SURVIVAL OF CLOSTRIDIUM PERFRINGENS ON CHICKEN PIECES COOKED WITH MICROWAVE ENERGY

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ABSTRACT

SURVIVAL OF CLOSTRIDIUM PERFRINGENS ON
CHICKEN PIECES COOKED WITH
MICROWAVE ENERGY

By

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This study was conducted to evaluate the influence of microwave cooking on survival of Clostridium perfringens vegetative cells and spores of a heat resistant strain and heat sensitive strain inoculated onto the surface of chicken thighs and drumsticks. Inoculated and uninoculated chicken pieces were cooked in a commercial belt fed microwave oven (915 MHz) with potable steam source and browned in heated vegetable oil. Other chicken pieces were cooked in a household microwave oven (2450 MHz) and evaluated. The effects of freezing, thawing, frozen storage and/or reheating were also examined. Viable cells and spores of C. perfringens were enumerated in a pouch system using modified Shahidi - Ferguson - Perfringens (SFP) agar.

Different antibiotic combinations were used in SFP to determine optimum recovery of heat injured vegetative cells. These antibiotics were; polymyxin B sulfate + sulfadiazine (SPS), kanamycin sulfate + polymyxin B sulfate (SFP) and D - cycloserine (TSC).

Microwave cooking resulted in a two log - cycle reduction in the number of vegetative cells. This degree of reduction applied to both resident cells and inoculated C. perfringens vegetative cells. Freezing and thawing prior to cooking in the microwave unit gave a greater reduction in the microbial load after cooking of both cells and spores of C. perfringens. Microwave cooking of unfrozen inoculated chicken pieces caused activation and germination of spores present; reduction of spores from these samples was minimal.

Cells and spores of C. perfringens were not recovered after microwave cooking followed by browning in heated vegetable oil.

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John F. Blanco

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TABLE OF CONTENTS

LIST OF FIGURES

Figure | Page

LIST OF APPENDICES

INTRODUCTION

The demand for quality convenience foods has brought about many new food products and processing innovations. Microwave energy is used for uniform heating, control of heat generation and depth of penetration without resultant surface crust formation. In a microwave system only the product heats and heat generation is controlled by an on-off switch. Microwave energy is more economical and provides greater efficiency than a conventional system for cooking poultry parts (Jeppson, 1964; May, 1969; Smith, 1969).

Dawson and Sison (1971b) reported that centralized processing of chicken, including precooking and freezing of breaded chicken pieces, offered one means for quality control by use of frozen product and provides continuous availability of uniform product with minimum loss as a result of fluctuating demand. Quality precooked fried chicken, similar to homecooked, can be served by avoiding refrying in fat before heating and serving (Hoffert, 1971).

Since variations occur in the precooking process, and in methods of handling and storage, different effects on the destruction of the microbial flora are expected. In particular, the effects of a process on food poisoning microorganisms should be determined. Thus the need exists to evaluate each particular precooking system to reduce or eliminate bacteria.

Detection of injured bacterial cells is important to the food microbiologist, as is knowledge of recovery of injured cells by the selective media and methods of isolation used. This is especially true when recovery of anaerobic cells is attempted.

In this study C. perfringens was selected as the organism of choice since it is ubiquitous in nature, a spore former, usually associated with food poisoning outbreaks in meals prepared in quantity, and sporulates on poultry meat.

In 1971 C. perfringens accounted for 25.3% of the reported food poisoning outbreaks and 32% of individuals involved in outbreaks of bacterial origin. During 1971, 33 of 51 reported food poisoning outbreaks due to C. perfringens involved meals served at restaurants, cafeterias, picnics, schools and churches.

The objective of the study was to evaluate the microbial problems associated with a large scale preparation of precooked fried chicken using microwave energy.

REVIEW OF LITERATURE

Microwave Energy

Heat generated by microwave energy is due to polarization of molecules within the material heated. Removal of the external field results in reversion of molecules to a random state. Return to the random state causes molecules to collide, releasing extra energy in the form of heat.

An analogy of this effect occurs when water molecules are placed between plates of a capacitor connected to a power supply. When the switch is closed the water molecules attempt to align to the proper pole. When the switch is opened the field relaxes and molecules return to a random orientation. Thus stored potential energy is emitted as heat. In a simple open-close operation the amount of energy is small but the effect is additive. In a microwave field this change from ordered to random state occurs several million times a second (Tinga, 1970).

Nature of Heating

Proctor and Goldbilth (1953) and Brown and Morrison (1954) indicated that the effect of microwave energy on bacteria was due to heat. Olson (1965) however, found no lethal effects of thermal energy, generated by a microwave source, on bread mold. He indicated that reduction of microbial loads was due solely to an inherent character-

istic of microwave energy. He also showed (1966) that microwave treated cells of Saccharomyces cerevisiae had a decreased respiration rate when compared to cells heated to the same temperature in a water bath.

Comparison of thermal death data following conventional heating and use of 2,450 MHz microwave energy showed no difference on microbial reduction of Bacillus subtilis to the same final temperature. Reduction of microbial levels from microwave energy was nearly identical to that expected for the same time-temperature exposure to conventional heating. To further substantiate these results ice was added to bacterial suspensions placed in the microwave oven. This lengthened the exposure time (100 sec.) but had little influence on bacterial viability. The highest temperature attained by the suspension during heating was 29 C (Goldblith and Wang, 1967).

Lechowich et al. (1968) examined the effects of microwave energy on Streptococcus faecalis and Saccharomyces cerevisiae. Their system included a Liebig condenser positioned in a 2,450 MHz microwave oven. The coolant in the jacket of the condenser was kerosene maintained at -25 C. A thermocouple relay system was used to measure the temperature of the suspension immediately after magnetron shut off. The use of microwave energy on S. faecalis and S. cerevisiae suspensions of 10^8 - 10^9 cells/ml effected a reduction of cell population solely by heat, as measured by the change in temperature. At non-lethal temperatures no effects of microwave energy were noted. Further, when

respiration rates of S. cerevisiae were compared before and after microwave heating, the decrease in respiration was due solely to a decrease in the viable count.

Bomar and Grunewald (1972) heated B. subtillis var. niger and B. stearothermophilus in nutrient broth and olive oil. The solutions were held in an ice water bath to maintain a specific temperature. When bacterial cells in various phases of the growth cycle were used in the microwave oven, no reduction was evident in their system. This was attributed to the lack of a temperature increase.

Reduction of Bacteria by Microwaves

Dessel et al. (1960) found that the microwave oven was more efficient than a conventional oven in reducing microbial loads. The lower counts from samples cooked in the microwave oven were thought to be due to more rapid heating and more even distribution of heat.

The effect of microwave energy on Salmonella typhimurium in chicken broth, in chicken sauce and on chicken parts was investigated by Woodburn et al. (1962). When 50 g of product containing 1.3×10^6 cells/g were exposed to microwave energy for 120 sec., the numbers of bacterial cells were reduced to levels of less than 10 cells/g. Similar findings were noted with Staphylococcus aureus at inoculum levels of 2.0×10^6 cells/g. Shorter exposure times did not reduce bacterial cells to this level.

B. subtillis spores, in mashed potatoes, were not completely destroyed when heated for three min. in a 2,450 MHz microwave oven.

The mean survival was approximately 16.4% of the original inoculum. Survivors were speculated to have resulted from nonuniform heating by an uneven distribution of microwave energy. Combinations of conventional heating to 65.5 C, infrared heating, and microwave heating resulted in no survivors (Lacy and Weiner, 1965). No attempts were made to allow for recovery of heat-damaged organisms.

Delaney et al. (1968) heated Aspergillus flavus spores and Escherichia coli in custard, reconstituted dry milk, 0.1 M sucrose and physiological saline and found little effect due to microwave energy. The level of survival depended on the composition of the heating media. Higher levels of organisms heated in custard and milk survived than when heated in 0.1 M sucrose or physiological saline. When heating for 10 min $\pm$ 30 sec using both 2,450 and 915 MHz sources of energy, no significant differences in degree of recovery were noted, and final temperatures were apparently similar.

Reduction of S. typhumurium populations by microwave energy in lemon and chocolate pies was studied by Baldwin et al. (1968). Pies were inoculated with cells from a 24 hr broth culture, prepared with sterile equipment under sanitary conditions, and evaluated before and after microwave cooking. Pies were also held for 24, 48, and 72 hours at 33 C. No survivors were found by plate count method in inoculated uncooked or cooked lemon pies. Survivors from lemon pies were detected only when the pies were sampled immediately after preparation before cooking by pre-enrichment technique using lactose broth. Only pre-enrichment methods detected survivors in chocolate pies after microwave cooking.

Clostridium perfringens

C. perfringens is a normal inhabitant of the intestinal tracts of man and animals. The organisms exist in sewage and soil in both vegetative and spore states. In normal individuals, the numbers of C. perfringens in the stool are low. The number in stools of individuals suffering from C. perfringens food poisoning are significantly higher. Examination of stool samples from 219 food handlers for the presence of food poisoning microorganisms (Hall and Hauser, 1966) indicated that 78% were positive for C. perfringens, and 35% contained strains that produced heat-resistant spores.

Various soils have been examined for the presence of C. perfringens. Taylor and Gordon (1940) reported that 97% of the soil samples tested contained C. perfringens, mostly type A. Levels varied from 10^2 to 10^4 C. perfringens cells/g, and black loams contained the greatest concentration (Smith, 1963). Eighty-five percent of dust samples from a commerical kitchen environment contained C. perfringens Type A (McKillop, 1959).

Hall and Angelotti (1965) examined 262 samples of retail cuts of meat for C. perfringens. Of this total, 167 samples were raw unprocessed beef, veal, lamb, pork and chicken, and 101 were processed meat and meat dishes. C. perfringens was isolated from 58% of the raw samples and from 20% of the cooked samples. Strong et al. (1963) isolated C. perfringens from 16% of 122 samples of raw meat, fish and poultry. The difference in values reported by these studies is probably due to the use of enrichment methods by Hall and Angelotti (1965).

Isolation of C. perfringens from retail outlets of two socio-economic areas in Cincinnati (Messer et al., 1970) showed that levels of C. perfringens from the two areas were similar. They reported the following percentage isolation of C. perfringens:

Chicken	16.7%
Green beans	3.3%
Hamburger	30.0%

Lillard (1971) surveyed three broiler processing plants and reported high levels of C. perfringens on chickens brought into the plants. Except for feathers, feet and ceca, the number of organisms isolated from birds after final washing or from chill tanks were low. With few exceptions C. perfringens was isolated from further processed chicken products only by enrichment methods and 2.6% of 118 samples contained C. perfringens. Mead and Impey (1970) found levels of 31-347 cells/g on neck skin and 1-3 cells/10 cm^2 on whole birds just prior to packaging. The types of C. perfringens found on the carcasses and in the gut were considered to be the same on the basis of α-hemolysis.

Effects of Cooking on Cells and Spores

Cooking can reduce the numbers of, or completely destroy, vegetative cells of C. perfringens, but spores may survive. Heat activates the majority of spores, and survivors may be stimulated to germinate. Heat also drives off oxygen and creates an anaerobic environment. Strong and Ripp (1967) inoculated hams and turkey rolls with cells of heat-sensitive and heat-resistant strains of C. perfringens.

Hams were baked as per directions on the can. The maximum internal temperature attained was 59 C after 28.4 min. and C. perfringens were recovered from both outer and inner slices. Turkey rolls were cooked to internal temperatures of 74 C and 85 C. "Apparent survival" was greatest when the final temperature was 85 C. This was believed to be due to heat activation of spores. Optimal activation of spores at temperatures of 75, 80, 85 and 90 C was reported by Roberts (1968). At these temperatures significant activation occurred, whereas at 95-100 C inactivation was proportionately more rapid.

Spores of heat-sensitive and heat-resistant strains can survive the cooking process. The degree of survival depends on the internal temperature and the consistency of the heating medium (Woodburn et al., 1967). Spores of a heat-sensitive strain survived in a bread and onion stuffing (cooked in a pan) when cooked to internal temperature of 73 C and 82.5 C. The number of heat-sensitive survivors was 60-110 cells/g and depended on the heating temperature.

Pivnick et al. (1968) showed that when raw chicken was inoculated with 10^4 spores/g of C. perfringens and cooked to internal temperatures of 85-90 C, the inoculum was reduced to 1.5 cells/g. Incubation of these pieces at 45 C showed an increase to 10^6 cells/g in 14.7 hours. Bacterial counts of chicken parts inoculated with 2.7 x 10^2 vegetative cells/g and held at 45 C increased to 10^7 cells/g in 6.3 hr.

Hobbs (1953) reported that heat-resistant strains were dominant in foods associated with food poisoning outbreaks. She attributed most cases of C. perfringens food poisoning to meat or poultry that

was cooled slowly or left overnight at room temperature. Heat resistance of C. perfrigens strains was reported by Collee et al. (1961) to be genetically determined and not dependent upon the presence of a large number of spores. Roberts (1968) showed that, in the absence of heating, low numbers of spores of heat-resistant strains (0.13 to 3.6%) produced colonies, compared to 36-41% from spores of heat-sensitive strains. Non-hemolytic strains had D_{100} values of 6-17 min, whereas B-hemolytic strains had values of 1 min with D-90 values of 3-5 min, z-value were 9-17 C and 6-7 C respectively.

Excellent growth and sporulation were reported (Mead, 1968) on raw chicken thighs and breasts at temperatures of 30-50 C. The pH of leg and breast was 6.5-6.7 and 5.6-5.7, respectively. Sporulation of C. perfringens cells does not occur at these pH values in laboratory media. Sporulation of several strains, including heat-resistant types, was 10-100 times greater in thigh than in breast muscle, probably due to higher pH values.

Effects on Low Temperatures

Recovery of unheated cells and spores of heat-sensitive and heat-resistant strains in various solutions was reported by Canada and Strong (1964). The level of vegetative cells was reduced to 0.6-0.3%; 4.0-9.0% and 0.3-1.1% when held at 7.0 C for 48 hrs in phosphate buffer, 0.1% peptone, and 2% sucrose solutions, respectively. They used SPS agar and incubated in an anaerobic incubator with 90% N_2; 10% CO_2. Storage at -17.7 C resulted in 0-0.4%, 0.2-0.4%, and 0.1-2.4%

recovery in phosphate buffer, 0.1% peptone and 2% sucrose solutions, respectively. The recovery of spores held at 7.1 C was from 21-41%. At -17.7 C, recovery ranged from 16-58% in the various solutions. The spore suspensions were not subjected to a heat shock treatment (Canada et al., 1964). Survival of vegetative cells in chicken gravy, held at -17.7 C was less than 20% in 24 hr, 80% in 10 days, and 1% in 180 days. Under similar conditions, spore survival was 50% after one day, showed a slight increase after 10 days, and decreased thereafter. After storage at -17.7 C, for 180 days recovery was less than 10% of the original inoculum (Canada and Strong, 1964). No reason was given for the increase that occurred after 10 days storage. Survival of C. perfringens in turkey rolls inoculated with a suspension that consisted of vegetative cells and spores was evaluated by Strong and Ripp (1967). Their recovery methods did not include a heat shock treatment. When turkey was cooked to 85 C and held at 10 C, the number decreased slightly after 24-48 hours. Survivors of the cooking process (1.7×10^4 cells/g) did not decrease significantly in 24 hr, but decreased to 8.7×10^3 cells/g in 48 hours. Cooking to an internal temperature of 74 C gave comparable results.

