00	05.00	-01.00	5.5
inoculated	10 C	4	56.0	01.00	05.00	00.50	05.00	-01.00	5.4
inoculated	10 C	8	50.0	01.00	06.00	01.00	05.00	+07.29	5.4
inoculated	10 C	12	39.0	01.00	06.00	01.00	06.00	-01.00	5.3
inoculated	10 C	16	43.0	01.50	06.00	01.00	06.00	-01.00	5.3
inoculated	10 C	20	40.0	01.00	05.00	00.50	04.00	-01.00	5.3

Appendix E. Treatments, storage temperatures, storage times, mean EKV values, dye reduction times, logs of bacterial numbers, and pH values for Trial III - A (light inoculation of S. faecalis).

Treatment	Storage temperature	Storage time - days	Mean EKV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH
control	2 C	0	60.0	02.00	03.00	01.00	03.00	-01.00	5.8
control	2 C	2	55.0	00.25	04.00	00.25	03.00	-01.00	5.7
control	2 C	4	50.0	00.50	08.00	00.50	03.00	-01.00	5.7
control	2 C	8	44.2	00.50	13.00	00.50	04.00	+00.69	5.7
control	2 C	12	48.5	01.00	06.00	01.00	06.00	+02.32	5.7
control	2 C	16	56.0	01.00	04.00	01.00	05.00	+03.44	5.6
control	2 C	20	57.0	01.00	03.00	01.00	04.00	+03.39	5.6
control	10 C	0	60.0	02.00	03.00	01.00	03.00	+01.13	5.8
control	10 C	2	48.0	00.25	01.00	00.25	03.00	+01.47	5.8
control	10 C	4	46.0	00.50	08.00	00.50	04.00	-01.00	5.7
control	10 C	8	42.5	00.50	03.00	00.50	04.00	-01.00	5.7
control	10 C	12	39.2	01.00	11.00	01.00	06.00	-01.00	5.7
control	10 C	16	46.5	01.00	23.00	01.00	05.00	-01.00	5.5
control	10 C	20	50.0	01.00	-----	01.00	04.00	-01.00	5.5
inoculated	2 C	0	61.5	01.00	03.00	01.00	02.00	+04.34	5.8
inoculated	2 C	2	39.5	00.25	04.00	00.25	03.00	+03.97	5.7
inoculated	2 C	4	41.0	00.50	04.00	00.50	03.00	+03.96	5.7
inoculated	2 C	8	41.0	00.50	05.00	00.50	04.00	+03.81	5.6
inoculated	2 C	12	30.5	01.00	05.00	01.00	05.00	+05.23	5.6
inoculated	2 C	16	33.0	01.00	04.00	01.00	05.00	+03.66	5.5
inoculated	2 C	20	35.0	01.00	05.00	01.00	04.00	+04.05	5.5
inoculated	10 C	0	61.5	01.00	03.00	01.00	02.00	+04.34	5.8
inoculated	10 C	2	38.5	00.25	04.00	00.25	03.00	+03.86	5.8
inoculated	10 C	4	40.0	00.50	04.00	00.50	03.00	+04.44	5.7
inoculated	10 C	8	37.0	00.50	04.00	00.50	03.00	+04.98	5.6
inoculated	10 C	12	32.0	01.00	05.00	01.00	05.00	+04.81	5.5
inoculated	10 C	16	34.5	01.00	03.00	01.00	05.00	+04.56	5.5
inoculated	10 C	20	34.0	01.00	03.00	01.00	04.00	+04.65	5.5

Appendix F. Treatments, storage temperatures, storage times, mean ERV values, dye reduction times, logs of bacterial numbers, and pH values for Trial III - B (heavy inoculation of *P. cerevisiae*).

