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EFFECT OF MICROBIAL LOAD ON VACUUM PACKAGED FRESH GROUND BEEF PATTIES SUBJECTED TO "IN PACKAGE" THERMAL TREATMENT

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EFFECT OF MICROBIAL LOAD ON VACUUM PACKAGED FRESH GROUND BEEF PATTIES SUBJECTED TO "IN PACKAGE" THERMAL TREATMENT.

BY

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ABSTRACT

EFFECT OF MICROBIAL LOAD ON VACUUM PACKAGED FRESH GROUND BEEF PATTIES SUBJECTED TO "IN PACKAGE" THERMAL TREATMENT.

By

Pakorn Manomaiwiboon

The microbial load of fresh, vacuum packaged, cook-in-bag uncured beef patties was determined in two film structures, a commercial (PE/EVOH), and super barrier (SiO_2 coated polyester) material. Packaged samples were cooked to internal temperatures of 71 and 82^{o}C for 30 min., and stored in temperature abused (23±2^{o}C) and refrigerated storage (4-6^{o}C). Barrier properties had a significant effect ($P<0.001$) on aerobic and mesophilic growth in the abused condition. Cooking temperatures had a statistically significant effect ($P<0.05$) on aerobic growth in the refrigerated condition. The growth of anaerobes and psychrophiles were not significantly effected by either variable. Storage time had the most significant effect ($P<0.001$) for all groups of microorganism.

The model organism, *C.sporogenes* ATCC7955 was used to study the growth of mesophilic anaerobic sporeformers. Storage time had highly significant effects on its growth ($P<0.001$) Cooking temperature had a significant effect on its growth in the abused condition ($P<0.05$). In a spore profile test, an initial load of 10^2 spores or less per patty was reduced to a nondetectable level for 60 days in refrigerated storage when samples were cooked at 71 or 82^{o}C for 30 minutes.

The physical properties of the commercial film (strength, thickness, and shrinkage) were changed after exposure to thermal treatment, while the super barrier package had virtually no change.

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INTRODUCTION

In today's market, there is potential demand for precooked, refrigerated uncured meat products because of possible energy savings, ease in preparation, and convenience in distribution, as compared to frozen products. However, there are microbial concerns with regard to the products' quality and safety. The initial microbial contamination of meat before processing is an important factor affecting microbiological stability of products throughout storage (Sutherland et al., 1982). Microorganisms from many sources such as the animal itself, environment (soil and water), man, and processing equipment (Johnston and Tompkin, 1984) may grow and cause quality changes, and create an unsafe product. Several types of microorganisms are reported to grow in precooked meats, if the products are cooked at inadequate thermal processing conditions, temperature abused, mishandled or are post-processing contaminated. For example, growth of psychrophilic microorganisms such as *Pseudomonas sp.* can cause meat spoilage in prolonged refrigerated storage condition. Some mesophilic microorganisms such as *Clostridium sp.* and *Staphylococcus sp.* can cause outbreaks of food-borne diseases by growing and producing toxin in these type of meat products.

Using vacuum packaging techniques for precooked meat products results in longer shelf life and better product quality, because growth of aerobic and some facultative anaerobe species are retarded by the low oxygen content of the surrounding atmosphere inside the package. Many types of meat products currently distributed in the U.S. market are packaged in vacuum form.

An important concern in vacuum packaged, precooked, uncured meat products is the potential growth and subsequent toxin production of *Clostridium botulinum*. Based on cultural and serological characteristics, there are 4 groups of *C. botulinum* (group I - IV), which are also classified as 7 serotypes from type A to G. (Eklund, 1988). *C. botulinum* serotype E has been used as a target group for creating thermal treatment conditions to destroy spore of *C. botulinum* in processing. This serotype may not be suitable for use as a target group because the study by Simunovic et al. (1985) reported that spores from serotype E had considerably lower heat resistance than spores from serotype B. Spores of *C. botulinum* which survive after thermal processing of products are likely to grow and produce a fatal neurotoxin because precooked, uncured meat has sufficient nutrients, favorable pH, little or no added antimicrobial substances, and there will be less growth competition because most other microorganisms are destroyed during processing. Vacuum packaging also creates an oxygen-free environment (anaerobic condition), which is required for *C. botulinum* to produce toxin (Mass et al., 1989). Generally, growth and toxin production will occur if products are held at temperatures higher than 10°C (Goldoni et al., 1980). There are some serotypes (B, E, and F) that can grow and produce toxin at temperatures as low as 3.3°C. (Genigeorgis, 1986). Based on its growth characteristics, and the deadly nature of the neurotoxin produced by *C. botulinum*, it appears that the hazard potential for *C. botulinum* growth in this type of meat products should be high. However, the reported incidence of foodborne disease caused by botulism toxin is low when compared to foodborne diseases caused by other pathogens (Johnston and Tompkin, 1984).

There are two packaging techniques used to manufacture vacuum, cook-in-package meat products. These are cook-in-strips, which use

different packages, one for processing and another for distribution; and cook-in-ship, which uses the same package throughout processing and distribution (Terlizzi et al., 1984). Advantages of vacuum packaged, cook-in-ship meat products with subsequent thermal treatment and refrigerated storage were reported by Simunovic (1985) to be: 1) effectively eliminate post-processing microbial contamination. 2) reduce moisture loss from product during processing because it is sealed tightly in barrier materials and 3) extend product shelf life by destroying or retarding growth of most pathogens and spoilage microorganisms.

Today, the cook-in-package technique called "Sous Vide", which originated in France, is widely used in Europe for premium refrigerated entrees (Swientek, 1989). In this technique, raw or precooked products are placed in heat stable, high barrier plastic bags or pouches, packaged, sealed under vacuum, and cooked to a time and temperature required for the individual foods. The products are then chilled rapidly in an ice water bath, and stored in controlled refrigerated conditions. Advantages for products prepared according to the "Sous Vide" technique are; 1) product shelf life is generally acceptable up to 2-3 weeks; 2) natural product flavor, aroma, and juice are sealed in; 3) overcooking problems found with retort processing are avoided; 4) preparation such as mixing ingredients is done at central locations, therefore, the quality and taste of products will be consistent; and 5) convenient to consumer because products need only to be reheated before consumption. (Raffael, 1984, Light et al., 1988)

In the U.S., "Sous Vide" products are available only at the foodservice level in some geographical areas. Products are manufactured under the Hazard Analysis Critical Control Point (HACCP) program, which requires strict control for processing and storage conditions (Swientek, 1989).

The Food and Drug Administration (FDA) has barred this product at the retail level because of the potential microbiological safety concerns associated with toxin production from *C. botulinum* if proper handling procedures are not strictly followed, or products are temperature abused (Anon. 1988). In the UK, distribution of Sous Vide products is not controlled. This can create false confidence for consumers because this product/package system is believed to be the perfect vessel to encourage growth of *C. botulinum* (Johnson and Mitchell, 1989).

The present study is done with three specific objectives; First to evaluate growth of microflora and *C. sporogenes* in two vacuum packaged, cook-in-bag uncured meat systems (noninoculated and inoculated with *C. sporogenes* PA 3679/ATCC 7955). The microbial load of each system is determined as influenced by: a) The effect of commercial pasteurization (71^{o}C) versus severe pasteurization. (82^{o}C). b) The barrier properties of a commercial packaging material used for cook-in-bag meat products, versus a superior barrier packaging material (Silicon Dioxide coated Polyester - SiO_2 coated PET). and c) The effect of storage times in prolonged refrigerated storage and room temperature abused storage. Second, to determine the level of mesophilie anaerobic spores (using *C. sporogenes* PA 3679/ATCC 7955 spores as a model) initially present in raw meat which can be destroyed using the vacuum, cook-in-bag technique and stored in a refrigerated condition. A final objective is to determine changes in the physical and barrier properties of cook-in-bag packaging materials after exposure to processing, handling and storage.

LITERATURE REVIEW

There are three important factors which effect the shelf life of vacuum packaged, cook-in-bag meat products system. These are: packaging material selection, thermal processing conditions, and storage conditions. Many studies have been done on each of these.

Effect of packaging material for vacuum packaged meat products.

Taclindo et al. (1967) examined 113 prepared food samples in plastic packages for growth of *C. botulinum* and found that the incidence of growth in these food samples was extremely low. A further experiment was conducted by inoculating a total of 64 food samples with *C. botulinum* type E spores, with storage in an anaerobic condition at 30^{o}C for 11 days. Growth and toxin production were examined in turkey rolls and soybean cake samples using conventional techniques. For the remaining 62 inoculated food samples, 60 samples showed the possibility of *C. botulinum* growth and toxin production if a media enrichment technique was used. Borgstrom (1968) reported that vacuum packaging was not able to inhibit microbial growth, but changed the nature of microflora in meat products. Baren et al. (1969) observed that growth of aerobes in vacuum packaged samples was retarded, while growth of anaerobes occurred faster in vacuum packaged ground beef at 5^{o}C storage. Arafa and Chen (1975) determined growth of microorganisms in vacuum packaged cut-up chickens. It was found that vacuum packaging did not confer any protection to fresh poultry in refrigerated storage. Goepfert and Kim (1975)

used two types of packaging materials, cellophane and saran, to create aerobic and anaerobic conditions for inoculated ground beef. The packaging materials had little or no influence on behavior of microorganisms during 14 days storage at temperatures ranging from 1 to 12.5°C.

Hanna et al. (1977), Blickstad and Molin (1983), and Simard et al. (1984), reported that packaging materials and techniques strongly influenced the type of microorganisms in meat, because they could alter the environment surrounding the product. Seideman et al. (1976) vacuum packaged different types of beef cuts (knuckles, ribs, chucks) in packages with oxygen transmission rates ranging from 0.41-2.28 cc/100 in^2/24hr., with storage at 1-3°C for 0-35 days. Microbial counts were slightly higher at the higher oxygen transmission rates, but the difference in number of psychrotrophic, mesophilic and lactobacillus organisms in the different packaged products was not statistically significant. Dainty (1979) reported that meat products vacuum packaged using materials with oxygen permeabilities ranging from 5 to 90 cc/m^2/d/atm had extended shelf lives up to 8 weeks in refrigerated storage, due to retardation of growth of some microorganisms, especially *Pseudomonas sp.*

It can be implied that barrier properties of packaging material influence on types and numbers of microorganisms presented in meat products. Therefore, the microbial load of precooked, uncured meat packaged in super barrier material will be evaluated compared to the same products packaged in commercial cook-in-bag material, which expected to have lower barrier properties. Some mechanical and physical properties of both packaging materials after exposed to processing will be tested because changes of these properties may effect barrier and performance of final package.

Packaging materials used for cook-in-bag meat products.

Bag materials for cook-in-bag meat products must be heat tolerant, impermeable to gas and moisture, and have sufficient strength and toughness to withstand the severe conditions during vacuuming, cooking, handling and shipping (Terlizzi et al.,1984). The authors suggested that the oxygen permeability for this type of material in prolonged refrigerated storage should be below 70 cc/mil/m^2/24hr/atm at 20^{o}C, 0%RH, to retard microbial growth, as well as fat and light induced color oxidation. Currently, these materials are manufactured as multilayer composites using coextrusion and lamination technology to combine two or more polymers together (Harte, 1987 and Rosinsky et al., 1989). Multilayer structures are designed to obtain specific barrier, strength, machinability, sealability, and thermal stability properties (Lundquist, 1987). Shrinkage of material after cooking at high temperature must be controllable because it influences package appearance, product yield, and adhesion properties between meat surface and the inner layer of the package. (Rosinsky et al., 1989)

There are many type of polymers approved by the FDA for cook-in-bag meat packaging. Multilayer packages are typically made by combining barrier layer to sealant layer. For the barrier layer, ethylene vinyl alcohol (EVOH) copolymer is widely used because of its excellent oxygen barrier properties. Bag material is made by sandwiching EVOH between two moisture barrier polymers (eg. polyethylene). Due to the hydrophilic nature of EVOH, loss of barrier properties can occur when the moisture content of the film is high. Polyvinylidene Chloride (Saran) copolymer has excellent moisture and oxygen barrier properties. Bag material is made by coating Saran onto other base polymers, such as polyester, polypropylene, and Nylon to increase physical properties and machinability. For the sealant layer, polyethylene is used alone

or mixed with ethylene vinyl acetate (EVA) copolymer or linear low density polyethylene (LLDPE) to improve mechanical and sealing properties. Surlyn', which is an ionomer, is the most commonly used sealant layer for vacuum packaging of meat products because it has a broad range of sealing temperatures (Terlizzi et al., 1984, Simms, 1985 and Lindquist, 1987).

Effect of cooking temperature on growth of *Clostridium sp.* and other microorganisms.

The United States Department of Agriculture (USDA) requires that perishable, uncured meat must be cooked to a minimum internal temperature of 62.8-76.7^{o}C (Tompkin, 1986). Sofos (1986) reported that many commercial cook-in-bag meat products are cooked to an internal temperature of 71^{o}C, which is intended to destroy most *Salmonella sp.,* but does not provide sufficient assurance of microbiological stability for all meat products. Cook-in-bag products are required by the USDA to be manufactured under HACCP control to assure product quality and consumer safety. Refrigerated warning labels are required on packages to warn retail outlets and consumers that products need to be kept at temperatures of 4^{o}C or lower to retard growth of surviving microorganisms (Tompkin, 1986 and Prabhu et al.,1988). Many of these products also have print expiration dates on packages to specify product shelf life (NFPA, 1988).