White and Hobbs (1963) reported that C. perfringens did not grow in cooked meat held at 1.6 C for 7 days. Barnes et al. (1963) showed that growth of C. perfringens is restricted below a temperature of 15-20 C.

Media and Methods for Isolation

Selective Media.--Media which provide presumptive evidence of the presence of clostridia contain various inhibitors to prevent the growth of other microorganisms. Inclusion of sulfite, which some organisms reduce to sulfides, results in black colonies. Presence of such colonies in selective media is presumptive evidence of C. perfringens. Fuch and Bonde (1957) reported that formation of black colonies depended on a minimal concentration of 2 µmole/ml of sulfite but that repeated transfers in peptone broth increased the threshold concentration of sulfite needed for ferrous sulfide formation. Some strains of C. perfringens eventually lost their ability to form ferrous sulfide. When meat extract and peptone agars were used, those strains regained their ability to reduce sulfite, this was due to presence of nicotinamide. The ability of an organism to reduce sulfite was reported to depend on the redox potential of the media (Mead, 1969). Thus, the greater the reduced environment of the media, the greater the possibility for formation of sulfides.

Sulfadiazine was incorporated into sulfite iron agar by Mossell (1956, 1959) and by Angelotti et al. (1961) to inhibit members of the family Enterobacteriaceae. Some non-sulfite reducing bacteria grew but did not form black colonies. Sulfite and neomycin were used by Marshall et al. (1965) in their medium, tryptone-sulfite-neomycin (TSN) to isolate C. perfringens. Spencer (1969) added 50 µg/ml of neomycin to blood and nagler agar as a selective agent for clostridia.

They reported that C. botulinum types A and B and heat resistant strains were not inhibited. Heat sensitive strains were found to be more susceptible to inhibition by this antibiotic than heat-resistant strains of C. perfringens. Shahidi and Ferguson (1971) incorporated polymyxin B sulfate and kanamycin sulfate as selective agents. Their medium also contained sodium meta-bisulfite and ferric ammonium citrate to indicate sulfite reduction, and egg yolk to indicate production of lecithinase. Recovery of C. perfringens on this medium was higher than on sulfite-polymyxin-sulfadiazine (SPS) agar. Shahidi-Ferguson-Perfringens (SFP), TSN, and SPS agars were evaluated by Harmon et al. (1971a) to determine which was most selective for C. perfringens and most inhibitory to other microorganisms. They found that SFP gave significantly higher recoveries of spores and vegetative cells than the other two, but SFP was least selective. TSN was found to be the most selective, however, incubation of SFP at 46 C enhanced its selectivity. SFP was later modified (Harmon et al., 1971b) by replacing the antibiotics with D-cycloserine. Their medium, tryptose-sulfite-cycloserine (TSC), was compared with SFP for recovery of vegetative cells of C. perfringens, and found to be more selective than SFP. A concentration of 400 µg/ml D-cycloserine inhibited most facultative anaerobes tested. Of facultative strains tested, only Serratia marcescens and Streptococcus lactis grew but they are non-sulfite reducers.

The antibiotics currently used in media for presumptive identification of clostridia are polymyxin B sulfate, kanamycin sulfate and

and D-cycloserine. Polymyxin B sulfate binds to the cytoplasmic membrane and disturbs its function. It has little effect on gram-positive organisms. This is believed to be due to the presence of a magnesium ribonucleate coating of the gram-positive cell membrane.

The action of kanamycin sulfate is similar to that of streptomycin. These antibiotics cause a marked inhibition of protein biosynthesis, believed to be due to their ability to hinder the formation of a "functional initiation complex" and the elongation of a previously initiated peptide chain. This effect is thought to be related to its ability to induce misreading of mRNA's.

D-cycloserine is a mimic for a D-alanine-D-alanine group. Incorporation of D-cycloserine into the peptidioglycan structure prevents linkage of peptidioglycan units. The peptidioglycan has been shown to be the basic unit of bacterial cell walls (Strominger and Ghuysen, 1967).

SFP, SPS, AND TSC provide only presumptive evidence of Clostridia. Specificity of the presence of C. perfringens can be determined by nitrate reduction, gelatin liquification, lecithinase production and motility. Angelotti et al. (1961) proposed the use of nitrate motility medium, sporulation broth and Noyer veal broth for toxin production to confirm black colonies on SPS as C. perfringens. Hall et al. (1969) suggested the use of SPS followed by examination of black colonies in nitrate-motility media, and lactose egg yolk agar (LEY) to confirm that an isolate was C. perfringens. Shahidi and Ferguson (1971a) proposed that SFP, when used with lactose-motility agar (LM) is sifficient to identify an orgamism as C. perfringens.

Diluents.--Diluents used by various investigators included standard phosphate buffer (APHA), 0.1% peptone, reconstituted clostridial medium (RCM) and prereduced anaerobically sterilized (PRAS) media. Rapid and extensive destruction of the natural mixed bacterial population of precooked frozen poultry has been shown to occur in diluents such as water and phosphate buffer. Peptone, however, provides full protection for up to one hour (Straka and Stokes, 1957). Phosphate buffer and peptone (0.1%) were evaluated by Hauschild et al. (1967) in combination with commercial SPS agar for recovery of C. perfringens. They reported that 0.1% peptone gave the best recovery of C. perfringens. Smith (1971) suggested that ordinary buffered salts contained trace quantities of heavy metals which affected cell recovery. He recommended PRAS diluent, a balanced salt solution containing 0.05% cysteine. Cysteine binds to heavy metals present. RCM was recommended by Hirsch and Grinsted (1954) as the diluent of choice for enumeration of anaerobic sporeformers. Since RCM is a very rich diluent, an increase in bacterial numbers reported may be possible due to multiplication in the diluent. In large plating studies this could be a significant factor.

Methods for Recovery of Bacteria from Poultry

A variety of methods have been advocated for recovery of bacteria from poultry carcasses. Walker and Ayres (1956) found a consistant relationship between the bacterial numbers removed by the cotton swab technique and those recovered by a rinse method. The

rinse method was recommended by Mallmann and Dawson (1958) on the basis of greater variance of results obtained with the cotton swab technique. Variation among methods is due to removal of bacteria from poultry surfaces. Fromm (1959) recommended a swab method because the scrubbing action removed more bacteria. He found that many bacteria remained on poultry surfaces after rinsing. He indicated that fat composition of poultry skin would inhibit the action of water in dislodging microorganisms from the skin. The swab method is simple, rapid and requires no special equipment.

Recovery of bacteria from surfaces of poultry held for 0-8 days at 4 C was found to be independent of the area of the template, medium or incubation temperature used. Areas of 2, 5, and 10 cm^2 were swabbed. Plates of tryptone-glucose-yeast extract, nutrient, heart infusion, Eugon, and trypticase soy agars, inoculated and incubated at 10 C for 10 days, 20 C for 4 days, and 30 C for 2 days (Kinsley and Mountney, 1966) showed no significant differences in numbers recovered.

Effects of Heat on Vegetative Bacterial Cells

The traditional theory of thermal death of bacteria is that it follows the first order kinetics of a unimolecular reaction. A first order reaction is one in which the rate is proportional to the number of molecules present. If thermal death of bacteria follows first order kinetics, death must result from inactivation of a single molecule or "site" per bacterial cell. This implies that the death

rate is highest when the sample is first subjected to heat, when the number of bacteria is greatest. This theory does not account for the frequently observed initial lag in bacterial death rate, or for sublethal injury (Moats, 1971).

Biochemical events that result from heat injury have been investigated. The effects of sublethal injury noted are an extended lag phase (Kaufmann, 1959), specificity for a richer media (Harris, 1963) and a change in salt tolerance (Iandolo and Ordal, 1966). The latter authors described the ability of heat-injured S. aureus to recover on media containing 7.5% salt.

The initial cause of heat injury to viable cells has not been specifically elucidated, but significant evidence has been accumulated to suggest possible mode(s) of action. Leakage of cytoplasmic constituents was noted by Russell and Harries (1967) after heat treatment. This indicated damage to the cytoplasmic membrane and subsequent leakage of cell nucleic acids as measured by an increase in absorbance at 260nm. Ribosomal RNA resynthesis is essential for recovery of S. aureus (Sogin and Ordal, 1967) and S. typhimurium (Clark et al., 1968). Heat degrades rRNA and growth does not proceed without recovery and is represented by an extended lag period (Sogin and Ordal, 1967; Tomlins and Ordal, 1971).

Recovery of Streptococcus faecalis, Staphylococcus aureus and Bacillus subtilis requires RNA synthesis (Sogin and Ordal, 1967; Clark et al., 1968; Miller and Ordal, 1972) whereas recovery of Salmonella typhimurium requires both protein and rRNA synthesis (Tomlins and Ordal, 1971). DNA does not appear to be involved in the recovery of sublethally heat-injured vegetative cells.

Recovery (not growth) of sublethally injured cells of S. aureus and S. typhimurium requires glucose, amino acids and inorganic phosphate. Recovery also occurred in media that would not support growth (Iandolo and Ordal, 1966).

Activation and Germination of Spores

Different terms have been used to explain the process of vegetative cell formation from the spore state. The explanations offered by Keynan and Evenchick (1969) will be used here. Activation was described as a state in which the spore is induced to germinate but does not lose its cryptobiotic (dormant) properties. Thus the spore is still refractile, heat resistant, radiation resistant and does not stain. Spores in this stage can revert to the dormant state when germination conditions are not adequate.

Germination is defined as that state where the spore loses its spore properties but still differs from the vegetative cell in that there is no new macromolecular systhesis. The spore becomes phase dark, stains and is sensitive to heat. Although no new synthesis occurs, an increase in respiratory activity is measurable. Outgrowth is the process of changing from a germinated spore to a vegetative cell, thus synthesis of new macromolecules occurs.

Exposure of spores to sublethal heat, low pH (1.0-1.5), thiol compounds or strong oxidizing agents induced activation. Heat will induce activation, however, too low a temperature or too short a time may induce a more dormant state (Keynan and Evenchick, 1969).

Spores can also become activated during prolonged storage by a process described as "aging". Aging has been equated to a sublethal heat treatment, since it induces loss of the dormant state. The only difference noted between aging and activation is that in the latter, dormancy is lost temporarily and in the former dormancy is lost irreversibly.

Germination may also occur without an activation step. Physiological germination is induced by specific nutrients required for germination, i.e. metabolized by spores, and the kinetics of germination is activation dependent. In non-physiolocial germination, activation is not required, and may result from exposure to surface active agents, chelating agents or by mechanical means. These latter effects neither depend on, nor are changed by, heat activation. Germination has been shown to occur in the presence of inhibitors of macromolecular systhesis, therefore no new macromolecules are formed. Since spore components are broken down and excreted into surroundings it is considered a degradation process during which spore metabolism is initiated. Bacillus cerus-T spores have been shown to germinate rapidly in inosine whether activated or not. Unactivated spores, however, require a higher concentration of the germinating agent for a given rate of germination (Gould, 1971).

Physiological germination is believed to involve induction of metabolism by a "triggering mechanism." Activation is required for this triggering, however, in those germination systems which circumvent the metabolic trigger, activation is not required.

Various agents and conditions are necessary for germination. Activation may be a prerequisite for germination of some organisms. The most commonly noted germination factors are amino acids, ribosides and glucose. The most commonly noted amino acid is L-alanine, the most commonly noted riboside, inosine. Germination factors however vary with species and strain. Putrefactive anaerobe 3679 was shown by Uehara and Frank (1965) to require L-alanine alone at 45 C in sodium pyrophosphate buffer (pH 8.5). D-Alanine competitively inhibited germination, thus the germinating pattern was similar to that shown by spores of many Bacillus spp. Germinating requirements for Clostridium spp. also differ from those of Bacillus spp. Germinating requirements for Clostridium spp. also differ from those of Bacillus spp. Bicarbonate induces germination in PA 3679 and various Clostridium botulinum strains but not Bacillus spp. spores (Gould, 1969).

Oxygen is detrimental to germination of some spores of anaerobes, but did not effect germination of PA 3679, C. welchii or C. botulinum (type A) when germination was measured in a rich or defined medium (Fujioka and Frank, 1966). These investigators showed that spores of PA 3679 were suppressed by oxygen in an alanine-deficient medium but not in a media with high levels of alanine. Thus, they suggested that germination by an alanine pathway was insensitive to the presence of oxygen.

METHODS AND MATERIALS

Research was conducted to evaluate the effectiveness of a commercial chicken cooking system using microwave energy in destroying C. perfringens cells and spores on chicken pieces. Fresh thighs and drumsticks were obtained from a commercial source for all tests.

Design of Experiments

Heat Injury of C. perfringens Vegetative Cells

Vegetative cells of a heat resistant strain, NCTC 8238 were heated for varying times at 60 C in reconstituted clostridial medium (RCM), cooled to 15 C, diluted in prereduced anaerobically sterilized medium (PRAS). Plate counts were made in modified Shahidi-Ferguson-Perfringens agar. Growth and/or injury were evaluated for the following:

1. Growth of heat injured vegetative cells on stress and non-stress media.
2. Heat injury as measured by loss of cell constituents.
3. Growth of heat injured vegetative cells at various incubation temperatures.
4. Growth of heat injured vegetative cells in the presence of selective agents.

Recovery of Bacterial Cells from Chicken Parts Uninoculated and Inoculated

Chicken pieces were processed and evaluated microbiologically after each step of the commercial cooking process. Variables included:

1. Chicken thighs inoculated with vegetative cells of a heat sensitive strain of C. perfringens. Commercial (915 MHz) microwave oven used to cook chicken.

    a. raw

    b. microwave cook

    c. microwave cook plus brown in oil

    d. microwave cook plus frozen storage

    e. microwave cook, plus brown in oil plus frozen storage plus reheat

    f. microwave cook, plus frozen storage plus reheat

    g. freeze and thaw at 4 C

    h. freeze and thaw at 4 C. plus microwave cook.

2. Chicken thighs inoculated with vegetative cells of a heat resistant strain of C. perfringens. Commercial (915 MHz) microwave oven used to cook chicken.

    a.-h. ibid.

3. Uninoculated chicken thighs. Commercial (915 MHz) microwave oven used to cook chicken.

    a.-h. ibid.

4. Chicken drumsticks inoculated with spores of a heat-resistant strain of C. perfringens cooked in household (2,450 MHz) microwave oven.

    a. raw

    b. microwave cook

    c. microwave cook plus freeze and thaw

    d. microwave cook and hold at 4 C for 12 hr

    e. microwave cook and hold at 4 C for 36 hr

5. Chicken drumsticks, inoculated with spores of a heat-resistant strain of C. perfringens, cooked in commercial belt-fed microwave oven (915 MHz).

    a. raw

    b. microwave cook

    c. microwave cook plus brown in oil

    d. microwave cook plus brown in oil, freeze and thaw at 4 C and reheat

    f. freeze, thaw

    g. freeze, thaw, microwave cook

Source and Preparation of Cells and Spores

Two strains of C. perfringens, one heat-sensitive (ATCC 3624) and one heat-resistant (NCTC 8238), were obtained from the Food Research Institute, University of Wisconsin. Each was checked for purity before use, by evaluating the strains for production of black colonies on SPS medium, lack of motility, and nitrite formation. Nitrite production and motility were evaluated in nitrate-motility media, and fermentation of lactose, production of lecthinase and ability to grow aerobically were determined. Lactose and lecthinase were evaluated with lactose-egg yolk agar as described by Hall et al. (1969).