Treatment	Storage temperature	Storage time - days	Mean ERV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH
control	2 C	0	56.5	00.25	01.75	00.75	04.00	-01.00	5.8
control	2 C	2	57.0	00.50	07.00	00.50	06.00	-01.00	5.7
control	2 C	4	58.5	00.75	10.00	00.75	08.00	-01.00	5.7
control	2 C	8	59.5	00.50	13.00	00.50	04.00	-01.00	5.7
control	2 C	12	64.5	01.00	12.50	01.00	06.50	-01.00	5.6
control	2 C	16	70.5	01.00	11.00	01.00	09.00	-01.00	5.6
control	2 C	20	70.0	01.00	10.00	01.00	06.00	-01.00	5.6
control	10 C	0	56.5	00.25	01.75	00.75	04.00	-01.00	5.8
control	10 C	2	54.0	00.50	07.00	00.50	06.00	-01.00	5.7
control	10 C	4	52.0	00.75	10.00	00.75	08.00	-01.00	5.7
control	10 C	8	47.0	00.50	13.00	00.50	04.00	-01.00	5.7
control	10 C	12	44.7	01.00	12.50	01.00	06.50	-01.00	5.7
control	10 C	16	64.0	01.00	11.00	01.00	09.00	-01.00	5.6
control	10 C	20	63.0	01.00	10.00	01.00	06.00	-01.00	5.6
inoculated	2 C	0	63.0	00.25	04.00	00.25	01.75	+04.41	5.8
inoculated	2 C	2	44.5	00.50	05.00	00.50	01.00	+04.63	5.7
inoculated	2 C	4	45.0	00.50	09.00	00.25	08.00	+04.90	5.5
inoculated	2 C	8	50.5	00.50	08.00	00.50	04.00	+05.33	5.4
inoculated	2 C	12	51.0	01.00	07.50	01.00	02.50	+06.10	5.4
inoculated	2 C	16	51.0	01.00	08.00	01.00	09.00	------	5.4
inoculated	2 C	20	50.0	01.00	02.00	01.00	04.00	+06.27	5.4
inoculated	10 C	0	63.0	00.25	04.00	00.25	01.75	+04.41	5.8
inoculated	10 C	2	50.0	00.50	05.00	00.50	01.00	+05.20	5.6
inoculated	10 C	4	48.0	00.50	09.00	00.25	08.00	+06.07	5.5
inoculated	10 C	8	43.2	00.50	08.00	00.50	04.00	+06.38	5.3
inoculated	10 C	12	34.0	01.00	07.50	01.00	02.50	+07.92	5.4
inoculated	10 C	16	46.0	01.00	08.00	01.00	09.00	+08.08	5.4
inoculated	10 C	20	44.0	01.00	02.00	01.00	04.00	+08.06	5.4

Appendix G. Treatments, storage temperatures, storage times, mean EKV values, dye reduction times, logs of bacterial numbers, and pH values for Trial IV - A (light inoculation of mixed culture).

Treatment	Storage temperature	Storage time - days	Mean EKV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH
control	2 C	0	61.5	00.50	03.00	01.00	08.00	+02.50	5.8
control	2 C	2	59.5	01.00	05.00	01.00	08.00	+02.00	5.8
control	2 C	4	58.5	01.00	08.00	01.00	09.00	+02.00	5.6
control	2 C	8	58.0	01.00	10.00	01.00	12.00	-01.00	5.6
control	2 C	12	49.5	01.00	09.00	01.00	10.00	-01.00	5.6
control	2 C	16	47.5	01.00	08.00	01.00	09.00	-01.00	5.6
control	2 C	20	49.5	01.00	08.00	01.00	09.00	-01.00	5.6
control	10 C	0	61.5	00.50	03.00	01.00	07.00	+02.50	5.8
control	10 C	2	57.0	01.00	04.00	01.00	07.00	+01.77	5.8
control	10 C	4	46.5	01.00	08.00	01.00	08.00	-01.00	5.6
control	10 C	8	48.5	01.00	09.00	01.00	10.00	-01.00	5.6
control	10 C	12	45.5	01.00	07.00	01.00	09.00	-01.00	5.7
control	10 C	16	52.0	01.00	08.00	01.00	07.00	-01.00	5.8
control	10 C	20	53.7	01.00	08.00	01.00	08.00	-01.00	5.8
inoculated	2 C	0	64.5	01.00	02.00	01.00	06.00	+03.86	5.8
inoculated	2 C	2	53.5	00.50	02.00	01.00	04.00	+04.49	5.7
inoculated	2 C	4	54.0	00.50	01.50	01.00	03.00	+05.30	5.8
inoculated	2 C	8	51.0	00.25	01.00	00.75	01.00	+08.07	5.5
inoculated	2 C	12	12.0	00.25	01.00	00.50	01.50	+10.18	6.1
inoculated	2 C	16	12.0	00.25	01.00	00.50	02.00	+09.07	6.2
inoculated	2 C	20	15.0	00.25	00.75	00.50	02.00	+09.96	6.2
inoculated	10 C	0	64.5	01.00	03.00	01.00	06.00	+03.86	5.8
inoculated	10 C	2	50.5	00.50	02.00	00.75	04.00	+05.43	5.7
inoculated	10 C	4	43.0	00.50	01.00	00.75	02.00	+08.06	5.7
inoculated	10 C	8	16.0	00.25	01.00	00.25	01.50	+10.03	6.0
inoculated	10 C	12	15.5	00.25	00.50	00.25	01.00	+10.35	6.3
inoculated	10 C	16	09.5	00.25	01.00	00.75	02.00	+09.72	6.4
inoculated	10 C	20	09.0	00.25	00.50	00.50	01.50	+09.91	6.6

