# THE UNIVERSITY OF MANITOBA

# PROSPECTS FOR SALMONELLA CONTROL IN LIQUID EGG WHITE BY A BACTERIAL FERMENTATION PROCESS

by

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# PROSPECTS FOR SALMONELLA CONTROL IN LIQUID EGG WHITE BY A BACTERIAL FERMENTATION PROCESS

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ABSTRACT

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In order for egg white powder to maintain its color, flavor, solubility, and odor the natural free glucose must be removed prior to drying. At the present time the best method for the removal of glucose is by controlled bacterial fermentation processes which are vulnerable to the invasion by Salmonella. In this study liquid egg white was desugared by controlled bacterial fermentation employing Leuconostoc mesenteroides NRRL B199, Leuconostoc mesenteroides ATCC 27258, and Streptococcus cremoris. Optimum fermentation temperature for Leuconostoc strains was found to be 32°C. An inoculum size of 10% (v/w) was shown to be adequate for egg white fermentations.

Leuconostoc mesenteroides NRRL B199 desugared egg white to the commonly accepted level in ca. 7 hours and produced a fermented product with a pleasant, sweet aroma. Fermentation of egg white with Leuconostoc mesenteroides ATCC 27258 required 8 hours and produced a fermented product with a slightly unpleasant odor. The Streptococci require

more than 9 hours to desugar egg white to the acceptable level and produced a product with strong objectionable odors.

Fermentation of egg white with Leuconostoc strains at temperatures higher than 32°C resulted in an increase in contaminating microorganisms. Fermentation with Leuconostoc mesenteroides NRRL B199 and Leuconostoc mesenteroides ATCC 27258 resulted in 24.8% and 38.0% of the microbial population to be atypical colony-forming bacteria respectively. Whereas fermentation of egg white at 32°C with L. mesenteroides NRRL B199 and L. mesenteroides ATCC 27258 resulted in 24.3% and 28.9% of the microbial population being atypical colony-forming bacteria respectively. This indicates that L. mesenteroides is a more effective fermentation organism as well as being capable of suppressing the growth of contaminating organisms.

In this study egg white fermented at 32°C and 37°C was artificially contaminated with ca. 10 and ca. 100 Salmonella per mL. With artificial contamination at a level of ca. 10 Salmonella per mL of egg white the organism was undetectable after the first fermentation. However, the fermentations containing ca. 100 Salmonella per mL egg white growth of Salmonella resulted during the fermentation. When fermented egg white containing more than 100 Salmonella per mL was used an inoculum for a fresh lot of egg white contamination occured with the Salmonella levels increased. After four culture transfers at 32°C and 37°C the Salmonella level reached $1.8 \times 10^5$ and $3.5 \times 10^5$ Salmonella per mL of fermented

egg white. At the end of seven culture transfers of the fermented egg white at 32°C and 37°C the Salmonella levels stabilized at $5.4 \times 10^5$ and $1.5 \times 10^6$ at 32°C and 37°C respectively.

TABLE OF CONTENTS

TABLE OF CONTENTS Continued

TABLE OF CONTENTS Continued

TABLE OF CONTENTS Continued

LIST OF TABLES

LIST OF FIGURES

# 1.0 INTRODUCTION

Eggs and egg whites are important as food ingredients because of their properties and uses. Egg white is an extremely multi-purpose food ingredient due to its functional properties as a binder, whipping agent, foaming agent, and because it coagulates easily. The bland flavor of egg white also facilitates its use in many food products, as it adds very little flavor to such products.

Production of dried egg white in Canada amounts to about one million pounds per year. According to Agriculture Canada Poultry Division (1981), the production in 1977 was 1,083,218 pounds, in 1978 it was 830,178 pounds, and in 1979 it stood at 943,449 pounds.

Research in the past has indicated that if dried egg white is to maintain its colour, flavor, odor, as well as its high quality, the free glucose content in the egg white must be removed, Stuart and Goresline (1942).

Over the years numerous methods have been employed to desugar egg white prior to dehydration by spray drying. The methods of desugaring egg whites are: natural fermentation, yeast fermentation, enzyme fermentation, and controlled bacterial fermentation.

Controlled bacterial fermentation is presently the

most widely used method in industry because it produces egg white powder with superior functional properties. It is also the most economical to perform.

Regardless of which desugaring process is employed, the proliferation of Salmonellae in egg white still remains a problem. Salmonellae is part of the natural microflora of eggs. Wells (1958) Northolt (1978) Aryes and Solberg (1949) Banwart and Aryes (1956).

During fermentation of egg whites, Salmonellae may grow to substantial numbers, Mickelson and Flippin (1960). The prupose of this investigation was to study ways to minimize Salmonellae contamination and growth during the desurgaring process employing Leuconostoc mesenteroides.

# 2.0 REVIEW OF LITERATURE

## 2.1.0 Uses of Egg White Powder

Egg white is an important food ingredient because of the many functional and nutritional properties it possesses. The proteins of egg white function as leavening agents by foam formation upon physical whipping; such as used in sponge and angel food cakes. Egg white also possesses a tenderizing function which is used in baked products such as cakes. Texture is also derived from the coagulation of egg white proteins by heating. Products such as wieners, sausages, and meat analog products use egg white powder as a binder. Nutritionally egg white is an excellent, well balanced source of the twenty amino acids required by man. However, care must be taken in processing and dehydrating this product to avoid adverse affect on the properties of egg white proteins.

According to Wells et al. (1958) Salmonellae are frequently isolated from eggs and egg products. Therefore pasteurization is an important process particularily for egg whites used in nougat cream, marshmallow whip, ice cream, sherbert, and other confectionaries where products are insufficiently cooked to destroy Salmonella and other pathogenic organisms.

## 2.2.0 Egg White Composition

Egg white consists primarily of proteins and water with a gross composition of 10% protein, 0.03% lipid, 0.4 - 0.9% carbohydrate, 0.5% ash, and 88% water. (Table I)

## 2.2.1 Egg White Proteins

Egg white contains as many as 40 proteins, more than half of which are minor components. At least 19 bands can be identified on starch gel electrophoratograms and of these, only 6 proteins exist in a concentration of 1% or greater (Lush, 1961).

Ovalbumin is a protein which constitutes slightly over one-half of the egg white proteins (Steven, 1961). Recently it has been determined that ovalbumin consists of three separate proteins. Ovalbumin is unique as an egg protein since it is free of sulfhydryl groups.

Lysozyme is a globular glycoprotein present in a fairly large quantity, 3.5% in fresh liquid egg white. This protein has lysogenic enzyme activity on Gram positive bacterial cell walls (Johnson, 1966).

Ovatransferrin (conalbumin) is another glycoprotein which constitutes 13% of the albumen fraction of eggs. This protein has a molecular weight of 68,000 to 95,000 and acts as a chelator. It is able to bind two atoms of iron per molecule of protein. The exact mechanism of iron binding appears to be mediated via two nitrogen and three phenolic groups and possibly a bicarbonate radical (Windle et al.

TABLE 1

Gross Composition of Egg White

| Composition | Percentage |
|---|---|
| water | 88 |
| protein | 10 |
| carbohydrate | 0.4 - 0.9 |
| ash | 0.5 |
| lipid | 0.03 |

Egg Science and Technology (1973)

1963). The binding affinity of metal ions for ovatransferrin is Fe, Co, Cu, Zn, and Al, with iron having the greatest affinity.

Avidin is the albumen protein which binds biotin (Eakin et al. 1941). This protein is only a minor constituent of egg white comprising only 0.05% of the total protein. It has a high binding capacity in that three moles of biotin can be bound to one mole of avidin, rendering biotin unavailable in egg white for use by microorganisms.

Ovomucin is the protein which gives egg white its characteristic viscous jelly-like and sticky properties. This protein precipitates out of solution of a pH of 6.0 or below as stringy white fibrils. Therefore, it is important that processes like fermentation of eggs do not decrease the pH below this critical level.

Ovomucoid is an egg white protein which is heat and acid stable. This protein was discovered by Lineweaver and Murray (1947) and was found to be a trypsin inhibitor with less than one molecule of ovomucoid required to reduce the activity of one molecule of trypsin by 50%. The quantity of this protein in egg white is about 1% to 2%.

Flavoprotein is another protein of egg white which is very heat stable being able to withstand temperatures of 100°C for 15 minutes without denaturation. Flavoprotein is one of the few proteins in nature having a vitamin bound directly to it. Riboflavin when bound to flavoprotein also forms a heat stable complex. In nature this vitamin-protein complex

is thought to ensure the transfer of riboflavin from blood serum to the albumin fraction upon formation of the egg.

### 2.2.2 Lipids in Egg White

The lipid content of albumen is considered negligible, consisting of only 0.02% to 0.03% of egg white and has never been well characterized (Vadehar and Nath 1973). Egg white may be contaminated with egg yolk lipids during the egg breaking and separation operations in commercial processing thus increasing the lipid level of the product.

The lipid content of egg yolk is about 29%, and therefore even minute amounts of yolk will greatly change the composition of the egg white. Bergquirst (1964) illustrated that addition of 0.03% yolk to egg white resulted in only a slight reduction in foaming ability of liquid egg white. The whipping properties of egg white solids with this amount of yolk were greatly reduced. Cotterill and Funk (1963) found that 0.02% yolk was detrimental in spray-dried egg white solids. It was found that in the presence of small quantities of egg yolk, the loss of whipping properties were greatly magnified upon spray drying the egg white. Minute quantities of fats derived from contaminating yolks are changed from a highly emulsified state to a free state during drying. These free fats could spread and coat the dried egg white particles, thus reducing the foaming ability of the egg white when reconstituted. Cotterill and Funk (1963) showed that pancreatic lipase improved the whipping properties of the egg white

contaminated with yolk. In commercial practice about 1 ounce of commercial lipase preparation per 1,000 pounds of egg white is used to improve the whipping properties of egg white.

### 2.2.3 Carbohydrates in Egg White

The amount of free carbohydrates in egg white is about 0.5% (Romanoff and Romanoff 1949) with more than 98% of the carbohydrate in the form of glucose. This small amount of glucose, however, is very important since it has been shown to affect the functionality and quality of egg white, (Wadehra and Nath 1973). In addition, 0.4% to 0.5% of the carbohydrate in egg white is in the form of glycoproteins which do not appear to affect the functionality, color, or flavor of the product.

The presence of free glucose in egg white can cause chemical changes during drying, hot room pasteurization and storage of egg white solids. Kline and Sonada (1951); and Kline et al. (1954) reported that if the egg product contains its natural glucose, it will be more adversely affected by drying and subsequent hot room treatment than if the glucose had been removed through fermentation.

Egg white containing free glucose when heated yields a reaction involving the reducing groups of glucose and the amino groups of proteins. Condensed brown pigments called melanoidins are formed (Kline and Stewart 1948). This is the "Maillard Reaction" which involves a condensation

reaction between the α amino groups of amino acids, known as the carboxylamino reaction. The initial condensation product is a Schiffs base which undergoes cyclization to a corresponding N- substituted glycosylamine. The reaction then undergoes an Amadori rearrangement with N- substituted glycosylamine being protonated to the cation of Schiff's base, which is unstable and loses a proton yielding N- substituted 1- amino 1- deoxy 2- ketose in the enor form. This product is tautomerized to the keto form, then cyclized to fructosamine acid. The fructosamine derivatives through poorly defined reactions produce brown pigments. These brown melanoidin pigments and the listed intermediates are known to add unwanted falvors and add to the insolubility of spray dried egg white. Therefore the removal of sugar from egg white is definitely essential to a high quality stable dried product.

## 2.3.0 Nutritional Value of Eggs

Eggs and especially egg whites are a valuble food in the human diet. Nutritionists have termed eggs nature's most nearly perfect food protein. Egg white protein is known as a balanced protein because it contains all of the amino considered essential for growth and maintenance of body tissues (Table 2). Two eggs may furnish from 35% to 121% of man's daily requirements of essential amino acids (Hunt and Morrison 1977). Egg white is also an excellent source of vitamins such as biotin, inositol, niacin, and riboflavin (Table 3).

TABLE 2

Amino Acid Content of Egg White Powder

| Amino Acid | g/100g powdered egg white |
|---|---|
| Alanine | 4.66 |
| Argenine | 4.42 |
| Aspartic acid | 8.17 |
| Cystine | 2.38 |
| Glutamic acid | 10.51 |
| Glycine | 2.57 |
| Histidine | 1.83 |
| Isoleucine | 3.97 |
| Leucine | 7.35 |
| Lysine | 5.36 |
| Methionine | 2.95 |
| Phenylalanine | 4.74 |
| Proline | 2.77 |
| Serine | 5.37 |
| Threonine | 3.55 |
| Tryptophan | 1.38 |
| Tyrosine | 3.20 |
| Valine | 5.16 |

Cotterill, Glaurent, and Froning (1978)

TABLE 3

Vitamin Content of Egg White Powder

| Vitamin | mg/100 g powdered egg white |
|---|---|
| $B_{12}$ | 00.21 |
| Biotin | 38.90 |
| Folic acid | 00.08 |
| Inositol | 51.30 |
| Niacin | 01.18 |
| Panthothenic acid | 00.67 |
| Riboflavin | 02.75 |
| Thiamine | 00.19 |

Cotterill, Glaurent, and Froning (1978)

Minerals which are supplied by egg white are potassium, sodium, and sulfur (Table 4).

Egg yolk is the source of the lipid portion of the egg. Egg lipids are easily digested and help provide energy for bodily needs. This portion of the egg is rich in essential fatty acids. The fat component of egg yolk contains a high proportion of unsaturated fatty acids. Egg yolk is also a good source of vitamins A, D, and $B_{12}$. Only vitamin C is not found in significant quantities in eggs, white or yolk. Egg yolk is also an excellent source of iron and phosphorous.

The egg as a whole is an excellent food because of the high nutrient content, low caloric content, approximately 80 calories for a Grade A Large egg and ease of digestability.

## 2.4.0 Antimicrobial Properties of Egg Whites

Proteins of egg white, while possessing interesting physical and chemical properties, have in addition several unique biological properties. The intrinsic antimicrobial agents found in egg white are of particular interest in the desugaring process during fermentation. These agents have been extensively studied, and their presence as antagonists has been appraised for their bacterostatic and bactericidal ability. The agents and their mode of actions have been thoroughly reviewed by Yadav and Yadehra (1977), Board (1968), Lineweaver and Murray (1947) and Yadehra and Nath (1973).

### 2.4.1 Lysozyme

Lysozyme is a protein in egg white which has a

TABLE 4

Ash Content of Egg White Powder

| Minerals | mg/100 g powdered egg white |
|---|---|
| Calcium | 53.70 |
| Chlorine | 1374.50 |
| Copper | 0.18 |
| Iodine | 0.03 |
| Iron | 0.05 |
| Magnesium | 88.70 |
| Manganese | 0.50 |
| Phosphorous | 114.00 |
| Potassium | 1290.00 |
| Sodium | 1280.00 |
| Sulfer | 1338.00 |
| Zinc | 0.10 |

Cotterill, Glaurent, and Froning (1978)

bacteriostatic activity on Gram positive cell walled bacteria. The mode of action for lysozyme is by hydrolysis, specifically between the $\beta$ (1-4) linkages of N- acetylmuraminic acid and N- acetylglucosamine (mucopeptides) of the bacterial cell wall (Board, 1968). Gram negative bacteria are generally resistant to lysozyme, due to the mucopeptides being protected by the laminae layer of lipoproteins and lypopolysaccharides (Burge and Draper, 1967). It has been suggested that lysozyme also act by flocculation of bacterial cells that penetrated into the egg. This action is thought to be related to the basic properties of lysozyme, as compared to the net negative charge on the bacterial cell wall (Wersen et al. 1971). The antibacterial properties of lysozyme are restricted to selected organisms possessing sufficient amounts of lysozyme substrates in their cell walls.

### 2.4.2 Ovatransferrin

Ovatransferrin is an organic ligand that forms complexes with iron, zinc and copper. The inhibition by ovatransferrin is expressed an an increased lag phase during growth, or as a decrease in the growth rate once growth is initiated. This is a bacteriostatic function presumably due to a decrease in the organisms ability for oxidative phosphorylation (Theodore and Schade 1965). This antibacterial property is, however, restricted (to bacteria in an iron deficient environment.) This interrelationship between ovotransferrin and iron in this situation is still not clear. It is possible that the organisms transport iron on their

surfaces which may be readily available to them in their microenvironment at the locale of growth (Vadehra and Nath 1973).

### 2.4.3 Avidin

Avidin is an egg protein which exerts an inhibitary action on bacteria by its ability to bind biotin (Eakin et al. 1940, 1941). Biotin is a growth factor which is required for lipid synthesis by some bacteria. The avidin-biotin complex is very stable and resistant to dissociating agents like urea and guanidinium chloride. However, due to the minute quantities of avidin present in albumen, the practical importance in preventing egg spoilage is miniscule.

### 2.4.4 Antibacterial Action of Egg White due to its Physio-chemical Properties

The physio-chemical properties of egg whites have been proposed as being antagonistic to bacteria. Due to the considerable changes in the pH of egg albumen during storage (pH 7.6 to 9.2) it has been suggested that pH has an influence on the growth of bacteria. However, most bacteria grow fairly well up to pH 9.0, and the extent of pH change if any, is of minor practical importance according to Vadehra and Nath (1973).

The physical structure of egg albumen has been proposed to be inhibiting to bacterial growth (Yadav and Vadehra 1977). They altered the physical structures of the proteins by sonic and papin treatments and observed a decrease in the

resistance of egg white to bacterial growth. They also proposed that in fresh egg white, the physical structure of the proteins themselves are probably the primary barrier to bacterial growth.

It has also been suggested by Vadehra and Nathe (1973) that the lack of available water may be a significant inhibitor of bacterial growth in albumen. This lack of available water in albumen was reported by Yadav and Vadehra (1977) who demonstrated a decrease in resistance to bacterial growth by addition of water to egg white at a ratio of 4:6 egg white/water, volume/volume.

## 2.5.0 Buffering Activity of Egg White Proteins

During the fermentation of egg white, microorganisms utilize the free glucose present and produce organic acids. The effect of acid production on the change in pH is usually observed so that a decrease in pH can be used as an indication of glucose degradation. Strong buffering capacities if present in egg white, would make pH change a poor indicator of glucose utilization.

According to Cotterill (1958) egg white is only weakly buffered between pH 7.0 and 9.0 while fresh egg white is strongly buffered in the regions of pH 6.4 and 10.3. Normal fermentation of egg white occurs between pH 9.0 and 6.5, therefore it is not affected by the buffering regions.

The age of the egg, or in effect, the loss of carbon dioxide from the egg, decreases the buffering capacity of egg

white. Thus there is a loss in buffering capacity upon aging of the egg. Carbon dioxide exists in various forms at different pH values. The buffering capacity of this system is maximal at pH 6.4 and 10.4 and minimal at pH 8.3. Approximately 6% of the carbon dioxide in egg white at pH 7.6 is theoretically in the form of carbonic acid, which easily decomposes to carbon dioxide and water. When the carbon dioxide is removed from the system the pH of the egg white rises.

Titration curves for egg white protein have been investigated by Greenberg (1951), Tanford (1955), Steinhardt and Zaiser (1955), Fox and Foster (1957), and Cotterill et al. (1958). Figure 1 illustrates a typical titration curve for egg white.