The thermal destruction conditions necessary to destroy *C. botulinum* in low acid foods were developed by the National Food Processors Association - NFPA. (originally National Canners Association - NCA). Tuna in oil (contained 20.5% fat) was reported to be the most protective medium tested. For *C. botulinum* type E strain saratoga, the $D_{82.2^{o}C}$ was 6.6 min (NCA 1973, 1976). Simunovic et al. (1985) reported that the $D_{82.2^{o}C}$ values for *C. botulinum*

type E were ranged from 0.15 min to greater than 4.9 min in reference media, and from 0.74 min to 6.6 min in foodstuffs.

To determine actual thermal treatment in actual processing, the use of *C. botulinum* as a tested organism is avoided, because of its hazardous potential if in-plant contamination by spores of *C.botulinum* were to occur. (Goldoni et al., 1980) An alternative microorganism, *C.sporogenes* PA 3679 /ATCC 7955, is a nontoxic strain that has many physical and biochemical characteristics similar to *C. botulinum.* This microorganism is used as a model for thermal process evaluation of many food products. In table 1, a comparison is made of physiological requirements for these two clostridium species. *C. sporogenes* is a putrefactive, gram positive, straight-rod-shape-anaerobe. Its spore is oval shaped with subterminal end location. Thermal death time curves for both clostridium species were reported by Stumbo et al. (1950). They found that *C sporogenes* PA 3679 spores had a higher heat resistance (as higher z-value) than *C. botulinum* spores, when suspended in various media prepared from many types of food. Comparison of the D values (destruction time of spores to 1 log cycle reduction) for many food products at temperatures ranging from 220-260^{o}F, demonstrated that spores of *C. sporogenes* have higher D values than spores of *C. botulinum.*

The microbial load of cook-in-bag meat products (noninoculated study) and *C. sporogenes* inoculated study (which is used as a model for *C. botulinum*) will be evaluated in this study using two cooking temperatures; 82.2^{o}C for 30 min, which is used as a target temperature to destroy C. *botulinum*; and 71^{o}C for 30 min, which is previously reported to be used for many cook-in-bag meat products (Sofos, 1986). For the effective of both cooking conditions to the destruction of *C. botulinum* spores, different spore concentration of model organism (*C. sporogenes*) will be evaluated.

TABLE1: Some physical characteristics of C. sporogenes and C. botulinum

Characteristics	*C. sporogenes*	*C .botulinum*
Casein digested	yes	yes
Lecithinase	no	no
Indole	no	no
Lipase	yes	yes
Glucose	yes	yes
Mannose	no	no
Maltose	variable	variable
Lactose	no	no
Salicin	n/d	variable
Produce H2S gas	yes	yes
Urease	no	no
Gelatin liquidfied	yes	yes
Nitrate reduced	no	no
Blood hemolyzed	yes	yes
Acetylmethylcarbinol	no	no
Toxin produced	no	yes
Pathogenic to animals	no	yes

n/d= need further tests to confirm

From: Bergey's Manual of Determinative Bacteriology (1974)

Cook-in-bag vacuum packaged meat and poultry products.

There have been several studies dealing with the microbiological stability of vacuum packaged fresh meat and poultry products, though studies on vacuum packaged, precooked, in-package thermally treated uncured meat and poultry products have been limited. Simunovic et al. (1985) tested restructured beef formulated with 1.5% sodium chloride and 0.4% sodium tripolyphosphate (STPP-antimicrobial agent), vacuum packaged, and processed in pressurized water at 96°C and 1.38×10^5 Pa, until the center of the meat was heated equivalent to 82.2°C for 15 min. No aerobic or anaerobic colony-forming units were detected, and the microbial numbers in the product remained stable for 16 weeks in a vacuum package, or for 2 weeks without vacuum packaged at 4.4°C storage. Prabhu et al. (1988) examined the shelf life of vacuum packaged pork chops inoculated with *C. sporogenes* PA 3679 and *S. aureus* z88. Product was cooked to internal temperatures of 66°C and 71°C in a temperature-controlled water bath set at 80°C. Product shelf life was 15-21 days at 2-4°C storage temperature and could be extended up to 60 days if product was exposed to secondary in-bag cooking to 66°C. In prolonged temperature abuse or mishandling, secondary cooking did not make the product more microbiologically safe. Pork chops treated by dipping into antimicrobial agents prior to packaging effectively prevented growth of microorganisms on the surface under a simulated abuse condition (24-25°C for 48 hrs.).

Refrigerated, vacuumed, cook-in-bag uncured turkey breast rolls were studied by Smith and Alvarez (1988). Turkey rolls were prepared, vacuum packaged, and cooked to an internal temperature of 71°C. No psychrotrophic aerobic growth was detected during storage at 4°C for 87 days. Mesophilic

anaerobic microorganisms were detected, but the number did not change significantly during storage. The implication was that these organisms can survive at this cooking condition, and may grow when a product has been temperature abused. Maas et al. (1989) studied the growth and toxin production of *C. botulinum* in comminuted raw turkey formulated with sodium chloride, sodium phosphate, and various levels of sodium lactate. Samples were inoculated with a mixture of *C. botulinum* type A and B spores, vacuum packaged, and cooked in hot water to an internal temperature of 71.1°C. *C. botulinum* growth and toxin production were detected in samples treated with 0% sodium lactate after 2 days and 4 days storage at 27°C, for the 160 and 28 spores/ml. inoculated samples respectively. *C. botulinum* growth was delayed to 4-5, 4-6, 7, and 7-8 days when the concentration of lactate present in the meat was 2.0, 2.5, 3.0, and 3.5%.

"Sous Vide" products were evaluated by Schafheitle and Light (1989) using chicken ballotine, as representative of a food product currently manufactured by this method. All ingredients (boneless chicken, vegetables, and seasonings) were placed into pouches, evacuated at a high setting, and then sealed immediately. Pouches were cooked using a convection wet steamer to an internal temperature at 80°C for 10 min., and then cooled in a chilling cabinet for 90 min. prior to storage at 0-3°C. Microbiological evaluation during 21 days storage found that no samples had mean spore counts (aerobes, anaerobes, anaerobic spores, and anaerobic spores) higher than 1.3×10^2 g^{-1}. Carefully controlled processing and chilling were the most important steps which maintained product shelf life for up to 21 days.

Cook-in-bag, smoked fish was processed using this technique (Eklund et al.,1988) to determine the feasibility of the process to inactivate growth and toxin production of *C. botulinum* types B and E. Smoked salmon

was inoculated with 10^6 spores of *C. botulinum* per sample, vacuum packaged and cooked using different time and temperature conditions. Cooking at 92.2°C for 45 min. and 55 min. prevented toxin production by type E during 120 days storage at 10°C and 21 days storage at 25°C, respectively. To avoid toxin production in products containing *C. botulinum* type B spores, longer cooking times at all temperatures were required, when compared to the cooking times used to destroy type E which is used as the target group for *C. botulinum* studies.

According to these studies on vacuum, cook-in-bag uncured meat products previously described, there are some variation on thermal treatment and storage condition conditions which can provide adequate shelf life with microbiologically safe for meat and poultry products. This study is intended to provide additional information on microbial safety and shelf life for these type of products, using the current thermal processing and storage conditions.

MATERIALS AND METHODS

Meat preparation. (Figure 1)

Fresh lean trimmings from beef chucks (approximately 85-90% lean) were obtained from the MSU Meat Laboratory. The beef was ground using a coarse plate (0.63 cm. plate), and then finely ground (0.16 cm. plate) using a Hobart grinder (Hobart Manufacturing, Troy, OH). The ground beef was removed from the grinder and weighed equally into two batches; one was used for the noninoculated study and the other for the *C. sporogenes* inoculation study. Each batch of ground beef was mixed using a Hobart Laboratory Mixer (Hobart Manufacturing, Troy, OH) for 5 minutes to obtain uniform texture. The noninoculated sample was mixed first , followed by the inoculated sample. Ten ml. of sterile 0.1% peptone water was added to the noninoculated batch during mixing. For the inoculated batch, a 10 ml. inoculum of *Clostridium sporogenes* ATCC 7955 spores (prepared according to the methods described below) was added to the beef instead of the peptone water. Each batch was removed from the mixer and stuffed into a 4.4 cm. diameter cellulose casing to get uniform patty diameter and portion control when sliced. Both samples were placed into a freezer (-30^{o}C) overnight before being sliced into patties.

C. sporogenes inoculum preparation

The spore inoculum of the model pathogen, *Clostridium sporogenes* ATCC 7955/ NCA 3679,was prepared by propagation in a sterile tube containing Beef Liver Medium (prepared according to the ATCC formula;

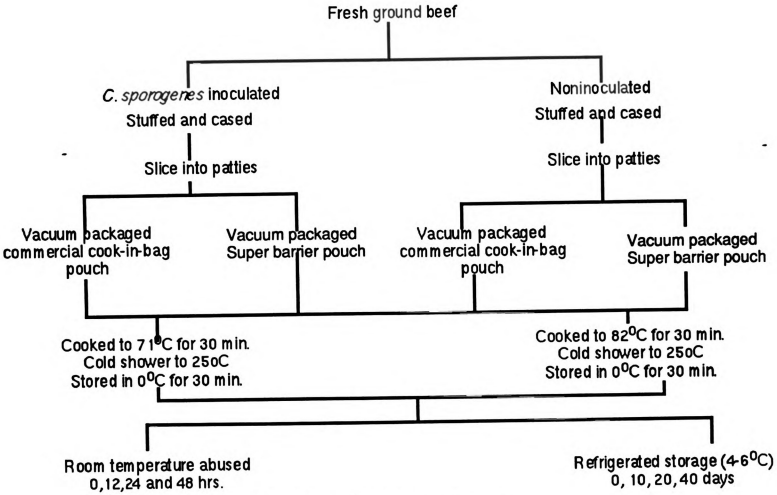

Figure.1: Sample preparation of cooked, uncured beef patties.

ATCC, 1982). The tube was incubated under anaerobic conditions at 37°C for 24-48 hrs prior to use. Anaerobic conditions were achieved by adding sterile mineral oil to the top of the liquid as a barrier layer to prevent oxygen contact. An inoculum was made by transferring the spore suspension from the culture media into the sterile tube, and 0.1 % sterile peptone water added to adjust total inoculum to 10 ml. An inoculum concentration of approximately 10^5 spores per meat patty was expected. The actual spore concentration was determined by doing plate counts of *C. sporogenes* on the inoculated, vacuum packaged, uncooked, fresh ground beef patties.

Packaging materials used for cook-in-bag study.

Two bag materials were used in this study, Both were supplied by commercial film manufactures. The first material, a shrinkable, multi-ply structure of polyolefin and ethylene vinyl alcohol, is currently used as a commercial packaging material for cook-in-bag meat products, and precooked meat products. The Oxygen Transmission rate of this material was reported by the manufacturer to be 20 cc/m^2 24 hrs atm at 23°C(73°F) 0% Relative Humidity (Anon., 1989) The second bag material was a silicon dioxide (SiO_2) coated polyester (PET), laminated to a heat sealable polypropylene. This material is currently used as a super barrier packaging material for some shelf stable retortable food and pharmaceutical products in Japan. Oxygen Transmission rate of this material was measured using the Oxtran-100 Oxygen permeability tester (Modern Controls Inc., Elk River, MN.) to be approximately 0.4 cc/m^2-24 hrs-atm at 23±2°C,100% RH. The SiO_2 coated layer is responsible for the superior barrier properties of this material. (Anon. 1988)

Packaging and processing.

The ground beef (in casings) was removed from the freezer and sliced into patties using a meat cutter (BIRO model 3334, BIRO Manufacturing Co. Marblehead, OH). Each patty was about 0.5 cm. (0.2 in.) thick, 4.4 cm. (1.75 in). diameter and weighted approximately 10 grams. Patties were individually placed into 10 x 15 cm. pouches made from both types of cook-in-bag packaging materials. Each filled package was vacuum heat sealed using a Multivac Vacuum Packaging Machine (Sepp Haggenmuller, W. Germany). Packages were labelled, divided into two sets, one for each cooking condition, and kept in a walk-in-cooler set at 0°C for approximately 30 minutes prior to be thermal processed.

The first set of sample packages was taken from the cooler and placed on racks inside the smokehouse with microprocessor controls (Drying Systems, Inc.). Beef patties were cooked using a forced air technique at 100% humidity until an internal patty temperature of 71°C (commercial pasteurization condition) was reached. The packaged products were then held at this temperature for 30 minutes. The second set was cooked using the same technique but to an internal temperature of 82°C (severe pasteurization). Internal temperature was measured as duplicate values in two patty samples placed in the center of the rack using two thermocouple probes, one connected to the smokehouse microprocessor control unit (PC 5809 Butcher & Packer Supply Co.), and the other to an external temperature reader (Honeywell Co. Minneapolis, MN). Each probe was inserted into the center of the patty before processing. Internal temperature from each patty was read every 5 minutes during processing. After cooking was completed, the packages were showered with cold water in the smokehouse until an internal temperature of 23-25°C was reached, and then immediately removed to cold storage (0°C) for 30 minutes to

thermally shock the remaining microorganisms and spores. Excess water on each package surface was wiped off before placing samples into the two different storage conditions.