Appendix H. Treatments, storage temperatures, storage times, mean ERV values, dye reduction times, logs of bacterial numbers, and pH values for Trial IV - B (heavy inoculation of mixed culture).

Treatment	Storage temperature	Storage time - days	Mean ERV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH
control	2 C	0	68.5	00.50	03.00	01.00	08.00	+02.36	5.8
control	2 C	2	65.0	01.00	08.00	01.00	08.00	+01.74	5.8
control	2 C	4	66.0	01.00	08.00	01.50	09.00	-01.00	5.8
control	2 C	8	60.5	01.00	10.00	01.00	10.00	-01.00	5.8
control	2 C	12	66.0	01.00	09.00	01.00	10.00	-01.00	5.7
control	2 C	16	50.5	01.50	08.00	01.00	09.00	-01.00	5.7
control	2 C	20	49.0	01.00	08.00	01.00	08.00	-01.00	5.7
control	10 C	0	68.5	00.50	03.00	01.00	08.00	+02.36	5.8
control	10 C	2	63.0	01.00	08.00	01.00	09.00	-01.00	5.7
control	10 C	4	62.0	01.00	07.50	02.00	09.00	-01.00	5.7
control	10 C	8	59.5	01.00	10.00	02.00	09.00	-01.00	5.7
control	10 C	12	63.5	02.00	09.00	01.00	09.00	-01.00	5.7
control	10 C	16	36.5	01.00	07.00	01.00	09.00	-01.00	5.7
control	10 C	20	35.0	01.00	04.00	01.00	08.00	-01.00	5.7
inoculated	2 C	0	68.0	00.75	03.00	01.00	07.00	+03.65	5.8
inoculated	2 C	2	63.0	01.00	04.00	01.00	05.00	+04.43	5.6
inoculated	2 C	4	57.0	00.75	03.00	01.00	06.00	+03.74	5.8
inoculated	2 C	8	56.5	00.50	04.00	01.00	05.50	+06.17	5.8
inoculated	2 C	12	53.0	00.50	03.00	01.00	04.00	+08.05	5.8
inoculated	2 C	16	40.0	00.50	03.00	01.00	04.00	+09.50	5.7
inoculated	2 C	20	34.5	00.50	03.00	01.00	03.00	+08.44	5.7
inoculated	10 C	0	68.0	00.75	03.00	01.00	07.00	+03.65	5.8
inoculated	10 C	2	57.0	00.50	04.00	01.00	06.00	+05.65	5.6
inoculated	10 C	4	50.5	00.50	04.00	01.00	03.00	+07.45	5.8
inoculated	10 C	8	43.5	00.50	03.00	01.00	02.00	+09.71	5.8
inoculated	10 C	12	28.5	00.50	02.50	01.00	02.50	+09.76	6.0
inoculated	10 C	16	26.5	00.50	04.00	00.50	03.00	+08.49	6.0
inoculated	10 C	20	29.0	00.25	04.00	05.00	02.00	+10.31	6.0

Appendix I. Treatments, storage temperatures, storage times, mean EKV values, dye reduction times, logs of bacterial numbers, and pH values for Trial V - A (light inoculation of C. perfringens).