Spores were produced from each strain and evaluated for heat resistance at 100 C to be certain that the heat-sensitive and heat-

resistant strains were properly labeled as received. Vegetative cells were grown from spores of each strain in cooked meat medium (Difco). After an 18-20 hr growth period, 0.5 ml aliquots were inoculated into tubes of thioglycollate broth, frozen and stored at -26 C. These frozen cultures served as stocks.

Vegetative cells of each strain were removed from the freezer, grown and transferred in thioglycollate broth 4 times prior to use. Each transfer was made after a growth period of 18-20 hr. After four transfers in thioglycollate broth, less than 10 spores/ml were found. Vegetative cells were grown in 400 ml of thioglycollate broth for 18-20 hr, centrifuged at 15 C for 20 min at 4080 x g in a Sorvall RC-2B (Ivan Sorvall Inc., Norwalk, Conn.), resuspended in 100 ml of 100 mM phosphate buffer at pH 7.6. Cells prepared in this matter were prepared the day of use and used for only one study.

To obtain spores from the heat-resistant strain, the vegetative cells were grown in thioglycollate medium for 18-20 hr, and an aliquot was transferred to a nutrient broth plus sodium thioglycollate (0.1%) and held for four hours. Thirty-three ml of the cell suspension were then transferred to 800 ml of DS medium (Duncan and Strong, 1968). The same procedure was followed to obtain heat-sensitive spores, except that they were grown in Ellner's medium (Ellner, 1956). Incubation temperature for spore production was 37 C.

All spore counts were made by heating a 2-ml aliquot of the suspension for 20 min at 75 C in a Bronwill immersion heater (Bronwill Scientific, Rochester, N. Y.). The spore solution was cooled in an

ice-bath at 0 C, plated on SFP agar, and incubated at 46 C for 24-48 hr.

Each crude spore suspension was centrifuged at 5860 x g for 20 min; the supernatant was discarded, and the pellet was washed 5 times in sterile water. The crude spore pellet was suspended in sterile water, and agitated for 16-20 hr at 4 C using a magnetic stirring plate. The resulting crude spore suspension was centrifuged, suspended in a lysozyme solution (0.75 g + 7.5 g KCl/1, to pH 7.0), and incubated at 37 C for 2 hr. Lysis of vegetative cells and sporangia was induced by ultrasonic oscillation of 50 ml aliquots of the mixture at 65.6 watts of power for 20 min, using a Bronson Sonifier (Heat System Ultrasonics, Plainview, N. Y.). The crude spore suspension was placed in an ice-water bath at 0 C, during the sonification.

The crude spore suspension was then centrifuged at 5860 x g and washed twice in sterile water and suspended in sterile water. Washed spore suspension was held at 4 C for 16-20 hr, agitated continuously with a magnetic stirrer. This spore suspension was centrifuged, resuspended in lysozyme, incubated at 37 C for 1 hr, sonified as before, washed with sterile water and suspended in sterile water. Stock spore suspensions were held at 4 C in sterile water (Grecz et al., 1962).

Chicken Preparation and Cooking

Inoculation and Preparation for Cooking.--Chicken thighs and drumsticks were inoculated by a dip technique, using vegetative cells or spores from a concentrated suspension. Cell suspensions were prepared the day of use. Spore suspensions were diluted from stock

solution. Cells were suspended in 100 mM phosphate buffer, adjusted to an absorbance of .22-.23 at 640 nm. Spore suspensions were adjusted to a concentration of 10^5 spores/ml.

After inoculation, excess solution was allowed to drain from each chicken piece. Each piece-thigh or drumstick was then placed on a paper plate in a paper bag prior to cooking in the microwave unit.

A maximum of 16 pieces of chicken were used for each cell or spore suspension. The 0-time samples were inoculated last and remained at room temperature until all other samples were microwave cooked, or approximately 1 hr and 20 min.

Cooking Studies with Microwave Energy

Cooking with commercial oven: Chicken pieces were cooked in a commercial belt-fed microwave oven at 915 MHz which had a maximum power of 25 kw (Cryodry Corporation, San Ramon, Ca.). A potable steam source was attached to the unit to reduce the weight loss of product as a result of condensation of moisture from chicken to oven walls. Chicken pieces were heated for 4 min at 5 kw power in this unit. During this time the temperature of the meat increased from 8 to 98-99 C and resulted in satisfactorily cooked product with the desired weight loss of approximately 9%. This relative weight loss was established in an earlier study (Dawson and Sison, 1973a) to provide the greatest yield of satisfactorily cooked products in a 915 MHz oven.

Cooking with household oven: The household unit was a 2,450 MHz oven (Litton Industries, Atherton Division, Minneapolis, Minn.).

This unit contained no moving belt and heating was not uniform within the chamber. Chicken was cooked for 1-1 1/4 min. When chicken was cooked to the same degree of "doneness" weight losses were greated from the chicken pieces cooked in the household unit than from those cooked in the commercial unit.

Post-cooking procedures.--Browning by DFF: A Presto-Canner model 7 B (National Presto Inc., Eau Claire, Wis.) was used. Four chicken pieces were DFF for 2 1/2 min at 190.5 C.

Packaging, freezing, storage and reheating: Four cooked pieces were put in Cryovac® bags (W. R. Grace & Co., Cedar Rapids, Iowa) and held in a moving air freezer at -30.6 C for 4 days. Temperature data were recorded by Honeywell Electronik 16 and 8 multipoint recording potentiometers (Honeywell, Brown Instrument Division, Philadelphia, Pa.) by inserting Thermocouple along bone axis. Frozen samples were allowed to thaw at 4 C for approximately 24 hr and sampled for number of bacteria present. Chicken pieces were reheated in a household microwave oven for 1 min to serving temperature (Dawson and Sison, 1973).

Freezing of raw chicken: Samples were frozen for 2 days, thawed at 4 C for 24 hr and evaluated. Similar samples were microwave cooked in the commercial microwave oven.

Enumeration of Bacteria

Sampling chicken pieces.--The swab sampling method used was recommended by Walker and Ayres (1956). Each sample represented swabs

from an area of 8 cm^2 using a 2 cm^2 template. An area of 4 cm^2 was swabbed per peice and 2 pieces of chicken were used per sample 2 samples/trial. All swabs were dipped in 0.1% peptone, excess fluid was removed by pressing against the wall of the tube prior to sampling. After swabbing, the swabs were placed in various diluents containing glass beads which were used to aid in dispersal of the cotton.

Swabs for total aerobic plate counts (TAPC) of uninoculated chicken pieces were placed in 0.1% peptone dilution blanks and held at 0 C in an ice-water bath. Subsequent dilutions were made in 0.1% peptone.

Swabs for counts of vegetative cells of C. perfringens were placed in PRAS (Smith, 1971) and held at 15 C to prevent low temperature injury (Canada and Strong, 1964). Subsequent dilutions were made in PRAS medium.

Spore counts were made by dividing swab samples into heat shocked and non-heat shocked swab samples. Heat shock treatment was 20 min at 75 C. Swab samples were placed in sterile water dilution blanks held at 0 C; subsequent dilutions were made in sterile water blanks.

Recovery of C. perfringens.--Dilutions for TAPC were made in 0.1% peptone. Pour plates were made with plate count agar (Difco) and incubated at 30 C for 48-72 hr (Kinsley and Mountney, 1966).

C. perfringens counts were made on a modified Shahidi-Ferguson-Perfringens (SPS) medium without egg yolk, (Shahidi and Ferguson, 1971) using a pouch method. Bladel and Greenburg (1965) suggested using

pouches made from oxygen impermeable material. Each pouch contained an area equal to the area of a standard 100 x 15 mm petri plate. Bags, IKD all vak 13, were obtained from international Kenfield Distributing Company, Broadview, Ill. They were made of low permeability film designed for use in vacuum packaging and were not affected by freezing or heating at boiling water temperatures. The modified SFP media for these studies were made the day prior to use. Media were autoclaved without antibiotics. Antibiotics were filter sterilized and added to sterilized media at a temperature of 50 C. Kanamycin sulfate and polymyxin B sulfate were obtained from Sigma Biochemicals (St. Louis, Mo.). Modified SFP medium with antibiotics was dispensed into premade pouches with a Cornwallis pipettor. Pouches containing media were held in a 55 C incubator (Thelco Precision incubator, Precision Scientific, Chicago, Ill.) until used. After inoculation and solidification the pouches were incubated at 46 C in a gravity flow aerobic incubator.

Confirmation of C. perfringens.--Representative black colonies from SFP were transferred to nitrate-motility agar tubes and lactose egg yolk agar plates, which were sealed with SFP basal media without egg yolk, and incubated at 37 C in an aerobic incubator for 24-48 hr. The air in the incubator was evacuated twice, each time the chamber was filled with nitrogen and carbon dioxide gas in a ratio of 9:1. Egg yolk emulsion for LEY agar was prepared as described by McClung and Toabe (1947).

RESULTS AND DISCUSSION

Heat Injury of C. perfringens Vegetative Cells

Growth of Heat Injured Vegetative Cells on Stress and Non-Stress Median

Initially studies were conducted to evaluate methods for the recovery of sublethally heat-injured vegetative cells of Clostridium perfringens.

Figure 1 shows the recovery of vegetative cells of a heat-resistant strain of C. perfringens. The vegetative cells were heated for 0 to 65 min in reconstituted clostridial media without agar (RCM) at 60 C and plated on basal Shahidi-Ferguson-Perfringens agar (BSFP) and BSFP + 2% NaCl. Neither egg yolk nor antibiotics were added to the media. It is assumed that the difference in counts obtained on the stress medium (BSFP + 2% NaCl) and non-stress medium (BSFP) is due to the recovery of the heat-injured population (Iandolo and Ordal, 1966). After heating for 30 min or less at 60 C, the counts obtained on BSFP + 2% NaCl and BSFP differed by less than one log-cycle and the difference increased to 1 1/2 log-cycles after 55 min of heating. Figure 1 shows that the injured cells were not able to recover to initial levels, however recovery of injured cells in media with and without inhibitory agents, (stress vs. non-stress media) were different.

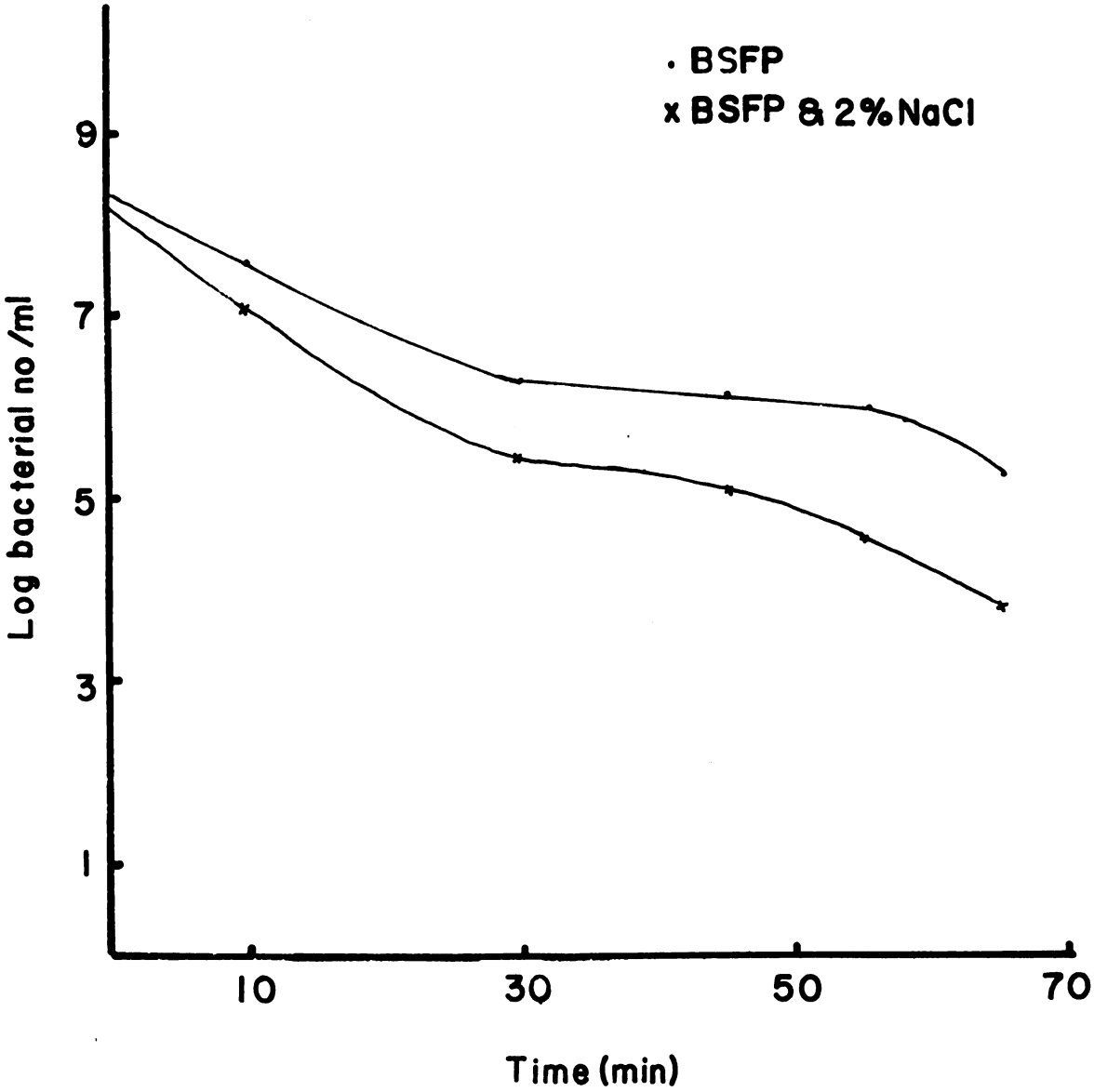

Figure 1.--Number of heat injured C. perfringens NCTC 8238 survivors. Vegetative cells heated in RCM broth for varying times at 60 C. Cell suspensions were cooled to 15 C prior to plating on BSFP and BSFP + 2% NaCl; incubated at 37 C, duplicate counts made.

A difference in numbers between sublethally heat injured and non-heat injured vegetative cells enumerated on solid media was anticipated. However, as shown in Figure 1, recovery on a non-stress medium (BSFP) was not sufficient to show more than a 1 1/2 log-cycle difference in vegetative cell counts from that found on stress medium (BSFP + 2% NaCl). This difference resulted after 55 min of heating at 60 C.

Some strains of C. perfringens grow at 50 C but not at 55 C. In view of the results reported in Figure 1, sufficient injury at 55 C would probably not have resulted to provide a significant difference between counts on stress and non-stress media. Both strains studies grew at 50 C.

A heating temperature of 65 C resulted in a marked decrease in the number of viable cells. No differences were shown in the ability of injured cells to recover on stress and non-stress media, which indicated that injury sustained could not be overcome in this non-stress environment (See Appendix Table 1).

Salt concentrations of 2.0 and 2.5% were used in BSFP as a stress environment. These levels did not inhibit growth of vegetative cells due to the salt concentration. Smith (1971) reported that the effect of NaCl concentrations on growth of C. perfringens is not great as long as it corresponds to a water activity value of not less than 0.98, equivalent to about 2.5% NaCl. Busta and Jezeski (1963) used 7.5% NaCl to distinguish between heat injured and non-heat injured S. aureus cells, however, this percent salt does not affect normal

growth of uninjured cells. Clark and Ordal (1969) used 2% NaCl to distinguish between heat-injured and non-heat-injured S. typhimurium cells. Mead (1969) reported growth of C. perfringens vegetative cells in salt (NaCl) concentrations of 5% from large inoculums in low redox environments and in 6% NaCl in 14 days, however, recovery was in a liquid or semi-solid (0.06% agar) medium.