Storage conditions

Equal numbers of packages from the noninoculated and inoculated study were kept in the two storage conditions; temperature abused and refrigerated. For temperature abused, samples were stored at room temperature (23±1°C) for 0, 12, 24, and 48 hours. Refrigerated storage samples were stored in a refrigerated walk-in cooler for 0, 10, 20, and 40 days. The temperature was chosen to fall within a range of 4-6°C to simulate refrigerated storage at the retail level. Packages stored in both conditions were taken out periodically as defined above and evaluated. For noninoculated samples, total numbers of aerobic, anaerobic, mesophilic, and psychrophilic microorganisms were determined. For inoculated samples, total numbers of *C. sporogenes* were determined.

Sample evaluation for noninoculated samples (Figure 2).

The total microbial count for each sample was determined using the APHA Standard Plate Count method (Busta et.al., 1984). Each package was torn open and a gram of meat was taken from the center of each patty. A 10^{-1} sample dilution was made by aseptically transferring meat from each sample into a tube containing 9 ml. of sterile 0.1% peptone water and mixed well. Ten-fold serial dilutions were made by aseptically transferring 1 ml. of the previous dilution into a tube containing 9 ml. of sterile 0.1% peptone water and mixing well. Serial dilutions for each sample were made from 10^{-1} to 10^{-4}, and sample dilutions from 10^{-2} to 10^{-4} were used for microbial enumeration. A one ml.

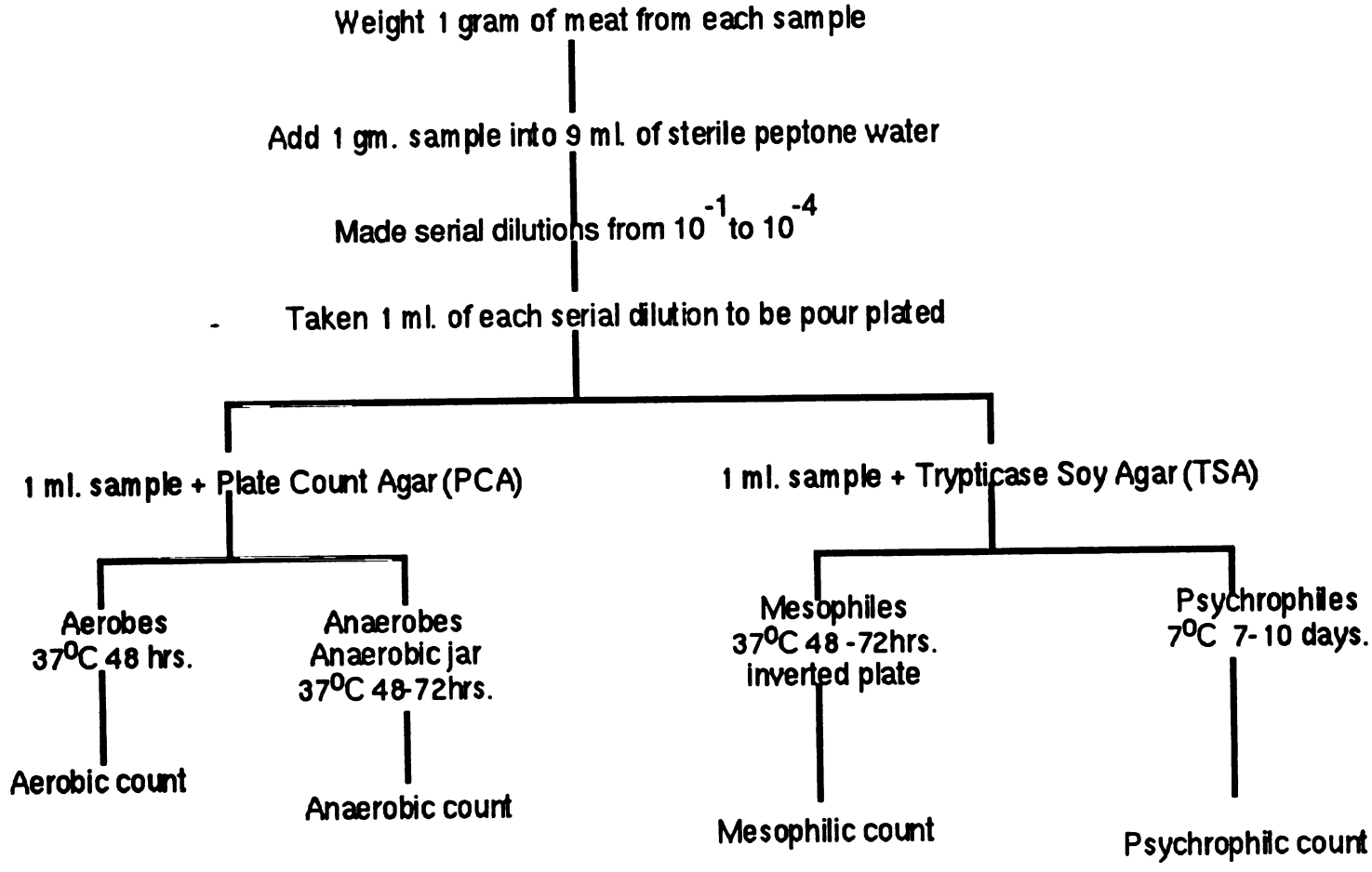

Figure 2: Microbial evaluation of noninoculated samples.

aliquot was taken from each dilution to determine microbial numbers for the four different groups of microorganisms (aerobes, anaerobes, mesophiles and psychrophiles), using standard pour plate techniques (Busta et al., 1984). Media and incubating conditions for each type of microbial group were as follows:

- Aerobic Plate Count: Plate Count Agar (PCA, Difco Co., Detroit, MI), with incubation at 37°C for 48-72 hours.
- Anaerobic Plate Count: Plate Count Agar with incubation at 37°C for 48-72 hours in anaerobic condition using GasPak 150 anaerobic jar with generator and indicator system (BBL Microbiology Systems, Cookeysville MD). Anaerobic condition was reached when the color of anaerobic indicator changed to colorless.
- Mesophilic Plate Count: Trypticase Soy Agar (TSA, Difco Co.), with incubation at 37°C for 48-72 hours. Plates were placed in an incubator for about an hour until agar solidified, then plates were inverted face down.
- Psychrophilc Plate Count: Trypticase Soy Agar, with incubation at 7°C for 7-10 days.

Colonies from each plate were counted using the method described in Appendix and calculated as the average colony forming units (CFU) per gram meat. This number was calculated and reported as $\log_{10}$CFU for each sample.

Sample evaluation for *C. sporogenes* inoculated samples (Figure 3).

The model pathogen (*Clostridium sporogenes*) was enumerated using the Standard Plate Count method as modified by Prabhu et.al. (1988). Sample preparation and serial dilutions were done using the same procedures as previously described for the noninoculated sample. The media used was

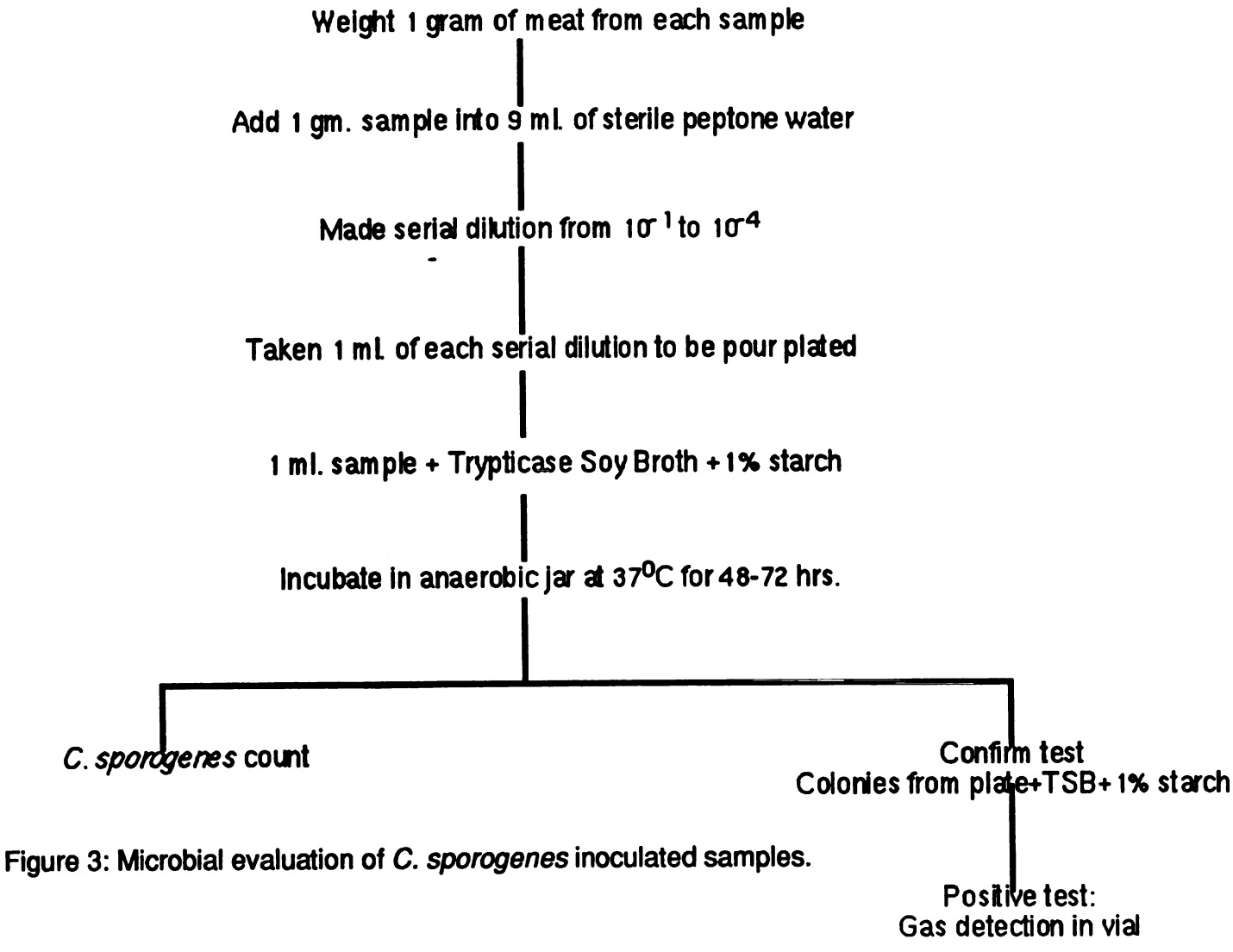

Figure 3: Microbial evaluation of *C. sporogenes* inoculated samples.

Trypticase Soy Agar (TSA) + 1% Soluble starch (Difco). Plates were incubated at 37°C for 72 hours in an anaerobic condition using a GasPak 150 anaerobic jar with generator and indicator system. Colonies on each plate were counted using the procedures described in Appendix. The CFU number (colonies per gram meat) was reported as $\log_{10}$CFU per gram sample.

C. sporogenes growth was confirmed by transferring colonies from plate samples into a tube with an inverted vial containing sterile Trypticase Soy Broth (TSB, Difco Co.) + 1% Soluble starch . A positive test is confirmed by detecting gas production inside the vial after incubation in an anaerobic condition for 48-72 hours at 37°C.

Statistical analysis of data.

A total of 4 replicates were done in each study (noninoculated and *C. sporogenes* inoculated beef) to obtain sufficient data for statistical analysis.Statistical analysis was evaluated based on three factors in the study. These factors were; 1) type of package (commercial and super barrier); 2) cooking temperatures (71 and 82oC); and 3) length of storage time in temperature abused (48 hours period), and refrigerated storage (40 days period). Data for each type of microorganism (as $\log_{10}$CFU value) was analyzed using a 3-way Analysis of Variance (ANOVA) technique. Procedures were obtained from "Design and Analysis of Experiments" by Gill (1987). Statistical results were calculated as mean square values of each single factor, and all possible combined interactions of 2 and 3 factors to be tested for significant. Significance differences of each factor was determined using the Fisher's variance ratio test (f-test). The f-value was calculated from the mean squares of the particular variable in question divided by the mean square error (total mean square).

pH of the meat

The pH of the meat was measured by blending meat in sterile peptone water. An approximately 1 gram sample was taken from the package, transferred into a tube containing 9 ml. of 0.1% sterile peptone water (pH 7.0± 0.1), and mixed well. The pH value was measured using a Corning pH Meter. The pH was reported as an average from the four replicates.

