EVALUATION OF PRESERVATIVE METHODS FOR LUMPFISH (*Cyclopterus lumpus*) CAVIAR

SANDRA WHITEWAY

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By

SANDRA WHITEWAY

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ABSTRACT

Lumpfish caviar was prepared using various preservation techniques including pasteurization, temperature control, and chemical additives. A shelf-life study was conducted on the various caviare samples during which microbial quality and proximate analyses was examined. The chemical composition was found to be similar to that of other commercial brands. The microbial quality indicated that the roe used for production of the finished caviare product may have been of poor quality. The lumpfish caviare was found to be free of most food borne pathogens. The predominant bacteria isolated was a gram positive cocci (~95%) which is probably a *Staphylococcus* species.

The average pH of the caviare was 5.9 and the chemical additives had limited effectiveness at this pH. Pasteurization was found to produce a poor aesthetical product which may be attributed to the raw product quality. Refrigeration was acceptable as a preservation technique for a limited period. Combination preservation techniques may offer the best method for extending the shelf-life of caviare while maintaining microbial quality and chemical stability.

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INTRODUCTION

1.1 Caviar

The term "caviar (caviare)" has traditionally been reserved for sturgeon eggs, the main source of which has been the Caspian Sea. The Caspian Sea is home to three sturgeon species (11), each producing distinctive caviar. The Beluga (*Husa husa*) has the largest eggs that are mostly black producing the finest grade and most expensive of the caviars. Osetrova eggs (*Acipenser rudiventris*) are grey or golden whereas Servrunga (*Acipenser stellatus*) yields the smallest grain caviar with a colour similar to that of Beluga Caviar.

1.2 Economics of Caviar

Supply shortages and associated high prices of sturgeon caviars has led to the development of caviar substitutes. The most important of these are Salmon, Whitefish and Lumpfish Caviars (11). Caviar substitutes must indicate on the label the source of the roes (17). Colouring is allowed in caviar substitutes only (4). Thus Lumpfish Caviar substitutes maybe dyed black to imitate Beluga Caviar, or red to imitate Salmon Caviar (11,41).

Caspian Caviar of either Russian or Iranian origin accounts for 98 percent of North American imports as of 1987. The remaining 2 percent was imported from China, Romania,

and/or Czechoslovakia. The average wholesale price for imported caviar in 1987 was $190.00 (US) per pound for pasteurized and vacuum-packed (Table 1.1). Domestic sturgeon caviar sold for half the price of imported at $95.00 (US) per pound. Lumpfish Caviar had a wholesale price of $15.00 (US) per pound, Whitefish Caviar was $16.00 (US) per pound and Salmon Caviar was $35.00 (US) per pound. The wide price differences reflect the different and distinct niches that these products occupy in the caviar market (11).

1.3 Newfoundland Roe Industry

Inception of the lumpfish roe industry for Newfoundland began in the late 1960's. It has continued to grow (table 1.1, 1.2) to such an extent that earnings from lumpfish fishery may represent a significant portion of a fisherman's income (11). Newfoundland and Labrador has become the world's leading producer and exporter of bulk salted roe (11). Before 1990, all of value-added conversion of bulk salted roe to bottled caviar (11, 8) has taken place outside Canada. At present, there are 3 or fewer producers (8) of the caviar in Newfoundland (DFO designation) Region.

Table 1.1: Newfoundland lumpfish roes landings 1970 - 1987.[a]
Landings in metric tonnes/values in $'000.

Year	Lumpfish Roe	
	Landings	Value
1970	21	5
1971	156	33
1972	204	53
1973	153	51
1974	60	23
1975	94	41
1976	320	408
1977	503	261
1978	942	577
1979	930	619
1980	577	399
1981	846	601
1982	795	565
1983	1,068	770
1984	938	680
1985	1,225	961
1986	2,048	2,997
1987[b]	3056	11,658

[a] 1970-1984 figures: Department of Fisheries and Oceans, Statistics Branch, Nfld Region
1985-1987 figures: Department of Fisheries and Oceans, Statistics Branch, Nfld and Gulf regions

[b] figures are preliminary and only include landing as of October 20, 1987.

Source: (10)

Table 1.2: Lumpfish Roe Production Newfoundland Region 1988-1992 (kilograms).

Year	Quantity	Product Form
1988	1,703,388	Brine cured lumpfish roe
1989	1,940,903	Brine cured lumpfish roe
1990	953,486	Brine cured lumpfish roe & caviar
1991	1,629,616	Brine cured lumpfish roe & caviar
1992[a]	1,619,025	Brine cured lumpfish roe, caviar, canned caviar and frozen roe.

[a]: preliminary and subjected to revision

Source: (6)

1.4 Roe Processing

Caviar yield and quality are dependant upon the fish handling practices (harvest to roe extraction), fishing season, fish size, fish species, area of catch and maturity (10, 14). The lumpfish gillnet fisheries occurs during the spawning period (mid-April on). Generally, nets of 10-11 inch mesh size are used in random, gangs or fleets (10). Male lumpfish are smaller than the females and can usually pass through the mesh. Should the males become

enmeshed, they are easily distinguished from the females by their red bellies and are returned to the water.

Roe is removed immediately after catch. The roe is removed by cutting open the belly of the female and carefully extracting the two sacs of eggs. It is important during the extraction procedure that the roe sacs remain intact and clean (11). If the sacs are removed intact, the roe remains relatively sterile. Gut fluids are not permitted in the roe holding containers since this fluid will introduce contamination. The removed roe is placed in containers which meet fish inspection regulations. The roe is iced. Care must be taken to ensure that a sufficient ice-to-roe ratio is achieved (10). Roe must be processed as soon as possible. Generally, insufficient ice is used to chill roe prior to arrival at the plant, thus reinforcing the need to process the roe immediately.

Upon receipt at plant, the roe is drained by placing approximately 23 kg of roe on a 1-1.5 mm mesh size screen. It is weighed. Roe should not be kept longer than overnight prior to processing (10).

1.4.1 Separation

The first step in lumpfish roe processing is the separation of the eggs from the sac. This can be preformed either manually or mechanically (10).

1.4.1.1 Manual Separation

The manual method of separation uses stainless steel screens set in a stainless steel frame. It is recommended that three screens of 10 mm mesh, 5 mm mesh and 3 mm mesh be used to produce a cleaner roe containing a minimal amount of blood, gut and sac (extraneous) material (10).

The roe is spread on the top screen and gently but firmly rubbed across the screen using the palm of the hand. Eggs separate from the sac and fall through to the second screen. Extraneous material may also pass through to the second screen. The remaining extraneous material will be screened out as the eggs pass through the second and third screens. Screens must be cleaned frequently to protect roe quality and limit contamination. Eggs fall to a draining screen and are covered with a plastic sheet to limit contamination as they drain (10).

1.4.1.2 Mechanical Separation

The mechanical separation method is preformed on a separating machine. The machine contains a stainless steel drum which has rows of 5 mm perforations. There are two to six paddles which rotate inside the drum and push the eggs through the perforations. Eggs fall on to a 2 mm mesh draining screen, while the extraneous material remain inside

the drum. The drum is cleaned frequently to protect roe quality and limit contamination (10).

1.4.2 Draining

Eggs are left on the draining screen for several hours to remove as much water as possible. During this time the draining screen is covered to protect the eggs from contamination. Eggs must not be piled more than 25 cm deep to ensure the bottom eggs are not damaged from pressure. Placing the draining screens on an incline speeds the draining process. The length of time the roe is drained will influence the amount of roe needed for topping-up prior to shipping. The better drained (longer the draining time) the roe is, the less roe that is needed for topping-up (10).

1.4.3 Mixing with Salt

After the roe is drained, the roe is mixed with fine fishery salt which acts as a preservative. The amount of salt used (12-20%) is considered borderline for the preservation purposes and thus the product must be kept chilled during and after curing to maintain product quality (10)..

The proper mixing of the salt and roe is essential to the final product quality. Mixing is preformed in three, four or five batches per barrel to ensure an even distribution of salt and roe. Roe and salt for each batch are separately weighed and mixed by hand. The salt-to-roe ratio is determined by buyer specifications and is usually between 12-20%. A minimum of greater than 9% salt is necessary to prevent spoilage of the product from microorganisms such as *Clostridium botulinium* and other halo-tolerant bacteria (10).

1.4.4 Filling the Barrels

Batches of the roe-salt mixture are placed in herring barrels. Barrels are filled, rims cleaned, covered and sealed. Barrels are stored in a chill room at 3-4 °C. Barrels should be left up right so the roe can settle and the following day the barrels should be topped-up with salted roe prepared the same day as those in the barrels. This is necessary because after 24 hours the contents of the barrel will shrink/ settle and occupy approximately ¾ of the volume of the barrel. The lid is replaced and the barrel is filled with a brine solution through the bung hole to expel any air present. Barrels should be left upright and topped-up next day to avoid problems. Air will react with natural oils in the roe and cause rancidity which is one of the major problems with lumpfish roe (10).

1.4.5 Curing

The barrels are left to cure for 12-14 days, during which they are rolled and topped-up with brine. Rolling the barrels helps to mix roe, salt and brine thereby preventing spoilage of the roe. Barrels or cured roe are kept chilled and during the first month of storage are rolled and topped-up with brine once a week. This process is then repeated once a month for the remaining storage period (10).

1.5 Caviar Processing

The production of caviar from cured roe requires a series of procedures that include salt reduction, roe colouration, pH adjustment, additive incorporation, packaging, pasteurization (if desired), labelling and storage. A flow diagram outlining the process is presented in Figure 1. The first step in the caviar process is to determine if the barrelled roe is suitable for processing. The following conditions must be met for the cured roe to be suitable for caviar processing:

- The temperature of the cured roe should be 0°- 3°C.
- No trace of rancid or off odours.
- The roe must be free of objectional matter such as blood, liver or sac material.
- There must be no foreign material present in the cured roe.

Figure legend for Figure 1.0.

Flow diagram of the lumpfish roe caviare process illustrating critical steps in the manufacturing process.

Source: Department of Fisheries. Government of Newfoundland and Labrador. 1989. Industry Support Services Report No. 43.

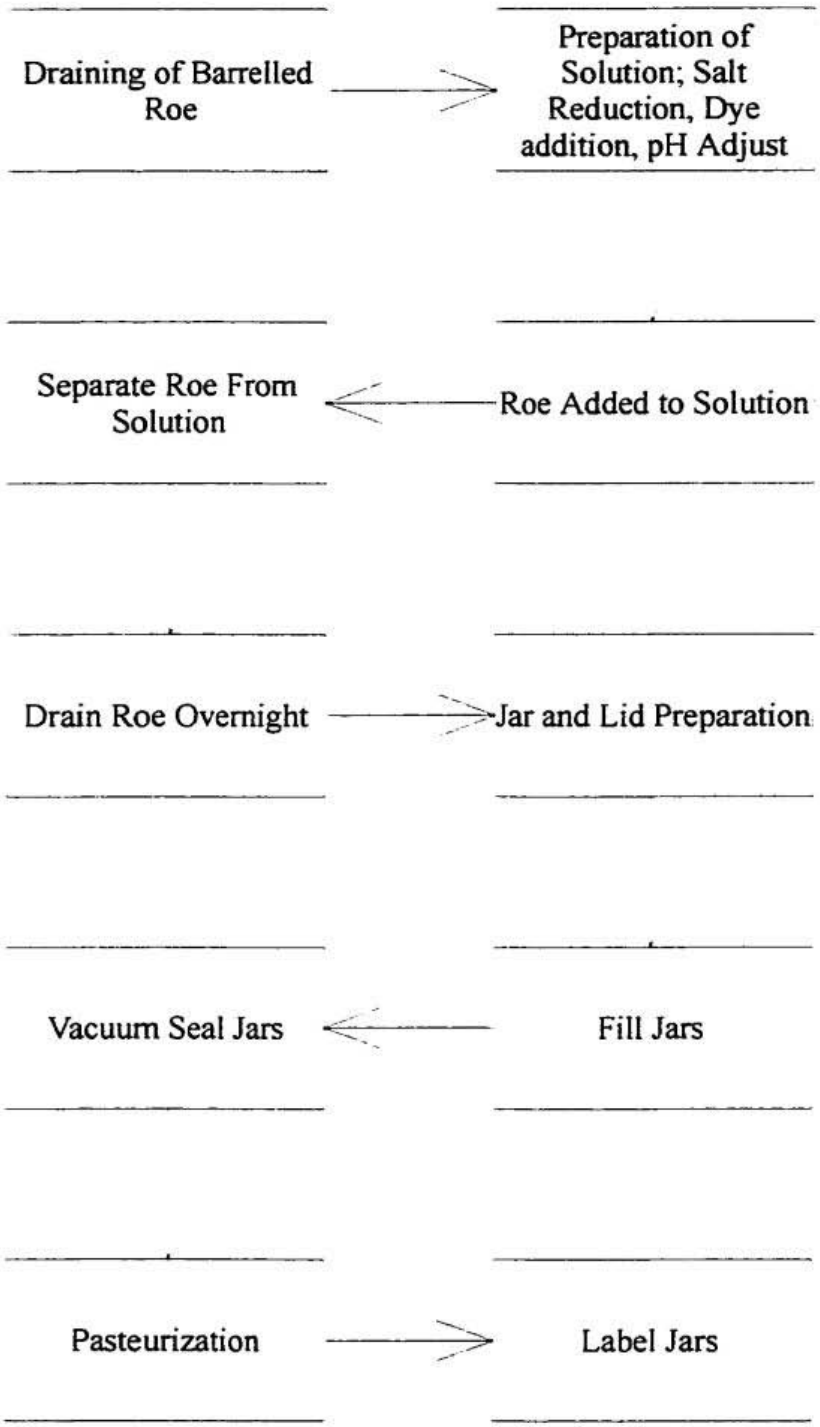

Figure 1.0 Lumpfish Roe Caviar Process

- The cured roe must be checked for the percentage of broken eggs.
- The salt content must be checked to determine the proper ratio of dilution for the desalting process.
- The pH must be measured to determine the amount of acidulent necessary for pH adjustment (10).

After a barrel of cured roe has been found acceptable for caviar processing it must be drained. A screen is clamped on in place of the lid. The barrel is inclined to allow excess brine to drain. This takes approximately 30 minutes. During this time, the results of the salt and pH content are used to calculate the volume of water, dye and acidulent necessary to adjust these factors to the desired level (10). The salt content is adjusted using the following formula:

$$\text{wt. of roe (kg)} \times 0.90 \times \frac{\text{salt content - desired salt content}}{\text{Desired salt content}} = \text{Water (L)}$$

This formula determines the amount of water in litres that is needed to dilute the roe. Dye is added (10).

The amount of acid/acidulent required to adjust the pH is determined by trial and error. The amount required will depend upon the pH of the dilution water, pH of cured roe and the ratio of roe to dilution solution. Citric acid is used as a acidulent and the pH is monitored until the desired pH level has been attained (10).

The dilution solution is poured into a large vat and the roe is added. The temperature must be maintained below 15°C. The roe remains in the dilution solution for 30-45 minutes depending upon the dye used. This is necessary to eliminate the possibility of inconsistent dyeing (10).

The roe and solution are slowly agitated to ensure proper and thorough mixing. Sac material, broken eggs and other debris will float and are skimmed of (10). The roe is now dyed, salt reduced and acid adjusted. The roe is poured onto drain screens which are capable of holding up to 25 kg of caviar material. A portion of the dilution solution is collected and reserved for rinsing out the vats. The screens with the draining roe are placed in a chill area on racks which are inclined at a 20° angle and do not allow draining from top trays to fall on lower trays. The roe should be left overnight to reduce the amount of free liquid (10).

Roe is removed form the chill area after draining has been completed. At this time desired additives are added to the caviar. Common additives are oil (which increases the product "flow", increases the jar filling capacity and gives the product a glossy appearance), antioxidants, flavour enhancers, spices, sugar, and/or preservatives. The additives used are often related to market requirements and opportunities (10).

Jars are cleaned by removing any debris, washed and dry. Lids are usually packaged under sanitary conditions and do not need any prewashing. Jars are fed to the filling

machine and lids are fed to the vacuum closing machine (10). Jars are usually filled by either an automated or semi-automated filler. Caviar fillers fill from the bottom of the jar to the rim. The methods of capping are screw-on screw-off and crimp-on screw-off. Lumpfish caviar are most commonly vacuum sealed by either a mechanical vacuum, steam evacuation or hermetical self sealing caps. Closed jars should be coded on closing or immediately after (10).

Closed jars periodically undergo a quality control check for weight and vacuum integrity. The number of jars which are checked should be predetermined according to a statistical sampling plan. Jars which weigh greater or less than established tolerance limits are rejected. It may be necessary to readjust the filling machine to ensure the appropriate volume is dispensed. Similarly , if there is a problem with the vacuum sealed lids, the vacuum sealing method must be checked, the problem identified and corrected (10). At this stage the caviar is ready to be labelled, cartoned and stored or if desired the caviar will undergo pasteurization.

1.5.1 Labelling, Cartoning and Storage

Labels for caviar sold in glass containers are generally applied to the lids. The labelling step usually occurs after the jars are cleaned and dried. Lumpfish caviar is usually packaged as 6 or 12 jars to a case. Once jars are packed in cartons the final product is held in storage

prior to shipping. Lumpfish caviar should be stored, shipped and handled at chill temperatures (2° - 4°C) at all times after processing (10).

1.6 Pasteurization

Pasteurization of capped caviar jars is done in either batches or continuously. Batch pasteurization requires that closed jars are collected in a basket and immersed into a temperature regulated bath. Continuous pasteurization requires that the closed jars be placed on a conveyor belt that passes through a pasteurization tunnel (10).

Regardless of the pasteurization method, the pasteurization process is followed by a cooling unit which uses lukewarm water to avoid cold shock cracking of the glass (10). Pasteurization temperatures and times generally used within the industry are presented in Table 1.3. Pasteurization depends on the come-up time. This is the time required to raise the temperature of the product at the centre of the jar to pasteurization temperature. The exact time and temperature depends upon the shape and dimensions of the container, the initial bacterial load and the ambient temperature (10).

Table 1.3 Pasteurization Time and Temperatures

Jar Size (oz)	Time (min)	Temperature (°C)	Internal Temperature (°C)
2 oz *	23	73.9°	65.5 °
4 oz *	41	73.9°	65.5 °
7 oz *	53	73.9°	65.5 °
14 oz *	91	73.9°	65.5 °
50 oz **	120	57 °	--
100 oz ***	36	75-80°	75°
50 oz***	60	71 °	--

* Source: Romanoff Food Inc.
** Source: Dr. Iredale, Freshwater Institute, Winnipeg Manitoba
*** Source: Dewar, Lipton and Mack, 1971

Table Source (10).

1.7 Microbiology of Caviare

Caviare may be preserved chemically, or through the use of temperature preservation methods such as refrigeration or pasteurization. These preservative methods may result in the reduction of bacterial loads, retard or inhibit microorganism growth thereby extending product shelflife and ensuring product safety. The intrinsic parameters (pH, salt, moisture, water activity, etc.) of many food products including caviare are such that bacterial growth and decomposition are not retarded and consequently neither are microorganisms responsible for food infections and intoxicants. The method of preservation chosen will

depend greatly upon the target shelflife, the intended consumer, product use, storage, shipping requirements, import country regulations, buyer specifications and the expected initial bacterial loads.

The availability of published literature on caviare microbiology is sparse (particularly when compared to other food products), dated and often found in obscure sources. A majority of the published material on caviare is based on Soviet research, does not deal with lumpfish caviare per say and is based on methodologies which are not necessarily comparable to North American standard methods. Information on microbial and chemical quality of caviare held by commercial producers would be confidential and therefore unavailable to the public. In addition, concerns have been raised by government agencies with respect to the lumpfish caviare product safety, and as such there is an identifable data gap.

Total coliforms, fecal coliforms, *Esherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus cereus* are known causative agents or indicators of food intoxications or infections. This study will undertake to determine the occurrence of the microorganisms in lumpfish caviare. The total aerobic and anaerobic content of lumpfish caviare will also be examined as an indicator of product quality, preservative effectiveness and shelflife stability.

1.8 Objectives

Caviar presently produced in Newfoundland uses sodium benzoate (40) for preservation. The EEC (15), Korea and other countries (40) prohibit the use of sodium benzoate as a preservative in caviar. The use of sodium benzoate in combination with potassium sorbate up to 400 ppm would be acceptable for most EEC countries under new guidelines. Therefore, to market caviar in these countries, preservation methods such as pasteurization, refrigeration or the use of acceptable chemical additives must be implemented.

The proposed study will investigate:

1. Alternate preservation methods for caviare.
2. Determine if alternate preservation methods would ensure acceptable microbiological and chemical quality.
3. Determine the most effective alternative preservation method.

This study will examine the following preservation methods:

1. Sodium benzoate.
2. Mixture of sodium benzoate (100 mg/kg) and potassium sorbate (300 mg/kg).
3. Mixture of sodium benzoate (200 mg/kg) and potassium sorbate (200 mg/kg).
4. Mixture of sodium benzoate (300 mg/kg) and potassium sorbate (100 mg/kg).
5. Refrigeration (4°C).

6. Pasteurization (55°C for 135 minutes).

7. Pasteurization (70°C for 45 minutes).

CHAPTER 2

MATERIALS AND METHODS

North Atlantic Packaging is a secondary processor of lumpfish roe caviar based in Newfoundland, producing 50 and 100 gram product in glass jars with sealed metal caps. The products are preserved with brine and sodium benzoate (0.08%). The products have the requirement for refrigeration upon opening. The products are retailed at a variety of outlets in Canada (40).

Present markets are limited to the Canadian retail market. A new potential market in South Korea does not permit the use of sodium benzoate. The South Koreans require all natural ingredients to be used (40). North Atlantic Packaging produced several experimental runs of lumpfish roe caviar to examine alternative preservative methods, determine if the alternative methods would ensure microbiological and chemical quality, and determine the most effective alternative method. The alternative methods examined were:

1. Pasteurization 55°C.
2. Pasteurization 70°C.
3. Refrigeration.
4. Combination of sodium benzoate/potassium sorbate (3:1).
5. Combination of sodium benzoate/potassium sorbate (1:3).
6. Combination of sodium benzoate/potassium sorbate (1:1).

The present preservative method using sodium benzoate (80 mg/kg) was also examined. Alternative methods 5 and 6 would be applicable for most EEC countries under proposed new guidelines (15).

2.1 Caviar Samples

Caviare samples were obtained from North Atlantic Packaging containing sodium benzoate at 80 mg/kg. North Atlantic Packaging produced special runs to make the caviare samples with the preservative levels at:

1. Mixture of sodium benzoate (100 mg/kg) and potassium sorbate (300 mg/kg).
2. Mixture of sodium benzoate (200 mg/kg) and potassium sorbate (200 mg/kg).
3. Mixture of sodium benzoate (300 mg/kg) and potassium sorbate (100 mg/kg).
4. Un-preserved caviare.

Caviar samples specially prepared for this study were collected from North Atlantic Packaging immediately after processing. The microbiological survey was initiated within three hours of processing. Samples that were chemically preserved or pasteurized were stored at 20°C (holding temperature) and 37°C (abusive temperature) for the duration of the study. Samples prepared with no preservative for refrigeration were stored in a refrigerator at 4°C (holding temperature) and 20°C (abusive temperature) for the duration of the study.