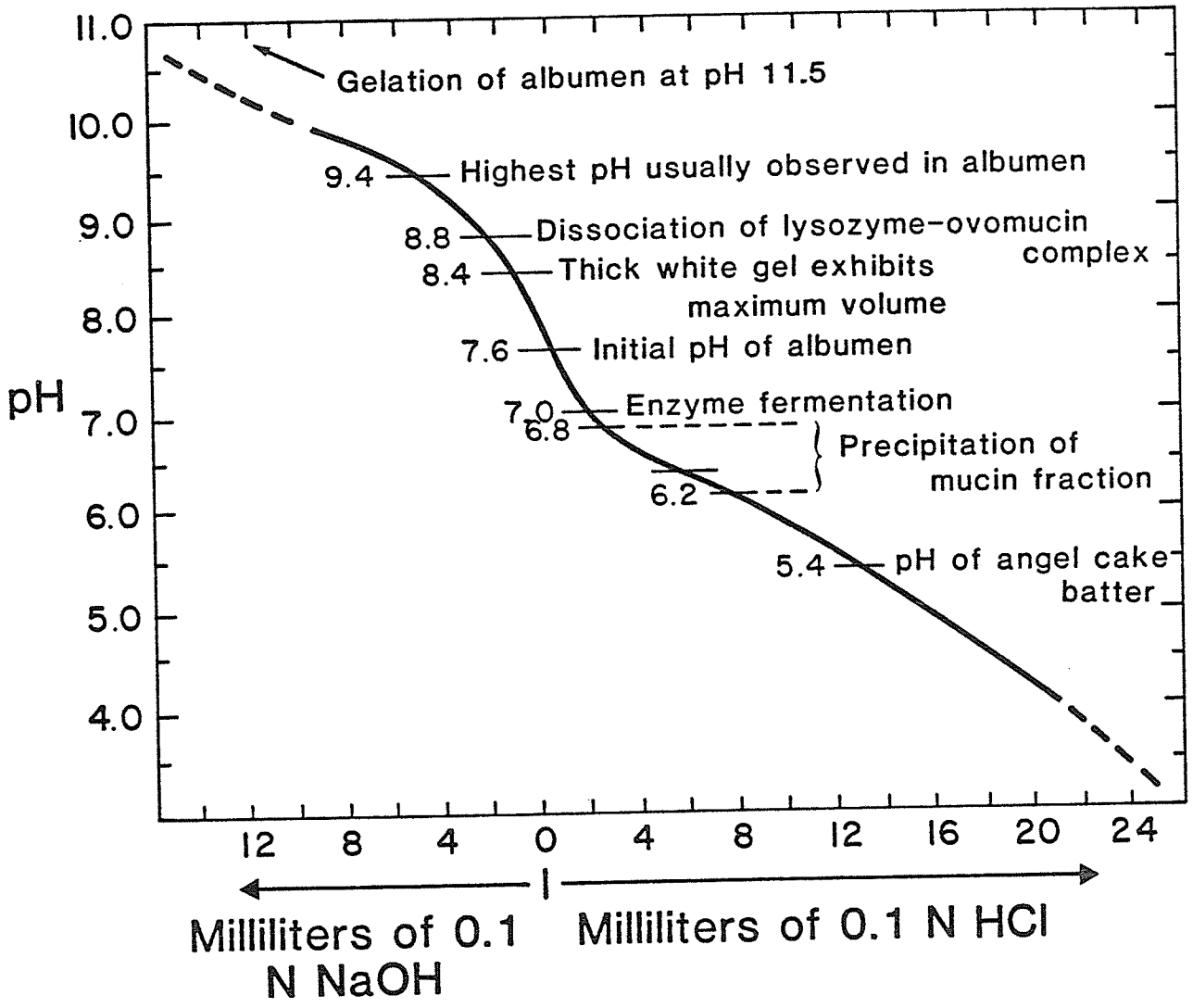
### 2.6.0 Desugaring of Egg White

The removal of free glucose from raw egg white is necessary to produce a high quality finished dried product. Stewart and Kline (1941) associated changes in color, flavor, and solubility because of the presence of small amounts of free glucose. A more stable product will result if most of the free glucose is removed. Sugar removal is essential for product stability (color, flavor, and solubility) that may occur during hot room pasteurization and subsequent storage.

In the past various methods of desugaring egg white have been proposed. The most primitive method being the natural fermentation first used in China 55 years ago (Egg Science and Technology, 1977). Other methods were yeast

Figure 1

Significant pH values superimposed
on egg white titration curve




Cotterill, O. J., F. A. Gordner, F. E. Cunningham, and E. M. Funk (1958)

fermentation, enzyme oxidation of glucose and controlled bacterial fermentation. The controlled bacterial fermentation method is a progression from the natural fermentation procedure and is more dependable resulting in a more consistent product.

### 2.6.1 Natural Fermentation

Natural fermentation of egg white was initiated in China in the 1920's. The eggs were broken and separated and the white was allowed to ferment naturally by the microflora found in the egg or contaminants from equipment, shells, and air. These organisms were used to liquify the thick white prior to pan drying in the sun. During the fermentation process carbon dioxide and ammonia are produced by the enzyme system. The egg white becomes alkaline, bacteria then grow and hydrolyze the proteins and carbohydrates producing organic acids. This fermentation was allowed to proceed for 30 to 60 hours at 70°F (21.1°C). A scum would rise to the top and a sludge settle at the bottom of the wooden vat. Both the top scum and settled sludge were discarded as unfit for drying. They made up approximately 8% of the total volume. In America, prior to the mid 1940's, fermentation of raw liquid egg white was conducted in a similar process, by contaminating microorganisms, giving rise to a natural fermentation".

Stuart and Kline (1941) studied the natural fermentation of egg white at temperatures between 23.9°C and 29.5°C for 2 to 7 days. They reported that the exact time

of fermentation depended on the type of organism and the amount of bacterial contamination. During the spontaneous process of fermentation, they observed thinning of the albumen and a gradual production of many organic acids, with mild characteristic odors. The effect of acid production mucin fibers in the thick egg white fraction to contract, causing flotation of the scum on top of the vat. In the natural fermentation process, the reaction was stopped after approximately 72 hours. The liquid egg white was then prepared for drying (Stuart and Goreslime 1942a).

Confusion as to the true purpose of fermentation arose when Epstein and Harris (1931) added glucose to delay proteolysis and putrifaction in liquid egg white during fermentation. Stuart and Goreslime (1942a) clarified the confusion from a manufacturer's viewpoint by suggesting that fermentation accomplishes two objectives. It removes sufficient carbohydrate so that the resulting product was stable thereby maintaining its quality during storage as well as making the produce thin enough to facilitate ease of handling and drying.

Stuart and Goreslime (1942a, 1942b) bacteriologically examined 8 samples of commercially fermented egg white and found the predominant organisms to be of the genera Enterobacter and Citrobacter. Egg white fermented by either of these organisms yielded a bright, crystaline and granular product after drying. However, if proteolytic bacteria such as Proteus, Serriatia, or Pseudomonas became established during the fermentation, an inferior product with off flavors and

odors resulted.

### 2.6.2 Enzyme Fermentation

The enzyme, glucose oxidase, discovered and isolated by Muller (1928) from the molds Aspergillus niger and penicillium glaucum, was shown to have the ability to oxidize glucose, with molecular oxygen, to produce gluconic acid. Frank and Lorenz (1937) using an enzyme preparation from Pencillium with greater purity detected by hydrogen peroxide formation when allowed to react with glucose, they also suggested the enzyme was a flavoprotein. Coulthard et al. (1942, 1945), Brugger et al. (1943), and Kielin and Hartree (1946, 1948) also investigated methods to produce a more purified glucose oxidize enzyme preparation.

Bentley and Neuberger (1949) characterized this enzyme as a glucose dehydrogenase, and using radioisotopic techniques, discovered that the oxygen atom in hydrogen peroxide was derived from molecular oxygen, and that the enzyme catalyses the transfer of hydrogen from glucose to oxygen. In practice glucose oxidase is used in association with the enzyme catalase to convert hydrogen peroxide to water and oxygen.

Shar and Roghavan (1949) indicated that $H_2O_2$ will oxidize, coagulate, or modify egg white performance. Their study supported the use of catalase to reduce the peroxide which was capable of denaturing egg white. Snider and Cotterill (1972) studied the effects of hydrogen peroxide on egg white. They found that hydrogen peroxide could oxidize certain amino

acid groups to a highly polar species which could then lead to coagulation by polymerization of proteins through extensive hydrogen bonding and/or electrostatic interactions.

On the commercial scale, egg white was first enzymatically desugared by Baldwin et al. (1953) using a commercial preparation of glucose oxidase catalase enzyme system isolated from Phascolus radiatus and Phascolus mungo. The process was reported to be easy to control, to reproduce, and was capable of producing a uniform powder with no objectionable odors, traceable to the desugaring process. Carlin and Ayres (1953) produced egg white powder from glucose oxidize treated albumen which had no off odors or flavors and produced acceptable angel food cakes.

Enzyme treatment of egg white is not commercially used today since the finished produce is functionally inferior as compared to the product produced by controlled bacterial fermentation.

The functional properties of enzyme treated egg white compared unfavorably to those of egg white fermented with Streptococcus faecalis (Mulder, 1981). With enzyme treated egg white the whipping time was 110 seconds as compared to 55 seconds for bacterially treated egg white. He also found that foam volumes were 800% and 1305% respectively and that foam stabilities were 72% and 95% respectively after one hour.

### 2.6.3 Yeast Fermentation

The use of pure yeast cultures to remove glucose from liquid egg white was introduced by Hawthorne and Brooks (1944) who desugared egg white by fermentation with the yeast Saccharomyces apicilatis using a 1% (2/2) inoculum and an incubation temperature of 37°C. This process reduced the free glucose level of the egg white from 0.5% to 0.05% in 3 hours. A disadvantage of this process was the presence of a yeasty flavor in the finished, dired product.

Ayres and Stewart (1947) used a smaller incoculum size of 0.5% (w/w) of Saccharomyces cerivesia with the addition of 0.1% yeast extract. The fermentation was performed at 30°C for 24 hours. This process was successful in removing all of the free glucose from egg white without destroying the mucin fraction (pH above 6.4); little yeastiness was noted in the finished product.

Jack et al. (1949) fermented whole egg with a 0.2 to 0.4% inoculum size (w/w) of Saccharomyces cerivesia at 22 to 23°C and reported that the free glucose was depleted in 2 to 4 hours. Kline et al. (1951, 1953), and Hawson and Kline (1954) suggested that yeast fermentation was practical for desugaring egg white. Carlin et al. (1954), however, reported that yeast-fermented, pan dried egg white, was inferior to enzyme treated egg white powder in angel cake tests. They stated the yeast fermentation had a drastic, adverse affect on the functionality of the egg white, as well as the production of off odor, and flavor.

### 2.6.4 Controlled Bacterial Fermentation

As previously stated, early bacterial fermentations were spontaneous or natural, the numbers and types of organisms which fermented the egg white where dependant upon the natural microflora of the egg, egg shells, breaking machinery, and the cleanliness of the fermentation vat. Stuart and Goresline (1942) used pure cultures of coliform organisms of the genus Enterobacter and Escherichia to desugar egg white. The conditions employed included temperatures of 30 to 33°C and fermentation times of 12 to 24 hours. This process yielded a finished product with a higher quality than the naturally fermented egg white.

Kaplain et al. (1950) reported a satisfactory fermentation scheme using a 1% (w/w) Streptoccus lactus inoculum. The fermentation process was carried out at 37°C for two hours, reaching a final pH of 6.5. This process of using bacterial concentrates was shown to reduce the contamination of the egg white and eliminate the need to prepare starter cultures. The fermentation was rapid and the products were free of objectionable flavors and odors. Recently, Galluzzo et al. (1974) fermented the glucose from whole egg using Streptococcus diacetilactus at 21.5°C. They noted, however, the production of a metalic odor and flavor when the pH of the whole egg was lowered with lactic acid. The culture used for the fermentation was grown and carried in 10% sterile non-fat dry milk containing 0.1% yeast extract. Although an inoculum rate of $5.0 \times 10^6$ to $3.0 \times 10^7$ cells per ml of whole egg was used, the presence of glucose was still detected

after 24 hours of fermentation. Galluzo used two Leuconostoc species that were identified as Leuconostoc citrovorium. The Leuconostoc cultures produced a weak fermentation odor at pH 6.5 and 6.0 resembling that of a weak solution of acetic acid and ethanol. There were no metallic odors nor diacetyl production by either culture. Their work illustrated that Leuconostoc do not generally produce diacetyl unless they cannot complete their metabolic process or are in a medium of low pH (Keenan and Bills 1968).

A potential drawback of using lactic cultures to desugar egg products is the production of off odors together with the presence of diacetyl. A strong metallic odor was also noted at times, suggesting the presence of acetaldehyde. Galluzzo (1974), Keenan et al (1966) found significant production of acetaldehyde by strains of Steptococcus cremoris, Steptococcus lactus, and Streptococcus diacetialactus.

Mulder (1981) stated that Streptococcus strains could be used to desugar liquid egg white and whole egg, although in high cell concentrations a slight off odor was produced. Streptococcus cultures, however, produced a very good quality product after drying based on functional properties assessed by the baking of angel food cakes.

Mulder (1981) fermented egg white with Streptococcus faecalis, Lactobacillus brevis, Lactobacillus casei, Lactobacillus fermentum, and Lactobacillus planitarium as well as by an enzyme fermentation. He observed that fermentations using the organism Lactobacillus brevis at 30°C for 36 hours

and Lactobacillus fermentum at 25°C for 48 hours produced the best finished product after the fermented products were dried. Mulder also found that glucose oxidase-catalase desugared egg white, produced a powder with the poorest functional properties. The organism Streptococcus faecalis fermented egg white in 22 hours at 37°C with a final pH of 5.0, however, no sensory evaluation was performed on the finished product.

Egg white is utilized primarily for its functional properties and not for its flavor properties, since egg white has either a bland flavor or no flavor at all. Since the ideal fermentation organisms should not impart any off flavors or odors, to the finished product, selected species of Leuconostoc have been employed commercially for desugaring egg white prior to drying for the manufacture of dried egg white.

Some of the work in the development of cultures for the bacterial fermentation of egg albumen has been carried out by individual companies in the egg processing industry. The results of their studies, and the cultures involved are generally regarded as confidential information and are closely guarded and usually not published. Consequently, there is a scarcity of recent information on this subject. Egg Science and Technology (1977).

## 2.7.0 Functional Properties of Egg White

The changes in egg white during fermentation, pumping, spray drying, and hot room pasteurization treatments are

mainly chemical in nature, however functional properties are usually used as an index of the extent to which the physical structures of egg proteins have been altered. Changes in protein structure are directly related to functional properties of egg white. The characteristic properties are: whipping, coagulation, binding, solubility, color, flavor, odor, and nutritional properties.

### 2.7.1 Whipping

Whipping or foam formation is an important functional property in products such as cakes and souffles. Whipped egg white proteins impart volume and lightness to food products. During the whipping process there is an apparent unfolding and spreading of protein which occurs at the air/liquid interface. Bergquist (1973) determined that proteins unfold or denature at the interface resulting in the formation of a relatively stable structure. MacDonnel et al. (1955) characterized the proteins primarily responsible for the functional properties of egg white when used in angel food cake. They concluded that ovoglobulin is the egg white protein largely responsible for the rapid whipping properties, while the protein ovomucin helps to stabilize the foam structure when it is formed.

Whipping is evaluated by the whip test developed by Gorman (1973). The test for dried products involves dissolving 43 grams of spray dried albumen in 430 ml of 70°F water and mixing in a Hobart electric mixer bowl on speed no. 1 (low) for 5 minutes prior to whipping on speed no. 2 for 90 seconds

and speed no. 3 for 90 seconds. The resulting foam is measured for foam depth, foam volume, and stability (volume of the foam 1 hour after whipping).

Coagulation is an important functional property of egg white. It allows for the fixation of food ingredients after coagulation by heating. The ovalbumen fraction is responsible for the structure of egg white by coagulation during backing (Vadehra and Nath 1973).

### 2.7.2 Binding

Egg proteins are becoming increasingly important as food binders in the rapidly growing fabricated food industry. The role of egg proteins in fabricated and simulated foods comes indirectly from their functional properties. Egg white is one of the best binders for holding vegetable proteins together in products like meat analogues. Egg proteins in addition also have the benefit of coagulation by heat. Thus egg proteins are capable of "glueing" the ingredients together and fixing them by coagulation upon heating (Vadehra and Nath, 1973). Egg white powder is used as a binder in products such as wieners, turkey and chicken rolls and loaves, sausages, etc.

### 2.7.3 Flavor

Eggs have a distinct not pronounced flavor by themselves and contribute to the bland flavor to the products in which they are used. The flavor of eggs can be changed by adverse drying conditions during storage of the dried egg

product. One of the most important factors contributing to loss of stability in storage of dried albumen is the presence of free glucose (Stewart and Kline 1941; Bate - Smith and Hawthorne, 1945). The most probable causes of off flavors in dried egg white are those produced by bacterial fermentation. Some fermenting organisms may produce compounds like diacetyl or acetaldhyde which have pronounced flavors. Whole egg and egg yolk powders are susceptible to oxidative deterioration due to phospholipids in the egg yolk and may result in more off-odor problems.

### 2.7.4 The Affect of Spray Drying on Functional Properties of Egg White

When egg white is dehydrated it remains primarily in the crystaline form until the water content is reduced below the 12% level. Further drying will cause the crystals to dry out and break up into granuals, and eventually to form a powdery material. In spray drying operations, egg white particles are dehydrated so rapidly that they apparently bypass the intermediate crystaline phases of drying. This results in the rapid formation of new surfaces which causes surface denaturation of the protein. MacDonnel et al. (1955), and Bergquist and Stewart (1952) illustrated that liquid egg white is quite sensitive to physical treatments such as pumping, and filtration, whereas whole egg and egg yolk is much more resistant to these shear forces. Bergquist and Stewart (1952) also observed denaturation of egg white during the homogenization and atomization steps in the production

of spray-dried egg white powder. They described surface denaturation as the irreversible unfolding of protein molecules at the solution surface.

## 2.8.0 Pasteurization of Eggs

Salmonellae are pathogenic microorganisms to man and animals, and constitute a health hazard when present in foods. Raw egg products are frequently contaminated with Salmonella and therefore must be pasteurized in order to protect the consumer from illness, Northolt et al. (1978). Pasteurization times and temperatures for the various egg products are listed in Table 5 and 6.

Liquid egg white, whole egg and egg yolk have been shown to exhibit different effects on the heat resistance of Salmonella. These differences are thought to be due to the difference in pH of these products. Osborne et al. (1954) determined that the heat destruction of different types of Salmonella at 140°F was 3 to 4 times greater in egg white (pH 9.0) than whole egg (pH 7.6) depending on the Salmonella serotype.

## 2.8.1 Pasteurization of Egg White Powder

The HTST pasteurization of liquid egg white prior to drying results in a loss of functional properties after drying according to Slosberg et al. (1948). As a result commercial processing involves some type of heat treatment of the dried egg white powder to eliminate Salmonella from the

TABLE 5

Pasteurization Requirements of Liquid Egg Products

| Liquid Product | Minimum Temperature Requirement | Holding Time Required Minutes |
|---|---|---|
| Albumen (without chemicals) | 57.0<br>55.5 | 3.5<br>6.2 |
| Albumen (lactic acid - $Al_2$ $[SO_4]_3$) | 60.0 - 61.5 | 3.5 |
| Albumen ($H_2O_2$) | 51.5 - 53.3 | 1.5 |
| Whole egg | 60.0 | 3.5 |
| Whole egg blends 2% non-egg ingredients (NEI) | 61.0<br>60.0 | 3.5<br>6.2 |
| Fortified whole egg and blends 24 - 38% egg solids, 2 - 12% NEI | 62.2<br>61.0 | 3.5<br>6.2 |
| Salted whole egg 2% or more NaCl | 63.3<br>62.2 | 3.5<br>6.2 |
| Sugar whole egg 2 - 12% sugar added | 61.0<br>60.0 | 3.5<br>6.2 |
| Plain yolk | 61.0<br>60.0 | 3.5<br>6.2 |
| Sugar yolk 2% or more sugar | 63.3<br>62.2 | 3.5<br>6.2 |
| Salted yolk 2 - 12% NaCl added | 63.3<br>62.6 | 3.5<br>6.2 |

Agriculture Canada, Poultry Division (1981)

TABLE 6

Pasteurization Requirements for Dried Egg Products

| Dried Product | Minimum Temperature Requirements °C | Minimum Time Required |
|---|---|---|
| Dried albumen[1] | 54 | 7 days |
| Dried whole egg[2] | 61 | 3.5 minutes |
| Dried egg yolk[2] | 61 | 3.5 minutes |
| Dried egg yolk with sugar and salt | 63 | 3.5 minutes |

[1]Pasteurized in dried form in 22.5 kg boxes in hot room.

[2]Pasteurized in liquid form with HTST treatment prior to dehydration

Agriculture Canada, Poultry Division (1981)

product with minimal loss of functional properties. This process is commonly referred to as hot room pasteurization.

Gibbons and Futton (1943) noted a two stage decline in total bacterial counts during hot room pasteurization. They observed a sharp initial decline then a gradual decline in bacterial numbers during the hot room treatment of egg white solids. At hot room pasteurization temperatures of 55°C for 5 weeks they found only spore forming bacteria surviving in dried egg white powder. Gibbons and Moore (1944) studying the destruction of low numbers of Salmonella in dried whole egg, also noted the two distinct death rates upon hot room storage of dried egg products. They reported that 99% of the organisms of the genus Salmonella in liquid whole egg were destroyed during the spray drying of the product.