Spore profile study for *C. sporogenes*

A *C. sporogenes* (ATCC 7955) spore inoculum was prepared using the procedures previously described to obtain six levels of spore concentrations ranging from approximately 1 (10^0) spore to 100,000 (10^5) spores per patty. Six batches of ground beef were prepared and inoculated with each level of spore dilution using the procedures described for the *C. sporogenes* inoculation study. Packaging and processing procedures used were described in the previous study except only one type of pouch (commercial cook-in-bag) was used as the package material. After processing, samples were kept at 4-6^{0}C. The total number of viable C. *sporogenes* was determined at 0, 10, 20, 40, and 60 days of storage for each sample, at each level of spore inoculum. The procedures used were identical to the *C. sporogenes* sample evaluation procedures previously described. Serial dilutions for spore profile samples were made from 10^{-1} to 10^{-3}. A one ml. aliquot was obtained from each dilution to be microbially evaluated. The procedures used for counting the numbers of sporogenes are followed the *C. sporogenes* evaluation. Each test was repeated four times.

Mechanical and physical properties of packaging materials

The commercial cook-in-bag and SiO_2 coated PET materials in sheet and pouch forms were thermally processed in both cooking conditions (71 and 82°C) for 30 minutes in the forced air smokehouse. The physical properties of the film were evaluated as roll stock and thermally processed as follows;

- Material thickness: Thickness of each film sample was determining using a Testing Machines' Micrometer, model 549M. (Testing Machines Inc., Amityville C.I., NY.). Thickness (in mil) was reported as an average from ten measurements.

- Percent material shrinkage after thermal processing: Surface area of each pouch before and after cooking was determined at both 71° and 82°C for 30 minutes using 5 sealed pouches per material type. Percent shrinkage was calculated as:

$$\text{Percent shrinkage} = 100 \times \frac{\text{surface area after cook}}{\text{surface area before cook}}$$

Shrinkage was reported as average percent shrinkage for five pouches cooked at each temperature.

- Package and seal leakage: An ARO non-porous package tester model F-099-1080 (The ARO Corp., Buffalo NY.) equipped with vacuum pump was used to detect material and seal integrity of the pouches. Five sealed pouches of each material were tested before and after thermal processing at both temperatures. Each pouch was submerged into the vacuum chamber, which was filled with water, and vacuum slowly applied by adjusting the vacuum level to a maximum level of 30 psi (2.18 kg/cm^2). The package was then held at this vacuum level continuously for 60 seconds. If there was no leakage (air bubbles) detected, the result was recorded as non-detectable (N/D). Pouches from each sample

material were tested five times.

- Oxygen permeability of the materials before and after cooking: Three sheets from each material (rollstock, 71°C cooked for 30 min., and 82°C cooked for 30 min.) were tested using the Oxtran-100 Oxygen permeability tester (Modern Controls Inc., Elk River, MN). All permeability tests were done at 23±2°C, 100%RH. Oxygen permeability was calculated and reported as an average from three samples in units of $cc/m^2/24$ hrs.atm.

- Strength of packaging material and seal (Tensile tests): The procedure followed is described in ASTM Standard D882-83. Film samples [Machine Direction - MD, and Cross Direction -CD] were cut into 1" wide strips using a precision sample cutter (Thwing-Albert Instrumental Co., PA). For sealed strips, seals were made in both MD and CD directions using an Impulse Heat Sealer (Sentinel Packaging Industries, Hyannis, MA) prior to cutting. The sealing conditions used were the same as used for pouch sealing in the previous study. Sample strips were tested using an INSTRON,tensile testing machine model 4201 (Instron Corp., Canton, MA.). Tensile strength was reported in kg/cm^2 unit, and Strain (change in length of sample) in percent.

RESULTS AND DISCUSSION

Microbial evaluation of noninoculated ground beef patties.

Uncured beef patties were vacuum packaged in two different cook-in-bag materials, processed at two temperatures, and stored in two conditions; room temperature abused and refrigerated storage. The mean and standard deviation values were reported to show some variations in microbial count within the four replicates. These variations may have been due to meat from different animals, and from unavoidable contamination during packaging, processing, and microbial evaluation. The aerobic and anaerobic plate count of vacuum, uncooked, fresh ground beef patties (raw material) were examined by randomly selecting patties prior to thermal treatment from each batch. The initial microbial population in the fresh patties ranged between nondetectable level (log_{10}CFU less than 2.0) and log_{10}CFU 2.5 per gram meat.

In this study, the smallest dilution used for microbial evaluation was 10^{-2}, and the largest dilution used was 10^{-4}. If there was no growth detected in the smallest dilution, the log_{10}CFU number was reported to be less than 2.0 (<2.0). This value was substituted as (log_{10}CFU) 2.0 in the mean and S.D. calculation, and statistical analysis, corresponding to the minimum number of microorganisms present in the meat. At the maximum dilution (10^{-4}), if the actual colony count in a plate was higher than 300 colonies, the log_{10}CFU number was reported to be higher than 6.48 ($log_{10}300x10^4 = 6.48$). This value was substituted as (log_{10}CFU) 6.48 in the mean and S.D. calculation, and statistical analysis, corresponding to the maximum number of microorganisms

which could be detected using this evaluation technique. Both minimum and maximum log_{10}CFU numbers were substituted to reduce deviation of data caused by outlier values.

In table 2 and 3 are shown the f-values calculated for all four types of microorganism in temperature abused and refrigerated storage conditions, respectively. The calculated f-value was compared to standard f-test distributions to obtain significance of the variables. Significance was reported as a probability value for each factor. In this study, 95% probability ($P<0.05$) was used as the minimum significant level. Several of the factors and their interactions significantly influenced microbial growth in either room temperature abused or refrigerated storage conditions.

a) Aerobes:

The average number of aerobic microorganisms in table 4 was used to construct curves versus storage times for both types of package. The plots are shown in figure 4 and 5 for room temperature abused and refrigerated storage respectively. In the abused condition (figure 4), growth of aerobes was higher in meat packaged in the commercial pouch than in the super barrier pouch after 24 hours storage. In refrigerated storage (figure 5), there was an approximately 1.5 log increases in aerobic growth during a 40 day period of refrigerated storage. The difference in barrier properties did not effect aerobic growth in refrigerated storage because the growth pattern and number of microorganisms in both packaging materials were not different.

Statistical analysis of factors effecting aerobic growth in the temperature abused condition (table 2) indicated that type of package (barrier properties) and storage time had strong influence on aerobic growth ($P< 0.001$). The interaction of both factors was also significant ($P<0.01$). The rapid increase in the number of aerobes in the commercial pouch was probably because the

TABLE 2: f-test values for temperature abused sample from 3-way ANOVA test.

Factors	D.F.	Aerobes	Anaerobes	Mesophiles	Psychrophiles	C.sporogenes
A(film)	1	17.53	2.50	10.12	3.27	0.00
B(Temp.)	1	0.47	2.93	1.12	3.55	6.40
C(Time)	3	70.59	91.14	65.94	23.36	302.77
AB interact.	1	1.65	1.86	0.35	0.36	0.36
AC interact.	3	5.65	1.29	3.82	0.73	0.54
BC interact.	3	0.47	0.07	0.35	1.18	1.18
ABC interact.	3	0.53	0.21	0.82	0.09	0.07

TABLE 3: f-test values for refrigerated storage sample from 3-way ANOVA test.

Factors	D.F.	Aerobes	Anaerobes	Mesophiles	Psychrophiles	C.sporogenes
A(film)	1	0.04	0.66	3.33	0.17	0.08
B(Temp.)	1	6.15	1.41	0.56	0.17	2.69
C(Time)	3	48.73	27.03	45.67	22.42	9.31
AB interact.	1	0.02	0.00	1.00	0.08	0.00
AC interact.	3	0.10	0.35	0.56	1.17	1.46
BC interact.	3	1.00	0.56	0.22	0.58	0.62
ABC interact.	3	0.01	0.52	0.11	0.50	0.15

<u>f-values:</u>

P<0.05	f(1,48)=	4.04	f(3,48)=	2.80
P<0.01	f(1,48)=	7.19	f(3,48)=	4.32
P<0.001	f(1,48)=	12.60	f(3,48)=	6.60

TABLE 4
Total count of aerobes in beef patties in two type of packages, subjected to two cooking conditions.
(average from four replicates)

	Log CFU per gram of meat sample			
Temp.	71 C		82 C	
	Mean	S.D.	Mean	S.D.
Commercial pouch				
Temp. Abused (hrs)				
0	2.33	0.24	2.08*	0.15*
12	2.68*	0.46*	2.33*	0.29*
24	3.03	0.17	2.73	0.26
48	4.36	0.52	4.73	0.78
Refrigerated storage (days)				
0	2.33	0.24	2.08*	0.15*
10	3.00	0.44	2.75	0.53
20	3.03	0.45	3.08	0.22
40	3.60	0.08	3.35	0.38
Super barrier pouch				
Temp. abused (hrs)				
0	2.33	0.24	2.08*	0.15*
12	2.2*	0.24*	2.40	0.34
24	2.38*	0.43*	2.58*	0.40*
48	3.50	0.53	3.60	0.68
Refrigerated storage (days)				
0	2.33	0.24	2.08*	0.15*
10	3.00	0.24	2.70	0.34
20	3.08	0.32	3.10	0.18
40	3.53	0.21	3.28	0.19

* value less than 2.0 was substituted by 2.0

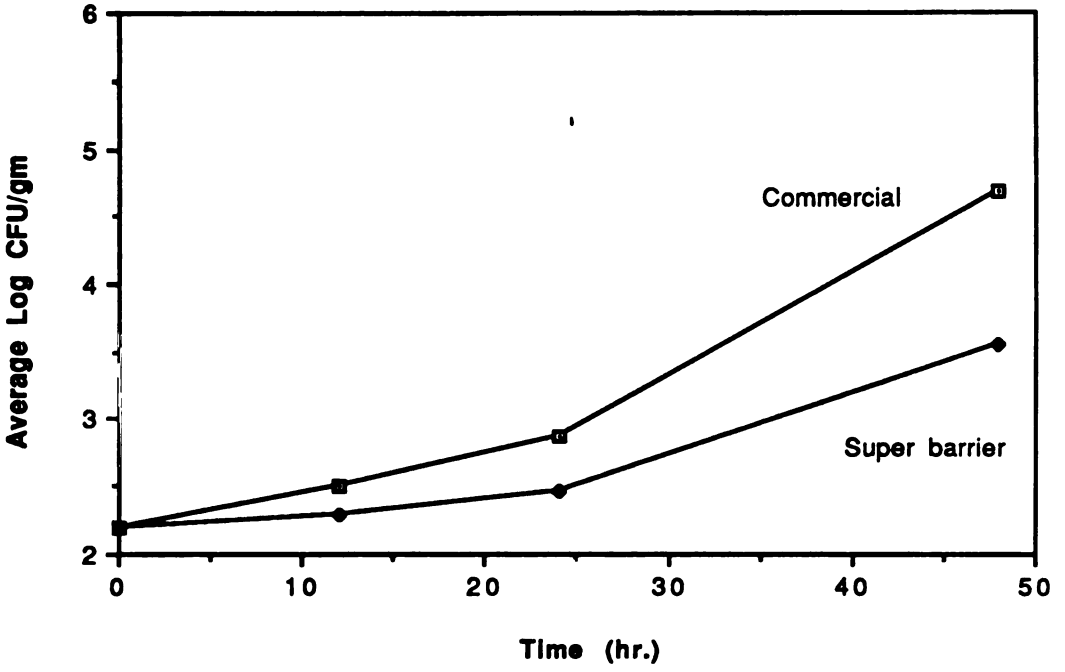

Figure 4: Aerobic count of two films in the temperature abused condition.

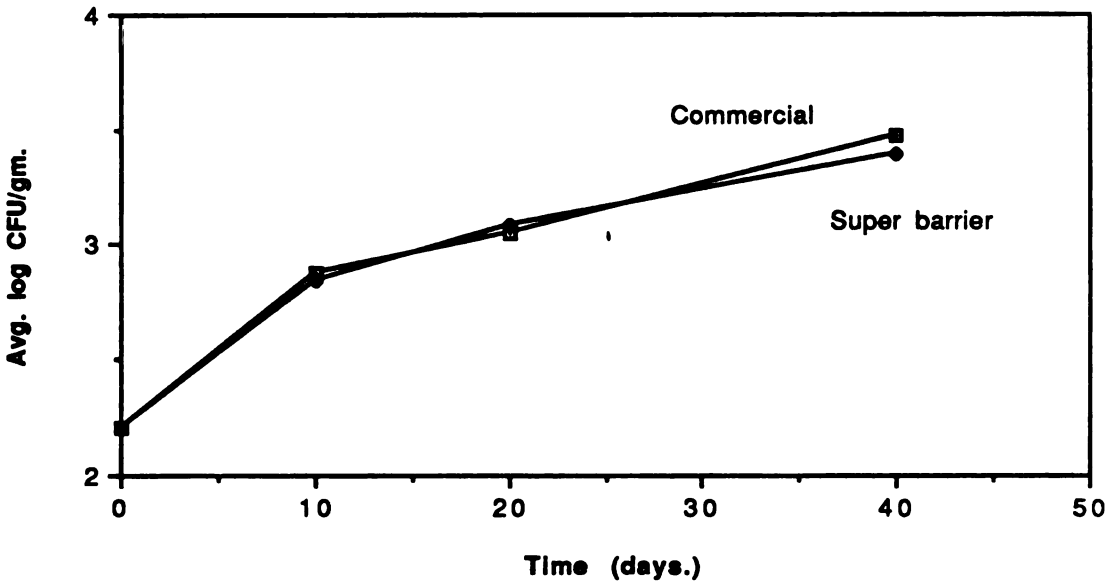

Figure 5: Aerobic count of two films in the refrigerated condition.

abused temperature was close to the optimum growth temperature of many aerobes. Ingram (1972) reported that growth of aerobic organisms is usually higher on the meat surface, which is directly in contact with the package surface when it is vacuumed. The amount of oxygen permeating through the commercial material would be higher than the amount of oxygen permeating through the super barrier material during the same period, because of the difference in barrier properties. The amount of oxygen inside the commercial pouch would support aerobic growth on the meat surface. Some of the growth microorganisms found in the super barrier pouch may have been microaerophiles or facultative anaerobes (eg. *Lactobacillus sp.*), which require only small amounts of oxygen to grow.