Commercial samples were obtained directly from the processing line and were stored at 20°C (holding temperature) and 37°C (abusive temperature).

Samples for pasteurization contained no preservatives and were obtained directly from the processing line. They were transported to the lab where they were placed in prepared water baths of 70°C and 55°C. The pasteurization process started within 15 minutes after removal from the processing line. Samples were pasteurized for 55 minutes at 70°C and 135 minutes at 55°C. Pasteurized samples were stored at 20°C and 37°C for the duration of the study.

2.2 Media

All media used in this project were of reagent or laboratory grade. Chemicals and media were obtained from Fisher Scientific Limited, Dartmouth, Nova Scotia or BDH Chemicals, Dartmouth, Nova Scotia. Potassium sorbate and sodium benzoate were of food grade quality and were provided by North Atlantic Packaging.

The following media and chemicals were obtained from Fisher Scientific Limited, Dartmouth, Nova Scotia: Listeria enrichment broth; UVM listeria enrichment broth; Oxford listeria selective agar and supplement; Typticase soy agar; coagulase plasma with EDTA; Baird Parker agar; nutrient broth; tetrathionate broth; brilliant green dye; potassium iodide;

iodine; bismuth sulfite agar; brilliant green sulfa agar; XLD agar; Hektoen Enteric agar; Egg yolk tellurite emulsion; egg yolk 50% emulsion; purple broth base; motility test medium; Levine's EMB agar; Gram stain test kit; trypticase soy broth; peptone; nutrient agar; tryptone bile agar; 0.45 micron 85 mm cellulosic membrane filters and yeast extract.

The following chemicals and media were obtained from BDH Chemicals, Dartmouth, Nova Scotia: PALCAM Listeria selective agar and supplement; sodium chloride; violet red bile agar; standard methods agar; Selenite Cysteine; Bacillus cereus agar; Anaerocult A and Anaerotest.

2.3 Reference Cultures

Listeria cultures were obtained from Dr. T. Patel, Memorial University of Newfoundland, St. John's, NF. The *Listeria* cultures used were *Listeria monocytogenes* ½b (HPB #395), *L. Innocua* (HPB #8) and *L. Ivanovii* (HPB #28). The *Bacillus cereus* (E 14579), *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (11775), *Clostridium sporogenes* (19404) and *Staphylococcus aureus* (E 12600) cultures were obtained from the Department of Biology, Memorial University culture stock collection.

2.4 Sample Analyses

Caviare samples were examined within 2 hours of processing, and after 2, 4, 8 and 16 weeks of storage of samples at normal (20°C, 4°C) and abusive (37°C, 20°C) conditions. Samples were examined for chemical and microbiological quality.

2.4.1 Chemical Analyses

Proximate (chemical) analyses were preformed as per standard protocols established by the Association of Official Analytical Chemists (1). The chemical quality was determined by examination of caviare samples for pH, salt, moisture, fat, protein, and water activity.

2.4.1.1 pH

Ten grams of caviar were homogenized with 90 ml of distilled water. The pH was read with a standardized pH meter (Orion, Fisher Scientific Ltd.). The probe was immersed in the samples and the digital readings recorded (1).

2.4.1.2 Salt

Ten grams of caviar were homogenized with 90 ml of distilled water (same one used for pH determination). Salt analyses were conducted using a SM10 salt meter (Presto-Tek Corporation) standardized with a reference salt solution. The probe was immersed in the samples and the analog readings recorded (1).

2.4.1.3 Ash

Two grams of caviar was placed in an ashing crucible and weighed. The crucible was placed in a muffle furnace at 525°C for 24 hours. The crucible was re-weighed and the ash content calculated (1).

2.4.1.4 Moisture

Two grams of caviar was placed in an aluminum planchet and weighed. The planchet was placed in a forced air convection oven at 100°C until consistent weights were obtained. The samples were cooled in a desiccator between weighing.. The loss in weight is reported as moisture loss (1).

2.4.1.5 Fat

Total Lipid (fat) contents (1) were determined by the soxhlet method (36) using the tecator soxhlet unit. Three grams of dried sample was placed into the thimble. The thimbles were attached to adapters and fat free cotton plugs were placed on top of the sample. The thimbles were inserted into the condenser. The condenser knobs were in the rinse position. The knobs were set to the boiling position so that magnet fastened to the thimble adapter. The knob was adjusted to the rinse position. Extraction cups of known weight containing boiling chips and 25-50 ml of hexane were placed into the condenser. The handle was lowered until the safety catch engaged (36).

The extraction knob was set to the boiling position. The thimbles were immersed into the hexane solvent. The sample was boiled for 1 hour and rinsed for 2 hours. The condenser valves are closed after the rinsing cycle is completed by turning a quarter turn. Upon collection of the remaining solvent in the condenser, the "AIR" button on the service unit was pressed and the "EVAPORATION" valve on the extraction unit was opened. The "EVAPORATION" valve was closed, extraction cups released and removed. The cups were placed in an oven at 80°C for 20 minutes. The thimbles were removed from the condenser using a thimble holder. The instrument was shut down (36).

The fat content was calculated as follows:

$$\text{Fat \%} = \frac{100 \times \text{Lipid Weight}}{\text{Sample Weight}}$$

2.4.1.6 Protein

The crude proteins (%N x 6.25) were determined (1) by the macro-Kjeldahl method (35). A tecator digester and distillation unit were employed to preform the macro-Kjeldahl (35).

A 2.5 gram sample was placed in the digestion flask. Added to the flask sequently were 15 g Na_2SO_4, 1 g $CuSO_4$, one or two selenized boiling granules and 25 mL of concentrated H_2SO_4. The mixture was digested until the solution was colourless or a light green (approximately 2 hours for inorganic material). The sample was cooled for an additional 30 minutes. Two hundred mL of water was cautiously added to the cooled sample. Additional boiling granules (if necessary) were added to prevent bumping (35).

One hundred mL of 0.1 N HCl were pipetted into a 500 mL Erlenmeyer flask, 1 mL of Conways indicator was added. The flask was placed under the condenser ensuring that the condenser tip was immersed in the acid solution. The Kjeldahl flask containing the digested sample was tilted and 100 mL of 50% NaOH solution was added without agitation. The

flask was immediately connected to the distilling bulb of the distillation apparatus. The flask was rotated to thoroughly mix contents (35).

The sample was heated until all ammonia had passed over the standard acid. Approximately 150 mL was collected and removed immediately. The tip of condenser was washed and excess standard HCl in distillate was titrated with NaOH standard solution (35).

The precent nitrogen (wet weight basis) was calculated as follows:

$$\% \text{ Nitrogen (wet)} = \frac{(A-B) \times 1.4007}{\text{Weight (g) of sample}}$$

where A = volume (mL) standard HCl x normality of standard HCl.

B = volume (mL) standard NaOH x normality of standard NaOH.

2.4.1.7 Water Activity

Samples of caviar were placed in the water activity (1) containers. The containers were placed in the CX-1 Decagon water activity unit (Decagon Devices Inc.). Efficiency of the water activity unit was verified with a KNO_3 solution which has a water activity of 0.936.

2.4.2 Microbiological Analyses

Microbiological analyses were preformed as per standard protocols established by either Health Protection Branch (HPB) of Health and Welfare Canada or Food and Drug Agency (FDA) of the United States. The microbiological quality was determined by the examination of caviare samples for total aerobic counts, total anaerobic counts, total coliform counts, fecal coliform counts (*E. coli*), *Bacillus cereus*, *Salmonella* spp., coagulase positive *Staphylococcus aureus* and *Listeria* spp.

2.4.2.1 Total Aerobic Counts

The total aerobic count was conducted as per HPB standard method MFHPB-18 (18) and the protocols outlined by the USFDA (37). Approximately 11.0 g samples of caviar were stomached in the Stomacher Lab Blender (Canlab Division, Baxter Corporation, Mount Pearl, NF) for 1 minute with 99 ml of 0.1 % sterile peptone water. Decimal dilutions were prepared from the 10^{-1} dilution (usually up to 10^{-6} dilution) by transferring 1.1 ml of the previous dilution into 9.9 ml of 0.1 % sterile peptone water in a test tube (18, 37).

Each dilution was agitated to resuspend material prior to plating. One ml or 0.1 ml of the required dilutions were pipetted into appropriate labelled duplicate petri dishes. Twelve (12) to fifteen (18) ml of tempered standard methods (plate count) agar were poured into

each plate. The plates were mixed by rotating and tilting. The plates were allowed to solidify. Plates were incubated in the inverted position at 30°C for 48 ± 2 hours. Colonies on the plates were enumerated using a quebec colony counter (Fisher Scientific Ltd, Dartmouth, NS). Total aerobic counts were determined using enumeration guidelines as per USFDA (1984) standard methods (18,37).

2.4.2.2 Total Anaerobic Counts

Approximately 11.0 g samples of caviar were stomached in the Stomacher Lab Blender (Canlab Division, Baxter Corporation, Mount Pearl, NF) for 1 minute with 99 ml of 0.1 % sterile peptone water. Decimal dilutions were prepared from the 10^{-1} dilution (usually up to 10^{-6} dilution) by transferring 1.1 ml of the previous dilution into 9.9 ml of 0.1 % sterile peptone water in a test tube.

One ml or 0.1 ml of the required dilutions were pipetted into appropriate labelled duplicate petri dishes. Twelve (12) to fifteen (15) ml of tempered trypticase soy agar were poured into each plate. The plates were mixed by rotating and tilting. The plates were allowed to solidify. Plates were placed inverted into an anaerobe jar with Anaerocult A gas package and an anaerobe condition indicator (Anaerotest). The anaerobe jars were incubated at 35°C for 48 ± 2 hours. Colonies on the plates were enumerated using a Quebec colony

counter (Fisher Scientific Ltd, Dartmouth, NS). Total anaerobic vegetative counts were determined using enumeration guidelines as per USFDA (1984) standard methods (37).

2.4.2.3 Total Coliform Counts

The total coliform analysis was conducted as per protocols outlined in HPB MFLP-43 (18). Approximately 11.0 g samples of caviar were stomached in the Stomacher Lab Blender (Canlab Division, Baxter Corporation, Mount Pearl, NF) for 1 minute with 99 ml of 0.1 % sterile peptone water. Decimal dilutions were prepared from the 10^{-1} dilution (usually up to 10^{-6} dilution) by transferring 1.1 ml of the previous dilution into 9.9 ml of 0.1 % sterile peptone water in a test tube (18).

Each dilution was agitated to resuspend material prior to plating. One ml or 0.1 ml of the required dilutions were pipetted into appropriate labelled duplicate petri dishes. Twelve (12) to fifteen (15) ml of tempered violet red bile agar were poured into each plate. The plates were mixed by rotating and tilting. The plates were allowed to solidify. Plates were incubated in the inverted position at 37°C for 48 ± 2 hours (18). Colonies on the plates were enumerated using a quebec colony counter (Fisher Scientific Ltd, Dartmouth, NS). Total coliform counts were determined as per guidelines used for total aerobic counts (18, 37).

A direct plating method for the determination of total coliforms was used instead of the standard Most Probable Number (MPN) method because of space and equipment considerations. The multiple tube method requires fifteen (15) tubes per dilution for each sample to be incubated in a coliform water bath. This would have required more water baths than were available, therefore a direct plating method was the only viable option.

2.4.2.4 Fecal Coliform Counts

Enumeration of Fecal Coliforms (E. coli) was conducted as per protocols outlined in HPB MFHPB-27 methodology (18). A direct plating method for the determination of fecal coliform (*Esherichia coli*) was used instead of the standard multiple tube method because of space and equipment considerations.

Approximately 11.0 g samples of caviar were stomached in the Stomacher Lab Blender (Canlab Division, Baxter Corporation, Mount Pearl, NF) for 1 minute with 99 ml of 0.1 % sterile peptone water. Decimal dilutions were prepared from the 10^{-1} dilution (usually up to 10^{-6} dilution) by transferring 1.1 ml of the previous dilution into 9.9 ml of 0.1 % sterile peptone water in a test tube (18).

Each dilution was agitated to resuspend material prior to plating. In duplicate, 0.5 ml of two consecutive decimal dilutions were plated on a membrane filter overlaying nutrient

agar. The innoculum was spread over the membrane filter with a glass spreader. Care was taken to spread the innoculum evenly without spilling it over the edge of the membrane filter. After the innoculum was absorbed, the plates were incubated right side up at 37°C for 4 hours (18).

The membrane filters were removed with sterile forceps after the incubation period of four hours. The filters were transferred to prepared and air dried tryptone bile agar plates. The plates were incubated upright at 44.5°C for 18-24 hours (18).

After incubation at 44.5°C, the petri dish covers were removed and wiped dry and 2.0 ml of indole reagent placed in each cover. The membrane filter was lifted and placed in its respective cover so that the entire undersurface is soaked with the reagent. The membrane filter and reagent are left for 20 minutes at room temperature. Remove the membrane filter by dragging it across the lip of the cover to remove excess indole reagent. Dry the filters under a germicidal UV lamp for 20 minutes. The pink to red colonies appearing on the membrane filters are indole producers and are enumerated as *E. coli* biotype I (18).

2.4.2.5 *Bacillus cereus*

Enumeration for *Bacillus cereus* was conducted as per protocols outlined by the USFDA (37). The USFDA method was used instead of the HPB method as a result of the inability

to obtain the base media due to back orders. Both methods are similar and either will identify and enumerate *B. cereus* (37).

Approximately 11.0 g samples of caviar were stomached in the Stomacher Lab Blender (Canlab Division, Baxter Corporation, Mount Pearl, NF) for 1 minute with 99 ml of 0.1 % sterile peptone water. Decimal dilutions were prepared from the 10^{-1} dilution (usually up to 10^{-6} dilution) by transferring 1.1 ml of the previous dilution into 9.9 ml of 0.1 % sterile peptone water in a test tube (18, 37).

Duplicate Mannitol-Egg Yolk-Polymyxin (MYP) plates per dilution were inoculated with 0.1 ml evenly distributed over the surface with a sterile glass spreading rod. Plates were incubated inverted at 30°C for 24 hours. Plates were checked for typical *B. cereus* colonies (pink colour with precipitate zone indicating lecithinase production). Plates with unclear reactions or no growth were incubated an additional 24 hours (37).

They were no colonies indicative of *B. cereus* on the plates, thus *B. cereus* were considered absent. Therefore, it was not necessary to conduct confirmation and differentiation analyses for *B. cereus*.

2.4.2.6 *Salmonella* spp.

Isolation and identification of *Salmonella* was conducted as per HPB MFHPB-20 protocols (18). Approximately 11.0 g samples of caviar were stomached in the Stomacher Lab Blender (Canlab Division, Baxter Corporation, Mount Pearl, NF) for 1 minute with 99 ml of nutrient broth as a pre-enrichment. The broth was incubated at 35°C for 18-24 hours. One (1) ml of the incubated pre-enrichment broth was transferred to 9 ml of selenite cysteine and tetrathionate broths. The selenite cysteine was incubated at 35°C and the tetrathionate broth at 43°C for 24 hours (18).

A loop from each of the selective enrichment broths were streaked onto bismuth sulfite agar, brilliant green sulfa agar, XLD agar, and hektoen enteric agar. All plates were incubated at 35°C for 24 hours. Plates were examined for colonies typical of *Salmonella* (18).

There were no colonies indicative of *Salmonella* on the plates, thus bacteria of the genus *Salmonella* were considered absent. Therefore, it was not necessary to conduct biochemical screening and serological identification.

2.4.2.7 Coagulase Positive *Staphylococcus aureus*

Enumeration of coagulase positive *Staphylococcus aureus* was conducted as per protocols outlined by the USFDA (37). Approximately 11.0 g samples of caviar were stomached in the Stomacher Lab Blender (Canlab Division, Baxter Corporation, Mount Pearl, NF) for 1 minute with 99 ml of 0.1 % sterile peptone water. Decimal dilutions were prepared from the 10^{-1} dilution (usually up to 10^{-6} dilution) by transferring 1.1 ml of the previous dilution into 9.9 ml of 0.1 % sterile peptone water in a test tube (18, 37).

For each dilution plated, 1.0 ml (0.3, 0.3 and 0.4 ml) of sample was distributed equally over 3 Baird-Parker egg yolk tellurite plates. Innoculum was spread over the surface of the plates using a sterile bent glass rod. Plates were retained upright until innoculum was absorbed (approximately 10 minutes). Plates were incubated inverted at 35°C for 45-48 hours (37).

Colonies typical of *S. aureus* (circular, smooth, moist, convex, 2-3 mm, gray to jet black, and frequently having a outer clear zone) were transferred to tubes containing 0.2-0.3 ml of brain heart infusion. The brain heart infusion tubes were incubated at 35°C for 18-24 hours. Reconstituted coagulase plasma with EDTA (0.5 ml) was added to the tubes. The tubes were re-incubated at 35°C for 6 hours and periodically examined for clot formation. Only firm and complete clots which stay in place upon tilting were considered positive (37).

2.4.2.8 *Listeria* spp.

Isolation of *Listeria monocytogenes* was conducted as per protocols outlined in HPB MFHPB-30. This method is based on the USFDA method with modifications based on research by Warburton et al (18).

Approximately 11.0 g samples of caviar were stomached in the Stomacher Lab Blender (Canlab Division, Baxter Corporation, Mount Pearl, NF) for 1 minute with 99 ml of *Listeria* enrichment broth (LEB). LEB cultures were incubated in the stomacher bag at 30°C for 48 hours. At 24 and 48 hours, the LEB culture was mixed and streaked onto Oxford agar (OXA) and PALCAM (PAL). Plates were incubated at 35°C for 24-48 hours. The inoculation of Modified Fraser broth step was not undertaken (18).

Plates were examined for typical *L. monocytogenes* growth characteristics. There were no colonies indicative of *L. monocytogenes* on the plates, thus *L. monocytogenes* were considered absent. Therefore, it was not necessary to conduct identification, confirmation and serological analyses for *L. monocytogenes*.

2.5 Isolation of Predominant Organism

The predominant organisms which accounted for approximately 90% of all organisms was isolated and purified. The caviare isolate was grown in Trypticase Soya Broth for 24 hoyurs at 30°C. These cultures served as the inocula for the growth profiles.

2.6 Growth profiles of Caviare Isolate for Various Preservative Methods

Growth profiles (determined by optical densities) of the caviare isolate under various preservative methods (salt, temperature, sodium benzoate, potassium sorbate, and a sodium benzoate/potassium sorbate mixture) were examined. Series of test tubes containing nine mL of trypticase soya broth with varying concentrations of the preservatives were prepared.

The tubes containing the chemical preservatives sodium benzoate and potassium sorbate were pH adjusted to pH 4.0, 5.0, 6.0 and 7.0 from an initial pH of 7.2.. The pH adjustment was achieved by the addition of 0.1 N HCl. The pH of the solutions were tested prior to sterilization. An additional tube was prepared to test the pH of the solutions after sterilization. All solutions maintained the pre-sterilization pH within 0.2 units and no additional adjustment was made.

Six tubes for each series were inoculated with 1 ml of the inocula. The inocula was enumerated by preparing serial dilutions up to 10^{-6} dilution. These dilutions were plated by the spread plate method on to prepared plates of standard method agar. The plates were incubated at 30°C for 48 hours and enumerated.

2.6.1 Temperature

Series of test tubes (8 test tubes) containing 9 ml of typticase soya broth were prepared. One ml of inoculum was dispensed into each test tube using a Eppendorf pipetter with sterile tips. The inocula were dispensed into the tubes immediately above the solution taking care not to touch the solution. Tubes were mixed on a vortex mixer. The initial optical densities were taken using one tube from each series. The optical densities were determined by a Shimatzu double beam spectrophotometer at a wavelength of 630 nm. An uninoculated tube (at the concentration being tested) was used to zero the spectrophotometer.

The remaining six tubes (one uninoculated tube) were incubated for 24 hours at 30°C. The optical densities were recorded and the results corrected for the initial inocula density.

2.6.2 Salt

Series of test tubes (8 test tubes) containing 9 ml of typticase soya broth with salt concentrations ranging from 0 to 24% were prepared. Six tubes per salt concentration were prepared; one tube for initial inoculum reading and five replicate samples.

One ml of inocula was dispensed into each test tube using a Eppendorf pipetter with sterile tips. The inocula was dispensed into the tubes immediately above the solution taking care not to touch the solution. The tip was changed for each concentration to ensure that there was no carryover of solution from a different concentration. Tips were changed if the solution was touched. Tubes were mixed on a vortex mixer. The initial optical densities were taken using one tube from each salt concentration. The optical densities were determined by a Shimatzu double beam spectrophotometer at a wavelength of 630 nm. An uninoculated tube (at the concentration being tested) was used to zero the spectrophotometer.

The remaining six tubes (one uninoculated tube) were incubated for 24 hours at 30°C. The optical densities were recorded and the results corrected for the initial inocula density.

2.6.3 Growth Profile for Sodium Benzoate

Series of test tubes (8 test tubes) containing 9 ml of trypticase soya broth with sodium benzoate concentrations of 0, 250, 500, 750 and 1000 ppm were prepared at pH's of 4, 5, 6, and 7. The pH adjustment was achieved by the addition of 0.1 N NaOH. The pH of the solutions were tested prior to sterilization and all pH's were confirmed after sterilization by testing the pH of one tube. All solutions maintained the pre-sterilization within 0.2 units. No additional pH adjustment was necessary.

One ml of inoculum was dispensed into each test tube using a Eppendorf pipetter with sterile tips. The inoculum was dispensed into the tubes immediately above the solution taking care not to touch the solution. The tip was changed for each concentration to ensure that there was no carryover of solution from a different concentration. Tips were changed if the solution was touched. Tubes were mixed on a vortex mixer. The initial optical densities were taken using one tube from each sodium benzoate concentration per pH level. The optical densities were determined by a Shimatzu double beam spectrophotometer at a wavelength of 630 nm. An uninoculated tube (at the concentration being tested) was used to zero the spectrophotometer.

The remaining six tubes (one uninoculated tube) were incubated for 24 hours at 30°C. The optical densities were recorded and the results corrected for the initial inocula density.

2.6.4 Growth Profile for Potassium Sorbate

Series of test tubes (8 test tubes) containing 9 ml of trypticase soya broth with potassium sorbate concentrations of 0, 250, 500, 750 and 1000 ppm were prepared at pH's of 4, 5, 6, and 7. The pH adjustment was achieved by the addition of 0.1 N NaOH. The pH of the solutions were tested prior to sterilization and all pH's were confirmed after sterilization by testing the pH of one tube. All solutions maintained the pre-sterilization within 0.2 units. No additional pH adjustment was necessary.

One ml of inocula was dispensed into each test tube using a Eppendorf pipetter with sterile tips. The inocula was dispensed into the tubes immediately above the solution taking care not to touch the solution. The tip was changed for each concentration to ensure that there was no carryover of solution from a different concentration. Tips were changed if the solution was touched. Tubes were mixed on a vortex mixer. The initial optical densities were taken using one tube from each potassium sorbate concentration per pH level. The optical densities were determined by a Shimatzu double beam spectrophotometer at a wavelength of 630 nm. An uninoculated tube (at the concentration being tested) was used to zero the spectrophotometer.