Barnwart and Aryes (1956) found that dried egg white artificially contaminated with Salmonella at levels between $10^1$ and $10^7$ per ml. could be treated by hot room pasteurization, making the product free of Salmonella without imparting significant losses in functional properties of the product. When the storage temperature was increased from 50°C to 70°C and the moisture content also increased from 1.5% to 3.0%, an increase in the death rate of Salmonella was observed. These investigators stored egg white in a hot room for up to 100 days and monitored the functional properties of the product, such as solubility, whip test, and Angel cake volume. They suggested that it was possible to store dried albumen

containing 1.5, 3.0, or 6.0% moisture at 50°C or albumen containing 1.5% or 3.0% moisture at 60° or 70°C, respectively, to eliminate large numbers of Salmonella without significant impairment to the functional properties of the product.

Ayes and Slosberg (1949) observed that Salmonella in dried egg white survived room temperature storage for 18 months, whereas powders, pasteurized by storage in hot rooms at 57.2, 54.4, and 48.9°C for 4, 8, and 20 days, respectively, were Salmonella-free.

Smith (1964) obtained a patent for a procedure involving storage of dried egg white solids for eight hours at 71°C and then one hour at 99°C to insure a Salmonella-free product.

Baldwin et al. (1967) studied the effect of hot room pasteurization temperature and pH on the functional properties of egg white. They found that elevated storage temperatures of 54, 60, 71, and 82°C reduced the foam inhibiting ability of egg white which was contaminated with small quantities of egg yolk as indicated by a reduction in whipping time. They reported that the functional properties of spray-dried egg white were not impaired after 60 days of storage at 54°C. However, egg white stored at 71°C or 82°C with their pH adjusted to an alkaline pH range of 8.5 to 9.5 exhibited significant denaturation.

Of all the different time-temperature combinations used for the pasteurization of egg white powder, Northolt et al. (1978) recognized the preferred method as the storage of

the dried product in a hot room under the conditions outlined by Ayes and Slosberg (1949). The product is heat treated in the package thus making recontamination impossible, Barnwart and Aryes (1956). Hot room pasteurization is now a common practice in the commercial production of spray dried egg white powder requiring a minimal treatment at 54°C for 5 days. However, some commercial firms commonly use hot room conditions of 68°C for 14 days.

### 2.9.1 Leuconostoc mesenteroides - The Organism

The species of the genus Leuconostoc have been known for many years as spoilage organisms in the sugar industry and as useful components of dairy starters. Reliable representatives of the different species for comparison with unknown strains are not easy to obtain and thus in the past many cultures have been wrongly classified as Leuconostoc.

### 2.9.2 Classification

According to Bergey's Manual of Determative Bacteriology (1974), the family Streptococcaecae can be delineated into five genera: Streptococcus, Leuconostoc, Pediococcus, Aerococcus, and Genella on the basis of the following characteristics.

a) Genus I Streptococcus

Glucose is fermented by the hexose monophosphate pathway yielding levarotatory lactic acid, carbon dioxide, ethanol and/or acetic

acid (heterofermentative). Cell division in one plane resulting in pairs and chains, catalase negative.

b) Genus II Genella

Respiritory system resistant to KCN is the distinguishing feature of this genus.

c) Genus III Pediococcus

Glucose fermented yielding dextrorotatory lactic acid (homofermentative). Cell division in two planes resulting in pairs and tetrads, catalase variable. Meat starter cultures.

d) Genus IV Aerococcus

Glucose is fermented but the products of fermentation have not yet been completely separated. Gram indeterminate, but cell wall structure is of the Gram positive type. Catalase negative.

e) Genus V Leuconostoc

Glucose is fermented yielding optically inactive lactic acid (homofermentative). Cell division is in two planes resulting in pairs and tetrads. Catalase variable.

Taxonomically, Leuconostoc mesenteroides is defined as the non-slime forming Leuconostoc. However, it has been reported that certain strains of this organism can be induced to produce slime. Therefore, it is not proper to base species on slime formation. The most widely used characteristic to distinguish Leuconostoc mesenteroides is the ability of

the organisms to produce acid from pentoses such as arabinose, Garvie (1960).

### 2.9.3 General Characteristics of Leuconostoc

Bergey's Manual of Determinative Bacteriology (1974) defines Lueconostoc mesenteroides as spheres or lenticular cells 0.5 - 0.7 by 0.7 - 1.2 um; in pairs or short chains forming white pin head 1mm colonies. Glucose is catabolized anaerobically by the pentose pathway yielding 1 mole each of D(-) lactic acid, ethanol and carbon dioxide. Phosphoketo-lactose is present, fructose 1.6 diphosphate aldolase is absent. Some strains also have an aerobic oxidative metabolism using 1 mole oxygen to 1 mole glucose yielding equimolar quantities of carbon dioxide, lactate and acetate. These strains also have peroxidase activity. Furthermore, acetate, lactate, and tartrate are not used as a sole carbon source. The number of amino acids required is small only valine and glutamic acid, which are present in sufficient amount in egg white proteins. Temperature range for growth is between 10 and 37°C, optimum temperature at 20 to 30°C. Actively growing Leuconostoc do not generally produce diacetyl unless they cannot complete their metabolic pathways or are in a low pH environment, Keenan and Bills (1968).

## 2.10 Salmonella-The Organism

### 2.10.1 History

Salmonella was first discovered by Drs. Salmon and

Smith (1885). The organism was believed to be the causative agent of hog cholerae (cholerae-suis). The genus name was given to the grop in honor of Dr. P. E. Salmon and the first organism of the group isolated was Salmonella cholora-suis.

To date more than 2,000 strains of the genus Salmonella have been identified. 11 of these are capable of causing gastro enteritis in man and animals. In 1955 White and Kaufman established the present method of antigenic analysis of the Salmonella groups. By using specific diagnostic antisera the "O" antigen (body antigen) and the "H" antigen (flagella antigen) can be detected and these are used to identify and classify the serotypes of the genera.

### 2.10.2 Classification

According to Bergey's Manual of Determinative Bacteriology (1974) the family Enterobacteracae is divided into twelve genuses: Eschenchia, Edwardsiella, Citrobacter, Salmonella, Shigella, Proteus, Yersinia, Erwinia, Klebsiella, Enterobacter, Hafnia, and Serratia.

Classification of the family Enterobacter is documented in Bergey's Manual of Determinative Bacterology (1974).

### 2.10.3 General Characteristics of Salmonella

Bergey's Manual of Determinative Bacterology (1974) defines the genus Salmonella as Gram negative, rods, usually motile by peritrichous flagella; non-motile mutants may occur and the types S. gallinarium and S. pullorum are always non-

motile. Colonies are generally 2-4 mm. in diameter but certain types S. abortus-equi, S. typhi-suis and S. abortus-suis produce colonies of about 1 mm. Most strains will grow on defined media without special growth factors, and are capable of using citrate as a sole source of carbon.

Most strains are aerogenic but S. typhi an important exception, never produces gas. Anaerogenic varients of normal gas producing serotypes are found in nature, this is particularly common with S. dublin. Due to the more than 2,000 serotypes of Salmonella there is no complete information available on the growth requirements, optimum pH, or optimum temperature for growth of Salmonella.

## 2.11.0 The Sources and Vectors of Salmonallae

Salmonella is a widespread group of organisms found everywhere man and animal exist. Medical and veterinary medical literature during the past few years shows a wide variety of birds, reptiles, domestic and wild animals, garden snakes, pet turtles, frogs, and family domestic pets have been found to harbor and transmit Salmonellae (Edwards and Foster 1971).

The Food and Drug Administration (FDA) Task Force Report (1973) stated "Salmonella exist in the environment of man in profuse numbers and may be isolated with relative ease from water, food, air, sewage, milk and other environmental sources". The list of food product recalls over the last 10 years further concludes that Salmonellae are indeed ubiquitous.

Salmonellae in food products became prominent during the Second World War when United States spray dried egg products exported to Britain were found to be contaminated with Salmonella according to Stadelman and Cotterill (1977). Contamination of the egg occurred primarily after laying according to Stuart and McNally (1943). The numbers of microorganisms per shell is estimated to vary from $9.5 \times 10^3$ to $3.1 \times 10^6$ Rosser (1942); Board and Wilson (1956). It has also been well established that bacteria and dirt on the shell surfaces are the major sources for contamination of egg meats when the eggs are broken, Kraft et al. (1967); Penneston (1947); Solowey et al. (1946). Gram negative bacteria were found to predominate on badly soiled eggs. Salmonellae organisms were shown to pass from the alimentary tract through the blood to the ovaries and contaminate the ova Garden and Tucker (1965). Chickens are frequently carriers of Salmonellae organisms, Simmons and Byrmes (1973), but shell contamination is considered to be the major route of contamination according to Ross et al. (1964); Muller and Banwart (1965).

## 2.12.0 The Effect of Spray Drying on Salmonellae

McBee and Cotterill (1970) found little or no effect on alternate drying conditions with respect to Salmonellae destruction. Temperature and resulting moisture levels had no significant effect on the number of S.oranienburg cells destroyed during the spray drying of artificially contaminated egg white. Their data showed a 99.98% destruction of

S.oranienburg cells and 85% destruction of non-salmonellae cells in the spray drying operation in a pilot plant employing a tower co-current spray dryer. The inlet temperature was 121°C, exhaust temperature 43°C, air flow 300 CFM and a feed rate of 300 ml/minute. Gibbons and Moore (1944) working with artificially infected whole eggs obtained a Salmonellae reduction of about four log cycles or 99.99% destruction during experimental spray drying. In both experiments total destruction of Salmonella was not achieved by the spray drying process.

Li Cari and Potter (1970) postulated that during spray drying, many organisms are dehydrated below lethal moisture levels. Once dried, these organisms became more resistant to heat, in addition to being protected from heat by the dried powder.

## 2.13.0 Salmonellosis Outbreaks

The fact that there are over 2,000 known serotypes of Salmonellae is of academic but not of practical interest in that between the years 1968 and 1972 only 10 serotypes accounted for 66 to 77.2% of the Salmonellae isolated from humans (Thompson 1975). An hypothesis to how Salmonellae contamination takes place in a food plant is that one, and possibly several, serotypes become part of the indigenous microflora of the food processing environment. That is the bacterium gains entrance to the food plant environment by some means, probably in the raw materials, establishes itself

and becomes part of the natural microflora. Therefore it is important to find the site in the plant where Salmonella harbored and eliminate the organisms through proper sanitation techniques. However, in some instances it is possible for the serotypes to reoccur to detectable levels some months or a year later as a result of relaxed sanitation or some change in processing. The point to be made is that Salmonella need not be ubiquitous in the food processing environment. There is a reason for the presence of the Salmonellae and through proper sanitation these organisms can be controlled.

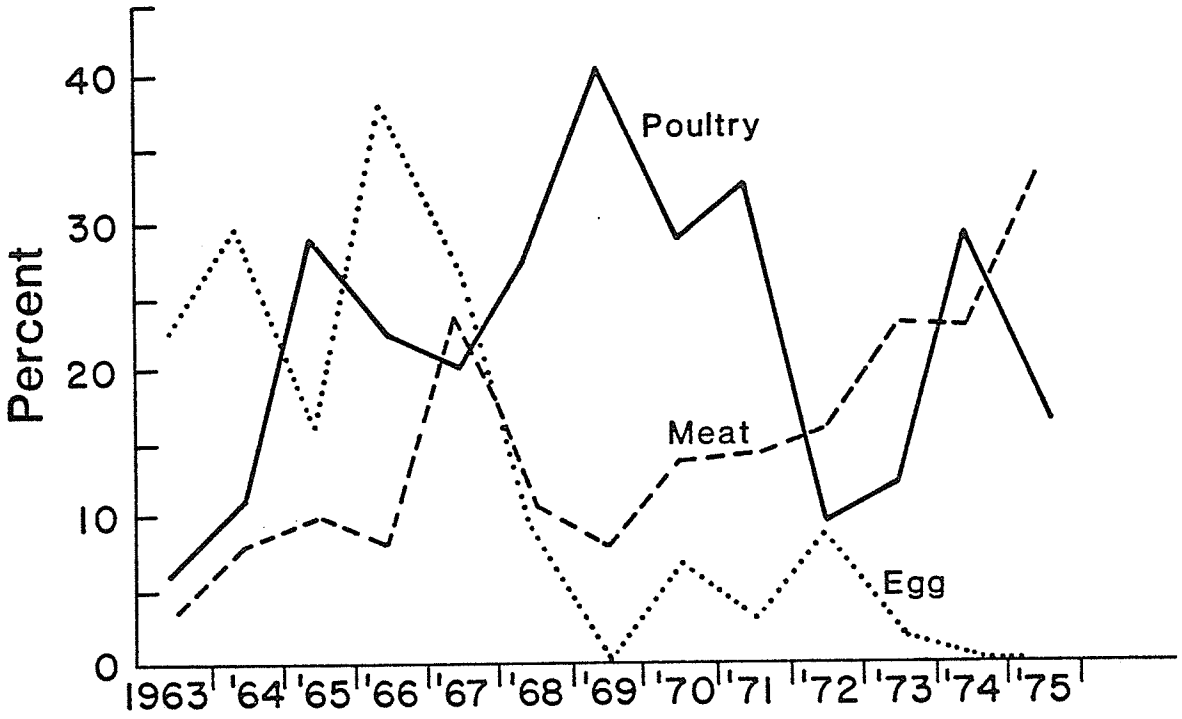
When Salmonella do become part of the processing environment and are undetected or uncontrolled there is a possibility of contamination through cross-contamination of the food product. When this occurs and Salmonella are present in the finished food product a Salmonellosis outbreak may occur. An outbreak is an incident in which two or more persons experience a similar illness after ingestion of a common vehicle (food) and epidemiological analysis implicates the vehicle as the source of illness, Todd (1975).

In 1978 Salmonella represented 29.2% of all confirmed food born disease outbreaks and cases, or 42.8% of the bacterial causes of food borne disease outbreaks CDC Food Borne Diseases (1981). These results thus implicate Salmonallosis as the number one food-borne pathogenic organism. Egg products, however, are not the main vector for Salmonellosis. A 1973 study of foods associated with food borne incidents and cases in Canada found that eggs account for only 1.5%

of all incidents and cases, Todd (1975). Bryan (1981) found eggs to be the vehicles of outbreaks in Salmonellosis in Canada, United States, and England/Wales to be 3.8%, 5.5% and 0.7% respectively. Table 7 shows the increase in reported incidences of human Salmonellosis in the United States from 1942 to 1972. Figure 2 shows the percentage of Salmonellosis outbreaks caused by poultry, meat, or eggs in the United States 1963 to 1975. The figure illustrates the effect of hygenic processing, pasteurization, and quality control on the reduction of Salmonella outbreaks in egg products, Cohn and Blake (1977). Pasteurized frozen and dried eggs have seldom been identified as vehicles of Salmonellosis outbreaks in recent years, Bryan (1981). Figure 2 shows the repeated food borne cases and outbreaks of Salmonellosis in Canada from 1969 to 1979.

Figure 2

Percentage of salmonellosis outbreaks
(outbreaks caused by unidentified vehicles
are eliminated) caused by poultry, meat, or eggs
United States 1963-1975




Cohen, M. L., and P. A. Blake (1977)

TABLE 7

Reported Incidence of Human Salmonellosis in the United States 1942-1972

| Year | Number of Isolations |
|---|---|
| 1942 | 400 |
| 1944 | 500 |
| 1946 | 600 |
| 1948 | 750 |
| 1950 | 1 000 |
| 1952 | 2 000 |
| 1954 | 5 000 |
| 1956 | 6 300 |
| 1958 | 6 000 |
| 1960 | 8 000 |
| 1962 | 10 000 |
| 1964 | 20 000 |
| 1966 | 19 000 |
| 1968 | 21 000 |
| 1970 | 23 000 |
| 1972 | 26 000 |

## SCOPE OF THE INVESTIGATION

This study was initiated to compare the effectiveness of Leuconostoc mesenteroides strains and a Streptococcus strain to desugar egg white by controlled bacterial fermentation. The effects of modified process control of the fermentation on Salmonellae contamination and proliferation were investigated. Specifically the following areas were explored:

a) Optimium fermentation temperatures to promote the growth of fermenting organisms and discourage the growth of contaminating microorganisms.

b) To study the relationship between the rate of arid production and glucose utilization by the test organism.

c) To determine the effect of fermentation temperature on the microbial population during fermentation.

d) Determine the proliferation of Salmonella when egg white is fermented using a repeatedly carried 10% starter culture.

# 3.0 METHODS AND MATERIALS

## 3.1 Sources of Egg White

Egg white used during this investigation was from one of three sources, depending on the experiment involved. Fresh eggs which were less than 24 hours old were obtained directly from the Department of Animal Science poultry barn on the University of Manitoba Campus. Washed eggs of unknown age similar to the eggs used to produce spray dried egg white powder were obtained commercially at the retail level. Pastuerized frozen egg white was obtained from Export Packers Ltd. Winnipeg, Manitoba in 38 pound tins. This egg white was certified Salmonella free to meet Canada Health Protection Branch standards.

## 3.2 Procedure for Aseptic Breaking and Separating of Shell Eggs

Stock egg white was prepared by a method similar to the procedure used by Cotterill (1968). Unwashed eggs from the University of Manitoba were broken according to the method outlined by Cotterill except for the omission of filtration through cheese cloth. Washed eggs obtained commercially were treated and broken according to Cotterill (1968) except that 100°C water was used in place of 200 ppm chlorine solution and eggs were not filtered. After aseptic

breaking and separating the egg white was blended at high speed on a Warring blender for 30 seconds and aseptically transferred in the required quantities into sterile foam stoppered erlenmeyer flasks. The flasks were then stored at 4°C until used. Cotterill (1968) stated that egg white produced by aseptic breaking could be stored at 4°C for at least two weeks without growth of bacteria. In this series of experiments, egg white was stored for only three days at 4°C, after which unused portions were discarded.

## 3.3 Procedure for Handling and Thawing of Pasteurized Frozen Egg White

The pasteruized frozen egg white obtained from Export Packers Ltd. in 38 pound tins was thawed by the procedure listed below. The cans contained the egg white in a plastic bag closed with a tie strip. Thawing of the frozen egg white was performed by placing the entire can in a Puffer Hubard refrigerated incubator at 7.2°C until the egg white was fully thawed. Once the pasteurized egg white was thawed, portions of about 3 L were aseptically transferred with a sterile stainless steel ladle into disposable 2.5 gallon dairy poly bags. The egg white in these poly bags then stored at 4.0°C when it was used immediately or refrozen for use at a later date.

### 3.4.1 Microbial Analysis of Aseptically Broken Eggs

### 3.4.2 Total Aerobic Mesophili Count

Enumeration of total Aerobic plate count was performed

according to A.P.H.A. (1978). Appropriate serial dilutions were made by transferring eleven ml of egg white into 99 ml of sterile 0.1% peptone water blanks. Dilution blanks used for $10^1$ dillution also contained about five 5 mm glass beads to disperse the egg white in the peptone water. Direct plating or $10^0$ dilution was also prepared with the sample spread over the bottom of the petri plate before tempered medium was poured to obtain the best homogenization with the egg white. Dilutions of 1:1 egg white/peptone water were also prepared in test tubes with glass beads and were vortexed at high speed for 1 second. Standard plate count agar-SPC (Difco) was prepared according to manufacture's specifications. The medium was sterilized by autoclaving at 121°C for 15 minutes in 200 ml. media bottles. After autoclaving the media was tempered at 45°C in a Blue M water bath. Duplicate plates were poured and incubated inverted for 48 hours at 32°C $\pm$ 0.1°C. Plates with between 30 and 300 colonies were counted on a Quebec colony counter.

### 3.4.3 Yeast and Mold

Appropriate serial dilutions were prepared by the same procedure as listed in section 17.1 Standard plate count. Potato Dextrose agar-PDA (Difco) was prepared according to manufacture's directions. Exactly 100 ml. of PDA media was poured into 200 ml. media bottles. The medium was then autoclaved at 121°C for 15 minutes, and tempered to 45°C in a Blue M water bath. Prior to pouring of plates, 1.5 ml. of sterile 10% tartaric acid was aseptically transfered

to each 100 ml. of sterilized PDA medium, and the bottle gently rotated to homogenize the medium and acid. The tartaric acid lowered the pH of the media to 3.5°C ± 0.1°C to inhibit the growth of bacteria, but allows yeast and molds to grow A.P.H.A. (1978). Duplicate plates are prepared according to the pour plate method. Plates were incubated at 21°C (room temperature) for 5 days in the inverted position.