Statistical analysis of the samples exposed to refrigerated storage are shown in table 3. Storage time had a strong influence ($P<0.001$) on growth of aerobes. No differences were found dependent on package type. Cooking temperature also had a significant effect ($P<0.05$), which may indicate that there were different number of aerobes remaining after patties were cooked at 71 and 82°C. After the 71°C cook, the number of surviving aerobes was higher than after the 82°C cook. Figure 6 showed the relationship between $\log_{10}$CFU numbers of samples and storage time in two cooking temperatures. The growth of aerobes in refrigerated storage was small probably because the low temperature can retarded growth.

b) Anaerobes:

From the average number of anaerobic growth in table 5, and the statistical results of anaerobes in table 2 and 3, it was found that storage time was the only factor that significantly influenced growth of anaerobes ($P<0.001$). The average numbers in table 5 were plotted versus storage times for temperature abused and refrigerated storage condition, and are shown in figure

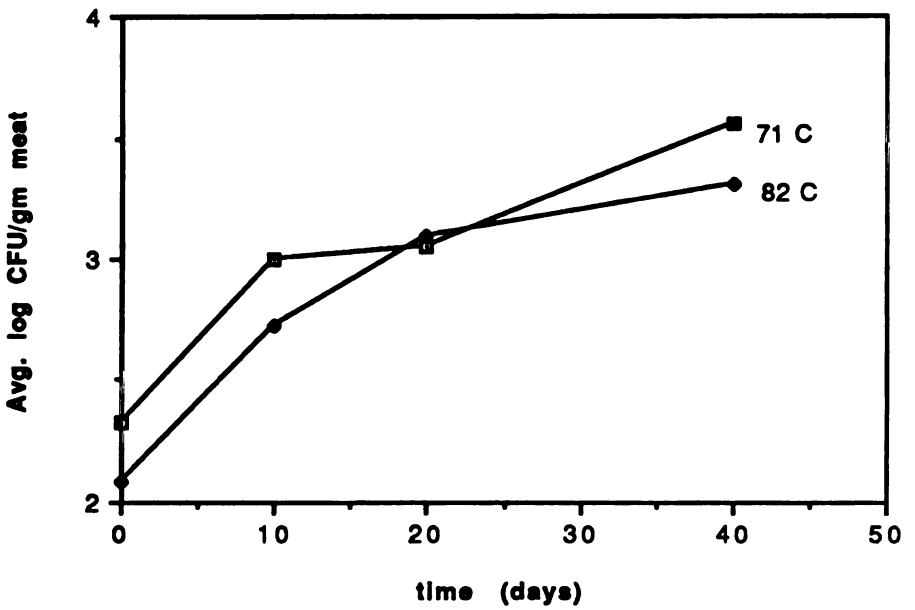

Fig. 6: Effect of cooking temp. on growth of aerobes in the refrigerated storage

TABLE 5
Total count of anaerobes in beef patties in two type of packages, subjected to two cooking conditions.
(average from four replicates)

	Log CFU per gram of meat sample			
Temp.	71 C		82 C	
	Mean	S.D.	Mean	S.D.
Commercial pouch				
Temp. Abused (hrs)				
0	2.28	0.21	2.00*	0.00*
12	2.45	0.31	2.23*	0.29*
24	2.80	0.16	2.40*	0.27*
48	4.55	0.60	4.15	0.24
Refrigerated storage (days)				
0	2.28	0.21	2.00*	0.00*
10	2.90	0.45	2.53	0.67
20	3.18	0.57	3.08	0.45
40	3.30	0.22	3.58	0.33
Super barrier pouch				
Temp. abused (hrs)				
0	2.28	0.21	2.00*	0.00*
12	2.23*	0.45*	2.25*	0.50*
24	2.58*	0.51*	2.58	0.42
48	3.90	0.35	3.88	0.46
Refrigerated storage (days)				
0	2.28	0.21	2.00*	0.00*
10	2.70	0.56	2.53*	0.56*
20	2.93	0.48	2.90	0.32
40	3.43	0.17	3.28	0.25

* value less than 2.0 was substituted by 2.0

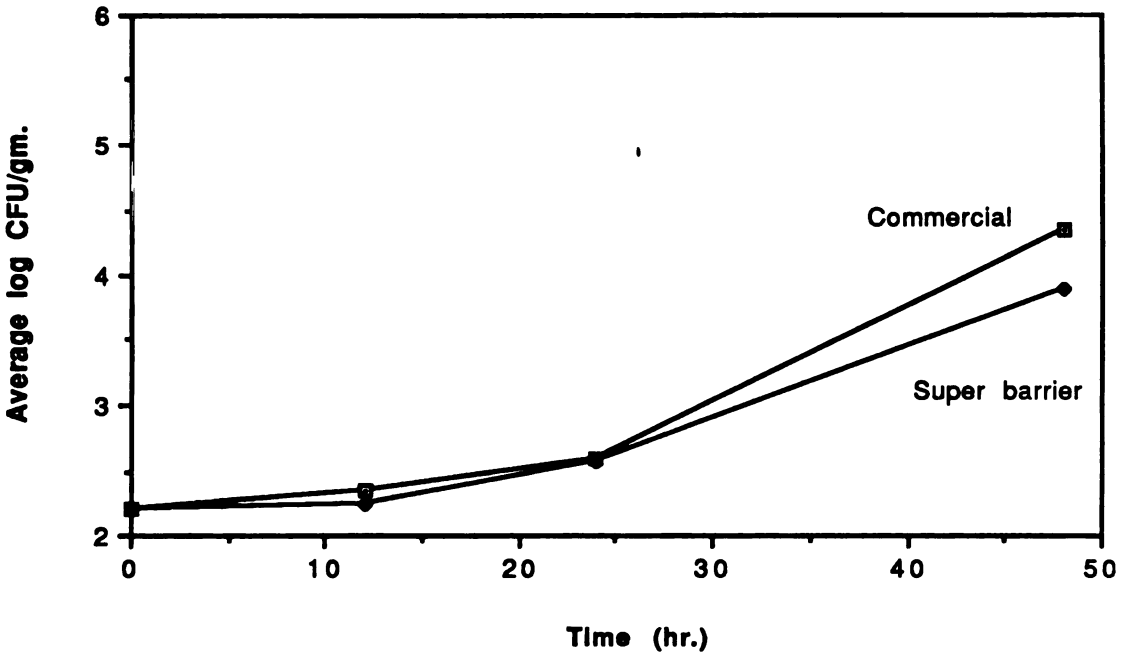

Figure 7: Anaerobic count of two films in the temperature abused condition.

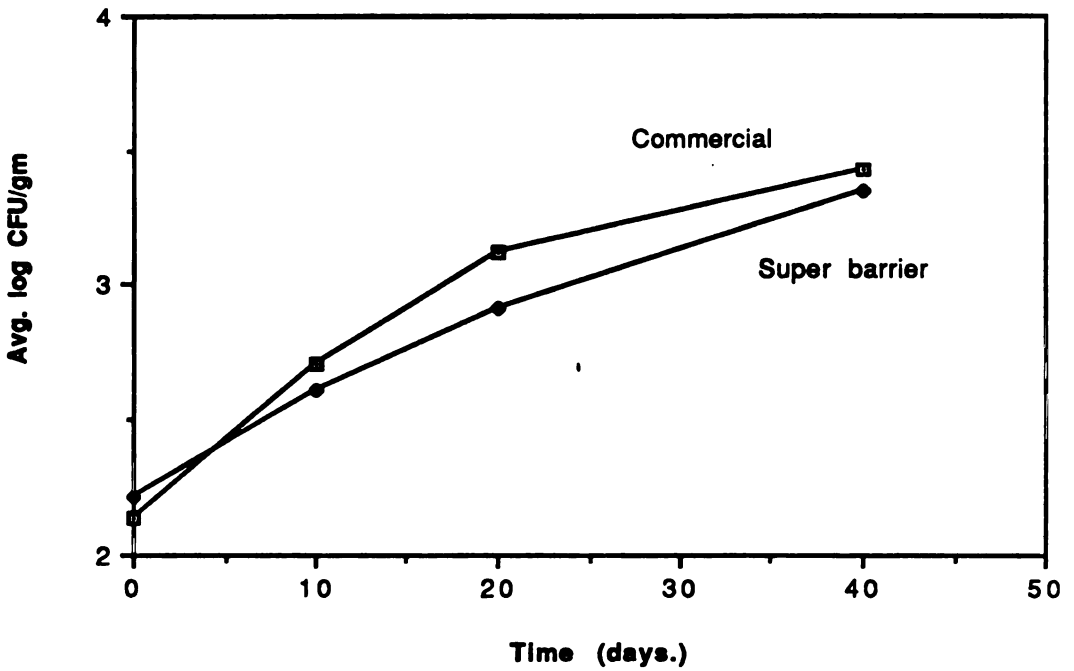

Figure 8: Anaerobic count of two films in the refrigerated storage.

7 and 8 respectively. Growth of anaerobes in both storage conditions was not significantly different for film type or cooking temperature in either storage condition. The number of anaerobes enumerated may include some facultative anaerobes since a small amount of oxygen probably remains inside the meat. Their growth occurs at a short distance beneath the surface.

c) Mesophiles:

Growth of some mesophilic sporeformers, such as *Bacillus sp.* and *Clostridium sp.*, have been reported as indicative of the sanitation quality of low acid food products, packed in hermetically sealed containers (Lake and Lynt 1984). Loss of quality in these products due to mesophilic growth caused by inadequate thermal processing condition and post-processing contamination. Therefore, the mesophilic count was evaluated to check the possibilities of both incidences during this study.

In figure 9 and 10, the relationship between average $\log_{10}$CFU numbers of mesophilic growth (table 6) and storage time were shown for both storage conditions. Statistical data in table 2 and 3 indicated that storage time was the most significant factor influencing mesophilic growth ($P<0.001$) in both storage conditions. Type of packaging material (barrier properties) was another factor that had significant influence ($P<0.01$) for temperature abused condition (table 2). Barrier properties of the material affect growth of mesophilic aerobes on the meat surface, because the oxygen permeating through the film allows growth to occur. In refrigerated storage condition, there was slightly difference of mesophilic growth for samples packaged in both films, which was not statistically different (table 3).

The potential of either inadequate thermal processing, or post-processing contamination caused by mesophilic microorganisms can be observed from the rapidly increase of microbial at a short period of storage time,

TABLE 6

Total count of mesophiles in beef patties in two type of packages, subjected to two cooking conditions.
(average from four replicates)

	Log CFU per gram of meat sample			
Temp.	71 C		82 C	
	Mean	S.D.	Mean	S.D.
Commercial pouch				
Temp. Abused (hrs)				
0	2.15*	0.17*	2.08*	0.15*
12	2.48*	0.34*	2.35*	0.26*
24	2.93	0.30	2.43*	0.35*
48	4.53	0.57	4.40	0.59
Refrigerated storage (days)				
0	2.15*	0.17*	2.08*	0.15*
10	2.88	0.30	2.85	0.31
20	3.10	0.49	3.03	0.36
40	3.43	0.25	3.53	0.21
Super barrier pouch				
Temp. abused (hrs)				
0	2.15*	0.17*	2.15*	0.17*
12	2.43	0.38	2.08*	0.15*
24	2.43	0.49	'2.55	0.38
48	3.53	0.63	2.35	0.75
Refrigerated storage (days)				
0	2.15*	0.17*	2.15*	0.17*
10	2.73	0.29	2.53*	0.51*
20	3.08	0.22	2.95	0.10
40	3.35	0.33	3.15	0.34

* value less than 2.0 was substituted by 2.0

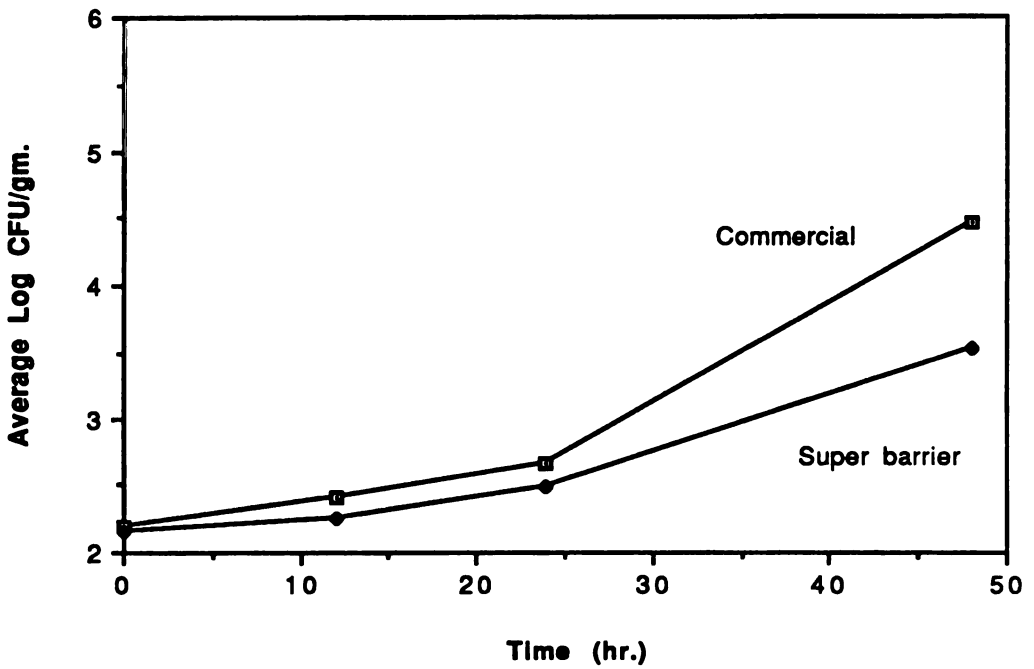

Figure 9: Mesophilic count of two films in the temperature abused storage.