The remaining six tubes (one uninoculated tube) were incubated for 24 hours at 30°C. The optical densities were recorded and the results corrected for the initial inocula density.

2.6.5 Growth Profile for Sodium Benzoate/Potassium Sorbate Combination Preservatives

Series of test tubes (8 test tubes) containing 9 ml of trypticase soya broth with sodium benzoate/potassium sorbate at a ratio of 1:1 were prepared. The final concentrations were 0, 250, 500, 750 and 1000 ppm at pH's of 4, 5, 6, and 7. The pH adjustment was achieved by the addition of 0.1 N NaOH. The pH of the solutions were tested prior to sterilization and all pH's were confirmed after sterilization by testing the pH of one tube. All solutions maintained the pre-sterilization within 0.2 units. No additional pH adjustment was necessary.

One ml of inocula was dispensed into each test tube using a Eppendorf pipetter with sterile tips. The inocula was dispensed into the tubes immediately above the solution taking care not to touch the solution. The tip was changed for each concentration to ensure that there was no carryover of solution from a different concentration. Tips were changed if the solution was touched. Tubes were mixed on a vortex mixer. The initial optical densities were taken using one tube from each sodium benzoate concentration per pH level. The optical densities were determined by a Shimatzu double beam spectrophotometer at a wavelength of 630 nm. An uninoculated tube (at the concentration being tested) was used to zero the spectrophotometer.

The remaining six tubes (one uninoculated tube) were incubated for 24 hours at 30°C. The optical densities were recorded and the results corrected for the initial inocula density.

2.7 Statistical Analyses

Statistical examination of the data was conducted using the computer statistical package Systat for Windows™, Version 5. The statistical analyses conducted were Analysis of Variance (ANOVA) and pairwise comparisons were conducted by Bonferroni Adjustment. Analyses of variance which is a classical statistical technique for analysing data which has a quantitative dependent variable and a categorical independent variable was conducted on data collected for aerobic and anaerobic standard plate counts at normal and abusive storage temperatures using the Systat program (42). The ANOVA procedure compares differences in means (42). ANOVA techniques compute the variability of each dependent value score from the "grand mean" of scores (42).

A pairwise comparison of the data using Bonferroni's Adjustment provides information which identifies the statistical differences between preservatives. The ANOVA tells us there are differences in the data and that it is due to the preservative methods. However, it does not tell us where or why the differences with the data are occurring. The Bonferroni Adjustment is a strong asset in making comparisons among simple pairs of means (42). For example, we are evaluating a number of preservative methods and we want to determine

which preforms significantly better than the others. Therefore, we wish to compare differences among all possible pairs of level means. To achieve this end we can apply the Bonferroni Adjustment or Tukey HSD test. The Bonferroni Adjustment tends to be more rigorous than the Tukey HSD Test. Initially both the Bonferroni Adjustment and the Tukey HSD were conducted and there were no differences between the two results obtained. Thus the Bonferroni Adjustment is reported as it is considered the more rigorous of the two methods.

CHAPTER 3

RESULTS

All samples were stored at 20°C to approximate room temperature and 37°C as an abusive temperature. The choice of the 20°C temperature was based on the assumption that retail outlets would try to maintain their temperatures at or near 20°C. The abusive temperature of 37°C was chosen since cargo containers, retail storage areas, and the South Korean climate could reach temperatures near 37°C under certain conditions. Refrigerated samples were stored at 4°C (normal conditions) and 20°C (abusive conditions).

Samples were examined for chemical and microbiological quality over a four month time period. The samples were analysed at 0, 14, 28, 56, and 112 days. Raw material used in the experimental runs were also examined. Three samples were analysed in duplicate for all microbiological and chemical parameters.

3.1 Chemical Analyses

The chemical parameters examined were:

1. pH.
2. Salt.
3. Water activity.
4. Ash.
5. Moisture.
6. Fat.
7. Protein.

The results for the chemical analyses are located in Appendix A (Tables A1 - A14). The chemical parameters showed little or no variation over time (Table 3.1).

Table 3.1: Proximate Analyses Results

Parameter	Range	Mean ± Standard Deviation
pH	4.97 - 6.24	5.902 ± 0.134
Salt (%)	4.95 - 7.14	5.88 ± 0.35
Moisture (%)	74.56 - 77.52	74.93 ± 1.00
Water Activity	0.920 - 0.939	0.934 ± 0.003
Ash (%)	5.20 - 6.95	5.92 ± 0.49
Fat (%)	1.28 - 4.09	2.15 ± 0.41
Protein (%)	11.21 - 15.61	13.88 ± 0.81

3.2 Microbiological Analyses

The microbial analyses conducted were:

1) Aerobic plate counts.
2) Anaerobic plate counts.
3) Anaerobic sporulative counts.
4) Total coliform.
5) Fecal coliform.
6) Coagulase positive *Staphylococcus aureus*.
7) Salmonella species.
8) Bacillus cereus.
9) Listeria monocytogenes.

The results for all microbial analyses conducted are presented in Appendix B (Tables B1 - B16). *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* species, and *Escherichia coli* were not detected in any samples. This indicates that the lumpfish roe caviar was free of a majority of the major food pathogens. The only food pathogens of worry would be anaerobic forms such as *Clostrida* spp.. Total coliforms when detected were usually below 500 cfu per gram and not considered a problem.

3.2.1 Preservatives and Storage Conditions

Bacterial growth in caviare samples exhibited the same pattern of growth regardless of the preservative regime (Figures 3.1 - 3.8). Bacterial loads increased from the initial load (day 0) to reach maximum bacterial loads between 14-28 days, then fell back to levels near the initial loads. The final bacterial loads (day 112) were more varaible than any other day pattern on the bacterial growth curves. Some preservative regimes had final bacterial loads that were slightly higher than the initial bacterial loads, others had essentially the same final load as the initial load, and others were lower than the initial loads. There was no discernable pattern to the final loads based on storage temperature of preservative method.

Temperature preservation techniques (pasteurization and refrigeration) generally achieved the highest bacterial loads earlier in the growth curve than did samples that were chemically preserved. Samples stored at 20°C generally achieved the highest bacterial loads earlier than samples stored at 37°C.

Graphic presentation of results for aerobic and anaerobic plates counts (log transformed) are presented in Figures 3.1 - 3.8. These Figures show that pasteurization at 55°C and sodium benzoate (80 mg/kg) are not effective preservation methods with respect to either anaerobe or aerobic plates counts.

Figure Legend for Figure 3.1.

Figure Legend Identification	Explanation
Sodium Benzoate/Potassium Sorbate 3:1	Sodium Benzoate (300 ppm) and Potassium Sorbate (100 ppm) mixture.
Sodium Benzoate/Potassium Sorbate 1:1	Sodium Benzoate (200 ppm) and Potassium Sorbate (200 ppm) mixture.
Sodium Benzoate/Potassium Sorbate 1:3	Sodium Benzoate (100 ppm) and Potassium Sorbate (300 ppm) mixture.
Sodium Benzoate	Sodium Benzoate (80 ppm) - Present commercial product.

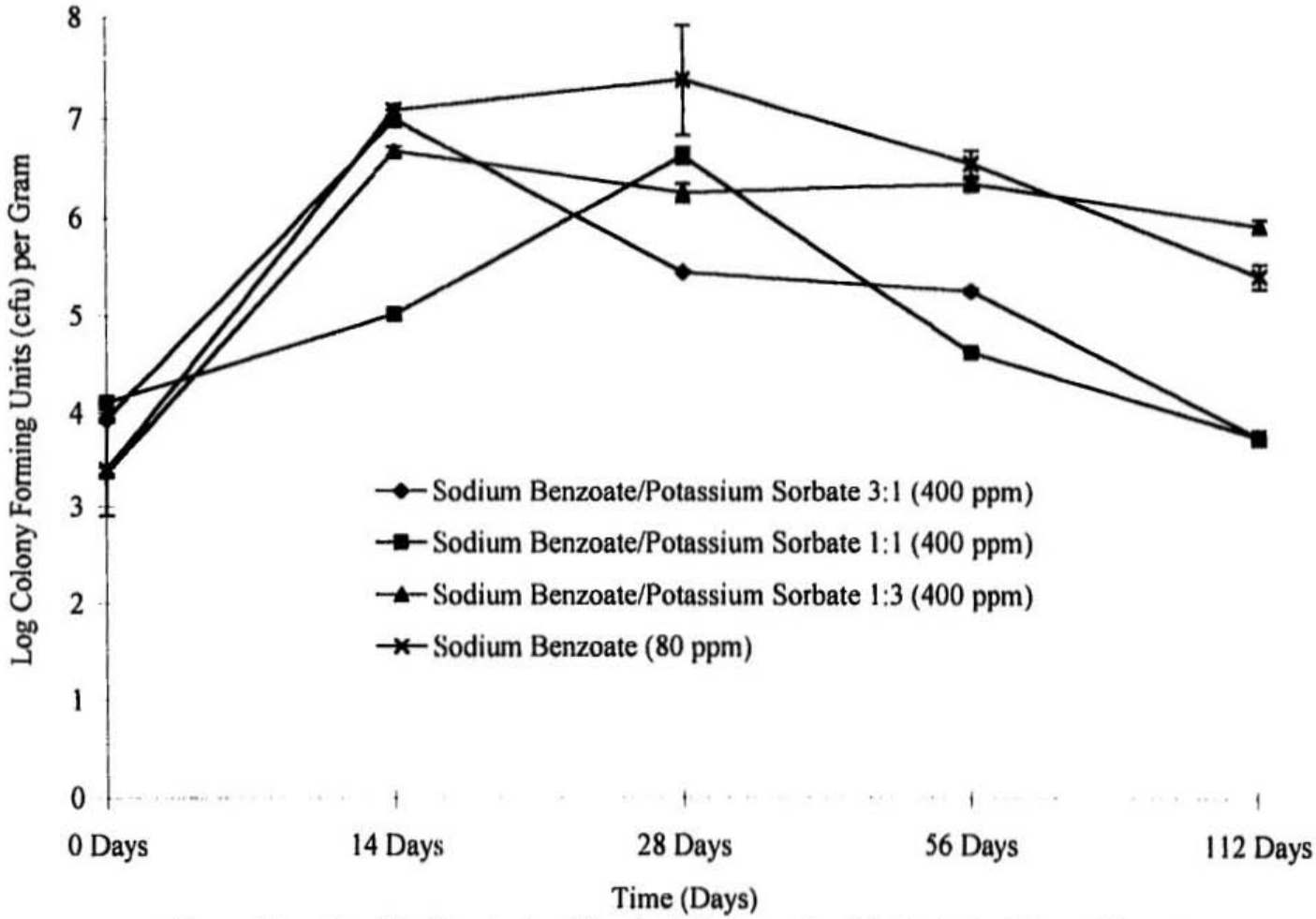

Figure 3.1: Aerobic Counts for Chemical Preservation Methods for Normal Storage Conditions.

Figure legend for Figure 3.2.

Figure Legend Identification	Explanation
Pasteurized 55°C	Samples were pasteurized at 55°C for 135 minutes.
Pasteurized 70°C	Samples were pasteurized at 70°C for 45 minutes.
Refrigerated	Samples were stored under refrigerated conditions at 4°C with neither pasteurization or chemical preservatives.

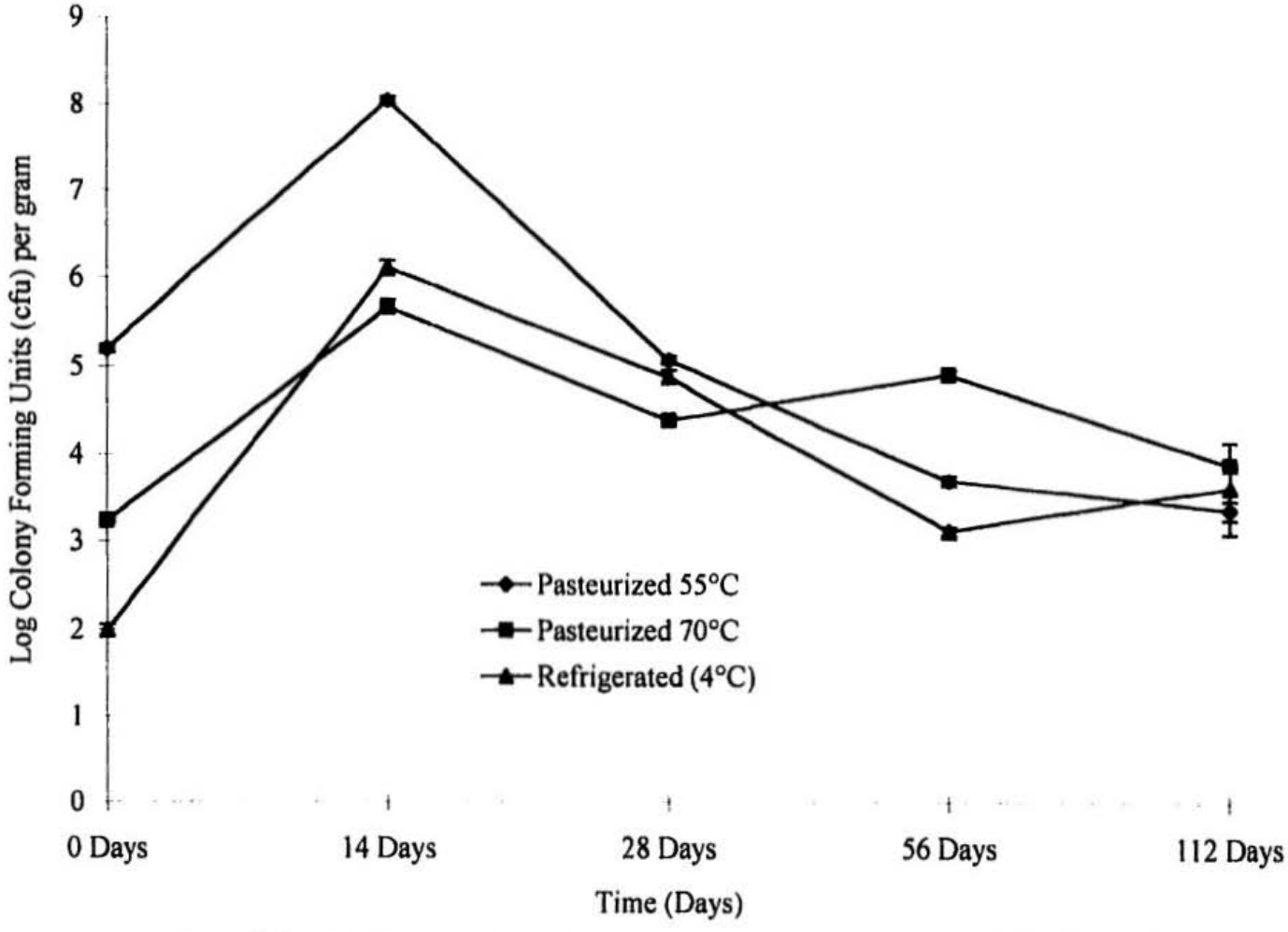

Figure 3.2: Aerobic Counts for Temperature Preservation Methods for Normal Storage Conditions.

Figure legend for Figure 3.3.

Figure Legend Identification	Explanation
Sodium Benzoate/Potassium Sorbate 3:1	Sodium Benzoate (300 ppm) and Potassium Sorbate (100 ppm) mixture.
Sodium Benzoate/Potassium Sorbate 1:1	Sodium Benzoate (200 ppm) and Potassium Sorbate (200 ppm) mixture.
Sodium Benzoate/Potassium Sorbate 1:3	Sodium Benzoate (100 ppm) and Potassium Sorbate (300 ppm) mixture.
Sodium Benzoate	Sodium Benzoate (80 ppm) - Present commercial product.

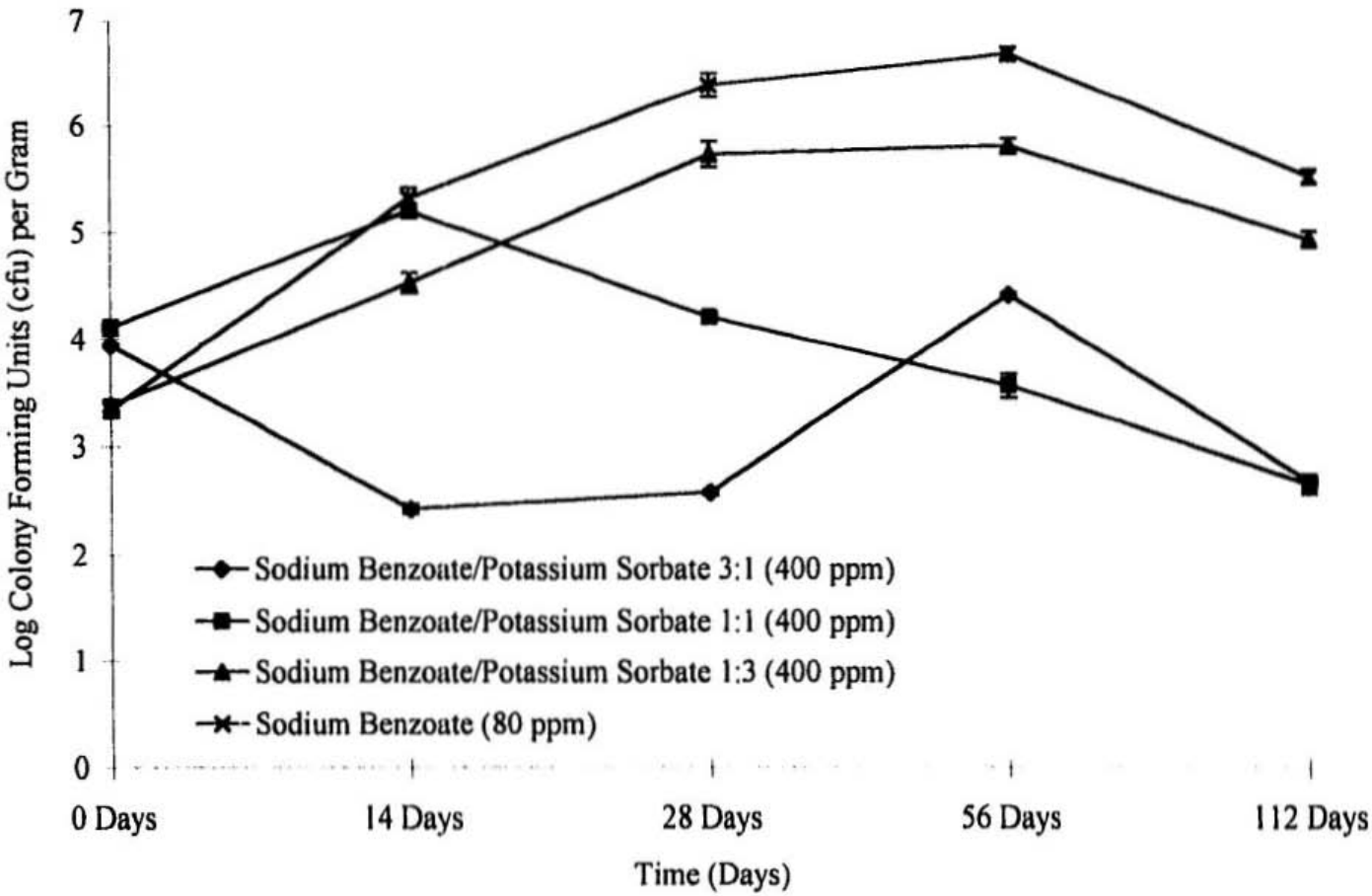

Figure 3.3: Aerobic Counts for Chemical Preservation methods for Abusive Storage Conditions.

Figure legend for Figure 3.4.

Figure Legend Identification	Explanation
Pasteurized 55°C	Samples were pasteurized at 55°C for 135 minutes.
Pasteurized 70°C	Samples were pasteurized at 70°C for 45 minutes.
Refrigerated	Samples were stored under refrigerated conditions at 4°C with neither pasteurization or chemical preservatives.

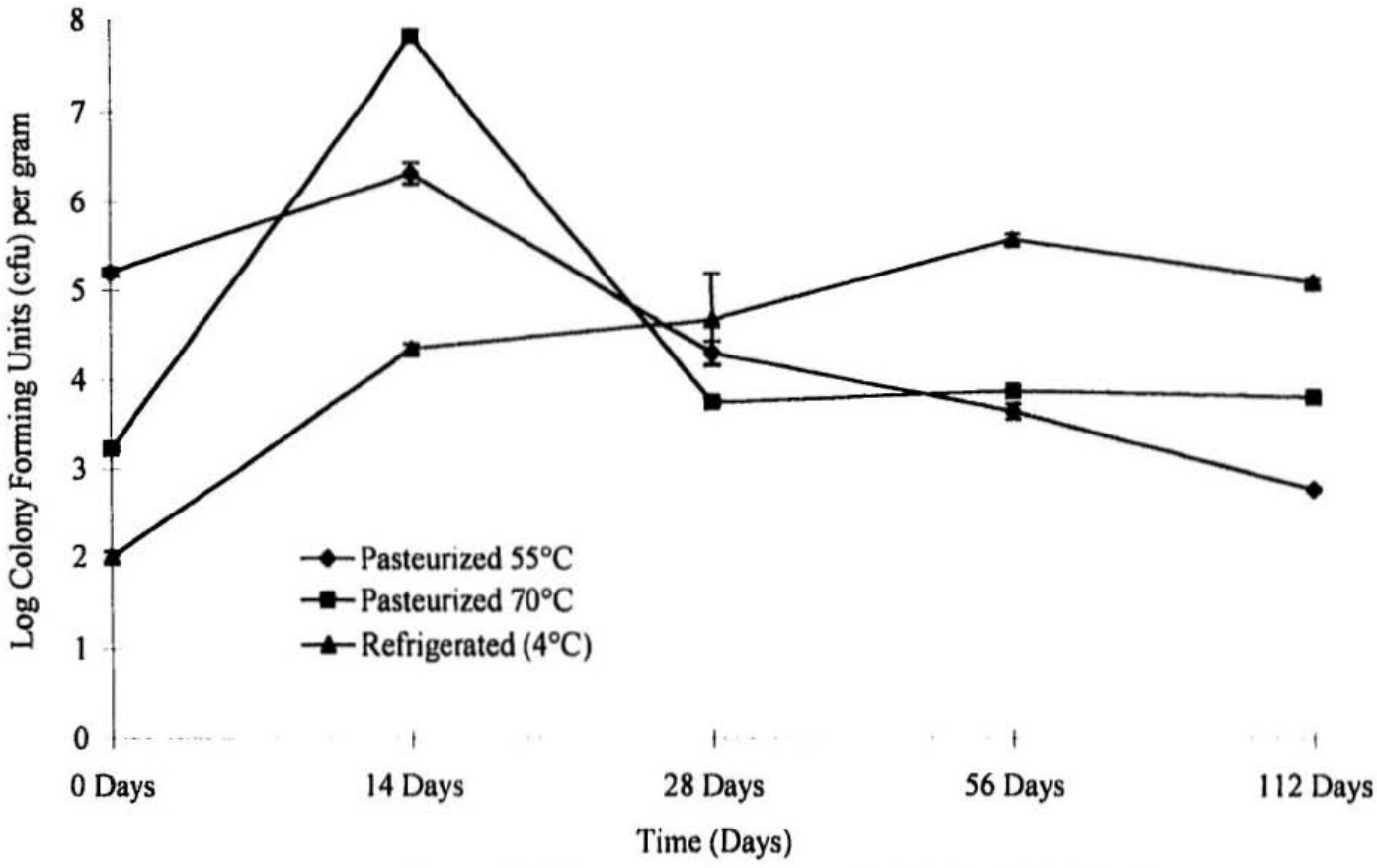

Figure 3.4: Aerobic Counts for Temperature Preservation Methods for Abusive Storage Conditions.