A study was conducted to determine if different media constituents would provide greater recovery of heat injured cells. Various media used for growth of clostridia were evaluated and are reported in Appendix Table II.

The recovery of sublethally injured cells was not sufficient to indicate a difference in counts between stress and non-stress media. Factors which may account for this are the lack of an essential nutrient in the media evaluated; BSFP, APT, brain heart infusion (BHI), blood agar base at pH 7.6 (BAB), BAB + yeast extract and reconstituted clostridial medium (RCM). The method used to dilute and transfer cells may not have been sufficiently anaerobic to allow recovery of sublethally injured cells. Ades and Pierson (1973) indicated that good anaerobic techniques are necessary to recover heat injured cells of C. perfringens. Extending these findings to the molecular level may indicate that re-establishment of anaerobic respiration by the heat damaged cells is the influencing factor for repair of heat injured vegetative cells of C. perfringens.

Heat Injury as Measured by Loss of Cell Constituents

Heat injury was also evaluated by measuring loss of 260 nm absorbing substances. Leakage of cell material from a heat-resistant strain and a heat-sensitive strain of C. perfringens, as determined by absorbance at 260 nm, for cells held at 60 C up to 65 min are shown in Figure 2. The losses of these substances increased gradually, and were greatest from heat-resistant cells after 15 min. The amount of leakage material from the heat-sensitive strain remained constant after 30 min, whereas that from heat resistant cells, plateaued from 20-45 min, then increased.

Loss of materials which absorb at 260 nm from the cells correlated with cell injury (Russell and Harries, 1967). These investigators heated Escherichia coli at 50 and 60 C and found that leakage of materials which absorb at 260 nm increased with heating time. They postulated that the presence of these substances and eventual loss of viability were due to damage of the cell membrane. Their conclusions were supported by Allwood and Russell (1968), who showed that 8-anilino-1-napthalene-sulphonic acid (ANS) penetrated heat treated cells but not unheated cells of S. aureus.

Growth of Heat Injured Cells at Various Incubation Temperatures

The ability of heat injured cells to recover at three temperatures is shown in Figure 3. Vegetative cells of the heat resistant strain were heated up to 60 min at 60 C in RCM, plated on BSFP and

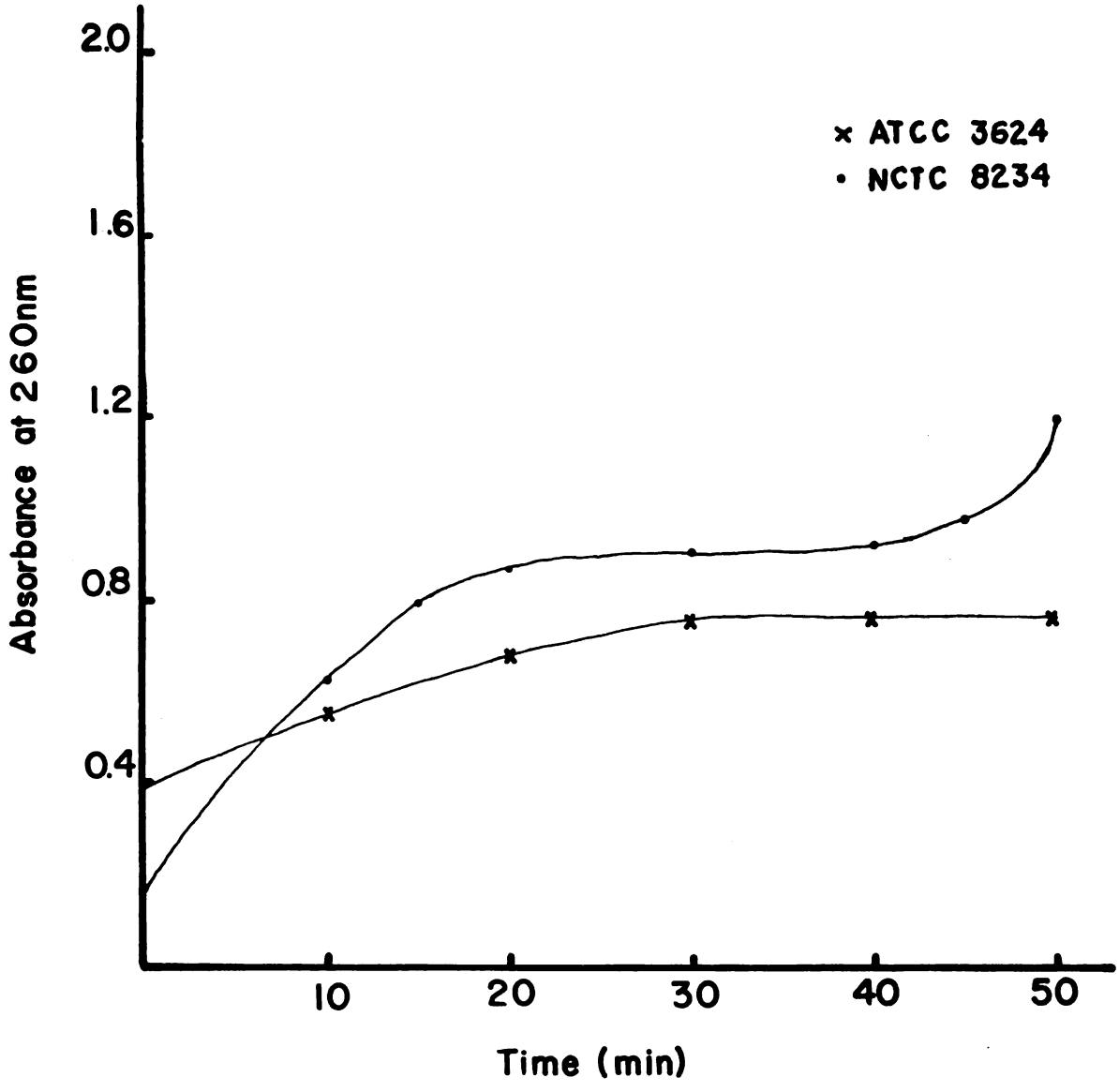

Figure 2.--Heat injury as measured by absorbance of cell leakage material at 260 nm. Vegetative cells of a heat resistant (8238) and heat sensitive (3624) strain were heated in RCM at 60 C. Cells removed by centrifugation and millipore filtration. Graphs show a 1:2 dilution.

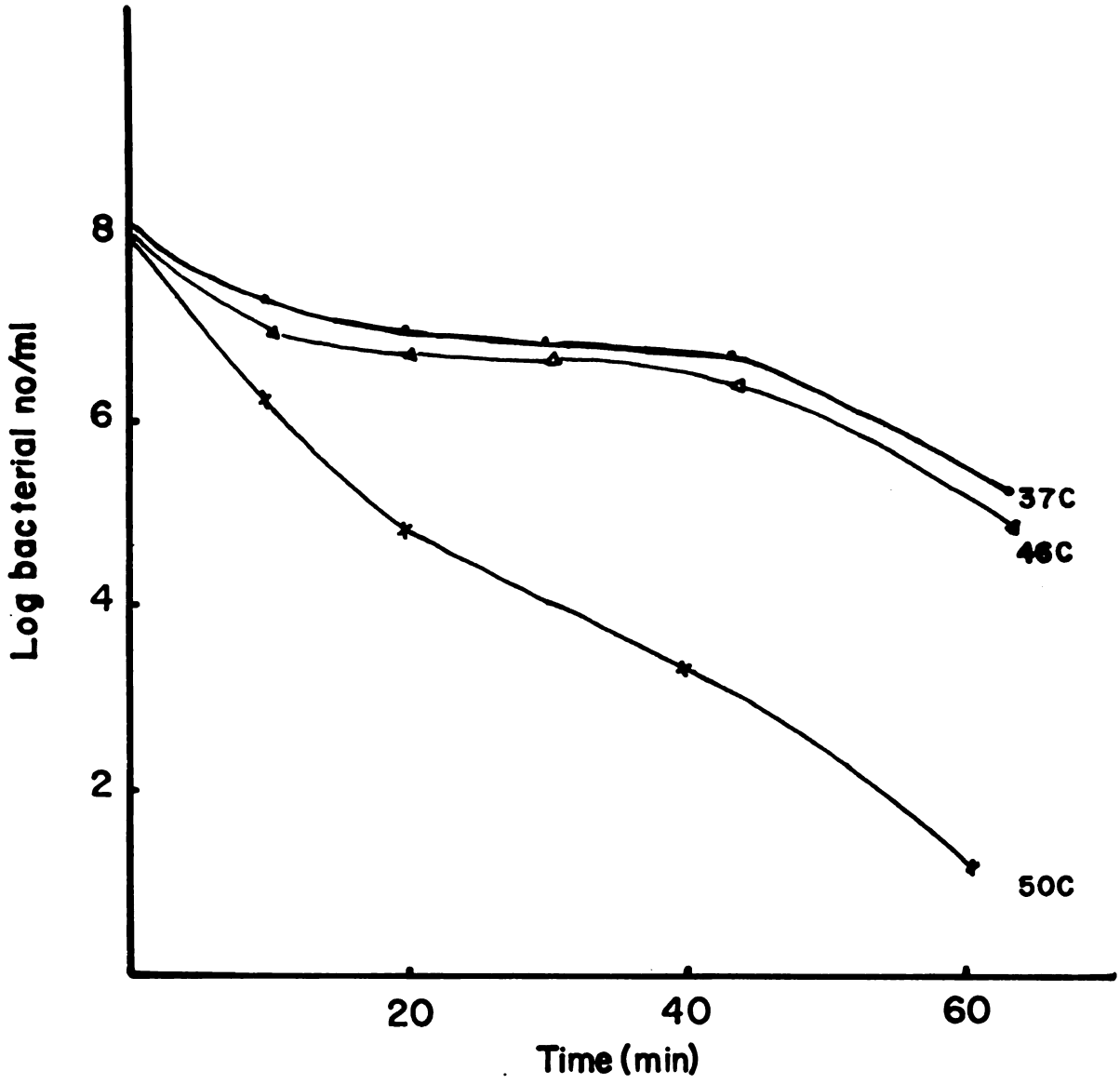

Figure 3.--Growth of heat injured C. perfringens (8238) vegetative cells at various growth temperatures. Vegetative cells heated in RCM broth for varying times at 60 C. Cell suspensions were cooled to 15 C prior to plating on BSFP, 37 & 46 C average two trials, 50 C one trial.

incubated at 37, 45 and 50 C in gravity flow and forced air incubators. The incubation temperatures did not affect unheated (0-time) samples. As the heating time increased, the ability of heat injured cells to recover at 50 C declined markedly. No significant difference between growth at 37 or 46 C was found. The low recoveries obtained at 50 C might be due to a number of factors. Campbell and Pace (1968) reported that the maximum growth temperatures of cultures may be related to the heat stability of essential cell components, such as ribonucleic acids and various cell enzymes. Morita and Burton (1963) used psychrophilic Pseudomonas spp and showed that heat destruction of respiratory enzymes was involved in determining the maximum growth temperatures of psychophilic Pseudomonas spp. Reduced levels of growth of injured organisms incubated on solid media, was noted by Brown and Melling (1971).

Growth of Heat Injured Vegetative Cells in the Presence of Selective Agents

The ability of heat injured vegetative cells of a heat-resistant strain of C. perfringens to grow in the presence of different inhibitory substances is shown in Figure 4. The antibiotics were those used in SPS, SFP, and tryptose-sulfite-cycloserine (TSC) media. Vegetative cells were heated at 60 C for different times in RCM and recovered on BSFP, BSFP plus polymyxin B sulfate + sulfadiazine, BSFP plus kanamycin sulfate + polymyxin B sulfate, and BSFP plus D-cycloserine media. The level of antibiotics incorporated were as specified for the particular media, SPS, SFP or TSC. Polymyxin B sulfate + sulfadiazine (SPS) or kanamycin sulfate + polymyxin B sulfate were least inhibitory to the recovery of heat injured vegetative cells of C. perfringens.

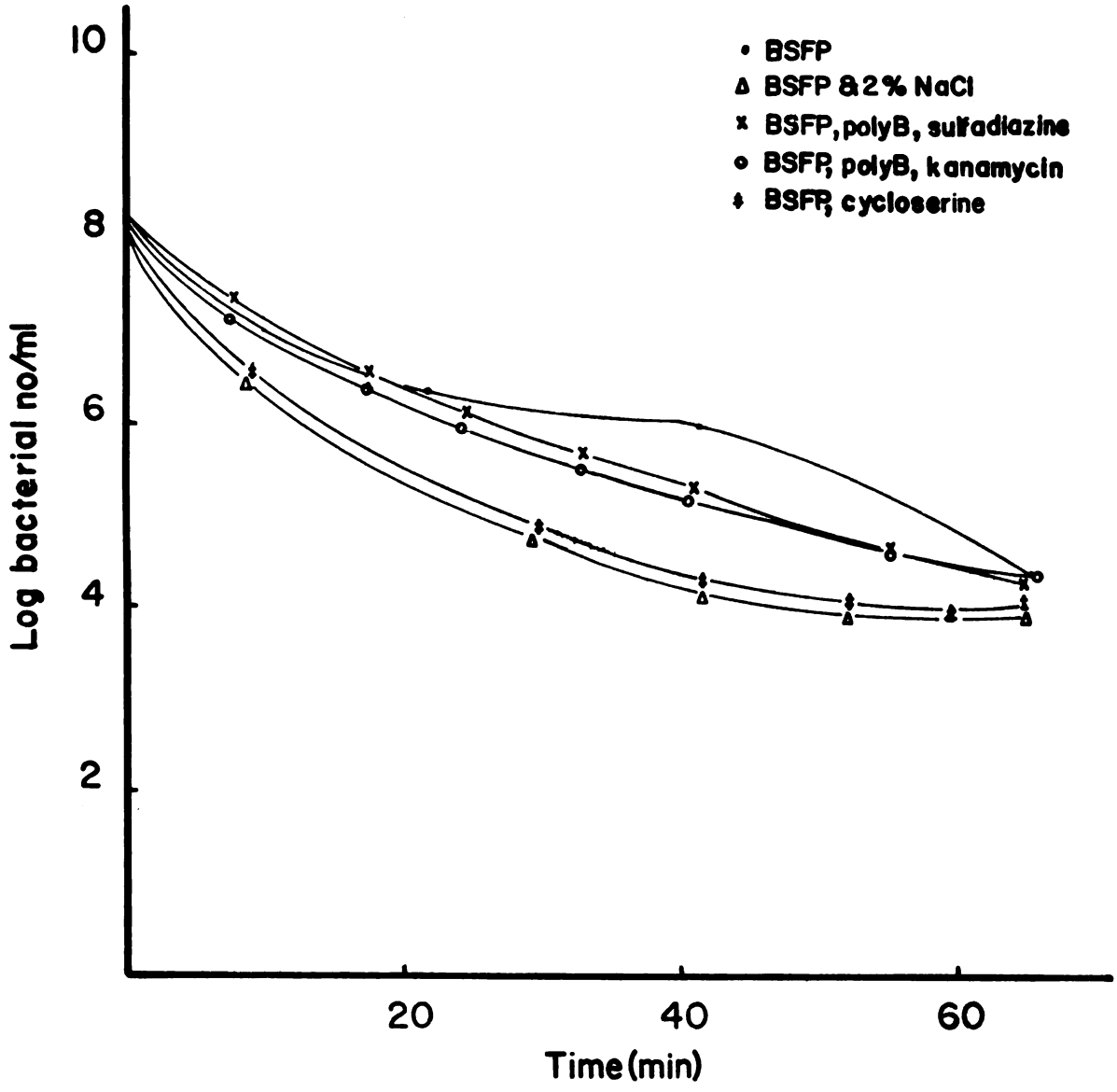

Figure 4.--Growth of heat injured C. perfringens vegetative cells (8238) in the presence of various selective agents used in SPS, SFP and TSC for selective identification of Clostridium. Cells heated in RCM and cooled to 15 C. Incubated at 46 C, duplicate counts.