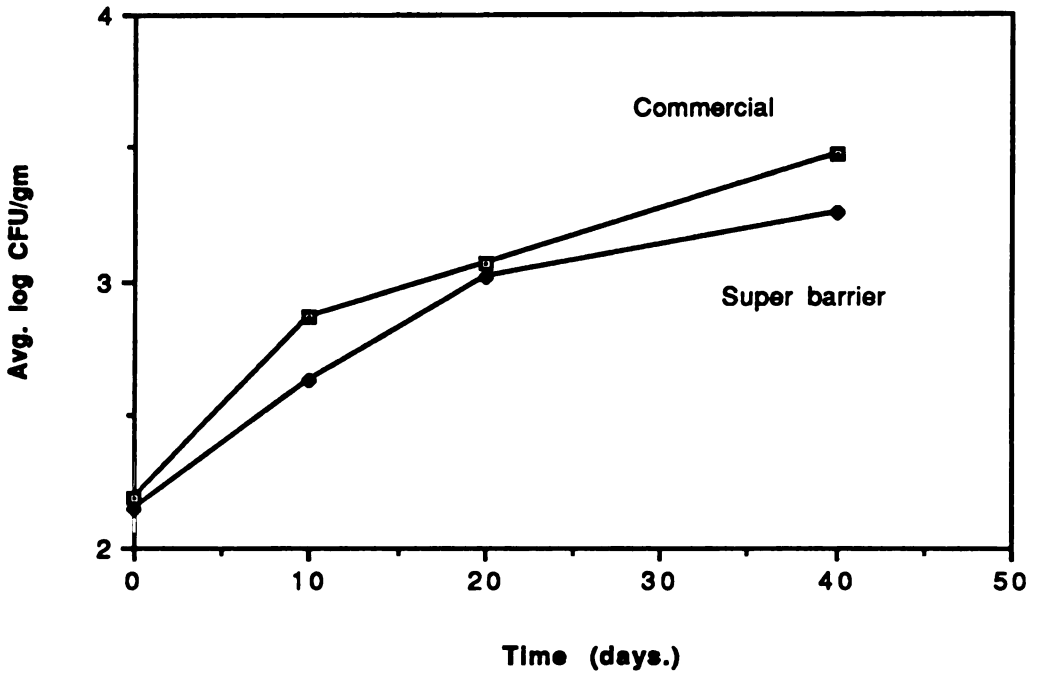

Figure 10: Mesophilic count of two films in the refrigrated storage.

especially in temperature abused condition. From figure 9 and 10, growth patterns of mesophiles for both type of packages did not indicate any rapidly increased of microorganisms. Therefore, it can imply that neither inadequate thermal processing, nor post-processing contamination was occurred in this study.

d) Psychrophiles:

The presence of psychrophile in cooked, uncured meat products can result in potential spoilage over prolonged storage for commercial refrigerated meat products. Growth of psychrophile also implies inadequate thermal treatment or post-processing contamination problems, since most psychrophile can be destroyed by mild heat treatments. (Gilliland et.al. 1984).

In table 7, the average $\log_{10}$CFU number of psychrophile at immediately after processing (t=0) was nondetectable ($\log_{10}$CFU <2.0). Plotting the average $\log_{10}$CFU of psychrophile versus storage time for both films (figure 11 and 12) showed that samples packed in commercial packages had slightly higher psychrophilic growth than samples packaged in the super barrier material after 24 hours in temperature abused (figure 11). Psychrophilic growth was slowly increased in both type of packages after approximately 25 days in refrigerated storage. At the end of both storage conditions, growth of psychrophile had increased approximately 1 log. From the statistical analysis (table 2 and 3), it can be seen that storage time was the main factor influencing microbial growth ($P<0.001$).

The possibility of post-processing contamination or inadequate thermal treatment caused by psychrophilic microorganisms can occurred in refrigerated condition, which is closed to their optimum growth conditions. The rapidly increase in psychrophilic numbers during storage period was observed if either incidence occurred. In this study, the increase in psychrophile during

TABLE 7
Total count of psychrophiles in beef patties in two type of packages, subjected to two cooking conditions.
(average from four replicates)

	Log CFU per gram of meat sample			
Temp.	71 C		82 C	
	Mean	S.D.	Mean	S.D.
Commercial pouch				
Temp. Abused (hrs)				
0	<2.00*	0.00*	<2.00*	0.00*
12	2.13*	0.25*	<2.00*	0.00*
24	2.38*	0.57*	2.20*	0.40*
48	3.28	0.33	2.75*	0.61*
Refrigerated storage (days)				
0	<2.00*	0.00*	<2.00*	0.00*
10	2.63	0.58	2.55	0.45
20	2.60	0.26	2.60	0.35
40	3.22	0.45	3.08	0.49
Super barrier pouch				
Temp. abused (hrs)				
0	<2.00*	0.00*	<2.00*	0.00*
12	2.00*	0.00*	2.00*	0.00*
24	2.13*	0.25*	2.00*	0.00*
48	2.85*	0.68*	2.55	0.48
Refrigerated storage (days)				
0	<2.00*	0.00*	<2.00*	0.00*
10	2.48*	0.59*	2.35*	0.43*
20	2.78	0.22	2.40*	0.49*
40	2.98	0.39	3.03	0.40

* value less than 2.0 was substituted by 2.0

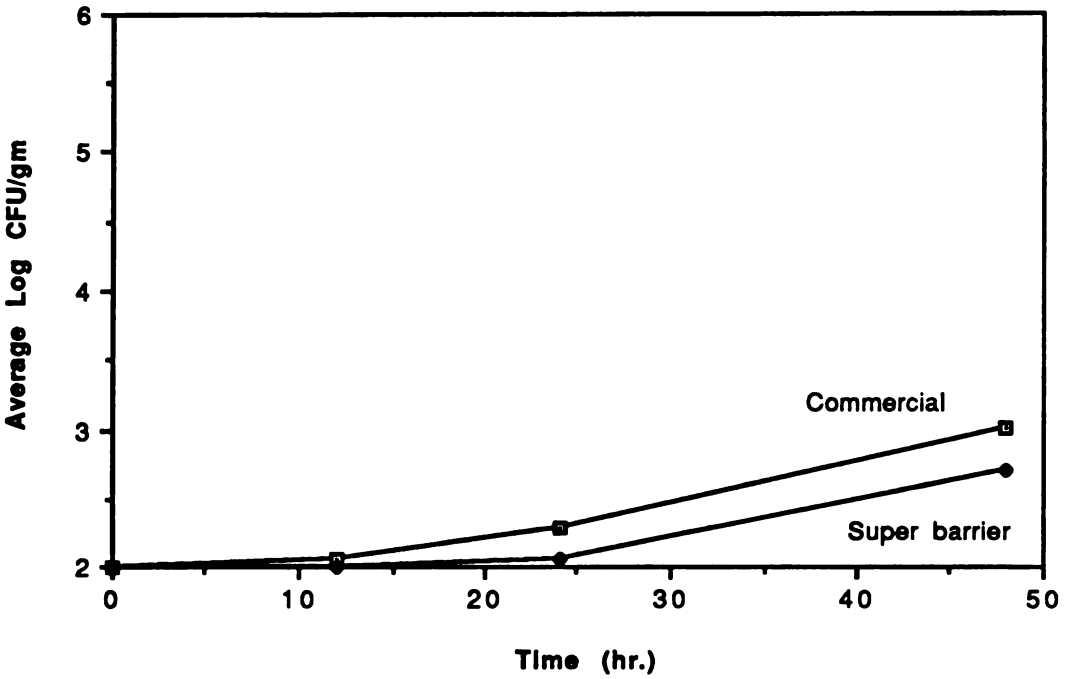

Figure 11: Psychrophilic count of two films in the temperature abused storage

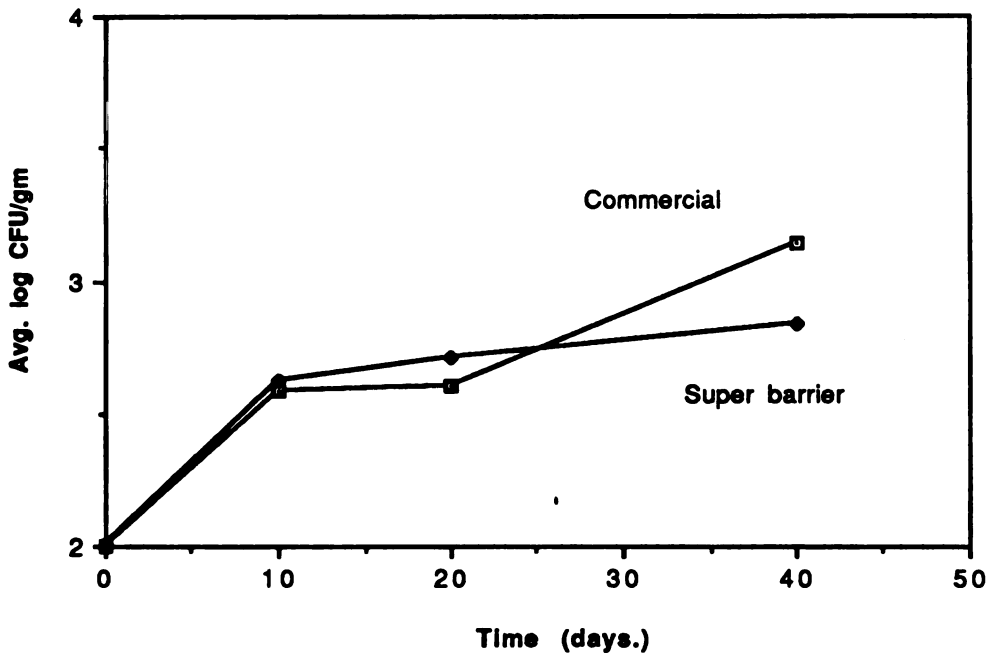

Figure 12: Psychrophilic count of two films in the refrigerated storage.

storage in both conditions was small in refrigerated storage (figure 12), and their growth patterns did not reveal any rapid increase. Therefore, neither inadequate thermal processing, nor post-processing contamination of psychrophile occurred.

Shelf life evaluation of cook-in-bag noninoculated ground beef patties.

A reasonable indicator of the basic quality of meat products can be implied using the total microbial count (Sutherland 1982). The total plate count on PCA at 35°C for fresh, prepared, cooked, uncured meat has been reported by the USDA to be 10^4 colonies or less (log CFU $\leq$ 4.0) per gram sample (Johnston and Tompkin, 1984). The unavoidable contamination during processing, packaging and handling may increase the number of microorganism on the product surface by levels of 10^1 to 10^2 per gram sample (Johnston and Tompkin, 1984).

In recommendation pertaining to meat products by USDA (Tompkin, 1986), and meat regulations defined by many states, the numbers of aerobic plate count (APC) allowable in cooked, uncured meat products is used as a controlling factor for product quality. Wehr (1978) reported that APC numbers ranged from less than 5.0×10^4 to 1.0×10^6 colonies per gram meat ($\log_{10}$CFU=4.7-6.0) in most regulations established by individual U.S. States (table 8).