Figure legend for Figure 3.5.

Figure Legend Identification	Explanation
Sodium Benzoate/Potassium Sorbate 3:1	Sodium Benzoate (300 ppm) and Potassium Sorbate (100 ppm) mixture.
Sodium Benzoate/Potassium Sorbate 1:1	Sodium Benzoate (200 ppm) and Potassium Sorbate (200 ppm) mixture.
Sodium Benzoate/Potassium Sorbate 1:3	Sodium Benzoate (100 ppm) and Potassium Sorbate (300 ppm) mixture.
Sodium Benzoate	Sodium Benzoate (80 ppm) - Present commercial product.

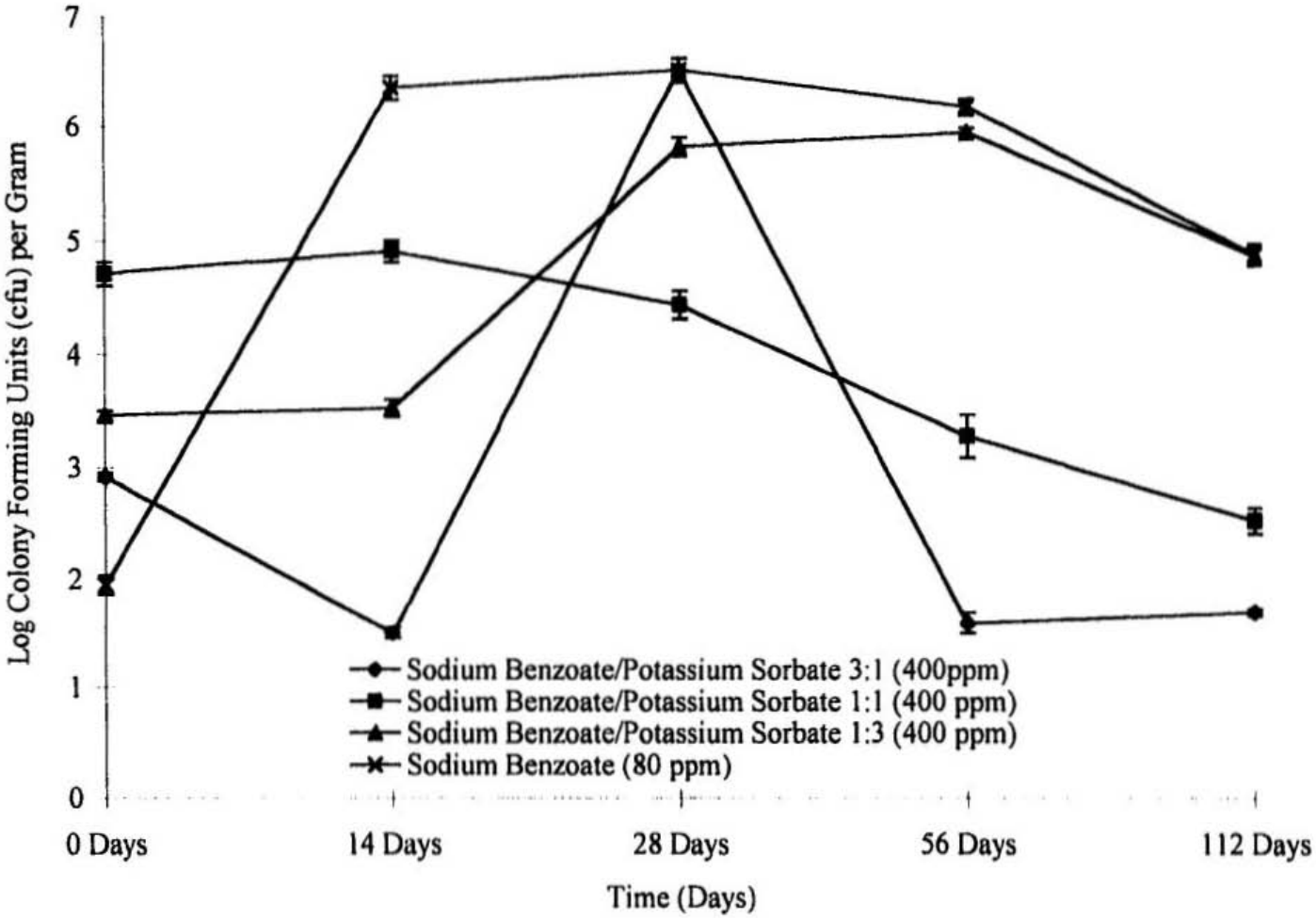

Figure 3.5: Anaerobic Counts for Chemical Preservation Methods for Normal Storage Conditions.

Figure legend for Figure 3.6.

Figure Legend Identification	Explanation
Pasteurized 55°C	Samples were pasteurized at 55°C for 135 minutes.
Pasteurized 70°C	Samples were pasteurized at 70°C for 45 minutes.
Refrigerated	Samples were stored under refrigerated conditions at 4°C with neither pasteurization or chemical preservatives.

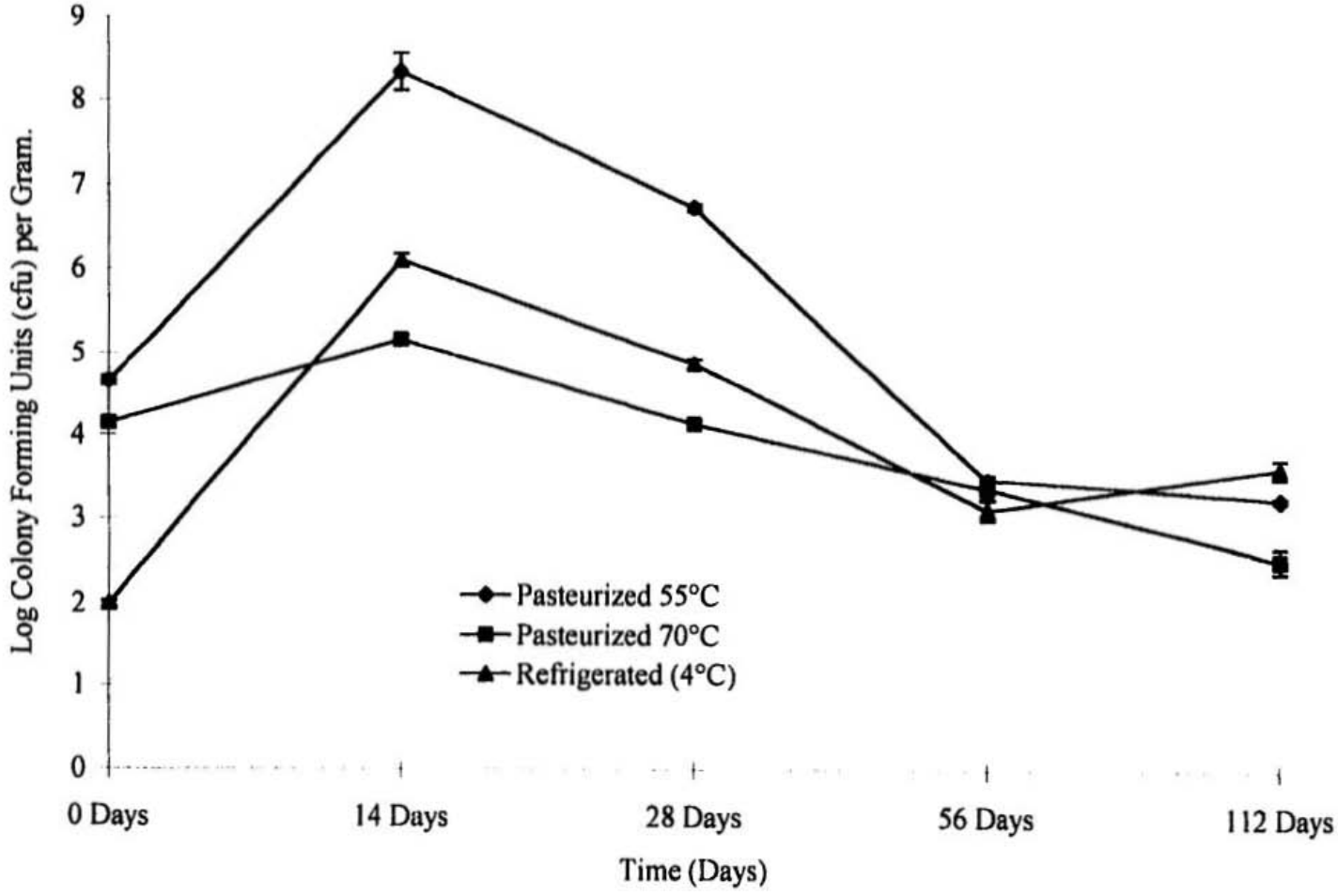

Figure 3.6: Anaerobic Counts for Temperature Preservation Methods for Normal Storage Conditions.

Figure legend for Figure 3.7.

Figure Legend Identification	Explanation
Sodium Benzoate/Potassium Sorbate 3:1	Sodium Benzoate (300 ppm) and Potassium Sorbate (100 ppm) mixture.
Sodium Benzoate/Potassium Sorbate 1:1	Sodium Benzoate (200 ppm) and Potassium Sorbate (200 ppm) mixture.
Sodium Benzoate/Potassium Sorbate 1:3	Sodium Benzoate (100 ppm) and Potassium Sorbate (300 ppm) mixture.
Sodium Benzoate	Sodium Benzoate (80 ppm) - Present commercial product.

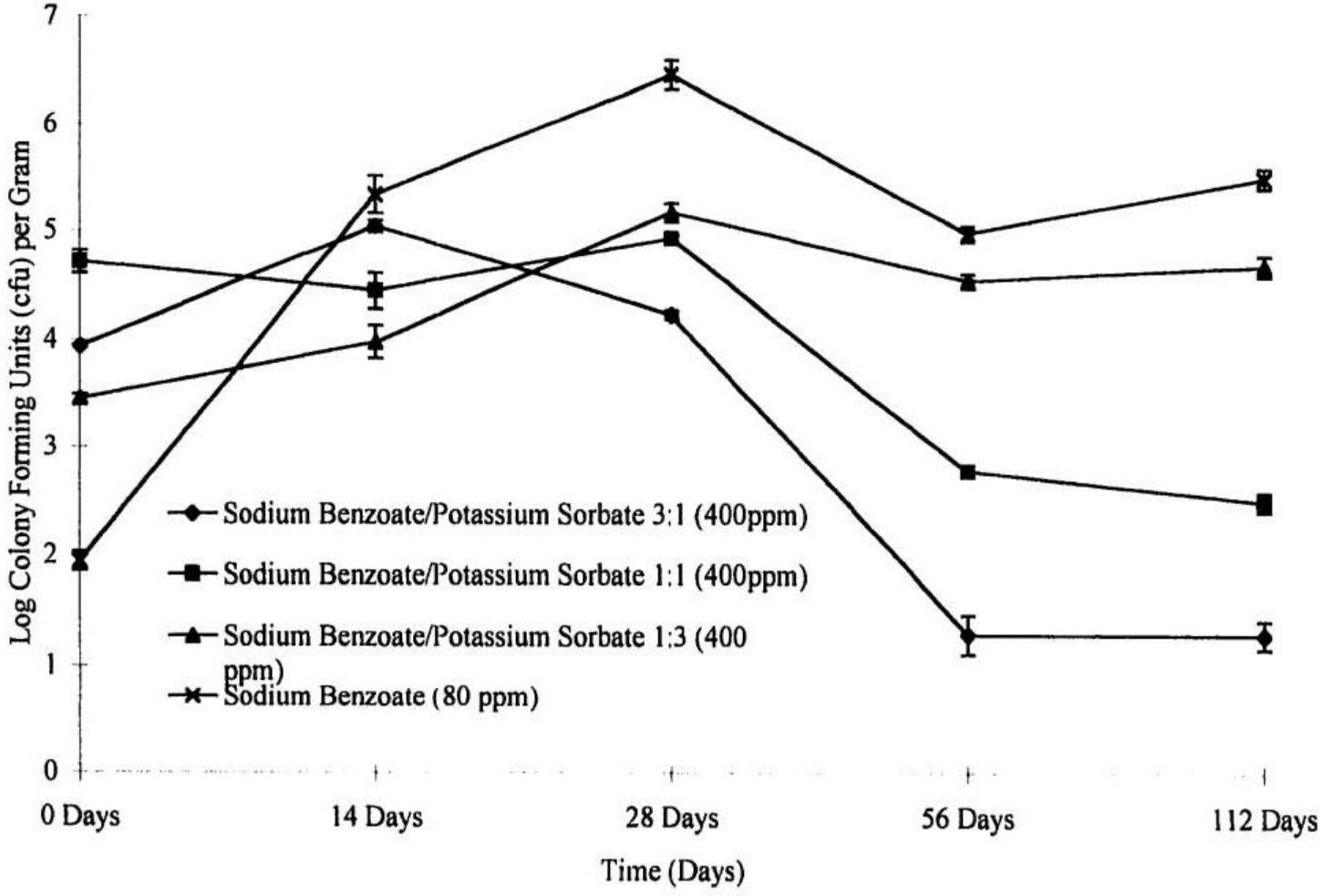

Figure 3.7: Anaerobic Counts for Chemical Preservation Methods for Abusive Storage Conditions.

Figure legend for Figure 3.8.

Figure Legend Identification	Explanation
Pasteurized 55°C	Samples were pasteurized at 55°C for 135 minutes.
Pasteurized 70°C	Samples were pasteurized at 70°C for 45 minutes.
Refrigerated	Samples were stored under refrigerated conditions at 4°C with neither pasteurization or chemical preservatives.

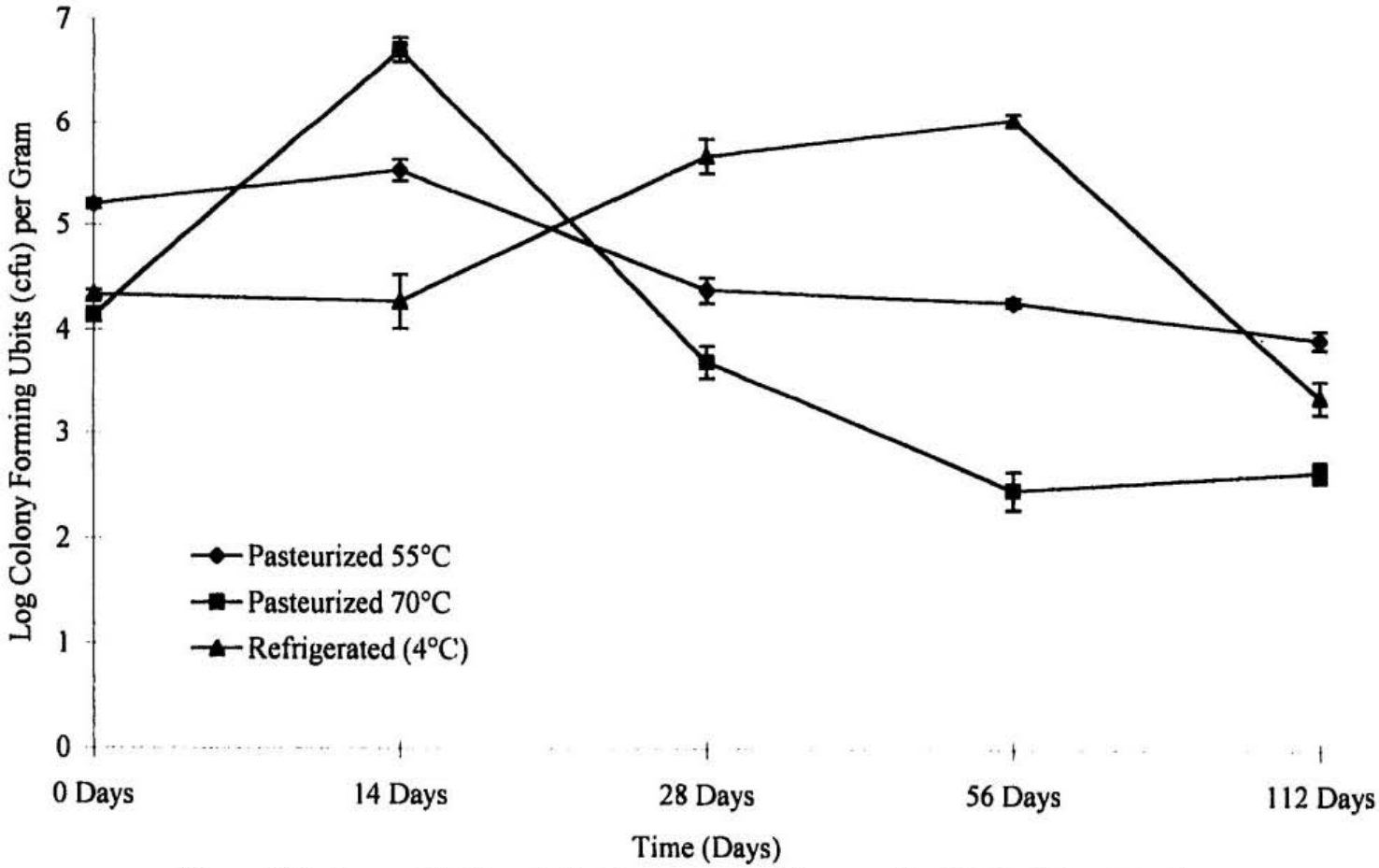

Figure 3.8 Anaerobic Counts for Temperature Preservation Methods for Abusive Storage Conditions.

3.2.2 Caviare Isolate Physical Parameters

There was one predominant bacteria type isolated from the caviar samples. The caviare isolate was purified and used in lab trials. The isolate grew at temperatures ranging from 5 °C to 45°C. (Figure 3.9). The isolates growth at 5°and 10°C was limited, although increasing the temperature from 5°C to 10°C result in a doubling of the optical density (approximately 0.01 for 5°and 0.02 for 10°C). Increasing the incubation temperature from 10° to 20°C also resulted in a doubling of the optical density from 0.02 at 10°C to 0.04 at 20°C. The largest increase in growth occurred between 20° and 35°C at which the optical density readings increased from approximately 0.04 to over 0.12. Optical density readings at 45°C was approximately 0.14, however there was a larger degree of error associated with this value than that of the value obtained at 35°C. This indicates that the optimum temperature for growth of the bacterial isolate is at or near 35°C.

The caviare isolate grew at salt concentrations ranging from 0% to 20% (Figure 3.10). Optimal growth occurred between 2% and 6%. Very limited, if any growth occurred at 22 and 24%. A steady decline in growth was observed from the 4% salt concentration with an optical density of 0.25 down to the 20% salt concentration which had an optical density of approximately 0.10. The isolate was a gram positive cocci which could utilize glucose, sucrose, and maltose as carbon sources.

Figure legend for Figure 3.9.

The caviare isolate was grown in test tubes containing 10 ml of trypticase soy broth under a variety of temperatures and growth was determined by optical density at 630 nm.

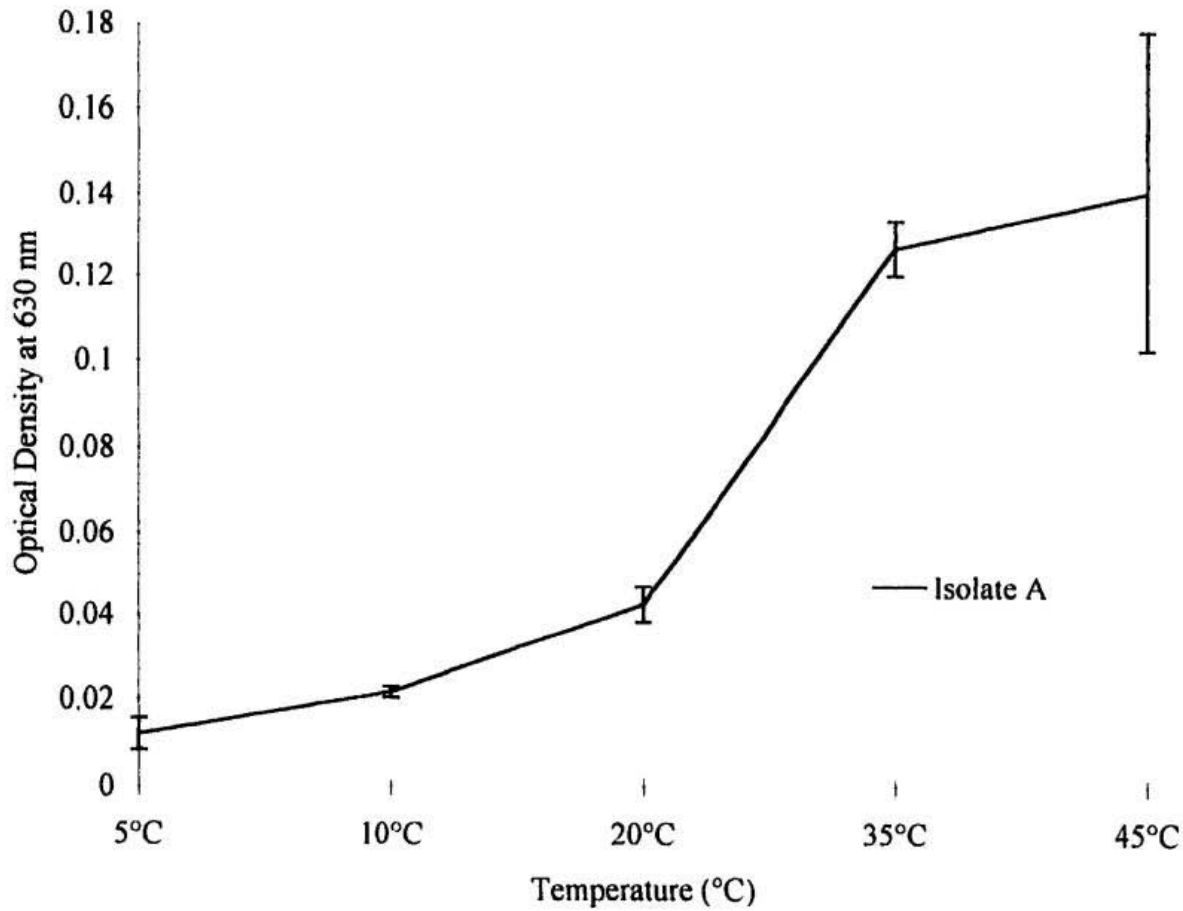

Figure 3.9: Temperature Growth Curve for Caviare Isolate

Figure legend for Figure 3.10.

The caviare isolate was grown in test tubes containing 10 ml of trypticase soy broth with various salt concentrations (0% to 24%) and growth was determined by optical density at 630 nm.