Harmon et al. (1971a) found kanamycin sulfate + polymyxin B sulfate the least inhibitory, of the antibiotic used, for selection of unheated vegetative cells of C. perfringens. In this study, similar responses were found with the antibiotic combinations of SFP and SPS. Selectivity of these antibiotic combinations of SFP and SPS. Selectivity of these antibiotic combinations were identical as measured by recovery of sublethally heat injured cells. D-cycloserine appears to provide as much stress, as 2% NaCl to heat injured cells.

The combination of antibiotics appeared to be mildly inhibitory to the recovery of heat treated C. perfringens cells (Figure 4). Based on the results of other investigators, (Iandolo and Ordal, 1966; Sogin and Ordal, 1967) concerning the recovery of heat injured vegetative cells lack of inhibition by kanamycin sulfate would have been expected. Kanamycin sulfate is an inhibitor of protein systhesis which has not been shown to be a prime factor in recovery of sublethally heat injured cells of S. aureus and Streptococcus faecalis (Iandolo and Ordal, 1966; Clark et al., 1968).

Cell wall formation has not been shown to be a factor in recovery of sublethally heat injured cells (Iandolo and Ordal, 1966). However, in this study, it appears that heat treated cells are more susceptible to the action of D-cycloserine. It is surprising that polymyxin B sulfate was not more inhibitory since membrane damage had been shown to occur on heating (Iandolo and Ordal, 1966; Allwood and Russell, 1968; Ordal, 1970). Polymyxin B sulfate probably does not affect gram-positive cells because of the magnesium ribonucleate

coating; this coating is not found in gram negative cells (Sebek, 1967). This lack of inhibition by polymyxin B sulfate may result from the amount incorporated into the media or because the majority of the magnesium ribonucleate coating found in gram-positive cell membranes was not destroyed or released from the cell membranes of subiethally heat injured cells.

Heating and Cooling Patterns of Microwave Cooked Chicken

Figures 5, 6, 7, and 8 show the heating curves for chicken drumsticks and thighs in the commercial microwave oven (915MHz), the cooking pattern of microwave cooked chicken at room temperature, the freezing pattern of chicken after various cooking treatments and the cooling pattern of chicken drumsticks cooked in a household microwave (2450 MHz) oven and held at 4 C, respectively. Cooling and freezing curves (Figures 6, 7, and 8) show the length of the time that inoculated pieces remained within the growth temperature range of C. perfringens. Figure 6 shows that the chicken pieces remained in the growth temperature range of C. perfringens for 50 min; however, the time at the optimum growth temperature, 45 C, was minimal.

The freezing curve for the deep-fat fried (DFF) product, Figure 7 shows that the maximum time the organism remained in a temperature growth range was 10 min. Figure 8 shows the cooling pattern of pieces cooked in the household microwave oven, cooled and held at 4 C. Chicken pieces remained in growth temperature range for C. perfringens for 30 min. The times at optimum regeneration temperature shown in

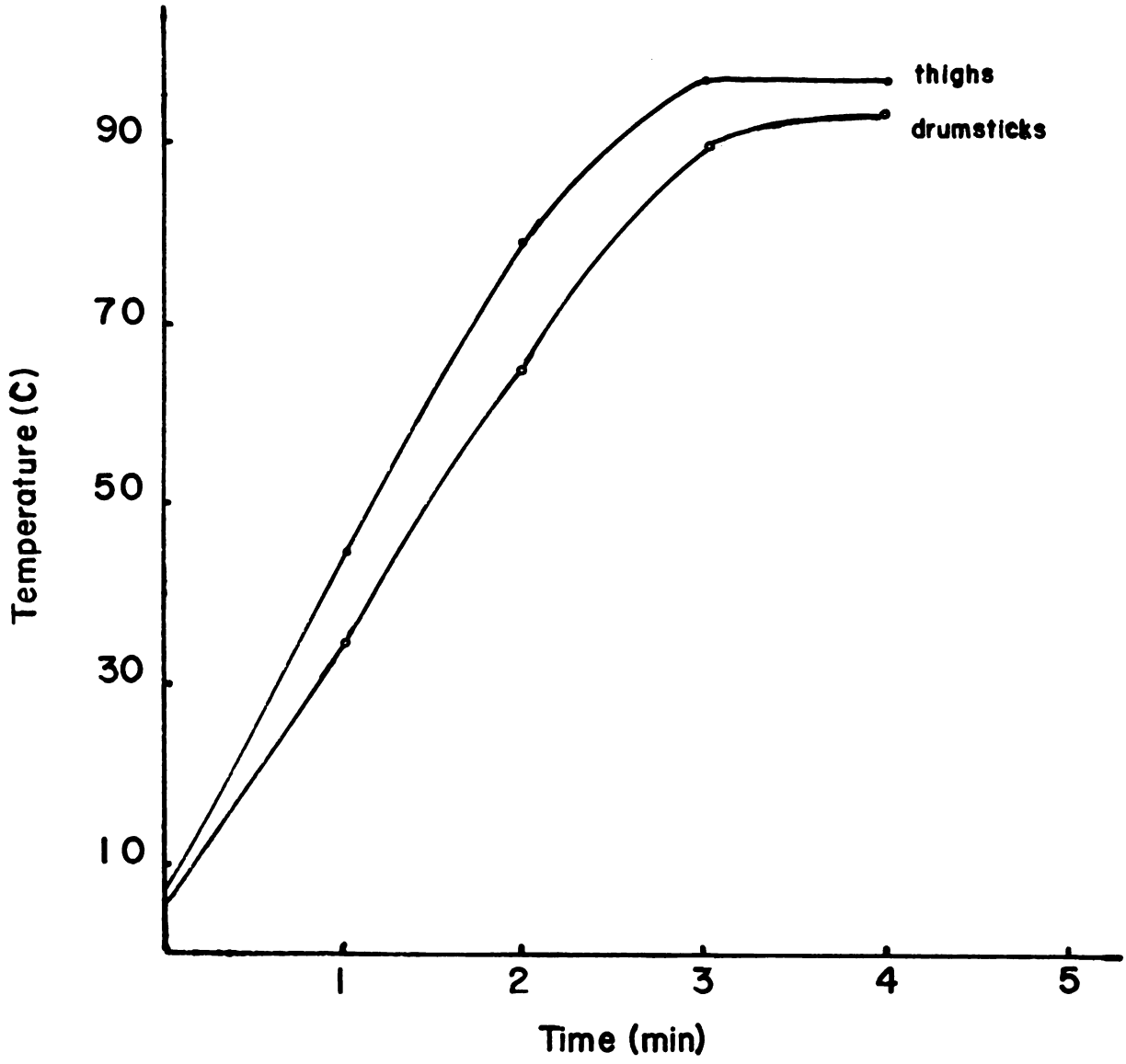

Figure 5.--Heating pattern of chicken thighs and drumsticks cooked in a commercial microwave oven (915 MHz) with attached potable steam source. Average of three trials.

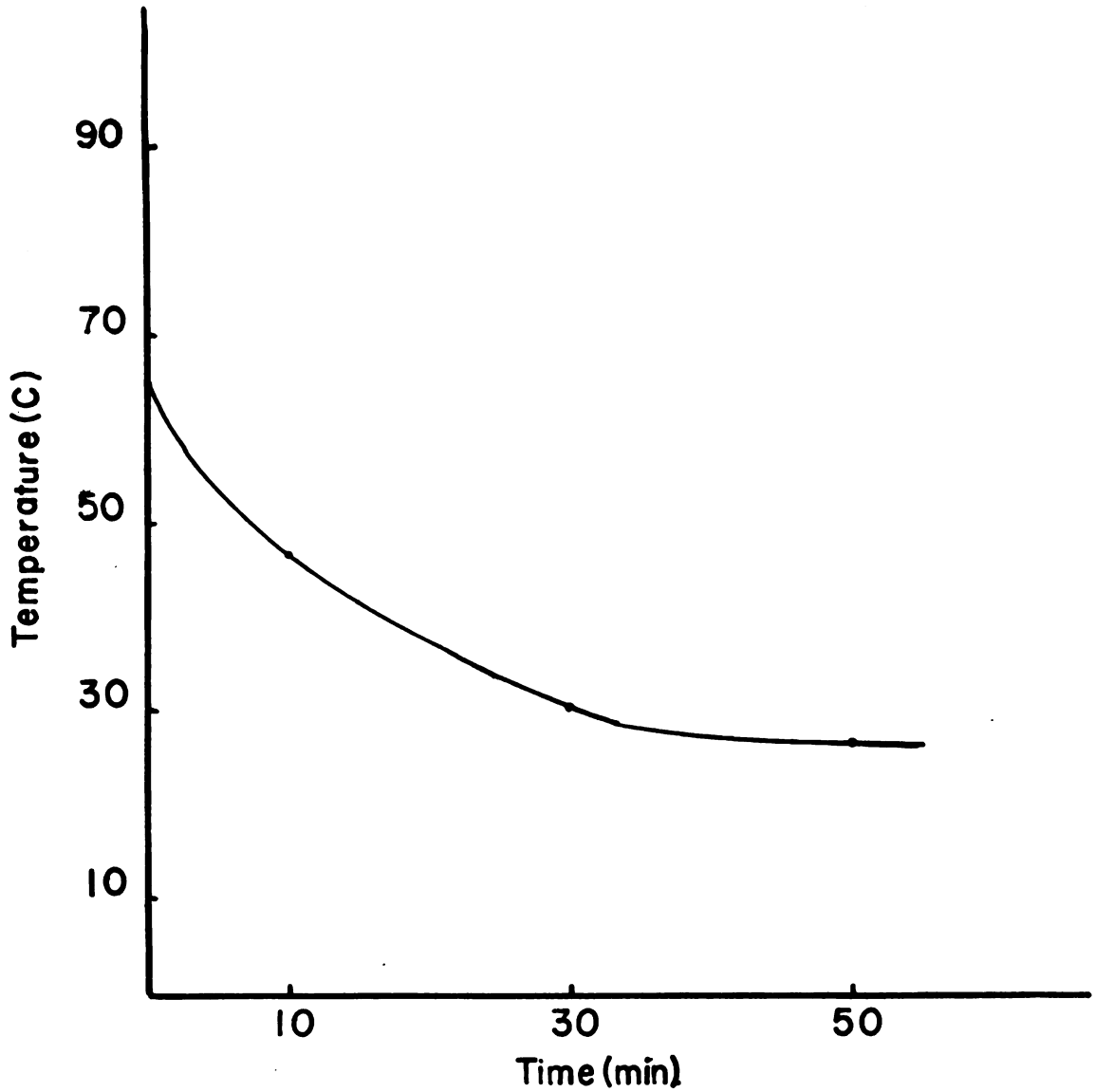

Figure 6.--Cooling pattern of chicken cooked in a commercial microwave unit with potable steam source attached, when held at room temperature.

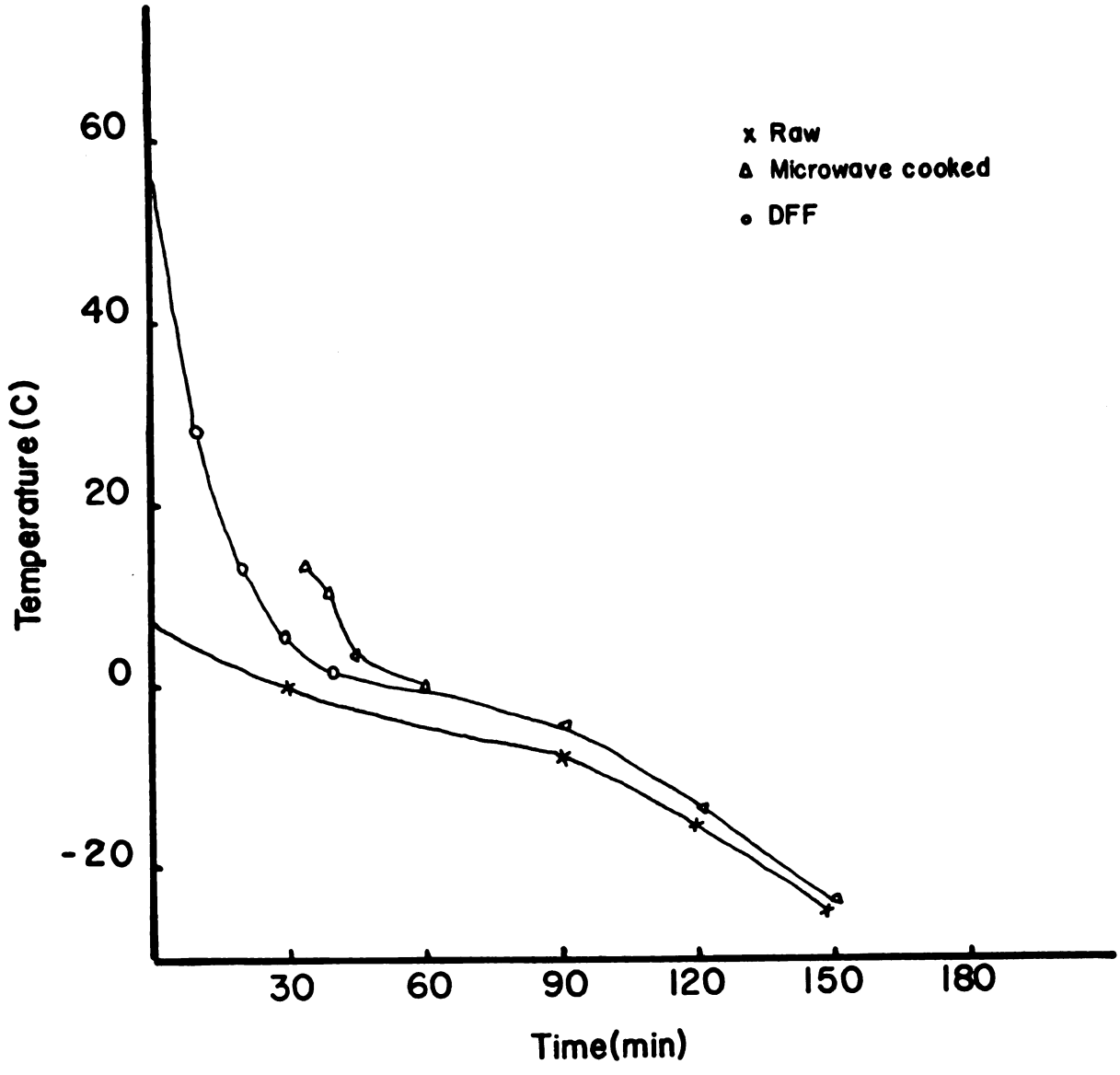

Figure 7.--Freezing patterns of raw, microwave cooked and browned chicken pieces at -30.6 C. Samples were packaged 4/bag.