### 3.4.4 Salmonella (Qualitative Analysis)

Qualitative analysis for Salmonella was performed on aseptically broken egg white by A.P.H.A. (1978) method. A 25 gram sample was pipetted into 225 ml of sterile nutrient broth (DIFCO) in a 500 ml blender jar. The sample was homogenized for one minute at high speed in a Waring Blender and then incubated for 24 hours at 37°C ± 0.1°C. From this step of non-selective enrichment 1 ml of the culture was transferred into 10 ml of Selenite Cystine broth (DIFCO) and 1 ml into 10 ml of tetrathionate broth (DIFCO). Each of the selective enrichment tubes were incubated for 24 hours at 37°C ± 0.1°C and the cultures were then streaked on Brilliant Green Sulphadiazine Agar - BGS (DIFCO) and Salmonella Shigella Agar - SS (BBL). Plates were incubated inverted for 24 hours at 37°C ± 0.1°C

i) Typical Salmonellae colonies on BGS agar are colorless, pink to fuchsia, or translucent to opaque with the surrounding medium pink to red.

ii) Typical Salmonellae on S.S. agar are uncolored to pale

pink, opaque to translucent.

Suspected Salmonellae colonies were selected from B.G.S. and S.S. agar plates and purified on MacConkey agar (DIFCO) and incubated for 24 hours at 37°C ± 0.1°C. Typical Salmonellae colonies of MacConkey agar have clearing and precipitated areas surrounding the colonies. Typical colonies were then streaked on nutrient agar plates (DIFCO) and incubated, inverted for 24 hours at 37°C ± 0.1°C.

Determination of presumptive Salmonellae was performed by inoculating the following media with the 24 hour purified culture.

i) one tube Triple Sugar Iron - TSI (DIFCO)

ii) one tube Lysine Iron agar - LIA (DIFCO)

Typical presumptive Salmonellae reactions on TSI are indicated by a red slant and yellow butt, with or without $H_2S$ production ($H_2S$ production is indicated by a blackening of medium). The typical reactions of presumptive Salmonellae and Arizona species on LIA are indicated by light purple slant and butt with production of $H_2S$ and sometimes gas.

### 3.4.5 Coliforms (Presumptive Plate Method)

Presumptive coliforms were enumerated using the direct plating method with Deoxycholate Lactose agar - DOC (DIFCO). Appropriate dilutions were prepared in the same way as listed in 3.4.2 Total Aerobic Mesophitic Plate Count. DOC medium was prepared according to manufacturers specifications and autoclaved at 121°C for 15 minutes and tempered at 45°C ± 0.1°C in a Blue M waterbath. Duplicate plates were poured

over each plate to prevent the formation of atypical colonies. Plates were incubated, inverted for 24 hours at 37°C ± 0.1°C.

Presumptive coliform colonies on DOC media are described as typical dark red colonies of a diameter of at least 0.5 mm and surrounded by a zone of precipitated bile salts.

3.4.6 Staphylococcus aureus (Presumptive analysis)

Baird - Parker agar - BP (OXOID) was prepared according to manufacturer's directions, autoclaved at 121°C for 15 minutes. The BP medium was tempered in a Blue M water bath to 50°C, just prior to pouring plates. Five mL of sterile Egg Yolk Tellurite emulsion (OCIOD) was aseptically transferred to 100 mL quantitites of tempered BP agar. The medium was then slowly rotated in the media bottle and care taken not to create excessive air bubbles. Plates were poured for use by spread plate Standard Methods technique using 15 mL of BP medium per plate and drying the plates in an incubator at 37°C for 10 minutes.

Aliquots of 0.1 mL of egg white samples were pipetted aseptically onto the surface of the solidified medium and surface spread. Plates were spread until the surface of each plate appeared dry. Duplicate plates of each dilution were prepared and incubated inverted at 37°C ± 0.1°C for 24 and 48 hours.

Typical presumptive Staphylococcus aureus colonies are black and shiny with narrow white margins, and are

surrounded by clear zones extending into the opaque medium.

### 3.5.0 Chemical Analysis of Egg White

The parameters which were monitored during fermentation of egg white were pH and the presence of free glucose. The determination of pH was used to monitor the changes in the fermentation process as well as an indication of the completion of fermentation. The determination of free glucose content in egg white reveals exactly how the desugaring was proceeding. This method of free glucose determination is complex and is not applicable for routine industrial uses. Therefore Clinstic (Ames Labs) reagent strips were also used to give an indication of the approximate sugar content of fermenting albumen.

### 3.5.1 pH

The pH of egg white, at the various stages of the fermentation process, was monitored by aseptically transfering 30 mL of egg white into a 40 mL beaker. A Fisher Accumet Model 520 Digital pH/ion meter equipped with an Accumet combination electrode was used for pH determination.

The pH meter was standardized before use with Fisher certified Gram - Pac buffer salt pH 6.86 $\pm$ 0.02 at 25°C. This buffer reagent was the closest to the pH of fermented egg white which has a pH of 6.5 and would therefore be the most accurate at the most critical pH.

### 3.5.2 Rapid Method for Glucose Determination in Egg White

The approximate amount of free glucose in egg white was rapidly determined using Clinistic reagent strips (Ames Labs) Galluzo et al. (1974) to indicate how well glucose degradation was proceeding during the fermentation process.

### 3.5.3 A.O.A.C. Official Method for Glucose Determination in Egg White

Samples of liquid egg white from fermentations experiments were analysed for free glucose content by washing 25 grams of sample into a 250 mL volumetric flask containing about 1 gram of $CaCO_3$. A 50 mL aliquot of 5 per cent NaCl solution was then pipetted into each flask along with a little (about 5 mL) of warm water. Exactly 130 mL of 95 per cent ethanol was added to each flask with continuous swirling, and incubated in a refrigerator at 7.2°C for 24 hours. The flasks were filled to the mark with deionized water and shaken. The samples were then filtered using Whatman 18.5 cm 2V folded filter paper. A 150 mL aliquot of the filtrate was heated in a 250 mL beaker on a steam bath to a final volume of 40 mL. The concentrated material was cooled and washed into a 100 mL volumetric flask with deionized water. One gram of phosphotungstic acid crystals (Fisher) were added to precipitate the remaining protein in the sample. The flask was then shaken and filled to the mark with deionized water. The contents of the flask were then filtered using Whatman number 12 folded filter paper. A 50 mL portion of the filtrate was placed in a 400 mL beaker. Exactly 25 mL

each of copper sulphate solution and alkaline tartrate solution were pipetted into the 50 mL of filtrate. The mixture in the 400 mL beaker was immediately placed on a heated electric hot plate (Fisher) so that the solution boiled within 4 minutes, and continued to boil for 2 minutes. The solution was then immediately filtered with preweighed Selas standard porcelain filter crucibles (gooch crucibles) using vacuum. The red precipitate was then washed into the crucibles with warm deionized water and dried for one hour at 100°C in a Blue M drying oven. The crucibles were cooled in a desiccator and reweighed on an analytical balance. The weight of the red $Cu_2O$ precipitate was converted to per cent reducing sugar expressed as glucose according to the Munson Walker Tables.

i) Copper sulphate solution - Dissolve 34.639 grams of $CuSO_4 \cdot 5H_2O$ (Fisher) in deionized water. Dilute to 500 mL in a volumetric flask. Filter through a glass filter pad in a buchner funnel under a vacuum.

ii) Alkaline tartrate solution - Dissolve 173 grams of KNa tartrate $\cdot 4H_2O$ (Fisher) and 50 grams NaOH in deionized water. Dilute to 500 mL in a volumeteric flask. Incubate the solution at room temperature to insure that total dissolution has occured. Fill flask to the mark and filter through a glass filter pad in a buchner funnel under vacuum.

## 3.6.0 Preparation of Bacterial Cultures

### 3.6.1 Reviving Freeze Dried Cultures

Cultures of Leuconostoc mesenteroides ATCC 27258 was obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, USA 20852 in freeze dried form.

The organism was removed from the double vial container by heating the pointed end of the outer container vigorously in a Bunsen flame. While the glass was still hot a drop of water was placed on the glass to cause fracturing. The tip of the container was then carefully removed with a sharp blow from a pencil. The asbestos plug removed and the cotton plugged inner vial removed.

Aseptically 0.3 to 0.4 mL of double strength Lactobacillus MRS broth (DIFCO) was added to the cotton plugged freeze dried culture powder using a sterile Pasteur pipette. The contents were mixed well and aseptically transferred to a test tube containing 6 to 6 mL of the double strength Lactobacillus MRS broth. The culture was then incubated at 32°C $\pm$ 0.1°C until turbid.

### 3.6.2 Maintenance of Cultures

Cultures of Leuconostoc mesenteroides ATCC27258 and Leuconostoc mesenteroides NRRL B199 were routinely maintained on Lactobacillus MRS broth fortified with 1.5 per cent agar to be used for slant preparation. The Leuconostoc organisms were grown on the slants at 32°C $\pm$ 0.1°C for 24 hours, then

stored at 7.2°C. The cultures were transferred to fresh slants every second day to maintain activity.

The slant cultures were periodicatlly streaked on Tomato Juice agar (DIFCO) plates and incubated inverted for 24 to 48 hours at 32°C ± 0.1°C. This step was used to check the purity of the cultures. Colonies were picked off the plates and the Gram stain performed. The stained slides were microscopically examined for Leuconostoc mesenteroides morphology.

Cultures of Salmonella typhimurium LH067981 were maintained on Nutrient agar (DIFCO) slants grown at 37°C ± 0.1°C for 24 hours and stored at 7.2°C. To maintain viability, and to reduce the risk of mutation, cultures were transferred every second day. The slant cultures were periodically streaked on S-S agar and incubated at 37°C ± 0.1°C for 24 hours. A colony was picked off and transferred to TSI agar slants and LIA slants in order to check for purity and typical salmonella reactions. Cultures were also stained and checked for Salmonella morphology.

Hansens cheese starter culture number 70 consisting of Streptococcus Cremoris (95%) and Betacoccus cremoris (5%) was maintained in 10 per cent non-fat dried milk which was heat treated in an sutoclave at 121°C for 10 minutes. Immediately after heat treatment the skim milk was cooled in cold flowing water to reduce denaturation of milk proteins and browning. The culture was maintained in 50 mL foam stoppered Erlenmeyer flasks containing 200 mL of 10 per cent reconstituted skim milk. An inoculum rate of 1.0 per cent was

used and incubation was carried out for 16 hours at 26°C ± 0.1°C. Cultures were stored at 7.2°C and transferred every day.

### 3.6.3 Preparation of Leuconostoc Strain Cultures in Egg White

Bulk starters cultures were prepared by transferring one loopful of Leuconostoc mesenteroides strain culture from a slant to 10 mL of Lactobacillus MRS broth and incubated for 24 hours at 32°C ± 0.1°C. This culture was then used to inoculate 500 mL of Lactobacillus MRS broth in a 1 L Erlenmeyer flask which was again incubated for 24 hours at 32°C ± 0.1°C. Leuconostoc meseteroides cells were harvested in sterile 250 mL centrifuge tubes and centrifuged at 3,000 xg. 4°C for 30 minutes in a Sorval Superspeed RC-2B centrifuge. The supernatent was aseptically decanted and the remaining pellet resuspended by Vortex in 20 mL of 0.1% peptone water. The resulting slurry of cells was then used to inoculate 400 g of egg white. Yeast extract was then added at a rate of 0.022 g yeast extract/gram egg white. The yeast extract was prepared by dissolving 2.2 g yeast extract in 100 mL deionized water in a milk dilution bottle and autoclaved at 121°C for 15 minutes. One milliliter of this yeast extract solution was added per 100 g of egg white. The egg white was then incubated at 32°C ± 0.1°C until the pH decreased to 6.5.

### 3.6.4 Preparation of Hansen Streptococcus Cultures in Egg White

Bulk starter cultures of Hansen Number 70 cheese culture

consisting of Streptococcus cremoris (95%) and Beta coccus cremoris (5%) synonymous with Leuconostoc cremoris by inoculating 200 grams of egg white with 10% (w/w) of the Streptococci culture which was carried in 10% reconstituted skim milk. To encourage growth of the organism the egg white was fortified with 0.1% yeast extract. The inoculated egg white was incubated at 32°C ± 0.1°C until a pH of 6.5 was obtained usually requiring about 72 hours. The culture was then transferred in egg white several times to insure activity. These subsequent fermentations achieved a pH of 6.5 within 9 to 10 hours.

### 3.7.0 Standardization of Salmonella for Artificial Contamination of Egg White

One loopful of culture was transferred from a nutrient agar slant culture of Salmonella typhimurium LHO 67981 to exactly 10 mL of nutrient broth and incubated for exactly 24 hours at 37°C ± 0.1°C. The culture was transferred daily for several days in nutrient broth using a 0.01 mL platinum inoculation loop. Exactly 1.0 mL of the standardized broth culture was serially diluted to $10^{-6}$ dilution in 99 mL dilution blank with 0.1% peptone water. When one mL of $10^{-6}$ diluted culture was added to 100 mL of egg white it would result in approximately 10 Salmonella per gram of egg white and one mL of the $10^{5}$ dilution resulted in approximately 100 Salmonella per gram of egg white and the $10^{-4}$ dilution about 1,000 Salmonella per gram of egg white.

Bismuth sulphite agar (DIFCO) was prepared according to manufacturer's direction and tempered at 52°C. The

appropriate dilution of the Salmonella culture, non-contaminated pasteurized egg white and artificially contaminated egg white were plated and incubated inverted at 37°C for 48 hours.

### 3.8.0 The Effect of Temperature on the pH of Fermenting Egg White at an Inoculum Size of 10%

Starter cultures of Leuconostoc mesenteroides NRRL B199 and Leuconostoc mesenteroides ATCC 27258 were prepared from slant cultures as described in section 3.6.3 of Methods and Materials. These cultures were transferred twice in egg white and grown at 32°C ± 0.1°C until a final pH of 6.5 was achieved to insure culture activity before they were used as starters. For each organism six sterile 500 mL Erlenmeyer flasks with foam stoppers were filled with 400 grams of egg white containing 0.022% yeast extract. Duplicate flasks for each test organism were inoculated at a rate of 10% (v/w and fermented at 25°C, 32°C, and 37°C respectively for a total of 9 hours. During the fermentation period 40 mL samples were withdrawn aseptically at time intervals of 0, 3, 6, 9, and 12 hours. The pH of each sample was determined by the procedure listed in section 3.5.1 of Methods and Materials.

### 3.9.0 The Effect of Inoculum Size on the pH of Egg White Fermented at 32°C

Starter cultures of Leuconostoc mesenteroides NRRL B199 and Leuconostoc mesenteroides ATCC 27258 were prepared by the same procedure as outlined in section 3.8.0 of Methods and Materials. Eighteen sterile 500 mL Erlenmeyer flasks

with foam stoppers were filled with 400 grams of egg white containing 0.22% yeast extract. The unfermented product in each of the eighteen flasks was tempered to 32°C prior to inoculation with different rates of starter cultures. Fermentations in duplicate were performed for each organism using inoculum rates of 0, 5, 10, 15, and 20 percent (v/w). All flasks were carefully swirled to disperse the starter in the egg white prior to incubation at 32°C ± 0.1°C for 12 hours. During the fermentation period 40 mL samples were withdrawn aseptically at time intervals of 0, 3, 6, 9, and 12 hours. The pH of each sample was performed by the procedure listed in section 3.5.1 of Methods and Materials.

### 3.10.0 The Effect of Inoculum Size on Rate of Glucose Utilization by Leuconostoc mesenteroides strains at 32°C

Starter cultures of Leuconostoc mesenteroides NRRL B199 and Leuconostoc mesenteroides ATCC 27258 were prepared as outlined in section 3.6.3 of Methods and Materials. Eighteen sterile 500 mL flasks with foam stoppers were filled with 400 grams of egg white enriched with 0.022% yeast extract. The egg white was tempered to 32°C ± 0.1°C prior to inoculation with the different rates of the starter cultures.

Fermentations were performed with each test organism using inoculum rates of 0, 10, 15, and 20 percent (v/w) starter culture. The contents of each flask were carefully swirled to disperse the starter in the egg white, and incubated at 32°C ± 0.1°C for 12 hours. Samples of 40 mL were aseptically withdrawn at time periods of 0, 3, 6, 9, and 12 hours

during the fermentation process. Each of the samples was placed in 200 mL Whirl-pak sample bags (Can Lab) and frozen for glucose analysis at a later date by the procedure listed in Section 3.5.3 of Methods and Materials.

### 3.11.0 Cell Count, pH, and Residual Glucose Analysis on Egg White Fermented with Leuconostoc mesenteroides strains at 32°C

Starter cultures of Leuconostoc mesenteroides NRRL B199 and Leuconostoc mesenteroides ATCC 27258 were prepared as outlined in section 3.6.3 of Methods and Materials. Four sterilized 1 litre Erlenmeyer flasks with foam stoppers were filled with 500 grams of egg white, which was enriched with 0.022% yeast extract. The contents of the flasks were tempered to 32°C prior to inoculation with the test organism.

Two flasks each were inoculated with L. mesenteroides NRRL B199 and L. mesenteroides ATCC 27258 respectively at a rate of 10% (v/w). All flasks were carefully swirled to disperse the starter in the egg white prior to incubation at 32°C $\pm$ 0.1°C for 9 hours.

Flasks were sampled at time intervals of 0, 3, 6, and 9 hours. Microbiological analysis for Leuconostoc organisms was conducted using Tomato Juice agar (DIFCO). The tomato juice agar was prepared according to manufacturer's specifications and tempered to 45°C $\pm$ 0.1°C. At the time of sampling, an eleven mL sample was aseptically pipetted from each flask and serially diluted according to the procedure outlined in section 3.4.2 of Methods and Materials. Duplicate plates

were poured and incubated inverted at 32°C ± 0.1°C for 48 hours. Also during the same sampling period an additional duplicate sample of 40 mL was withdrawn for analysis of pH and residual glucose.

### 3.12.0 Comparison of the Efficiency of Fermentation of Egg White with Leuconostoc mesenteroides Strains and Hansen's Streptococci at 32°C

Starter cultures of Leuconostoc mesenteroides NRRL B199 and Leuconostoc mesenteroides ATCC 27258 were prepared from slant cultures as described in section 3.6.3 of Methods and Materials. A starter culture of Hansen's Streptococci was also prepared according to the procedure outlined in section 3.6.4 of Methods and Materials. All cultures were grown at 32°C ± 0.1°C and transferred at a rate of 10% (v/w).

The egg white used for all fermentations was fortified with 0.022% yeast extract, except that for the Hansen's Streptococci culture fortification was with 0.022% (LYE) and 0.1% (HYE) yeast extract. Kaplan et al. (1950) reported that yeast extract concentrations of 0.1% were required for growth of Streptococci in egg white.

Duplicate fermentations with each test organism were performed in 500 mL Erlenmeyer flasks containing 400 grams of egg white tempered at 32°C. Inoculation rates used for all three test organisms were at a rate of 10% and all fermentation flasks were incubated at 32°C ± for 0.1°C for 9 hours. Forty millilitre samples were withdrawn after 0, 3, 6, and 9 hours of fermentation. The samples were analyzed

for pH, residual glucose, and an odor evaluation.

### 3.13.0 The Fate of Salmonella Organisms in Fermenting Egg White Following Repeated Transfers

Fermenting egg white was artifically contaminated with Salmonella typhimurium LHO 67981. The fermented culture was transferred at a rate of 10% and fermented at temperatures of 32°C and 37°C respectively.

A starter culture of Leuconostoc mesenteroides NRRL B199 was prepared as outlined in section 3.6.3 of Methods and Materials. The egg white was artificially contaminated with two levels of organisms, approximately 10 and 100 Salmonella cells per gram of egg white. This was achieved by the method presented in section 20.0 Standardization of Salmonella for Artificial Contamination in egg white listed in Methods and Materials.

Duplicate fermentations of L. mesenteroides NRRL B199 were fermented at 32°C ± 0.1°C and 37°C ± 0.1°C respectively until a pH of 6.5 was obtained. At the end of the fermentation period an eleven Ml sample was aseptically transferred into a 99 mL dilution blank. The samples were serially diluted and plated by the pour plate technique A.P.H.A. (1978) using Bismuth Sulfate - BS agar (DIFCO) prepared according to manufacturer's specifications and tempered at 53°C ± 0.1°C. Plates were poured and carefully swirled to produce separate colonies of Salmonella typhimurium LHO 67981. The plates were incubated inverted at 37°C ±

0.1°C for 48 hours. Colonies on BS agar were black and 2 to 3 mm in diameter typical of Salmonella morphology.

After each fermentation was completed and Salmonella determination was performed on the contents, the fermented product was then rapidly cooled in an ice water bath and then stored at 7.2°C, overnight. The following day the flasks were removed from the refrigerator and tempered to 32°C in preparation for its use as starter cultures to inoculate a fresh lot of egg white. Inoculation was at a rate of 10% (v/w). This procedure of carrying the culture was repeated continuously for seven transfers and the level of Salmonella typhimurium LHO 67981 in the product analyzed after each fermentation.