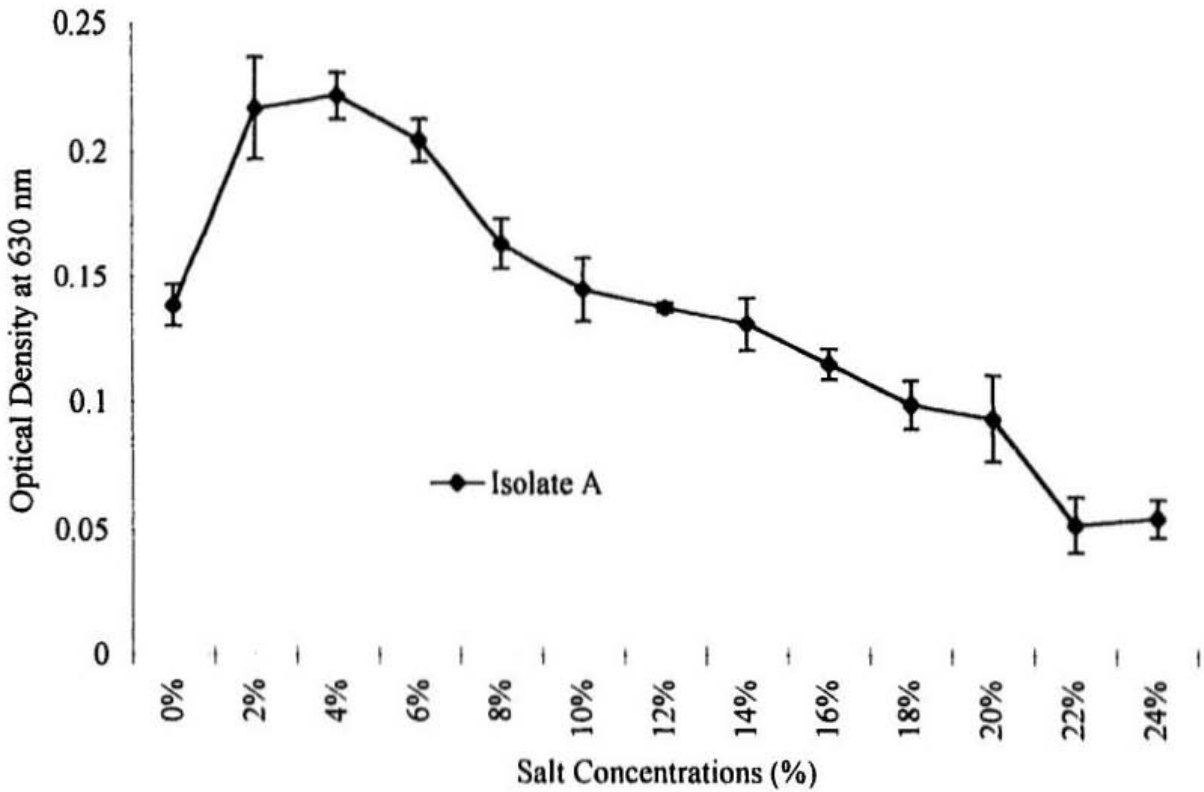

Figure 3.10: Growth Curve of Caviare Isolate in Various Salt Concentrations

3.2.3 Caviare Isolate Growth Profiles

The caviare isolate was used in laboratory tests to determine growth profiles in a variety of preservatives (sodium benzoate, potassium sorbate, and a combination of sodium benzoate and potassium sorbate at a 1:1 ratio). These profiles were conducted at 4 pH levels (pH 4, 5, 6, and 7). The different pH levels were incorporated into these laboratory trials to determine the pH level for which the preservative would be the most effective.

The growth curves for sodium benzoate (Figure 3.11) at pH 6.0 and 7.0 were flat indicating that sodium benzoate had no effect on the caviare isolate at these pH levels. There was limited bacterial reduction at pH 5.0 at concentrations above 500 ppm sodium benzoate. Bacterial loads were halved at 250 ppm sodium benzoate (pH 4.0), a 2/3 reduction was observed at 500 ppm (pH 4.0) and almost total inhibition was observed for the 750 and 1000 ppm levels of sodium benzoate at pH 4.0. The growth profile for sodium benzoate indicated that this preservative was the most effective at pH 4.0 and at levels above 500 ppm (figure 3.11). The average pH level for the caviare was 5.9 and sodium benzoate was found to have little or no effect at this level (figure 3.11).

The growth curves for potassium sorbate (Figure 3.12) at pH 5.0, 6.0 and 7.0 were flat indicating that potassium sorbate had no effect on the caviare isolate at these pH levels. Bacterial loads were almost totally inhibited at 250 ppm potassium sorbate (pH 4.0) and

Figure legend for Figure 3.11.

The caviare isolate was grown in test tubes containing 10 ml of trypticase soy broth with a variety of sodium benzoate concentrations and at various pH's. Growth was determined by optical density at 630 nm.

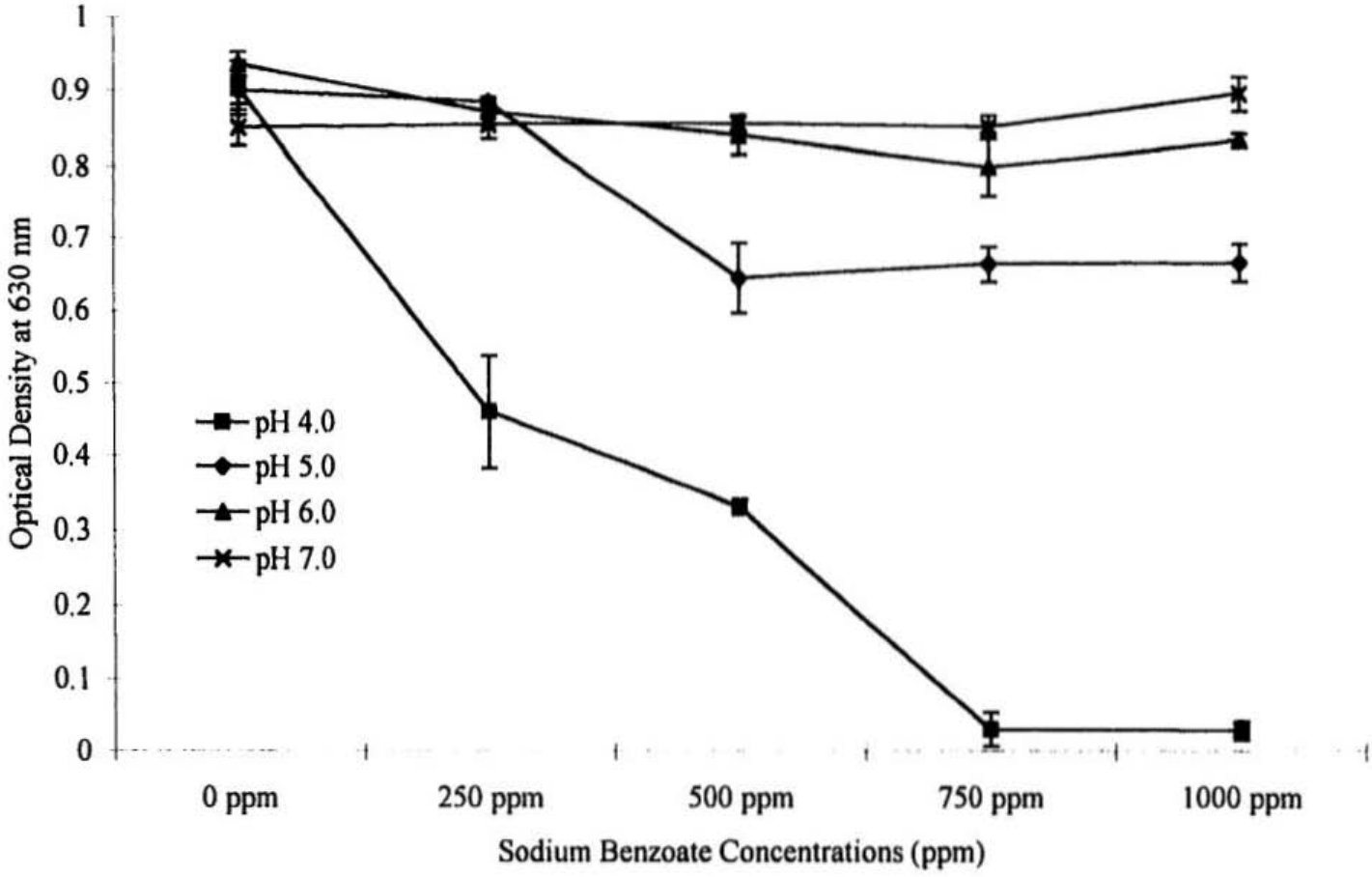

Figure 3.11: Growth Curve of Caviare Isolate in Sodium Benzoate

Figure legend for Figure 3.12.

The caviare isolate was grown in test tubes containing 10 ml of trypticase soy broth with a variety of potassium sorbate concentrations and at various pH's. Growth was determined by optical density at 630 nm.

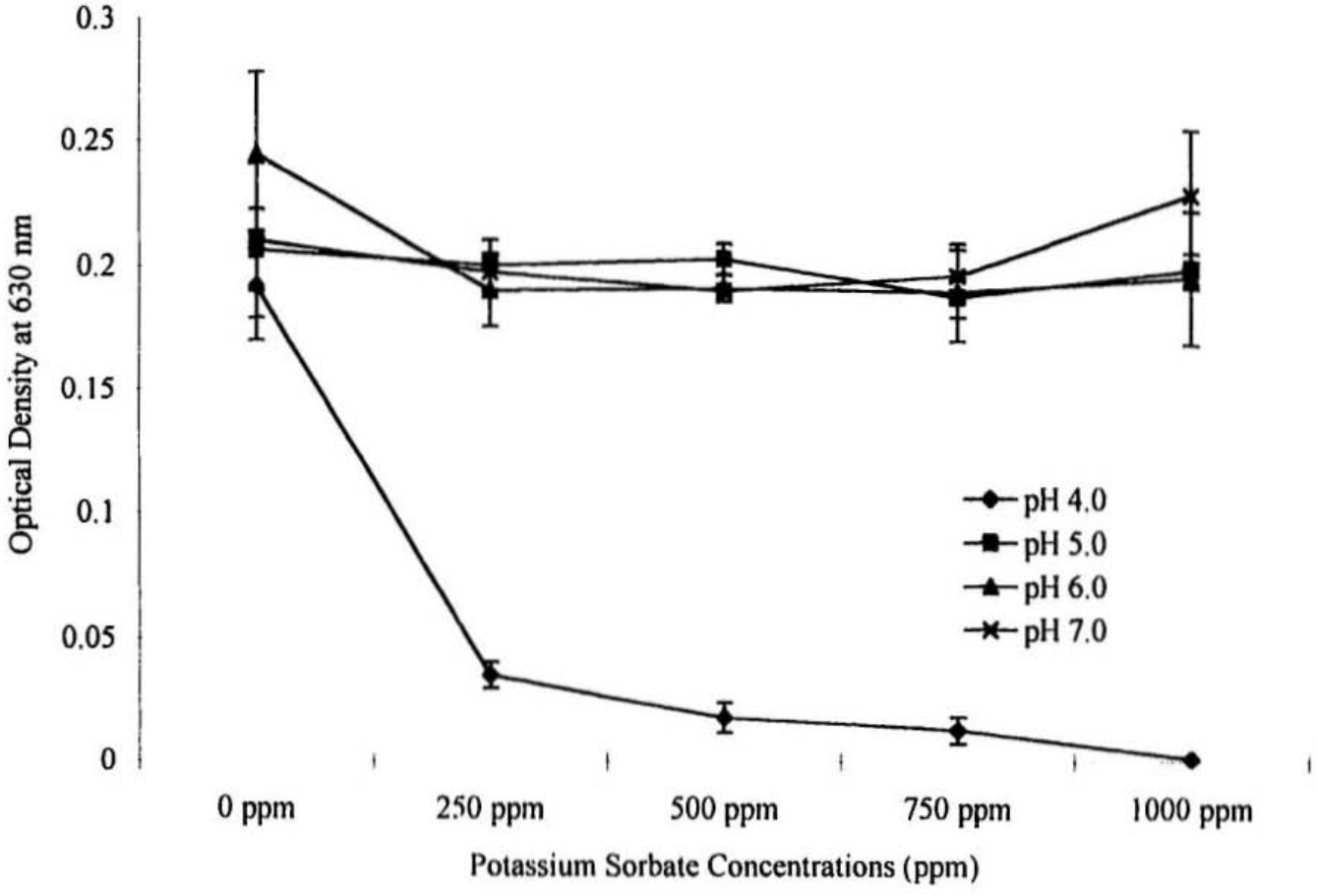

Figure 3.12: Growth Curve of Caviare Isolate in Potassium Sorbate

were totally inhibited by 1000 ppm (potassium sorbate at pH 4.0). The growth profile for potassium sorbate indicated that this preservative is the most effective at pH 4.0 and at levels of 250 ppm or above (figure 3.12). The average pH level for the caviare was 5.9 and potassium sorbate was found to have little or no effect at this level (figure 3.12).

The growth curves for sodium benzoate/potassium sorbate mixture at a 1:1 ratio (Figure 3.13) for pH 6.0 and 7.0 were flat indicating that preservative mixture had no effect on the caviare isolate at these pH levels. Bacterial loads were cut by 1/3 by the preservative mixture at 250 ppm (pH 5.0) and further reduced by at 750 and 500 ppm. Bacterial loads were reduced by 2/3 at 250 ppm and almost totally inhibited at 1000 ppm by the preservative mixture at pH 4.0. The growth profile for the preservative mixture indicated that this combination was the most effective at pH 4.0 and at levels above 500 ppm (figure 3.13). However the observed effect may in reality be due to the action of potassium sorbate and not the mixture. The average pH level for the caviare was 5.9 and the preservative mixture was found to have little or no effect at this level (figure 3.13).

3.2.4 Statistical Results for Preservatives and Storage Conditions

The ANOVA results are presented in Table 3.2 and a statistically significant difference was detected between preservation methods. The ANOVA results indicated that there was a significant difference between the ability of the different preservatives to control microbial

Figure legend for Figure 3.13.

The sodium benzoate/potassium sorbate mixture was prepared at a ratio of 1:1, thus if the concentration of the mixture was 250 ppm, then 125 ppm of sodium benzoate and 125 ppm of potassium sorbate was used. Growth was determined by optical density at 630 nm. The mixture concentrations used were as follows:

Concentration	Components
0 ppm	0 ppm sodium benzoate, 0 ppm potassium sorbate
250 ppm	125 ppm sodium benzoate, 125 ppm potassium sorbate
500 ppm	250 ppm sodium benzoate, 250 ppm potassium sorbate
750 ppm	375 ppm sodium benzoate, 375 ppm potassium sorbate
1000 ppm	500 ppm sodium benzoate, 500 ppm potassium sorbate

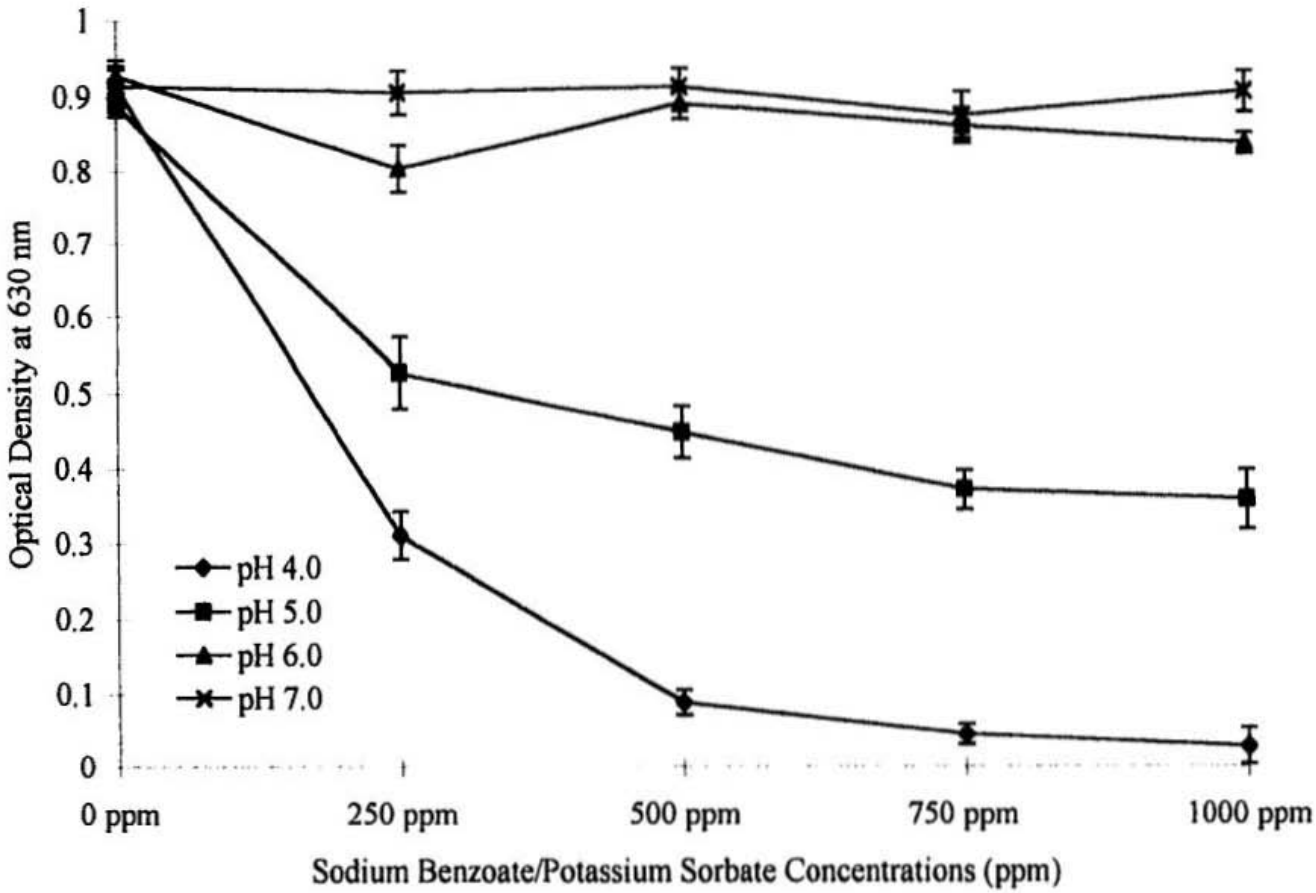

Figure 3.13: Growth Curve for Caviare Isolate in Sodium Benzoate and Potassium Sorbate Mixture

growth but it did not indicate which preservatives were responsible for the observed differences.

Table 3.2: ANOVA Results for Aerobic and Anaerobic Standard Plate Counts.

Analysis	F Ratio	Probability	Interpretation
Anaerobic (Normal Temperature)	5.136	0.000	Significant
Anaerobic (Abusive Temperature)	3.639	0.003	Significant
Aerobic (Normal Temperature)	4.154	0.001	Significant
Aerobic (Abusive Temperature)	5.132	0.000	Significant

The results of the Bonferroni Adjustment were that significant differences were detected between the following preservative methods:

1) Anaerobic Standard Plate Count Stored at Normal Temperatures;

- Pasteurization (55°C) and Sodium Benzoate/Potassium Sorbate Mixture (3:1 ratio);
- Sodium Benzoate (80 ppm) and Sodium Benzoate/Potassium Sorbate Mixture (3:1 ratio)differed significantly ; and
- Sodium Benzoate/Potassium Sorbate Mixture (1:3 ratio) and Sodium Benzoate/Potassium Sorbate Mixture (3:1 ratio) differed significantly.

2) Anaerobic Standard Plate Count Stored at Abusive Temperatures;

- Pasteurization (55°C) and Sodium Benzoate/Potassium Sorbate Mixture (3:1 ratio) differed significantly;
- refrigeration and Sodium Benzoate/Potassium Sorbate Mixture (3:1 ratio) differed significantly; and
- Sodium Benzoate (80 ppm) and Sodium Benzoate/Potassium Sorbate Mixture (3:1 ratio) differed significantly.

3) Aerobic Standard Plate Count Stored at Normal Temperatures; and

- Pasteurization (70°C) and Sodium Benzoate (80 ppm) differed significantly;
- Refrigeration (4°C) and Sodium Benzoate (80 ppm) differed significantly; and
- Refrigeration (4°C) and Sodium Benzoate/Potassium Sorbate Mixture (1:3 ratio) differed significantly.

4) Aerobic Standard Plate Count Stored at Abusive Temperatures.

- Sodium Benzoate (80 ppm) and Sodium Benzoate/Potassium Sorbate Mixture (1:1 ratio) differed significantly;
- Sodium Benzoate (80 ppm) and Sodium Benzoate/Potassium Sorbate Mixture (3:1 ratio) differed significantly; and

- Sodium Benzoate/Potassium Sorbate Mixture (1:3 ratio) and Sodium Benzoate/Potassium Sorbate Mixture (3:1 ratio) differed significantly.

The statistical results compared well to general trend observations from the graphical presentation of the results. The statistical analyses supported the observation that the best overall preservative method is the chemical preservative mixture of sodium benzoate (300 ppm) and potassium sorbate (100 ppm). Pasteurization (55°C) and Sodium Benzoate 80 ppm (commercial product) seemed to provide the lowest amount of microbial inhibition. Significant differences were not detected (pairwise comparisons) for a variety of preservatives and the differences detected did not always show patterns between analyses and storage temperatures. The pairwise comparison probabilities are presented in Appendix C and probabilities ≤ 0.050 are considered significant.

3.2.5 Statistical Results for Bacterial Isolate Physical Parameters

Analyses of variance was conducted on data collected for the bacterial isolate growth profiles for temperature and salt concentrations. The ANOVA results are presented in Table 3.3 and a statistically significant difference was detected for the growth at various temperatures and salt concentrations.

Table 3.3: ANOVA Results for Temperature and Salt Growth Profiles of Bacterial Isolate.

Physical Characteristics	F Ratio	Probability	Interpretation
Temperature Ranges	60.311	0.000	Significant
Salt Concentrations	124.976	0.000	Significant

The ANOVA results indicated that there was a significant difference between the ability of the caviare isolate to grow at different temperatures and salt concentrations but it did not indicate which temperatures and salt concentrations were responsible for the observed differences. A pairwise comparison of the data using Bonferroni's Adjustment detected statistical differences between the following temperatures and salt concentrations for the associated growth profiles:

1) Temperature
    - 5°C, 10°C and 20°C versus 35°C and 45°C were significantly different.

2) Salt Concentrations.
    - 0 ppm versus 2, 4, 6, 8, 18, 20, 22, and 24 ppm were significantly different;
    - 2 ppm versus 8 thru 24 ppm were significantly different;
    - 4 ppm versus 8 thru 24 ppm were significantly different;
    - 6 ppm versus 8 thru 24 ppm were significantly different;
    - 8 ppm versus 10 thru 24 ppm were significantly different;
    - 10 ppm versus 16 thru 24 ppm were significantly different;

- 12 ppm versus 18 thru 24 ppm were significantly different;
- 14 ppm versus 18 thru 24 ppm were significantly different;
- 16 ppm versus 22 and 24 ppm were significantly different;
- 18 ppm versus 22 and 24 ppm were significantly different; and
- 20 ppm versus 22 and 24 ppm were significantly different.