Treatment	Storage temperature	Storage time - days	Mean EKV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH	Logban
control	2 C	0	57.5	00.50	10.00	00.50	07.00	----	5.8	+03.92
control	2 C	2	53.0	01.00	09.00	01.00	09.00	-01.00	5.8	-01.00
control	2 C	4	50.5	01.00	10.00	01.00	10.00	-01.00	5.7	-01.00
control	2 C	8	53.5	01.00	09.00	02.00	10.00	-01.00	6.0	-01.00
control	2 C	12	52.5	01.00	09.00	02.00	09.00	-01.00	5.8	-01.00
control	2 C	16	48.0	01.00	08.00	01.00	10.00	-01.00	5.8	-01.00
control	2 C	20	52.5	00.50	08.00	01.00	09.00	-01.00	5.8	-01.00
control	10 C	0	57.5	00.50	10.00	00.50	07.00	----	5.8	+03.92
control	10 C	2	56.5	01.00	09.00	01.00	09.00	-01.00	5.8	+02.00
control	10 C	4	47.5	01.00	10.00	01.00	10.00	-01.00	5.7	-01.00
control	10 C	8	52.5	02.00	10.00	02.00	08.00	-01.00	6.0	+02.30
control	10 C	12	36.5	01.00	09.00	01.00	10.00	-01.00	5.8	-01.00
control	10 C	16	41.5	01.00	09.00	02.00	10.00	-01.00	5.7	-01.00
control	10 C	20	37.0	01.00	08.00	01.00	10.00	-01.00	5.8	-01.00
inoculated	2 C	0	54.5	00.50	09.00	00.50	06.00	----	5.8	+04.51
inoculated	2 C	2	53.5	01.00	05.00	01.00	05.00	-01.00	5.6	-01.00
inoculated	2 C	4	49.5	01.00	04.00	01.00	02.00	-01.00	5.5	-01.00
inoculated	2 C	8	49.0	00.50	07.00	01.00	08.00	-01.00	5.8	-01.00
inoculated	2 C	12	44.0	00.50	06.00	01.00	07.00	+04.03	5.8	-01.00
inoculated	2 C	16	41.0	01.00	08.00	02.00	10.00	-01.00	6.0	-01.00
inoculated	2 C	20	45.0	00.50	06.00	01.00	08.00	+02.00	6.0	-01.00
inoculated	10 C	0	54.5	00.50	06.00	00.50	06.00	----	5.8	+04.51
inoculated	10 C	2	47.5	01.00	05.00	01.00	05.00	-01.00	5.5	-01.00
inoculated	10 C	4	53.0	01.00	04.00	01.00	02.00	-01.00	5.4	-01.00
inoculated	10 C	8	48.0	00.50	07.00	01.00	05.00	-01.00	5.7	-01.00
inoculated	10 C	12	46.0	00.50	07.00	01.00	05.00	-01.00	6.1	-01.00
inoculated	10 C	16	40.0	00.50	05.00	01.00	08.00	-01.00	5.9	-01.00
inoculated	10 C	20	30.5	00.50	04.00	00.50	06.00	+02.00	6.1	-01.00

Appendix J. Treatments, storage temperatures, storage times, mean EKV values, dye reduction times, logs of bacterial numbers, and pH values for Trial V - B (heavy inoculation of C. perfringens).