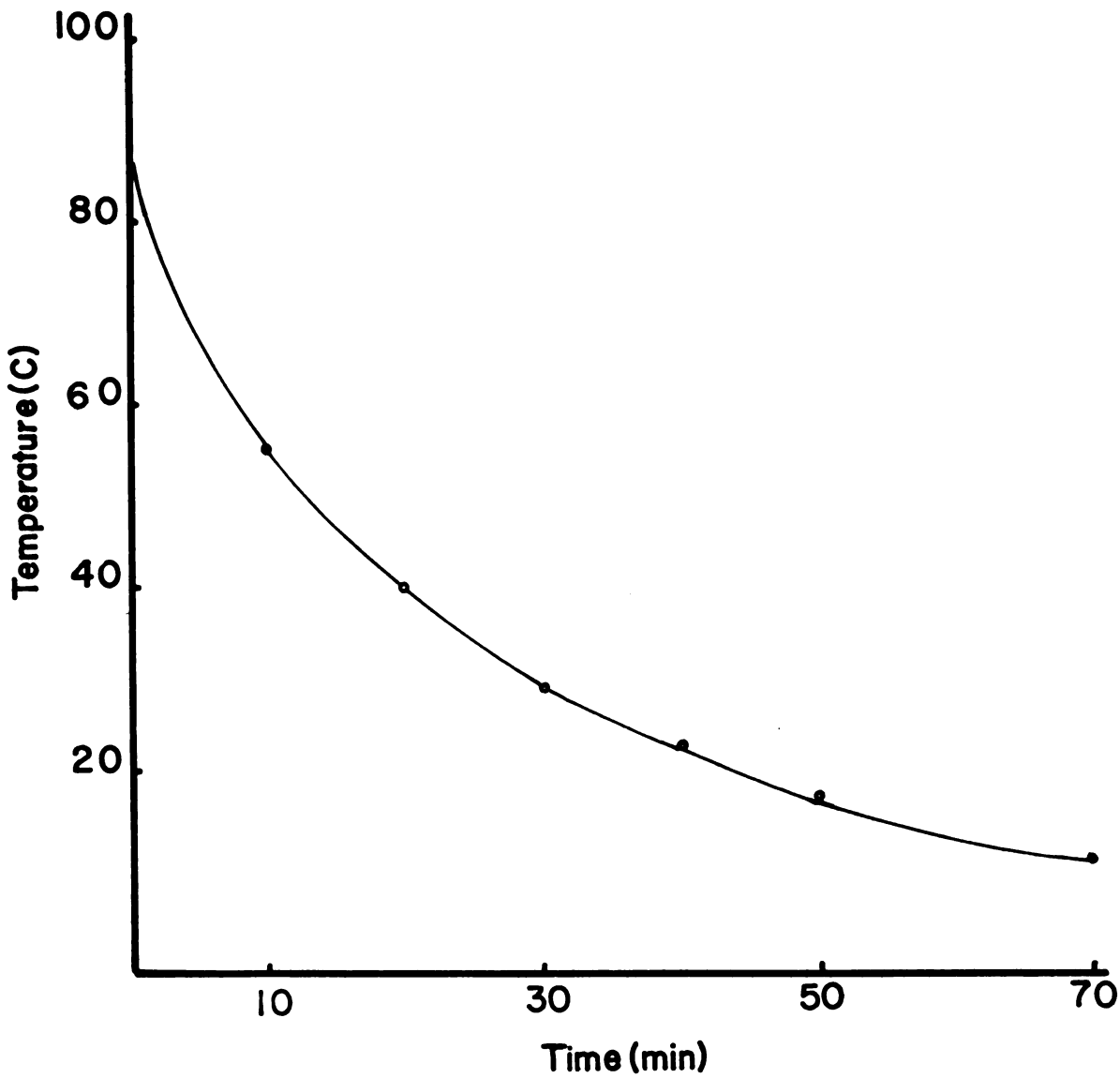

Figure 8.--Cooling pattern of chicken pieces cooked in a household (2450 MHz) microwave oven and held at 4 C.

Figures 6, 7 and 8 are not considered significant, since an extended lag time has been shown for heat injured microorganisms (Kaufmann et al., 1959; Clark and Ordal, 1965).

Recovery of Bacterial Cells from Chicken Parts Uninoculated and Inoculated

Effect of Various Treatments of a Commercial Chicken Cooking System on Recovery of C. perfringens

Figures 9 and 10 report the recovery of heat-sensitive and heat-resistant vegetative cells of C. perfringens on chicken pieces after various treatments. Samples cooked in the commercial microwave oven showed a two log-cycle reduction which represented approximately 0.38% survival of both the heat sensitive and heat resistant vegetative cells. Microwave cooked samples were considered done when 9% weight loss was achieved (see Appendix Table 111). Longer cooking times would probably result in a greater reduction of viable cells but also a greater weight loss of the final product. Considering the reduction of cell numbers that occurs during browning a greater degree of cooking in the microwave unit is not necessary. Browning in oil (DFF) for 2 1/2 min proved to be most efficient in destruction of vegetative cells of C. perfringens. Thermal injury did not predispose vegetative cells (heat-sensitive or heat-resistant strain) to greater sensitivity to the freezing and thawing procedures. Chicken pieces were thawed at 4 C for 24 hr and reported as "D", Figures 9 and 10. These results differ from those reports of Canada and Strong (1964). They found that 24 hr frozen storage of cell suspensions of C. perfringens, which had

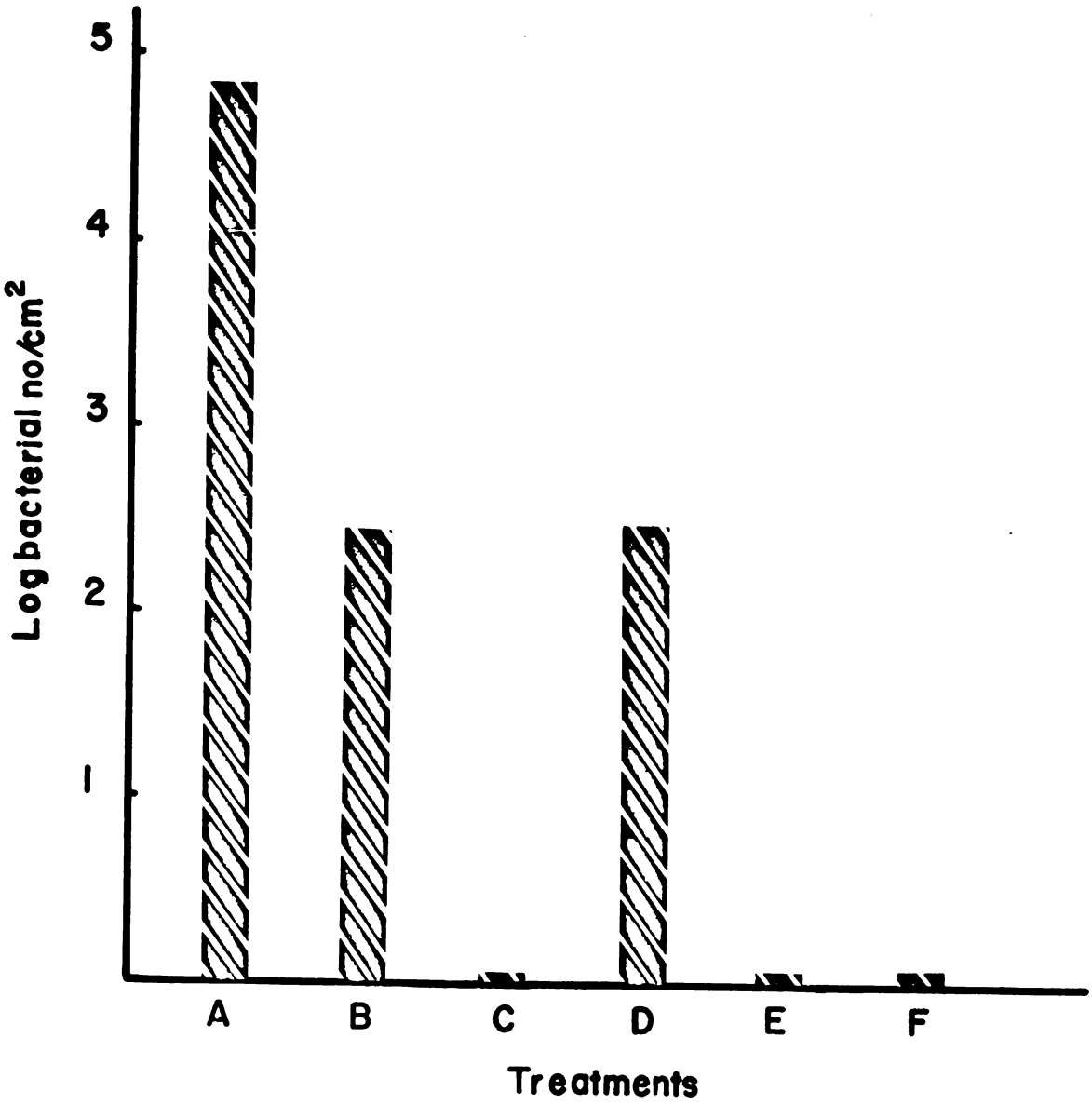

Figure 9.--Effect of various processing steps of a commercial chicken cooking operation on the reduction of heat sensitive vegetative cells of C. perfringens from chicken pieces cooked in 915 MHz oven. A-control; B-microwave cook; C-microwave cook + brown; D-microwave + freeze + thaw; E-microwave + brown + freeze; F-microwave + brown + freeze + reheat. Pouch method with SFP, incubated at 46 C, average 2 trials.

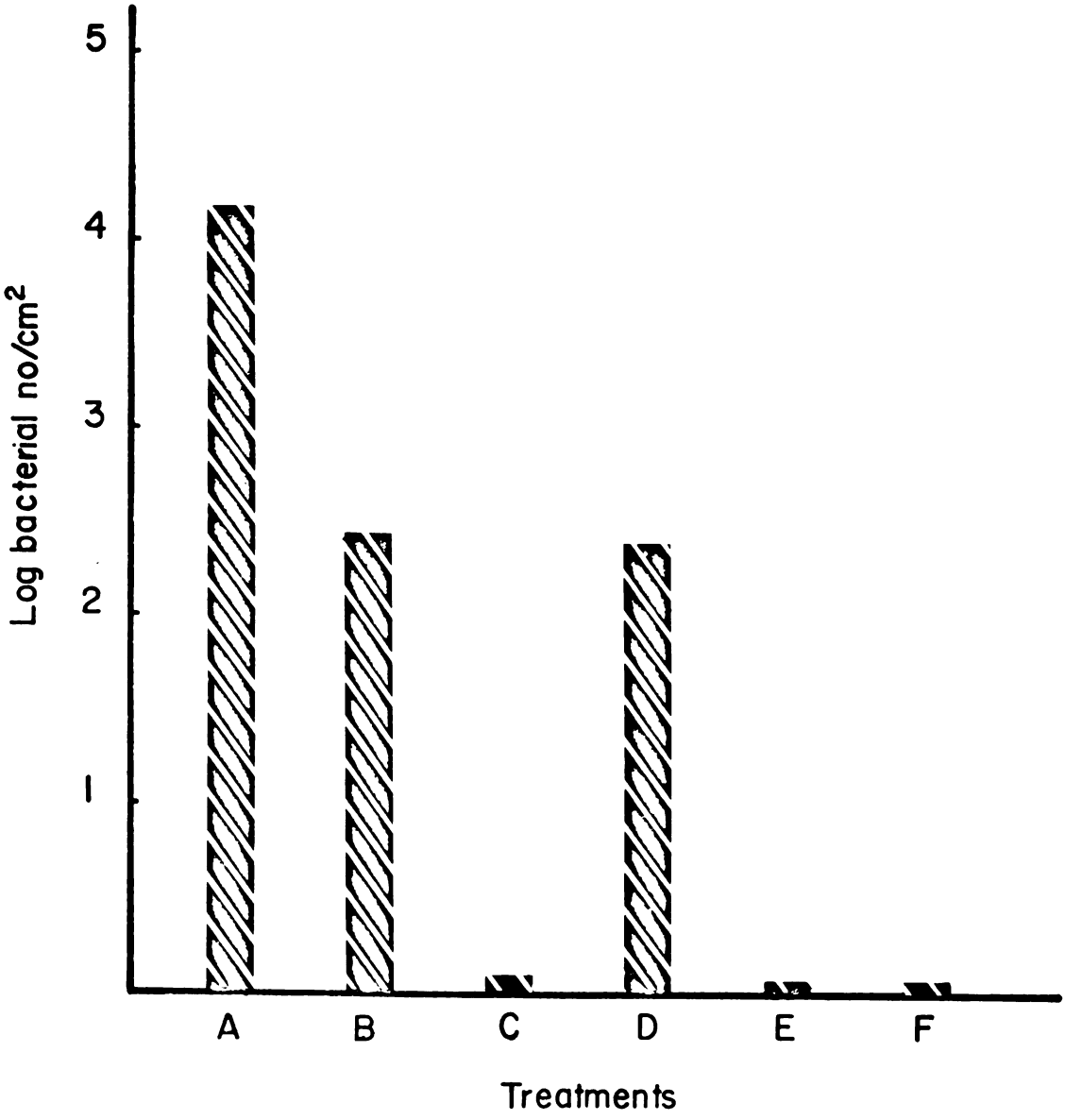

Figure 10.--Effect of various processing steps of a commercial chicken cooking operation on the reduction of vegetative cells of a heat resistant strain of C. perfringens from chicken pieces cooked in 915 MHz oven. A-control; B-microwave cook; C-microwave cook + brown; D-microwave + freeze + thaw; E-microwave + brown + freeze; F-microwave + brown + freeze + reheat. Pouch method with SFP, incubated at 46 C, average 2 trials.

been subjected to a cooking process, resulted in 80% reduction in viable count. In this study no viable cells were recovered from samples which received the browning treatment (DFF).

Effect of Freezing and Thawing on Vegetative Cells of C. perfringens

Figure 11 reports the microbial counts on chicken thighs inoculated with vegetative cells of heat-sensitive or heat-resistant strains that were subjected to freezing, thawing and microwave cooking in the commercial unit. Samples were frozen at -30.6 C for 48 hr, thawed at 4 C for 24 hr. Figure 11 reports results of the numbers present on the unfrozen inoculated control, frozen thawed controls and frozen thawed microwave cooked samples.

Freezing prior to cooking had no effect on the level of viable cells from both strains of C. perfringens present on chicken surfaces (Figure 11). The combination of freezing and thawing, prior to cooking, may induce greater susceptibility to some vegetative cells to heat as indicated in the results from inoculated samples that were frozen and microwave cooked (Figure 11). Recovery of vegetative heat sensitive cells (N) was less than $10/cm^2$.