The statistical results compared well to general observations obtained from the graphical presentation of the results. The statistical analyses supported the observation that the caviare isolate optimal growth occurred at temperatures of 35°C and 45°C. Although the caviare isolate grew at salt concentrations ranging from 0% to 20%, statistical analyses supported the observation that optimal growth occurred between 2% and 6% salt. Statistical analyses supported the observation that limited, if any growth occurred at 22 and 24%. Statistical analyses indicated that although the caviare isolate grew in broth with no salt, the addition of minimal salt (2%) stimulated its growth significantly. The pairwise comparison probabilities are presented in Appendix C and probabilities ≤ 0.050 are considered significant.

3.2.6 Statistical Results for Caviare Isolate Growth Profiles

Analyses of variance was conducted on data collected for the caviare isolate growth profiles for various chemical preservative concentrations and under various pH regimes.

The ANOVA results are presented in Table 3.4 and a statistically significant differences were detected for the various chemical preservative preservatives.

Table 3.4: ANOVA Results for Aerobic and Anaerobic Standard Plate Counts.

Affect	F Ratio	Probability	Interpretation
pH Affect			
Sodium Benzoate	38.681	0.000	Significant
Potassium Sorbate	42.073	0.000	Significant
Potassium Sorbate/Sodium Benzoate Mixture (1:1 ratio)	55.259	0.000	Significant
Preservative Concentration Affect			
Sodium Benzoate	5.584	0.000	Significant
Potassium Sorbate	5.170	0.001	Significant
Potassium Sorbate/Sodium Benzoate Mixture (1:1 ratio)	5.479	0.001	Significant

The ANOVA results indicated that there were significant differences between the affect of the preservative concentrations and the affect of the preservative at various pH levels on the growth of the caviare isolate but it did not indicate which preservative concentrations or pH levels were responsible for the observed differences. A pairwise comparison of the data using Bonferroni's Adjustment detected the following statistical differences between the pH's and the preservative concentrations:

1) pH Effect on the Preservative

- Potassium Sorbate and the Potassium Sorbate/Sodium Benzoate Mixture both exhibited significant differences between pH 4.0 versus pH 5.0, 6.0 and 7.0; and pH 5.0 versus pH 6.0 and 7.0.
- Sodium Benzoate exhibited significant differences between pH 4.0 versus pH 5.0; and pH 5.0 versus pH 6.0 and 7.0.

2) Preservative Concentration Effects.

- Sodium Benzoate exhibited significant differences between 1000 ppm versus 0 and 250 ppm.
- Potassium Sorbate and the Potassium Sorbate/Sodium Benzoate Mixture both exhibited significant differences between 0 ppm and 250, 500, 750, and 1000 ppm.

The statistical results compared well to general observations obtained from the graphical presentation of the results. The statistical analyses supported the observation that sodium benzoate, potassium sorbate and the sodium benzoate/potassium sorbate mixture were the most effective at pH 4.0. Potassium sorbate and the potassium sorbate/sodium benzoate mixtures were also found to be effective at pH 5.0. Figures 3.12 and 3.13 illustrates that these chemical were not as effective at pH 5.0 as they were at pH 4.0.

The statistical analyses found that sodium benzoate was effective at concentration levels above 750 ppm. Examination of Figure 3.12 would suggest that sodium benzoate was effective at levels above 500 ppm. This suggest that although there is reduction at 500 ppm sodium benzoate, the observed reduction is not significant. The statistical analyses found that both potassium sorbate and the sodium benzoate/potassium sorbate mixture was effective at 250 ppm or above. These results compared well to the graphical observations.

The statistical results and graphical presentation of the data found that the sample pH is the primary factor affecting the preservative effectiveness. This was expected since sodium benzoate is effective up to pH 4.0 and is not recommended for use over pH 4.5; and potassium sorbate is effective up to pH 6.0-6.5 and exhibits optimal effectiveness at or below pH 6.0 (5). The pH of the caviare was 5.9, thus sodium benzoate would have little or no effect and potassium sorbate would be more effective.

The pairwise comparison probabilities are presented in Appendix C (C1-C12) and probabilities of ≤ 0.050 are considered significant.

3.2.7 Caviare Isolate Characterization

Caviare isolate characterization was not one of the objectives of this project, however there was one predominant isolate and a preliminary characterization was undertaken. A

second specimen was isolated during week one analyses, however it did not take to purification and isolation procedures suggesting that there were some micronutrients required for its growth that were contained within the caviar but not the bacterial media. Microscopic examination suggested it was an Actinomycetes species (26) and it is suspected that this was the organism which has been found to occur in seafood samples occasionally (27). This isolate was black in colour on Baird-Parker media and grey and Standard plate count with a rocket shaped appearance.

The caviare isolate was subjected to biochemical characterization to help classify the organisms. All analyses were conducted in triplicate and isolates from both aerobic and anaerobic conditions were examined. Table 3.5 presents the results of the preliminary biochemical characterization. No positive identification can be made based on these results, however it is known that the isolate is a gram positive cocci, facultative anaerobe, mesophyll which can grow in elevated salt concentrations and exhibits reduced growth under anaerobic conditions (colony size is reduced). The colonies are ovoid, mucoid with a regular edge and some elevation. The colonies are cream in color on most commonly used media.

Table 3.5: Biochemical Characterization of the Caviare Isolate.

Parameter	Response
Gram Stain	Positive
Shape	Cocci
Spores	Negative
Growth	Aerobic/Anaerobic
Colony Morphology	Opaque cream with regular edges, convex
Biochemical Analyses	
Citrate fermentation	Negative
Glucose fermentation	Positive
Saccharose (sucrose) fermentation	Positive
Mannitol fermentation	Negative
Inositol fermentation	Negative
Sorbitol fermentation	Negative
Rhamnose fermentation	Negative
Esculin fermentation	Negative
Arabinose fermentation	Negative
Maltose fermentation	Positive
Melibiose fermentation	Negative
O-nitrophenyl-β-d-galactoside	Negative
Arginine dihydrolase	Positive
Lysine decarboxylase	Negative
Ornithine decarboxylase	Negative
Hydrogen Sulfide Production	Negative
Urea hydrolysis	Positive
Indole production	Negative
Vogues Proskauer test	Positive
Gelatin hydrolysis	Positive
Amygdalin fermentation	Negative
$NO_3 \rightarrow NO_2$ reduction	Positive
$NO_3 \rightarrow N_2$ reduction	Negative

CHAPTER 4

DISCUSSION

Popularity, high prices and a growing demand led to the development of caviare substitutes. Lumpfish caviare became one of the most popular substitutes resulting in a growing demand for lumpfish resources. Newfoundland became one of the leading producers of lumpfish roe but secondary processing of lumpfish caviare is limited.

Lumpfish caviare destined for the retail market is expected to have a shelf-life of at least one year (12, 14). Extending the shelf-life of a product is achieved through preservation techniques used individually or in combination. The choice of preservative methods are often dictated by regulatory and/or buyers requirements.

4.1 Chemical Composition

Commercial samples of lumpfish roe caviar produced outside of Newfoundland were examined by Department of Fisheries (10). The salt content ranged from 4.49-12.06% and the pH ranged from 4.9 to 6.0 (10). The caviare produced by North Atlantic Packaging was found to be similar to other commercial brands.

Cantoni et al. (4) found the following values for the chemical parameters in lumpfish roe caviar produced in Iceland, Germany and Denmark:

1) Moisture (%) 71 - 75.
2) Ash (%) 4 - 7.
3) Fats (%) 2 - 6.8.
4) Proteins (%) 14 - 16.

The caviare used in this study was found to be comparable to the European products for chemical composition.

4.2 Microbiological Quality

The best overall method with respect to microbial quality appears to be pasteurization at 70°C. However, pasteurization at this temperature produced a poor quality product with respect to appearance. The eggs were dried out and clumped together. Water originally contained within the eggs had pooled in the bottom of the jar producing overall poor aesthetical quality and appearance. Although the pasteurization temperature regime of 70°C for 45 minutes was based on commercial pasteurization used by Romanoff (10), it is a well known fact that at 60°C certain undesirable irreversible changes occur (12). Iredale and York reported that changes occur at temperatures as low as 55°C (20).

Commercial caviare used in this study was preserved with 80 ppm sodium benzoate and this method was found to be the least effective of the preservative methods examined. A review of all the figures indicates that the best overall preservative method appears to be a mixture of sodium benzoate (300 ppm) and potassium sorbate (100 ppm). The mixture of sodium benzoate (200 ppm) and potassium sorbate (200 ppm) provided similar results. However, no method completely inhibited growth of organisms in the caviare samples. The study results indicated that refrigeration of sample limited the growth of bacteria better than some of the chemical preservative methods employed such as sodium benzoate (80 ppm), and sodium benzoate/potassium sorbate mixture at a 3:1 ratio.

Laboratory experiments using the caviare isolate in solutions containing various levels of sodium benzoate, potassium sorbate and a mixture of sodium benzoate and potassium sorbate at different pH was examined. The results concurred with common knowledge on the effectiveness of these chemical preservatives. Sodium benzoate was effective against the caviare isolate, primarily at pH 4.0 and above 500 ppm. Limited effectiveness was observed at 250 ppm. Research by various researchers has found that the inhibitory action of benzoic acid against microorganisms varied from 20-1800 ppm (6). The inhibitory effect is dependant upon the organism and the product pH.

Potassium sorbate was effective against the caviare isolate at pH 4.0 and at concentrations of 250 ppm and above. The sodium benzoate/potassium sorbate mixture at

a ratio of 1:1 was most effective at pH 4.0 and at concentration levels of 500 ppm. Some effect was observed at pH 5.0 and a limited effect was observed at pH 6.0.

The caviare isolate grew well at salt concentrations of 2-6% and exhibited minor growth at salt concentrations of 18% and above (Figure 3.10). The caviare isolate exhibited slight growth at 5°C (figure 3.9) optimal growth at around 35°C, and the ability to grow at 45°C..

There is limited published research readily available into the microbial quality of caviare (12, 14, 17, 30, 38). The microbial research available from outside of North American often can not be equated or are extremely difficult to equate with standard North American practices (38). It is suspected that commercial producers have unpublished information available, the vast majority of which would be proprietory and confidential information which is not available to the public. The majority of publish literature deals with the chemical composition of caviare (5, 12, 14, 13, 16, 24, 28, 30, 38, 41) and caviare processing techniques (10, 12, 13, 14, 20, 21, 22, 25, 28, 40, 41).

4.3 Product Quality

A product's shelf-life is dependant upon the initial product quality, additive sterility, adherence to production processes and storage quality. The microbial quality can be measured by total plate counts which are an internationally recognized method (14). No

total plate count limits have been established for caviare. Nevertheless, caviare with total plate counts of 10^4-10^6 are an indication of low quality product (14). The highest grades of caviare tend to have a total plate count of $\leq$ 50 per gram (14).

The results indicate that the caviare produced was of low quality based on the criteria cited in Duncan (14). Raw product (no preservatives, dyes and/or additives) had total plate counts in the 10^2-10^4 range. Research on the lumpfish roe (10) during curing found total plate counts ranged from 10^3 to 10^5 and cured product was in the range of 10^3 to 10^4. This indicated that the raw product obtained from a variety of different processors was of low quality prior to secondary processing. The finished product (containing preservatives, dyes and/or additives) had total plate counts in the 10^2-10^5 range.

An examination of the raw and finished product microbial quality indicated that the addition of preservatives, additives (salt, spices) and/or dyes may have contributed to the microbial load by a factor of 10^1-10^3. This coupled with the salt tolerance of the caviare isolate which was responsible for approximately 90% of isolated organisms indicated that the salt may be responsible for some of the microbial load.

Only pasteurized caviare tolerates room temperatures for short periods of time (14). Duncan (14) states that the best storage temperatures for caviare product are those below freezing and short periods of abusive temperatures may trigger spoilage due to microbial

growth. Constant refrigeration at the lowest possible temperature should be applied to caviare regardless the preservative method (chemical or pasteurization). The practice of refrigeration regardless the preservative method is not practised by the commercial producers. A vast majority of lumpfish caviare sold in North America is preserved with sodium benzoate and does not indicate that the product should be refrigerated.

Sternin and Hori (34) during a salmon caviare seminar indicated that the shelf-life of salmon caviare depends upon the product (non-preserved, preserved chemically or pasteurized and how it is stored. They suggest that caviare is normally stored refrigerated, salmon caviare anyway. Examination of commercially produced lumpfish caviare indicates that it is seldom stored refrigerated and many brands do not indicate a requirement for refrigeration on the label. The shelf-life of salmon caviare (34) can vary from 24 months for pasteurized caviare stored at -2 to -4°C to 3-4 months for pasteurized caviare stored at 10-18°C. Salmon caviare un-preserved and stored at -2 to -4°C has a shelf-life of 3-4 months.

4.4 Caviare Isolate

The caviare isolate was a gram positive, facultative cocci which was able to with stand elevated concentrations of salt (Figure3.10). Research conducted by Scheen (31) on colorant decomposition found that the responsible bacterial strain was capable of growth at relativley

low pH and high salt concentrations. The caviare isolate exhibited good growth in media at pH 4.0 and in media with elevated salt concentrations (18-20%). Pace et al. (25) found that post-pasteurization bacterial flora on oysters were primarily dominated by gram positive bacteria. The majority of bacteria surviving the pasteurization process were from the genus *Bacillus*, *Clostridium*, *Corynebacterium*, *Listeria*, *Peprostreptococcus* and *Staphylococcus*.

The caviare isolate could be a member of the Staphylococcus genus, whose members are gram positive, facultative anaerobic non-spore forming cocci with opaque colonies white, cream or yellow in colour. Members of this genus can grow in the presence of 10% salt and growth at 15% salt is not unusual. The optimum temperature is between 30 - 37°C with good growth at 45°C common. The caviare isolate fits all these characteristics but a proper identification requires a more in-depth characterization which was not one of the objectives of the study.

4.5 Preservation Methods

The retardation or inhibition of microorganisms (2) can extend the shelf-life of food products. Retardation or inhibition of microorganisms depends on the ability of microbial growth factors (such as temperature, water activity, acidity, oxidation-reduction potential and chemical inhibitors) to be altered. Most methods of food preservation try to prevent or

delay microbial decomposition by hindering the growth and activity of microorganisms. The preservation methods examined in this study were:

1. Refrigeration.
2. Pasteurization.
3. The use of chemical additives (sodium benzoate and potassium sorbate).

4.5.1 Refrigeration

Temperature is one of the most important environmental factors (2) which influence the growth and activity of microorganisms. Temperature is not only related to the growth of organisms, but also to its ability to survive. The temperature has an effect on cell size, metabolic products, nutritional requirements, enzymatic reactions, and the chemical composition of cells. Lowering the temperature of a food product by holding it at refrigerated or freezer temperatures can reduce the microbial and biochemical activity. The lower the temperature, the lower the rate of biochemical reactions and/or microbial activity.

Refrigeration generally refers to temperatures below 10°C (2), thus mesophiles (organisms with an optimum temperature range of 25°- 45°C) will not grow and are not a problem. The mesophiles consist of two groups of microorganisms:

1. The saprophytic organisms which have an optimum temperature of 25°- 30°C.
2. Potential pathogens with an optimum temperature range of 35°- 45°C.

This is important since a majority of microorganisms (table 4.1) causing food-borne illness belong to the mesophile group.

Table 4.1: Temperature Ranges of Selected Microorganisms

Microorganism	Temperature (°C)		
	Minimum	Optimum	Maximum
Bacteria			
Acinetobacter	5	-	50
Aeromonas	0-5	25-30	38-41
Bacillus cereus[a]	10	28-35	50
Clostridium	0-45	-	60
C. botulinum[a]	3.3-10	30-40	-
C. perfringens[a]	15-20	30-40	45-50
Escherichia coli[a]	5-10	37	-
Lactobacillus	5	30-40	53
Leuconostoc	10	20-30	40
Micrococcus	10	25-30	45
P. fluorescens	0-4	20-25	40
Salmonella[a]	5-10	35-37	46
S. aureus[a]	5-10	35-39	48
Vibrio	-	10-37	-
Yeasts			
Candida	0	-	29-48
Saccaromyces	0-7	20-30	40

[a] Microorganisms which may cause foodborne illnesses.

Source: Banwart, G.J. (2).

The main organisms of concern on refrigerated foods (2) are psychrophiles which can grow at temperatures as low as -15°C and often have an optimum growth temperature as low as 10°C. Some microorganisms causing food-borne illness are psychrotrophic, however most will not grow or produce toxins below 4.4°C. Thus for safety, refrigerated foods should be held below 4.4°C.

It is usual for other methods of food preservation to be used with refrigeration. Salting, curing, smoking or chemical additives may be used to inhibit or reduce the microorganisms on refrigerated food products (2). International experience has shown that the refrigeration of caviare at 0°- 2°C will have a shelf-life of approximately 4 months (14). However, the sources on which this shelf-life has been based have not referred to the salinity or the grade of the product, both which can greatly influence the shelf-life estimates.

Ushakova and Daniliuk's (38) study into "Novinka" pike caviare pasteurized at 60°C for 150 minutes and stored at -2° to -4°C for 3.5 months had bacterial counts in the 10^5 range. Caviare which was washed three times with water at 90-98°C and stored at -2° to -4°C for 5 months had bacterial counts in the 10^6 range. Caviare which was washed two times with water at 85°c and stored at -2° to -4°C for 3.5 months had bacterial counts in the 10^7 range. Organoleptic evaluations conducted on these caviare samples indicated that all were still considered to be of "palatable" quality (38). The growth curves of the "Novinka" caviare

exhibited the same trends as was found in this study on lumpfish caviare (Figures 3.1 - 3.8), except that the time period was extended due to the lower storage temperature.

4.5.2 Pasteurization

Pasteurization is the heat treatment of food products below temperatures needed for sterilization. Generally, temperature treatment below 100°C is called pasteurization, while temperature treatment above 100°C is called sterilization (2). Most pasteurization processes use heat treatment between 60°C and 85°C for a few seconds up to an hour.

The best preservative method from a microbial quality point of view during this study was pasteurization at 70°C (figures 3.1 - 3.8). Pasteurization has been found to extend the shelf-life of caviare. The storage method after pasteurization will greatly affect the shelf-life and quality of the product. Pasteurized product held at room temperature has a shelf-life of approximately 3 months compared to 8 months when stored at 2°C (14). "Novinka" pasteurized pike caviare held at -2° to -4°C had a shelf-life of 15 months (38).

Pasteurization is a costly process which can also effect the organoleptic properties of the product. The pasteurization temperature used for lumpfish caviare should range between 55 - 70°C (14). Only the best grade of caviare should be pasteurized as poor quality eggs may produce an un-edible produce due to broken eggs and strong odours. This phenomenon

(broken eggs) was observed during this study for product pasteurized at 70°C and to a lesser extent for product pasteurized at 55°C.

The pasteurization regimes of 70° for 45 minutes and 55°C for 135 minutes were chosen after advice was obtained from G. Whiteway (41) and G. Churchill (7), both of whom have experience in lumpfish caviare production and research. These temperatures and times (see table 1.3) were similiar to those used by commercial lumpfish caviare producers and other researchers. "Novinka" pike caviare product produced in Russia is pasteurized at 60°C for 150 minutes (38).

Duncan (14) suggests a pasteurization temperature of 65 - 69°C for an exposure time of 60-120 minutes. The use of 120 minutes at 70°C for pasteurization may have improved the microbial quality of our product. However, the impaired organoleptic quality (broken eggs) would not be improved by increased time. It may well have increased the unacceptable organoleptic quality of the product.

4.5.3 Chemical Additives

A chemical preservative is defined as "a substance that is capable of inhibiting, retarding or arresting the decomposition of food, but does not include common salt, sugars, vinegars, spices or oils extracted from spices, substances added to food by direct exposure to wood

smoke, or chemicals applied for their respective insecticidal or herbicidal properties" (17). Chemical preservation should be used when other methods for the control of microorganisms are lacking, damaging to the product or are expensive. Chemical preservation adds a margin of safety from possible abuses at the post processing stages. Table 4.2 outlines the requirements for chemical preservatives (2).

Ideally the chemical preservatives will inhibit or kill the important microorganisms and then break down to harmless, non toxic substances. The chemical should not decompose so fast that it is ineffective and slow inactivation of microorganisms can lead to unsuccessful preservation. The degree of inhibition varies with the chemical preservative and the amount of inhibition influenced by the concentration of the chemical (2).

4.5.3.1 Activity of Preservatives

The factors affecting the antimicrobial activity of chemical preservatives include the type of chemical and its concentration, the type of organisms and their physiological state, numbers of organisms, the composition of food, pH of food, and the temperature of storage. The rule of thumb is the higher the microbial load, the greater the amount of chemical preservative necessary to accomplish inhibition or death of the cells. Many preservatives

Table 4.2: Requirements for Chemical Preservatives.

1:	Provide an economical means of preservation.
2:	Be used only when other preservation methods are inadequate or not available.
3:	Extend the shelf-life of the product.
4:	Be readily soluble.
5:	Exhibit antimicrobial properties over the pH range of the product.
6:	Be safe at all levels.
7:	Be readily identified by chemical analyses.
8:	Not retard the action of digestive enzymes.
9:	Not decompose or react to form compounds of greater toxicity.
10:	Not lower the quality (colour, flavour, odour) of the product.
11:	Be easily controlled and uniformly distributed in the product.
12:	Have a wide antimicrobial spectrum that includes the spoilage types of organisms associated with the product to be preserved.

Source: Banwart, G.J. (2)

have increased activity in acid foods. Liquid foods allow better contact between inhibitor and the microorganism than do solid foods (2,17).

Increasing the temperature often increases the effect of preservatives on microorganisms. However, if a low temperature is increased toward the optimum for growth of a microorganism, then the stimulatory effect on growth may outweigh the increased

action of the preservative. When the temperature is above the optimum for growth, the increased preservative effect is more pronounced (2).

Chemical preservatives may inhibit the growth (bacteriostat, fungistat) or kill (bactericide, fungicide, sporicide, or virucide) microorganisms. In dilute amounts some chemicals may act as a food source for microorganisms. Increasing levels may be inhibitory, while still higher levels may kill some or all of the microbial cells. Generally, the more concentrated the chemical agent, the more effective the action. However, very high levels are not desired due to potential adverse effects on food quality or toxicity to humans (2).

4.5.3.2 Mode of Action of Chemical Preservatives

The mode of action generally falls into one of three categories:

1. reaction with the cell membrane, causing increased permeability and loss of cellular constituents.
2. Inactivation of essential enzymes.
3. Destruction or functional inactivation of genetic material.

An antimicrobial which acts on the membrane in a nonspecific fashion has the widest overall spectrum of activity. Such membrane activity, may decrease the effectiveness of the chemical preservative against certain microorganisms (4).

4.5.3.3 Acids as Chemical Preservatives.

Acids serve a variety of functions in foods. Acidic condition tends to be unfavourable for the growth of microorganisms. The preservative effect of acids may be due to the pH, the undissociated molecule or the anion. At low pH levels, the undissociated molecules of the weak, short chain organic acids enter the cell and interfere with intracellular enzymes. The ionic form does not pass through the cell wall as does the undissociated form (2).

The pH and the type of acid are important in the inhibitory or lethal action of these chemicals. The exact order of effectiveness depends on a variety of factors such as the type of microorganism, whether inhibition or death is desired, the pH, temperature, other environmental conditions of the substrate, and the concentration of acid used (2).