Treatment	Storage temperature	Storage time - days	Mean EKV - ml	Res 1 - hours	Res 2 - hours	Tet 1 - hours	Tet 2 - hours	Logba	pH	Logban
control	2 C	0	53.0	00.50	09.00	00.50	07.00	-01.00	5.8	-01.00
control	2 C	2	50.0	00.50	10.00	01.00	10.00	-01.00	5.8	-01.00
control	2 C	4	49.0	01.00	09.00	01.00	09.00	-01.00	5.9	-01.00
control	2 C	8	39.5	01.00	09.00	00.50	07.00	+02.60	5.8	+03.35
control	2 C	12	45.5	02.00	08.00	01.00	07.00	-01.00	5.7	-01.00
control	2 C	16	44.0	02.00	08.00	01.00	10.00	-01.00	5.7	-01.00
control	2 C	20	42.0	02.00	10.00	01.00	10.00	-01.00	5.7	-01.00
control	10 C	0	53.0	00.25	09.00	00.50	08.00	-01.00	5.8	-01.00
control	10 C	2	45.0	00.50	10.00	00.50	09.00	-01.00	5.8	-01.00
control	10 C	4	39.5	00.50	08.00	01.00	09.00	-01.00	5.8	+02.00
control	10 C	8	37.5	01.00	09.00	01.00	08.00	+03.74	5.7	+02.60
control	10 C	12	33.5	01.00	08.00	01.00	06.00	-01.00	5.7	-01.00
control	10 C	16	37.0	02.00	09.00	01.00	09.00	-01.00	5.7	-01.00
control	10 C	20	37.0	01.00	09.00	01.00	10.00	-01.00	5.7	-01.00
inoculated	2 C	0	54.0	00.50	09.00	00.50	11.00	-01.00	5.8	-01.00
inoculated	2 C	2	50.5	00.50	08.00	00.50	09.00	-01.00	5.8	-01.00
inoculated	2 C	4	49.5	01.00	10.00	01.00	10.00	-01.00	5.8	-01.00
inoculated	2 C	8	46.0	01.00	09.00	01.00	07.00	+04.04	5.9	+03.53
inoculated	2 C	12	47.0	02.00	05.00	00.50	06.00	-01.00	6.0	-01.00
inoculated	2 C	16	45.0	02.00	06.00	01.00	07.00	-01.00	6.0	-01.00
inoculated	2 C	20	42.0	02.00	06.00	01.00	08.00	-01.00	5.9	-01.00
inoculated	10 C	0	54.0	00.50	08.00	00.50	08.00	-01.00	5.8	-01.00
inoculated	10 C	2	49.5	00.50	08.00	00.50	07.00	-01.00	5.8	-01.00
inoculated	10 C	4	49.5	01.00	08.00	02.00	06.00	-01.00	5.7	-01.00
inoculated	10 C	8	40.5	01.00	06.00	01.00	07.00	+03.00	5.9	+03.79
inoculated	10 C	12	34.5	01.00	05.00	01.00	04.00	-01.00	5.9	-01.00
inoculated	10 C	16	35.0	02.00	07.00	01.00	06.00	-01.00	5.9	-01.00
inoculated	10 C	20	31.5	01.00	05.00	01.00	08.00	-01.00	5.9	-01.00

Appendix K. Analysis of variance tables for Res 2, Tet 2, ERV, and Logba among two treatments, two inoculation levels, two storage temperatures, and seven storage times for Trial I (*P. fluorescens* inoculation).

table a. Dependent variable = Res 2

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	12.54	1	12.54	15.77**
treatment	121.54	1	121.54	152.85**
temperature	0.75	1	0.75	0.95
time	37.30	6	6.22	7.82**
inoculation level x treatment	2.79	1	2.79	3.51
inoculation level x temperature	0.22	1	0.22	0.28
inoculation level x time	4.80	6	0.80	1.01
treatment x temperature	1.61	1	1.61	2.03
treatment x time	21.68	6	3.61	4.54*
temperature x time	4.84	6	0.81	1.01
remaining error	19.88	25	0.79	
total	227.96	55		

** = significant at the 0.1% level ($P < .001$)
* = significant at the 1.0% level ($P < .01$)

table b. Dependent variable = Tet 2

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	26.47	1	26.47	37.76**
treatment	202.54	1	202.54	288.97**
temperature	6.79	1	6.79	4.69*
time	52.68	6	8.78	12.53**
inoculation level x treatment	2.36	1	2.36	3.37
inoculation level x temperature	0.11	1	0.11	0.16
inoculation level x time	14.38	6	2.40	3.42
treatment x temperature	3.25	1	3.25	4.64
treatment x time	20.18	6	3.36	4.80*
temperature x time	2.68	6	0.45	0.64
remaining error	17.52	25	0.70	
total	348.96	55		