# RESULTS AND DISCUSSION

## 4.1 Analysis of Egg White

The egg white used to study the growth characteristics of the fermenting organisms had to be of low microbial content and free from Salmonella. This was obtained by breaking and separating the contents of the eggs into sterile flasks under aseptic conditions. In this study it was established that egg white prepared in this manner could be stored at 4°C for one week without significant increase in microbial growth. Lots of egg white were analyzed for total plate count, coliform count, Staphylococcus aureus, yeast and molds, Salmonella, pH and per cent residual glucose. All analyses was conducted immediately after breaking as well as after one week storage at 4°C. The results are presented in table 8.

Egg white broken and separated aseptically from fresh or washed and sanitized eggs had a total mesophilic aerobic plate count of less than 10 cells per mL after breaking as well as after storage at 4°C for seven days. No presemptive coliforms were detected by direct plating on deoxycholate agar after breaking or storage. Yeast and molds were also not detected in egg white during the period of analysis. The results also indicated all samples of egg white tested were free of presumptive Salmonellae and presumptive Staphylococcus

TABLE 8

ANALYSIS OF EGG WHITE

| Trial | Storage Time Days | Standard Plate Count per mL | Coliform per mL | Yeast and Mold per mL | Staphycoccus aureus per mL | Salmonellae per mL | pH | Percent Residual Glucose |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | <10 | <10 | <10 | (-) | (-) | 8.60 | 0.354 |
| 1 | 7 | <10 | <10 | <10 | (-) | (-) | 8.70 | 0.354 |
| 2 | 0 | <10 | <10 | <10 | (-) | (-) | 8.70 | 0.352 |
| 2 | 7 | <10 | <10 | <10 | (-) | (-) | 9.00 | 0.352 |
| 3 | 0 | <10 | <10 | <10 | (-) | (-) | 7.95 | 0.334 |
| 3 | 7 | <10 | <10 | <10 | (-) | (-) | 8.20 | 0.334 |
| 4 | 0 | <10 | <10 | <10 | (-) | (-) | 8.20 | 0.380 |
| 4 | 7 | <10 | <10 | <10 | (-) | (-) | 8.25 | 0.380 |

aureus. The results indicated that the method employed to obtain egg white for future fermentation experiments was acceptable from the microbiological point of view. The quantity of glucose in egg white remained relatively constant during the storage of the egg white at 4°C. This further indicated that total sugars were not utilized microbiologically or enzymaticaly during the storage. The egg white during storage maintained its natural thick viscosity and mild pleasant odor.

However, an increase in pH was observed during the storage of the egg white from an initial pH ranging from 7.95 to 8.60 to a high of 8.25 to 9.00 after seven days. These results are apparently quite normal and are due to the dynamics of egg white during storage. Sharp and Powell (1931) reported that the pH of stored albumen increases at a temperature dependant rate to a maximum value of 9.7. They observed that after three days of storage at 3°C the pH of the albumen increased to 9.18 and after twenty-one days of storage the pH had risen to 9.4.

This rise in the albumen pH is caused by a loss of carbon dioxide from the egg through the 7,000 to 17,000 funnel shaped pores in the egg shell. The pH of the albumen is dependent on the equilibrium between the dissolved carbon dioxide, bicarbonate ion, and egg proteins according to Brooks and Pace (1938). Cotterill et al. (1959). The concentration of the bicarbonate and carbonate ions are governed by the partial pressure of the carbon dioxide in the surrounding

external environment. The variation in the initial pH of the egg white was probably due to the different ages of eggs used in this study.

## 4.2 The Free Glucose Content of Liquid Egg White

The glucose content of raw egg white was monitored to give an indication of the variability of the free glucose content in egg white and also the quantity of glucose which must be removed by the controlled bacterial fermentation process. Samples of egg white were tested for free glucose content at regular intervals during this study. The results obtained are presented in table 9.

The average free glucose level obtained from samples taken between October 1980 and March 1981 was 0.349%. This value in in accordance with the levels reported by Rómanoff and Romanoff (1949). The glucose content of egg white is affected by external factors such as feed, age of hen, and species of hen (Cunningham et al. 1962). The range of free glucose levels in the egg white used throughout this study varied between 0.316 and 0.412%.

These results indicated that there is a great variability in the glucose content of liquid egg white. Since glucose is the primary substrate of the test organism during fermentation, this variation in glucose content could be one of the reasons for the variations observed in the extent of fermentation, or the amount of time required for the organisms to use up the glucose to the commercially acceptable level of 0.1% glucose before drying.

TABLE 9

FREE GLUCOSE CONTENT OF LIQUID EGG WHITE

| Sample Number* | Average Free Glucose in Egg White, Per Cent |
|---|---|
| 1 | 0.334 |
| 2 | 0.380 |
| 3 | 0.352 |
| 4 | 0.354 |
| 5 | 0.331 |
| 6 | 0.343 |
| 7 | 0.327 |
| 8 | 0.325 |
| 9 | 0.344 |
| 10 | 0.402 |
| 11 | 0.412 |
| 12 | 0.316 |
| 13 | 0.321 |

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* in triplicate

Range 0.316 - 0.412
Mean 0.3493
Standard Deviation 0.0307

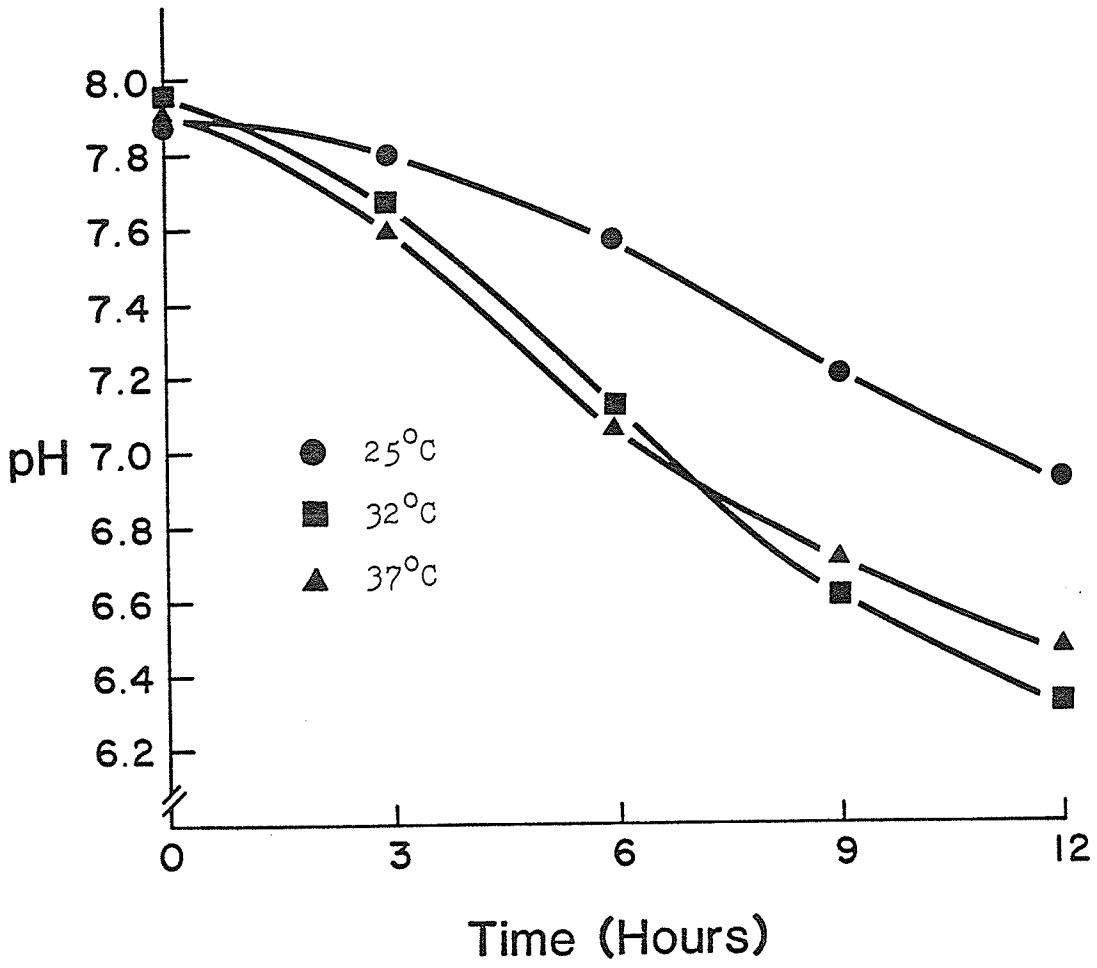
## 4.3 The Effect of Temperature on the pH of Fermenting Egg White at an Inoculum Rate of 10%

In commercial egg white fermentation processes using Leuconostoc mesenteroides strains, the product is usually fermented at a temperature of 37°C. It was postulated that this temperature may be too high for the optimum growth of Leuconostoc organisms as well as encouraging the growth of contaminating organisms primarily Enterobacteriaceae that are normally found in commercial egg white. Consequently, the affects of various fermentation temperature, including 25°C, 32°C, and 37°C on acid production by Leuconostoc mesenteroides NRRL B199 and Leuconostoc mesenteroides ATCC 27258 were investigated. The change in pH was monitored at 3 hour intervals to indicate the extent of acid production.

As cited previously in the Review of the Literature, when one mole of glucose is metabolized anaerobically by Leuconostoc mesenteroides spp., one mole each of D(-)-lactic acid, ethanol, and carbon dioxide is produced. Figure 3 (Appendix, table 1A) presents fermentation of egg white by L. mesenteroides NRRL B199 using an inoculum level of 10%. The results of the fermentation show that the pH of the egg white was lowered to a level of 6.5 in Ca, 10 hours when grown at 32°C. Whereas when a temperature of 37°C was used, 11.5 hours was required to reach a pH of 6.5. On the other hand at 25°C even after 12 hours of fermentation, the pH was only lowered to 6.87. Figure 3 also shows that the fermentation at 37°C initially had the greatest rate of acid production. However, after 7 hours of fermentation the rate

Figure 3

The Effect of Fermentation Temperature on Acid Production of Leuconostoc mesenteroides NRRL B199 in Egg White
10% Inoculum Size



of acid production at 37°C decreased and was less than that produced by the fermentation at 32°C.

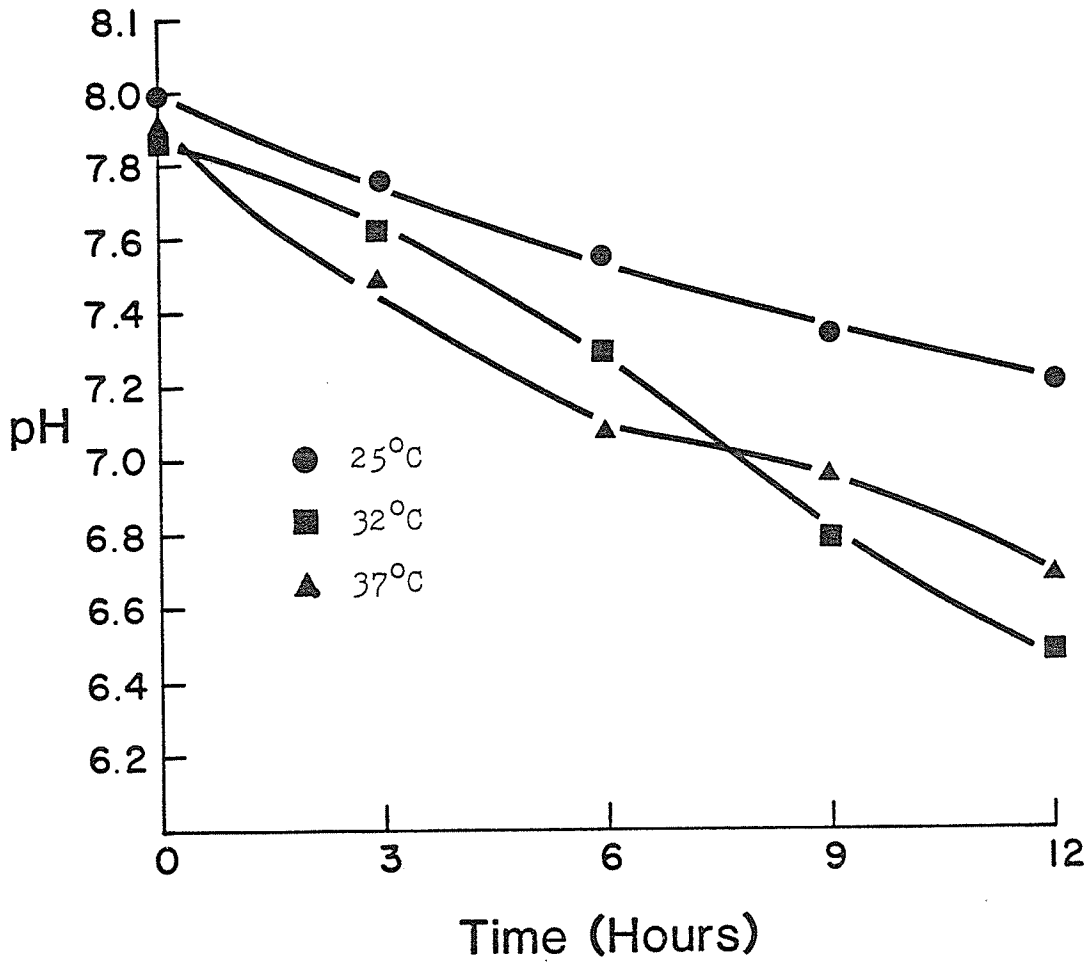
The temperature range between 25°C and 37°C was investigated using L. mesenteroides ATCC 27258 by the same procedure used for L. mesenteroides NRRL B199. The results are presented in figure 4, (Appendix, table 1B). The results indicate that after 12 hours of fermentation at 32°C the pH had decreased to 6.53 whereas at 37°C the pH had decreased to 6.7 and at 25°C a pH of 7.13 was obtained.

Figure 4 also showed the same effect as was observed in figure 3, that is fermentation at 37°C initially produced the most rapid acid production, then after approximately 8 hours of fermentation L. mesenteroides ATCC 27258 grown at 32°C surpassed this rate. Further comparison between figures 3 and 4 also shows that L. mesenteroides NRRL B199 produced more acid in egg white than by L. mesenteroides ATCC 27258 when grown under the same conditions.

From the results of figures 3 and 4 it would appear that the optimum fermentation temperature for egg white with Leuconostoc mesenteroides strains is more effective at 32°C than at 37°C as presently being used in the dried egg white industry. These findings are supported by Garvie (1960) who reported the otpimum growth temperature of L. mesenteroides strains to be between 20°C and 30°C rather than at higher temperatures.

Figure 4

The Effect of Fermentation Temperature
on Acid Production of Leuconostoc mesenteroides
ATCC 27258 in Egg White
10% Inoculum Size



## 4.4 The Effect of Inoculum Size on Acid Production by Leuconostoc mesenteroides NRRL B199 Grown at 32°C in Egg White

The inoculum sizes used by researchers Mulder (1981), Galluzzo et al. (1974), Kaplan et al. (1950) in the past have usually been expressed as the number of cells per mL of liquid egg. The average inoculum rates employed by these investigators, about $10^7$ - $10^8$ microorganisms per mL of product. The expression of inoculum sizes as cell count may be acceptable for academic publications, however, for industrial applications, the inoculum sizes are normally presented as a percentage of the total volume or weight of egg product. In order to keep this research on the same bases as practiced in industrial operations, inoculum sizes were expressed as per cent to weight (v/w).

The standard practice in commercial egg fermentation is to use an inoculum size of 10% (v/w). This volume of starter will provide the required number of organisms to ferment the egg white. This study investigated the prospects of using inoculum sizes varying from 5% to 20%.

The effect of inoculum sizes of 5%, 10%, 15%, and 20% of L. mesenteroides NRRL B199 was studied with respect to acid production as indicated by a decrease in pH. The starter culture used was grown in egg white at 32°C with 0.022% yeast extract added, and grown to a final pH of 6.5. The results are presented in Figure 5 (appendix table 2). The results indicate that within the first 3 hours of fermentation there is a noticable difference in the rate of acid

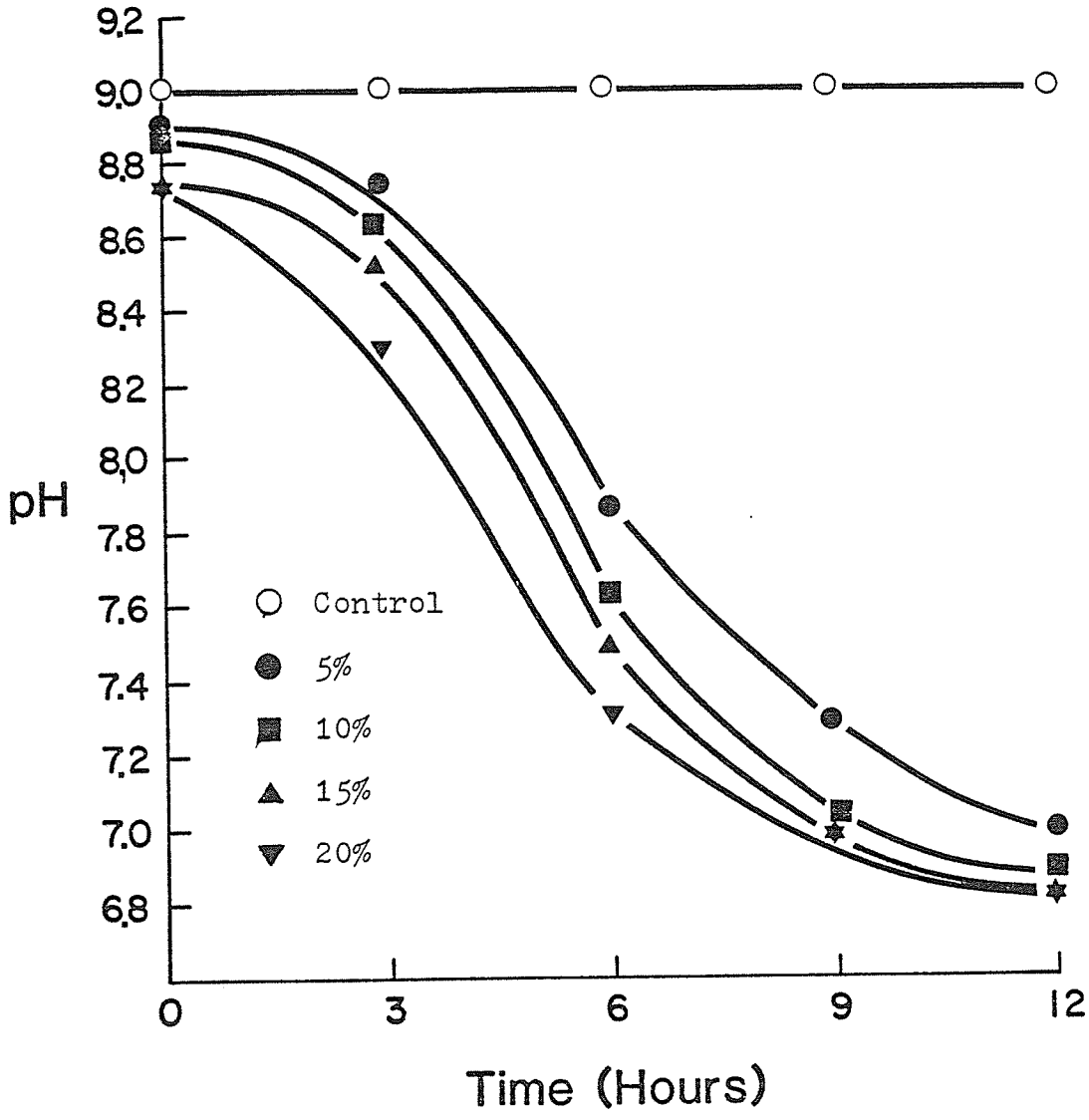
production by the four different levels of inocula used. Kaplan et al. (1950) using inoculum sizes of 0.10%, 0.25%, 0.50%, 0.75%, and 1.00% (weight/weight) resting cells of Streptococci, found that the inoculum size had an effect on the rate of acid production. However due to the very large inoculum number $10^{11}$ to $10^{12}$ a very rapid rate of acid production occurred, (Ayres and Stewart 1947). Our data indicate that the greater the inoculum size, the more rapid is the acid production. Between 3 and 6 hours of fermentation from visual observations of graphs there appeared to be very little difference in acid production as indicated by the slopes of all the lines being rather similar. After 6 hours of fermentation, with an inoculum level of 20% there was a very pronounced increase in the rate of acid production as indicated by rapid decrease in pH. Inoculum sizes of 10% and 15% showed similar acid production rates but to a lesser extent at 15% and a much lesser rate at the 10% level. With an inoculum level of 5% there was only a slight rate of acid production after 12 hours of fermentation.