4.5.4 Benzoic Acid

Benzoic acid is one of the oldest chemical preservatives used in the cosmetic, drug and food industries. Its preservative action was first described in 1875, and introduced for food preservation around 1900. The advantages of its low cost, ease of incorporation into products, lack of colour, and relative low toxicity has caused benzoic acid to become one of the most widely used preservatives in the world (6).

4.5.4.1 Chemical Properties

Benzoic acid also called phenylformic acid or benzenecarboxylic acid occurs in pure form as colourless or white needles or leaflets. It has a limited solubility in water and thus the sodium salt form (sodium benzoate) is preferred for commercial applications. Benzoic acid occurs naturally in cranberries, prunes, greengage plums, cinnamon, ripe cloves and apples. Sodium benzoate is a white granular or crystalline powder which is easily dissolved in water (6).

4.5.4.2 Antimicrobial Activity

The undissociated molecular of benzoic acid are responsible for the antimicrobial activity (Table 4.3). The antimicrobial effect of benzoic acid is nearly 100 times as efficient in strong acid solutions as in neutral solutions. The toxicity of sodium benzoate in solution was due to the undissociated benzoic acid molecule. The strong dependence of uptake on pH is due to the relative distribution of undissociated and dissociated forms in solution and not to pH itself. The effect of temperature on the uptake is similar to that of enzymatic reactions, thus an increase in temperature often increases the effectiveness of the preservative action (6).

It has been suggested that benzoic acids inhibit or kill microorganisms by interfering with the permeability of the microbial cell membrane, causing uncoupling of both substrate transport and oxidative phosphorylation from the electron transport system. Benzoate inhibits amino acid uptake in *Bacillus subtilis, Penicillium chrysogenum, Escherichia coli, and Pseudomonas aeruginosa*. Studies suggest that the undissociated form of benzoic acid may diffuse freely through the cell membrane and then ionize in the cell to yield protons that acidify the alkaline interior of the cell (6).

Benzoic acid and sodium benzoate can inhibit specific enzyme systems within cells including acetic acid metabolism and oxidative phosphorylation. Alpha-ketogluturate and succinate dehydrogenases appear to be sensitive to action by benzoates. Aflatoxin production may be greatly reduced by the presence of benzoates. Furthermore benzoate may serve as a scavenger for free radicals, as an inhibitor of D-amino acid oxidases, a weak inhibitor of poly(ADP ribose)polymerase, and as an inhibitor of passive anion transport (6).

The use of benzoic acid and sodium benzoate as a food preservative has been limited to those products which are acidic in nature. Currently, most yeast and moulds are inhibited by 0.05-0.1% undissociated acid, and food poisoning and sporeforming bacteria by 0.01-0.02% undissociated acid. However, many spoilage bacteria are more resistant. Therefore,

benzoates cannot be relied upon to effectively preserve foods capable of supporting bacterial growth. Minimum inhibitory concentrations for some microorganisms involved in food poisoning and food spoilage are given in table 4.4 (6).

Table 4.3: The pH values needed for various levels of undissociated organic acids.

Undissociated Acid (%)	Acids	
	Benzoic	Sorbic
99	2.19	2.75
95	2.91	3.47
90	3.24	3.80
80	3.59	4.15
70	3.82	4.38
60	4.01	4.57
50 (pK)	4.19	4.75
40	4.37	4.93
30	4.56	5.12
20	4.79	5.35
10	5.14	5.70
1	6.19	6.75
0.5	6.49	7.05
pKa	4.19	4.75

Source: Chipley, J.R. (6)

No preservative is completely effective against all microorganisms; thus one should combine various preservatives having different modes of action to compensate for this deficiency. Then it would be possible to achieve a broader spectrum of action or increased antimicrobial action. Combinations of benzoic acid and sorbic acid inhibit several strains of bacteria better than either chemical preservative used alone. Furthermore it may be advantageous to combine several preservative methods with physical methods of food preservation, such as heating, pasteurization, refrigeration, irradiation or drying (6).

Benzoic acid and sodium benzoate are most suitable for foods and beverages that are in the pH range below 4.5 or which can be brought into that range by acidification. Laboratory experimentation with the caviare isolate concurred with these findings (Figure 3.11). The main advantages of benzoates as a chemical preservative are low cost, ease of incorporation into products and lack of colour. The disadvantages are the narrow pH range in which they are effective, the off-flavour they may impart to foods,
and toxicological properties (6).

4.5.5 Sorbic Acid

Sorbic acid and its salts, particularly potassium sorbate, are known as "Sorbates". Sorbic acid was isolated in 1859 by A.W. Hoffmann from the unripened berries of the mountain ash tree. The structure of sorbic acid was determined between 1870-1890, however, its

Table 4.4: Inhibitory action of benzoic acid on microorganisms.

Name of test organism	pH value	Minimum inhibitory concentration (ppm)
Pseudomonas sp.	6.0	200-480
Micrococcus sp.	5.5-5.6	50-100
Streptococcus sp.	5.2-5.6	200-400
Lactobacillus sp.	4.3-6.0	300-1800
Escherichia coli	5.2-5.6	50-120
Bacillus cereus	6.3	500
Sporogenic yeasts	2.6-4.5	20-200
Asporogenic yeasts	4.0-5.0	70-150
Penicillium sp.	2.6-5.0	30-280
Aspergillus sp.	3.0-5.0	20-300
Aspergillus niger	5.0	0.20 (a)

(a): values reported in percent.

source: Chipley,J.R. (6).

antimicrobial properties were not recognized until the late 1940's. Research since the 1950's has concentrated on the application of sorbates as a preservative, heatlh aspects, methods of analysis, manufacturing of sorbates and mechanisms of antimicrobial activity (32).

4.5.5.1 Chemical Properties

Sorbic acid is a straight chain, monocarboxylic, trans-trans unsaturated fatty acid (2,4 - hexadienoic acid). Potassium sorbate is the potassium salt form of sorbic acid which has a water solubility that far exceeds that of sorbic acid. The antimicrobial potency of potassium sorbate is about 74% of sorbic acid on a weight basis (6).

Sorbic acid and its derivatives in the powder form are stable to oxidation, whereas aqueous solutions are somewhat unstable and degrade. Molecules with oxidizing capacities attacked sorbates at the double bond forming peroxides, followed by degradation and polymerization (6).

Loss of sorbic acid during the storage of food may occur with the amount of loss depended upon storage temperature and time, sorbate content, moisture content, nature of food material, pH, packaging material, processing conditioned and other additives present (23). Results of loss of sorbates in actual food systems are conflicting. Some studies report significant losses during the storage of certain foods (6).

Commercial sorbates are available in a variety of forms including crystals, granules, suspensions or solutions. The acid when recrystallized produces a colourless crystal that has an acrid odour and sour taste while the potassium salt (a powder form) has a mild non

objectionable odour. Both forms are produced as highly refined (98-99% pure) white flowing powders or granules (6).

4.5.5.2 Antimicrobial Activity

Sorbates have been found to delay the growth of many microorganisms, including yeasts, moulds, and bacteria. Sorbate concentrations used in foods are usually static in antimicrobial activity, while higher levels may be cidal. Sorbate inhibition of microorganisms is generally more pronounced against yeast and mould as compared to bacteria. There are many yeast and mould species inhibited than bacteria giving the impression that sorbates are only fungal static agents (6).

The most important use of sorbates is for the inhibition of moulds in food products including mycotoxin-producing species and strains. The inhibition concentration varies dependent upon intrinsic parameters of the substrate and the target mould species (6,32). The minimum inhibition concentration may be as low as 500 ppm for *Aspergillus* spp. or as high as 12,000 ppm for some *Penicillium* spp (32).

Moulds that grow on foods may produce mycotoxins and thus it is important to examine the potential of mycotoxin formation in foods preserved with sorbates. Studies have reported that sorbate levels of 0.01 to 0.3% inhibit growth and mycotoxin formation by

moulds in cultured media and foods (6,23). Some studies have shown that under certain conditions, subinhibitory levels of sorbate may stimulate production of mycotoxins. Yousef and Marth observed an increase in aflatoxin production by *A. parasiticus* in the presence of sublethal amounts of sorbate over media that was free of sorbates (43). Furthermore, sorbates may inhibit mould growth but have no influence on the formation of mycotoxins such as patulin and aflatoxin (6). However, for the most part sorbates are very effective in the inhibition of the growth of moulds (6,23).

Inhibition of moulds was found to occur during all stages of their development, including spore germination, growth initiation and mycelial growth. Inhibition of mycotoxin biosynthesis by sorbate may be due to inhibition of transfer of substances from the growth substrate into the cell (6).

Inhibition concentrations for sorbates against yeasts depend upon various factors including species, strains, and substrate pH. Yeasts can be inhibited by sorbates in the range of 0.0025 to 0.20%, with the majority of yeast inhibition occurring in the range of 0.010 to 0.20% (6). Beuchat (3) found that the presence of sorbate in recovery medium influences the ability of yeast exposed to heat to form colonies. The results strongly suggest that yeasts possess a wide range of physiological characteristics which are subject to heat injury. Furthermore, potassium sorbate enhances injury during heating and retards or prevents repair

of injured cells during recovery. Sensitivity to heat generally increases as the pH of the heating medium decreased from pH 7.0 to 2.5 (3).

Presence of oxygen in the atmosphere may influence the inhibitory activity of sorbate against yeast. The inhibitory effect on *Candida albicans* was stronger under anaerobic than aerobic conditions. The decreased inhibition under aerobic condition was attributed to a detoxification effect on sorbate by the yeast culture (6).

Information on sorbate activity to inhibit the growth of bacteria is not as detailed as for other microorganisms. However, it is known that a great variety of bacteria are inhibited by sorbate including gram positive and gram negative spoilage and pathogenic organisms. Important bacteria inhibited by sorbates include *Salmonella* spp., *Escherichia coli*, *Staphylococcus* spp., *Vibrio parahaemolyticus*, *Bacillus* spp. and *Clostridium botulinum* (6).

Sorbate concentrations required to inhibit bacteria range from 0.001% to 0.01%, with some species more resistant than others (6). The sorbate concentration used will depend on environmental factors such as pH, water activity, temperature, atmosphere conditions, initial bacterial load, type of micro-flora (23) and whether complete inactivation or partial inhibition is desired.

Spore-forming bacteria such as *Clostridium botulinum* may have their spore germination, outgrowth, and/or cell division affected by sorbates. The majority of studies undertaken do not report the step in the life cycle that is being inhibited (6).

Wagner and Busta (39) found that potassium sorbate was a strong inhibitor of germination at pH 5.7, and had reduced effectiveness at higher pH's of 6.2 and 6.7. The concentration of potassium sorbate used in the study was 0.26% (39). It should be noted that the blocking of germination by sorbate in *C. botulinum* cells will greatly depend on the strains present and blanketed interpretations of the various results given by different authors should not occur.

The ability of sorbate to inhibit emergence of vegetative cells from spores (outgrowth) and inhibit cell division has been studied. The general findings from the studies indicate that sorbate can inhibit both outgrowth and cell division depending on sorbate concentration and media pH (6).

The process by which sorbate inhibits bacterial growth is not clear or well defined. The potential mechanisms of inhibition can be viewed from the following prospectives:

1. Germination.
2. Cell growth.

Retardation of germination in spore forming bacteria, such as *C. botulinum* and *B. cereus*, appears when the activated spore is exposed to sorbates. It has been suggested that sorbates act as a competitive and reversible inhibitor of amino acid - induced germination (33). Specifically it has been postulated that sorbates compete directly with germinant (L-amino acids) for a binding site on the germination - trigger - receptor site or for an active site on an enzyme involved in the germination process (33).

Another proposed mechanism for sorbate retardation of the spore germination process is by inhibiting the activity of enzymes within the spore. Enzymes that may be interfered with include serine or sulfhydryl proteases (6). During cell growth it has been shown that sorbates have an effect on metabolic functions involving enzymes and ATP. Inhibition of sulfhydryl enzymes has been attributed to binding of sorbates with the sulfhydryl groups, which reduce the number of active sulfhydryl groups on the enzyme (6,23). Deactivating this sulfhydryl group renders the enzyme inactive.

Studies into the inhibitory effect of sorbates on the enzyme catalase have provided some answers to the mechanics of enzyme deactivation. The formation of sorbyl peroxides are credited with inactivating the catalase or the coenzymes vital to cell development. One study concluded that coenzyme A was the factor inhibited by the sorbates (6).

Research indicates that decreased levels of ATP may account for delayed growth and reduced respiration within microbes. Przybylski and Bullerman (29) proposed that dissociation of sorbic acid in the cells increased intercellular cation concentrations. Attempts by the cell to maintain ion balance may result in some ATP depletion because of the primary sodium/hydrogen pump being directly linked to hydrolysis of ATP. Due to excess hydrogen intake, the pH gradient required for ATP formation is disturbed; thus a reduction in ATP production occurs (29).

Microorganisms vary greatly in their ability to survive and grow in the presence of sorbates, some may even metabolize sorbates. *Staphylococcus* spp. were the most resistant to the sorbate followed by *Pseudomonas* spp., *Acinetobacter* spp. and *Moraxella* spp (6,23). Certain bacteria are not only resistant to sorbates they are able to metabolize and degrade the compound. Lactic acid producing bacteria are known for utilizing sublethal concentrations of sorbates as a carbon source, converting it to hexadienol. Though the majority of researchers believe that sorbates are metabolized by lactic acid bacteria some reports exist to the contrary (6,23).

A variety of yeast and moulds are resistant to sorbates or may acquire such resistance. Some osmophilic yeast may grow and cause spoilage of foods having reduced water activity, low pH and sorbates present. Although the general rule is that increasing the sucrose

concentration, decreasing pH, and increasing the sorbate content enhances inhibition of yeast species, these osmophilic yeast are, or have become, resistant to inhibition by sorbates (6).

Moulds, to a lesser extent, can grow in the presence of sorbates and some can even metabolize the compound. This is accounted for by the occasional spoilage of sorbate - treated foods. Variation in sensitivity to sorbate among moulds has been attributed to the ability of certain moulds to metabolize sorbates under certain conditions. Products of sorbate metabolism by moulds include carbon dioxide and water as a result of β - oxidation, and 1,3 - pentadiene, methyl ketones, trans-4-hexenol and ethyl sorbate (6).

Foods containing preservatives such as sorbates should be processed, packaged, and stored under conditions that minimize contamination by bacteria, yeast and moulds acclimated to the preservative (17).

The maximum pH for inhibition by sorbate is in the range of pH 6.0 - 6.5 with the optimal effect at pH 6.0 and below. Laboratory experimentation with the caviare isolate concurred with these findings (Figure 3.12, 3.13). It is ineffective at pH 7.0 and above. The pH at which inhibition by sorbates becomes significant is dependant upon a variety of factors such as sorbate concentration, species and strains of microorganisms and storage temperature (33).

4.5.6 Sodium Chloride

Sodium Chloride (salt) has been used since biblical times to flavour and preserve a variety of food substances. Today, it is most commonly used in combination with other antimicrobial or preservation techniques (9).

Salt lowers the water activity (a_w) of solutions, which is most likely the primary cause for its antimicrobial action. Toxic effect of salt may include factors such as removal of oxygen, or alteration of pH, sodium or chloride ion concentrations. The primary reason for inhibition by salt is most probably its plasmolytic effect. Other mechanisms may include dehydration, limiting oxygen solubility, interference with enzymes, and loss of magnesium ions (9).

The salt content of the caviare was 5.9% and this would limit the growth of some microorganisms but not the caviare isolate. Thus the preservative effect salt has on caviare is limited but may have an important synergistic effect when used in combination with other preservative methods.

CHAPTER 5

CONCLUSIONS

The most effective alternative preservative method examined in this study was sodium benzoate (300 ppm) and potassium sorbate (100 ppm) with similiar results obtained for sodium benzoate (200 ppm) and potassium sorbate (200 ppm). The commercially produced caviare which had sodium benzoate at 80 ppm as a preservative was found to be the least effective of the preservation methods examined. Pasteurization at 70°C was the most effective from a microbiological view point. However, the product quality was unacceptable and resulted in a high percentage of broken eggs which produced a runny and un-aesthetical product.

The use of chemical additives tends to be a cheap and effective means by which shelf-life may be extended and product quality ensured. Sodium benzoate is most effective at pH below 4.0 and potassium sorbate is most effective below pH 6.0 with limited effectiveness between pH 6.0 - 6.5. The pH of commercial brands generally range from 4.9 to 6.0, with the pH of the caviare used during the study being 5.9. Thus the use of sodium benzoate is ineffective and limited at caviare pH and its use in this product is questioned. The use of potassium sorbate and sodium benzoate in combination proved to be a more effective and suitable preservation method than that presently used.

The aim of pasteurization is to provide a longer shelf-life by the reduction of microbial load, inactivation of fermentative processes and a reduction of spore development. Pasteurization is an effective means to extend shelf-life and product quality, however it is a costly process that can affect the organoleptic properties of the product. Only the highest grades of caviare (maturity and freshness) should be used for pasteurization (14). Pasteurization of poor quality caviare may produce inedible product due to broken eggs and odours (14) and will result in an unacceptable product, aesthetically and chemically. Caviare product pasteurized for this study (both at 55° and 70°C) resulted in a high percentage of broken eggs which produced a runny and un-aesthetical product. Furthermore, it is a well known fact that at 60°C certain undesirable and irreversible changes occur (12) in fish products and Iredale and York (20) reported that these changes can occur at temperatures as low as 55°C. Thus the use of pasteurization for caviare should be limited to highest quality roe and further research into temperatures and times regimes, particularly at or near 55°C for caviare products could result in the production of an aesthetical product regardless of the initial bacterial load.

Duncan (14) states that caviare should be refrigerated regardless of the initial preservative method employed. This most likely would have improved the caviare quality and when incorporated with an appropriate chemical preservative or pasteurization the shelf-life could be extended to an acceptable time.

The development of guidelines incorporating organoleptic properties, chemical composition and microbial loads are essential for the production of a quality product. Duncan (14) presented some criteria on which product quality can be based, however there is presently no accepted standard. Using the criteria suggested by Duncan (14), the caviare product used for this study was of low quality and would result in an unacceptable product if pasteurized. The results of this study support this statement. The need for guidelines is further demonstrated by the fact that some researchers have found spoiled samples on retail shelves, no use of "best before" dates, and conflicting label instructions (17).

Processing of salt and other additives at 150-160°C for 2 hours is an added precaution against possible microbial contamination (14). The processing of salt and additives can be done at the primary processing (product curing) and the secondary processing (caviare product) stages. This may help to limit the introduction of microorganisms from additives and increase product quality.

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Appendix A

Chemical Analyses - Proximate

Table A.1: pH Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.96	5.93	5.98	5.95	5.95
5.92	5.95	5.98	5.84	5.93
5.91	5.97	5.99	5.87	5.94
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.87	5.88	5.79	5.91	5.76
5.93	5.84	5.79	5.93	5.74
5.9	5.85	5.79	5.84	5.75
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.98	5.41	5.74	5.89	5.82
5.98	5.43	5.78	5.83	5.78
5.95	5.34	5.91	5.86	5.66
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.88	5.84	5.89	5.84	5.9
5.86	5.78	5.86	5.89	5.92
5.9	5.8	5.85	5.77	5.87
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.83	5.93	5.96	5.99	5.93
5.79	5.93	5.96	5.92	5.84
5.81	5.87	5.96	5.96	5.95
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.92	5.92	5.82	6.05	5.98
5.93	5.89	5.83	5.98	5.96
5.91	5.85	5.7	6.03	5.87
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.82	5.9	5.8	5.94	5.92
5.81	5.8	5.76	5.94	5.83
5.83	5.87	5.8	5.94	5.76

Table A.2: pH Raw Data for Abusive Storage Temperature

(Normal Temperature 37°C for all samples except Refrigeration (20°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.94	5.97	5.84	5.93	5.49
5.94	5.95	5.88	5.91	5.64
5.94	5.99	5.86	5.92	5.58
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.23	5.9	2.99	5.63	5.97
6.2	5.89	2.98	5.63	5.97
6.19	5.89	5.91	5.66	5.98
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.98	6.23	6	6.01	5.92
5.98	6.22	6.01	6.03	5.9
5.95	6.17	6	5.94	5.84
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.88	5.83	5.95	5.93	5.89
5.86	5.83	5.95	5.92	5.92
5.9	5.79	5.94	5.87	5.87
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.83	5.75	5.94	5.87	5.91
5.93	5.78	5.96	5.84	5.93
5.81	5.72	5.93	5.8	5.88
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.92	5.99	5.93	5.99	5.87
5.93	5.92	5.99	5.99	5.83
5.91	5.9	6.04	6	5.74
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.82	5.86	5.82	5.95	5.84
5.81	5.87	5.83	5.95	5.84
5.83	5.85	5.73	5.91	5.84

Table A.3: Water Activity (a_w) Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.937	0.937	0.935	0.94	0.935
0.937	0.936	0.935	0.938	0.933
0.937	0.938	0.935	0.939	0.934
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.935	0.934	0.935	0.937	0.933
0.935	0.933	0.935	0.937	0.934
0.936	0.934	0.935	0.937	0.935
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.927	0.934	0.932	0.931	0.933
0.927	0.934	0.932	0.931	0.934
0.928	0.934	0.932	0.932	0.933
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.939	0.935	0.939	0.937	0.936
0.941	0.935	0.939	0.937	0.935
0.938	0.935	0.938	0.937	0.935
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.932	0.938	0.93	0.931	0.938
0.931	0.937	0.93	0.931	0.932
0.932	0.93	0.93	0.932	0.934
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.929	0.931	0.934	0.932	0.934
0.93	0.931	0.935	0.932	0.934
0.929	0.931	0.934	0.931	0.934
Refrigerated (4°C)				
0 Days	14 Day	28 Days	56 Days	112 Days
0.93	0.931	0.934	0.933	0.932
0.931	0.931	0.934	0.932	0.932
0.929	0.931	0.934	0.932	0.932

Table A.4: Water Activity (a_w) Raw Data for Abusive Storage Temperature

(Normal Temperature 37°C for all samples except Refrigeration (20°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.937	0.937	0.935	0.937	0.938
0.937	0.937	0.935	0.937	0.939
0.937	0.937	0.935	0.938	0.938
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.934	0.934	0.932	0.933	0.935
0.935	0.934	0.932	0.934	0.935
0.933	0.934	0.932	0.931	0.935
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.927	0.932	0.933	0.93	0.929
0.927	0.933	0.932	0.93	0.929
0.928	0.932	0.932	0.93	0.929
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.939	0.936	0.936	0.936	0.938
0.941	0.936	0.937	0.935	0.938
0.938	0.937	0.937	0.936	0.938
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.932	0.931	0.93	0.928	0.931
0.931	0.932	0.93	0.93	0.931
0.932	0.933	0.93	0.926	0.931
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.929	0.931	0.93	0.928	0.933
0.93	0.931	0.93	0.928	0.932
0.929	0.931	0.93	0.928	0.932
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
0.93	0.931	0.934	0.931	0.93
0.931	0.932	0.934	0.931	0.93
0.929	0.934	0.933	0.931	0.93