** = significant at the 0.1% level ($P < .001$)
* = significant at the 1.0% level ($P < .01$)

Appendix K. cont'd.

table c. Dependent variable = ERV

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	2463.18	1	2463.18	332.20**
treatment	146.58	1	146.58	19.77**
temperature	37.79	1	37.79	5.10
time	2584.14	6	430.69	58.09**
inoculation level x treatment	10.63	1	10.63	1.43
inoculation level x temperature	15.02	1	15.02	2.03
inoculation level x time	423.32	6	70.55	9.52**
treatment x temperature	21.88	1	21.88	2.95
treatment x time	152.22	6	25.37	3.42
temperature x time	154.96	6	25.83	3.48
remaining error	185.37	25	7.41	
total	6196.08	55		

** = significant at the 0.1% level ($P < .001$)
* = significant at the 1.0% level ($P < .01$)

table d. Dependent variable = Logba

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	10.95	1	10.95	30.27**
treatment	850.04	1	850.04	2350.52**
temperature	4.83	1	4.83	13.35**
time	64.28	6	10.71	29.62**
inoculation level x treatment	46.23	1	46.23	127.83**
inoculation level x temperature	1.91	1	1.91	5.28
inoculation level x time	15.81	6	2.64	7.29**
treatment x temperature	0.85	1	0.85	2.34
treatment x time	7.39	6	1.23	3.40
temperature x time	3.11	6	0.52	1.43
remaining error	9.04	25	0.36	
total	1014.43	55		

** = significant at the 0.1% level ($P < .001$)
* = significant at the 1.0% level ($P < .01$)

Appendix L. Analysis of variance tables for Res 2, Tet 2, ERV, and Logba among two treatments, two inoculation levels, two storage temperatures, and seven storage times for Trial III (S. faecalis and P. cerevisiae inoculation).

table a. Dependent variable = Res 2

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	91.29	1	91.29	8.08
treatment	107.25	1	107.25	9.49*
temperature	0.29	1	0.29	0.03
time	284.53	6	47.42	4.20*
inoculation level x treatment	1.61	1	1.61	0.14
inoculation level x temperature	0.29	1	0.29	0.03
inoculation level x time	28.74	6	4.79	0.42
treatment x temperature	2.57	1	2.57	0.23
treatment x time	79.78	6	13.30	1.18
temperature x time	62.71	6	10.45	0.92
remaining error	282.56	25	11.30	
total	941.62	55		

** = significant at the 0.1% level ($P < .001$)
* = significant at the 1.0% level ($P < .01$)

table b. Dependent variable = Tet 2

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	27.86	1	27.86	52.75**
treatment	18.86	1	18.86	35.71**
temperature	0.00	1	0.00	0.00
time	103.81	6	17.30	32.76**
inoculation level x treatment	7.50	1	7.50	14.21**
inoculation level x temperature	0.00	1	0.00	0.00
inoculation level x time	54.17	6	9.03	17.09**
treatment x temperature	0.07	1	0.07	0.14
treatment x time	13.67	6	2.27	4.31*
temperature x time	0.25	6	0.04	0.08
remaining error	13.21	25	0.53	
total	239.41	55		

** - significant at the 0.1% level ($P < .001$)
* - significant at the 1.0% level ($P < .01$)

Appendix L. cont'd.

table c. Dependent variable = ERV

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	1021.73	1	1021.73	124.33**
treatment	1382.06	1	1382.06	168.18**
temperature	278.13	1	287.13	33.84**
time	1432.30	6	238.72	29.05**
inoculation level x treatment	1.65	1	1.65	0.20
inoculation level x temperature	28.29	1	28.29	3.44
inoculation level x time	340.33	6	56.72	6.90**
treatment x temperature	70.43	1	70.43	8.57*
treatment x time	759.04	6	126.51	15.39**
temperature x time	167.27	6	27.88	3.39
remaining error	205.44	25	8.22	
total	5686.65	55		