The purpose of temperature abusing samples was to create a situation where meat was mishandled by holding at temperatures higher than in normal refrigerated conditions. Such temperatures have been reported to result in the majority of food poisoning outbreaks, and are common to food handling (Buchanan, 1986). By examining the aerobic total count (table 4), the microbial shelf life of this vacuum packaged, cook-in-bag meat product subjected to

TABLE 8: Microbial guidelines and standards for cooked meat products established by some states (1977)

State	Product(s)	APC per gram meat	$\log_{10}$APC
Idaho	cooked meat	$<1x10^6$	6.0
Massachusetts	cooked meat	$<5x10^4$	4.7
Nabraska	delicatessen	$<1x10^5$	5.0
	smoked or heat treated	$<1x10^6$	6.0
North Dakota	cooked or smoked meat	$<1x10^6$	6.0
Oregon	cooked meat	$<1x10^6$	6.0
Tennessee	processed meat	$<5x10^6$	6.7
East Virginia	delicatessen	$<1x10^4$	4.0

Source: Wehr (1978) Food Technology Vol. 31 (1): 64

temperature abuse (23±2°C) can be considered. The growth of aerobes in cooked uncured meat, packaged in the commercial pouch increased approximately 2.5-3 log (figure 4) to log_{10}CFU 5.0 after 48 hours abuse, while samples packaged in the high barrier material increased about 1.5 log. Average number of aerobes in commercial package was exceed the allowable APC number indicated in table 8. Therefore, if these types of products are left at abusive temperatures longer than 24 hours, their microbial shelf life may be not acceptable.

Refrigerated storage was used to create retail and conventional storage conditions. The temperature used in this study (4-6°C) retarded the growth of the surviving microorganisms. The average APC number was slightly increased from log_{10}CFU 2.0 to log_{10}CFU 3.5 after 40 days of storage. Refrigerated storage also eliminated the difference in barrier properties of the packaging materials In refrigerated storage (fig. 5). Using the regulations stated in table 8 as the basis, the microbial shelf life of products in refrigerated storage was still acceptable, after 40 days at 4-6°C storage.

Growth of microbial in cook-in-bag uncured meat products was caused by some groups of microorganisms, mainly thermophiles and sporeformer groups, which can survive after products are thermally treated. They adjust to the new environment during the first period of storage, and will use nutrients from meat to grow and produce biochemical substances such as acids or toxin. The process can occur quickly at room temperature, which is close to the optimum growth temperature for these microorganisms. Some of the substances produced can cause food poisoning and loss of meat quality resulting from odor, discoloration, flavor change etc.

Microbial evaluation of C. *sporogenes* inoculated ground beef patties.

The actual concentration of *C. sporogenes* inoculated into each batch was counted from vacuum, uncooked, inoculated, fresh ground beef patties to be in range of $5x10^4$-$1x10^5$ spores (log_{10}CFU 4.7-5.0) per patty. This concentration was chosen to insure that the model organism was the dominant species in the meat, and to avoid the competitive growth of other microorganisms present in the meat. The confirmatory test for *C. sporogenes* (detection of gas in liquid media) was done on all plates which containing suspected *C. sporogenes* colonies (visual observation).

The average log_{10}CFU number of C. *sporogenes* after thermal processing is shown in table 9. Number of *C. sporogenes* was reduced by both thermal treatments conditions by 2.5-3.0 log immediately after samples were thermal processed (t=0). Figure 13 and 14 to show the relationship between average log_{10}CFU number of *C. sporogenes* and storage time in both storage conditions. In the temperature abused condition, number of *C. sporogenes* increased rapidly after 24 hours (figure 13). In refrigerated storage (figure 14), growth of *C. sporogenes* was increased less than 1 log. Therefore, refrigerated storage effectively retarded growth of surviving *C. sporogenes* after cooking.

Growth of *C. sporogenes* (table 6) was statistically analyzed using the same procedures as described for the noninoculated study. Storage time was the most significant factor (table 2 and 3) effecting growth of *C. sporogenes* ($P<0.001$). Cooking temperature was another factor that showed significant difference for the temperature abused samples ($P<0.05$), but not for the refrigerated storage samples. The numbers of *C. sporogenes* after cooking versus storage time at the two temperatures in the abuse condition is shown in figure 15. At the time immediately after cooking (t=0), the average number of *C. sporogenes* in sample cooking at 71°C was reduced from log_{10}CFU 5.0 to 2.4,

TABLE 9

Total count of C. sporogenes in beef patties in two type of packages, subjected to two cooking conditions.
(average from four replicates)

	Log CFU per gram of meat sample			
Temp.	71 C		82 C	
	Mean	S.D.	Mean	S.D.
Commercial pouch				
Temp. Abused (hrs)				
0	2.40	0.11	2.10	0.14
12	2.43	0.29	2.08	0.15
24	3.28	0.43	2.75	0.52
48	5.60	0.25	5.60	0.41
Refrigerated storage (days)				
0	2.40	0.11	2.03*	0.05*
10	2.68	0.24	2.78	0.22
20	2.73	0.57	2.65	0.47
40	2.90	0.08	2.70	0.08
Super barrier pouch				
Temp. abused (hrs)				
0	2.40	0.11	2.10	0.14
12	2.33*	0.25*	2.2*	0.24*
24	3.03	0.55	2.65	0.62
48	5.73	0.32	5.78**	0.66**
Refrigerated storage (days)				
0	2.40	0.11	2.10	0.14
10	2.43	0.34	2.35	0.44
20	2.83	0.57	2.68	0.59
40	3.00	0.32	2.90	0.54

* value less than 2.0 was substituted by 2.0

** value higher than 6.48 was substituted by 6.48

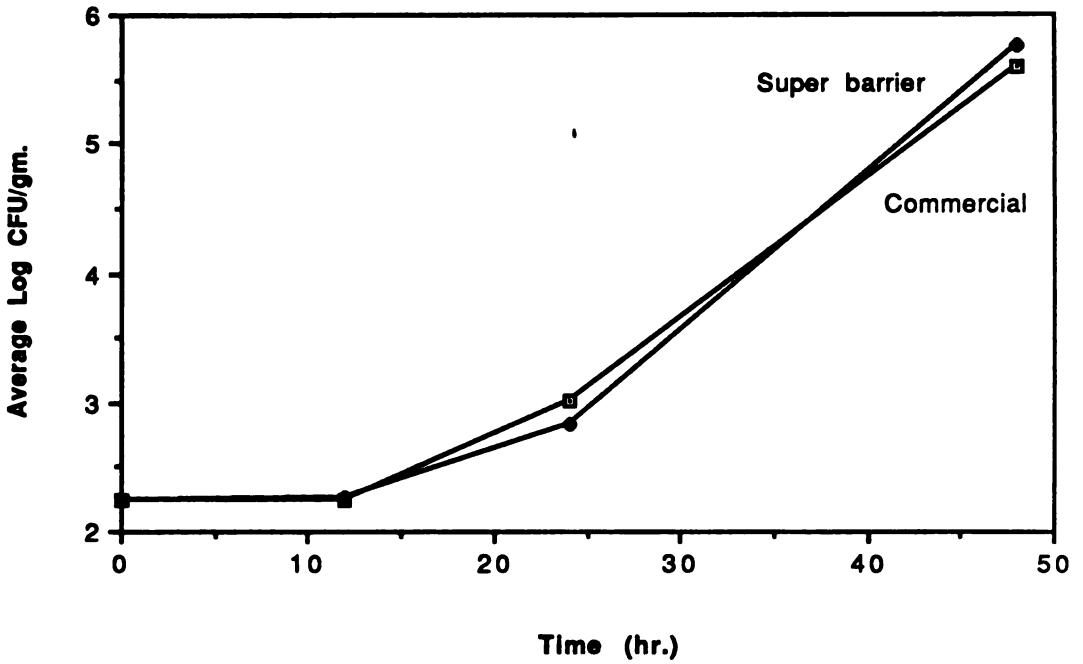

Figure 13: C. sporogenes count of two films in the temperature abused storage.

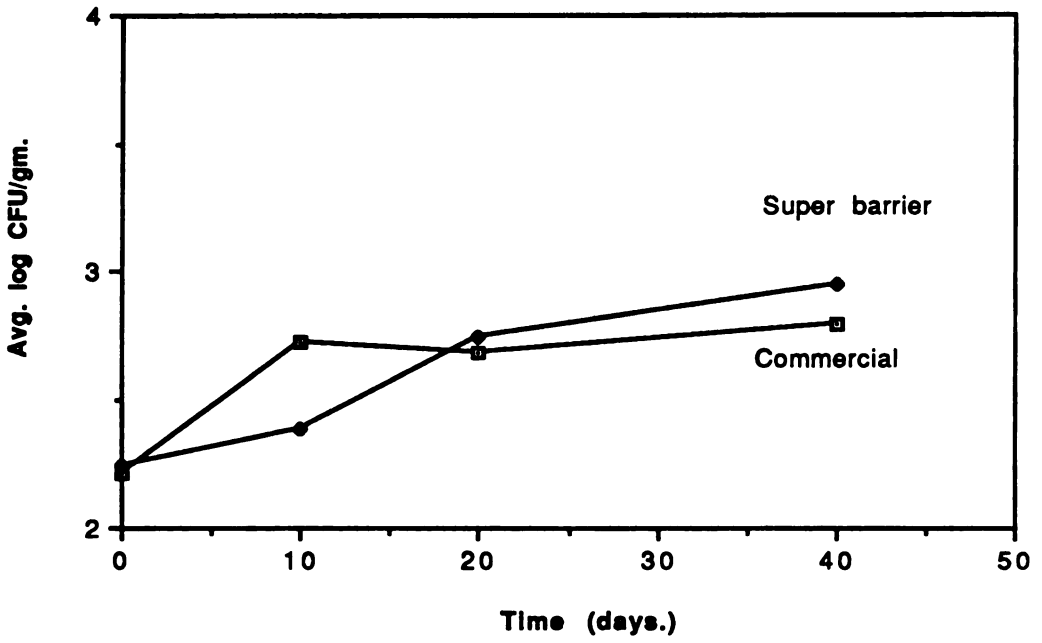

Figure 14: C. sporogenes count of two films in the refrigerated storage.

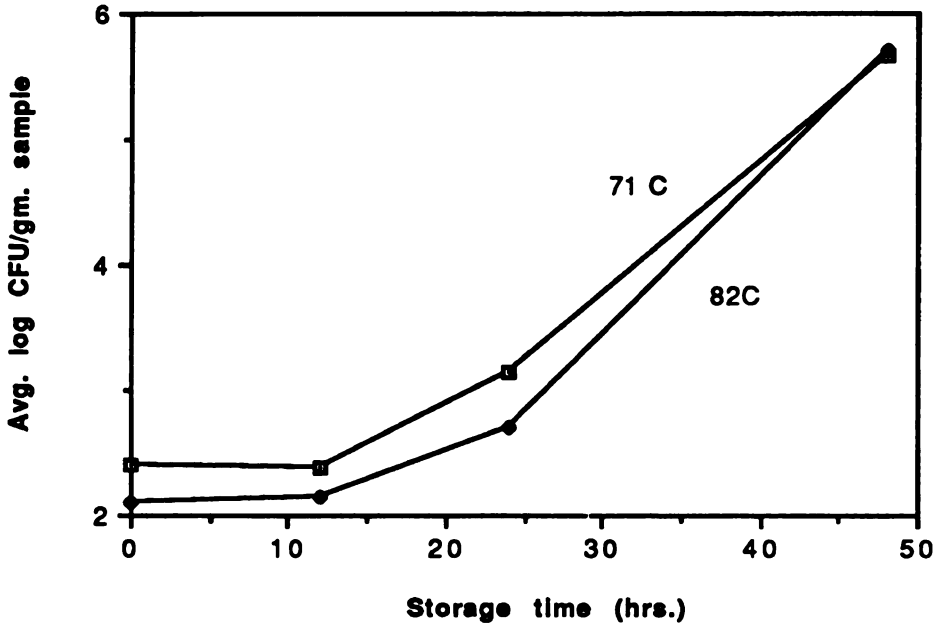

Fig. 15 Effect of cooking temperatures on sporogenes growth in the temperature abused condition

while cooking at 82°C reduced it to 2.1. At 48 hours of abusive temperature storage, the number of *C. sporogenes* was not different in either cooking condition. This may be do to the fact that the spores subjected to 82°C cooking could grow with less competition from other microorganisms.

Shelf life of the product based on growth of *C. sporogenes* in a retail, refrigerated condition (4-6°C) would probably be acceptable for 40 days storage, since most of the inoculated spores (approximately 2.5-3 log) were destroyed after cooking and the growth of the surviving spores was very limited at low temperature. Although the microbial shelf life of this products based on model organism would be acceptable, growth and toxin production of *C. botulinum* could be a problem at this refrigerated temperature, because some serotypes (B, E, F) have previously been reported to grow and produce toxin at temperatures as low as 3.3°C. Other factors also effect shelf life, such as change in physical properties, appearance, and/or flavor.

Changes in pH of meat

The pH of noninoculated and inoculated meat samples were measured during storage in both conditions. The data shown in table 10 were averaged from all four replicates tested. The pH of noninoculated and *C. sporogenes* inoculated samples showed small difference in both storage conditions within range of 5.95-6.22 and 5.92-6.21, respectively.