The pH after 12 hours of fermentation at inocula of 5%, 10%, 15%, and 20% were 7.02, 6.94, 6.86, and 6.85 respectively. By using pH as an indication of sugar breakdown, it was difficult to differentiate whether or not there was a substantial difference in fermentation with different levels of inoculum.

The reduction in the rate of acid production during the latter stages with high inoculum sizes was probably due

Figure 5

The Effect of Inoculum Size on Rate of Acid Production by Leuconostoc mesenteroides NRRL B199 in Egg White at 32°C



to most of the glucose having consumed. Thus, with less substrate available for the growth of the organism, the production of acid was slowed or inhibited.

The results presented indicate a reasonably rapid fermentation with an inoculum size of 5%. However, during the course of this research many failures were encountered with inoculum sizes of 10%. The reasons for starter failures and slow fermentations were not resolved during this study. However, it is possible that poor growth of the fermenting organisms was caused by the antimicrobial nature of the egg white proteins; when these problems occured they were related to a specific lot of eggs. Similar failures are also encountered in industrial fermentations using a 10% (v/w) inoculum size. When problems occur at the industrial level an additional 10% of starter culture is added to the product in order to increase the number of fermenting organisms thereby accelerating the fermentation rate and process.

For the reasons mentioned above, an inoculum size of 10% was used for subsequent studies to comply more closely to the industrial processing of controlled industrial fermentations. Using inoculum sizes of greater than 10% is economically not feasible, and an inoculum size of 5% produces too much of an initial lag phase, therefore, an inoculum rate of 10% was used throughtout the remained of the study.

## 4.5 The Effect of Inoculum Size on Residual Glucose in Egg White Fermented by L. mesenteroides NRRL B199

The effect of inoculum size on the rate of free glucose

utilization was determined during the fermentation of egg white with Leuconostoc mesenteroides NRRL B199. Duplicate samples of 40 mL of egg white were aseptically removed from the fermentation flask at 3 hour intervals and frozen in whirl-pak bags for analysis of residual free glucose as outlined in section 3.5.3. Inoculum sizes of 0%, 10%, 15%, and 20% were investigated, and all fermentations were conducted at 32°C ± 0.1°C. The results are presented in Figure 6 (Appendix table 3).

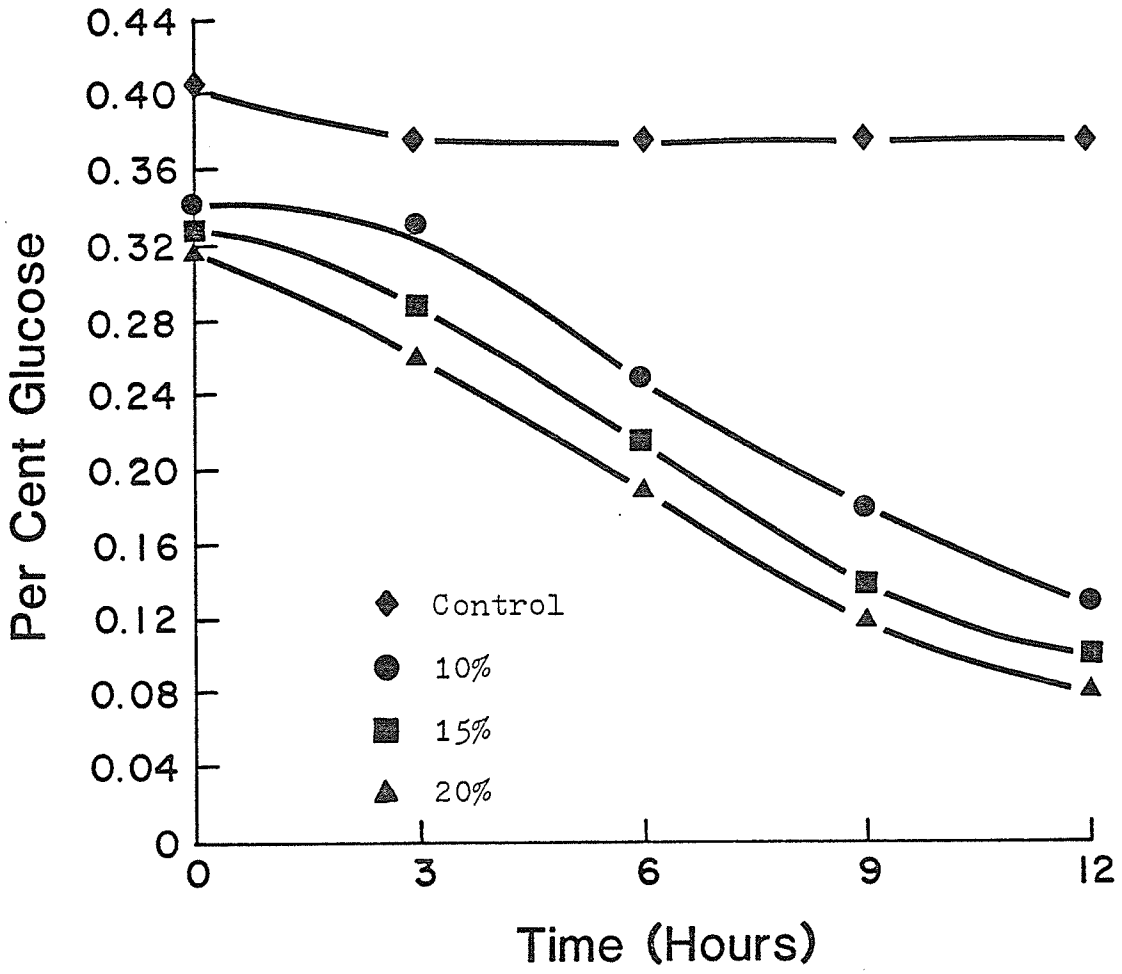
The effect of inoculum size on the rate of glucose utilization revealed that the most pronounced difference occured during the first 3 hours of fermentation. A substantial lag in glucose utilization was noted at the inoculum rate of 10%. After 3 hours of fermentation, using an inoculum size of 10%, only 0.0122% of glucose was utilized by L. mesenteroides NRRL B199. At the inoculum rate of 15% only a slight lag was present, after 3 hours of fermentation 0.0408% glucose was utilized. No lag phase at all was present when the inoculum level was raised to 20%, as after 3 hours of fermentation, a reduction of 0.0569% in free glucose was achieved.

After 12 hours of fermentation, using an inoculum level of 10% there was a 0.2096% reduction in free glucose. With an inoculum size of 15% and 20% there was a 0.2189% and 0.2319% reduction in free glucose respectively after 12 hours of fermentation.

The results indicate that at lower inoculum sizes there was a lag period where perhaps the cell number had to increase to a maximum level before glucose utilization could

Figure 6

The Effect of Inoculum Size on Rate of Glucose Utilization by Leuconostoc mesenteroides NRRL B199 in Egg White at 32°C



proceed at a higher rate. The above observation is supported by the findings of Kaplan et al. (1950), Galluzo et al. (1974), and Ayres and Stewart (1947).

## 4.6 Cell Population Analysis of Fermentation of Egg White with Leuconostoc mesenteroides Strains

This phase of research was initiated to study the growth characteristic of Leuconostoc mesenteroides strains in egg white. No published studies on the growth of Leuconostoc in egg white were found in the literature, however, Mulder (1981), Kaplan et al. (1950), Galluzzo et al. (1974) reported fermentation of egg white with the following organisms: Lactobacillus brevis, Lactobacillus casei, Lactobacillus, fementum, Lactobacillus plantarum, and Streptococcus faecalis.

In this study, egg white was fermented with Leuconostoc mesenteroides ATCC 27258 and Leuconostoc mesenteroides NRRL B199. During the fermentation at intervals of 0, 3, 6, and 9 hours, samples were aseptically taken for pH, free glucose and cell number determination. The fermentation patterns for Leuconostoc mesenteroides NRRL B100 are presented in Figure 7, (Appendix Table 4), and results for fermentation with Leuconostoc mesenteroides ATCC 27258 are presented in Figure 8 (Appendix Table 4). On the basis of acid production it was observed that L. mesenteroides NRRL B199 reached a final pH of 6.32 after 9 hours of fermentation, whereas during the same period L. mesenteroides ATTCC 27258 achieved a final pH of 6.51, both fermentations having started at an initial pH of 8.86 thus indicating that

Figure 7

Cell Count, pH, and Residual Glucose Analysis on Egg White Fermented With *Leuconostoc mesenteroides* NRRL B199 at 32°C

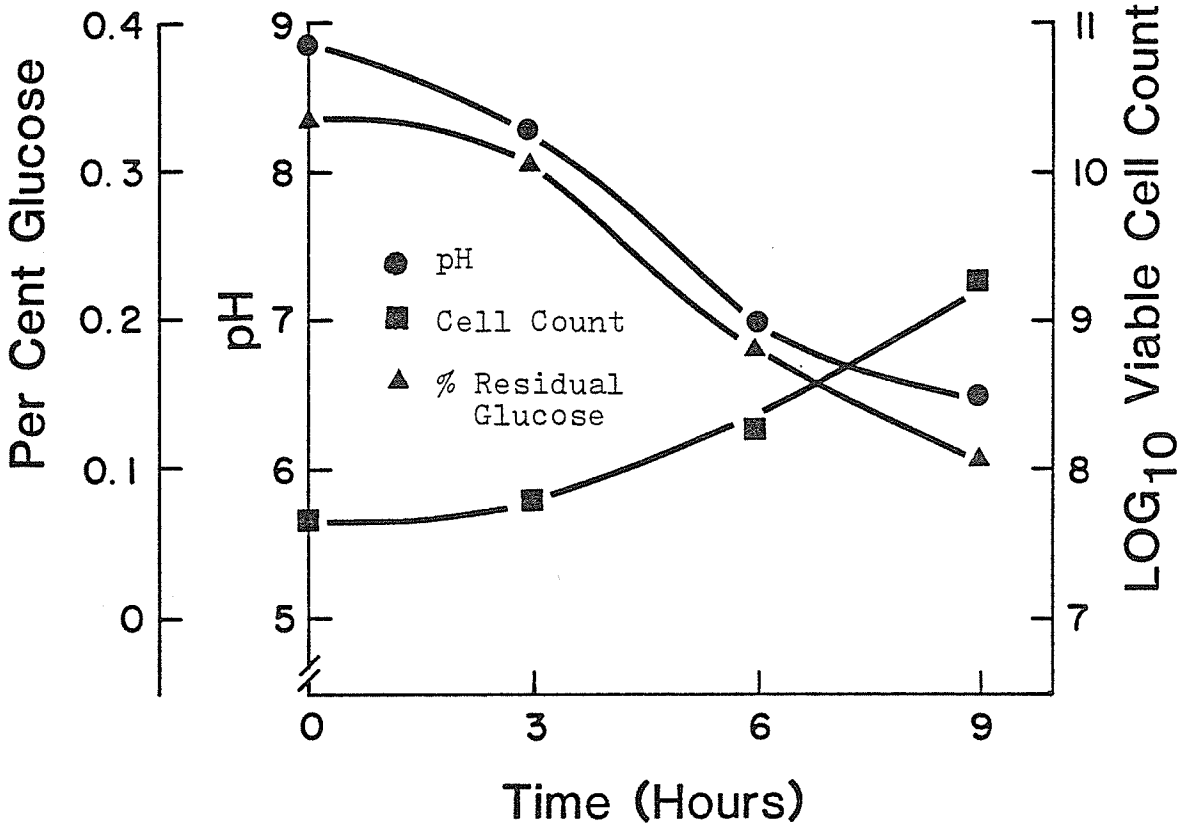
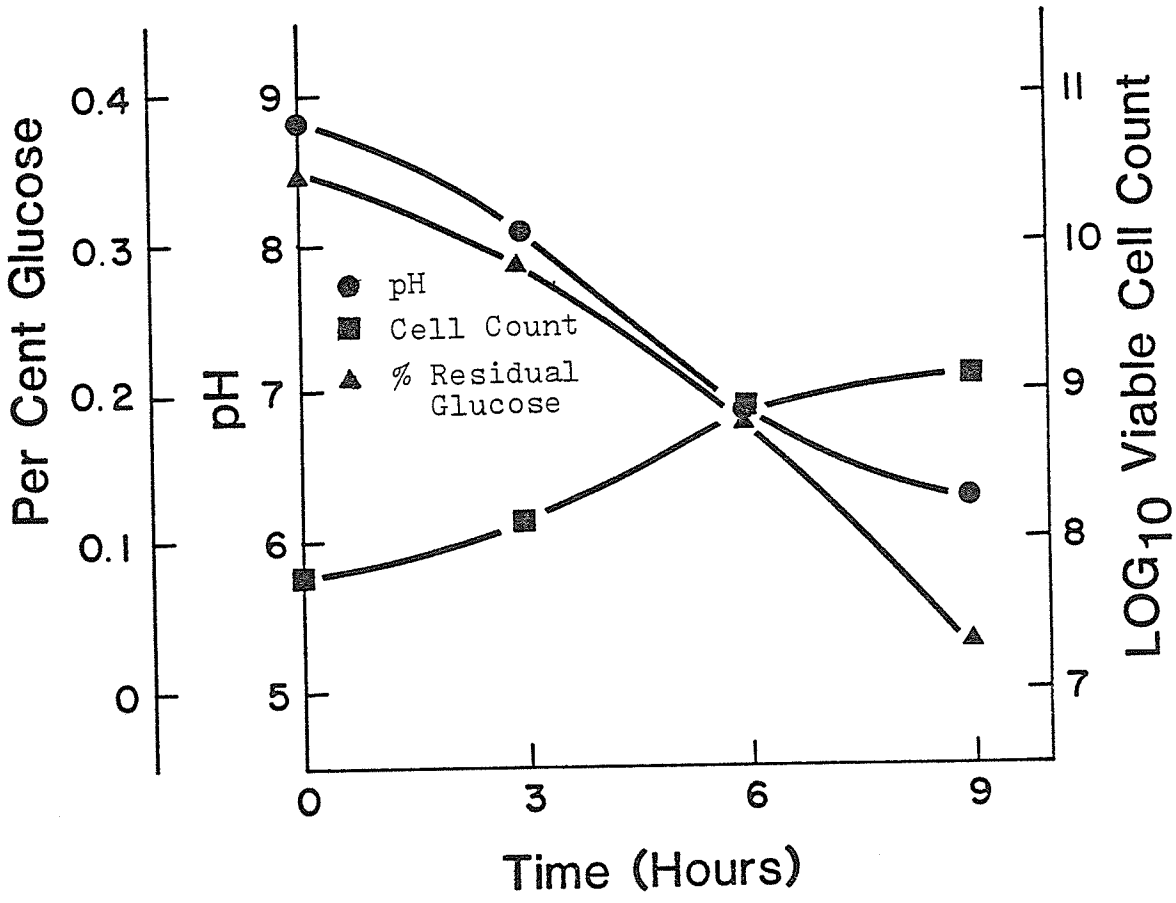


Figure 8

Cell Count, pH, and Residual Glucose Analysis on Egg White Fermented With Leuconostoc mesenteroides ATCC 27258 at 32°C



within the same period, culture NRRL B199 was slightly more efficient in acid production.

The reduction in free glucose further substantiates that L. mesenteroides NRRL B199 is the more efficient organism in the utilization of glucose. As after 9 hours of fermentation 0.3068% and 0.2271% of residued glucose was utilized by Leuconostoc mesenteroides NRRL B199 and Leuconostoc mesenteroides ATCC 27258 respectively.

These results indicate that L. mesenteroides NRRL B199 is more capable of growth in egg white than is L. mesenteroides ATCC 27258. Initial cell numbers of each organism at zero time were $4.3 \times 10^7$ per mL of egg white for L. mesenteroides ATCC 27258, and $5.6 \times 10^7$ per mL of egg white for L. mesenteroides NRRL B100. After 6 hours of fermentation the cell count for L. mesenteroides NRRL B199 was $7.6 \times 10^8$ per mL of egg white, whereas with L. mesenteroides ATCC 27258 a cell count of $1.7 \times 10^8$ per mL was achieved. At the end of the fermentation period the cell counts were $1.7 \times 10^9$ of L. mesenteroides NRRL B199 per mL egg white and $1.3 \times 10^9$ of L. mesenteroides ATCC 27258 per mL of egg white. This data can also be expressed as an increase of 1.61 log cycle for L. mesenteroides NRRL B199 cells during the 9 hours of fermentation, and an increase of 1.37 log cycle for L. mesenteroides ATCC 27258 for the same period.

The overall assessment is that L. mesenteroides NRRL B199 grows more readily in egg white thereby utilizing more free glucose; and producing more organic acids as a

result of the fermentation process. The fermentation patterns for the two L. mesenteroides strains (Figure 7 & 8) are quite similar, in that in both cases the cell numbers increased, acids were produced by the metabolism of the glucose and as a result the pH decreased.

Mulder (1981) in his recent studies of controlled bacterial fermentation by different organisms reported a 1.3 log cycle increase in growth of Lactobacillus brevis in egg white in 36 hours at 30°C, unfortunately residual glucose levels of the fermented products were not reported. He further reported that: L. casei, L. fermentum, and L. planterum did not exhibit the same extent of increase in cell number and that an average increase of less than 0.5 log cycles was shown for this organisms. Mulder (1981) also reported that fermentation of egg white with Streptococus faecalis grown for 30 hours at 37°C only exhibited a 0.4 log cycle increase in cell number. Fermentations were performed on egg white fortified with 0.1% bacteriological peptone (OXOID).

The data presented in Figures 7 and 8 were subjected to statistical analysis and correlations between decrease in pH and residual free glucose were determined. The results indicate r=0.97615 for L. mesenteroides NRRL B199 and 4=0.94365 for L. mesenteroides ATCC 27258. The analysis are tabulated in table 10.

An additional factor that was observed furing this study was the aromas and odors produced by the organisms when

TABLE 10

CORRELATION BETWEEN pH AND FREE GLUCOSE
DURING FERMENTATION (PEARSON CORRELATION)

| Organism | Correlation(R) | Significance |
|---|---|---|
| L. mesenteroides NRRL B 199 | 0.97615 | 0.0238 |
| L. mesenteroides ATCC 27258 | 0.94365 | 0.0563 |

grown in egg white. Fermentation with L. mesenteroides NRRL B199 resulted in a sweet, pleasant odor resembling the aroma of freshly risen bread. Whereas with L. mesenteroides ATCC 27258, the odor produced after fermentation was less pleasant and slightly fruity. Both of these aromas were undetectable after storage of the fermented egg white for 24 hours at 7.2°C. The only odor present after storage was the typical odor of liquid egg white. It would appear that the odors that were detected immediately after fermentation were volatile in nature.

## 4.7 Comparison Between Fermentation of Egg White with Leuconostoc mesenteroides Strains and Streptococcus cremoris at 32°C and an Inoculum Rate of 10%

The objective of this study was to ferment egg white with a Streptococci and compare the process with the fermentation conducted with Leuconostoc mesenteroides strains. In the past Streptococci have been used to ferment egg white, Kaplan et al. (1950) and Mulder (1981).