** = significant at the 0.1% level ($P < .001$)
* = significant at the 1.0% level ($P < .01$)

table d. Dependent variable = Logba

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	0.99	1	0.99	0.71
treatment	359.41	1	359.41	256.40**
temperature	0.05	1	0.05	0.04
time	6.12	6	1.02	0.73
inoculation level x treatment	30.98	1	30.98	22.10**
inoculation level x temperature	13.22	1	13.22	9.43*
inoculation level x time	4.97	6	0.83	0.59
treatment x temperature	18.80	1	18.80	13.41**
treatment x time	8.91	6	1.48	1.06
temperature x time	3.64	6	0.61	0.43
remaining error	35.04	25	1.40	
total	482.14	55		

** = significant at the 0.1% level ($P < .001$)
* = significant at the 1.0% level ($P < .01$)

Appendix M. Analysis of variance tables for Res 2, Tet 2, ERV, and Logba among two treatments, two inoculation levels, two storage temperatures, and seven storage times for Trial IV (mixed culture inoculation).

table a. Dependent variable = Res 2

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	20.34	1	20.34	23.55**
treatment	324.24	1	324.24	375.45**
temperature	1.22	1	1.22	1.41
time	43.88	6	7.31	8.47**
inoculation level x treatment	10.94	1	10.94	12.67*
inoculation level x temperature	< 0.01	1	< 0.01	<0.01
inoculation level x time	8.29	6	1.38	1.60
treatment x temperature	2.06	1	2.06	2.39
treatment x time	70.39	6	11.73	13.58**
temperature x time	1.98	6	0.33	0.38
remaining error	21.59	25	0.86	
total	504.94	55		

** = significant at the 0.1% level (P <.001)
* = significant at the 1.0% level (P <.01)

table b. Dependent variable = Tet 2

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	10.72	1	10.72	17.73**
treatment	383.25	1	383.25	634.04**
temperature	7.50	1	7.50	12.42*
time	18.49	6	3.08	5.10*
inoculation level x treatment	7.50	1	7.50	12.42*
inoculation level x temperature	<0.01	1	<0.01	<0.01
inoculation level x time	2.31	6	0.39	0.64
treatment x temperature	<0.01	1	<0.01	<0.01
treatment x time	62.53	6	10.42	17.24**
temperature x time	4.28	6	0.71	1.18
remaining error	15.11	25	0.60	
total	511.71	55		

** = significant at the 0.1% level (P <.001)
* = significant at the 1.0% level (P <.01)

Appendix M. cont'd.

table c. Dependent variable = ERV

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	1299.86	1	1299.86	25.26**
treatment	3108.14	1	3108.14	60.40**
temperature	577.29	1	577.29	11.22**
time	7051.01	6	1175.17	22.84**
inoculation level x treatment	351.00	1	351.00	6.82*
inoculation level x temperature	19.68	1	19.68	0.38
inoculation level x time	422.93	6	70.49	1.37
treatment x temperature	78.26	1	78.26	1.52
treatment x time	1614.40	6	269.07	5.23**
temperature x time	246.08	6	41.01	0.80
remaining error	1286.50	25	51.46	
total	16055.16	55		

** = significant at the 0.1% level ($P < .001$)
* = significant at the 1.0% level ($P < .01$)

table d. Dependent variable = Logba

Source	Sum of squares	Degrees freedom	Mean square	F value
inoculation level	4.35	1	4.35	5.79
treatment	787.95	1	787.95	1050.34**
temperature	2.36	1	2.36	3.15
time	17.88	6	2.98	3.97*
inoculation level x treatment	0.16	1	0.16	0.21
inoculation level x temperature	0.46	1	0.46	0.62
inoculation level x time	1.86	6	0.31	0.41
treatment x temperature	9.81	1	9.81	13.08**
treatment x time	166.48	6	27.75	36.99**
temperature x time	3.88	6	0.65	0.86
remaining error	18.75	25	0.75	
total	1013.95	55		

** = significant at the 0.1% level ($P < .001$)
* = significant at the 1.0% level ($P < .01$)