Spore profile test *C. sporogenes.*

Different spore levels of the model organism (*C. sporogenes* ATCC 7955) were used as inoculum to study the number of mesophilic anaerobic sporeformers (such as *C. botulinum*) initially present in meat, which could be destroyed using both thermal treatments conditions to the

Table 10: pH of meat sample during 2 storage conditions subjected to 2 cooking temperatures. (measured in 0.1% sterile peptone water)

Temp. abused			pH	
Time (hrs.)	Material	Cooking temp.(C)	Noninoculated	Inoculated
0	Commercial	71	6.22	6.17
		82	6.21	6.19
12	Commercial	71	6.15	6.16
		82	6.18	6.18
	Super barrier	71	6.20	6.15
		82	6.18	6.18
24	Commercial	71	5.95	5.99
		82	5.98	6.02
	Super barrier	71	5.98	6.03
		82	6.00	6.06
48	Commercial	71	5.96	5.89
		82	5.98	5.96
	Super barrier	71	5.99	5.97
		82	6.00	5.92
Refrigerated storage Time (days)				
0	Commercial	71	6.22	6.17
		82	6.21	6.19
10	Commercial	71	6.14	6.15
		82	6.17	6.19
	Super barrier	71	6.15	6.16
		82	6.18	6.16
20	Commercial	71	6.11	6.09
		82	6.13	6.13
	Super barrier	71	6.13	6.14
		82	6.13	6.14
40	Commercial	71	6.19	6.18
		82	6.20	6.21
	Super barrier	71	6.20	6.19
		82	6.21	6.20

nondetectable level (in this study, log_{10}CFU <1.0 was the minimum sensitivity level). Growth of the remaining spores of *C. sporogenes* in refrigerated conditions (4-6°C) was evaluated for 60 days period. No growth of microorganism could be detected in samples which had less than 100 spores per patty initially during 60 days of storage (table 11). Growth of C. *sporogenes* was detected in samples which had an initial number of 1000 spores per patty at 60 days of refrigerated storage for both 71 and 82°C cooking. The destructive effect of different cooking temperatures on *C. sporogenes* spore reduction was evident for samples having an initial number of 10,000 spores per patty and above. The number of spores remaining in patties cooked at 82°C was initially lower than in patties cooked at 71°C.

For samples immediately evaluated after thermal processing (t=0), it was observed that both cooking conditions reduced the number of spores by 3 log. Growth of the surviving spores in samples cooked at 71°C increased approximately 1 log cycle during 60 days of refrigerated storage at 4-6°C. Growth of *C. sporogenes* was increased in samples cooked at 82°C to be closed to samples cooked at 71°C after 40 days of storage, probably due to the lack of competition from other microorganisms.

The possibility of contamination by anaerobic sporeformers from C. *botulinum* in a conventional processing plant (for cook-in-bag meat products) is probably low due to; 1) the use of strict regulations and inspection of meat in the HACCP program for vacuum packaged, cook-in-package uncured meat products; 2) the lack of anaerobic conditions in most equipment in food processing plant cause the nonconductive conditions for growth and sporulation of anaerobes (Lake and Lynt, 1984); and 3) the occurrence of C. botulinum spores in meat and meat products has been reported to be very low, approximately 1-2 spores per 10 kg. of meat (Simunovic et al., 1985). When

TABLE 11: Destruction of C. sporogenes and growth in refrigerated storage in the commercial barrier material.

Log10No. of spore per patty	Log CFU per gram of meat sample					
	5	4	3	2	1	0
Storage time (Days)						
71C Cooking:						
0	2.17	1.30	n/d	n/d	n/d	n/d
10	1.95	1.40	1.00	n/d	n/d	n/d
20	2.38	1.68	n/d	n/d	n/d	n/d
40	2.85	1.74	n/d	n/d	n/d	n/d
60	2.95	2.27	1.44	n/d	n/d	n/d
82C Cooking:						
0	1.90	n/d	n/d	n/d	n/d	n/d
10	1.98	n/d	n/d	n/d	n/d	n/d
20	2.08	1.48	n/d	n/d	n/d	n/d
40	2.52	1.67	n/d	n/d	n/d	n/d
60	3.03	2.01	1.48	n/d	n/d	n/d

n/d = nondetectable growth (less than log10=1.00 per gm.)

compare the spore profile study of model mesophilic sporeformer (*C. sporogenes*) to *C. botulinum* , thermal processing to internal temperatures of 71°C or 82°C for 30 minutes would be enough to reduce spores of *C. botulinum* to an undetectable level if the raw meat has an initial number of spores less than 100 per gram. Outbreaks caused by botulism in cook-in-bag meat products would be at low risk, unless samples are abused or mishandled.

Mechanical and physical properties of packaging materials.

The mechanical and physical properties of the commercial and super barrier packaging materials were tested after 3 treatments: rollstock, heat treated at 71°C and heat treated at 82°C for 30 minutes to observe changes after exposure to thermal treatments. The data are shown in table 12, and are discussed as follows:

a) Material thickness: Thickness of the commercial package material increased proportionally with increasing cooking temperature, which may also due to shrinkage following cooking. There were no changes in thickness of the super barrier package material. Change in thickness effects many of the mechanical and physical properties of packaging materials.

b) Shrinkage of pouch surface area: The percent shrinkage of the commercial package increased proportionally with increasing cooking temperature, while the super barrier package showed no change in dimensions at both cooking temperatures. Shrinkage of cook-in-bag material must be controllable because it influences final package appearance and cost of material. In more severe cooking conditions such as retorting, the commercial cook-in-bag package might not suitable.

c) Package and seal leakage test: All tested pouches from both packaging materials withstood pressure up to 30 psi for 60 seconds with no leakage

TABLE 12
Mechanical and physical properties of packaging materials.

	Commercial package			Superbarrier package		
Test performed	Uncooked	71 C cooked	82 C cooked	Uncooked	71 C cooked	82 C cooked
Thickness (mil.)	2.5	3.7	4	3.5	3.5	3.5
%Surface area shrinkage (after cooked)	0	30.5	46.5	0	0	0
Package/seal integrity(@30mmHg)	N/D	N/D	N/D	N/D	N/D	N/D
Oxygen permeability (cc/m2 24hr atm @ 23 C 100%RH)	82.07	68.23	87.68	0.4659	N/D *	0.5575
Material:						
Tensile (kg/cm2) MD	555.8	415.5	488.6	655.2	631.5	691.1
CD	491.6	355.4	368.2	659.9	654.5	654.4
%Strain MD	171.05	177.3	286.6	76.33	67.53	93.38
CD	103.9	172.55	241.15	136.05	117.38	113.13
Seal						
Tensile (kg/cm2 seal area) MD	2599.5	2010.2	1362.9	7084.5	7582.4	5921.3
	3786.6	1653.9	1227.6	3914.1	4463.9	4704
%Strain MD	58.88	32.7	39.23	11.45	8.05	8.55
CD	20.15	21.13	37.08	15.3	13.15	16.38

* Test was not conducted, O2 permeability was expected to be 0.5 cc/m2 atm 24 hr.

occurring. This test evaluated the ability of material and seal to withstand the severe conditions which pouches were subjected to, in vacuum packaging, at pressures of 20-25 psi. This test can be used to check pinholes in the seal and package surface caused when packages are subjected to severe cooking conditions or material and seal defects. Leakage of seal and package can cause loss of vacuum and post-processing contamination.

d) Oxygen barrier properties: Due to the amount of aerobic growth, it could be predicted that the amount of oxygen permeating through the commercial package material would be higher than that permeating through the super barrier package material. This can influence the number of aerobes in beef patties packaged in the commercial material. The oxygen transmission rate of the commercial packaging material in rollstock form was 82.1 cc/m^2 atm 24 hrs. at 23±2^oC 100%RH, as determined using the Oxtran-100 Oxygen Permeability tester. An oxygen transmission rate of 20 cc/m^2 24 hrs atm at 23^oC (73^oF), 0% RH was provided by the manufacture of this commercial packaging material (Anon, 1989). The barrier properties of the commercial package were considerably less at the high humidity condition. This may be because the barrier layer of the commercial package is ethylene vinyl alcohol, which losses some of its barrier properties at high humidities. For the thermally treated commercial package at both cooking temperatures, barrier measurements were difficult to obtain because of the change in film thickness and surface area due to shrinkage. For the super barrier package, there was almost no change in the oxygen barrier properties after thermally processing at both cooking temperatures. The oxygen transmission rate of this material was very low (0.4-0.5 cc/m^2 atm 24 hrs. at 23±2^oC 100%RH) , which is approximately 150-180 times lower than the commercial package. The excellent barrier properties of this material are due to the SiO_2 coated layer.

e) Tensile tests: Tensile tests were conducted to observe changes in the material and seal strength of the materials before and after heat treatment. For the commercial package, tensile values were reported (table 12) at the breakage point of the material or the separation point of seal strips. For the super barrier package material, it was observed that the film separated into two layers during the test. One layer (polypropylene sealant layer) continue to elongate until breakage, while the other layer (SiO_2 coated PET) stretched slowly without breakage. Tensile values were reported (table 12) at the point where the fracture completely occurred at the center of sealant layer. Commercial package material showed loss of tensile strength in both machine and cross direction after cooking. Changes in material and seal strength of the commercial package material was difficult to measure for the heat treated samples, due to material shrinkage and change of thickness. The super barrier package material had a higher tensile strength than the commercial package material. Changes of tensile strength of samples after heat treated were fluctuated, which may cause by some structural changes of the sealent layer. Low strain of this material was observed because of the inability of the barrier layer (SiO_2 coated PET) to expand.

CONCLUSIONS

The results of microbial evaluation on vacuum packaged cook-in-bag uncured beef patties for noninoculated and *C. sporogenes* inoculated samples were statistically analyzed. Also, the changes of packages and packaging materials after thermal processing was evaluated. It can be concluded that:

1) The effect of two different cooking temperatures (71 and 82°C) was demonstrated by the reduction of microorganisms in the meat after cooking. For aerobic growth in refrigerated storage, and for *C. sporogenes* growth in temperature abused condition, a significant difference ($P<0.05$) was found. The internal cooking temperatures at 82°C resulted in lower numbers of microorganism or spores remaining in the products after thermally processing than cooking at 71°C.

2) The effect of different cook-in-bag packaging materials was significant at the level of $P<0.001$ for aerobic growth in temperature abused condition. This factor was also significant for mesophilic aerobes. Growth of aerobes in the patties rapidly increased in samples packaged in the commercial pouch (PE/EVOH), due to a high amount of oxygen permeating through the package surface. Comparing the oxygen transmission rates (OTR) showed that the commercial package had OTR approximately 150-180 times higher than the super barrier package (SiO_2 coated PET), before and after exposure to the defined thermal processing conditions used in this study. In refrigerated storage, the barrier properties of material showed no significant effect on aerobic growth.

3) Storage time was the most significant factor effecting the number of microorganism for all types of microbial groups, at both temperature abused and refrigerated storage conditions ($P<0.001$). This could be used to predict microbial shelf life for the vacuum packaged, cook-in-bag meat products by comparing the number of microorganisms at the defined storage times to the allowable number of microorganisms defined in meat products regulations by USDA or many states. If the microbial load is close to or exceeds the defined number, this product would not be acceptable. The microbial load of meat products subjected to temperature abuse is of concern because growth of the remaining microorganisms (after cooking) increases rapidly within a short period of time (24 hr.). At refrigerated storage (4-6^{o}C), growth of microorganisms was low, so, products might be considered acceptable for 40 days of storage.

4) The spore profile test with the model organism, *C. sporogenes*, showed that different temperatures used for thermal processing had different capacity to destroy the same number of mesophilic anaerobic spores in the same cooking period. An initial concentration of 10^2 *C. sporogenes* spores per patty or less can be reduced to nondetectable levels for 60 days of refrigerated storage (4-6^{o}C) when the meat patties are thermally processed at 71 or 82^{o}C for 30 minutes.

5) The commercial package (PE/EVOH) showed changes in most physical properties after exposure to thermal processing. Loss of mechanical strength after cooking was observed. Oxygen permeability of this material was inconsistent after cooking because of the surface area changes (shrinkage) and increased thickness. There were small changes in both mechanical and barrier properties of the super barrier package after cooking. Therefore, change in material properties must be considered when selecting the appropriate

packaging materials to be used for cook-in-package meat products.

APPENDIX

APPENDIX

Standard Colony Count Methods

Each sample in the study contained 3 plates form 10^{-2} to 10^{-4} dilution. Plates from the same sample were individually counted and calculated as units of CFU per gram meat sample following these steps. Reported number of CFU per gram sample was averaged from the CFU value calculated from each plate of the same sample.

1) If there was no colony growth on all plates at the same dilution - the CFU number was reported as less than 100 CFU per gram.

2) If colony growth in each plate was less than 25 colonies in all dilutions - the actual number of colonies appearing on each plate was counted and reported as estimated CFU per gram sample.

3) If colony growth in each plate ranged between 25-300 colonies - the number of colonies on plate was counted and reported as CFU per gram sample.

4) If the growth in all plates was more than 300 colonies - then it was reported as more than 3.0×10^6 CFU per gram. An estimated number of colonies in plate was obtained from the average count of 4 (four) 1x1 cm squares selected randomly in a 10^{-4} dilution, then multiplying this number by the plate surface area.

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