Cell counts of the heat-resistant strain after cooking (N) showed approximately a three log-cycle reduction or 0.036% survival based on counts before freezing. The results indicated that the freeze-thaw process induced greater sensitivity of vegetative cells of heat sensitive strain of C. perfringens to heating Sample (N). A lower

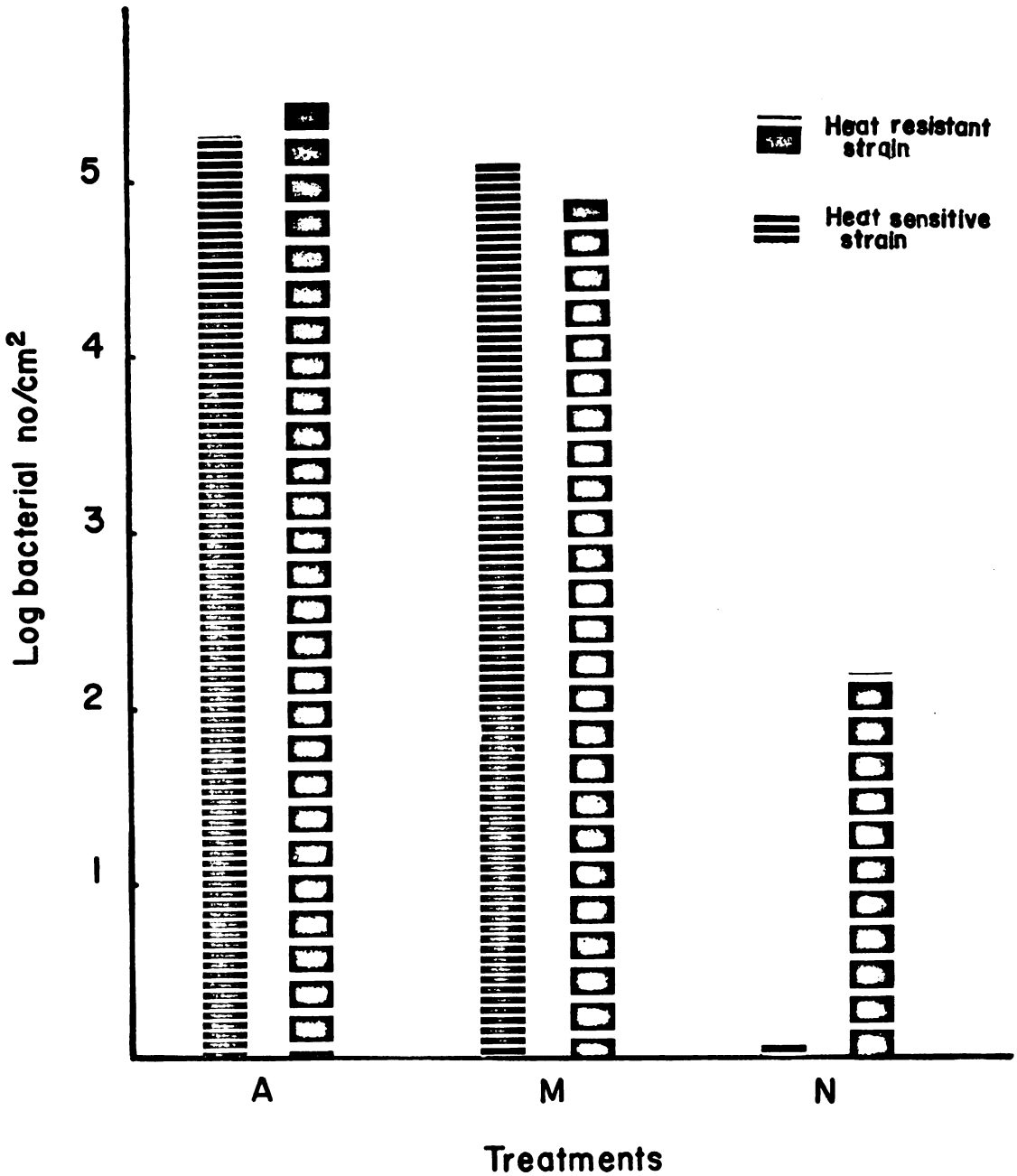

Figure 11.--Effect of freezing, thawing and microwave cooking on vegetative cells of C. perfringens on chicken pieces cooked in 915 MHz oven. A-control; M-freeze + thaw; N-freeze + thaw + microwave cook. Pouch method with SFP, incubated at 46 C, average 2 trials.

number of heat-sensitive cells recovered than the heat-resistant cells, after cooking (Figure 11) may indicate a strain difference.

Effect of Various Treatments of a Commercial Cooking System on Total Aerobic Counts of Chicken Pieces

Figure 12 shows the numbers of resident organisms (total aerobic count) on chicken thighs after various treatments. Microwave cooking of unfrozen chicken thighs in the commercial unit reduced total aerobic plate counts (TAPC) by approximately 2 log-cycles which represented 0.49% survival. Browning in oil (DFF) reduced counts below detectable levels. Chicken thighs which were frozen after microwave cooking and thawed at 4 C had TAPC's about one log-cycle higher than those not frozen. An increase in the resident microbial population, Figure 12, of those samples microwave cooked, frozen and thawed at 4 C for 24 hrs is to be expected since the majority of cells found naturally on processed chicken are psychrophiles (Shantz et al., 1969).

These results agree with the results of Kraft and Ayres (1966) who found that an increase in the resident microbial population did not influence the recovery of pathogens. They showed that over growth of Pseudomonas fluorescens occurred at 5 C even though originally S. aureus was present in much higher numbers (100:1) than Ps. fluorescens. Little change occurred in the initial population of S. aureus when held for as long as one week at 5 C.

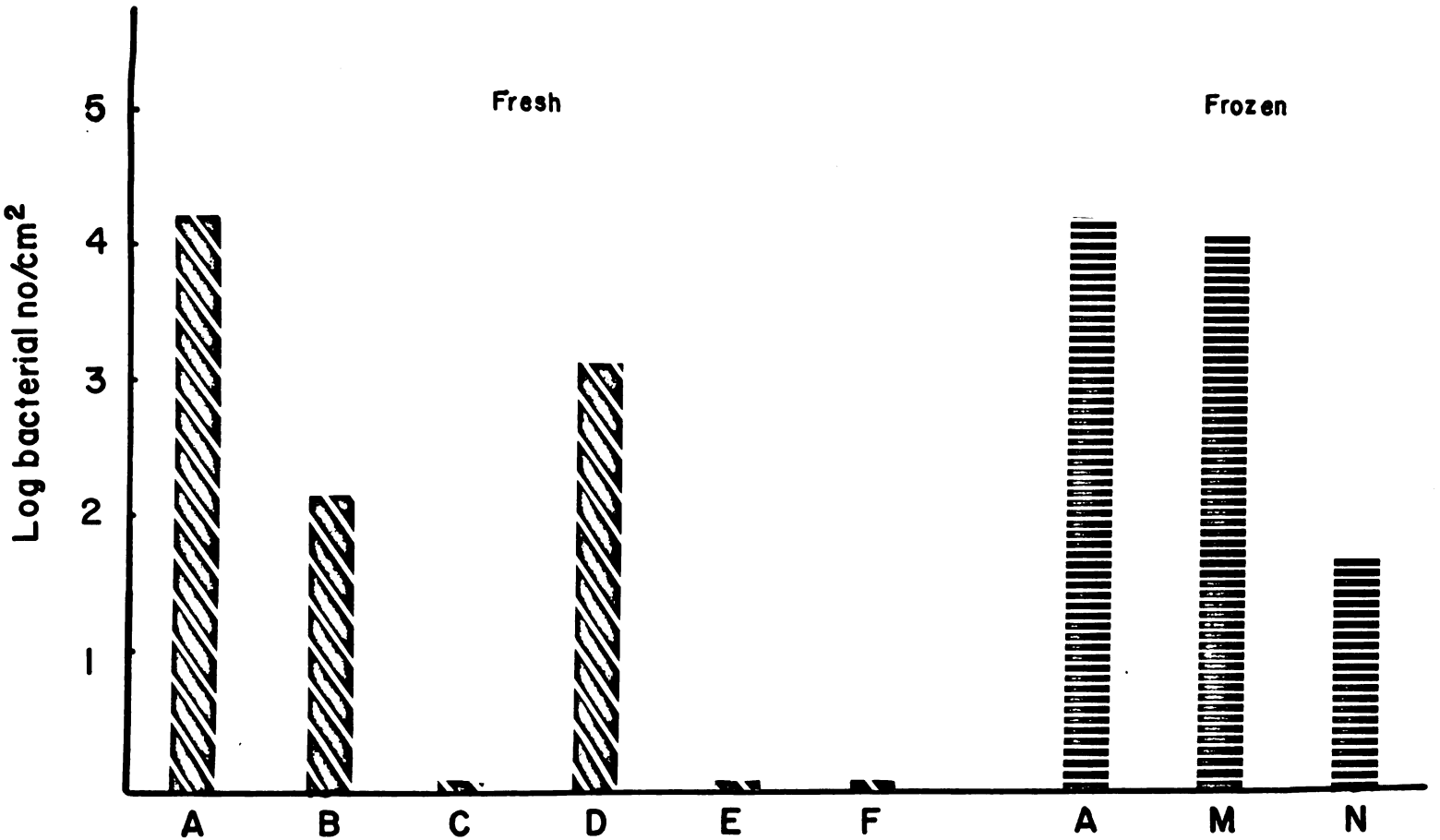

Figure 12.--Effect of various processing steps of a commercial chicken cooking operation, freezing and thawing on reduction of normal flora of chicken pieces cooked in 915 MHz oven. A-control; B-microwave cook; C-microwave cook + brown; D-microwave + freeze + thaw; E-microwave + brown + freeze; F-microwave + brown + freeze + reheat. Plated on PCA at 30 C for 48-72 hrs, average 2 trials.

Freezing of uninoculated chicken thighs prior to cooking, Figure 12, gave results similar to those on frozen inoculated samples. Thus freezing had no effect on reduction of natural cell population. Microwave cooking of the previously frozen and thawed pieces resulted in approximately a 3 log-cycle reduction or a 0.030% survival of original resident population.

DFF at 190.5 C for 2 1/2 min was effective in destroying bacterial populations on the surface (Figure 9, 10 and 12). Mabee and Mountney (1970) inoculated Salmonella senftenberg intramuscularly at levels of 10^9 cells/g. Chicken parts were pressure fried or fried at atmospheric pressure to doneness. Cooking times varied since some samples were cooked from a frozen state, but they were unable to detect S. senftenberg by enrichment techniques.

The chicken thighs that were DFF in these experiments contained low numbers of C. perfringens (10^2 cells/cm^2). It is not known whether higher numbers would be similarly reduced by browning for the same length of time, however higher levels of contamination would usually be present only on spoiled chicken. Thus the levels used may reflect natural conditions of fresh poultry parts.

Effect of Microwave Energy on Spores of C. perfringens

Figure 13 shows the spore counts of chicken pieces inoculated with heat resistant spores and cooked in the household microwave unit for 1-1.25 min. Spore counts were determined by two procedures, one in which the spores recovered from chicken pieces (on swabs) were

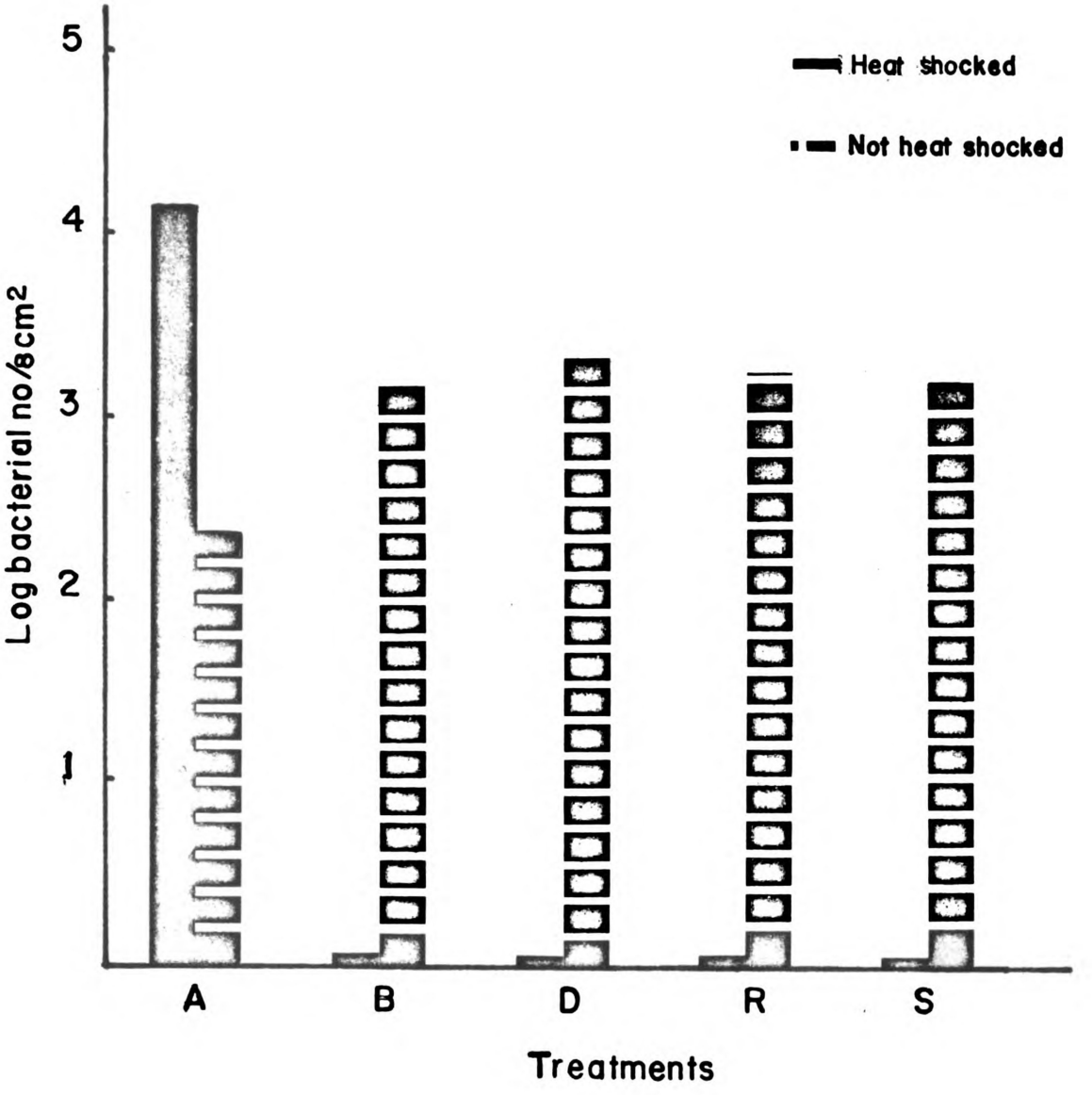

Figure 13.--Effects of microwave energy on spores of a heat resistant strain of C. perfringens. A-control; B-microwave cook; D-microwave cook + freeze + thaw; R-microwave cook + store 12 hrs at 4 C; S-microwave cook + store 36 hrs at 46 C, average 2 trials.

heated (20 min at 75 C) and the other not heated. The initial level of heat resistant spores is represented by the shaded area of the before treatment samples (A). The stripped area (SA) before treatment samples (A) show those spores that were able to grow out without an activation step. The amount of outgrowth shown in Figure 13 (SA) before treatment samples, correlates well with levels of outgrowth of unheated spores reported by Roberts (1967) for various heat resistant strains of C. perfringens.

Examination of data from samples which were cooked and heat-shocked (Figure 13) indicates that the cooking process and the environment provided an efficient activation and germination step for C. perfringens spores. Activation was provided by the cooking treatment and may have caused a change in the level of germinants needed for germination and/or the germination rate. Germination may have been induced by the environmental or compositional changes that occurred during the cooking of chicken.

Sogin et al. (1972) showed that heat activation decreased the amount of various germinating agents needed for germination of Bacillus cerus T spores. This is a well recognized trend with spores of other species (Gould, 1969). Thus activation by heat (cooking) would lower the level of a particular germinant or combination of germinants needed for germination.

The unheated counts (SA) in Figure 13 indicate that the cooking process (heat) did not sufficiently injure the majority of spores present to prevent outgrowth at 46 C but induced activated and germi-

nated forms. This further substatiates the fact that germination occurred, since greater numbers were recovered from the non-heat-shocked samples microwave cooked (B, D, R, S) than from the uncooked (A) samples (SA). The increase in counts of samples B, D, R and S (SA) is due outgrowth of spores that were activated and germinated.