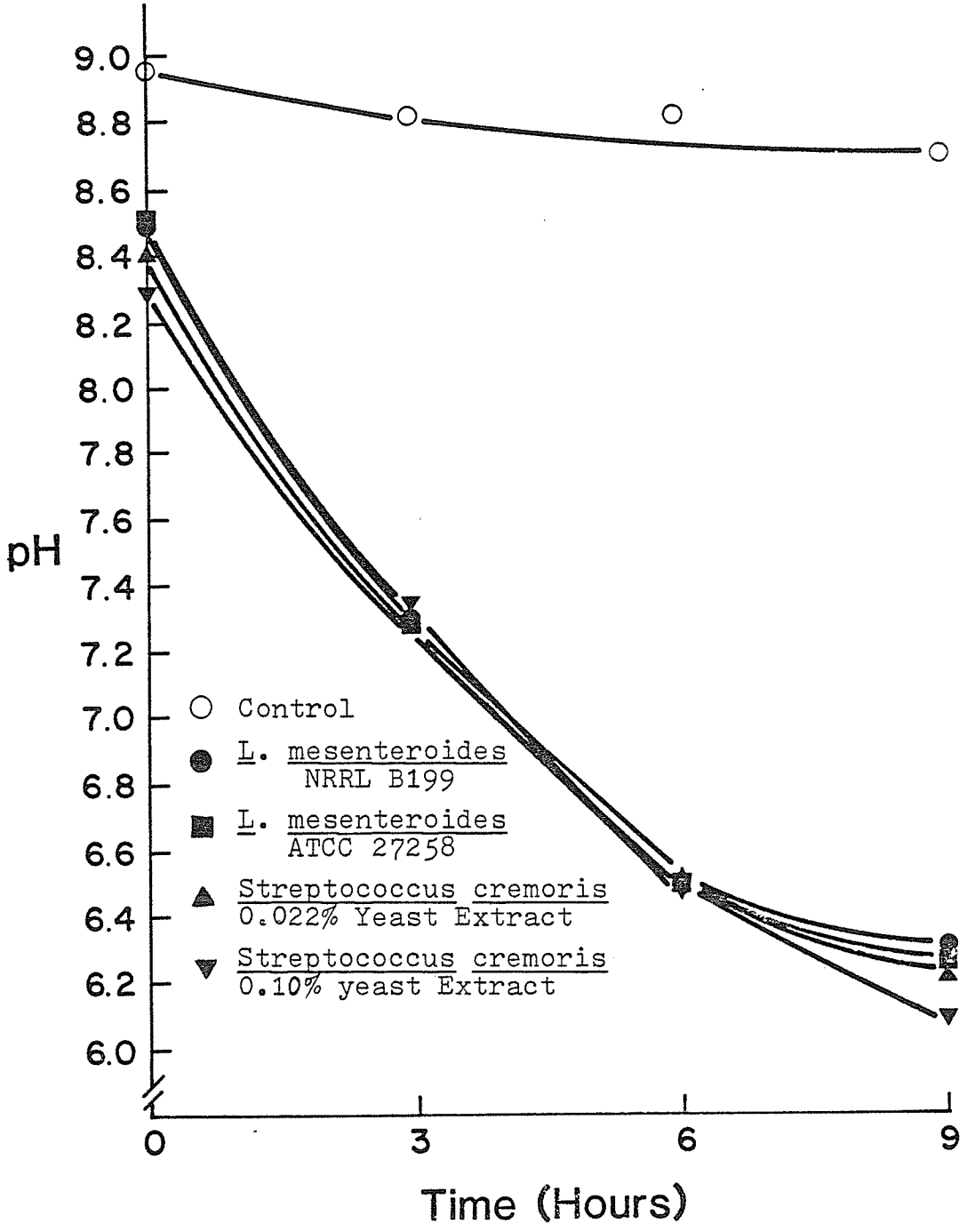
In this experiment two strains of Leuconostoc mesenteroides were used to desugar egg white, as well as a Hansen cheese starter culture consisting of Streptococcus cremoris (95%) and Betacoccus cremoric (5%) syn. to Leuconostoc cremoris. This culture was used primarily for its rapid acid production in milk during the manufacture of cheddar cheese. Fermentation with Leuconostoc organisms was performed on egg white fortified with 0.022% yeast extract. Whereas fermentations with the Hansen Streptococci culture

were performed on egg white fortified with an additional 0.1% yeast extract. Gulluzzo (1974), Kaplan et al. (1950) found it necessary to fortify egg white with 0.1% yeast extract in order for the Streptococci to grow in egg white or whole egg.

All fermentations were performed at 32°C using an inoculum level of 10% from a culture carried in egg white. Acid production by all organisms during fermentation are presented in figure 9. The data were subjected to analysis of variance A.N.O.V.A. (Appendix Table 6), and the results revealed no significant difference at the 1% confidence level between the two species on the basis of acid production as indicated by a change in pH. The results also showed that there was very little effect when the higher yeast extract level was used (on the growth of the Streptococci in eggs). Kaplan et al. (1950) and Galluzzo et al. (1974) suggested the need for the higher yeast extract level to promote the growth of the Streptococci. It was observed that Streptococci starter cultures cultured in 10% reconstituted skim milk and inoculated in egg white resulted in long lag phases of about 72 hours, even when the medium was fortified with 0.1% yeast extract. No growth was observed when the egg white was fortified with 0.022% yeast extract. The work of Kaplan et al. (1950) regarding the growth of Streptococcus liqufaciens and Streptococcus lactis resting cell suspensions reported that yeast extract concentrations of 0.1% was required to reduce the long lag phases of the cultures.

Figure 9

The Effect of Fermentation Organism on Rate of Acid Production in Egg White at 32°C



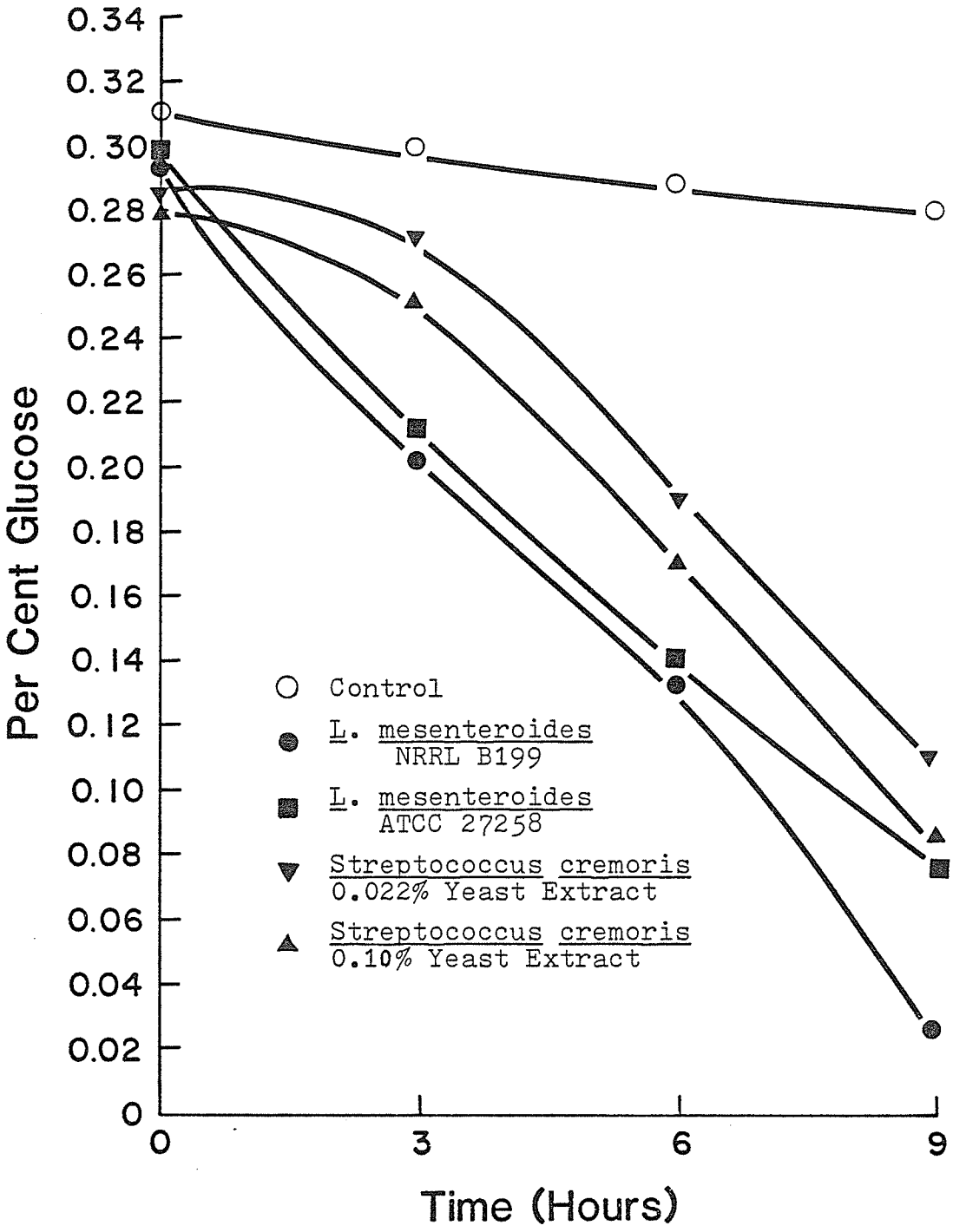
The problem of the long lag phase of Streptococcus cultures when grown in skim milk and then inoculated into egg white was overcome when the culture was grown in egg white for at least two transfers. It would appear the long lag phase was perhaps due to the difference in growth environment between skim milk and egg whites as well as the fact that the organisms require a period of adaptation before they can resume their active state of growth. The work of Mulder (1981) appears to support this explanation to a certain extent. His use of bacteriological peptone rather than yeast extract used in all other studies perhaps accounts for the remainder of the discrepancy between the fermentation times using Streptococci.

Microscopic examination of egg white fermented with the Hansen culture revealed the fermenting organism had typical Streptococci morphology, thus substaining that it was a Streptococci and not the Betacoccus responsible for the fermentation.

With respect to pH changes it was observed that acid production during and after fermentation was slightly greater for the Hansen Streptococci culture than for the Leuconostoc cultures. The difference between the species in their ability to utilize glucose in egg white is presented in figure 10. The analysis of variance, A.N.O.V.A. (Appendix table 7) on acid production and glucose utilization is presented in appendix tables 6 and 7. Although there is no significant difference between the Leuconostoc and Streptococci cultures

Figure 10

The Effect of Fermentation Organism on Rate of Glucose Utilization in Egg White at 32°C



with respect to acid production, there is a significant difference between the two species in the utilization of glucose, indicating that the analysis of the free glucose content in egg white is a more accurate method of determining the extent and completeness of fermentation than pH.

The graphs in figure 9 and 10 indicate that there is a distinct difference between the two methods of monitoring the fermentation process. The results in figure 9 do not show a significant difference between the two strains of L. mesenteroides or the Streptococci with respect to acid production as indicated by the change in pH. However, the analysis of residual glucose in the fermented product does show that there is a distinct difference between the ability of the organisms to utilize glucose. The results also showed the existence of a small difference in glucose utilization by the two strains of Leuconostoc mesenteroides as presented in figure 10 which is not evident in figure 9. Furthermore figure 10 shows that the Streptococci utilized glucose in egg white at a much slower rate that the two Leuconostoc strains. This was not evident by the analysis of acid production if figure 9. The results in figure 9 indicate that the Streptococci produce more acid than the Leuconostoc strains. However, the results in figure 10 indicates that the two Leuconostoc mesenteroides strains utilize glucose more rapidly than the Streptococci. The Leuconostoc strains did not show a lag phase as was observed during the first 3 hours of fermentation with the Streptococci.

The difference in the relationship between pH and glucose utilization is probably due to the Leuconostoc being homofermentive, and the Streptococci being heterofermentive, Bergys Manual of Determinative Bacterology (1974). The different fermentative pathways result in different end products produced during glucose metabolism. This difference could be detected organoleptically after fermentation. Fermentation with Leuconostoc mesenteroides NRRL B199 produced a sweet, oror resembling fresh risen bread. The odor produced by fermentation with Leuconostoc mesenteroides ATCC 27258 was slightly unpleasant and fruity. However, fermentation with Hansen Streptococci resulted in extremely objectionable putride odors. This is due to the production of many different compounds probably acealdehyde or compounds of egg protein proteolysis.

Kaplan et al. (1950) desugared egg white with resting cells of Streptococcus liquifaciens and Streptococcus lactis and reported no objectable odors of flavours, whith is contrary to the findings presented. Galluzzo et al. (1974) found S. diacetilactus incapable of completely desugaring whole egg, and strains of S. cremoris, S. lactis, and S. diacetilactus produce significant quantitites of acetaldehyde resulting in off odors and a "green" flavor in the whole egg. Mulder (1981) used Streptococcus cremoris and Betacoccus cremoris to desugar egg white, however, no data on odors or flavors produced were reported. It would appear that perhaps only some species or strains of Streptococci produce off odors and flavors in egg white. However, because the

Streptococcus strain used was less efficient at desugaring egg white, much research would have to be performed to find a Streptococcus strain superior to L. mesenteroides NRRL B199.

## 4.8 The Effect of Temperature on the Microbial Population of Fermented Egg White

Egg white fermented at 25°C, 32°C, and 37°C with L. mesenteroides NRRL B199 and L. mesenteroides ATCC 27258 were plated on tomato juice agar after fermentation. Starter cultures used were carried at the respective temperatures for 3 successive fermentations before plate counts were performed. This procedure allowed for the development of typical microflora upon carrying of the culture as used in industrial applications. The results are presented in Table 11.

As described in the Review of the Literature section Leuconostoc mesenteroides spp. for typical colonies that are of "pinhead" morphology, with a diameter of about 1 mm. Colonies with different morphology were counted as atypical of non-leuconostoc organisms.

As the fermentation temperature increased, the level of atypical colonies in the fermenting population increased. The increase in these atypical organisms were particularly prevalent in fermentations with L. mesenteroides ATCC 27258. At a fermentation temperature of 25°C the level of atypical colonies in egg white after reaching pH 6.5 was 21.6%, whereas the fermentation with L. mesenteroides NRRL B199 only 18.2% atypical colonies were found at the end of the

Figure 11

The Effect of Fermentation Temperature on per cent atypical Colony Forming Bacteria in Egg White

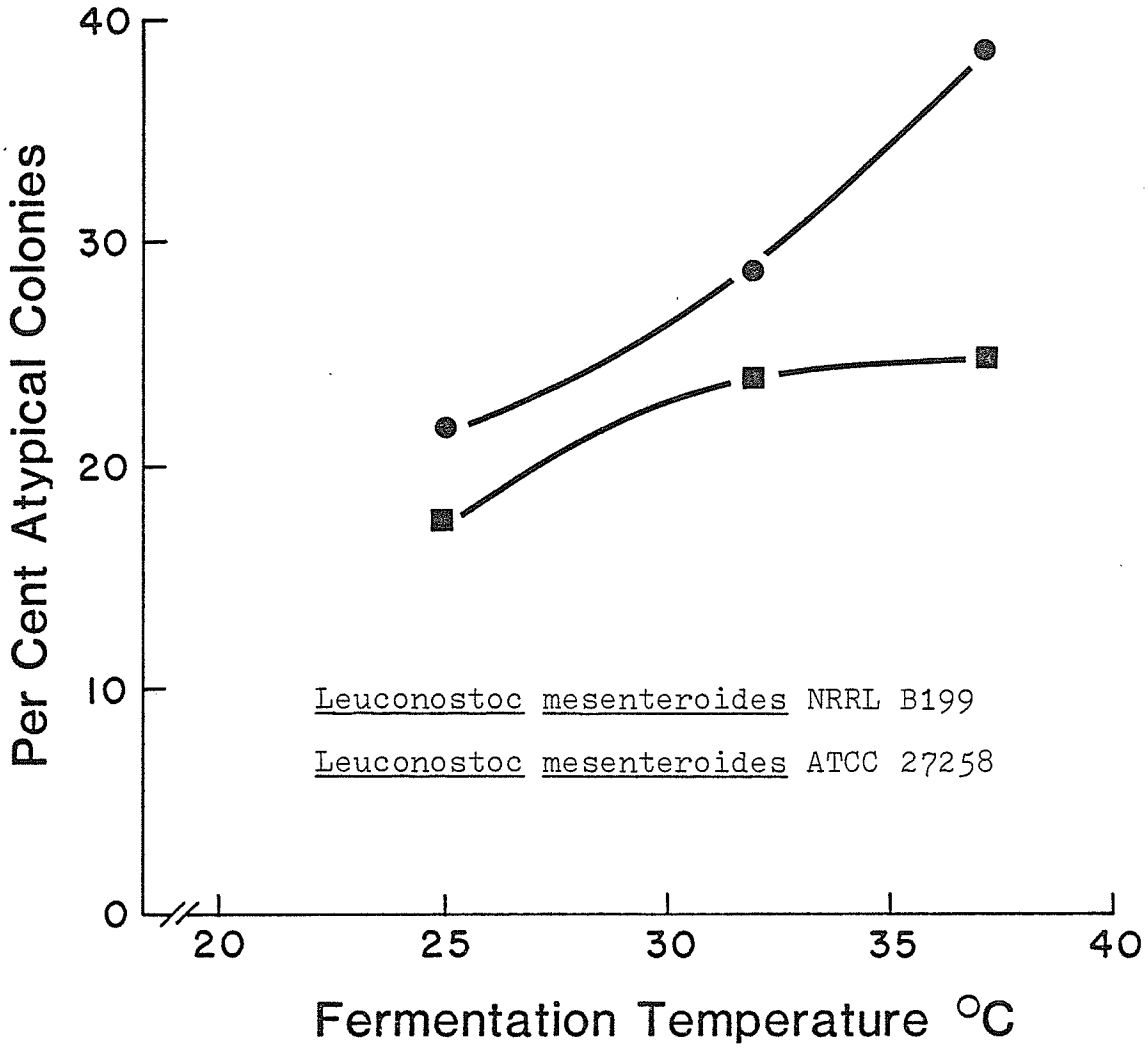


TABLE 11

THE EFFECT OF FERMENTATION TEMPERATURE ON THE PER CENT ATYPICAL COLONY FORMONG BACTERIA IN EGG WHITE

| Fermenting Organism | Fermentation Temperature | Per Cent Atypical Colonies |
|---|---|---|
| L. mesenteroides ATCC 27258 | 25 | 21.6 |
| L. mesenteroides NRRL B199 | 25 | 18.2 |
| L. mesenteroides ATCC 27258 | 32 | 28.9 |
| L. mesenteroides NRRL B199 | 32 | 24.3 |
| L. mesenteroides ATCC 27258 | 37 | 38.0 |
| L. mesenteroides NRRL B199 | 37 | 24.8 |

fermentation. This observation was further magnified as the fermentation temperature was increased. At 32°C L. mesenteroides ATCC 27258 had 28.9% atypical colonies whereas L. mesenteroides NRRL B199 showed only 24.3% atypical colonies at the end of the fermentation. As the fermentation temperature is increased higher to 37°C atypical colonies represented 38.0% of the fermentation population with L. mesenteroides ATCC 27258, and only 24.8% of the population were atypical when L. mesenteroides NRRL B199 was the fermenting organism.

The effect of temperature on atypical colony development is more visible when the results are graphed as presented in Figure 11. L. mesenteroides NRRL B199 appears to show an inhibitory effect against contaminating organisms as compared to L. mesenteroides ATCC 27258. Inhibitory effects have been reported in the literature for Leuconostoc citrovorum. Sorrells and Spech (1970) reported inhibition of Salmonella gallinarium at pH of 4.4 by a metabolite produced by Leuconostoc citrovorum. They attributed the primary inhibitory effect to acetic acid and a slightly inhibitory effect to lactic acid. Radick et al. (1969) reported that cultures of L. citrovorum extended the shelf life of cottage cheese, meat, and numerous other food products, presumably by preventing the growth of Pseudomonas species. The mechanism by which L. citrovorum inhibits microbial growth is still unresolved. Several workers have attributed the inhibitory activity to organic acids. Daly et al. (1972), Pinheiro et al. (1968), Sorrells and Speck (1970). Other researchers have suggested that hydrogen

peroxide contributes to the activity, Daly et al. (1972). However, Collins (1961), Daly et al. (1971, 1972), Mather and Babel (1959), Pinheiro et al. (1961) have suggested that the production of antimicrobial agents by L. citrovorum. Brannen et al. (1975) attributed most antimicrobial activity to organic acids as well as isolating an extracellular, water soluble, low molecular weight compound with antimicrobial activity.

These antimicrobial properties in L. citovorum are perhaps also produced by some strains of L. mesenteroides. At the present time there is no information on antimicrobial activity by L. mesenteroides strains. This is probably because L. citrovorum is used in cheese cultures and is studied more than L. mesenteroides which is used by a smaller number of egg processing plant to desugar egg white. The results obtained with L. mesenteroides ATCC 27258 and L. mesenteroides NRRL B199 do suggest some inhibitory effect on suppression of contaminating organisms, especially with L. mesenteroides NRRL B199.

## 4.9 The Effect of Culture Transfer on the Proliferation of Salmonella typhimurium LHO 67981 in Fermenting Egg White

In an attempt to determine the extent of proliferation of Salmonellae in egg white during fermentation egg white fermented with Leuconostoc mesenteroides NRRL B199 was artificially contaminated with Salmonella typhimurium LHO 67981 at levels of 10 and 100 Salmonella cells per mL of egg white. This was accomplished by the procedure as outlined

in section of Methods and Materials. The starter cultures used were 10% (v/w) aliquots obtained from a previously fermented egg white.