Table A.5: Salt (%) Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.07	6.11	6.21	6.15	6.49
6.03	6.11	6.25	6.16	6.45
6.11	6.12	6.24	6.14	6.41
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6	6.21	6.25	6.04	5.97
6	6.22	6.25	6.07	5.97
5.99	6.26	6.25	6.04	5.97
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.16	5.99	6.21	5.98	5.93
6.17	6	6.21	5.93	5.95
6.18	5.99	6.2	6.02	5.95
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.01	5.04	5.24	5.34	5.46
5.02	5.04	5.21	5.36	5.46
5.01	5.04	5.26	5.33	5.45
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.65	6.41	5.86	5.88	5.89
5.65	6.41	5.87	5.88	6.02
5.62	6.4	5.86	5.88	6.03
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.87	5.93	5.91	6	5.94
5.88	5.91	5.89	6	5.94
5.87	5.91	5.88	6.01	5.93
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.42	5.55	5.46	5.58	5.43
5.43	5.56	5.48	5.59	5.46
5.41	5.55	5.45	5.55	5.41

Table A.6: Salt (%) Raw Data for Abusive Storage Temperature

(Normal Temperature 37°C for all samples except Refrigeration (20°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.23	6.19	6.35	6.08	6.49
6.22	6.14	6.31	6.08	6.47
6.24	6.11	6.36	6.07	6.38
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.29	6.28	6.35	6.21	6.14
6.29	6.29	6.33	6.1	6.18
6.26	6.25	6.34	6.13	5.96
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.16	6.21	5.94	6.18	6.02
6.17	6.24	5.96	6.14	6.02
6.18	6.18	6.06	6.04	6.03
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.01	4.96	5.35	5.86	5.5
5.02	5	5.37	5.92	5.48
5.01	4.9	5.25	5.89	5.47
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.65	6.11	5.87	5.77	6.02
5.65	6.09	5.89	5.76	6.03
5.67	6.13	5.83	5.67	5.97
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.87	5.95	5.78	5.91	5.97
5.85	5.93	5.76	5.92	5.97
5.87	5.94	5.73	5.87	5.93
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.42	5.65	5.49	5.49	5.75
5.43	5.65	5.5	5.49	5.71
5.41	5.61	5.46	5.5	5.74

Table A.7: Moisture (%) Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
73.42	7502	75.83	75.36	74.3
73.39	75	75.86	75.35	74.3
73.26	74.84	75.44	75.23	74.27
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
74.26	74.68	74.36	73.35	74.84
74.24	74.69	74.31	73.26	74.31
74.25	74.56	74.27	73.25	74.52
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
74.18	74.39	75.18	74.2	75.28
74.09	74.36	75.16	74.2	75.25
74.05	74.37	75.14	74.22	75.2
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
77.28	77.21	77.43	74.69	74.53
77.24	77.18	77.4	74.68	74.54
77.26	77.13	77.38	74.66	74.51
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
74.53	74.62	74.1	74.35	74.35
74.51	74.58	74.08	74.26	74.37
74.42	74.49	73.94	74.24	74.32
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
74.68	74.2	73.9	74.1	74.75
74.71	74.25	73.73	74.09	74.82
74.51	74.28	73.84	74.23	74.48
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
75.26	77.02	74.8	75.36	75.89
75.3	77	74.72	75.34	75.93
75.17	77.04	74.75	75.34	75.86

Table A.8: Moisture (%) Raw Data for Abusive Storage Temperature

(Normal Temperature 37°C for all samples except Refrigeration (20°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
75.43	74.72	75.03	74.11	74.18
75.58	74.68	75.01	73.86	74.19
75.49	74.5	74.88	73.9	74.18
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
74.39	74.92	74.71	73.81	73.81
74.39	74.82	74.69	73.9	73.81
74.35	74.85	74.53	73.65	73.8
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
74.18	75	75.01	73.94	75.54
74.09	74.93	75.01	73.98	75.72
74.05	74.87	75	74.08	75.74
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
77.28	75.57	77.06	75.3	74.6
77.24	75.48	77.02	75.18	74.62
77.26	75.51	76.95	75.14	74.41
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
74.53	74.39	74.73	74.66	74.22
74.51	74.38	74.67	74.68	74.17
74.42	74.35	74.53	74.68	74.14
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
74.68	77.2	74.2	74.7	76.37
74.71	77.13	74.22	74.69	76.42
74.51	77.17	74.17	74.65	76.24
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
75.26	74.83	74.69	74.98	75.83
75.3	74.92	74.62	74.92	75.75
75.17	74.51	74.53	74.83	75.69

Table A.9: Ash (%) Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.62	6.25	6.76	5.47	5
6.63	6.26	6.76	5.43	5
6.57	6.18	6.75	5.44	5
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.99	6.28	5.8	5.48	5
6.03	6.28	5.79	5.44	5
5.96	6.27	5.72	5.47	5.01
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.65	6.51	6	5.27	5
6.64	6.51	5.92	5.25	5.03
6.57	6.51	5.86	5.2	4.96
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.42	5.43	5.67	5.49	4.98
5.4	5.42	5.67	5.49	4.96
5.34	5.43	5.53	5.48	5.05
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.54	6.02	6.0.	5.86	5.96
5.53	6.03	6.03	5.82	5.96
5.51	6.01	6.03	5.83	5.93
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.93	6.36	6.12	5.83	5
5.94	6.32	6.1	5.83	5
5.93	6.33	6.13	5.84	5.01
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.62	5.98	5.69	5.83	5
5.62	5.98	5.7	5.82	5
5.64	5.97	5.63	5.82	5

Table A.10: Ash (%) Raw Data for Abusive Storage Temperature

(Normal Temperature 37°C for all samples except Refrigeration (20°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.5	6.37	6.23	6.53	6.51
6.45	6.38	6.23	6.5	6.52
6.4	6.36	6.22	6.48	6.51
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.3	5.48	6.37	6.22	6.56
6.24	5.46	6.36	6.24	6.46
6.26	5.41	6.37	6.23	6.52
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
6.65	6.58	6.38	6	6.5
6.64	6.57	5.43	5.82	6.51
6.57	6.48	5.43	5.89	6.51
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.42	5.22	5.67	6.33	6.48
5.4	5.21	5.69	6.3	6.52
5.34	5.18	5.6	6.21	6.53
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.54	6.12	6.11	6.09	6.24
5.53	6.09	6.11	6.1	6.23
5.51	6.1	6.12	6.1	6.18
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.93	5.86	6.05	6.22	6.52
5.94	5.86	6.02	6.26	6.5
5.93	5.82	6.02	6.2	6.51
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
5.62	5.26	5.49	6.24	6.51
5.62	5.22	5.5	6.23	6.51
5.64	5.23	5.49	6.2	6.51

Table A.11: Fat (%) Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
1.96	1.88	2.23	1.84	3
1.96	1.87	2.21	1.81	2.98
1.88	1.83	2.17	1.64	2.88
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
2.18	1.95	2.63	2.43	2.7
2.2	1.84	2.59	2.4	2.71
2.14	1.93	2.45	2.35	2.71
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
2.27	3.1	2.34	2.2	2.1
2.25	3.08	2.3	2.19	2.01
2.18	2.98	2.3	2.2	2.08
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
1.32	1.37	1.35	2.1	1.92
1.3	1.37	1.37	2.14	1.89
1.27	1.36	1.24	2.1	1.85
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
1.82	2.39	2.9	2.49	2.37
1.72	2.34	2.85	2.48	2.37
1.76	2.39	2.87	2.39	2.35
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
2.01	2.01	1.32	2.68	2.3
1.99	2.01	1.41	2.69	2.27
1.98	2.04	1.39	2.54	2.23
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
1.93	2.24	2.09	2.65	1.75
1.93	2.32	2.03	2.65	1.76
1.94	2.2	2.1	2.59	1.75

Table A.12: Fat (%) Raw Data for Abusive Storage Temperature

(Normal Temperature 37°C for all samples except Refrigeration (20°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
2.53	1.89	2.21	2.47	1.98
2.52	1.92	2.2	2.44	1.93
2.49	1.82	2.17	2.53	1.95
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
2.18	2.42	2.3	2.18	2.04
2.2	2.45	2.29	2.2	2.05
2.14	2.35	2.25	2.08	2.01
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
2.27	2.87	2.09	1.89	2.56
2.25	2.76	2.09	1.9	2.56
2.18	2.8	2.1	1.83	2.48
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
1.3	1.35	1.78	2	1.96
1.32	1.35	1.79	2.03	1.96
1.23	1.25	1.7	1.98	1.92
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
1.82	2.98	2.7	2.78	2.63
1.76	2.95	2.7	2.8	2.68
1.72	2.9	2.7	2.68	2.59
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
2.01	1.08	2.23	2.03	2.34
1.99	1.09	2.22	2.02	2.3
1.98	1.05	2.23	1.97	2.21
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
2.18	2.39	1.9	2.11	1.98
2.19	2.4	1.91	2.1	2
2.13	2.39	1.81	2.11	1.94

Table A.13: Protein (%) Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
13.91	14.07	14.69	14.9	14.42
13.93	14.07	14.68	14.92	14.39
13.82	14.06	14.66	14.83	14.34
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
14.95	15.63	15.49	14.57	14.42
14.86	15.61	15.5	14.57	14.4
14.85	15.62	15.43	14.52	14.35
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
13.9	14.07	13.98	14.53	14.4
13.92	14.06	14	14.56	14.4
13.85	14.06	13.87	14.47	14.35
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
11.27	12.46	12.76	13	14.4
11.18	12.43	12.75	13.01	14.43
11.17	12.41	12.68	13.01	14.37
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
13.59	13.68	14.42	13.16	14
13.6	13.59	14.45	13.17	14
13.54	13.68	14.34	13.15	14.02
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
14.6	14.2	14.56	14.37	14.38
14.01	14.2	14.5	14.34	14.38
13.94	14.19	14.51	14.23	14.39
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
13.47	13.31	14.89	13.96	14.42
13.49	13.33	14.9	13.94	14.37
13.4	13.23	14.89	13.93	14.35

Table A.14: Protein (%) Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
14.1	14.27	14.38	14.75	14.07
14.12	14.27	14.39	14.76	14.06
14.02	14.1	14.27	14.69	14
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
14.95	14.1	14.78	13.75	14.05
14.85	14.1	14.79	13.78	14.02
14.86	13.98	14.68	13.67	14.05
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
13.9	12.07	13.82	13.46	14.06
13.92	12	13.78	13.47	14.06
13.85	11.98	13.75	13.4	14
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
11.27	12.63	12.15	14.32	14.08
11.18	12.65	12.16	14.27	14.1
11.17	12.57	12.08	14.18	13.95
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
13.59	13.32	13.78	13.93	13.87
13.6	13.3	13.77	13.95	13.9
13.54	13.17	13.74	13.86	13.76
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
14.6	14.45	14.4	13.31	14.04
14.01	14.52	14.41	13.3	14.03
13.94	14.6	14.34	13.27	14.06
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
13.47	13.31	13.74	13.9	14.06
13.4	13.35	13.74	14.02	14.08
13.49	13.21	13.65	13.98	13.98

Appendix B

Microbial Quality

Table B.1: Aerobic Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
8600	8000000	270000	168000	5600
8900	10800000	286000	184000	5600
8900	11200000	284000	188000	5900
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
15300	182000	5100000	39000	6200
12400	141000	4000000	42000	6500
11300	157000	3500000	45000	4700
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
2100	5100000	1410000	2450000	740000
2460	4100000	2120000	1980000	790000
2640	4900000	1870000	2170000	990000
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
9800	11300000	99000000	2500000	190000
1340	10900000	12300000	4200000	340000
1280	11300000	10800000	4100000	280000
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
178000	98000000	119000	5600	1700
156000	121000000	134000	4300	2400
146000	111000000	107000	5100	2800
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
1790	430000	23000	72000	8100
1610	580000	28000	87000	7200
1700	430000	24000	84000	8100
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
87	1560000	83000	1180	1010
115	1280000	62000	1470	8800
98	1060000	86000	1250	8100

Table B.2: Aerobic Raw Data for Abusive Storage Temperature

(Abusive Temperature 37°C for all samples except Refrigeration (20°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
8600	300	400	27300	510
8900	260	360	24800	460
8900	250	380	25900	470
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
15300	137000	16300	4500	540
12400	176000	14500	3900	480
11300	167000	17200	2700	360
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
2100	41000	390000	530000	92000
2460	34000	640000	660000	70000
2640	27000	590000	730000	96000
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
2500	198000	2900000	4200000	380000
1800	261000	1800000	5600000	280000
2300	171000	2800000	4900000	330000
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
178000	1500000	25000	3500	610
156000	2500000	14000	4600	570
146000	2300000	21000	5100	590
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
1790	57000000	5800	5800	6800
1610	60000000	5600	5600	5500
1700	78000000	5100	6800	6600
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
87	19000	95000	420000	131000
115	23000	11700	370000	116000
98	24000	88000	320000	113000

Table B.3: Anaerobic Raw Data for Normal Storage Temperature

(Normal Temperature 20°C for all samples except Refrigeration (4°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
910	28	2400000	51	53
770	35	3900000	35	46
810	33	3600000	34	51
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
55000	98000	37000	2900	420
62000	63000	21000	1200	250
39000	85000	26000	1900	350
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
3100	3200	720000	950000	77000
2600	4100	530000	800000	62000
3000	2900	730000	890000	83000
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
110	2900000	4100000	1670000	81000
85	2500000	3300000	1220000	59000
75	1800000	2500000	1610000	88000
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
53000	130000000	6000000	2600	1910
42000	230000000	4900000	2900	1720
43000	360000000	5600000	3500	1770
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
14700	134000	15900	1700	420
12000	168000	13200	3000	240
15300	148000	12900	2500	420
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
19800	128000	67000	4600	610
23700	89000	51000	2800	370
22500	113000	56000	4300	460

Table B.4: Anaerobic Raw Data for Abusive Storage Temperature

(Abusive Temperature 37°C for all samples except Refrigeration (20°C))

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
910	127000	18500	31	18
770	102000	15400	17	15
810	101000	17100	14	27
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
55000	33000	91000	700	390
62000	18000	78000	590	260
39000	36000	83000	540	310
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
3100	9000	175000	40000	37000
2600	14000	157000	33000	56000
3000	7000	118000	30000	51000
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
110	250000	3800000	111000	380000
85	300000	2100000	94000	250000
75	140000	3100000	80000	330000
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
53000	260000	18000	21100	9800
42000	390000	31000	17600	6700
43000	400000	26000	18300	9000
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
14700	6700000	6400	180	530
12000	4000000	5900	380	350
15300	5500000	3000	370	470
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
19800	32000	630000	1260000	1500
23700	10000	310000	940000	3100
22500	21000	560000	1100000	2600

Table B.5: Total Coliform Data for Normal Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
20	ND	ND	ND	ND
26	ND	ND	ND	ND
14	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	135	ND
ND	ND	ND	95	ND
ND	ND	ND	100	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.6: Total Coliform Data for Abusive Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
18	ND	ND	ND	ND
24	ND	ND	ND	ND
18	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
48	ND	ND	ND	ND
39	ND	ND	ND	ND
63	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
26	ND	ND	ND	ND
16	ND	ND	ND	ND
18	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.7: *Escherichia coli* Data for Normal Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.8: *Escherichia coli* Data for Abusive Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.9: *Staphylococcus aureus* Data for Normal Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.10: *Staphylococcus aureus* Data for Abusive Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.11: *Bacillus cereus* Data for Normal Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.12: *Bacillus cereus* Data for Abusive Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.13: *Listeria monocytogenes* Data for Normal Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.14: *Listeria monocytogenes* Data for Abusive Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.15: *Salmonella* spp. Data for Normal Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Table B.16: *Salmonella* spp. Data for Abusive Storage Temperature

Sodium Benzoate: Potassium Sorbate 3:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:1 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate: Potassium Sorbate 1:3 (400 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Sodium Benzoate (80 ppm)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (55°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Pasteurized (70°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
Refrigerated (4°C)				
0 Days	14 Days	28 Days	56 Days	112 Days
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND

ND - Not Detected

Appendix C

Post Hoc Test Results

Bonferroni Adjustment

Table C.1 Bonferonni Adjustment Pairwise Comparison Probabilities

(Aerobic Raw Data for Normal Storage Temperatures).

Samples	Past. 55°C	Past. 70°C	Fridge 4°C	SB80	SBPS1:1	SBPS1:3	SBPS3:1
Past55°C	1						
Past70°C	1	1					
Fridge4°C	0.453	1	1				
SB80	1	0.04	0.001	1			
SBPS1:1	1	1	1	0.52	1		
SBPS1:3	1	0.187	0.009	1	1	1	
SBPS3:1	1	1	0.44	1	1	1	1

SB - Sodium Benzoate SBPS - Sodium Benzoate/Potassium Sorbate Mixtures

1:1 - Ratio of the Mixture Past - Pasteurization

Table C.2 Bonferonni Adjustment Pairwise Comparison Probabilities

(Aerobic Raw Data for Abusive Storage Temperatures).

Samples	Past. 55°C	Past. 70°C	Fridge 4°C	SB80	SBPS1:1	SBPS1:3	SBPS3:1
Past55°C	1						
Past70°C	1	1					
Fridge4°C	1	1	1				
SB80	0.5	0.648	0.256	1			
SBPS1:1	1	1	1	0	1		
SBPS1:3	1	1	1	1	0.783	1	
SBPS3:1	0.125	0.092	0.257	0	1	0.005	1

SB - Sodium Benzoate SBPS - Sodium Benzoate/Potassium Sorbate Mixtures

1:1 - Ratio of the Mixture Past - Pasteurization

Table C.3 Bonferonni Adjustment Pairwise Comparison Probabilities

(Anaerobic Raw Data for Normal Storage Temperatures).

Samples	Past. 55°C	Past. 70°C	Fridge 4°C	SB80	SBPS1:1	SBPS1:3	SBPS3:1
Past55°C	1						
Past70°C	0.192	1					
Fridge4°C	0.522	1	1				
SB80	1	0.349	0.892	1			
SBPS1:1	0.309	1	1	0.55	1		
SBPS1:3	1	1	1	1	1	1	
SBPS3:1	0	1	0.481	0	0.791	0.014	1

SB - Sodium Benzoate SBPS - Sodium Benzoate/Potassium Sorbate Mixtures

1:1 - Ratio of the Mixture Past - Pasteurization

Table C.4 Bonferonni Adjustment Pairwise Comparison Probabilities

(Anaerobic Raw Data for Abusive Storage Temperatures).

Samples	Past. 55°C	Past. 70°C	Fridge 4°C	SB80	SBPS1:1	SBPS1:3	SBPS3:1
Past55°C	1						
Past70°C	1	1					
Fridge4°C	1	1	1				
SB80	1	0.966	1	1			
SBPS1:1	1	1	1	0.69	1		
SBPS1:3	1	1	1	1	1	1	
SBPS3:1	0.024	1	0.014	0	1	0.181	1

SB - Sodium Benzoate SBPS - Sodium Benzoate/Potassium Sorbate Mixtures

1:1 - Ratio of the Mixture Past - Pasteurization

Table C.5 Bonferonni Adjustment Pairwise Comparison Probabilities (Isolate Tempature Profile).

Temperatures	5°C	10°C	20°C	35°C	45°C
5°C	1				
10°C	1	1			
20°C	0.12	0.732	1		
35°C	0	0	0	1	
45°C	0	0	0	1	1

Table C.6 Bonferonni Adjustment Pairwise Comparison Probabilities (Isolate Utilization of Salt Profile).

Salt%	0%	2%	4%	6%	8%	10%	12%	14%	16%	18%	20%	22%	24%
0%	1												
2%	0	1											
4%	0	1	1										
6%	0	1	1	1									
8%	0.05	0	0	0	1								
10%	1	0	0	0	0.7	1							
12%	1	0	0	0	0	1	1						
14%	1	0	0	0	0	1	1	1					
16%	0.119	0	0	0	0	0	0.17	1	1				
18%	0	0	0	0	0	0	0	0	1	1			
20%	0	0	0	0	0	0	0	0	0.3	1	1		
22%	0	0	0	0	0	0	0	0	0	0	0	1	
24%	0	0	0	0	0	0	0	0	0	0	0	1	1

Table C.7 Bonferonni Adjustment Pairwise Comparison Probabilities (Isolate Growth in Sodium Benzoate at various concentrations (ppm)).

Concentrations (ppm)	0	250	500	750	1000
0	1				
250	1	1			
500	1	1	1		
750	0.707	0.213	1	1	
1000	0	0.001	0.1	0.608	1

Table C.8 Bonferonni Adjustment Pairwise Comparison Probabilities (Isolate Growth in Potassium Sorbate at various concentrations (ppm)).

Concentrations (ppm)	0	250	500	750	1000
0	1				
250	0.039	1			
500	0	1	1		
750	0	1	1	1	
1000	0.018	1	1	1	1

Table C.9 Bonferonni Adjustment Pairwise Comparison Probabilities (Isolate Growth in Sodium Benzoate/Potassium Sorbate (1:1 ratio) at various concentrations (ppm)).

Concentrations (ppm)	0	250	500	750	1000
0	1				
250	0.047	1			
500	0.01	1	1		
750	0	1	1	1	
1000	0	1	1	1	1

Table C.10 Bonferonni Adjustment Pairwise Comparison Probabilities (Isolate Growth in Sodium Benzoate at various pH concentrations).

pH Units	4	5	6	7
4	1			
5	0	1		
6	0.284	0	1	
7	0.225	0	1	1

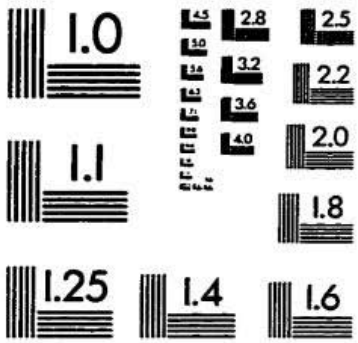
Table C.11 Bonferonni Adjustment Pairwise Comparison Probabilities (Isolate Growth in Potassium Sorbate at various pH concentrations).

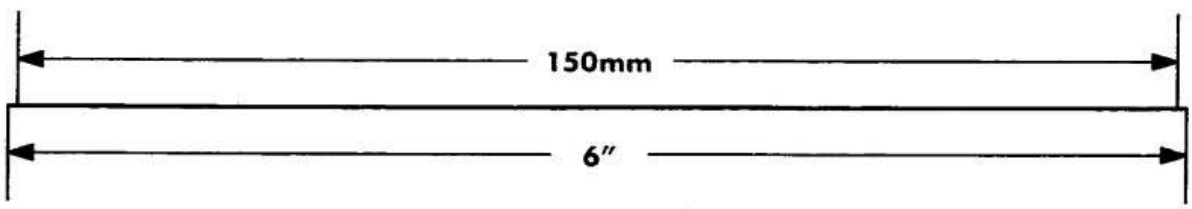
pH Units	4	5	6	7
4	1			
5	0	1		
6	0	0	1	
7	0	0	1	1

Table C.12 Bonferonni Adjustment Pairwise Comparison Probabilities (Isolate Growth in Sodium Benzoate/Potassium Sorbate Mixture (1:1 Ratio) at various pH concentrations).

pH Units	4	5	6	7
4	1			
5	0	1		
6	0	0	1	
7	0	0	1	1

TEST TARGET (QA-3)





APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989