Microwave cooking plus freezing to -30.6 C or holding at 4 C for various times did not cause a reversion to the dormant state. A return to the dormant state would have been indicated by a greater number of heat resistant forms as represented by an increase in the solid areas in Figure 13.

Keynan and Evenchick (1969) described the effect of heating and holding at various temperatures on activation and reversion of B. cerus T spores to the dormant state. They showed that, after a 24 hr period, some reversion could be noted at 4 C and 28 C. However, no reversion to the dormant state was found in the present study after 36 hrs at 4 C, or after 4 days storage at -20 C.

Such a finding is significant if precooked chicken parts are to be frozen and stored as part of an overall centralized chicken cooking system. Formation of heat-sensitive forms in the microwave cooking step without reversion to a heat-resistant state increases the amount of reduction in a subsequent cooking step. This would thus serve as an additional safety margin for a centralized precooking operation.

The number of spores of the heat resistant strain of C. perfringens recovered from chicken pieces after inoculation, cooked in a commercial microwave oven, browned in oil, frozen and stored and

reheated are reported in Figure 14. The solid area, before treatment sample (A) indicates the number of heat resistant spores initially present. The stripped area (SA) indicates the number which were recovered without an activation step. The microwave cooked (B) samples (solid area) indicates that a significant number of spores were either not affected or only activated and indicates the number of heat-resistant forms present. After microwave cooking in the commercial unit, a significant number of heat resistant forms were recovered. This may be due to the slower heating rate in the commercial unit. The pieces were cooked for 4 min in the commercial microwave oven to a weight loss of ca9%. The heating pattern of chicken pieces cooked in the commercial unit is shown in Figure 5. A longer cooking time in the commercial unit would have resulted in a greater number of heat sensitive forms. However, in view of the subsequent reduction of viable cells due to browning step, longer cooking in the microwave is not considered necessary.

The increase in outgrowth of non-heat-shocked samples (SA) again indicates that activation and germination occurred as a result of microwave cooking and possibly the presence of a favorable environment.

The browning procedure, in which chicken pieces were heated in vegetable oil at 190.5 C (Figure 14) destroyed both germinated and ungerminated spores of C. perfringens.

Figure 15 shows the effect of a freezing step prior to microwave cooking in commercial unit. Samples were inoculated, frozen and held for 4 days at -30.6 C. The samples were then thawed at 4 C for

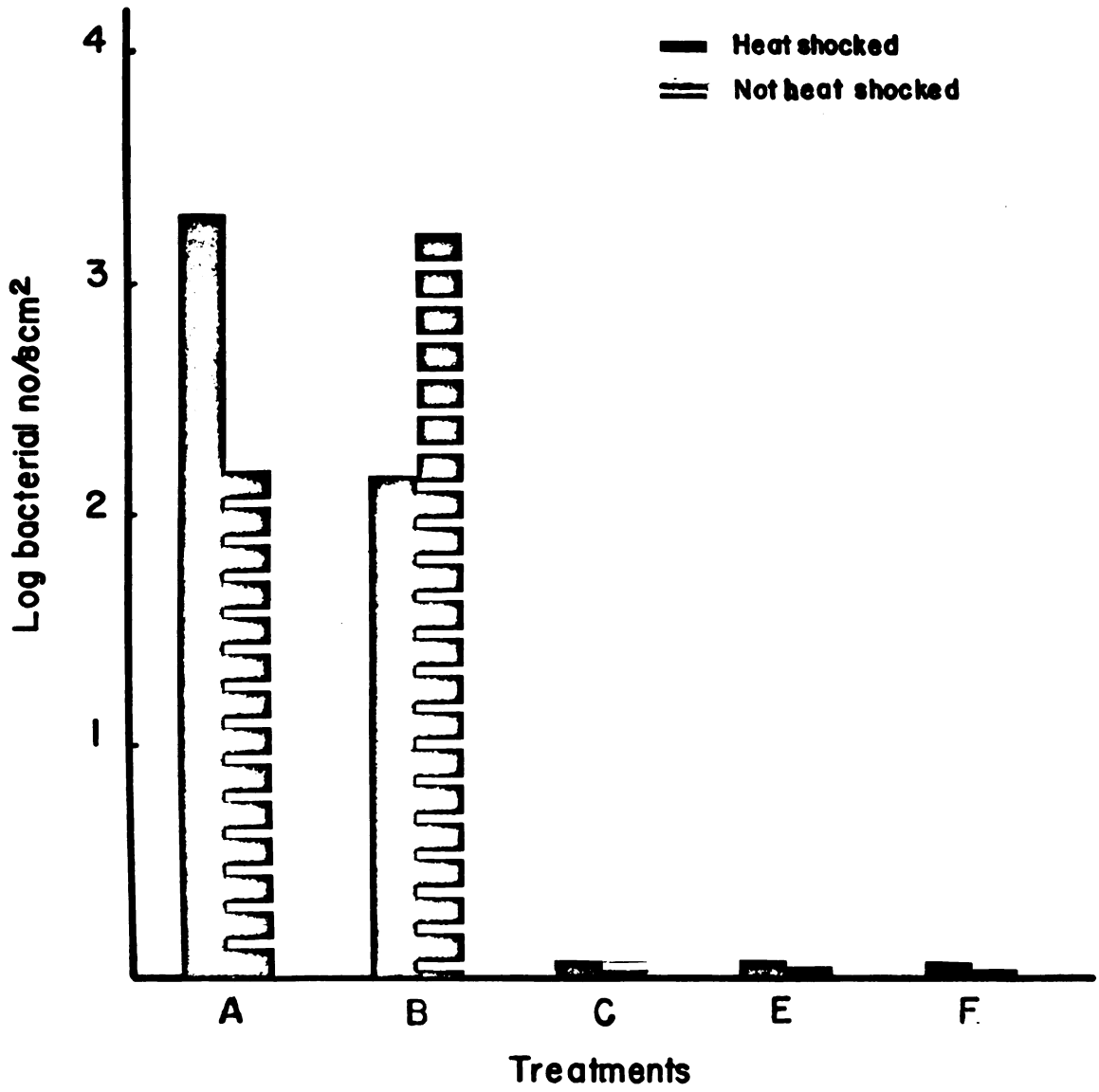

Figure 14.--Effects of various processing steps of a commercial chicken cooking operation on reduction of spores of a heat resistant strain of C. perfringens from chicken pieces cooked in 915 MHz oven. A-control; B-microwave cook; C-microwave cook + brown; E-microwave cook + brown + freeze; F-microwave + brown + freeze + reheat. Pouch method with SFP, incubated at 46 C, average 2 trials.

24 hr. The initial level of organisms present before freezing, the number recovered after freezing, and the numbers recovered after microwave cooking are reported in Figure 15.

The number of heat resistant and non-heat-resistant spore forms present before treatment was identical in the control (A) and inoculated, frozen, thawed (M) samples. The lack of recovery of heat resistant spore forms after microwave cooking indicates that the freeze-thaw step plus microwave heating induced the spores to germinate. It is possible that the freeze-thaw step provided a type of mechanical activation. The lack of heat-resistant forms after microwave cooking indicates that germination occurred prior to or during cooking. Germination may have occurred due to presence of the spores in a favorable environment. Germination without activation in strains of C. perfringens has not been evaluated nor has the effect of freezing and thawing on the dormant state of spores. The data presented in Figure 15 indicate that a freeze-thaw step plus heating will induce formation of heat-sensitive forms. That activation occurred by microwave heating is significant in view of the short cooking time (Figure 5). This step may have merit in formation of heat-sensitive forms which are then more easily destroyed by a heating process.

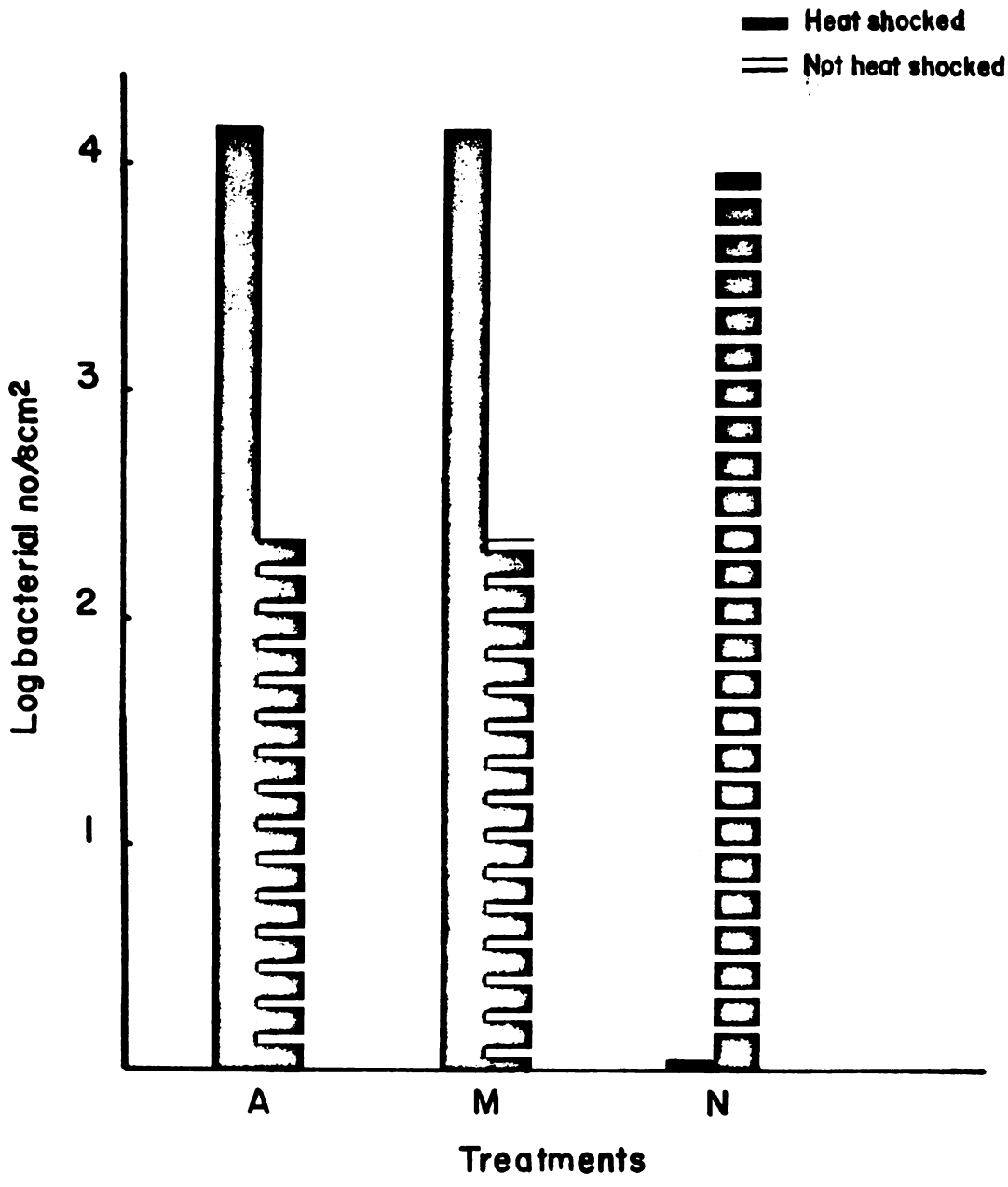

Figure 15.--Effect of freezing, thawing and microwave cooking on spores of heat resistant strain of C. perfringens from chicken pieces cooked in 915 MHz oven. A-control; M-freeze + thaw; N-freeze + thaw + microwave cook. Pouch method with SFP, incubated at 46 C, average 2 trials.

SUMMARY AND CONCLUSIONS

An evaluation of microbial reduction in a centralized cooking system for production of fried chicken was studied. Results show that chicken products of low bacterial levels are produced from such a process.

The recovery of heat-injured Clostridium perfringens cells at various incubation temperatures and on various selective media was determined. Incubation temperatures of 37 and 46 C had little or no effect on recovery of injured cells. A temperature of 50 C was inhibitory to growth of heat-injured cells. The antibiotic combinations normally used for selective isolation of clostridia proved only mildly inhibitory; however, the antibiotic combinations of SPS (Polymyxin B Sulfate + Sulfadiazine) and SFP (Kanamycin Sulfate + Polymyxin B Sulfate) were the least inhibitory.

Rapid cooling or freezing of microwave-cooked products prevented significant growth of C. perfringens. The microwave cooking step did not destroy the vegetative cells present, but provided approximately a 2 log-cycle decrease in cell count. Spores were not destroyed by microwave cooking but may have been converted to heat sensitive forms.

Freezing and storage did not affect the recovery of resident microbial populations or added vegetative cells and spored of C. perfringens.

The process of browning chicken in heated oil at 190.5 C was the most efficient step of the process for the elimination of vegetative cells (resident and added) and spores of C. perfringens.

Freezing and thawing of inoculated chicken before microwave cooking affected the number of vegetative cells (resident and added) and spores of C. perfringens. This step, prior to microwave cooking, resulted in greater reduction of vegetative cells of the heat sensitive strain and formation of heat sensitive spores of C. perfringens after microwave cooking.

The final food product, from such a system, contains few bacteria and is safe for human consumption. Distribution of this product after DFF could prove dangerous if the product is mistreated since all competition has been eliminated.

Assuming that mishandling of such precooked products is inevitable if this system were used for centralized processing and distribution, the safest product would result by DFF at the serving location. The recommendation is that such products be microwave cooked, frozen and stored. Browning at the serving location would minimize food poisoning due to C. perfringens.

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APPENDICES

Appendix I.--Growth of vegetative cells of C. perfringens heated at 65 C in RCM broth, cooled to 15 C and recovered on BSFP and BSFP + 2.5% NaCl at 37 C.

Heating time (min)	BSFP (c/ml)	BSFP + 2.5% NaCl (c/ml)
0	2.4×10^8	2.5×10^8
10	1.0×10^4	7.5×10^3
20	2.2×10^4	2.0×10^4
30	3.4×10^4	4.1×10^4
40	2.5×10^4	3.0×10^4
50	---	---

Appendix II.--Evaluation of various media for growth of heat injured *C. perfringens* cells. Cells heated at 60 C, cooled to 15 C, diluted in PRAS and incubated at 46 C for 24-48 hr.

Time	BSFP	RCM	BHI
0	1.8×10^8	1.7×10^8	1.8×10^8
10	1.8×10^5	1.6×10^5	1.2×10^5
20	6.9×10^4	8.5×10^4	2.8×10^4
40	1.1×10^4	2.3×10^4	6.0×10^4
50	1.9×10^4	1.5×10^3	2.7×10^3

	BAB to pH 7.6	BAB + yeast extract	APT
0	3.7×10^8	4.0×10^8	4.7×10^8
10	3.3×10^5	4.8×10^5	3.2×10^5
30	1.5×10^5	1.2×10^5	6.3×10^4
45	8.5×10^4	8.7×10^4	1.6×10^4
65	4.4×10^3	1.5×10^4	1.7×10^3

Appendix III.--Weight loss of chicken pieces cooked in commercial belt-fed microwave unit, (915 MHz) with potable steam source. Initial temperature 6-7 C.

Initial weight (g)	Final weight (g)	Loss (%)
75	70	6
75	65	13.4
110	100	9.1
90	80	11.1
60	55	8.0
95	90	5.3
90	85	5.5
100	89	11.0
95	86	9.5
85	76	10.5
	Average	8.9