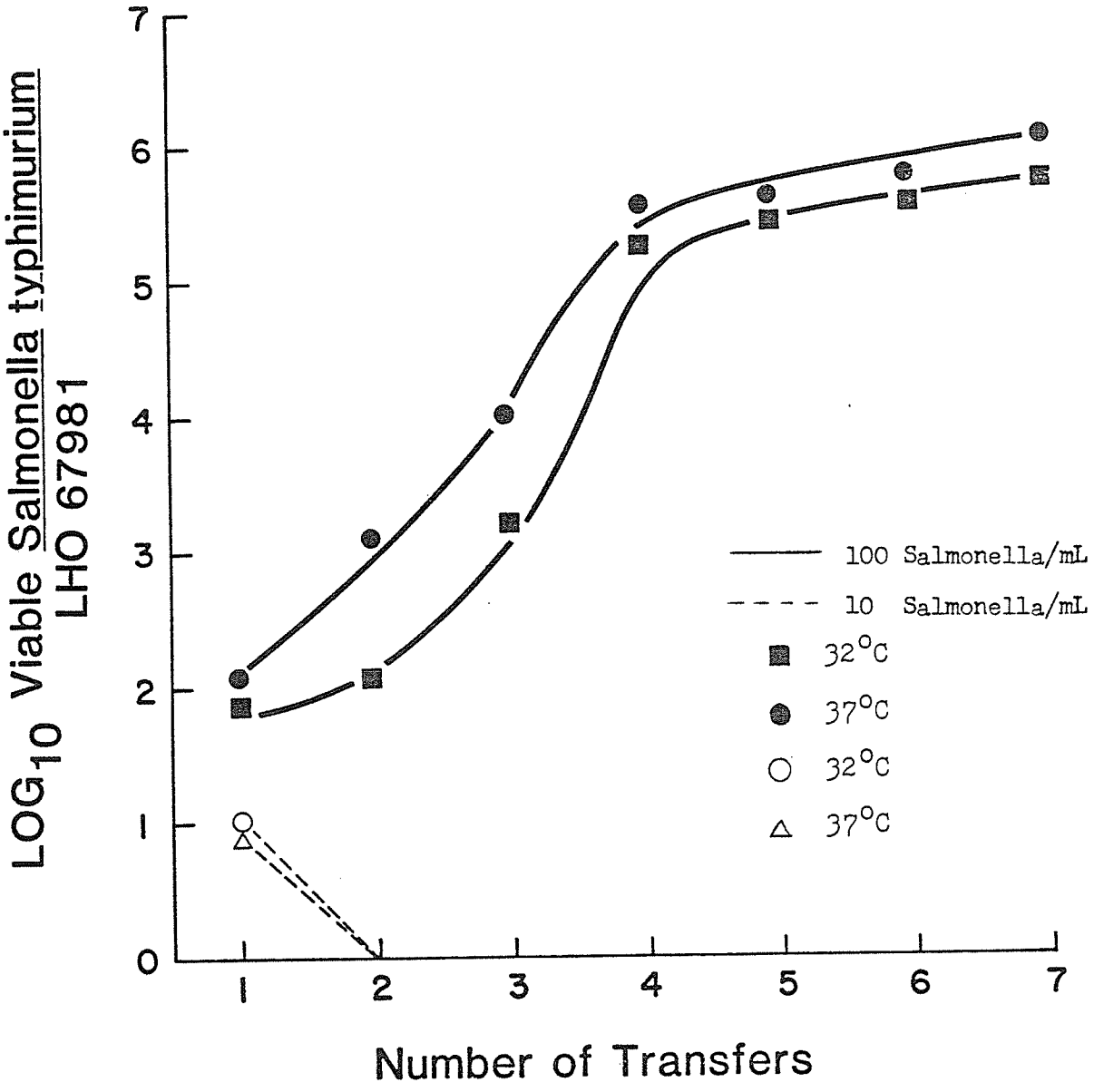
Figure 12 presents the proliferation of Salmonella as the culture is carried from one fermentation to the other. At a contamination level of about 100 Salmonella per mL of egg white there is a slight resistance of growth of Salmonella in egg white during the first transfer. After the second transfer, however, the Salmonella have adapted to growth in egg white and there is a substantial increase in number of organism. The growth of S. typhimurium in fermenting egg white is greater at 37°C than at 32°C. This is expected as studies on the effect of temperature on microbial population showed more growth of non-Leuconostoc types at 37°C. As well as the optimum temperature for growth of Salmonella to be about 37°C.

After the fourth transfer the proliferation of S. typhimurium begins to level off with counts of $3.5 \times 10^5$ Salmonella per mL of egg white at fermentation temperature 37°C, and $1.8 \times 10^5$ Salmonella per mL of egg white at fermentation temperature of 32°C. At the end of the seventh transferred fermentation the Salmonella level reached a maximum level of $1.15 \times 10^6$ Salmonella per mL of egg white at a fermentation temperature of 37°C, and $5.4 \times 10^5$ Salmonella per mL of egg white at 32°C.

When fermenting egg white was artificially contaminated with about 10 Salmonella per mL of fermenting egg white, the

Figure 12

The Effect of Culture Transfer on the Number of Salmonella typhimurium LHO 67981 in Fermenting Egg White



organism failed to establish itself at both 32°C and 37°C. That is, it was not detected after completion of the first fermentation, or any subsequent fermentations using a 10% aliquot as a starter. The loss of the organism at the low level of about 10 cells per mL of fermenting egg white may be due to the harsh growth environment of the egg white. The antimicrobial agents discussed in the Literature Review may have destroyed the Salmonella or injured them to a level that they were not detected by the sampling procedure used in this experiment.

The overall results indicate that at fermentation temperatures of 32°C or more time and more transfers are required for Salmonella to reach the same level as fermentation at 37°C. Although there is less proliferation of Salmonella at 32°C than at 37°C, fermentation temperature at 32°C does not solve the problem. The answer to the problem of Salmonella proliferation in fermenting egg white is to minimize the level of Salmonella in the raw egg white, and use starter culture which are free of Salmonella.

The present method for eliminating the Salmonella problem in dried egg white is to subject the product to a hot room pasteurization treatment, Northolt et al. (1978), McBee and Cotterill (1970). The minimum required hot room pasteurization treatment is for 7 days at 54°C. However, it is common in Canadian industry to use temperatures up to 62°C and times of 14 days. Even after this more severe heat treatment Salmonellae are sometimes detected in the

product. When this occurs, the product is once more subjected to a cycle of hot room pasteurization. The hot room process has also been found to be detrimental to functional properties if extreme heat is required to destroy the Salmonellae, Baldwin et al. (1967). Since McBee and Cotterill (1970) showed the reduction of Salmonella in egg powder to be directly related to the initial number of Salmonella in the egg powder. The greater the microbial contamination the more severe the heat treatment required to render the egg white powder Salmonella-free, that is if the Salmonella contamination in the finished product is minimized a less severe heat treatment will be required and the product will maintain its functionality.

Our study indicated that it is essential that only pure starter cultures free of pathogenic microorganisms be used for inoculating egg white, and there should be no culture transfer in the egg white fermentation process. Since occurance of cross-contamination during such practice is evident, as well as proliferation of Salmonella during the fermentation process.

# CONCLUSION

This investigation was initiated to study and optimize the controlled bacterial fermentation of egg white, and to minimize Salmonella growth during the process. Most research in the field has been conducted by private industry and information on the process is deemed strictly confidential. The findings of this study include:

1) The contents of shell egg are of low microbial count, less than 10 cells per mL when broken aseptically. An increase in pH is observed as egg white is aged. Egg white can be stored at 4.0°C for 7 days without significant bacterial growth.

2) The free glucose content of egg white used between October 5, 1980 and March 6, 1981 had a mean value of 0.3493 per cent, with a range from 0.316 to 0.412 per cent.

3) Optimum fermentation temperature for fermentation of egg white with Leuconostoc mesenteroides NRRL B199 and Leuconostoc mesenteroides ATCC 27258 was found to be 32°C as compared to 37°C used in commercial operations. Therefore it is recommended that fermentation with Leuconostoc mesenteroides strains be performed at 32°C.

4) Fermentation with Leuconostoc mesenteroides NRR1 B199 resulted in a more rapid rate of glucose utilization as compared to fermentation with Leuconostoc mesenteroides

ATCC 27258. L. mesenteroides NRRL B199 produced a fermented product with a pleasant aroma, whereas fermentation with L. mesenteroides ATCC 27258 produced a slight pungent aroma, and the Hansen #70 Streptococci culture producing extremely objectionable off odors.

5) Inoculum size did not significantly effect the rate of acid production by L. mesenteroides NRRL B199 at 32°C. Therefore it established that an inoculum size of 10% is adequate to desugar egg white.

6) Inoculum size also did not have a significant effect on the rate of glucose utilization upon fermentation with L. mesenteroides NRRL B199. Inoculum sizes of greater than 10% exhibited less of an initial lag during the first 3 hours of fermentation, however after this period sugar breakdown proceeded at a relatively constant rate independant of inoculum size.

7) Comparison between the two strains of Leuconostoc revealed that L. mesenteroides NRRL B199 was able to grow more rapidly and to a higher cell number than L. mesenteroides ATCC 27258 under similar conditions. The result of the faster growth rate and higher cell number of L. mesenteroides NRRL B199 was reflected in the utilization of glucose at a more rapid rate and production of more organic acid primarily lactic acid. L. mesenteroides NRRL B199 was capable of desugaring egg white to the acceptable level of less than 0.1% glucose in 9 hours whereas L. mesenteroides ATCC 27258 required at least 10 hours and 30 minutes to achieve this under the same conditions.

8) When the fermentation temperature was increased from 32°C to 37°C in fermenting egg white the number of non-leuconostoc or atypical colony forming organisms increased. L. mesenteroides NRRL B199 showed more inhibition to the growth of atypical colony forming organisms during fermentation than did L. mesenteroides ATCC 27258. This repressive action is thought to be due to the organic acids produced by the Leuconostoc, or perhaps was due to a low molecular weight metabolite with an inhibitory function against primarily gram negative microorganisms.

9) When egg white fermented with L. mesenteroides NRRL B199 was artificially contaminated with about 100 Salmonella typhimurium LHO 67981 cells per mL of egg white, the Salmonella organisms flourished and increased in number. The Salmonella increased to $1.8 \times 10^5$/mL after four transfers at a rate of 10% and at a fermentation temperature of 32°C, and to $3.5 \times 10^5$ per mL at a fermentation temperature of 37°C.

10) When fermenting egg white was artificially contaminated with about 10 Salmonella typhimurium LHO 67981 per mL, these Salmonella organisms were undetectable after the completion of the first fermentation, and subsequent transfers of fermented product to egg white failed to reveal any Salmonella organisms. This is perhaps due to destruction or injury of the Salmonella cells to an extent that they are not detectable by the sampling procedures used in this experiment. Thus there appears to be a threshold

level of Salmonella contamination required before the organism will establish growth and flourished in the fermenting egg white. The value is between 10 and 100 Salmonella per mL of egg white using the processes outlined in this study.

11) The recommendation of this research is to use Salmonella free starter cultures carefully prepared from pasteurized egg white. This would eliminate the possibilities of cross contamination. As well strict sanitation should be employed in the breaking and handling of the eggs and egg products prior to and during the fermentation process.

12) A prospect for further research and development would be to employ direct vat set technology to the fermentation of egg white. This would eliminate the need for production of starter cultures, and eliminate the risk of cross contamination of Salmonella and other pathogenic bacteria during the desugaring fermentation.

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# APPENDIX 1A

## THE EFFECT OF TEMPERATURE ON THE pH OF FERMENTING EGG WHITE AT AN INOCULUM RATE OF 10%

Fermenting Organism : L. mesenteroides NRRL B199
*Average values of duplicate samples

| Time Hours | Trial | pH 25°C | pH 32°C | pH 37°C |
|---|---|---|---|---|
| 0 | 1 | 7.80 | 7.90 | 7.90 |
| | 2 | 7.95 | 7.95 | 7.94 |
| 3 | 1 | 7.65 | 7.65 | 7.50 |
| | 2 | 7.92 | 7.67 | 7.73 |
| 6 | 1 | 7.30 | 7.00 | 6.80 |
| | 2 | 7.85 | 7.31 | 7.45 |
| 9 | 1 | 6.95 | 6.60 | 6.00 |
| | 2 | 7.47 | 6.65 | 6.85 |
| 12 | 1 | 6.70 | 6.40 | 6.55 |
| | 2 | 7.04 | 6.29 | 6.34 |

# APPENDIX 1B

## THE EFFECT OF TEMPERATURE ON THE pH OF FERMENTING EGG WHITE AT AN INOCULUM RATE OF 10%

Fermenting Organism: L. mesenteroides ATCC 27258
*Average value of duplicate samples

| Time Hours | Trial | pH 25°C | pH 32°C | pH 37°C |
|---|---|---|---|---|
| 0 | 1 | 8.00 | 7.80 | 7.55 |
| | 2 | 7.94 | 7.93 | 7.54 |
| 3 | 1 | 7.75 | 7.60 | 7.30 |
| | 2 | 7.73 | 7.67 | 7.69 |
| 6 | 1 | 7.60 | 6.95 | 6.75 |
| | 2 | 7.75 | 7.61 | 7.41 |
| 9 | 1 | 7.05 | 6.50 | 6.70 |
| | 2 | 7.65 | 7.09 | 7.15 |
| 12 | 1 | 6.90 | 6.50 | 6.60 |
| | 2 | 7.36 | 6.55 | 6.81 |

# APPENDIX 2

## THE EFFECT OF INOCULUM SIZE ON ACID PRODUCTION BY LEUCONOSTOC MESENTEROIDES NRRL B199 GROWN AT 32°C IN EGG WHITE

*Average value of duplicate samples

| Time Hours | Trial | pH Inoculum Size | | | | |
|---|---|---|---|---|---|---|
| | | Control | 5% | 10% | 15% | 20% |
| 0 | 1 | 9.01 | 8.90 | 8.85 | 8.75 | 8.69 |
| | 2 | 9.04 | 8.89 | 8.88 | 8.77 | 8.83 |
| 3 | 1 | 8.97 | 8.75 | 8.61 | 8.45 | 8.18 |
| | 2 | 9.05 | 8.73 | 8.71 | 8.64 | 8.50 |
| 6 | 1 | 9.08 | 7.90 | 7.56 | 7.35 | 7.22 |
| | 2 | 9.09 | 7.77 | 7.76 | 7.53 | 7.42 |
| 9 | 1 | 9.08 | 7.38 | 7.07 | 6.95 | 6.90 |
| | 2 | 9.11 | 7.16 | 7.15 | 7.04 | 7.02 |
| 12 | 1 | 9.12 | 7.10 | 6.93 | 6.86 | 6.78 |
| | 2 | 9.14 | 6.94 | 6.95 | 6.86 | 6.92 |

# APPENDIX 3

## THE EFFECT OF INOCULUM SIZE (%) ON RESIDUE GLUCOSE IN EGG WHITE FERMENTED BY L. MESENTEROIDES NRRL B199

*Average values of duplicate samples

| Time Hours | Trial | Per Cent Free Glucose | | | |
|---|---|---|---|---|---|
| | | Control | 10% | 15% | 20% |
| 0 | 1 | 0.405 | 0.375 | 0.367 | 0.358 |
| | 2 | 0.407 | 0.324 | 0.310 | 0.282 |
| 3 | 1 | 0.364 | 0.336 | 0.309 | 0.308 |
| | 2 | 0.366 | 0.329 | 0.270 | 0.268 |
| 6 | 1 | 0.369 | 0.255 | 0.201 | 0.211 |
| | 2 | 0.385 | 0.247 | 0.241 | 0.160 |
| 9 | 1 | 0.364 | 0.159 | 0.155 | 0.147 |
| | 2 | 0.382 | 0.211 | 0.130 | 0.126 |
| 12 | 1 | 0.377 | 0.115 | 0.107 | 0.089 |
| | 2 | 0.377 | 0.152 | 0.117 | 0.102 |

# APPENDIX 4

## CELL POPULATION ANALYSIS OF FERMENTATION OF EGG WHITE WITH LEUCONOSTOC MESENTEROIDES STRAINS

*Average values of duplicate samples

| Time Hours | Trial | Log Cell Number Control | L. mesenteroides NRRL B199 | L. mesenteroides ATCC 27258 |
|---|---|---|---|---|
| 0 | 1 | 1.46 | 7.53 | 7.79 |
| | 2 | 1.48 | 7.72 | 7.69 |
| 3 | 1 | 1.89 | 7.67 | 7.98 |
| | 2 | 1.93 | 7.98 | 8.29 |
| 6 | 1 | 2.69 | 8.34 | 8.70 |
| | 2 | 2.76 | 8.07 | 9.00 |
| 9 | 1 | 4.84 | 9.22 | 9.13 |
| | 2 | 3.89 | 9.26 | 9.11 |

| Time Hours | Trial | pH Control | L. mesenteroides NRRL B199 | L. mesenteroides ATCC 27258 |
|---|---|---|---|---|
| 0 | 1 | 8.85 | 8.85 | 8.85 |
| | 2 | 8.86 | 8.86 | 8.86 |
| 3 | 1 | 8.77 | 8.23 | 8.11 |
| | 2 | 8.82 | 8.10 | 8.33 |
| 6 | 1 | 8.79 | 6.47 | 6.91 |
| | 2 | 8.84 | 6.76 | 7.02 |
| 9 | 1 | 8.82 | 6.47 | 6.36 |
| | 2 | 8.85 | 6.28 | 6.54 |

| Time Hours | Trial | Per Cent Residual Glucose Control | L. mesenteroides NRRL B199 | L. mesenteroides ATCC 27258 |
|---|---|---|---|---|
| 0 | 1 | 0.3256 | 0.3254 | 0.3253 |
| | 2 | 0.3438 | 0.3438 | 0.3438 |
| 3 | 1 | 0.3256 | 0.2764 | 0.2959 |
| | 2 | 0.3410 | 0.2880 | 0.3413 |
| 6 | 1 | 0.3253 | 0.1370 | 0.1676 |
| | 2 | 0.3361 | 0.2391 | 0.2296 |
| 9 | 1 | 0.3249 | 0.0030 | 0.0050 |
| | 2 | 0.3304 | 0.550 | 0.1286 |

# APPENDIX 5

## THE EFFECT OF CULTURE TRANSFER ON THE PROLIFERATION OF *Salmonella typhimurium* LHO 67981 IN FERMENTING EGG WHITE

FERMENTING ORGANISM : *Leuconostoc meseteroides* NRRL B199

$Log_{10}$ *Salmonella typhimurium* LHO 67981

| FERMENTATION TEMPERATURE | ARTIFICIAL CONTAMINATION LEVEL | TRANSFER NUMBER | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| 32°C | 0.98 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32°C | 1.76 | 2.03 | 3.18 | 5.26 | 5.44 | 5.54 | 5.73 |
| 37°C | 0.85 | 0 | 0 | 0 | 0 | 0 | 0 |
| 37°C | 1.93 | 3.10 | 3.98 | 5.54 | 5.57 | 5.74 | 6.06 |

# APPENDIX 6

## Statistical Analysis of Acid Production During Egg White Fermentation with Leuconostoc mesenteroides NRRL B199, Leuconostoc mesenteroides ATCC 27258, and Streptococcus cremoris

### ANALYSIS OF THE VARIANCE (ANOVA)

### EFFECT OF ACID PRODUCTION

| Source | df | SS | MS | F | PR F |
|---|---|---|---|---|---|
| Runs | 1 | 0.8686 | 3.0448 | 141.87 | 0.0001 |
| Organisms | 2 | 0.0286 | | | 0.5202* |
| Time | 3 | 35.5475 | | | 0.0001 |
| Org* Time | 6 | 0.1034 | | | 0.5743* |
| Error | 35 | 0.7512 | 0.0215 | | |
| Total | 47 | 37.2993 | | | |

* No Significant Difference

# APPENDIX 7

## Statistical Analysis of Glucose Utilization During Fermentation of Egg White by Leuconostoc mesenteroides NRRL B199, Leuconostoc mesenteroides ATCC 27258, and Streptococcus cremoris

### ANALYSIS OF THE VARIENCE (ANOVA)

### EFFECT OF GLUCOSE UTILIZATION

| Source | df | SS | MS | F | PR F |
|---|---|---|---|---|---|
| Runs | 1 | 0.0001 | 0.0359 | 162.48 | 0.8844 |
| Organisms | 2 | 0.0090 | | | 0.0001* |
| Time | 3 | 0.3732 | | | 0.0001 |
| Org* Time | 6 | 0.4928 | | | 0.0001* |
| Error | 35 | 0.4393 | 0.0002 | | |
| Total | 47 | 1.3144 | | | |

*Significant Difference